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**Isolation and Utilization of Bacteriocin-Producing Lactic  
Acid Bacteria as a Bio-Preservative Agent in Soft Cheese**

**By**

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## APPROVAL SHEET

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**/12/2017**

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## ABSTRACT

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### Abstract

Isolation and utilization of bacteriocin-producing lactic acid bacteria (LAB) as a bio-preservative agent in soft cheese was studied. The results exerted that thirty-four isolates from 514 showed antimicrobial activity against *Lactobacillus bulgaricus* 340 after incubation for 24 hrs at 37 °C. Moreover, five isolates were active against *Listeria monocytogenes* EGDEe107776. Molecular biology of the five LAB isolates, that had antimicrobial activity against *Listeria monocytogenes* EGDEe107776 were identified as *Lactococcus lactis* subsp. *lactis* A15, *Lactococcus lactis* subsp. *lactis* A16, *Enterococcus faecium* A15, *Enterococcus faecium* A16 and *Enterococcus faecium* A17 by 16S rRNA gene sequences. *Lactococcus lactis* subsp. *lactis* A15 had produced only bacteriocin while *Enterococcus faecium* A15 produced other antimicrobial substances, such as H<sub>2</sub>O<sub>2</sub> beside bacteriocin. *Lactococcus lactis* subsp. *lactis* A15 and *Enterococcus faecium* A15 were more active between pH 5 and 8 and that activity was decreased at pH 2 and pH 10.

The effect of heat treatment and time on the bacteriocin activity showed no effect up to 100 °C for 10 min. in case of *Lactococcus lactis* subsp. *lactis* A15 and 20 min. for *Enterococcus faecium* A15. Maximum bacteriocin activity produced by *Lactococcus lactis* subsp. *lactis* A15 was observed at the end of logarithmic phase (at 37 °C for 11 hrs) and still stable until the end of incubation (48 hrs). On the other hand, maximum bacteriocin production for *Enterococcus faecium* A15 was clear in the logarithmic phase at 37 °C for 6 hrs, and consequently decreased until 24 hrs of incubation. Structural gene study of the produced bacteriocin for *Lactococcus lactis* subsp. *lactis* A15 and *Enterococcus faecium* A15 was defined as nisin Z and enterocin B, respectively. PCR analysis and haemolytic activity did not show presence of the (Cytolysin A) *cyl A* gene and  $\gamma$ -haemolytic for both. The results also, showed no gelatin lysis by *Lactococcus lactis* subsp. *lactis* A15 but had gelatin lysis by *Enterococcus faecium* A15. The effect of bacteriocinogenic strains *Lactococcus lactis* subsp. *lactis* A15 and *Enterococcus faecium* A15 as protective cultures to control growth of pathogenic bacteria (*Listeria monocytogenes*) in UHT milk, was more efficient on BHI broth media. In all cases, the number of *Listeria monocytogenes* was increased after 8 hrs when purified bacteriocin was used. The resultant white soft cheese acceptability and quality was significantly improved by both *Lactococcus lactis* subsp. *lactis* A15 strain and enterocin when compared with control cheese.

It can be concluded that *Lactococcus lactis* subsp. *lactis* A15 can be used as a culture or co-culture for improving white soft cheese quality. Moreover, enterocin produced by *Enterococcus faecium* A15 can be also used as a bio-preservative agent for improving cheese quality.

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