



Isolation of lactic acid bacteria from mung bean (*vigna radiata*) for production of bacteriocin and their antibacterial activity

Gunasekaran S, Selvi M^{*},

Department of Biotechnology, Sri Ganesh College of Arts and Science, Salem, Tamil Nadu, India

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ABSTRACT

The *lactobacillus* sp producing ribosomally synthesized antimicrobial peptides (bacteriocin) have exhibited antibacterial activity against food-borne pathogenic bacteria. In this study, *lactobacillus* sp was isolated from the mung bean (*vigna radiata*) seeds. Isolated bacterial strains were performed Gram staining, biochemical and sugar fermentation patterns to be followed Bergey's Manual of Systematic Bacteriology. Ammonium sulphate precipitation was used to separation of cured bacteriocin and then further molecular weight was determined by SDS-PAGE analysis. The antibacterial activity was performed against pathogenic bacteria of Gram positive (*S. aureus* and *S. pneumoniae*) and Gram negative bacteria (*E. coli* and *S. dysenteriae*) by using agar well diffusion method. The potential bactericidal effect was observed Gram negative bacteria of *E. coli* and *S. dysenteriae* then compared to Gram positive bacteria of *S. aureus* and *S. pneumonia*, the bactericidal activity based on the bacterial membrane interaction to peptide molecules. Overall, the result was suggested that the bacteriocin may lead to develop promising bio-preservative material for prevention of food-borne pathogens.

Corresponding author at.: Department of Biotechnology, Sri Ganesh College of Arts and Science, Salem, Tamil Nadu, India. *Email address:* selvim92@gmail.com (Selvi M)

1. Introduction

Probiotic terms derived from Greek words (*favor*) and bios (Life) probiotics are defined as live microbial feeds supplement that improve the health of man by its valuable secondary products. The probiotic bacteria secreted secondary by product of bacteriocins, it is used variety of beneficial roles for the human body, including maintenance of the normal intestinal microbiota, pathogen interference, antagonist activity, immune stimulation and immune modulation, anti-carcinogenic as well as deconjugation of bile acids (Rojo-Bezares et al., 2006). Bacteriocins production was number of studies focused on the Lactic acid bacteria (LAB) such as *Lactobacillus*, *Lactococci*, *Bifidobacteria*, *Saccharomyces* (Shankar et al, 2012; Ahmad et al., 2017; Alvarez-Sieiro et al., 2016). The gram positive lactic acid bacteria secreted various biological compounds such as diacetyl hydrogen peroxide, bactericidal proteins of bacteriocins are producing during the fermentation process (Alpay *et al*, 2003; Bollenbach, 2015). The bacteriocins have generally bacteria. The bacteriocins belong to ribosomally family, and generally define as peptides synthesized by bacteria that killing or inhibiting the pathogenic microorganisms (Cotter et al., 2005; Lourenço et al., 2017). For the few last decades, bacteriocins have been used many industrial application of natural food preservation, pharmaceutical and animal growth feed due their non-Immunogenicity properties and easily digested by the gastrointestinal tract.

The bacteriocins are classified four major groups based on the protein molecules, the first classification proposed by Klaenhammer et al., 1993. The class I bacteriocins are low molecular peptides (2-5 kDa) and post-translationally modified

bacteriocins, it's also called as lantibiotics are produced by gram – positive bacteria. This bacteriocins are differentiates from other them due their presence of enzymatically modified amino groups such as dehydroalanine, lanthionine, methyllanthionine, and dehydrobutyrine. Class II bacteriocins are small amino acids peptides, its positive charge, non-lantibiotics, and thermo-stability with an amphiphilic helical structure and that contain unmodified amino acid residues. The class II divided into three major sub-classes such as IIa, IIb, IIc. Bacteriocins peptides have amino acid sequence of YGNGVXC it's showed at N-terminal and strong bactericidal activity against the food pathogen of *Listeria monocytogenes*. The class IIb bacteriocins unmodified heterogeneous linear peptides it differed from pediocin family. Class IIc bacteriocins good bactericidal activities that have circular peptide of bacteriocins, such as carnocyclin A and enterocin AS-48 (Mathur et al., 2017; Lianou et al., 2017).

In this present investigation, *Lactobacillus* bacteria were isolated from mung bean sprout sample. The strains were further conformed by biochemical and gram staining test. The bacteriocin was separated and purified by ammonium sulphate precipitation method then its bactericidal activity was conformed agar wheel diffusion techniques against food born pathogenic bacteria. The isolated *Lactobacillus* sp potential inhibition against food pathogenic bacteria, so concern as a antibiotic and food feed for animals health.

2. Materials and methods

2.1 Procurement of materials and cultures

All the chemicals and Mueller Hinton agar were procurement from Himedia laboratory Mumbai, India. The bacterial species like

Staphylococcus aureus and *E.coli* were purchased from IMTECH, Chandigarh, India.

2.2 Sample collection and isolation of bacterial strains

The seeds sample of mung bean (*Vigna radiata*) was collected from Attayampatty, Salem and washed with sterile distilled water then macerated at 37 °C for 48hrs. After the incubation sample was grained with mortar pestle. Then 1gm of sample was dissolved in 100 mL of distilled water and then serially diluted from 10^{-1} to 10^{-7} . The 10^{-4} to 10^{-6} diluted 0.1mL of seed sample was spread on MRS (de Mann Rogosa Sharpe medium). The plate was incubated at 37 °C for 24 h, the creamy white color colonies were formed on the MRS plate.

2.3 Biochemical analysis of bacterial strains

The isolated bacterial strains of *Lactobacillus* sp were further conformed according to their morphological and biochemical characteristics by the procedures as described in Bergey's Manual of Systematic Bacteriology. The selected bacterial strains were carried out such as Gram stain, Indole, Methyl Red (MR), Voges-Proskauer (VP), Oxidase, Catalase and Citrate utilization test. Carbohydrate fermentation of sugars such as Arabinose, Mannitol, Lactose, Sucrose, Maltose and Dextrose were studied.

2.4 Extraction of bacteriocin

The isolated bacterial strains were inoculated into the 100 mL of MRS broth (pH 7.0) and incubated at 37°C for overnight. Then bacterial cultures were centrifuged around 10,000 rpm for 20 min at 4°C. After that procedure cell free supernatant was collected and adjusted pH at 7.0 by using of 1N NaOH. The supernatant solution

was added 80% of ammonium sulphate and maintained under stirred at 4°C for overnight. The supernatant precipitated was centrifuged at 10,000 rpm further analysis of agar well diffusion methods.

2.5 Antibacterial activity

Antibacterial activity was performed isolated probiotic bacteria of *Lactobacillus* sp against human pathogenic organisms of Gram negative and Gram positive bacterial such as *Escherichia coli*, *Shigella dysenteriae*, *Staphylococcus aureus* and *S. pneumoniae* by agar well diffusion method. 0.1 mL of overnight cultures were spread on the Mueller Hinton agar medium with the help of sterile swap and then created sterile wells. After that different concentration of partially separated bacteriocin suspension was added and then incubated at 37°C for 24 h, the zone of inhibition was measured in mm.

2.6 Molecular weight identification by Tricine SDS-PAGE

10 µl of partially separated bacteriocin from isolated lactic acid bacteria of *Lactobacillus* sp and 5 µl of low molecular weight standard marker were loaded to the electrophoresis gel, it was performed in a vertical slab gel apparatus at a constant voltage based on the gel thickness at approximately 5hrs. After that process gel was carefully removed from the glass plate and stained by 0.025% of coomassie dye in 10% acetic acid for 15 to 60min, and then treated gel was observed in white light illuminator.

3.0 Results and Discussion

3.1 Isolation and characterization of strains

The bacteriocin producing *Lactobacillus* sp (GG1) strain was isolated from the mung bean (*Vigna radiata*) on MRS medium. The selected bacterial strains were identified as LAB based on the physiological and biochemical characterization.

Antibacterial activity was performed against pathogenic bacteria of Gram positive (*S. aureus* and *S. pneumoniae*) and Gram negative (*E. coli* and *S. dysenteriae*) by agar well diffusion method shows in Fig.3.

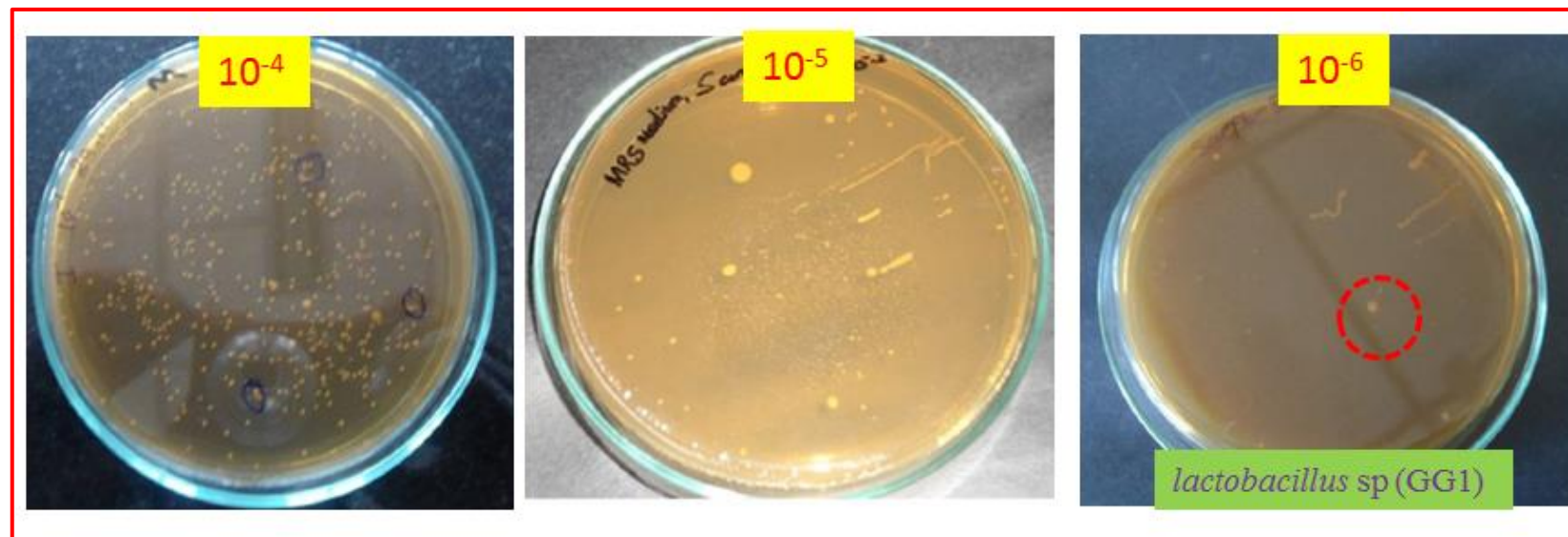


Figure 1: *Lactobacillus* sp (GG1) on MRS plates

Agreement with previous research reports Rodriguez-Bano et al., 2018. The isolated strains were showed white, centrally slightly raised and round colonies, the surface thick smooth and opaque (Fig. 1). The Gram staining also revealed gram positive spherical as well as long rod-shaped and arranged in clusters, catalase and sporulation negative. The sugar fermentation result shows in Fig.2. Therefore, preliminary screening to be confirmed the isolated bacterial strains as *Lactobacillus*.

3.2 Antibacterial activity against pathogenic bacteria

The maximum inhibition was observed against *E. coli* and *S. dysenteriae* then compared to Gram positive bacteria of *S. aureus* and *S. pneumoniae*. In last twenty decade, scientists are trying to develop novel natural antibiotic. Nowadays, the syntheses of antibiotic are using natural source of bacterial, fungi and phytocopounds separated from medicinal plants, it also potential used to prevention of pathogenic bacteria of human and animals (Kang et al., 2017; Jean et al., 2016; Kumar et al., 2016). The *Lactobacillus* sp (GG) produced bacteriocin was potential

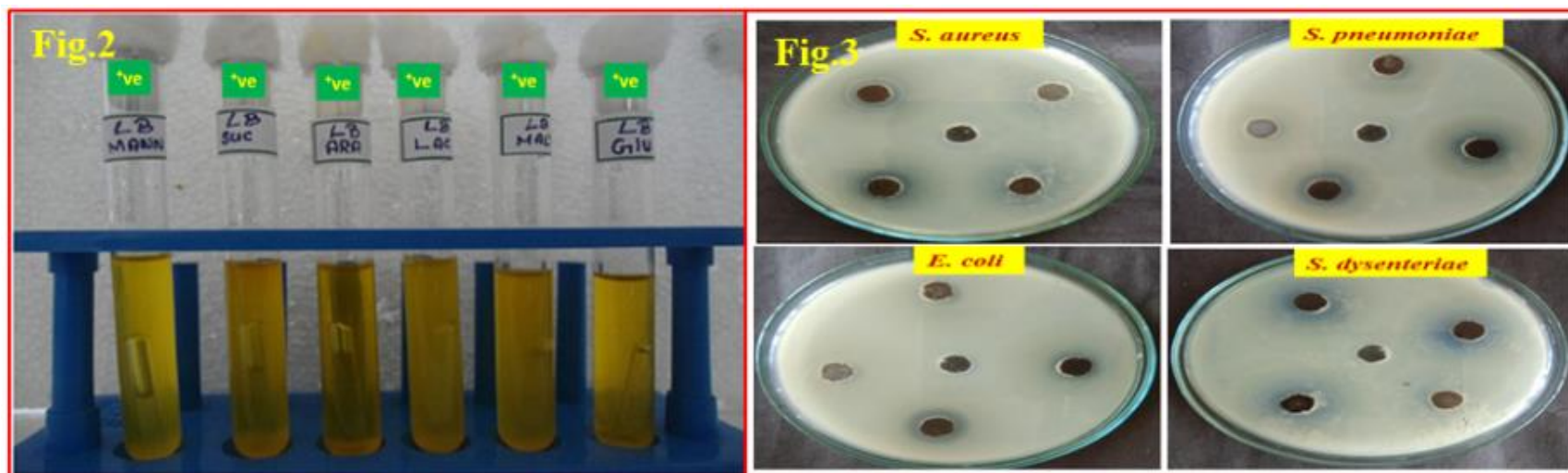


Figure 2: Carbohydrate sugar fermentation test of *Lactobacillus* sp (GG1), **Figure 3:** Antibacterial activity of bacteriocin producing *Lactobacillus* sp (GG1) Against pathogenic bacteria.

preventing intestinal infection, lowering cholesterol, reducing risk of colon cancer and also enhancing immune system. The previous researchers are reported that *lactobacillus* strains isolated from dairy products which suggested highest inhibitory effect that against the growth of *P. aeruginosa*, *E. coli*, *Salmonella typhimurium*, and *S. aureus*. Ogunbanwo et al., have reported highest bactericidal activity against *Bacillus cereus*. In current research studies, increasing number of multi-drug resistant pathogenic bacteria has been serious problem in the worldwide, so immediately need to new generation of antimicrobial agents. Bacteriocins are potential antimicrobial compounds against antibiotic resistant bacteria of *S. aureus*, Lactic acid bacteria was producing bacteriocin compounds

have used for many beneficial applications of animal feed and therapeutic purpose of human health (Lai et al., 2016).

3.3 Molecular weight determination by SDS-PAGE

The purified bacteriocin from *lactobacillus* sp (GG1) revealed homogeneity of a single protein band on 15% native PAGE. Its molecular weight was estimated at 10 kDa by SDS-PAGE result shows in (Fig. 4). Similar results were recorded by Dimov *et al.* (2005), Dobson et al., (2012) and Barbosa *et al.* (2015). In conclusion therefore, the peculiar antimicrobial characteristics of *lactobacillus* sp (GG1) can positively have impact on their use as

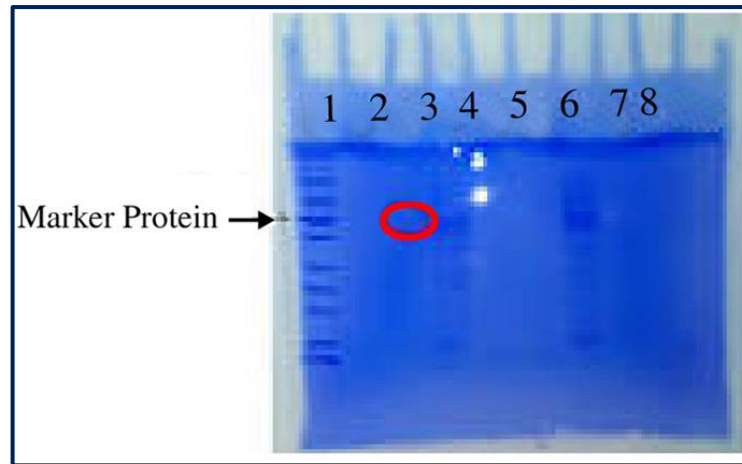


Figure 4: SDS-PAGE of bacteriocin separated from *Lactobacillus* sp (GG1)

starter cultures for traditional fermented foods, with a view to improving the hygiene and safety of the food products so produced.

4. Conclusion

In this study, investigated bacteriocin producing lactic acid bacteria of *lactobacillus* sp (GG1) was isolated and characterized from the mung bean seeds. The partially separated bacteriocin was tested against pathogenic bacteria of Gram positive (*S. aureus* and *S. pneumoniae*) and Gram negative bacteria (*E. coli* and *S. dysenteriae*). The good bactericidal activity was observed in Gram negative bacteria of *E. coli* and *S. dysenteriae* then compared to Gram positive *S. aureus* and *S. pneumonia*. The bacteriocin was separated by using of ammonium sulphate precipitation and molecular weight to be identified SDS-PAGE

analysis. Therefore, the conclusion of the study bacteriocin of antibacterial compounds from *Lactobacillus* sp (GG1) has used for potential bio-preservative in the food industry to inhibit the bacterial pathogen as well as newly development of antibiotic to against multi-drug resistant pathogenic bacterial.

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Conflicts of Interest: None

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