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Genomics update

Probiotics genomics

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We were sitting in the Irish pub on quiz night, dumbfounded by trivia questions about ingredients of Mornay sauce and best-selling Boy Bands, when the following question came up: What are 'Live microorganisms which when administered in adequate amounts confer a health benefit on the host?' At long last, we had a correct answer: PROBIOTICS! The quizmaster was totally disinterested in our extensive elaboration on this topic, so we offer it to the readers of this Genomics Update.

The Russian Noble Prize winner Elie Metchinkoff first suggested that certain bacteria could modify the composition of the gut flora (Metchnikoff, 1907). He suggested that the longevity of Bulgarians and Russians of the Steppes was due to their consumption of 'sour milk' containing beneficial microbes, which in fact probably were lactic acid bacteria (LAB) such as *Lactobacillus bulgaricus*. Henry Tissier of the Pasteur Institute isolated bacteria (now called *Bifidobacterium bifidum*) from the faeces of healthy breast-fed infants and recommended giving it to babies suffering from diarrhoea (Tissier, 1900). In 1935, Minoru Shirota in Japan developed the first commercial probiotic drink called Yakult, which contains *Lactobacillus casei* Shirota that can survive the passage through the stomach and colonize the intestine. The probiotic market is now estimated to be worth about $6 000 000 000 a year and is growing at around 10% annually (UBIC-Consulting, 2008). Since 1981 there have been over 2000 patent applications on probiotics filed (with 'probiotic' mentioned in patent somewhere) and some 524 granted (in the USA and Europe). The two most commonly used probiotics in commercial products are lactobacilli, members of the LAB, and bifidobacteria, but some yeasts and other bacteria have been claimed to have probiotic potential. See Table 1 and Ouwehand and colleagues (2002) for an overview of commercially used strains and their claimed probiotic effects.

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Probiotic mechanisms

What do probiotics actually do? What is the meaning of 'confer a health benefit'? Probiotics are most commonly known as yoghurts or yoghurt-type drinks that people ingest. The consumption of probiotics by humans is intended to improve or maintain a healthy intestine. The claimed modes of action of probiotics include strengthening of the intestinal barrier function, modulation of immune responses, supply of vitamins, and antagonism of pathogens (or other commensals) either by producing antimicrobials or by binding to the mucosa (so called competitive exclusion). (For recent reviews see Marco *et al.*, 2006; Ventura *et al.*, 2007; Kalliomaki *et al.*, 2008; Lebeer *et al.*, 2008; Kleerebezem and Vaughan, 2009.) In general, desired attributes of probiotic strains include adequate survival of the stomach passage (i.e. low pH stability), and adaptation to the host gut environment, including stress response, active and synergistic metabolism, and adherence to the intestinal mucosa and mucus. Probiotics are presumed to have an ecological advantage owing to their capacity to metabolize complex sugars that are derived from the diet as well as from the host. Sugar metabolism enzymes include various glycosyl hydrolases (GHs) which can degrade plant-derived dietary fibres or complex host carbohydrate structures. Bacteriocin production may enhance their competitiveness in the gut. From an industrial perspective, crucial attributes of probiotic strains are good technological properties for production and storage and low health risk to consumers.

Probiotics need not be restricted to food applications or oral delivery. Some can be applied to the skin as lotions or cream (Krutmann, 2009) and have been used to treat vaginal infections (Reid, 2008). Probiotics are also added to animal and fish feed to enhance growth, replacing the banned additive antibiotics or growth hormones (Gatesoupe, 2008; Higuchi *et al.*, 2008; Wynn, 2009). They appear to work by inhibiting/reducing the pathogenic bacterial load that some animals or fish carry. There is evidence for all of these probiotic modes,

Table 1. Examples of commercial probiotic strains and products (adapted from http://en.wikipedia.org/wiki/Probiotic#cite_note-48).

Species/strain	Brand name	Producer	Claimed effect in humans/animals
Bacillus coagulans GBI-30, 6086	GanedenBC[30]	Ganeden Biotech	Improves abdominal pain and bloating in IBS patients. Increases immune response to viral challenge
Bifidobacterium animalis ssp. *lactis* BB-12	BB-12	Chr. Hansen	Reduction in *Strept. mutans* in mouth; IBS amelioration in a multispecies trial
Bifidobacterium animalis ssp. *lactis* HN019 (DR10)	Howaru Bifido	Danisco	Reduced prevalence of atopy and eczema in the first 2 years of life
Bifidobacterium breve Yakult	Bifiene	Yakult	Ulcerative colitis amelioration
Bifidobacterium infantis 35624	Align	Procter & Gamble	Irritable bowel syndrome treatment
Bifidobacterium longum BB536	BB536	Morinaga	Treatment of allergy, especially Japanese cedar pollinosis
Escherichia coli M-17	ProBactrix	BioBalance	Irritable bowel syndrome treatment
Escherichia coli Nissle 1917	Mutaflor	Ardeypharm	Enterocolitis, remission of ulcerative colitis
Lactobacillus acidophilus DDS-1	DDS-1	Nebraska Cultures	Alleviation of traveller's diarrhoea; vitamin production
Lactobacillus acidophilus LA-5	LA-5	Chr. Hansen	Alleviation of acute diarrhoea
Lactobacillus acidophilus NCFM	Howaru acidophilus	Danisco	Improvement of intestinal health, treatment of vaginal/urogential infections
Lactobacillus acidophilus GAL-2	Ghenisson 22	GHEN Co	Improves digestive health in poultry
Lactobacillus brevis KB290	LABRE	Kagome	Improvement of bowel movement, enhances NK activity and interferon-α activity
Lactobacillus casei DN114-001	Actimel, DanActive	Danone	Acute diarrhoea treatment; infection prevention; gut development
Lactobacillus casei CRL431	CRL431	Chr. Hansen	Immune stimulation, Alleviation of acute diarrhoea
Lactobacillus casei F19	Cultura	Arla Foods	Improvement in bowel function
Lactobacillus casei Shirota	Yakult	Yakult	Alleviation of acute diarrhoea
Lactobacillus paracasei St11	Lactobacillus fortis	Nestlé	Natural defence/immune system, gut health
Lactobacillus johnsonii NCC533	LC1 range	Nestlé	Immunomodulation; pathogen inhibition
Lactococcus lactis L1A	VERUM HÄLSOFIL	Norrmejerier	Immune stimulation; improves digestive health; reduces antibiotic-associated diarrhoea
Lactobacillus plantarum 299v	GoodBelly, ProViva, TuZen	NextFoods, Probi, Ferring	Iron absorption
Lactobacillus reuteri ATTC 55730	L. reuteri Protectis	BioGaia Biologics	Diarrhoea prevention and mitigation; eradication of *H. pylori* infection; amelioration of gingivitis.
Lactobacillus rhamnosus GG	Vifit and others	Valio	Immune stimulation; alleviates atopic eczema; prevents diarrhoea in children and many other types of diarrhoea
Lactobacillus rhamnosus LB21	Verum	Norrmejerier	
Lactobacillus rhamnosus GR-1 & *Lactobacillus reuteri* RC-14	Bion, Flore, Intime, Jarrow, Fem-Dophilu	Chr. Hansen	Vaginal colonization and prevention of vaginitis
Lactobacillus acidophilus NCFM & *Bifidobacterium bifidum* BB-12	Florajen3	American Lifeline, Inc	Reduction of *C. difficile*–associated disease (CDAD)
Lactobacillus acidophilus CL1285 & *Lactobacillus casei*	Bio-K+ CL1285	Bio-K+ International	Improves digestive health; prevents Antiobic Associated Diarrhea (AAD; inhibition of pathogens
Lactobacillus acidophilus MNFLM01 & *Enterococcus faecium*	LAB-MOS	Alltech	Lowers pathogen numbers in lamb intestine
Lactobacillus helveticus R0052 & *Lactobacillus rhamnosus* R0011	A'Biotica and others	Institut Rosell	*Helicobacter pylori* inhibition

For several other products with mixtures of probiotic bacteria see http://en.wikipedia.org/wiki/Probiotic#cite_note-48.

but the exact mechanisms of action are still not very clear. Genome-scale analyses of health-promoting bacteria, also coined 'probiogenomics' (Ventura *et al.*, 2009), should provide clues for probiotic mechanisms and potential. Here, we provide an update of recent genomics studies in this field.

Genome sequencing

Table 2 and Fig. 1 give an overview of genome sequencing of putative probiotic bacteria that are publicly available, and Table 3 gives examples of proprietary sequences of commercial probiotics. By far the most

Table 2. Publicly available sequenced complete genomes of (putative) probiotic bacteria (adapted from the GOLD Database (http://www.genomesonline.org; October 2009).

Species	Strain	Accession	Isolation source	Reference
ACTINOBACTERIA				
Bifidobacterium adolescentis	ATCC 15703	NC_008618	Human faeces	Unpublished; Gifu University, Japan
Bifidobacterium animalis ssp. *lactis*	AD011	NC_011835	Human infant faeces	Kim *et al.* (2009)
Bifidobacterium animalis ssp. *lactis*	ATCC SD5219	NC_012814	Human infant faeces	Barrangou *et al.* (2009)
Bifidobacterium animalis ssp. *lactis*	DSM 10140	NC_012815	Swiss yoghurt	Barrangou *et al.* (2009)
Bifidobacterium breve	UCC203			Leahy *et al.* (2005)
Bifidobacterium longum	NCC2705	NC_004307	Human infant faeces	Schell *et al.* (2002)
Bifidobacterium longum	DJO10A	NC_010816	Human adolescent faeces	Lee *et al.* (2008)
Bifidobacterium longum ssp. *infantis*	ATCC 15697	NC_011593	Human infant faeces	Sela *et al.* (2008)
Propionibacterium freundenreichii	ATCC9614		Swiss cheese	Unpublished; INRA, Rennes, France
FIRMICUTES				
Lactobacillus acidophilus	NCFM	NC_006814	Human intestine	Altermann *et al.* (2005)
Lactobacillus casei	ATCC 334	NC_008526	Emmental cheese	Makarova *et al.* (2006)
Lactobacillus casei	BL23	NC_010999		Unpublished; INRA, Jouy-en-Josas, France
Lactobacillus delbrueckii ssp. *bulgaricus*	ATCC BAA-365	NC_008529	French starter culture	Makarova *et al.* (2006)
Lactobacillus delbrueckii ssp. *bulgaricus*	ATCC 11842	NC_008054	Bulgarian yoghurt	van de Guchte *et al.* (2006)
Lactobacillus fermentum	IFO 3956	NC_010610	Japanese fermented plant	Morita *et al.* (2008)
Lactobacillus gasseri	ATCC 33323	NC_008530	Human intestine	Makarova *et al.* (2006)
Lactobacillus helveticus	DPC 4571	NC_010080	Swiss cheese	Callanan *et al.* (2008)
Lactobacillus johnsonii	NCC533	NC_005362	Human intestine	Pridmore *et al.* (2004)
Lactobacillus johnsonii	FI9785	FN298497	Poultry	Wegmann *et al.* (2009)
Lactobacillus plantarum	WCFS1	NC_004567	Human saliva	Kleerebezem *et al.* (2003)
Lactobacillus plantarum	JDM1	NC_012984		Zhang *et al.* (2009)
Lactobacillus reuteri	F275, JCM1112	NC_010609	Human adult intestine	Morita *et al.* (2008)
Lactobacillus rhamnosus	GG	NC_013198	Human faeces	Kankainen *et al.* (2009)
Lactobacillus rhamnosus	ATCC53103	AP011548	Human intestine	Morita *et al.* (2009)
Lactobacillus salivarius	UCC118	NC_007929	Human small intestine	Claesson *et al.* (2006)
Leuconostoc citreum	KM20	NC_010471	Korean fermented vegetables	Kim *et al.* (2008a)

In the 'Ongoing genome sequencing projects' (http://www.genomesonline.org/gold.cgi?want=Bacterial+Ongoing+Genomes#) in the GOLD database, another 45 *Bifidobacterium* and 98 *Lactobacillus* strains are listed; incomplete genome sequence data is already publicly available for 10 and 34 of these strains respectively. Although many are gut isolates, not all will represent probiotic strains.

used probiotics and the ones which have their genomes sequenced are those associated with gut health. Details of genomes sequenced before 2009 have been summarized by Mayo and colleagues (2008) and Ventura and colleagues (2009). Infants are born with a sterile gastrointestinal (GI) tract but in breast-fed babies colonization by bifidobacteria is rapidly seen. It is thought that these bacteria confer a health benefit to the infant. The first colonizer is *Bifidobacterium longum* ssp. *infantis*, which has the largest genome of any sequenced bifidobacteria at 2.83 Mb (Sela *et al*., 2008). The genome has complete pathways for the synthesis of some vitamins and a novel 43 kb gene cluster encoding a system for the import and degradation of human milk oligosaccharides (HMOs). After weaning, the numbers of this bifidobacterium decline but others become more dominant. *Bifidobacterium animalis* ssp. *lactis*, a resident of the GI tract and the most commonly used probiotic in Europe and North America, has a genome size of only 1.9 Mb. These bifidobacteria lack the HMO cluster as presumably post-weaned animals no longer require this functionality. They do, however, contain the *fos* gene cluster necessary to produce the enzymes to break down and utilize health-promoting fructo-oligosaccharides, a well-known prebiotic and bifidogenic factor.

Several new genome sequences of probiotics have been released in 2009. *Bifidobacterium animalis* ssp. *lactis* AD011, isolated from a healthy breast-fed infant, has a high level of immunomodulatory activity (Kim *et al*., 2008b). Its genome encodes multiple glycosylases than can degrade plant- or milk-derived oligosaccharides, and the *fos* gene cluster for processing of fructo-oligosaccharides (Kim *et al*., 2009). *Bifidobacterium animalis* ssp. *lactis* strains B1-04 and DSM10140, both from commercial probiotic products, differ only by 47 single nucleotide polymorphisms and four small indels, of which one indel in a CRISPR (Barrangou *et al*., 2009). *Lactobacillus johnsonii* FI9785 is a competitive exclusion agent against pathogens in poultry (Wegmann *et al*., 2009). *Lactobacillus plantarum* JDM1 is a widely used Chinese commercial probiotic strain which appears to have lost 100 kb relative to the non-commercial strain WCFS1, encoding sugar transport and metabolism, possibly due to prolonged growth of this probiotic strain in rich medium (Zhang *et al*., 2009). *Lactobacillus rhamnosus* GG and *Lactobacillus rhamnosus* ATCC53103, probiotic strains

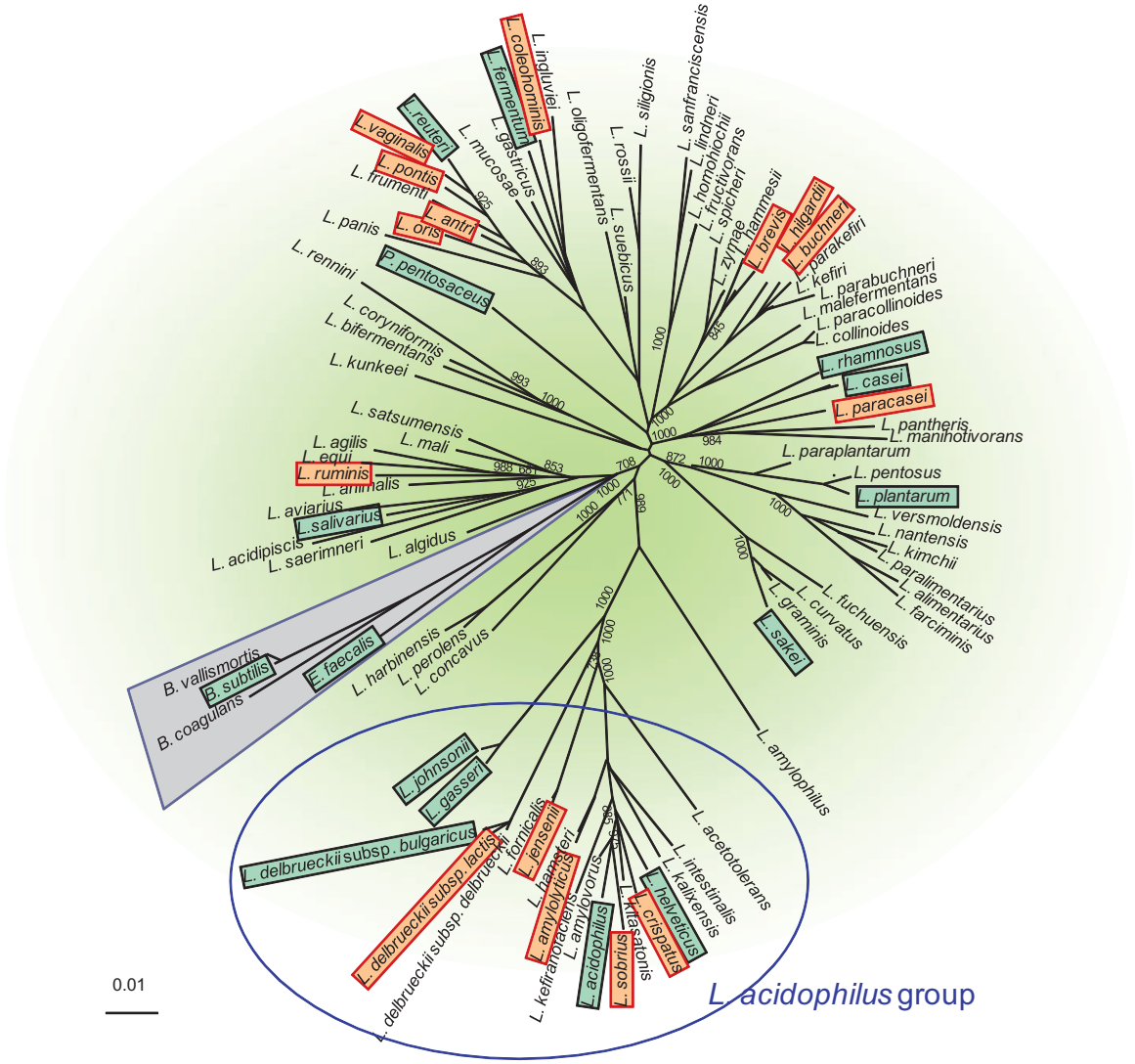

Fig. 1. Evolutionary relationships between the main gastrointestinal tract commensal lactobacilli, based on a neighbour-joining tree of 16S rRNA gene sequences. Bootstrap values above 600 are indicated. Bacterial taxa for which whole genome sequences are available are shaded in green. The outgroup is shaded in grey. Lactobacilli for which genome sequencing is ongoing/incomplete are shaded in red. Reproduced and adapted from Ventura and colleagues (2009), with permission from Macmillan Publishers Limited, 2009.

used widely for nearly 20 years in a variety of functional foods, differ only by deletion of 5 kb in ATCC53103, and an inversion of 8.9 kb (Kankainen *et al.*, 2009; Morita *et al.*, 2009). Compared with other sequenced intestinal lactobacilli, both *Lb. rhamnosus* genomes have a relatively high number of proteins involved in carbohydrate and amino acid metabolism and transport, and defence mechanisms. In particular, 28 complete PTS-type transporters and 25 putative GHs are encoded, including the alpha-L-fucosidase (GH29; see Cazy database http://www.cazy.org) and alpha-mannosidase (GH38) families, which are not found in other sequenced lactobacilli. In addition, these *Lb. rhamnosus* genomes have three gene clusters encoding proteins with WxL domains which can attach to the peptidoglycan on cell surfaces (Siezen *et al.*, 2006; Brinster *et al.*, 2007); again, these gene clusters have not been found in other intestinal lactobacilli, but rather in plant-associated Gram-positive bacteria (Siezen *et al.*, 2006). Most novel is the finding that *Lb. rhamnosus* GG has a gene cluster *spaCBA*, encoding three secreted pilin proteins with LPxTG-type peptidoglycan anchors, which is not present in the highly syntenous genome of *Lb. rhamnosus* LC705 (Kankainen *et al.*, 2009). SpaA is the major scaffolding protein upon which the minor pili proteins SpaB and SpaC are attached. Using insertional inactivation of *spaC*, a truncated SpaC protein was produced which resulted in cells with a greatly reduced binding to human mucus (Kankainen *et al.*, 2009). The authors suggest that the presence of SpaC-containing pili (Fig. 2) may possibly explain the longer persistence of this

Table 3. Proprietary genome sequences of commercial (putative) probiotic bacteria.

Species	Strain	Genome size (Mb)	Company	Reference
ACTINOBACTERIA				
Bifidobacterium animalis ssp. *lactis*	BB-12	2.0	Chr. Hansen, Denmark	christel.garrigues@dk.chr-hansen.com
Bifidobacterium breve	Yakult	2.35	Yakult, Japan	yukio-shirasawa@yakult.co.jp
Bifidobacterium breve	M-16V	2.3	Morinaga Milk, Japan	k_nanba@morinagamilk.co.jp
Bifidobacterium longum biot *infantis*	M-63	2.8	Morinaga Milk, Japan	k_nanba@morinagamilk.co.jp
Bifidobacterium longum	BB536	2.5	Morinaga Milk, Japan	k_nanba@morinagamilk.co.jp
Bifidobacterium lactis		1.94	Danone, France	tamara.smokvina@danone.com
FIRMICUTES				
Lactobacillus brevis	KB290	2.49	Kagome, Japan	masanori_fukao@kagome.co.jp
Lactobacillus casei	Shirota	3.03	Yakult, Japan	yukio-shirasawa@yakult.co.jp
Lactobacillus casei		3.14	Danone, France	tamara.smokvina@danone.com
Lactobacillus reuteri	ATCC55730	2.0	SLU, Sweden	klara.bath@mikrob.slu.se

Source: Abstracts Symposium on Lactic Acid Bacteria 2005 and 2008, Egmond aan Zee, the Netherlands.

strain in the GI tract than strain LC705. Together with the high potential for sugar uptake and metabolism, this may explain probiotic effects of these *Lb. rhamnosus* strains.

Experimental omics exploration of molecular mechanisms

Ingested probiotic microbes themselves will react to the new environment of the intestine and change their gene expression accordingly. Transcriptional responses of bifidobacteria to human and formula milk have been described in *in vitro* and *in vivo* experiments, the latter from faecal samples of infants (Gonzalez *et al*., 2008; Klaassens *et al*., 2009). Carbohydrate metabolism genes are commonly upregulated, and include enzymes for degradation of complex plant carbohydrates, which are poorly digested by the host or other intestinal microbes (Klaassens *et al*., 2009), and for metabolism of mucin and HMOs (Gonzalez *et al*., 2008). In addition, putative genes were upregulated for cell-surface type 2 glycoprotein-

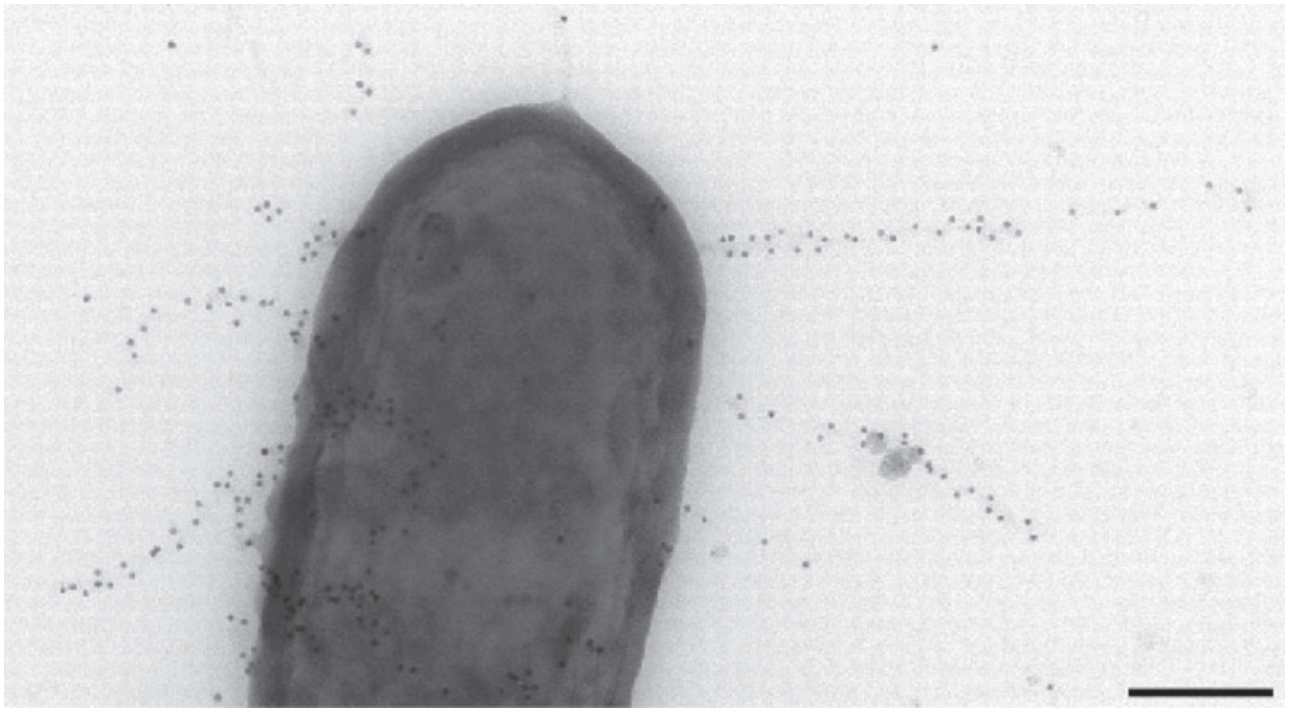

Fig. 2. Identification of pili in *L. rhamnosus* GG by immunogold high-resolution electron micrography. Multiple pili are shown with gold-labelled SpaC proteins. Reproduced with permission from Kankainen and colleagues (2009).

binding fimbriae that are implicated in attachment and colonization in the intestine (Gonzalez *et al*., 2008).

In vitro transcriptional response of *Lactobacillus reuteri* ATCC55730, a strain marketed for probiotic usage, to bile stress has been described (Whitehead *et al*., 2008). Upregulation was seen for genes involved in multidrug transport, membrane/cell wall stress, oxidative stress, DNA damage and protein denaturation. Transcription and comparative genomics analysis of *Lb. johnsonii* NCC533, an isolate characterized by long gut persistence, identified three genetic loci that were specifically expressed in the jejunum of mice mono-colonized with this strain, encoding a PTS-type sugar transporter, glycosyltransferases and an IgA-type protease (Denou *et al*., 2008). Several years ago, a very elegant resolvase-based *in vivo* expression technology was developed to study specific *in vivo* gene expression in *L. plantarum* WCFS1, using the mouse GI tract as a model system (Bron *et al*., 2004). This has now been followed up by whole genome transcriptome profiling of strain WCFS1 during colonization of the caeca of germ-free mice fed either standard low-fat rodent diet rich in complex plant polysaccharides or a Western diet rich in simple sugars and fats (Marco *et al*., 2009). Numerous carbon metabolism pathways of *L. plantarum* were upregulated on both diets, including uptake and utilization of raffinose, cellulose, maltose, lactose/galactose, sucrose, melibiose, sugar alcohols and sialic acid. Sialic acid is a common component of (human) gut glycoproteins.

Host responses to potential probiotics have recently been described in intervention studies in healthy human volunteers. Duodenal mucosa was sampled after intraduodenal infusion (Troost *et al*., 2008) or oral ingestion (van Baarlen *et al*., 2009) of *L. plantarum* WCFS1. The continuous perfusion study showed that after prolonged exposure, mucosal cells switched to a more proliferative phase with upregulation of genes involved in lipid metabolism, cellular growth and development. Cell death and immune responses were triggered, but cell-death executing cells or inflammatory signals were not expressed. In the second study, consumption of live *L. plantarum* cells showed striking modulation of NF-κB-dependent pathways in mucosal cells, and identified cellular pathways that correlated with the establishment of immune tolerance in healthy adults (van Baarlen *et al*., 2009). Figure 3 summarizes some of the mechanistic events underlying probiotic effects that are beginning to be understood from these *in vitro* and *in vivo* studies.

Adaptation of probiotic strains

Bifidobacterium longum DJO10A, an intestinal isolate, was shown to lose functionality by gene loss after prolonged pure culture (Lee *et al*., 2008). It would appear that when growing in the competitive environment of the colon the cells retained some important functionalities predicted to be involved in diverse traits pertinent to the human intestinal environment, specifically oligosaccharide and polyol utilization, arsenic resistance and bacteriocin production. The targeted loss of genomic regions was experimentally validated when growth of the intestinal *B. longum* in the laboratory for 1000 generations resulted in two large deletions, one in a bacteriocin-encoding region, analogous to a predicted deletion event in the commercial strain *B. longum* NCC2705 (O'Sullivan, 2008). This deletion strain showed a significantly reduced competitive ability against *Clostridium difficile* and *Escherichia coli*. The deleted region was between two IS30 elements which were experimentally demonstrated to be hyperactive within the genome. Hence, deletion of genomic regions, often facilitated by mobile elements, allows bifidobacteria to adapt to fermentation environments in a very rapid manner (two genome deletions per 1000 generations) and the concomitant loss of possible competitive abilities in the gut. This has implications for industry, because the claims for the use of a probiotic need to be fully substantiated.

Future

One of the most remarkable probiotic discoveries was made by the German Alfred Nissle in 1917 in World War I. Life in the trenches was dangerous and not just from the fighting. Disease was rife, especially enterocolitis (inflammation of the small and large intestine) caused by outbreaks of shigellosis. One soldier did not succumb to the disease and Nissle isolated from his faeces a bacterium with which he successfully treated other soldiers. *Escherichia coli* Nissle 1917 is still in use and is one of the few examples of a non-LAB probiotic (Mutaflor) (Table 1). At present, many of the commercial probiotic strains originate from the intestine of healthy infants and adults. Current research focuses on the determination of the characteristics these bacteria use to survive and compete successfully in the intestine, and with this knowledge more effective probiotic strains can be identified. To speed up this search, numerous gut metagenomic sequencing efforts are ongoing world-wide to identify potential new probiotic candidates (Gill *et al*., 2006; Kurokawa *et al*., 2007). See also the Human Gut Metagenome Initiative (http://www.international.inra.fr/press/mapping_the_human_intestinal_metagenome) and the Human Gut Microbiome Initiative (Gordon *et al*., 2006) (http://genomeold.wustl.edu/hgm/HGM_frontpage.cgi).

Perhaps the future will bring us health-promoting drinks containing mixtures of many probiotic strains, much like the cocktails used these days for vaccination against infectious diseases. And what will be the next hype? Memory-enhancing drinks would definitely be a commercial success on quiz night in the pub!

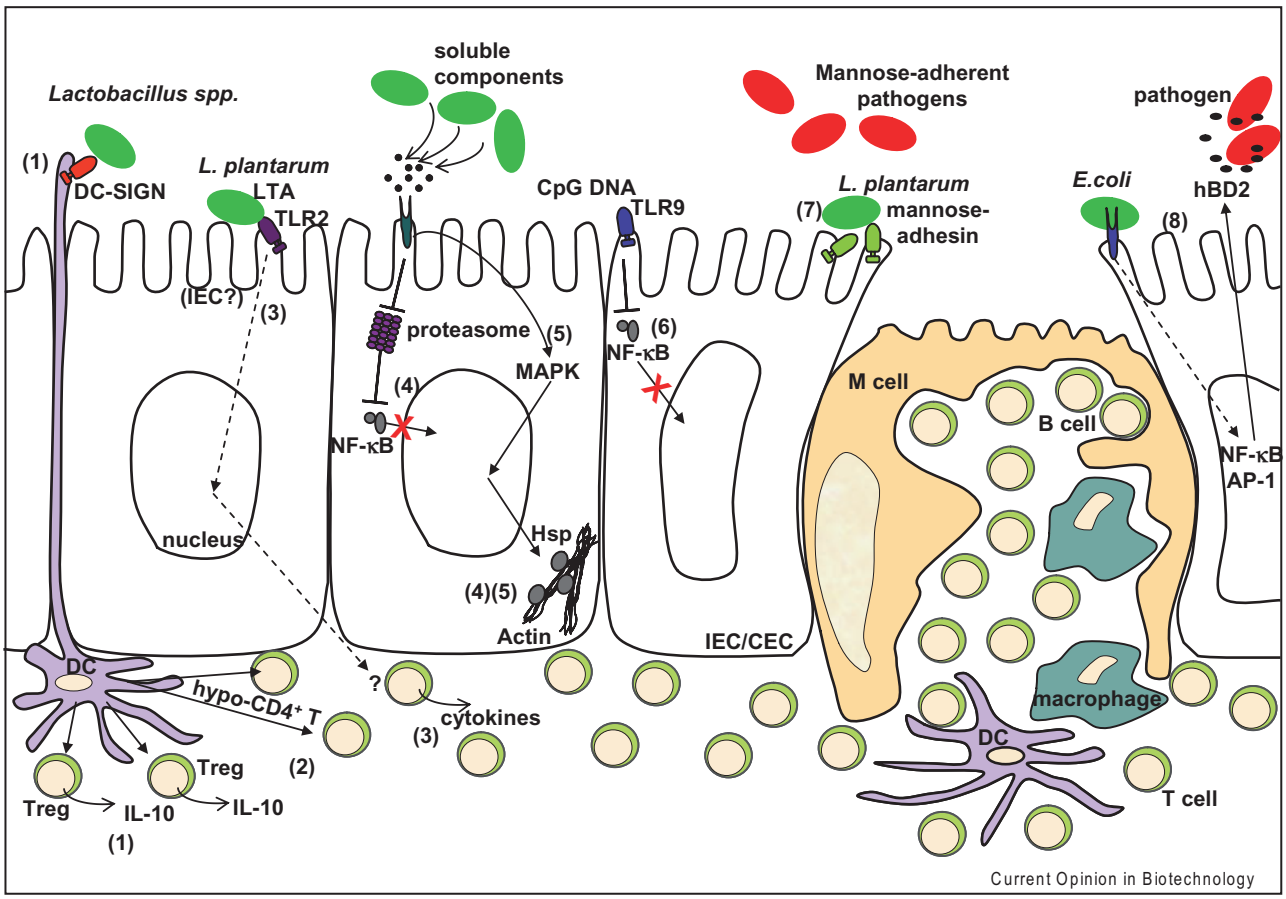

Fig. 3. Bacterial and host effector molecules with potential probiotic effects. *Lactobacillus* strains are able to induce IL-10-producing, regulatory T cells (T reg) through DC-SIGN interaction (1). They can also induce hyporesponsive $CD4^+$ T-cell populations after DC interaction (2). Lipoteichoic acid (LTA) composition is responsible for the differential modulation of cytokine production (3). Modulation of inflammatory responses by inactivation of the NF-κB signalling pathway is achieved through proteasome inhibition after IEC recognition of soluble probiotic components (4) or after recognition of bacterial motifs (e.g. CpG DNA by TLR9 receptors) (6). The induction of Hsps (either via 4 or 5) stabilizing the actin cytoskeleton would strengthen the mucosal barrier. Pathogen attachment and growth could be counteracted by strains possessing mannose adhesins (7) or by induction of hBD2 in IECs (8). M cell is an epithelial cell specialized in antigen uptake and transport. Reproduced and adapted with permission from Marco and colleagues (2006), Elsevier Ltd.

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References

Altermann, E., Russell, W.M., Azcarate-Peril, M.A., Barrangou, R., Buck, B.L., McAuliffe, O., *et al.* (2005) Complete genome sequence of the probiotic lactic acid bacterium *Lactobacillus acidophilus* NCFM. *Proc Natl Acad Sci USA* **102:** 3906–3912.

van Baarlen, P., Troost, F.J., van Hemert, S., van der Meer, C., de Vos, W.M., de Groot, P.J., *et al.* (2009) Differential NF-kappaB pathways induction by *Lactobacillus plantarum* in the duodenum of healthy humans correlating with immune tolerance. *Proc Natl Acad Sci USA* **106:** 2371–2376.

Barrangou, R., Briczinski, E.P., Traeger, L.L., Loquasto, J.R., Richards, M., Horvath, P., *et al.* (2009) Comparison of the complete genome sequences of *Bifidobacterium animalis* subsp. *lactis* DSM 10140 and Bl-04. *J Bacteriol* **191:** 4144–4151.

Brinster, S., Furlan, S., and Serror, P. (2007) C-terminal WxL domain mediates cell wall binding in *Enterococcus faecalis* and other gram-positive bacteria. *J Bacteriol* **189:** 1244–1253.

Bron, P.A., Grangette, C., Mercenier, A., de Vos, W.M., and Kleerebezem, M. (2004) Identification of *Lactobacillus plantarum* genes that are induced in the gastrointestinal tract of mice. *J Bacteriol* **186:** 5721–5729.

Callanan, M., Kaleta, P., O'Callaghan, J., O'Sullivan, O., Jordan, K., McAuliffe, O., *et al.* (2008) Genome sequence of *Lactobacillus helveticus*, an organism distinguished by

selective gene loss and insertion sequence element expansion. *J Bacteriol* **190:** 727–735.

Claesson, M.J., Li, Y., Leahy, S., Canchaya, C., van Pijkeren, J.P., Cerdeno-Tarraga, A.M., *et al.* (2006) Multireplicon genome architecture of *Lactobacillus salivarius*. *Proc Natl Acad Sci USA* **103:** 6718–6723.

Denou, E., Pridmore, R.D., Berger, B., Panoff, J.M., Arigoni, F., and Brussow, H. (2008) Identification of genes associated with the long-gut-persistence phenotype of the probiotic *Lactobacillus johnsonii* strain NCC533 using a combination of genomics and transcriptome analysis. *J Bacteriol* **190:** 3161–3168.

Gatesoupe, F.J. (2008) Updating the importance of lactic acid bacteria in fish farming: natural occurrence and probiotic treatments. *J Mol Microbiol Biotechnol* **14:** 107–114.

Gill, S.R., Pop, M., DeBoy, R.T., Eckburg, P.B., Turnbaugh, P.J., Samuel, B.S., *et al.* (2006) Metagenomic analysis of the human distal gut microbiome. *Science* **312:** 1355–1359.

Gonzalez, R., Klaassens, E.S., Malinen, E., de Vos, W.M., and Vaughan, E.E. (2008) Differential transcriptional response of *Bifidobacterium longum* to human milk, formula milk, and galactooligosaccharide. *Appl Environ Microbiol* **74:** 4686–4694.

Gordon, J.I., Ley, R.E., Wilson, R., Mardis, E.J.X., Fraser, C.M., and Relman, D.A. (2006) *Extending our view of self: the Human Gut Microbiome Initiative (HGMI)* [WWW document]. URL http://www.genome.gov/Pages/Research/Sequencing/SeqProposals/HGMISeq.pdf.

van de Guchte, M., Penaud, S., Grimaldi, C., Barbe, V., Bryson, K., Nicolas, P., *et al.* (2006) The complete genome sequence of *Lactobacillus bulgaricus* reveals extensive and ongoing reductive evolution. *Proc Natl Acad Sci USA* **103:** 9274–9279.

Higuchi, W., Muramatsu, M., Dohmae, S., Takano, T., Isobe, H., Yabe, S., *et al.* (2008) Identification of probiotic lactobacilli used for animal feeds on the basis of 16S ribosomal RNA gene sequence. *Microbiol Immunol* **52:** 559–563.

Kalliomaki, M., Salminen, S., and Isolauri, E. (2008) Positive interactions with the microbiota: probiotics. *Adv Exp Med Biol* **635:** 57–66.

Kankainen, M., Paulin, L., Tynkkynen, S., von Ossowski, I., Reunanen, J., Partanen, P., *et al.* (2009) Comparative genomic analysis of *Lactobacillus rhamnosus* GG reveals pili containing a human-mucus binding protein. *Proc Natl Acad Sci USA* **106:** 17193–17198.

Kim, J.F., Jeong, H., Lee, J.S., Choi, S.H., Ha, M., Hur, C.G., *et al.* (2008a) Complete genome sequence of *Leuconostoc citreum* KM20. *J Bacteriol* **190:** 3093–3094.

Kim, J.F., Jeong, H., Yu, D.S., Choi, S.H., Hur, C.G., Park, M.S., *et al.* (2009) Genome sequence of the probiotic bacterium *Bifidobacterium animalis* subsp. *lactis* AD011. *J Bacteriol* **191:** 678–679.

Kim, J.Y., Choi, Y.O., and Ji, G.E. (2008b) Effect of oral probiotics (*Bifidobacterium lactis* AD011 and *Lactobacillus acidophilus* AD031) administration on ovalbumin-induced food allergy mouse model. *J Microbiol Biotechnol* **18:** 1393–1400.

Klaassens, E.S., Boesten, R.J., Haarman, M., Knol, J., Schuren, F.H., Vaughan, E.E., and de Vos, W.M. (2009) Mixed-species genomic microarray analysis of fecal samples reveals differential transcriptional responses of bifidobacteria in breast- and formula-fed infants. *Appl Environ Microbiol* **75:** 2668–2676.

Kleerebezem, M., and Vaughan, E.E. (2009) Probiotic and gut lactobacilli and bifidobacteria: molecular approaches to study diversity and activity. *Ann Rev Microbiol* **63:** 269–290.

Kleerebezem, M., Boekhorst, J., van Kranenburg, R., Molenaar, D., Kuipers, O.P., Leer, R., *et al.* (2003) Complete genome sequence of *Lactobacillus plantarum* WCFS1. *Proc Natl Acad Sci USA* **100:** 1990–1995.

Krutmann, J. (2009) Pre- and probiotics for human skin. *J Dermatol Sci* **54:** 1–5.

Kurokawa, K., Itoh, T., Kuwahara, T., Oshima, K., Toh, H., Toyoda, A., *et al.* (2007) Comparative metagenomics revealed commonly enriched gene sets in human gut microbiomes. *DNA Res* **14:** 169–181.

Leahy, S.C., Higgins, D.G., Fitzgerald, G.F., and van Sinderen, D. (2005) Getting better with bifidobacteria. *J Appl Microbiol* **98:** 1303–1315.

Lebeer, S., Vanderleyden, J., and De Keersmaecker, S.C. (2008) Genes and molecules of lactobacilli supporting probiotic action. *Microbiol Mol Biol Rev* **72:** 728–764.

Lee, J.H., Karamychev, V.N., Kozyavkin, S.A., Mills, D., Pavlov, A.R., Pavlova, N.V., *et al.* (2008) Comparative genomic analysis of the gut bacterium *Bifidobacterium longum* reveals loci susceptible to deletion during pure culture growth. *BMC Genomics* **9:** 247.

Makarova, K., Slesarev, A., Wolf, Y., Sorokin, A., Mirkin, B., Koonin, E., *et al.* (2006) Comparative genomics of the lactic acid bacteria. *Proc Natl Acad Sci USA* **103:** 15611–15616.

Marco, M.L., Pavan, S., and Kleerebezem, M. (2006) Towards understanding molecular modes of probiotic action. *Curr Opin Biotechnol* **17:** 204–210.

Marco, M.L., Peters, T.H., Bongers, R.S., Molenaar, D., van Hemert, S., Sonnenburg, J.L., *et al.* (2009) Lifestyle of *Lactobacillus plantarum* in the mouse caecum. *Environ Microbiol* **11:** 2747–2757.

Mayo, B., van Sinderen, D., and Ventura, M. (2008) Genome analysis of food grade lactic acid-producing bacteria: from basics to applications. *Curr Genomics* **9:** 169–183.

Metchnikoff, E. (1907) *Essais Optimistes. The Prolongation of Life Optimistic Studies*. London, UK: Heinemann.

Morita, H., Toh, H., Fukuda, S., Horikawa, H., Oshima, K., Suzuki, T., *et al.* (2008) Comparative genome analysis of *Lactobacillus reuteri* and *Lactobacillus fermentum* reveal a genomic island for reuterin and cobalamin production. *DNA Res* **15:** 151–161.

Morita, H., Toh, H., Oshima, K., Murakami, M., Taylor, T.D., Igimi, S., and Hattori, M. (2009) Complete genome sequence of probiotic *Lactobacillus rhamnosus* ATCC 53103. *J Bacteriol* [Epub ahead of print].

O'Sullivan, D.J. (2008) Genomics can advance the potential for probiotic cultures to improve liver and overall health. *Curr Pharm Des* **14:** 1376–1381.

Ouwehand, A.C., Salminen, S., and Isolauri, E. (2002) Probiotics: an overview of beneficial effects. *Antonie Van Leeuwenhoek* **82:** 279–289.

Pridmore, R.D., Berger, B., Desiere, F., Vilanova, D., Barretto, C., Pittet, A.C., *et al.* (2004) The genome sequence of the probiotic intestinal bacterium *Lactobacillus johnsonii* NCC 533. *Proc Natl Acad Sci USA* **101:** 2512–2517.

Reid, G. (2008) Probiotic lactobacilli for urogenital health in women. *J Clin Gastroenterol* **42** (Suppl. 3 Part 2)**:** S234–S236.

Schell, M.A., Karmirantzou, M., Snel, B., Vilanova, D., Berger, B., Pessi, G., *et al.* (2002) The genome sequence of *Bifidobacterium longum* reflects its adaptation to the human gastrointestinal tract. *Proc Natl Acad Sci USA* **99:** 14422–14427.

Sela, D.A., Chapman, J., Adeuya, A., Kim, J.H., Chen, F., Whitehead, T.R., *et al.* (2008) The genome sequence of *Bifidobacterium longum* subsp. *infantis* reveals adaptations for milk utilization within the infant microbiome. *Proc Natl Acad Sci USA* **105:** 18964–18969.

Siezen, R., Boekhorst, J., Muscariello, L., Molenaar, D., Renckens, B., and Kleerebezem, M. (2006) *Lactobacillus plantarum* gene clusters encoding putative cell-surface protein complexes for carbohydrate utilization are conserved in specific gram-positive bacteria. *BMC Genomics* **7:** 126.

Tissier, H. (1900) *Recherchers sur la flora intestinale normale et pathologique du nourisson*. Paris, France: University of Paris.

Troost, F.J., van Baarlen, P., Lindsey, P., Kodde, A., de Vos, W.M., Kleerebezem, M., and Brummer, R.J. (2008) Identification of the transcriptional response of human intestinal mucosa to *Lactobacillus plantarum* WCFS1 in vivo. *BMC Genomics* **9:** 374.

UBIC-Consulting (2008) *The World Probiotic Ingredient Market 2005–2007* [WWW document]. URL http://www.ubic-consulting.com/template/fs/documents/Nutraceuticals/Probiotic-synbiotic-Ingredient-Market.pdf.

Ventura, M., O'Connell-Motherway, M., Leahy, S., Moreno-Munoz, J.A., Fitzgerald, G.F., and van Sinderen, D. (2007) From bacterial genome to functionality; case bifidobacteria. *Int J Food Microbiol* **120:** 2–12.

Ventura, M., O'Flaherty, S., Claesson, M.J., Turroni, F., Klaenhammer, T.R., van Sinderen, D., and O'Toole, P.W. (2009) Genome-scale analyses of health-promoting bacteria: probiogenomics. *Nat Rev Microbiol* **7:** 61–71.

Wegmann, U., Overweg, K., Horn, N., Goesmann, A., Narbad, A., Gasson, M.J., and Shearman, C. (2009) The complete genome sequence of *Lactobacillus johnsonii* FI9785, a competitive exclusion agent against pathogens in poultry. *J Bacteriol* **191:** 7142–7143.

Whitehead, K., Versalovic, J., Roos, S., and Britton, R.A. (2008) Genomic and genetic characterization of the bile stress response of probiotic *Lactobacillus reuteri* ATCC 55730. *Appl Environ Microbiol* **74:** 1812–1819.

Wynn, S.G. (2009) Probiotics in veterinary practice. *J Am Vet Med Assoc* **234:** 606–613.

Zhang, Z.Y., Liu, C., Zhu, Y.Z., Zhong, Y., Zhu, Y.Q., Zheng, H.J., *et al.* (2009) Complete genome sequence of *Lactobacillus plantarum* JDM1. *J Bacteriol* **191:** 5020–5021.

