

PURIFICATION AND CHARACTERIZATION OF NISIN PRODUCED BY LACTOCOCCUS LACTIS ISOLATED FROM INDIAN CURD

Saba A. Mahdy*, Qusay J. Rasheed** and P.T. Kalaichelvan*

* Centre for Advanced Studies in Botany, Guindy Campus, University of Madras, Chennai 600 025, Tamil Nadu, India

**Department of Chemical Engineering, University of Technology, Baghdad, Iraq

* Corresponding author Email: engineerqusay@yahoo.com

ABSTRACT: *Lactococcus lactis* isolated from traditional dairy Indian curd. Strains were preliminarily identified by PCR analysis and partial 16S rRNA confirmed that N5 were 100% identical to *Lactococcus lactis* sp. *lactis*. The results revealed that only the bacteriocin produced from strain N5 was shown as being active against mostly gram positive bacteria. The bacteriocin produced purified by precipitation followed by loading with gel chromatography. The partially purified bacteriocin was found to be stable over a wide range of pH, temperature and enzymes. The molecular weight of the peptide was judged to be 3.5 kDa by SDS-polyacrylamide gel electrophoresis and conform to the result of mass spectrometry by maldi-tof test which calculated the mass of 3354.07 Da for nisin. These results indicate that bacteriocin produced by *L. lactis* sp. *lactis* N5 is a nisin.

Keywords: Food pathogens, lactic acid bacteria, bacteriocins, nisin.

INTRODUCTION

LAB have for centuries been responsible for the fermentative processing and preservation of many food products including dairy, meat, vegetables and bakery products¹. *Lactococcus lactis* has been traditionally used as starter in the manufacture of cheese and fermented milk products on account of their function of preservation and contribution to flavor and aroma. Selected strains are used as combined cultures, single or as mixture of single cultures. Preservation of fermented foods is due primarily to the conversion of sugars in organic acids with a concomitant lowering of the pH and removal of large amounts of carbohydrates as nutrient sources. These effects extend the shelf life and safety of the final product^{2,3,4}.

Nisin, which is secreted by *Lactococcus lactis* and⁵ although nisin has been used as a food preservative for more than fifty years, no significant bacterial resistance against nisin has been reported⁶. Nisin is one of several ribosomally synthesized small protein antibiotics that contain dehydro residues (dehydroalanine [DHA] and dehydrobutyryne [DHB] and thioether cross-linkages (lanthionine and, B-methylanthionine) that are introduced by posttranslational modifications of ordinary amino acids (serine, threonine, and cysteine). Although these antibiotics are produced by a disparate group of gram-positive bacteria^{7,8}. Nowadays, production of highly purified nisin preparations and enhancement by chelators has led to interest in the use of nisin for human ulcer therapy and mastitis control in the cattle⁹.

Nisin from *L. lactis* can be purified directly from the culture medium^{10,11}. It is a cationic peptide and therefore commonly purified using gel permeable chromatography at acidic pH, using high salt concentration for elution, typically a single step elution with 1M NaCl^{12,13, and 14}.

Nisin shows promising activity towards clinical isolates of the Methicillin resistant *Staphylococcus aureus* (MRSA) bacterium, *Streptococcus pyogenes* and several of the most severe human pathogens, including the multi-resistant *Streptococcus pneumoniae* and vancomycin-resistant *Ent. faecium* or *Ent. faecalis*, against which new effective antibiotics are most urgently needed^{15,16}. In all these studies purified nisin was used and the bactericidal activity of nisin was measured by we describe the isolation and detection of nisin from *L. lactis* strain N5. Nisin was tested for bactericidal activity using the nisin-sensitive strains through all steps of purification.