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Recombinant expression and purification of functional XorI and XorII from *Xanthomonas oryzae* pv. *oryzae* (*Xoo*)

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Objectives

We have tried to produce 2 *Xoo* restriction endonuclease as recombinant proteins from *E. coli*. The first enzyme, XorI, has been known to bind PstI recognition site, but the actual cleavage site has not been revealed. The second enzyme, XorII, has been known as an isoschizomer of PvuI.

Materials and Methods

1. Material

   E.coli-Rosetta(DE3) harboring another plasmid, pLysS.

   DNA-*Xoo* chromosomal DNA, pET28a (Novagen)

2. Methods:

   The genes coding XorI and XorII were amplified, and inserted into pET28a vector. The resulting plasmids were brought into the expression host, Rosetta(DE3) harboring pLysS. The protein production was induced by adding IPTG. The recombinant proteins were purified by IMAC. The qualitative activity was measured by digesting Yep24 and pBluscriptKSII(+) by XorI and XorII, respectively.

Results and Discussion

The genes of two restriction endonucleases, XorI and XorII from *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), have been isolated and inserted into the expression vector, pET28a. The enzymes were successfully overexpressed in Rosetta(DE3) carrying another plasmid, pLysS, and purified by using the immobilized metal affinity chromatography. With enough column washing, the proteins were almost pure.The purified XorI and XorII were functional, and were shown to be the isoschizomers of PstI and PvuI, respectively. This work will provide better quality and quantity of the currently available commercial enzymes.

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