CHAPTER 1.2.1

The Genera *Staphylococcus* and *Macrococcus*

FRIEDRICH GÖTZ, TAMMY BANNERMAN AND KARL-HEINZ SCHLEIFER

Introduction

The name *Staphylococcus* (staphyle, bunch of grapes) was introduced by Ogston (1883) for the group micrococci causing inflammation and suppuration. He was the first to differentiate two kinds of pyogenic cocci: one arranged in groups or masses was called "*Staphylococcus*" and another arranged in chains was named "Billroth's *Streptococcus*." A formal description of the genus *Staphylococcus* was provided by Rosenbach (1884). He divided the genus into the two species *Staphylococcus aureus* and *S. albus*. Zopf (1885) placed the mass-forming staphylococci and tetrad-forming micrococci in the genus *Micrococcus*. In 1886, the genus *Staphylococcus* was separated from *Micrococcus* by Flügge (1886). He differentiated the two genera mainly on the basis of their action on gelatin and on relation to their hosts. Staphylococci liquefied gelatin and were parasitic or pathogenic or both whereas micrococci were variable in their action on gelatin and were saprophytic. The genera *Staphylococcus*, *Micrococcus* and *Planococcus*, containing Gram-positive, catalase-positive cocci, were later placed in the family Micrococcaceae. Evans et al. (1955) proposed separating staphylococci from micrococci on the basis of their relation to oxygen. The facultative anaerobic cocci were placed in the genus *Staphylococcus* and the obligate aerobic cocci in the genus *Micrococcus*. By the mid-1960s, a clear distinction could be made between staphylococci and micrococci on the basis of their DNA base composition (Silvestri and Hill, 1965). Members of the genus *Staphylococcus* have a DNA G+C content of 33–40 mol%, whereas members of the genus *Micrococcus* have a high G+C content of around 70 mol%. Further studies have shown that staphylococci can be distinguished from micrococci and other catalase-positive cocci on the basis of their cell wall composition (Schleifer and Kandler, 1972; Endl et al., 1983), cytochrome profile (Faller et al., 1980) and menaquinone pattern (Collins and Jones, 1981), susceptibility to lysostaphin and erythromycin (Schleifer and Kloos, 1975b), bacitracin (Falk and Guering, 1983), and fura-zolidone (Baker, 1984). Comparative immunochemical studies of catalases (Schleifer, 1986), DNA-DNA hybridization studies, DNA-rRNA hybridization studies (Schleifer et al., 1979; Kilpper et al., 1980), and comparative oligonucleotide cataloguing of 16S rRNA (Ludwig et al., 1981) clearly demonstrated the epigenetic and genetic difference of staphylococci and micrococci. Members of the genus *Staphylococcus* form a coherent and well-defined group of related species that is widely divergent from those of the genus *Micrococcus*. Until the early 1970s, the genus *Staphylococcus* consisted of three species: the coagulase-positive species *S. aureus* and the coagulase-negative species *S. epidermidis* and *S. saprophyticus*, but a deeper look into the chemotaxonomic and genotypic properties of staphylococci led to the description of many new staphylococcal species. Currently, 36 species and several subspecies are recognized in the genus *Staphylococcus* (Table 1).

The genus *Macrococcus* has been described on the basis of comparative 16S rRNA analysis, DNA-DNA hybridization studies, ribotype patterns, cell wall composition, and phenotypic characteristics (Kloos et al., 1998a). Macrococci can be separated from staphylococci on the basis of a generally higher DNA G+C content (38–45 mol%), absence of cell wall teichoic acids (with the possible exception of *M. caseolyticus*), unique ribotype patterns, and generally larger cells. Members of the genus *Macrococcus* are also oxidase positive whereas most staphylococci (exceptions: *S. lentus*, *S. sciuri* and *S. vitulus*) are oxidase negative. There are four species in the genus *Macrococcus* (Table 1).

Isolation Techniques

Isolation of *S. aureus* from Foods

Staphylococcus aureus has been confirmed to be the causative agent of many cases of severe food poisoning; therefore, its presence in foods is of major concern. *Staphylococcus aureus* is very susceptible to heat treatment and most sanitiz-

Table 1. List of species and subspecies in the genera *Macrococcus* and *Staphylococcus*.

Species name	References[a]
Macrococcus bovicus	Kloos et al., 1998a
M. carouselicus	Kloos et al., 1998a
M. caseolyticus	Schleifer et al., 1982
	Kloos et al., 1998a
M. equipercicus[T]	Kloos et al., 1998a
Staphylococcus arlettae	Schleifer et al., 1984
S. *auricularis*	Kloos and Schleifer, 1983a
S. *aureus*[T]	Rosenbach, 1884
S. *aureus* subsp. *anaerobius*	De la Fuente et al., 1985
S. *aureus* subsp. *aureus*	De la Fuente et al., 1985
S. *capitis*	Kloos and Schleifer, 1975b
S. *capitis* subsp. *capitis*	Bannerman and Kloos, 1991
S. *capitis* subsp. *urealyticus*	Bannerman and Kloos, 1991
S. *caprae*	Devriese et al., 1983
S. *carnosus*	Schleifer and Fischer, 1982
S. *carnosus* subsp. *carnosus*	Probst et al., 1998
S. *carnosus* subsp. *utilis*	Probst et al., 1998
S. *chromogenes*	Devriese et al., 1978
S. *cohnii*	Schleifer and Kloos, 1975c
S. *cohnii* subsp. *cohnii*	Kloos and Wolfshohl, 1983b
S. *cohnii* subsp. *urealyticus*	Kloos and Wolfshohl, 1983b
S. *condimenti*	Probst et al., 1998
S. *delphini*	Varaldo et al., 1988
S. *epidermidis*	Winslow and Winslow, 1908
S. *equorum*	Schleifer et al., 1984
S. *felis*	Igimi et al., 1989
S. *fleurettii*	Vernozy-Rozand et al., 2000
S. *gallinarum*	Devriese et al., 1983
S. *haemolyticus*	Schleifer and Kloos, 1975c
S. *hominis*	Kloos and Schleifer, 1975b
S. *hominis* subsp. *hominis*	Kloos et al., 1998b
S. *hominis* subsp. *novobiosepticus*	Kloos et al., 1998b
S. *hyicus*	Devriese et al., 1978
S. *intermedius*	Hájek, 1976a
S. *kloosii*	Schleifer et al., 1984
S. *lugdunensis*	Freney et al., 1988
S. *lutrae*	Foster et al., 1997
S. *muscae*	Hájek et al., 1992
S. *pasteuri*	Chesneau et al., 1993
S. *piscifermentans*	Tanasupawat et al., 1992
S. *pulverei* (= *S. vitulinus*)	Petras, 1998
	Zakrzewska-Czerwinska et al., 1995
S. *saccharolyticus*	Kilpper-Bälz and Schleifer, 1981
S. *saprophyticus*	Schleifer and Kloos, 1975c
S. *saprophyticus* subsp. *bovis*	Hájek et al., 1996
S. *saprophyticus* subsp. *saprophyticus*	Hájek et al., 1996
S. *schleiferi*	Freney et al., 1988
S. *schleiferi* subsp. *coagulans*	Igimi et al., 1990
S. *schleiferi subsp. schleiferi*	Igimi et al., 1990
S. *sciuri*	Kloos et al., 1976a
S. *sciuri* subsp. *carnaticus*	Kloos et al., 1997
S. *sciuri* subsp. *lentus*	Kloos et al., 1997
S. *sciuri* subsp. *rodentium*	Kloos et al., 1997
S. *sciuri* subsp. *sciuri*	Kloos et al., 1997
S. *simulans*	Kloos and Schleifer, 1975b
S. *succinus*	Lambert et al., 1998
S. *vitulinus*	Webster et al., 1994
S. *warneri*	Kloos and Schleifer, 1975b
S. *xylosus*	Schleifer and Kloos, 1975c

Abbreviation: [T], type species.

[a]First description.

ing agents. Hence, when it or its enterotoxins are found in processed foods, poor sanitation is usually indicated. Detailed procedures for preparing food samples for analysis, isolating and enumerating *S. aureus*, and detecting staphylococcal enterotoxins in foods can be found in the following texts: *Compendium of Methods for the Microbiological Examination of Foods* (Downes and Ito, 2001), *Official Methods of Analysis of AOAC International* (Horowitz, 2000), and *Bacteriological Analytical Manual* (BAM; United States Food and Drug Administration, 1995). In addition to the AOAC (Association of Official Analytical Chemists) approved microslide test method (Horowitz, 2000), it is possible to detect enterotoxins directly in culture and in contaminated foods by the following rapid methods: radioimmunoassay (RIA) (Miller et al., 1978), enzyme-linked immunosorbent assay (ELISA), and reverse passive latex agglutination (RPLA). An ELISA kit, available from Tecra Diagnostics (Roseville, Australia), is distributed by International Bioproducts, Inc. (Redmond, WA, USA), and an RPLA is available from Oxoid (Columbia, MD, USA). Molecular methods are being investigated for their usefulness in detecting staphylococcal enterotoxin. For example, Western immunoblotting has been used to detect staphylococcal enterotoxin A (Rasooly and Rasooly, 1998). In this procedure, the staphylococcal enterotoxin A (native or heat-denatured) is separated by size using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), transferred to a membrane and then the membrane is probed with antibodies. The polymerase chain reaction (PCR) is an additional molecular method being studied for its ability to detect *S. aureus* and staphylococcal enterotoxins from food samples (McLauchlin et al., 2000; Atanassova et al., 2001; Tamarapu et al., 2001). One potential problem for PCR is the possibility of false-negative reactions due to PCR inhibitors that might be present in some foods.

Several conventional procedures for isolating *S. aureus* from foods are described below.

NONSELECTIVE ENRICHMENT PROCEDURES It is often necessary to use nonselective enrichment procedures for the detection of *S. aureus* in processed foods, especially when it is suspected that the food contains a small number of cells that may have been injured, e.g., as a result of heating, freezing, desiccation, or storage, and whose growth could be inhibited by toxic components of a selective enrichment media. The following nonselective (repair) enrichment procedure is appropriate for this use:

Nonselective Enrichment of *S. aureus* (Downes and Ito, 2001).

Transfer a 50-ml aliquot of a 1:10 dilution of the food sample homogenate to 50 ml of double-strength trypticase soy broth (TSB). Incubate the preparation for 3 h at 35–37°C. Then add 100 ml of a single-strength TSB containing 20% NaCl. Incubate for 24 ± 2 h at 35–37°C. Transfer 0.1-ml aliquots of the culture to each of duplicate Baird-Parker agar plates, and spread the inoculum, so as to obtain isolated colonies. Incubate the inoculated plates for 46 ± 2 h at 35–37°C. Select two or more colonies suspected to be *S. aureus* from each plate. *Staphylococcus aureus* colonies are usually 1.5 mm in diameter, jet-black to dark gray, smooth, convex, have entire margins and off-white edges, and may show an opaque zone and/or a clear halo extending beyond the opaque zone. Test selected colonies for coagulase activity. Results should be reported as *S. aureus* present or absent in 5 g of food, following the results of coagulase testing or clumping factor testing.

Coagulase Test (Horowitz, 2000; Downes and Ito, 2001).

Transfer colonies to tubes containing 0.2 ml of brain heart infusion (BHI) broth. Incubate culture suspensions 18–24 h at 35–37°C. Add 0.5 ml of reconstituted coagulase plasma with ethylenediaminetetraacetic acid (EDTA) and mix thoroughly. (The plasma is reconstituted according to manufacturer's directions. If not available, desiccated coagulase plasma (rabbit) is reconstituted and disodium dihydrate EDTA is added to final concentration of 0.1% in reconstituted plasma.) Incubate at 35–37°C and examine periodically during a 6-h interval for clot formation. Any degree of clot formation is considered a positive reaction. Small or poorly organized clots may be observed by gently tipping tube so that liquid portion of reaction mixture approaches lip of tube; clots will protrude above liquid surface. Coagulase-positive cultures are considered to be *S. aureus*. Test positive and negative controls simultaneously with cultures of unknown coagulase reactivity. Recheck doubtful coagulase test results on BHI cultures which have been incubated at 35–37°C for > 18 but ≤ 48 h.

This procedure is recommended by the AOAC for the identification of *S. aureus* isolated from foods. With this procedure, false-positive tests may occur with mixed cultures, but this will probably be avoided if only well-isolated colonies typical of *S. aureus* are chosen. On rare occasions, coagulase-negative mutants of *S. aureus* may be present in foods and overlooked by the above procedures, and the presence of other coagulase-positive staphylococci, such as *S. intermedius* and certain strains of *S. hyicus*, may be misrepresented as *S. aureus* using the coagulase test alone.

Baird-Parker agar base when supplemented with egg yolk tellurite enrichment is recommended for the detection and enumeration of coagulase-positive staphylococci in foods.

Baird-Parker Agar

Basal medium:	
Tryptone	10.0 g
Beef extract	5.0 g
Yeast extract	1.0 g
Glycine	12.0 g
Lithium chloride · 6H$_2$O	5.0 g
Agar	20.0 g

This basal medium may be special-ordered from Difco Laboratories, Detroit, MI. Suspend ingredients in 950 ml distilled water. Boil to dissolve completely. Dispense 95.0 ml portions in screw-capped bottles. Autoclave 15 min at 121°C. Adjust final pH to 6.8–7.2 at 25°C.

Egg Yolk Tellurite Enrichment

Soak eggs in aqueous mercuric chloride 1:1000 for not less than one min. Rinse in sterile water and dry with a sterile cloth. Aseptically crack eggs and separate whites and yolks. Blend yolk and sterile physiological saline solution (3 + 7 v/v) in high-speed sterile blender for 5 s. Mix 50.0 ml of blended egg yolk with 10.0 ml of filter-sterilized 1% potassium tellurite. Mix and store at 2–8°C. Bacto egg-tellurite enrichment is a commercial preparation available from Difco Laboratories.

Preparation of Plates

Add 5.0 ml of prewarmed (45–50°C) enrichment to 95 ml of melted basal medium, which has been adjusted to 45–50°C. Mix well (avoiding bubbles), and pour 15.0–18.0 ml into sterile 15 × 100 mm Petri dishes. Plates can be stored at 2–8°C in plastic bags for 4 weeks. Immediately prior to use, spread 0.5 ml per plate of 20% solution of (membrane) filter-sterilized sodium pyruvate and dry plates at 50°C for 2 h or 4 h at 35°C with agar surface uppermost.

SELECTIVE ENRICHMENT PROCEDURES Selective enrichment is recommended for raw food ingredients and unprocessed foods expected to contain < 100 *S. aureus* cells/g and a large population of competing species. The recommended procedure of the AOAC is widely accepted and uses the most probable number technique.

Most Probable Number (MPN) Technique (Horowitz, 2000).

Inoculate three tubes of trypticase soy broth with 10% NaCl and 1% sodium pyruvate at each test dilution with 1-ml aliquots of decimal dilutions of sample. Highest dilution of sample must give a negative endpoint. Incubate 48 h ± 2 h at 35°C. Using 3-mm loop, transfer 1 loopful from each tube showing growth to dried Baird-Parker medium. Vortex-mix tubes before streaking if growth is visible only on bottom or sides of tubes. Streak inoculum to obtain isolated colonies. Incubate 48 h at 35–37°C. For each plate showing growth, transfer 1 colony suspected to be *S. aureus* to BHI broth. With sterile needle, transfer

colonies to tubes containing 0.2 ml of brain heart infusion (BHI) broth and to agar slants containing any suitable maintenance medium, e.g., trypticase soy agar, standard plate-count agar, etc. Incubate BHI culture suspensions and slants 18–24 h at 35°C. The BHI culture suspensions are used as inocula for the coagulase test (described above), and the slant cultures are used for ancillary tests or repeats of the coagulase test, if results are questionable. Report *S. aureus*/g as MPN/g, according to tables of MPN values (United States Food and Drug Administration, 1995).

Direct Surface Plating Procedures These procedures are sometimes preferred over the MPN technique for the detection of *S. aureus* in raw or unprocessed foods as they are more rapid and are regarded by some investigators to be more accurate than MPN.

Surface Plating Procedure for the Enumeration of S. aureus *(Horowitz, 2000)* For each dilution to be plated, aseptically transfer 1 ml of sample suspension to triplicate plates of Baird-Parker agar and distribute the 1 ml of inoculum equally over the triplicate plates (e.g., 0.4, 0.3, and 0.3 ml). Aseptically, spread the inoculum over the surface of the agar. Avoid the extreme edges of the plate. Maintain the plates in an upright position until the inoculum is adsorbed by the medium (about 10 min on properly dried plates). If the inoculum is not readily adsorbed, plates may be placed in an incubator in an upright position for about 1 h before inverting. Invert plates and incubate 45–48 h at 35–37°C. Select plates containing 20–200 colonies unless plates at only lower dilutions (> 200 colonies) have colonies with the typical appearance of *S. aureus*. If several types of colonies appear to be *S. aureus*, count the number of colonies of each type and record counts separately. When plates at the lowest dilution plated contain < 20 colonies, they may be used. If plates containing > 200 colonies have colonies with the typical appearance of *S. aureus* and typical colonies do not appear on plates at higher dilutions, use these plates for enumeration of *S. aureus*, but do not count non-typical colonies. Select one or more colonies of each type counted and test for coagulase production. Coagulase-positive cultures may be considered to be *S. aureus*. Add the number of colonies on triplicate plates represented by colonies giving a positive coagulase test, and multiply the total by the same dilution factor. Report this number as *S. aureus* per gram of product tested. The sensitivity of this procedure may be increased by using larger volumes (> 1 ml) distributed over > 3 replicate plates. Plating of two

or more decimal dilutions may be required to obtain plates with the desired number of colonies per plate.

Direct enumeration of coagulase-positive *S. aureus* can be made on Baird-Parker agar containing rabbit plasma-fibrinogen tellurite (Boothby et al., 1979) or Baird-Parker agar without egg yolk to which a tempered pork plasma-fibrinogen overlay agar has been added (Hauschild et al., 1979). In place of Baird-Parker agar, some laboratories have reported the successful use of tellurite polymyxin egg yolk agar (Crisley et al., 1964), Kaliumrhodanid (i.e., potassium thiocyanate)-Actidione-Natriumazid (i.e., sodium azide)-Egg yolk-Pyruvate (KRA-NEP) agar (Sinell and Baumgart, 1966), and Schleifer-Krämer (SK) agar (Schleifer and Krämer, 1980) for the selective isolation and enumeration of staphylococci from foods.

SK Agar for Selective Isolation of Staphylococci (Schleifer and Krämer, 1980)

Basal medium:	
Tryptone or peptone from casein	10.0 g
Beef extract	5.0 g
Yeast extract	3.0 g
Glycerol	10.0 g
Sodium pyruvate	10.0 g
Glycine	0.5 g
KSCN	2.25 g
$NaH_2PO_4 \cdot H_2O$	0.6 g
$Na_2HPO_4 \cdot 2H_2O$	0.9 g
LiCl	2.0 g
Agar	13.0 g
Distilled H_2O	1 liter

Adjust pH to 7.2. Autoclave at 121°C for 15 min, cool down in water bath to 45°C and add 10 ml of a 0.45% sterile-filtered solution of sodium azide. Mix medium thoroughly and pour immediately into Petri dishes. The medium can be stored at 4°C for at least one week. Staphylococci can be detected in various foods at levels as low as 100 colony-forming-units (CFU)/g of food. As in the case of KRANEP agar, the addition of egg yolk or pork plasma to the basal medium can provide a basis for distinguishing *S. aureus* from the coagulase-negative staphylococci.

The recovery of some animal species (e.g., *S. caprae* and *S. chromogenes*) can be improved by adding 5% sheep blood and/or reducing the level of sodium azide in SK agar from 45 to 15 mg/liter (Harvey and Gilmour, 1988).

Isolation of Staphylococci from Clinical Specimens

The isolation and enumeration of staphylococci from clinical specimens are routine operations in the hospital and veterinary clinical laboratory. Procedures for handling specimens and isolating and enumerating staphylococci can be found in the following texts: American Society for Micro-

biology (ASM) *Manual of Clinical Microbiology*, seventh edition (Murray et al., 1999), *A Guide to Specimen Management in Clinical Microbiology*, second edition (Miller, 1998) and *Clinical Microbiology Procedures Handbook* (Isenberg, 1994).

Staphylococci from a variety of clinical specimens are usually isolated in primary culture on blood agar and in a fluid medium such as thioglycolate broth. A general discussion of the preparation of specimens for primary culturing and inoculation of media and colony isolation methodology can be found in the *ASM Manual of Clinical Microbiology* (Murray et al., 1999).

Isolation and Culture from Clinical Specimens (Kloos and Bannerman, 1999) Every specimen should be plated onto blood agar (preferably sheep blood agar) and other media as indicated. On blood agar, abundant growth of most staphylococcal species occurs within 18–24 h. Since most species cannot be distinguished from one another during this time period, colonies should be picked at this time only for preliminary identification testing (e.g., when specimens are taken from patients with acute infections). Colonies should be allowed to grow for at least an additional two to three days before the primary isolation plate is confirmed for species or strain composition (Kloos and Schleifer, 1975b; Kloos and Schleifer, 1975c). This growth period is particularly important when the sampling of more than one colony is needed to obtain sufficient inocula for determining the predominant organism or to obtain a pure culture. Failure to hold plates for 72 h, can result in 1) selection of more than one species or strain if sampling yields an inoculum of two or more colonies, 2) selection of an organism(s) not producing the infection, if the specimen contains two or more different species or strains, and 3) incorrectly labeling a mixed culture as a pure culture. Colonies should be Gram-stained, subcultured, and tested for genus, species, and strain properties. It should be noted that most staphylococci of major medical interest produce growth in the upper as well as the lower anaerobic portions of thioglycolate broth or semisolid agar (Kloos and Schleifer, 1975c).

Fecal specimens suspected of containing infecting staphylococci (e.g., associated with staphylococcal enterocolitis) and other specimens from potentially heavily contaminated sources should also be inoculated on a selective medium such as SK agar (described above), Columbia CNA agar, lipase-salt-mannitol agar (LSM; Remel, Lenexa, KS, USA), tellurite glycine agar, phenylethyl alcohol agar, or mannitol salt agar. These media inhibit the growth of Gram-negative bacteria in addition to some other contaminating species. Incubation of these cultures should be for at least 48–72 h for discernible colony development.

Blood Cultures and Catheter Tips Blood cultures are usually indicated when there is a sudden increase in the pulse rate and temperature of the patient, a change in sensorium, and the onset of chills, prostration, and hypotension. Timing of collection is usually not critical when bacteremia is expected to be continuous; however, bacteremia often is intermittent. In these cases, timing may be very important, and bacteremia may precede the onset of fever or chill by as much as 1 h. Staphylococci are one of the major groups of bacteria that can produce a serious bacteremia.

Ideally, blood specimens should be collected before administration of antimicrobial agents and as close as possible to a fever spike. Blood for culture should be collected aseptically, first by cleaning the venipuncture site with 70% alcohol and then with an iodine preparation in a concentric fashion. The iodine should be allowed to dry. The venipuncture site should not be touched after cleansing. After venipuncture, the iodine should be removed from the skin with alcohol. It is recommended that 10–20 ml/set be collected in adults and 1–10 ml/set in infants. Also for acute sepsis, 2 or 3 sets should be collected from separate sites all within 10 min; for acute endocarditis, 3 sets should be collected from separate sites within 1–2 h; and for subacute endocarditis, 3 sets should be collected from separate sites taken 15 min apart. Initial processing of a blood specimen depends on the culture system used. All bacterial blood cultures should be incubated at 35°C for up to 7 days for evidence of growth. Gram-stained smears and subcultures of suspected positive cultures should be prepared immediately.

Culture of catheter tips may be performed to determine the source of bacteremia. The most commonly used method is the semi-quantitative method (Maki et al., 1977). A 5-cm segment of the distal tip is rolled across a blood agar plate four times. Cultures yielding > 15 bacterial colonies are considered clinically significant for a catheter-related infection.

Preparation of Body Fluid Cultures Staphylococci may infect a variety of body fluids in addition to blood, such as cerebrospinal fluid (CSF) and joint, intraocular, pericardial, peritoneal, and pleural space fluids. It usually is easiest to establish the etiological agent in infections of normally sterile body sites, provided puncture and handling of the specimen are performed under conditions of strict asepsis. The skin

should be disinfected with a 2% solution of tincture of iodine. The specimen should be injected immediately into a sterile (screw-cap) tube or bottle. The specimen should be transported immediately to the laboratory for testing. Since only a small number of microorganisms may be present in clear or slightly cloudy fluids, volumes > 1 ml should be centrifuged to concentrate the organisms. A portion of the sediment is used for Gram staining and to inoculate the media.

PREPARATION OF URINE CULTURES Staphylococcal urinary tract infections in humans are commonly caused by *S. saprophyticus*, *S. epidermidis* and *S. aureus*. Acceptable methods for urine collection include midstream clean catch, catheterization, and suprapubic aspiration. At least 1 ml of midstream urine should be collected in a sterile, wide-mouth container. Unpreserved specimens should be cultured within 2 h of collection or stored in a refrigerator for no more than 24 h. Most references to diagnostic criteria state that a significant bacteriuria occurs when there are 100,000 cells or more per ml in a clean-voided, midstream specimen obtained from asymptomatic patients. With acute dysuria and frequency in young, sexually active females, a colony count as low as 100 per ml may be a useful criterion. Many significant urinary tract infections due to *S. saprophyticus* are associated with only 100 to 10,000 colonies per ml. If significant, these low counts may be substantiated by repetition of the procedure. Significant bacteriuria may also be determined by microscopic examination of a Gram-stained smear of uncentrifuged urine. The presence of at least two bacteria per 1000-X microscopic field of the Gram-stained smear is approximately equal to 100,000 or more cells per ml (Pollock, 1983).

ISOLATION OF STAPHYLOCOCCI FROM SKIN AND MUCOUS MEMBRANES Several basic methods are available for isolating staphylococci and other aerobic bacteria from skin and the adjacent mucous membranes (reviewed by Noble and Somerville, 1974). Washing or swabbing methods disperse cutaneous bacteria to provide samples of uniform composition. They break up large aggregates or microcolonies on skin into smaller colony-forming units (CFU) and in some cases single cells. Impression methods estimate the number of microcolonies or aggregates of bacteria on the skin surface. Biopsy methods can determine the location of bacteria in microniches on skin. Most of the sampling of aerobic bacteria on skin and mucous membranes has been performed using scrubbing and swabbing methods. The swab technique described by Kloos and

Musselwhite (1975a) is suitable for use with human as well as other mammalian skin. The medium most widely used for the isolation and culture of natural populations of staphylococci is P agar (Kloos et al., 1974).

P Agar

Peptone	10.0 g
Yeast extract	5.0 g
Sodium chloride	5.0 g
Glucose	1.0 g
Agar	15.0 g
Distilled water	1 liter

Adjust pH to 7.5 before autoclaving at 121°C for 15 min.

From each inoculated swab, a series of dilutions are prepared and plated on standard size (15 × 100 mm) P agar plates, in an attempt to obtain 50–300 isolated colonies on a plate for identification and enumeration. Inoculated plates are incubated at 34–35°C for 3–4 days and then held at room temperature for an additional 2 days. Each colony type is enumerated and one or two representatives of each type per plate are examined further for distinguishing genus, species, and strain characteristics. Colony morphology can be a useful supplementary character in the identification of species and strains. Selective media may be used in addition to the nonselective P agar if bacterial and/or fungal populations are very large (e.g., from the human inguinal and perineal area or from the skin of certain animals) and/or if the cutaneous flora contains species producing large, spreading colonies. Pigment production of colonies may be enhanced by the addition of milk, fat, glycerol monoacetate, or soaps to P agar or heart infusion agar (Willis et al., 1966).

Isolation of Staphylococci from Water

The presence of potentially pathogenic staphylococci in recreational waters, swimming pools, water that might be added to foods, and hydrotherapy pools poses a threat to human health (reviewed by Evans, 1977). Most attention is focused on the presence in water of *S. aureus* although several other staphylococcal species are also opportunistic pathogens. Staphylococci are somewhat resistant to halogen disinfectants. For this reason, significant numbers of these organisms can remain viable for extended periods of time in inadequately treated bathing places. Methods for the recovery and enumeration of pathogens from water are described in the manual jointly published by the American Public Health Association (APHA) and Water Environment Federation entitled *Standard Methods for the Examination of Water and Wastewater*, twentieth edition (Clesceri et al., 1998).

Phylogeny

On the basis of comparative 16S rRNA sequence studies, the genera *Staphylococcus* and *Macrococcus* belong to the Gram-positive bacteria with a low DNA G+C content. They are closely related to bacilli and other Gram-positive bacteria with low DNA G+C content such as enterococci, streptococci, lactobacilli and listeria (Fig. 1). Combining *Staphylococcus*, *Gemella*, *Macrococcus* and *Salinicoccus* within the family Staphylococcaceae has been proposed (Garrity and Holt, 2001).

The genera *Staphylococcus* and *Macrococcus* are monophyletic (Fig. 2) and well separated from each other with intergenera 16S rRNA sequence similarities of 93.4–95.3%. The intragenus similarities are significantly higher with at least 96.5% for staphylococci and 97.7% for macrococci. On the basis of DNA-DNA hybridization studies, staphylococcal species can be grouped (Kloos et al., 1992). The most important ones are the two coagulase-negative and novobiocin-susceptible species groups *S. epidermidis* (e.g., *S. capitis*, *S. caprae*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. saccharolyticus* and *S. warneri*) and *S. simulans* (e.g., *S. carnosos* and *S. simulans)*; the two coagulase-negative and

novobiocin-resistant species groups *S. saprophyticus* (e.g., *S. cohnii*, *S. saprophyticus* and *S. xylosus*) and *S. sciuri* (e.g., *S. lentus*, *S. sciuri* and *S. vitulus*), and the two coagulase-positive and novobiocin-susceptible species groups *S. intermedius* (e.g., *S. delphini* and *S. intermedius*) and *S. aureus* (e.g., *S. aureus* and *S. aureus* subsp. *anaerobius*).

On the basis of DNA-DNA hybridization studies, the species *M. equipercicus*, *M. bovicus* and *M. carouselicus* were more closely related to one another than to *M. caseolyticus* (Kloos et al., 1998a). Both DNA-DNA hybridization studies and 16S rRNA sequence analysis indicate a closer relationship of the genus *Macrococcus* to the *S. sciuri* species group than to other staphylococcal species.

Identification

General Properties

Members of the genus *Staphylococcus* are Gram-positive cocci (0.5–1.5 μm in diameter) that occur singly, in pairs, tetrads, short chains (three or four cells), and irregular grape-like clusters. They are nonmotile, nonsporeforming, and usually are unencapsulated or have limited capsule formation. Most species are facultative anaerobes and they are positive for the catalase and benzidine tests. With the exception of *S. saccharolyticus* and *S. aureus* subsp. *anaerobius*, growth is more rapid and abundant under aerobic conditions. These exceptional staphylococci are also catalase-negative. Most species contain *a*- and *b*-type cytochromes. The exceptional species *S. lentus*, *S. sciuri* and *S. vitulus* contain *a*-, *b*- and *c*-type cytochromes. Menaquinones are unsaturated (normal). The G+C content of DNA is in the range of 30–39 mol%. The genome size is in the range of 2000–3000 kb (George and Kloos, 1994; Kloos et al., 1998b). Staphylococci are generally susceptible to lysostaphin (some species more than others), furazolidone and nitrofuran, and resistant to erythromycin and bacitracin at low levels. In the laboratory routine, rapid distinction of staphylococci from micrococci can be made by demonstrating the susceptibility of staphylococci to 200 μg of lysostaphin per ml and resistance to erythromycin at 0.04 μg per ml, plus the production of acid from glycerol (Schleifer and Kloos, 1975b) or, alternatively, demonstrating susceptibility of staphylococci to a 100 μg furazolidone disk and resistance to a 0.04-unit bacitracin disk (Baker, 1984). Furthermore, staphylococci, with the exceptions of *S. lentus*, *S. sciuri* and *S. vitulus* exhibit a negative reaction with the rapid modified oxidase test, whereas micrococci are positive for this test (Faller and Schleifer, 1981).
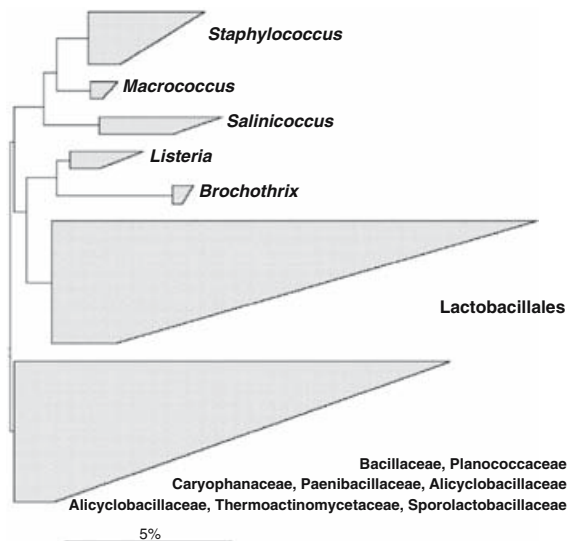

Fig. 1. A 16S rRNA-based tree reflecting the relationships of the *Staphylococcus-Macrococcus-Salinicoccus* cluster and its sister groups among the bacilli. The tree was reconstructed by a maximum likelihood analysis of all currently available almost complete (at least 80% with respect to the *E. coli* reference) small subunit rRNA sequences. Only alignment positions at which at least 50% of all included members share the same characters were included for tree calculation. The bar length indicates 5% estimated sequence divergence.
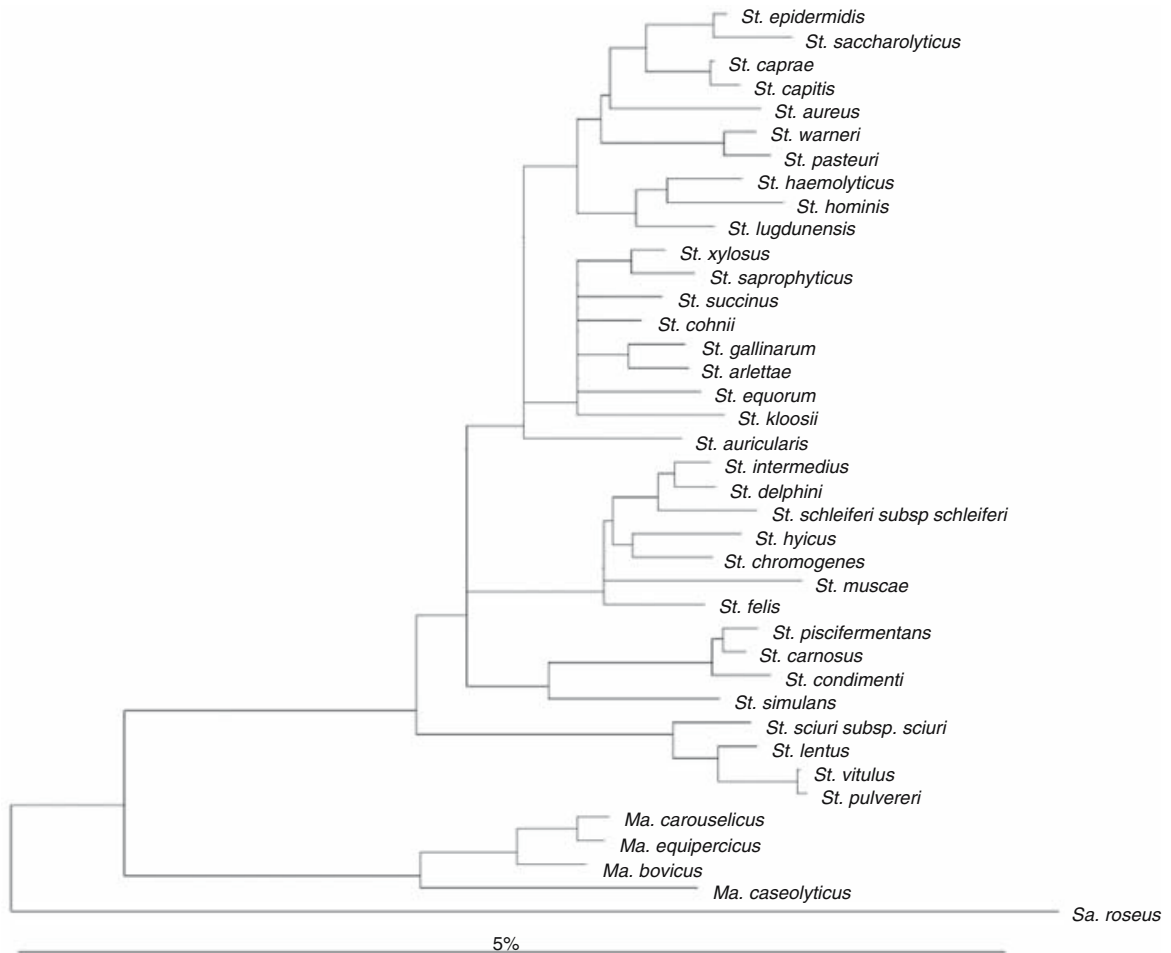
Fig. 2. A 16S rRNA-based tree reflecting the relationships of staphylococci, macrococci and salinicocci. The tree was reconstructed by a maximum likelihood analysis of all currently available almost complete (at least 80% with respect to the *E. coli* reference) small subunit rRNA sequences. Alignment positions at which the primary structures of less than 50% of all included members of the staphylococci, macrococci and salinicocci share a common character were excluded from the calculations. The tree topology was evaluated and corrected according to the results obtained by applying distance matrix and maximum likelihood approaches. (All available rRNA sequences from representatives of the Firmicutes and a selection of sequences representing the bacterial and archaeal phyla [1970 sequences] were used for distance matrix analyses, whereas the respective sequences from all staphylococci, macrococci and salinicocci in combination with a small selection of reference sequences of members of the sister groups [122 sequences] were analyzed by applying maximum likelihood procedures.) Multifurcations indicate that common relative branching order could not be unambiguously determined or was not supported when applying the alternative treeing approaches. The bar length indicates 5% estimated sequence divergence.

Members of the genus *Macrococcus* are Gram-positive cocci (1.1–2.5 μm in diameter) that occur mostly in pairs and tetrads, and occasionally single and arranged in short chains (Kloos et al., 1998a). They are nonmotile, non-sporeforming, and usually are unencapsulated. Macrococci are marginally facultative anaerobes; growth occurs better under aerobic conditions. They are positive for catalase and oxidase activities. They are resistant to bacitracin and lysozyme (25 μg/ml), and susceptible to furazolidone. Macrococci can contain *a*-, *b*- and/or *c*-type cytochromes. The G+C content of DNA is in the range of 38–45 mol%. The genome size is in the range of 1500–1800 kb.

Cell Wall Composition

The ultrastructure and chemical composition of the cell wall of staphylococci is similar to that of other Gram-positive bacteria. It consists of a thick (usually 60–80 nm), rather homogeneous, and not very electron-dense layer. It is made up of peptidoglycan, teichoic acid, and protein (Schleifer, 1983). Macrococci, with the possible

exception of *M. caseolyticus*, do not have detectable levels of teichoic acids.

A characteristic feature of the peptidoglycan of staphylococci is the occurrence of glycine-rich interpeptide bridges. Penta- and hexaglycine interpeptide bridges are found in about half of the staphylococcal species (peptidoglycan type: Lys-Gly$_{5-6}$). In most of the other half, a minor part of the glycine residues can be replaced with L-serine (peptidoglycan type: Lys-Gly$_4$, Ser). *Staphylococcus sciuri*, *S. lentus*, *S fleuretti* and *S. vitulus* may have an L-alanine instead of a glycine residue bound to lysine of the peptide subunit (peptidoglycan type: Lys-Ala-Gly$_4$). The peptidoglycan types for macrococci can be either Lys-Gly$_{3-4}$, L-Ser (*M. caseolyticus*, *M. equipercicus* and *M. carouselicus*) or Lys-Gly$_3$, L-Ser (*M. bovicus*).

Staphylococcal cell wall teichoic acids are water-soluble polymers containing repeating phosphodiester groups that are covalently linked to peptidoglycan. They consist of polyol (glycerol and ribitol) sugars and/or *N*-acetylamino sugars. Most staphylococci contain glycerol or ribitol teichoic acids. The teichoic acids consist of polymerized polyol phosphates that are substituted with various combinations of sugars and/or *N*-acetylamino sugar residues, and also ester-linked D-alanine residues. In some cases, *N*-acetylamino sugar residues can also form an integral part of the polymer chain (Endl et al., 1983; Endl et al., 1984). The occurrence of the same major components does not always mean that the structure of teichoic acid is identical; for example, the teichoic acids of *S. capitis* and *S. hyicus* show a similar composition but their structures are quite different.

Differentiation of Species and Subspecies

The classification of species and subspecies of staphylococci and macrococci can be based on a variety of phenotypic character analyses and DNA-DNA (genomic) relationships. In addition, rRNA analysis and ribotyping may be used to describe the relationship of reference and new species. DNA similarity (> 70%) is the criterion that has been used to determine species boundaries in the formal classification of staphylococcal species and most subspecies. Selected phenotypic characters are also useful in classification because they had a high predictive value in identifying DNA similarity groups. Recently, recommended minimal standards for the description of new staphylococcal species have been published (Freney et al., 1988). Characters studied at the cellular and population levels, including morphological and physiological properties, enzyme reactions, and intrinsic resistance to certain antibiotics, have been included in practical identification schemes as shown in Tables 2–5. Molecular studies of phenotypic characters have also provided a basis for determining epigenetic relationships.

CONVENTIONAL METHODS Conventional methods for the determination of phenotypic characters at the cellular and population levels were developed first and then examined for their correlation to DNA relatedness (reviewed by Kloos

Table 2. Differentiation of coagulase/clumping factor-positive *Staphylococcus* species and subspecies.

Character	*S. aureus*	*S. aureus* subsp. *anaerobius*	*S. delphini*	*S. hyicus*	*S. intermedius*	*S. lugdunensis*	*S. lutrae*	*S. sciuri* subsp. *carnaticus*	*S. sciuri* subsp. *rodentium*	*S. schleiferi*	*S. schleiferi* subsp. *coagulans*
Colony size ±6mm	+	−	+	+	+	d	−	−	d	−	d
Colony pigment	+	−	−	−	−	d	−	d	d	−	−
Anaerobic growth	+	(+)	(+)	+	(+)	+	+	(d)	(d)	+	+
Aerobic growth	+	()	+	+	+	+	+	+	+	+	+
Staphylocoagulase	+	+	+	d	+	−	+	−	−	−	+
Clumping factor	+	−	−	−	d	(+)	−	d	+	+	−
Thermonuclease	+	+	−	+	+	−	()	−	−	+	+
Hemolysis	+	+	+	−	d	(+)	+	()	()	(+)	(+)
Catalase	+	−	+	+	+	+	+	+	+	+	+
Modified oxidase	−	−	−	−	−	−	−	+	+	−	−
Alkaline phosphatase	+	+	+	+	+	−	+	d	d	+	+
Pyrrolidonyl arylamidase	−	ND	ND	−	+	+	ND	−	−	+	ND
Ornithine decarboxylase	−	ND	ND	−	−	+	ND	−	−	−	ND
Urease	d	ND	+	d	+	d	+	−	−	−	+

Table 2. *Continued*

Character	*S. aureus*	*S. aureus* subsp. *anaerobius*	*S. delphini*	*S. hyicus*	*S. intermedius*	*S. lugdunensis*	*S. lutrae*	*S. sciuri* subsp. *carnaticus*	*S. sciuri* subsp. *rodentium*	*S. schleiferi*	*S. schleiferi* subsp. *coagulans*
β-Glucosidase	+	−	ND	d	d	+	ND	+	+	−	ND
β-Glucuronidase	−	−	ND	+	−	−	ND	−	−	−	ND
β-Galactosidase	−	−	ND	−	+	−	+	−	−	(+)	ND
Arginine dihydrolase	+	ND	+	+	d	−	−	−	−	+	+
Acetoin production	+	−	−	−	−	+	−	−	−	+	+
Nitrate reduction	+	−	+	+	+	+	+	+	+	+	+
Esculin hydrolysis	−	−	ND	−	−	−	ND	+	+	−	ND
Novobiocin resistance	−	−	−	−	−	−	−	+	+	−	−
Acid (aerobically) from:											
D-Trehalose	+	−	−	+	+	+	+	+	(+)	d	−
D-Mannitol	+	ND	(+)	−	(d)	−	d	+	+	−	d
D-Mannose	+	−	+	+	+	+	+	(d)	(+)	+	+
D-Turanose	+	ND	ND	−	d	(d)	ND	ND	ND	−	ND
D-Xylose	−	−	−	−	−	−	+	+	(d)	−	−
D-Cellobiose	−	−	ND	−	−	−	ND	(d)	d	−	−
L-Arabinose	−	−	−	−	−	−	ND	d	(d)	−	−
Maltose	+	+	+	−	()	+	+	(d)	(d)	−	−
Sucrose	+	+	+	+	+	+	ND	+	+	−	d
N-Acetylglucosamine	+	−	ND	+	+	+	ND	−	−	(+)	ND
Raffinose	−	−	ND	−	−	−	ND	−	−	−	−

Symbols: +, 90% or more strains; −, 90% or more strains negative; d, 11 to 89% of strains positive; ND, not determined. () indicate a delayed reaction.
Adapted from Kloos and Bannerman (1999); Kloos and Scheifer (1986); Schleifer (1986).

Table 3. Differentiation of coagulase-negative, novobiocin-susceptible *Staphylococcus* species and subspecies.

Character	*S. auricularis*	*S. capitis*	*S. capitis* subsp. *ureolyticus*	*S. caprae*	*S. carnosus*	*S. carnosus* subsp. *utilis*	*S. chromogenes*	*S. condimenti*	*S. epidermidis*	*S. felis*	*S. haemolyticus*	*S. hominis*	*S. lugdunensis*	*S. muscae*	*S. pasteuri*	*S. piscifermentans*	*S. saccharolyticus*	*S. schleiferi*	*S. simulans*	*S. succinus*	*S. warneri*
Colony size ±6mm	−	−		d	+	−	+	ND	−	+	+	−	d	d	d	ND	−	−	+	ND	d
Colony pigment	−	−	(d)	−	−	−	+	d	−	−	d	d	−	d	d	−	−	−	−	−	d
Anaerobic growth	(+)	(+)	(+)	(+)	+	+	+	+	+	+	(+)	()	+	+	+	+	+	+	+	−	+
Aerobic growth	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	()	+	+	+	+
Staphylocoagulase	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	ND	−
Clumping factor	−	−	−	−	−	ND	−	ND	−	−	−	−	(+)	−	−	ND	−	+	−	ND	−
Thermonuclease	−	−	−	−	−	ND	−	ND	−	−	−	−	−	−	−	+	−	+	−	ND	−
Hemolysis	−	(d)	(d)	(d)	ND	ND	ND	−	(d)	(d)	(+)	−	(+)	(+)	(d)	−	−	(+)	(d)	ND	(d)
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Modified oxidase	−	−	−	−	−	ND	−	ND	−	−	−	−	−	−	−	−	−	−	−	−	−
Alkaline phosphatase	−	−	−	(+)	+	−	+	+	+	+	−	−	−	+	−	+	d	+	(d)	+	−
Pyrrolidonyl arylamidase	d	−	(d)	d	+	ND	d	ND	−	ND	+	−	+	ND	−	ND	ND	+	+	−	−
Ornithine decarboxylase	−	−	−	−	−	ND	−	ND	(d)	ND	−	−	+	−	−	ND	−	−	−	−	−
Urease	−	−	+	+	−	−	+	+	+	+	−	+	d	−	+	+	ND	−	+	+	+
β-Glucosidase	−	−	−	−	−	ND	d	ND	(d)	−	d	−	+	ND	+	+	ND	−	−	ND	+
β-Glucuronidase	−	−	−	−	−	−	−	−	−	−	d	−	+	ND	−	−	ND	−	d	ND	d
β-Galactosidase	(d)	−	−	−	+	−	−	+	−	+	−	−	−	−	−	−	ND	(+)	+	ND	−
Arginine dihydrolase	d	d	+	+	+	+	+	+	d	+	+	d	−	−	d	+	+	+	+	−	d
Acetoin production	−	d	d	+	+	ND	−	ND	+	−	+	d	+	−	d	−	ND	+	d	−	+

Table 3. *Continued*

Character	S. auricularis	S. capitis	S. capitis subsp. ureolyticus	S. caprae	S. carnosus	S. carnosus subsp. utilis	S. chromogenes	S. condimenti	S. epidermidis	S. felis	S. haemolyticus	S. hominis	S. lugdunensis	S. muscae	S. pasteuri	S. piscifermentans	S. saccharolyticus	S. schleiferi	S. simulans	S. succinus	S. warneri
Nitrate reduction	(d)	d	+	+	+	d	+	+	+	+	+	d	+	ND	d	+	+	+	+	−	d
Esculin hydrolysis	−	−	−	−	−	−	−	−	−	ND	−	−	−	ND	−	+	ND	−	−	ND	−
Novobiocin resistance	−	−	−	−	−	ND	−	ND	−	−	−	−	−	−	−	−	−	−	−	ND	−
Acid (aerobically) from:																					
D-Trehalose	(+)	−	−	(+)	d	d	+	+	−	+	+	d	+	+	+	+	−	d	d	+	+
D-Mannitol	−	+	+	d	+	−	d	+	−	+	d	−	−	−	d	d	−	−	+	ND	d
D-Mannose	−	+	+	+	+	−	+	+	(+)	+	−	−	+	−	−	−	(+)	+	d	+	−
D-Turanose	(d)	−	−	−	−	−	d	(d)	−	ND	(d)	+	(d)	+	(d)	−	ND	−	−	ND	(d)
D-Xylose	−	−	−	−	−	ND	−	ND	−	−	−	−	−	+	−	−	−	−	−	ND	−
D-Cellobiose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	ND	−
L-Arabinose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	ND	−
Maltose	(+)	−	+	(d)	−	−	d	−	+	−	+	+	+	−	(d)	d	−	−	()	ND	(+)
Sucrose	d	(+)	+	−	−	−	+	−	+	d	+	(+)	+	+	+	d	−	−	+	ND	+
N-Acetylglucosamine	−	−	−	−	ND	ND	d	ND	−	+	+	d	+	ND	ND	ND	ND	(+)	+	ND	−
Raffinose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	(d)	−

Symbols: +, 90% or more strains; −, 90% or more strains negative; d, 11 to 89% of strains positive; ND, not determined. () indicate a delayed reaction.

Adapted from Kloos and Bannerman (1999); Kloos and Scheifer (1986); Schleifer (1986).

Table 4. Differentiation of novobiocin-resistant *Staphylococcus* species and subspecies.

Character	S. arlettae	S. cohnii	S. cohnii subsp. urealyticus	S. equorum	S. fleurettii	S. gallinarum	S. hominis subsp. novobiosepticus	S. kloosii	S. lentus	S. saprophyticus	S. saprophyticus subsp. bovis	S. sciuri	S. sciuri subsp. carnaticus	S. sciuri subsp. rodentium	S. xylosus	S. vitulinus
Colony size ±6mm	d	d	+	−	−	+	−	d	−	+	−	+	−	d	+	−
Colony pigment	+	−	d	−	−	d	−	d	d	d	+	d	d	d	d	+
Anaerobic growth	−	d	(+)	−	+	(+)	−	−	()	(+)	+	(+)	(d)	(d)	d	−
Aerobic growth	+	+	+	(+)	+	+	+	+	(+)	+	+	+	+	+	+	(+)
Staphylocoagulase	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Clumping factor	−	−	−	−	ND	−	−	−	−	−	−	−	d	+	−	−
Thermonuclease	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Hemolysis	−	(d)	(d)	(d)	ND	(d)	−	(d)	−	−	−	()	()	()	−	−
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Modified oxidase	−	−	−	−	+	−	−	−	+	−	−	+	+	+	−	+
Alkaline phosphatase	(+)	−	+	(+)	d	(+)	−	d	()	−	−	+	d	d	d	−
Pyrrolidonyl arylamidase	−	−	d	−	−	−	−	d	−	−	+	−	−	−	d	−
Ornithine decarboxylase	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Urease	−	−	+	+	−	+	+	d	−	+	+	−	−	−	+	−
β-Glucosidase	ND	−	−	ND	ND	+	−	d	+	d	d	+	+	+	+	d
β-Glucuronidase	+	−	+	+	−	d	−	d	−	−	−	−	−	−	+	−
β-Galactosidase	d	−	+	d	−	d	−	d	−	+	d	−	−	−	+	−
Arginine dihydrolase	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Acetoin production	−	d	d	−	d	−	d	d	−	+	d	−	−	−	d	−
Nitrate reduction	−	−	−	+	+	+	d	−	+	−	+	+	+	+	d	+
Esculin hydrolysis	−	−	−	d	d	+	−	d	+	−	−	+	+	+	d	d

(*Continued*)

Table 4. *Continued*

Character	*S. arlettae*	*S. cohnii*	*S. cohnii* subsp. *urealyticus*	*S. equorum*	*S. fleurettii*	*S. gallinarum*	*S. hominis* subsp. *novobiosepticus*	*S. kloosii*	*S. lentus*	*S. saprophyticus*	*S. saprophyticus* subsp. *bovis*	*S. sciuri*	*S. sciuri* subsp. *carnaticus*	*S. sciuri* subsp. *rodentium*	*S. xylosus*	*S. vitulinus*
Acid (aerobically) from:																
D-Trehalose	+	+	+	+	+	+	−	+	+	+	+	+	+	(+)	+	(d)
D-Mannitol	+	d	+	+	ND	+	−	+	+	d	+	+	+	+	+	+
D-Mannose	+	(d)	+	+	+	+	−	−	(+)	−	−	(d)	(d)	(+)	+	−
D-Turanose	+	−	−	d	+	+	ND	−	()	+	+	()	ND	ND	d	−
D-Xylose	+	−	−	+	d	+	−	(d)	()	−	−	(d)	+	(d)	+	d
D-Cellobiose	−	−	−	(d)	−	+	−	−	+	−	−	+	(d)	d	−	d
L-Arabinose	+	−	−	+	d	+	−	d	d	−	−	d	d	(d)	d	−
Maltose	+	(d)	(+)	d	+	+	+	d	d	+	+	(d)	(d)	(d)	+	−
Sucrose	+	−	−	+	+	+	(+)	()	+	+	+	+	+	+	+	+
N-Acetylglucosamine	−	−	d	d	ND	+	−	−	d	d	+	d	−	−	+	−
Raffinose	+	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−

Symbols: +, 90% or more strains; −, 90% or more strains negative; d, 11 to 89% of strains positive; ND, not determined. () indicate a delayed reaction.
Adapted from Kloos and Bannerman (1999); Kloos and Scheifer (1986); Schleifer (1986).

Table 5. Conventional identification methods useful for the differentiation of *Macrococcus* species.

Character	*M. caseolyticus*	*M. equipercicus*	*M. bovicus*	*M. carouselicus*	*S. sciuri* subsp. *sciuri*	*S. sciuri* subsp. *carnaticus*	*S. sciuri* subsp. *rodentium*	*S. lentus*	*S. fleuretti*	*S. vitulus*
Anaerobic growth	d	−	−	−	(+)	(d)	(d)	()	+	−
Heat-stable nuclease	d	−	d	+	−	−	−	−	−	−
Alkaline phosphatase	−	−	−	−	+	d	d	()	d	−
Pyrrolidonyl arylamidase	+	−	−	−	−	−	−	−	−	−
Urease	−	d	d	−	−	−	−	−	−	−
β-Glucosidase	−	−	d	−	+	+	+	+	ND	d
Acetoin production	+	−	−	−	−	−	−	−	d	−
Nitrate reduction	+	−	−	−	+	+	+	+	+	+
Esculin hydrolysis	d	d	−	+	+	+	+	+	d	d
Acid (aerobically) from:										
D-Mannitol	−	+	+	d	+	+	+	+	ND	+
D-Mannose	−	−	−	−	(d)	(d)	(+)	(+)	+	−
D-Turanose	−	−	−	−	()	ND	ND	()	+	−
D-Xylose	−	−	−	−	(d)	+	(d)	()	d	d
D-Cellobiose	−	−	−	−	+	(d)	d	+	−	d
L-Arabinose	−	−	−	−	d	d	(d)	d	d	−
Maltose	+	d	d	−	(d)	(d)	(d)	d	+	−
Sucrose	d	−	d	d	+	+	+	+	+	+
Raffinose	−	−	−	−	−	−	−	+	−	−

Symbols: +, 90% or more of strains positive; −, 90% or more strains negative; d, 11 to 89% of strains positive; ND, not determined. () indicate a delayed reaction.

and Schleifer [1986] and Schleifer [1986]). Key characters now used for species and subspecies identification include the following: colony morphology, oxygen requirements, coagulase, clumping factor, heat-stable nuclease (thermonuclease), hemolysins, catalase, oxidase, alkaline phosphatase, urease, ornithine decarboxylase, pyrrolidonyl arylamidase, β-galactosidase, acetoin production, nitrate reduction, esculin hydrolysis, aerobic acid production from a variety of carbohydrates including D-trehalose, D-mannitol, D-mannose, D-turanose, D-xylose, D-cellobiose, L-arabinose, maltose, α-lactose, sucrose, and raffinose, and intrinsic resistance to novobiocin and polymyxin B (reviewed by Kloos and Bannerman, 1999). Some conventional methods may require up to three to five days before a final result can be obtained, while others only require several hours for interpretation. They are usually quite reliable and have served as a reference for more recent studies aimed at simplifying and expediting character analyses.

RAPID IDENTIFICATION SYSTEM To facilitate identification in the routine or clinical laboratory, several manufacturers have developed rapid species identification kits or automated systems requiring only a few hours to one day for the completion of tests. Identification of a number of the *Staphylococcus* species can be made with an accuracy of 70 to > 90% using the commercial systems (Kloos and Bannerman, 1994). Since their introduction, these systems have been improved and expanded to include more species. Their reliability will continue to increase as the result of a growing database and the addition of more discriminating tests. *Staphylococcus aureus*, *S. epidermidis*, *S. capitis*, *S. haemolyticus*, *S. saprophyticus*, *S. simulans* and *S. intermedius* can be identified reliably by most of the commercial systems now available. For some systems, reliability depends upon additional testing as suggested by the manufacturer and/or by published evaluations of the product. Some identification systems now available include the following: RAPIDEC Staph (for identification of *S. aureus*, *S. epidermidis* and *S. saprophyticus*) and API STAPH (bioMérieux Vitek, Inc., Hazelwood, MO, USA); VITEK, a fully automated microbiology system that uses a Gram-positive identification (GPI) card (bioMérieux Vitek); MicroScan Pos ID panel (read manually or on MicroScan instrumentation) and MicroScan Rapid Pos ID panel (read by the WalkAway systems; in addition, the ID panels are available with antimicrobial agents for susceptibility testing; Dade MicroScan, Inc., West Sacramento, CA, USA); Crystal Gram-Positive Identification System, Crystal Rapid Gram-Positive Identification System, Pasco MIC/ID Gram-Positive Panel, and the Phoenix, an automated identification system (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA); GP MicroPlate test panel (read manually, using Biolog MicroLog system, or automatically with the Biolog MicroStation system; Biolog, Haywood, CA, USA); MIDI Sherlock Identification System Microbial Identification System (MIS) that automates microbial identification by combining cellular fatty acid analysis with computerized high-resolution gas chromatography (MIDI, Newark, DE, USA); and RiboPrinter Microbial Characterization System (Qualicon, Inc. Wilmington, DE, USA), based on ribotype pattern analysis.

Rapid detection of the species *S. aureus* can be made using the AccuProbe culture identification test for *S. aureus* (Gen-Probe, Inc., San Diego, CA, USA). This test is a DNA probe assay directed against rRNA, and it is very accurate (100% specificity). Tube coagulase-negative and slide test-negative strains of *S. aureus* should be identified correctly by the AccuProbe test. Additionally, PCR analysis of the 16S–23S rRNA intergenic spacer region has preliminarily shown successful results in discriminating among 31 *Staphylococcus* species (Mendoza et al., 1998). PCR analysis allows for the identification of pure culture staphylococci within 24–48 h.

Physiology

Sugar Transport

Sugar transport, metabolism, and catabolite repression of staphylococci is best studied in the pathogenic species *S. aureus* and the nonpathogenic species *S. xylosus*. Two types of carbohydrate transport have been identified and studied in staphylococci: 1) the phosphoenolpyruvate (PEP):sugar phosphotransferase system (PTS), which is responsible for the binding, transmembrane transport, and phosphorylation of numerous sugar substrates, and 2) the PTS-independent carbohydrate transport in which the sugar is transported via a permease and is subsequently phosphorylated by an ATP-dependent kinase.

The known sugar transport systems in *S. aureus* and *S. xylosus* are summarized in Table 6. The type of transport used depends on the sugar and staphylococcal species. For some sugars, e.g., glucose, both types of transport systems are present in one species to ensure efficient transport, and they are functional under specific environmental conditions. Studies with PTS-deficient strains of *S. aureus* (Reizer et al., 1988), for example, have demonstrated the utilization of glucose by both PTS-dependent and PTS-independent mechanisms.

THE PHOSPHOTRANSFERASE SYSTEM (PTS) The PTS delivers exogenous carbohydrates as phos-

Table 6. Carbohydrate transport systems in *S. aureus* and *S. xylosus*.

Carbohydrate	*S. aureus*		*S. xylosus*	
	PTS	PTS-independent	PTS	PTS-independent
Glucose	+	+	+	+
Galactose	+	ND	−	+
Fructose	ND	ND	+	ND
N-Acetylglucosamine	+	ND	ND	ND
Mannitol	+	ND	+	ND
Mannose	+	ND	ND	ND
Trehalose	ND	ND	+	ND
Lactose	+	ND	−	+
Maltose	ND	ND	+	ND
Sucrose	ND	ND	+	ND
Xylose	ND	−	−	+
Ribose	ND	ND	−	+
Arabinose	ND	−	−	+

Symbols and abbreviations: +, described transporters; −, not present; PTS, phosphoenolpyruvate (PEP): sugar phosphotransferase system; and ND, not determined.

phate esters to the cell cytoplasm. It consists of two nonspecific, energy-coupling components, enzyme I (EI, *ptsI*) and a heat-stable phospho-carrier protein (HPr, *ptsH*), as well as several sugar-specific multiprotein permeases known as enzymes II (EIIA, B and C). In most cases, enzymes IIA and IIB are located in the cytoplasm, while enzyme IIC forms a membrane channel.

EI and HPr play a central role in the PTS-mediated uptake of most sugars; both enzymes are soluble. The 80-kDa EI of *S. aureus* has been partially purified and appears to be monomeric. The EI protein is phosphorylated by PEP, a reaction that requires Mg^{+2}. The phosphorylation occurs at a histidine residue in the N3 position. HPr is a small protein with a M_r of 8300; its amino acid sequence has been determined (Bey-reuther et al., 1977). Also, HPr is phosphorylated by EI-P in position N1 of His-15 (Schrecker et al., 1975). A comparison of the primary structures of HPr proteins of various Gram-positive and -negative bacteria revealed a similar size and three highly conserved centers (Reizer et al., 1988).

GLUCOSE-SPECIFIC PTS The data available on staphylococcal PTS-dependent glucose uptake are restricted to *S. carnosus*. Two genes located next to each other, *glcA* and *glcB*, have been cloned; the genes complement an *Escherichia coli* mutant strain deficient in glucose uptake (Christiansen and Hengstenberg, 1996). The GlcA and GlcB proteins are highly similar to each other (69% identity) and to glucose-specific enzyme II proteins from *Bacillus subtilis* and *E. coli*.

The two *S. carnosus* PTS EII permeases have fused EII domains in the order EIICBA. Glu-cose is the primary substrate of both permeases, but various glucosides may also be recognized (Christiansen and Hengstenberg, 1999). The gene *glcT* is immediately upstream of *glcA* and the deduced amino acid sequence of its protein GlcT shows a high degree of similarity to bacterial regulators involved in antitermination (Stülke et al., 1998). Interestingly, the activity of these regulators is controlled by PTS-mediated phosphorylation. A putative transcriptional terminator partially overlapping an inverted repeat, which could be the target site for the antiterminator protein GlcT, is found in the *glcT-glcA* intergenic region. This organization resembles the *glcT-ptsG* region of *B. subtilis*, encoding the GlcT antiterminator protein and the glucose-specific enzyme II, respectively (Stülke and Hillen, 1999). Therefore, *glcA* expression in *S. carnosus* is most likely controlled by antitermination. Studies of *S. carnosus* GlcT activity in the heterologous host *B. subtilis* have indicated that the protein is indeed able to cause antitermination (Knezevic et al., 2000).

In *S. aureus* as well as in *S. xylosus*, genes encoding glucose-specific PTS permeases are not arranged in tandem. *Staphylococcus carnosus* may therefore be an exception among the staphylococci. As already mentioned, glucose uptake does not solely rely on PTS activity since HPr or EI mutants of *S. aureus* still ferment glucose. The same is also true for *S. xylosus* (Jankovic and Brückner, 2002) and *S. carnosus* (Brückner, 1997); the entry of glucose into the cells by two different mechanisms may thus be a general phenomenon.

LACTOSE-SPECIFIC PTS The EIIA^lac protein of the lactose-specific PTS of *S. aureus* consists of 103 amino acids (Stüber et al., 1985). Each of the

three subunits is phosphorylated via HPr-P at the N3 position of His-82 (Sobek et al., 1984). The lactose-specific enzyme EIICB^{lac} of *S. aureus* couples translocation to phosphorylation of the transported lactose. It is composed of the N-terminal membrane-bound IIC domain, which includes the sugar-binding site, and the C-terminal IIB domain, which contains the phosphorylation site at Cys-476 (Peters et al., 1995). The kinetics of various EIICB^{lac} fusion constructs have been investigated (Kowolik and Hengstenberg, 1998). The protein, which normally functions as a trimer, is believed to separate into its subunits after phosphorylation. Some of its structural features, like the presence of two histidine residues at the active site, seem to be common to all enzymes, although there is no overall structural similarity to any PTS proteins or to any other proteins in the Protein Data Bank (Sliz et al., 1997). Relevant PTS genes annotated in the genome of *S. aureus* N315 are shown in Table 7.

PTS-INDEPENDENT SUGAR TRANSPORT Only little is known about PTS-independent sugar transport in *S. aureus*. The situation is better with *S. xylosus*, in which sugar transport and its regulation has been investigated by the group of R. Brückner (Tübingen).

In *S. xylosus*, GlcU and GlkA constitute a glucose utilization system enabling glucose to be catabolized independently from the PTS. Glucose is transported through the cell membrane via GlcU in a nonphosphorylated form and is subsequently phosphorylated by GlkA. This PTS-independent system substantially contributes to glucose catabolism in *S. xylosus*. The novel, non-PTS glucose uptake gene *glcU* and the downstream gene *gdh*, which encodes glucose dehydrogenase, are found in a bicistronic operon (Fiegler et al., 1999). A similar organization has also been found in *B. subtilis*. The glucose kinase gene, *glkA*, is not part of the operon in *S. xylosus* (Wagner et al., 1995). The *glcU* and *glkA* genes were first detected by using Tn*917* insertions that resulted in a defect in glucose-specific catabolite repression of the lactose operon. Several other catabolic operons are also affected, which suggests the involvement of GlcU and GlkA in catabolite repression. There is evidence that the GlcU permease is functional at high extracellular glucose concentrations; the highly efficient glucose-specific PTS recognizes and transports glucose preferentially at low external glucose concentrations. The mechanism by which GlcU allows glucose to enter the cells remains to be elucidated. In the genome sequence of *S. aureus*, homologs of *glcU* and *glkA* are present, which strongly suggests that

Table 7. PTS and non-PTS sugar transporters of *S. aureus* N315 and *S. aureus* NCTC 8325.

Gene designation	Encoded protein
General PTS genes (organized in an operon)	
ptsH	Phosphocarrier protein HPr
ptsI	Phosphoenolpyruvate-protein phosphatase, enzyme I
Glucose-specific PTS genes	
ptsG	Glucose-specific IIABC component
glcA	Glucose-specific IIA homolog
—	Glucose-specific IIA homolog
Other sugar specific PTS genes	
mtlF	Mannitol-specific IIBC component
mtlA	Mannitol-specific IIA component
lacE	Lactose-specific IIBC component
lacF	Lactose-specific IIA component
scrA	Sucrose-specific IIBC component
ptaA	*N*-Acetylglucosamine-specific IIABC component
—	Maltose- and glucose-specific II homolog
—	Similar to fructose-specific IIBC
gatC	Similar to galactitol-specific IIB
—	Galactitol-specific enzyme IIC homolog
—	β-Glucoside-specific II ABC
glvC	Arbutin-like IIBC component
treP	Trehalose-specific enzyme II
Non-PTS transporters	
gntP	Gluconate permease
uhpT	Hexose phosphate transport protein
rbsD	Ribose permease
glpT	Glycerol-3-phosphate transporter

Symbol and abbreviation: PTS, phosphoenolpyruvate (PEP):sugar phosphotransferase system; and −, not yet assigned.
[a]Genome sequenced by Kuroda et al. (2001).
[b]Sequenced at the University of Oklahoma Health Sciences Center.

also in this and perhaps other staphylococcal species, glucose is taken up by both PTS-dependent and PTS-independent mechanisms.

Staphylococcus xylosus is unable to utilize arbutin, raffinose, cellobiose, sorbitol, rhamnose and fucose, and fermentation of salicin and ribose is very weak. Sucrose and fructose are transported exclusively via the PTS system, and PTS plays a central role in trehalose, mannose, maltose, maltotriose, and mannitol transport. Galactose and lactose (Bassias and Brückner, 1998), the pentoses xylose, ribose and arabinose, the corresponding pentitols, and glycerol are clearly non-PTS sugars (Lehmer and Schleifer, 1980; Table 6). The lactose utilization enzymes of *S. xylosus* comprise LacP (lactose permease) and LacH (β-galactosidase). The *lacR* gene, found upstream of *lacPH* encodes the activator (Bassias and Brückner, 1998). Lactose transport and β-galactosidase activity are induced by the addition of lactose to the growth medium. The

lacPH promoter is also subject to carbon catabolite repression mediated by the catabolite control protein CcpA. Relevant PTS-independent transporter genes annotated in the genome of *S. aureus* N315 are shown in Table 7.

THE GLYCOLYTIC PATHWAY   Staphylococci are facultatively anaerobic microorganisms. The fructose 1,6-bisphosphate (FBP) and the oxidative

Table 8. Genes of the FBP pathway of *S. aureus* N315 and *S. aureus* NCTC 8325.

Gene designation	Encoded protein
—	Similar to glucokinase
pgi	Glucose-6-phosphate isomerase A
pfk	6-Phosphofructokinase
fbaA	Fructose-bisphosphate aldolase
tpi	Triosephosphate isomerase
gap	Glyceraldehyde-3-phosphate dehydrogenase
pgk	Phosphoglycerate kinase
pgm	2,3-Diphosphoglycerate-independent phosphoglycerate mutase
eno	Enolase (2-phosphoglycerate dehydrogenase)
pykA	Pyruvate kinase

Symbol and abbreviation: –, not yet assigned; and FBP, fructose 1,6-bisphosphate.
[a]Genome sequenced by Kuroda et al. (2001).
[b]Sequenced at the University of Oklahoma Health Sciences Center.

pentose phosphate (PP) pathways are the two central routes of glucose metabolism. There is no evidence for the existence of the Entner-Doudoroff pathway. Earlier studies of glucose metabolism, mainly confined to the oxidative aspects in *S. aureus*, are reviewed by Blumenthal (1972).

The *S. aureus* N315 genome sequence is available (Kuroda et al., 2001), allowing all genes of the FBP glycolytic pathway to be annotated. The genes are listed in the order of the enzymatic steps of the pathway in Table 8.

GALACTOSE AND LACTOSE METABOLISM (THE LELOIR AND THE TAGATOSE-6-P PATHWAYS)   In many microorganisms, galactose is usually metabolized via the Leloir pathway (Bissett and Anderson, 1973; Bissett and Anderson, 1974). In *S. aureus*, galactose is converted to D-galactose 6-P, which is further metabolized through tagatose derivatives (Fig. 3). A study of the distribution of the tagatose-6-P pathway in various staphylococcal species has revealed that the key enzyme of this pathway, tagatose-6-P kinase, is found in *S. aureus*, *S. epidermidis* and *S. hominis*. These species do not possess enzymes of the Leloir pathway. The tagatose-6-P kinase is inducible by growth on galactose or lactose. In contrast, *S. intermedius*, *S. saprophyticus* and *S. xylosus* use galactose only via the Leloir pathway and not via the tagatose-6-P pathway (Schleifer
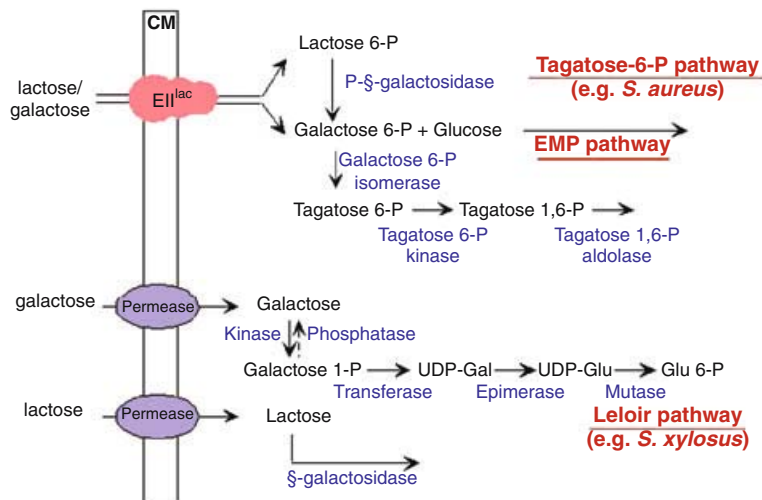
Fig. 3. Lactose and galactose pathways of staphylococci. Transport of lactose and galactose and their catabolism are shown. In *S. aureus*, lactose and galactose are transported by the phosphoenolpyruvate:carbohydrate phosphotransferase system (PTS). Internalized lactose 6-phosphate is hydrolyzed by a phospho-β-galactosidase to yield galactose 6-phosphate and glucose. Galactose 6-phosphate is catabolized through the tagatose 6-phosphate pathway. This pathway most likely exists in staphylococci exhibiting lactose PTS activity. In *S. xylosus* and probably other staphylococcal species that do not possess a lactose PTS, a permease is responsible for the transport of lactose. Galactose uptake has not been studied in these species. Nonphosphorylated lactose is hydrolyzed by a β-galactosidase to yield glucose and galactose. Galactose is likely catabolized through the Leloir pathway. In both staphylococcal species, glucose 6-phosphate, produced by a glucose kinase, enters the FBP pathway, the main glycolytic pathway in staphylococci. Only the encoded products of the galactoside-specific genes are shown. CM, cytoplasmic membrane; EII[lac], lactose-specific enzyme II; UDP-Gal, UDP-galactose; UDP-Glc, UDP-glucose; and Glc 6-P, glucose 6-P.

et al., 1978). The cloned lactose operon (Fig. 4) of *S. aureus* contains not only the phospho-β-galactosidase gene and genes involved in lactose uptake, but also the genes of the tagatose-6-P pathway (Breidt et al., 1987; Oskouian and Stewart, 1987). The corresponding genes are arranged in a heptacistronic operon (*lacABCD-FEG*). The locus was originally defined by mutations abolishing lactose utilization. The genes *lacFE* code for the galactoside-specific PTS permease, enzyme IIA, and enzyme IICB (Breidt and Stewart, 1986). The last gene, *lacG*, encodes the phospho-β-galactosidase. The *lacABCD* genes specify the enzymes of the tagatose-6-P pathway, with *lacAB* coding for galactose-6-P isomerase, *lacC* for tagatose-6-P kinase, and *lacD* for tagatose-1,6-diP aldolase (Rosey et al., 1991). The gene encoding the lactose operon repressor, *lacR*, is upstream of the *lacABCD-FEG* operon (Oskouian and Stewart, 1990a; Oskouian et al., 1990b). Utilization of lactose and galactose in *S. aureus* relies on the PTS-dependent uptake and phosphorylation of the sugars, resulting in lactose 6-P and galactose 6-P, respectively. Glucose and galactose 6-P are produced from intracellular lactose 6-P by a phospho-β-galactosidase (Hengstenberg et al., 1993). Glucose is metabolized via the FBP pathway, whereas galactose 6-P is degraded via the tagatose-6-P pathway.

Supplementation of the growth medium with lactose or galactose results in the induction of the *lac* genes, with galactose 6-P being the intracellular inducer. Apart from this sugar-specific regulation, the lactose operon of *S. aureus* is also subject to global carbon catabolite repression, but the nature of the regulatory mechanism remains unclear (Oskouian and Stewart, 1990a). It is conceivable that part of the described catabolite repression of the *lac* operon of *S. aureus* is actually caused by glucose-mediated inducer exclusion (Reizer et al., 1989; Saier et al., 1996).

The lactose metabolism pathway described for *S. aureus* is not universal for all staphylococci.

In *S. xylosus*, for example, lactose is taken up in the nonphosphorylated form by a lactose permease, a member of the galactoside-pentoside-hexuronide (GPH) cation symporter protein family (Poolman et al., 1996). The lactose permease is encoded by *lacP* and the β-galactosidase by *lacH* (Bassias and Brückner, 1998). After lactose hydrolysis, glucose is phosphorylated by a glucose kinase and catabolized through the FBP pathway, and galactose is likely catabolized through the Leloir pathway (Fig. 3). The genes encoding the enzymes for the Leloir pathway from *S. carnosus* have recently been cloned (B. Krismer, unpublished data), and a gene encoding galactokinase has been isolated from an *S. xylosus* gene library (Brückner et al., 1993). The *lacR* gene of *S. xylosus*, upstream and in the opposite orientation of the *lacPH* operon, encodes an activator belonging to the AraC/XylS family. In contrast to *S. aureus*, *S. xylosus lacPH* operon is induced by only lactose, but not galactose, in the culture medium (Bassias and Brückner, 1998).

METABOLISM OF SUCROSE, MALTOSE, MANNITOL, RIBOSE AND XYLOSE The sucrose PTS permease of *S. xylosus*, encoded by *scrA*, is composed of fused EIIBC domains (Wagner et al., 1993). The EIIA domain, which is essential for PTS-mediated sucrose uptake, has not been identified. On the basis of sucrose utilization in *E. coli* and *B. subtilis*, it appears questionable whether a separate sucrose-specific EIIA protein exists. In both of these organisms, the EIIA domain specific for glucose, either as an independent enzyme or fused to the IICB domains of the glucose permease, serves as phosphoryl donor for the sucrose-specific EII enzymes. The GlcA and GlcB proteins mentioned above would thus be good candidates for sucrose uptake factors.

Internalized sucrose 6-P is cleaved by sucrose-phosphate hydrolase, the gene product of *scrB*, to yield glucose 6-P and fructose (Brückner et al., 1993). Fructose is subsequently phosphorylated

Staphylococcus aureus lactose operon

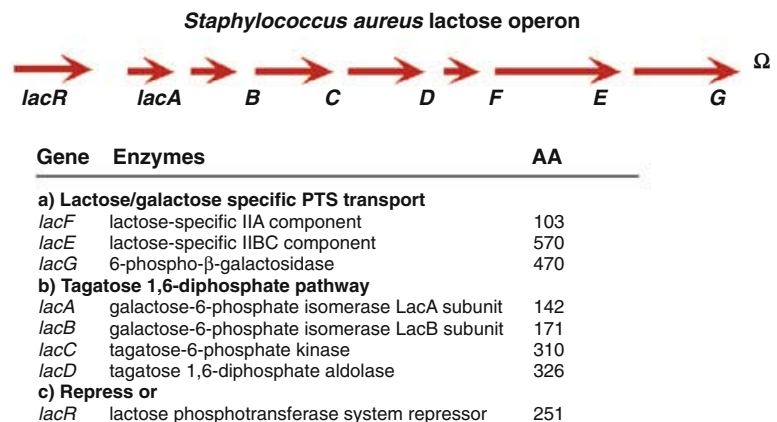| Gene	Enzymes	AA
a) Lactose/galactose specific PTS transport		
lacF	lactose-specific IIA component	103
lacE	lactose-specific IIBC component	570
lacG	6-phospho-β-galactosidase	470
b) Tagatose 1,6-diphosphate pathway		
lacA	galactose-6-phosphate isomerase LacA subunit	142
lacB	galactose-6-phosphate isomerase LacB subunit	171
lacC	tagatose-6-phosphate kinase	310
lacD	tagatose 1,6-diphosphate aldolase	326
c) Repress or		
lacR	lactose phosphotransferase system repressor	251

Fig. 4. Lactose operon of *Staphylococcus aureus* (from Oskouian and Stewart [1990a] and Oskouian et al. [1990b]).

by a fructokinase encoded by *scrK* (R. Brückner, personal communication). The genes *scrB* and *scrK* are located next to each other and form an operon. The sucrose permease gene *scrA* is not located near the *scrBK* genes. Expression of *scrA* as well as *scrB* is induced by sucrose in the medium. Regulation is dependent on the LacI/GalR-type repressor ScrR, whose gene *scrR* is found upstream of *scrB* (Gering and Brückner, 1996). In addition to sucrose-specific ScrR-dependent regulation, the sucrose permease gene *scrA* is subject to carbon catabolite repression by the catabolite control protein CcpA (R. Brückner, personal communication).

An scr gene cluster, consisting of *scrR*, *scrB* and *scrK*, is also present in *S. aureus*. It is located next to *agrA*, the gene encoding the response regulator of the accessory gene regulator system (Agr; Novick et al., 1995). The gene for the sucrose permease has not yet been detected.

Maltose utilization in *S. xylosus* is dependent on an α-glucosidase (maltase), whose gene, *malA*, is found in the *malRA* operon; *malR* encodes a regulator belonging to the LacI/GalR family (Egeter and Brückner, 1995). The same enzymatic activity as mediated by MalA in *S. xylosus* has been characterized in *S. aureus*, and a gene with high sequence similarity has been identified in the *S. aureus* genome sequence. Therefore, both staphylococcal species probably use this enzyme to cleave maltose. However, the mechanism of maltose transport remains to be elucidated. In any case, at least one glucose moiety has to be phosphorylated by the glucose kinase for complete maltose catabolism.

The mannitol phosphotransferase system of *S. carnosus* has been described. The system consists of an EIICB enzyme (encoded by *mtlA*) and of EIIA (encoded by *mtlF*), which together form the mannitol-specific PTS permease. The *mtlA* and *mtlF* genes are clustered on the *S. carnosus* chromosome with *mtlD*, the gene for mannitol-1-phosphate dehydrogenase, which produces fructose 6-P. The gene *mtlA* is about 2 kb away from *mtlF* and *mtlD* (Fischer et al., 1989; Fischer and Hengstenberg, 1992). The nucleotide sequence of this intervening region has not been determined. *Staphylococcus aureus* possesses an EIIA protein specific for mannitol with the same apparent molecular mass as the *S. carnosus* enzyme and virtually identical amino acid sequence at the amino terminus and around the phosphorylation site (Reiche et al., 1988). Thus, the *mtl* systems in the two organisms appear to be very similar.

The uptake of pentoses and pentitols is PTS-independent (Lehmer and Schleifer, 1980). Many staphylococcal species are unable to ferment pentoses, such as D-ribose, L-arabinose, and D-xylose, but are capable of pentose uptake.

For example, anaerobically grown *S. epidermidis* cells are unable to ferment ribose, mannitol, and lactose, but transport measurements confirm that ribose is taken up (Sivakanesan and Dawes, 1980). The genes encoding the enzymes for ribose utilization in *S. hyicus* have been cloned in *S. carnosus*, which thereby obtained the ability to utilize D-ribose (Keller et al., 1984). The *S. hyicus* DNA donor strain possessed D-ribokinase and D-ribose-5-P isomerase, which are absent in *S. carnosus*. Although the parent *S. carnosus* strain was unable to utilize ribose, the strain possessed a D-ribose-inducible uptake system that was severely repressed by the addition of glucose.

The xylose degradation genes of *S. xylosus* are organized in the operon *xylABR*, consisting of *xylR* (encoding the Xyl repressor), *xylA* (encoding xylose isomerase), and *xylB* (encoding xylulokinase; Sizemore et al., 1991). A xylose transporter is not part of the operon. The XylR protein acts as a repressor that binds to an operator site identified by footprint analysis (Sizemore et al., 1992). An interesting finding is that xylose is the inducer; gel-mobility shift experiments have shown that XylR is inactivated only in the presence of xylose, but not of xylose-P or other phosphorylated sugars. The *xyl* operon is also subject to glucose catabolite repression at the level of transcription; this control is independent of a functional *xylR* gene. Indeed, apart from the XylR operator sequence, a catabolite responsive element (*cre*) is also in front of *xylA*; *cre* is involved in glucose repression by binding activated CcpA (Hueck et al., 1994; Egeter and Brückner, 1996).

Catabolite Regulation

The major end product of anaerobic glucose metabolism in *S. aureus* is lactate (73–94%); acetate (4–7%) and traces of pyruvate are also formed (Theodore and Schade, 1965). During aerobic growth, acetate and CO_2 are the predominant end products; only 5–10% of the glucose carbon appears as lactate (Strasters and Winkler, 1963). In the presence of glucose, glycolysis is enhanced, and many enzymes of the pentose phosphate (PP) pathway and the tricarboxylic acid (TCA) cycle are suppressed; furthermore, the oxidation of pyruvate and the cytochrome content are decreased in glucose-grown *S. aureus* cells. Glucose-mediated catabolite repression is markedly pronounced in staphylococci.

The addition of glucose to aerobically grown *S. aureus* cells reduces both glucose degradation via the PP pathway and the subsequent oxidation of pyruvate via the TCA cycle. An investigation of the specific activity of various PP enzymes has

revealed no marked differences in extracts of *S. aureus* grown with or without glucose in nutrient broth. However, the specific activities of two enzymes of the FBP pathway (glyceraldehyde-3-P dehydrogenase and lactate dehydrogenase) are markedly increased in the presence of glucose. Furthermore, in the presence of glucose, the specific activities of the TCA cycle enzymes (succinate dehydrogenase and fumarase) are markedly decreased and fumarase activity is not detectable.

The reduced activities of the TCA cycle enzymes are very likely due to a repression of their biosynthesis; this phenomenon is referred to as the "glucose effect" or "carbon catabolite repression." The glucose-mediated inhibition of the TCA cycle activity could be triggered by the observed increase in the ATP pool. Citrate synthases of staphylococci are not affected by reduced nicotinamide adenine dinucleotide (NADH), but are severely inhibited by ATP (Hoo et al., 1971). In the presence of glucose, the nicotinamide adenine dinucleotide (NAD)-dependent L-lactate dehydrogenase of *S. aureus* has considerable activity during aerobic growth; although its activity is about tenfold less than that under anoxic conditions. It is therefore not surprising that in the presence of glucose, some lactic acid is produced even under oxic conditions (Garrard and Lascelles, 1968). During aerobic growth in the absence of glucose, an oxidation of acetate, succinate and malate by resting *S. aureus* cell suspensions has been observed manometrically (Collins and Lascelles, 1962; Strasters and Winkler, 1963). However, when glucose or galactose (0.04 M) is present in the growth medium, the oxidation of these substrates is abolished. Growth in the presence of glucose also results in a 40-fold decrease in the cytochrome content (Strasters and Winkler, 1963). The presence of a pyruvate dismutation system in staphylococci was first described by Krebs (1937). Pyruvate dehydrogenase activity has been demonstrated in *S. aureus* and *S. epidermidis* (Sivakanesan and Dawes, 1980).

REGULATION OF LACTATE DEHYDROGENASE ACTIVITY Anaerobically grown *S. epidermidis* cells ferment glucose with the production of lactate and trace amounts of acetate, formate, and CO_2. In *S. aureus*, glucose is metabolized principally via glycolysis and to a limited extent by the PP oxidative pathway (Sivakanesan and Dawes, 1980). However, certain staphylococcal species, such as *S. epidermidis* and *S. intermedius*, are distinguished by a fructose-1,6-bisphosphate (FBP)-activated, NAD-dependent L-lactate dehydrogenase (LDH; Götz and Schleifer, 1975; Götz and Schleifer, 1976). The enzyme has a total M_r of 130,000 and is composed of four subunits.

Physiological studies with *S. epidermidis* have shown that the intracellular concentration of FBP influences the LDH activity. During anaerobic growth and in the presence of glucose, a high intracellular concentration of FBP is reached, resulting in maximal LDH activity. In cells grown anaerobically in a glucose-limited medium, the FBP pool is exhausted because of glucose limitation. In this case, LDH is not fully saturated with FBP and its activity is not maximal. Similar results have been obtained with cells grown aerobically in a glucose-excess medium (Götz and Schleifer, 1978).

CLASS I ALDOLASE Aldolase is one of the key enzymes of the glycolytic pathway. There are two forms of fructose-1,6-bisphosphate aldolase which can be differentiated by their catalytic and structural properties. Class I aldolases function via the formation of a Schiff base intermediate between the substrate and a lysine amino group of the enzyme. Class II aldolases do not form a Schiff base intermediate, but contain an essential divalent cation, such as Zn^{+2}, Ca^{+2} or Fe^{+2}, and can be inhibited by EDTA. Since class I aldolases are typical for higher animals and plants and are only found in a few bacteria, it is surprising that nearly all staphylococcal species studied so far possess a class I aldolase (Götz et al., 1980). The only exceptions are *S. intermedius* and *S. hyicus*, which possess both classes of aldolases, and *S. caseolyticus* (reclassified as *Macrococcus caseolyticus*), which possesses only a class II aldolase (Fischer et al., 1982). The 33-kDa class I aldolase of *S. aureus* strain ATCC 12600 has been purified; it appears to be active as a monomer (Götz et al., 1980). Later, the *S. carnosus* TM300 *fda* gene, encoding the glycolytic fructose-1,6-bisphosphate aldolase (Fda), was cloned in *E. coli*. The 296-amino-acid protein has a M_r of 32,855. The *S. carnosus* FDA is also a class I aldolase (EC 4.1.2.13), which does not need divalent metal ions for catalytic activity. Cloning of *fda* back into *S. carnosus* led to a sixfold increase of aldolase production and activity. Glucose in the growth medium has a stimulating effect on aldolase production or activity (Witke and Götz, 1993).

CARBON CATABOLITE REPRESSION The availability of carbohydrates, especially of glucose, leads to regulatory processes often referred to as the "glucose effect" or "carbon catabolite repression" (Saier et al., 1995; Saier et al., 1996). Numerous publications on *S. aureus* describe the influence of glucose on a variety of cellular processes, such as utilization of alternative carbon sources, production of extracellular enzymes, activity of glycolytic enzymes, and

cytochrome content. Especially the production of potential virulence factors has attracted considerable attention. However, the mechanism by which glucose exerts its regulatory effect has not been elucidated. The analysis of catabolite repression of inducible systems is quite often complicated by the inability of the inducer to enter the cells in appreciable amounts, when glucose is also present in the medium. In addition to this process, referred to as "inducer exclusion/inducer expulsion," the rapid removal of internalized inducer, has been described for a number of Gram-positive bacteria (Saier et al., 1996). While inducer exclusion is found in a wide variety of bacteria, inducer expulsion is encountered less frequently. The latter does not appear to operate in *S. aureus*. The molecular mechanisms leading to inducer exclusion in Gram-positive bacteria are not yet completely understood.

In Gram-positive bacteria, one form of carbon catabolite repression relies on a transcriptional regulator, termed "catabolite control protein A" (CcpA; Henkin, 1996), a member of the GalR-LacI family of transcription factors (Weickert and Adhya, 1992). CcpA-dependent carbon catabolite repression has been analyzed in some detail in *S. xylosus* (Egeter and Brückner, 1996). Inactivation of the *ccpA* gene by insertion of a resistance cassette leads to a pleiotropic loss of transcriptional regulation at several promoters, including the *ccpA* promoters. This autoregulation of *ccpA* apparently reflects the need of *S. xylosus* to balance regulation by CcpA carefully. CcpA shows a relatively weak affinity for its cognate operator sites, termed "catabolite responsive elements" (*cre*; Hueck et al., 1994), and must, therefore, be activated to bind efficiently to *cre*. The signal enabling CcpA to bind to DNA is a phosphorylated form of HPr, the phosphocarrier protein of the PTS, which is produced by the HPr kinase (HPrK; Huynh et al., 2000). HPr is phosphorylated at Ser-46 in an ATP-dependent manner and can also be dephosphorylated by the same enzyme. Thus, HPrK constitutes a bifunctional HPr kinase/phosphatase. HPr as well as HPr kinase are absolutely required for CcpA activity in *S. xylosus* (Jankovic and Brückner, 2002). The genetic organization around the *hprK* gene of *S. xylosus* is identical to that in *S. epidermidis* and *S. aureus* (Huynh et al., 2000).

In addition to the loss of carbon catabolite repression, the *S. xylosus* HPr-kinase-deficient strain shows an unexpected phenotype. Its growth is inhibited by glucose. The mutant strain transports glucose at much higher rates than the wildtype strain, and it also produces methylglyoxal, which indicates unbalanced glucose metabolism. Therefore, HPr kinase is not only

important to trigger carbon catabolite repression, it is also needed to balance carbohydrate uptake and catabolic capacities of the cell. A summary of the current knowledge of CcpA-dependent catabolite repression in staphylococci and other Gram-positive bacteria with low DNA G+C content is shown in Fig. 5.

Gluconeogenesis

In the absence of a fermentable sugar source, heterotrophic microorganisms normally have to synthesize glucose or glucose 6-P. Pyruvate usually serves as a starting substrate. The pathway is essentially a reversal of glycolysis. Most of the glycolytic enzymes can also catalyze the reverse reaction. In gluconeogenesis, however, three bypass steps are involved: 1) pyruvate to phosphoenolpyruvate, 2) fructose 1,6-bisphosphate to fructose 6-P, and 3) glucose 6-P to glucose. The latter bypass is mostly found in mammals, where glucose has both signal and regulatory functions. Indeed, a glucose-6-phosphatase homolog has not yet been annotated and is very likely not necessary in staphylococci. Enzymes encoded by *pycA*, *pckA* and *fbp* represent the bypass enzymes, while the other enzymes belong to the FBP (fructose 1,6-bisphosphate) pathway and catalyze the reverse reaction.

The postulated gluconeogenesis pathway, according to the genes identified in the *S. aureus* N315 genome sequence (Kuroda et al., 2001), is shown in Fig. 6.

Oxidative Pentose Phosphate Cycle

The PP cycle is an essential cycle since it serves as the source of pentose phosphates, of which 5-phospho-ribosyl-1-pyrophosphate is required for the biosynthesis of nucleotides. According to the *S. aureus* N315 genome sequence, the relevant genes are present; only the lactonase gene has not yet been annotated. In Table 9, the corresponding annotated genes are listed.

Aerobic Respiration

Tricarboxylic Acid Cycle Not much is known about the biochemical properties or enzyme activities of the TCA cycle enzymes in staphylococci under various conditions. Most enzymatic activities were identified, e.g., aconitase (Somerville et al., 2002), isocitrate dehydrogenase, succinate dehydrogenase (Solozhenkin et al., 1991), fumarase, or malate dehydrogenase (Tynecka and Gajdzinska, 1967). In the genome sequence of *S. aureus* strains N315 and 8325, all the classical TCA cycle genes were annotated (Table 10). One therefore can assume that staphylococci
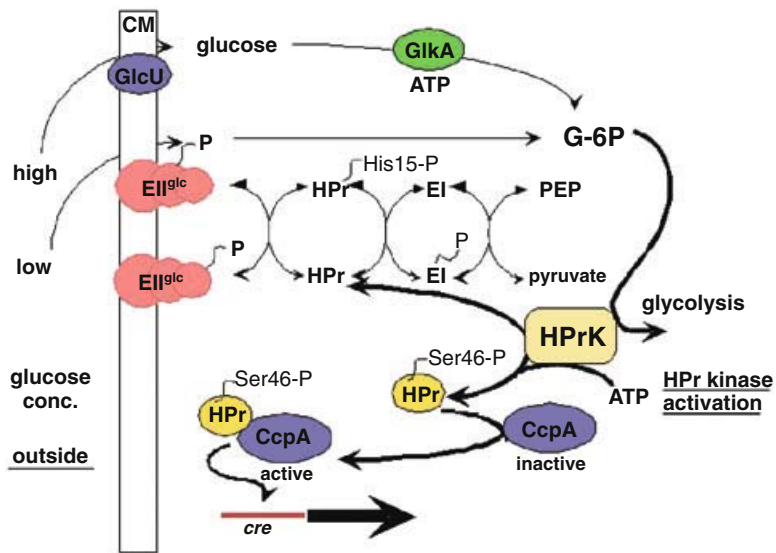
Fig. 5. Summary of the current knowledge on CcpA-dependent catabolite repression in staphylococci and other Gram-positive bacteria with low G+C content. When provided with a rapidly metabolizable phosphotransferase system (PTS) sugar (e.g., glucose), bacteria transport it via the PTS. During the process of sugar transport, the phosphoryl group is transferred to the entering sugar, and the PTS proteins are predominantly left in a non-phosphorylated form. Heat-stable phosphocarrier (HPr) kinase senses the presence of glucose in the cell, most probably via intermediates of glycolysis, and phosphorylates non-phosphorylated HPr at Ser-46 in an ATP-dependent manner. By interacting with the central regulator CcpA, P-Ser-46-HPr increases its affinity for cre operators. The complex CcpA/HPr-Ser46-P/*cre* blocks or activates the transcription of different operons. In the absence of glucose, the activity of HPrK shifts towards P-Ser-HPr phosphatase. Since there is no available sugar and thus no phosphoryl group transfer by the PTS, the HPr protein remains phosphorylated at His-15. Without P-Ser-46-HPr as a co-effector, CcpA shows very low affinity for binding to *cre* elements, and as a consequence, CcpA does not regulate the catabolic operons. It is assumed that in the presence of high glucose concentrations, glucose is mainly transported independently from the PTS via the permease GlcU in a non-phosphorylated form and is subsequently phosphorylated by glucose kinase, GlkA.

Table 9. Genes of the oxidative pentose phosphate cycle in *S. aureus* N315 and *S. aureus* NCTC 8325.

Gene designation	Encoded enzyme
—	Glucose-6-phosphate dehydrogenase
gnd	Phosphogluconate dehydrogenase (decarboxylating)
rpi	Similar to ribose-5-phosphate isomerase
cfxE	Ribulose-5-phosphate 3-epimerase homolog
—	Similar to transaldolase
tkt	Transketolase

Symbol: –, not yet assigned.
[a]Genome sequenced by Kuroda et al. (2001).
[b]Sequenced at the University of Oklahoma Health Sciences Center.

Table 10. Tricarboxylic acid cycle genes.

Gene designation	Gene function
citZ	Citrate synthase II
citB	Aconitate hydratase
citC	Isocitrate dehydrogenase
odhA	2-Oxoglutarate dehydrogenase E1
odhB	Dihydrolipoamide succinyltransferase
sucC	Succinyl-CoA synthetase (β-subunit)
sucD	Succinyl-CoA synthetase (α-subunit)
sdhC	Succinate dehydrogenase cytochrome b-558
sdhA	Succinate dehydrogenase flavoprotein subunit
sdhB	Succinate dehydrogenase iron-sulfur protein subunit
citG	Fumarate hydratase, class-II
mdh	Malate dehydrogenase homolog

From Kuroda et al. (2001).

have in principle a complete TCA cycle; however, bottlenecks exist.

The TCA cycle is fuelled by acetyl CoA and the responsible enzyme is the pyruvate dehydrogenase (PDH), which catalyzes the oxidative decarboxylation of pyruvate, to form acetyl-CoA. The *S. aureus* PDH complex has been isolated and the last gene of the PDH operon, *pdh*D, which encodes the lipoamide dehydrogenase (LPD) has been sequenced (Hemila, 1991).

The *S. aureus* genome sequence revealed the E_2 core complex subunit and the peripheral subunits E_1 and E_3 (Table 11). The *S. aureus* genome contains also a typical pyruvate carboxylase gene that is necessary for initiation of the TCA cycle (Table 6). The activity of this enzyme has been verified.

Pyruvate

ATP ⟶ + HCO_3^-		**Pyruvate**
ADP ⟵		**carboxylase (pycA)**

Oxaloacetate

A(G)TP ⟶	**PEP-**
A(G)DP ⟵	**carboxykinase (pckA)**

PEP

⇅   **Enolase**
     Mutase

3-P-glycerate

ATP ⟶	**P-glycerate**
ADP ⟵	**kinase**

1,3-P-glycerate

⟶ NADH	**GAP-DH**
⟵ NAD+	**TPI**
	Aldolase

Fructose-1,6-P

ATP ⟶	**Fructose-**
ADP ⟵	**bisphosphatase**

Fructose-6-P

⇅   **Isomerase**

Glucose-6-P

⋮
↓

(Glucose ?)
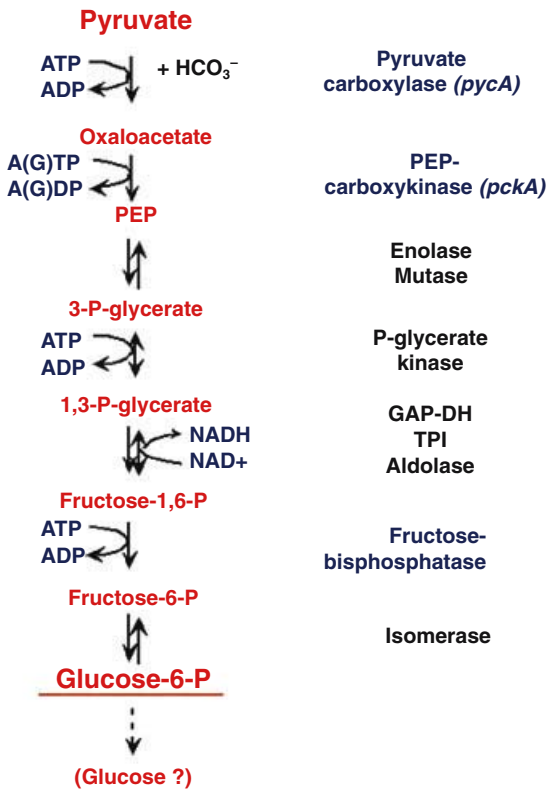
Fig. 6. Proposed gluconeogenesis pathway in *S. aureus*. PEP, phosphoenol pyruvate; GAP-DH, D-glyceraldehyde-3-phosphate dehydrogenase; and TPI, triose phosphate isomerase.

Table 11. Entrance into the TCA-cycle.

Gene designation	Gene function
1. Pyruvate-DH (E1, E2 and E3 components) Pyruvate → acetyl-CoA + CO_2	
phdB	Pyruvate dehydrogenase E1 component β-subunit
pdhC	Dihydrolipoamide S-acetyltransferase E2 component[a]
	Acetyl-CoA + dihydrolipoamide ↔ CoA + S-acetyldihydrolipoamide
pdhD	Dihydrolipoamide dehydrogenase component E3
2. Pyruvate carboxylase Pyruvate + HCO_3^- + ATP ↔ Oxalacetate + ADP + Pi	
pycA	Pyruvate carboxylase

[a]Hemila (1991).
From Kuroda et al. (2001).

RESPIRATORY CHAIN Taber and Morrison (1964) identified three cytochromes in *S. aureus*: cytochrome *a*-602 and cytochromes *b*-555 and *b*-557. The two *b*-type cytochromes have different reactivities. Cytochrome *b*-557 is reduced in the presence of 2-heptyl-4-hydroxyquinoline-*N*-oxide (HOQNO), while all the other hemoproteins of the respiratory chain remain oxidized. In contrast to cytochromes *a*-602 and *b*-555, cytochrome *b*-557 is not reduced by ascorbate-dichlorophenol-indophenol, suggesting that it has a lower reduction potential than the other cytochromes. The most significant difference, however, is that cytochrome *b*-557, unlike cytochromes *b*-555 and *a*-602, does not react with carbon monoxide. Cytochrome *b*-557 appears to be an intermediate electron carrier of the cytochrome *b* or *b₁*-type. Cytochrome *b*-555, which is also found in *S. epidermidis*, binds carbon monoxide and probably is the major terminal oxidase; it is therefore, now referred to as "cytochrome *O*." There are some indications that the prosthetic groups of cytochromes *O* and *b*-557 are protohemins. The role of cytochrome *a*-602 as a terminal oxidase is less clear. It may be involved with nitrate reductase activity. In particulate enzyme preparations from *S. aureus*, succinate oxidase, NAD-linked ethanol oxidase, and NADH-oxidase activities are detectable. Succinate oxidase activity is inhibited by ultraviolet (UV) light (340 nm) and surprisingly by amytal (barbiturate A), which inhibits electron transfer from NADH to ubiquinone in higher organisms. The two oxidation-reduction dyes, methylene blue and 2,6-dichlorophenol-indophenol interact with the electron transfer system. The reduced form of 2,6-dichlorophenol-indophenol reduces cytochrome *a*-602 and cytochrome *O* without reducing cytochrome *b*-557. It was found that trimethylamine-*N*-oxide (TMAO) inhibited the growth of *S. aureus* but not of *S. epidermidis*. This selective inhibition of *S. aureus* was based on the inhibition of the electron transport system by oxidizing the cytochromes in *S. aureus*. It was suggested that the inhibition occurred between cytochrome *b* and cytochrome *O* (Suzuki et al., 1992).

Analysis of cytochrome spectra of *S. aureus* and other staphylococcal species revealed two more minor cytochromes of the *b*-type in addition to cytochromes *a*-602, *b*-557, and *O*-555 (Faller et al., 1980). Cytochrome *b*-552 is found in all staphylococcal species except *S. sciuri*. Cytochromes *b*-560 and *b*-566 are widely distributed in staphylococci. Cytochromes of the *c*-type (e.g., *c*-549 and *c*-554) have been only found so far in *S. sciuri*, *S. lentus* and *Macrococcus caseolyticus*.

On the basis of the presence of cytochrome *c*, modified oxidase and benzidine tests have been developed which allow rapid differentiation between staphylococci on one hand and micrococci and macrococci on the other hand (Faller and Schleifer, 1981).

Staphylococci possess menaquinones (MK, vitamin K_2) as their sole isoprenoid quinones.

There is a species-specific variation with regard to the length of the isoprenoid side chains. The two principal menaquinones have seven to eight isoprene units (Collins and Jones, 1981). Menaquinones are located in the cytoplasmic membrane (White and Frerman, 1967) and play important roles in electron transport and oxidative phosphorylation.

Staphylococcus aureus uses aerobically very likely menaquinone oxidase(s) and at least four quinol oxidase-like genes (*qox*A, B, C and D) were identified. Since the *S. aureus qoxA* to *qoxD* genes are very similar to the corresponding *B. subtilis* genes, we expect these genes will have a similar function. As all analyzed staphylococcal species possess a cytochrome *a*-605, we believe that this cytochrome is part of the *S. aureus* Qox system and functions as a second terminal oxidase. Very likely the ubiquitous cytochrome *b*-552 (Faller et al., 1980) is associated with cytochrome *a*-605.

Moreover, the *S. aureus* genome sequences revealed two putative cytochrome *d* menaquinol oxidases: subunit I homolog (*cydA*) and subunit II homolog (*cydB*). Since a *d*-type cytochrome has never been observed in the staphylococcal cytochrome spectrum (Faller et al., 1980), it is possible that CydAB complex represents the cytochrome *O*-555 menaquinol oxidase. The very few bits of information on the respiratory components of staphylococci suggest that they have a branched respiratory system consisting of two alternative and menaquinol-dependent terminal oxidases, a cytochrome *bo* and a cytochrome *aa3* oxidase. The still limited knowledge on the overall electron transfer sequence is illustrated in Fig. 7. The scheme is necessarily incomplete since the components of the respiratory chains of *S. aureus* and other staphylococci are not yet fully identified.

The *S. aureus* genome sequence revealed the presence of a typical F_0F_1-ATP synthase [EC:3.6.3.14] with the following gene order of the various subunits: epsilon (*atpC*), beta (*atpD*), gamma (*atpG*), alpha (*atpA*), delta (*atpH*), b (*atpF*), c (*atpE*), and a (*atpB*) subunits.

Anaerobic Respiration

In respiration, nitrate is used as an alternative electron acceptor when oxygen is not available. The enzymes for this pathway are only found in bacteria. Two main forms have been described so far; in both, nitrate reduction is coupled to the generation of a proton motive force (p; Boonstra and Konings, 1977; Jones et al., 1980), which is directly utilized as a source of energy or transformed to ATP by a membrane-associated ATPase.

Despite its broad application in food technology, little was known about the biochemistry and genetics of nitrate reduction in *S. carnosus*. A physiological characterization of nitrate and nitrite reduction in *S. carnosus* (Neubauer and Götz, 1996) revealed the presence of a typical dissimilatory nitrate reductase with the following features: 1) repression and inhibition by oxygen, 2) induction by anaerobiosis and nitrate or nitrite, 3) no inhibition by ammonia, 4) energy gain under anaerobic conditions, and 5) localization in the membrane.

Nitrite reductase in *S. carnosus* is a NADH-dependent cytosolic enzyme that forms ammonia as the only end product. The enzyme is regulated similar to dissimilatory enzymes, i.e., it is 1) repressed and inhibited by oxygen, 2) induced by anaerobiosis and nitrate or nitrite, and 3) not inhibited by its end product ammonia. The relatively low energy gain with nitrite under anaerobic conditions points towards a function as terminal electron acceptor, which might be important for the anaerobic recycling of NADH (Neubauer and Götz, 1996). In cells of *S. carnosus*, nitrate is reduced to ammonia in two steps. The first step includes uptake of nitrate, its reduction to nitrite and the subsequent excretion of nitrite. The second step takes place after depletion of nitrate: the accumulated nitrite is imported and reduced to ammonia, which accumulates in the medium. In growth yield studies, nitrate reduction appeared energetically more favorable than nitrite reduction, which makes sense since nitrate reduction is most likely coupled to the generation of a proton motive force. A comparison of the turnover rates in whole cells with glucose as electron donor reveals that nitrate reduction is 10-fold faster than nitrite reduction. For the inhibition of nitrite reduction by nitrate, two possible mechanisms are discussed which are not mutually exclusive. Both are based on the higher activity of nitrate compared to nitrite reductase. Both enzymes compete for NADH, which is in vivo the most important electron
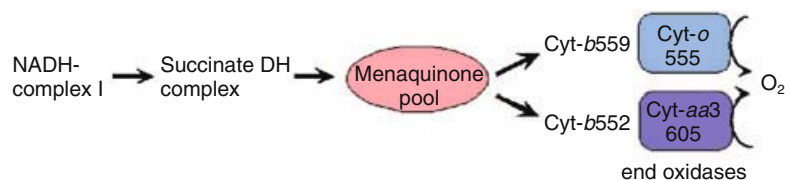

Fig. 7. Proposed respiratory system in staphylococci.

donor. Owing to its higher specific activity, nitrate reductase is expected to perform the bulk of NADH oxidation. In addition, the high rate of nitrate reduction could lead to an internal accumulation of nitrite, which could be the result of a less efficient nitrite reduction or export. High concentrations of nitrite (100 mM) effectively inhibit nitrite reductase but not nitrate reductase activity (Neubauer and Götz, 1996).
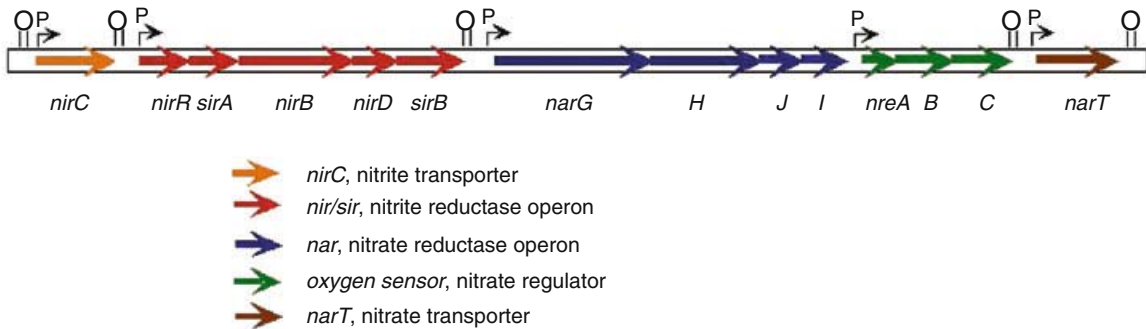
THE NITRATE REDUCTASE OPERON (NARGHJI) The enzyme that catalyzes the energy-gaining reduction of nitrate to nitrite is well characterized in *E. coli*. The membrane-bound dissimilatory nitrate reductase (NRA) is an enzyme complex composed of three subunits ($a_2b_2g_4$). The corresponding genes are organized in an operon, *narGHJI* and *narZYWV*. The NarJ protein is believed to be involved in the assembly of the native enzyme complex (Dubourdieu and DeMoss, 1992). The γ-subunit (NarV), a *b*-type cytochrome, receives electrons from the quinone pool. The electrons are then transferred via the iron-sulfur clusters of the β-subunit (NarY) to the molybdenum cofactor bound to the α-

subunit (NarG and NarZ, respectively; Blasco et al., 1989).

To gain more insight into the genetic basis of dissimilatory nitrate reduction, a transposon Tn*917* mutagenesis in *S. carnosus* TM300 was performed and mutants defective in the conversion of nitrate to nitrite were selected and analyzed. Various nitrate-reductase-negative mutants led to the identification of a similar nitrate reductase operon, *narGHJI*, as described for *E. coli* (Pantel et al., 1998). Unlike in *E. coli*, in *S. carnosus* there is no evidence for a second operon. The organization of genes and operons involved in dissimilatory nitrate/nitrite reduction are shown in Fig. 8.

Transcription from the *nar* promoter is induced by anaerobiosis, nitrate and nitrite. This is in accordance with the nitrate reductase activities determined with benzyl viologen as electron donor. However, in the presence of oxygen and nitrate, high transcription initiation but low nitrate reductase activity was observed. Since the nitrate reductase formed during anaerobic growth was insensitive to oxygen, other oxygen-sensitive steps (e.g., posttranscriptional mechanisms and molybdenum cofactor biosynthesis)

A) Genetic organization of the nitrite/nitrate gene locus

nirC   nirR sirA   nirB   nirD sirB   narG   H   J I   nreA B   C   narT

nirC, nitrite transporter
nir/sir, nitrite reductase operon
nar, nitrate reductase operon
oxygen sensor, nitrate regulator
narT, nitrate transporter

B) Genetic organization of the molybdo-cofactor biosynthesis genes

P   modA   B   C   P   moeB   moaB   moaC   moeA   mobB   moaE D   mobA   moaA

mod, molybdate transport
moa and *moa* genes most likely involved in biosynthesis of molybdopterin
mob genes most likely involved in insertion of GMP
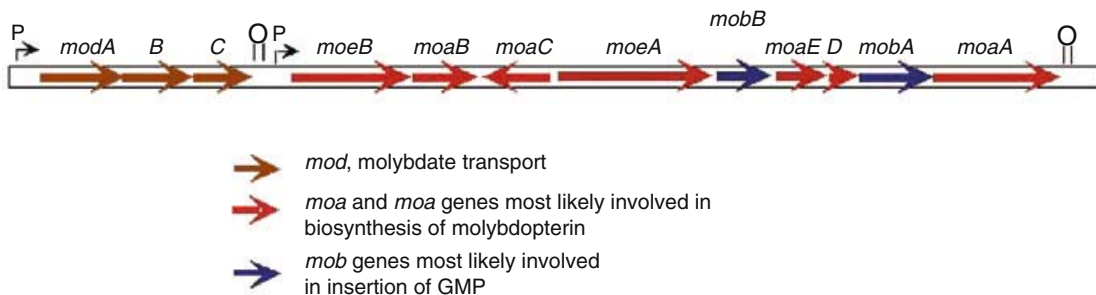
Fig. 8. Genetic organization of genes and operons involved in the dissimilatory nitrate/nitrite reduction in *Staphylococcus carnosus*.

must be involved. In *E. coli*, the *nar* promoter is induced by the *trans*-acting factors ferredoxin-NADP reductase (Fnr) and phosphorylated NarL; Fnr is activated in response to oxygen deprivation and NarL is the response regulator of a two-component system that signals the presence of nitrate. In the *S. carnosus nar* promoter, no conspicuous Fnr and integration host factor (IHF) recognition sites are present; there is only one site that is related to the *E. coli* NarL consensus sequence.

THE NITRITE-REDUCING SYSTEM Characterization of a nitrite-reductase-negative *S. carnosus* Tn*917* mutants led to the identification of the *nir* operon, which is comprised of five genes: *nirR*, *sirA*, *nirB*, *nirD* and *sirB* (Neubauer et al., 1999a). The NirBD proteins represent the dissimilatory NADH-dependent nitrite reductase, SirA and SirB (both necessary for biosynthesis of the siroheme prosthetic group), the putative oxidase/chelatase and the uroporphyrinogen III methylase, respectively, and NirR, a protein with known function. It was suggested that NirR is essential for *nir* promoter activity (Neubauer et al., 1999a). In the absence of NirR, a weak promoter upstream of *sirA* seems to drive transcription of *sirA*, *nirB*, *nirD* and *sirB* in the stationary growth phase. Nitrite reduction in *S. carnosus* does not occur in the presence of oxygen and nitrate (Neubauer and Götz, 1996), which is in agreement with the fact that anaerobiosis, nitrite, and nitrate induce the *nir* promoter. Although a transcript is detectable, no nitrite reduction occurs in cells grown aerobically with nitrate or nitrite, indicating an additional oxygen-controlled step at the level of translation, enzyme folding, assembly, or insertion of prosthetic groups. The nitrite-reducing activity expressed during anaerobiosis is switched off reversibly when the oxygen tension increases, most likely owing to the competition for electrons with the aerobic respiratory chain. Another gene, *nirC*, is located upstream of the *nir* operon and encodes a putative integral membrane-spanning protein with unknown function. A *nirC* mutant showed no distinct phenotype. In *S. carnosus*, nitrate is first reduced to nitrite evidently by a dissimilatory enzyme. Nitrite accumulates in the growth medium and is further reduced by a dissimilatory NADH-dependent nitrite reductase. Nitrite reduction only occurs in the absence of nitrate.

The *narT* mutant displayed wildtype levels of nitrate reductase activity but, unlike the wildtype strain, does not take up nitrate and accumulate nitrite when grown in the presence of nitrate under anaerobic conditions (Fast et al., 1997). The 41-kDa NarT is a highly hydrophobic trans-membrane protein of 388 amino acids. Its protein sequence shows similarity to *B. subtilis* NasA (25.8% identity) and *E. coli* NarK (22.8% identity).

MOLYBDOPTERIN COFACTOR BIOSYNTHESIS All molybdoenzymes except nitrogenase contain a unique form of molybdopterin as cofactor, sometimes conjugated to a nucleoside monophosphate (Rajagopalan and Johnson, 1992). At least five different loci in *E. coli* are involved in molybdate transport and processing, synthesis of molybdopterin, and conversion to the dinucleotide form (Rajagopalan, 1996).

In *S. carnosus*, nine genes were identified (Fig. 8), all of which appear to be involved in molybdenum cofactor biosynthesis (Neubauer et al., 1998). The regulation and function of one of the proteins, MoeB, was further analyzed. In addition, *moeB* Tn*917*-insertion mutants showed no nitrate reductase activity because they were molybdenum-cofactor-deficient. However, nitrate reductase activity in cell-free extracts could be reconstituted with a low-molecular-weight component (most likely free molybdenum cofactor) from an *S. carnosus* mutant that is defective in the nitrate reductase structural genes. Expression studies of *moeB* indicated that anaerobiosis and nitrate each enhance transcription of *moeB* (Neubauer et al., 1998).

MOLYBDATE TRANSPORT SYSTEM MODABC In *S. carnosus* three genes, *modABC*, were identified which encode an ATP-binding cassette (ABC) transporter that is involved in molybdate transport (Neubauer et al., 1999b). These genes are located directly upstream of *moeB* and seem to be part of the molybdenum cofactor biosynthesis gene cluster. The *mod* mutants are devoid of nitrate reductase activity. It was shown by [^{14}C]-palmitate labeling that ModA represents a lipoprotein that, in Gram-positive bacteria, is the counterpart of the periplasmic binding proteins of Gram-negative organisms. The sequence characteristics identify ModB as the integral-membrane, channel-forming protein and ModC as the ATP-binding energizer for the transport system. Mutants defective in *modABC* revealed only 0.4% of wildtype nitrate reductase activity. Molybdate at a nonphysiologically high concentration (100 μM) fully restored nitrate reductase activity, suggesting that at least one other system is able to transport molybdate but with lower affinity. The expression of *modA* (and most likely *modBC*) was independent from oxygen and nitrate. To date, there are no indications for molybdate-specific regulation of *modABC* expression since in a *modB* mutant, *modA*

expression was unchanged and no different from that in the wildtype strain (Neubauer et al., 1999b).

Recently in *S. carnosus*, the *nreABC* (for nitrogen regulation) genes were identified and shown to link the nitrate reductase operon (*narGHJI*) and the putative nitrate transporter gene *narT*. An *nreABC* deletion mutant was dramatically affected in nitrate and nitrite reduction and growth. The data provide evidence for a global regulatory system important for aerobic and anaerobic metabolism, with NreB and NreC forming a classical two-component system and NreB acting as a sensor protein with oxygen as the effector molecule (Fedtke et al., 2002).

Homologous sequences of the described nitrate/nitrite reductase systems in *S. carnosus* are also identifiable in the *S. aureus* N315 and *S. epidermidis* RP62A genomes. Thus the ability to use nitrate as an alternative terminal electron donor appears to be widely distributed among staphylococci.

Cell Wall Peptidoglycan

Staphylococcus aureus was one of the first bacterial species where peptidoglycan biosynthesis was studied and pioneering results achieved. A comprehensive description is presented in the monographs by Rogers et al. (1980b) and Ghuysen and Hakenbeck (1994). The staphylococcal peptidoglycan is a heteropolymer consisting of glycan strands crosslinked by peptides. According to the classification by Schleifer and Kandler (1972), *S. aureus* has an A3α peptidoglycan type. The peptide chain length is generally long in Gram-positive bacteria, yet the most extensively crosslinked peptidoglycan, that of *S. aureus*, has an average of about 15 repeating units and a maximal chain length of 30–40 peptide units (Snowden and Perkins, 1990; Henze et al., 1993). In exponentially grown cells in rich medium, more than 95% of the subunits are crosslinked in *S. aureus*. This extremely high degree of crosslinking is possible only because their long and flexible pentaglycine interpeptide bridges are able to span distances between peptides otherwise much too far apart to be crosslinked (Ghuysen and Hakenbeck, 1994).

The peptidoglycan of staphylococci is also exceptional in that, apart from the small proportion of residual uncross-linked primary pentapeptide side-chains, it has almost no free carboxyl groups, since the D-glutamic acid α-carboxyl group is amidated (Tipper et al., 1967; Schleifer, 1975a; Pucci et al., 1995).

Staphylococcus aureus also provides an example of the second type of peptidoglycan modification, the presence of *O*-acetyl substituents on the *N*-acetylmuramic acid residues. In this organism about 50% of the muramic acid residues are present as the 4-*N*, 6-*O*-diacetylderivative (Tipper et al., 1971). This substitution has the effect of making the peptidoglycan resistant to the muramidase egg-white lysozyme (Warren and Gray, 1965).

Additions to the peptidoglycan of *S. aureus* also occur in the form of covalently linked wall teichoic acid. They are attached by phosphodiester linkages on C_6 of some of the muramic acid residues (Hay et al., 1965). In *S. aureus*, approximately 7.7% of the muramic acid is phosphorylated. Suggesting that on approximately every thirteenth muramic acid a wall teichoic acid is covalently linked.

As in all staphylococci, consecutive cell divisions are initiated at an angle of 90 degrees in three dimensions, sometimes even before completion of the cell separation process of the first division plane. Cell division is achieved by the formation of a highly organized cross wall, which is initiated asymmetrically and eventually fuses in the center of the cell to form a complete cross wall. On the basis of distinct morphological features, as revealed by electron microscopy, involvement of several autolytic systems has been suggested in cell division process. One of these, the splitting system, appears as a ring of periodically arranged tubules in the center of the cross wall (Giesbrecht et al., 1998).

Teichoic acid-like material has been chemically associated with the splitting system (Morioka et al., 1987). During isolation and purification of the peptidoglycan, the morphological appearance of the splitting system remains detectable until the final purification step, i.e., removal of the teichoic acid. If the splitting system can no longer be detected, two separated pieces of cross wall are visible. Once the cross wall has been completed, cell separation is initiated by highly organized entities called "murosomes," which punch tiny holes into the peripheral wall along the division plane (Giesbrecht et al., 1998).

BIOSYNTHESIS OF PEPTIDOGLYCAN The overall peptidoglycan synthesis can be divided into three distinct stages (Rogers et al., 1980b): 1) The formation of the nucleotide sugar-linked precursors, UDP-*N*-acetylglucosamine, UDP-*N*-acetylmuramic acid and UDP-*N*-acetylmuramyl-pentapeptide; 2) the transfer of phospho-*N*-acetylmuramyl-pentapeptide and *N*-acetylglucosamine to the lipophilic carrier, undecaprenyl phosphate, to yield a disaccharide-(pentapeptide)-pyrophosphate-undecaprenol; and 3) the transfer of this complete subunit to the growing peptidoglycan. At this stage, cross-bridge formation occurs together with secondary modification of the newly synthesized peptidoglycan. Known genes

(enzymes) involved in the pathway of peptidoglycan biosynthesis are shown in Fig. 9, and in Table 12, annotated peptidoglycan biosynthesis genes of *S. aureus* N315 are listed.

UDP-*N*-acetylmuramic acid is formed from UDP-*N*-acetylglucosamine (UDP-Glc*N*ac), UTP and *N*-acetylglucosamine-1-P. The synthesis of UDP-Glc*N*ac starts from fructose-6-P and involves UTP-glucose-1-P uridyltransferase (GtaB), glucosamine-fructose-6-P aminotransferase (GlmS), and phosphoglucosamine-mutase GlmM (FemD). The UDP-*N*-acetylglucosamine-pyrophosphorylase reaction is also involved in the synthesis of many other nucleotide-linked sugars. The next step is the formation of the first intermediate UDP-*N*-acetylmuramic acid. These reactions involve the transfer of a pyruvate enol ether from phosphoenolpyruvate to UDP-*N*-acetylglucosamine and its subsequent reduction to yield UDP-*N*-acetylmuramic acid (Wickus et al., 1973). The enzyme involved is UDP-*N*-acetylglucosamine 1-carboxyvinyl transferase. In the genome sequence, there are two transferases present, MurA and MurZ (MurB). The transferase activity was initially detected in cell-free

BIOSYNTHESIS OF PEPTIDOGLYCAN
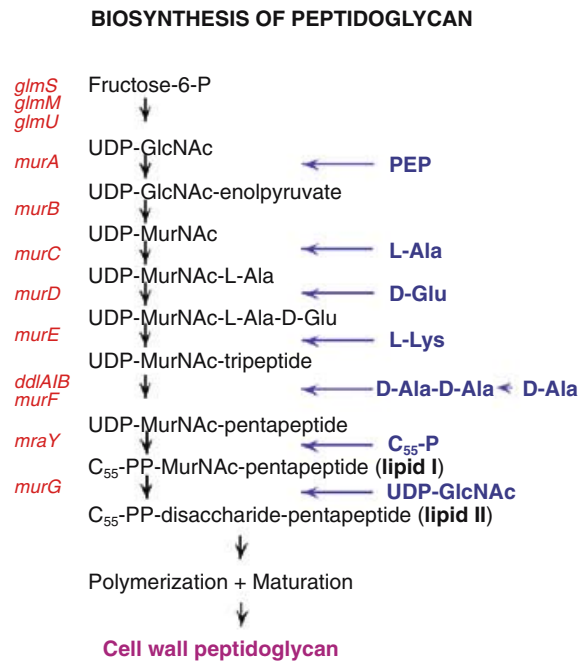
Fig. 9. Pathway of peptidoglycan biosynthesis in staphylococci.

Table 12. Annotated peptidoglycan biosynthesis genes of *S. aureus* N315.

Gene designation	Encoded enzyme
glmS	Glucosamine-fructose-6-phosphate aminotransferase
glmM (*femD*)	Phosphoglucosamine-mutase
gtaB	UTP-glucose-1-phosphate uridyltransferase
murA	UDP-*N*-acetylglucosamine 1-carboxyvinyl transferase 1
murZ	UDP-*N*-acetylglucosamine 1-carboxylvinyl transferase 2
murC	UDP-*N*-acetylmuramate-alanine ligase
ddlA	D-Alanine-D-alanine ligase
murF	UDP-*N*-acetylmuramoylalanyl-D-glutamyl-2,6-diaminopimelate-D-alanyl-D-alanyl ligase
murI	Glutamate racemase
murD	UDP-*N*-acetylmuramoylalanine-D-glutamate ligase
mraY	Phospho-*N*-muramic acid-pentapeptide translocase
murG	Undecaprenyl-PP-Mur*N*Ac-pentapeptide-UDPGlc*N*Ac Glc*N*Ac transferase
murE	UDP-*N*-acetylmuramoylalanyl-D-glutamate-2,6-diaminopimelate ligase
sgtA	Probable transglycosylase
sgtB	Hypothetical protein, similar to penicillin-binding protein 1A/1B
uppS	Undecaprenyl pyrophosphatase synthetase
pbp4	Penicillin binding protein 4
bacA	Bacitracin resistance protein (putative undecaprenol kinase) homologue
femA	Factor essential for expression of methicillin resistance
femB	FemB protein
uppS	Undecaprenyl pyrophosphatase synthetase
pbpA	Penicillin-binding protein 1
pbp2	Penicillin-binding protein 2
pbp3	Penicillin-binding protein 3
sgtB	Hypothetical protein, similar to penicillin-binding protein 1A/1B
NN	Similar to UDP-*N*-acetylglucosamine pyrophosphorylase
mraY	Phospho-*N*-muramic acid-pentapeptide translocase
fmhA	FmhA protein
fmhB	FmhB protein
fmhC (*eprh*)	FmhC protein
srtA	Sortase
drp35	Drp35
ebsB	Cell wall enzyme

From Kuroda et al. (2001).

extracts from *S. aureus* (Strominger, 1965). It was partially purified from *S. epidermidis* (Wickus et al., 1973). The reaction is reversible and moreover specifically and irreversibly inhibited by fosfomycin. The UDP-GlcNAc-enolpyruvate reductase was also purified from *S. aureus* (Wickus et al., 1973). In all cases, NADPH is used as co-factor. The synthetases that catalyze the assembly of the peptide moiety of the peptidoglycan unit are cytoplasmic.

The UDP-MurNAc pentapeptide is formed by the sequential addition of L-alanine, D-glutamic acid, L-lysine and D-alanyl-D-alanine to the D-lactyl group of UDP-MurNAc. Each step is catalyzed by a specific synthetase using ATP. The involved enzymes are MurC, MurD, MurE and DdlA (see Table 9). The end product is UDP-MurNAc-L-Ala-D-*iso*-Glu-L-Lys-D-Ala-D-Ala (also named "UDP-MurNAc-pentapeptide" and "Park's nucleotide").

The conversion of L-Glu to D-Glu is catalyzed by MurI. The conversion of L-Ala to D-Ala is catalyzed by alanine-racemase, an enzyme studied in detail in *S. aureus* (Cheung et al., 1983; Kullik et al., 1998b). D-Alanyl-D-alanine is formed by the dimerization of D-alanine. The enzyme involved, D-alanine:D-alanine ligase, has been already purified from *S. aureus* by Ito and Strominger (1973). The enzyme requires a "heat-stable co-factor," Mg^{+2} (or Mn^{+2}), K^+ and ATP for activity (for a review, see Rogers et al., 1980a).

Under certain conditions, glycine has been shown to replace both alanine isomers in UDP-*N*-acetylmuramyl-pentapeptide. Growth of *S. aureus* in the presence of high concentrations of glycine resulted in the accumulation of modified UDP-*N*-acetylmuramyl-pentapeptides containing both glycyl-D-alanine and D-alanyl-glycine as the terminal dipeptide (Hammes et al., 1973). Thus, under these rather special conditions the substrate specificities of both the D-alanine ligase and the UDP-*N*-acetylmuramyl-3-peptide:D-Ala-D-Ala-ligase have been overridden. The synthesis and subsequent incorporation into peptidoglycan of such natural precursors may contribute to the inhibitory effects of glycine and D-amino acids on many bacteria.

BIOSYNTHESIS OF THE LIPID II PRECURSORS Undecaprenol, originally present either as the free alcohol or as a phosphorylated derivative has been isolated in pure form from *S. aureus* (Higashi et al., 1970). The 17-kDa phosphokinase of *S. aureus* is soluble in several organic solvents but insoluble in water and has an unusually high content (58%) of nonpolar amino acids. Subsequent purification allows the separation of an apoprotein to which activity can be restored

by a wide range of natural and synthetic phospholipids and detergents. Initially it was suggested that phosphatidylglycerol or diphosphatidylglycerol (cardiolipin) were specific lipid co-factors. However, enzymatic activity appears to be more dependent on "lipid hydration" rather than lipid viscosity or the actual chemical structure of the polar group of the activating lipid (Sanderman, 1976). In stationary-phase cultures, free undecaprenol was present in large excess, representing approximately 80% of the total peptidoglycan lipid.

UDP-MurNAc-pentapeptide is phosphodiester linked to an undecaprenyl-pyrophosphate carrier molecule at the expense of UDP to yield C_{55}–PP-MurNAc-L-Ala-D-*i*Gln-L-Lys-D-Ala-D-Ala, or lipid I (Higashi et al., 1970). UDP-GlcNAc is linked to the muramoyl moiety to generate the disaccharide lipid II precursor [C_{55}-PP-MurNAc(-L-Ala–D-*i*Gln–L-Lys(Gly5)-D-Ala-D-Ala)-β1-4-GlcNAc]. Lipid I and lipid II precursors are hooked at the inner side of the cytoplasmic membrane where also very likely the synthesis of the pentaglycine interpeptide takes place. Recently it was shown that lipid II is a peptidoglycan substrate for sortase-catalyzed surface protein anchoring (Perry et al., 2002).

FORMATION OF PENTAGLYCINE INTERPEPTIDE Synthesis of the pentaglycine chain occurs at the membrane-bound lipid II precursor GlcNAc-(β-1,4)-*N*-acetylmuramic acid(-L-Ala-D-*i*Gln-L-Lys-D-Ala-D-Ala)-pyrophosphoryl-undecaprenol by sequential addition of glycine to the ε-amino group of lysine, using glycyl-tRNA as donor, in a ribosome independent fashion (Kamiryo and Matsuhashi, 1972). First hints for an involvement of glycyl-tRNA were based on the finding that incorporation of glycine into polymeric peptidoglycan was prevented by ribonuclease. Under identical conditions, the incorporation of *N*-acetylmuramyl-peptide from the nucleotide precursor was unaffected. Five glycine residues activated by tRNA are added initially to the ε-NH_2 group of lysine and then subsequently to the N-terminus of the growing peptide chain (Kamiryo and Matsuhashi, 1972). This mechanism is in direct contrast to that of protein synthesis, which occurs by addition at the carboxyl terminus. The residues appeared to be added singly with no evidence for the involvement of peptidyl tRNA intermediates. The tRNA of *S. aureus* has been found to contain at least three species of glycyl-tRNA (Gly-tRNA). They can be used for the incorporation of glycine into peptidoglycan, whereas one of the species is inactive in protein synthesis. The formation of all Gly-tRNA is catalyzed by a single Gly-tRNA synthetase, which has been purified (Niyomporn et al., 1968). A more complex situation has been found

in *S. epidermidis* where four different pentapeptide cross-bridges have been shown to be present (Tipper and Berman, 1969). Each contains glycine and L-serine residues in the ratio 3:2 in a characteristic sequence where glycine is always the initial substituent of the ε-amino group of L-lysine. Addition of the amino acids occur at the level of lipid intermediates, the transfer of glycine and serine from their respective tRNAs being catalyzed by membrane-bound enzymes. The incorporation of glycine is independent of that of serine, whereas maximum serine incorporation requires the simultaneous incorporation of glycine. Fractionation of tRNA of *S. epidermidis* has demonstrated the presence of four glycyl- and four seryl-tRNAs. In each case, all species, including one species of each type that did not participate in protein synthesis, were active in cross-bridge synthesis. The glycyl-tRNA with apparent specificity for peptidoglycan synthesis has been purified and shown to be made up of two distinct *iso*-accepting species (Roberts et al., 1974). The two sequences define six spaces and the insertion of an additional base in the dehydrouridine loop of one. Thus, *S. epidermidis* appears to have developed a means of supplying glycine residues for cross-bridge synthesis without involving the machinery of protein synthesis.

THE FEM FACTORS The expression of methicillin resistance depends on several genes named "*fem*" (*f*actors *e*ssential for *m*ethicillin resistance; Berger-Bächi, 1994). Recently, it was demonstrated that FmhB (FemX) links the first glycine residue to the ε-amino group of lysine (Rohrer et al., 1999). Evidence for this function was that, under conditions where *fmhB* is down-regulated, unsubstituted peptidoglycan monomer precursors accumulate. Since FmhB is essential in *S. aureus*, it represents an ideal drug target. While FmhB incorporates the first glycyl residue, FemA incorporates glycyl residues 2 and 3 (Stranden et al., 1997), and FemB, glycyl residues 4 and 5 (Henze et al., 1993). Although FemA and FemB have a relatively high amino acid sequence identity (40%) and similarity (64%), they are specific and cannot substitute for each other. Null mutants of *femAB* are barely viable and depend on compensatory mutations for survival (Ling and Berger-Bächi, 1998). Interestingly, *S. aureus* contains one glycyl-tRNA gene for protein biosynthesis and three non-proteinogenic glycyl-tRNA genes that are involved in cell wall biosynthesis (Green and Vold, 1993). It is tempting to speculate that the two Fem factors and FmhB may preferentially recognize one of the three glycyl-tRNA species. The site of glycine incorporation in the peptidoglycan is shown in Fig. 10.
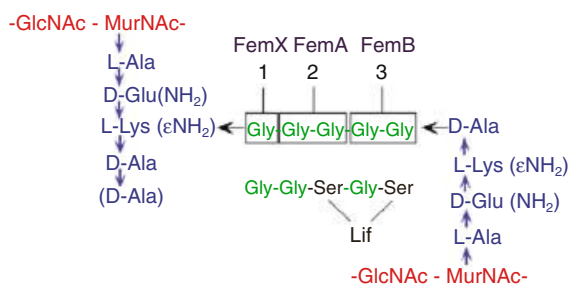
Fig. 10. Pentaglycine biosynthesis of *Staphylococcus aureus* peptidoglycan. Methicillin resistance factor FemX adds the first glycine, FemA adds glycines 2 and 3, and FemB adds glycines 4 and 5. Lif was identified in *S. simulans* var. *staphylolyticus* and is responsible for incorporation of serine residues in positions 3 and 5. Lif, lysostaphin immunity factor; Mur*N*Ac, *N*-acetylmuramic acid; and Glc*N*Ac, *N*-acetylglucosamine.

Other FemAB-like factors have been identified in staphylococci, such as *l*ysostaphin *i*mmunity *f*actor (Lif) in *S. simulans* biovar *staphylolyticus* (Thumm and Götz, 1997) and Epr in *S. capitis* (Sugai et al., 1998), which protect these strains from their own glycyl-glycine endopeptidase (Thumm and Götz, 1997).

The expression of *lif* in *S. carnosus* led to an increase of the serine-glycine ratio of the interpeptide bridges from 2 to 35%, suggesting that lysostaphin immunity depends on serine incorporation into the interpeptide bridge. If in addition to *lif*, *lss* is coexpressed, the serine-glycine ratio is further increased to 58%, suggesting that Lss selects for optimal serine incorporation. Lif shows similarity to FemA and FemB proteins, which are involved in the biosynthesis of the glycine interpeptide bridge of staphylococcal peptidoglycan. In contrast to Lif, the production of FemA and FemB in *S. carnosus* does not cause lysostaphin immunity (Thumm and Götz, 1997). The putative tRNA^{Ser} gene located downstream of *lss* had no recognizable influence on lysostaphin immunity. The *lss* and *lif* genes are flanked by insertion sequences, suggesting that *S. simulans* biovar *staphylolyticus* received *lif* and *lss* by horizontal gene transfer. Recently, it was shown that Lif and Epr lead to incorporation of serine residues into the staphylococcal peptidoglycan interpeptide bridges specifically at positions 3 and 5 and that this incorporation requires the presence of FemA and/or FemB (Ehlert et al., 2000).

TRANSLOCATION OF LIPID II-PENTAGLYCINE In the second stage of the biosynthetic process, the lipid II-pentaglycine is translocated from the inner side of the cytoplasmic membrane to the outer side where the incorporation into the growing peptidoglycan occurs. The initial reaction

involves the transfer of phospho-*N*-acetyl-muramyl-pentapeptide from the nucleotide to undecaprenyl phosphate with the formation of undecaprenyl-P-P-*N*-acetylmuramyl-pentapeptide and UMP. This reaction is catalyzed by a translocase. Incorporation of undecaprenyl-pyrophosphate in UDP-*N*-acetylmuramyl-pentapeptide is inhibited by tunicamycin. In a second step *N*-acetyl-glucosamine is glycosidically linked to the *N*-acetyl-muramyl residue to form undecaprenyl-P-P-*N*-acetylmuramyl (pentapeptide)-*N*-acetylglucosamine and UDP (Anderson et al., 1967). Since these reactions involve both hydrophilic and hydrophobic substrates, it seems likely that both enzymes act at the cytoplasmic lumen of the membrane.

The translocase (phospho-*N*-acetylmuramyl-pentapeptide translocase), which has been extensively studied by Neuhaus and his colleagues (Pless and Neuhaus, 1973), can be conveniently assayed either in the forward direction as a transferase or in the reverse direction, by the exchange reaction following the incorporation of radioactivity from UMP into UDP-*N*-acetyl-muramyl-pentapeptide. The enzyme has been obtained in soluble form from *S. aureus* by treatment of membrane preparations with the non-ionic detergent Triton X-100 (Pless and Neuhaus, 1973; Weppner and Neuhaus, 1977). The enzyme is stimulated by the addition of polar lipid fraction containing phosphatidylglycerol. However, phosphatidylserine, phosphatidylethanolamine and phosphatidylinositol were equally capable of restoring enzyme activity. Thus, the reactivation of the translocase appeared to result from the provision of a lipid microenvironment rather than a requirement for a specific phospholipid. In vivo, the microenvironment of the translocase is the lipids of the membrane with which it interacts.

Investigation of the microenvironment in membranes of the fluorescent lipid intermediate undecaprenyl-*N*-acetylmuramyl-(*N*-dansyl)-pentapeptide suggests that the lipid intermediate is immobilized within hydrophobic environment close to the membrane surface. This observation is supported by the earlier finding that spin-labelled lipid intermediate could still form complexes with vancomycin and ristocetin (Johnston and Neuhaus, 1975). It was proposed that at least the terminal D-alanyl-D-alanine-dipeptide of the pentapeptide side chain has access to the aqueous phase. Vancomycin and ristocetin mimics the effect of detergents. At low concentrations, the antibiotic stimulates the transfer but not the exchange reaction. Hammes and coworkers (Hammes and Neuhaus, 1974) have investigated the specificity of phospho-*N*-acetylmuramyl-pentapeptide translocase from *S. aureus*. They could show that the translocase

has 24% activity when the terminal D-alanine is missing, 60% activity when the terminal amino acid is glycine instead of D-alanine, and 57% activity when *meso*-diaminopimelic acid (mA$_2$pm) instead of L-lysine is present.

In many organisms, the lipid-linked disaccharide pentapeptide units are utilized directly for synthesis of peptidoglycan. In *S. aureus*, the carboxyl group of D-isoglutamic acid is amidated. The reaction is catalyzed by a membrane-bound enzyme. The enzyme can utilize either glutamine or NH$_4^+$, and both undecaprenyl-P-P-*N*-acetyl-muramyl-pentapeptide and disaccharide-pentapeptide act as substrates, whereas a nucleotide precursor UDP-*N*-acetylmuramyl-pentapeptide is completely inactive. The reaction involves hydrolysis of ATP to ADP. It seems likely that many other minor differences found in the structure of peptidoglycan such as the O-acetylation of muramic acid residues in *S. aureus* (Ghuysen and Hakenbeck, 1994) and the addition to the peptide side chain of single amino acid residues, not involved in interpeptide-bridge formation, occurs at the level of lipid intermediates.

The final reaction of the membrane-mediated stage of peptidoglycan synthesis is also the initial reaction in the third stage, i.e., the incorporation of the newly synthesized disaccharide peptide unit into the growing peptidoglycan. The presence of uncross-linked side chains in all peptidoglycans examined argues strongly that polymerization occurs by extension of the glycan chain (transglycosylation) prior to peptide-bond formation (transpeptidation). The finding of free reducing groups of muramic acid in the walls of *S. aureus* (Ward and Perkins, 1973) suggests that there must exist some mechanisms to terminate synthesis of a particular glycan chain by detaching the lipid carrier. At present, the nature of this process is not evident.

TRANSPEPTIDATION: THE FORMATION OF CROSSLINKS By the mid-1960s, detailed studies of the chemical structure of several peptidoglycans established the presence of crosslinked peptides. It was suggested that D-alanyl-D-alanine-dipeptide appeared to be involved in the formation of these cross-linkages. When sublethal concentrations of penicillin were applied, the walls contained an increase in the free amino groups of glycine and an excess of alanine over those walls prepared from organisms grown in the absence of penicillin. These observations were independently confirmed by direct chemical analysis of staphylococcal walls prepared from organisms grown under similar conditions (Tipper and Strominger, 1968). In the presence of penicillin, an increased synthesis of uncross-

linked peptide monomer units was observed. These units retained the complete D-alanyl-D-alanine terminus of the nucleotide precursor and were substituted with the pentaglycine cross-bridge peptide on the ε-amino group of L-lysine. During subsequent growth of the organism in the absence of penicillin, these monomer units did not become crosslinked. To explain these observations, it was proposed that incorporation of the newly synthesized disaccharide-peptide occurred at the wall-membrane interface with almost immediate formation of crosslinks to adjacent peptide side chains. In staphylococci, units synthesized in the presence of penicillin could not form crosslinks on the subsequent removal of the antibiotic because continued synthesis of linear uncross-linked peptidoglycan during the initial penicillin treatment moved these units outside the reach of the transpeptidase molecules. These in vivo studies supported the conclusion that crosslinkage in peptidoglycan occurred by means of a transpeptidation reaction inhibited by β-lactam antibiotics.

In vitro, cross-link studies with cell wall preparations from *S. aureus* (Mirelman and Sharon, 1972) were shown to retain some cytoplasmic membrane not removed by extensive washing. These preparations catalyzed the incorporation of radioactivity from UDP-*N*-acetylmuramyl([^{14}C]-L-lysine)pentapeptide into both peptidoglycan and lipid intermediates. The penicillin sensitive release of D-alanine occurred when the wall-membrane preparation was incubated with the two nucleotide precursors and glycine, whereas no such release occurred in the preparations incubated in the absence of UDP-*N*-acetylglucosamine. Glycine incorporation was sensitive to ribonuclease. Penicillin also inhibited the incorporation of glycine, but in contrast to release of D-alanine, this was never complete.

AUTOLYSINS AND TURNOVER OF BACTERIAL WALL POLYMERS There are various autolytic enzymes present in most staphylococci:

1) An enzyme that hydrolyzes the *N*-acetyl-muramyl-1,4-β-*N*-acetylglucosamine bonds in the glycans to liberate free-reducing groups of *N*-acetylmuramic acid; since this enzyme is only active with micrococcal but not with staphylococcal peptidoglycan, we postulate that it plays a role before the peptidoglycan becomes O-acetylated

2) A β-*N*-acetylglucosaminidase that liberates the free reducing groups of *N*-acetylglucosamine

3) An *N*-acetylmuramyl-L-alanine amidase (amidase) that hydrolyzes the bond between the glycan chains and the peptide subunits

4) Endopeptidases that can hydrolyze some of the main peptides and the bridge peptide when they occur between the C-terminal D-alanine and the amino group of a contiguous peptide chain

5) Commonly occurring in many organisms are D-alanine-carboxypeptidases that can cleave off any carboxyl terminal D-alanyl residues of the peptidoglycan. These enzymes, however, are not known to act as autolysins and therefore will not be considered.

Staphylococcus aureus does not exhibit a lysozyme like muramidase (EC 3.2.1.17), which cleaves its O-acetylated peptidoglycan.

Pulse chase studies indicated that walls of many organisms such as bacilli, lactobacilli or staphylococci are in a state of flux. Wall synthesis and degradation are balanced by a so-called "turnover of the cell wall." If wall synthesis is inhibited in such situations, without a corresponding inhibition of either the formation or action of the autolysins, cell lysis might be expected and indeed occurs. Antibiotics that inhibit wall synthesis are bactericidal, unlike most of those inhibiting protein synthesis, which are bacteriostatic.

There are still several open questions: Is the inhibition of wall synthesis sufficient to kill bacteria? Do cells lyse because their walls can no longer expand so that the cell bursts? Is autolytic action essential? One possible way to answer these questions would be to stop the formation of autolysins by blocking protein synthesis. A combination of chloramphenicol and penicillins is not bactericidal for *E. coli* or for staphylococci (Rogers et al., 1980b). Lysis of staphylococci could also be stopped, as with *B. subtilis* and *Enterococcus faecalis*, by the inhibition of protein synthesis. However, to date the answers to these questions have been inconclusive. The observation that an autolytic deficient strain of *S. aureus* failed to show wall turnover, although the wildtype turnover rate was rather slow (Chatterjee et al., 1976) should be reevaluated.

Suginaka et al. (1979) have shown that the addition of homologous lipoteichoic acid to cultures of *S. aureus* treated with penicillin prevents lysis and death of the bacteria. Forssman antigen (lipoteichoic acid from pneumococci) can also prevent penicillin-induced lysis in *S. aureus*. Lipoteichoic acids or lipids certainly regulate the action of autolysins in vivo. It was also found that wall autolysis is inhibited by sodium polyanethole sulfonate "liquoid" (Wecke et al., 1986). The inhibition is not due to direct interaction with the autolysins, and liquoid blocks very likely the target structures of autolysins, especially the wall teichoic acid (WTA).

BIOLOGICAL ACTIVITY OF PEPTIDOGLYCAN It was early recognized that cell wall muramyl peptides exert excellent adjuvant properties, and consequently a variety of muramyl derivatives with biological potentials as adjuvant have been synthesized and investigated (Adam et al., 1975). In a somewhat analogous manner, another interesting biological property (i.e., the somnogenic effects of muramyl compounds) was investigated by Krueger and coworkers (Johannsen et al., 1989; Johannsen et al., 1991a; Johannsen et al., 1991b). It is of interest that macrophages produce somnogenic and pyrogenic muramyl peptides during digestion of staphylococci. Somnogenic compounds of bacterial origin are obviously small enough to cross the blood-brain barrier, and it is of interest to speculate that lysozyme capable of digesting the insoluble wall peptidoglycan polymer could have a role in the production of suitably sized substances with such properties.

Obviously the immune system recognizes that muramyl peptides are products of bacteria, and responds by becoming activated to resist infection. This resistance to infection is nonspecific and extends to unrelated species of bacteria, fungi, and viruses. A key mechanism of the resistance to infection is activation of macrophages. Macrophage activation results in increased production of microbicidal oxygen radicals like superoxide and peroxide, and in increased secretion of inflammatory cytokines like interleukin-1β and tumor necrosis factor-α. These cytokines, besides activating neutrophils, B lymphocytes, and T lymphocytes, act on the central nervous system to induce physiological responses like fever and sleep. These physiological responses also aid in combating infection. Muramyl peptides activate macrophages and other cells of the immune system to kill cancer cells. It is therefore believed that muramyl peptides and similar agents will become more important as therapeutic agents in the future, owing to increasing resistance of microbes to antibiotics, and increasing numbers of patients with immunodeficiencies (Pabst et al., 1999).

Mammals have a peptidoglycan recognition protein (PGRP) which binds to peptidoglycan (PG) or live bacteria and which is upregulated by PG. This PGRP is ubiquitous and involved in innate immunity. Tag7, a novel cytokine, is also induced by bacterial products; Tag7 is apoptotic to murine L929 cells in a NF-κB-independent manner. Both the *PGRP* and *tag7* genes are expressed in brain, lymphatic and hematopoietic tissues. Recently, it was found that murine *PGRP* and *tag7* encode identical transcripts, and their corresponding proteins have structural relationships to lysozymes. Furthermore, the cDNA of rat PGRP was cloned and expressed in brains of sleep-deprived and control rats. It turned out that PGRP expression was upregulated by sleep deprivation, suggesting a role for PGRP in a homeostatic regulation of sleep (Rehman et al., 2001).

Antibiotics Affecting Bacterial Peptidoglycan Synthesis

PHOSPHONOMYCIN (FOSFOMYCIN) This antibiotic, the isolation and characterization of which were first described in 1969, inhibits what might be regarded as the earliest step in peptidoglycan synthesis. It inhibits the transfer of the enolpyruvate residue from phosphoenol pyruvate to UDP-*N*-acetylglucosamine (UDP-*N*-acetylglucosamine-3-*O*-enolpyruvyl transferase). Phosphonomycin binds covalently to cysteine residues of the transferase. Resistance is achieved by mutations in the transport systems. For review of the cell wall antibiotics, see Rogers et al. (1980a).

CYCLOSERINE, O-CARBAMOYL-D-SERINE, ALAPHOSPHIN, AND THE HALOALANINES Antibiotics that inhibit D-alanine metabolism are cycloserine, O-carbamoyl-D-serine, alaphosphin (L-alanyl-L-1-aminoethyl phosphonic acid) and the haloalanines. Alanine racemase is competitively and irreversibly inhibited by cycloserine, O-carbamoyl-D-serine and the haloalanines.

The inhibiting effect of D-cycloserine on bacterial growth can be reversed by the addition of D-alanine. The presence of D-alanine prevents conversion to spheroplasts of several bacteria incubated with cycloserine in osmotically stabilized media. Cycloserine acts by inhibiting the alanine racemase and thus the incorporation of D-alanine into the peptidoglycan. As a consequence, UDP-*N*-acetylmuramyl-L-alanine-D-isoglutamyl-L-lysine accumulates. O-carbamoyl-D-serine, alaphosphin (L-alanyl-L-1-aminoethyl phosphonic acid) and the haloalanines act in a similar way. While D-cycloserine alone appears to inhibit D-alanine:D-alanine ligase, the D-alanyl-D-alanine adding enzyme (UDP-*N*-acetylmuramyl-L-alanine-D-isoglu-L-lys:D-Ala-D-Ala ligase [ADP]) is not affected by any of these antibiotics. D-Cycloserine is a competitor of the substrate for both alanine racemase and D-alanine:D-alanine ligase. The inhibition constant of D-cycloserine for alanine racemase is 5×10^{-5}. Resistance to D-cycloserine can arise either by elevated racemase and ligase activity or by inability to transport the antibiotic.

BACITRACIN Bacitracin specifically inhibits the dephosphorylation of undecaprenyl-pyrophosphate.

TUNICAMYCIN Initially it was observed that tunicamycin inhibits glycoprotein synthesis in Newcastle disease virus-infected cells (Takatsuki and Tamura, 1971). In bacteria, it inhibits the phospho-*N*-acetylmuramyl-pentapeptide translocase, i.e., the formation of the first lipid intermediate in peptidoglycan synthesis. In *S. aureus* and several bacilli, it inhibits also the linking of WTA to peptidoglycan (Bracha and Glaser, 1976; Bracha et al., 1978).

VANCOMYCIN AND RISTOCETIN B Inhibition occurs very rapidly and binding of the antibiotic to bacteria takes place within 20 seconds of treatment. In *S. aureus*, approximately 10^7 molecules are bound per cell (although this number might be too high because of aggregate formation). Both vancomycin and ristocetin B bind preferentially to the terminal D-alanyl-D-alanine peptide of the UDP-*N*-acetylmuramyl-pentapeptide. The binding site of ramoplanin appears to be different from that of vancomycin (Nieto et al., 1972; Somner and Reynolds, 1990).

Glycopeptide resistance in *S. aureus* is poorly understood. Characterization of vancomycin resistant *S. aureus* (VRSA) strains from all over the world confirmed that emergence of vancomycin resistance in *S. aureus* is a global issue. A certain group of *S. aureus*, designated "heteroVRSA," frequently generates VRSA upon exposure to vancomycin, and is associated with infections that are potentially refractory to vancomycin therapy (Hiramatsu, 2001). Vancomycin resistance is acquired via mutation and cell wall thickening, due to accumulation of excess amounts of peptidoglycan. This seems to be a common resistance mechanism for all VRSA strains isolated so far (Geisel et al., 2001; Komatsuzawa et al., 2002). By exposure to vancomycin, a resistant *S. aureus* COL mutant was isolated. This mutant showed decreased susceptibility to teicoplanin (8-fold), methicillin (2-fold), macarbomycin (8-fold), and moenomycin (16-fold). Macarbomycin and moenomycin are thought to directly inhibit transglycosylase activity. Characterization of the mutant revealed a thickened cell wall and suppression of penicillin-induced lysis, although the amounts of the five penicillin-binding proteins (PBPs 1, 2, 3 and 4) and MecA, and the profiles of peptidoglycan hydrolases were not altered. Analysis of muropeptide profile and glycan chain length distribution by reverse-phase high-pressure liquid chromatography revealed slightly decreased peptide crosslinking and an increased average glycan chain length compared to those of the parent. These results together suggest that a transglycosylase activity was enhanced in the mutant. This may represent a novel mechanism of glycopep-

tide resistance in *S. aureus* (Komatsuzawa et al., 2002).

In June 2002, a VRSA was isolated from a swab obtained from a catheter exit site of a Michigan resident aged 40 years with diabetes, peripheral vascular disease, and chronic renal failure. The patient received dialysis at an outpatient facility (Centers for Disease Control and Prevention, 2002). In June, the patient developed a suspected catheter exit-site infection, and the temporary dialysis catheter was removed; cultures of the exit site and catheter tip subsequently grew *S. aureus* resistant to oxacillin (minimal inhibitory concentration [MIC] > 16 µg/ml) and vancomycin (MIC > 128 µg/ml). It turned out that the isolate contained the *vanA* vancomycin resistance genes from enterococci, which is consistent with the glycopeptide MIC profiles. Transfer of *vanA* genes from *Enterococcus faecalis*, with which the patient was also infected, to *S. aureus* is strongly indicated. And to make it worse, the *S. aureus* isolate contained in addition the oxacillin/methicillin-resistance gene *mecA*. This report describes the first clinical isolate of *S. aureus* that is fully resistant to vancomycin (Centers for Disease Control and Prevention, 2002). Transfer of *van* resistance genes from enterococci to *S. aureus* has long been predicted, with such a conjugative transfer having been demonstrated in vitro (Noble et al., 1992).

β-LACTAM AND METHICILLIN RESISTANCE Three basic enzymes have been described which are subject to penicillin-inhibition: transpeptidases, D-alanine carboxypeptidases, and endopeptidases. The blanket resistance of methicillin-resistant *S. aureus* to all β-lactam antibiotics is related to the properties of the key component of this resistance mechanism: the "acquired" penicillin-binding protein MecA, which has unusual low affinity for all β-lactam antibiotics. Until now, the accepted model of resistance had implied that in the presence of β-lactam antibiotics, MecA must take over the biosynthesis of staphylococcal cell wall from the four native staphylococcal PBPs (PBP1, PBP2, PBP3 and PBP4) because the latter become rapidly acylated and inactivated at even low concentrations of the antibiotic. However, recent observations indicate that this model requires revision. Inactivation of the transglycosylase domain, but not the transpeptidase domain, of MecA of *S. aureus* prevents expression of β-lactam resistance, despite the presence of the low-affinity MecA. The observations suggest that cell-wall synthesis in the presence of β-lactam antibiotics requires the cooperative functioning of the transglycosylase domain of the native staphylococcal PBP2

and the transpeptidase domain of the MecA (Pinho et al., 2001). There are indications that the *mecA* gene was acquired from *S. sciuri* which is, however, uniformly susceptible to β-lactam antibiotics. Recently, it was shown that the *mecA* homologue in *S. sciuri* is hardly expressed but is well expressed by a single mutation in the promoter region and provides methicillin resistance in a susceptible *S. aureus* strain. This supports the proposition that the *mecA* homologue ubiquitous in the antibiotic-susceptible animal species (*S. sciuri*) may indeed be an evolutionary precursor of the methicillin resistance gene *mecA* of the pathogenic strains of MRSA (Wu et al., 2001).

The peptidoglycan isolated from methicillin resistant *S. aureus* (MRSA) strains that contain *mecA* did not differ from that of the susceptible *S. aureus* isolates, suggesting that the activity of MecA cannot be distinguished from that of existing penicillin binding proteins (PBPs), or that it is not functioning in cell wall biosynthesis, as long as these strains are cultivated in the absence of β-lactam antibiotics (de Jonge et al., 1992; Labischinski and Maidhof, 1994). When β-lactam antibiotics saturate the normal set of staphylococcal PBPs, the cell wall is still produced due to the presence of MecA but is drastically hypocrosslinked. The expression of methicillin resistance does depend on *fem* genes (Berger-Bächi, 1994). The inactivation of *femA* and *femB* results in loss of resistance of MRSA strains and induction of hypersensitivity of sensitive staphylococci.

ACQUISITION OF *mecA* AND ITS REGULATION Both annotated *S. aureus* N315 and Mu50 genomes contain a so-called "staphylococcal cassette chromosome mec" (SCCmec) which encodes resistance to β-lactams, bleomycin, macrolide-lincosamide streptogramin B, aminoglycosides (tobramycin and amikacin), and spectinomycin (Kuroda et al., 2001). One can also consider the 40–60-kb long SCCmec as a resistance island, which was acquired very likely by horizontal gene transfer.

In opposite orientation to *mecA* are two cotranscribed genes, *mecR1* and *mecI*. The *mecR1* gene encodes a membrane-bound signal transduction protein (MecR1), while *mecI* encodes a transcriptional regulator (MecI). Between *mecA* and *mecR1* are the promoters for these genes and an operator region that encompasses the –10 sequence of *mecA* and the –35 sequence of *mecR1* (Sharma et al., 1998). MecR1 and MecI have high protein sequence homology with the proteins, BlaR1 and BlaI, respectively, that are involved in the inducible expression of the plasmid-mediated staphylococcal β-lactamase gene, *blaZ*. MecI is a tight regulator of *mecA*

transcription, and most β-lactam antibiotics do not efficiently activate MecR1 (Kuwahara-Arai et al., 1996). Consequently, some isolates, referred to as "pre-MRSA," are methicillin-sensitive despite carrying the *mecA* gene. However, selective pressure through antibiotic usage has promoted *S. aureus* isolates that have mutations or deletions in *mecI* or the *mecA* promoter/operator region, giving rise to an inactive repressor and constitutive *mecA* expression. These mutants can display homogeneous (all cells are resistant to high concentrations of methicillin [>128 μg/liter]) or heterogeneous (only a small minority of cells exhibit high-level methicillin resistance) methicillin resistance phenotypes (Kondo et al., 2001).

Other Cell Wall Components

CELL WALL TEICHOIC ACID The staphylococcal cell wall consists not only of a thick peptidoglycan fabric but also of polymers of alternating phosphate and alditol groups called "teichoic acids." The discovery of the teichoic acids resulted from the identification of ribitol in a cytidine nucleotide isolated and characterized by Baddiley and coworkers already in the mid-1950s. The size of ribitol teichoic acids and the nature of their linkage to glycosaminopeptides was described in 1965 (Hay et al., 1965).

There are two teichoic acid types in staphylococci: a wall teichoic acid (WTA), which is covalently bound to the peptidoglycan and which is, depending on the species, composed of ribitol and/or glycerol teichoic acids (Pooley and Karamata, 1994). The WTA is distinct from the subsequently discovered lipoteichoic acids (LTAs). LTA contains only glycerol phosphate and is anchored in the cytoplasmic membranes of Gram-positive bacteria (Fischer, 1988). The alditol units of WTAs and the LTAs contain normal substituents of which D-alanine and glycosyl residues are most common. The structures of the *S. aureus* WTA and LTA are shown in Fig. 11.

STRUCTURE AND BIOSYNTHESIS OF WALL TEICHOIC ACID The cell wall teichoic acid structures of 13 staphylococcal type strains were determined (Endl et al., 1983). Most of the strains contain a poly(polyolphosphate) teichoic acid with glycerol and/or ribitol as polyol component. The polyolphosphate backbone is partially substituted with various combinations of sugars and/or amino sugars. Most of the substituents occur in a monomeric form, but some strains also contain dimers of *N*-acetylglucosamine as substituents. *Staphylococcus hyicus* subsp. *hyicus* and *S. sciuri* revealed rather complex cell wall teichoic acids. They consist of repeating sequences of phos-
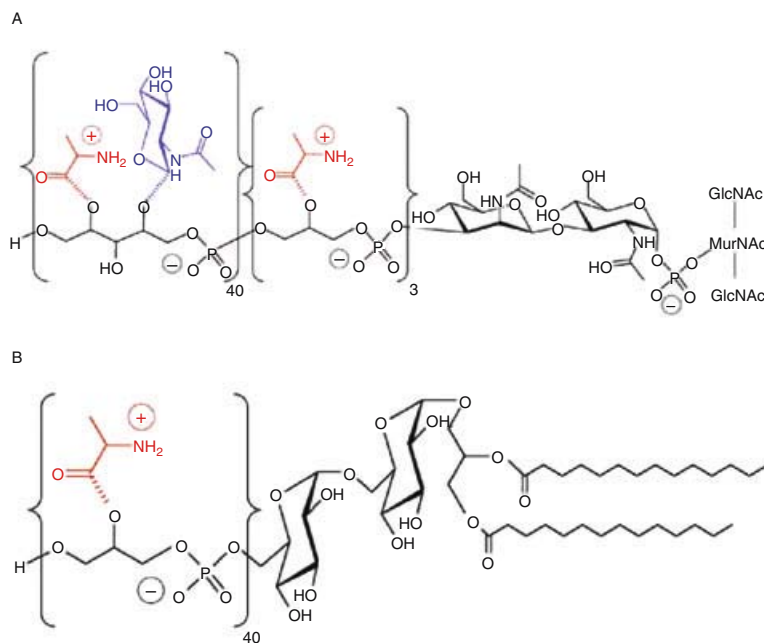
Fig. 11. A) Structure of the *S. aureus* wall teichoic acid (WTA). The WTA is linked via a phosphodiester bond to the 6 OH-group of muramic acid residue in the glycan strand. 1,3-Man*N*Ac-Glc*N*Ac-PP-prenol serves as substrate. The 2 OH-group of Man*N*Ac is phosphodiester linked with three units of D-alanylated glycerol-phosphates to which approximately 40 units of ribitol-phosphates (substituted with D-alanine and/or Glc*N*Ac) are linked. B) Structure of the *S. aureus* lipoteichoic acid (LTA). The membrane anchor region is a diacylglycerol which is C1 linked to the disaccharide Glc(ß1,6); in the second glucosyl residue of the disaccharide, approximately 40 units of glycerol phosphates are linked via the 6 OH-group. Position 2 of the glycerol phosphate residues is in part substituted either with D-alanine or D-alanine ester and glycosyl (mostly Glc*N*Ac) residues. Man*N*Ac, *N*-acetylmannosamine; Glc*N*Ac, glucosamine; PP, pentose phosphate. Structure was drawn by Peschel (2002).

phate-glycerol-phosphate-*N*-acetylglucosamine. The amino sugar component is present in this case as a monomer or an oligomer. Moreover, the glycerol residues are partially substituted with *N*-acetylglucosamine. The cell wall teichoic acid of *S. auricularis* is a poly(*N*-acetylglucosaminyl-phosphate) polymer similar to that found in *Macrococcus caseolyticus*.

Biosynthesis of ribitol teichoic acids was studied using membrane fractions of *S. aureus* H (Yokoyama et al., 1986). Incubation of *S. aureus* membranes with CDP-glycerol and Man*N*Ac-[^{14}C]Glc*N*Ac-PP-prenol led to synthesis of (glycerol phosphate)-1-3-Man*N*Ac-[^{14}C]Glc*N*Ac-PP-prenol. In *S. aureus* (glycerol phosphate)-2-Man*N*Ac-[^{14}C]Glc*N*Ac-PP-prenol as well as (glycerol phosphate)-3-Man*N*Ac-[^{14}C]Glc*N*Ac-PP-prenol served as an acceptor for ribitol phosphate units, but (glycerol phosphate)-Man*N*Ac-[^{14}C]Glc*N*Ac-PP-prenol did not. The covalent attachment of WTA to peptidoglycan does not appear to play a role in the maintenance of the shape or rigidity of the cell wall, since their extraction with trichloracetic acid (TCA) leaves an essentially intact residual wall structure (Baddiley, 1972). While the cell-biological functions of

LTA are manifold, not much is known about WTA, which however plays an important role as bacteriophage receptor (Schleifer and Steber, 1974). It was for a long time an open question whether WTA is essential for growth. Recently in the group of A. Peschel, a completely WTA negative deletion mutant was isolated which exhibits normal growth but became resistant to a number of bacteriophages was impaired in adherence to nasal cells, and was completely unable to colonize cotton rat nares (Weidenmaier et al., 2004).

LIPOTEICHOIC ACID (LTA) With a pure (>99%) LTA preparation from *S. aureus*, nuclear magnetic resonance and mass spectrometry analyses were carried out (Morath et al., 2001). The results essentially corroborate the structure described by Fischer (1994). The average chain length of polyglycerol phosphate was 45–50 units; 70% of the glycerophosphate units were esterified with D-alanine, 15% bore α-D-*N*-acetylglucosamine, and 15% had no substituents. The fatty acids had an average chain length of $C_{15}H_{31}$. Fischer found that the alanine esters are rapidly lost from completed LTA and he hypoth-

esized that by spontaneous hydrolysis of the labile ester bond, D-alanine is transferred to the WTA. The D-alanine loss in LTA is compensated by incorporation of new alanine ester at a rate adjusted to the velocity of loss (Fischer, 1994).

Biological Functions It has been proposed (Heptinstall et al., 1970) that teichoic acids (which are highly negatively charged polymers) play an important role in divalent cation sequestration at the bacterial surface. Indeed it was found that the alanine ester content influences the magnesium binding capacity of walls of *S. aureus* H grown at different pH values (Archibald et al., 1973).

Fischer and coworkers investigated the influence of the degree of D-alanine esterification of staphylococcal LTA on autolysin activity using extracellular autolysin from *S. aureus* (Fischer et al., 1981). It turned out that the inhibitory activity was highest with D-alanine-free LTA. Glycosylation of LTA up to an extent of 0.5 did not depress inhibitory activity. They hypothesized that the anti-autolytic activity of LTA resides in a sequence of glycerophosphate units and that the negative charges of appropriately spaced phosphodiester groups play a crucial role. This work shows that autolysis is regulated in vivo by the alanine ester content of the LTA, and very likely by the WTA too, which also carries ester bound D-alanine. LTA is also an important antigen (Knox and Wicken, 1973). Some of the biological properties of the staphylococcal envelope components are listed in Table 13.

Table 13. Some biological properties of staphylococcal envelope components.

Envelope components	Property or activity
Peptidoglycan	Cell shape
	Osmotic stability
	Antigenic
	Pyrogenic
Peptidoglycolipid	Adjuvant
Muramylpeptides	Somnogenic
WTA	Antigenic
	Phage receptor
	Divalent cation binding
	Autolysis inhibition/regulation
LTA	Immunogenic
	Cell adhesion
	Phage receptor
	Complement activation
	Autolysis inhibition/regulation
Lipoprotein	Cell binding
	Immunogenic
	Mitogenic

Abbreviations: WTA, wall teichoic acid; and LTA, lipoteichoic acid.
[a]From Salton (1994).

Apart from lethal toxicity and pyrogenicity, LTA appeared to share many of the biological properties exhibited by lipopolysaccharides (Wicken and Knox, 1980). Critical micelle concentrations of various LTAs has been determined to be 1–10 µg/liter, suggesting that acylated LTAs in their monomer forms may represent the major configuration of extracellular LTAs in bacterial culture fluids (Wicken et al., 1986).

Great care has to be taken when analyzing commercial LTA preparations for immune stimulating activity. One must be aware that frequently these preparations are inhomogeneous and contain decomposed LTA and endotoxins of more than 10 ng of LPS/mg of LTA (Morath et al., 2002). A novel isolation procedure has been worked out that leads to a pure (> 99%) biologically active LTA, allowing the first structural analysis by nuclear magnetic resonance and mass spectrometry. A comparison with LTA purified by standard techniques revealed that alanine substituents are lost during standard purification, resulting in attenuated cytokine induction activity. In line with this finding, hydrolysis of alanine substituents of active LTA eliminated cytokine induction. Purified *S. aureus* LTA induced the release of TNF-α, IL-1β, IL-6 and IL-10 in human whole blood. Soluble CD14 (sCD14) inhibited monokine induction by LTA but failed to confer LTA responsiveness for IL-6 and IL-8 release of human umbilical vein endothelial cells (HUVECs). In a competitive LPS-binding protein (LBP) binding assay, the IC_{50} of the tested LTA preparations was up to 3230-fold higher than for LPS. The LBP enhanced TNF-α release of human peripheral blood mononuclear cells (PBMCs) upon LPS but not LTA stimulation. These data demonstrate a differential role for the serum proteins LBP and sCD14 in the recognition of LPS and LTA (Hermann et al., 2002). There is evidence that the recognition sites of CD14 for LPS and LTA are distinct with a partial overlap. While the maximal achievable monokine release in response to LTA or to LPS was comparable, all LTA induced significantly less IL-12 and IFN-γ. IL-12 substitution increased LTA-inducible IFN-γ release up to 180-fold, suggesting a critical role of poor LTA-inducible IL-12 for IFN-γ formation. Pretreatment with IFN-γ rendered galactosamine-sensitized mice sensitive to challenge with LTA. When compared to LPS, LTA (a major immunostimulatory component of *S. aureus*) is a weak inducer of IL-12 and subsequent IFN-γ formation, which might explain the lower toxicity in vivo.

In a recent study, the effects of LPS from *E. coli*, LTA and peptidoglycan (PG) from *S. aureus* and live *S. aureus* on leukocyte-endothelial interactions in vivo have been investigated (Yipp et

al., 2002). It was found that local administration of LPS into muscle induced significant leukocyte rolling, adhesion, and emigration in postcapillary venules at the site of injection. Given systemically, LPS caused circulating leukocyte counts to drop dramatically and neutrophil counts to increase in the lung. However, the drop in circulating leukocytes was not associated with leukocyte sequestration to the site of injection (peritoneum) or to peripheral microvessels in muscles. Unlike LPS, LTA had no systemic and very minor local effect on leukocyte-endothelium interactions, even at high doses and prolonged exposure. LPS, but not LTA, potently activated human endothelium to recruit leukocytes under flow conditions in vitro. Endothelial adhesion molecule expression was also increased extensively with LPS, but not LTA. Interestingly, systemic administration of live *S. aureus* induced leukocyte-endothelial cell responses similar to LPS. PG was able to induce leukocyte-endothelial interactions in muscle and peritoneum, but had no effect systemically (no increase in neutrophils in lungs and no decrease in circulating neutrophil counts). These results demonstrate that: 1) LPS has potent, but divergent local and systemic effects on leukocyte-endothelial interactions; and 2) *S. aureus* can induce a systemic response similar to LPS, but this response is unlikely to be due to LTA, but more likely to be mediated in part by PG.

Toll-like receptors (TLRs) are involved in cellular activation by microbial products, including lipopolysaccharide, lipoproteins, and peptidoglycan. Although for these ligands the specific transmembrane signal transducers TLR-4, TLR-2, or TLR-2 and -6 have now been identified, the molecular basis of recognition of LTA and related glycolipids has not been completely understood. Purified LTAs from *S. aureus* and *B. subtilis* exhibited TLR-2 dependence in nuclear factor κB activation and cytokine induction. The signaling molecules MyD88 and NIK appear to be also involved in cell stimulation by LTA (Opitz et al., 2001). The results presented here suggest that TLR-2 is the main receptor for the LTA-mediated inflammatory response.

Alanine Esterification and Virulence Staphylococcus aureus and *S. xylosus* normally tolerate high concentrations of several positively charged antimicrobial peptides. Andreas Peschel isolated staphylococcal transposon mutants that were not only hypersensitive to lantibiotics such as gallidermin (Götz and Jung, 2001a) but also to human defensin HNP1- 3, animal-derived protegrins, and other antimicrobial peptides (Peschel et al., 1999). All these substances are *c*ationic *a*nti*m*icrobial *p*eptides (CAMPs), many of which have membrane-damaging activity.

One mutant type lacked ester-bound D-alanine in the teichoic acids of both WTA and LTA. As a result the cells carry an increased negative surface charge and bound fewer anionic, but more positively charged peptides and proteins (Peschel et al., 1999). In addition, they became much more sensitive to human defensins, animal-derived protegrins, tachyplesins, and magainin II, and to the lantibiotics gallidermin and nisin. The mutation was located within the *dlt* genes involved in the transfer of D-alanine into teichoic acids. The fact that high concentrations of magnesium ions compromised the protective properties of D-alanine esters corroborates the hypothesis that the increased negative charge of the cell wall is responsible for the hypersensitivity against positively charged antimicrobial peptides. These results also show that the D-alanine-esterification of teichoic acids (which occurs in many Gram-positive bacteria) is not essential for growth but is essential in the protection against human and animal defense systems (Peschel et al., 1999). The innate immune system is largely based on the release of defensins by immune cells and various tissue cells such as epithelial or endothelial cells. Therefore, *dltA* mutants should be less virulent than wild type cells. Indeed it was demonstrated that the *S. aureus dltA* mutant lacking D-alanine modifications of teichoic acids is highly susceptible to neutrophil killing and is virulence-attenuated in mice (Collins et al., 2002). These results clearly show that D-alanine-esterification of teichoic acids is a virulence factor.

Substances such as defensins from the granules of phagocytes, epithelial surfaces, and skin (Ganz and Lehrer, 1994) share an amphiphilic cationic structure and a membrane-damaging activity by forming pores or disintegrating the cytoplasmic membrane bilayer. They are not only produced by mammals but also by insects and bacteria (Sahl and Bierbaum, 1998). CAMPs represent an important human defense mechanism, protecting skin and epithelia against invading microorganisms and assisting neutrophils and platelets. *Staphylococcus aureus* and other bacterial pathogens have evolved countermeasures to limit the effectiveness of CAMPs, including the repulsion of CAMPs by reducing the net negative charge of the bacterial cell envelope through covalent modification of anionic molecules (e.g., teichoic acids, phospholipids and lipid A). Mutants susceptible to CAMPs are more efficiently inactivated by phagocytes and are virulence-attenuated, indicating that CAMP resistance plays a key role in bacterial infections as reviewed by Peschel (2002).

Another biological effect of the *S. aureus dltA* mutant was that it became biofilm-negative. The underlying mechanism is its attenuated adher-

ence to artificial surfaces that play a key role in the first step of biofilm formation. The lack of D-alanine esters with the associated increase of negative surface charge affected colonization of polystyrene or glass (Gross et al., 2001), while PIA (polysaccharide intercellular adhesion) production, necessary for accumulated growth was only marginally influenced in the mutant. The data suggest that repulsive electrostatic forces can lead to reduced staphylococcal biofilm formation. Interestingly, the addition of >10 mM $MgCl_2$ but not $CaCl_2$ completely restored biofilm formation, again corroborating the important role of teichoic acids in scavenging Mg^{+2} ions.

Cell Wall Bound Proteins

Many surface proteins of Gram-positive bacteria are anchored to the cell wall envelope by a transpeptidation mechanism, requiring a C-terminal sorting signal with a conserved LPXTG motif (leucine-proline-unknown-threonine-glycine). Pioneering work in the identification and characterization of the enzyme involved in protein anchoring and the underlying mechanism was carried out in the group of Olaf Schneewind. In a recent review, the main achievements are summarized (Mazmanian et al., 2001).

PROTEIN A—A CELL WALL-ANCHORED SURFACE PROTEIN Protein A, an immunoglobulin G (IgG) binding protein, was the first identified surface protein of *S. aureus* (Sjödahl, 1977). Protein A is covalently bound via its C-terminal end to the pentaglycine interpeptide bridge of peptidoglycans and can be released by treatment with lysostaphin, a glycyl-glycine endopeptidase. Protein A (Spa) was one of the first proteins shown to be covalently anchored to the peptidoglycan. Protein A is synthesized as a precursor, containing an N-terminal signal peptide, a pro-peptide region, five IgG-binding domains (Moks et al., 1986), a cell-wall spanning region, and finally a 35-residue cell wall sorting (cws) sequence which is conserved in surface anchored proteins of most Gram-positive bacteria (Schneewind et al., 1992). The cws consists of the typical LPXTG motif, which is followed by a hydrophobic domain and a tail of mostly positively charged residues. Mutations at the LPXTG motif lead normally to the secretion of the mutant protein into the extracellular medium. Deletion of this motif prevents cleavage and cell wall anchoring of protein A. The cws sequence of protein A is representative (Fig. 12).

Apart from *spa*, there are in *S. aureus* several other proteins with a typical cws sequence (Table 14).

SORTASE Sortase (SrtA) cleaves polypeptides between the threonine and the glycine of the LPXTG motif (peptidase reaction) and catalyzes the formation of an amide bond between the carboxyl-group of threonine and the amino group of peptidoglycan crossbridges (transpeptidation reaction; Mazmanian et al., 2001). Even if the pentaglycine anchor structure of the cell wall is altered by incorporation of serine residues, anchoring to the cell wall is not affected (Strauss et al., 1998).

Staphylococcus aureus mutants lacking the *srtA* gene fail to anchor and display some surface proteins (mostly protein A and fibronectin-binding protein) and are impaired in the ability to cause animal infections (Mazmanian et al.,

Fig. 12. Cell wall sorting (cws) sequence of protein A.

Table 14. *Staphylococcus aureus* surface proteins.

Protein	Length (aa)	Ligand	References
Spa	508	IgG, vWBF	Uhlen et al., 1984
FnbA	1018	Fibronectin or fibrinogen	Signäs et al., 1989
FnbB	914	Fibronectin or fibrinogen	Jönsson et al., 1991
ClfA	933	Fibrinogen	McDevitt et al., 1994
ClfB	913	Fibrinogen	Ni Eidhin et al., 1998
Cna	1183	Collagen	Patti et al., 1994
SdrC	947	Unknown	Josefsson et al., 1998a
SdrD	1315	Calcium	Josefsson et al., 1998b
SdrE	1166	Bone sialoprotein	Tung et al., 2000
Pls	1637	None[a]	Savolainen et al., 2001
SasA to SasJ	ND	Unknown	Genome sequence

Abbreviation: aa, number of amino acids; vWBF, von Willebrand-Factor; Sas, *S. aureus* surface proteins; and ND, not determined.

[a]Inhibits adhesion to fibronectin or fibrinogen.

2000). Sortase acts on surface proteins that are normally secreted by the classical secretory "sec" pathway and whose signal peptide is removed by the signal peptidase.

Recently, a second sortase gene (*srtB*) was identified. SrtB appears to be required for anchoring of a surface protein with an asparagine-proline-glutamine-threonine-asparagine (NPQTN) motif. An *srtB* mutant is defective in the persistence of animal infections. The *srtB* gene is part of an iron-regulated locus called "iron-responsive surface determinants" (*isd*), which also contains a ferrichrome transporter and surface proteins with NPQTN and LPXTG motifs. It is suggested that SrtB might be involved in iron acquisition (Mazmanian et al., 2002).

When tethered to the C-terminus of other proteins bearing N-terminal signal peptides, the cws promotes anchoring of hybrid protein to the cell wall (Schneewind et al., 1993; Navarre et al., 1996). It was also possible to anchor enzymes such as lipases or β-lactamases in active form to the staphylococcal cell wall, provided that a cell wall spanning region is upstream of the LPXTG motif (Strauss and Götz, 1996).

Slime and Biofilm Formation

The molecular and biochemical basis of biofilm formation in *S. aureus* and *S. epidermidis* has been recently reviewed (Götz, 2002). The first reports that slime-producing *S. epidermidis* strains are involved in catheter-associated sepsis and infections came from the groups of Peters (Peters et al., 1981), Christensen (Christensen et al., 1982), and Costerton (Marrie et al., 1982b). Mostly *S. epidermidis*, but sometimes also mixed cultures have been isolated from colonized catheters recovered from patients. The bacteria appeared to be closely packed and cemented together by a slimy matrix (Peters et al., 1981). Transmission and scanning electron micrographs showed again that *S. epidermidis* forms a confluent biofilm, in which the cells are embedded in an amorphous material referred to as "slime" (Christensen et al., 1982). These early observations and pioneering work indicated that 1) the skin bacterium *S. epidermidis*, once considered to be harmless, is an opportunistic pathogen that could cause chronic staphylococcal infection and 2) in certain strains, slime (an extracellular polysaccharide) is produced and contributes to the formation of a confluent biofilm. As summarized recently, *S. epidermidis* can form a biofilm on nearly any synthetic polymers used as prosthetic devices, and in addition, they can bind to blood proteins, matrix proteins, and human cell receptors (Götz and Peters, 2000a; Götz et al., 2000b).

Today *S. epidermidis* is regarded as a leading species in causing chronic polymer-associated clinical infection. Microscopy of biofilm formation in vitro suggests that two steps are involved: 1) the attachment of the bacterial cells to the polymer surface, and 2) the growth-dependent accumulation to form multilayered cell clusters surrounded by a slimy matrix (Fig. 13).

The slime consists of a polysaccharide composed of β-1,6-linked *N*-acetylglucosamine, 15–20% of which is de-acetylated and therefore positively charged (Mack et al., 1996). Also identified was another polysaccharide II (<20%), which is structurally related, but has a lower content of non-*N*-acetylated D-glucosaminyl residues and contains phosphate and ester-linked succinate, rendering it anionic. The structure of this polysaccharide is unique, and according
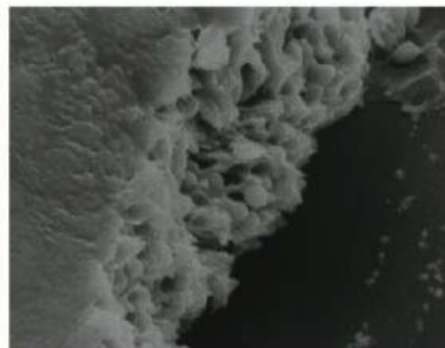
2-step model of biofilm formation in staphylococci

1. Adhesion of bacterial cells to a surface
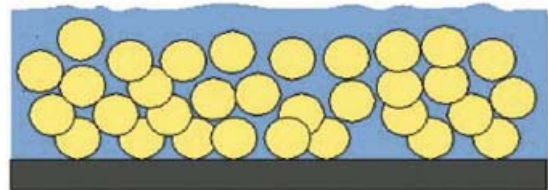
2. Proliferation of cells and embedding in a slimy matrix, mainly based on the polyglucosamine (PIA)

3. EM photograph of a *S. epidermidis* biofilm developed on plastic surface

Fig. 13. Two-step model of staphylococcal biofilm formation. The first step is the adherence of the bacterial cells to a surface. The second is the embedding of the cells in a thick slime matrix (biofilm). Within the biofilm, cells appear to have reduced physiological activity in an anoxic environment, and exhibit a decreased sensitivity to many antibiotics, compared with their planktonic counterparts. See also Götz and Peters (2000a).

to its function in intercellular aggregation, it was referred to as "*polysaccharide intercellular adhesin*" (PIA; Mack et al., 1996). A recently described polysaccharide PNSG (poly-*N*-succinyl β-1-6 glucosamine; McKenney et al., 1999) from *S. aureus* MN8N, containing *N*-acetylglucosamine residues regarded as completely succinylated, turned out to be an artefact (G. Pier, personal communication). PIA has several functions (Götz, 2002): it is a major factor of biofilm formation, contributes to the pathogenesis of biomaterial-associated infections (Rupp et al., 2001), is immunogenic, is involved in hemagglutination, binds to hydrophilic surfaces, and finally causes intercellular adhesion. Detection methods for PIA and biofilm formation have been recently described (Cramton et al., 2000; Leriche et al., 2000).

Polysaccharide Intercellular Adhesion Biosynthesis The genes responsible for biofilm formation were identified by isolating and analyzing biofilm-negative transposon-insertion mutants of *S. epidermidis* O-47 (Heilmann et al., 1996a). In this way, a number of genes could be identified which are involved in biofilm formation. There is the *ica* (*intercellular adhesion*) operon, which is responsible for the biosynthesis of PIA and its transcriptional control (Heilmann et al., 1996b). The operon is composed of the *icaR* (regulatory) gene and *icaADBC* (biosynthesis) genes. The in vitro biosynthesis of PIA has been analyzed using the membrane fraction or cellular or extracellular extracts (Gerke et al., 1998). IcaA has *N*-acetylglucosaminyl-transferase activity with UDP-*N*-acetyl-glucosamine as substrate. IcaD might be a chaperone that directs the correct folding and membrane insertion of IcaA and, in addition, might act as a link between IcaA and IcaC. The role of IcaC is still unclear.

Other biofilm-negative staphylococcal mutants were defective in adherence to polymers and extracellular matrix. The deletion of the major autolysin *atlE* gene revealed a pleiotropic effect (Heilmann et al., 1997): Because of their amidase deficiency, the mutant cells form huge cell clusters that resist even detergent treatment, which points to a defect in cell separation. Owing to extracellular processing of the precursor AtlE, five surface-bound proteins were missing. Binding to vitronectin and fibronectin was reduced, and finally, adherence to and biofilm-formation on hydrophobic surfaces was markedly decreased.

In a search for *S. aureus* mutants hypersensitive to positively charged antimicrobial peptides, mutants with an altered teichoic acid structure have been isolated (Peschel et al., 1999). The mutations are in the *dltA* operon, and the teichoic acids lack D-alanine. It has been postulated that the increased negative charge of the cell surface of the mutants leads to an increased scavenging of positively charged antimicrobial peptides, thus leading to the observed hypersensitivity. The *dltA* mutant is also biofilm-negative even though PIA production appears to be unchanged. The mutant is severely affected in adherence to polystyrene or glass surfaces (Gross et al., 2001), the first step of biofilm formation. In this respect, the *dltA* mutant resembles the *atlE* mutant described above, with one exception: the *atlE* mutant is still able to form a biofilm on a glass surface. That biofilm formation of the *dltA* mutant can be completely restored by the addition of Mg^{+2} ions corroborates the biological importance of the charge balance of the Gram-positive cell surface.

Other proteins can also contribute to biofilm formation in staphylococci, such as the accumulation-associated protein (AAP; Hussain et al., 1997) and the biofilm-associated protein (Bap; Cucarella et al., 2001). Especially *S. aureus* is capable of adhering to a large variety of matrix components to initiate colonization. This adherence is frequently mediated by protein adhesins of the MSCRAMM (microbial surface components recognizing adhesive matrix molecules) family, which in many cases are covalently anchored to the cell wall peptidoglycan (Foster and Höök, 1998). Very likely, surface proteins such as fibronectin-binding protein A and B (FnBPA and FnBPB), the collagen-binding protein Cna, and the fibrinogen-binding proteins, clumping factor A and B (ClfA and ClfB), contribute to adherence and thus to biofilm formation.

Slime production and biofilm formation are regulated by a number of environmental parameters and stress factors, which were summarized by Götz (2002). Among them, anaerobiosis clearly induces ica expression (Cramton et al., 2001). It was also shown that IS*256* integrates into the ica operon and can also be precisely excised (Ziebuhr et al., 1999; Ziebuhr et al., 2000a; Ziebuhr et al., 2000b). The role of PIA in pathogenicity is also corroborated by the finding that an intact *ica* operon is more prevalent in clinical (septicemic disease and shunt-associated meningitis) *S. epidermidis* isolates than in skin isolates of nonhospitalized persons (Ziebuhr et al., 1997).

Biofilm formation in staphylococci is multifactorial, and the ability to form a biofilm makes the strains much better able to survive in the normally hostile environment of tissue and blood. Biofilm formation appears to be a bacterial survival strategy that is turned on when, for example, oxygen and Fe ions become limited, when sublethal concentrations of certain antibiotics are present, or other stress

situations emerge. The modulation of biofilm formation by various environmental conditions appears to be an advantage for successful infection.

NEW MEMBRANE COMPONENTS Another mutant type was hypersensitive to CAMPs (Peschel et al., 2001) and identified with a novel staphylococcal gene, *mprF*. The *mprF* mutant strain was killed considerably faster by human neutrophils and exhibited attenuated virulence in mice, indicating a key role for defensin resistance in the pathogenicity of *S. aureus*. In the *mprF* mutant, the predominant membrane component phosphatidylglycerol lacks L-lysine modification. The structure of lysylphosphatidylglycerol (L-PG) is shown in Fig. 14. As this unusual modification leads to a reduced negative charge of the membrane surface, MprF-mediated peptide resistance is most likely based on repulsion of the cationic peptides. Accordingly, inactivation of *mprF* led to increased binding of antimicrobial peptides by the bacteria. The *mprF* gene has no similarity with genes of known function, but related genes were identified in the genomes of several pathogens including *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*. MprF thus constitutes a novel virulence factor, which may be of general relevance for bacterial pathogens and represents a new target for attacking multidrug resistant bacteria.

Protein Secretion in Staphylococci

The *S. aureus* genome was screened for secretion genes of the classical sec pathway (Kuroda et al., 2001; Mazmanian et al., 2001). Apart from the *E. coli* specific *secB* and *secF* genes, all other genes (*secA*, *D*, *E*, *G*, *Y*; *yajC*; *ffh*; *ftsY*, and *lepB*) are also present in the *S. aureus* genome. In addition, the *S. aureus* genomes revealed two *secA* (*secA*-1 and *secA*-2) and two *secY* genes (*secY*-1 and *secY*-2). The presence of two homologous genes is unclear; however, one can speculate that they play a role for a specific set of translocated proteins, like for example *srtB*, whose function differs apparently from that of *srtA*. The presence of most of the *sec* genes suggests that in *S. aureus*, the sec pathway is essentially similar to that described for *E. coli*. Maybe this is the reason why protein secretion is hardly studied in staphylococci. However, it was shown that many of the secreted proteins in staphylococci possess a so-called "pro-peptide region" and the role of this region in protein expression and secretion has been studied in more detail.

ROLE OF THE PRO-PEPTIDE REGION IN EXOPROTEINS Since the *S. hyicus* specific lipase (SHL) was the first bacterial lipase gene ever to be cloned and sequenced (Götz et al., 1985), both the enzymatic properties and the role of the pro-peptide was investigated in more detail with this enzyme (reviewed in Götz and Rosenstein, 2001b).

According to sequence comparisons, all staphylococcal lipases are predicted to be primarily pre-pro-lipases. A structural organization of some of the lipases is shown in Fig. 15. The various pro-peptides have no striking similarities at the sequence level but are distinguished by
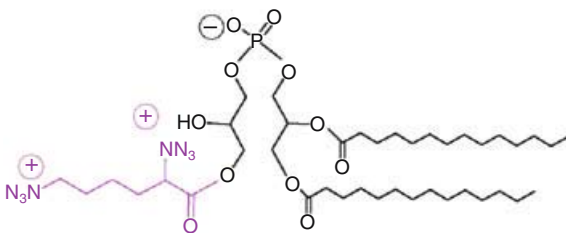
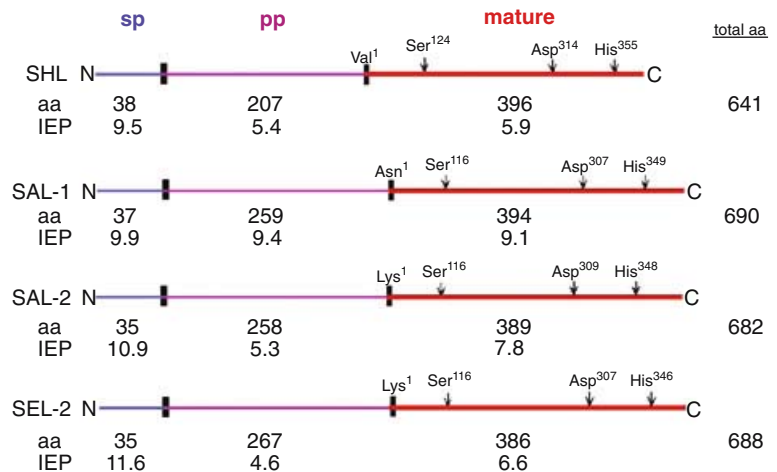Fig. 14. Structure of lysylphosphatidylglycerol (L-PG).

Fig. 15. Organization of staphylococcal (phospho)lipases as pre-pro-enzymes. SHL, (phospho) lipase from *S. hyicus* subsp. *hyicus* DSM 20459; SAL-2, lipase from *S. aureus* NCTC 8530; SAL-1, lipase from S. aureus PS54; SEL-2, lipase from *S. epidermidis* RP62A. sp, signal peptide; and pp, pro-peptide region. The proposed active site amino acids are indicated in the mature form of each enzyme. aa, amino acid number or position; and IEP, isoelectric point. From Götz et al. (1998).

their overall hydrophilic character. To get more insight into the function of the pro-peptide, internal deletions within this region were made and analyzed (Demleitner and Götz, 1994). The results obtained with these lipase mutants indicate that the SHL pro-peptide may have two functional domains, with each one located in one half of the pro-region. The N-terminal part seems to be important for lipase activity and the C-terminal part, for translocation and stability. A stabilizing effect of the SHL pro-peptide has also been observed in an experiment where OmpA of *E. coli* is fused to the pre-pro portion of SHL; without the SHL pro-peptide, massive proteolytic degradation occurred after secretion by *S. carnosus* (Meens et al., 1997). A number of experiments designed to address the question whether the pro-peptide could act also in *trans* indicated that it has no *trans* activity. It exerts its beneficial effects on translocation, stability, and activity only intramolecularly (G. Thumm et al., unpublished observation). All studies carried with the pro-peptide speak in favor of an intramolecular chaperon-like function. Maybe it compensates in *Staphylococcus* and also in *Bacillus* for the lack of the SecB homolog.

The proteolytic processing of pro-SHL has been studied in more detail (Liebl and Götz, 1986; Götz, 1991; Demleitner and Götz, 1994). Since the 86-kDa pro-SHL purified from *S. carnosus* is processed by culture supernatants of *S. hyicus*, it became apparent that the processing of the pro-region by an extracellular protease occurs after secretion. The proteolytic activities in the supernatant of *S. hyicus* were analyzed, and two proteases (ShpI and ShpII) were identified; ShpII proved to be involved in pro-SHL processing (Ayora and Götz, 1994a; Ayora et al., 1994b).

THE USE OF SHL AS EXPRESSION AND SECRETION SYSTEM The beneficial role of the pro-peptide region of SHL for efficient secretion of heterologous proteins and enzymes was already described earlier (Liebl and Götz, 1986). The results support a dual role for the SHL pro-peptide: an involvement in protein translocation and a role in stabilization against proteolytic degradation.

Because of these encouraging results, the pre-pro-portion of SHL was fused to several other heterologous proteins such as human growth hormone (Sturmfels et al., 2001) and malaria antigen (Samuelson et al., 1995). In all cases, the heterologous proteins were successfully and in good quantities secreted by *S. carnosus*, indicating a general application of this strategy.

An alignment of the lipase signal peptides shows a remarkable motif containing the perfectly conserved residues Ser, Ile, Arg and Lys, designated as the "SIRK-motif" (Götz et al., 1985; Nikoleit et al., 1995). This motif is present in many exoproteins of Gram-positive bacteria. Its function is still unclear.

Genomic Analysis of Virulence Genes

Several staphylococcal strains are completely sequenced but so far only the *S. aureus* strains N315 and Mu50 (Kuroda et al., 2001) are annotated and permit a general protein search. Some general features of *S. aureus* N315 genome are listed in Table 15.

To see whether and to which extent the genomes of *S. aureus* N315 (causing acute infections) and *S. epidermidis* RP62A (causing chronic infections) differ from each other, an arbitrary list of 125 virulence or fitness genes of the *S. aureus* N315 genome were investigated for the presence of homologous genes in *S. epidermidis* RP62A (Nerz et al., 2002). Genes or operons were selected that are involved in the production of exoenzyme, toxins, adhesins including biofilm formation, Fe-uptake, resistance to various antibiotics, and other functions. The comparison of both genomes was complicated by the fact that the RP62A sequence was

Table 15. General features of *Staphylococcus aureus* N315 genome.

Chromosome	
Length of sequence (bp)	2,813,641
G+C content (total genome)	32.8%
ORFs Percentage coding	84.5%
Protein coding regions	2595
Ribosomal RNAs	
16S	5
23S	5
5S	6
t-RNAs	62
tmRNAs	1
Insertion sequences	
IS*1181*	8
IS*431*	2
Others	10
Transposons	
Tn*554*	5
Others	0
Bacteriophages	1
SCCmec	1
Pathogenicity islands	**3**
Plasmid	
Length of sequence (bp)	2465

Abbreviations: tmRNA, RNA that has dual tRNA-like and mRNA-like nature; IS, insertion sequence; Tn, transposon; SCCmec, staphylococcal cassette chromosome mec; and mec, methicillin-resistance component.
From Kuroda et al. (2001).

not yet annotated. The selected virulence genes were localized on the *S. aureus* N315 genome (at the DOGAN-Server) by their ID-number and translated into amino acid sequence. The protein sequences were used as queries in a tBLASTn search of the *S. epidermidis* RP62A genome at The Institute for Genomic Research (TIGR) Web site. We also checked the almost finished genome sequence of the food-grade *Staphylococcus carnosus* TM300 (R. Rosenstein, C. Nerz, and F. Götz). To evaluate whether the resulting matches could be regarded as significant and thus represent homologous genes, they were checked carefully for length of alignment, percentage of similarity/identity and presence of functional protein domains. For this analysis, we used the following Web sites/tools:

In Tables 16 to 20, the genes identified in *S. aureus* N315 and the corresponding protein names (if available) are indicated. Those genes that have homologous counterparts in *S. carnosus* TM300 are highlighted in red.

Of the 159 selected virulence genes in *S. aureus* N315, only 37 genes (18%) had a homologous equivalent in *S. epidermidis* RP62A and 31 genes had homologous equivalent in *S. carnosus* TM300 (Rosenstein, Nerz, and Götz). This clearly reflects the difference in the pathogenic potential of the three species representatives and explains why *S. aureus* is rather an aggressive pathogen, *S. epidermidis* a mild pathogen and *S. carnosus* is non-pathogenic. Of the 40 toxin genes in *S. aureus*, only 3 and 5, respectively, were identified in the *S. epidermidis* and *S. carnosus* genome. This finding is in agreement with the earlier observations of decreased toxin production of *S. epidermidis* and corroborates well with the decreased severity of an *S. epidermidis*

Table 16. Presence of exoenzymes in *S. aureus* N315/Mu50 and *S. carnosus* TM300.

Exoenzymes	ORFs	Gene name	Function	Similarity (%)
Staphylocoagulase	SA0222	*coa*	Possible coagulation in tissues (agr-controlled)	—
Possible staphylocoagulase	SA0743	None	Unknown	—
Thermonase	SA1160	*nuc*	Degradation of host's nucleic acid	77
Staphylococcal nuclease Micrococcal nuclease[a]	SA0746	*nuc*	Degradation of host's nucleic acid (agr-controlled)	—
Probable 59-nucleotidase	SA0022	None	Unknown	62
Lipase	SA0309	*geh*	Hydrolytic degradation of lipids	—
Triacylglycerol lipase	SA2463	*lip*	Hydrolytic degradation of lipids	56
Similar to lipase LipA	SA0610	None	Unknown	77
Probable lipase	SA2323		Unknown	—
1-Phosphatidylinositol phosphodiesterase	SA0091	*plc*	Hydrolysis of phosphatidylinositol	—
Hyaluronate lyase[b]	SA2003	*hysA*	Degradation of host's hyaluronic acid	—
Serine protease[c]	SA0901	*sspA*	Proteolytic destruction of host tissues (agr-controlled)	—
Serine protease	SA0879	None	Proteolytic destruction of host	—
Probable serine protease	SA1627, 1628, 1629, 1630, and 1631 Location: SaPIn3/ SaPIm3	*splA*, *splB*, *splC*, *splD*, and *splF*	Unknown	—
Cysteine protease	SA0900	*sspB*	Proteolytic destruction of host tissues	—
Staphopain[d]	SA1725	None	Proteolytic destruction of host tissues	—
Zinc metalloproteinase aureolysin	SA2430	*aur*	Proteolysis of host tissues	—
Staphylokinase[e]	SA1758 Location: N315/ Mu50A	*sak*	Proteolytic destruction of host tissues	—

Symbol and abbreviations: −, not determined; ORFs, open reading frames; agr, accessory gene regulator; and Tnase, thermonuclease.

[a]EC-Number 3.1.31.1; typical for *S. aureus*.
[b]Hyaluronidase.
[c]V8 protease.
[d]Cysteine proteinase.
[e]Protease III.
Blue indicates newly identified ORFs.
Red indicates counterpart in the *S. carnosus* TM300 genome.

Table 17. Presence of toxins in *S. aureus* N315/Mu50 and *S. carnosus* TM300.

Toxins	ORFs	Gene name	Function	Similarity (%)
α-Hemolysin	SA1007	*hla*	Destruction of blood and tissue cells (agr-controlled)	—
γ-Hemolysin components	SA2207, 2208, and 2209	*hlgA*, *hlgC*, and *hlgB*	Destruction of blood cells	—
δ-Hemolysin	SAS065	*hld*	Destruction of blood and tissue cells (agr-controlled)	77
Leukotoxins	SA1637 and 1638 Location: SaPIn3/SaPIm3	*lukD* and *lukE*	Destruction of white blood cells	—
Possible leukocidin	SA1813	*lukM*	Unknown	—
Possible hemolysin	SA0657	None	Unknown	89
Possible hemolysin	SA0780	None	Unknown	82
Similar to exotoxin 1	SA1009	None	Unknown	55
Possible hemolysin	SA1812	None	Unknown	—
Similar to hemolysin III	SA1973	None	Unknown	75
Possible exotoxins	SA0357	None	Unknown	—
	SA0382, 0383, 0384 Location: SaPIn2/SaPIm2	*set6*, *set7*, *set8*, *set9*	Unknown	—
	0385, 0386, 0387	*set10*, *set11*, *set12*		—
	0388, 0389, 0390, and 0393	*set13*, *set14*		—
	SA1009, 1010, and 1011		Unknown	—
Superantigens Enterotoxins	SA1642, 1643, 1644, 1645, 1646, 1647, and 1648	*seg*, *sen*, *ent2*, *ent1*, *sei*, *sem*, and *seo*	Food poisoning, superantigen (agr-controlled)	—
Enterotoxin P	SA1761(N315)	*sep*	Food poisoning, superantigen	—
Enterotoxin A	SAV119(Mu50)	*sea*	Food poisoning, superantigen	—
Enterotoxins	SA1816 and 1817 Location: SaPIn1/SaPIm1	*sel* and *sec3*	Food poisoning, superantigen	—
Toxic shock syndrome toxin 1	SA1819 Location: SaPIn1/SaPIm1	*tst*	Fever, shock, skin rash, superantigen (agr-controlled)	—
Probable enterotoxin	SA1430	None	Unknown	—

Symbol and abbreviation: –, not determined; and agr, accessory gene regulator.
Blue indicates newly identified ORFs.
Red indicates counterpart in the *S. carnosus* TM300 genome.

Table 18. Presence of adhesins in *S. aureus* N315/Mu50 and *S. carnosus* TM300.

Adhesins	ORFs	Gene name	Function	Similarity (%)
IgG-binding protein A	SA0107	*spa*	Potential immune disorder in host (agr-controlled)	—
IgG-binding protein SBI	SA2206	*sbi*	Potential immune disorder in host	—
Ser-Asp-rich fibrinogen-binding proteins	SA0742 and 2423	*clfA, clfB*	Cellular adhesion onto host tissues	—
Possible fibrinogen-binding proteins	SA1000, 1003, and 1004	None	Unknown	—
Fibronectin-binding proteins	SA2290 and 2291	*fnbB* and *fnbA*	Cellular adhesion onto host tissues (agr-controlled)	—
Ser-Asp-rich proteins	0519, 0520 and 0521	*sdrC*, *sdrD* and *sdrE*	Cellular adhesion onto host tissues	—
Possible extracellular matrix binding proteins	SA0744 and 0745		Cellular adhesion onto host tissues	—
Probable extracellular matrix binding proteins	SA1267 and 1268	*ebhA* and *ebhB*	Unknown	—
Sortase A	SA2316	*srtA*	Covalent linkage of binding proteins to the peptidoglycan	74[a]
Sortase B	SA0982	*srtB*	Covalent linkage of binding Fe-protein to the peptidoglycan	—

Table 18. *Continued*

Adhesins	ORFs	Gene name	Function	Similarity (%)
Proteins with the cell wall sorting signals (LPXTG)	SA0976, 0977, 1552, 1577, 1888, 1964, 2284, 2381, and 2447	None	Possible pathogenic factors	—
Probable adhesin	SA0587	None	Cellular adhesion onto host tissues	83
Elastin-binding protein	SA1312	*ebpS*	Cellular adhesion onto host tissues	—

Symbol and abbreviations: –, not determined; IgG, immunoglobulin G; and LPXTG, leucine-proline-unknown-threonine-glycine.

[a]Similarity: 77% (ubiquitous).

Blue indicates newly identified ORFs.

Red indicates counterpart in the *S. carnosus* TM300 genome.

Table 19. Presence of Fe-uptake and other virulence genes in *S. aureus* N315/Mu50 and *S. carnosus* TM300.

Fe-uptake	ORFs	Gene name	Function	Similarity (%)
Possible siderophore biosynthesis proteins	SA0116 and 0117	None	Iron uptake	—
Possible iron-binding protein	SA0217	None	Iron uptake	—
Possible iron permease components	SA0566 and 0567	None	Iron uptake	73, 60
Possible ferrichrome ABC transporter	SA0891	None	Iron uptake	60
Possible ferrichrome ABC transporter components	SA0980, 0981	None	Iron uptake	—
Possible ferritin	SA1709	None	Maintenance of cellular iron	—
Possible ferrichrome ABC transporter components	SA1977, 1978, and 1979	None	Iron uptake	77, 74, and 71
Ferrichrome ABC transporter FhuD homolog	SA2079	*fhuD*	Iron uptake	84
Possible iron transport proteins	SA2337 and 2369		Iron uptake	80, 70
Ferrichrome ABC transporter FhuD homolog	SAV0812	SaGIm[a]	Iron uptake	—

Others	ORFs	Gene name	Function	Similarity
Capsular polysaccharide synthesis proteins	SA0144-159	*cap A-P*	Possible escape from immune system	72–89 cap A-D, M,P
Probable capsular polysaccharide synthesis proteins	SA0126, 0127		Unknown	—
Intercellular adhesion proteins	SA2459, 2460, 2461, and 2462	*icaA*, *D*, *B* and *C*	Cell-cell aggregation on infected tissues	—
MHC class II-like protein Map-w	SA0841, 1750–51, 2006		Unknown	—
Myosin-crossreactive MHC class II-like protein	SA0102		Potential immune disorder in host	—
Synthesis of lysyl-phosphatidyl glyceride	SA1193	*mprF* (*fmtC*)	Resistance to cationic antimicrobial peptides[b]	75
D-Alanyl esterification of teichoic acids	SA0793-96	*dltA*, *B*, *C* and *D*	Resistance to cationic antimicrobial peptides[b]	89, 84, 99, and 84

Symbol and abbreviations: –, not determined; ORFs, open reading frames; ABC, ATP-binding cassette; and MHC, major histocompatibility complex.

[a]SaGIm is the genomic island name.

[b]Gene is widespread.

Blue indicates newly identified ORFs.

Red indicates counterpart in the *S. carnosus* TM300 genome.

Table 20. Presence of antibiotic resistance genes in *S. aureus* N315/Mu50 and *S. carnosus* TM300.

Resistance genes	ORF	Gene name	Function	Similarity (%)
Penicillin binding protein 29	SA0038	*mecA*	Pc resistance	—
β-Lactamase	SAP010	*blaZ*	Pc resistance[a]	—
rRNA adenine *N*-6-methyltransferase	SA0048	*ermA*	Em resistance[b]	—
rRNA adenine *N*-6-methyltransferase	SA0766	*ermA*	Em resistance	—
rRNA adenine *N*-6-methyltransferase	SA1480	*ermA*	Em resistance	—
rRNA adenine *N*-6-methyltransferase	SA1951	*ermA*	Em resistance	—
rRNA adenine *N*-6-methyltransferase	SA2348	*ermA*	Em resistance	—
O-nucleotidyltransferase	SA0049	*ant*	Ags resistance[b]	—
O-nucleotidyltransferase	SA0765	*ant*	Ags resistance	—
O-nucleotidyltransferase	SA1481	*ant*	Ags resistance	—
O-nucleotidyltransferase	SA1952	*ant*	Ags resistance	—
O-nucleotidyltransferase	SA2385	*ant*	Ags resistance	—
Bifunctional AAC/APH protein	SAVP026	*aacA*	Ags resistance	—
Tetracycline resistance protein	SAV0387	*tetM*	Tc resistance	—
Bleomycin resistance protein	SA0032	*bleO*	Ble resistance	—

Symbol and abbreviations: –, not determined; ORF, open reading frame; Pc, penicillin; Em, erythromycin; Ags, aminoglycoside; Tc, tetracycline; Ble, bleomycin; and AAC/APH, 6′-aminoglycoside-*N*-acetyltransferase and 2′-aminoglycoside phosphotransferase.
[a]Two copies.
[b]Three copies.

infection. Compared to *S. aureus*, *S. carnosus* was also found to have a much decreased number of other potential virulence factors such as exoenzymes and adhesins.

This is only a preliminary study since the *S. epidermidis* and *S. carnosus* genomes were only screened for the presence of known *S. aureus* virulence genes. On the other hand, not many virulence genes are known in *S. epidermidis*. However, this first analysis may shed some light why *S. epidermidis* causes rather mild or chronic infections. In the future, we shall get a more complete picture if the genomes of other staphylococcal species are included in the comparative genome analysis.

The Regulatory Network in Staphylococci

Accessory Gene Regulation

The initial step in an infectious disease is often adhesion to and colonization of host tissue surfaces. *Staphylococcus aureus* has been shown to bind to several host matrix proteins and plasma proteins, such as fibronectin (Flock et al., 1987; Froman et al., 1987; Jonsson et al., 1991), fibrinogen (Boden and Flock, 1989), collagen (Foster and Höök, 1998), elastin (Park et al., 1991), laminin (Vercellotti et al., 1985; Herrmann et al., 1988), prothrombin (Kawabata et al., 1985), thrombospondin (Herrmann et al., 1991), bone sialoprotein (Ryden et al., 1990; Yacoub et al., 1994), and vitronectin (Liang et al., 1995; Heilmann et al., 1997). For each of these binding functions, a corresponding surface-associated protein has been identified. The existence of an *S. aureus* extracellular matrix binding protein with broad specificity that is capable of binding several extracellular glycoproteins has also been reported (McGavin et al., 1993). The role of some of these proteins in the pathogenesis of staphylococcal infections has been shown in animal models (Foster and McDevitt, 1994; Moreillon et al., 1995; Flock et al., 1996; Palma et al., 1996).

The expression of many matrix-binding proteins is controlled by the agr (accessory gene regulator) system. Many of the genes coding for extracellular toxins, enzymes, and cell surface proteins in *S. aureus* are regulated by a 510-nucleotide RNA molecule, RNAIII. Transcription of genes encoding secreted toxins and enzymes (including *hla* [α-toxin], *saeB* [enterotoxin B], *tst* [toxic shock syndrome toxin 1], and *ssp* [serine protease]) is stimulated, while transcription of genes encoding cell surface proteins (like *spa* [protein A] and *fnb* [fibronectin binding proteins]), is repressed (Tegmark et al., 1998).

In *S. aureus*, transcription of at least 15 virulence genes, encoding extracellular toxins, enzymes, and cell surface proteins, is also regulated (Janzon and Arvidson, 1990; Novick et al., 1993). Production of toxins and enzymes is generally positively controlled, while that of cell surface proteins is negatively controlled (Janzon et al., 1989; Kornblum et al., 1990). In Table 21, agr-controlled genes are classified according to their degree of regulation (Kornblum et al., 1990). For example, the expression of α-hemolysin, serine protease, toxic shock syndrome toxin (TSST-1), and δ-lysin is enhanced in an agr+ and very low

Table 21. Classification of agr-regulated extracellular protein expression in *Staphylococcus aureus*.

Class	Exoprotein	Gene	agr$^+$	agr$^-$
I	α-hemolysine	*hla*	high	undetectable
	serine protease	*spr* (V8)	high	undetectable
	TSST-1	*tst*	high	undetectable
	δ-hemolysin	*hld*	high	undetectable
II	nuclease	*nuc*	high	low-moderate
	β-hemolysin	*hlb*	high	low-moderate
	Enterotoxin B	*entB*	high	low-moderate
III	Protein A	*spa*	low	high
	Coagulase	*coa*	low	high
	FibronectinBP	*fbn*	low	high
IV	β-Lactamase	*bla*	no effect	
	Enterotoxin A	*entA*	no effect	

in an agr⁻ background, while the expression of the cell wall-bound proteins such as protein A, coagulase, and fibronectin BP is downregulated.

Synthesis of RNAIII is induced when the concentration of an autocrine octapeptide in the environment has reached a certain level (Ji et al., 1995; Ji et al., 1997). Generally, this happens during the late exponential phase of growth in laboratory cultures, which means that cell surface proteins are produced during the early exponential phase, while secreted toxins and enzymes are produced mainly during the post-exponential phase of growth (Björklind and Arvidson, 1980; Vandenesch et al., 1993; Lebeau et al., 1994).

The *agr* locus contains two divergent transcription units, RNAII and RNAIII, driven by the promoters P2 and P3, both of which are active only from the mid-exponential phase of growth (Novick et al., 1995). RNAII contains four genes: *agrA*, *agrB*, *agrC* and *agrD*. The *agrC* and *agrA* genes code for the components of a classical two-component signal transduction system, where AgrC is the membrane-bound signal receptor and AgrA is the response regulator, which is required for transcription of the RNAIII molecule and the *agr* operon itself (Novick et al., 1993; Novick et al., 1995).

The autoinducing peptide (AIP) is encoded in and derived from the AgrD propeptide that is in an unknown way post-translationally processed, modified and secreted. Very likely AgrB (Ji et al., 1995; Ji et al., 1997), an integral membrane protein, is involved besides additional factors (Saenz et al., 2000). The resultant mature AIP is the ligand that binds to and activates the phosphorylation of AgrC (Lina et al., 1998), which in turn is thought to phosphorylate AgrA, and phosphorylated AgrA found to induce RNAII and RNAIII synthesis (Ji et al., 1995). RNAIII causes an upregulation of secreted virulence factors as well as the downregulation of surface proteins. The gene organization of the agr system is shown in Fig. 16.
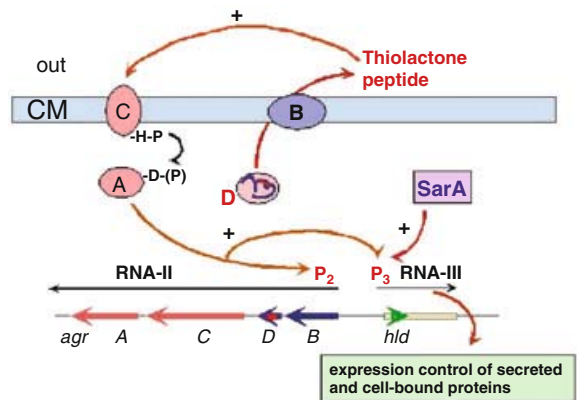
Fig. 16. Accessory gene regulatory (agr) genes and regulation cascade. From promoter region (P2), the RNA-II is transcribed encoding the genes *agrB*, *agrD*, *agrD*, *agrC* and *agrA*. The thiolactone peptide acts as an autoinducer that is sensed by the two component proteins AgrC and AgrA; the latter in its phosphorylated state activates transcription from P2 and P3. RNA-III controls gene expression of secreted and cell-bound proteins in an essentially unknown manner. RNA-III transcription is also positively controlled by the staphylococcal accessory regulator (SarA).

Quorum Sensing

The synthesis of RNAIII is regulated by a quorum sensing mechanism. The AIP molecules accumulate in the culture supernatant, and when a certain threshold concentration is reached, they interact with AgrC and induce as a consequence both RNAII and RNAIII transcription. Since transcription of P2 requires P2 operon products, the P2 operon is autocatalytic, and is thus suited for rapid production of secreted proteins at a time when overall growth is coming to a halt.

RNA III is the actual effector of the agr response, and incidentally encodes the agr-regulated peptide δ-lysin (Janzon et al., 1989), which is a 26-amino acid polypeptide that can form pores in membranes and lyse erythrocytes

(Freer and Birkbeck, 1982) but is not required for the regulation of target genes by RNAIII. How RNAIII interacts with target gene promoters is unknown.

Distribution of the Agr Locus

The *agr* locus, organized in the same way as that of *S. aureus*, has been demonstrated in the coagulase-negative *S. lugdunensis* (Vandenesch et al., 1993). However, RNAIII from *S. lugdunensis* does not code for δ-lysin, and its role in gene regulation is not known. The *agr* genes are also present in other coagulase-negative staphylococci such as *S. epidermidis*, *S. simulans* and *S. warneri* where RNAIII and δ-lysin homologues have been identified (Otto et al., 1998; Tegmark et al., 1998). In all RNAIII molecules, the first 50 and last 150 nucleotides were highly conserved, suggesting that these regions are important for the regulatory function.

Agr-specific Peptide Pheromones are Thiolactones

The agr-specific peptide pheromones are referred in the literature as AIP. However, the latter designation, does not consider the findings that these peptide pheromones not only induce the native agr system but also act at the same time as inhibitors of foreign agr systems. Therefore, the less restrictive term "peptide pheromone" is preferred.

The first hint that a peptide acts as an autoinducer of the agr system in *S. aureus* comes from the group of R. Novick. It turned out that the staphylococcal peptide pheromone sequences that are encoded in *agrD* gene vary from species to species. Even within *S. aureus*, four subtypes were identified (Ji et al., 1997). The first structure of a pheromone peptide and its biological activity was described in 1998 (Otto et al., 1998). The peptide sequence, DSVCASYF, was derived from AgrD of *S. epidermidis*. This synthetic octapeptide revealed only autoinducing activity when it contained the thiolactone ring between the central cysteine and the C-terminal carboxyl group (DSVc[CASYF]). The structure of the peptide pheromone of *S. epidermidis* is shown in Fig. 17. Unmodified peptides showed no activity (Otto et al., 1998). The receptor for the peptide pheromones, AgrC, must be very sensitive since the thiolactones are active at nanomolar concentrations. The N-terminus of the peptide pheromones is crucial for biological activity and specificity (Otto et al., 1998).

Interestingly, the *S. epidermidis* specific peptide pheromone is a potent inhibitor of the *S. aureus* agr system (Otto et al., 1999). However, while the correct N-terminus and the thiolactone
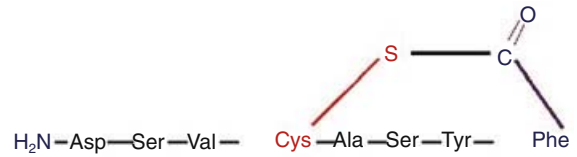
Fig. 17. Structure of agr pheromone (autoinducing peptide, AIP) of *S. epidermidis*. The agr pheromones are distinguished by a thiolester linkage between the central cysteine and the C-terminal carboxyl group. From Otto et al. (1998).

structure were absolute prerequisites for an agr-activating effect in *S. epidermidis*, inhibition of the *S. aureus* agr system showed a lesser structural stringency. Another important finding was that the corresponding lactone- and lactam-containing peptides DSVc[SASYF] and DSVc[DprASYF] inhibit the *S. aureus* agr system, but have lost autoinducing activity in its native *S. epidermidis* agr system (Otto et al., 1999). The *S. epidermidis* thiolactone inhibits the agr subgroups 1–3 but not 4 (Otto et al., 2001). An explanation for this could be that subgroup 4 thiolactone is very similar to that of *S. epidermidis*. A series of autoinducing peptide analogues (including the L-alanine and D-amino acid scanned peptides) were synthesized to determine the functionally critical residues within the *S. aureus* group I. It was found that 1) the group I autoinducing peptide (YSTc[CDFIM]) is inactivated in culture supernatants by the formation of the corresponding methionyl sulfoxide, and 2) that the lactam analogue retains the capacity to activate agr, suggesting that the thiolactone structure, which would allow covalent modification of the AgrC receptor, is not a necessary prerequisite for agr activation. Replacement of the endocyclic amino acid residue (D-aspartate) with alanine converted the group I thiolactone from an activator to a potent inhibitor. The addition of exogenous agr inhibitors to *S. aureus* decreased the production of TSST-1 and enterotoxin C3, confirming the potential of quorum-sensing blockade as a therapeutic strategy (Dowell et al., 2001).

It is very likely that in nature, there is a fierce competition between *S. aureus* and *S. epidermidis* and it looks like quorum-sensing cross talk generally favors survival of *S. epidermidis*, which might explain the predominance of *S. epidermidis* on the skin and in infections on indwelling medical devices (Götz, 2002).

Agr and Pathogenicity

In *agr* mutants, decrease in pathogenicity is very likely due to decreased synthesis of extracellular toxins and enzymes, such as α-, β-, and

δ-hemolysins, leucocidin, lipase, hyaluronate lyase, and proteases. In a murine arthritis model, the virulence was investigated of *S. aureus* 8325-4 and two *agr/hld* mutants derived from it (Abdelnour et al., 1993). Sixty percent of the mice injected with the wildtype strain developed arthritis, whereas *agrA* and *hld* mutants displayed joint involvement in only 10 and 30%, respectively. The question is also whether agr inhibiting thiolactone peptides can be therapeutically used. First tests showed that agr inhibitors exhibited biological activity in vivo in a mouse protection test (Mayville et al., 1999).

Staphylococcal Accessory Regulator

In *S. aureus*, the production of virulence factors such as cell wall adhesins and exotoxins during the growth cycle has been shown to be under the control of at least two regulator genes, *agr* and staphylococcal accessory regulator (*sar*; Manna et al., 1998). The global agr regulatory system is itself controlled by another regulator, namely the sar regulation system. This regulation system was first identified by Ambrose Cheung and his colleagues (Cheung et al., 1992). The sar locus consists of the 372-bp *sarA* preceded by a triple promoter region interspersed with two smaller open reading frames (ORFs) ORF3 and ORF4 (Bayer et al., 1996). The triple promoter system yields three overlapping sar transcripts, *sarA*, *sarC* and *sarB* of 0.56, 0.8 and 1.2 kb, respectively (Fig. 18). Expression studies with a *sigB* mutant revealed that the P3 promoter is SigB dependent (Manna et al., 1998). The protein sequence of the 14-kDa SarA shares sequence similarity with VirF of *Shigella flexneri* (Cheung and Projan, 1994).

Mutations in *sarA* resulted in a decreased expression of several extracellular (especially α-hemolysin) and cell wall proteins. This can be explained by the fact that transcription of RNAII and RNAIII of the agr system is also decreased.
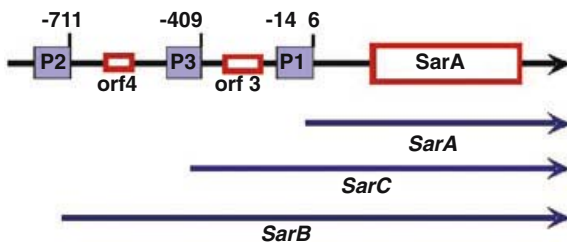
Fig. 18. Staphylococcal accessory regulator (sar) locus. The sar locus consists of the 372-bp *sarA* preceded by a triple promoter region (P1-P3) interspersed with two smaller open reading frames (ORF3 and ORF4; Bayer et al., 1996). The triple promoter system yields three overlapping sar transcripts, *sarA*, *sarC* and *sarB* of 0.56, 0.8 and 1.2 kb, respectively.

The *sarA* mutants can be complemented in part by plasmids expressing SarA, however, a full complementation was only achieved if *sarB* or *sarC* is expressed. These data suggest that ORF3, and to a lesser degree ORF4, may affect *agr* expression by modulating SarA protein expression. SarA binds to the P2 promoter region of *agr*, thus leading to transcription of RNAII and, subsequently, RNAIII, the two major transcripts encoded within the *agr* locus (Chien et al., 1998). The reduction in SarA expression correlated with a lower level of *agr* activation in the corresponding *sar* mutant clone.

However, in addition to controlling target gene expression via agr (e.g., α-hemolysin), the sar locus can also regulate target gene transcription via agr-independent mechanisms. In particular, it was found that SarA binds to conserved sequences homologous to the SarA-binding site on the agr promoter, upstream of the –35 promoter boxes of several target genes including *hla* (α-hemolysin gene), *spa* (protein A gene), *fnb* (fibronectin-binding protein genes), and *sec* (enterotoxin C gene; Chien et al., 1999). Deletion of the SarA recognition motif in the promoter regions of *agr* and *hla* in shuttle plasmids rendered the transcription of these genes undetectable in *agr* and hla mutants, respectively. Likewise, the transcription activity of *spa* (a gene normally repressed by *sar*) became derepressed in a wildtype strain containing a shuttle plasmid in which the SarA recognition site had been deleted from the *spa* promoter region. It is assumed that SarA is a regulatory protein that binds to its consensus recognition motif to activate (e.g., *hla*) or repress (e.g., *spa*) the transcription of *sar* target genes, thus accounting for both *agr*-dependent and *agr*-independent mode of regulation (Chien et al., 1999).

With a number of selected promoter regions, the one under control of SarA was investigated. Of the seven σ(A)-dependent promoters that were tested, SarA repressed transcription from agrP2, agrP3, *cna*, *sarP1*, and *sea* promoters and did not affect *sec* and *znt* promoters. Furthermore, SarA had no effect on transcription from the σ(B)-dependent *sar*P3 promoter. In vitro experimental data presented in this report suggest that SarA expression is autoregulated (Chakrabarti and Misra, 2000). SarA binds to conserved DNA motifs immediately upstream of both positively and negatively regulated promoters.

SarA interacts with a series of heptad repeats (AGTTAAG) within the agr promoter. Subsequent DNA-binding studies revealed that SarA binds readily to multiple AT-rich sequences of variable lengths. Crystal structure analysis of SarA and a SarA-DNA complex brought some insight to bear on the conformational changes

resulting from SarA encasement of DNA. SarA recognizes an AT-rich site in which the DNA is highly overwound and adopts a D-DNA-like conformation by indirect readout (Schumacher et al., 2001).

SarA Protein Family

The staphylococcal genome sequences revealed a number of SarA homologs. In search of additional regulators that could explain the differential effects of RNAIII and SarA, four differently regulated genes (*hla*, α-toxin; *hld*, RNAIII; *spa*, protein A; and *ssp*, serine protease) were analyzed for binding of potential regulatory proteins to the corresponding promoter DNA fragments linked to magnetic beads. One protein (29 kDa), with affinity for all four promoters, showed a high degree of similarity to SarA and was named "SarH1" (Sar homolog 1). Expression of *sarH1* was strongly repressed by *sarA* and *agr*. Further analysis revealed that *sarH1* has a strong repressive effect on *hla* and an activating effect on *spa* transcription (Tegmark et al., 2000). Furthermore, the production of several other exoproteins was affected by *sarH1*.

The level of SarA is partially controlled by the differential activation of sar promoters. With a DNA-specific column containing a sar P2 promoter fragment, a protein was purified which is encoded by *sarR*. SarR is a 13.6-kDa protein with homology to SarA. SarR binds to sar P1, P2, and P3 promoter fragments and a *sarR* mutant expressed a higher level of P1 transcript than the parent. As the P1 transcript is the predominant sar transcript, it was proposed that SarR is a sarA repressor protein that binds to the sar promoters to downregulate P1 transcription and, in consequence, SarA expression (Manna and Cheung, 2001).

The genome sequence revealed another SarA homolog, the 250-amino-acid (aa) SarS. Its gene is upstream of the *spa* gene. The expression of *sarS* was almost undetectable in parental *S. aureus* strain but was highly expressed in agr and *sarA* mutants, strains normally expressing a high level of protein A. Interestingly, protein A expression was decreased in a *sarS* mutant. The enhancement in *spa* expression in an *agr* mutant returned to a near-parental level in the *agr-sarS* double mutant, but not in the *sarA-sarS* double mutant. All the data indicated that *agr* probably mediates *spa* repression by suppressing the transcription of *sarS*, an activator of *spa* expression. However, the pathway by which the *sarA* downregulates *spa* expression is *sarS* independent (Cheung et al., 2001).

Another SarA homolog is the 118-aa long and basic SarT. Expression of *sarT* is repressed by *sarA* and *agr*. In a *sarT* mutant, the RNAIII level was notably increased particularly in the postex-

ponential phase. SarT repressed the expression of *hla* (α-hemolysin) similar to the repression in *agr* and *sarA* mutants. This finding suggests that *sarA*, contrary to the regulatory action of *agr*, induced α-hemolysin production by repressing *sarT*, a repressor of *hla* transcription (Schmidt et al., 2001).

Many of the SarA protein family members are either small basic proteins (<153 residues) or two-domain proteins in which a single domain has sequence similarity with one of the small basic proteins. Because of its structure and unique mode of DNA binding, SarR, and possibly other SarA family members, may belong to a new functional class of the winged-helix family, accommodating a long stretch of DNA with bending points. On the basis of sequence homology, it is hypothesized that the SarA protein family may entail homologous structures with similar DNA-binding motifs but divergent activation domains. An understanding of how these regulators interact with each other in vivo and how they sense environmental signals to control virulence gene expression (e.g., α-hemolysin) will provide more insight in the complex regulatory network (Cheung and Zhang, 2002).

Exoprotein Gene Regulator

In addition to *agr* and *sar*, another exoprotein gene regulator (*sae*) is involved in the expression of extracellular and cell surface proteins. In *sae* mutants, the production of α- and β-hemolysins and coagulase are drastically diminished and to a lesser extent also protein A. The *sae* locus regulates these exoprotein genes at transcriptional level (Giraudo et al., 1997). The *sae* mutation does not affect the expression of *agr* or *sar*. A *sae-agr* double mutant expressed reduced or null levels of α-, β-, and δ-hemolysins, coagulase, and high levels of protein A. The corresponding genes are not transcribed, while *spa* is transcribed at high levels. It is assumed that *sae* and *agr* interact in a complex way in the control of the expression of the genes of several exoproteins. The *sae* locus consists of two genes, designated "*saeR*" and "*saeS*," encoding a typical response regulator and histidine protein kinase, respectively (Giraudo et al., 1999).

In a guinea pig model of device-related infection, the impact of *agr*, *sarA* and *sae* mutants on the induction of *hla* (α-toxin gene) transcription was studied (Goerke et al., 2001). *Staphylococcus aureus* strains RN6390 and Newman expressed considerably smaller amounts of RNAIII in the guinea pig than during in vitro growth. Highest RNAIII and *hla* expression was detected in both strains early in infection, decreased during the course of infection that suggests that it was negatively correlated with

bacterial densities. The *agr* and *sarA* mutants of strains Newman and RN6390 did not affect *hla* expression in vivo, while in *sae* mutants, *hla* is severely downregulated in vitro as well as in vivo. This study suggests that *S. aureus* seems to be provided with regulatory circuits different from those characterized in vitro to ensure α-toxin synthesis during infections (Goerke et al., 2001).

Repressor of Toxins

Recently a gene called "repressor of toxins" (*rot*) was identified in *S. aureus* RN6390 that shows homology with *agrA* and *sarA* (McNamara et al., 2000). In a *rot-agr* double mutant the expression of protease and α-toxin is restored. This phenotype suggests that Rot acts as a transcriptional repressor of *hla*. Whether other genes are controlled by Rot is unknown.

The *arlS-arlR* Two-component System

In a *S. aureus* 8325 derivative, another two-component system, *arlS-arlR*, was identified which is involved in regulation of exoproteins (Fournier and Hooper, 2000a). ArlS is the sensor protein while ArlR represents the response regulator. An *arlS* mutant showed pleiotropic effects. It formed a biofilm on a polystyrene surface unlike the parent strain and the complemented mutant strain. Biofilm formation was associated with increased primary adherence to polystyrene, whereas cellular adhesion was only slightly decreased. In addition, the *arlS* mutant exhibited increased autolysis and altered peptidoglycan hydrolase activity. Another effect of the *arlS* mutant is that the activity of multidrug resistance efflux pump, NorA, is increased (Fournier et al., 2000b). While in the *arlS* or *arlR* mutants, the extracellular proteolytic activity, including serine protease activity, is dramatically decreased (Fournier and Hooper, 2000a), and transcription and production of other secreted proteins such as α- and β-hemolysins, lipase, coagulase, or the cell-wall bound protein A are increased (Fournier et al., 2001). The *arl* operon decreases the production of virulence factors by transcriptional downregulation of the corresponding genes. Since the *arl* mutation did not change *spa* expression in an *agr* or *sarA* mutant, it is suggested that arl acts indirectly on virulence gene expression through *agr* and *sar*. This is supported by the finding that RNAII and RNAIII are decreased by ArlS-ArlR, while *sarA* expression is increased.

Staphylococcal Virulence Regulator

The staphylococcal virulence regulator gene *svrA* was originally identified in *S. aureus* by signature-tagged mutagenesis as necessary for virulence. SvrA is a membrane-associated protein, having two regions with six membrane-spanning domains, separated by an extended hydrophilic loop (Garvis et al., 2002). The *svrA* mutant expressed greatly reduced amounts of α-, β- and δ-toxins and an increased amount of protein A; the regulation occurred at the transcriptional level. As the agr-specific RNAII and RNAIII were absent in the *svrA* mutant, it is assumed that SvrA is, like many other regulators, required for expression of agr.

The SrrA-SrrB Two-component System

It was found that the expression of toxic shock syndrome toxin 1 (TSST-1) by *S. aureus* in liquid culture consumes oxygen. In the course of studies examining the mechanism by which oxygen might regulate toxin production, the *srrAB* (staphylococcal respiratory response) genes were identified. The genes are homologous to the *B. subtilis resDE* genes. ResD-ResE represents a global regulator of aerobic and anaerobic respiratory metabolism in *B. subtilis*. The two-component regulatory system SrrA-SrrB very likely acts in anaerobic repression of staphylococcal virulence factors (Yarwood et al., 2001). In *srrB* mutants, RNAIII synthesis was upregulated while exotoxin TSST-1 synthesis was downregulated under microaerobic conditions and, to a lesser extent, under aerobic conditions as well. At the same time, protein A production was upregulated in microaerobic conditions and decreased in aerobic conditions. Overexpression of *srrAB* resulted in nearly complete repression of TSST-1 production in both microaerobic and aerobic conditions. It is assumed that SrrA-SrrB acts in the global regulation of staphylococcal virulence factors, and may repress virulence factors under low-oxygen conditions. Furthermore, *srrAB* may provide a mechanistic link between respiratory metabolism, environmental signals, and regulation of virulence factors in *S. aureus* (Yarwood et al., 2001). This regulation is mediated in part by agr.

The Alternative Sigma Factor: Sigma B

In *S. aureus*, homologous genes to the *sigB* operon of the stationary-phase σ factor SigB (σB) of *Bacillus subtilis* were identified (Kullik and Giachino, 1997). The *sigB* region contains a total of six ORFs of which orf2, orf3, orf4 and orf5 show 64, 67, 71 and 77% similarity to the *B. subtilis* proteins RsbU, RsbV, RsbW and SigB, respectively, with SigB representing the σ factor and the Rsb proteins representing regulators of SigB. The organization of the *sigB* operon is shown in Fig. 19.
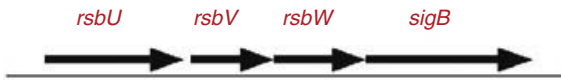
rsbU   rsbV   rsbW   sigB

Fig. 19. Organization and proposed function of the *sigB* operon in staphylococci. SigB is already present in unstressed cells, but it is sequestered by the anti-σ factor RsbW, which has RsbV kinase activity. RsbV is an anti-anti-σ factor, which can bind in its phosphorylated form to RsbW thus causing release of SigB from SigB-RsbW complex. RsbU has phosphatase activity. When RsbU gets activated, P-RsbV is dephosphorylated causing SigB release and interaction with the sigB specific promoters.

Very likely we have in *S. aureus* a similar sigB regulation cascade as described for *B. subtilis* (for review, see Mittenhuber, 2002). In *B. subtilis*, SigB is already present in unstressed cells (e.g., in exponential growth phase), but it is bound and sequestered by the anti-σ factor RsbW and therefore unable to interact with the core-enzyme of RNA polymerase (Hecker and Volker, 1998; Scott et al., 2000). A third protein, the anti-anti σ factor RsbV, which can also bind to RsbW, accomplishes release of SigB from RsbW. The phosphorylation status of RsbV is critical for its binding to RsbW. In its unmodified form, RsbV forms a complex with RsbW, whereas P-RsbV is unable to bind to RsbW that can then form a complex with SigB. This novel regulatory principle was called "partner switching" (Alper et al., 1994). Antagonistic activities of a kinase and two phosphatases control the phosphorylation status of RsbV. In addition, RsbW is also an RsbV kinase, whereas RsbU (phosphatase) dephosphorylates P-RsbV. Depending on the nature of the growth-restricting factor, RsbU is activated after imposition of environmental (physical) stress.

However, in *B. subtilis*, other genes are involved in stress regulation (e.g., *rsbS*, *rsbR*, *rsbT* and *rsbX*) that are not identified in *S. aureus* so far. Therefore, one has to be cautious in the unexamined functional adaptation of the homologous Rsb proteins.

Deletion of *sigB* in *S. aureus* revealed its function as a global regulator of virulence genes (Kullik et al., 1998a). The *sigB* mutants showed reduced pigmentation, accelerated sedimentation, and increased sensitivity to hydrogen peroxide during the stationary growth phase. Furthermore, the cytoplasmic alkaline shock protein 23 and pigmentation were undetectable, while some secreted enzymes such as thermonuclease and lipase were increased in the mutant. The *S. aureus rsbW* encodes an anti-σ factor of SigB and acts as a negative posttranslational regulator (Miyazaki et al., 1999). In an in vitro transcription runoff assay, RsbW prevented SigB-directed transcription from the sarA-P3 promoter, a known SigB-dependent promoter.

To find out the influence of a functional *sigB* on the global regulators sar and agr, studies were carried out in the *sigB*-positive MSSA1112 and Newman strains carrying the wildtype *rsbU* allele (Bischoff et al., 2001). The SigB concentration reached a maximum in the late exponential phase and declined towards the stationary phase when bacteria were grown in Luria-Bertani medium. In *sar*P1-3 reporter fusion studies, a strong SigB and growth phase-dependent increase in *sar* expression that was totally absent in either *rsbU* or *rsbU-sigB* deletion mutants was revealed. In contrast, expression of agr RNAIII is increased in the *sigB* mutant. Thus, SigB increases sar expression while simultaneously reducing the RNAIII level in a growth phase-dependent manner.

Interestingly, most *sigB* deletion phenotypes were only seen in *S. aureus* COL and Newman and not in 8325, which was found to contain an 11-bp deletion in the regulator gene *rsbU* and behaves phenotypically like a *sigB*-defective mutant. Since hydrogen peroxide (H_2O_2) represents an important stress factor for *S. aureus* during infection, it is postulated that *sigB* is important to survive high concentrations of peroxide especially during late stationary phase. The genes that are controlled by SigB (such as *asp23*) contain a SigB-specific promoter consensus sequence.

Critical Remarks

There is no doubt that the accessory gene regulator (agr) and the staphylococcal accessory regulator (sar) are central regulatory elements that control the production of virulence factors. Most of the functions of these global regulators have been defined using *S. aureus* RN6390, which is a representative of the laboratory strain 8325-4. However, RN6390 has a mutation in *rsbU* that results in a phenotype resembling that of a *sigB* mutant (Kullik et al., 1998b). For that reason, it remains unclear whether the regulatory characteristics described for RN6390 are representative for clinical isolates. To address this question, mutations were generated in the *sarA* and *agr* loci of three laboratory strains (RN6390, Newman, and S6C) and four clinical isolates (UAMS-1, UAMS-601, DB, and SC-1) and tested for collagen binding (*cna*-expression). The *sarA* mutants of strains UAMS-1 and UAMS-601 showed an increased capacity to bind collagen, while mutation of agr had little impact. Northern blot analysis confirmed that the increase in collagen binding was due to increased *cna* transcription. Without exception, an increased production

of proteases and a decreased capacity to bind fibronectin was revealed in *sarA* mutants. Mutation of *agr* had the opposite effect. Although *sarA* mutants had slightly reduced *fnbA* transcription, changes in the ability to bind fibronectin appeared to be more directly correlated with changes in protease activity. Lipase production was reduced in both *sarA* and *agr* mutants.

In contrast to the SigB-positive strains, mutation of *sarA* in RN6390 (affected in *rsbU*) resulted in a reduced hemolytic activity. The levels of the *sarC* transcript also seemed to be reduced in RN6390, but there was no difference in the overall pattern of *sarA* transcription or the production of SarA. Taken together, these results suggest that studies defining the regulatory roles of *sarA* and *agr* by using RN6390 are not always representative of the events that occur in clinical isolates of *S. aureus* (Blevins et al., 2002).

Ecology

Members of the genus *Staphylococcus* are widespread in nature and occupy a variety of niches. As a result of their ubiquity and adaptability, staphylococci are a major group of bacteria inhabiting the skin, skin glands, and mucous membranes of humans, other mammals, and birds. A variety of habitats present in the human cutaneous ecosystem can be distinguished by differences in the density and structure of the microbial communities inhabiting them, as well as by their anatomical and physiological properties. For example, skin regions supplied with large numbers of pilosebaceous units, sweat glands, and mucous membranes surrounding openings to the body surface contain the largest populations of staphylococci. In addition, staphylococcal communities may be found living in the follicular canals, the openings to sweat glands, the capacious lumen of sebaceous follicles, and on the surface of and beneath desquamating epithelial scales (Noble and Somerville, 1974; Noble and Pitcher, 1978).

Staphylococci may be found on the skin as residents or transients; as a result, one must use stringent criteria based on temporal studies and population size to estimate residency status and host range (Price, 1938; Noble and Somerville, 1974; Kloos and Musselwhite, 1975a). Resident bacteria are indigenous to the host, maintain relatively stable populations, and increase in numbers mainly by multiplication of those already present. Transient bacteria are derived from exogenous sources, found primarily on exposed skin, and may be easily washed away. Cross-contamination of staphylococci can occur readily where different host species come in contact with one another, but where host specificity is high, the transient organisms will usually be eliminated within several hours or days, unless the normal defense barriers are compromised. Ideally, determination of natural host range should be made with host species that are relatively isolated in nature. Most of the ecological studies reported fall short of this ideal situation, but a few have indicated some clear patterns of host and niche preferences for certain *Staphylococcus* species.

Staphylococcus epidermidis (Schleifer and Kloos, 1975b) is the most prevalent and persistent *Staphylococcus* species on human skin. It is found over much of the body surface and produces the largest populations where moisture content and nutrition are high, such as in the anterior nares, axillae, inguinal and perineal area, and toe webs. This species may be found occasionally on other hosts, such as domestic animals, but it is presumably transferred there from human sources. *Staphylococcus hominis* (Kloos and Schleifer, 1975b) is also prevalent on human skin. Its population size is usually second or equal to *S. epidermidis* on skin sites where apocrine glands are numerous (e.g., in the axillae and inguinal and perineal areas). It can also colonize the drier regions of skin (e.g., on the extremities) more successfully than other species. *Staphylococcus haemolyticus* (Schleifer and Kloos, 1975c) shares many of the habitats of *S. hominis*, but it is usually found in smaller populations. Some individuals may carry unusually large populations of *S. haemolyticus*. A different subspecies of *S. haemolyticus* found living on nonhuman primate skin (e.g., *Pan*, *Macaca*, *Cercocebus*, *Erythrocebus*, *Microcebus* and *Lemur* skin) can be distinguished from the human-adapted subspecies on the basis of DNA-DNA hybridization (Kloos and Wolfshohl, 1979). Since it is difficult to distinguish colonies of the nonhuman primate *S. haemolyticus* subspecies from a sibling species, provisionally designated "*S. simians*," found also on nonhuman primate skin, adequate enumeration of this subspecies is not yet possible. *Staphylococcus warneri* (Kloos and Schleifer, 1975b) is found usually in small numbers on human skin, though a few individuals may carry unusually large populations. *Staphylococcus warneri* is a major species on nonhuman primates, especially on the more advanced Cercopithecoidea and Pongidae. Occasionally, small transient populations of *S. haemolyticus* or *S. warneri* may be isolated from domestic animals. *Staphylococcus capitis* (Kloos and Schleifer, 1975b) produces large populations on the human scalp following puberty. It is also found on other regions of the adult head, e.g., forehead, face, eyebrows, and external auditory

meatus in moderate-sized to large populations. The largest populations are found in areas where sebaceous glands are numerous and well developed. *Staphylococcus capitis* subsp. *ureolyticus* is present on regions of the head in rather small populations and, like *S. capitis*, may be found only occasionally on other body sites (Bannerman and Kloos, 1991). This subspecies has been isolated from both human and nonhuman primate skin (e.g., *Pan* skin).

Staphylococcus caprae, originally isolated from the skin of domestic goats or in their milk (Devriese et al., 1983; Poutrel, 1984), has been isolated from human clinical specimens (Vandenesch et al., 1995; Kawamura et al., 1998). *Staphylococcus hominis* subsp. *novobiosepticus*, *S. lugdunensis*, *S. pasteuri*, *S. schleiferi* are other clinically significant species isolated from human specimens (Freney et al., 1988; Chesneau et al., 1993; Kloos et al., 1998b). Their original niche preference and prevalence is undetermined.

Staphylococcus auricularis is one of the major species found living in the adult human, external auditory meatus and demonstrates a strong preference for this niche (Kloos and Schleifer, 1983a). A different subspecies of *S. auricularis* is found in the ear and specialized scent (or marking) glands of nonhuman primates (e.g., *Pan*, *Pongo*, *Cercopithecus*, *Lemur*, *Galago* and *Microcebus*; Kloos, 1985; Kloos and Schleifer, 1986).

Staphylococcus aureus is a major species of primates, though specific ecovars or biotypes can be found occasionally living on different domestic animals or birds (Meyer, 1967; Kloos, 1980). This species is found infrequently on nonprimate wild animals. In humans, *S. aureus* has a niche preference for the anterior nares, especially in the adult. Here it can exist as a resident or as a transient member of the normal flora. *Staphylococcus aureus* selectively adheres to nasal epithelial (mucosal) cells (Aly et al., 1981). Nasal carrier rates range from less than 10% to more than 40% in normal adult human populations residing outside of the hospital (Noble and Somerville, 1974). The nasal adherence of *S. aureus* is significantly greater for carriers of this species than for noncarriers. *Staphylococcus aureus* subsp. *anaerobius* is found living on sheep (De la Fuente et al., 1985).

The host range of *Staphylococcus saprophyticus* and similar species varies from humans to lower mammals and birds (Kloos, 1980; Devriese, 1986). As a group, these staphylococci are most prevalent on lower primates and mammals. Those species found most frequently on primates include *S. saprophyticus*, *S. cohnii* and *S. xylosus*. *Staphylococcus saprophyticus* is found usually in small, transient populations on the skin of humans or other primates. This species possesses surface properties that allow it to adhere readily to urogenital cells (Colleen et al., 1979). It may also be isolated from lower mammals and environmental sources. *Staphylococcus saprophyticus* subsp. *bovis* is found in the anterior nares of cows (Hájek et al., 1996). *Staphylococcus cohnii* (Schleifer and Kloos, 1975c) is found as a temporary resident or transient on human skin, and *S. cohnii* subsp. *urealyticus* (Kloos and Wolfshohl, 1991) is sometimes found on human skin, but it is often one of the major species and subspecies of nonhuman primates, especially the lower primates. The largest populations of subspecies are found living on the Tupaiidae, Prosimii, and Ceboidea (Kloos and Wolfshohl, 1983b). A third subspecies is also found on the Ceboidea. *Staphylococcus xylosus* (Schleifer and Kloos, 1975c) is often found as a transient on the skin of lower primates and other mammals, and occasionally on birds (Kloos et al., 1976b; Akatov et al., 1985; Devriese et al., 1985). The related species *S. kloosii* has been found living on a variety of lower mammals including wild marsupials, rodents and carnivores, and less frequently on domestic animals (Schleifer et al., 1984; Kloos, 1980). *Staphylococcus arlettae* has been isolated from poultry and goats, *S. equorum* from horses, and *S. gallinarum* from poultry (Devriese et al., 1983; Schleifer et al., 1984).

Staphylococcus intermedius is a major species of the domestic dog (Hájek and Marsalek, 1976b; Krogh and Kristensen, 1976). This species can be found in relatively large populations on canine skin and can on occasion be transferred to the skin of human handlers (Kloos et al., 1976b). *Staphylococcus intermedius* appears to be also indigenous to a variety of other carnivores, including the mink (*Mustela*; Hájek et al., 1972; Oeding et al., 1973; Hájek, 1976a), fox (*Vulpes*; Hájek, 1976a), and raccoon (*Procyon*; Kloos et al., 1976b). It has also been isolated from horses and pigeons (Hájek and Marsalek, 1971; Hájek, 1976a). *Staphylococcus felis* is one of the major species of the domestic cat (Igimi et al., 1989). *Staphylococcus schleiferi* subsp. *coagulans* is a coagulase-positive species, which has been isolated from the external auditory meatus of dogs with ear infections (Igimi et al., 1990). Other coagulase-positive species isolated from animals include *S. delphini* (Varaldo et al., 1988) and *S. lutrae* (Foster et al., 1997). *Staphylococcus sciuri*, *S. sciuri* subsp. *carnaticus* and *S. sciuri* subsp. *rodentium* have been isolated from a variety of lower mammals and domestic animals (Kloos et al., 1976a; Kloos et al., 1997). In addition, the *S. sciuri* subspecies may be isolated from human clinical specimens (Marsou et al.,

1999). *Staphylococcus scuiri* appears to be a natural reservoir of methicillin resistance and staphylolytic enzyme genes (Kloos et al., 1997). Their role, if any, in the appearance of methicillin-resistance in the more pathogenic species *S. aureus* has not yet been determined. *Staphylococcus hyicus* and *S. chromogenes* are found predominantly on domestic ungulates such as pigs, cattle and horses (Devriese et al., 1978; Devriese, 1979a; Devriese, 1986; Phillips et al., 1980; Hájek et al., 1986). *Staphylococcus lentus* (Schleifer et al., 1983) has been isolated in large populations from domestic sheep and goats (Kloos et al., 1976a; Kloos et al., 1976b), occasionally from other domestic animals (Devriese et al., 1985), and is a major bacterium in saliva of rabbits (Kanda et al., 2001). Members of the newly described genus *Macrococcus* are found primarily on domestic animals (Kloos et al., 1998a).

Staphylococci have been isolated sporadically from a wide variety of environmental sources such as soil, beach sand, seawater, fresh water, plant surfaces and products, feeds, meat and poultry, dairy products, and on the surfaces of cooking ware, utensils, furniture, clothing, blankets, carpets, linens, paper currency, and dust and air in various inhabited areas. *Staphylococcus carnosus*, *S. carnosus* subsp. *utilis*, *S. condimenti*, *S. fleurettii*, *S. piscifermentans* and *S. vitulus* have been isolated from various food products (Schleifer and Fischer, 1982; Tanasupawat et al., 1992; Webster et al., 1994; Probst et al., 1998; Vernozy-Rozand et al., 2000). Some of the staphylococci may be involved in food poisoning and spoilage or in desirable food fermentation. Recently, a staphylococcal species from Dominican amber, *S. succinus*, has been described (Lambert et al., 1998).

With the exception of the animal products, most environmental sources contain small, transient populations of staphylococci, many of which are probably contaminants disseminated by human, animal, or bird host carriers. Possibly, certain species (e.g., *S. sciuri* and *S. xylosus*) can grow in habitats containing only an inorganic nitrogen source, and thus might be more free-living than other staphylococci (Emmett and Kloos, 1975; Emmett and Kloos, 1979). These species have been isolated in small numbers from beach sand, natural waters, and marsh grass (Kloos and Schleifer, 1981) and also from plant products (Bucher et al., 1980; Pioch et al., 1988). Flies of the genera *Musca*, *Fannia* and *Stomoxys*, commonly found in human and animal habitations, can carry populations of staphylococci (e.g., *S. muscae*; Hájek et al., 1992) and appear to be significant vectors of these organisms in an epizootiological chain (Hájek and Balusek, 1985).

Opportunistic Pathogens

The coagulase-positive species *S. aureus*, *S. intermedius*, *S. delphini*, *S. schleiferi* subsp. *coagulans* and the coagulase-variable species *S. hyicus* are regarded as potentially serious pathogens. *Staphylococcus aureus*, since its early discovery as an opportunistic pathogen, continues to be a major cause of mortality and is responsible for a variety of infections. In the late 1950s and early 1960s, *S. aureus* caused considerable morbidity and mortality as a nosocomial pathogen. Among the major human infections caused by this species are furuncles, carbuncles, impetigo, toxic epidermal necrolysis (scalded skin syndrome), pneumonia, osteomyelitis, acute endocarditis, myocarditis, pericarditis, enterocolitis, mastitis, cystitis, prostatitis, cervicitis, cerebritis, meningitis, bacteremia, toxic shock syndrome, and abscesses of the muscle, skin, urogenital tract, central nervous system, and various intraabdominal organs. In addition, staphylococcal enterotoxin is involved in food poisoning. Methicillin-resistant *S. aureus* (MRSA) strains have emerged in the 1980s as a major clinical and epidemiological problem in hospitals. These strains are beginning to spread out of the hospitals and into communities. The origin and future significance of these isolates to the community has yet to be determined.

Staphylococcus aureus can also infect a variety of other mammals and birds. The more common natural infections include mastitis, synovitis, arthritis, endometritis, furuncles, suppurative dermatitis, pyemia, and septicemia. Staphylococcal mastitis in either a clinical or subclinical form may have considerable economic consequences in the dairy industry. *Staphylococcus aureus* subsp. *anaerobius* is the etiologic agent of an abscess disease in sheep, symptomatically similar to caseous lymphadenitis (De la Fuente et al., 1985). Hagan and Bruner's *Microbiology and Infectious Diseases of Domestic Animals* (Timoney et al., 1988) may be referenced for further information on the nature of *S. aureus* infections in animals.

Staphylococcus intermedius is a serious opportunistic pathogen of dogs and may cause otitis externa, pyoderma, abscesses, reproductive tract infections, mastitis, and purulent wound infections. *Staphylococcus hyicus* has been implicated as the etiologic agent of infectious exudative epidermitis (greasy pig disease) and septic polyarthritis of pigs, skin lesions in cattle and horses, osteomyelitis in poultry and cattle, and occasionally associated with mastitis in cattle. *Staphylococcus delphini* has been implicated in purulent skin lesions of dolphins (Varaldo et al., 1988). *Staphylococcus schleiferi* subsp. *coagulans* is

associated with external auditory meatus of dogs (Igimi et al., 1990).

Although the coagulase-negative staphylococcal species constitute a major component of the normal microflora in humans, their role (especially that of *S. epidermidis*) in causing nosocomial infections has been recognized and well documented over the last two decades. The increase in infections by these organisms has been correlated with the wide medical use of prosthetic and indwelling devices and the growing number of immunocompromised patients in hospitals. Infectious processes may result from the introduction of endogenous staphylococci beyond the normal integumentary barriers. *Staphylococcus epidermidis* appears to have the greatest pathogenic potential and adaptive diversity. This species has been implicated in bacteremia, native and prosthetic valve endocarditis, osteomyelitis, pyoarthritis, peritonitis during continuous ambulatory dialysis, mediastinitis, infections of permanent pacemakers, vascular grafts, cerebrospinal fluid shunts, prosthetic joints, and a variety of orthopedic devices, and urinary tract infections including cystitis, urethritis, and pyelonephritis. Recent reviews have been published on the nature of human infections caused by *S. epidermidis* and other coagulase-negative species (Kloos and Bannerman, 1994; Rupp and Archer, 1994; Crossley and Archer, 1997). Nosocomial methicillin-resistant *S. epidermidis* (MRSE) strains became a serious clinical problem in the 1980s, especially in patients with prosthetic heart valves or who had undergone other forms of cardiac surgery (Archer and Tenenbaum, 1980; Karchmer et al., 1983). *Staphylococcus epidermidis* has also been occasionally associated with mastitis in cattle (Baba et al., 1980; Devriese and De Keyser, 1980; Holmberg, 1986).

Certain other coagulase-negative species have been associated with infections in humans and animals. *Staphylococcus haemolyticus* is the second most frequently encountered species of this group found in human clinical infections. It has been implicated in native valve endocarditis, septicemia, peritonitis, and urinary tract infections, and is occasionally associated with wound, bone, and joint infections. *Staphylococcus haemolyticus* has been occasionally associated with mastitis in cattle (Baba et al., 1980). *Staphylococcus caprae*, previously misidentified as *S. haemolyticus*, *S. hominis* and *S. warneri*, is widely distributed in human clinical specimens (Kawamura et al., 1998) and has been implicated in cases of infective endocarditis, bacteremia, and urinary tract infections. *Staphylococcus lugdunensis* has been implicated in native and prosthetic valve endocarditis, septicemia, brain abscess, and chronic osteoarthritis

and infections of soft tissues, bone, peritoneal fluid, and catheters, especially in patients with underlying diseases. *Staphylococcus schleiferi* has been implicated in human brain empyema, osteoarithritis, bacteremia, wound infections, and infections associated with a cranial drain and jugular catheter. This species occurs less frequently than *S. lugdunensis* in the hospital environment and human infections. *Staphylococcus saprophyticus* is an important opportunistic pathogen in human urinary tract infections, especially in young, sexually active females. It is considered to be the second most common cause of urinary tract infections, such as acute cystitis or pyelonephritis, in these patients. This species can also produce in men urinary tract infections, which (unlike those in women) occur most commonly in the elderly with predisposing diseases of the urinary tract (Marrie et al., 1982a; Hovelius et al., 1984; Hovelius, 1986). *Staphylococcus saprophyticus* has occasionally been isolated from wound infections and septicemia (Marsik and Brake, 1982; Fleurette et al., 1987).

Several other coagulase-negative species have been implicated at low incidence in a variety of human infections. In most cases, patients with these infections had predisposing or underlying diseases that drastically altered their immune systems, and had also experienced surgery or intravascular manipulations. *Staphylococcus warneri* has been on occasion the etiologic agent of vertebral osteomyelitis, native valve endocarditis, and urinary tract infections in males and females. This species has been associated with mastitis in cattle (Devriese and Derycke, 1979b; Devriese and De Keyser, 1980). *Staphylococcus simulans* has been associated with human chronic osteomyelitis, pyarthrosis, and bovine mastitis. *Staphylococcus felis*, a relative of *S. simulans*, has been isolated from clinical infections in cats, including external ear otitis, cystitis, abscesses, wounds, and other skin infections (Igimi et al., 1989). *Staphylococcus capitis* has been implicated in endocarditis, septicemia, and catheter infections. *Staphylococcus hominis* has been associated with human endocarditis, peritonitis, septicemia, and arthritis. Some of the earlier reports indicating an association of this species with infections were in error, owing to the misidentification of phosphatase-negative strains of *S. epidermidis* as *S. hominis*. *Staphylococcus cohnii* has been associated with urinary tract infections and arthritis. *Staphylococcus chromogenes*, a close relative of *S. hyicus*, is commonly isolated from the milk of cows suffering from mastitis, although its role as an etiologic agent is questionable (Devriese and De Keyser, 1980; Langlois et al., 1983; Watts et al., 1984). *Staphylococcus sciuri* subspecies has been iso-

lated from wound, skin, and soft tissue infections (Marsou et al., 1999).

Members of the *Macrococcus* genus constitute a portion of the normal microflora of cattle, horses and ponies (Kloos et al., 1998a). *Macrococcus caseolyticus* has been isolated from abscesses of slaughtered lambs (de la Fuente et al., 1992).

Applications

It has been known that Gram-positive and catalase-positive cocci play an important role in the ripening process of dry sausages (Lerche and Sinell, 1955; Niinivaara and Pohja, 1956). The predominant microorganism in fermented meat is *S. carnosus*, appearing in the early literature as *Micrococcus* (Schleifer and Fischer, 1982). For more than 50 years, *S. carnosus* has been used alone or in combination with lactobacilli or pediococci, as a starter culture for the production of raw fermented sausages. One of the main advantages of starter cultures in fermented food processing is that the fermentation and ripening process can be carried out under controlled conditions. In this way, food-poisoning and food-spoilage microorganisms can be suppressed, and the course of the fermentation process and its termination more reliably monitored. During the ripening process of dry sausage, *S. carnosus* exerts several desired functions (Liepe and Porobic, 1983; Götz, 1990). First, *S. carnosus* gradually reduces nitrate to nitrite and nitrite to ammonia. The advantage of this reaction is that the nitrate concentration is lowered, and that nitrite can combine with myoglobin to form nitrosomyoglobin, which gives the typical red color. As outlined above, nitrite is further reduced to the ammonia thus lowering the unbound nitrite concentration (Neubauer and Götz, 1996). Other advantages are development of characteristic flavor, lowering the pH moderately, and the capacity to reduce hydrogen peroxide produced by the catalase-negative lactobacilli, thus preventing odors.

Another application of *S. carnosus* is as an alternative host organism for the production of heterologous proteins or hormones. Transformation systems (Götz and Schumacher, 1987; Augustin and Götz, 1990) have been worked out and useful plasmid vectors have been constructed (Wieland et al., 1995; Peschel et al., 1996). With the aid of the lipase propeptide, an efficient secretion system has been worked out (Götz and Rosenstein, 2001b), which has been used successfully for secretion of heterologous proteins such as the human growth hormone (Sturmfels et al., 2001).

Literature Cited

Abdelnour, A., S. Arvidson, T. Bremell, C. Ryden, and A. Tarkowski. 1993. The accessory gene regulator (agr) controls Staphylococcus aureus virulence in a murine arthritis model. Infect. Immun. 61:3879–3885.

Adam, A., F. Ellouz, R. Ciorbaru, J. F. Petit, and E. Lederer. 1975. Peptidoglycan adjuvants: Minimal structure required for activity. Z. Immunitatsforsch. Exp. Klin. Immunol. 149:341–348.

Akatov, A. K., V. Hájek, T. M. Samsonova, and J. Balusek. 1985. Classification and drug resistance of coagulase-negative staphylococci isolated from wild birds. *In:* J. Jeljaszewicz (Ed.) The Staphylococci: Proceedings of the 5th International Symposium on Staphylococci and Staphylococcal Infections. Gustav-Fischer Verlag. Stuttgart, Germany. 125–127.

Alper, S., L. Duncan, and R. Losick. 1994. An adenosine nucleotide switch controlling the activity of a cell type-specific transcription factor in B. subtilis. Cell 77:195–205.

Aly, R., H. R. Shinefield, and H. I. Maibach. 1981. Staphylococcus aureus adherence to nasal epithelial cells: Studies of some parameters. *In:* H. I. Maibach and R. Aly (Eds.) Skin Microbiology: Relevance to Clinical Infection. Springer-Verlag. New York, NY. 171–179.

Anderson, J. S., M. Matsuhashi, M. A. Haskin, and J. L. Strominger. 1967. Biosynthesis of the peptidoglycan of bacterial cell walls. II: Phospholipid carriers in the reaction sequence. J. Biol. Chem. 242:3180–3190.

Archer, G. L., and M. J. Tenenbaum. 1980. Antibiotic-resistant Staphylococcus epidermidis in patients undergoing cardiac surgery. Antimicrob. Agents Chemother. 17:269–272.

Archibald, A. R., J. Baddiley, and S. Heptinstall. 1973. The alanine ester content and magnesium binding capacity of walls of Staphylococcus aureus H grown at different pH values. Biochim. Biophys. Acta 291:629–634.

Atanassova, V., A. Meindl, and C. Ring. 2001. Prevalence of Staphylococcus aureus and staphylococcal enterotoxins in raw pork and uncooked smoked ham: A comparison of classical culturing detection and RFLP-PCR. Int. J. Food Microbiol. 68:105–113.

Augustin, J., and F. Götz. 1990. Transformation of Staphylococcus epidermidis and other staphylococcal species with DNA by electroporation. FEMS Microbiol. Lett. 66:203–208.

Ayora, S., and F. Götz. 1994a. Genetic and biochemical properties of an extracellular neutral metalloprotease from Staphylococcus hyicus subsp. hyicus. Molec. Gen. Genet. 242:421–430.

Ayora, S., P. E. Lindgren, and F. Götz. 1994b. Biochemical properties of a novel metalloprotease from Staphylococcus hyicus subsp. hyicus involved in extracellular lipase processing. J. Bacteriol. 176:3218–3223.

Baba, E., T. Fukata, and H. Matsumoto. 1980. Ecological studies on coagulase-negative staphylococci in and around udder. Bull. Univ. Osaka Pref. Ser. B 32:70–75.

Baddiley, J. 1972. Teichoic acids in cell walls and membranes of bacteria. Essays Biochem. 8:35–77.

Baker, J. S. 1984. Comparison of various methods for differentiation of staphylococci and micrococci. J. Clin. Microbiol. 19:875–879.

Bannerman, T. L., and W. E. Kloos. 1991. Staphylococcus capitis subsp. ureolyticus subsp. nov. from human strain. Int. J. Syst. Bacteriol. 41:144–147.

Bassias, J., and R. Brückner. 1998. Regulation of lactose utilization genes in Staphylococcus xylosus. J. Bacteriol. 180:2273–2279.

Bayer, M. G., J. H. Heinrichs, and A. L. Cheung. 1996. The molecular architecture of the sar locus in Staphylococcus aureus. J. Bacteriol. 178:4563–4570.

Berger-Bächi, B. 1994. Expression of resistance to methicillin. Trends Microbiol. 2:389–393.

Beyreuther, K., H. Raufuss, O. Schrecker, and W. Hengstenberg. 1977. The phosphoenolpyruvate-dependent phosphotransferase system of Staphylococcus aureus. I: Amino-acid sequence of the phosphocarrier protein HPr. Eur. J. Biochem. 75:275–286.

Bischoff, M., J. M. Entenza, and P. Giachino. 2001. Influence of a functional sigB operon on the global regulators sar and agr in Staphylococcus aureus. J. Bacteriol. 183:5171–5179.

Bissett, D. L., and R. L. Anderson. 1973. Lactose and D-galactose metabolism in Staphylococcus aureus: Pathway of D-galactose 6-phosphate degradation. Biochem. Biophys. Res. Commun. 52:641–647.

Bissett, D. L., and R. L. Anderson. 1974. Lactose and D-galactose metabolism in group N streptococci: Presence of enzymes for both the D-galactose 1-phosphate and D-tagatose 6-phosphate pathways. J. Bacteriol. 117:318–320.

Björklind, A., and S. Arvidson. 1980. Mutants of Staphylococcus aureus affected in the regulation of exoprotein synthesis. FEMS Microbiol. Lett. 7:202–206.

Blasco, F., C. Iobbi, G. Giordano, M. Chippaux, and V. Bonnefoy. 1989. Nitrate reductase of Escherichia coli: Completion of the nucleotide sequence of the nar operon and reassessment of the role of the α and β subunits in iron binding and electron transfer. Molec. Gen. Genet. 218:249–256.

Blevins, J. S., K. E. Beenken, M. O. Elasri, B. K. Hurlburt, and M. S. Smeltzer. 2002. Strain-dependent differences in the regulatory roles of sarA and agr in Staphylococcus aureus. Infect. Immun. 70:470–480.

Blumenthal, H. J. 1972. Glucose catabolism in staphylococci. *In:* J. O. Cohen (Ed.) The Staphylococci. Wiley Interscience. New York, NY. 111–135.

Boden, M. K., and J.-I. Flock. 1989. Fibrinogen-binding protein/clumping factor from Staphylococcus aureus. Infect. Immun. 57:2358–2363.

Boonstra, J., and W. N. Konings. 1977. Generation of an electrochemical proton gradient by nitrate respiration in membrane vesicles from anaerobically grown Escherichia coli. Eur. J. Biochem. 78:361–368.

Boothby, J., C. Genigeorgis, and M. J. Fanelli. 1979. Tandem coagulase/thermonuclease agar method for the detection of Staphylococcus aureus. Appl. Environ. Microbiol. 37:298–302.

Bracha, R., and L. Glaser. 1976. In vitro system for the synthesis of teichoic acid linked to peptidoglycan. J. Bacteriol. 125:872–879.

Bracha, R., R. Davidson, and D. Mirelman. 1978. Defect in biosynthesis of the linkage unit between peptidoglycan and teichoic acid in a bacteriophage-resistant mutant of Staphylococcus aureus. J. Bacteriol. 134:412–417.

Breidt Jr., F., and G. C. Stewart. 1986. Cloning and expression of the phospho-beta-galactosidase gene of Staphylococcus aureus in Escherichia coli. J. Bacteriol. 166:1061–1066.

Breidt Jr., F., W. Hengstenberg, U. Finkeldei, and G. C. Stewart. 1987. Identification of the genes for the lactose-specific components of the phosphotransferase system in the lac operon of Staphylococcus aureus. J. Biol. Chem. 262:16444–16449.

Brückner, R., E. Wagner, and F. Götz. 1993. Characterization of a sucrase gene from Staphylococcus xylosus. J. Bacteriol. 175:851–857.

Brückner, R. 1997. Gene replacement in Staphylococcus carnosus and Staphylococcus xylosus. FEMS Microbiol. Lett. 151:1–8.

Bucher, E., G. Beck, and K.-H. Schleifer. 1980. Vorkommen und Verteilung von Staphylokokken und Mikrokokken in Sojaextraktionsschoten. Zentralbl. Bakteriol. Hyg. Abt. 1. Orig. C 1:320–329.

Centers for Disease Control, and Prevention. 2002. Vancomycin Resistant Staphylococcus aureus. CDC. Pennsylvania, PA. 2116.

Chakrabarti, S. K., and T. K. Misra. 2000. SarA represses agr operon expression in a purified in vitro Staphylococcus aureus transcription system. J. Bacteriol. 182:5893–5897.

Chatterjee, A. N., W. Wong, F. E. Young, and R. W. Gilpin. 1976. Isolation and characterization of a mutant of Staphylococcus aureus deficient in autolytic activity. J. Bacteriol. 125:961–967.

Chesneau, O., A. Morvan, F. Grimont, H. Labischinski, and N. el Solh. 1993. Staphylococcus pasteuri sp. nov., isolated from human, animal, and food specimens. Int. J. Syst. Bacteriol. 43:237–244.

Cheung, K. S., S. A. Wasserman, E. Dudek, S. A. Lerner, and M. Johnston. 1983. Chloralanyl and propargylglycyl dipeptides: Suicide substrate containing antibacterials. J. Med. Chem. 26:1733–1741.

Cheung, A. L., J. M. Koomey, C. A. Butler, S. J. Projan, and V. A. Fischetti. 1992. Regulation of exoprotein expression in Staphylococcus aureus by a locus (sar) distinct from agr. Proc. Natl. Acad. Sci. USA 89:6462–6466.

Cheung, A. L., and S. J. Projan. 1994. Cloning and sequencing of sarA of Staphylococcus aureus, a gene required for the expression of agr. J. Bacteriol. 176:4168–4172.

Cheung, A. L., K. Schmidt, B. Bateman, and A. C. Manna. 2001. SarS, a SarA homolog repressible by agr, is an activator of protein A synthesis in Staphylococcus aureus. Infect. Immun. 69:2448–2455.

Cheung, A. L., and G. Zhang. 2002. Global regulation of virulence determinants in Staphylococcus aureus by the SarA protein family. Front. Biosci. 7:1825–1842.

Chien, Y., A. C. Manna, and A. L. Cheung. 1998. SarA level is a determinant of agr activation in Staphylococcus aureus. Molec. Microbiol. 30:991–1001.

Chien, Y., A. C. Manna, S. J. Projan, and A. L. Cheung. 1999. SarA, a global regulator of virulence determinants in Staphylococcus aureus, binds to a conserved motif essential for sar-dependent gene regulation. J. Biol. Chem. 274:37169–37176.

Christensen, G. D., W. A. Simpson, A. L. Bisno, and E. H. Beachey. 1982. Adherence of slime-producing strains of Staphylococcus epidermidis to smooth surfaces. Infect. Immun. 37:318–326.

Christiansen, I., and W. Hengstenberg. 1996. Cloning and sequencing of two genes from Staphylococcus carnosus

coding for glucose-specific PTS and their expression in Escherichia coli K-12. Molec. Gen. Genet. 250:375–379.

Christiansen, I., and W. Hengstenberg. 1999. Staphylococcal phosphoenolpyruvate-dependent phosphotransferase system: Two highly similar glucose permeases in Staphylococcus carnosus with different glucoside specificity—protein engineering in vivo?. Microbiology 145:2881–2889.

Clesceri, L. S., A. E. Greenberg, and A. D. Eaton. 1998. Standard Methods for the Examination of Water and Wastewater. American Public Health Association. Washington, DC.

Colleen, S., B. Hovelius, A. Wieslander, and P. A. Mardh. 1979. Surface properties of Staphylococcus saprophyticus and Staphylococcus epidermidis as studied by adherence tests and two-polymer, aqueous phase systems. Acta Pathol. Microbiol. Scand. B 87:321–328.

Collins, F. M., and J. Lascelles. 1962. The effect of growth conditions on oxidation and dehydrogenase activity in Staphylococcus aureus. J. Gen. Microbiol. 29:531–535.

Collins, M. D., and D. Jones. 1981. Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implication. Microbiol. Rev. 45:316–354.

Collins, L. V., S. A. Kristian, C. Weidenmaier, M. Faigle, K. P. Van Kessel, J. A. Van Strijp, F. Götz, B. Neumeister, and A. Peschel. 2002. Staphylococcus aureus strains lacking D-alanine modifications of teichoic acids are highly susceptible to human neutrophil killing and are virulence attenuated in mice. J. Infect. Dis. 186:214–219.

Cramton, S. E., N. F. Schnell, F. Götz, and R. Brückner. 2000. Identification of a new repetitive element in Staphylococcus aureus. Infect. Immun. 68:2344–2348.

Cramton, S. E., M. Ulrich, F. Götz, and G. Döring. 2001. Anaerobic conditions induce expression of polysaccharide intercellular adhesin in Staphylococcus aureus and Staphylococcus epidermidis. Infect. Immun. 69:4079–4085.

Crisley, F. D., R. Angelotti, and M. J. Foter. 1964. Multiplication of Staphylococcus aureus in synthetic cream fillings and pies. Pub. Health Rep. 79:369–376.

Crossley, K. B., and G. L. Archer. 1997. The Staphylococci in Human Disease. Churchill Livingston. New York, NY.

Cucarella, C., C. Solano, J. Valle, B. Amorena, I. Lasa, and J. R. Penades. 2001. Bap, a Staphylococcus aureus surface protein involved in biofilm formation. J. Bacteriol. 183:2888–2896.

de Jonge, B. L., Y. S. Chang, D. Gage, and A. Tomasz. 1992. Peptidoglycan composition of a highly methicillin-resistant Staphylococcus aureus strain: The role of penicillin binding protein 2A. J. Biol. Chem. 267:11248–11254.

de la Fuente, R., G. Suarez, and K. H. Schleifer. 1985. Staphylococcus aureus subsp. anaerobius subsp. nov., the causal agent of abscess disease in sheep. Int. J. Syst. Bacteriol. 35:99–102.

de la Fuente, R., G. Suarez, J. A. Ruiz Santa Quiteria, H. Meugnier, M. Bes, J. Freney, and J. Fleurette. 1992. Identification of coagulase negative staphylococci isolated from lambs as Staphylococcus caseolyticus. Comp. Immunol. Microbiol. Infect. Dis. 15:47–52.

Demleitner, G., and F. Götz. 1994. Evidence for importance of the Staphylococcus hyicus lipase pro-peptide in lipase secretion, stability and activity. FEMS Microbiol. Lett. 121:189–197.

Devriese, L. A., V. Hájek, P. Oeding, S. A. Meyer, and K.-H. Schleifer. 1978. Staphylococcus hyicus (Sompolinsky 1953) comb. nov. and Staphylococcus hyicus subsp. chromogenes subsp. nov. Int. J. Syst. Bacteriol. 28:482–490.

Devriese, L. A. 1979a. Identification of clumping-factor-negative staphylococci isolated from cows' udders. Res. Vet. Sci. 27:313–320.

Devriese, L. A., and J. Derycke. 1979b. Staphylococcus hyicus in cattle. Res. Vet. Sci. 26:356–358.

Devriese, L. A., and H. De Keyser. 1980. Prevalence of different species of coagulase-negative staphylococci on teats and in milk samples from dairy cows. J. Dairy Res. 47:155–158.

Devriese, L. A., B. Poutrel, R. Kilpper-Bälz, and K.-H. Schleifer. 1983. Staphylococcus gallinarum and Staphylococcus caprae, two new species from animals. Int. J. Syst. Bacteriol. 33:480–486.

Devriese, L. A., K.-H. Schleifer, and G. O. Adegoke. 1985. Identification of coagulase-negative staphylococci from farm animals. J. Appl. Bacteriol. 58:45–55.

Devriese, L. A. 1986. Coagulase-negative staphylococci in animals. In: P.-A. Mardh and K.-H. Schleifer (Eds.) Coagulase-negative Staphylococci. Almquist and Wiksell International. Stockholm, Sweden. 51–57.

Dowell, P., Z. Affas, C. Reynolds, M. T. Holden, S. J. Wood, S. Saint, A. Cockayne, P. J. Hill, C. E. Dodd, B. W. Bycroft, W. C. Chan, and P. Williams. 2001. Structure, activity and evolution of the group I thiolactone peptide quorum-sensing system of Staphylococcus aureus. Molec. Microbiol. 41:503–512.

Downes, F. P., and K. Ito. 2001. Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association.

Dubourdieu, M., and J. A. DeMoss. 1992. The narJ gene product is required for biogenesis of respiratory nitrate reductase in Escherichia coli. J. Bacteriol. 174:867–872.

Egeter, O., and R. Brückner. 1995. Characterization of a genetic locus essential for maltose-maltotriose utilization in Staphylococcus xylosus. J. Bacteriol. 177:2408–2415.

Egeter, O., and R. Brückner. 1996. Catabolite repression mediated by the catabolite control protein CcpA in Staphylococcus xylosus. Molec. Microbiol. 21:739–749.

Ehlert, K., M. Tschierske, C. Mori, W. Schroder, and B. Berger-Bächi. 2000. Site-specific serine incorporation by Lif and Epr into positions 3 and 5 of the staphylococcal peptidoglycan interpeptide bridge. J. Bacteriol. 182:2635–2638.

Emmett, M., and W. E. Kloos. 1975. Amino acid requirements of staphylococci isolated from human skin. Can. J. Microbiol. 21:729–733.

Emmett, M., and W. E. Kloos. 1979. The nature of arginine auxotrophy in cutaneous populations of staphylococci. J. Gen. Microbiol. 110:305–314.

Endl, J., H. P. Seidl, F. Fiedler, and K.-H. Schleifer. 1983. Chemical composition and structure of cell wall teichoic acids of staphylococci. Arch. Microbiol. 135:215–223.

Endl, J., P. H. Seidl, F. Fiedler, and K.-H. Schleifer. 1984. Determination of cell wall teichoic acid structure of staphylococci by rapid chemical and

serological screening methods. Arch. Microbiol. 137:272–280.

Evans, J. B., W. L. J. Bradford, and C. F. Niven. 1955. Comments concerning the taxonomy of the genera Micrococcus and Staphylococcus. Int. Bull. Bacteriol. Nomencl. Taxon. 5:61–66.

Evans, J. B. 1977. Coagulase-positive staphylococci as indicators of potential health hazards from water. In: A. W. Hoadley and B. J. Dutka (Eds.) Bacterial Indicators and Health Hazards Associated with Water. American Society for Testing and Materials. Philadelphia, PA. 126–130.

Falk, D., and S. J. Guering. 1983. Differentiation of Staphylococcus and Micrococcus spp. with the Taxo A bacitracin disk. J. Clin. Microbiol. 18:719–721.

Faller, A. H., F. Götz, and K.-H. Schleifer. 1980. Cytochrome patterns of staphylococci and micrococci and their taxonomic implications. Zentralbl. Bakteriol. Hyg. Abt. 1 Orig. C 1:26–39.

Faller, A., and K.-H. Schleifer. 1981. Modified oxidase and benzidine tests for separation of staphylococci from micrococci. J. Clin. Microbiol. 13:1031–1035.

Fast, B., P.-E. Lindgren, and F. Götz. 1997. Cloning, sequencing, and characterization of a gene (narT) encoding a transport protein involved in dissimilatory nitrate reduction in Staphylococcus carnosus. Arch. Microbiol. 166:361–367.

Fedtke, I., A. Kamps, B. Krismer, and F. Götz. 2002. The nitrate reductase and nitrite reductase operons and the narT gene of Staphylococcus carnosus are positively controlled by the novel two-component system NreBC. J. Bacteriol. 184:6624–6634.

Fiegler, H., J. Bassias, I. Jankovic, and R. Brückner. 1999. Identification of a gene in Staphylococcus xylosus encoding a novel glucose uptake protein. J. Bacteriol. 181:4929–4936.

Fischer, W., P. Rosel, and H. U. Koch. 1981. Effect of alanine ester substitution and other structural features of lipoteichoic acids on their inhibitory activity against autolysins of Staphylococcus aureus. J. Bacteriol. 146:467–475.

Fischer, S., H. Luczak, and K. H. Schleifer. 1982. Improved methods for the detection of class I and II fructose-1,6-bis-phosphate aldolases in bacteria. FEMS Microbiol. Lett. 15:103–108.

Fischer, W. 1988. Physiology of lipoteichoic acids in bacteria. Adv. Microb. Physiol. 29:233–302.

Fischer, R., R. Eisermann, B. Reiche, and W. Hengstenberg. 1989. Cloning, sequencing and overexpression of the mannitol-specific enzyme-III-encoding gene of Staphylococcus carnosus. Gene 82:249–257.

Fischer, R., and W. Hengstenberg. 1992. Mannitol-specific enzyme II of the phosphoenolpyruvate-dependent phosphotransferase system of Staphylococcus carnosus: Sequence and expression in Escherichia coli and structural comparison with the enzyme IImannitol of Escherichia coli. Eur. J. Biochem. 204:963–969.

Fischer, W. 1994. Lipoteichoic acids and lipoglycans. In: J. M. Ghuysen and R. Hakenbeck (Eds.) Bacterial Cell Wall. Elsevier. London, UK. 199–215.

Fleurette, J., Y. Brun, M. Bes, M. Coulet, and F. Forey. 1987. Infections caused by coagulase-negative staphylococci other than S. epidermidis and S. saprophyticus. In: G. Peters (Ed.) Pathogenicity and Clinical Significance of Coagulase-negative Staphylococci. Gustav Fischer-Verlag. Stuttgart, Germany. 195–208.

Flock, J. I., G. Froman, K. Jonsson, B. Guss, C. Signas, B. Nilsson, G. Raucci, M. Hook, T. Wadstrom, and M. Lindberg. 1987. Cloning and expression of the gene for a fibronectin-binding protein from Staphylococcus aureus. Embo J. 6:2351–2357.

Flock, J. I., S. A. Hienz, A. Heimdahl, and T. Schennings. 1996. Reconsideration of the role of fibronectin binding in endocarditis caused by Staphylococcus aureus. Infect. Immun. 64:1876–1878.

Flügge, C. 1886. Die Mikroorganismen. F. C. W. Vogel. Leipzig, Germany.

Foster, T. J., and D. McDevitt. 1994.. Surface-associated proteins of Staphylococcus aureus: Their possible roles in virulence. FEMS Microbiol. Lett. 118:199–205.

Foster, G., H. M. Ross, R. A. Hutson, and M. D. Collins. 1997. Staphylococcus lutrae sp. nov., a new coagulase-positive species isolated from otters. Int. J. Syst. Bacteriol. 47:724–726.

Foster, T. J., and M. Höök. 1998. Surface protein adhesins of Staphylococcus aureus. Trends Microbiol. 6:484–488.

Fournier, B., and D. C. Hooper. 2000a. A new two-component regulatory system involved in adhesion, autolysis, and extracellular proteolytic activity of Staphylococcus aureus. J. Bacteriol. 182:3955–3964.

Fournier, B., R. Aras, and D. C. Hooper. 2000b. Expression of the multidrug resistance transporter NorA from Staphylococcus aureus is modified by a two-component regulatory system. J. Bacteriol. 182:664–671.

Fournier, B., A. Klier, and G. Rapoport. 2001. The two-component system ArlS-ArlR is a regulator of virulence gene expression in Staphylococcus aureus. Molec. Microbiol. 41:247–261.

Freer, J. H., and T. H. Birkbeck. 1982. Possible conformation of delta-lysin, a membrane-damaging peptide of Staphylococcus aureus. J. Theor. Biol. 94:535–540.

Freney, J., Y. Brun, M. Bes, H. Meugneir, F. Grimont, P. A. D. Grimont, C. Nervi, and J. Fleurette. 1988. Staphylococcus lugdunensis sp. nov. and Staphylococcus schleiferi sp. nov., two species from human clinical specimens. Int. J. Syst. Bacteriol. 38:168–172.

Froman, G., L. M. Switalski, P. Speziale, and M. Hook. 1987. Isolation and characterization of a fibronectin receptor from Staphylococcus aureus. J. Biol. Chem. 262:6564–6571.

Ganz, T., and R. I. Lehrer. 1994. Defensins. Curr. Opin. Immunol. 6:584–589.

Garrard, W., and J. Lascelles. 1968. Regulation of Staphylococcus aureus lactate dehydrogenase. J. Bacteriol. 95:152–156.

Garrity, G. M., and J. G. Holt. 2001. The road map to the manual. In: R. D. Boone, R. W. Castenholz, and G. M. Garrity (Eds.) Bergey's Manual of Systematic Bacteriology, 2nd ed. Springer-Verlag. New York, NY. 119–166.

Garvis, S., J. M. Mei, J. Ruiz-Albert, and D. W. Holden. 2002. Staphylococcus aureus svrA: A gene required for virulence and expression of the agr locus. Microbiology 148:3235–3243.

Geisel, R., F. J. Schmitz, A. C. Fluit, and H. Labischinski. 2001. Emergence, mechanism, and clinical implications of reduced glycopeptide susceptibility in Staphylococcus aureus. Eur. J. Clin. Microbiol. Infect. Dis. 20:685–697.

George, C. G., and W. E. Kloos. 1994. Comparison of the SmaI-digested chromosomes of Staphylococcus epider-

midis and the closely related species Staphylococcus capitis and Staphylococcus caprae. Int. J. Syst. Bacteriol. 44:404–409.

Gering, M., and R. Brückner. 1996. Transcriptional regulation of the sucrase gene of Staphylococcus xylosus by the repressor ScrR. J. Bacteriol. 178:462–469.

Gerke, C., A. Kraft, R. Süssmuth, O. Schweitzer, and F. Götz. 1998. Characterization of the N-acetylglucosaminyltransferase activity involved in the biosynthesis of the Staphylococcus epidermidis polysaccharide intercellular adhesin. J. Biol. Chem. 273:18586–18593.

Ghuysen, J.-M., and R. Hakenbeck. 1994. Bacterial Cell Wall. Elsevier. London, UK.

Giesbrecht, P., T. Kersten, H. Maidhof, and J. Wecke. 1998. Staphylococcal cell wall: Morphogenesis and fatal variations in the presence of penicillin. Microbiol. Molec. Biol. Rev. 62:1371–1414.

Giraudo, A. T., A. L. Cheung, and R. Nagel. 1997. The sae locus of Staphylococcus aureus controls exoprotein synthesis at the transcriptional level. Arch. Microbiol. 168:53–58.

Giraudo, A. T., A. Calzolari, A. A. Cataldi, C. Bogni, and R. Nagel. 1999. The sae locus of Staphylococcus aureus encodes a two-component regulatory system. FEMS Microbiol. Lett. 177:15–22.

Goerke, C., U. Flückiger, A. Steinhuber, W. Zimmerli, and C. Wolz. 2001. Impact of the regulatory loci agr, sarA and sae of Staphylococcus aureus on the induction of alpha-toxin during device-related infection resolved by direct quantitative transcript analysis. Molec. Microbiol. 40:1439–1447.

Götz, F., and K.-H. Schleifer. 1975. Purification and properties of a fructose-1,6-diphosphate activated L-lactate dehydrogenase from Staphylococcus epidermidis. Arch. Microbiol. 105:303–312.

Götz, F., and K.-H. Schleifer. 1976. Comparative biochemistry of lactate dehydrogenases from staphylococci. In: J. Jeljaszewicz (Ed.) Staphylococci and Staphylococcal Diseases. Gustav Fischer-Verlag. Stuttgart, Germany. 245–252.

Götz, F., and K.-H. Schleifer. 1978. Biochemical properties and the physiological role of the fructose-1,6-bisphosphate activated L-lactate dehydrogenase from Staphylococcus epidermidis. Eur. J. Biochem. 90:555–561.

Götz, F., S. Fischer, and K.-H. Schleifer. 1980. Purification and characterization of an unusually heat-stable and acid/base stable class I fructose-1,6-bisphosphate aldolase from Staphylococcus aureus. Eur. J. Biochem. 108:295–301.

Götz, F., F. Popp, E. Korn, and K.-H. Schleifer. 1985. Complete nucleotide sequence of the lipase gene from Staphylococcus hyicus cloned in Staphylococcus carnosus. Nucleic Acids Res. 13:5895–5906.

Götz, F., and B. Schumacher. 1987. Improvements of protoplast transformation in Staphylococcus carnosus. FEMS Microbiol. Lett. 40:285–288.

Götz, F. 1990. Staphylococcus carnosus: A new host organism for gene cloning and protein production. J. Appl. Bacteriol. Symp. Suppl. 69:49–53.

Götz, F. 1991. Staphylococcal lipases and phospholipases. In: L. Alberghina, R. D. Schmid, and R. Verger (Eds.) Lipases: Structure, Mechanism and Genetic Engineering. VCH Publishers. Weinheim, Germany. 285–292.

Götz, F., H. M. Verheij, and R. Rosenstein. 1998. Staphylococcal lipases: Molecular characterisation, secretion, and processing. Chem. Phys. Lipids 93:15–25.

Götz, F., and G. Peters. 2000a. Colonization of medical devices by coagulase-negative staphylococci. In: F. A. Waldvogel and A. L. Bisno (Eds.) Infections Associated with Indwelling Medical Devices. ASM Press. Washington, DC. 55–88.

Götz, F., C. Heilmann, and S. E. Cramton. 2000b. Molecular basis of catheter associated infections by staphylococci. In: L. Emödy (Ed.) Genes and Proteins Underlying Microbial Urinary Tract Virulence. Kluwer Academic/Plenum Publishers. Dordrecht, The Netherlands. 103–111.

Götz, F., and G. Jung. 2001a. Biosynthesis of the lantibiotics epidermin and gallidermin. In: V. Braun and F. Götz (Eds.) Microbial Fundamentals of Biotechnology. Wiley-VCH Verlag. Weinheim, Germany. 52–92.

Götz, F., and R. Rosenstein. 2001b. Staphylococcal lipases: Molecular characterization and use as an expression and secretion system. In: V. Braun and F. Götz (Eds.) Microbial Fundamentals of Biotechnology. Wiley-VCH Verlag. Weinheim, Germany. 238–248.

Götz, F. 2002. Staphylococcus and biofilms. Molec. Microbiol. 43:1367–1378.

Green, C. J., and B. S. Vold. 1993. Staphylococcus aureus has clustered tRNA genes. J. Bacteriol. 175:5091–5096.

Gross, M., S. E. Cramton, F. Götz, and A. Peschel. 2001. Key role of teichoic acid net charge in Staphylococcus aureus colonization of artificial surfaces. Infect. Immun. 69:3423–5426.

Hájek, V., and E. Marsalek. 1971. A study of staphylococci isolated from the upper respiratory tract of different animal species. IV: Physiological properties of Staphylococcus aureus strains of hare origin. Zentralbl. Bakteriol. [Orig.] 216:168–174.

Hájek, V., E. Marsalek, and J. Hubacek. 1972. A study of staphylococci isolated from the upper respiratory tract of different animal species. V: Physiological properties of Staphylococcus aureus strains from mink. Zentralbl. Bakteriol. [Orig. A] 222:194–199.

Hájek, V. 1976a. Staphylococcus intermedius, a new species isolated from animals. Int. J. Syst. Bacteriol. 26:401–408.

Hájek, V., and E. Marsalek. 1976b. Staphylococci outside the hospital: Staphylococcus aureus in sheep. Zentralbl. Bakteriol. [Orig. B] 161:455–461.

Hájek, V., and J. Balusek. 1985. Staphylococci from flies of different environments. In: J. Jeljaszewicz (Ed.) The Staphylococci: Proceedings of the 5th International Symposium on Staphylococci and Staphylococcal Infections. Gustav Fischer-Verlag. Stuttgart, Germany. 129–133.

Hájek, V., L. A. Devriese, M. Mordarski, M. Goodfellow, G. Pulverer, and P. E. Veraldo. 1986. Elevation of Staphylococcus hyicus subsp. chromogenes (Devriese et. al., 1978) to species status: Staphylococcus chromogenes (Devriese et al., 1978) comb. nov. Syst. Appl. Microbiol. 8:169–173.

Hájek, V., W. Ludwig, K. H. Schleifer, N. Springer, W. Zitzelsberger, R. M. Kroppenstedt, and M. Kocur. 1992. Staphylococcus muscae, a new species isolated from flies. Int. J. Syst. Bacteriol. 42:97–101.

Hájek, V., H. Meugnier, M. Bes, Y. Brun, F. Fiedler, Z. Chmela, Y. Lasne, J. Fleurette, and J. Freney. 1996.

Staphylococcus saprophyticus subsp. bovis subsp. nov., isolated from bovine nostrils. Int. J. Syst. Bacteriol. 46:792–796.

Hammes, W., K.-H. Schleifer, and O. Kandler. 1973. Mode of action of glycine on the biosynthesis of peptidoglycan. J. Bacteriol. 116:1029–1053.

Hammes, W. P., and F. C. Neuhaus. 1974. On the specificity of phospho-N-acetylmuramyl-pentapeptide translocase: The peptide subunit of uridine diphosphate-N-actylmuramyl-pentapeptide. J. Biol. Chem. 249:3140–3150.

Harvey, J., and A. Gilmour. 1988. Isolation and characterization of staphylococci from goats milk produced in Northern Ireland. Lett. Appl. Microbiol. 7:79–82.

Hauschild, A. H., C. E. Park, and R. Hilsheimer. 1979. A modified pork plasma agar for the enumeration of Staphylococcus aureus in foods. Can. J. Microbiol. 25:1052–1057.

Hay, J. B., A. R. Archibald, and J. Baddiley. 1965. The molecular structure of bacterial walls: The size of ribitol teichoic acids and the nature of their linkage to glycosaminopeptides. Biochem. J. 97:723–730.

Hecker, M., and U. Volker. 1998. Non-specific, general and multiple stress resistance of growth- restricted Bacillus subtilis cells by the expression of the sigmaB regulon. Molec. Microbiol. 29:1129–1136.

Heilmann, C., C. Gerke, F. Perdreau-Remington, and F. Götz. 1996a. Characterization of Tn917 insertion mutants of Staphylococcus epidermidis affected in biofilm formation. Infect. Immun. 64:277–282.

Heilmann, C., O. Schweitzer, C. Gerke, N. Vanittanakom, D. Mack, and F. Götz. 1996b. Molecular basis of intercellular adhesion in the biofilm-forming Staphylococcus epidermidis. Molec. Microbiol. 20:1083–1091.

Heilmann, C., M. Hussain, G. Peters, and F. Götz. 1997. Evidence for autolysin-mediated primary attachment of Staphylococcus epidermidis to a polystyrene surface. Molec. Microbiol. 24:1013–1024.

Hemila, H. 1991. Lipoamide dehydrogenase of Staphylococcus aureus: Nucleotide sequence and sequence analysis. Biochim. Biophys. Acta 1129:119–123.

Hengstenberg, W., D. Kohlbrecher, E. Witt, R. Kruse, I. Christiansen, D. Peters, R. Pogge von Strandmann, P. Stadtler, B. Koch, and H. R. Kalbitzer. 1993. Structure and function of proteins of the phosphotransferase system and of 6-phospho-β-glycosidases in Gram-positive bacteria. FEMS Microbiol. Rev. 12:149–163.

Henkin, T. M. 1996. The role of CcpA transcriptional regulator in carbon metabolism in Bacillus subtilis. FEMS Microbiol. Lett. 135:9–15.

Henze, U., T. Sidow, J. Wecke, H. Labischinski, and B. Berger-Bächi. 1993. Influence of femB on methicillin resistance and peptidoglycan metabolism in Staphylococcus aureus. J. Bacteriol. 175:1612–1620.

Heptinstall, S., A. R. Archibald, and J. Baddiley. 1970. Teichoic acids and membrane function in bacteria. Nature 225:519–521.

Hermann, C., I. Spreitzer, N. W. Schroder, S. Morath, M. D. Lehner, W. Fischer, C. Schutt, R. R. Schumann, and T. Hartung. 2002. Cytokine induction by purified lipoteichoic acids from various bacterial species—role of LBP, sCD14, CD14 and failure to induce IL-12 and subsequent IFN-gamma release. Eur. J. Immunol. 32:541–551.

Herrmann, M., P. E. Vaudaux, D. Pittet, R. Auckenthaler, P. D. Lew, F. Schumacher-Perdreau, G. Peters, and F. A. Waldvogel. 1988. Fibronectin, fibrinogen, and laminin act as mediators of adherence of clinical staphylococcal isolates to foreign material. J. Infect. Dis. 158:693–701.

Herrmann, M., S. J. Suchard, L. A. Boxer, F. A. Waldvogel, and P. D. Lew. 1991. Thrombospondin binds to Staphylococcus aureus and promotes staphylococcal adherence to surfaces. Infect. Immun. 59:279–288.

Higashi, Y., J. L. Strominger, and C. C. Sweeley. 1970. Biosynthesis of the peptidoglycan of bacterial cell walls. XXI: Isolation of free C55-isoprenoid alcohol and of lipid intermediates in peptidoglycan synthesis from Staphylococcus aureus. J. Biol. Chem. 245:3697–3702.

Hiramatsu, K. 2001. Vancomycin-resistant Staphylococcus aureus: A new model of antibiotic resistance. Lancet Infect Dis. 1:147–155.

Holmberg, O. 1986. Coagulase-negative staphylococci in bovine mastitis. In: P.-A. Mardh and K.-H. Schleifer (Eds.) Coagulase-negative Staphylococci. Almquist and Wiksell International. Stockholm, Sweden. 203–211.

Horowitz, W. 2000. Official Methods of Analysis of AOAC International. Gaithersburg, MD.

Hovelius, B., S. Colleen, and P.-A. Mardh. 1984. Urinary tract infections in men caused by Staphylococcus saprophyticus. Scand. J. Infect. Dis. 16:37–41.

Hovelius, B. 1986. Epidermiological and clinical aspects of urinary tract infections caused by Staphylococcus saprophyticus. In: P.-A. Mardh and K.-H. Schleifer (Eds.) Coagulase-negative Staphylococci. Almquist and Wiksell International. Stockholm, Sweden. 195–202.

Hueck, C., A. Kraus, and W. Hillen. 1994. Sequences of ccpA and two downstream Bacillus megaterium genes with homology to the motAB operon from Bacillus subtilis. Gene 143:147–148.

Hussain, M., M. Herrmann, C. von Eiff, F. Perdreau-Remington, and G. Peters. 1997. A 140-kilodalton extracellular protein is essential for the accumulation of Staphylococcus epidermidis strains on surfaces. Infect. Immun. 65:519–524.

Huynh, P. L., I. Jankovic, N. F. Schnell, and R. Brückner. 2000. Characterization of an HPr kinase mutant of Staphylococcus xylosus. J. Bacteriol. 182:1895–1902.

Igimi, S., S. Kawamura, E. Takahashi, and T. Mitsuoka. 1989. Staphylococcus felis, a new species from clinical specimens from cats. Int. J. Syst. Bacteriol. 39:373–377.

Igimi, S., E. Takahashi, and T. Mitsuoka. 1990. Staphylococcus schleiferi subsp. coagulans subsp. nov., isolated from the external auditory meatus of dogs with external ear otitis. Int. J. Syst. Bacteriol. 40:409–411.

Isenberg, H. D. 1994. Clinical Microbiology Procedures Handbook. American Society for Microbiology. Washington, DC.

Ito, E., and J. L. Strominger. 1973. Enzymatic synthesis of the peptide in bacterial uridine nucleotides. VII: Comparative biochemistry. J. Biol. Chem. 248:3131–3136.

Jankovic, I., and R. Brückner. 2002. Carbon catabolite repression by the catabolite control protein CcpA in Staphylococcus xylosus. J. Molec. Microbiol. Biotechnol. 4:309–314.

Janzon, L., S. Lofdahl, and S. Arvidson. 1989. Identification and nucleotide sequence of the delta-lysin gene, hld, adjacent to the accessory gene regulator (agr) of Staphylococcus aureus. Molec. Gen. Genet. 219:480–485.

Janzon, L., and S. Arvidson. 1990. The role of the delta-lysin gene (hld) in the regulation of virulence genes by the accessory gene regulator (agr) in Staphylococcus aureus. Embo J. 9:1391–1399.

Ji, G., R. C. Beavis, and R. P. Novick. 1995. Cell density control of staphylococcal virulence mediated by an octapeptide pheromone. Proc. Natl. Acad. Sci. USA 92:12055–12059.

Ji, G., R. Beavis, and R. P. Novick. 1997. Bacterial interference caused by autoinducing peptide variants. Science 276:2027–2030.

Johannsen, L., R. S. Rosenthal, S. A. Martin, A. B. Cady, F. Obal Jr., M. Guinand, and J. M. Krueger. 1989. Somnogenic activity of O-acetylated and dimeric muramyl peptides. Infect. Immun. 57:2726–2732.

Johannsen, L., H. Labischinski, and J. M. Krueger. 1991a. Somnogenic activity of pseudomurein in rabbits. Infect. Immun. 59:2502–2504.

Johannsen, L., J. Wecke, F. Obal Jr., and J. M. Krueger. 1991b. Macrophages produce somnogenic and pyrogenic muramyl peptides during digestion of staphylococci. Am. J. Physiol. 260:R126–R133.

Johnston, L. S., and F. C. Neuhaus. 1975. Initial membrane reaction in the biosynthesis of peptidoglycan: Spin-labeled intermediates as receptors for vancomycin and ristocetin. Biochemistry 14:2754–2760.

Jones, R. W., A. Lamont, and P. B. Garland. 1980. The mechanism of proton translocation driven by the respiratory nitrate reductase complex of Escherichia coli. Biochem. J. 190:79–94.

Jönsson, K., C. Signäs, H.-P. Müller, and M. Lindberg. 1991. Two different genes encode fibronectin binding proteins in Staphylococcus aureus: The complete nucleotide sequence and characterization of the second gene. Eur. J. Biochem. 202:1041–1048.

Josefsson, E., K. W. McCrea, D. Ni Eidhin, D. O'Connell, J. Cox, M. Hook, and T. J. Foster. 1998a. Three new members of the serine-aspartate repeat protein multigene family of Staphylococcus aureus. Microbiology 144:3387–3395.

Josefsson, E., D. O'Connell, T. J. Foster, I. Durussel, and J. A. Cox. 1998b. The binding of calcium to the B-repeat segment of SdrD, a cell surface protein of Staphylococcus aureus. J. Biol. Chem. 273:31145–31152.

Kamiryo, T., and M. Matsuhashi. 1972. The biosynthesis of the cross-linking peptides in the cell wall peptidoglycan of Staphylococcus aureus. J. Biol. Chem. 247:6306–6311.

Kanda, M., H. Inoue, T. Fukuizumi, T. Tsujisawa, K. Tominaga, and J. Fukuda. 2001. Detection and rapid increase of salivary antibodies to Staphylococcus lentus, an indigenous bacterium in rabbit saliva, through a single tonsillar application of bacterial cells. Oral Microbiol. Immunol. 16:257–264.

Karchmer, A. W., G. L. Archer, and W. E. Dismukes. 1983. Staphylococcus epidermidis causing prosthetic valve endocarditis: Microbiologic and clinical observations as guides to therapy. Ann. Intern. Med. 98:447–455.

Kawabata, S., T. Morita, S. Iwanaga, and H. Igarashi. 1985. Enzymatic properties of staphylothrombin, an active molecular complex formed between staphylocoagulase and human prothrombin. J. Biochem. (Tokyo) 98:1603–1614.

Kawamura, Y., X. G. Hou, F. Sultana, K. Hirose, M. Miyake, S. E. Shu, and T. Ezaki. 1998. Distribution of Staphylococcus species among human clinical specimens and emended description of Staphylococcus caprae. J. Clin. Microbiol. 36:2038–2042.

Keller, G., K. H. Schleifer, and F. Götz. 1984. Cloning of the ribokinase gene of Staphylococcus hyicus subsp. hyicus in Staphylococcus carnosus. Arch. Microbiol. 140:218–224.

Kilpper, R., U. Buhl, and K.-H. Schleifer. 1980. Nucleic acid homology studies between Peptococcus saccharolyticus and various anaerobic and facultative anaerobic Gram-positive cocci. FEMS Microbiol. Lett. 8:205–210.

Kilpper-Bälz, R., and K.-H. Schleifer. 1981. Transfer of Peptococcus saccharolyticus Foubert and Douglas to the genus Staphylococcus: Staphylococcus saccharolyticus (Foubert and Douglas) comb. nov. Zentralbl. Bakteriol. Mikrobiol. Hyg. Abt. 1 Orig. C 2:324–331.

Kloos, W. E., T. G. Tornabene, and K.-H. Schleifer. 1974. Isolation and characterization of micrococci from human skin, including two new species: Micrococcus lylae and Micrococcus kristinae. Int. J. Syst. Bacteriol. 24:79–101.

Kloos, W. E., and M. S. Musselwhite. 1975a. Distribution and persistence of Staphylococcus and Micrococcus species and other aerobic bacteria on human skin. Appl. Microbiol. 30:381–385.

Kloos, W. E., and K.-H. Schleifer. 1975b. Isolation and characterization of staphylococci from human skin. II: Description of four new species: Staphylococcus warneri, Staphylococcus capitis, Staphylococcus hominis, and Staphylococcus simulans. Int. J. Syst. Bacteriol. 25:62–79.

Kloos, W. E., and K.-H. Schleifer. 1975c. Simplified scheme for routine identification of human Staphylococcus species. J. Clin. Microbiol. 1:82–88.

Kloos, W. E., K.-H. Schleifer, and R. F. Smith. 1976a. Characterization of Staphylococcus sciuri sp. nov. and its subspecies. Int. J. Syst. Bacteriol. 26:22–37.

Kloos, W. E., R. J. Zimmerman, and R. F. Smith. 1976b. Preliminary studies on the characterization and distribution of Staphylococcus and Micrococcus species on animal skin. Appl. Environ. Microbiol. 31:53–59.

Kloos, W. E., and J. F. Wolfshohl. 1979. Evidence for deoxyribonucleotide sequence divergence between staphylococci living on human and other primate skin. Curr. Microbiol. 3:167–172.

Kloos, W. E. 1980. Natural populations of the genus Staphylococcus. Ann. Rev. Microbiol. 34:559–592.

Kloos, W. E., and K.-H. Schleifer. 1981. The genus Staphylococcus. *In:* M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schegel (Eds.) The Prokaryotes. Springer-Verlag. New York, NY. 1548–1569.

Kloos, W. E., and K.-H. Schleifer. 1983a. Staphylococcus auricularis sp. nov.: An inhabitant of the human external ear. Int. J. Syst. Bacteriol. 33:9–14.

Kloos, W. E., and J. F. Wolfshohl. 1983b. Deoxyribonucleotide sequence divergence between Staphylococcus cohnii subspecies populations living on primate skin. Curr. Microbiol. 8:115–121.

Kloos, W. E. 1985. Staphylococcus. *In:* S. P. Parker (Ed.) McGraw-Hill Book 1986 Yearbook of Science and Technology. McGraw-Hill Book. New York, NY. 431–434.

Kloos, W. E., and K.-H. Schleifer. 1986. Genus 4. Staphylococcus Rosenbach 1984. *In:* J. G. Holt, P. H. A. Sneath, N. S. Mair, and M. S. Sharpe (Eds.) Bergey's Manual of Systematic Bacteriology. Williams and Wilkins. Baltimore, MD. 2:1013–1035.

Kloos, W. E., and J. F. Wolfshohl. 1991. Staphylococcus cohnii subspecies: Staphylococcus cohnii subsp. cohnii subsp. nov. and Staphylococcus cohnii subsp. urealyticum subsp. nov. Int. J. Syst. Bacteriol. 41:284–289.

Kloos, W., K. H. Schleifer, and F. Götz. 1992. The genus Staphylococcus. *In:* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer (Eds.) The Prokaryotes. Springer-Verlag, New York, NY. 1369–1420.

Kloos, W. E., and T. L. Bannerman. 1994. Update on clinical significance of coagulase-negative staphylococci. Clin. Microbiol. Rev. 7:117–140.

Kloos, W. E., D. N. Ballard, J. A. Webster, R. J. Hübner, A. Tomasz, I. Couto, G. L. Sloan, H. P. Dehart, F. Fiedler, K. Schubert, H. de Lencastre, I. S. Sanches, H. E. Heath, P. A. Leblanc, and A. Ljungh. 1997. Ribotype delineation and description of Staphylococcus sciuri subspecies and their potential as reservoirs of methicillin resistance and staphylolytic enzyme genes. Int. J. Syst. Bacteriol. 47:313–323.

Kloos, W. E., D. N. Ballard, C. G. George, J. A. Webster, R. J. Hubner, W. Ludwig, K. H. Schleifer, F. Fiedler, and K. Schubert. 1998a. Delimiting the genus Staphylococcus through description of Macrococcus caseolyticus gen. nov., comb. nov. and Macrococcus equipercicus sp. nov., and Macrococcus bovicus sp. no. and Macrococcus carouselicus sp. nov. Int. J. Syst. Bacteriol. 48:859–877.

Kloos, W. E., C. G. George, J. S. Olgiate, L. Van Pelt, M. L. McKinnon, B. L. Zimmer, E. Muller, M. P. Weinstein, and S. Mirrett. 1998b. Staphylococcus hominis subsp. novobiosepticus subsp. nov., a novel trehalose- and N-acetyl-D-glucosamine-negative, novobiocin- and multiple-antibiotic-resistant subspecies isolated from human blood cultures. Int. J. Syst. Bacteriol. 48 Pt 3:799–812.

Kloos, W. E., and T. L. Bannerman. 1999. Staphylococcus and Micrococcus. *In:* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (Eds.) Manual of Clinical Microbiology. American Society for Microbiology. Washington, DC. 276.

Knezevic, I., S. Bachem, A. Sickmann, H. E. Meyer, J. Stulke, and W. Hengstenberg. 2000. Regulation of the glucose-specific phosphotransferase system (PTS) of Staphylococcus carnosus by the antiterminator protein GlcT. Microbiology 146:2333–2342.

Knox, K. W., and A. J. Wicken. 1973. Immunological properties of teichoic acids. Bacteriol. Rev. 37:215–257.

Komatsuzawa, H., K. Ohta, S. Yamada, K. Ehlert, H. Labischinski, J. Kajimura, T. Fujiwara, and M. Sugai. 2002. Increased glycan chain length distribution and decreased susceptibility to moenomycin in a vancomycin-resistant Staphylococcus aureus mutant. Antimicrob. Agents Chemother. 46:75–81.

Kondo, N., K. Kuwahara-Arai, H. Kuroda-Murakami, E. Tateda-Suzuki, and K. Hiramatsu. 2001. Eagle-type methicillin resistance: New phenotype of high methicillin resistance under mec regulator gene

control. Antimicrob. Agents Chemother. 45:815–824.

Kornblum, J., B. Kreiswirth, S. J. Projan, H. Ross, and R. Novick. 1990. Agr: A polycystronic locus regulating exoprotein synthesis in Staphylococcus aureus. *In:* R. Novick (Ed.) Molecular Biology of Staphylococci. VCH Publishers. New York, NY. 373–402.

Kowolik, C. M., and W. Hengstenberg. 1998. The lactose transporter of Staphylococcus aureus: Overexpression, purification and characterization of the histidine-tagged domains IIC and IIB. Eur. J. Biochem. 257:389–394.

Krebs, H. A. 1937. Dismutation of pyruvic acid in Gonococcus and Staphylococcus. Biochem. J. 31:661–671.

Krogh, H. V., and S. Kristensen. 1976. A study of skin diseases in dogs and cats. II: Microflora of the normal skin of dogs and cats. Nord. Vet. Med. 28:459–463.

Kullik, I., and P. Giachino. 1997. The alternative sigma factor sigmaB in Staphylococcus aureus: Regulation of the sigB operon in response to growth phase and heat shock. Arch. Microbiol. 167:151–159.

Kullik, I., P. Giachino, and T. Fuchs. 1998a. Deletion of the alternative sigma factor sigmaB in Staphylococcus aureus reveals its function as a global regulator of virulence genes. J. Bacteriol. 180:4814–4820.

Kullik, I., R. Jenni, and B. Berger-Bächi. 1998b. Sequence of the putative alanine racemase operon in Staphylococcus aureus: Insertional interruption of this operon reduces D-alanine substitution of lipoteichoic acid and autolysis. Gene 219:9–17.

Kuroda, M., T. Ohta, I. Uchiyama, T. Baba, H. Yuzawa, I. Kobayashi, L. Cui, A. Oguchi, K. Aoki, Y. Nagai, J. Lian, T. Ito, M. Kanamori, H. Matsumaru, A. Maruyama, H. Murakami, A. Hosoyama, Y. Mizutani-Ui, N. K. Takahashi, T. Sawano, R. Inoue, C. Kaito, K. Sekimizu, H. Hirakawa, S. Kuhara, S. Goto, J. Yabuzaki, M. Kanehisa, A. Yamashita, K. Oshima, K. Furuya, C. Yoshino, T. Shiba, M. Hattori, N. Ogasawara, H. Hayashi, and K. Hiramatsu. 2001. Whole genome sequencing of methicillin-resistant Staphylococcus aureus. Lancet 357:1225–1240.

Kuwahara-Arai, K., N. Kondo, S. Hori, E. Tateda-Suzuki, and K. Hiramatsu. 1996. Suppression of methicillin resistance in a mecA containing pre-methicillin-resistant Staphylococcus aureus strain is caused by the mecI-mediated repression of PBP 2′ production. Antimicrob. Agents Chemother. 40:2680–2685.

Labischinski, H., and H. Maidhof. 1994. Bacterial peptidoglycan: Overview and evolving concepts. *In:* J. M. Ghuysen and R. Hakenbeck (Eds.) Bacterial Cell Wall. Elsevier. London, UK. 23–38.

Lambert, L. H., T. Cox, K. Mitchell, R. A. Rossello-Mora, C. Del Cueto, D. E. Dodge, P. Orkand, and R. J. Cano. 1998. Staphylococcus succinus sp. nov., isolated from Dominican amber. Int. J. Syst. Bacteriol. 48 Pt 2:511–518.

Langlois, B. E., R. J. Harmon, and K. Akers. 1983. Identification of Staphylococcus species of bovine origin with the API Staph-Ident system. J. Clin. Microbiol. 18:1212–1219.

Lebeau, C., F. Vandenesch, T. Greenland, R. P. Novick, and J. Etienne. 1994. Coagulase expression in Staphylococcus aureus is positively and negatively modulated by an agr-dependent mechanism. J. Bacteriol. 176:5534–5536.

Lehmer, A., and K.-H. Schleifer. 1980. Metabolism of pentose and pentitols by Staphylococcus xylosus and

Staphylococcus saprophyticus. Zentralbl. Bakteriol. Mikrobiol. Hyg. Abt. 1 Orig. C 1:109–123.

Lerche, M., and H. J. Sinell. 1955. Zum Vorkommen von Kokken in Rohwürsten. Archiv Lebensmittelhyg. 6:194–198.

Leriche, V., P. Sibille, and B. Carpentier. 2000. Use of an enzyme-linked lectinsorbent assay to monitor the shift in polysaccharide composition in bacterial biofilms. Appl. Environ. Microbiol. 66:1851–1856.

Liang, O. D., J. I. Flock, and T. Wadstrom. 1995. Isolation and characterisation of a vitronectin-binding surface protein from Staphylococcus aureus. Biochim. Biophys. Acta 1250:110–116.

Liebl, W., and F. Götz. 1986. Studies on lipase directed export of Escherichia coli beta-lactamase in Staphylococcus carnosus. Molec. Gen. Genet. 204:166–173.

Liepe, H.-U., and R. Porobic. 1983. Influence of storage conditions on survival rates and fermentative activity of lyophilized staphylococci. Fleischwirtschaft 63:1756–1757.

Lina, G., S. Jarraud, G. Ji, T. Greenland, A. Pedraza, J. Etienne, R. P. Novick, and F. Vandenesch. 1998. Transmembrane topology and histidine protein kinase activity of AgrC, the agr signal receptor in Staphylococcus aureus. Molec. Microbiol. 28:655–662.

Ling, B., and B. Berger-Bächi. 1998. Increased overall antibiotic susceptibility in Staphylococcus aureus femAB null mutants. Antimicrob. Agents Chemother. 42:936–938.

Ludwig, W., K.-H. Schleifer, G. E. Fox, E. Seewaldt, and E. Stackebrandt. 1981. A phylogenetic analysis of staphylococci, Peptococcus saccharolyticus and Micrococcus mucilaginosus. J. Gen. Microbiol. 125: 357–366.

Mack, D., W. Fischer, A. Krokotsch, K. Leopold, R. Hartmann, H. Egge, and R. Laufs. 1996. The intercellular adhesin involved in biofilm accumulation of Staphylococcus epidermidis is a linear beta-1,6-linked glucosaminoglycan: Purification and structural analysis. J. Bacteriol. 178:175–183.

Maki, D. G., C. E. Weise, and H. W. Sarafin. 1977. A semiquantitative culture method for identifying intravenous-catheter-related infection. N. Engl. J. Med. 296:1305–1309.

Manna, A. C., M. G. Bayer, and A. L. Cheung. 1998. Transcriptional analysis of different promoters in the sar locus in Staphylococcus aureus. J. Bacteriol. 180:3828–3836.

Manna, A., and A. L. Cheung. 2001. Characterization of sarR, a modulator of sar expression in Staphylococcus aureus. Infect. Immun. 69:885–896.

Marrie, T. J., C. Kwan, M. A. Noble, A. West, and L. Duffield. 1982a. Staphylococcus saprophyticus as a cause of urinary tract infections. J. Clin. Microbiol. 16:427–431.

Marrie, T. J., J. Nelligan, and J. W. Costerton. 1982b. A scanning and transmission electron microscopic study of an infected endocardial pacemaker lead. Circulation 66:1339–1341.

Marsik, F. J., and S. Brake. 1982. Species identification and susceptibility to 17 antibiotics of coagulase-negative staphylococci isolated from clinical specimens. J. Clin. Microbiol. 15:640–645.

Marsou, R., M. Bes, M. Boudouma, Y. Brun, H. Meugnier, J. Freney, F. Vandenesch, and J. Etienne. 1999. Distribution of Staphylococcus sciuri subspecies among human clinical specimens, and profile of antibiotic resistance. Res. Microbiol. 150:531–541.

Mayville, P., G. Ji, R. Beavis, H. Yang, M. Goger, R. P. Novick, and T. W. Muir. 1999. Structure-activity analysis of synthetic autoinducing thiolactone peptides from Staphylococcus aureus responsible for virulence. Proc. Natl. Acad. Sci. USA 96:1218–1223.

Mazmanian, S. K., G. Liu, E. R. Jensen, E. Lenoy, and O. Schneewind. 2000. Staphylococcus aureus sortase mutants defective in the display of surface proteins and in the pathogenesis of animal infections. Proc. Natl. Acad. Sci. USA 97:5510–5515.

Mazmanian, S. K., H. Ton-That, and O. Schneewind. 2001. Sortase-catalysed anchoring of surface proteins to the cell wall of Staphylococcus aureus. Molec. Microbiol. 40:1049–1057.

Mazmanian, S. K., H. Ton-That, K. Su, and O. Schneewind. 2002. An iron-regulated sortase anchors a class of surface protein during Staphylococcus aureus pathogenesis. Proc. Natl. Acad. Sci. USA 99:2293–2298.

McDevitt, D., P. Francois, P. Vaudaux, and T. J. Foster. 1994. Molecular characterization of the clumping factor (fibrinogen receptor) of Staphylococcus aureus. Molec. Microbiol. 11:237–248.

McGavin, M. H., D. Krajewska-Pietrasik, C. Ryden, and M. Höök. 1993. Identification of a Staphylococcus aureus extracellular matrix-binding protein with broad specificity. Infect. Immun. 61:2479–2485.

McKenney, D., K. L. Pouliot, Y. Wang, V. Murthy, M. Ulrich, G. Döring, J. C. Lee, D. A. Goldmann, and G. B. Pier. 1999. Broadly protective vaccine for Staphylococcus aureus based on an in vivo-expressed antigen. Science 284:1523–1527.

McLauchlin, J., G. L. Narayanan, V. Mithani, and G. O'Neill. 2000. The detection of enterotoxins and toxic shock syndrome toxin genes in Staphylococcus aureus by polymerase chain reaction. J. Food Prot. 63:479–488.

McNamara, P. J., K. C. Milligan-Monroe, S. Khalili, and R. A. Proctor. 2000. Identification, cloning, and initial characterization of rot, a locus encoding a regulator of virulence factor expression in Staphylococcus aureus. J. Bacteriol. 182:3197–3203.

Meens, J., M. Herbort, M. Klein, and R. Freudl. 1997. Use of the pre-pro part of Staphylococcus hyicus lipase as a carrier for secretion of Escherichia coli outer membrane protein A (OmpA) prevents proteolytic degradation of OmpA by cell-associated protease(s) in two different Gram-positive bacteria. Appl. Environ. Microbiol. 63:2814–2820.

Mendoza, M., H. Meugnier, M. Bes, J. Etienne, and J. Freney. 1998. Identification of Staphylococcus species by 16S-23S rDNA intergenic spacer PCR analysis. Int. J. Syst. Bacteriol. 48 Pt 3:1049–1055.

Meyer, W. 1967. A proposal for subdividing the species Staphylococcus aureus. Int. J. Syst. Bacteriol. 17:387–389.

Miller, B. A., R. F. Reiser, and M. S. Bergdoll. 1978. Detection of staphylococcal enterotoxins A, B, C, D, and E in foods by radioimnunoassay, using staphyloccal cells containing protein A as immunoadsorbent. Appl. Environ. Microbiol. 36:421–426.

Miller, J. M. 1998. A Guide to Specimen Management in Clinical Microbiology. American Society for Microbiology. Washington, DC.

Mirelman, D., and N. Sharon. 1972. Biosynthesis of peptidoglycan by a cell wall preparation of Staphylococcus

aureus and its inhibition by penicillin. Biochem. Biophys. Res. Commun. 46:1909–1917.

Mittenhuber, G. 2002. A phylogenomic study of the general stress response sigma factor sigmaB of Bacillus subtilis and its regulatory proteins. J. Molec. Microbiol. Biotechnol. 4:427–452.

Miyazaki, E., J. M. Chen, C. Ko, and W. R. Bishai. 1999. The Staphylococcus aureus rsbW (orf159) gene encodes an anti-sigma factor of SigB. J. Bacteriol. 181:2846–2851.

Moks, T., L. Abrahmsen, B. Nilsson, U. Hellman, J. Sjöquist, and M. Uhlen. 1986. Staphylococcal protein A consists of five IgG-binding domains. Eur. J. Biochem. 156:637–643.

Morath, S., A. Geyer, and T. Hartung. 2001. Structure-function relationship of cytokine induction by lipoteichoic acid from Staphylococcus aureus. J. Exp. Med. 193:393–397.

Morath, S., A. Geyer, I. Spreitzer, C. Hermann, and T. Hartung. 2002. Structural decomposition and heterogeneity of commercial lipoteichoic acid preparations. Infect. Immun. 70:938–944.

Moreillon, P., J. M. Entenza, P. Francioli, D. McDevitt, T. J. Foster, P. Francois, and P. Vaudaux. 1995. Role of Staphylococcus aureus coagulase and clumping factor in pathogenesis of experimental endocarditis. Infect. Immun. 63:4738–4743.

Morioka, H., M. Tachibana, and A. Suganuma. 1987. Ultrastructural localization of carbohydrates on thin sections of Staphylococcus aureus with silver methenamine and wheat germ agglutinin-gold complex. J. Bacteriol. 169:1358–1362.

Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken. 1999. In: Manual of Clinical Microbiology. American Society for Microbiology. Washington, DC.

Navarre, W. W., S. Daefler, and O. Schneewind. 1996. Cell wall sorting of lipoproteins in Staphylococcus aureus. J. Bacteriol. 178:441–446.

Nerz, J., R. Rosenstein, and F. Götz. 2002. Comparison of virulence factors in Staphylococcus aureus N315 and Staphylococcus epidermidis RP62A. In: International Conference: Genomics in Infectious Diseases, Würzburg. Würzburg, Germany.

Neubauer, H., and F. Götz. 1996. Physiology and interaction of nitrate and nitrite reduction in Staphylococcus carnosus. J. Bacteriol. 178:2005–2009.

Neubauer, H., I. Pantel, and F. Götz. 1998. Characterization of moeB—part of the molybdenum cofactor biosynthesis gene cluster in Staphylococcus carnosus. FEMS Microbiol. Lett. 164:55–62.

Neubauer, H., I. Pantel, and F. Götz. 1999a. Molecular characterization of the nitrite-reducing system of Staphylococcus carnosus. J. Bacteriol. 181:1481–1488.

Neubauer, H., I. Pantel, P. E. Lindgren, and F. Götz. 1999b. Characterization of the molybdate transport system ModABC of Staphylococcus carnosus. Arch. Microbiol. 172:109–115.

Ni Eidhin, D., S. Perkins, P. Francois, P. Vaudaux, M. Höök, and T. J. Foster. 1998. Clumping factor B (ClfB), a new surface-located fibrinogen-binding adhesin of Staphylococcus aureus. Molec. Microbiol. 30:245–257.

Nieto, M., H. R. Perkins, and P. E. Reynolds. 1972. Reversal by a specific peptide (diacetyl-alpha gamma-L-diaminobutyryl-D-alanyl-D-alanine) of vancomycin inhibition in intact bacteria and cell-free preparations. Biochem. J. 126:139–149.

Niinivaara, F. P., and M. S. Pohja. 1956. Über die Reifung der Rohwurst. I. Mitt.: Die Veränderung der Bakterienflora während der Reifung. Zeitschr. Lebensm. Untersuch. Forsch. 104:413–422.

Nikoleit, K., R. Rosenstein, H. M. Verheij, and F. Götz. 1995. Comparative biochemical and molecular analysis of the Staphylococcus hyicus, Staphylococcus aureus and a hybrid lipase. Indication for a C-terminal phospholipase domain. Eur. J. Biochem. 228:732–738.

Niyomporn, B., J. L. Dahl, and J. L. Stromimger. 1968. Biosynthesis of the peptidoglycan of bacterial cell walls. IX: Purification and properties of glycyl transfer ribonucleic acid synthetase from Staphylococcus aureus. J. Biol. Chem. 243:773–778.

Noble, W. C., and D. A. Somerville. 1974. Microbiology of Human Skin. W. B. Saunders. London, UK.

Noble, W. C., and D. G. Pitcher. 1978. Microbial ecology of the human skin. Adv. Microb. Ecol. 2:245–289.

Noble, W. C., Z. Virani, and R. G. Cree. 1992. Co-transfer of vancomycin and other resistance genes from Enterococcus faecalis NCTC 12201 to Staphylococcus aureus. FEMS Microbiol. Lett. 72:195–198.

Novick, R. P., H. F. Ross, S. J. Projan, J. Kornblum, B. Kreiswirth, and S. Moghazeh. 1993. Synthesis of staphylococcal virulence factors is controlled by a regulatory RNA molecule. EMBO J. 12:3967–3975.

Novick, R. P., S. J. Projan, J. Kornblum, H. F. Ross, G. Ji, B. Kreiswirth, F. Vandenesch, and S. Moghazeh. 1995. The agr P2 operon: An autocatalytic sensory transduction system in Staphylococcus aureus. Molec. Gen. Genet. 248:446–458.

Oeding, P., V. Hajek, and E. Marsalek. 1973. A comparison of antigenic structure and phage pattern with biochemical properties of Staphylococcus aureus strains isolated from hares and mink. Acta Pathol. Microbiol. Scand. [B] Microbiol. Immunol. 81:567–570.

Ogston, A. 1883. Micrococcus poisoning. J. Anat. Physiol. (London) 17:24–58.

Opitz, B., N. W. Schröder, I. Spreitzer, K. S. Michelsen, C. J. Kirschning, W. Hallatschek, U. Zähringer, T. Hartung, U. B. Gobel, and R. R. Schumann. 2001. Toll-like receptor-2 mediates Treponema glycolipid and lipoteichoic acid-induced NF-kappaB translocation. J. Biol. Chem. 276:22041–22047.

Oskouian, B., and G. C. Stewart. 1987. Cloning and characterization of the repressor gene of the Staphylococcus aureus lactose operon. J. Bacteriol. 169:5459–5465.

Oskouian, B., and G. C. Stewart. 1990a. Repression and catabolite repression of the lactose operon of Staphylococcus aureus. J. Bacteriol. 172:3804–3812.

Oskouian, B., E. L. Rosy, F. Breidt Jr., and G. C. Stewart. 1990b. The lactose operon of Staphylococcus aureus. In: R. Novick (Ed.) Molecular Biology of the Staphylococci. VCH Publishers. New York, NY.

Otto, M., R. Süssmuth, G. Jung, and F. Götz. 1998. Structure of the pheromone peptide of the Staphylococcus epidermidis agr system. FEBS Lett. 424:89–94.

Otto, M., R. Süssmuth, C. Vuong, G. Jung, and F. Götz. 1999. Inhibition of virulence factor expression in Staphylococcus aureus by the Staphylococcus epidermidis agr pheromone and derivatives. FEBS Lett. 450:257–262.

Otto, M., H. Echner, W. Voelter, and F. Götz. 2001. Pheromone cross-inhibition between Staphylococcus aureus

and Staphylococcus epidermidis. Infect. Immun. 69:1957–1960.

Pabst, M. J., S. Beranova-Giorgianni, and J. M. Krueger. 1999. Effects of muramyl peptides on macrophages, monokines, and sleep. Neuroimmunomodulation 6:261–283.

Palma, M., S. Nozohoor, T. Schennings, A. Heimdahl, and J. I. Flock. 1996. Lack of the extracellular 19-kilodalton fibrinogen-binding protein from Staphylococcus aureus decreases virulence in experimental wound infection. Infect. Immun. 64:5284–5289.

Pantel, I., P. E. Lindgren, H. Neubauer, and F. Götz. 1998. Identification and characterization of the Staphylococcus carnosus nitrate reductase operon. Molec. Gen. Genet. 259:105–114.

Park, P. W., D. D. Roberts, L. E. Grosso, W. C. Parks, J. Rosenbloom, W. R. Abrams, and R. P. Mecham. 1991. Binding of elastin to Staphylococcus aureus. J. Biol. Chem. 266:23399–23406.

Patti, J. M., T. Bremell, D. Krajewska-Pietrasik, A. Abdelnour, A. Tarkowski, C. Ryden, and M. Höök. 1994. The Staphylococcus aureus collagen adhesin is a virulence determinant in experimental septic arthritis. Infect. Immun. 62:152–161.

Perry, A. M., H. Ton-That, S. K. Mazmanian, and O. Schneewind. 2002. Anchoring of surface proteins to the cell wall of Staphylococcus aureus III: Lipid II is an in vivo peptidoglycan substrate for sortase-catalyzed surface protein anchoring. J. Biol. Chem. 277:16241–16248.

Peschel, A., B. Ottenwälder, and F. Götz. 1996. Inducible production and cellular location of the epidermin biosynthetic enzyme EpiB using an improved staphylococcal expression system. FEMS Microbiol. Lett. 137:279–284.

Peschel, A., M. Otto, R. W. Jack, H. Kalbacher, G. Jung, and F. Götz. 1999. Inactivation of the dlt operon in Staphylococcus aureus confers sensitivity to defensins, protegrins, and other antimicrobial peptides. J. Biol. Chem. 274:8405–8410.

Peschel, A., R. W. Jack, M. Otto, L. V. Collins, P. Staubitz, G. Nicholson, H. Kalbacher, W. F. Nieuwenhuizen, G. Jung, A. Tarkowski, K. P. van Kessel, and J. A. van Strijp. 2001. Staphylococcus aureus resistance to human defensins and evasion of neutrophil killing via the novel virulence factor MprF is based on modification of membrane lipids with L-lysine. J. Exp. Med. 193:1067–1076.

Peschel, A. 2002. How do bacteria resist human antimicrobial peptides?. Trends Microbiol. 10:179–186.

Peters, G., R. Locci, and G. Pulverer. 1981. Microbial colonization of prosthetic devices. II: Scanning electron microscopy of naturally infected intravenous catheters. Zentralbl. Bakteriol. Mikrobiol. Hyg. B 173:293–299.

Peters, D., R. Frank, and W. Hengstenberg. 1995. Lactose-specific enzyme II of the phosphoenolpyruvate-dependent phosphotransferase system of Staphylococcus aureus. Purification of the histidine-tagged transmembrane component IICBLac and its hydrophilic IIB domain by metal-affinity chromatography, and functional characterization. Eur. J. Biochem. 228:798–804.

Petras, P. 1998. Staphylococcus pulvereri = Staphylococcus vitulus?. Int. J. Syst. Bacteriol. 48 Pt 2:617–618.

Phillips, W. E., R. E. King, and W. E. Kloos. 1980. Isolation of Staphylococcus hyicus subsp hyicus from a pig with septic polyarthritis. Am. J. Vet. Res. 41:274–276.

Pinho, M. G., H. de Lencastre, and A. Tomasz. 2001. An acquired and a native penicillin-binding protein cooperate in building the cell wall of drug-resistant staphylococci. Proc. Natl. Acad. Sci. USA 98:10886–10891.

Pioch, G., H. Heyne, and W. Witte. 1988. Koagulase-negative Staphylokokken-Spezies in Mischfuttermitteln und auf Getreide. Zentralbl Mikrobiol 143:157–171.

Pless, D. D., and F. C. Neuhaus. 1973. Initial membrane reaction in peptidoglycan synthesis: Lipid dependence of phospho-n-acetylmuramyl-pentapeptide translocase (exchange reaction). J. Biol. Chem. 248:1568–1576.

Pollock, H. M. 1983. Laboratory techniques for detection of urinary tract infection and assessment of value. Am. J. Med. 75:79–84.

Pooley, H. M., and D. Karamata. 1994. Teichoic acid synthesis in Bacillus subtilis: Genetic organization and biological roles. *In:* J.-M. Ghuysen and R. Hakenbeck (Eds.) Bacterial Cell Wall. Elsevier Science. Amsterdam, The Netherlands.

Poolman, B., J. Knol, C. van der Does, P. J. Henderson, W. J. Liang, G. Leblanc, T. Pourcher, and I. Mus-Veteau. 1996. Cation and sugar selectivity determinants in a novel family of transport proteins. Molec. Microbiol. 19:911–922.

Poutrel, B. 1984. Udder infection of goats by coagulase-negative staphylococci. Vet. Microbiol. 9:131–137.

Price, P. B. 1938. The bacteriology of normal skin: A new quantitative test applied to a study of the bacterial flora and the disinfectant action of mechanical cleansing. J. Infect. Dis. 63:301–318.

Probst, A. J., C. Hertel, L. Richter, L. Wassill, W. Ludwig, and W. P. Hammes. 1998. Staphylococcus condimenti sp. nov., from soy sauce mash, and Staphylococcus carnosus (Schleifer and Fischer 1982) subsp. utilis subsp. nov. Int. J. Syst. Bacteriol. 48 Pt 3:651–658.

Pucci, M. J., J. A. Thanassi, H. T. Ho, P. J. Falk, and T. J. Dougherty. 1995. Staphylococcus haemolyticus contains two D-glutamic acid biosynthetic activities, a glutamate racemase and a D-amino acid transaminase. J. Bacteriol. 177:336–342.

Rajagopalan, K. V., and J. L. Johnson. 1992. The pterin molybdenum cofactors. J. Biol. Chem. 267:10199–10202.

Rajagopalan, K. V. 1996. Biosynthesis of the molybdenum cofactor. *In:* Escherichia coli and Salmonella, F. C. Neidhardt (Ed.). ASM Press. Washington, DC. 1:674–679.

Rasooly, A., and R. S. Rasooly. 1998. Detection and analysis of Staphylococcal enterotoxin A in food by Western immunoblotting. Int. J. Food Microbiol. 41:205–212.

Rehman, A., P. Taishi, J. Fang, J. A. Majde, and J. M. Krueger. 2001. The cloning of a rat peptidoglycan recognition protein (PGRP) and its induction in brain by sleep deprivation. Cytokine 13:8–17.

Reiche, B., R. Frank, J. Deutscher, N. Meyer, and W. Hengstenberg. 1988. Staphylococcal phosphoenolpyruvate-dependent phosphotransferase system: Purification and characterization of the mannitol-specific enzyme IIImtl of Staphylococcus aureus and Staphylococcus carnosus and homology with the enzyme IImtl of Escherichia coli. Biochemistry 27:6512–6516.

Reizer, J., M. H. Saier, Jr., J. Deutscher, F. Grenier, J. Thompson, and W. Hengstenberg. 1988. The phosphoenolpyruvate:sugar phosphotransferase system in Gram-positive bacteria: Properties, mechanism, and regulation. Crit. Rev. Microbiol. 15:297–338.

Reizer, J., S. L. Sutrina, M. H. Saier, G. C. Stewart, A. Peterkofsky, and P. Reddy. 1989. Mechanistic and physiological consequences of HPr(ser) phosphorylation on the activities of the phosphoenolpyruvate:sugar phosphotransferase system in Gram-positive bacteria: Studies with site-specific mutants of HPr. EMBO J. 8:2111–2120.

Roberts, R. J., G. G. Lovinger, T. Tamura, and J. L. Strominger. 1974. Staphylococcal transfer ribonucleic acids. I. Isolation and purification of the isoaccepting glycine transfer ribonucleic acids from Staphylococcus epidermidis Texas 26. J. Biol. Chem. 249:4781–4786.

Rogers, H. J., H. R. Perkins, and J. B. Ward. 1980a. Antibiotics affecting bacterial wall synthesis. In: H. J. Rogers, H. R. Perkins, and J. B. Ward (Eds.) Microbial Cell Walls and Membranes. Chapman and Hall. London, UK. 298–382.

Rogers, H. J., H. R. Perkins, and J. B. Ward (Eds.). 1980b. Microbial Cell Walls and Membranes. Chapman and Hall. London, UK.

Rohrer, S., K. Ehlert, M. Tschierske, H. Labischinski, and B. Berger-Bächi. 1999. The essential Staphylococcus aureus gene fmhB is involved in the first step of peptidoglycan pentaglycine interpeptide formation. Proc. Natl. Acad. Sci. USA 96:9351–9356.

Rosenbach, F. J. 1884. Mikroorganismen bei den Wundinfections-Krankheiten des Menschen. Wiesbaden, Germany.

Rosey, E. L., B. Oskouian, and G. C. Stewart. 1991. Lactose metabolism by Staphylococcus aureus: Characterization of lacABCD, the structural genes of the tagatose 6-phosphate pathway. J. Bacteriol. 173:5992–5998.

Rupp, M. E., and G. L. Archer. 1994. Coagulase-negative staphylococci: Pathogens associated with medical progress. Clin. Infect. Dis. 19:231–243.

Rupp, M. E., P. D. Fey, C. Heilmann, and F. Götz. 2001. Characterization of the importance of Staphylococcus epidermidis autolysin and polysaccharide intercellular adhesin in the pathogenesis of intravascular catheter-associated infection in a rat model. J. Infect. Dis. 183:1038–1042.

Ryden, C., A. Yacoub, G. Hirsch, M. Wendel, A. Oldberg, and A. Ljungh. 1990. Binding of bone sialoprotein to Staphylococcus epidermidis isolated from a patient with chronic recurrent multifocal osteomyelitis. J. Infect. Dis. 161:814–815.

Saenz, H. L., V. Augsburger, C. Vuong, R. W. Jack, F. Götz, and M. Otto. 2000. Inducible expression and cellular location of AgrB, a protein involved in the maturation of the staphylococcal quorum-sensing pheromone. Arch. Microbiol. 174:452–455.

Sahl, H. G., and G. Bierbaum. 1998. Lantibiotics: Biosynthesis and biological activities of uniquely modified peptides from Gram-positive bacteria. Ann. Rev. Microbiol. 52:41–79.

Saier Jr., M. H., S. Chauvaux, J. Deutscher, J. Reizer, and J. J. Ye. 1995. Protein phosphorylation and regulation of carbon metabolism in Gram-negative versus Gram-positive bacteria. Trends Biochem. Sci. 20:267–271.

Saier Jr., M. H., S. Chauvaux, G. M. Cook, J. Deutscher, I. T. Paulsen, J. Reizer, and J. J. Ye. 1996. Catabolite repression and inducer control in Gram-positive bacteria. Microbiology 142:217–230.

Salton, M. R. J. 1994. The bacterial cell envelope: A historical perspective. In: J. M. Ghuysen and R. Hakenbeck (Eds.) Bacterial Cell Wall. Elsevier. London, UK.

Samuelson, P., M. Hansson, N. Ahlborg, C. Andreoni, F. Götz, T. Bächi, T. N. Nguyen, H. Binz, M. Uhlen, and S. Stahl. 1995. Cell surface display of recombinant proteins on Staphylococcus carnosus. J. Bacteriol. 177:1470–1476.

Sanderman Jr., H. 1976. Moenomycin: An inhibitor of c55-isoprenoid-alcohol kinase from Staphylococcus aureus. Biochim. Biophys. Acta 444:783–788.

Savolainen, K., L. Paulin, B. Westerlund-Wikstrom, T. J. Foster, T. K. Korhonen, and P. Kuusela. 2001. Expression of pls, a gene closely associated with the mecA gene of methicillin-resistant Staphylococcus aureus, prevents bacterial adhesion in vitro. Infect. Immun. 69:3013–3020.

Schleifer, K.-H., and O. Kandler. 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriol. Rev. 36:407–477.

Schleifer, K.-H., and J. Steber. 1974. Chemical studies on the phage receptor of Staphylococcus epidermidis. Arch. Microbiol. 98:251–270.

Schleifer, K.-H. 1975a. Chemical structure of the peptidoglycan, its modifiability and relation to the biological activity. Zeitschr. Immunitatsforsch. Exp. Klin. Immunol. 149:104–117.

Schleifer, K.-H., and W. E. Kloos. 1975b. A simple test system for the separation of staphylococci from micrococci. J. Clin. Microbiol. 1:337–338.

Schleifer, K.-H., and W. E. Kloos. 1975c. Isolation and characterization of staphylococci from human skin. I: Amended descriptions of Staphylococcus saprophyticus and descriptions of three new species: Staphylococcus cohnii, Staphylococcus haemolyticus, and Staphylococcus xylosus. Int. J. Syst. Bacteriol. 25:50–61.

Schleifer, K.-H., A. Hartinger, and F. Götz. 1978. Occurrence of D-tagatose-6-phosphate pathway of D-galactose metabolism among staphylococci. FEMS Microbiol. Lett. 3:9–11.

Schleifer, K.-H., S. A. Meyer, and M. Rupprecht. 1979. Relatedness among coagulase-negative staphylococci: Deoxyribonucleic acid reassociation and comparative immunological studies. Arch. Microbiol. 122:93–101.

Schleifer, K.-H., and E. Krämer. 1980. Selective medium for isolating staphylococci. Zentralbl. Bakteriol. Hyg. Abt. 1 Orig C 1:270–280.

Schleifer, K.-H., and U. Fischer. 1982. Description of a new species of the genus Staphylococcus: Staphylococcus carnosus. Int. J. Syst. Bacteriol. 32:153–156.

Schleifer, K.-H. 1983. The Cell Envelope. Academic Press. London, UK. 358–428.

Schleifer, K.-H., R. Kilpper-Bälz, and L. A. Devriese. 1984. Staphylococcus arlettae sp. nov., S. equorum sp. nov., and S. kloosii sp. nov.: Three new coagulase-negative staphylococci from animals. Syst. Appl. Microbiol. 5:501–509.

Schleifer, K.-H. 1986. Taxonomy of coagulase-negative staphylococci. In: P.-A. Mardh and K.-H. Schleifer (Eds.) Coagulase-negative Staphylococci. Almquist and Wiksell International. Stockholm, Sweden. 11–26.

Schmidt, K. A., A. C. Manna, S. Gill, and A. L. Cheung. 2001. SarT, a repressor of alpha-hemolysin in Staphylococcus aureus. Infect. Immun. 69:4749–4758.

Schneewind, O., P. Model, and V. A. Fischetti. 1992. Sorting of protein A to the staphylococcal cell wall. Cell 70:267–281.

Schneewind, O., D. Mihaylova-Petkov, and P. Model. 1993. Cell wall sorting signals in surface proteins of Gram-positive bacteria. EMBO J. 12:4803–4811.

Schrecker, O., R. Stein, W. Hengstenberg, M. Gassner, and D. Stehlik. 1975. The staphylococcal PEP dependent phosphotransferase system, proton magnetic resonance (PMR) studies on the phosphoryl carrier protein HPr: Evidence for a phosphohistidine residue in the intact phospho-HPr molecule. FEBS Lett. 51:309–312.

Schumacher, M. A., B. K. Hurlburt, and R. G. Brennan. 2001. Crystal structures of SarA, a pleiotropic regulator of virulence genes in S. aureus. Nature 409:215–219.

Scott, J. M., T. Mitchell, and W. G. Haldenwang. 2000. Stress triggers a process that limits activation of the Bacillus subtilis stress transcription factor sigma(B). J. Bacteriol. 182:1452–1456.

Sharma, V. K., C. J. Hackbarth, T. M. Dickinson, and G. L. Archer. 1998. Interaction of native and mutant MecI repressors with sequences that regulate mecA, the gene encoding penicillin binding protein 2a in methicillin-resistant staphylococci. J. Bacteriol. 180:2160–2166.

Signäs, C., G. Raucci, K. Jonsson, P. E. Lindgren, G. M. Anantharamaiah, M. Hook, and M. Lindberg. 1989. Nucleotide sequence of the gene for a fibronectin-binding protein from Staphylococcus aureus: Use of this peptide sequence in the synthesis of biologically active peptides. Proc. Natl. Acad. Sci. USA 86:699–703.

Silvestri, L. G., and L. R. Hill. 1965. Agreement between deoxyroibonucleic acid base composition and taxonomic classification of Gram-positive cocci. J. Bacteriol. 90:136–140.

Sinell, H. J., and J. Baumgart. 1966. Selektivnährböden zur Isolierung von Staphylokokken aus Lebensmitteln. Zentralbl. Bakteriol. Abt. 1 Orig 197:447–461.

Sivakanesan, R., and E. A. Dawes. 1980. Anaerobic glucose and serine metabolism in Staphylococcus epidermidis. J. Gen. Microbiol. 118:143–157.

Sizemore, C., E. Buchner, T. Rygus, C. Witke, F. Götz, and W. Hillen. 1991. Organization, promoter analysis and transcriptional regulation of the Staphylococcus xylosus xylose utilization operon. Molec. Gen. Genet. 227:377–384.

Sizemore, C., B. Wieland, F. Götz, and W. Hillen. 1992. Regulation of Staphylococcus xylosus xylose utilization genes at the molecular level. J. Bacteriol. 174:3042–3048.

Sjödahl, J. 1977. Repetitive sequences in protein A from Staphylococcus aureus: Arrangement of five regions within the protein, four being highly homologous and Fc-binding. Eur. J. Biochem. 73:343–351.

Sliz, P., R. Engelmann, W. Hengstenberg, and E. F. Pai. 1997. The structure of enzyme IIAlactose from Lactococcus lactis reveals a new fold and points to possible interactions of a multicomponent system. Structure 5:775–788.

Snowden, M. A., and H. R. Perkins. 1990. Peptidoglycan cross-linking in Staphylococcus aureus: An apparent random polymerisation process. Eur. J. Biochem. 191:373–377.

Sobek, H. M., K. Stüber, K. Beyreuther, W. Hengstenberg, and J. Deutscher. 1984. Staphylococcal phospho-enolpyruvate-dependent phosphotransferase system: Purification and characterization of a defective lactose-specific Factor III protein. Biochemistry 23:4460–4464.

Solozhenkin, I. P., N. V. Kukushkina, R. T. Kutsemako, L. M. Kotelevets, V. A. Ermachenko, M. A. Lukoianova, and D. Burbaev. 1991. Iron-sulfur centers in the Staphylococcus aureus respiratory chain. Biokhimiia 56:1259–1263.

Somerville, G. A., S. B. Beres, J. R. Fitzgerald, F. R. DeLeo, R. L. Cole, J. S. Hoff, and J. M. Musser. 2002. In vitro serial passage of Staphylococcus aureus: Changes in physiology, virulence factor production, and agr nucleotide sequence. J. Bacteriol. 184:1430–1437.

Somner, E. A., and P. E. Reynolds. 1990. Inhibition of peptidoglycan biosynthesis by ramoplanin. Antimicrob. Agents Chemother. 34:413–419.

Stranden, A. M., K. Ehlert, H. Labischinski, and B. Berger-Bächi. 1997. Cell wall monoglycine cross-bridges and methicillin hypersusceptibility in a femAB null mutant of methicillin-resistant Staphylococcus aureus. J. Bacteriol. 179:9–16.

Strasters, K. C., and K. C. Winkler. 1963. Carbohydrate metabolism of Staphylococcus hyicus. J. Gen. Microbiol. 33:213–229.

Strauss, A., and F. Götz. 1996. In vivo immobilization of enzymatically active polypeptides on the cell surface of Staphylococcus carnosus. Molec. Microbiol. 21:491–500.

Strauss, A., G. Thumm, and F. Götz. 1998. Influence of Lif, the lysostaphin immunity factor, on acceptors of surface proteins and cell wall sorting efficiency in Staphylococcus carnosus. J. Bacteriol. 180:4960–4962.

Strominger, J. L. 1965. Biochemistry of the cell wall of Staphylococcus aureus. Ann. NY Acad. Sci. 128:59–61.

Stüber, K., J. Deutscher, H. M. Sobek, W. Hengstenberg, and K. Beyreuther. 1985. Amino acid sequence of the amphiphilic phosphocarrier protein factor IIILac of the lactose-specific phosphotransferase system of Staphylococcus. Biochemistry 24:1164–1168.

Stülke, J., M. Arnaud, G. Rapoport, and I. Martin-Verstraete. 1998. PRD—a protein domain involved in PTS-dependent induction and carbon catabolite repression of catabolic operons in bacteria. Molec. Microbiol. 28:865–874.

Stülke, J., and W. Hillen. 1999. Carbon catabolite repression in bacteria. Curr. Opin. Microbiol. 2:195–201.

Sturmfels, A., F. Götz, and A. Peschel. 2001. Secretion of human growth hormone by the food-grade bacterium Staphylococcus carnosus requires a propeptide irrespective of the signal peptide used. Arch. Microbiol. 175:295–300.

Sugai, M., T. Fujiwara, H. Komatsuzawa, and H. Suginaka. 1998. Identification and molecular characterization of a gene homologous to epr (endopeptidase resistance gene) in Staphylococcus aureus. Gene 224:67–75.

Suginaka, H., M. Shimatani, M. Ogawa, and S. Kotani. 1979. Prevention of penicillin-induced lysis of Staphylococcus aureus by cellular lipoteichoic acid. J. Antibiot. (Tokyo) 32:73–77.

Suzuki, S., A. Kubo, H. Shinano, and K. Takama. 1992. Inhibition of the electron transport system in Staphylococcus aureus by trimethylamine-N-oxide. Microbios 71:145–148.

Taber, H. W., and M. Morrison. 1964. Electron transport in staphylococci: Properties of a particle preparation from exponential phase Staphylococcus aureus. Biochem. Biophys. 105:367–379.

Takatsuki, A., and G. Tamura. 1971. Tunicamycin, a new antibiotic. II: Some biological properties of the antiviral activity of tunicamycin. J. Antibiot. (Tokyo) 24:224–231.

Tamarapu, S., J. L. McKillip, and M. Drake. 2001. Development of a multiplex polymerase chain reaction assay for detection and differentiation of Staphylococcus aureus in dairy products. J. Food Prot. 64:664–668.

Tanasupawat, S., Y. Hashimoto, T. Ezaki, M. Kozaki, and K. Komagata. 1992. Staphylococcus piscifermentans sp. nov., from fermented fish in Thailand. Int. J. Syst. Bacteriol. 42:577–581.

Tegmark, K., E. Morfeldt, and S. Arvidson. 1998. Regulation of agr-dependent virulence genes in Staphylococcus aureus by RNAIII from coagulase-negative staphylococci. J. Bacteriol. 180:3181–3186.

Tegmark, K., A. Karlsson, and S. Arvidson. 2000. Identification and characterization of SarH1, a new global regulator of virulence gene expression in Staphylococcus aureus. Molec. Microbiol. 37:398–409.

Theodore, T. S., and A. L. Schade. 1965. Carbohydrate metabolism of iron-rich and iron-poor Staphylococcus aureus. J. Gen. Microbiol. 40:385–395.

Thumm, G., and F. Götz. 1997. Studies on prolysostaphin processing and characterization of the lysostaphin immunity factor (Lif) of Staphylococcus simulans biovar staphylolyticus. Molec. Microbiol. 23:1251–1265.

Timoney, J. F., J. H. Gillespie, F. W. Scott, and J. E. Barlough. 1988. Hagan and Bruner's Microbiology and Infectious Diseases of Domestic Animals. Comstock Publishing. Ithaca, NY.

Tipper, D. J., W. Katz, J. L. Strominger, and J. M. Ghuysen. 1967. Substituents on the alpha-carboxyl group of D-glutamic acid in the peptidoglycan of several bacterial cell walls. Biochemistry 6:921–929.

Tipper, D. J., and J. L. Strominger. 1968. Biosynthesis of the peptidoglycan of bacterial cell walls. XII: Inhibition of cross-linking by penicillins and cephalosporins: Studies in Staphylococcus aureus in vivo. J. Biol. Chem. 243:3169–3179.

Tipper, D. J., and M. F. Berman. 1969. Structures of the cell wall peptidoglycans of Staphylococcus epidermidis Texas 26 and Staphylococcus aureus Copenhagen. I: Chain length and average sequence of cross-bridge peptides. Biochemistry 8:2183–2192.

Tipper, D. J., M. Tomoeda, and J. L. Strominger. 1971. Isolation and characterization of 1,4-N-acetylmuramyl-N-acetylglucosamine and its O-acetyl derivative. Biochemistry 10:4683–4690.

Tung, H., B. Guss, U. Hellman, L. Persson, K. Rubin, and C. Ryden. 2000. A bone sialoprotein-binding protein from Staphylococcus aureus: A member of the staphylococcal Sdr family. Biochem. J. 345:611–619.

Tynecka, Z., and M. Gajdzinska. 1967. The activity of lactate, succinate and malate dehydrogenase in staphylococci. Acta Microbiol. Pol. 16:43–51.

Uhlen, M., B. Guss, B. Nilsson, S. Gatenbeck, L. Philipson, and M. Lindberg. 1984. Complete sequence of the staphylococcal gene encoding protein A. J. Biol. Chem. 259:1695–1702.

United States Food, and Drug Administration. 1995. Bacteriological Analytical Manual. Center for Food and Safety and Applied Nutrition. Washington, DC. Appendix 2.

Vandenesch, F., S. J. Projan, B. Kreiswirth, J. Etienne, and R. P. Novick. 1993. Agr-related sequences in Staphylococcus lugdunensis. FEMS Microbiol. Lett. 111:115–122.

Vandenesch, F., S. J. Eykyn, M. Bes, H. Meugnier, J. Fleurette, and J. Etienne. 1995. Identification and ribotypes of Staphylococcus caprae isolates isolated as human pathogens and from goat milk. J. Clin. Microbiol. 33:888–892.

Varaldo, P. E., R. Kilpper-Bälz, F. Biavasco, G. Satta, and K.-H. Schleifer. 1988. Staphylococcus delphini sp. nov., a coagulase-positive species isolated from dolphins. Int. J. Syst. Bacteriol. 38:436–439.

Vercellotti, G. M., J. B. McCarthy, P. Lindholm, P. K. Peterson, H. S. Jacob, and L. T. Furcht. 1985. Extracellular matrix proteins (fibronectin, laminin, and type IV collagen) bind and aggregate bacteria. Am. J. Pathol. 120:13–21.

Vernozy-Rozand, C., C. Mazuy, H. Meugnier, M. Bes, Y. Lasne, F. Fiedler, J. Etienne, and J. Freney. 2000. Staphylococcus fleurettii sp. nov., isolated from goat's milk cheeses. Int. J. Syst. Evol. Microbiol. 50 Pt 4:1521–1527.

Wagner, E., F. Götz, and R. Brückner. 1993. Cloning and characterization of the scrA gene encoding the sucrose-specific Enzyme II of the phosphotransferase system from Staphylococcus xylosus. Molec. Gen. Genet. 241:33–41.

Wagner, E., S. Marcandier, O. Egeter, J. Deutscher, F. Götz, and R. Brückner. 1995. Glucose kinase-dependent catabolite repression in Staphylococcus xylosus. J. Bacteriol. 177:6144–6152.

Ward, J. B., and H. R. Perkins. 1973. The direction of glycan synthesis in a bacterial peptidoglycan. Biochem. J. 135:721–728.

Warren, G. H., and J. Gray. 1965. Effect of sublethal concentrations of penicillins on the lysis of bacteria by lysozyme and trypsin. Proc. Soc. Exp. Biol. Med. 120:504–511.

Watts, J. L., J. W. Pankey, and S. C. Nickerson. 1984. Evaluation of the Staph-Ident and STAPHase systems for identification of staphylococci from bovine intramammary infections. J. Clin. Microbiol. 20:448–452.

Webster, J. A., T. L. Bannerman, R. J. Hubner, D. N. Ballard, E. M. Cole, J. L. Bruce, F. Fiedler, K. Schubert, and W. E. Kloos. 1994. Identification of the Staphylococcus sciuri species group with EcoRI fragments containing rRNA sequences and description of Staphylococcus vitulus sp. nov. Int. J. Syst. Bacteriol. 44:454–460.

Wecke, J., M. Lahav, I. Ginsburg, E. Kwa, and P. Giesbrecht. 1986. Inhibition of wall autolysis of staphylococci by sodium polyanethole sulfonate "liquoid." Arch. Microbiol. 144:110–115.

Weickert, M. J., and S. Adhya. 1992. A family of bacterial regulators homologous to Gal and Lac repressors. J. Biol. Chem. 267:15869–15874.

Weidenmaier, C., Kokai-Kun, J. F., Kristian, S. A., Chanturiya, T., Kalbacher, H., Gross, M., Nicholson, G., Neumeister, B., Mond, J. J., and Peschel, A. 2004. Role of teichoic acids in Staphylococcus aureus nasal colonization, a major risk factor in nosocomial infections. Nat. Med. 10:243–245.

Weppner, W. A., and F. C. Neuhaus. 1977. Fluorescent substrate for nascent peptidoglycan synthesis: Uridinediphosphate-N-acetylmuramyl-(Nepsilon-5-dimethylaminonaphthalene-1-sulfonyl)pentapeptide. J. Biol. Chem. 252:2296–2303.

White, D. C., and F. E. Frerman. 1967. Extraction, characterization, and cellular localization of the lipids of Staphylococcus aureus. J. Bacteriol. 94:1854–1867.

Wicken, A. J., and K. W. Knox. 1980. Bacterial cell surface amphiphiles. Biochim. Biophys. Acta 604:1–26.

Wicken, A. J., J. D. Evans, and K. W. Knox. 1986. Critical micelle concentrations of lipoteichoic acids. J. Bacteriol. 166:72–77.

Wickus, G. G., P. A. Rubenstein, A. D. Warth, and J. L. Strominger. 1973. Partial purification and some properties of the uridine diphospho-N-acetylglucosamine-enolpyruvate reductase from Staphylococcus epidermidis. J. Bacteriol. 113:291–294.

Wieland, K. P., B. Wieland, and F. Götz. 1995. A promoter-screening plasmid and xylose-inducible, glucose-repressible expression vectors for Staphylococcus carnosus. Gene 158:91–96.

Willis, A. T., J. J. O'Connor, and J. A. Smith. 1966. Colonial pigmentation of Staphylococcus aureus. J. Pathol. Bacteriol. 92:97–106.

Winslow, C. E. A., and A. R. Winslow. 1908. The Systematic Relationships of the Coccaceae. John Wiley. New York, NY.

Witke, C., and F. Götz. 1993. Cloning, sequencing, and characterization of the gene encoding the class I fructose-1,6-bisphosphate aldolase of Staphylococcus carnosus. J. Bacteriol. 175:7495–7499.

Wu, S. W., H. de Lencastre, and A. Tomasz. 2001. Recruitment of the mecA gene homologue of Staphylococcus sciuri into a resistance determinant and expression of the resistant phenotype in Staphylococcus aureus. J. Bacteriol. 183:2417–2424.

Yacoub, A., P. Lindahl, K. Rubin, M. Wendel, D. Heinegard, and C. Ryden. 1994. Purification of a bone sialoprotein-binding protein from Staphylococcus aureus. Eur. J. Biochem. 222:919–925.

Yarwood, J. M., J. K. McCormick, and P. M. Schlievert. 2001. Identification of a novel two-component regulatory system that acts in global regulation of virulence factors of Staphylococcus aureus. J. Bacteriol. 183:1113–1123.

Yipp, B. G., G. Andonegui, C. J. Howlett, S. M. Robbins, T. Hartung, M. Ho, and P. Kubes. 2002. Profound differences in leukocyte-endothelial cell responses to lipopolysaccharide versus lipoteichoic acid. J. Immunol. 168:4650–4658.

Yokoyama, K., T. Miyashita, Y. Araki, and E. Ito. 1986. Structure and functions of linkage unit intermediates in the biosynthesis of ribitol teichoic acids in Staphylococcus aureus H and Bacillus subtilis W23. Eur. J. Biochem. 161:479–489.

Zakrzewska-Czerwinska, J., A. Gaszewska-Mastalarz, B. Lis, A. Gamian, and M. Mordarski. 1995. Staphylococcus pulvereri sp. nov., isolated from human and animal specimens. Int. J. Syst. Bacteriol. 45:169–172.

Ziebuhr, W., C. Heilmann, F. Götz, P. Meyer, K. Wilms, E. Straube, and J. Hacker. 1997. Detection of the intercellular adhesion gene cluster (ica) and phase variation in Staphylococcus epidermidis blood culture strains and mucosal isolates. Infect. Immun. 65:890–896.

Ziebuhr, W., V. Krimmer, S. Rachid, I. Lößner, F. Götz, and J. Hacker. 1999. A novel mechanism of phase variation of virulence in Staphylococcus epidermidis: Evidence for control of the polysaccharide intercellular adhesin synthesis by alternating insertion and excision of the insertion sequence element IS256. Molec. Microbiol. 32:345–356.

Ziebuhr, W., K. Dietrich, M. Trautmann, and M. Wilhelm. 2000a. Chromosomal rearrangements affecting biofilm production and antibiotic resistance in a Staphylococcus epidermidis strain causing shunt-associated ventriculitis. Int. J. Med. Microbiol. 290:115–120.

Ziebuhr, W., I. Lössner, S. Rachid, K. Dietrich, F. Götz, and J. Hacker. 2000b. Modulation of the polysaccharide intercellular adhesin (PIA) expression in biofilm forming Staphylococcus epidermidis: Analysis of genetic mechanisms. Adv. Exp. Med. Biol. 485:151–157.

Zopf, W. 1885. Die Spaltpilze. E. Trewendt. Breslau, Poland.