IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 10, Issue 4 Ver. IV (Jul - Aug. 2015), PP 11-12
www.iosrjournals.org

Isolation of Bacteriocin Producing *Lactobacillus* species from fermented food like *Idli* and their antibacterial assay

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Abstract: *In present study bacteriocin producing Lactobacillus species were isolated from idli and bacteriocin was produced from Lactobacillus spp. by fermentation. After isolation of bacteriocin it was tested for antibacterial activity against five food spoilage causing and human pathogenic bacteria i.e., Bacillus licheniformis, Streptococcus thermophillus, Streptococcus acidophilus, Escherichia coli and Zymomonas anaerobia. In antibacterial assay bacterocin has showed as a strong antibacterial agent against all five bacteria by forming zone of inhibition of 18mm, 22mm, 19mm, 17mm and 21mm respectively. Standard antibiotic Erythromycin was used as a positive control and DMSO was used as negative control. This study revealed the possibility of using bacteriocin as biopreservative to control food spoilage causing bacteria*
Keywords*: Bacteriocin, biopreservative, idli, Lactobacillus spp.*

I. Introduction

Idli is a famous fermented food of India which is prepared by fermentation of black gram (Phaseolus mungo L.) and rice (Oryza sativa L.). The idli is the most preferred breakfast product due to its soft texture, mild pleasant flavor and aroma, easy digestibility and known health and nutritional benefits.

Lactic acid bacteria (LAB) are non pathogenic organism widely distributed in nature have an important role in the preservation of foods and fermented products and are designated as GRAS (Generally regarded as safe). LAB are present in idli and this LAB produces bacteriocin which acts as biopreservative [1].

II. Materials and Methods

2.1. Experimental bacteria

The experimental bacteria namely Bacillus licheniformis, Streptococcus thermophillus, Streptococcus acidophilus, Escherichia coli and Zymomonas anaerobia were procured from National Chemical Laboratory (NCL), Pune (Maharashtra), India.

2.2. Isolation of LAB from

Idli was prepared in Department of Post-Harvest and Food Biotechnology, K. K. Wagh College of Agricultural Biotechnology, Nashik, using rice and blackgram by fermentation process. Plate count agar (PCA) and (Mans Ragosa and Sharpe) MRS broth were used for isolation of LAB from idli [2]. The idli sample were homogenized using sterile glass rod in taste tube and distilled water is added drop by drop while homogenization. Then homogenized sample were used for serial dilution and dilutions were plated on PCA plates. After inoculation plates were incubated at 37 ^{0}C for 48 hours. After incubation colonies with typical white color characteristics were picked from plate and inoculated in MRS broth. LAB were identified by biochemical, microbiological, physiological and morphological characteristics. 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 [3].

2.3. Maintenance of bacteria

The cultures of LAB were maintained at 4 ^{0}C in MRS broth. Experimental bacteria were maintained at 4 ^{0}C on nutrient agar slants. All the bacterial cultures were subcultured at 15- 20 days interval.

2.4. Production of Bacteriocin

Isolated LAB were inoculated in 200 ml of MRS broth and incubated at 37 ^{0}C for 48 hours. The LAB cells were killed by heating at 80 ^{0}C for 10 minutes followed by centrifugation at 8000 rpm for 30 minutes. The resultant cell debris was discarded and cell free supernatant was collected. The pH of collected supernatant adjusted to 5.0 with 1N NaOH, then extract was concentrated using rotary flash evaporator and the solution thus obtained is crude bacteriocin. For preservation of crude bacteriocin it was mixed with DMSO and filter sterilized using 0.22µm membrane filter paper [4].

2.5. Agar well diffusion assay

200µl of the 18 hours old test cultures were inoculated on nutrient agar plates by spread plate method. Four 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 ^{o}C for 24hours. The inhibition zone was measured in millimeter scale using antibiotic zone scale [5].

III. Result and Discussion

Among 10 positive colonies picked from MRS broth 3 were identified as LAB by biochemical and microbiological tests. Result of biochemical analysis is shown in Table 1. Antimicrobial activity of bacteriocin among 3 isolates of LAB, Lactobacillus delbrueckii was selected for the production of bacteriocin and antimicrobial study against Bacillus licheniformis, Streptococcus thermophillus, Streptococcus acidophilus, Escherichia coli and Zymomonas anaerobi. Table 2 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 idli has antimicrobial property due to bacteriocin produced by Lactobacillus spp. present in idli. This bacteriocin also acts as a biopreservative by inhibiting growth of food spoilage causing bacteria [6].

Acknowledgement

The authors would like to thank Chairman Hon. Balasaheb Wagh and Principal Prof. N. S. Pachpor of K. K. Wagh College of Agricultural Biotechnology, Nashik for the support of chemicals and equipments for this research.

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Table 1. Biochemical analysis of Lactobacillus spp. isolated from Idli

Sr. No.	Name of test	Test result
1.	Gram staining	+
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	-

[Result: - Positive: (+), Negative: (-)]

Table 2. Antibacterial activity of Bacteriocin produced by Lactobacillus spp.

Sr. No.	Name of test bacteria	Zone of inhibition formed by Bacteriocin	Standard antibiotic in mm (Erythromycin)
1.	Bacillus licheniformis	18	23
2.	Streptococcus thermophillus	22	21
3.	Streptococcus acidophilus	19	19
4.	Escherichia coli	17	18
5.	Zymomonas anaerobi	21	19