Minireview

Adhesion determinants of the *Streptococcus* species

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Summary

Streptococci are clinically important Gram-positive bacteria that are capable to cause a wide variety of diseases in humans and animals. Phylogenetic analyses based on 16S rRNA sequences of the streptococcal species reveal a clustering pattern, reflecting, with a few exceptions, their pathogenic potential and ecological preferences. Microbial adhesion to host tissues is the initial critical event in the pathogenesis of most infections. Streptococci use multiple adhesins to attach to the epithelium, and their expression is regulated in response to environmental and growth conditions. Bacterial adhesins recognize and bind cell surface molecules and extracellular matrix components through specific domains that for certain adhesin families have been well defined and found conserved across the streptococcal species. In this review, we present the different streptococcal adhesin families categorized on the basis of their adhesive properties and structural characteristics, and, when available, we focus the attention on conserved functional domains.

Introduction

Streptococcal species

The genus *Streptococcus* comprises a wide variety of species among which many are known important human pathogens. The most notable examples are *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Streptococcus agalactiae.* Although related, these species differ greatly in their spectrum of diseases. *Streptococcus pyogenes*, also known as beta-haemolytic group A streptococcus, causes pharyngitis, scarlet fever as well as skin

infections like erysipelas and impetigo; moreover, it can lead to severe invasive infections like bacteraemia, infective endocarditis, cellulitis, and necrotizing fasciitis with high mortality due to streptococcal toxic shock syndrome. Both *S. pneumoniae* and *S. agalactiae* are known as classical causes of pneumoniae, sepsis, meningitis etc., but, remarkably, the molecular mechanisms of their pathogenesis are very different. *Streptococcus pneumoniae* colonizes the nasopharynx in up to 40% of healthy subjects, and is a leading cause of middle ear infections, meningitis and pneumonia in children and of pneumonia and sepsis in the elderly or immunocompromised. *Streptococcus agalactiae* (group B streptococcus: GBS) is a common cause of neonatal pneumonia, sometimes progressing to invasive disease as a result of the aspiration of infected amniotic fluid within the uterus or vaginal secretions during parturition. More recently also other species such as *S. gordonii*, *S. parasanguinis* or *S. sanguinis* have emerged as important human pathogens causing infective endocarditis or acting as colonizers of dental plaque (Moreillon *et al.*, 2002). Moreover, various streptococci are known as the cause of zoonoses in domestic and wild animals (Timoney, 2004; Agnew and Barnes, 2007; Lun *et al.*, 2007).

Oral streptococci (approximately 20% of the oral bacteria) are primary colonizers of the tooth surface and are abundant in dental plaque biofilms, where more than 500 species or phylotypes have been identified. Bacteria growing in these relatively dense, surface-associated communities are phenotypically quite distinct from their planktonic counterparts. The streptococci compete for adhesion binding sites on the saliva-coated tooth surface and are able to produce antimicrobial compounds. *Streptococcus mutans* can, however, become dominant in oral biofilms, leading to dental caries development. *Streptococcus mutans* dominance depends on competition with other bacteria like *S. sanguinis* and *S. gordonii*, and is influenced by bacterial production of antimicrobial compounds, the bacteriocins (Kreth *et al.*, 2008).

The nomenclature of *Streptococcus* species is rather complex as it is not only based on the species names but, for historical reasons, designations are used that are based on haemolysis type or serological grouping. In particular, Lancefield typing based on specific antisera
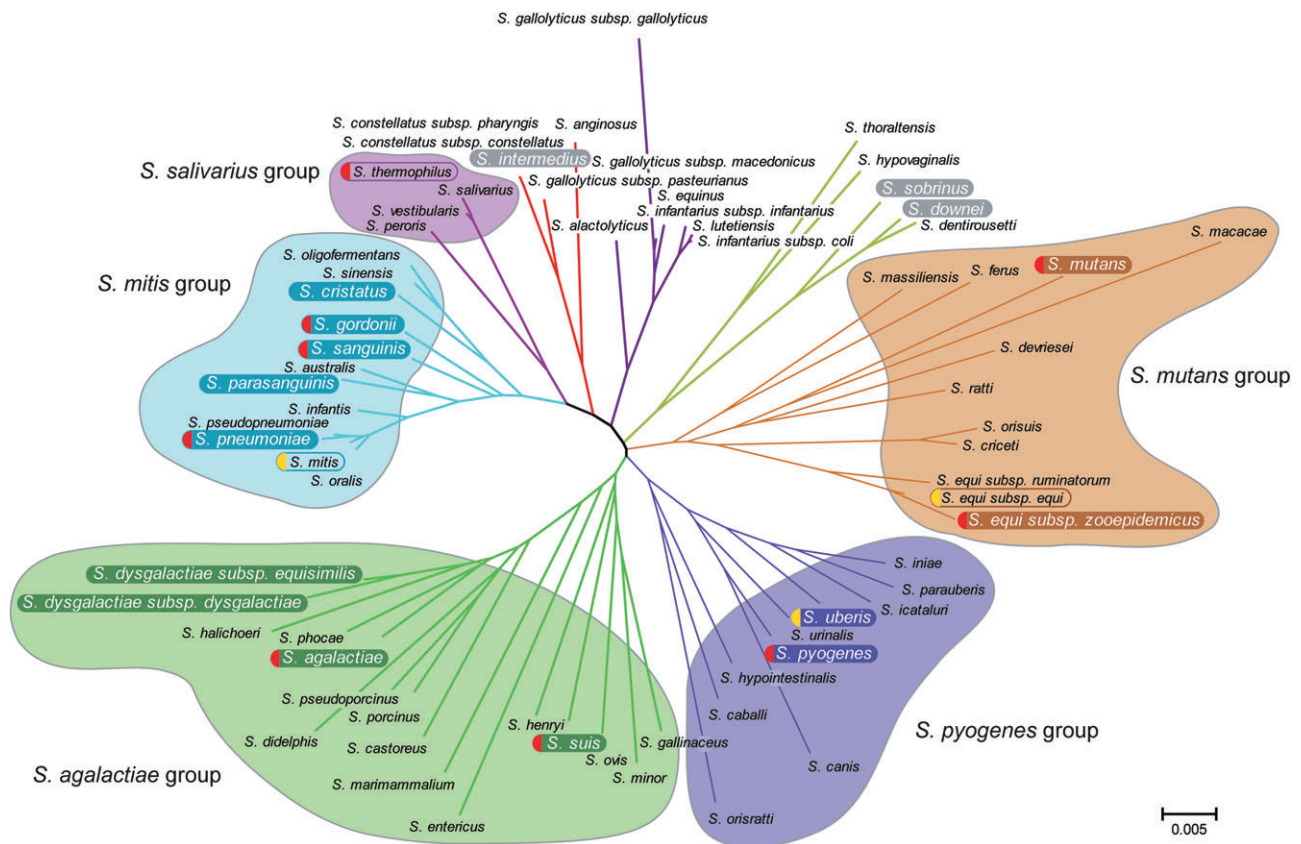
Fig. 1. Phylogeny of *Streptococcus* species based on 16S rRNA conservation. Phylogenetic and molecular evolutionary analyses were conducted using *MEGA* version 4 (Tamura *et al.*, 2004). Main groups are indicated by coloured areas. Streptococcal species in which adhesins were described are marked by filled boxes. Those species for which the complete genome is available are marked with red or yellow half circles. Red indicates that the sequence can be retrieved from NCBI, yellow indicates that the sequence is available either from The Sanger Institute or from the J.C. Venter Institute.

against 'group-specific' carbohydrate or lipoteichoic acid (Group D) antigens has been and is widely used to classify beta-haemolytic streptococci. As an example it turned out, however, that *S. pyogenes*, for which often the terminus GAS (group A *Streptococcus*) is used as a synonym, is not the only *Streptococcus* that may possess the group A antigen because also *S. anginosus* or *S. dysgalactiae* ssp. *equisimilis* may carry the A antigen. On the other hand, a given streptococcal species may have several diverse types of Lancefield antigen e.g. *Streptococcus dysgalactiae* ssp. *equisimilis* may be a carrier of A, C, G or L antigens (Facklam, 2002). In the last decades phylogenetic typing based on 16S rRNA (Fig. 1) has helped the accurate identification of bacterial isolates and the discovery of novel bacteria in clinical microbiology laboratories to form a clearer picture of streptococcal species relationships. In Fig. 1 we identified in the genus *Streptococcus* five major clusters: *S. mitis*, *S. mutans*, *S. salivarius* (viridians streptococci), *S. agalactiae* and *S. pyogenes* (pyogenic group). This analysis reveals a clustering pattern reflecting the pathogenic potential and ecological preferences of the streptococcal species (Kawamura *et al.*,

1995). One exception is the mitis group, which contains one of the leading pathogens, *S. pneumoniae*, along with other species that are prototype commensals of the upper respiratory tract (such as *S. mitis*, *S. oralis* or *S. infantis*). Recent works (Kilian *et al.*, 2008; Bishop *et al.*, 2009) propose the use of concatenated sequences of conserved housekeeping genes in addition to the analysis of presence/absence of virulence-specific genes to better assign newly discovered atypical isolates, identify eventual new species, discriminate between species that are really close in terms of 16S rRNA sequences, and define in this way a standard taxonomic procedure for the genus *Streptococcus*.

Adhesion in streptococci. The process of microbial infection involves a complex series of events that may result in host tissue malfunction and/or destruction. Bacterial adherence to host tissues represents a critical step in the pathogenic process and is usually mediated by bacterial surface-exposed proteins. The latter, organized as either single molecules or ordered structures such as pili or fimbriae, are usually considered potential virulence

factors and key players in the infectious process (Facklam, 2002; Mandlik *et al.*, 2008; Proft and Baker, 2009). Most pathogenic *Streptococcus* species have been shown to bind to host extracellular matrix components (ECM) and cell surface molecules. In many cases, bacterial gene products involved in this interaction have not been well characterized or identified. Host ECM components known to be involved in bacterial adherence include fibronectin (Fn), collagen (usually type I, II and IV), fibrinogen (Fg), fibrin, elastin, vitronectin, laminin (Lm), as well as decorin and heparin sulfate-containing proteoglycans (Joh *et al.*, 1998; Moreillon *et al.*, 2002) and salivary agglutinin glycoprotein, a constituent of the salivary film or pellicle that coats the tooth and is thought to be involved in streptococcal oral biofilm formation (dental plaque). Bacterial adhesins that specifically bind to ECM molecules are collectively termed Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs) (Patti *et al.*, 1994). According to this definition, all bacterial adhesins involved in host interaction through ECM proteins, and not through a specific receptor, can be considered MSCRAMMs, despite their differences in primary sequence and molecular organization. In a single bacterial species several proteins can contribute to bacterial adhesion displaying similar adhesive properties and host protein specificity (Kreikemeyer *et al.*, 2004, Table 1). Therefore, the ubiquitous nature of these proteins may act synergistically or as additive factors in a multifaceted adhesion process. Some of these molecules have been characterized in detail and share a significant degree of inter- and intra-species sequence similarity. In this respect, especially among genetically related species like streptococci, bacterial genome dissection and sequence similarity analyses have become powerful tools to predict potential adhesin function. Here, streptococcal adhesins will be reviewed on the basis of their sequence characteristics, structural and adhesive properties, as well as their distribution among streptococcal species.

Major adhesin families

Fibronectin binding

An important ubiquitous extracellular host protein targeted by a variety of streptococcal adhesins is Fn. The interaction is mediated by bacterial Fn binding proteins (FnBPs) that belong to the MSCRAMM family of adhesins (Patti *et al.*, 1994). FnBPs in streptococci have a similar molecular organization, although the sequence length can vary considerably. They all contain an N-terminal signal sequence, a C-terminal cell wall LPXTG-anchoring motif and a short positively charged C-terminal tail (Schwarz-Linek *et al.*, 2006). The Fn binding activity is located in the C-terminal half of the molecule, where sequence repeats of 30–40 residues can be found (Fn binding repeats,

FnBRs); FnBRs target the N-terminal domain (NTD) of Fn (Fig. 2A). In addition, some proteins contain an upstream Fn binding domain. Interestingly, FnBRs are intrinsically disordered regions within FnBPs. They were shown to adopt a more ordered conformation upon binding to the NTD of Fn (House-Pompeo *et al.*, 1996), thus defining a novel type of protein–protein interaction named 'tandem β-zipper' (Schwarz-Linek *et al.*, 2003). Each of the repeats can potentially bind to an Fn dimer, which contains two RGD binding sites for integrins. Fibronectin, in this model of interaction, functions as a molecular bridge between the bacteria and the host cell integrins in the FnBP-mediated bacteria internalization (Ozeri *et al.*, 1998). It is not clear if the number of FnBRs in FnBPs is correlated to adhesion or to invasion efficiency.

In *S. pyogenes* was identified a diverse number of FnBPs, not widespread in all the isolates tested. The occurrence of several different FnBPs in *S. pyogenes* may be linked to the ability of the bacteria to colonize different sites in the infected host. Moreover, the combination of Fn binding with other binding activities in different FnBPs could be the basis for differential tissue tropism (Courtney *et al.*, 2002; Kreikemeyer *et al.*, 2004).

Among *S. pyogenes* FnBPs, protein F1/SfbI is the best characterized and its activity has been unequivocally linked to *S. pyogenes* adherence and internalization (Talay *et al.*, 1994; Hanski *et al.*, 1996). SfbI is present in approximately 50% of the isolates tested and contains two Fn binding domains, a repeated domain (RD2) at the C-terminus and an additional domain located immediately N-terminal to the RD2 (Ozeri *et al.*, 1996). Similarly to other FnBPs, SfbI associates with host integrins through Fn, mechanism resulting in streptococcal invasion of eukaryotic cells (Cue *et al.*, 1998). SfbI was also demonstrated to be protective in an animal model of infection (McArthur *et al.*, 2004).

Protein F2, also called Pfbp, is different from Protein F1, it is present in most *S. pyogenes* strains that lack Protein F1 and binds Fn with high affinity. As demonstrated for protein F1, two distinct domains are responsible for its Fn binding activity (Hanski *et al.*, 1996; Jaffe *et al.*, 1996).

Interestingly, as reported below, protein F1 and F2 are both part of the pilus-encoding locus of *S. pyogenes*. Possible implications of this genetic architecture are currently under investigation (Falugi *et al.*, 2008).

An additional *S. pyogenes* Fg/Fn binding protein is SOF (serum opacity factor), also known as SfbII (Kreikemeyer *et al.*, 1995; Jeng *et al.*, 2003), a cell wall-anchored protein able to render human serum opaque by cleaving human serum apolipoprotein. Serum opacity factor-encoding genes exist in approximately 50% of the isolates. The SOF was demonstrated to be a *S. pyogenes* virulence factor in a murine infection model, and to be

Table 1. Streptococcal adhesins.

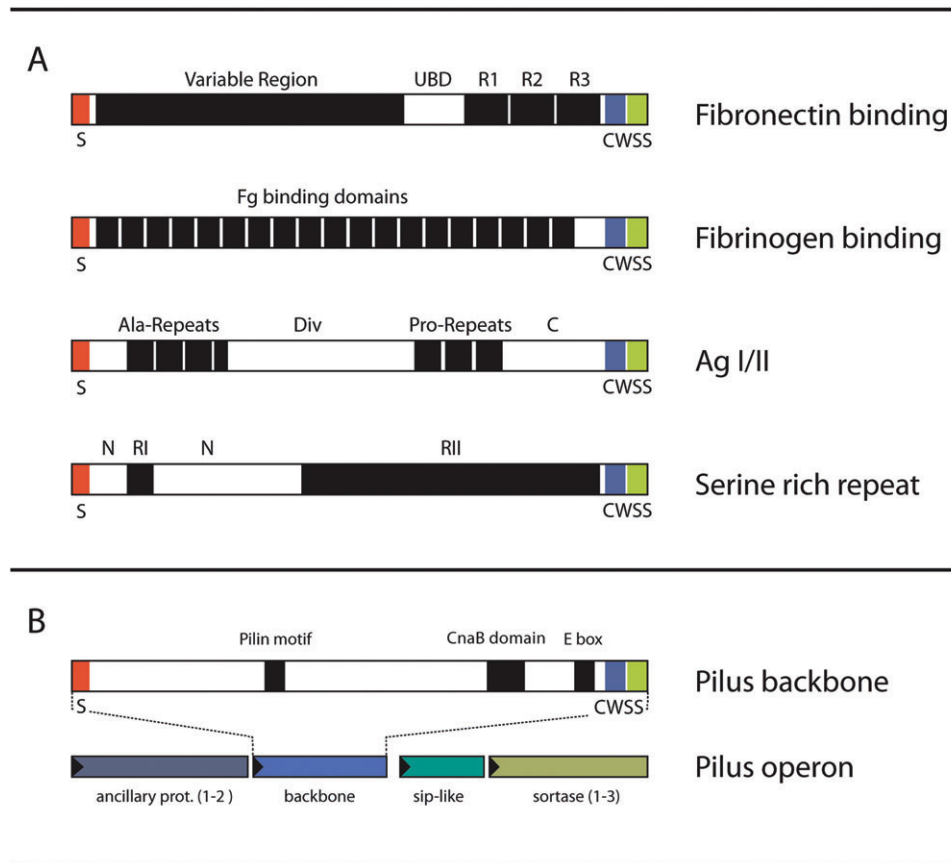| Species	Protein	References
S. mitis group		
S. pneumoniae	Pilus-1, RrgA	Nelson *et al.* (2007); Hilleringmann *et al.* (2008)
	PsaA	Anderton *et al.* (2007); Rajam *et al.* (2008)
	PspC	Iannelli *et al.* (2002); Luo *et al.* (2005)
	GtS	Mizrachi *et al.* (2007)
	FBA	Blau *et al.* (2007)
	6PGD	Daniely *et al.* (2006)
	PavA	Holmes *et al.* (2001)
	Psrp	Obert *et al.* (2006)
S. parasanguinis	Fap-1	Wu and Fives-Taylor (1999); Froeliger and Fives-Taylor (2001)
	FimA	Oligino and Fives-Taylor (1993); Burnette-Curley *et al.* (1995)
S. sanguinis	CshA	Black *et al.* (2004)
	PAAP	Gong *et al.* (1995)
	SrpA	Plummer *et al.* (2005)
	FimA	Xu *et al.* (2007)
S. gordonii	Has	Takahashi *et al.* (2004); Yajima *et al.* (2008)
	GspB	Takamatsu *et al.* (2004)
	CshA, CshB	McNab *et al.* (1995); Giomarelli *et al.* (2006)
	SspA, SspB	Egland *et al.* (2001); Nobbs *et al.* (2007)
	AbpA	Rogers *et al.* (2001)
	GTF	Vacca-Smith *et al.* (1994)
	FbpA	Christie *et al.* (2002)
S. cristatus	SrpA	Handley *et al.* (2005)
S. pyogenes group		
S. pyogenes	Cpa, ancillary protein pilus subunit	Kreikemeyer *et al.* (2005); Abbot *et al.* (2007)
	F1/sfbI	Talay *et al.* (1994); Hanski *et al.* (1996)
	F2/PFBP	Hanski *et al.* (1996); Jaffe *et al.* (1996)
	SfbII/SOF	Kreikemeyer *et al.* (1995); Timmer *et al.* (2006)
	Fbp54	Courtney *et al.* (1996)
	Fba, FbaB	Terao *et al.* (2001); Terao *et al.* (2002b)
	SfbX	Jeng *et al.* (2003); Almengor *et al.* (2006)
	GAPDH	Pancholi and Fischetti (1992)
	28 kDa protein	Courtney *et al.* (1992)
	Lbp	Terao *et al.* (2002a)
	SpeB	Stockbauer *et al.* (1999); Hytonen *et al.* (2001)
	M protein	Courtney *et al.* (2002); Carlsson *et al.* (2005)
S. uberis	SUAM	Almeida *et al.* (2006); Luther *et al.* (2008)
	Lbp	Moshynskyy *et al.* (2003)
S. agalactiae group		
S. agalactiae	Pilus	Dramsi *et al.* (2006)
	BibA	Santi *et al.* (2007)
	ScpB (streptococcal C5a peptidase)	Beckmann *et al.* (2002); Tamura *et al.* (2006)
	Lmb	Spellerberg *et al.* (1999)
	FbsA	Tenenbaum *et al.* (2005)
	Srr-1, Srr-2	Samen *et al.* (2007)
S. suis	GAPDH	Brassard *et al.* (2004)
	Galactosyl-alpha 1-4-galactose binding adhesin	Tikkanen *et al.* (1995)
S. dysgalactiae ssp. dysgalactiae	FnbB	Visai *et al.* (2003)
	FnbA	Joh *et al.* (1998)
S. dysgalactiae ssp. equisimilis	FnB	Lindgren *et al.* (1994)
	GfbA	Kline *et al.* (1996)
	FOG/M protein	Nitsche *et al.* (2006)
S. mutans group		
S. mutans	Adhesin P1, cell-surface adhesion protein SA I/II	Hajishengallis *et al.* (1994); Lee and Boran (2003)
	Cnm	Sato *et al.* (2004)
S. equi ssp. equi	SFS	Lindmark and Guss (1999)
S. equi ssp. zooepidemicus	SzP	Fan *et al.* (2008)
	SFS	Lindmark and Guss (1999)
	FnZ, FnZ2	Lindmark *et al.* (1996); Hong (2005)
S. intermedius	Cell-surface adhesion protein SA I/II	Petersen *et al.* (2001; 2002)
S. sobrinus	SpaA/PAg	Okahashi *et al.* (1993); Kuykindoll and Holt (1996)
S. downei	PAh	Tamura *et al.* (2008)

Fig. 2. A. Domain structure of streptococcal adhesins. Functional elements are shown as black boxes while conserved signal sequences are drawn in colour. Signal peptidase recognition sequences are filled in orange, sortase recognition sequences and membrane anchor sequences are blue and green respectively. Length of boxes does not reflect the actual scale. Designations of functional elements were adapted for fibronectin binding proteins (Courtney *et al.*, 1996), fibrinogen binding proteins (Rivera *et al.*, 2007), AgI/II proteins (Jenkinson and Demuth, 1997), serine-rich proteins (Seifert *et al.*, 2006).
B. Pilus islet genes and conserved elements in pilus backbone proteins. Conserved elements and signal sequences of the backbone protein are labelled as in A. Genes are shown as boxes. In general, pilus islets encode one or two ancillary proteins, one backbone protein and one to three sortase genes. Some islets but not all encode also a Sip-like protein.

important in the adhesion of *S. pyogenes* to HEp-2 human pharyngeal epithelial cells (Timmer *et al.*, 2006). The role of SOF Fg binding activity in virulence has not been determined.

An additional FnBP, SfbX, is encoded by a gene immediately downstream of *sof*. The two genes were found expressed in the same transcription unit and *sfbX* was present only in *sof* positive isolates. Recombinant SfbX bound to immobilized Fn and partially inhibited *S. pyogenes* adherence to Fn (Jeng *et al.*, 2003; Almengor *et al.*, 2006).

In *S. dysgalactiae*, a species frequently associated with bovine mastitis, two FnBPs have been reported, FnbA and FnbB, homologues to *S. pyogenes* SfbII and Protein F2 respectively. Both proteins are able to bind Fn at multiple sites (Lindgren *et al.*, 1993; Joh *et al.*, 1998; Visai *et al.*, 2003).

Similarly, in *S. dysgalactiae* ssp. *equisimilis* there is evidence for the presence of two FnBP, FnB and GfbA

(Lindgren *et al.*, 1994; Kline *et al.*, 1996). FnB, a protein of about 120 kDa, is homologous to the corresponding FnbB of *S. dysgalactiae* and possesses a C-terminal region containing three repeat units with Fn binding activity (Lindgren *et al.*, 1994). GfbA, present in about 36% of *S. dysgalactiae* ssp. *equisimilis* isolates tested, is homologous to SfbI of *S. pyogenes*, contains FnBRs and mediates adherence of *S. dysgalactiae* ssp. *equisimilis* to human skin fibroblasts (Kline *et al.*, 1996).

The obligate parasitic bacterium *S. equi* ssp. *equi* is the causative agent of strangles, a serious disease of the upper respiratory tract in horses. SFS represents a new type of FnBP, and is present in all *S. equi* ssp. *equi* isolates and in the majority of *S. equi* ssp. *zooepidemicus* tested. Interestingly, protein SFS was found to inhibit the binding between Fn and collagen in a concentration-dependent manner (Lindmark and Guss, 1999).

Streptococcus equi ssp. *zooepidemicus* is frequently isolated from clinical cases of equine pneumonia and

pleuropneumonia. In *S. equi* ssp. *zooepidemicus* a major FnBP is FnZ, a protein of approximately 61 kDa with a structural organization similar to that of other cell surface proteins of streptococci and staphylococci. FnZ shows a high degree of sequence similarity with SfbI of *S. pyogenes*. The Fn binding activity is localized to two domains in the C-terminal part of the protein, one domain composed of five repeats (Lindmark *et al.*, 1996). Moreover, in strains carrying FnZ, an additional FnBP was found and named FnZ2. Like FnZ, FnZ2 has the Fn binding domain localized at the C-terminus; for this protein both Fn and collagen binding activities were demonstrated (Hong, 2005).

Several FnBPs are also known to possess additional activities, like interaction with the host immune system and specifically with complement or Fg. In this respect, the streptococcal C5a-peptidase ScpB of GBS is a multifunctional protein found in all GBS clinical isolates and required for mucosal colonization in a neonatal mouse model of infection. ScpB is reported to inhibit neutrophil chemotaxis by enzymatically cleaving the complement component C5a and to bind to immobilized Fn (Tamura *et al.*, 2006). Moreover, an isogenic ScpB mutant was significantly less adherent to Fn than the wild-type strain (Beckmann *et al.*, 2002).

PavA is a pneumococcal protein associated with pneumococcal adhesion and virulence (PavA). PavA, despite the lack of a leader peptide and a membrane-anchoring sequence, is surface displayed. PavA specifically binds to immobilized human Fn (Holmes *et al.*, 2001), is widespread and conserved in the pneumococcal species and homologue to *S. pyogenes* Fbp54 and *S. gordonii* FbpA (Fig. 3). Unlike classical FnBPs (see above) PavA does not possess FnBRs. PavA is responsible for approximately 50% of the *S. pneumoniae* Fn binding activity, suggesting the presence of additional Fn adhesins. Moreover, PavA has been shown to be critical for invasive diseases such as sepsis and meningitis (Holmes *et al.*, 2001).

FBP54 is another FnBP expressed on the surface of *S. pyogenes* and widely distributed among the *S. pyogenes* population. The Fn binding domain is localized at the N-terminus of the protein, and shows no significant sequence similarity with other known Fn binding regions (Courtney *et al.*, 1996). FBP54 blocked streptococcal adhesion to human buccal cells but had no effect on the adhesion to HEp-2 cells.

Fn binding activity of *S. gordonii* is mediated by the high-molecular-mass protein CshA (as described below) and by FbpA (a PavA and Fbp54 homologue), a surface-exposed protein of about 63 kDa. Purified recombinant FbpA has been shown to bind to human immobilized Fn, while isogenic *S. gordonii* deletion mutants were impaired in their binding to Fn. Moreover, FbpA is expressed only

during exponential growth of bacteria and there is evidence that FbpA itself probably plays a regulatory role in CshA expression (Christie *et al.*, 2002).

Interestingly, a Fn binding activity has been recognized also for proteins predicted to have an enzymatic activity and cytosolic localization, like glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The GAPDH was described as a cytosolic enzyme, and also found on the surface of *S. pyogenes* and *S. suis.* In *S. pyogenes*, GAPDH, present as a tetramer on the surface, has both an enzymatic activity and binding capacity for a variety of proteins. The GAPDH is present in all *S. pyogenes* strains and binds to Fn and lysozyme, as well as to the cytoskeletal proteins myosin and actin. This multifunctional protein may play a role in colonization, internalization and subsequent proliferation of group A streptococci (Pancholi and Fischetti, 1992). Similarly, *S. suis* GAPDH is surface exposed and pre-incubation of porcine tracheal rings with recombinant GAPDH inhibited *S. suis* adhesion, indicating a possible involvement of GAPDH in the first steps of bacterial adhesion to host cells (Brassard *et al.*, 2004).

Fibrinogen binding

Fibrinogen is the major clotting protein in the blood plasma, and is critical for blood coagulation and thrombosis. Fibrinogen is primarily synthesized under normal conditions, but its synthesis is dramatically upregulated during stress and inflammation. Bacterial interaction with Fg seems to be important during infections like infective endocarditis, a disease caused by streptococci and staphylococci. The crucial step in infective endocarditis is bacterial-induced platelet activation and subsequent aggregation that leads to plaque formation on endocardium and valve leaflets and hence to endocarditis (Fitzgerald *et al.*, 2006; Rivera *et al.*, 2007). Bacteria can interact with platelets directly, as below described for the *S. gordonii* serine-rich proteins Hsa and GspB, or indirectly involving host proteins such as Fg that forms a link between a bacterial component and a platelet receptor.

In recent years, a number of Fg binding proteins have been described, among which one of the best characterized is the M protein of *S. pyogenes*. The M protein is a major virulence factor in *S. pyogenes* infections and is expressed in all *S. pyogenes* isolates. The M protein is a filamentous coiled coil dimer attached to the surface through the C-terminus; the Fg binding domain is localized in the β-repeat region near the N-terminus of the protein. The β-repeat region is composed of a variable number of repeats, with an α-helical coiled coil conserved structure. The M protein has an anti-phagocytic role and there is evidence that binding of Fg may be an important step in allowing *S. pyogenes* to escape neutrophil phagocytosis (Carlsson *et al.*, 2005). Fg interaction is also

Fig. 3. Streptococcal adhesin homologues. Protein homologues across the *Streptococcus* genus are presented. A protein sequence similarity search was performed at http://blast.ncbi.nlm.nih.gov/Blast.cgi against all the streptococcal protein sequences present in the NCBI database, by using as query sequences those listed in the first column (accession numbers are reported under brackets). Proteins reported in literature to be adhesins, are marked in bold. Streptococcal species are divided on the basis of the phylogenetic analysis reported in Fig. 1. Asterisks indicate the species for which complete genome sequences are present in the NCBI database. Pilus and fimbrial components are marked in orange and dark yellow respectively.

involved in platelet thrombus formation, mediating platelet–M protein adhesion (Sjobring *et al.*, 2002). Notably, in addition to Fg, M proteins can interact with different host serum factors including Fn, immunoglobulin, plasminogen, factor H, collagen IV and the complement regulator C4 binding protein (Courtney *et al.*, 2002). Similarly to group A streptococci, group C and G also bind Fg trough M proteins (Schnitzler *et al.*, 1995; Talay *et al.*, 1996).

The FbsA protein is the Fg binding protein of *S. agalactiae*. The Fg binding domain has been localized in a tandem repeat region. The number of repeats varies and there is no significant sequence similarity to other streptococcal Fg binding motifs. This suggests that probably FbsA binds Fg by a unique mechanism. FbsA can also bind epithelial cells directly and block bacteria from adhering and invading epithelial cells (Tenenbaum *et al.*, 2005). As for *S. pyogenes* M. protein, *S. agalactiae* platelet-induced aggregation requires FbsA and is mediated by Fg (Pietrocola *et al.*, 2005).

The group G streptococci are also equipped with Fg binding capabilities. *Streptococcus dysgalactiae* ssp. *equisimilis* (group G streptococcus: GGS) is both a human and animal pathogen of emerging clinical significance, causing skin and soft tissue infections, and occasionally resulting in sepsis and necrotizing fasciitis. The M-like fibrinogen binding protein of GGS (FOG) is a surface protein most notably involved with Fg and collagen binding, as well as in evasion of the host immune system. Moreover, FOG expressing isolates adhere more efficiently to human skin than a FOG-deficient strain (Nitsche *et al.*, 2006).

Laminin binding

In the process of adhesion, ECM proteins such as Fn and Lm serve as mediators between the bacteria and host cells. Laminin, a 900 kDa glycoprotein, is a major component of the basement membrane. It is composed of three distinct polypeptide chains (A, B1 and B2) which reversibly assemble to form a macromolecular structure. Functions of Lm include the formation of the basement membrane by interaction with other basement membrane components and the development and maintenance of cellular organization. Several bacteria were demonstrated to damage the pulmonary epithelium, exposing underlying basement membrane structures. Thus, adhesion to basement membrane components may be critical for bacterial colonization of damaged epithelium and invasion of bacteria into the bloodstream.

Specific Lm binding proteins have been described for *S. pyogenes* and *S. agalactiae*. Both bacteria have been reported to directly bind to Lm. In particular, in *S. agalactiae*, Lmb is a lipoprotein of approximately 35 kDa,

present and conserved in all the serotypes tested; its protein sequence exhibits significant similarity to the streptococcal LraI protein family, a family of surface-associated lipoproteins involved in co-aggregation and adherence of different streptococci. Pre-incubation of immobilized Lm with recombinant Lmb significantly reduced adherence of *S. agalactiae* to Lm, indicating that Lmb mediates the attachment of *S. agalactiae* to human Lm (Spellerberg *et al.*, 1999).

In *S. pyogenes* two distinct Lm binding proteins have been identified thus far, Lbp and SpeB (streptococcal pyrogenic exotoxin B). Lbp is a lipoprotein exhibiting a high degree of sequence similarity with GBS Lmb. Recombinant Lbp directly binds to purified human Lm and Lbp-deficient mutants showed a significantly lower efficiency of adhesion to HEp-2 cells than did the wild-type strains (Terao *et al.*, 2002a).

Streptococcal pyrogenic exotoxin B is an extracellular cysteine protease existing as three variants, one of which contains an Arg-Gly-Asp (RGD) sequence, a tripeptide motif that is commonly recognized by integrin receptors (Stockbauer *et al.*, 1999). The SpeB is an extracellular protease; however, it has been shown to be surface exposed with binding activity to Lm and other glycoproteins (Hytonen *et al.*, 2001). In addition, different activities were ascribed to SpeB: it was demonstrated to directly bind to integrins, and, as a secreted molecule to degrade host ECM proteins, thus contributing to bacterial dissemination, colonization and invasion (Kapur *et al.*, 1993; Lukomski *et al.*, 1998). BLAST sequence analysis revealed that SpeB is specific for *S. pyogenes*, whereas Lmb/Lbp homologues, with an extremely high degree of conservation, are present in several other *Streptococcus* species, including *S. pneumoniae*, *S. gordonii* and *S. sanguinis* (Fig. 3).

AgI/II family proteins

AgI/II family proteins are expressed by most oral streptococci and mediate interactions with salivary constituents, host cell matrix proteins, such as Fn, Fg and collagen, and with other oral bacteria, thus probably playing a role in interspecies interactions during oral biofilm formation (Jenkinson and Demuth, 1997). AgI/II consist of a protease-sensitive N-terminal region, AgI, and a protease-resistant AgII region, represented by the C-terminal and membrane-proximal third of the molecule (Russell *et al.*, 1980). The streptococcal AgI/II proteins consist of 1310–1653 amino acid residues and are structurally conserved, with seven discrete regions as deduced from the primary sequence (Fig. 2A). These include a signal (leader) peptide, an N-terminal region (N), alanine-rich repeat blocks (A), a divergent or variable central region (V), proline-rich repeat blocks (P), a C-terminal region contain-

ing a cell wall-spanning region, a membrane-spanning region, a cell wall anchor domain and a cytoplasmatic tail (Jenkinson and Demuth, 1997).

Streptococcus mutans, which plays a major role in tooth decay, is equipped with a cell wall-associated multifunctional protein P1, referred to by various research groups as antigen I/II, antigen B and Pac. This protein seems to provide *S. mutans* with the ability to attach to the salivary pellicle on teeth (Hajishengallis *et al.,* 1994). Structurally, P1 has a 38-amino-acid-residue signal sequence (N), and an A and P region (proline rich) with each region composed of three tandem repeats. P1-deficient cells lost the ability to aggregate in the presence of clarified whole saliva or fluid phase salivary agglutinin, and failed to adhere to saliva- or agglutinin-coated hydroxyapatite beads (Lee *et al.*, 1989; Koga *et al.*, 1990). Crowley and colleagues (2008) identified the A region as the salivary agglutinin binding domain within the P1 molecule. Moreover, P1 interacts with Fn and Fg forming a β-structure upon interaction with both proteins (Kelemen *et al.*, 2004).

Streptococcus intermedius is often associated with deep-seeded purulent infections. In *S. intermedius* a 160 kDa cell surface-associated protein antigen I/II homologue to *S. mutans* P1 was identified (Fig. 3); unlike P1, it is not involved in adhesion to a salivary film under flowing conditions, and neither in binding to rat collagen type I. Binding to human Fn and Lm is a common function associated with the *S. mutans* and *S. intermedius* antigen I/II. In addition *S. intermedius* antigen I/II was demonstrated to induce interleukin 8 release by THP-1 monocytic cells (Petersen *et al.*, 2001; 2002; Jakubovics *et al.*, 2005a).

SspA and SspB are two antigen I/II surface-exposed proteins encoded by a tandem arrangement of genes in *S. gordonii.* The SspA and SspB proteins are > 96% identical in the N, A, P and C regions, but share only 26% identical amino acid residues in their V regions. SspA and SspB polypeptides are independently expressed and both mediate bacterial binding to surface immobilized salivary agglutinin glycoprotein and are probably involved in adhesion and initial biofilm formation on teeth by regulating the expression of multiple adhesin genes (El-Sabaeny *et al.*, 2000; Jakubovics *et al.*, 2005a,b; Zhang *et al.*, 2005). In addition, an *S. gordonii* deletion mutant for both SspA and SspB was reduced in adherence to HEp-2 cells and was not internalized. Recombinant SspA and SspB polypeptides bound to purified human α5β1 integrin through sequences present within the N-terminal region of the AgI/II polypeptide. Moreover, adherence and internalization of *S. gordonii* by HEp-2 cells involves β1 integrin recognition but is not Fn-dependent (Nobbs *et al.*, 2007).

The SpaA protein (also refereed as PAg), a major surface protein antigen of *S. sobrinus*, and the *S. mutans* P1 protein, share 66% sequence identity at the amino acid level. As demonstrated for P1 in S. *mutans*, SpaA

plays a role in the virulence of *S. sobrinus* (mostly associated with human dental caries) by specifically interacting with fluid-phase salivary agglutinin to mediate sucrose-dependent cell aggregation (Kuykindoll and Holt, 1996).

The *pah* gene of the cariogenic organism *S. downei* encodes a cell wall-anchoring protein, PAh, containing 1565 amino acids. PAh shows a strong similarity to SpaA of *S. sobrinus* (97.6% identity). PAh is associated with the adhesion of cells to abiotic surfaces and whole saliva, as demonstrated in assays performed with wild-type *S. downei* and PAh knockout mutants (Tamura *et al.*, 2008).

Other species-specific adhesins

Streptococcus pneumoniae

In *S. pneumoniae*, one of the most unique group of cell wall-associated proteins involved in pathogenesis are the choline binding proteins (CBP). Pneumococci contain phosphorylcholine on both the cell wall teichoic acid and the membrane-associated lipoteichoic acid. The presence of choline in the cell wall was thought to be unique to *S. pneumoniae*. However, recent data have indicated the presence of choline on the surface of a number of other respiratory tract pathogens like *S. oralis*, *S. mitis*, *S. constellatus*, *Clostridium* strain NI-4, *C. beijerinckii*, *Neisseria meningitidis*, *Pseudomonas aeruginosa* and *Haemophilus influenzae* (Gosink *et al.*, 2000). In pneumococci, the CBPs bind to the phosphorylcholine of the cell wall non-covalently through a choline binding domain consisting of 2 –10 repeats of a 20-amino-acid sequence. CbpA (also referred to as PspC, SpsA Hic and PbcA) is the largest and the most abundant of the 15 Cbps identified in the genome of *S. pneumoniae* so far (Tettelin *et al.*, 2001). CbpA is known for its antigenic properties, its capability to bind secretory IgA (Hammerschmidt *et al.*, 1997) and complement factor H (Janulczyk *et al.*, 2000), its activity as an adhesin (Rosenow *et al.*, 1997) and in promoting invasion of epithelial cells (Zhang *et al.*, 2000). CbpA binds specifically to the ectodomain (secretory component) of polymeric immunoglobulin receptor of epithelial cells (Zhang *et al.*, 2000) through conserved hexapeptide motifs Y/R RNYPT located in two N-terminal repeated domains (Elm *et al.*, 2004). Recently, an important role in biofilm formation has been proposed for CbpA and other CBPs (LytC, PcpA, PspA) (Moscoso *et al.*, 2006). In addition, CbpA inhibits both the classical and the alternative complement pathway declining C3 and C3b activation and deposition (Ren *et al.*, 2003; 2004; Guiral *et al.*, 2005).

Pneumococcal surface adhesin A is a surface-exposed multi-functional lipoprotein of about 37 kDa, highly conserved in the pneumococcal population and across the majority of the streptococcal species (Fig. 3). However, specific data regarding this protein are present only for *S.*

pneumoniae, where PsaA represents one of the major and best characterized adhesins. This lipoprotein belongs to the ABC-type transport protein complex that transports Mn^{2+} and Zn^{2+}. PsaA deletion mutants are significantly less virulent than wild type, are growth-impaired in Mn^{2+}-deficient environments, hypersensitive to oxidative stress, and have reduced capacity to adhere to epithelial cells (Johnston *et al.*, 2004; Rajam *et al.*, 2008). Moreover, Anderton *et al.* demonstrated that E-cadherin, the cell junction protein in respiratory epithelial cells, is a receptor for PsaA (Anderton *et al.*, 2007).

Recently, three additional surface proteins were shown to have a role in *S. pneumoniae* adhesion to epithelial cells *in vitro:* glutamyl tRNA synthetase (GtS), a protein of about 56 kDa and two cell wall-localized lectins, bisphosphate aldolase (FBA) and 6-phosphogluconate dehydrogenase (6PGD) (Daniely *et al.*, 2006; Blau *et al.*, 2007; Mizrachi *et al.*, 2007).

Streptococcus agalactiae

Recently, a novel immunogenic adhesin with antiphagocytic activity (BibA) was identified in *S. agalactiae*. BibA is a multifunctional protein of approximately 65 kDa, containing a helix-rich NTD, a proline-rich region and a canonical LPXTG cell wall-anchoring domain. BibA specifically binds to human C4 binding protein, a regulator of the classic complement pathway, and its deletion severely reduces the capacity of GBS to survive in human blood and to resist opsonophagocytic killing by human neutrophils. In addition, BibA knockout mutants are impaired in adhesion to cervical epithelial cells (Santi *et al.*, 2007).

Streptococcus gordonii

In *S. gordonii* AbpA functions as an adhesin to amylase-coated hydroxyapatite, in salivary-amylase-mediated catabolism of dietary starches and in human saliva-supported biofilm formation. Amylase is the predominant salivary enzyme in humans and binds to *S. gordonii*, a primary colonizer of the teeth; this interaction is thought to be important in establishment and ecology of the oral microflora (Rogers *et al.*, 2001).

Streptococcus gordonii is also implicated in infective endocarditis, where one of the proteins thought to play a major role in adhesion is a surface-exposed glucosyltransferase (GTF), which mediates adhesion of *S. gordonii* to human umbilical vein endothelial cells *in vitro* and may provide a mechanism for colonization of the endocardium in infective endocarditis (Vacca-Smith *et al.*, 1994).

Streptococcus sanguinis

Like *S. gordonii*, *S. sanguinis* is implicated in infective endocarditis, accounting for as many as half of the cases of native valve endocarditis. Certain strains of *S. sanguinis* can adhere to human platelets and induce platelets to aggregate into macroscopic thrombi *in vitro*. The ability of *S. sanguinis* to induce platelets to aggregate is strongly suggested to be conferred by several separate surface components one of which, antigenically distinct, is the platelet aggregation-associated protein (PAAP). The PAAP contains a collagen-like immunodeterminant (PGE(P/Q)GPK) which probably interacts with a signal transducing receptor on the platelet, inducing platelet–platelet cross-linking (Gong *et al.*, 1995).

Streptococcus equi ssp. zooepidemicus

The M-like protein expressed on the surface of *S. equi* ssp. *zooepidemicus* (*S. zooepidemicus*) is also known as SzP. SzP is similar to the M protein of group A *Streptococcus* in the structure and characteristics of antiphagocytosis. Like the *S. pyogenes* M protein, also Szp of *S. zooepidemicus* functions as an adhesin, and is responsible for mediating *S. zooepidemicus* binding to HEp-2 cells (Fan *et al.*, 2008).

Streptococcus suis

Streptococcus suis causes meningitis, sepsis and other serious infections in newborn and young pigs and in adult humans. *Streptococcus suis* adhesion to host cells was found to be mediated by the recognition of the disaccharide galactosyl-α1-4-galactose through the Galα1-4Gal binding adhesin. This adhesin, as recombinant protein, bound to latex particles inducing haemagglutination, specifically inhibited with the same inhibitors as haemagglutination stimulated by the intact bacteria (Tikkanen *et al.*, 1995).

Streptococcus uberis

Streptococcus uberis is an important environmental pathogen that causes subclinical and clinical mastitis in lactating and non-lactating cows throughout the world. In *S. uberis* mastitis key players are the bacterial lactoferrin binding activity and its capacity to adhere to bovine mammary epithelial cells. Both activities were reported for a surface protein of about 112 kDa, SUAM, whereas, Lbp was only reported to bind to bovine lactoferrin (Moshynskyy *et al.*, 2003; Almeida *et al.*, 2006). Lbp was present on the surface of *S. uberis* in monomeric and dimeric form; analysis of the primary and secondary structure suggested that Lbp is an M-like protein.

Streptococcus mutans

Cnm is a 120 kDa protein detected in certain strains of *S. mutans* and involved in the cold-agglutination. Recombi-

nant Cnm was demonstrated to bind to immobilized collagen and Lm but not to Fn (Sato *et al.*, 2004). Cnm homologues were found by NCBI BLAST analyses in other species of the *S. mutans* group (*S. equi* ssp. *zooepidemicus* and *S. equi* ssp. *equi*) and in *S. dysgalactiae* (Fig. 3).

Fimbriae

Serine-rich proteins

Serine-rich proteins are present in many streptococcal species. In *S. parasanguinis* (Fap1) and *S. cristatus* (SrpA), they are large glycosylated surface-exposed proteins, demonstrated to form fimbrial-like structures of about 400 nm in length (Handley *et al.*, 2005) and typically involved in adhesion and virulence. These proteins are located within operons encoding a dedicated secretion system (SecA2, SecY2) and glycosyl transferases, responsible for their specific translocation and glycosylation (Bensing and Sullam, 2002).

Serine-rich proteins (Fig. 2A) consist of an N-terminal region predicted to contain an atypically long signal peptide, a serine-rich region (SRR1), a region rich in basic amino acid residues (basic region), a second serine-rich region (SRR2), and a C-terminal cell wall sorting signal that includes an LPXTG motif (Takahashi *et al.*, 2004).

In *S. parasanguinis*, isolated from human throat, blood and urine, Fap1 (fimbrial associated protein 1) is a 200 kDa protein forming long peritrichous fimbriae and responsible for *S. parasanguinis* adhesion to saliva-coated hydroxyapatite. Fimbriae, composed by multiple Fap1 subunits, essentially mediate adhesion of *S. parasanguinis* in an *in vitro* tooth model and are essential for the formation of biofilm, *in vivo* known as dental plaque (Wu *et al.*, 1998; Froeliger and Fives-Taylor, 2001).

In *S. sanguinis*, a serine-rich high-molecular-weight glycoprotein (SrpA) mediates adhesion to glycocalacin. *Streptococcus sanguinis* lacking SrpA showed significantly reduced binding to glycocalacin, reduced adherence to platelets and a prolonged lag time to platelet aggregation (Plummer *et al.*, 2005).

Similarly, the surface of the oral plaque bacterium *S. cristatus* is decorated with a lateral tuft of fibrils, involved in the adhesion of *S. cristatus* to heterologous bacterial species in the plaque biofilm and composed by the serine-rich protein SrpA (Handley *et al.*, 2005).

In *S. gordonii*, two allelic variants of serine-rich proteins have been identified, GspB and Hsa. GspB and Hsa surface expression are associated with a significant binding to human platelets (central mechanism in the pathogenesis of bacterial endocarditis) of M99 and DL1 strains respectively (Bensing and Sullam, 2002; Takahashi *et al.*, 2002; 2004). Moreover, by binding erythrocyte surface sialoglycoproteins, these serine-rich proteins are also involved in *S. gordonii*-induced haemagglutination, which may act as an additive factor to increase the severity of endocarditis disease. More in detail, Hsa specifically binds to GPA and band 3, α 2-3-linked sialic acid membrane glycoproteins present on the surface of erythrocytes (Yajima *et al.*, 2008).

Two different serine-rich protein isoforms (Srr-1 and Srr-2), showing substantial differences in their primary sequences, have been reported in *S. agalactiae*. Adhesion data are reported only for Srr-1; this protein was shown to interact with a 62 kDa protein in human saliva, which was identified as human keratin 4 and is involved in *S. agalactiae* adherence to epithelial HEp-2 cells (Samen *et al.*, 2007).

In *S. pneumoniae* only a certain number of isolates encode two sets of SecA and SecY homologues, one of which, based on sequence similarity, may constitute a specialized system for the transport of a very large serine-rich repeat protein, named PsrP (i.e. SP1772 of *S. pneumoniae* TIGR4). Disruption of *psrP* affects the ability of the bacteria to progress into the bloodstream, presumably from the lungs, and not colonization or survival in blood (Obert *et al.*, 2006).

Other fimbrial proteins

In *S. parasanguinis* FimA is a lipoprotein receptor antigen I (LraI), reported as additional component (different from the above mentioned Fap1) of surface-exposed fimbriae. FimA, as Fap1, is involved in *S. parasanguinis* attachment to saliva-coated hydroxyapatite (Oligino and Fives-Taylor, 1993). Strains mutated in *fimA* continued to produce fimbriae, indicating that FimA was not the fimbrial structural subunit. Immunoelectron microscopy revealed that FimA was localized at the tips of the fimbriae (Fenno *et al.*, 1995). Moreover, FimA was demonstrated to be an important virulence determinant of *S. parasanguinis*-induced endocarditis, maybe playing a role in adherence to fibrin deposits associated with damaged cardiac valves (Burnette-Curley *et al.*, 1995).

Interestingly, protein BLAST search performed by using *S. sanguinis* FimA identified as homologue proteins, pilus components (GBS) or pilus hypothetical components (*S. equi* and *S. suis*) (Fig. 3). The analysis of *S. sanguinis* genome sequence revealed that FimA is included in an apparent pilus-encoding locus, comprising four genes encoding for LPXTG surface-anchored proteins (SSA_1632 to SSA_1635) flanked by a SrtC (Xu *et al.*, 2007).

Surface fibrils of approximately 60 nm long have been identified in *S. gordonii*, are encoded by major cell surface polypeptides, and are termed CshA and CshB. CshA and CshB-deficient mutants are markedly reduced in hydrophobicity, deficient in binding to oral *Actinomyces* species,

to human Fn and to collagen I and II, and unable to colonize the oral cavities of mice (McNab *et al.*, 1996; 1999; Giomarelli *et al.*, 2006). Moreover, antibodies raised against *S. gordonii* CshA recognized on the surface of *S. sanguinis* and *S. oralis* short peritrichous fibrils, demonstrating the widespread nature of Csh-like proteins among streptococcal species, data also confirmed by sequence similarity analysis (Fig. 3) (Elliott *et al.*, 2003).

In the same context, an *in vitro* biofilm model of *S. sanguinis*, reproducing dental plaque biofilms, demonstrated that *S. sanguinis* CshA expression was significantly upregulated with respect to planktonic conditions (Black *et al.*, 2004).

Pili

While pili in Gram-negative bacteria have been extensively studied and recognized as important virulence factors, pili in Gram-positive bacteria are a quite recent discovery and their distribution and role in pathogenesis is still under investigation (Mandlik *et al.*, 2008; Proft and Baker, 2009).

Since the first identification of pili on the surface of *Corynebacterium diphtheriae* (Yanagawa and Honda, 1976; Ton-That and Schneewind, 2003), pili have been found on the surface of pathogenic streptococci, such as *S. pneumoniae*, *S. agalactiae* and *S. pyogenes*, where they appear as long, flexible filaments protruding up to 3 μm from the cell surface (Lauer *et al.*, 2005; Mora *et al.*, 2005; Barocchi *et al.*, 2006). Gram-positive pili are composed of covalently linked subunits, the backbone pilin forming the pilus shaft and the accessory pilins, known as ancillary proteins, distributed at the pilus base and as clusters or single molecules all along the pilus filaments. The ancillary proteins are not required for pilus assembly but are involved in adhesion and pilus-related pathogenic mechanisms (Kreikemeyer *et al.*, 2005; Dramsi *et al.*, 2006; LeMieux *et al.*, 2006; Nelson *et al.*, 2007).

Streptococcal pili are encoded by diverse genetic elements, inserted into specific genomic regions; in some streptococcal species like *S. pneumoniae* or *S. agalactiae* different pilus-encoding islets are inserted at diverse separate loci in the genome (Bagnoli *et al.*, 2008; Moschioni *et al.*, 2008; Margarit *et al.*, 2009). These islets vary in length and genetic organization, but share characteristic features. In fact, as shown in Fig. 2B, all pilus-encoding islets contain genes encoding for LPXTG or LPXTG-like surface-anchored proteins (showing intra and inter-species homology), and for sortases, specialized transpeptidases involved in pilus assembly. Moreover, some islets, e.g. PI-2 of *S. pneumoniae* and three FCT regions of *S. pyogenes*, contain a *sipA/lepA* gene, coding for a signal peptidase-like protein and essential for pilus assembly (Bagnoli *et al.*, 2008; Zahner and Scott, 2008).

Interestingly, these proteins lack essential active site residues, therefore their role in pilus biogenesis is still not clear. Detailed comparative analyses of pilin protein sequences revealed the presence of conserved characteristic sequence signatures necessary for a correct sortase-mediated assembly (Fig. 2B). Pilin protein precursors contain: (i) an N-terminal signal peptide for Sec-dependent secretion, (ii) a C-terminal cell anchor domain consisting of a membrane-spanning region preceded by the sortase recognition motif LPXTG (or a variant thereof) followed by a hydrophobic domain and a positively charged tail, (iii) a specific amino acid sequence (WXXXVXVYPKN) termed the pilin motif, where the lysine (K) residue is required for subunit polymerization providing the ε-amino group for intermolecular iso-peptide bond formation, and (iv) an E-BOX motif containing an invariant glutamate (E) demonstrated to be important for a correct pilus assembly. Following secretion of the N-terminal part and removal of the signal peptide, the protein remains anchored to the cell membrane via the hydrophobic cell anchor domain. In a successive step sortase specifically cleaves at the LPXTG motif forming an iso-peptide bond between C-terminal threonyl and the epsilon amino group of the lysine residue (K) of the pilin motif linking one subunit to the other and forming the polymer (Ton-That and Schneewind, 2004; Manzano *et al.*, 2008; Oh *et al.*, 2008).

Based on these data, the simplest way to identify novel pilus-encoding islets is through a genomic approach and sequence analysis, followed by biological confirmation. Consequently, two recent publications, based on genome analyses, reported the identification of one hypothetical pilus-encoding islet in *S. suis* (Fittipaldi *et al.*, 2007), and three new islets in *S. zooepidemicus* (Beres *et al.*, 2008), termed FimI, II and III.

Molecular epidemiology and genomic analyses demonstrated that all *S. pyogenes* and GBS isolates encode for a functional pilus, whereas in *S. pneumoniae*, only a minority of the isolates tested carry PI-1 or PI-2 (*S. pneumoniae* pilus-encoding islets) suggesting possible different roles for pili in the disease outcome of these major streptococcal pathogens (Basset *et al.*, 2007; Falugi *et al.*, 2008; Moschioni *et al.*, 2008; Margarit *et al.*, 2009).

In particular, pili in *S. pyogenes* are encoded by at least nine different islets always inserted into the same genomic region, although differently organized and demonstrating only a low degree of sequence similarity (Falugi *et al.*, 2008). Pili are involved in the *S. pyogenes* adhesion processes; in fact, *S. pyogenes* pilus-deficient mutants are impaired in adhesion to human tonsil epithelium and primary human keratinocytes. The pilus adhesive properties are probably triggered by the Cpa protein, accessory component of *S. pyogenes* pilus; in fact, Cpa was already known as selective Collagen I binding protein before pili

discovery (Kreikemeyer *et al.*, 2005). Furthermore, *S. pyogenes* pili were shown to contribute to bacterial cell aggregation in liquid culture, the formation of microcolonies on human cells and the formation of biofilms (Manetti *et al.*, 2007). Pili also contribute to *S. pyogenes* cell aggregation in saliva, through the saliva component Gp340, a process that is thought to play a role in host defence mechanisms (Edwards *et al.*, 2008).

Interestingly, two well-studied *S. pyogenes* FnBPs, Protein F1 and F2 (see above), are part of the pilus-encoding islet but were not found associated with the pilus.

In *S. pneumoniae*, pili seem to be involved in bacterial adhesion to epithelial cells (Nelson *et al.*, 2007; Hilleringmann *et al.*, 2008). Bacteria lacking either PI-1 or PI-2 are adhesion impaired in *in vitro* experiments (Barocchi *et al.*, 2006; Bagnoli *et al.*, 2008); PI-1 deletion mutants are also less virulent in murine models of colonization, pneumonia and bacteraemia. In pilus-1 (also called *rlrA* pilus) the component involved in adhesion properties of the pilus is the ancillary protein RrgA, present in clusters at the tip and along pili filaments. Recombinant RrgA and purified pili adhered in *in vitro* assays to ECM components, like collagen I, Fn and Lm, thus confirming MSCRAMM properties for this protein as already revealed by detection of significant sequence similarity (Hava and Camilli, 2002; Hava *et al.*, 2003; Nelson *et al.*, 2007; Hilleringmann *et al.*, 2008). Recent reports indicate a role for RrgA but not for the pilus per se in biofilm formation (Munoz-Elias *et al.*, 2008).

In GBS, two islets, one of which exists in two variants, code for functional pili. Recently, a potential role for the minor pilus protein of islet 2 as adhesin has been demonstrated by Dramsi *et al.*, who showed that deletion of gbs1474, predominantly located at the pilus base but also randomly along the pilus backbone, in strain NEM316 resulted in a significant reduction of adherence to lung and cervical epithelial cells (Dramsi *et al.*, 2006). These data taken together suggest that streptococcal pili are usually associated with virulence and adherence. Nonetheless, pili 'per se' are not required for host cell adhesion; in fact there is evidence that the minor pilins act as adhesins, favoured by, but independent from the pilus structure.

Conclusions

Analysis of streptococcal pathogenesis determinants has provided insight into how bacteria adhere to and invade cells, move intracellularly, and disseminate in tissues; information that has contributed to the generation of tools which address fundamental processes in cell biology. Exploring these strategies will help in the design of more technologically robust and effective biotech applications. Moreover, the plethora of experimental information gath-

ered through in-depth comparative genomic analyses, and in *vivo/in vitro* studies, makes bacterial adhesins one of the best-studied components important for tissue-tropisms and bacterial survival within the host. Identification of these protein components may be critical for the development of new drugs, inhibitors and vaccines.

Streptococcal adhesins have been grouped into larger families, based on the substrate with which they interact; most have distinct structures, while overlapping functions. This redundancy is not unique in nature, and may allow the bacterium to colonize or infect various hosts. The discovery and characterization of streptococcal adhesins may therefore be applied for the identification of small molecules directly targeting and inhibiting their function, thereby opening access to a more general target for therapeutic intervention. Potential synthetic adhesins could be used in the biotechnology industry as carrier molecules that deliver 'cargo' to specific targets, or as 'sticky' surfaces in aqueous preparations. These molecules may also be exploited in commercial dentistry products, as inhibitors of dental plaques, often caused by streptococcal species. Moreover, as recently suggested for streptococcal pilus subunits and several others streptococcal adhesins, these proteins, being surface exposed and expressed during crucial steps of the pathogenetic process, could be selected as vaccine components to prevent colonization and severe disease outcomes (Mora *et al.*, 2005; Terao *et al.*, 2005; Tai, 2006; Gianfaldoni *et al.*, 2007; Timoney *et al.*, 2007; Margarit *et al.*, 2009). In addition, one may envisage novel biotechnological applications for these adhesins. Whichever their application, the role and potential applications of adhesins will be a fascinating topic to investigate in the future.

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