MINIREVIEW

The life history of *Lactobacillus acidophilus* as a probiotic: a tale of revisionary taxonomy, misidentification and commercial success

Matthew Bull[1], Sue Plummer[2], Julian Marchesi[1,3] & Eshwar Mahenthiralingam[1]

[1]Organisms and Environment Division, Cardiff School of Biosciences, Cardiff University, Cardiff, UK; [2]Obsidian Research Ltd., Port Talbot, UK; and [3]Department of Hepatology and Gastroenterology, St Mary's Hospital, Imperial College London, London, UK

Correspondence: Eshwar Mahenthiralingam, Cardiff School of Biosciences, Cardiff University, Room 0.23 Main Building, Museum Avenue, Cardiff, CF10 3AT, UK.
Tel. +44 (0)29 20875875;
fax: +44 (0)29 20874305;
e-mail: MahenthiralingamE@cardiff.ac.uk

Received 2 August 2013; revised 30 September 2013; accepted 2 October 2013. Final version published online 24 October 2013.

DOI: 10.1111/1574-6968.12293

Editor: Craig Winstanley

Keywords
Lactobacillus acidophilus; food microbiology; probiotics; taxonomy; genomics; identification.

Abstract

Lactobacillus acidophilus is a commercially significant bacterial probiotic, originally isolated from the human gastrointestinal tract and designated *Bacillus acidophilus* in 1900. Throughout the development of methods to identify and characterise bacteria, *L. acidophilus* has undergone multiple taxonomic revisions and is now the type species of a phylogenetic subgroup in the highly diverse and heterogeneous *Lactobacillus* genus. As a result of the limitations of differentiating phenotypically similar species by morphological and biochemical means and revisionary nature of *Lactobacillus* taxonomy, the characterisation of *L. acidophilus* has struggled with misidentification and misrepresentation. In contrast, due to its global use as a probiotic supplement in functional foods, *L. acidophilus sensu stricto* is now one of the most well-characterised *Lactobacillus* species. Here, we establish the provenance of *L. acidophilus* strains, unpicking historical and current misidentifications of *L. acidophilus*, and reviewing the probiotic, genomic and physiological characteristics of this important *Lactobacillus* species.

Introduction

Lactic acid bacteria (LAB) constitute a diverse group of Gram-positive, nonsporulating, catalase-negative organisms that are found in a number of habitats (Carr *et al.*, 2002). LAB comprise multiple genera within the order *Lactobacilliales* that are acid tolerant, of which *Enterococcus*, *Streptococcus* and *Lactobacillus* species are among the most well characterised. They are known constituents of the human gut (Arumugam *et al.*, 2011) and also occur widely in dairy, meat, plants and fermented products of commercial value (Carr *et al.*, 2002). As a result of their ancient anthropological use in food preservation and their ability to rapidly ferment carbohydrates to lactic acid, they have become industrially important bacteria and are used in a myriad of food and agricultural fermentations worldwide. Their growth causes acidification of food material, preserving the product and imparting unique textures and flavours (Kleerebezem & Hugenholtz, 2003).

Probiotics are 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host' (FAO/WHO, 2002). *Lactobacillus acidophilus* is widely recognised to have probiotic effects and is one of the most commonly suggested organism for dietary use (Shah, 2007). It is frequently added to yoghurt and fermented milk products, with *c.* 80% of the yoghurts produced in the United States containing *L. acidophilus* (Sanders, 2003). *Lactobacillus acidophilus* isolates also form part of the natural human microbiota and have been cultured from the oral (Ahrné *et al.*, 1998), digestive (Kulp & Rettger, 1924) and vaginal (Rogosa & Sharpe, 1960) tracts. Here, we summarise key research on *L. acidophilus,* spanning its original isolation as normal human microbiota (Fig. 1) and describing its genomic, biosynthetic and probiotic characteristics (Fig. 2). In addition, we emphasise a need for rigour in describing *L. acidophilus* isolates by highlighting recent studies that incorrectly report the identity of isolates.

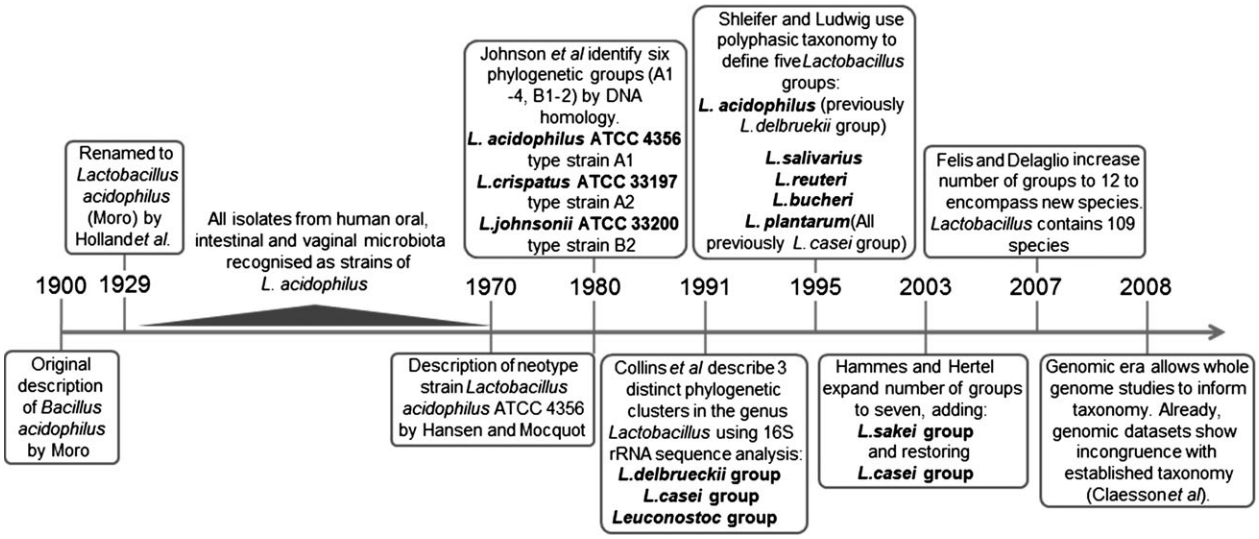

Fig. 1. History of *Lactobacillus acidophilus.* Major milestones in the development of *Lactobacillus* taxonomy, and the resulting effects on the taxonomic placement of *Lactobacillus acidophilus*.

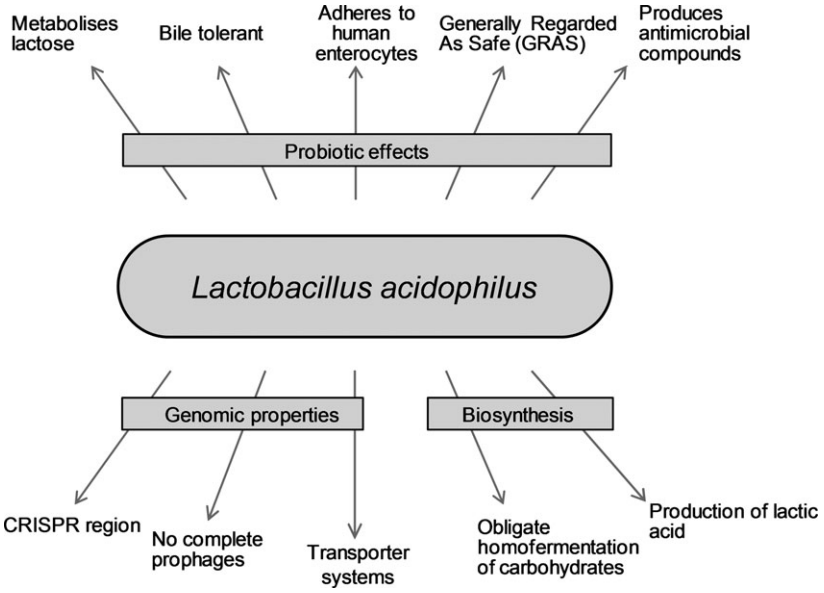

Fig. 2. Major genomic, biosynthetic and probiotic characteristics of *Lactobacillus acidophilus.* Historically, *Lactobacillus acidophilus* has been known for its probiotic effects in humans. Through further characterisation of this effect, and the determination of the genome sequence of *Lactobacillus acidophilus* NCFM, many biosynthetic capabilities of *Lactobacillus acidophilus* have been described.

Taxonomy of the lactobacilli

Lactobacillus is a highly heterogeneous genus, encompassing bacteria with a wide range of biochemical and physiological properties (Felis & Dellaglio, 2007). The genus *Lactobacillus* is the largest of those that belong to the LAB, with 185 species validly described at the time of writing, and increasing substantially from 145 in 2008 as a result of the reclassification of multiple species (Euzéby, 1997; Claesson *et al.*, 2008). From the initial description of the species *Lactobacillus acidophilus* in 1920 (Holland) until around 1970, many *Lactobacillus* isolates from human mucosal surfaces were collectively identified as *L. acidophilus* (Fig. 1). The identification of isolates using traditional phenotypic characteristics such as the fermentation of carbohydrates and cellular morphology, combined with the lack of a robust taxonomical framework, had historically led to such *Lactobacillus* isolates being incorrectly designated at the genus and species level. At the last review, the taxonomy of the genus *Lactobacillus,* it consisted of 14 phylogenetic subgroups (Felis & Dellaglio, 2007).

The *L. acidophilus* group is one of the most well-defined and deep-branching *Lactobacillus* phylogenetic subgroups (Fig. 3). Although its definition is partially based on DNA-DNA homology, the genomic GC content of constituent species ranges from 32% to 50% (Felis & Dellaglio, 2007),

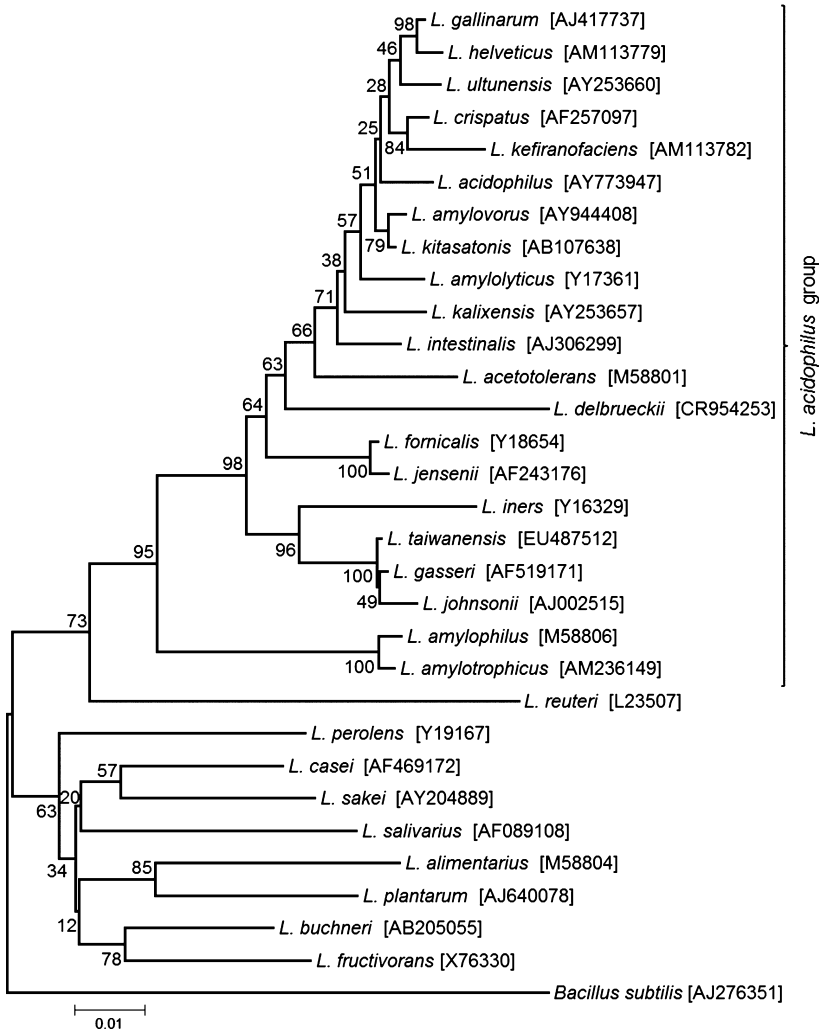

Fig. 3. Phylogenetic placement of the *Lactobacillus acidophilus* phylogenetic subgroup within the *Lactobacillus* genus. A phylogenetic tree of aligned 16S rRNA gene sequences from type strains of the *Lactobacillus acidophilus* phylogenetic subgroup (indicated with a brace) and representative type strains from the other *Lactobacillus* phylogenetic subgroups. The tree was rooted with the 16S rRNA gene from *Bacillus subtilis* DSM10. The genetic distance scale, bootstrap values and GenBank nucleotide accession numbers are indicated.

which is much larger than normally accepted for well-defined bacterial genera (Schleifer & Ludwig, 1995). The dawning of the postgenomic era has now added more tools to the taxonomist's toolkit, providing clarification and as well as further insight into how the taxonomy of the most challenging and complex bacterial groups can be resolved. Recent research into the relatedness of species in the *L. acidophilus* group has used polyphasic taxonomy, combining traditional phenotypic characteristics, such as sugar fermentation patterns (Yeung *et al.*, 2004), sequence analyses of genes, such as 16S rRNA, *rpoA*, *pheS* (Naser *et al.*, 2007), *groEL* (Claesson *et al.*, 2008), *tuf* (Ventura *et al.*, 2003), DNA fingerprinting methods such as rep-PCR (Gevers *et al.*, 2001) and pulsed-field gel electrophoresis (PFGE; Yeung *et al.*, 2004). These analyses have shown remarkable congruence with genome microarrays and genomic sequence comparisons, indicating that the *L. acidophilus* phylogenetic subgroup is a natural bacterial group. Genome sequencing now offers a definitive means to identify *Lactobacillus* species and strains (Claesson *et al.*, 2007, 2008; Felis & Dellaglio, 2007; Bull *et al.*, 2012).

Lactobacillus acidophilus strains and their history

Within the *L. acidophilus* group, there are some 20 species additional to *L. acidophilus sensu stricto* (Fig. 3). It is vital at this point to distinguish between the strain- and species-level classifications of constituent isolates within this group. Many of the early research into the *L. acidophilus* group blurs the lines between bacterial 'strains' of the *L. acidophilus* phylogenetic subgroup (many would now be considered as species that belong to the *L. acidophilus* group) and the present definition of a bacterial strain, which is deemed to be a subspecies level taxonomic unit (Klein *et al.*, 1998; Kullen *et al.*, 2000).

A lack of rigour and historical understanding of the literature surrounding *L. acidophilus* taxonomy may have

also contributed to confusion in species and strain identification. The reassignment, for example, of a strain once belonging to *L. acidophilus* (Tuomola & Salminen, 1998) to *Lactobacillus johnsonii*, as an entirely separate species (Pridmore *et al.*, 2004), had sound systematic support although some later studies have failed to adopt the correct taxonomic nomenclature (Pimentel *et al.*, 2012). The variety of names that may be attributed to a single strain (Table 1), from both culture collections and commercial trademarks, has also potentially led to multiple groups unknowingly working with the same strain referred to by a different name (Yeung *et al.*, 2002). The commercial success *of L. acidophilus* may have also contributed to the widespread industrial use of what appear to be identical strains because their proprietary protection and use within multiple functional foods or probiotic supplements.

Worrying recent examples of incorrect reporting of *L. acidophilus* include a genome sequence announcement for '*L. acidophilus*' strain 30SC (Oh *et al.*, 2011). Straightforward bioinformatic characterisation of the 16S rRNA and *gyrB* genes from the 30SC genome demonstrated the sequence was most likely derived from *L. amylovorus* (Bull *et al.*, 2012). This misidentification was further corroborated by evolutionary analysis of LAB metabolic pathways which showed those in strain 30SC were also more closely related to *L. amylovorus* (Salvetti *et al.*, 2013). Another strain that may have been misclassified in the published literature is *L. acidophilus* LAB20 (Tang *et al.*, 2012; Tang & Saris, 2013). This strain was isolated as a dominant LAB from the gastrointestinal tract of a dog (Tang *et al.*, 2012). Subsequent development of LAB20 strain-specific markers using an S-layer protein gene actually showed this selected marker was phylogenetically more closely related to *L. crispatus* than a validated *L. acidophilus sensu stricto* strain (Tang & Saris, 2013). In completing our review, we have collated only publications related to *L. acidophilus sensu stricto.*

Lactobacillus acidophilus was first isolated in 1900 (Moro) from infant faeces and at the time was designated as *Bacillus acidophilus*. The multiple strain names of the most commonly encountered *L. acidophilus* strains are listed in Table 1. The variety of strain names that have been be given to a single isolate deposited in multiple locations further complicates establishing the provenance of a particular strain. The StrainInfo database allows users to visually trace the history of a particular strain and can be used to resolve confusion in many cases (Dawyndt *et al.*, 2005). Fortunately, much of the body of work on *L. acidophilus*, particularly concerning its probiotic effects, has been undertaken on one particular strain: *L. acidophilus* NCFM. Although the depth of information available on NCFM has ensured that it is very well characterised as a true strain of *L. acidophilus*, it still has not escaped the confusion of being known by multiple strain names and may exist in the literature as NCFM, N2, NCK56, NCK45 and RL8K (Table 1). The large body of information concerning *L. acidophilus* NCFM has

Table 1. *Lactobacillus acidophilus* strains and their pseudonyms

ATCC*	DSMZ†	BCCM/LMG‡	NCIMB§	Other key names	Notes
ATCC 314		LMG 11467			
ATCC 832		LMG 11428	NCIMB 1723		
ATCC 4355		LMG 11469			
ATCC 4356^T	DSM 20079 T	LMG 13550^T	NCIMB 701748 T	NCFB 1748 T	Neotype strain (Hansen & Mocquot, 1970)
		LMG 7943 T	NCIMB 8690 T	NCTC 12980 T	
		LMG 8150 T			
		LMG 9433 T			
ATCC 4357	DSM 20242	LMG 11430	NCIMB 8607		
		LMG 13003			
ATCC 4796		LMG 11470			Draft genome sequence (Human Microbiome Project; Turnbaugh *et al.*, 2007)
ATCC 9224		LMG 11429	NCIMB 8116		
		LMG 11472			
		LMG 19170			
ATCC 13651	DSM 9126	LMG 11466	NCIMB 701360		
ATCC 700396				NCFM, N2, NCK56, NCK45, RL8K	Genome sequence (Altermann *et al.*, 2005)

*American Type Culture Collection, USA.
†Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany.
‡Belgian Co-ordinated Collections of Microorganisms, Belgium.
§National Collection of Industrial, Food and Marine Bacteria, UK.

contributed to it being deemed generally regarded as safe (GRAS) by the US Food and Drug Administration, as an approved ingredient in dairy products, functional beverages and nutritional powders (Bernardeau *et al.*, 2006).

Basic features of *L. acidophilus*

Lactobacillus acidophilus is a short (2–10 μm) Gram-positive rod that grows optimally from 37 to 42 °C (Altermann *et al.*, 2005) and is able to grow at temperatures as high as 45 °C. The species achieves its highest growth rates in slightly acidic media of pH 5.5–6.0, and growth ceases below pH 4.0 (Shah, 2007). It is an obligate homofermenter producing lactic acid from fermentation of carbohydrates and is among the least oxygen tolerant lactobacilli (Archibald & Fridovich, 1981; Claesson *et al.*, 2007).

From examination of the biosynthetic pathways encoded within its genome, *L. acidophilus* is auxotrophic for 14 amino acids and seems unable to synthesise multiple cofactors and vitamins including riboflavin, vitamin B6, nicotinate, nicotinamide, biotin and folate (Altermann *et al.*, 2005). These deficits in anabolic capacity are exemplified by the need to use nutrient-rich media such as deMan, Rogosa and Sharpe (MRS) agar (de Man *et al.*, 1960; Morishita *et al.*, 1981) for its routine culture. *Lactobacillus acidophilus* forms at least two colony morphotypes when grown under standard culture conditions on MRS agar, referred to as rough and smooth colonies. The proportion of rough to smooth colony morphotypes exhibited by *L. acidophilus* is influenced by exposure to antibiotics such as Penicillin G (Khaleghi *et al.*, 2011) or bile (Khaleghi *et al.*, 2010), which both cause a dose-dependent shift towards the smooth morphotype.

Although *L. acidophilus* has been isolated from multiple human-associated sources, recent phylogenomic characterisation by Claesson *et al.* (2008) established that the most likely environmental niche of *L. acidophilus* was the GI tract, with other lactobacilli broadly inhabiting plants and meat. The neotype *L. acidophilus* strain ATCC 4356 was described as isolated from the human microbiota although the records do not give the precise bodily location from where it was isolated. Metagenomic studies indicate that lactobacilli may compose just 0.2–1% of the total microbiota in the human colon and faeces and also show that their prevalence is highly variable between individuals (Walter, 2008; Kleerebezem & Vaughan, 2009). *Lactobacillus acidophilus* may be just a small and variable fraction of this low overall carriage of the genus. Culture-independent studies from other hosts also show wide variations in the prevalence of this LAB species. For example, *L. acidophilus* was present as the most abundant member of the lactobacilli in broiler chickens (Lu *et al.*, 2003), while in contrast, a total absence of *L. acidophilus* was found in pigs (Leser *et al.*, 2002). Culture-dependent analysis of lactobacilli within the pig GIT suggests they are largely comprised of the *L. acidophilus* group although no *L. acidophilus* isolates were specifically recovered (Korhonen *et al.*, 2007). Overall, gut carriage of *L. acidophilus* appears highly variable.

Human gut passage of *L. acidophilus* has been modelled in a probiotic capsule feeding study (Mahenthiralingam *et al.*, 2009). Participants were prescreened for faecal presence of *L. acidophilus* using culture-based methods in tandem with DNA fingerprinting to identify the *Lactobacillus* strain being administered. Three of the 12 participants were found to be culture positive for *L. acidophilus* prior to probiotic feeding, indicating faecal carriage of *L. acidophilus* in humans is not universal (Mahenthiralingam *et al.*, 2009). After feeding (5.6×10^9 viable bacteria per capsule which was taken daily), the administered *L. acidophilus* strain was detected in 10 of the 12 subjects, reaching cultivatable levels as high as 10^7 colony-forming units per gram of faeces in three of the volunteers (Mahenthiralingam *et al.*, 2009). Long-term carriage of *L. acidophilus* for 28 days postfeeding was detected in three subjects, who notably did not culture positive for *L. acidophilus* before feeding. Overall, these results suggest that dietary intake is a major influence on the human carriage of *L. acidophilus*.

Food and industrial use of *L. acidophilus*

Lactobacillus acidophilus is a major commercial species of the lactic acid bacteria (LAB), available in products including milk, yoghurt and toddler formula, as well as in dietary supplements with reported probiotic effects (Sanders & Klaenhammer, 2001; Altermann *et al.*, 2005). It is part of many undefined starter cultures for milk fermentation, a preservation process developed in the Early Neolithic era and used in the production of traditional fermented foods for more than 10 000 years (Tamime, 2002). Its slow growth in milk (Azcarate-Peril *et al.*, 2009) means that most of the fermentation in milk products is achieved with a yoghurt starter culture (e.g. *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) and *L. acidophilus* is subsequently added for additional probiotic value (Shah, 2000).

Probiotic strains of *L. acidophilus*

Probiotic bacterial strains are commonly mislabelled or unlabelled in products, often due to the difficulties in discerning both species and strains of *Lactobacillus* (Yeung *et al.*, 2002). The primary commercial probiotic strains of *L. acidophilus* are described by Shah (2007) and include

L. acidophilus LA-1 and LA-5 (Chr. Hansen, Denmark), NCFM (Dansico, Madison), DDS-1 (Nebraska Cultures, Nebraska) and SBT-2026 (Snow Brand Milk Products, Tokyo, Japan). *Lactobacillus acidophilus* NCFM, a major commercial strain, has identical fermentation and growth characteristics to the Type strain ATCC 4356^T and is also closely related to PFGE profile (Sanders & Klaenhammer, 2001). *Lactobacillus acidophilus* isolated from products claimed to contain strain LA-5 also produce DNA fingerprints with a high degree of similarity (91.9%) to the *L. acidophilus* ATCC 4356^T by randomly amplified polymorphic DNA (RAPD) fingerprint analysis (Schillinger *et al.*, 2003). *Lactobacillus acidophilus* LA-1 is no longer available as a product from Chr. Hansen. A wealth of research dedicated to '*L. acidophilus* La1' a commercial strain marketed by Nestlé may also be found in the published literature (Link-Amster *et al.*, 1994). However, this strain has subsequently been taxonomically reassigned to *L. johnsonii* and has a genome sequence available as *L. johnsonii* NCC 533 (Pridmore *et al.*, 2004). Comparative information on the differences in probiotic effect between each commercial strain is not available, however, it is recognised that different *Lactobacillus* species may display similar probiotic effects *in vitro,* yet have markedly divergent properties when assessed *in vivo* (Ibnou-Zekri *et al.*, 2003).

Probiotic characteristics and physiology

The probiotic effects of *L. acidophilus* NCFM are well characterised, aided recently by the availability of its genome sequence and the necessity of in-depth characterisation for application for GRAS status. Although a genome sequence is not (yet) available, *L. acidophilus* LA-5 is similarly characterised for patent claim information. The characterisation of probiotic strains may be broadly divided into two categories. The first is desirable probiotic physiology demonstrable *in vitro* such as stability in products (Shah, 2000), resistance to bile (Pfeiler *et al.*, 2007; Pfeiler & Klaenhammer, 2009; Khaleghi *et al.*, 2010) and tolerance to low pH (Azcarate-Peril *et al.*, 2004, 2005), adherence to human colonocytes in cell culture (Buck *et al.*, 2005), antimicrobial production (Sanders & Klaenhammer, 2001; Tabasco *et al.*, 2009) and lactase activity (Sanders *et al.*, 1996). The second category encompasses the gross probiotic effect observable in the context of feeding studies such as mediation of host immune response (Bron *et al.*, 2012), lowering host serum cholesterol (Shah, 2007), improving host lactose metabolism (Gilliland, 1989) and preventing or treating infection (Wang *et al.*, 2004). Several recent clinical trials have also shown that consumption of probiotics containing *L. acidophilus* NCFM in combination with *Bifidobacterium* species can produce health benefits, the 'gold standard' for a probiotic claim. For example, they reduce bloating in adults with functional bowel disorders (Ringel-Kulka *et al.*, 2011) and suppress cold and influenza-like symptoms in children (Leyer *et al.*, 2009).

Analysis of the *L. acidophilus* NCFM genome sequence has directly facilitated the functional characterisation of its ability to tolerate exposure to both low pH and bile, important factors for a probiotic organism that must pass through the gastrointestinal tract. Functional microarray experiments with *L. acidophilus* NCFM showed upregulation of transcripts from three transporter genes [two major facilitator (MFC) superfamily and the permease component of an ABC transporter] in the presence of bile (Pfeiler *et al.*, 2007). Similar transporters had previously been shown in other species to be involved in bile efflux from the cell (Solheim *et al.*, 2007). Furthermore, a study that generated deletion mutants lacking these three transporter genes showed a significant decrease in their ability to survive in bile (Pfeiler & Klaenhammer, 2009). *Lactobacillus acidophilus* NCFM is also able to survive exposure to pH 3.0 for 5 h with no loss of viability (Azcarate-Peril *et al.*, 2004).

Lactobacillus acidophilus is able to utilise a variety of carbon sources for growth (Sanders & Klaenhammer, 2001; Yeung *et al.*, 2004), but a comprehensive understanding of the mechanisms behind the uptake and metabolism of carbon sources has not yet been achieved. A study describing several genetic loci responsible for carbohydrate metabolism again demonstrated the utility of the *L. acidophilus* complete genome sequence (Barrangou *et al.*, 2006). Several classes of transporter (ATP-binding cassette, phosphoenol-pyruvate phosphotransferase system and galactoside pentose hexuronide permease) were found to be induced in the presence of their respective substrates but repressed in the presence glucose, suggesting that carbohydrate metabolism in *L. acidophilus* is strongly regulated by catabolite repression. The strong link between carbohydrate source and regulation of sugar uptake and metabolism genes likely contributes to the competitive ability of *L. acidophilus* in the human gastrointestinal tract. The metabolism of these complex carbohydrates also provides a function that is not present in humans and other microbiota, potentially enriching the growth of *L. acidophilus* and other probiotic LAB in the human gastrointestinal tract (Zhu *et al.*, 2009). Studies have demonstrated the ability of *L. acidophilus* to adhere to human Caco-2 colonocytes *in vitro*. An analysis of the adhesion factors involved in *L. acidophilus* NCFM-Caco-2 epithelial cell interaction found significant involvement in S-layer proteins, linked to the gene *slpA*, fibronectin-binding protein (FbpA) and mucin-binding protein (Mub; Buck *et al.*, 2005).

Genomic features

The genome sequence of *L. acidophilus* NCFM was the third of the *Lactobacillus* genomes to be published, behind *Lactobacillus plantarum* WCFS1 (Kleerebezem *et al.*, 2003) and *L. johnsonii* NCC 533 (Pridmore *et al.*, 2004), and the first genome sequence from an *L. acidophilus* phylogenetic subgroup species (Table 2). *In silico* analyses of the *L. acidophilus* NCFM genome shows it is able to synthesise a limited number of amino acids (cysteine, serine and aspartate) and to compensate it's genome is enriched in genes coding for amino acid transport and fermentative functions (Altermann *et al.*, 2005). The comparatively small (1 993 564 bp) genome of *L. acidophilus* has a low (35%) average GC content, compared with other members of the *L. acidophilus* phylogenetic subgroup (mean GC content = 40%), which have an upper range of 50% GC (*L. delbrueckii* subsp. *bulgaricus*). The GC content of the *L. acidophilus* genome is inherently higher (up to 50%) in the four regions containing rRNA genes as expected (Altermann *et al.*, 2005). Other than GC content, basic genomic attributes such as size and gene content do not vary significantly from other member of the *L. acidophilus* group.

Plasmids are also common features of members of the *L. acidophilus* group, present in seven of the 16 strains detailed in Table 2. Their distribution is heterogeneous, with multiple strains of some species with the same number of plasmids (*L. amylovorus*), some species showing strains with and without plasmids (*L. johnsonii* and *L. helveticus*) and others showing no evidence of plasmids at all (*L. acidophilus* and *L. gasseri*). Despite the lack of *L. acidophilus* NCFM and *L. johnsonii* NCC 533 plasmids, a recent study examining phylogenetic trees of 401 proteins identified horizontal gene transfer (HGT) of up to 40% of the core genome genes between the two species, causing an unprecedented level of phylogenetic incongruence (Nicolas *et al.*, 2007).

One genomic feature that does vary considerably across Lactobacillus genomes is clustered regularly spaced short palindromic repeat (CRISPR) regions. CRISPRs are commonly identified in *Lactobacillus* genomes from the *L. acidophilus* phylogenetic subgroup (Table 2) and beyond, with approximately half (26/53) of the sequenced *Lactobacillus* genomes possessing CRISPR regions, as identified by BlastP (Koonin & Makarova, 2009). The *L. acidophilus* NCFM CRISPR region has features characteristic of these regions, being *c.* 1.5 kb in size and composed of 32

Table 2. Completed and published genome sequences from the *Lactobacillus acidophilus* group

Organism	Strain	Origin/Use	GC (mol %)	Genome size (Mb)	Gene count	CRISPR count	Coding base count%	Plasmids	Publication
Lactobacillus acidophilus	NCFM	Probiotic	35	1.99	1970	1	89.64	0	Altermann *et al.* (2005)
Lactobacillus amylovorus	GRL 1118	Pig intestine	38	1.98	1994	3	86.86	2	Kant *et al.* (2011a)
Lactobacillus amylovorus	GRL 1112	Pig intestine	38	2.13	2193	0	86.99	2	Kant *et al.* (2011b)
Lactobacillus crispatus	ST1	Chicken	37	2.04	2100	3	89.37	0	Ojala *et al.* (2010)
Lactobacillus delbrueckii subsp. *bulgaricus*	ATCC 11842	Yoghurt	50	1.86	2234	1	76.01	0	van de Guchte *et al.* (2006)
Lactobacillus delbrueckii subsp. *bulgaricus*	ATCC BAA-365	Cheese, yoghurt	50	1.86	1865	1	79.63	0	Makarova *et al.* (2006)
Lactobacillus delbrueckii subsp. *bulgaricus*	2038	Milk, Probiotic	50	1.87	1907	1	84.52	0	Hao *et al.* (2011)
Lactobacillus delbrueckii subsp. *bulgaricus*	ND02	Milk, Probiotic	50	2.13	2139	2	84.82	1	Sun *et al.* (2011)
Lactobacillus gasseri	ATCC 33323	Human, probiotic	35	1.89	1874	0	90.11	0	Makarova *et al.* (2006)
Lactobacillus helveticus	DPC 4571	Cheese	37	2.08	1830	1	74.8	0	Callanan *et al.* (2008)
Lactobacillus helveticus	R0052	Probiotic	37	2.13	2084	0	80.22	1	Tompkins *et al.* (2012)
Lactobacillus helveticus	H10	Fermented milk	37	2.17	2052	2	81.32	1	Zhao *et al.* (2011)
Lactobacillus johnsonii	DPC 6026	Human	35	1.97	1840	2	88.6	0	Guinane *et al.* (2011)
Lactobacillus johnsonii	FI9785	Human	34	1.79	1804	0	89.64	2	Wegmann *et al.* (2009)
Lactobacillus johnsonii	NCC 533	Probiotic	35	1.99	1941	0	91.09	0	Pridmore *et al.* (2004)
Lactobacillus kefiranofaciens	ZW3	Kefir, Probiotic	37	2.35	2222	3	80.76	2	Wang *et al.* (2011)

near-perfect 29 base repeats, interspersed with unique 32 base spacer DNAs (Altermann *et al.*, 2005). No physiological function was attributed to CRISPR regions at the time of the NCFM genome publication (Altermann *et al.*, 2005), however, subsequent observations that the unique CRISPR spacer sequences were almost identical to fragments of virus and plasmid genes led to the hypothesis that CRISPR regions may be involved in defence against selfish DNA elements (Makarova *et al.*, 2011). This hypothesis has been validated by the demonstration that a short phage-like sequence inserted into the CRISPR locus of *Streptococcus thermophilus* conferred resistance against its cognate phage (Barrangou *et al.*, 2007).

Prophages and phage interactions are commonly encountered in both the study of LAB genomics and the large-scale manufacture of fermented products by LAB (Mahony *et al.*, 2012), where as a result of the economic implications of phage contamination in dairy fermentations, many LAB phages have been well characterised (Brüssow, 2001). The genome sequence of *L. acidophilus* NCFM revealed evidence of three isolated phage remnants, or potential autonomous units (PAUs) designated PauLA-I-III. Each PAU is composed of seven core ORFs, with synteny and ORF size highly conserved between PauLA-I and PauLA-II, with PauLA-III lacking a single ORF of hypothetical function. The high degree of similarity between PauLA-I and PauLA-II suggests that these may have been formed following a duplication event, and PauLA-III was evolved in a different organism and was integrated at a different time to the progenitor or PauLA-I and PauLA-II (Altermann *et al.*, 2005). Interestingly, there is an absence of literature on functional bacteriophages capable of infecting strains of *L. acidophilus sensu stricto* compared with other members of *L. acidophilus* phylogenetic subgroup.

Conclusions and perspective

Lactobacillus acidophilus is an important commercial bacterium with a long history that plays a pivotal role in the characterisation of the genus *Lactobacillus*. However, given the highly progressive nature of *Lactobacillus* taxonomy, *L. acidophilus* as a species has struggled with being misidentified and misrepresented in its past characterisation. Given the increased regulatory criteria being placed on the definition and sale of microbial species as probiotics, *L. acidophilus* strain NCFM has emerged as one of the most well-characterised probiotics within this species. However, for other areas of study such as the investigation of environmental niches or microbial composition of fermented foods, care should be taken to clearly identify whether *L. acidophilus sensu stricto* strains are present. Going forward, it will be important to clarify data provided for both (i) the species level *Lactobacillus* identification, by ensuring new publications are not made with references to old taxonomic names and (ii) the strain level identification of *L. acidophilus*, by conducting comparisons to well-characterised control strains. Ensuring that these parameters are clearly defined for *L. acidophilus* will overcome problems with the multiple strain names used for the same original 'isolate' greatly improve our understanding of this biotechnologically important *Lactobacillus* species.

Acknowledgements

M.B. acknowledges funding from the Biotechnology and Biological Sciences Research Council PhD funding with CASE sponsorship from Cultech Ltd., Baglan, Wales, UK. SP is a member of the board of directors at Cultech Ltd. and declares a conflict of interest in relation to co-sponsorship of these research studies.

References

Ahrné S, Nobaek S, Jeppsson B, Adlerberth I, Wold AE & Molin G (1998) The normal *Lactobacillus* flora of healthy human rectal and oral mucosa. *J Appl Microbiol* **85**: 88–94.

Altermann E, Russell WM, Azcarate-Peril MA *et al.* (2005) Complete genome sequence of the probiotic lactic acid bacterium *Lactobacillus acidophilus* NCFM. *P Natl Acad Sci USA* **102**: 3906–3912.

Archibald FS & Fridovich I (1981) Manganese, superoxide dismutase, and oxygen tolerance in some lactic acid bacteria. *J Bacteriol* **146**: 928–936.

Arumugam M, Raes J, Pelletier E *et al.* (2011) Enterotypes of the human gut microbiome. *Nature* **473**: 174–180.

Azcarate-Peril MA, Altermann E, Hoover-Fitzula RL, Cano RJ & Klaenhammer TR (2004) Identification and inactivation of genetic loci involved with *Lactobacillus acidophilus* acid tolerance. *Appl Environ Microbiol* **70**: 5315–5322.

Azcarate-Peril MA, McAuliffe O, Altermann E, Lick S, Russell WM & Klaenhammer TR (2005) Microarray analysis of a two-component regulatory system involved in acid resistance and proteolytic activity in *Lactobacillus acidophilus*. *Appl Environ Microbiol* **71**: 5794–5804.

Azcarate-Peril MA, Tallon R & Klaenhammer TR (2009) Temporal gene expression and probiotic attributes of *Lactobacillus acidophilus* during growth in milk. *J Dairy Sci* **92**: 870–886.

Barrangou R, Azcarate-Peril MA, Duong T, Conners SB, Kelly RM & Klaenhammer TR (2006) Global analysis of carbohydrate utilization by *Lactobacillus acidophilus* using cDNA microarrays. *P Natl Acad Sci USA* **103**: 3816–3821.

Barrangou R, Fremaux C, Deveau H *et al.* (2007) CRISPR provides acquired resistance against viruses in prokaryotes. *Science* **315**: 1709–1712.

Bernardeau M, Guguen M & Vernoux JP (2006) Beneficial lactobacilli in food and feed: long-term use, biodiversity and proposals for specific and realistic safety assessments. *FEMS Microbiol Rev* **30**: 487–513.

Bron PA, van Baarlen P & Kleerebezem M (2012) Emerging molecular insights into the interaction between probiotics and the host intestinal mucosa. *Nat Rev Microbiol* **10**: 66–78.

Brüssow H (2001) Phages of dairy bacteria. *Annu Rev Microbiol* **55**: 283–303.

Buck BL, Altermann E, Svingerud T & Klaenhammer TR (2005) Functional analysis of putative adhesion factors in *Lactobacillus acidophilus* NCFM. *Appl Environ Microbiol* **71**: 8344–8351.

Bull MJ, Marchesi JR, Vandamme P, Plummer S & Mahenthiralingam E (2012) Minimum taxonomic criteria for bacterial genome sequence depositions and announcements. *J Microbiol Methods* **89**: 18–21.

Callanan M, Kaleta P, O'Callaghan J *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.

Carr FJ, Chill D & Maida N (2002) The lactic acid bacteria: a literature survey. *Crit Rev Microbiol* **28**: 281–370.

Claesson MJ, van Sinderen D & O'Toole PW (2007) The genus *Lactobacillus* – a genomic basis for understanding its diversity. *FEMS Microbiol Lett* **269**: 22–28.

Claesson MJ, van Sinderen D & O'Toole PW (2008) *Lactobacillus* phylogenomics – towards a reclassification of the genus. *Int J Syst Evol Microbiol* **58**: 2945–2954.

Dawyndt P, Vancanneyt M, de Meyer H & Swings J (2005) Knowledge accumulation and resolution of data inconsistencies during the integration of microbial information sources. *IEEE Trans Knowl Data Eng* **17**: 1111–1126.

de Man JC, Rogosa M & Sharpe ME (1960) A medium for the cultivation of lactobacilli. *J Appl Microbiol* **23**: 130–135.

Euzéby JP (1997) List of bacterial names with standing in nomenclature: a folder available on the internet. *Int J Syst Bacteriol* **47**: 590–592.

FAO/WHO (2002) *Guidelines for the Evaluation of Probiotics in Food*. FAO/WHO, London, Ontario.

Felis GE & Dellaglio F (2007) Taxonomy of lactobacilli and bifidobacteria. *Curr Issues Intest Microbiol* **8**: 44–61.

Gevers D, Huys G & Swings J (2001) Applicability of rep-PCR fingerprinting for identification of *Lactobacillus* species. *FEMS Microbiol Lett* **205**: 31–36.

Gilliland SE (1989) Acidophilus milk products: a review of potential benefits to consumers. *J Dairy Sci* **72**: 2483–2494.

Guinane CM, Kent RM, Norberg S, Hill C, Fitzgerald GF, Stanton C & Ross RP (2011) Host specific diversity in *Lactobacillus johnsonii* as evidenced by a major chromosomal inversion and phage resistance mechanisms. *PLoS ONE* **6**: e18740.

Hansen PA & Mocquot G (1970) *Lactobacillus acidophilus* (Moro) comb. nov. *Int J Syst Bacteriol* **20**: 325–327.

Hao P, Zheng H, Yu Y *et al.* (2011) Complete sequencing and pan-genomic analysis of *Lactobacillus delbrueckii* subsp. *bulgaricus* reveal its genetic basis for industrial yogurt production. *PLoS ONE* **6**: e15964.

Holland D (1920) Generic index of the commoner forms of bacteria. *J Bacteriol* **5**: 215–229.

Ibnou-Zekri N, Blum S, Schiffrin EJ & Tvd W (2003) Divergent patterns of colonization and immune response elicited from two intestinal *Lactobacillus* strains that display similar properties *in vitro*. *Infect Immun* **71**: 428–436.

Kant R, Paulin L, Alatalo E, de Vos WM & Palva A (2011a) Genome sequence of *Lactobacillus amylovorus* GRL1118, isolated from pig ileum. *J Bacteriol* **193**: 3147–3148.

Kant R, Paulin L, Alatalo E, de Vos WM & Palva A (2011b) Genome sequence of *Lactobacillus amylovorus* GRL1112. *J Bacteriol* **193**: 789–790.

Khaleghi M, Kermanshahi RK, Yaghoobi MM, Zarkesh-Esfahani SH & Baghizadeh A (2010) Assessment of bile salt effects on s-layer production, *slp* gene expression and some physicochemical properties of *Lactobacillus acidophilus* ATCC 4356. *J Microbiol Biotechnol* **20**: 749–756.

Khaleghi M, Kasra Kermanshahi R & Zarkesh-Esfahani SH (2011) Effects of penicillin G on morphology and certain physiological parameters of *Lactobacillus acidophilus* ATCC 4356. *J Microbiol Biotechnol* **21**: 822–829.

Kleerebezem M & Hugenholtz J (2003) Metabolic pathway engineering in lactic acid bacteria. *Curr Opin Biotechnol* **14**: 232–237.

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

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

Klein G, Pack A, Bonaparte C & Reuter G (1998) Taxonomy and physiology of probiotic lactic acid bacteria. *Int J Food Microbiol* **41**: 103–125.

Koonin EV & Makarova KS (2009) CRISPR-Cas: an adaptive immunity system in prokaryotes. *F1000 Biol Rep* **1**: 95.

Korhonen JM, Sclivagnotis Y & von Wright A (2007) Characterization of dominant cultivable lactobacilli and their antibiotic resistance profiles from faecal samples of weaning piglets. *J Appl Microbiol* **103**: 2496–2503.

Kullen MJ, Sanozky-Dawes RB, Crowell DC & Klaenhammer TR (2000) Use of the DNA sequence of variable regions of the 16S rRNA gene for rapid and accurate identification of bacteria in the *Lactobacillus acidophilus* complex. *J Appl Microbiol* **89**: 511–516.

Kulp WL & Rettger LF (1924) Comparative study of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus*. *J Bacteriol* **9**: 357–395.

Leser TD, Amenuvor JZ, Jensen TK, Lindecrona RH, Boye M & Møller K (2002) Culture-independent analysis of gut bacteria: the pig gastrointestinal tract microbiota revisited. *Appl Environ Microbiol* **68**: 673–690.

Leyer GJ, Li S, Mubasher ME, Reifer C & Ouwehand AC (2009) Probiotic effects on cold and influenza-like symptom incidence and duration in children. *Pediatrics* **124**: e172–e179.

Link-Amster H, Rochat F, Saudan KY, Mignot O & Aeschlimann JM (1994) Modulation of a specific humoral immune response and changes in intestinal flora mediated through fermented milk intake. *FEMS Immunol Med Microbiol* **10**: 55–63.

Lu J, Idris U, Harmon B, Hofacre C, Maurer JJ & Lee MD (2003) Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. *Appl Environ Microbiol* **69**: 6816–6824.

Mahenthiralingam E, Marchbank A, Drevinek P, Garaiova I & Plummer S (2009) Use of colony-based bacterial strain typing for tracking the fate of *Lactobacillus* strains during human consumption. *BMC Microbiol* **9**: 251.

Mahony J, Ainsworth S, Stockdale S & van Sinderen D (2012) Phages of lactic acid bacteria: the role of genetics in understanding phage-host interactions and their co-evolutionary processes. *Virology* **434**: 143–150.

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

Makarova KS, Haft DH, Barrangou R *et al.* (2011) Evolution and classification of the CRISPR–Cas systems. *Nat Rev Microbiol* **9**: 467–477.

Morishita T, Deguchi Y, Yajima M, Sakurai T & Yura T (1981) Multiple nutritional requirements of lactobacilli: genetic lesions affecting amino acid biosynthetic pathways. *J Bacteriol* **148**: 64–71.

Moro E (1900) Ueber die nach Gram farbbaren bacillen des säuglingsstuhles. *Wien Klin Wochenschr* **13**: 114–115.

Naser SM, Dawyndt P, Hoste B *et al.* (2007) Identification of lactobacilli by *pheS* and *rpoA* gene sequence analyses. *Int J Syst Evol Microbiol* **57**: 2777–2789.

Nicolas P, Bessières P, Ehrlich SD, Maguin E & van de Guchte M (2007) Extensive horizontal transfer of core genome genes between two *Lactobacillus* species found in the gastrointestinal tract. *BMC Evol Biol* **7**: 141.

Oh S, Roh H, Ko HJ *et al.* (2011) Complete genome sequencing of *Lactobacillus acidophilus* 30SC, isolated from swine intestine. *J Bacteriol* **193**: 2882–2883.

Ojala T, Kuparinen V, Koskinen JP *et al.* (2010) Genome sequence of *Lactobacillus crispatus* ST1. *J Bacteriol* **192**: 3547–3548.

Pfeiler EA & Klaenhammer TR (2009) Role of transporter proteins in bile tolerance of *Lactobacillus acidophilus*. *Appl Environ Microbiol* **75**: 6013–6016.

Pfeiler EA, Azcarate-Peril MA & Klaenhammer TR (2007) Characterization of a novel bile-inducible operon encoding a two-component regulatory system in *Lactobacillus acidophilus*. *J Bacteriol* **189**: 4624–4634.

Pimentel LL, Mättö J, Malcata FX, Pintado ME & Saarela M (2012) Survival of potentially probiotic enterococci in dairy matrices and in the human gastrointestinal tract. *Int Dairy J* **27**: 53–57.

Pridmore RD, Berger B, Desiere F *et al.* (2004) The genome sequence of the probiotic intestinal bacterium *Lactobacillus johnsonii* NCC 533. *P Natl Acad Sci USA* **101**: 2512–2517.

Ringel-Kulka T, Palsson OS, Maier D, Carroll I, Galanko JA, Leyer G & Ringel Y (2011) Probiotic bacteria *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* Bi-07 versus placebo for the symptoms of bloating in patients with functional bowel disorders: a double-blind study. *J Clin Gastroenterol* **45**: 518–525.

Rogosa M & Sharpe ME (1960) Species differentiation of human vaginal lactobacilli. *J Gen Microbiol* **23**: 197–201.

Salvetti E, Fondi M, Fani R, Torriani S & Felis GE (2013) Evolution of lactic acid bacteria in the order *Lactobacillales* as depicted by analysis of glycolysis and pentose phosphate pathways. *Syst Appl Microbiol* **36**: 291–305.

Sanders ME (2003) Probiotics: considerations for human health. *Nutr Rev* **61**: 91–99.

Sanders ME & Klaenhammer TR (2001) Invited review: the scientific basis of *Lactobacillus acidophilus* NCFM functionality as a probiotic. *J Dairy Sci* **84**: 319–331.

Sanders ME, Walker DC, Walker KM, Aoyama K & Klaenhammer TR (1996) Performance of commercial cultures in fluid milk applications. *J Dairy Sci* **79**: 943–955.

Schillinger U, Yousif NMK, Sesar L & Franz CMAP (2003) Use of group-specific and RAPD-PCR analyses for rapid differentiation of *Lactobacillus* strains from probiotic yogurts. *Curr Microbiol* **47**: 453–456.

Schleifer K-H & Ludwig W (1995) Phylogeny of the genus *Lactobacillus* and related genera. *Syst Appl Microbiol* **18**: 461–467.

Shah NP (2000) Probiotic bacteria: selective enumeration and survival in dairy foods. *J Dairy Sci* **83**: 894–907.

Shah NP (2007) Functional cultures and health benefits. *Int Dairy J* **17**: 1262–1277.

Solheim M, Aakra A, Veb H, Snipen L & Nes IF (2007) Transcriptional responses of *Enterococcus faecalis* v583 to bovine bile and sodium dodecyl sulfate. *Appl Environ Microbiol* **73**: 5767–5774.

Sun Z, Chen X, Wang J *et al.* (2011) Complete genome sequence of *Lactobacillus delbrueckii* subsp. *bulgaricus* strain ND02. *J Bacteriol* **193**: 3426–3427.

Tabasco R, García-Cayuela T, Peláez C & Requena T (2009) *Lactobacillus acidophilus* La-5 increases lactacin B production when it senses live target bacteria. *Int J Food Microbiol* **132**: 109–116.

Tamime AY (2002) Fermented milks: a historical food with modern applications – a review. *Eur J Clin Nutr* **56**: 2–15.

Tang Y & Saris PE (2013) Strain-specific detection of orally administered canine jejunum-dominated *Lactobacillus acidophilus* LAB20 in dog faeces by real-time PCR targeted to the novel surface layer protein. *Lett Appl Microbiol* **57**: 330–335.

Tang Y, Manninen TJ & Saris PE (2012) Dominance of *Lactobacillus acidophilus* in the facultative jejunal *Lactobacillus* microbiota of fistulated beagles. *Appl Environ Microbiol* **78**: 7156–7159.

Tompkins TA, Barreau G & Broadbent JR (2012) Complete genome sequence of *Lactobacillus helveticus* R0052, a commercial probiotic strain. *J Bacteriol* **194**: 6349.

Tuomola EM & Salminen SJ (1998) Adhesion of some probiotic and dairy *Lactobacillus* strains to Caco-2 cell cultures. *Int J Food Microbiol* **41**: 45–51.

Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R & Gordon JI (2007) The human microbiome project. *Nature* **449**: 804–810.

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

Ventura M, Canchaya C, Meylan V, Klaenhammer TR & Zink R (2003) Analysis, characterization, and loci of the *tuf* genes in *Lactobacillus* and *Bifidobacterium* species and their direct application for species identification. *Appl Environ Microbiol* **69**: 6908–6922.

Walter J (2008) Ecological role of lactobacilli in the gastrointestinal tract: implications for fundamental and biomedical research. *Appl Environ Microbiol* **74**: 4985–4996.

Wang K-Y, Li S-N, Liu C-S *et al.* (2004) Effects of ingesting *Lactobacillus*- and *Bifidobacterium*-containing yogurt in subjects with colonized *Helicobacter pylori*. *Am J Clin Nutr* **80**: 737–741.

Wang Y, Wang J, Ahmed Z, Bai X & Wang J (2011) Complete genome sequence of *Lactobacillus kefiranofaciens* ZW3. *J Bacteriol* **193**: 4280–4281.

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

Yeung PSM, Sanders ME, Kitts CL, Cano R & Tong PS (2002) Species-specific identification of commercial probiotic strains. *J Dairy Sci* **85**: 1039–1051.

Yeung PSM, Kitts CL, Cano R, Tong PS & Sanders ME (2004) Application of genotypic and phenotypic analyses to commercial probiotic strain identity and relatedness. *J Appl Microbiol* **97**: 1095–1104.

Zhao W, Chen Y, Sun Z *et al.* (2011) Complete genome sequence of *Lactobacillus helveticus* H10. *J Bacteriol* **193**: 2666–2667.

Zhu Y, Zhang Y & Li Y (2009) Understanding the industrial application potential of lactic acid bacteria through genomics. *Appl Microbiol Biotechnol* **83**: 597–610.