

RESEARCH ARTICLE

Production and optimization of bacteriocin from *Lactococcus lactis*

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Abstract

An extracellular bacteriocin producing strain was isolated from yoghurt sample collected from local super market. The isolate was identified as *Lactococcus lactis* by 16S-rDNA sequence analysis. Experiments were set to observe the effects of various pH, temperature, incubation time, carbon and nitrogen sources on the growth of the isolate and its bacteriocin activity. Optimum biomass and bacteriocin activity was achieved after 96 h of incubation, at 30°C and pH 6. Among the various nitrogen sources investigated peptone was found to be the best inducer of bacteriocin, among the carbon sources tested, xylose maximized the bacteriocin production. Thus, *L. lactis* was proved to be an addition to the existing pool of extremophilic bacteria of industrial importance.

Keywords: Bacteriocin, yoghurt, *Lactococcus lactis*, pH, temperature, incubation time, carbon, nitrogen.

Introduction

Bacteriocins are the natural antimicrobial peptides produced by bacteria belonging to different taxonomic branches. The production of such antibiotic peptides is a common defense strategy against bacteria. This specific defence mechanism is not only possessed by microorganisms, but also by animals and plants (Chin *et al.*, 2001). Since bacteriocins are proteinecious substances, they were inactivated by variety of proteolytic enzymes. The pattern of protease sensitivity of the bacteriocin helps in their classification. The bacteriocin possess various advantages such as they improves the nutritional quality of the food, stabilizes the intestinal microflora by reducing pathogens, protects against intestinal and Urinary tract infections and also acts as preservative (Garneau *et al.*, 2002). Lactic acid bacteria are the gram positive, non-sporulating bacteria hold a prominent place in the production of bacteriocins. Phylogenetically, lactic acid bacteria belong to the gram positive *Clostridium-Bacillus* sub-class. Traditionally, the lactic acid bacteria have been used as starter cultures for the fermentation of various foods and beverages since they contribute to flavour and aroma and retard spoilage (Hechard and Sahl, 2002). Lactic acid bacteria are non-pathogenic grows rapidly, requires very cheap substrates. Hence, lactic acid bacteria were used as industrial microorganisms for the synthesis of various chemicals, pharmaceuticals, and other products useful to humans (Jack *et al.*, 1995). In recent years, the researchers paid much attention to isolate large variety of bacteriocins producing lactic acid bacteria from different sources, for their applications as food bio-preservatives, since they can be degraded only by gastrointestinal protease (Jamuna and Jeevaratnam, 2004).

Although, lactic acid bacteria show a high impact on effective protection to human health, there is obvious evidence that lactic acid bacteria from different sources possess antimicrobial properties at different extent. Against these backdrops, this study deals with the isolation bacteriocins producing LAB isolates from yoghurt and also to evaluate the production of these bacteriocins under different physical and cultural conditions like pH, temperature, carbon and nitrogen sources.

Materials and methods

Isolation and screening for lactic acid bacteria: Yoghurt samples were collected from various super markets in Chennai district, Tamil Nadu. The collected samples were suspended and 1g of sample was serially diluted up to 10⁻⁷ concentration. About 100 µL of each dilution were spread on MRS agar plates and then incubated at 37°C for 24 h. The isolated colonies were sub-cultured and stored at 4°C until further use.

Detection/assay of bacteriocin activity: The isolated LABs were screened for antimicrobial activity against indicator strain, *Micrococcus luteus* using agar well diffusion method on MRS agar plates (Utar Yaakoubi *et al.*, 2009). Indicator bacteria was cultured in nutrient broth for 24 h at 37°C and seeded onto the surface of MRS agar plates. Wells were created on the seeded MRS agar plates and 30 µL of LAB culture supernatants obtained through centrifugation was poured to each well. The plates were observed for the presence of zone of inhibition (mm) of the indicator bacteria.

Identification of bacteria: The bacterial isolates were presumptively identified by means of morphological examination and preliminary biochemical characterizations. The plates were stained by gram staining and endospore staining for identification of bacterial. The biochemical parameters investigated included indole test, methyl red test, Vogues-Proskauer test, citrate utilization test, catalase test, oxidase test, motility test, by standards methods (Noonpakdee *et al.*, 2003). The 16S-rDNA analysis was conducted in order to investigate the species of the potential isolate based on molecular assay. The isolation of genomic DNA from the isolate was done with CTAB method followed by the amplification of 16S-rRNA gene was done by PCR using specific primers (63f 5'-CAGGCCTAACACATGCAAGTC-3' and 1387r 5'-GGGCGGWGTGTACAAGGC-3') (Wahyudi *et al.*, 2010). The PCR product was purified and sequenced. Similarity of the 16S-rRNA sequence was aligned against GenBank database by using BLASTN program.

Production of bacteriocins: The mass production of bacteriocin by the isolate was carried out by submerged fermentation. The sterilized production media (composition (g/L): Peptone; 10.0, Meat extract; 10.0, Yeast extract; 05.0, D-glucose; 20.0, Tween-80; 01.0, K₂HPO₄; 02.0, Sodium acetate; 05.0, Tri-ammonium citrate; 02.0, MgSO₄.7H₂O; 0.2, MnSO₄.4H₂O; 0.05) (Mollendorff *et al.*, 2009) was inoculated with 1 mL of bacterial culture and incubated in a rotary shaker for 48 h at 4°C.

Optimization of culture conditions on bacteriocin activity: In order to determine the effects of physio-chemical parameters on bacteriocins production, the selected bacterial isolate was grown in production medium and incubated at various parameters. The influence of all factors on bacteriocins activity was determined by measuring its activity at varying pH values from 5.5 to 7.5 and temperature varying from 25 to 45°C and incubation period varying from 12 to 72 h. Both carbon and nitrogen sources have wide variety of impacts on the various organisms and enzyme production. Carbon and nitrogen sources have been replaced with various substances. Carbon sources such as fructose, glucose, lactose, maltose, xylose and sucrose and nitrogen sources such as peptone, casein, sodium nitrate, potassium nitrate, ammonium sulphate and urea. All factors influencing bacteriocin activity was determined by measuring zone of inhibition of indicator strain by the cell free extract of production medium. The bacterial biomass was determined by measuring the growth in the production medium at 600 nm in spectrometer (Beckman DU-64) (Henroette *et al.*, 1993).

Results and discussion

This study deals with the isolation, characterization and optimization of bacteriocin produced by *Lactococcus lactis* from yoghurt. Lactic acid bacteria were sought from the yoghurt samples collected from Chennai, TN.

Fig. 1. Isolation of bacteria using MRS agar plates.



Fig. 2. Effect of incubation time on bacteriocin production.

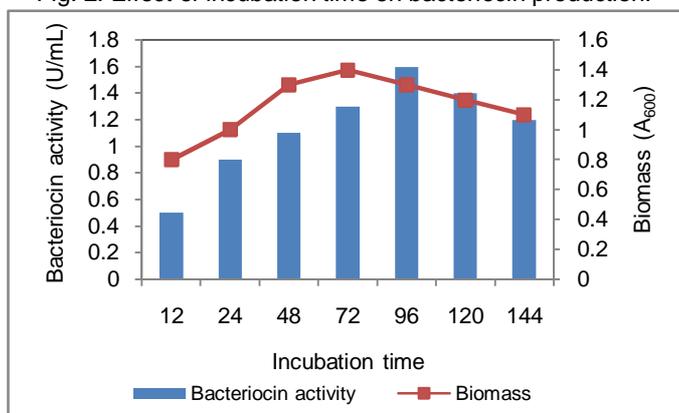


Fig. 3. Effect of temperature on bacteriocin production.

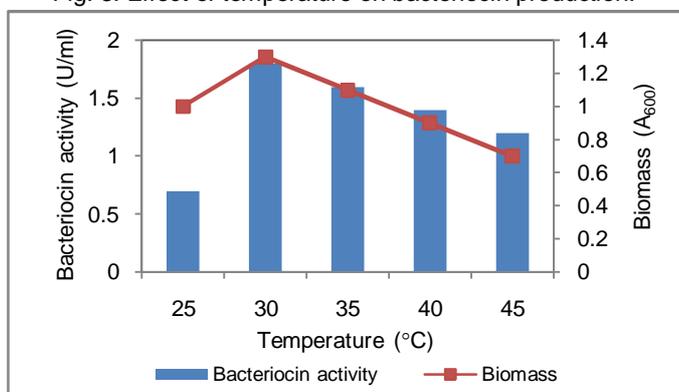
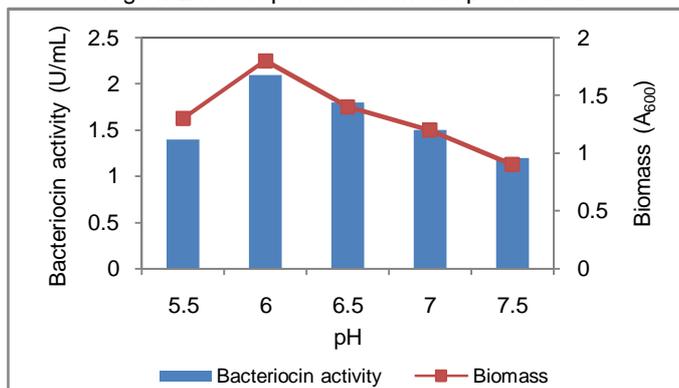


Fig. 4. Effect of pH on bacteriocin production.



Appropriate dilutions of each sample were placed on MRS agar plates (Fig. 1). Creamy white clones showing good colonial development was transferred to fresh MRS plates. A total of 13 LAB colonies were thus selected in the first round of screening. Their bacteriocin activities were confirmed by the agar well diffusion method on MRS agar medium against Indicator bacteria (*Micrococcus luteus*).

The bacteriocins activities were examined and compared among other strains isolated. Finally, the strain showing relatively higher bacteriocins activity was selected. The inhibitory effect demonstrated by isolate against the indicator bacteria is an indication of possession of antibacterial activity. Results also revealed the presence of the bacteriocin in the test isolate. Bacteriocins have been well reported to possess inhibitory activity against several bacteria (Todorov and Dicks, 2005). The production of bacteriocin the bacteria indicates its application as probiotic and as bio-preservative. Morphological and cultural studies revealed that the isolate were gram positive and cocci shaped bacteria. It was also confirmed as catalase-positive, aerobic, moderate thermophiles. The bacterial isolate was finally identified by 16S-rRNA gene sequence analysis. The amplified 16S-rDNA sequence was entered into the nucleotide-nucleotide BLAST (NCBI) system, and percentage identities were established. The highest identity for the isolate was found to be as 100% with *L. lactis*. Based on their morphological, physiological, and genetic data, the bacterial isolate was designated as *L. lactis*.

The production medium should be optimized for the better production of bacteriocin. The parameters such as incubation time, pH, temperature, carbon and nitrogen sources were considered to be optimized primarily as reported by many researchers (Trinetta *et al.*, 2008; Wiese *et al.*, 2010). *Lactococcus lactis* was tested for its growth under various parameters like temperature, pH, carbon and nitrogen sources. pH and temperature played an important role in cell growth and bacteriocin production. The bacteriocin activity was tested with different temperatures and maximum bacteriocin activity was recorded during 96th h of incubation (Fig. 2) at 30°C (Fig. 3). The maximum bacteriocin activity was recorded at pH 6 (Fig. 4).

Bacteriocin production strongly depends on pH, nutrients source and temperature (Todorov and Dicks, 2004). Among the various carbon sources tested, xylose was found to be supports the best bacteriocin production (Fig. 5). It was also observed that the production of bacteriocin was not greatly affected other carbon sources. In addition, good bacteriocin activity was also observed in the media supplemented with glucose. Such enhancement of bacteriocin using glucose (Bing Han *et al.*, 2011; Meera and Charitha Devi, 2012) has been reported earlier.

Fig. 5. Effect of carbon source on bacteriocin production.

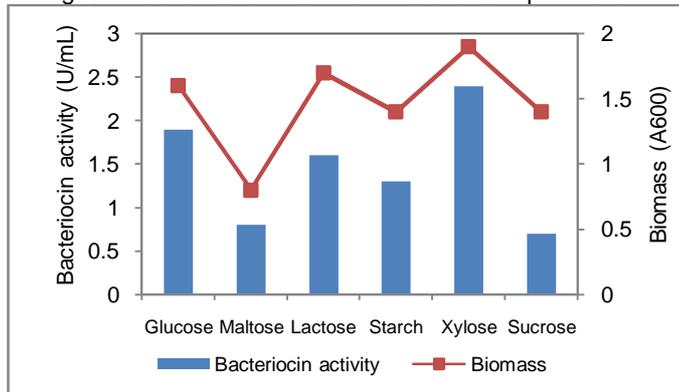
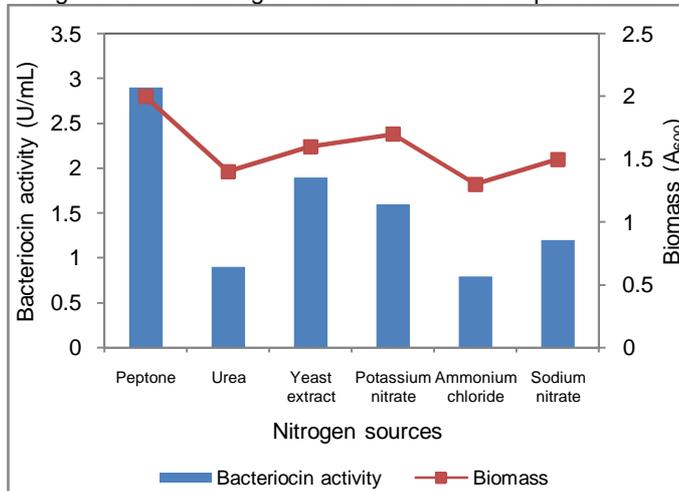


Fig. 6. Effect of nitrogen source on bacteriocin production.



The repression effect of sucrose was consistent with the findings of the study on bacteriocin production. Among the used nitrogen sources, peptone was found to have significant effect on the production of the bacteriocin (Fig. 6). Ammonium chloride exhibited poor effect on the bacteriocin production in *L. lactis*. Inorganic nitrogen sources caused significant reduction in the bacteriocin yields. In some earlier reports, it was found that different nitrogen sources such as yeast extract (Bing Han *et al.*, 2011; Meera and Charitha Devi, 2012) were effective ingredients for the bacteriocin production.

Conclusion

The bacteriocin produced from *Lactococcus lactis* can be considered as a possible candidate for the cost-effective due to use of inexpensive substrates such as lactose and peptone. Considering the characteristics of *L. lactis*, and its bacteriocin activity in a wide range of pH, this strain could be a potential source of bacteriocin to be used as additive in bio-preservative formulation. The production process can be commercialized for enhanced bacteriocin production and its characterization in future.

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