Trends in Life Sciences
An International Peer-reviewed Journal
www.sciencejournal.in

ANTIMICROBIAL ACTIVITY OF LACTOBACILLUS SPECIES ISOLATED FROM YOGHURT AND CHEESE AGAINST FOOD PATHOGENS

Rohini Kulkarni (Pandhare) [1] Gothoskar Vandan[1]

[1]Department of Microbiology, Government College of Arts, Science & Commerce, Aurangabad, (M.S.), India.
[2] Department of Microbiology, Shivchhatrapati College, Aurangabad, (M.S.), India.

ABSTRACT

Food contamination by food pathogens (*Escherichia coli* and *Staphylococcus aureus*) is a major problem for consumer's health in society especially during rainy and summer season. The use of bacterial interaction is a new way to control the growth of pathogenic germs. Detection of antimicrobial substances produced by lactic acid bacteria against undesirable food pathogens is the objective of this work. Microbiological and biochemical methods were used to identify lactic acid bacteria having antimicrobial activity. An isolate of lactic acid bacteria obtained from yoghurt (fermented milk products) was identified the dominant species belonging to lactobacillus genera are *L. rhamnosus*, *L .plantarum*, *L. casei*, *L. paracasi*, *L. acidophilus*, *L .delbruckii* sub species *lactis*, *L. fermentum*, *L.paraplantarum*, *L.sakei* subspecies *sakei*. The study reveals that the two species of Lactobacillus viz *L.plantarum* and *L.casei* are able to inhibit the growth of *Escherichia coli* and *Staphylococcus aureus*.

KEY WORDS: Yoghurt and Cheese, Lactic acid bacteria, interaction, food pathogens

INTRODUCTION

Control of both pathogenic and spoilage microbes in variety of foods are important to guarantee food quality and safety. Recently bio preservation has become a topic of interest. This technique is used as an alternative to chemical additives for increasing shelf-life storage and enhancing safety of food by using natural microflora and their antimicrobial products.

Biopreservation refers to extended storage life and enhanced safety of foods using the natural microflora and (or) their antibacterial products. Lactic acid bacteria have a major potential for use in biopreservation because they are safe to consume and during storage they naturally dominate the microflora of many foods. In milk, brined vegetables, many cereal products and meats with added carbohydrate, the growth of lactic acid bacteria produces a new food product. In raw meats and fish that are chilled stored under vacuum or in an environment with elevated carbon dioxide concentration, the lactic acid bacteria become the dominant population and preserve the meat with a 'hidden' fermentation.

Lactic acid bacteria (LAB) are the most common types of microbes used. LAB has been used in the food industry for many years, because they are able to convert sugar (including lactose) and other carbohydrates into lactic acid. They are also used for production of cheese (Desmazeaud, 1996). This not only provides the characteristic sour taste of fermented dairy & food products but also lowering pH may create fewer opportunities for spoilage microorganisms to grow, hence creating possible health benefits on preventing gastrointestinal infections. Strains of the genera Lactobacillus are the most widely used probiotic bacteria.

Probiotics are dietary supplement containing potentially beneficial bacteria or yeasts. According to FAO/WHO, probiotics are: Live microorganisms which when administered in adequate amount confer a health benefits on host. Probiotic bacterial culture is intended to assist the body's naturally occurring gut flora. They are sometimes recommended by doctors & more frequently by nutritionists, after a course of antibiotics, or as part of the treatment for gut related candidiasis. Probiotics strengthen the immune system to combat allergies, excessive alcohol intake, stress, exposure to toxic substances & other diseases

Lactic acid bacteria are believed to be safe because they have been long established as normal flora in fermented food, thus they have great potential for use in bio preservation. The preserving effect of lactic acid bacteria are due to the production of antimicrobial agents such as organic acids, hydrogen peroxide and bacteriocin or related substances. Bacteriocins are proteinaceous compounds that mainly inhibit closely related species. Some bacteriocins have been shown to possess the ability to inhibit the actions of unrelated genera such as *Clostridia, Listeria,* Enteropathogenic

PRINCIPAL
Govt. College of Arts & Science
Aurangabad

bacteria and Gram negative bacteria. For these reasons bacteria are promising candidates for bio preservation of food. Several lactobacillus strains are an important diary culture starter and for manufacture of fermented food.

This discovery of bacteriocins gave a new way for food development in better hygienic quality. In recent years, there have been many reports on bacteriocins that are produced by lactic acid bacteria (Rodney *et. al.,* 2014). However most reports deal with bacteriocins that are produced by various *Lactococci*, *Pediococci*, *Leuconostoc*, *Enterococci* and *Lactobacillus*. The search for new strains of lactic acid bacteria that produce antimicrobial substances is an universal objective for the creation of new cultures with a high biosafety for fermented food. In order to avoid the side effect of chemical preservatives recently bacteriocins or strains of lactic acid bacteria are used for applications. The objectives of this work are the isolation of lactic acid bacteria that produce antimicrobial substances belonging to bacteriocin type able to inhibit the bacteria which causes food poisoning. In the study of Cocolin *et al.* (2007) two strains of *Enterococcus faecium*, M241 and M249, isolated from goat milk, were studied for their capability to produce antibacterial compounds. It was determined that the bacteriocins produced by both strains were active towards *Listeria monocytogenes* and *Clostridium butyricum,* and they did not have any activity with respect to other species of lactic acid bacteria.

MATERIALS AND METHODS

1. Isolation of Lactobacillus spp.

The species of lactobacillus were isolated from yoghurt and cheese on MRS agar. The pathogenic bacteria responsible of food infection (*Escherichia coli* and *Staphylococcus aureus)* were obtained from the department of Microbiology Shivchhatrapati College, Aurangabad.

The species of lactobacilli were inoculated in liquid MRS with pH 5.0, and then incubated at 30°C for 72hrs. The selective enumeration of *Escherichia coli* and *Staphylococcus aureus* is carried out on nutrient agar at 37°C for 24hrs.

2. Antibiotic bioassay

The isolated lactobacillus strains were selected as bacteriocin producers because of their broad antimicrobial activity and subjected to phenotypic identification. Cell morphology and gram staining reaction were examined by light microscopy. Test for catalase activity and fermentation of different sugars were also tested as described by Berge's Manual.

The Lactobacillus containing MRS broth was taken in 1ml of quantity and centrifuged at 8000rpm for 10 mins. The supernatant obtained was kept for evaporation at 37°C for 24 hrs. The evaporated extract was dissolved in 5% trichloro acetic acid. Then swabbing cultures of food pathogens (*Escherichia coli* and *Staphylococcus aureus)* was done on Muller Hinton agar plates. Then two wells each for supernatant and evaporated extract were made. Then 100ul of supernatant and evaporated extract were poured in separate wells and incubated at30°C for 24hrs. After incubation, during 24 hrs at 30°C, the bacterial lawn was examined for zone of inhibition. Inhibition was recorded as positive if a clear zone around the colonies was produced and measured for zone of inhibition

RESULTS AND DISCUSSIONS

A total number of five strains of lactobacilli were isolated from yoghurt and cheese samples. The strains which produce antimicrobial substances were detected by conforming on solid culture medium (Table 1) From five strains one strain showed an inhibiting activity. The strain was identified to species level by microbial and biochemical methods as described characterisation of the isolates (Bergeys manual of deenminative bacteriology). Grams nature of the isolated lactobacillus was shown in Fig 1 as well as growth of lactobacilli is observed in MRS broth (Fig 1).

Table 1:- Morphological characters of the Lactobacilli isolated from yoghurt and cheese.

Strains code	Cells form & arrangement.
Lab 1	Long rod in chain (*Lactobacillus casei)*
Lab 2	Rod diplobacilli in chain (*Lactobacillus acidophilus)*
Lab 3	Short rods, diplobacilli (*Lactobacillus plantrum)*
Lab 4	Short rods, diplobacilli in chain, (*Lactobacillus fermentum)*
Lab 5	Short rods arranged in chain, (*Lactobacillus rhamnous)*.

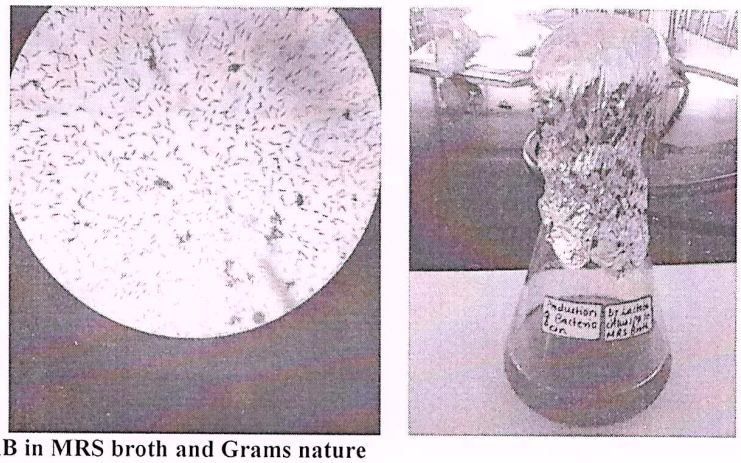

Fig: 1 Growth of LAB in MRS broth and Grams nature

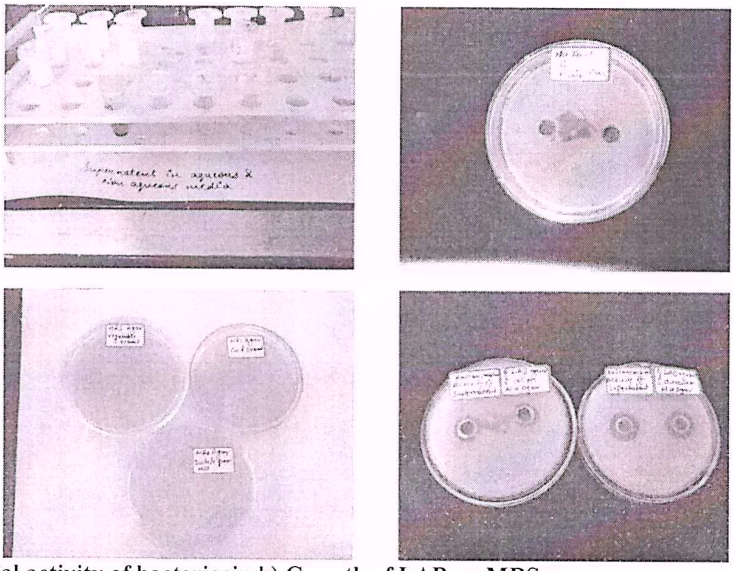

Fig 2: a) Antimicrobial activity of bacteriocin b) Growth of LAB on MRS agar

The stains isolated showed colonies of approximately 2mm of diameter, with white, milky or light yellow in colour, smooth surface and regular circular circumstances were observed on solid medium.(Fig. 2) The microscopic examination reveals that tested stains are gram positive, with cellular rod from associated in pairs or in chains. And the bactereriocin production against the test organism is presented in (Fig 2). Salasiah Kormin *et al* (2001) observed bacteriocin production which exhibited inhibitory activity was found to be Gram positive, catalase negative, short rod and non-motile. The culture was able to grow at 10°C, 15°C, 45°C and on acetate agar. Ability to grow in MRS-6.5% NaCl and in pH 9.6 were other characteristics of this producer strain. These results showed that the isolate was homofermentative *Lactobacillus* species.

CONCLUSION

Lactic acid bacteria originally isolated form yoghurt and cheese are probably the best candidates for improving the safety of traditional fermented foods of diary origin. The selective use of this bacteriocinogenic strain may improve the microbiological quantity of such foods. *Streptococcus thermophilus* SBT1277 or its bacteriocin which has a wide inhibitory spectrum has a potential use as a bio preservative in dairy products (Stiles,1996).

Kabuki *et al* (2007) produced Thermophilin 1277, using *S. thermophilus* SBT1277, showed an antimicrobial activity against several food spoilage bacteria including Clostridium butylicum, *C. sprogenes* and Bacillus cereus. The effects of culture condition for the bacteriocin production by *S. thermophilus* SBT1277 were studied. During the batch fermentation, using M17 broth, bacteriocin production was detected in pH controlled ST broth at pH values of 5.5-6.5.

Bacteriocins from microorganisms are Generally Recognized as Safe LAB have continued to gain great interest among an increasing number of research groups due to their huge application potential both in food, and in pharmaceutical industries. In the food industry, bacteriocins have long been proposed as a solution to the problems of food spoilage and food-borne infections.

Bacteriocins are antibacterial proteins produced by bacteria that kill or inhibit the growth of other bacteria (Cleveland, 2001) Many lactic acid bacteria (LAB) produce a high diversity of different bacteriocins. Though these bacteriocins are produced by LAB found in numerous fermented and non-fermented foods. Many bacteriocins have been characterized biochemically and genetically (Klaenhammer,1993) and though there is a basic understanding of their structure-function, biosynthesis, and mode of action, many aspects of these compounds are still unknown. Research and their long-time intentional use strongly suggest that bacteriocins can be safely used. Thus from the research work it was concluded that the isolated strains of Lactobacilli have capacity to produce bacteriocin it can be exploited for further studies.

REFERENCES

Bergeys David hendricks (1923). Bergeys manual of Determinative Bacteriology

Cleveland J., Montville T.J., Nes I.F. and Chikindas M.L. (2001). Bacteriocins safe natural antimicrobials for food preservation *Int. J. Food Microbiol.* 71:1-20.

Cocolin L., Foschino R., Comi G. and Fortina M.G. (2007). Decription of bacteriocins produced by two strains of *Enterococcus faecium* isolated from Italian goat milk. *Food Microbiol.* 31:753-758.

Desmazeaud M. and Cogan T.M. (1996). Role of cultures in cheese ripening in dairy starter cultures, VCH publishers Inc. New York, pp.207-231.

Kabuki T., Uenishi H., Watanale M., Seto H. and Nakajima H. (2007). Characterization of bacteriocin, thermophilin 1277, produced by *Streptococcus thermophiles* SB1277, *Letters Applied Microbiol.* 102, 971-980.

Klaenhammer T.R. (1993). Genetics of bcteriocins produced by lactic acid bacteria. *Microbiol. Rev.* 12:39-46.

Stiles M.E. (1996). Biopreservation by lactic acid bacteria, Antoni Lewenhock Journal 70, 331-345.

Salasiah Kormin, Gulam Rusul, Son Rodu and Foo Hooi Ling (2001). Bacteriocin-producing Lactic acid Bacteria isolated from traditional fermented food. *Malays J. Med* (1) 63-68.

Rodney H Perez, Takeshi Zendo and Kenji Sonomotor (2014). Novel bacteriocin from lactic acid bacteria: various structure and applications, Microbial cell factories published online

PRINCIPAL
Govt. College of Arts &
Aurang

