

Comparative Characteristics of Bacteriocins from Endemic Lactic Acid Bacteria

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Abstract : Introduction: Globalization of the food supply has created the conditions favorable for the emergence and spread of food-borne and especially dangerous pathogens (EDP) in developing countries. The fresh-cut fruit and vegetable industry is searching for alternatives to replace chemical treatments with biopreservative approaches that ensure the safety of the processed foods product. Antimicrobial compounds of lactic acid bacteria (LAB) possess bactericidal or bacteriostatic activity against intestinal pathogens, spoilage organisms and food-borne pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus* and *Salmonella*. Endemic strains of LAB were isolated. The strains, showing broad spectrum of antimicrobial activity against food spoiling microorganisms, were selected. The genotyping by 16S rRNA sequencing, GS-PCR, RAPD PCR methods showed that they were presented by *Lactobacillus rhamnosus* 109, *L. plantarum* 65, *L. plantarum* 66 and *Enterococcus faecium* 64 species. LAB are deposited in "Microbial Depository Center" (MDC) SPC "Armbiotechnology". Methods: LAB strains were isolated from different dairy products from rural households from the highland regions of Armenia. Serially diluted samples were spread on MRS (Merck, Germany) and hydrolyzed milk agar (1,2 % w/v). Single colonies from each LAB were individually inoculated in liquid MRS medium and incubated at 37°C for 24 hours. Culture broth with biomass was centrifuged at 10,000 g during 20 min for obtaining of cell free culture broth (CFC). The antimicrobial substances from CFC broth were purified by the combination of adsorption-desorption and ion-exchange chromatography methods. Separation of bacteriocins was performed using a HPLC method on "Avex ODS" C18 column. Mass analysis of peptides recorded on the device API 4000 in the electron ionization mode. The spot-on-lawn method on the test culture plated in the solid medium was applied. The antimicrobial activity is expressed in arbitrary units (AU/ml). Results. Purification of CFC broth of LAB allowed to obtain partially purified antimicrobial preparations which contains bacteriocins with broad spectrum of antimicrobial activity. Investigation of their main biochemical properties shown, that inhibitory activity of preparations is partially reduced after treatment with proteinase K, trypsin, pepsin, suggesting a proteinaceous nature of bacteriocin-like substances containing in CFC broth. Preparations preserved their activity after heat treatment (50-121 °C, 20 min) and were stable in the pH range 3-8. The results of SDS PAAG electrophoresis show that *L. plantarum* 66 and *Ent. faecium* 64 strains have one bacteriocin (BCN) with maximal antimicrobial activity with approximate molecular weight 2.0-3.0 kDa. From *L. rhamnosus* 109 two BCNs were obtained. Mass spectral analysis indicates that these bacteriocins have peptide bonds and molecular weight of BCN 1 and BCN 2 are approximately 1.5 kDa and 700 Da. Discussion: Thus, our experimental data shown, that isolated endemic strains of LAB are able to produce bacteriocins with high and different inhibitory activity against broad spectrum of microorganisms of different taxonomic group, such as *Salmonella* sp., *Escherichia coli*, *Bacillus* sp., *L. monocytogenes*, *Proteus mirabilis*, *Staph. aureus*, *Ps. aeruginosa*. Obtained results proved the perspectives for use of endemic strains in the preservation of foodstuffs. Acknowledgments: This work was realized with financial support of the Project Global Initiatives for Proliferation Prevention (GIPP) T2- 298, ISTC A-1866.

Keywords : antimicrobial activity, bacteriocins, endemic strains, food safety

Conference Title : ICFSN 2015 : International Conference on Food Security and Nutrition

Conference Location : Athens, Greece

Conference Dates : July 20-21, 2015