Anti-Microbial Activity of probiotic Lactobacilli and Optimization of Bacteriocin Production

P. Pradeepa[1] M.P. Prasad[2] V. Aswini[3] Moneesha Rexon[4]
[2,3]Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, India
[1, 2, 3, 4]San Genomics Research Labs, Bangalore, India

Abstract— The present study is about the anti-microbial activity of the bacteriocin producing lactobacilli and optimization of bacteriocin production. Bacteriocin was extracted by solvent extraction with chloroform and the antimicrobial activity was tested against 5 different pathogens by agar spotting method. Optimization of bacteriocin production was done for 4 different parameters such as pH, Temperature, Carbon source and Nitrogen source and the anti-microbial activity was tested against the following 5 different pathogens and the results were observed and diameter of the zone of inhibition was measured and tabulated. From the results of the study it was found that bacteriocin produced from lactobacilli has good antimicrobial activity.

I. INTRODUCTION

A. *Lactic Acid Bacteria*

A set of bacteria which are commonly present in dairy foods such as milk, curd are the lactic acid bacteria. It has been found that these bacteria produce a number of compounds that are antagonistic to other harmful micro-organisms [1]. The Lactobacilli are found to produce proteinases which could be extra and intra-cellular. Of these, some preferably hydrolyse the casein yielding peptides which are further broken down by peptidases present inside the Lactobacilli cell [2]. A lot of disorders do occur due to the lack of these bacteria such as vaginal infections. These can be rectified by prescriptions that can restore the vaginal Lactobacilli. Recently these vaginal Lactobacilli has been found to produce hydrogen peroxide and the prescriptions were studied. Of these, 62% of them were found to produce hydrogen peroxide [3]. Due to such properties, Lactobacilli have been considered as a species worth studying. And these previous studies potentially increased the need of further studies about the bacteria.

B. *Bacteriocins*

Lactic acid bacteria are found to produce bacteriocins which act against a set of closely related pathogens. Hence these are mainly found in the dairy products helping their preservation. Hence, with respect to probiotics, particular strains of bacteria are found inhibit the growth of harmful organisms [4]. Hence the Lactobacilli strains are being used in chemical, biochemical, food and pharmaceutical industries. They also play a very important role in the stabilization of intestinal micro flora by preventing the entry and growth of pathogens [5]. Nowadays in commercial products such as cheese, milk these strains are intentionally added due to their probiotic properties [6]. The main reason for these properties is the bacteriocins present in the bacteria. These are nothing but the anti-bacterial proteins that potentially inhibit the growth of pathogens. The bacteriocins have been found in numerous fermented and non-fermented foods among which nisin is the only bacteriocin that is extensively used as a preservative. Though there is a basic understanding of the structure, function and properties of the bacteriocins, many of the attributes are however still unknown [7]. Bacteriocins have also been used in the field of medicine and have been suggested in the treatment of cancer [8]. Further the extraction of these bacteriocins gained importance and a novel method to assist was in need. It was found that the solvent extraction was a flexible way of extraction of these bacteriocins [9]. There was also a need to compute the extent of the anti-microbial activity of them and for this; the effective method was agar spotting assay. The diameter of the zone of inhibition was usually taken as the measure of the bacteriocin's anti-microbial activity [10]. Hence, based on these studies, there is a need for the optimization of the bacteriocin from Lactobacilli and the assay of their respective anti-microbial properties.

II. MATERIALS AND METHODS

A. *Optimization of Bacteriocin Production*

Optimization is done for temperature, pH, carbon and nitrogen source.

1) *Optimization of temperature*

100 ml MRS broth was taken in 2 different conical flasks and was kept in an autoclave for 15 minutes at 15 lbs pressure. Then after cooling, it was inoculated with 2ml of mother culture (isolated from curd) in each conical flask and was incubated for 24 hours at 2 different temperatures such as 35˚c, 40˚c. After incubation the MRS broth was centrifuged and supernatant was collected. It was then mixed with half the volume of chloroform and shaken vigorously using a magnetic stirrer for 20 minutes after that centrifugation was carried out at 7000 rpm for 20 minutes. The aqueous layer was separated by holding back the floating interfacial precipitate. The interfacial layer was transferred in another bottle and it was dissolved in 0.1M buffer at pH 7. It was mixed & centrifuged again at 6800 rpm for 15 minutes. The supernatant was poured off and the pellet was dissolved in 0.1 M tris buffer at pH 7.The bacteriocin extracted was tested against the 5 different pathogens by spotting technique and was incubated for 24 hours and the result was observed and the diameter of zone of inhibition was measured and tabulated.

2) *Optimization of pH*

100 ml MRS broth was taken in 2 different conical flasks and was kept in an autoclave for 15 minutes at 15 lbs pressure. Then after cooling it was inoculated with 2ml of mother culture (isolated from curd) in each conical flask and

was Incubated for 24 hours at 2 different pH such as 3, 5. After incubation the MRS broth was centrifuged and supernatant was collected. The bacteriocin was extracted with chloroform as described earlier. The bacteriocin extracted was tested against the 5 different pathogens by spotting technique and was incubated for 24 hours. The result was observed and the diameter of zone of inhibition was measured and tabulated.

3) Optimization of carbon and nitrogen sources

For carbon source, different carbon sources like dextrose, glucose, maltose, and lactose were taken in 4 different conical flasks and 5 different nitrogen sources like ammonium citrate, ammonium nitrate, ammonium sulphate, ammonium acetate were taken. The media was inoculated with 2ml of the culture. After incubation the MRS broth was centrifuged and supernatant was collected. The bacteriocin was extracted by solvent extraction and further tested against the 5 different pathogens by spotting technique and was incubated at 37˚c for 24 hours and the result was observed and the diameter of zone of inhibition was measured and tabulated.

B. Mass production

1) Preparation of production media

500ml of MRS broth was prepared for the production of bacteriocin from isolated Lactobacilli and kept at 37˚C for 24 hours incubation in shaker incubator.

2) Product recovery - Centrifugation

Media was taken out from the flask and was centrifuged at 10000 rpm for 15 minutes.

3) Solvent extraction and Antimicrobial assay

Bacteriocin was extracted by chloroform extraction. Antimicrobial activity was determined by agar spotting method and the plates were incubated at 37˚C for 24 hours. The result was observed and the diameter of cleared zones was measured in mm. The resulting zone diameter is shown in the table. The transparently cleared zones micro colonies showed bacteriostatic activity.

III. RESULTS AND DISCUSSIONS

	Zone Diameter (mm)			
ORGANISM NAME	35˚C	40˚C	3 pH	5 pH
B.subtilis	8	13	10	11
S.aureus	-	10	16	12
Klebsiella pneumonia	9	11	14	8
Salmonella typhi	-	10	14	11
Proteus Sps.	16	12	14	12

Table 1: Temperature and pH Optimization

	Zone Diameter (mm)			
ORGANISM NAME	DEXTROSE	GLUCOSE	MALTOSE	LACTOSE
B.subtilis	-	-	13	-
S.aureus	16	21	15	-
Klebsiella pneumonia	14	-	12	8
Salmonella typhi	15	13	13	-
Proteus Sps.	11	-	8	16

Table 2 Optimization of Carbon Sources

Thus from the above table it was concluded that the bacteriocin was maximum produced at 40˚C and 3 pH by lactobacilli

	Zone Diameter (mm)			
ORGANISM NAME	AMMONIUM CITRATE	AMMONIUM NITRATE	AMMONIUM SULPHATE	AMMONIUM ACETATE
B.subtilis	11	-	13	11
S.aureus	13	-	10	-
Klebsiella pneumonia	-	11	14	11
Salmonella typhi	-	14	14	15
Proteus Sps.	9	12	12	13

Table 3: Optimization of Nitrogen Sources

Thus from the above table it was concluded that the bacteriocin was maximum produced with ammonium sulphate as its nitrogen source.

A. Mass Production

The weight of the crude bacteriocin extracted during large scale production under the above optimized conditions was found to be 11.57 grams.

IV. CONCLUSION

It was hence found that the Lactobacilli from the curd showed considerable anti-microbial activity against the following pathogens: Bacillus subtilis, Streptococcus aureus, Klebsiella pnuemoniae, Salmonella typhi, and proteus sps. The bacteriocin production was optimized under different carbon sources, nitrogen sources, pH and temperature. Under the optimized conditions, large scale production was done followed by the extraction of crude bacteriocin.

ACKNOWLEDGEMENT

The authors sincerely thank the San Genomics Research Laboratory, Bangalore, India for rendering constant support, guidance and infra-structure required for the successful conduct of research.

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