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UTILIZATION OF CHITINOUS SUBSTANCES FOR THE OPTIMIZED BIOPRODUCTION OF ANTIFUNGAL CHITINASE BY *Paenibacillus tylopili*

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Abstract

Chitin is the major source of natural organic compounds. This long biopolymer chain contains N-acetyl-D-glucosamine (GLcNAc) monomers forming covalent β-1,4 linkages. It is widely dispersed in the structural components of numerous organisms including crustacean and mollusc shells, arthropod exoskeletons as well as fungal cell walls. Chitin is created from marine food production waste, e.g. shrimp and crab shells or krill. Approximately 75% of the total weight of shellfish such as shrimp, crab and krill is considered waste, and chitin represents from 20% to 58% of the dry weight of this waste. Both degradation and recycling of chitin constitute an important phase in maintaining the global circulation of carbon and nitrogen. Chitinases play an important role in the decomposition of chitin and potentially in the utilization of chitin as a renewable resource, this is why they are widely applied.

Four *Bacillus* strains were screened from soil and rhizosphere in the centre of Poland on chitin medium. They were identified as *Bacillus licheniformis*, *Bacillus pumilus*, *Bacillus thuringiensis* and *Paenibacillus tylopili*. The identification was based on standard biochemical tests and analysis of the 16S rRNA gene sequence. The chitinase activity was detected after the second day: it increased gradually and reached its maximum after six days of cultivation. Shrimp shell waste was the finest inducer for the synthesis of chitinases. Under the experimental conditions tested, *Paenibacillus tylopil* was selected as the best enzyme producer. Chitinase was purified from a culture medium by fractionation with ammonium sulphate and chitin affinity chromatography. Purified proteins produced were subjected to identification by mass spectrometry. The molecular weight of the purified enzyme as determined by SDS-PAGE was approximately 55 kDa. The optimum temperature of the chitinase proved to be 45°C. The enzyme was characterised by thermostability at 40°C and 45°C during 180 minutes of preincubation. The activity of the enzyme was strongly inhibited in the presence of Hg^{2+} and Pb^{2+} and stabilized by the ions Mg^{2+}. Purified chitinase inhibited growth of fungal phytopathogenes: *Alternaria alternata*. Additionally, the crude chitinase inhibited the growth of potential phytopathogenes *Penicillium purpurogenum* and *Penillium* sp.