INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES

(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com

Review Article

IDENTIFICATION AND PROBIOTICS PROPERTIES OF *LACTOBACILLUS*

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Abstract

Lactobacillus strains are a major part of the micorflora of the gut and of fermented dairy products, and are found in a variety of environments. Lactobacillus casei, Lactobacillus paracasei, Lactobacillus rhamnosus and Lactobacillus zeae form closely related taxonomic group within the facultatively heterofermentative lactobacilli. The classification and nomenclature of these bacteria are controversial. In this paper, relationships between these species were reviewed, examined with DNA-based techniques and conventional carbohydrate tests. Carbohydrates fermentation patterns gave poor discrimination of some species, but PCR using specific primers targeted to sequences of the 16S rRNA gene discriminated 4 types consistent with the currently recognized species. Pulsed-field agarose gel electrophoresis of chromosomal Not I restriction fragments identified 18 different band patterns from independent Lactobacillus isolates and confirmed the identity of L.casei strains from 2 cultures collections (CSCC 5203 and ASCC 290), both representing the type strains of L. casei. Some isolates were reclassified as L. rhamnosus as a natural component of the microflora of dairy foods and dairy environments. These methods can provide a practical basis for discrimination of the species and identification of individual industrial strains.

Keywords: Lactobacillus, taxonomic group, 16S rRNA gene.

Introduction

According to Sgouras *et al. (*2004), Lactic acid bacteria including *Lactobacillus* spp. Such as *Lactobacillus casei, Lactobacillus acidophilus,* and *Bifidobacterium* spp. are becoming very popular in dairy industries due to their therapeutic benefits. Some health benefits include improvement in intestinal disorders and lactose intolerance, altered vitamin content of milk, antagonism against various pathogenic organisms and antimutagenic and anti-carcinogenic activities. These bacteria are widely used in the production of fermented foods and beverages and contribute both sensory qualities of the food and the prevention of spoilage. These organisms have added a new dimension to the importance of fermented milks in human nutrition and health. Moreover, they are present in large numbers in the normal human and animal gastrointestinal flora Health promoting benefits of consumption of LAB have been known for several years, first attributed longevity of Bulgarian peasants to consumption of fermented milks. they defined probiotics as 'a preparation or product containing viable, defined micro-organisms in sufficient numbers that alter the micorflora in a compartment of the host and by that exerts health effects in this host'. Recent research has credited several health benefits to probiotic organisms that are indigenous to the gastrointestinal tract, as well as those consumed through probiotic products. These include their ability to relieve symptoms of lactose intolerance ,increase immune function, cholesterol lowering potential (Noh *et al.,* 1997), antimutagenic activity and treatment of diarrhea (Guandilini *et al.,* 2000) to name a few. The 'natural' target of ingested probiotics is the intestine, its microflora and the associated immune system, and therefore investigations and clinical studies of non-intestinal infections are rather scarce (de Verse and Schrenzenmeir, 2002). Therapeutic activity of probiotic bacteria can be due to competition with pathogens for nutrients and mucosal adherence, production of antimicrobial substances, and modulation of mucosal immune functions (O'sullivan *et al.,* 2005).

The health benefits derived by the consumption of foods containing probiotic bacteria are well documented and more than 90 probiotic products are available worldwide (Shah, 2000). To provide health benefits, the suggested concentration for probiotic bacteria is 106 cfu/g of a product (Shah, 2000). A number of factors have been claimed to affect the viability of probiotic bacteria in fermented food including acid and hydrogen peroxide produced by bacteria, oxygen content in the product, and oxygen permeation through the package (Shah, 2000).

History of *Lactobacillus*

Historical background of *Lactobacillus*

The genus *Lactobacillus* is by far the largest of the genera included in lactic acid bacteria. It is also very heterogeneous, encompassing species with a large variety of phenotypic, biochemical, and physiological properties. The heterogeneity and the large number of species are due to definition of the genus, which essentially are rod-shaped lactic acid bacteria. Such a definition is comparable to an arrangement where the entire coccoid lactic acid bacteria were included in one genus. Lactobacilli are wide spread in nature, and many species have found applications in the food industry. They are Gram-positive, non-spore-forming, rods or coccobacilli with a G + C content of DNA usually <50mol%. Lactic acid bacteria due to their higher molecular percentage of G + C contents in DNA and are placed in the Actinomycete branch as presented Table 1.4, while other lactic acid bacteria are placed in the *Clostridium* branch. Presented in Tables 1.3 and 1.4 compares the G + C contents in DNA of several genus of lactic acid bacteria and phylogenetic relationship of lactic acid bacteria according to G + C mol percent content in DNA, respectively.

Grouping of *Lactobacillus*

The latest grouping of lactobacilli by Kandler and Weiss (1986) relies on biochemical-physiological criteria and neglects classical criteria of Orla-Jensen such as morphology and growth temperature since many of recently described species did not fit into the traditional classification scheme. Unfortunately, the description of new species usually does not include the analysis of the end products derived from the fermentation of pentoses, and therefore, the enzymes of the pentose phosphate pathway may be present permitting a homofermentative metabolism of pentose in lactobacilli. Nevertheless, maintaining the traditional terms is justified with regards to hexose utilization. However, at low substrate concentration and under strictly anaerobic conditions, some facultatively heterofermentative species may produce acetate, ethanol and formate instead of lactate from pyruvate. Thus, the definitions have to be used in awareness of their limitations.

Gut Microflora

The human colon is the body's most metabolically active organ. This is because of the resident microbiota, which comprises 1012 bacterial cells for every gram of gut contents. There are numerous publications purporting that probiotic are active in the gut after ingestion, others have questioned such claims and the beneficial effects that probiotics are said to confer their hosts. In terms of the microbiology of different digestive tract areas, there is variability both in terms of composition and activity. The lumen of the human stomach is essentially sterile due to a low gastric pH. However, micro-organisms are known to reside in the mucosal layer that overlies the gastric epithelium. This includes *Helicobacter pylori*, which has attracted a great deal of research interest. This organism uses its flagellae to invade the gastric mucus layer and thereafter adhere to epithelial cells. In conjunction with a production of ammonia, this allows effective colonization of the stomach (Rathbone and Heatley, 1992).

Microbiological aspects of large intestine

The large intestine harbours the largest and most complex microbial ecosystem associated with the human body, consisting of several hundred different strains of anaerobic bacteria, with numbers exceeding 1011/g of intestinal, as the large intestine usually contains about 200 g of contents, there is enormous biological activity. The fact that these activities can be modulated or perhaps even controlled through diet is of high relevance (Gibson *et al*., 2000). The microbiota is involved in the catabolism of a vast range of dietary and endogenously secreted compounds. The products of these biotransformations are often toxicological significance to the host. For example, the occurrence of colon cancer is greatly influenced by diet, while metabolism of dietary components by intestinal bacteria has been demonstrated to be an important factor in tumour initiation. The colonic microflora may be involved in the aetiology of large bowel cancer by chemical modification or activation of a wide variety of chemical agents with carcinogenic or co-carcinogenic potential. Exposure of the intestinal microbiota to potential toxicants may occur due to their presence in the diet by biliary excretion of endogenously metabolised substances into the intestine, enzymic activation of procarcinogens by the gut microflora or by direct production of mutagenic substances by intestinal microorganisms (McBain and

Macfarlane, 1998). In terms of functionality, the human colon is the body's most metabolically active organ.

Structure and function of the gastrointestinal tract (GI)

The gastrointestinal tract is a tube extending from the lips to the anus and is divided into various well-defined anatomical regions. The digestive and absorptive functions are well known but, in addition to being an organ in the body, the intestine acts as a container for the most intimate portion of the chemical environment. Assimilation of food is not the only physiological function of the alimentary tract. It is also concerned with the excretion of chemical waste, the control of body metabolism and immune response. Furthermore, the gut harbours a complex ecosystem.

Cancer of the large intestine

The large intestine is the second most common site for carcinoma in man and faeces from individuals living in Western societies frequently contain mutagenic substances as indicated by the Ames test. There is no general agreement regarding the aetiology of bowel cancer, although factors such as diet, environment and genetics have been implicated. It has been speculated that tumours occur 100 times more often in the hindgut than in the small intestine, indicating that the colonic microbiota plays an important role in carcinogenesis. It has been suggested that a mechanism whereby intestinal bacteria may be involved in these processes is by the production of carcinogenic metabolites from non-toxic precursor molecules, and a variety of hydrolytic and reductive enzymes responsible for carcinogen production are produced by colonic micro-organisms (McBain and Macfarlane, 1998).

Natural microflora in gastrointestinal tract

Many different types of bacteria representing most bacterial groups have at some time been isolated from the intestine. Those isolated most frequently can be considered as members of the resident flora or as contaminants from the environment. The number of bacterial groups that may be detected is related to the methods used for their detection. Very few investigators have attempted a systematic investigation of the intestinal bacteria and so any list of the species present in the gut must be provisional. Numerically, the most important genus of intestinal bacteria in animals and man is *Bacteroides*. This along with *Fusobacterium*, which contains pathogenic species, and *Leptotrichia*, which is also found in the mouth, comprise the famil *Bacteroidaceae* which also contains members of the former genus *Sphaerophorus*. These are all Gram-negative, strictly anaerobic, non-sporing rods, although some may show varying degrees of polymorphism. *B. fragilis* is ubiquitous in animals and man.

Probiotics

One manner in which modulation of the gut microbiota composition has been attempted is through the use of live microbial dietary additions, as probiotics. The word probiotic is translated from the Greek meaning 'for life'. An early definition was given by Parker (1974): 'Organisms and substances which contribute to intestinal microbial balance.' However, this was subsequently refined by Fuller (1989) as: 'a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance.' This latter version is the most widely used definition and has gained widespread scientific acceptability. A probiotic would therefore incorporate living micro-organisms, seen as beneficial for gut health, into diet.

Probiotics has a long history. In fact, the first records of intake of bacterial drinks by humans are over 2000 years old. However, at the beginning of this century probiotics were first put onto a scientific basis by the work of Metchnikoff at the Pasteur Institute in Paris. Metchnikoff (1907) observed longevity in Bulgarian peasants and associated this with their elevated intake of soured milks. During these studies, he hypothesized that the normal gut Microflora could exert adverse effects on the host and that consumption of certain bacteria could reverse this effect. Metchnikoff refined the treatment by using pure cultures of what is now called *Lactobacillus delbruckeii* subsp. *bulgaricus*, which, with *Streptococcus salivarius* subsp. *thermophilus*, is used to ferment milk in the production of traditional yoghurt.

Subsequent research has been directed towards the use of intestinal isolates of bacteria as probiotics (Fernandes *et al*., 1987). Over the years many species of micro-organisms have been used. They mainly consist of lactic acid producing bacteria (lactobacilli, streptococci, enterococci, lactococci, bifidobacteria) but also *Bacillus* spp. and fungi such as *Saccharomyces* spp. and *Aspergillus* spp.

Properties required for probiotics to be effective in nutritional and therapeutic settings

A probiotic can be used exogenously or endogenously to enhance nutritional status and/or the health of the host. In the case of exogenous use, microorganisms are most commonly used to ferment various foods and by this process can preserve and make nutrients bioavailable. In addition, microorganisms can metabolise sugars, such as lactose in yoghurt, making this food more acceptable for consumption by individuals suffering from lactose intolerance. However, the most interesting properties that probiotics acting exogenously can have is the production of substances that may be antibiotics, anticarcinogens or have other pharmaceutical properties. The properties required for exogenously derived benefits from probiotics are the ability to grow in the food or the media in which the organism is placed, and the specific metabolic properties which result in the potential beneficial effects stated above. The selection of organisms that can be helpful therapeutically and nutritionally would be based on specific properties that are desired.

Requirements for probiotics

It is of high importance that the probiotic strain can survive the location where it is presumed to be active. For a longer and perhaps higher activity, it is necessary that the strain can proliferate and colonise at this specific location. Probably only host-specific microbial strains are able to compete with the indigenous microflora and to colonise the niches. Besides, the probiotic strain must be tolerated by the immune system and not provoke the formation of antibodies against the probiotic strain. So, the host must be immuno-tolerant to the probiotic. On the other hand, the probiotic strain can act as an adjuvant and stimulate the immune system against pathogenic microorganisms. It goes without saying that a probiotic has to be harmless to the host: there must be no local or general pathogenic, allergic or mutagenic/carcinogenic reactions provoked by the microorganism itself, its fermentation products or its cell components after decrease of the bacteria.

Viability of probiotic organisms

Microorganisms introduced orally have to, at least, transiently survive in the stomach and small intestine. Although this appears to be a rather minimal requirement, many bacteria including the yoghurt-producing bacteria *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* often do not survive to reach the lower small intestine. The reason for this appears to be low pH of the stomach. In fasting individuals, the pH of the stomach is between 1.0 and 2.0 and most microorganisms, including lactobacilli, can only survive from 30 seconds to several minutes under these conditions. Therefore, in order for a probiotic to be effective, even the selection of strains that can survive in acid at pH 3.0 for sometime would have to be introduced in a buffered system such as milk, yoghurt or other food.

Antimicrobial properties

As indicated previously, the intestinal microflora is a complex ecosystem. Introducing new organisms into this highly competitive environment is difficult. Thus organisms that can produce a product or products that will inhibit the growth or kill existing organisms in the intestinal milieu have a distinct advantage. The growth media filtrates and sonicates from the bacterial cells of prospective probiotics should be tested for bactericidal and bacteriostatic activity in well-plates against a wide variety of pathogens. The ability of probiotics to establish in the gastrointestinal tract will be enhanced by their ability to eliminate competitors.

Acid and bile tolerance

One of the most important criteria for selection of probiotic organisms is their ability to survive in the acidic environment of the product and in the stomach, where the pH can reach as low as 1.5. Similarly, the organisms must be able to survive in the bile concentrations encountered in the intestine. Lankaputhra and Shah (1995) showed that, among several strains of *L. acidophilus* and *Bifidobacterium* sp. studied, only a few strains survived under the acidic conditions and bile concentrations normally encountered in fermented products and in the gastrointestinal tract, respectively. Therefore, it cannot be generalised that all probiotic strains are acid and bile tolerant. Clark *et al*. (1993) and Lankaputhra and Shah (1995) showed that *Bifidobacterium longum* survives better in acidic conditions and is able to tolerate a bile concentration as high as 4%. Acid and bile tolerance is strain dependent, and care should be taken to select strains based on these attributes.

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Antagonism among bacteria

Bifidobacteria produce acetic and lactic acids in a molar ratio of 3:2. *L. acidophilus* and *L. casei* produce lactic acid as the main end product of fermentation. In addition to lactic and acetic acids, probiotic organisms produce other acids, such as hippuric and citric acid. Lactic acid bacteria also produce hydrogen peroxide, diacetyl and bacteriocin as antimicrobial substances. These inhibitory substances create antagonistic environments for foodborne pathogens and spoilage organisms. Yoghurt bacteria are reported to produce bacteriocin against probiotic bacteria and vice versa (Dave and Shah, 1997).

Anticarcinogenic properties

In the last two decades, the number of people suffering from colon cancer has been gradually increasing, particularly in industrialised countries (Moore and Moore, 1995) have indicated that diet and antibiotics can lower the generation of carcinogens in the colon and reduce chemically induced tumours. These effects appear to be mediated through the intestinal microflora. Additional studies have shown that the introduction of *L. acidophilus* into the diet lowered the incidence of chemically induced colon tumours in rats (Goldin and Gorbach, 1980). A possible mechanism for these anticancer effects relies on inhibiting intestinal bacterial enzymes that convert procarcinogens to more proximal carcinogens. This technique can be expanded in the future by testing probiotics for their ability to inhibit the growth or organisms normally found in the flora that have high activities of enzymes such as β-glucuronidase (Reddy *et al*., 1974), nitroreductase, azoreductase and β-glycosidase or the capability for nitrososation. The ability of probiotics to deactivate faecal mutagens can also be a marker used to introduce organisms that lower cancer risk.

Adherence of probiotic bacteria

It is not clear if adhesion to the intestinal epithelium is essential for the persistence of a probiotic in the human intestinal tract. However, adhesion seems to be a property that enhances long-term survival. The ability of microorganisms to adhere to epithelial cells is to a large extent species specific, although this may be relative. Screening of organisms for their ability to survive in the human gastrointestinal tract is not difficult. The selection of human bacterial isolates will enhance the possibility of finding organisms that will survive. The isolates can then be tested by administering orally between 109 and 1011 viable organism in a single dose with an appropriate buffering agent and the bacterial counts of the specific organism are then measured in the faeces over a several week period. This technique is most successful if the natural flora does not contain the organism being tested or only in small numbers. The first question of transient survival can be established in 48 to 96 h. The evaluation of the ability of the organism to permanently establish in the gastrointestinal tract, by proliferation, can be established by continuous appearance in the faeces over several weeks to several months. The faecal counts should exceed 106/g of faeces. The application of this screen for selecting probiotics should be encouraged in the future. There are several tests for determining if a prospective probiotic can bind to intestinal epithelium. Radiolabelling the microorganisms with an amino acid and then counting for adhering radioactivity in either ileal cells recovered from ileostroma effluent or from buccal cells obtained by gently scraping the inside of the cheek are effective methods. Good adhesion properties should enhance the possibility of long-term survival of the organism in the intestinal tract by countering the peristaltic action of the intestine.

Immunological enhancement

In recent years there have been several reports indicating that lactobacilli used in dairy products can enhance the immune response of the host. Organisms that have been identified as having this property are *Bifidobacterium longum*, *L. acidophilus*, *L. casei* subsp. *rhamnosum* and *L. helveticus* (Isolauri *et al*., 2001). In the future, prospective probiotics, in the appropriate settings (anticancer or infection resistance), should be tested for enhancement of the immunological response. The measurements that should be considered are lymphocyte proliferation, interleukin 1, 2 and 6, tumour necrosis factor, prostaglandin E production and serum total protein, albumin, globulin and gamma interferon.

Application of probiotics

Importance of probiotic consumption in humans

The number of food and other dietary adjuncts products containing live *Bifidobacterium* and *Lactobacillus* bacteria have significantly increased over the last 20 years due in part to the beneficial effects these probiotic organisms are believed to provide (Laroia and Martin, 1990). Presented in Table 1.16. is a listing of bacterial species used as probiotic cultures in food products. Although research is ongoing, the available evidence indicates that ingestion of probiotic bacteria may promote desirable changes in the gastrointestinal tract of humans (Kaplan and Hutkins, 2000).

Prebiotics

There is currently much interest in the concept of actively improving the host health by managing the colonic microflora. Traditionally, this has been attempted by using probiotics. An alternative approach is the consumption of food ingredients known as prebiotics (Rycroft *et al*., 2001). Prebiotics, as currently conceived of, are all carbohydrates of relatively short chain length

(Cummings *et al*., 2001), additionally carbohydrates that have escaped digestion in the upper gastrointestinal tract form the predominant substrates for bacterial growth in the colon (Roberfroid *et al*., 1998). Present evidence concerning the two most studied prebiotics, fructooligosaccharides and inulin, is consistent with their resisting digestion by gastric juice and pancreatic enzymes *in vivo*. In the large intestine, prebiotics, in addition to their selective effects on bifidobacteria and lactobacilli, influence many aspects of bowel function through fermentation (Alles, 1998). Short-chain fatty acids are a major product of prebiotic breakdown, but as yet, no characteristic pattern of fermentation has been identified. Through stimulation of bacterial growth and fermentation, prebiotics affect bowel habit and are mildly laxative (Cummings *et al*., 2001).

References

Dave, R. I. and N. P. Shah 1996. Evaluation of media for selective enumeration of *S. thermophilus, L. delbrueckii ssp. bulgaricus, L. aidophilus* and *bifidobacteria*. *J Dairy Sci.* 79: 1529-1536.

Dave, R. I. and N. P. Shah 1997. Characteristics of bacteriocin produced by *Lactobacillus acidophilus* LA-1 *Int Dairy J.* 7: 707-715.

Dave, R. I . and N. P. Shah 1997a. Viability of yoghurt and probiotic bacteria in yoghurt made from commercial starter cultures *Int Dairy J.* 7: 31-41.

Dave, R. I. and N. P. Shah 1998. Ingredient supplementation effects on viability of probiotic bacteria in yoghurt *J Dairy Sci.* 81: 2804-2816.

Gibson, G.R., P.B. Ottaway and R. A. Rastall 2000. Prebiotics. Chandos publishing (oxford) Ltd, Oxford, England**:** 1-20.

Gibson, G.R. and M.B. Robertfroid 1995. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics *Am J Clin Nutr.* 1401-1412.

Goldin, B. R. and S. L. Gorbach 1992. Probiotics for humans: Probiotics. R. Fuller (Ed.). Blackie Academic and Professional, London, United kingdom**:** 355-376.

Goldin, G.R., S. L. Gorbach, M. Saxelin, S. Barakat, L. Gualtieri and S. Salminen 1992. Survival of *Lactobacillus* species (Strain GG) in human gastrointestinal tract *Dig Dis Sci.* 37(1): 121-128.

Roy, D., P. Chevalier and L. Savoie 1991. Sugars fermented by *Bifidobacterium infantis* ATCC 27920 in relation to growth and a-galactosidase activity. *Appl Microbiol Biotechnol.* 34: 653-655.