



Molecular biological and biochemical characteristics of extracellular proteases of thermophilic bacterial strains

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Abstract

The use of individual and complex enzyme preparations, including proteolytic action, is one of the most important parts of industrial technologies, molecular biological research, medical diagnostics and therapy. Microorganisms attract particular attention as sources of enzymes, since they differ in great diversity, simple cultivation and susceptibility to genetic modification as compared to sources of plant and animal origin. Enzymes isolated from thermophilic bacteria are characterized by a suitable set of technological properties, such as thermal stability, resistance to chemical agents and to extreme pH values. For example, *Bacillus* proteases have high efficiency and wide substrate specificity and are successfully used in detergent industry, pharmaceutical, food and feed industry. In this work a study of the profile of proteases secreted by thermophilic bacteria strains was carried out, as well as a search for genes responsible for the synthesis of extracellular proteolytic enzymes. It was stated that thermophilic strain 8, strain 33 and strain 35, previously identified as bacteria of the genus *Bacillus*, secrete a broad range of proteases in range of 15 to 63 kDa, among which were found enzymes that are capable to hydrolyze gelatin, casein and hemoglobin with different efficiency. During the inhibitory analysis, serine proteases in range of 33.5-57.0 kDa and a metalloprotease of 28.2 kDa were identified in the studied strains. Using real-time PCR, genetic screening of thermophilic strain 8, strain 33 and strain 35 for the presence of genes encoding extracellular proteolytic enzymes was carried out, as a result of which the genes responsible for production of bacillopeptidase F (*bpr*), subtilisin E (*aprE*), glutamyl-specific endopeptidase (*mpr*), and neutral zinc-dependent protease (*nprB*), which are widespread in bacteria *Bacillus subtilis* and related species, were found.

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