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# Evaluation of Fungistatic Property of Indigenous Lactic Acid Bacteria Isolated From Dairy Source

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**Abstract** **Original Research Article**

Probiotics are live microorganisms which impart into gastro-intestinal tract and are beneficial to the health of consumer. Some probiotics have potential to boost the immunity as well as inhibit the growth of bacteria and fungi. This study was designed to isolate and identify potential Lactobacillus isolates from dairy product and also evaluate the fungistatic activity of these bacteria against environmental isolates which are involved in food poisoning; Candida specie and Aspergillus niger. Food spoilage by fungi and human fungal infection are now considered as a major problem and cause great economic losses. The chemical which are used in food preservation are very expensive and unaffordable, so, to get rid of these problems we are now moving to bio preservation and there is a high demand of consumers for natural preserved food products which protects the food and human health as well. In this study, lactobacilli were isolated from different dairy product (yogurt and raw milk) which is the cheapest source to isolate fungi inhibitors. The fungistatic activities of these isolates were evaluated by Agar well-diffusion method. Zone of inhibition were measured in millimeter (mm), the highest inhibition zone was observed for Aspergillus niger that is 24.5mm, while 25.5mm was the highest for Candida specie. This indicates that the lactobacillus possess fungistatic property and contain antifungal compound which can inhibit the growth of fungi. The isolates of lactobacillus were more effective against Candida spp. The result of present study revealed that fungal spores and fungal growth was lessen by the cell-free neutralized filtrates (CFNF) of Lactic acid bacteria, so by using these antifungal agents we can preserve the food product for long time economically and can boost the immunity and health of an individual as well.

**Keywords:** Probiotics, Cell Free Supernatant, Fungistatic, Preservatives, Generally Recognized as Safe (GRAS).



## INTRODUCTION

Food spoilage is a main issue in Pakistan that had not only affects public health but also badly influence the country's exports. It is estimated that about 20-25% of the fruits are destroyed by pathogens which influence the fruits economic value and human health [1]. All over the world, food spoilage by fungi is very common and it is highly responsible for food waste and economical losses [2]. The major issue of fungal growth in food is of incredible threat in the food industries and numerous specialists have been adopted to limit this issue. There is an immediate need to stop fungus toxicity of foods, since it could cause financial and additionally health threats because of misfortune in dietary and organoleptic properties or potentially generation of mycotoxins. Food spoilage because of fungus is one of the largest and world-wide problems [3]. Aspergillus nigar & Candida specie produce spores and mycotoxins that cause serious health hazards to human beings. During the most recent couple of years there has been a developing enthusiasm for preservation by microbes, the application of microorganisms and/or their metabolites to stop decay and to broaden the preservation time of sustenance. Lactobacillus is becoming most desire organism in the preservation of food and feed because they produce antimicrobial compounds like lactic acid, acetic acid, and bacteriocin. While many other researches focused on antibacterial activities of lactobacillus [4]. There are relatively few researches on antifungal effects. El-Gendy and Marth researches proved that Lactobacillus stops the growth and the compound aflatoxin production of Aspergillus spp. Many researches exhibited that the antifungal compounds such as phenyllactic acid and 4-hydrophenyllactic acid were extracted from microorganism Lactobacillus spp [5]. Probiotics are live microorganisms which travel through the gastrointestinal tract (GIT) and in doing as such; advantage the healthiness of customer. During the last ten years, the utilization of probiotics for human has gotten expanding consideration as logical proof keeps on

gathering on the properties, usefulness, and useful impacts of probiotic microscopic organisms on people [6]. It is already studied that some of the infections and disturbance in the body of human being, such as abdominal pain and diarrhea or constipation, inflammatory bowel disease, could be because of reduced intestinal micro flora, and probiotics have been viewed as one of the infection control methodologies to conquer such illness. Hence, probiotics have become demanding microorganisms for utilization in the food industry. Lactic acid bacteria, especially Lactobacillus are the most generally utilized organism as probiotics because of its ability that they are prudent members of human intestinal micro flora and these bacteria are "Generally Recognized as Safe" for consumption. The present study was conducted to isolate and identify the Lactic acid bacteria from dairy source and assess their antifungal activity against different fungal species which are involved in food spoilage.

## MATERIAL AND METHODS

### Isolation of *Lactobacillus*

Four yogurt and four raw milk samples were collected from different places and localities of Karachi city, for the isolation of *lactobacillus* and to get a more extensive assorted variety of *lactobacillus*. Each yogurt and milk sample was collected in a sterile bottle independently and put in a polyethylene sack during transportation to the research laboratory. One gram of curd sample and 1mL of raw milk sample was quickly processed under germ free environment by suspending in 9mL of normal saline (0.85%) and was vortexed for proper homogenization. After vortex, mixture were serially diluted and transferred in to the MRS broth which is selective for the better growth of *lactobacillus*. MRS broths were incubated anaerobically in jar for 24h at 37 °C. Prior to immunization of test, the pH of MRS broth was changed to 6.5± 0.2. Growth was checked after 24 h, if visible growths were not obtained then again incubated the broths for next 24 h. After incubation the enriched samples were streaked on the Selective MRS agar plates. Four ways streaking was done and MRS plates along with control plates were incubated anaerobically for 48h at 37 °C. After 48 h the plates were observed for colonies of *lactobacillus* [7].

### Identification of *Lactobacilli*

Further recognizable proof of *Lactobacillus* isolates developed on MRS agar was done basically with the assistance of the different tests: Microscopic examination, Biochemical test (Catalase test, Nitrate Reduction Test) Carbohydrate Utilization test, and Citrate Utilization test, Growth on different temperatures 10 + 1 º C and/or 42 + 1 º C), growth under aerobic and anaerobic conditions.

### Agar Well Diffusion Method

To test antifungal activity of Lactobacillus first we need cell free supernatant which was obtained by centrifugation. For this purpose, we took eppendorf, added suspension of lactobacillus and placed it in micro centrifuge machine for 20 min at 10,000 rpm. Afterwards, filtered the suspension by filter paper and obtained cell free supernatant (CFS). The pure Cell Free Neutralized Supernatant (CFNS) were kept at 4°C and Freeze-dried samples were matched with 0.5 McFarland and then mixed (to a 10-fold concentration) in 20 mM citrate buffer (pH 7.0). The fungal spore suspension was obtained by adding tween 20 in sterile saline tube; spores of fungus were added and homogenized by centrifuge for few min and wait for 5min to allow settlement of hyphae. Two plates of PDA, two Plates of SDA were taken and 1mL of Spore suspension of Aspergillus nigar and Candida specie was poured separately on PDA and SDA plates respectively. After pouring, wells (5 mm diameter) were made on agar plates using a sterile corn borer, and sealed with a drop of sterile molten agar to avoid leakage. 100 µL of neutralized cell-free culture supernatant fluid of each Lactic acid bacteria was placed into each well. Plates were pre-incubated for 2 h at 4°C to permit a dissemination of the CFNS and after that incubated at 25°C for 24 to 48 h. The antifungal activity of Lactobacillus was determined by measuring the inhibition zone formed around the wells [8].

## RESULTS

All of the eight isolates collected from different dairy sources were identified as *lactobacillus* on the basis of morphology, growth and biochemical tests. They are seen as gram positive bacteria and short rods under microscope, all of them are catalase, nitrate, and citrate negative. All eight isolates utilize carbohydrate (glucose, sucrose, lactose, and mannitol) as their carbon source and produce acid and gas as the byproducts. The inhibitory action shown by these *Lactobacillus* species demonstrate that the cell free solution of detached *Lactobacillus* species had the ability to hinder the development of some fungi. This experiment clearly indicates that the inhibitory metabolites produced by isolated *Lactobacillus* species were extracellular and diffusible. The results are presented in Fig. 1-4 and Table 1.

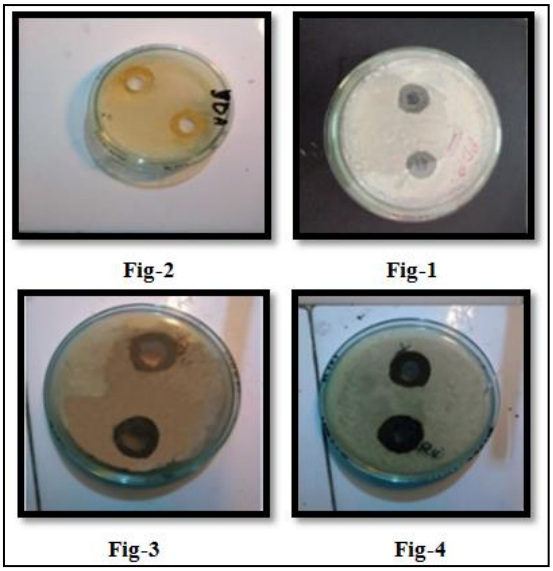


Fig. 1: Represents the inhibitory effects against *A. niger* on PDA Fig. 2: Represents the zone of inhibition against *A. niger* on SDA. Fig. 3: Shows the zone of inhibition against *Candida spp*. on PDA and Fig. 4: Represents the inhibitory effects against *Candida spp*. on SDA.

**Table-1: Indicates the zone of inhibition (in mm) of eight *lactobacillus* isolates against *A.niger* and *Candida* on PDA and SDA plates**

| S.no. | No. of isolate | Source | Indicator Organism | | | |
|---|---|---|---|---|---|---|
| | | | ***Aspergillus niger*** | | ***Candida spp.*** | |
| | | | **PDA** | **SDA** | **PDA** | **SDA** |
| **1** | M1 | Milk | 24.5mm | No clear zone | 22.5mm | 25mm |
| **2** | M2 | Milk | 24mm | 20mm | 23.5mm | 22.5mm |
| **3** | M3 | Milk | 20mm | 21mm | 20mm | 21mm |
| **4** | M4 | Milk | 20mm | 21mm | 20mm | 21mm |
| **5** | Y1 | Yogurt | 23.5mm | 19.1mm | No clear zone | 25.5mm |
| **6** | Y2 | Yogurt | 22.5mm | 7mm | 21mm | 22mm |
| **7** | Y3 | Yogurt | 20mm | 21mm | 20mm | 21mm |
| **8** | Y4 | Yogurt | 20mm | 21mm | 20mm | 21mm |

## DISCUSSION

In the present study the initial screening of Lactobacillus which is use to check fungistatic property revealed that, Lactobacillus strains are exhibit antimicrobial compound and contain antifungal/fungistatic activities. The antipathetic action was generated by catalase-treated, neutralized cell-free neutralized supernatants (CFNS), which shows that the inhibitory action was not because of the activity of organic acid or hydrogen-peroxide created by these Lactic acid bacteria. The fungistatic impact is due to the production of bacteriocins and phenyllactic which is produce by the Lactobacillus. To check the antifungal activity of Lactobacillus we followed the Agar well-diffusion method which is most recommended method to check the antimicrobial activity of LAB. An inhibitory zone was seen within 48 hours of incubation time period on indicator fungus. On PDA plates we observed zone of inhibition against both the fungus (Aspergillus niger,and Candida spp.) but the highest zone was recorded for Candida spp. as compared to Aspergillus niger. Zone of inhibition against Candida spp. were very clear and observed easily. On SDA plates Lactobacillus showed more clear and effective result against Candida spp. then against Aspergillus niger. Lactobacillus inhibits the growth of these fungi because it contains antifungal compounds such as phenyllactic acid, Bacteriocin and 4-hydrophenyllactic acid [10].This research showed that production of contagious fungal spores and fungal compounds were basically decreased by the presence of Lactobacillus as well as cell free filtrates of lactobacillus. Growth inhibition of Candida specie was more detectable in this study than Aspergillus niger.

## CONCLUSION

The result of present investigation suggests that the *lactobacillus* isolateed from different indigenous dairy products have high potential as antifungal/fungistatic agents. These potential agents can be useful to save many foods and food products from spoilage. They can be used as bio-preservatives for

increasing the shelf life of food products. They can also help to treat infectious disease and considered as a good probiotic candidates. Further in-vivo studies and clinical trials are expected to be done for the confirmation of these *Lactobacillus* isolates as a potential antifungal agent.

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