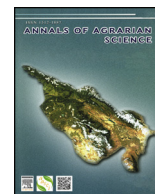




Contents lists available at ScienceDirect

Annals of Agrarian Science

journal homepage: www.elsevier.com/locate/aasci

Production optimization of broad spectrum bacteriocin of three strains of *Lactococcus lactis* isolated from homemade buttermilk

Soma Barman^a, Ranjan Ghosh^b, Narayan C. Mandal^{c,*}

^a National Post-Doctoral Fellow, Soil and Agro-Bioengineering Laboratory, Department of Environmental Science, Tezpur University, Sonitpur, Napaam, Tezpur, Assam 784028, India

^b Guest Teacher, Department of Botany, Visva-Bharati, Santiniketan 731235, West Bengal, India

^c Professor, Mycology and Plant Pathology Laboratory, Department of Botany, Visva-Bharati, Santiniketan 731235, West Bengal, India

ARTICLE INFO

Keywords:

Lactococcus lactis
bacteriocin
Antibacterial spectrum
bacteriocidal
Scanning electron microscope

ABSTRACT

Three potent strains of lactic acid bacteria (LAB) with bacteriocin producing abilities have been isolated from homemade buttermilk. They can produce proteinaceous bacteriocins in the medium which are resistant to heat (121 °C for 15 min) and can tolerate range of pH from 3.0 to 11.0. They were found to be Gram positive, non-motile, non-endospore forming, coccoid structures. The strains LABW1 and LABW3 were present in chains while LABW4 were present singly or in pairs. By considering the 16S rDNA sequence homologies and phylogenetic analysis LABW1 and LABW3 were identified as *Lactococcus lactis* subsp. *hordniae* where as LABW4 was identified as *Lactococcus lactis* subsp. *lactis*. Maximum extracellular bacteriocin production by the isolates takes place in MRS medium (pH 5.0–7.0) at 28 °C and it showed strong correlation with bacterial growth. They started producing bacteriocin at early exponential phase (400 AU/ml) and reached its peak at the early stationary phase (12800 AU/ml for LABW1 and LABW3 and 25600 AU/ml for LABW4) which remain unchanged up to late stationary phase. The bacteriocin of the isolates had an antibacterial spectrum both against Gram positive as well as Gram negative pathogenic bacterial strains. The inhibition of Gram negative bacteria by bacteriocin of LAB is an unusual phenomenon. It exhibits bacteriocidal action on *Staphylococcus aureus* MTCC96 and *Pseudomonas aeruginosa* MTCC741. Prominent morphological aberrations, cell shrinkages and pore formations on cell wall of pathogenic bacterial cells upon bacteriocin treatment as evidenced from scanning electron microscope (SEM).

Introduction

Antibiotic resistance of pathogenic bacteria is a major problem in the recent year due to extensive use of classical antibiotics to overcome pathogenic human and animal diseases [1,2]. As a result, multiple antibiotic resistant strains have developed and spread that restricted the use of classical antibiotics. As for example resistance to broad-spectrum antibiotic cephalosporins is a constant challenge for managing the infections caused by *Pseudomonas aeruginosa*. Similarly methicillin resistance in *Staphylococcus aureus* and vancomycin resistance in enterococci are also creating notorious problems in hospitals, nursing homes or in other medical sectors. Therefore, demands of developing new antimicrobial agents to combat pathogenic organisms with multiple drug resistance are increasing in recent years [3,4].

To control the offensive use of classical antibiotics in food and feed, applications of some bacterial peptides with antimicrobial potential are considered nowadays as a suitable alternative. Among them,

bacteriocins are highly important due to their high therapeutic indices and nontoxic nature. Bacteriocins produced by lactic acid bacteria (LAB) are ribosomally synthesized, small thermostable or large thermolabile proteins or protein complexes that display antimicrobial properties against other bacteria even closely related to the producer strain [5]. Most of the LAB are as generally recognized as safe (GRAS) by the United States Food and Drug Administration's (USFDA). They exist in most of the fermented foods either as the natural microflora or as starter cultures added under controlled conditions. Bacteriocins are of interest for prospective applications in the food industries because of their potential to increase safety and to extend shelf life of food [6]. Antimicrobial potential of bacteriocin could also be used as a therapy for the cure of human ulcer disease, which links with colonization by *Helicobacter pylori*, a Gram negative bacterium. *Helicobacter pylori* was found to be sensitive to nisin produced by *Lactococcus lactis* *in vitro* in the presence of chelator [7]. In addition to nisin, pediocin produced by *Pediococcus acidilactici* also showed good potential as a therapeutic

Peer review under responsibility of Journal Annals of Agrarian Science.

* Corresponding author.

E-mail addresses: vb.somabarman@gmail.com (S. Barman), vbranjana@gmail.com (R. Ghosh), mandalnc@visva-bharati.ac.in, mandalnc@rediffmail.com (N.C. Mandal).

<https://doi.org/10.1016/j.aasci.2018.05.004>

Received 25 August 2017; Received in revised form 11 May 2018; Accepted 12 May 2018

1512-1887/ © 2018 Published by Elsevier B.V. on behalf of Agricultural University of Georgia This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

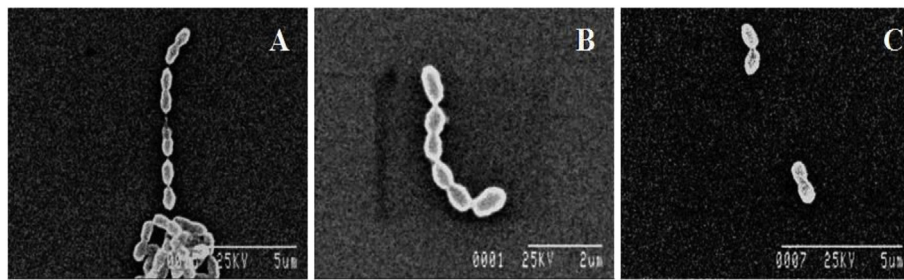


Fig. 1. Scanning electron micrograph of the isolated LAB strains. A. *Lactococcus lactis* subsp. *hordniae* LABW1, B. *Lactococcus lactis* subsp. *hordniae* LABW3, C. *Lactococcus lactis* subsp. *lactis* LABW4.

Table 1

Morphological and biochemical properties of isolated LAB strains.

Characteristics	Results		
	LABW1	LABW3	LABW4
Colony morphology	Small, creamish white, round, smooth	Small, creamish white, round, smooth	Small, creamish white, round, smooth
Shape	Coccioid	Coccioid	Coccioid
Gram nature	Gram positive	Gram positive	Gram positive
Cell size (nm)	0.68–0.75 × 1.28–1.36	0.57–0.60 × 0.86–0.99	0.68–0.73 × 0.90–1.02
Optimum growth temperature	28 °C	28 °C	28 °C
Motility	Non-motile	Non-motile	Non-motile
Catalase	–	–	–
Nitrate reductase	+	+	+
MR-VP	+	+	+
Indole	–	–	–
Urease	+	+	+
CO ₂ Production	–	–	–
Gelatin	–	–	–
Lactose	+	+	+
Xylose	–	–	–
Maltose	+	+	+
Fructose	+	+	+
Dextrose	+	+	+
Galactose	–	–	–
Raffinose	–	–	–
Trehalose	+	+	+
Melibiose	–	–	–
Sucrose	+	+	+
D-Arabinose	–	–	+
Mannose	+	+	+
Inulin	+	+	+
Cellobiose	+	+	+
Mannitol	–	–	+
Salicin	+	+	+
Esculin hydrolysis	+	+	+

‘+’ = positive reaction; ‘–’ = negative reaction.

agent in the treatment of bovine mastitis, topical skin infections and multiple-drug-resistant systemic infections [8,9]. On the other hand, a nisin-based mouth rinse appeared to prevent both the build-up of plaque and gingival inflammation in beagle dogs [10].

Although several LAB strains have been reported to produce antimicrobial bacteriocin but the production of bacteriocins is dependent on multiple factors such as potent LAB strain, pH, temperature, medium compositions and bacterial growth conditions. In recent years, studies are focused on isolation of potent LAB strains and optimization of bacteriocin production conditions to achieve maximum bacteriocin production for individual strains [11–16]. In the present endeavor potent LAB strains were isolated from good quality homemade buttermilk with effective bacteriocin producing capabilities and antimicrobial potential. In addition attempts were also taken to find out the optimized condition for maximum bacteriocin production as well as to find out their probable mode of action against pathogenic microorganisms.

Materials and methods

Bacterial strains and media

The LAB strains were cultured in *Lactobacillus* MRS medium (HiMedia, Mumbai, India) [17] and were maintained in 10% glycerol-skimmed milk at 4 °C. Bacteriocin sensitive indicator organism *Enterococcus faecalis* MB1 was kindly provided by Prof. Bibek Ray, Wyoming University, USA. Pathogenic bacterial strains used in anti-bacterial assay (Tables 3 and 4) were procured from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India and were maintained in nutrient agar medium (HiMedia, Mumbai, India) in slants at 4 °C as per MTCC guidelines.

Isolation of LAB

The LAB strains were isolated from homemade naturally fermented milk product (buttermilk) on *Lactobacillus* MRS [17] (HiMedia, Mumbai, India) agar plates after serial dilutions. Plates were incubated

Download English Version:

<https://daneshyari.com/en/article/10226644>

Download Persian Version:

<https://daneshyari.com/article/10226644>

[Daneshyari.com](https://daneshyari.com)