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Antimicrobial Activity of Cell Free Extract of Lactic Acid Bacteria Isolates from Spoiled Fruits and Vegetables

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ABSTRACT

The spoiled fruits and vegetables were collected from the local market of Malkapur, Maharashtra, India and were subjected to the isolation of Lactic Acid Bacteria by enrichment culture technique. The isolates were designated and studied for their cultural, morphological, and biochemical characterization. The isolates were tentatively identified up to species level. The cell-free extracts of the isolates were subjected to evaluation of antimicrobial potential against some laboratory pathogenic strains of bacteria. Out of eight bacterial isolates four isolates were lactic acid bacteria among them LAB-1 and LAB-2 were tentatively identified as Lactobacillus bulgaricus, while LAB-3, and LAB-4 were tentatively identified as strains of Lactobacillus acidophilus. The zone of inhibition was found for a cell-free extract of LAB-1 and LAB-2 against all test organisms. The cell-free extract of LAB-3 and LAB-4 did not show antimicrobial activity against the test organisms. The LAB-1 and 2 have potential to control common pathogens like Proteus vulgaris, E. coli, Klebsiella spp. and Candida albicans.

***Key words**: Lactic Acid Bacteria, spoiled fruits and vegetables, Lactobacillus bulgaricus, Lactobacillus acidophilus, Zone of Inhibition*

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INTRODUCTION

Lactic acid bacteria (LAB) can be isolated from spoiled fruits and vegetables, as they are commonly found in them. The isolates obtained from spoiled fruits and vegetables can be novel LAB strains having enhanced metabolites with characteristics could be useful in food industry. The plant diversity becomes challenge for LAB strains to adapt to fruits and vegetable environments [1]. And hence, a vast diversity of LAB can be found in fresh as well as spoiled fruits and vegetables. Some of the LAB isolated from fresh fruits and vegetables are *Lactobacillus plantarum* [2], *Lactiplanti bacillus plantarum* [3], *Leuconostoc pseudomesenteroides* [4], *Weissella, confusa* [5] and *Leuconostoc mesenteroides* [6].

For the food protection Lactic Acid Bacteria are being studied extensively [2,7,8]. The biocontrol organisms are obtained from apples and lettuce to control pathogenic and spoilage causing microorganisms. If desired nutritional, rheological, and sensory characteristics of fruits and vegetables are maintained the shelf life of fruits and vegetables can be increased by using starter LAB isolated from these fruits and vegetables [9]. Many workers have reported novel strains of LAB which can be used as fermentation starters [7,10,11,12,13]. Instead of using deleterious chemicals to control undesirable microorganisms in preparation of fermented foods, use of antagonistic LAB will be an eco-friendly approach to maintain food safety [14].

There are various strains of LAB with industrial potential yet to be revealed. This project was focused mainly to evaluate antimicrobial potential of LAB strains isolated from spoiled fruits and vegetable, to examine their ability for technological applications as substitutive sources for the food industry.

MATERIALS AND METHODS

The spoiled fruits (Apples, Oranges and Pomegranates) and vegetables (Tomato, Chilly and Spinach) were collected from local market of Malkapur, Maharashtra, India in sterile polythene bags. The spoiled fruits and vegetables were brought immediately to laboratory for further studies.

Isolation of Lactic Acid Bacteria

Lactic acid bacteria were isolated by enrichment culture technique where 1g of spoiled fruits and vegetables material were separately inoculated into 50ml flasks containing sterile De-man, Rogosa and Sharpe broth medium (MRS) incubated at 28^0c for 48-h and then the loopful of enriched material were streaked on sterile MRS agar plates, and were incubated at 28^0c for 24-48 h. The colonies of different

morphologies were selected and their cultural, morphological, biochemical characteristics were studied with reference to Bergey's manual of determinative bacteriology [15].
The colony characters of different isolates were recorded after incubation and different morphological characteristics viz. Gram staining [16], Motility (hanging drop method), Spore staining [17] were studied. Different biochemical characteristics viz. catalase test, oxidase test, urea hydrolysis test, arginine hydrolysis test, H_2S production test, nitrate reduction test, starch hydrolysis test, casein hydrolysis test, gelatine liquefaction test of different isolates were studied. The isolates were preserved at refrigeration till further use.

Cultivation of Lactic Acid Bacteria and Preparation of Cell Free Extract

For the cultivation of Lactic acid bacteria, 10 mL of sterile MRS broth tubes, for each isolate one, were prepared and a loopful of suspensions of different isolates were inoculated & were incubated at 28^0c for 48 h. Incubated MRS broths were centrifuged aseptically at 10000 rpm for 10 min and supernatants were collected in sterile tubes, labelled and used as cell free extracts for further work. [18]

Evaluation of Antimicrobial Properties

The evaluation of antimicrobial properties of cell free extract was carried out against laboratory pathogenic strains viz. *Proteus vulgaris, E. coli, Klebsiella spp, Candida albicans* by Agar-well diffusion method. In brief, 100 µl of cell free extracts were added in wells and were allowed to diffuse at 4°C for 10 min. The plates were incubated in upright position at 37°C for 24 h. After incubation plates were observed for zone of inhibition and zones of inhibition were measured. [18]

RESULTS AND DISCUSSION

Isolation of Lactic Acid Bacteria

Total 8 isolates were obtained on MRS agar from spoiled fruits (Apple, Oranges Pomegranates) and vegetables (Tomato, Chilli and Spinach) samples and of which four LAB isolates were designated as LAB-1, LAB-2, LAB-3 and LAB-4. The isolates were purified and maintained on the slants of MRS agar at refrigeration till further use.

Characterization of the Isolates

The results of characterization of the isolates are shown in the Tables 1, Table 2 and Photoplate 1

Biochemical Characteristics of the Isolates

Biochemical characteristics viz. catalase test, oxidase test, urea hydrolysis test, arginine hydrolysis test, H_2S production test, nitrate reduction test, starch hydrolysis test, casein hydrolysis test, gelatine liquefaction test of bacterial isolates were recorded in Table 2.
From the cultural, morphological and biochemical characteristics of the isolates LAB-1 and LAB-2 were tentatively identified as *Lactobacillus bulgaricus,* while LAB-3 and LAB-4 were tentatively identified as *Lactobacillus acidophilus.*

Evaluation of Antimicrobial Properties of the Isolates

Results of antimicrobial properties of cell free extracts of isolates by agar well diffusion method were recorded in Table-3, Fig-1 and photoplate-2. The cell free extracts of LAB-1 and LAB-2 showed antimicrobial activity against all test pathogens while the cell free extracts of LAB-3 and LAB-4 were not showed any antimicrobial activity against any test pathogens used in experimentation.

Table-1: The Morphological Properties of LAB Isolates

Colony characters	Isolates			
	LAB-1	**LAB-2**	**LAB-3**	**LAB-4**
Size	1mm	0.9mm	1mm	0.5
Shape	Circular	Circular	Circular	Circular
Color	White creamy	White	Off White	White
Margin	Entire	Entire	Entire	Entire
Elevation	Convex	Flat	Flat	Convex
Opacity	Opaque	Opaque	Opaque	Opaque
Consistency	Moist	Moist	Moist	Moist
Gram staining	Gram Positive	Gram Positive	Gram Positive	Gram Positive
Motility	Non motile	Non motile	Non motile	Non motile
Endospore	Negative	Negative	Negative	Negative

Table 2: Results of biochemical characterisation of the Isolates

Name of Test	Isolates			
	LAB-1	LAB-2	LAB-3	LAB-4
Catalase Test	-	-	-	-
Oxidase Test	+	+	+	+
Urea Hydrolysis Test	-	+	+	-
Nitrate Reduction Test	+	-	+	-
Starch Hydrolysis Test	+	+	+	+
Casein Hydrolysis Test	+	+	+	+
Gelatin Hydrolysis Test	+	+	-	-
Arginine Hydrolysis Test	+	-	+	-
H_2S Production Test	-	-	-	-

* (+ = positive test) (- = negative test)

Table 3: Zone of Inhibition

Name of the test organism	Zone of inhibition in mm			
	Cell extract of LAB-1	Cell extract of LAB- 2	Cell extract of LAB- 3	Cell extract of LAB- 4
Proteus vulgaris	11	12	0	0
Klebsilla spp	13	11	0	0
E. Coli	10	10	0	0
Candida albicans	12	8	0	0

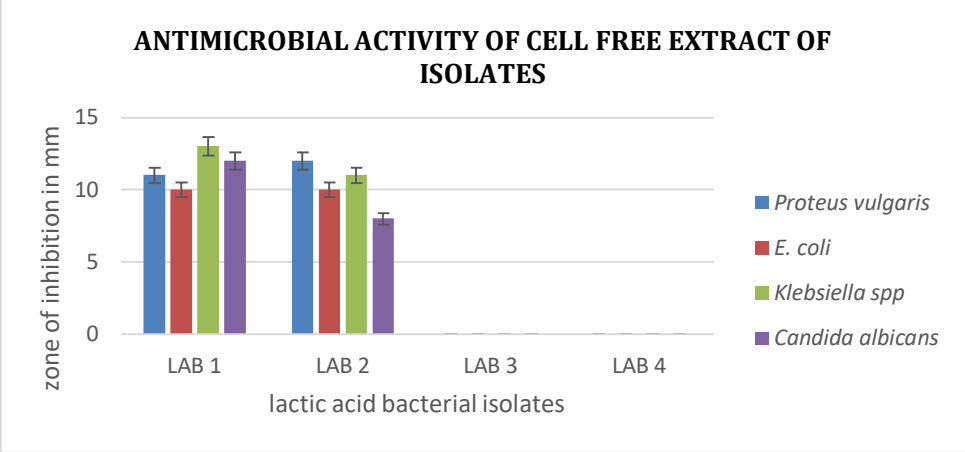
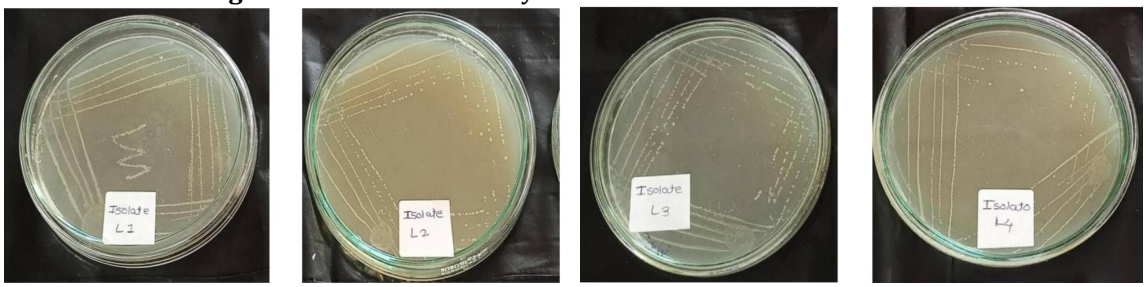
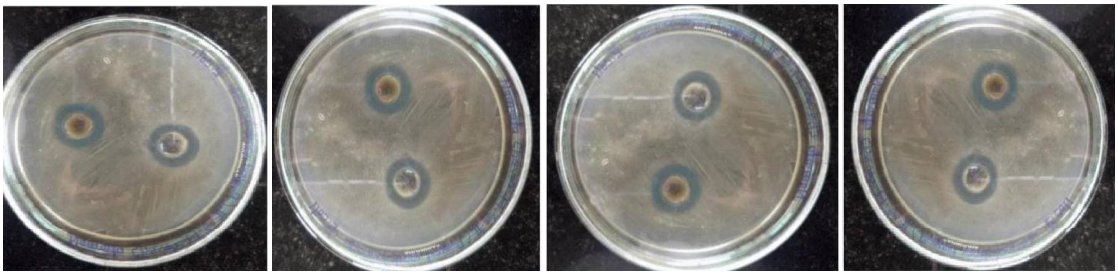


Fig. 1: Antimicrobial Activity of Cell Free Extract of LAB isolates.



Photoplate 1 : Colonial Morphology of Isolates on MRS Agar at 280C for 48-h Under Microaerophilic Conditions (Anaerobic jar).



Photoplate 2: Zone of Inhibition of Cell Free Extract of LAB-1 and LAB-2 Isolates against *Proteus vulgaris, E. coli, Klebsiella spp,* and *Candida albicans.*

CONCLUSION

Lactobacillus bulgaricus isolates showed the antimicrobial potential against *Proteus vulgaris, E. coli, Klebsiella spp., and Candida albicans. pathogens* and hence can be used for control of such pathogens.

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