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Research Article

CHALLENGING APPROACHES OF LACTIC ACID BACTERIA FOR THE PRESERVATION OF SELECTED FRUITS

Hasen Mothi M

PG and Research Department of Microbiology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi, Thanjavur, Tamil Nadu, India

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ABSTRACT

Edible fruits and vegetables are easily degraded or denatured by several reasons especially from environmental factors. Due that the shelf life of plant materials are drastically reduced. It may affect the economic value of individual persons. From this research work was undertaken few selected curd samples, tomato samples and grapes from various areas for selecting significant lactic acid bacteria (LAB). Screening of probiotics was done by the detection of antagonistic activities, acid and bile salt tolerance test, antibiotic resistance test and sensitivity pattern and antimicrobial activity study. In the present studies, total 52 fruit flora bacterial cultures were isolated from two different site oriented tomato and grapes.LAB colonies in 10^{-5} dilution were expressed good loading capacity in curd sample, grapes and tomato. In antagonistic activity, *Lactobacillus delburecki* and *Lactobacillus* sp. were observed highest zone against *Shigella sp*. From this, *Lacto bacillus delburecki* and *Lactobacillus* sp. were actively preserved the selected vegetables. They were increased the shelf-life and reduced the degradation of the samples.

Keywords: Edible fruits, LAB, *Lactobacillus delburecki*, Bile salt tolerance test, Antagonistic activity.

INTRODUCTION

Most of the vegetables and fruits are prone to deterioration as early as possible due to moisture content and it attracts more number of bacterial and fungal groups. In which, their life span or shelf life are very short. Also, during transportation of the materials; respiration and maturation are play a major role to increase the metabolism, it increase the rate of maturity and senescence of the fruits and vegetables. Unfavourable environmental conditions such as method of harvest, post-harvest treatment, and climatic condition are also decrease the shelf-life of the living materials. It declines the nutritional quality of the fruits and other plant origins (Shafiur-Rahman, 2007). Almost 25 to 40% of vegetables and fruits are lost before consumed by the consumers because of poor management (José R. Linares-Morales *et al., 2018).* Post-harvest management is very much important to preserve the agricultural products from the rate of respiration and transpiration, provide the external membrane protection and reduce the microbial contamination (Bisen *et al*., 2010). Perishable plant products are treated immediately after the post-harvest storage, which improve the shelf life of fruits and vegetables (Lal Basediya *et al*., 2013). Synthetic chemicals and fumigation may reduce the losses of vegetables and fruits wastages and also extend the shelf life.

To overwhelmed this problem, lactic acid bacteria (LAB) is a good source for the application of antimicrobial peptides for reducing the target pathogens without any toxic or adverse effects and maintains the stability between beneficial and harmful bacteria (Pringsulaka *et al*., 2015). LAB is a suitable candidate to preserve the fruits and vegetables, these genera comes under "Generally Recognize as Safe" (GRAS) category (Bouchard *et al*., 2015). It is a good producer for the different antimicrobial agents and metabolites which may give more attention due to their application in food preservation techniques for the preservation of food with safe and natural ways (De Vuyst and Leroy, 2007). Extent the shelf life and increase the

*Corresponding Author: Hasen Mothi M, PG and Research Department of Microbiology, A.V.V.M. Sri Pushpam College (Autonomous) Poondi, Thanjavur, Tamil Nadu, India, Email: hasenmothi@mail.com.

duration of food spoilage is a novel approach using LAB, in our study we approach this idea in selected fruits.

MATERIALS AND METHODS

Isolation of Lactic acid bacteria

Two Curd samples were collected from the different local market in Thanjavur, Tamil Nadu, India. After that the samples were transported to the laboratory and kept in 4°C for futuristic purposes. The isolation and identification were performed by routine microbiological procedure and inoculation was carried on solid MRS media.

Identification of LAB

According to the Sneath *et al*., (1986), standard morphological, cultural and biochemical reactions were adapted for the identification of isolates of LAB.

Screening for probiotics

The standard procedures of Tambekar and Bhutada (2010) was adapted with slight changes for the screening of probiotic, detection of antagonistic activities, acid and bile salt tolerance, antibiotic resistance and sensitivity pattern and antimicrobial activity.

Isolation of bacterial flora from spoiled fruits

Collection of bacterial flora present on the spoiled fruits: totally ten samples of spoiled tomato and grapes were collected in sterile polyethylene bags from the local market for the present investigation. The bacterial flora was collected from spoiled fruits by swabbing method using sterilized cotton buds. These swabs were added with saline solutions (0.85%) of various dilutions under sterile conditions and 0.2 mL from each dilution was inoculated innutrient agar 0 and they were incubated at 37°C for 24 h. After the incubation, different colonies were isolated and CFU were counted. On the basis of standard procedure, morphology, cultural characteristics and identified for the screening and selection of distinct colonies.

Antagonistic activities of LAB against fruit flora

LAB candidates were inoculated in the MRS broth and incubated for 48 h at 37 °C. Then, each broths were centrifuged at 5000 rpm for 15 min and the supernatant was taken into sterile container. Bacteriocin-Like Inhibitory Substance (BLIS) was determined by well-diffusion method for the antagonistic activity of selected LAB (Vishal Dhundale *et al*., 2018).

Self-life of fruits preserve by biofilm of LAB (supernatant)

Slightly modified method of Saha *et al*., (2014), LAB culture was inoculated in 100 mL MRS broth and incubated at 37°C for 48 h. Then, the broth was centrifuged at 10,000 rpm for 20 min. The supernatant was collected and used as a source of biofilm coating agent. The experimental fruits were dipped in collected supernatant for the coating of biofilm while control group was uncoated with biofilm. Then, the fruits were air dried to remove the surface moisture for 1 h. Both coated and uncoated fruits were kept approximately at 35°C in the room temperature for several days and recorded the spoiling of fruits (Figure 1).

RESULTS AND DISCUSSION

Spoilage of fruits and other food is a biggest problem in the world, we must take care and need necessary scientific action. Preservation of quality and improve the tolerant capacity of fruits from microbes are essential and post harvesting preservation is very important to package and storage. Lots of methods are available today, though the preservation or increasing the shelf life is challengeable. In the present studies, approaches the probiotic use in fruit preservation and improve the shelf-life. Lactic acid bacteria were isolated from the selected curd samples after the standard procedure adopted to scrutinize the active LAB for further studies. Table 1 shows the isolation of LAB counts with different dilution in different fruits were collected from different parts of Thanjavur district of Tamil Nadu.LAB colonies were found in curd sample 1with 28, 23 and 20 cfu/mlcolonies and curd sample 2 with 24, 18 and 15 were observed from10^{-4}, 10^{-5} and 10^{-6} dilution factors respectively. Tomato sample 2 were shown highest isolates represented as 52, 46 and 30 cfu/ml in all dilution factors whereas in grape sample 4,was observed the LAB 43, 47 and 23 cfu/ml. Overall, 10^{-5} dilution was expressed good loading capacity of LAB. Similar results were observed in Tambekar and Bhuta (2010).

Identification and characterization of bacteria are playing an important role in human health. Table 2 and 3 shows the isolation and identification of LAB from the selected curd and fruits. Cultural and morphological characteristics of LAB with different isolates and colony characteristic like medium, colony morphology, gram stain, shape and arrangement, endospore and motility, shape with cocci, diplococci, rod were observed. From the response of LAB in biochemical test of indole, methyl red, vogesproskauer, catalase, oxidase and carboxyl rate fermentation test were noted and the bacteria were identified as *Lactobacillus delbrueckii*, *Lactobacillus* sp., *Bacillus* sp., *Salmonella* sp., *Shigella* sp., *E.coli*, *Staphylococcus aureus*, *P.auriginosa*, *Bacillus* sp. and *E.coli*. In the current study of isolates of lactic acid producing bacteria was maximum total number of colonies in 10^{-5} dilution factors were recognised from curd Tomato and Grapes sample respectively.

Effect of pH on the growth and cell density were studied in LAB. Finally, *Lactobacillus delbrueckii* and *Lactobacillus* sp. were grown successfully with different pH level. After 3h of incubation in different pH 3.0, 4.0, 5.0, the final cell density (cfu/ml) of *Lactobacillus delbrueckii* was 248×10^5, 210.2×10^5, 165.3×10^5 and *Lactobacillus sp.* was 252×10^5, 225×10^5, 170.2×10^5 respectively (Table 4).

Table 1. Number of LAB from curd and spoiled fruit samples in different dilution factor.

Samples	LAB colonies (cfu/ml) in different dilution factor		
	10^{-4}	10^{-5}	10^{-6}
Curd sample 1	28	23	20
Curd Sample 2	24	18	15
Tomato 1	50	42	38
Tomato 2	52	46	30
Tomato 3	22	27	16
Tomato 4	33	42	30
Grapes 1	43	42	26
Grapes 2	36	38	30
Grapes 3	37	43	36
Grapes 4	43	47	27

Table 2. Cultural and morphological characteristics of lactic acid bacteria.

Isolate	Medium	Colony morphology	Gram stain	Shape and arrangement	Endospore	Motility
C1	MRS	White cream, irregular, radiate, Mucoid	+	Short rod, Single, diplobacilli.	-	-
C2	MRS	White cream, irregular, radiate, mucoid	+	Short rod, Single, diplobacilli	-	-
T1	N.A	White attached spreading with Crenate margin, dry.	+	Long rods with round edges inscattered arrangement.	-	Motile
T2	N.A	White, slimy, Transclucent and Raise growth.	-	Short rod inscattered arrangement	-	Non motile
T3	N.A	-	-	-	-	Non motile
T4	N.A	White, opaque, Large, smooth, Flat,moist.	-	Short rod inscattered arrangement	-	Motile
G1	N.A	Golden yellow, circular, large, opaque, convex, smooth,shiny	+	Cocci inbunches	-	Non motile
G2	N.A	-	-	-	-	Motile
G3	N.A	White attached spreading with Crenate margin, dry.	+	Long rods withround edges inscattered arrangement		
G4	N.A	White, opaque, Large, smooth, Flat, moist.	-	Short rod inscattered arrangement	-	Motile

(C1-C2: curd sample T1 – T4: Tamato, G1 – G4 Grapes) (+-positive, _ negative); NA: Nutrient Agar

Table 3.Biochemical characterization of isolated bacteria.

S.No	Biochemical test	C1	C2	T1	T2	T3	T4	G1	G2	G3	G4
1	Indole	-	-	-	+	+/-	+	-	-	-	+
2	MethylRed	+	+	-	+	+	+	+	-	-	+

3	Voges proskauer	-	-	-	+	-	-	+	-	-	-
4	Catalase	-	-	-	-	+	+	+	+	-	+
5	Oxidase	-	-	+	-	-	-	-	+	+	-
6	Carbohydrate fermentation										
	a)glucose	A	A	AG	AG	-	AG	A	-	AG	AG
	b)sucrose	A	A	-	AG	-	A	A	-	-	A
	c)lactose	-	-	A	A	-	AG	A	-	A	AG
	d)maltose	A	A	-	A	-	A	A	-	-	A
Identified bacteria		*L. delbrueckii*	*L.* sp.	*Bacillus* sp	*Salmonella* sp	*Shigella* sp	*E.coli*	*Staphylococcus aureus*	*P.aurignosa*	*Bacillus* sp	*E.coli*

(+) – Present, (-) – absent (T1 – T4, Tamato, G1 – G4 Grapes)

Table 4. Screening of LAB cultures in pH(after 3hrs of incubation).

Isolate	pH	Initial cell density (cfu/ml)	Final cell density (cfu/ml)
Lactobacillus delbrueckii	3.0	77.2×10^5	248×10^5
	4.0	60.0×10^5	210.2×10^5
	5.0	46.4×10^5	165.3×10^5
Lactobacillus sp.	3.0	80.2×10^5	252×10^5
	4.0	68.2×10^5	225×10^5
	5.0	42.2×10^5	170.2×10^5

Table 5.Screening of LAB cultures in bile salts tolerance (OD values at 620nm).

Isolate	Bile concentration	Initial 0 hincubation	After 3h incubation
Lactobacillus delbrueckii	0.3 %	0.072	0.202
	0.4 %	0.048	0.740
	0.7 %	0.041	0.073
Lactobacillus sp.	0.3 %	0.070	0.215
	0.4 %	0.053	0.734
	0.7 %	0.050	0.07

Table 6. Zone of Inhibition of LAB against standard antibiotics.

Antibiotics	**Zone of Inhibition in diameter (mm)**	
	Lactobacillus delbrueckii	***Lactobacillus* sp**
Ampicillin	12.0 ± 0.12	13.0 ± 0.12
Erythromycin	15.6 ± 0.10	12.3 ± 0.10
Neomycin	14.6 ± 0.32	11.3 ± 0.21
Streptomycin	17.6 ± 0.25	13.0 ± 0.32

Mean ± SE

After the analysis of growth and development of LAB at different concentration of bile was calculated at 3%, 4%, 7% and it was observed from initial o hrs to 3 h of incubation. It was recorded in *Lactobacillus delbrueckii* 0.202, 0.740, 0.073, and in *Lactobacillus* sp. 0.215, 0.734 and 0.074 at 3 h incubation (Table 5). Similarly, Reid, (1999) demonstrated the tolerance to bile salts for the growth and metabolic activity of bacteria in the intestine of the host. Efficacy of antibiotic resistance was identified for the LAB candidate against the commercial antibiotic discs such as Ampicillin, Erythromycin, Neomycin and Streptomycin on MRS plate. The zone of inhibition in

Lactobacillus delbrueckii was 12.0 ± 0.12, 15.6 ± 0.10, 14.6 ± 0.32 and 17.6 ± 0.25 mm and in *Lactobacillus* sp. of 13.0 ± 0.12, 12.3 ± 0.10, 11.3 ± 0.21 and 13.0 ± 0.32 were recorded respectively against the standard antibiotics (Table 6). Vishal Dhundale *et al*., (2018), were studied and stated the similar results of antibiotic profile.

The antagonistic effect of isolated LAB has more potential against the selected bacterial pathogens and it was performed in well diffusion method. The zone of inhibition was exhibited against *Bacillus* sp., *E. coli, Klebsiella* sp., *Shigella* sp., *Pseudomonas* sp., and *Staphylococcus* sp.(Table 7). Antibacterial activity was shown the notable response by LAB, especially *Lactobacillus delburecki* and *Lactobacillus* sp. were observed highest zone against Shigella sp. (23.5 ± 2.12 and 23.2 ± 2.10 respectively). Our study was shown the similar results of Tambekar and Bhutada (2010) who studied in *Proteus, S. aureus, L. rhamnosus* and *L. plantarum. Lactobacillus delbrueckii* and *Lactobacillus* sp. provide the strongest antibacterial activity against selected bacteria.

Table 7. Antagonistic activity of LAB against selected bacteria.

Antibiotics	Zone of Inhibition in diameter (mm)	
	Lactobacillus delbrueckii	*Lactobacillus* sp
Bacillus sp.	18.0 ± 2.12	13.0 ± 0.12
E. coli	15.6 ± 1.10	12.3 ± 0.10
*Klebsiella*sp.	14.6 ± 0.13	11.3 ± 0.21
Pseudomonas sp.	21.6 ± 0.30	20.2 ± 0.32
*Shigella*sp.	23.5 ± 2.12	23.5 ± 2.12
Staphylococcus sp.	20.3 ± 2.10	16.3 ± 0.10

Mean ± SE

Table 8. Antagonistic activity of LAB against selected fungi.

Fungi	*Lactobacillus delbruecki*	*Lactobacillus* sp.
A. awamori	5.03 ± 0.47,	09.3 ± 2.04
A. flavus	11.1 ± 1.04	18.5 ± 3.08
A. fumigatus	6.02 ± 0.37	08.3 ± 3.04
A. terreus	7.05 ± 2.84	11.0 ± 2.05
A. niger	8.05 ± 2.19	13.0 ± 3.31

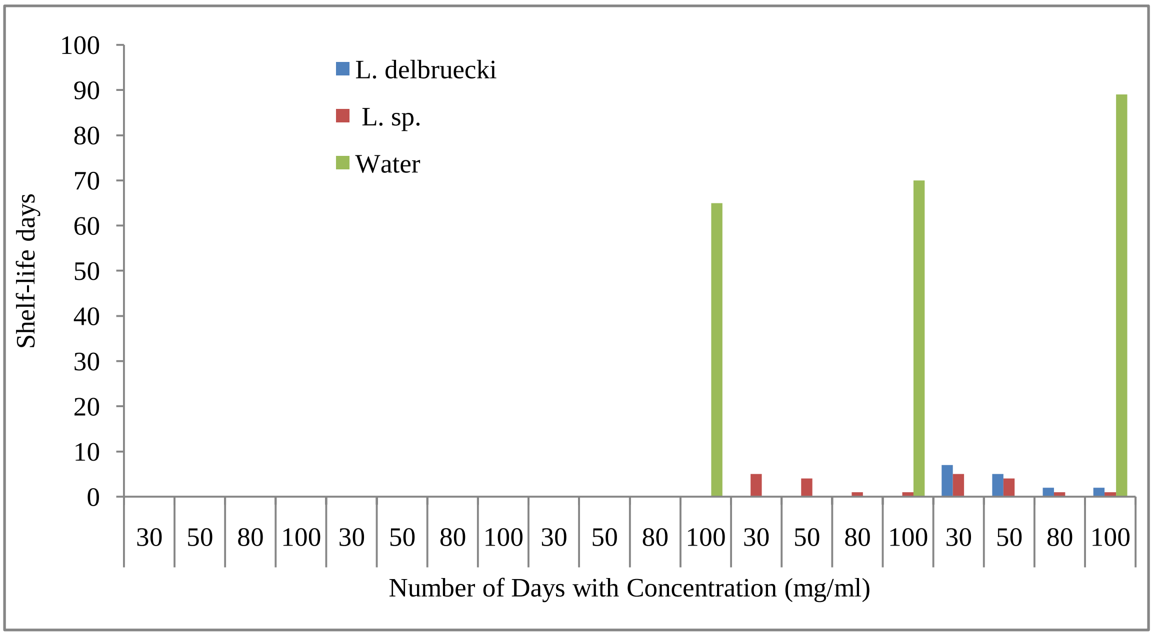

Figure1. Extending and preserved shelf-life of both Tomato and Grapes by LAB.

Table 8 was reflected the effect of antifungal activity of LAB against some selected fungus like *Aspergillus awamori, A. flavus, A. fumigatus, A.terreus and A.niger*. Interestingly, *A. flavus* (11.1 ± 1.04 and 18.5 ± 3.08) was shown more response and then *A. niger* (8.05 ± 2.19 and 13.0 ± 3.31) respectively by *Lactobacillus delburecki* and *Lactobacillus* sp. Shelf-life was extended and improved their self-life in both tomato and grapes (Figure 1). The quality of the fruits should be maintain and improve their preservation in the laboratory condition. In the present investigation, water is used a control to evaluate the shelf-life of the tomato and grapes. The shelf-life was increased when we used the LAB dipped fruits than the water dipped fruits. This study was conducted for 10 days, the decaying of fruits were observed in 8 days in water dipped fruits, at the same time in *Lactobacillus delburecki* and *Lactobacillus* sp. supernatant dipped fruits were spoiled after reaching 10^{th} day. In Ghavidel *et al*. (2013), studied were performed in Coatings constituted by soy protein isolate, whey protein concentrate, alginates and carrageen and were characterized as coatings. Coma *et al*., (2001) was proved the shelf life and improve the quality of fruits by applying the edible films on fruits to prevent the entry of moisture and gases. Romanazzi *et al*. (2017) was extended the shelf-life of fruits and vegetables using chitosan treatment.

CONCLUSION

Major challenges of the food industry are to satisfy consumers demand for better tasting foods and preservation. In the present studies, total 52 fruit flora bacterial cultures were isolated from two different site oriented tomato and grapes. Two active LAB species were identified and they were used to improve the preservation and shelf-life of the selected fruits. These selected *Lactobacillus delburecki* and *Lactobacillus* sp. were further analysed and to study with some more preservation studies for other fruits and vegetables.

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