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Studies on β-1,3-glucanase and Chitinase from *Arthrobacter* sp. NHB-10

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Arthrobacter sp. NHB-10 was isolated as a bacterium degrading nigeran that is a water-insoluble D-glucan found in the hypal wall of fungi such as *Apergillus niger*. Recently the strain has lytic activity on *Asp. niger* and *Asp. japonicus* cells. As β-1,3-glucan and chitin are also the main structural components of fungal cell wall, β-1,3-glucanase [EC 3.2. 1.39] and chitinase [EC 3.2. 1.14] seem to be key enzymes in the lysis of the cell walls. This study describes purification, properties and gene cloning of β-1,3-glucanase and chtinase from *Arthrobacter* sp. NHB-10.

A β-1,3-glucanase was purified from the culture filtrate of *Arthrobacter* sp. NHB-10 by column chromatographies on DEAE-Sepharose, Superose 12 and Mono Q. The enzyme preparation showed a single band with a molecular mass of about 73,000 on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and with an isoelectric point of 3.0 on thin-layer agarose gel for electorofocusing. The purified enzyme specifically hydrolyzed the laminarin and its oligosaccharides (laminaritriose to laminariheptaose) by an endo-type action, but not laminaribiose and lichenan. The optimum pH and temperature for the enzyme activity were 6.5 and 50℃, respectively. The enzyme was stable in the pH range from 4.0 to 11.0 and up to 45℃.

A chitinase was purified from the culture filtrate of *Arthrobacter* sp. NHB-10 by precipitation with ammonium sulfate and column chromatographies on DEAE-Sephadex A-50 and Superose 12. The final preparation was homogenous in polyacrylamide gel electrophoresis. The molecular weight of the purified enzyme was 30,000 and its isoelectric point was 6.8. The optimum pH and temperature for the enzyme activity were 5.0 and 45℃, respectively. The enzyme was stable from pH 3 to 7 and up to 55℃. The enzyme activity was inhibited by Hg^{2+} and *p*-chloromercuribenzoic acid.

The N-terminal and internal amino acid sequences of the purified β-1,3-glucanase were Glu-Pro-Ala-Pro-Asp-Pro-Asp-Leu-Gly-Pro-Asn-Val-Val-Phe-Ile-Asp-Asp and Leu-Asp-Thr-Thr-Pro-Leu-Ser-Arg-Glu-Ala-Pro, respectively. Two internal amino acid sequences of the purified chitinase were Ala-Gly-Pro-Gln-Leu-Leu-Thr-Gly-Tyr-Tyr and Ile-Gly-Gly-Val-Met-Thr. On the basis of these amino acid sequences, several oligonucleotide probes were synthesized and then labeled with digoxigenin. Chromosomal DNA was prepared from *Arthrobacter* sp. NHB-10, partially digested with *Pst* I and then ligated into *Pst* I site of pUC 19. Southern hybridization of plasmids DNA from the transformants demonstrated several positive clones carrying β-1,3-glucanase and chitinase genes.