Antifungal and Antibacterial Activity of Lactic Acid Bacteria isolated from Fermented Foods and Plant Sources

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Abstract

Lactic acid bacteria (LAB) are group of Gram positive bacteria that produce lactic acid as their main fermentation end product. LAB have long history of being consumed as part of traditional fermented foods. Six different isolates of lactic acid bacteria isolated from various sources such as curd, dosa batter, jalebi batter, leaves of custard apple, guava, and pomegranate were studied for antifungal and antimicrobial activity. All isolates exhibited inhibitory activity against *Aspergillus sp* and *Penicillium sp* and also against bacterial pathogens like *Staphylococcus aureus, Escherichia coli, Klebsiella sp and Salmonella sp.* For antifungal and antimicrobial activity agar overlay method was used. These results suggest that LAB could be useful as probiotic to increase immunity of host through dietary supplementation; it can also be used to increase shelf life in food, feed and pharmaceutical preparations. Antifungal LAB products can be used against spoilage causing fungi in agriculture products like fruits and vegetables and help in reduction of use of chemical fungicides thereby reducing pollution hazards.

KEYWORDS: Lactic Acid Bacteria, Antibacterial Activity, Antifungal Activity, Preservation, Probiotic

Introduction:

Lactic acid bacteria (LAB) are group of Gram positive bacteria that produce lactic acid as their main fermentation end product. LAB have long history of being consumed as part of traditional fermented foods. Lactobacilli are naturally present or deliberately added as starter cultures in unpasteurized milk and dairy products such as cheese, yogurt and fermented milks. LAB have been used in domestic food production for centuries not only because they allow food to be stored for a long period of time but also because of multiple antagonistic activities. As a result of their extensive use in traditional fermented products, most of the LAB are Generally Regarded as Safe (GRAS), granted by the American Food and Drug Agency (FDA). The European Food Safety Authority (EFSA) also granted the Qualified Presumption of Safety (QPS) status to most of the LAB genera, such as *Lactococcus, Lactobacillus, Leuconostoc, Pediococcus,* and some *Streptococcus* (EFSA, 2007).

LAB are among the most powerful prokaryotes when it comes to antimicrobial potential. These bacteria not only produce several antimicrobials during carbon source metabolism,

they also compete with other species by acidifying their environment and by rapidly depleting the nutrients. The list of antimicrobial metabolites synthesized by LAB includes organic acids, hydrogen peroxide, various lipids, carbon dioxide, diacetyl and bacteriocins. It is also significant to define the role of metals in production of antimicrobial and antifungal components (Mickaël Claudel et al ,2020). Bacteriocins producing LAB can be applied as a starter culture in food fermentation or added to fresh food as bio-preservatives. Some lactic acid bacteria also produce potent antibiotic compounds via complex secondary metabolism pathways. These include bacteriocin antibiotics and small antibiotic like molecules such as reuterin. Among them, bacteriocins produced by lactic acid bacteria have attracted increasing attention, since they are active in a nanomolar range and have no toxicity (José Luis Parada et al, 2007).

Bacteriocins are protein or peptides, which do not harm producer strain but have lethal antibacterial activity against food spoilers and/or food borne pathogens. Most of the bacteriocins are from species of the genus *Lactobacillus*. Bacteriocin produced by these bacteria can inhibit pathogenic and spoilage micro-organisms extending the shelf-life and enhancing the safety of food products. Bacteriocins are synthesized in ribosomes of Gram positive bacteria (Dzung B Diep et al,2002) . Isolation of lactic acid bacteria to be used as health promoting ingredients in food and pharmaceutical preparation is under study **(**Bintsis T, 2018). The quantity and types of antibacterial components depends on taxonomic position of LAB, composition of nutrient medium and cultivation conditions. The expansion of the phenomenon of multi-resistance of pathogenic agent against contemporary conventional antibiotics, the emergence of new infectious agents, the reinforced requirements of microbiological security in food supply, discovery of new techniques of food bio preservation, new antibiotics, new targets and the development of novel probiotic preparations form new challenges for modern biosciences(Veer Pal Singh,2018). The methods for reducing contamination by various pathogenic bacteria, moulds and yeast in the production and storage of food and feed is of paramount importance and the task is to develop the efficient and safe possibilities for this purpose. In recent years, special interest in the antagonistic activity of bacterial communities is on an increase. Various scenarios are possible in these associations which include competition for nutrients, inhibition of necessary action due to the use of bacterial components instead of preliminary nutrients etc (Lusine Matevosyan, 2019)

Unfortunately, less data on the pathway of metabolism association is known. The study of mixed LAB cultures would explain the various microbial-microbial interactions that can be used in the production of food and drug. Based on current literature, it can be concluded that antifungal compounds of lactic acid bacteria do exist and have potential for being effective in combating food borne yeasts and moulds (Cassandra De Muynck et al, 2004). Most of the antifungal capacity of the lactic acid bacteria described in these reports is due to the production of an antifungal protein or proteinaceous compounds. Food and feed spoiling moulds and yeasts can be potential hazards and cause great economic losses worldwide. The reduction of mould and yeasts growth in food and feed production and their storage is of the primary importance. The application of biological preservation has received much attention in recent years.

The best candidates satisfying these demands are lactic acid bacteria. Inside gastrointestinal tract LAB supress unwanted microbiota growth due to production of various antimicrobial substances and ability to lower the environmental pH. LAB offers the host protection against disease and promotes normal intestinal function. These microorganisms survive the passage through the gastrointestinal tract and eventually establish in colon. Their benefits are related to the prevention of growth of harmful bacteria by competition, exclusion and by the production of organic compounds **(**Bermudez-Brito M et al, 2012). Associated effects of probiotic include prevention and treatment of diarrhoea, alleviation of allergies in children. The beneficial effects of probiotics depend on their colonization of the gut and their effect on harmful bacteria for which certain functional properties are necessary (Fergus Shanahan, 2004). The strains evince good gut tolerance capacity, antioxidant activity and adherence properties, which are characteristics of probiotic bacteria and thus are candidates for therapeutic uses and also to be used as starter cultures (Arup Jyoti Das et al, 2019).

Materials and Methods:

LAB were isolated from fermented food sources such as dosa batter, jalebi batter, curd, and natural habitats like leaves of guava plant, pomegranate plant and custard apple plant. Isolates were cultivated in de Man, Rogosa, and Sharpe (MRS) medium at room temperature for 24-48h.

Laboratory cultures of *Staphylococcus aureus, Escherichia coli, Klebsiella sp.* and *Salmonella sp.* were cultivated on nutrient agar for study of antibacterial activity. These cultures were transferred and maintained on MacConkey's agar.

For the study of antifungal activity laboratory cultures of *Aspergillus niger, Aspergillus flavus and Penicillium spp* were taken, cultivated and maintained in Sabouraud dextrose agar.

Determination of antibacterial activity

The antibacterial activity of 6 LAB isolates was determined by agar overlay method.LAB isolates were streaked on MRS medium in two parallel streaks. After incubating for 24-48 hours and checking for growth of LAB, Nutrient Agar medium was overlaid and allowed to solidify. Then culture of the test organism, *Escherichia coli, Staphylococcus aureus, Salmonella sp.,*and *Klebsiellia sp.* was spread and incubated for 24-48 h. Zone of inhibition was observed and antibacterial activity confirmed.

Determination of antifungal activity

Antifungal properties were determined by agar overlay method. Various groups of fungi were used as test-organism: *Aspergillus niger, Aspergillus flavus* and *Pencillium spp.*LAB Isolates were streaked on MRS medium in two parallel streaks,incubated for 24-48 hours and the growth was checked. Sabouraud dextrose agar was overlayed and then fungal culture was spore suspension was spread. It was kept for incubation for 48 hours or until fungal growth was well observed. Zone of inhibition was observed and antifungal activity confirmed.

Results and Discussion:

All the isolates obtained in this study were considered LAB, based on their positive Gram reaction, non-motility, absence of catalase activity, no spore formation, and the rod or coccus shape.

The various isolates obtained from different sources are as follows:

Table1: Sources of LAB Isolates

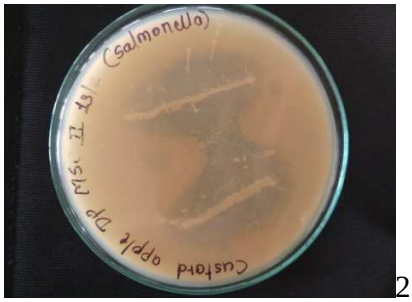
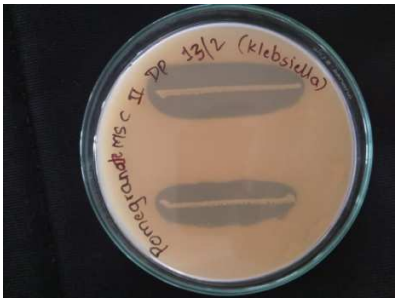
LAB	**Source**
LAB1	Dosa Batter
LAB2	Jalebi Batter
LAB3	Curd
LAB4	Custard Apple plant leaves
LAB5	Guava plant leaves
LAB6	Pomegranate plant leaves

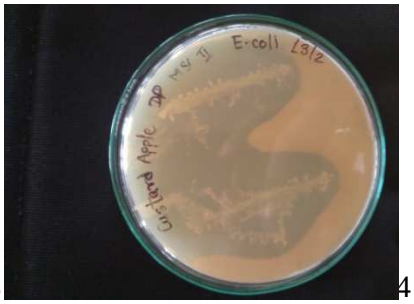
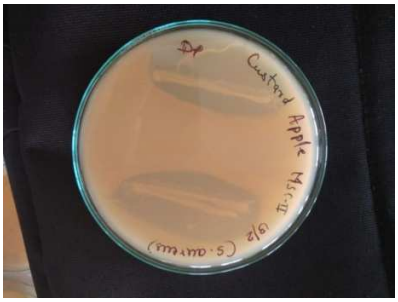
Antibacterial activity:
Most LAB strains showed significant inhibitory effect against bacterial cultures taken. There were three Gram negative bacteria *E. coli, Salmonella spp* and *Klebsiella spp.* One Gram positive, *S. aureus was used.* Isolates LAB 1, LAB 4 and LAB 6 showed best activity against all bacterial cultures. Isolate LAB 5 showed inhibitory effect against *Klebsiella spp* and *E. coli.* Isolate LAB 2 and LAB 3 has not shown any inhibitory effect against single bacterial culture. Individual results of LAB are shown in table 2.

Table2: Antibacterial Activity of LAB Isolates

LAB Isolates	**Bacterial Cultures**			
	S. aureus	*Salmonella spp*	*Klebsiella spp*	*E. coli*
LAB1	+	+	+	+
LAB2	-	-	-	-
LAB3	-	-	-	-
LAB4	+	+	+	+
LAB5	-	-	+	+
LAB6	+	+	+	+

(+)-presence of antibacterial activity (-)-absence of antibacterial activity

1  2 

3  4 

Photographs:

1. Antibacterial activity of LAB isolated from Custard Apple plant leaves against *Salmonella sp.*

2. Antibacterial activity of LAB isolated from Pomegranate plant leaves against *Klebsiella sp.*

3. Antibacterial activity of LAB isolated from Pomegranate plant leaves against *E.coli.*

4. Antibacterial activity of LAB isolated from Pomegranate plant leaves against *S.aureus.*

Antifungal activity
Most LAB strains showed significant inhibitory effect against fungal cultures taken. The isolates LAB 1 and LAB 4 demonstrate antifungal activity all the three fungal isolates used i.e *A. niger*, *A. flavus* and *Penicillium spp.* The isolate LAB 3 has shown activity against *A. flavus* and *Pencillium spp.* The isolate LAB 5 has shown activity against *A. niger* and *A. flavus.*
LAB2 and LAB6 have not shown any inhibitory activity against any of the fungal isolates used

Individual results of LAB are shown in table 3.

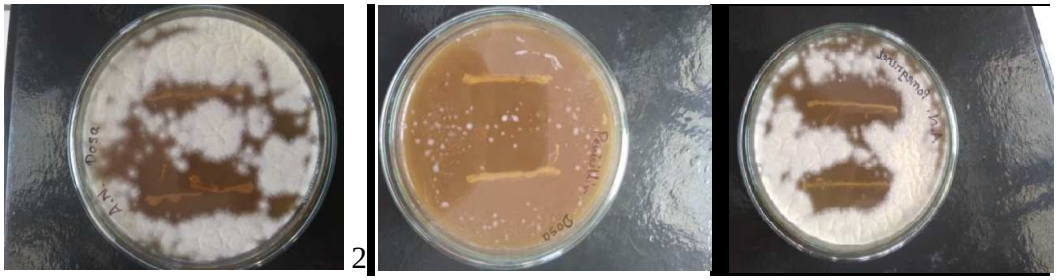
Table3: Antifungal Activity of LAB Isolates

LAB Isolates	**Fungal Cultures**		
	A.flavus	***A.niger***	***Penicillium sp.***
LAB1	+	+	+
LAB2	-	-	-
LAB3	-	+	+

LAB4	+	+	+
LAB5	+	+	-
LAB6	-	-	-

(+)-presence of antifungal activity (-)-absence of antifungal activity

1 2



Photographs:

1. Antifungal activity of LAB isolated from dosa batter against *Aspergillus niger.*

2. Antifungal activity of LAB isolated from Dosa batter against *Penicillium sp.*

3. Antifungal activity of LAB isolated from Pomegranate plant leaves against *Aspergillus niger*.

The results indicate presence of Lactic Acid Bacteria in conventional sources like the fermented foods and more importantly on non conventional sources for lactic acid bacteria like plants. Though Lactic Acid Bacteria have been isolated from plant sources (Immacolata Anacarso et al, 2015) fermented foods still remain the chief source for their isolation and study.

Antibacterial and antifungal activity was observed for almost all the isolates irrespective of the source from where they were isolated. The isolates showed good antibacterial activity against the pathogenic bacteria. LAB metabolic products, such as acid, hydrogen peroxide, and bacteriocin, can inhibit some bacteria. Therefore, LAB with high antibacterial activities should be screened, and their antibacterial components should be analyzed. The results of the growth inhibition experiment of pathogenic bacteria have confirmed that LAB produce certain supernatant fluid that inhibits bacterial growth.(Dayong Ren et al, 2015). The antimicrobial-producing LAB may be used as protective cultures to improve the microbial safety of foods and they also play an important role in the preservation of fermented foods (Pınar Şanlıbaba and Yalçın Güçer,2015).

Antibacterial and antifungal abilities of LAB were depended on the LAB strain and on the indicator microorganism species and strain. The LAB application is wide-ranging and most promising if one considers that the indicator microorganisms isolated from various vegetables, grain, fruit, meat, oil and water were suppressed. These studies highlight the possibility that food safety and food quality can dramatically be improved by using the LAB with antimicrobial activities as starter for food fermentation (Dalia Cizeikiene et al, 2012)

Conclusion:

It can be concluded that LAB isolates revealed strong inhibitory effects. Sources like jalebi batter, dosa batter, and curd, leaves of custard apple, pomegranate and guava gave good isolation of LAB. LAB isolates have showed inhibitory effect against pathogenic bacterial cultures such as *Staphylococcus aureus, Salmonella spp, Klebsiella spp* and *Escherchia coli,* and fungal cultures such as *A. niger, A. flavus* and *Penicillium spp.*

From the results obtained it can be said that LAB isolated from various dairy and plant sources show antibacterial and antifungal activity.

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