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Amplification of D-Xylose and D-Glucose Isomerase Activities in *Escherichia coli* by Gene Cloning

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A recombinant plasmid, designated pUC1002, was constructed by ligation of a *Hin*dIII restriction endonuclease fragment of *Escherichia coli* chromosomal DNA to vector plasmid pMB9. Strains carrying this plasmid were selected by transformation of an *E. coli* strain bearing the *xyl-7* mutation to a xylose-positive (Xyl^+) phenotype. Strains containing pUC1002 produced coordinately elevated levels of D-xylose isomerase and D-xylulose kinase. Under appropriate conditions, the isomerase also efficiently catalyzed the conversion of D-glucose to D-fructose.

A number of bacteria, including *Escherichia coli*, contain D-xylose isomerases which function physiologically to catalyze the first reaction in the catabolism of D-xylose but which also convert D-glucose to D-fructose under certain conditions (24). The latter reaction is the basis for industrial processes currently used to produce very large quantities of high-fructose syrups (4). This use, as well as interest in the use of the enzyme to promote efficient fermentation of D-xylose to ethanol (5, 12, 21, 22; T. W. Jeffries and S. Choi, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, O34, p. 193), led us to try to increase the yield of the enzyme by gene cloning. This report describes the construction and selection of a recombinant plasmid coding for both *E. coli* D-xylose isomerase and *E. coli* D-xylulose kinase, the second enzyme in the pathway for D-xylose utilization by *E. coli*. We also describe the production of the enzymes and the D-glucose isomerase activity in strains bearing the plasmid.

The recipient strain used in the cloning was JC1553 (6), an *E. coli* K-12 derivative obtained from the *E. coli* Genetic Stock Center, Yale University School of Medicine, New Haven, Conn. This strain carries the *xyl-7* mutation, a genetic lesion uncharacterized beyond the fact that it results in a xylose-negative (Xyl^-) phenotype; i.e., cells with this mutation cannot use D-xylose as the sole source of carbon and energy. Several genetic defects could result in a Xyl^- phenotype, including blocks in the isomerase, D-xylulose kinase, or a D-xylose transport protein. However, since evidence suggests that genes for these functions are organized as an operon in *E. coli* (9), just as they are in *Salmonella typhimurium* (18), it seemed likely that selection by complementation of this lesion might, depending on the restriction enzyme used and the size of the cloned DNA, result in the cloning of the entire operon.

Chromosomal DNA from Xyl^+ *E. coli* K-12 strain K37 (11) was isolated by the Marmur procedure (15). The multicopy vector plasmid pMB9 (3) was isolated from crude lysates of *E. coli* by centrifugation in a cesium chloride-ethidium bromide density gradient (17). A 3:1 mixture of chromosomal DNA and pMB9 was cleaved with a twofold excess of *Hin*dIII (New England Biolabs, Beverly, Mass.) and then treated with T4 ligase (New England Biolabs); both reactions were carried out under conditions described by the supplier. The ligated DNA (12 μg/ml) was then used to transform *E. coli* JC1553 as previously described (7). The transformed cells were grown at 37°C for 1 h in L-broth (13) and then plated for selection of transformants on the minimal salts medium of Vogel and Bonner (20) supplemented with L-arginine, L-leucine, L-histidine, and L-methionine (each at 40 μg/ml), 0.2% xylose as the sole carbon source, and tetracycline (5 μg/ml).

Plasmid DNA was isolated from several clones which grew on the plates and was found, when digested with *Hin*dIII, to consist of a fragment the size of pMB9 plus an additional 8.8-megadalton fragment (Fig. 1). The size of the additional *Hin*dIII fragment was determined by observing its electrophoretic mobility on agarose gel relative to that of *Hin*dIII-cut λ DNA (16) and by velocity sedimentation of the intact plasmid in a 5 to 20% (wt/vol) sucrose density gradient. The 8.8-megadalton DNA segment hybridized strongly to *E. coli* K37 chromosomal DNA when tested by the Southern technique

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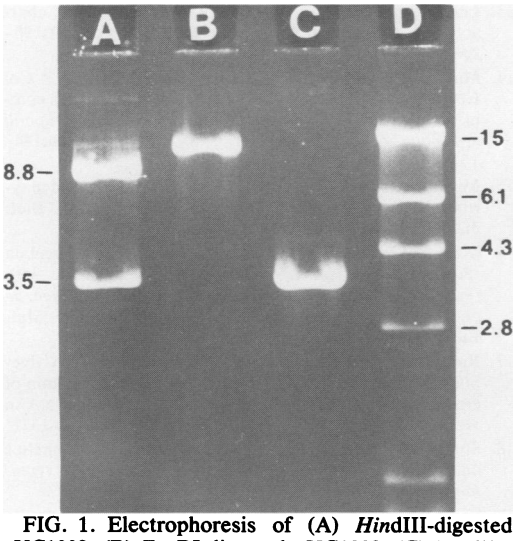

FIG. 1. Electrophoresis of (A) *Hin*dIII-digested pUC1002, (B) *Eco*RI-digested pUC1002, (C) *Hin*dIII-digested pMB9, and (D) *Hin*dIII-digested λ DNA on a 0.7% agarose gel. Numbers indicate the molecular weights ($\times 10^6$) of the various fragments.

(19). When this composite plasmid, designated pUC1002, was used to transform JC1553, all of the tetracycline-resistant transformants were also found to be Xyl^+. This indicated that the K37 chromosomal genes complementing the *xyl-7* mutation were carried on the inserted DNA segment.

Cultures of K37, JC1553, and JC1553 (pUC1002) were grown overnight at 37°C in the glycerol-based medium of Fraser and Jerrel (10), and portions of each (5% [vol/vol]) were used to inoculate fresh flasks of this medium supplemented with xylose at 0.2 g/liter. After growth for 7 h, the cells were collected by centrifugation, resuspended in cold 0.03 M potassium phosphate buffer, pH 7.2, and then subjected to sonic disruption. The crude lysates were centrifuged at 5,000 × *g* for 5 min, and the supernatants were assayed for D-xylose (8, 23) and D-glucose (see Table 1, footnote *a*) isomerase activities and D-xylulose kinase (2).

Extracts of the recipient strain grown in the presence of D-xylose have no detectable D-xylose isomerase activity and only very low levels of D-xylulose kinase activity, perhaps reflecting a polar mutation in the operon, a regulatory mutation, or a defect in a carrier protein involved in transport of a common inducer (Table 1). In contrast, the activities were induced in JC1553(pUC1002) and K37. Furthermore, in extracts of transformed cells, each of the activities was amplified more than fivefold, relative to activities in K37. Glucose isomerase activity also increased proportionately in the induced strain bearing pUC1002. None of the activities was present in extracts of any culture grown in the absence of D-xylose. Like many microbial isomerases (23), the *E. coli* enzyme had a very low affinity for glucose (apparent K_m, 0.5 M) and required cobalt ions for maximum conversion of this substrate. It was also quite heat stable in the presence of high substrate concentrations, with an optimum reaction temperature of about 60°C at 1 M glucose.

The glucose isomerases in *E. coli* strains containing pUC1002 were found to be comparable in specific activity to those in several bacterial cultures currently used for commercial production of the enzyme (data not shown), and it is reasonable to expect that much greater yields can be achieved in *E. coli* by subsequent in vitro manipulations of the cloned genes. We are also studying expression of these genes in other microbial systems.

Recently, there have been reports describing other hybrid plasmids which carry *E. coli* genes coding for D-xylose isomerase production (14; J. Polaina, M. Wiggs, S. Lastick, R. H. Villet, and K. Grohmann, Abstr. Annu. Meet. Am. Chem. Soc. 1982, CARB 030). One of these hybrids, pRM10, was isolated from an *E. coli* K-12 gene bank by complementation of D-xylose-negative mutants and, like pUC1002, codes for the production of D-xylulose kinase. However, strains bearing this plasmid appear to produce the enzymes quite differently in pattern and level, compared with pUC1002-containing strains. Maleszka et al. (14) found that only the D-xylulose kinase is increased, relative to the level of activity in untransformed wild-type cultures, whereas we observed similar amplifications of both enzymes. This may reflect either differences in expression of the xylose isomerase genes in the K-12 strains or, perhaps more likely, that the isomerase gene was somehow affected in the construction of pRM10.

TABLE 1. D-Xylose isomerase, D-xylulose kinase, and D-glucose isomerase activities in crude extracts of *E. coli* K37, JC1553, and JC1553(pUC1002)

Strain	Activity (nmol formed/min/mg of protein)		
	D-Xylose isomerase	D-Xylulose kinase	D-Glucose isomerase[a]
K37	107	135	356
JC1553	<10	30	<10
JC1553(pUC1002)	560	1,082	1,363

[a] D-Glucose isomerase assays were carried out at 60°C with 20 mM potassium phosphate, pH 7.2, 2.5 mM $CoCl_2$, 1 M D-glucose, and crude extract in a volume of 1 ml. The accumulation of fructose was measured by the resorcinol method (1).

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