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Editorial

Lactic acid bacteria: life after genomics

As powerhouses for the food industry, lactic acid bacteria continue to be the focus of considerable interest. While traditionally applied in industrial dairy and other food fermentations, lactic acid bacteria are now used in a great variety of drinks, yoghurts or other products that are marketed with specific health benefits. This has been the largest growth segment in the functional foods market with an annual sales value of over a billion Euro. The lactic acid bacteria in these products include notably species of *Lactobacillus* and *Bifidobacterium* that are marketed as probiotics (Saxelin *et al*., 2005). Hence it is no surprise that these species are actively studied and often considered together as will be detailed below. The attention for these groups of lactic acid bacteria represents a marked shift in the attention from the traditional focus on strains of notably *Lactococcus* and *Streptococcus* spp. that are used as starters in important milk fermentations, resulting in commodity products, such as cheese and yoghurt. However, *Lactococcus lactis* can still be seen as a model for lactic acid bacteria as will be discussed below. All these genera belong to the Gram-positive bacteria and produce lactic acid from sugars. *Bifidobacteria* belong to the *Actinobacteria* and contain genomic DNA with a high G+C content, while the other lactic acid bacteria are phylogenetically closely related and belong to the *Firmicutes* that are characterized by genomes with a low G + C content. It is of more than anecdotal importance to note here that the bacteria belonging to the *Actinobacteria* and *Firmicutes* make up the vast majority of microbial taxa present in our intestinal tract (Zoetendal *et al*., 2008). Both *Lactobacillus* and *Bifidobacterium* spp. are early colonizers and belong to our microbes inside where – in adult life – they are notably present in the upper and lower intestinal tract respectively (Kleerebezem and Vaughan, 2009). This explains why the growing interest in these different groups of lactic acid bacteria and their application in health-promoting products is paralleled by attention for their activity and diversity in the human body. The impact of consumed lactobacilli has recently been established in pioneering studies addressing the human response in the upper intestinal tract (van Baarlen *et al*., 2009; 2011). The results show specific and strain-dependent transcriptional host responses that are dependent on the physiological state of the consumed cells. In this way, the provide support for the functionality of probiotics, which are often defined as microbial cultures providing when consumed in adequate amounts a health benefit to the host (Saxelin *et al*., 2005).

In line with the industrial importance and scientific interest, this issue of *Microbial Biotechnology* addresses various aspects of *Lactobacillus* and *Bifidobacterium* spp. and the genetic model *L. lactis*. All contributions capitalize in different forms on the post-genomic progress that set off a revolution in the understanding of the lactic acid bacteria (De Vos, 2001). A decade ago, the 2 Mb genome was characterized of a plasmid-free *L. lactis* strain (Bolotin *et al*., 2001) and the first complete genome sequence of a *Lactobacillus* strain was reported with the 3.3-Mb-large genome of *Lactobacillus plantarum* (Kleerebezem *et al*., 2003). Subsequently, a great number of industrial and model strains have been characterized at the genome level. These include representatives of the over 100 *Lactobacillus* spp., as well as different strains belonging to the same species. However, many genomes have only been partially sequenced and have some gaps as the genomes are notably highly repetitive because of the presence of IS elements, repeated domains or other unusual sequences. These include several intestinal isolates that recently have been reported as part of the Human Microbiome Jumpstart Initiative (Nelson *et al*., 2010). In a review article, Ravi Kant and colleagues focus on the 20 completely genomes of *Lactobacillus* spp. and describe their comparative bioinformatic analysis (Kant *et al*., 2011). These genomes varied in size between 1.8 and 3.3 Mb, reflecting the large variation of niches occupied by these lactic acid bacteria. The deduced *Lactobacillus* pangenome of over 14 000 protein-encoding genes was predicted together with the *Lactobacillus* Core Genome that includes several hundreds of orthologous genes. This detailed analysis provides a platform for functional and comparative genomics of presently known lactobacilli as well as future analysis of new *Lactobacillus* genomes.

Knowledge of a genome sequence does not directly provide insight in the activity or behaviour of an organism. The relations between the genomic blueprint and the apparent functional properties can be unravelled using functional genomic approaches that target different molecular levels, i.e. transcriptomics, proteomics and

metabolomics. Transcriptome platforms have been developed for several lactic acid bacteria and bifidobacteria and have accelerated gene-function annotation and enabled holistic approaches towards adaptive behaviour under different environmental conditions. Transcriptome analyses under multiple conditions allow for data driven reconstruction of regulatory networks as is illustrated by Michiel Wels and co-workers, who reconstructed the gene regulatory networks and cognate *cis* acting elements that control gene expression in *L. plantarum* (Wels *et al*., 2011). Use was made of a large amount of transcriptional data sets that were collected under different growth conditions. Over 40 sets of co-regulated genes were discovered enhancing the insight in the metabolic response of *L. plantarum* that has developed into one of probiotic paradigms. Validation of these and other gene-regulation networks by mutation analysis of the regulator involved is often required to further specify their regulons and mode of control. This approach is taken by Ida Rudd and colleagues who targeted the *cggR* gene encoding the central glycolytic gene regulator that is conserved in lactobacilli and several other Gram-positive bacteria (Rud *et al*., 2011). The impact of this mutation on the transcriptome was studied in two strains of *L. plantarum* (WCFS1 and NC8), showing that the role of CggR in glycolysis control is rather strain-specific. This notion underpins earlier findings in *L. lactis* that identified an impressive degree of diversity in the environmental control of gene expression among individual strains (Bachmann *et al*., 2009).

For the lactic acid bacteria that exert their relevant health-promoting function in the intestine of the consumer, functional genomics approaches are faced with the relative inaccessibility and physicochemical complexity of this niche. Nevertheless, recent *in situ* transcriptome studies succeeded to elucidate the convergence of metabolic and cell-wall architecture adaptations elicited in *L. plantarum* when it resides in the mouse and human gastrointestinal tract (Marco *et al*., 2010). The observed adaptations of the cell-wall architecture of this microbe during its transit through the intestine may have a profound impact on host responses since the extracellular properties of bacteria are commonly considered to be of great importance in molecular communication with specific host cells (Kleerebezem *et al*., 2010). This is clearly illustrated by the differential responses measured in human immature dendritic cells (iDC) upon their interaction with wild-type and mutant lactobacilli that harbour mutations in genes encoding specific cell surface molecules or their biosynthetic pathway (Grangette *et al*., 2005; Konstantinov *et al*., 2008; Meijerink *et al*., 2010). Inversely, cell-surface properties and architecture may influence the impact of host-derived antimicrobial factors on bacterial physiology. Many lactic acid bacteria produce extracellular polysaccharides and these have been studied extensively in lactic acid bacteria, notably with attention for their biosynthesis, structure and function, and engineering (Van Kranenburg *et al*., 1999). The importance of these molecules in host–microbe interactions is evidenced by the work presented by Sarah Lebeer and colleagues, showing that the extracellular polysaccharides provide a surface layer that protects *Lactobacillus rhamnosus* against the inactivating capacities of human innate immunity activity (Lebeer *et al*., 2011). This explains the observation that a mutant defective in the production of extracellular polysaccharides shows much less survival than the wild-type *L. rhamnosus* GG. Moreover, it was observed that the production of extracellular polysaccharides is induced by innate defence molecules. *Lactobacillus rhamnosus* GG is among the most widely used probiotic strains (Saxelin *et al*., 2005) and known to carry a genomic island coding for the production of extracellular polysaccharides (Kankainen *et al*., 2009). The now reported observations provide a molecular basis for understanding the dynamic role of extracellular polysaccharides produced by an important probiotic strain in the human host that at the one hand provides protection and at the other hand prevents other surface molecules to be exposed.

A variety of effective genetic engineering systems for lactic acid bacteria have been developed during the past decades (De Vos, 1999; 2001). However, this toolbox is constantly expanded by novel cloning and expression vectors with specific and attractive features as is exemplified by the novel gene expression tools for lactobacilli presented by Duong and colleagues (2011). They used a transcriptome-based approach to identify strongly regulated promoters and control elements in *L. acidophilus* and developed these into versatile inducible and strong constitutive expression vectors by using the broad-host-range pWV01 replicon (De Vos, 1987). Their functionality and further application potential was exemplified by the controlled overproduction of the oxalate degradation pathway into various intestinal lactobacilli. This is of great interest as lactic acid bacteria that degrade oxalate have potential as candidate biotherapeutics for the treatment of human oxalate-related disorders. Another relevant group of biotherapeutics is formed by several of the lantibiotics produced by lactic acid bacteria. Its paradigm is nisin A, a 34-residue peptide produced by *L. lactis* that is both an antimicrobial targeting a wide range of Gram-positive bacteria and an auto-inducing signal molecule (Kleerebezem *et al*., 1997). Hence it is produced in a density-dependent way by some strains of *L. lactis.* There are wide applications in the food industry both for nisin A and for nisin Z, a naturally occurring variant differing from nisin A in a single residue that affects greatly its solubility and activity (De Vos *et al*., 1993). Moreover, a variety of engineered nisin derivatives have been generated following the first protein-engineering study addressing its structure–

function relation (Kuipers *et al*., 1992). However, there is a need for improved derivatives, notably those that have an increased level and spectrum of bioactivity. This has been addressed in the study by Claire Piper and co-workers, who generated isogenic *L. lactis* strains producing the naturally occurring nisin A, Z, Q and F (Piper *et al*., 2011). This was realized by using as a host the *L. lactis* strain NZ9800 carrying a deletion in the structural gene for nisin A (Kuipers *et al*., 1993). Both producing strains and the purified peptides were tested – the latter as to eliminate any confounding effect of the auto-inducing capacity of the nisin derivatives that is known to vary (Kleerebezem *et al*., 1997). Following analysis of a wide range of clinically relevant indicator bacteria, it was found that nisin F was the most active antimicrobial against multidrug-resistant *Staphylococcus aureus* strains. Nisin F showed minimal inhibitory concentrations of some staphylococci of as low as 1.3 $\mu g\ ml^{-1}$. It differs from nisin A in the substitution His27Asn (like in nisin Z) and Ile30Val; hence, the antistaphylococcal activity resides in the C-terminal part of the molecule and this knowledge may form the basis for new biotherapeutic use of nisin derivatives.

As is evident from the work summarized above, *L. lactis* is still used as a model for lactic acid bacteria. While its genome was the first of the lactic acid bacteria to be characterized (Bolotin *et al*., 2001) its comparative genomics lags behind that of lactobacilli that are much more diverse. In fact, only three subspecies of *L. lactis* are known (ssp. *lactis, cremoris* and *hordniae*) and in the study by Roland Siezen and co-workers, a series of 39 strains from dairy and plant origin was compared using comparative genome hybridization (CGH) and extensive functional analysis (Siezen *et al*., 2011). The use of CGH in differentiating *L. plantarum* strains has been described more than 5 years ago (Molenaar *et al*., 2005) and has shown considerable power in gene–trait matching searches for probiotic properties as discussed above (Pretzer *et al*., 2005; Meijerink *et al*., 2010). While it is known that lactococci have a multitude of plasmids that code for industrially relevant properties, the CGH analysis of *L. lactis* strains extends this now to its genomic traits and illustrates the flexibility of the lactococcal genomes. A considerable variation was observed in the degradation of plant-derived polysaccharides, such as various α-galactosides, arabinose and xylan, and galacturonate. Moreover, the production of extracellular polysaccharides, defence systems (including nisin production) and stress response systems were found to be variable in the studied strains. This is of practical relevance as transfer of those properties to industrial strains by known and efficient genomic conjugation systems may extend their substrate use. Transfer of the 70 kb sucrose-nisin conjugative transposons has allowed new starters to be developed that are industrially used as non-genetic modification is involved (Rauch *et al*., 1994). Similarly, a 51 kb conjugal transposon that codes for the use of α-galactosides has recently been discovered in a specific plant isolate (Machielsen *et al*., 2011). The presented CGH shows the presence of α-galactosides transposon-like structures in various other strains, allowing an extended range of potentially donor strains.

Bifidobacteria are among the first intestinal colonizers in human life, explaining the great interest in their function, diagnostics and development as probiotic bacteria. The latter relates to the potential health-promoting effects that can be ascribed to *Bifidobacteria* (Boesten and De Vos, 2008). Moreover, the activity of *Bifidobacteria* can be modulated by the consumption of specific oligo- and polysaccharides. This reflects the natural function of *Bifidobacteria* that predominantly reside in the colon where they are involved in the degradation of plant or host-derived sugars. Hence, analysis of the sugar degradation capacity is a recurrent theme in *Bifidobacteria* research that has been facilitated by genomic characterization of some species that all have small genomes with a size of around 2 Mb (Ventura *et al*., 2009). The genome of *Bifidobacterium breve* UCC2003 has recently been completed and served as a basis for molecular studies on the metabolism of the plant-derived galactan, a galactose-containing polysaccharide, as reported by O'Connell Motherway and colleagues (2011). They focused on the *B. breve gal* locus and showed this to encode the complete galactan degradation pathway and included genes for an extracellular endogalactanase, an ABC transporter and a β-galactosidase. Moreover, the control of gene expression by the galactan was studied and found to involve the LacI-like repressor GalR. As galactan is an important component of potato tubers, the information on its degradation provides opportunities to develop dedicated and improved prebiotics, defined as selectively fermented food ingredients that allow specific changes, both in the composition and/or in the activity in the gastrointestinal microbiota that confer benefits upon the host' well-being and health. A variety of prebiotics have been developed for all stages of life and many include plant-derived oligosaccharides that promote the growth of specific groups of *Bifidobacteria*. These and other oligosaccharides are increasingly used in infant formulas as it has been noted that their content is much higher in human than in bovine milk. The effect of the consumption of such prebiotics on the development of the microbiota in infants was determined in the study described by Boesten and colleagues (2011). They used a specific bifidobacterial mixed-species microarray capable of detecting the relevant species in intestinal *Bifidobacteria*. This was used to address the composition and development of bifidobacteria in a period of 2 years since birth. The developed approach did not rely on any PCR amplification and

hence has advantages over other presently used methods, including next-generation sequencing technology. It was observed that prebiotic intervention indeed affected the number and species diversity of *Bifidobacteria* opening avenues for further optimizing infant formulas.

All present studies on lactic acid bacteria are strongly genome-driven. This allows to address global effects as well as permits the detailed analysis of basic mechanisms underlying the industrial properties of the used strains. The future developments in this area will be strongly influenced by the increasing number of sequenced genomes of strains within a species, which will expand rapidly through the application of next-generation sequencing technologies. It is expected that this may enable the sequence-based and strain-specific reconstruction of gene-regulation networks as an integral part of a systems biology approach to mathematically describe microbial behaviour at a molecular level and extending the currently available genome-based metabolic models (Teusink *et al*., 2006).

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