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Concluding remarks

The focus of this Symposium was on bifidobacteria and propionibacteria for dairy and probiotic applications. Both genera have many similarities: being phylogenetically closely grouped within the Actinobacteria, they are high G+C branch Gram-positive bacteria, and share several physiological properties, despite having different industrial applications.

It was highlighted in this Symposium that, from the point of view of consumers, consumer organizations, and government agencies, clarity regarding the quality and the label correctness of these commercial probiotic products is often lacking. Some papers have demonstrated that the recovery of the incorporated probiotic organisms is often poor, sometimes below the levels established by legislative rules, especially if the bacteria used are not biologically stable in the particular food formulation. The extreme sensitivity or thermal tolerance of *Bifidobacterium* to spray-drying and storage temperature has proven to be a major impediment to the effective application of these bacteria in functional foods. Other papers concluded that more attention should be directed to the identity, safety and functionality of these strains. This is also of interest to both industry and consumers alike.

For this reason, a significant number of presentations during this meeting were related to the identification of propionibacteria and bifidobacteria at species or strain level, as probiotic characteristics are strain-dependent. From a scientific, technological and regulation point of view, it is important to identify, characterize and recover strains from products correctly. Besides, since bifidobacteria and propionibacteria naturally occur in complex environments, such as food and the intestine of animals and man, their final identification, based on phenotypic patterns, have proven to be difficult, time-consuming and not conclusive.

Modern tools of rapid detection of bifidobacteria and propionibacteria, and quantification and study of population growth dynamics of mixed or individual strains, based mainly on molecular methods, were presented. The developed methods include the amplification of encoded 16S rRNA, DNA sequences of specific genes (L-lactate deshydrogenase – *ldh*, *recA*, 60-kDa heat shock protein – *hsp*60, etc.), PCR-RAPD and Real-Time PCR, Denaturant Gradient Gel Electrophoresis (DGGE) and Pulsed Field Gel Electrophoresis (PFGE). It was shown that DGGE analysis of 16S rDNA amplicons gives an accurate quantitative, fast and reliable analysis of any probiotic sample at the species level and that Real-Time PCR will be very popular for detection, identification and quantification of *Bifidobacterium*.

These methodologies facilitate the elucidation of the identity of species and strains used in dairy products, especially for those that have been very hard to distinguish by phenotypic patterns, such as *B. animalis* and *B. lactis*, the most widely used strains in dairy products worldwide, and different species of *Propionibacterium,* alone and in mixed populations. They enable both absolute and relative quantifications.

As bifidobacteria are the main group present in faeces of both animals and man, it was proposed that the use of bifidobacteria as faecal indicator organisms in raw milk products would be possible to definitively determine by PCR of the *hsp60* gene the human or animal origin of contamination. Besides, faecal samples are considered to be more suitable for the application of proteomic approaches due to the relative stability of proteins. Examples of such metaproteomics were presented, focusing on the temporary development of bifidobacteria in newborn infants or specific bifidobacteria populations isolated by flow cytometry.

So, from a technological point of view, the molecular techniques are essential for reliable identification and genetic improvement of bifidobacteria and propionibacteria strains, and open up a very exciting possibility for the expression of pharmacologically and functionally important gene products.

To exert health benefits, probiotic strains must survive in the adverse conditions of the upper part of the digestive tract, such as low pH and high bile salt concentrations, in order to colonize the gastrointestinal tract (GIT). A cytoplasmic pH homeostasis system may function in the acid tolerance of these bacteria. It was also presented that the acquisition of bile salts tolerance can change the enzymatic activities of bifidobacteria, promoting a change in the fermentation patterns of carbohydrates, directed to a more efficient use of some prebiotics. This type of physiological screening should be done for each new isolated strain.

A symbiotic product, containing the probiotic bacteria and the prebiotic in a single food, can improve the survival of bifidobacteria during the storage of the product and during the passage to the intestinal tract, and also reduce the competition with GIT microorganisms. The enzymes β-fructofuranosidase and phosphoketolase produced by these bacteria were characterized and were shown to cleave a variety of fructooligosacharides which are used as prebiotics. It was observed that *B. longum* has an excessive number of genes associated with oligosaccharide metabolism, comprising >8% of the genome.

Researches concerning the type of food carrying probiotic cultures were also reported regarding how it influences the level of their viability and activity. A number of papers concerned other food products containing live bifidobacteria (orange juice, ice cream and chocolate filling) and propionibacteria (tomato juice enriched in vitamin B12). New uses of *Propionibacterium* strains were presented, which included the production of exopolysaccharides in fermented vegetable juices to improve the sensory profile, and in bread products, to increase the rate of dough fermentation, reduce the amount of yeast and to improve the taste and aroma of bread. As bifidobacteria grow weakly in milk due to their poor proteolytic activity and the presence of other strains of bacteria, the supplementation of milk with casein peptone and cysteine is necessary to increase both growth and lactate production. Dairy products containing probiotics provide not only viable bacteria, but also additional nutritional benefits for consumers and, at the same time, the natural buffering of stomach acid by the food carrier would enhance the stability of the probiotics after consumption.

Yoghurt remains the product of choice in several countries but there is an expanding demand for products such as frozen yoghurt-like products, cheese and fermented milk containing these bacteria. Scientific data presented during this meeting indicate that some technological and chemical factors can influence their viability in fermented milks during refrigerated storage such as: the composition and heat treatment of milk and fermented milks; the way the culture of bifidobacteria is used (loss of cell viability due to freeze-drying is greater than with fresh cultures); the use of ultrafiltered cheese with whey protein concentrate can increase the viability of *B. lactis* BB12, which remains stable during the storage period of cow's and goat's fermented milks. Fermented milk supplementation with propionibacteria influenced positively the nutritive and dietetic aspects due to the increase in vitamin B12 and extended survival of *Bifidobacterium* and starter culture cells. Furthermore, an antibacterial action was observed against *Yersinia enterocolitica* and *Escherichia coli*.

Cheese was considered to have a higher efficiency in delivering viable bacteria encapsulated or not to the intestine than fermented milk, presumably because cheese has a higher buffering capacity, increasing the anaerobic environment; especially UF cheeses, with a high lipid content, which can protect the probiotic during processing and digestion. Furthermore, *B. bifidum* could decrease the coliform counts in cheese.

The product diversity is greater than that presented in this Symposium focused on dairy products. Different categories of probiotic products exist, such as breakfast cereal, nutrition bars and vegetable extract ("soy milk") as well as dietary supplements. Some of these products are not currently available but offer attractive possibilities for future probiotic products and are already produced by several companies worldwide.

In parallel to the propagation of the probiotic concept, propionibacteria and, to a much greater extent bifidobacteria, were investigated for their probiotic functions in dairy and vegetable products. Such functions are, among others, attributed to bacteriocins and organic acids produced by propionibacteria and by bifidobacteria, which can exhibit antagonistic activity toward several pathogens and fungi, and have been genetically characterized. Synergistic effects were also seen between the identified antimicrobial metabolites of propionibacteria and lactobacilli, leading to a strong enhancement of antagonistic features. Progress has been made on their biochemical and genetic characterization, making them available for a more properly use in foods it is specially the case for propiocin T1.

Some important indicators of quality in fermented milks include texture and viscosity. Besides the improvement of organoleptic and rheological properties of fermented milk products, the exopolysaccharides (EPS), produced by bifidobacteria, may be involved in modulating the host immune response, cholesterol-lowering, toxin binding, anti-tumoral and, as a prebiotic, stimulating probiotic growth. As important differences have been observed between EPS gene sequences with the sequenced *B. longum* genome, further sequencing will be used to corroborate the EPS structural analysis, to design probes that will be useful for revealing the EPS-producing potential of new bacterial isolates as well as to follow gene expression under various conditions. More genetic information is necessary to establish the effects on human health.

Dairy propionibacteria are important in the food industry and biotechnology, producing flavor in Swiss-type cheeses, vitamin B12, trehalose and propionic acid. New inputs on culture media for optimization of vitamin B12 industrial production by selected highly-producing *Propionibacterium* strains were also presented during this Symposium. The genetic tools for improving the production of flavor by these bacteria classify them as GMOs, limiting current possible utilization.

The study of the interaction of exogenous bifidobacteria and propionibacteria with human targets in the intestine of individuals is of great interest. For this, genomic research is considered to be prerequisite and has been performed by several research groups, which is expected to explain probiotic and so far hypothetical or novel metabolic functions. The first complete genome sequence publicly available to date is that of *B. longum* NCC2705. It was elucidated by the bioinformatic analysis of the 2.25 Mb genome sequence of this bacterium, and revealed several physiological traits that could partially explain the successful adaptation of this bacterium to the human intestinal tract, such as the catabolism of a variety of oligosaccharides which contributes to the competitiveness and persistence of bifidobacteria in the colon. The genome sequencing of *B. breve* is currently also under way. Developments in genome and post-genomics approaches to these bacteria will be very important in strain characterization and analysis of their functionality in the human intestine. The genome of *P. freudenreichii* subsp. *freudenreichii* was sequenced and data on transcriptase analysis about vitamin B12 and production of flavor in Swiss cheese were obtained.

Some cloning vectors have been constructed for bifidobacteria and propionibacteria; they can be used for probiotic and industrial applications, and to understand gene functions in the original host background. Different previously characterized marker genes were proposed, such as *bsh* (bile salt hydrolases), *rep*, tet(w)-tetracycline resistance, *cat* (chloramphenicol-acetyltransferase) and *pcf*A-propiocin F.

With such vectors, the overexpression of heterologous proteins can be achieved, several resulting in products with great technological interest. Some examples are: cholesterol-oxidase from *Streptomyces*, human interleukin 18, β-galactosidase, amylase, tetrapyrrole compounds, vitamin B12 and lactic acid developed by *Propionibacterium*. Through these systems many other useful proteins can be expressed in probiotic bacteria, as well as in other bacteria, such as *Corynebacterium glutamicum* and the acetic acid producer *Gluconobacter*

oxydans, also presented in the Symposium. Safe systems obtained for *Bifidobacterium* can be used to deliver vaccines or anticarcinogenic polypeptides into the human intestinal tract.

Several presentations suggested that probiotic cultures can mediate a variety of health effects through various proposed mechanisms. Theses included: potential benefit of propionibacteria in colonic mucosae healing, decrease in serum total cholesterol, possibility of controlling the Tuberculosis process by *Propionibacterium*, anti-diarrheal effects, decrease in mycotoxins, anti-cancer effects and immunomodulation. Several possible mechanisms of probiotic action are proposed in order to explain these health responses. Despite the massive popularity of the probiotic products, many of the mechanisms involved in the health claims associated with them are still currently undetermined. Some of the potential health effects are scientifically quite well established while others may show promising results in animal models but need additional investigation in humans.

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