Note

Extracellular Production of Xylanase L in *Escherichia coli* using Excretion Vector pEAP2

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In the previous study, plasmid pEAP2 containing the penicillinase gene of alkalophilic *Bacillus* sp. strain 170 was constructed.¹⁾ It was found that most of the plasmid-encoded penicillinase was in the culture medium of *Escherichia coli* carrying the pEAP2^{1,2)} and the penicillinase excretion was caused by a change in the permeability of the outer membrane of *E. coli*.

In our laboratory, we have cloned several genes of alkalophilic microorganisms and expressed them in *E. coli*. Alkalophilic *Aeromonas* sp. strain 212 secreted xylanase L into the culture medium.³⁾ This xylanase L gene was cloned from alkalophilic *Aeromonas* sp. strain 212 by using pBR322 as a vector. The hybrid plasmid, pAX1, consisted of pBR322 and a 6.0 kb fragment containing the xylanase L gene inserted into the *Hind*III site.⁴⁾ The plasmid-encoded xylanase expressed in *E. coli* was located in the periplasmic and cellular fractions, and not found in the extracellular fraction at all. These results suggested that the xylanase L was a secretory protein.

To develop an *E. coli* excretion system, we attempted the extracellular production of xylanase L in *E. coli* by using pEAP2. This paper deals with the construction of a recombinant plasmid, pXP102, which consists of pEAP2 and a 4.0 kb *Bgl*II fragment containing the xylanase L gene, and the extracellular production of xylanase L in *E. coli*.

E. coli K-12 HB-101 (*pro leuB B1 lacY hsdR hsdM ara14 galK2 xyl5 mtl1 supE44 F⁻ endoA recA Str^r*)⁵⁾ and plasmid pEAP2¹⁾ were used throughout. LB-broth contained 10 g tryptone (Difco Laboratories, Detroit, Mich.), 5 g yeast extract, 1 g glucose and 10 g NaCl in 1 l deionized water; the pH was adjusted to 7.0 with NaOH and the medium solidified if necessary with 20 g agar. Xylanase activity was measured as described previously.⁴⁾ One unit of xylanase activity is defined as the amount of enzyme that produces 1 mg reducing sugars (calculated as xylose)/min at 40°C. β -Galactosidase activity was assayed as described previously.¹⁾

Plasmid pAX1 was digested with *Bgl*II and the 4.0 kb

*Bgl*II fragment containing the xylanase L gene was isolated (Fig. 1). This fragment was ligated to *Bam*HI digested pEAP2. Transformation was performed as described by Lederberg and Cohen.⁶⁾ The plasmid, pXP102, thus constructed, was obtained from Ap^r, Xyl⁺ transformants. The construction scheme for plasmid pXP102 is shown in Fig. 1.

E. coli HB101 carrying pAX1 or pXP102 was inoculated into LB-broth and cultured aerobically at 37°C. A 2 ml aliquot of the broth was withdrawn from the culture at 4 hr-intervals. Cell growth was measured at 660 nm, and extracellular, periplasmic and cellular fractions were prepared by the osmotic shock method.⁷⁾ As shown in Fig. 2, most of the xylanase activities of *E. coli* HB101 carrying pXP102 were detected in the extracellular fraction, while the xylanase activities of *E. coli* HB101 carrying pAX1 were detected in the periplasmic and cellular fractions. Most of the β -galactosidase activity was found in the cellular fraction (more than 90%) in *E. coli* HB101 carrying pAX1 or pXP102. These results suggested that the xylanase L was excreted into the medium like periplasmic proteins due to a permeability change of the outer membrane of *E. coli*. Extracellular production of proteins from *E. coli* has not been successful except for that of few proteins (colicin, hemolysin, etc.). The results above showed that the plasmid encoded xylanase L was excreted into the culture medium with excretion vector pEAP2, and suggested the possibility of the extracellular production of

FIG. 1. Construction of Plasmid pXP102.

The thin line represents a 2.4 kb *Hind*III fragment containing the penicillinase gene and the thick line a 4.0 kb *Bgl*II fragment containing the xylanase L gene.

FIG. 2. Bacterial Growth and Xylanase Production by *E. coli* HB101 Carrying pAX1 (Left) and pXP102 (Right).

E. coli HB101 (pAX1 or pXP102) was inoculated into LB-broth and cultured at 37°C on a rotary shaker. Bacterial growth (absorbancy at 660 nm, ●), xylanase activities (extracellular, ○—○; periplasmic, △—△; and cellular, □—□), and B-galactosidase activities (extracellular, ○---○; periplasmic, △---△; and cellular, □---□) were determined.

other useful proteins with the use of this excretion vector. Subcloning experiments indicated that the penicillinase was not necessary for the permeability change of the outer membrane of *E. coli* (unpublished data). Therefore, the construction of a more useful excretion vector is now in progress.

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