ABSTRACT

PhD Thesis

CHARACTERIZATION AND PURIFICATION OF BACTERIOCINS PRODUCED BY THERMOPHILIC BACTERIA ISOLATED FROM VARIOUS NATURAL SPRINGS

Gamze BAŞBÜLBÜL

Adnan Menderes Üniversity Graduate School of Naturel and Applied Sciences Department of Biology

Supervisor: Asst. Prof. Dr. H.Halil BIYIK

In this study thermophilic bacteria were isolated from water, soil and mud samples from various natural hot springs around Aydin and the other provinces around. Two strains with broadest antimicrobial spectrum were selected among 208 isolates and isolation, characterization and purification of their bacteriocins achieved. According to their 16S rRNA sequences, it was found that both of two isolates showed maximum similarity (99 %) with *Geobacillus toebii*. Antibacterial substances produced by isolates were found to be effective especially against Gram positive bacteria, they didn't effect any of Gram negative bacteria used in this study. Culture supernatants from isolates are found to be sensitive to proteolytic enzymes.

It was determined that production of bacteriocin for HBB-218 starts at logarithmic growth phase and it reaches maximum level at the end of the stationary phase. Optimum bacteriocin production were observed at 60 °C and pH 6.0. Soja peptone was found to be best nitrogen source for bacteriocin production among the other sources, while galactose was found to be best sugar source. Bacteriocin from HBB-218 was purified to homogenity by using, ammonium sulphate precipitation, dialyse, gel filtration and anion exchange chromotography. Molecular weight of the bacteriocin produced by strain HBB-218 was determined by Tricine SDS-PAGE method and it was found about 5.5 kDa.

The bacteriocin of HBB-247 was found to be produce at logarithmic growth phase and it reaches maximum level at the end of the logarithmic growth phase. Optimum bacteriocin production were observed at 60 °C and pH 6.5. Maximum bacteriocin production were determined in the presence of soja peptone and fructose. Proteins obtained from anion exchange chromotography were runned electrophoretically and two protein bants were detected. Molecular weight of the bacteriocins produced by strain HBB-247 was found about 33 and 42 kDa by Tricine SDS-PAGE method.

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Keywords

Bacteriocin, Geobacillus, isolation, optimization