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Lactic Acid Bacteria: A Potential Tool in Biological Preservation of Food.

Shashank Maurya, Tanwi Priya, Adarsh Tripathi, Jayanthi S, and Vimala R*.

School of Biosciences and Technology, VIT University, Vellore-632014, Tamil Nadu, India.

ABSTRACT

Lactic acid bacteria (LAB) have a major role in biopreservation of foods because they are considered safe and are designated as GRAS (Generally Recognized as Safe) organisms. The preservative property of LAB is attributed to the combined action of a range of antimicrobial substances viz. lactic acid, acetic acid, propionic acid, carbon dioxide, hydrogen peroxide, bacteriocins and antifungal peptides. Acids produced by these bacteria reduce the pH and inhibit the growth of a wide range of microbes such as Gram positive and Gram negative bacteria, yeast and moulds. The secondary metabolite reuterin, has been reported to have a broad spectrum of activity and inhibits fungi, protozoa and bacteria. Phenyllactic acid which is one of the major compounds produced by some strains of LAB possesses antifungal activity. These antimicrobial metabolites represent a promising natural device for controlling fungal contaminants in different food systems including vegetables and fruits, dairy and bakery products. In this review the data available on various antimicrobial metabolites produced by different strains of LAB has been compiled. The potential application of antifungal LAB has also been discussed.

Keywords: Biopreservation, lactic acid bacteria, antimicrobial metabolites, reuterin, phenyllactic acid

Corresponding author

INTRODUCTION

The food industry has been undergoing well pronounced advancements during the recent years. Still there are few problems which occupy the core of concerns in this ever growing field. One such cause of concern is the spoilage of food materials and thus food preservation has been a challenge for the food industry. Lactic Acid Bacteria (LAB) is a frequently used organism in food industry because of its capability to cause characteristic flavour changes. It has been reported to have additional applications as bio-preservative. LAB produces either lactic acid or acetic acid which in turn leads to the production of other inhibitory substances such as hydrogen peroxide, diacetyl, 3-hydroxypropionaldehyde (reuterin) and bacteriocins. Reuterin is a broad-spectrum antimicrobial agent produced by *Lactobacillus reuteri* from glycerol. It is active towards fungi, protozoa, viruses and enteropathogens. Bacteriocins have also been reported to exhibit antibacterial effect against a narrow spectrum of closely related bacteria. Most of these bacteriocins have narrow antibacterial spectrum and few others are active against *Listeria* and *Enterococcus* species. Nisin is active against *Clostridium botulinum*. Thus, lactic acid bacteria, in addition to the enhancement of nutritive value, also act as potential biopreservative agents [1].

The spoilage caused by fungi is another cause of concern in the food industry. The presence of molds and yeasts in the stored and processed food poses serious problems which lead to economic loss and health concerns. This is mainly because of the mycotoxins produced by the molds. This problem has severely affected the dairy industry which has faced losses due to spoilage of cheese by fungi such as *Aspergillus sp.*, *Fusarium sp.* and *Rhizopus sp*. Lactic acid bacteria holds promise in this regard too because of the presence of antimycotic componds such as organic acids, cyclin dipeptides and fatty acids in it [2].

In several of the experiments, LAB from vacuum packaged meat preserved at 4° C produced some antagonistic substances which showed activity against closely related bacteria. The production of these antagonistic compounds was observed to change with pH, growth medium and temperature [3]. Another study led to an observation that when chilled meat was packaged under vaccum with high levels of CO_2 then its microflora mainly constitutes of LABs. This can be utilized to develop new packaging technologies which will lead to better microbial control and thus extended shelf life and increased microbiological safety of meat products. Thus, bacteriocins and bacteriocinogenic LABs from meat can assist this recent approach to meat preservation [4]. This review discusses the antimicrobial metabolites produced by LAB and its potential application against fungi in various food systems.

ANTIMICROBIAL METABOLITES ASSOCIATED WITH LACTIC ACID BACTERIA

With the growing demand for safe and minimally processed products the use of chemical preservatives has been largely reduced and it has been replaced by biological antimicrobial systems. An example for one such biological agent is lactic acid bacteria which are known to have positive impact on the human gastrointestinal tract. Since LAB have GRAS (generally regarded as safe) status which makes them easier to be utilized for preservation purposes. A wide variety of antifungal compounds viz. organic acids, fatty acids, phenyllactic acid, reuterin and cyclic dipeptides are produced by this group of bacteria which are responsible for its activity.

Organic acids

The common organic acids produced by LAB are lactic acid, propionic acid and acetic acid. These are end products of the fermentation and they result in creating an acidic environment which inhibits growth of bacteria as well as fungi [5]. These acids reduce the pH levels to such low levels where growth cannot occur and metabolism gets inhibited. Acetic acid acts synergistically with lactic acid in order to prevent fungal growth but due to high pKa value of acetic acid, it is more potent [2]. The anti-yeast activity of propionic acid is much higher because of its pKa value which is above that of acetic acid. In an experiment broad spectrum anti-mould activity of *Leuconostoc sanfranciscensis* CB1 was observed which was because of a mixture of acetic, propionic, butyric, caproic, formic and n-valeric acid [6]. In another study recently, *Leuconostoc citreum* and *Weisella confuse* were observed to produce acetic acid as the primary anti-fungal compound. This acid retarded growth of *Clostridium sp* YS1 and *Penicillium crutosum* YS2 at higher concentrations [7]. In addition to this, several carboxylic acids such as cinnamic acid derivatives, salicylic acid, D-glucuronic acid isolated from *Lactobacillum amylovirus* DSM 19280 have also shown antifungal activit [8].

Phenyllactic acid

Phenyllactic acid is one of the most extensively studied antimicrobial compounds because of its broad activity and antifungal action. Its activity has been observed both against gram positive and gram negative bacteria. It mainly acts synergistically with other metabolites [8-11]. The main characteristics of phenyllactic acid which makes it a good choice for the control of food spoilage microorganisms are its lack of odour and zero toxicity. It was first isolated together with its 4-hydroxy derivative from the cell free supernatant of *Lactobacillus plantarum* 21B and was found to inhibit the bread spoilage fungi *Aspergillus, Fusarium, Penicillium* and *Eurotium*. [12]. It has been observed that the production of phenyllactic acid from *Lactobacillus plantarum* strain could be improved on increasing the concentration of phenylalanine in culture and in the presence of low amounts of tyrosine [13]. The naturally produced antimicrobial phenyllactic acid alone has been reported to strongly inhibit *Listeria monocytogenes*, *Salmonella enterica*, *Staphylococcus aureus*, and *Escherichia coli* O157:H7 in laboratory broth and in cream of chicken soup [14]. Phenyllactic acid in milk, cheese, and cultured medium was shown to inhibit the growth of *Listeria monocytogenes* [15]. Schwenninger et al [16] demonstrated that PLA is effective against *Rhodotorula mucilaginosa, Candida pulcherrima,* and *Candida parapsilosis.* The ability of phenyllactic acid to act as a fungicide and delay the growth of fungal contaminants in foods opens new avenues for research in extending the shelf life of foods.

Reuterin

Reuterin is another low molecular weight compound which shows a broad spectrum antimicrobial activity against a range of bacteria such as *Salmonella typhimurium* and *Escherichia coli* K12. It is strongly antagonistic against a wide range of Gram-positive and Gram-negative bacteria, yeast, moulds and protozoa probably due to the inhibition of ribonucleotide reductase. The possible use of reuterin for biopreservation has been investigated in meat, milk and cottage cheese [17]. Spoilage organisms sensitive to reuterin include species of *Salmonella*, *Shigella*, *Clostridium*, *Staphylococcus, Listeria* and *Trypanosoma* [18].

It also has the potential to inhibit growth of moulds and yeast such as *Candida albicans* and *Aspergillus flavus* [19, 20]. Addition of glycerol to the growth medium of certain *Lactobacillus cornyformis* strains has been reported to result in an increased antifungal activity [11, 21]. This increased activity has been later attributed to the production of reuterin because of the breakdown products of glycerol degradation such as 1, 3-propanediol and 3-HPA. The mechanism involved in this has been deciphered in a recent study where it was shown that the aldehyde group of reuterin is highly reactive and it interacts with the thiol groups of small proteins and other molecules which cause oxidative stress in the cells leading to growth inhibition [22, 23].

Cyclic dipeptides

The cyclic dipeptides (2, 5 dioxopiperazines) are one of the most common peptide derivative found in nature. They also have potential antitumoral activity in addition to antimicrobial activity and involvement in quorum sensing [24]. When isolated from *Lactobacillus plantarum* VTT E-78076, it reported to retard the growth of gram negative bacterium *Pantoea agglomerans* and the cereal mould *Fusarium avenaceum* [25, 26]. The main dipeptides responsible for these activities are cyclo (L-Phe-L-Pro) and cyclo (L-Phe-trans-4-OH-L-Pro). They can also act synergistically with phenyllactic acid. The production of cyclic dipeptides is controlled by temperature and acidification. The biochemical pathways involved in its production are yet to be explored completely [9].

Fatty acids

The antimicrobial and antifungal potential of fatty acids have been demonstrated in several studies. It has been observed that chain length played an important role in controlling the extent of inhibition. Lauric acid and capric acid are the most potent fatty acids as per several studies [27]. Four antifungal compounds, namely, 3-(R)-hydroxydecanoic acid, 3-hydroxy-5-cis-dodecenoic acid, 3-(R)-hydroxydodecanoic acid and 3-(R)-hydroxytetradecanoic acid were obtained from *Lactobacillus plantarum* MiLAB 14 [28]. Although the activity has been observed against a number of yeasts and moulds, it is more pronounced against yeasts because of their hypersensitivity towards such hydroxylated fatty acids. LAB have been documented to produce hydroxyl fatty acids from linoleic acid [29]. The conversion of linoleic acid to a monohydroxy octadecanoic fatty acid by *Lactobacillus hammesi* DSM 16381, which displayed antifungal characteristics against *Aspergillus niger* has been reported by Black et al [30].

Antimicrobial proteinaceous compounds

The antibacterial activity of proteinaceous compounds has been observed recently and studies on such compounds are not that intense as compared to other such compounds discussed earlier. Several species belonging to genera such as *Lactobacillus*, *Lactococcus*, *Streptococcus* and *Pediococcus* have shown production of broad spectrum antifungal compounds. Among these, *Lactobacillus* is the most efficient group of bacteria involved in the production of antibacterial proteinaceous compounds. Several antimycotic peptides have been isolated from *Lactobacillus brevis* AM7 and they inhibited growth of *Penicillium roqueforti* DPPMAF1. One of the isolated peptide had similarity to the defensin-like protein present in pear and the other isolated peptide was found to be similar to anti-hypersentivity and anti-microbial peptides found in caseins [31]. Similar compounds were obtained from *Lactobacillus plantarum* with minimum inhibitory concentration (MIC) values between 2.5 and 10 mg per ml. One of these compounds had homology to the lantibiotic lacticin 3147 [32]. Since the mode of action is still not clear, further research is required in order to decipher the underlying mechanism.

POTENTIAL APPLICATION OF ANTIFUNGAL LAB IN FOODS

The food industry has been suffering huge losses because of microbial spoilage. Filamentous fungi are one of the main groups of spoilage microorganisms contributing to significant economic losses and human health risks and a remedial strategy which is as per the standards of regulatory authorities is needed. The use of LAB provides one such strategy which is considered safe by the consumers and as well as the regulatory bodies. These organisms have been given GRAS (Generally Recognized as Safe) status but further testing is necessary for reliability. They can be used as bioprotectants or in association with chemical preservatives in food items as they have been observed to prevent fungal spoilage.

Vegetables and Fruits

The main spoilage organism in case of fruits and vegetables are species of the genera *Fusarium*, *Penicillium*, *Alternaria* and *Botrytis* [33]. *Lactobacillus plantarum* CUK 501 inhibits growth of all these fungi [34]. It delays spoilage of apples, pears, grapes etc. by *Penicillium* sp. Another culture filtrate of *Lactobacillus planarum* IMAU10012 was reported to inhibit growth of *Botrytis cinerea* on tomato leaves. Another advanced strain F3C2 was produced artificially through genome reshuffling and it eliminated growth of *Penicillium digitatum* KM08 on certain fruits such as kumquats [35]. The research done so far has confirmed the potential of lactic acid bacteria as an antifungal agent and further confirmation of regulatory bodies is required in order to diversify their application in the food industry.

Dairy products

The primary dairy products cheese and yoghurt are vulnerable to fungal attack and for this reason lactic acid bacteria are used in the starter culture in order to prevent fungal contamination. Yoghurt is more prone to fungal attack because its low pH, low storage temperature etc. favour the growth of yeast. A mixed culture of *Lactobacillus paracasei* subsp *paracasei* and *Propionibacterium jensenji* inhibits growth of *Candida* sp. on the surface of cheese [36]. Some isolates of *Lactobacillus plantarum* have shown antifungal properties and they are used as adjuncts during the production of cheddar cheese [37]. The use of LAB as an antifungal agent also improves shelf life of processed cheese [38]. A broad spectrum of antifungal activity of four different strains of *Lactobacillus brevis* against members of genera *Aspergillus, Fusarium, Penicillium* and *Trichoderma* has been reported [39]. The advent of LAB as a natural preservative has limited the use of other preservatives such as sodium benzoate, sorbic acid and natamycin in dairy industry.

Bakery products

Bakery products such as bread have short shelf life and they get spoiled very easily because of the fungal growth on the surface. It leads to both economic and health concerns. Some studies of the past have reported that on using *Lactobacillus plantarum* 21B and *Saccharomyces cerevisiae* together, the growth of

Aspergillus niger FTDC3227 was inhibited to a considerable extent over a period of seven days of storage [12]. Other strains of *Lactobacillus* include *Lactobacillus rossiae* LD108 and *Lactobacillus paralimentarius* PB127 which were observed to prevent the growth of *Aspergillus japonicus* in bread and increased the shelf life to a minimum of 11 and maximum of 32 days [40]. Several strains of *Pediococcus* such as *Pediococcus acidilactici* KTU05-7, *Pediococcus pentosaceus* KTU05-8 and KTU05-10 inhibit growth of mould on the surface of bread [41].

CONCLUSIONS

The application of lactic acid bacteria as an antimicrobial agent has experienced several advancements, but there are some issues which need to be addressed in order to make it a more potent bio-preservative agent. The research studies conducted till date have concentrated on the inhibitory effect of LAB and reports on the final quality of the product are limited. The possibility of LAB in having an impact on the organoleptic properties of food has to be disproved by research studies. More emphasis could be placed on the identification of new antimicrobial metabolites. Analysis of potential safety and health issues associated with the use of LAB in food items need to be focused. Further approach should consider the synergistic effects of these antimicrobial metabolites with other advanced technologies. LAB has the potential to completely replace chemical preservatives as bio-preservative agents in food items. Development of genomic tools and microarray technology can be utilized to develop better and more efficient strains of LAB.

REFERENCES

[1] Stiles ME, A Van Leeuw J Microb 1996; 70: 331-345.
[2] Batish, VK, Roy U, Lal R, Grover S, Crit Rev Biotechnol 1997; 17: 209-225.
[3] Ahn C, Stiles ME, J Appl Bacteriol 1990; 69: 302-310.
[4] Stiles, ME, Hastings JW, Trends Food Sci Tech 1990; 2: 247-251.
[5] Ross RP, Morgan S, Hill C, Int J Food Microbiol 2003; 79(1-2): 3-16.
[6] Corsetti A, Gobbetti M, Rossi J, Damiani P, Appl Microbiol Biot 1998; 50(2): 253-256.
[7] Baek E, Kim H, Choi H, Yoon S, Kim J, J Microbiol 2012; 50(5): 842-848.
[8] Ryan LA, Zannini E, Bello FD, Pawlowska A, Koehler P, Arendt EK, Int J Food Microbiol 2011; 146: 276-283.
[9] Bello FD, Clarke CI, Ryan LAM, Ulmer H, Schober TJ, Strom K, Sjogren J, Sinderen DV, Schnurer J, Arendt EK, J Cereal Sci 2007; 45(3): 309-318.
[10] Rizzello CG, Cassone A, Coda R, Gobbetti M, Food Chem 2011; 127: 952-959.
[11] Magnusson J, Strom K, Roos S, Sjogren J, Schnurer J, FEMS Microbiol Lett 2003; 219: 129-135.
[12] Lavermicocca P, Valerio F, Evidente A, Lazzaroni S, Corsetti A, Gobbetti M, Appl Environ Microb 2000; 66: 4084-4090.
[13] Valerio F, Lavermicocca P, Pascale M, Visconti A, FEMS Microbiol Lett 2004; 233: 289-295.
[14] Manu DK, Graduate Theses, 2012; Iowa State University, Ames, Iowa.
[15] Dieuleveux V, Gueguen M, J Food Prot 2012; 61:1281-1285.
[16] Schwenninger SM, Lacroix C, Truttmann S, Jans C, Sporndli C, Bigler L, Meile L, J Food Prot 2008; 71:2481–2487.
[17] Rasch M, Int J Food Microbiol 2002; 72:225-231
[18] Davidson PM, Branen AL, 2005. Antimicrobials in food. CRC Press, Boca Raton, FL, 2005, pp. 209-303
[19] Talarico TL, Casas IA, Chung TC, Dobrogosz WJ, Antimicrob Agents Ch 1988; 32(12): 1854-1858.
[20] Axelsson LT, Chung TC, Dobrogosz, WJ, Lindgren SE, Microb Ecol Health D 1989; 2: 131-136
[21] Nakanishi K, Tokuda H, Ando T, Yajima M, Nakajima T, Tanaka O, Ohmomo S, Jpn J Lactic Acid Bacter 2002; 13(1): 37-45.
[22] Tanaka O, Komatsu T, Oshibe A, Cai Y, Miyazaki S, Nakanishi K Biosci Biotech Bioch 2009; 73(7): 1494-1499.
[23] Schaefer L, Auchtung TA, Hermans KE, Whitehead D, Borhan B, Britton RA, Microbiology, 2010; 156: 1589-1599.
[24] Rhee KH, Int. J. Antimicrob. Ag. 2004; 24: 423-427.
[25] Niku-Paavola ML, Laitila A, Mattila-Sandholm T, Haikara A, J Appl Microbiol 1999; 86: 29-35.
[26] Strom K, Sjogren J, Broberg A, Schnurer J, Appl Environ Microb 2002; 68:4322-4327.
[27] Bergsson G, Arnfinnsson J, Steingrímsson O, Thormar H, Antimicrob Agents Ch 2001; 45(11):3209-3212.

[28] Sjogren J, Magnusson J, Broberg A, Schnurer J, Kenne L, Appl. Environ. Microb. 2003; 69: 7554-7557.
[29] Kishimoto N, Yamamoto I, Toraishi K, Yoshiok, S, Saito K, Masuda H, Fujita T, Lipids, 2003; 38: 1269-1274.
[30] Black BA, Zannini E, Curtis JM, Ganzle MG, Appl Environ Microb 2013; 79: 1866-73.
[31] Coda R, Rizzello CG, Nigro F, De Angelis M, Arnault P, Gobbetti M, Appl Environ Microb 2008; 74: 7391-9398.
[32] Coda R, Cassone A, Rizzello CG, Nionelli L, Cardinali G, Gobbetti M, Appl Environ Microb 2011, 77: 3484-3492.
[33] Sathe SJ, Nawani, NN, Dhakephalkar PK, Kapadnis BP, J Appl Microbiol 2007; 103: 2622-2628.
[34] Crowley S, Mahony J, van Sinderen D, J Appl Microbiol 2012; 113: 1417-1427.
[35] Wang H, Shen F, Xiao R, Zhou Y, Dai Y. Ann Microbiol 2013; 63: 1075-1081.
[36] Schwenninger SM, Meile L, Syst Appl Microbiol 2004; 27: 229-237.
[37] Zhao D, Ph. D. thesis. 2011, USA: California Polytechnic State University.
[38] Garcha S, Natt NK, J Food Sci Technol 2012; 49: 643-648.
[39] Tropcheva R, Nikolova D, Evstatieva Y, Danova S, Anaerobe, 2014; 28: 78-84.
[40] Garofalo C, Zannini E, Aquilanti L, Silvestri G, Fierro O, Picariello G, Clementi F, J Agr Food Chem. 2012; 60:7719-7728.
[41] Cizeikiene D, Juodeikiene, G, Paskevicius A, Bartkiene E, Food Control, 2013; 31: 539-545.