

## Isolation of Bacteriocin Producing *Lactobacillus SPP* from fermented food like *Dhokla* and their antimicrobial assay

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**Abstract:** In present study bacteriocin producing *Lactobacillus spp* was isolated from *dhokla* and bacteriocin was produced from *Lactobacillus spp* by fermentation. Bacteriocin shows strong antimicrobial activity against *Bacillus licheniformis*, *Streptococcus thermophilus*, *Streptococcus acidophilus*, *Escherichia coli* and *Zymomonas anaerobia*. This study revealed the possibility of using bacteriocin as biopreservative to control food spoilage and pathogenic bacteria

**Keywords:** bacteriocin, *Dhokla*, biopreservative, *Lactobacillus spp*.

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### I. Introduction

Dhokla is a traditional fermented food of India and is prepared in almost every household of India. Origin of Dhokla is Gujarat, India. Dhokla has antimicrobial property as Lactic acid bacteria (LAB) spp present in Dhokla produces bacteriocin. Bacteriocin also acts as biopreservative (Saavadoo Aly *et al.*, 2006).

### II. Materials and Methods

#### Test microorganism.

The test microorganisms namely *Bacillus licheniformis*, *Streptococcus thermophilus*, *Streptococcus acidophilus*, *Escherichia coli* and *Zymomonas anaerobia* were procured from National Chemical Laboratory (NCL), Pune, Maharashtra, India

#### Isolation of LAB from Dhokla

Dhokla was procured from Local market of Nashik, India. Plate count agar (PCA) and (Mans Ragoza and Sharpe) MRS broth were used for isolation of LAB (De Man *et al.*, 1960).

The Dhokla sample were homogenized using mortar and pestle using distilled water and after serial dilution pour plated on PCA plates. Inoculated PCA plates were incubated at 37°C for 72 h. Isolated colonies with typical white colour characteristics were picked from plated and inoculated in MRS broth.

The cultures of LAB were identified by biochemical, microbiological, physiological and morphological characteristics. (Neetu Singh *et al.*, 2013). The biochemical tests used were Indole production, Methyl red, Voges-Proskauer, citrate utilization and production of Catalase among microbiological test gram staining and motility tests were conducted. (De Vuyst *et al.*, 2007).

#### Maintenance of microorganisms

The cultures of LAB were maintained at 4°C in MRS broth. Test organisms were maintained at 4°C on nutrient agar slants. All the bacterial cultures were subcultured at 20 days interval.

#### Production of Bacteriocin

Isolated LAB cultures were inoculated in 150ml of MRS broth and incubated anaerobically at 37°C for 36h. The LAB cells were killed by heating at 80°C for 10minutes

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followed by centrifugation of broth at 8000 rpm for 30 minutes. The resultant cell debris was discarded and cell free supernatant was collected. The pH of collected supernatant was then adjusted to 5.0 with 1N NaOH, then extract was concentrated using rotary flash evaporator and the solution thus obtained is crude bacteriocin. For synergistic activity bacteriocin was mixed with 1 ml of DMSO and filter sterilized using 0.22µm membrane filter paper (Vijay Pal *et al.*, 2005)

### Agar well diffusion assay

200µl of the 18h old test cultures were inoculated on nutrient agar plates by spread plate method. 4 wells of diameter 8mm were made in each of the plates. These plates were filled with 100µl of concentrated bacteriocin and the plates were incubated at 37°C for 24 hours (Schillinger and Lucke, 1989). The inhibition zone was measured in millimeter scale using antibiotic zone scale (HiMedia, Mumbai)

### III. Result and Discussion

Among 17 positive colonies picked from MRS broth 4 were identified as LAB by biochemical and microbiological tests.

#### Antimicrobial activity of bacteriocin

Among 4 isolates of LAB, *Lactobacillus delbrueckii* was selected for the production of bacteriocin and antimicrobial study against *Bacillus licheniformis*, *Streptococcus thermophilus*, *Streptococcus acidophilus*, *Escherichia coli* and *Zymomonas anaerobi*. Table 3 shows the antibiogram of bacteriocin isolated from *Lactobacillus delbrueckii*. Standard antibiotic Erythromycin was used as a positive control and DMSO was used as negative control. This study proves that fermented food like Dhokla has antimicrobial property due to bacteriocin produced by *Lactobacillus spp.* This bacteriocin also acts as a biopreservative by inhibiting growth of food spoilage causing bacteria. (Bozaris and Adams, 1999).

**Table 1 Inhibition zone of antimicrobial activity**

**Table 2 Biochemical test of bacterial organisms**

Sr No	Test Name	II 10 <sup>-3</sup>
1	Gram staining	+

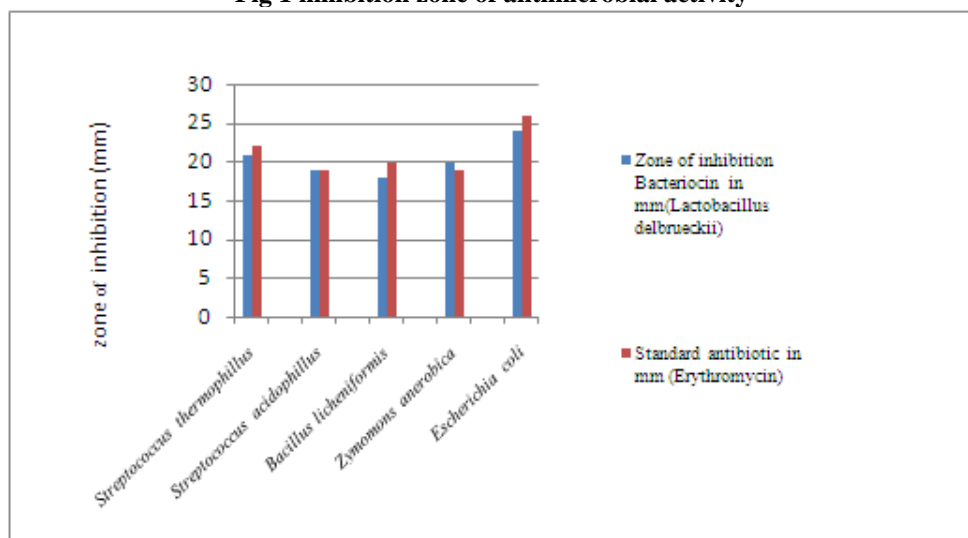
No	Name of microorganism	Zone of inhibition Bacteriocin in mm (Lactobacillus delbrueckii)	Standard antibiotic in mm (Erythromycin)
1.	<i>Streptococcus thermophilus</i>	21	22
2.	<i>Streptococcus acidophilus</i>	19	19
3.	<i>Bacillus licheniformis</i>	18	20
4.	<i>Zymomonas anaerobica</i>	20	19
5.	<i>Escherichia coli</i>	24	26

2	Indole test	+
3	Methyl red test	+
4	Voges - proskauer test	+
5	Citrate utilization test	-
6	Motility test	-
7	Catalase test	-
8	Glucose fermentation test	-
9	Mannitol test	-

(+ Positive; - Negative)

Fig 1 inhibition zone of antimicrobial activity



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