

Characterization and optimization of bacteriocin from *Lactobacillus plantarum* isolated from fermented beef

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Abstract

Many carboxylic acid bacteria (LAB) were isolated from 'Shermout', a well-liked Sudanese fermented beef product intended for long storage. An isolate that demonstrated significant antibacterial activity was identified *Lactobacillus plantarum*

PM4 supported phenotypic, physiological and biochemical characteristics and carbohydrate utilization patterns. The inhibitory activity of the partially purified bacteriocin was completely arrested by the proteolytic enzymes proteinase-k and pepsin but not by α -amylase, asserting its proteinaceous nature. The activity wasn't thanks to H₂O₂ as similar inhibition was obtained by cell-free supernatant (CFS) produced under anaerobic conditions. The bacteriocin showed a relative molecular mass within the range of 3-5 kDa and had a bactericidal mode of action. No significant reduction in activity was observed on heating at 60°C for 60 min, but activity was lost on heating at 100°C or autoclaving. Highest inhibitory activity was at pH 5.5 and there was appreciable reduction in activity at pH 3, 7 or 9. There was no drop-in activity at -80 or -20°C up to four weeks of storage. However, at 4 and 35°C a gradual decline in activity was observed. *L. plantarum* PM4 exhibited bactericidal activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli* ATCC25922, *Klebsiella pneumoniae* and *Proteus vulgaris*. Bacteriocin production generally coincided with the phase of maximum growth and therefore the best combination for max production of inhibitory activity was at pH 5.5 for 48 hours, further incubation at 25, 30 or 37°C. *L. plantarum* PM4 showed promise as a starter culture within the fermentation of preserved meat products.

Lactic acid bacteria (LAB) may be a group of Gram positive facultative anaerobic bacteria that are ready to produce antagonistic molecules in their growth medium which will be used as antimicrobials and preservatives. These antagonistic properties of LAB are allied to their safe history of use in traditional fermented food products that make them very attractive as biopreservatives which will replace or allow reduction of chemical additives. LAB is employed in food biopreservation because they're safe for human consumption enjoying the status of GRAS (Generally Recognized as Safe) and are the prevalent indigenous microflora in many foods. Accordingly, a good sort of LAB strains are routinely employed as starter cultures within the manufacture of meat, dairy, vegetable and bakery products. one among the foremost important contributions of

those bacteria—whether indigenous or added as starters—is the extension of time period of the fermented products through inhibition of the expansion of spoilage and pathogenic bacteria in these foods thanks to competition for nutrients and therefore the presence of the antagonistic molecules like carboxylic acid, peroxide, diacetyl and bacteriocins. Moreover, health benefits acclaimed to be offered by LAB include production of vitamins, immunomodulation, reduction within the risk of diarrhea, and a decrease in serum cholesterol. Among the antagonistic molecules produced by LAB are bacteriocins which are antimicrobial peptides or proteins produced by strains of diverse bacterial species. The antimicrobial activity of this group of natural substances against foodborne pathogens, also as spoilage bacteria, has raised considerable interest for his or her application in food preservation.

Within LAB, the lactobacilli are an important group recognized for their fermentative ability as well as health and nutritional benefits. In this group, *Lactobacillus plantarum* is one of the most widely distributed in nature, and is one of the most versatile species, used both as starter and probiotic. *Lb. plantarum* has been isolated from various habitats, and bacteriocins have been described for strains from fermented meat products. It is one of the most important LAB strains used for the production of fermented meat products. Over the past few decades, there has been an increasing research interest in the development of nitrite-free meat curing systems. The principle concern with the use of nitrite for curing of meat is the eventual formation of carcinogenic N-nitrosamines. Consumers are increasingly demanding food that is free from pathogens, with minimal processing and fewer chemical preservatives and additives. Thus biopreservation has gained increasing attention as means of naturally controlling the shelf life and safety of meat products. In recent years bacteriocins of lactic acid bacteria have attracted the attention of many investigators because of their use as a natural food preservative with probiotic capability within the human body after ingestion of food.

Shermout is sun-dried lean beef strips widely used for prolonged storage. It has unique sensory characteristics and is very popular in Sudan and neighboring countries. It is very similar to "kaddid" and jerky except that no or little salt is added and the product undergoes mild fermentation by indigenous microbial flora, mainly LAB. The process is artisanal in nature, with no bacterial starters added and is usually subject to microbial deterioration. Various gram positive and gram negative

bacteria like *Salmonella typhi*, *Bacillus subtilis* and staphylococci are the main causative organisms. The objectives of this study were the isolation and identification of *Lb. plantarum* from local Sudanese fermented beef (shermout), characterization of the bacteriocin it produces and determination of its antibacterial activity, study of the bacteriocin kinetics and determination of the optimum growth conditions for bacteriocin production. Ten g of traditional Sudanese fermented beef (shermout) samples were aseptically added to 90 mL sterile peptone water (10 g peptone/L distilled H₂O), carefully shaken and were left to homogenize for 1 h. Serial decimal dilutions were prepared from the sample homogenate, and were streaked onto duplicate plates of MRS medium to which 0.1% (w/v) nystatin had been added to inhibit fungal growth. The streaked plates were incubated anaerobically at 30°C for 2 - 3 days in an anaerobic jar system (GasPak; BBL Microbiology Systems, Cock-

eysville, Maryland, USA) with a gas-generating kit (BR0038B, Oxoid, Hampshire, UK). The pure colonies obtained were examined for Gram reaction, catalase activity, and spore formation. A Gram-positive, catalase-negative, non-spore-forming rod was selected and identified as *Lactobacillus plantarum* by use of the fermentation pattern from KB009 HiCarbohydrate identification kit (HiMedia Laboratories, Mumbai, India) in conjunction with other tests which included growth at 10 and 45 °C, tolerance of 6.5% NaCl, pH (4.4 and 9.6) and gas production from glucose.

Biography

Idress Hamad Attitalla has a Medical Degree from the Federal University of Sao Carlos and is a former resident in Internal Medicine from Santa Casa de Sao Paulo, Brazil. She is now applying for Medical Residency in the United States.

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