



Stimulation of the growth of two probiotic bacteria, *Lactobacillus Acidophilus* NCFM and *Bifidobacterium Lactis* BL-04, by selected prebiotic candidates

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centuries and consumed because of an inherent belief that they promote health and well-being.

The probiotic properties of LAB isolated from boza, kefir, traditional beer, African fermented milk, molasses were investigated and good adhesion to Caco-2 and HT-29 cell lines observed. This LAB was showing a good survival in presence of low pH and high concentrations of ox-bile. Susceptibility to antibiotics and growth in presence of commercial medication was studied.

LAB has a long history in the preparation of TFFP and most of them have an accepted GRAS status. Together with their importance as a starter culture in the fermentation process, LAB may also be effective bacteriocin producers and potential probiotics. TFFP need to be re-evaluated in view of the potential source of probiotic LAB and as a valuable source of their delivery to humans.

MEDICINAL PLANTS IN FOOD SUPPLEMENTS – THEIR USE, BENEFITS AND SAFETY ASSESSMENT

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Key Words: botanicals, phytopharmaceuticals, secondary metabolites, doses

Many medicinal plants, other botanicals and derived preparations (made from plants, algae, fungi or lichens) have a long history of use in Europe and all over the world and are widely available on the market. Some botanicals are considered as traditional herbal medicinal plants and are used both in medicinal products, mostly in over-the-counter phytopharmaceuticals, and in food supplements. Such products are typically labelled as natural foods and a variety of claims are made regarding possible health benefits. These claims are mainly based on the content of secondary metabolites in these products and their beneficial influences on physiological functions.

In the European Union, the classification of a botanical as a medicine or a food supplement is up to each Member State, which determines whether or not a botanical can be marketed as a foodstuff or as a medicine on its territory. The European Medicines Agency (EMA) is responsible for assessing both the safety and efficacy of herbal preparations when used as medicines. The European Food Safety Authority (EFSA) is currently developing a framework for the safety assessment of botanical ingredients in food supplements that will consider data reasonably required to conclude on safe food use.

STIMULATION OF THE GROWTH OF TWO PROBIOTIC BACTERIA, LACTOBACILLUS ACIDOPHILUS NCFM AND BIFIDOBACTERIUM LACTIS BL-04, BY SELECTED PREBIOTIC CANDIDATES

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Key Words: Probiotics, Prebiotics

Introduction: Prebiotics have been shown to support the growth of probiotic bacteria thereby having a beneficial effect on human health. The aim of this work was to evaluate selected and structurally different carbohydrate prebiotic candidates by measuring their capability to stimulate the growth of the two probiotic bacteria *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* BL-04.

Methods: A Bioscreen instrument was used to screen the pre- and probiotics under anaerobic conditions and the optical density at 600 nm was measured to monitor the growth of isolated cultures of the probiotic strains, *L. acidophilus* NCFM and *B. lactis* BL-04, in combination with different prebiotic candidates (1%). By using the area under the growth curves obtained by measuring OD₆₀₀ during 24 h fermentations, the growth of the two probiotic bacteria on the different prebiotic candidates was compared to the growth supported by a known prebiotic, galactooligosaccharide (GOS).

Results: For *L. acidophilus* NCFM raffinose, isomaltulose, panose, isomaltose, FOS (fructooligosaccharide), galactose, stachyose, trehalose and verbascose supported growth to the same extent as for GOS whilst for gentiobiose and

cellobiose the growth was 30 and 35 % higher than GOS, respectively. For *B. lactis* BL-04 isomaltose, melibiose, stachyose and verbascose supported growth to the same extent as GOS whilst for panose, raffinose and maltotriose prepared from hydrolyzed pullulan the growth was 27, 28 and 41 % higher than for GOS, respectively.

Discussion: The fermentation patterns of both Lactobacilli and Bifidobacteria are strain and species specific. *L. acidophilus* is previously shown to ferment a wide variety of different carbohydrates, but though a gene for a putative transporter of gentiobiose and cellobiose has been identified for *L. acidophilus* NCFM it has not previously been shown that this strain is able to utilize gentiobiose. Most Bifidobacteria are able to utilize raffinose but to our knowledge it has not previously been shown that *B. lactis* BL-04 is able to utilize panose and maltotriose. The most promising combinations of pre- and probiotics will be further investigated by differential proteomics to give more understanding of the interactions between the pre- and probiotics. Moreover some of the pre-/probiotic combinations will be used in a colon simulator to simulate their effects on human health.

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EXAMPLES OF PROBIOTICS SELECTION AND HEALTH EFFECTS IN CHILDREN

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Key words:

Probiotics are live micro-organisms which when ingested in adequate amounts confer a health benefit to the host (FAO/WHO, 2002). Some lactic acid bacteria, such as *Lactobacillus casei* DN-114 001, *Bifidobacterium breve* or *Lactobacillus rhamnosus* LGG, can be ideal candidates as probiotics in foods, such as yoghurts. These strains have indeed a long history of safety and beneficial effects have been described in children, adults and seniors.

Probiotics are selected amongst many different strains deposited in culture collections. Early selection is based on generic methods which allow strain characterization at the molecular level, safety tests, as well as evaluation of technological properties such as the property to ferment milk and the ability of the strain to survive during transit. Proof of concept includes tests which evaluate functional properties *in vitro* and *in vivo*. For example, strains can be tested for anti- or pro-inflammatory properties *in vitro* using Caco-2 cell cultures and read-outs on NF-κB and IL-8. When looking for effects on allergy, strains can be tested for their capacity to inhibit mast cell-dependent activation. Effects observed *in vitro* can then be confirmed *in vivo* in, for example, an ovalbumine sensitized mouse model for allergic asthma. When looking for effects on infections, strains can be tested in infection models or vaccination models, like for example an Influenza mouse vaccination model. Quite interesting is the observation that synergistic effects can be obtained when combining certain prebiotics with probiotics.

Good probiotic candidates are then formulated into products taking into account sensorial aspects as well. Proof of product must always be demonstrated in randomised clinical trials. The overall body of evidences, including *in vitro*, *in vivo* and clinical data should follow the transational research process as recommended by WHO and ILSI.

Health benefits have been demonstrated in children drinking a fermented probiotic dairy drink on several occasions and some examples are given herein. In a study in Italy, which included 187 children, 2 to 5 years old, a probiotic drink reduced the number of episodes of allergic rhinitis during a one year consumption period (Giovannini et al 2007). In another study including 59 children on triple antibiotic therapy, the probiotic drink enhanced *H. pylori* eradication when consumed for 14 days (Sykora et al 2005). The same drink had already been shown to reduce the incidence or duration of diarrhea in infants (Pedone et al 1999, Pedone et al 2000). Recently, large randomised clinical trials conducted during the 2006-2007 winter season in 600 children, 3 to 6 years old, in the USA and Russia, showed that the probiotic could reduce the cumulated number of common infections in the USA and the incidence of rhinopharyngitis in Russia. These results confirm the potential of probiotics applications in foods or drinks for children.