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Prospects of lactic acid bacteria of marine origin

K Kathiresan and G Thiruneelakandan

Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai 608 502, India

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Lactic acid bacteria (LAB) are beneficial bacteria. They are known for their human gut floral maintenance, fermentative activity and food preservative capacity. The marine strains may have better potential than their terrestrial counterparts. This is due to essential nutrients provided by marine biotopes for nurturing the LAB, and to extreme environmental niches. The marine LAB and their by-products may have potential values in food processing, fermentation, pharmaceutical and biopolymer industries.

Keywords: Lactic acid bacteria, marine environment, lactobacilli, bacteriocin, probiotics

Introduction

Lactic acid bacteria (LAB) are among the best studied microbes, constituting a large group of beneficial bacteria that produce lactic acid from fermentable carbohydrates. LAB are Gram-positive, aero-tolerant anaerobes, non-sporulating rods and/or cocci frequently occurring in chains. They are wide spread in nature and can be isolated from grains, green plants, fermenting vegetables, mucosal surface of animals, dairy and meat products[1] and also from fresh and decomposing sponges, seaweeds, shellfish, crabs and fishes of marine environment[2-4]. In spite of voluminous information about LAB, only a few studies are available for the LAB of marine origin[2-5]. The marine LAB could play a significant role in transformation of organic matter, especially in the surface of deep marine sediments. This review highlights the prospects of marine LAB for their utility in food processing, fermentation and pharmaceutical industries.

Diversity and Characteristics of Marine LAB

Marine bacteria, so far known, are mostly the species of *Pseudomonas, Vibrio, Flavobacterium, Alcaligenes, Xanthomonas, Micrococcus, Lactobacillus,* and *Achromobacter*[6-9]. The bacteria belonging to the phyla Firmicutes, Cytophaga, Spirochaetes and Proteobacteria are abundant in deep marine sediments[10,11]. However, only a few strains of LAB are known from marine environment, mostly from the Pacific Ocean region of Japan[5]. The LAB have been retrieved from coastal and estuarine sediments especially where decaying organic matter is present or sewage disposal occurs[12]. Two species, namely *Carnobacterium funditum* and *C. alterfunditum*, are reported from the Ace Lake in Antarctica[13]. One novel species, *Marinilactobacillus psychrotolerance* has been isolated from dead and living marine organisms at Miura Peninsula, in Japan[4]. Several members of the genus *Marinilactobacillus* have also been obtained from coastal sub-seafloor sediments of the Okhotsk Sea in Japan[14] and *Carnobacterium*-like strains have been retrieved from the Nankai Trough, in South-east of Japan[15]. Another novel species, *M. piezotolerans* has been obtained from a sediment core collected at 4.15 m below the seafloor from a water depth of 4790.7 m at the Nankai trough[16]. Two new species of LAB, namely *Halolactibacillus halophilus* and *H. miurensis*, have been found from living and decaying marine organisms collected from a temperate area of Japan[17]. Recently, another novel species *Trichococcus patagoniensis* is reported as an extremely psychrotolerant LAB strain, discovered from penguins (*Spheniscus magellanicus*) in Chilean Patagonia[18].

Marine LAB have unique features which are, different from terrestrial LAB. The *Carnobacterium* species from the Antarctic lake are neutrophilic, growing best at *p*H 7.0. When this is grown in broth culture with *p*H 8.5, the final *p*H reduced to as low as 4.7-5.2. This low *p*H may exclude other bacteria

*Author for correspondence:
Tel: 91-4144-243223 extn. 210; Fax: 91-4144-238419
E-mail: cdl_aucasmb@sancharnet.in; kathirsum@rediffmail.com

present in seawater-associated alkaline environments[13]. However, the marine strain of *M. psychrotolerans* prefers alkaline to acidic conditions with a *p*H range from 6.0 to 10.0. Thus, it is unable to produce acidic conditions like almost other terrestrial LAB, which prefer weakly acidic reactions and suppress other microorganisms in their niche by making their surroundings acidic. *M. psychrotolerans* also grows under a wide range of temperatures, such as at -1.8°C and from the freezing point of seawater to 40-45°C with an optimum temperature requirement of 37-40°C. Thus, the optimum and maximum temperatures for its growth are markedly higher than those of usual marine bacteria. The strain is halotolerant and can grow in 0 to 20.5% concentration of NaCl, having an optimum growth concentration of 2.0-3.75%. Further, it is hetero-fermentative, producing 87-100% of lactate per mole of glucose consumed, besides the production of formate, acetate and ethanol with a molar ratio of 2:1:1. The product composition is markedly affected by the *p*H of fermentation medium; at higher *p*H, the yield of lactate decreased and that of other products increased conversely[4]. A similar trend is also exhibited by *H. halophilus* and *H. miurensis*[17].

Another LAB, *M. piezotolerans* grows at optimum pressure of 0.1 MPa, tolerating up to 30 MPa. It is mesophilic and able to grow in 0-12% concentration of NaCl, with an optimum concentration range of 1-2%. Further, the strain has 4 to 50°C as range of temperature for growth, with 37-40°C as optimal range. The *M. piezotolerans* is closely related to *M. psychrotolerans* in the 16sRNA gene sequence; however, DNA-DNA hybridization level between the two species was less than 20%. Hence, the species is proposed to be novel[16]. Similarly, *T. patagoniensis* has 100% 16sRNA gene sequence similarity with *T. collinsii*, but DNA-DNA hybridization between them exhibits relatedness value of <45%; while the strain has 99.85% similarity in 16S rRNA sequencing with *T. pasteurii* and 47% DNA-DNA hybridization relatedness. *T. patagoniensis* does not require NaCl, as it grows in the presence of 0-6.5% NaCl with optimum growth at 0.5%. It is sensitive to ampicillin, tetracycline, chloramphenicol, rifampicin, kanamycin and gentamicin[18].

LAB in Human Health

The LAB constitute an important microbiota in gastro-intestinal and uro-genital regions of man, and in maintaining and promoting human health. Prominent members of the intestinal flora are *Lactobacillus* spp. They cause reduced lactose intolerance, alleviation of diarrhoea induced by antibiotics and viral infections, lowered blood cholesterol, increased immune responses, and prevention of cancer[19-22]. The lactobacilli suppress the growth of pathogens at the mucosal surface by out-competing them for nutrients or by producing antibacterial compounds[23,24]. The LAB reduce the toxicity of intestinal contents by suppressing the levels of the bacterial enzymes, namely B-glucoronidase, nitro-reductase azo-reductase and urease that activate pro-carcinogens[23-25]. Many LAB that produce metabolic products, like butyrate/butyric acid, have antitumorigenic activities *in vitro*[26]. *Lactobacillus casei shirota* when orally administered, reduces the recurrence of superficial bladder carcinoma in humans[27,28]. The LAB can modulate host immune response through increased production of immunoglobulins, interleukins 6 to 10, gamma interferon, tumour necrosis factor-α and increased phagocytic activity[27,28]. When lactobacilli are abundant, the person is healthy and when they are depleted due to antibiotics, couching, sexual practice or other pathogenic organisms, such as Gram-negative anaerobes, take-over. This leads to a condition known as bacterial vaginosis that may cause serious impacts on pregnancy and acquisition of HIV[29,30]. Probiotic lactobacilli are suggested as appropriate bio-therapeutic remedy for bacterial vaginosis[31].

Lactobacilli play an important role in the maintenance of vaginal health of women[32,33]. This may be due to their ability to provide physical protection to vagina against pathogens, and to produce antimicrobial proteins; production of the proteins increases when NaCl is added at 1%[34]. Three strains of *Lactobacillus* (*L. acidophilus, L. gasseri & L. jensenii*), isolated from vaginas of healthy pre-menopausal women, have been studied for their adherence to vaginal epithelial cells and interaction with uro-pathogens. All the strains protect the vaginal epithelium by formation of a barrier through self-aggregation, and adherence of the strains to epithelial vaginal cells due to glycoprotein. The LAB inhibit growth of *Escherichia coli* and *Streptococcus agalactate* due to their binding with glycolipids, which are receptor binding sites for pathogens[35].

The LAB are resistant to antimicrobial substances. Even though the amino-glycoside antibiotics have broad-spectrum activities, they are ineffective against

LAB because of membrane impermeability of the bacteria due to presence of multiple cationic charges. The LAB are also resistant to bile acids and to largely soluble and negatively charged taurocholic acid than to a strongly hydrophobic and neutral cholic acid[36]. Being less potentially toxic than current antimicrobial agents, LAB and their by-products are used as probiotics as they can benefit the host by improving intestinal and vaginal well-being. In order to have potent probiotic strains, strict attention to strain selection is required. The selection criteria for probiotic LAB include: safety; viability in delivery vehicles; resistance to acid and bile; adherence to gut epithelial tissue; ability to colonize gut; production of antimicrobial substances; ability to stimulate a host immune response; and the ability to influence metabolic activities, such as vitamin production, cholesterol assimilation and lactose activity[22,37,38].

Lactobacilli have been tested for their potential as probiotics. *L. acidophilus* exhibits good acid tolerance and produces a heat stable antimicrobial protein, which is active over a wide *p*H range and inhibits a number of Gram-positive bacteria, including *Listeria ivanovii* and pathogenic strains. Its utility is known in the manufacturing of dairy foods and dietary supplements[39]. Probiotic strain of *L. rhamnosus* gets attached to human intestinal mucosa persistently[40]. Adhesion property of *L. casei shirota* and *L. acidophilus* to an artificial caries model has been demonstrated[41]. By virtue of its ability to persist in the human gastro-intestinal tract, *L. plantarum* is preferred for its use as a delivery vehicle for therapeutic compounds including vaccines[42]. The protective effect of *L. acidophilus* and *L. casei*, isolated from fresh cow milk, has been demonstrated to improve the liver functions and maintain the gut flora in the rats dosed with *Lactobacillus* isolates and simultaneously with *E. coli*[43]. The world-wide market for the probiotics continues to increase with our health consciousness. There is a need to explore novel potent strains from marine biotope.

Prebiotics are slow or non-absorbable complex carbohydrates that can be assimilated by probiotic bacteria, such as *Lactobacillus* and *Bifidobacteria* spp. The prebiotic substrates are inulin, lactose, various galacto, fructo, xylo-oligosaccharides and sugar alcohols, such as lactitol and xylitol[23]. A combination of a probiotic culture with prebiotic substrate, which is referred to as 'synbiotic' is shown to reduce significantly the rate of infection and restore health quickly in sick and post-operative patients. This is especially important as pharmaceutical treatment, including the use of antibiotics, has largely failed, and the medical world is in much need of new treatment strategy[44].

LAB in Fish and Animal Health

The LAB are known to improve fish health. For example, *L. rhamnosus* induces immuno-stimulatory effect in rainbow trout fish, *Oncorhynchus mykiss* by increasing plasma immunoglobulin level[45]. However, it is also known that some of the LAB have become a major source of concern for aquaculture. *S. iniae* is a pathogen of both humans and fish from both fresh water and salt water environment[46-48]. This was originally identified from subcutaneous abscesses on the Amazon fresh water dolphins[49]. This species has been recognized as one of the most serious causes of diseases in fish raised in aquaculture, causing 30-50% mortality in affected fish ponds[50], and is capable of causing disease in humans who have handled infected fish from aquaculture farms[51,52]. Other pathogenic LAB, such as *L. piscicola, L. garvieae* and *Lactococcus piscium*, have been reported to produce occasional fish mortalities[53].

By virtue of enzyme production, the LAB are beneficial to the animal nutrition. *L. acidophilus* produces high amylase activity, hydrolysing large amounts of corn starch. The pigs injected with the culture of *L. acidophilus* (3×10^9 cells in 10 mL) have been found to improve the starch digestibility, which otherwise is extremely low in baby pigs due to their lack of ability to produce enough pancreatic amylase. This has resulted in improved growth rates and feed efficiency in the pigs[45].

An important problem in the field of animal nutrition is the preservation of fodder, which is done in silos for maintaining the nutritive value of the fodders. In anaerobic conditions, the LAB (*L. plantarum*) ferments the glucosides in the silo, leading to the formation of lactic and acetic acids, which cause the *p*H to decrease and inhibit the development of butyric bacteria. The enrichment of the ensiled fodders with probiotic products confers on them the role of maintaining a microbial flora inside the digestive tract favourable for farm animal health[54].

LAB in Synthesis of Biopolymers

Being biodegradable, the polymers of lactic acids have medical applications as sutures, controlled drug release, orthopaedic implants in filling the gaps in

bones and joining membranes and skins[55]. Calcium lactate can be used for calcium deficiency therapy and as anti-caries agent. In recently issued patents, a polymer of lactic acid, extracted from an osmo-tolerent strain of LAB, and its use in electrodialysis for desalting and water splitting have been proposed[56]. Another important property of polylactic acid is its strength against UV-radiation. Thus, lactic acid polymers for medical and agricultural applications and as packaging material gained importance due to environmental issues[57]. In the international market, natural form of polymers is preferred to be used for medical purposes compared to that produced by chemical or enzymatic processes.

LAB in Food Industries

LAB are of great economic significance and well-known for fermentation of milk products to yield yoghurt, cheese, butter and butter milk; as well for pickling of vegetables, baking and wine-making; and curing fish, meats and sausages. Lactic acid gives fermented milk a tart taste and acetaldehyde provides aroma of yoghurt, while diacetyl imparts a buttery taste to other fermented milks. Lactobacilli are also involved in the manufacture of fermented fish and marine mammals.

Biopreservatives are more preferred to chemical preservatives[58]. As a result, there is an increased interest in preservation through LAB because of their safe association with human fermented foods. Lactobacilli are resistant to stress conditions especially to heat treatment or a sub-lethal osmotic shock which improves the resistance to subsequent freeze-drying and storage. Thus, they can be preconditioned to withstand stress conditions associated with industrial processes, for improving both stress resistance and long-term storage stability of LAB culture concentrates[59].

LAB and their metabolites have potential as natural preservatives to control the growth of spoilage and pathogenic bacteria in foods. Several metabolic products produced by these bacteria, including organic acids, fatty acids, hydrogen peroxide, carbon dioxide and diacetyl, have antimicrobial effects[60,61]. However, great attention has been focused on the ability of LAB to produce specific proteinaceous substances known as bacteriocins, which inhibit the growth of pathogens, such as *Listeria, Clostridium, Staphylococcus, Bacillus* and *Enterococcus* spp., and thus enhance the shelf-life of foods[62].

The LAB bacterocins are of four classes, namely (i) lantibiotics, (ii) small hydrophobic heat stable peptides (<13 kDa), (iii) large (30 kDa) heat liable proteins, and (iv) complex proteins that require additional carbohydrates or lipid moieties to attain anti-microbial activity. To date, nisin produced by *L. lactis* sub sp. *lactis* strains[63,64] is the only bacteriocin that has found practical applications as a direct human food ingredient and as a preservative in milk and dairy products, canned foods, cured meats and other segments of fermentation industry[65]. The nisin has a broad inhibitory spectrum against Gram-positive bacteria and can prevent out-growth of spores of *Bacillus* and *Clostridium*. However, it is not effective against Gram-negative bacteria, yeasts and moulds[66]. Its primary target is the cell membrane of the host where it creates pores in the membranes, interferes with energy supply and dissipates the membrane potential[67]. As compared to nisin, the pediocin-like bacteriocins are the most effective against *Literia monocytogenes*, which causes severe disease in the elders, pregnant women and new-borns with chronic illnesses, such as diabetes, cancer and AIDS[65,68,69].

In recent years, several bacteriocins have been purified and characterized, such as amylovorin from *L. amylovorus*[70] and bacteriocin-like molecules from *Vagococcus carniphilus* and *Lactococcus gravieae* isolated from a meat processing industry[71]. Bacteriocins produced by lactobacilli isolated from fermented food products are highly stable to temperature and *p*H. The bacteriocins isolated from *L. brevis* and *L. plantarum* maintained full stability after storage for 60 d at -20°C as well at 121^{0}C for up to 1 h[72]. However, applications of bacteriocins in food have some limitations, such as narrow activity spectrum, inactivation by proteolytic enzymes, binding to food components and low production. These obstacles may be overcome by extracting bacteriocins of marine origin.

Biotechnological Studies of LAB

Now, genome sequencing and functional genomics studies of a variety of LAB are rapidly providing insights into their diversity and evolution, and revealing the molecular basis for important traits like flavour formation, sugar metabolism, stress response, adaptation and interactions. Bioinformatics plays a key role in handling, integrating and analyzing the large 'omics' data being generated. Reconstruction of metabolic potential using bioinformatics tools and databases, followed by experimental verification and

exploration of the metabolic and regulatory network properties, are the present challenges that should lead to improved exploitation of LAB[73] .

Progress has been made in genetic modification of LAB for food grade quality, which opened new potential application in various industries[74]. Lactic acid exists in two optically active isomeric forms, L (+) and D (-). Of these, L (+) form is of great application for its ability to tolerate high concentration of hydrogen ions[75]. A few attempts have been made to improve L (+) lactic acid production by metabolic engineering in lactobacilli *(L. helveticus)* in different ways: (i) inactivation of *idh*D (D-lactate dehydrogenase gene); (ii) internal deletion of the promoter region for *idh*D; and (iii) replacement of *idh*D gene with *idh*L. The effort has resulted respectively in 2-fold, and increase of L-lactate dehydrogenase activity (93%) as compared to the wild type strain (53%)[76]. *L. rhamnosus* produces L (+) lactic acid and ethanol under anaerobic conditions. An *adh*-mutant of *L. rhamnosus* has been developed by chemical mutagenesis, which could produce pure L (+) lactic acid as the only product. Although biomass yield is similar in both wild type and mutant strains, lactic acid is higher by 6.6% in mutant strain[77]. A complete genomic sequence of *L. plantarum*, isolated from human saliva, is known to contain 3,308,274 bp and 3,052 protein encoding genes. This species has one of the largest genomes known in any LAB and that makes the species flexible and adaptable to many environmental niches[42]. Recent years have seen an explosion in the number of genomic sequences of LAB that are used for developing designed probiotics and products that impact gut health to increase the animal and human health[78]. In order to design the probiotic lactobacilli for treating women, the nature of lactobacilli in vaginal micro-biota of women has been studied by using the 16sRNA gene sequencing technique[79-81].

Future Prospects on Marine LAB

The recent advances in biotechnology have significantly increased the production of high quality, nutritional and tasteful foods that remain fresh for longer time. The potential application of bacteriocins as biopreservatives either in the form of protective cultures or as additives is significant. Being less toxic or carcinogenic than current antimicrobial agents, LAB and their by-products have been shown to be more effective and flexible in several applications[82]. However, understanding of LAB present in ocean, their interaction with marine organisms and their function in the ocean is far from clear. It is necessary to undertake morphological, physiological, biochemical and genetic characterization studies on the lactobacilli strains isolated from seawater sediments, and fresh and decomposing marine organisms of varied marine biotopes. Being extremophils, marine LAB, unlike terrestrial LAB, would produce novel chemicals with unique structural characteristics and potential biological activities. Bio-prospecting of marine LAB will provide high value products of pharmaceutical, food and biopolymer applications.

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