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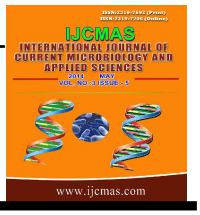

Original Research Article

Isolation, Characterization and Estimation of Antimicrobial Activity of Novel Bacteriocin from *Lactobacillus plantarum*

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K e y w o r d s

Food borne pathogens; *Salmonella typhi; Bacillus subtilis*

A B S T R A C T

Food borne pathogens are becoming a matter of great concern in various industries like diary, egg and other food industries. Various gram positive and gram negative bacteria like *Salmonella typhi*, *Bacillus subtilis* are the main causative organisms. The present study is based on isolation, identification and screening of major food borne pathogens and novel bacteriocin producing strain of Lactobacillus plantarum. Investigation was conducted on 389 food samples and 12 different genus of bacteria were identified and isolated. From the 62 isolates of Lactobacillus sp. isolated, 22 isolates were Lactobacillus plantarum and of which 14% (3 isolates) were found capable of producing bacteriocin in large amounts. The isolates were grown for 96 hours and the bacteriocin was extracted by centrifugation. Ammonium sulphate precipitation at 70% level of saturation was performed and the precipitate was centrifuged. Well diffusion assay of the extracted protein was performed on different isolated food borne pathogens and the diameter of the zones of inhibition was recorded. The study reveals the extensive scope of bacteriocin in the field of food industry as preservatives and the need for further research in the field

Introduction

The risk of contamination by pathogens mostly food borne microorganism is becoming a matter of great concern in the day to day world. This decreases the quality control of the food products and thereby decreasing consumer demand for the product in the market. The Food and Drug Administration (FDA) have prioritized the matter as top level and have listed the food borne pathogens in Bacteriological Analytical Manual, FDA (FDA, 2012). The list include various microorganisms like *Escherichia coli*, *Salmonella sp.*, *Shigella sp.*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Bacillus subtilis*. The pathogens recorded previously of causing contamination in food industry are *Staphylococcus* and *Streptococcus* species. Bacteriocins are classified as antibacterial

peptides or proteins that are synthesised by bacteria as a microbial defence mechanism. At the molecular level, they are synthesized by small ribosomes and they can permeabilize through membrane and are cationic in nature (Klaenhammer[a], 1993; Jack *et al*., 1995; Thompson *et al.*, 1996). Previous studies have found the antimicrobial nature of the peptides. Despite of their different source, structure, mode of action and specificity, any molecule of protein that is secreted by the bacteria and has antimicrobial activity is considered to be a bacteriocin (Rammelsberg and Radler, 1990). These bacteria inhibits gram positive bacteria and food spoilage bacteria (Klaenhammer[b], 1988, Caslae*t al*., 1996, Ennane*t al*., 1996, Contreras *et al*., 1997, Messi*et al*., 2001) and gram negative bacteria (Lewuse*t al*., 1991, Stevens *et al*., 1991, Messi*et al*., 2001).

Lactic acid bacteria (LAB) are characterised as gram positive bacteria and they are cocci or rod shaped. The genus is anaerobic but can tolerate and grow in the presence of air. These bacteria produce antagonist substances called bacteriocins which have high antimicrobial activity in low concentration (Klaenhammer[a], 1993, Moronoe*t al.,* 2006), The antimicrobial activity of these compounds have increased the scope of research and interest in the isolation of *Lactobacillus sp.* producing bacteriocin and characterisation of these peptides (Deraze*t al.,* 2005).

Materials and Methods

Bacterial Strains Isolation and Culture Conditions

389 samples were collected from randomly selected from various retail shops in Erode, Tiruppur, Namakkal and Coimbatore districts of Tamil Nadu, India and immediately to laboratory conditions for isolation. The various samples procured were meat, fish products, milk, dairy products, raw vegetables, bakery products, beverage and fermented rice products. The food samples were aseptically inoculated into freshly made and sterile Brain Heart Infusion broth (Hi-Media Laboratories, Mumbai) test tubes and is maintained aerobically at fermented material and the food samples from BHI broth were inoculated into culture specific medium De man Rogosa Sharpe (Hi-media Laboratories), Tryptone Soy Agar (Hi-media Laboratories), Sheep Blood Agar (Hi-media Laboratories), MacConkey Agar (Hi-media Laboratories), Xylose lysine deoxycholate Agar (Hi-media Laboratories) and incubate characters were selected randomly and repeated streaking in fresh agar culture was carried out each time until pure culture is obtained. The pure cultures were regrown in Nutrient Agar characterisation according to Bergey's manual of determinative bacteriology (Holt *et al.,* 1994).

Screening of Isolates for Bacteriocin Production

The bacteriocin produced by 22 isolates of *Lactobacillus plantarum* was checked for activity by screening against maximum amount of food borne pathogens isolated. The isolates of anaerobically. The culture was centrifuged at 10000xg for 15 mins and the supernatant was collected. The crude bacteriocin was tested for activity by well diffusion method. The well dimensions was maintained at 7mm in diameter and 5mm deep in each culture and 35µl sample was added to each vessel. The diameter of the zones were

Table.1.1 Morphological Characteristics of isolated bacteria (Suspected)

Bacteria Investigated	Motility Test	Gram Staining	Flagella Staining	Endospore Staining
Aeromonassorbia	Motile	Gram Negative	Single Polar Flagella	No Endospore
Bacillus cereus	Motile	Gram Positive, Rod	Peritrichous Flagella	Central
Bacillus subtilis	Motile	Gram Positive, Rod	Peritrichous Flagella	Subterminal
Escherichia coli	Motile	Gram Negative, Rod	Peritrichous Flagella	No Endospore
Klebsiellaoxytoca	Non-Motile	Gram Negative, Rod	No Flagella	No Endospore
Klebsiella pneumonia	Non-Motile	Gram Negative, Rod	No Flagella	No Endospore
Listeria monocytogenes	Motile	Gram Positive, Cocci	Peritrichous Flagella	No Endospore
Salmonella enterica	Motile	Gram Negative, Rod	Peritrichous Flagella	No Endospore
Staphylococcus aureus	Non-Motile	Gram Positive, Cocci	No Flagella	No Endospore
Streptococcus agalactiae	Non-Motile	Gram Positive, Cocci	No Flagella	No Endospore

Table.1.2 Percentage of Isolated Tested Positive for Biochemical Tests

Bacteria Investigated	No. of Isolates	NR	SH	CT	CA	OX	UR
Aeromonassorbia	34	97	-	85	-	84	-
Bacillus cereus	22	92	89	100	85	-	59
Bacillus subtilis	31	*1	86	99	90	64	-
Escherichia coli	86	100	-	-	85	-	-
Klebsiellaoxytoca	12	95	-	99	80	-	89
Klebsiella pneumonia	21	95	-	98	84	-	85
Listeria monocytogenes	82	-	-	-	99	-	-
Salmonella enterica	8	95	-	-	88	-	-
Staphylococcus aureus	11	100	-	99	85	-	-
Streptococcus agalactiae	54	-	-	-	-	-	-

NR-Nitrate Reduction, SH-Starch Hydrolysis, CT-Citrate, CA-Catalase, OX-Oxidase, UR-Urease

Table.2.1 Antimicrobial Activity of Crude and Partially Purified Bacteriocin

Isolated Strains	Zone of Inhibition (Crude)mm			Zone of Inhibition(Partially Purified)mm		
	LP-FP-6	LP-BR-3	LP-CD-12	LP-FP-6	LP-BR-3	LP-CD-12
Aeromonassorbia	6	6	5	7	9	7
Bacillus cereus	7	8	7	10	10	11
Bacillus subtilis	8	8	7	13	12	10
Escherichia coli	6	5	4	9	9	9
Klebsiellaoxytoca	0	0	0	0	0	0
Klebsiella pneumonia	2	3	2	5	8	5
Listeria monocytogenes	6	8	6	7	10	9
Salmonella enterica	2	5	2	5	8	4
Staphylococcus aureus	9	12	10	13	13	12
Streptococcus agalactiae	5	8	5	8	9	8

recorded for maximum number of indicator bacterial isolates to select the best strains.

Extraction of Bacteriocin:

250 ml of MRS broth was inoculated with the best strains of *Lactobacillus plantarum*

Antimicrobial Assay of purified bacteriocin

Antimicrobial property of the purified bacteriocin against various food borne pathogen isolates by well diffusion assay (Sharma *et al.*, 2011). 1 ml of the indicator bacteria was swabbed on a previously poured and sterilised nutrient agar plates using sterile cotton buds.

Results and Discussion

Isolation and Identification of Bacteria

From a total of 389 samples were investigated and from which a total of 688 species were isolated. The suspected bacterial colonies were purified by repeated streaking in selective agar media plates until the pure cultures were obtained. The morphological and biochemical characteristics were recorded.

Antimicrobial Assay

Antimicrobial assay was performed with both crude and partially purified bacteriocin. The partially purified bacteriocin displayed a large zone of inhibition in microorganisms like *Staphylococcus aureus* and *Bacillus aureus.* The results were almost similar to the study done by Dhanpathi*et al*., 2008. The bacteriocin produced by *lactobacillus plantarum* was found to be effective against both gram positive and gram negative bacteria. The comparison of crude and purified bacteriocin revealed an increase in activity. The study reveals a positive co-relationship with the study done by Bizani*et al.*, 2002.

Figure.2.2 Comparison of Zones of Inhibition of Crude and Partially Purified

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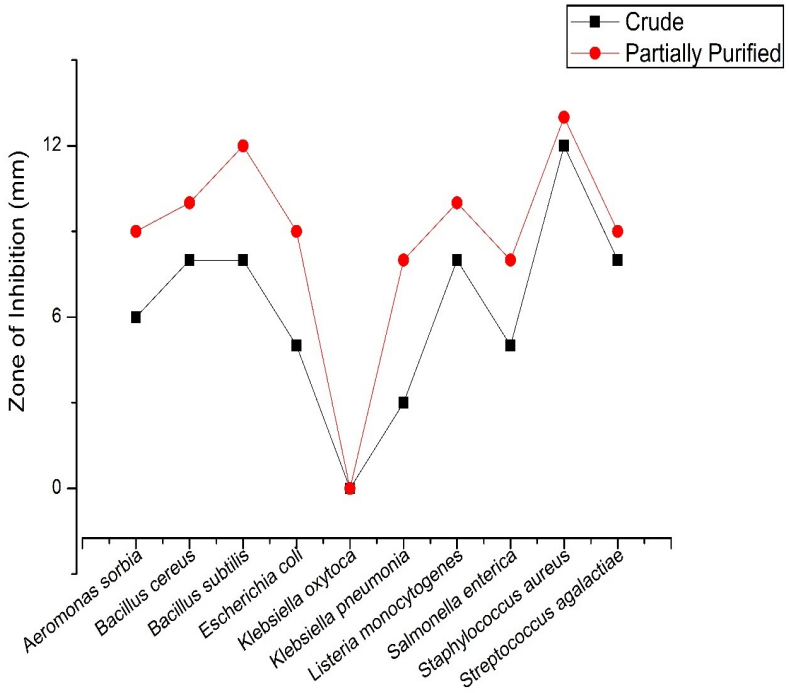

The presence of various microorganism like *Aeromonas, Bacillus, Escherichia, Klebsiella, Listeria, Staphylococcus, Salmonella and Streptococcus* was confirmed but the present study in different food, fermented and dairy products. The strains of *Lactobacillus plantarum* which produces bacteriocins were isolated and the crude and partially purified bacteriocins were tested for antimicrobial activity successfully. The inhibition was found to be more active in gram positive bacteria and the prospects of the usage of these bacteriocins in the food industry as preservatives is large. Further studies like large scale production and structural analysis can be implored on and way could be paved for profound research

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