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# Molecular cloning, expression and purification of a novel chitinase from marine bacterium, *Pseudomonas* sp. BK-1

Moon-Sun Jang, Nam-Young Kang, Yong-Lark Choi, Young-Su Cho, Cheorl-Ho Kim[1] and Young-Choon Lee

Faculty of Biotechnology, Dong-A University, Busan 604-714, Korea
[1]Dept. of Bichemistry and Molecular Biology, College of Oriental Medicine, Dongguk University, Kyungbuk 780-714, Korea

The pchi gene encoding a novel chitinase (pchi) from a marine bacterium, *Pseudomonas* sp. BK-1 was cloned, expressed and purified. The chitinase gene (pchi) revealed a single open reading frame of pchi comprised of 1,605 nucleotide base pairs and 534 deduced amino acids with a molecular weight of 55.372 Da. The deduced amino acid sequence showed 32% and 26% overall homology to the chitinase from *Bacillus circulans* and *Pseudomonas aeruginosa*, respectively. The deduced pchi was a modular enzyme composed of a glycoside hydrolase family 18 catalytic domain that was responsible for the chitinase activity, the chitin binding domain and the carbohydrate binding module.

Open reading frame fragment was inserted into a expression vector, pET-29a(+). Recombinant pchi was overproduced in *Escherichia coli* BL21(DE3) in an insoluble form and the solubilized protein with 8M urea was purified by anion-exchange chromatography on DEAE-Sepharose in a urea-denatured form and refolded by removing urea. Finally, the refolded protein (pchi) was purified by gel fiteration chromatography.