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Challenges & Opportunities Before Indian Business Environment

α-Galactosidase Producing Probiotics Bacteria and Their Health Implications

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ABSTRACT

Nowadays, people are aware that diet plays a major role in preventing diseases and promoting health. Therefore there is an increasing trend for functional foods containing probiotic culture. "Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host". Some LAB positively influence human health mainly by improving the composition of intestinal micro biota and for this reason, they are called probiotics. The increasing cost of health care, the steady increase in life expectancy and the desire of the elderly for improved quality of life **research and development required in the area of probiotics. The concept of providing functional foods including beneficial components rather than removing potentially harmful components. Soybeans and other pulses contain oligosaccharides which may cause intestinal disturbances such as flatulence. This study was undertaken to investigate α-galactosidase-producing probiotics bacteria. The enzymes and cultures can be added to foods in order to enhance the digestibility of carbohydrates in the gastrointestinal tract. However since many of these bacteria are reported for probiotic properties that support and induced health benefits to the consumer. The study provides data on the stability of α-galactosidase, which could potentially be added to food matrices containing stachyose or raffinose such as beans, soya and other pulses and could be an alternative or remedies of oligosaccharides intolerance.**

1 INTRODUCTION A plethora of studies are in progress to evaluate and improve the health benefits attributed by Lactic Acid Bacteria (LAB) because of the century old hypothesis that some specific dairy products fermented by LAB may provide health benefits (Vadeboncoeur & Moineau, 2004). LAB are generally regarded as safe (GRAS) and therefore the metabolites derived from them might be used without extensive purifications (Vasiljevic & Jelen, 2002; Vinderola & Reinheimer, 2003). LAB are gram-positive, catalase

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negative, anaerobic but aero-tolerant, non spore forming rods or cocci that produce lactic acid as the major end product from the fermentation of carbohydrates (Trachoo & Boudreaux, 2006). LAB are commonly known to produce antimicrobial substances such as bacteriocins a antimicrobial compounds in foods and thus possessing a great potential to be used as food biopreservatives (Pal et al., 2005). Inspite of that LAB are also reported to produce some functional enzymes viz. α-galactosidase and β-galactosidase. The antimicrobial potential of lactic acid bacteria has been appreciated for more than 10,000 years and has enabled to extend the shelf life of many foods (Savadogo et al., 2004).

Nowadays, people are aware that diet plays a major role in preventing diseases and promoting health. Therefore there is an increasing trend for foods containing probiotic cultures (Soomro et al., 2002). "Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2002). Some LAB positively influence human health mainly by improving the composition of intestinal micro biota, immunomodulaters, decreasing chlosterol level and for this reason, they are called probiotics (Grajek et al., 2005). The increasing cost of health care and the steady increase in life expectancy and the desire of the elderly for improved quality of their lives are driving factors for research and development in the area of probiotics and also the concept of providing functional foods including beneficial components rather than removing potentially harmful components (Azizpour et al., 2009).

Probiotics LAB that are widely used in food fermentations and able to survive in the gastrointestinal tract of consumers can be used as vehicles for delivery of biologically active proteins like β-galactosidase, α-galactosidase etc. (Connes et al., 2002). α-galactosidase (α-Gal) catalyses the hydrolysis of α-galactosides which are present in plants as storage molecules like melibiose, raffinose and stachyose (Naumoff, 2004). Soya and its derivatives are nutritionally high quality food products but they are rich in α-galactosides which are not digested in small intestine of mammals due to absence of α-galactosidase enzyme. The passage of these carbohydrates to large intestine makes them available for fermentation by gas-producing bacteria leading to intestinal flatulence (LeBlanc et al., 2008).

α -Galactosidase has been isolated from many plant sources and it also has been reported in few genera of bacteria, fungi and yeast (Marraccini et al., 2005; Brouns et al., 2006). It has been reported that some LAB like *Lactobacillus plantarum*, *L. fermentum*, *L. brevis*, *L. buchneri* and *L. reuteri* are able to hydrolyze α-galactosides into digestible carbohydrates. Thus, the use of LAB expressing α-Gal is a promising solution for the elimination of these α- galactosides before they reach the large intestine (LeBlanc et al., 2004a). LAB can be used in two different strategies to remove or degrade α- galactosides present in soy products: First is fermentation of soy-products by LAB previous to consumption and second is to use LAB as vehicle for delivering α-Gal to the small intestine for *in situ* degradation of α- galactosides (LeBlanc et al., 2008).

Therefore, the utilization of LAB strains that are considered to be probiotic and can carry out both fermentation as well as α-galactosides removal is economically attractive. The present study will investigate the production and optimization of α- galactosidase by probiotic LAB, which will open new door to many applications in the development of new food products with improved nutritional value.

2. LACTIC ACID BACTERIA

Lactic Acid Bacteria (LAB) consist of a number of bacterial genera within the phylum Firmicutes. The *genera Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Lactosphaera, Leuconostoc, Melissococcus, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus* and *Weisella* are recognised as LAB (Holzapfel et al., 2001).

In general, LAB are gram-positive, catalase negative, nonaerobic but aerotolerant, nonspore forming rods or cocci that produce lactic acid as the major end product from the energy yielding fermentation of carbohydrates (Trachoo & Boudreaux, 2006). LAB were first isolated from milk and occur naturally in fermented food like milk products, vegetables, beverages and bakery products (Carr et al., 2002; O'Sullivan et al., 2002). There is a long history of health claims concerning living microorganisms in food, particularly lactic acid bacteria. The acidification and enzymatic processes accompanying the growth of LAB impart key flavour, texture and preservative qualities to a variety of fermented foods, predominantly milk and dairy products (Klaenhammer et al., 2005). Lactic Acid Bacteria (LAB) show a special promise for selection and implementation as protective cultures in food as they are considered "food grade" organisms and lactic acid fermentations are believed to be oldest means of food preservation known to mankind (Klaenhammer et al., 2005 ;Benekerroum, et al., 2007) . Lactic Acid bacteria such as *Latococcus lactis* and *Streptococcus thermophilus* preserves the nutritive qualities of food material for an extended shelf life by inhibiting food spoilage and growth of pathogenic bacteria (O'Sullivan et al., 2002; Heller, 2001). Some workers have reported that metabolites from LAB can be exploited as biological preservatives in food packaging materials (Scannel et al., 2000; Pirttijarvi et al., 1999).

LAB and their food products confer a variety of important nutritional and therapeutic benefits in humans such as inhibition of pathogenic organism, improvement of microbial balance in the intestine, immune system modulation, alleviation of lactose intolerance and reduction of blood cholesterol, etc. (Rashid et al., 2007). Recently LAB has been the focus of extensive research because of their potential significance in fermentation, bio processing, agriculture, food and medicine. The β- galactosidase enzyme from LAB is widely used in dairy industry for reducing lactose intolerance (Nguyen et al., 2007; Ghettanchi et al., 2010).

2.1 Lactic Acid Bacteria: Antimicrobial Prospective

LAB has been shown to possess inhibitory activity towards the growth of enteropathogenic bacteria such as *Listeria monocytogenes*, enterohemorrhagic *Escherichia coli* (EHEC) and *Salmonella* species (Moghaddam et al., 2006). The inhibition of pathogens by LAB could be due to the production of inhibitory compounds during fermentation such as organic acids, hydrogen peroxide, diacetyl and bacteriocins (Hirano et al., 2003). It has been reported that bacteriocins produced by LAB are extremely important in preventing the growth of spoilage and pathogenic bacteria, and have been a subject of extensive studies in recent years because of their potential use as novel, natural food bio-preservatives (Sezer & Guven, 2009). Bio-preservation is a widely accepted conservation system, since it is a natural procedure to control pathogenic and deteriorating microorganisms in foods. It also provides conditions to extend shelf-life and to increase food safety (Fiorentini et al., 2001).

According to (Chen & Hoover, 2003) bacteriocins are ribosomally synthesized polypeptides which possess bactericidal activity and are rapidly digested by proteases in the human digestive tract. The bacteriocins produced by lactic acid bacteria are categorised in four major classes : **Class I** bacteriocins *(lantibiotics***)** contain an unusual amino acid, lanthionone and include small membrane-active, single or two-peptide bacteriocins of molecular weight less than 5 KDa, **Class II** bacteriocins are small, heat-stable, *non- lanthionine* containing, membrane-active peptides of molecular weight less than 10 KDa, **Class III** bacteriocins include heat-labile, lytic proteins (often murein hydrolases) of large molecular mass and **Class IV** bacteriocins are a group of complex heat stable proteins that are relatively hydrophobic (Alpay et al., 2003; Vuyst & Leroy, 2007; Moghaddam et al., 2006) .

2.2Probiotics: The Good Bugs

The word Probiotics is derived from Greek words: pro and bios which are translated as "for life" (Hamilton et al., 2003). The definition of probiotics has been constantly evolved but in 2002, the Food and Agriculture Organisation (FAO) of the United Nations and World Health Organization (WHO) established the general definition of Probiotics. According to their guidelines, Probiotics are defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host".

Elie Metchnikoff is considered to be the inventor of probiotics. In 1908, he suggested that the longevity of the Caucasian population is due to frequent consumption of fermented milks. Metchnikoff proposed that

the acid-producing organisms in fermented dairy products could prevent "fouling" in the large intestine and thus lead to prolongation of the life span of the consumer. His ideas were clearly related to lactic acid bacteria in dairy products and this generated great interest of other scientists in potential health benefits of lactic acid bacteria (Heller, 2001). Probiotics commonly stem from the category of Lactic Acid Bacteria, especially of genera *Lactobacillus and Bifidobacterium.* A few of the Lab that are used as probiotics are *Lactobacillus acidophilus, Lactobacillus amylovorus, Lactobacillus casei, Lactobacillus delbrueckii, Lactobacillus johnsonoo, Lactobacillus reuteri, Lactobacillus rhamnosus,* etc. (Anal & Singh, 2007). The metabolites produced by them are considered to be quite important in their beneficial effects. These include organic acids (especially lactic acid), hydrogen peroxide deconjugated bile salts, glycosidases and bacteriocins (Gomes & Malcata, 1999).

2.3Probiotics: Health Benefits

Since Metchnikoff's era, a number of health benefits have been contributed to products containing probiotic organisms. Some of these benefits have been well documented while others have shown a promising potential in animal models (Azizpour *et al.,* 2009). The host benefits that have been attributed to consumption of probiotic microorganisms are diverse and include immune modulation (Gill et al., 2001), decrease in colon cancer risk (Capurso et al., 2006), reduction in symptoms of inflammatory disorders, such as inflammatory bowel diseases i.e. ulcerative colitis, chron's disease and pouchitis (Ishikawa et al., 2003; Gionchetti et al., 2003) alleviation of some symptoms of irritable bowel syndrome (Whorwell et al., 2006), alleviation of lactose intolerance (Adolfsson et al., 2004) prevention of allergic symptoms (Krijavainen et al., 2003) enhanced nutrient value (Stanton et al., 2005).

2.4 Probiotics – Selection Criteria

A group of requirements have been identified for a microorganism to be defined as an effective probiotic (Azizpour *et al.,* 2009; Harish & Varghese, 2006; Park et al., 2002).

These requirements are given as follows:

- It must be able to survive transit through the gastrointestinal tract.
- It must be able to adhere to host cells.
- It must be able to persist and multiply.
- It must produce antimicrobial substances to inhibit the growth of pathogens.
- It must be safe, noninvasive, noncarcinogenic and non-pathogenic.
- It must be able to form a normal balanced flora.

3.α- GALACTOSIDASE

The α-galactosidase (α-Gal; α-D-galactoside galactohydrolase) is an exoglycosidase that catalyses the hydrolysis of terminal α-1-6 galactosidic bonds present in oligosaccharides (α-galactosides) of raffinose family sugars such as melibiose, raffinose, stachyose and polymeric galactomannans and guar gum (Naumoff, 2004). α - Galactosidase has been isolated and characterized from many plant sources like sunflower seeds (Kim et al., 2003) tomato fruit (Feurtado et al., 2001) grape flesh (Bryant & Rao, 2000), peanuts and germinating seeds of coffee beans (Marraccini et al., 2005). It has also been reported that few genera of bacteria, fungi and yeast produce α–Gal enzyme for degrading α-galactosides to obtain energy from these storage molecules of plants (Brouns et al., 2006). This enzyme is industrially important because it is applied in food and feed industries for hydrolysing raffinose series oligosaccharides (RO) that are the factors primarily responsible for flatulence upon ingestion of soybean-derived products (Liu et al., 2007). Recently, Ramalingam et al. (2007) have reported that α-Gal obtained from hyperthermophilic microorganisms has potential biotechnological applications in hydraulic manufacturing of oil and gas wells.

3.1 Soy: Nutrition Rich Food

Consumption of soy-derived food products is increasing steadily worldwide due to the distrust of consumers towards animal derived food products, due to the healthy status of soy and most importantly the amino acid profile of soy protein corresponds more closely to human requirements than most other plant proteins. Thus, the nutritional value of soya-derived products is high (Connes et al., 2002; LeBlanc et al., 2008). Soy contains polyunsaturated fatty acids which lower cholesterol levels in consumers and it is rich in isoflavones which are recognized for their potential health benefits like prevention of cancers, cardio-vascular diseases and osteoporosis (Lopez-Lazaro & Akiyama, 2002; Hermansen et al., 2003). Sacks et al. (2006) have also reported that soy proteins could be used to increase total dietary protein intake and can reduce carbohydrate or fat intake.

3.2Soy Food: Limitations

Human Consumption of soy-derived products has been limited by the presence of non-digestible oligosaccharides (NDO), such as α-galactosides like raffinose and stachyose (LeBlanc et al., 2004b). Hydrolytic digestion of these galactosides is relatively weak in mammals (including human) because they do not possess enzyme α-galactosidase (α-Gal) in the upper gastrointestinal tract which is necessary to hydrolyze α -1, 6 linkages found in these sugars (LeBlanc et al., 2005). The indigestibility of these carbohydrates results in their delivery into the colon where they are rapidly fermented by the resident microbiota resulting in the production of large amounts of gas, thereby creating intestinal disorders such as flatulence (Connes et al., 2002).

To overcome these drawbacks of soy products and to boost the consumption of these highly nutritional food products, many attempts have been made to eliminate the α-galactosides from soybeans. These consist of tedious physical methods, including bean soaking, bean germination, water extraction and ultrafiltration but the use of microorganisms expressing α-Gal is a promising solution for elimination of these oligosaccharides before they reach the large intestine (Silvestroni et al., 2002).

3.3 Lactic Acid Bacteria: α- Galactosidase Producers

Lactic acid bacteria have long been used in food processing and some of these are able to produce α-Gal, thus making them good candidate to degrade α- galactosides (Carrera-Silva et al., 2006). It has been reported that LAB such as *Lactobacillus plantarum*, *L. fermentum*, *L. brevis*, *L. buchneri* and *L. reuteri* are able to hydrolyze α- galactosides into digestible carbohydrates due to their α-Gal activity and because of this activity they are used in vegetable fermentations (LeBlanc et al., 2005). Lactic acid bacteria have three excellent potential features which make them suitable to overcome the nutritional drawback of soy foods. First is their ability to convert sugars into lactic acid; second, LAB are used as starters in various fermented products where they are present at high levels (10^9- 10^{10} bacteria/gram) and third, some LAB are able to survive in the gastrointestinal tract of humans and exhibit various metabolic activities there (Connes et al., 2002; Vesa et al., 2000).

LAB can be used in two different strategies to remove or degrade α-galactosides present in soy products. The first strategy is fermentation of soy-products by LAB previous to consumption and second is to use LAB as vehicle for delivering α-Gal to the small intestine for *in situ* degradation of α- galactosides, thus preventing their delivery in the large intestine where they would otherwise be fermented (LeBlanc et al., 2008). Thus, the use of LAB α-Gal is a promising solution for the degradation of α-galactosides in soya products (LeBlanc et al., 2005).

3.4 α- Galactosidase: Potential Applications

α-galactosidase has many important applications in biotechnology and in food and feed processing. The α-galactosidase preparations are available as dietary supplements in human's diets to reduce the problems related to flatulence and it is also used in the processing of legume-based foods, sugar beet molasses and guar gum. it has also been reported that α-galactosidases could enhance the bleaching effect of β-1, 4 mannanase on soft wood kraft pulp in paper and pulp industry (Clarke et al., 2000). In medicine, it plays a crucial role in the treatment of Fabry's disease (Breunig et al., 2003). Recently, have been reported that α-Gal obtained from hyperthermophilic microorganisms has potential biotechnological applications in hydraulic manufacturing of

oil and gas wells (LeBlanc et al., 2005).

4.CONCLUSION

Probiotics is a biological preparation mainly LAB and upon ingestion it confers health benefits to the host. Although probiotics seem to have beneficial effects in a large number of clinical conditions viz. Conditioning gastrointestinal tract, boosting immunity, lowering blood cholesterol etc. Also, it has been reported that some probiotics LAB combat oligosaccharide intolerance by digesting soya products with the production of enzyme α-gal. Thus far there is strong evidence to support their use in treatment of acute diarrheal diseases, prevention of antibiotic-associated diarrhea, lowering cholesterol level and inducing immunity. Although probiotics α-gal producer LAB are generally regarded safe and can be used in specific subgroups of patients such as the immunocompromised, the elderly, the young and patients with indwelling intravenous catheters.

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