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Determination of antibacterial Activity of Bacteriocin of Lactic Acid Bacteria Isolated from Meat Samples

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ABSTRACT

The present study is aimed at determination of antimicrobial activity of lactic acid bacteria isolated from meat samples against common food borne pathogens. The meat samples were collected from local meat shops and were inoculated in sterile saline to make suspension. The isolates were enriched in tomato juice at acidic pH and then the bacteriocin producing lactic acid bacteria were isolated by using de-Man Rogosa Sharpe (MRS) agar supplemented with 0.01% sodium azide and 2% β Glycerophosphate. The plates were overlaid with the same media and incubated at 35 ^{0}C for 48- h. The production of crude bacteriocin was carried out by growing isolated strain in MRS broth, which was then centrifuged at 10000 rpm for 15 min at 4 ^{0}C to get the bacteriocin fraction in the cell free supernatant. The crude bacteriocin preparation was subjected to characterization with respect to heat stability, pH, susceptibility to denaturation by enzymes and detergents. Antagonistic activity of bacteriocin was studied by agar well diffusion method against different microorganisms such as *B. subtilis, B. cereus, S. typhi, M. luteus, S. aureus, E.coli* and *P vulgaris*. The result showed that bacteriocin producing lactobacilli obtained from meat samples have potential to inhibit bacteria such as *B.cereus, S. typhi, S. aureus, E.coli , P. vulgaris.*

Key words : *Bacteriocin, Lactic acid bacteria, Antagonistic activity, Food pathogens*

Introduction

Bacteriocins are antimicrobial peptides which are produced by bacteria. Deficiency of nutrients in the environment triggers variety of bacteriocin production for competition of space and resources. To obtain more nutrients and living space in environment, the killing ability of bacteriocins is considered a successful strategy for maintaining population and reducing the numbers of competitors (Soltani *et al.,* 2021). Bacteriocins are low molecular weight compounds and can be easily degraded by proteolytic enzymes especially proteases of the mammalian GI tract which make them safe for human consumption. Bacteriocin contains an excess of lysyland arginyl residues which give them cationic and amphipathic nature. When bacteriocins are exposed to a structure promoting solvents such as triofluroethanol or anionic phospholipid membrane they form a helical structure, when incorporated in aqueous solution they are usually unstructured. Due to the production of bacteriocins Lactic Acid Bacteria have gained particular attention nowadays among the Gram positive bacteria. Bacteriocins can be used as natural preservatives in food industry (Zacharof, 2012).

Materials and Methods

Sample collection

Samples were collected from local meat shop situated at Malkapur, Karad Dist. Satara, MH. Samples were collected in clean plastic bags and stored at 4

^{0}C until used.

Enrichment and isolation of *Lactobacillus*

Tomato juice broth was used for enriching *Lactobacillus* species. Meat samples (1g) were inoculated in sterile saline (10 ml) and 0.1 ml suspension was inoculated in sterile tomato juice broth and incubated in anaerobic jar for 24 h. Enriched medium was then streak inoculated on tomato juice agar plates and colonies were studied for morphological characteristics.

Isolation of bacteriocin producing *Lactic Acid Bacteria*

For detection of antagonistic activity sandwich test was used.

The dilutions of lactobacillus colonies were inoculated onto MRS agar supplemented with 0.01% sodium azide to inhibit Gram negative bacteria. To rule out any inhibition due to pH reduction caused by organic acid production, 2% sodium â glycerophosphate was added to MRS agar. The plates were overlaid with the same media to create microaerophilic condition.

After the incubation period plates were overlaid with 4.5 ml of soft nutrient agar, seeded with 0.5 ml of *E.coli* suspension. The plates were incubated at 35 ^{0}C for 24-h. Lysis of the indicator strains resulted in a clear zone.

Colonies showing zones of inhibition were transferred to MRS agar and incubated at 30^0 C for upto 72-h.

The cultures were purified by repeated sub culturing on MRS agar plates and incubated at 30 ^{0}C for 18- h.

The purified isolates were examined by Gram staining and catalase production and also for biochemical tests for identification to genus level of Lactobacillus.

Study of biochemical characteristics of isolates

Enzymatic activities of isolates

In the studies on physiological and biochemical characteristics following tests were performed with reference to Bergey's manual of systematic bacteriology volume II (1986)

Catalase test, oxidase, urea hydrolysis, arginine hydrolysis, H_2S production, nitrate reduction and sugar fermentation tests.

Production of crude bacteriocin

The isolate ML4 was grown in MRS broth (pH 6) seeded with 5% inoculum of overnight culture and maintained anaerobically at 30 ^{0}C for 48-h.

After incubation cells were removed from the growth medium by centrifugation at 10000 ×g for 15 min, 4 ^{0}C. The cell free supernatant was adjusted to pH 6.0 using 1N NaOH and was used as a crude bacteriocin.

Detection of antagonistic activity

Bacteriocin production by the lactic acid bacteria isolated above mentioned method was assayed by the agar well diffusion method against following organisms.

Bacillus subtilis, Bacillus cereus, Salmonella typhi, Micrococcus luteus, Staphylococcus aureus, Escherichia coli and Proteus vulgaris.

Petri dishes were filled with nutrient agar seeded with test organism to a thickness of 5mm. The wells were punched out of the agar (4 in no.), by using a cork borer and then standardized quantities (0.1 ml) of bacteriocin preparations were added to the wells.

The plates were incubated at 4 ^{0}C for 1 to 2-h to allow the diffusion of the bacteriocin into the medium and then incubated at 37 ^{0}C for 24-h.

The plates were examined for zone of inhibition around the wells. The zones of clearance around wells was taken as indication of presence of bacteriocin.

Characterization of bacteriocin

Crude bacteriocin preparation was subjected to characterization with respect to heat stability, pH stability, susceptibility to denaturation by enzymes and detergents.

Heat Resistance

Crude bacteriocin preparation was exposed to various heat treatments as 80 ^{0}C, 100 ^{0}C and 121 ^{0}C

Aliquot volumes of each fraction were then assayed for bacteriocin activity by agar well diffusion method.

pH sensitivity

Crude bacteriocin preparation were adjusted to pH 2,4,6,8 and 10 with hydrochloric acid (HCL) (1N) and sodium hydroxide (NaOH)(1N), incubated for 4- h at room temperature and assayed by agar well diffusion method.

Effect of enzymes and detergents

The sensitivity of the active substance to enzymes was tested on cell free supernatants.

The supernatant was treated for 2- h with 1ml of the following enzymes at final concentration of 1mg/ml: protease, amylase, pepsin (Hi media) and assayed as above.

The surfactant tested were sodium dodecyl sulphate (SDS), Tween 80, and EDTA at final concentration of 1% and assayed as above.

Results and Discussion

From a total of 5 different fresh meat samples analysed, 5 isolates of lactic acid bacteria were obtained and designated as ML1, ML2, ML3, ML4, ML5 and colony characteristics, Gram nature and motility of different isolates are presented in Table-1 and were subjected to their antagonistic activity against *E. coli*.

It was observed from colony characteristics of all 5 isolates that there were not much difference except few in terms of color and consistency. The isolates are Gram positive short to large rods in nature.

Isolates were studied for biochemical tests, from these tests it was found that all isolates were not able to give positive results for biochemicals except isolate ML1 and ML4 which shows positive result for arginine hydrolysis (Table 2).

Table 1. List of isolates obtained from different sources

Meat samples	Name of Isolates
1	ML1
2	ML2
3	ML3
4	ML4
5	ML5

Table 4. Details of sugar fermentation test (Isolate ML4)

Sr. No.	Sugar	Results (Acid production)
1	Arabinose	Negative
2	Fructose	Positive
3	Galactose	Positive
4	Lactose	Positive
5	Maltose	Positive
6	Mannitol	Negative
7	Ribose	Negative
8	Sucrose	Positive
9	Glucose	Positive
10	Xylose	Negative

Table 5. Antagonistic activity of lactic acid bacterial isolates on *E. coli*

Sr. No.	Isolates	Antagonistic activity (Positive/ Negative
1	ML1	+
2	ML2	+
3	ML3	+
4	ML4	+++
5	ML5	+

+ = Minimum antagonistic activity
+++ = Maximum antagonistic activity

Table 2. Details of colony characteristics, Gram nature and motility of lactic acid bacteria isolated from meat samples.

Codes of isolates	Size	Shape	Colour	Margin	Elevation	Opacity	Consistancy	Gram nature and morphology	Motility
ML1	2mm	Circular	White	Entire	Convex	Opaque	Moist	Gram positive rod	Non motile
ML2	2mm	Circular	White	Entire	Convex	Opaque	Moist	Gram positive rod	Non motile
ML3	2mm	Circular	White	Entire	Convex	Opaque	Mucoid	Gram positive rod	Non motile
ML4	1mm	Circular	Off White	Entire	Convex	Opaque	Mucoid	Gram positive rod	Non motile
ML5	2mm	Circular	White	Entire	Convex	Opaque	Moist	Gram positive rod	Non motile

Table 3. Details of distinctive biochemical features of isolates

Codes of isolates	Catalase production	Oxidase production	Urea hydrolysis	Arginine hydrolysis	H_2S production	Nitrate reduction
ML1	Negative	Negative	Negative	Positive	Negative	Negative
ML2	Negative	Negative	Negative	Negative	Negative	Negative
ML3	Negative	Negative	Negative	Negative	Negative	Negative
ML4	Negative	Negative	Negative	Positive	Negative	Negative
ML5	Negative	Negative	Negative	Negative	Negative	Negative

Table 6. Details of effect of crude bacteriocin preparation from ML-4 on growth of selected test organisms

Sr. No.	Test Organism	Zone of inhibition (positive/ Negative)
1	*Bacillus cereus*	Positive
2	*Bacillus subtilis*	Positive
3	*Salmonella typhi*	Positive
4	*Micrococcus luteus*	Negative
5	*Staphylococcus aureus*	Positive
6	*E. coli*	Positive
7	*Proteus vulgaris*	Positive

Table 7. Effect of heat and pH on activity of crude bacteriocin using *E.coli* as indicator organism

	Heat			pH				
Zone of inhibition	80 ^{0}C	100 ^{0}C	121 ^{0}C	2	4	6	8	10
	Positive	Positive	Negative	Negative	Positive	Positive	Positive	Negative

Table 8. Effect of enzymes and detergents on the activity of crude bacteriocin against *E.coli*

	Enzymes			Detergents		
	Protease	Amylase	Pepsin	SDS	Tween 80	EDTA
Zone of inhibition	Negative	Negative	Positive	Positive	Positive	Negative

Effect of crude bacteriocin on growth of different microorganisms was studied, it was found that bacteriocin inhibited all 7 microorganisms except *Micrococcus luteus* (Table 4).

Effect of heat and pH on activity of crude bacteriocin against *E. coli* was studied, Zone of inhibition was observed at 80 ^{0}C, 100 ^{0}C and at pH 4, 6, 8, indicating heat and pH tolerance of bacteriocin (Table 5).

Effect of enzymes and detergents were observed which shows zone of inhibition after exposure to pepsin, SDS and Tween 80.

Conclusion

1) The results showed that bacteriocin producing lactic acid bacteria obtained from meat samples have potential to inhibit bacteria such as *Bacillus subtilis, B. cereus, Salmonella typhi, Staphylococcus aureus, E. coli* and *Proteus vulgaris.*
2) Isolate ML-4 was potent bacteriocin producer out of five lactic acid bacterial isolates.
3) It indicates that bacteriocin possesses protein and carbohydrate moieties and was resistant to SDS and Tween 80 but sensitive to EDTA.

Conflict of interest: There is no conflict of interest among authors.

References

Barefoot, S.F. and Klaenhammer, T.R. 1984. Purification and characterization of the Lactobacillus acidophilus bacteriocin lactacin B. *Antimicrobial Agents and Chemotherapy*. 26(3) : 328-334.

Benkerroum, N., Ghouati, Y., Sandine, W.E. and Tantaoui Elaraki, A. 1993. Methods to demonstrate the bactericidal activity of bacteriocins. *Letters in Applied Microbiology*. 17(2) : 78-81.

Bergey, D.H. 1994. *Bergey's Manual of Determinative Bacteriology*. Lippincott Williams & Wilkins.

Messi, P., Bondi, M., Sabia, C., Battini, R., Manicardi, G. 2001. Detection and preliminary characterization of a bacteriocin (plantaricin 35d) produced by a Lactobacillus plantarum strain. *International Journal of Food Microbiology*. 64(1-2) : 193-198.

Piard, J.C. and Desmazeaud, M. 1992. Inhibiting factors produced by lactic acid bacteria. 2. Bacteriocins and other antibacterial substances. *Le lait*. 72(2) : 113-142.

Soltani, S., Hammami, R., Cotter, P.D., Rebuffat, S., Said, L.B., Gaudreau, H., Bédard, F., Biron, E., Drider, D. and Fliss, I. 2021. Bacteriocins as a new generation of antimicrobials: Toxicity aspects and regulations. *FEMS Microbiology Reviews*. 45(1): fuaa039.

Zacharof, M.P. and Lovitt, R.W. 2012. An innovative growth strategy for propagation and bacteriocin production of Lactobacilli. In: *Microbes in Applied Research: Current Advances and Challenges*. pp. 321-326.