

Molecular cloning and heterologous expression of phospholipase D from *Streptomyces* sp. YU100

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A phospholipase D (PLD) gene was cloned from the *Streptomyces* sp. YU100 genomic DNA library and heterologously expressed in *Escherichia coli*. Analysis of DNA sequence data revealed an open reading frame encoding a 540-amino acid protein that included 33-amino acid signal peptide. The deduced amino acid sequence of the cloned PLD was highly homologous to PLDs from *S. halstedii* and *S. septatus* with a homology of 77 and 75%, respectively, and showed the presence of highly conserved sequence motifs, namely the HKD motifs, which may be important in the catalytic function. The mature PLD gene was subcloned in a pET expression vector containing the pelB signal sequence to promote the secretion of the protein into the medium. The recombinant PLD was expressed in *E. coli* BL21(DE3)pLysS cells after induction with IPTG, detected in the culture supernatant by Western blot analysis, and purified by nickel affinity chromatography.

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References

1. Lim, S. K., J. W. Choi, E. T. Lee, Y. H. Khang, S. D. Kim, and D. H. Nam (2001) *J. Microbiol. Biotechnol.* **12**: 71-76.
2. Lim, S. K., J. W. Choi, M. H. Chung, E. T. Lee, Y. H. Khang, S. D. Kim, and D. H. Nam (2001) *J. Microbiol. Biotechnol.* **12**: 189-195.
3. Iwasaki, Y., Nakano, H, and Yamane, T. (1994) *Appl. Microbiol. Biotechnol.*, **42**: 290-299.

4. Hatanaka, T., Negidhi, T., Kubota-Akizawa, M., and Hagishita, T. (2002) *Enz. Microb. Technol.*, **31**: 233-241.
5. Yang, H. and Roberts, M. (2002) *Prot. Sci.*, **11**: 2958-2968.\
6. Ogino, C., Y. Negi, T. Matsumiya, K. Nakaoka, A. Kondo, S. Kuroda, S. Tokuyama, U. Kikkawa, T. Yamane, and H. Fukuda (1999) *J. Biochem.* **125**: 263-269.