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IS2 Activates the *ilvA* Gene of *Pseudomonas cepacia* in *Escherichia coli*

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The *ilvA* gene of *Pseudomonas cepacia* was expressed poorly in *Escherichia coli*. Insertion of IS2 upstream of the cloned gene dramatically increased its transcription, resulting in an 85-fold increase in threonine dehydratase (deaminase) specific activity. The results confirm earlier reports that IS2 promotes efficient expression of foreign genes in *E. coli*.

Pseudomonas cepacia, a bacterium notable for its extraordinary catabolic potential and adaptability (1, 10), forms an atypically stable, isoleucine-sensitive L-threonine dehydratase (deaminase) (11). The enzyme, which is formed constitutively at high levels, can participate in catabolism of L-threonine as well as biosynthesis of L-isoleucine (19). In the course of cloning the *ilvA* gene which encodes this enzyme, we noted that it was expressed poorly in *Escherichia coli*. We report here a case of strong activation of the *P. cepacia ilvA* gene in *E. coli* by IS2.

To clone the *ilvA* gene, we ligated fragments of the *P. cepacia* chromosome into the plasmid vector pACYC184 (6) and selected for recombinant plasmids able to complement the isoleucine auxotroph *E. coli* CU406 (14). Chromosomal DNA was prepared by the procedure of Marmur (13) and partially digested with *Sau*3A. Preparations enriched for fragments of between 5 and 12 kilobases (kb) were obtained by electroelution of DNA from agarose gels. These were ligated with *Bam*HI-digested, alkaline phosphatase-treated pACYC184 DNA (12) and introduced into strain CU406 by transformation (9). Plasmid DNA was prepared by the procedure of Birnboim and Doly (2). Selection was for transformants able to grow without isoleucine in inorganic salts medium (19) containing 0.5% glucose and 24 μg of chloramphenicol per ml.

The initial transformants grew slowly unless the medium was supplemented with isoleucine. One such transformant contained the 8.7-kb recombinant plasmid pTGL114. This plasmid was isolated and used to retransform strain CU406 to chloramphenicol resistance in medium supplemented with 1% yeast extract and 25 μg of chloramphenicol per ml. The transformants were shown subsequently to grow in the absence of isoleucine, confirming that pTGL114 was responsible for complementing the *ilvA* mutation of strain CU406. Bacteria containing pTGL114 gave rise spontaneously to variants which grew rapidly without isoleucine. One such variant contained the 10.05-kb plasmid pTGL116, a derivative of pTGL114 in which a 1.35-kb element subsequently identified as IS2 had inserted into the 4.7-kb *P. cepacia* sequence containing the putative *ilvA* gene.

In Table 1, the threonine dehydratase levels of extracts of the transformants containing pTGL114 and pTGL116 are compared with those of extracts of *P. cepacia* 249 and the *E. coli* strains CU406 and K-12S. Threonine dehydratase activity of the pTGL114-containing transformant was comparable to that of the *E. coli* wild-type K-12S. The level of activity in the pTGL116-containing transformant was almost 100-fold higher and was comparable to that of *P. cepacia* 249. The activity in extracts of the pTGL114- and pTGL116-containing derivatives of strain CU406 (unlike that of *E. coli* K-12S, the Ilv$^+$ parent of strain CU406) persisted during several days of storage at 4°C. The results confirmed that the sequence cloned in pTGL114 contained the *P. cepacia ilvA* gene.

The 1.35 kb of DNA added to pTGL114 in the formation of pTGL116 contained *Hin*dIII, *Sma*I, and *Hpa*I sites but no *Sal*I or *Eco*RI sites. The *Sal*I fragment patterns of pTGL114 and pTGL116 are compared in Fig. 1A. *Sal*I cut pTGl114 into five fragments. The largest (4.25 kb) contained primarily pACYC184 DNA. The others (1.5, 1.3, 0.7, and 0.6 kb) represented segments of the cloned *P. cepacia* sequence. In pTGL116, the 1.3-kb fragment was replaced by a 2.65-kb fragment. This alteration accounted fully for the difference in size between the two plasmids. The size of the inserted element (1.35 kb) and its complement of restriction enzyme sites (8) suggested that it was IS2.

The results of a Southern blot hybridization experiment (16) which confirmed that pTGL116 contained IS2 are shown in Fig. 1B. In this experiment, *Eco*RI digests of coliphage lambda and of the IS2-containing variant lambda r32 (3) were resolved on an agarose gel and probed with nick-translated pTGL116 DNA. pTGL116 hybridized with only one of the

TABLE 1. L-Threonine dehydratase levels in *E. coli* CU406 derivatives containing the *P. cepacia ilvA* gene[a]

Strain	L-Threonine dehydratase specific activity (nmol of α-ketobutyrate formed/min per mg of protein)
P. cepacia 249	1,421
E. coli K-12S	18
E. coli CU406	<1
E. coli CU406(pTGL114)[b]	13
E. coli CU406(pTGL116)[c]	1,106

[a] The bacteria were grown in inorganic salts medium (19) containing 0.5% glucose and 50 μg of L-isoleucine per ml and disrupted by sonic treatment. Cell extracts were assayed for L-threonine dehydratase (EC 4.2.1.16) at 37°C (11).

[b] A pACYC184 recombinant containing a 4.7-kb insert of chromosomal DNA from *P. cepacia* 249.

[c] pTGL114::IS2.

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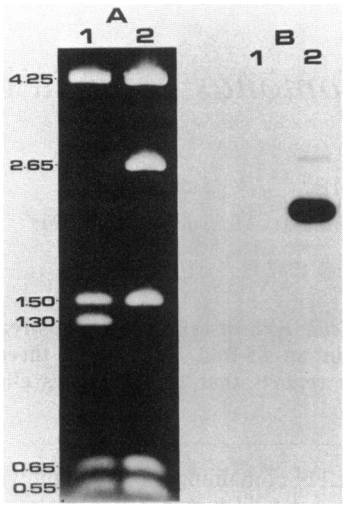

FIG. 1. Identification of IS2 as the *ilvA*-activating element on pTGL116. (A) *Sal*I digests of plasmids pTGL114 and pTGL116 resolved electrophoretically in 0.7% agarose (15). The gel was poststained with ethidium bromide (0.5 μg/ml) to visualize the *Sal*I fragments. (B) Southern blot hybridization (16) of ^{32}P-labeled denatured pTGL116 DNA (10^8 cpm/μg of DNA) with *Eco*RI fragments of coliphage lambda and its IS2-containing variant r32 (3) immobilized on a Gene Screen Plus membrane (New England Nuclear Corp., Boston, Mass.). Conditions were those recommended by the supplier. Plasmid pTGL116 failed to hybridize with any of the coliphage lambda fragments (lane 1) but exhibited strong homology with the 9-kb IS2-containing fragment of lambda r32 (lane 2).

*Eco*RI fragments of lambda r32 (the 9-kb IS2-containing fragment in lane 2). It did not hybridize with any of the six fragments of coliphage lambda DNA (lane 1).

Restriction maps of plasmids pTGL114 and pTGL116 are shown in Fig. 2. The orientation of IS2 in pTGL116 was determined by defining the positions of the *Hin*dIII and *Hpa*I sites within this element relative to the *Hin*dIII site within the pACYC184 moiety of this plasmid. *Hin*dIII cut pTGL116 into two fragments of 6.1 and 3.9 kb. The latter contained the 1.5-kb *Sal*I fragment common to pTGL114 and pTGL116. Double digestion of pTGL116 with *Hin*dIII and *Hpa*I generated fragments of 6.1, 3.2, and 0.7 kb, indicating that IS2 had inserted in orientation II relative to the 1.5-kb fragment.

Northern blot hybridization experiments (17) indicated that the increase in threonine deaminase specific activity observed in the pTGL116-containing derivative of strain CU406 reflected a similarly dramatic increase in the level of *ilvA* mRNA. Figure 3 shows the results of an experiment in which a preparation of nick-translated DNA containing only the 1.3- and 1.5-kb *Sal*I fragments of pTGL114 was used to probe resolved RNA species from derivatives of strain CU406 containing pTGL114 or pTGL116. Two transcripts of 1.5 and 2.5 kb were detected in the RNA preparation from the pTGL116-containing strain (lane 4). Only traces of these two transcripts were detected in RNA from the strain containing pTGL114 (lane 3). In this and in other experiments not shown here, the 2.5-kb species appeared to be the primary *ilvA* transcript in *P. cepacia* (lane 2). The relatively large size of this species suggests that it is a polycistronic transcript. The occurrence of two prominent *ilvA* transcripts in *E. coli* might reflect the presence of a transcription stop signal recognized in *E. coli* but not in *P. cepacia*. It should be noted that the smaller transcript was sufficiently large to encode threonine deaminase.

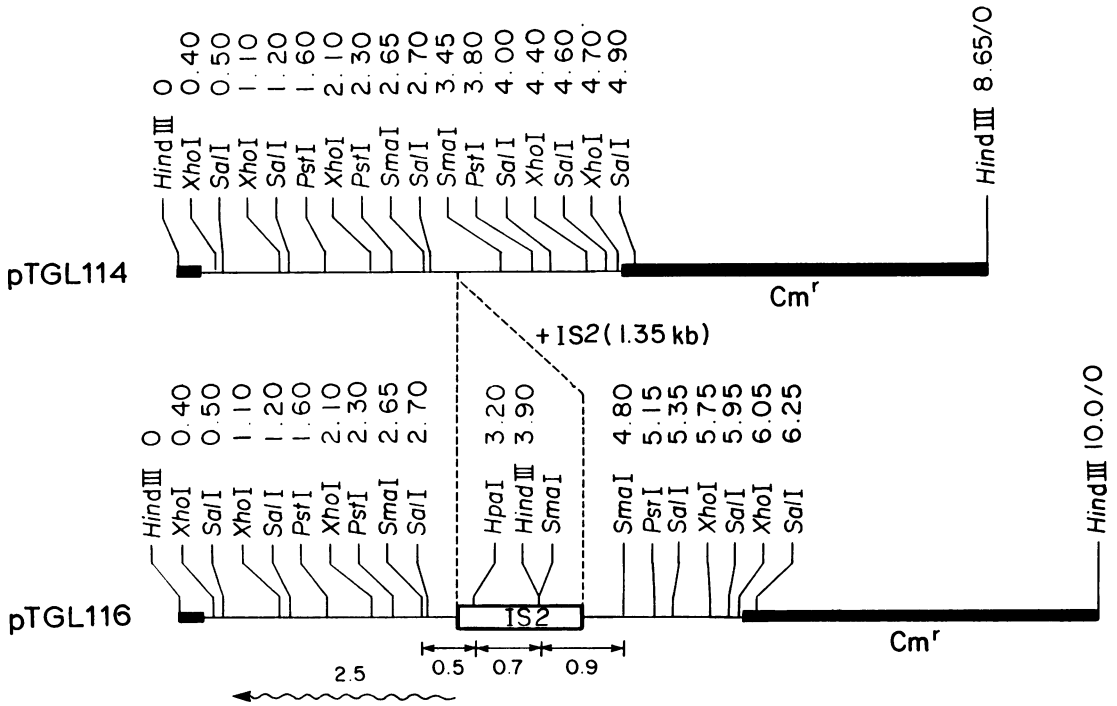

FIG. 2. Restriction maps of pTGL114 and pTGL116. The heavy lines indicate pACYC184 sequences. The distances of various restriction enzyme sites from the *Hin*dIII site of pACYC184 are indicated in kilobases. IS2 inserted into the 1.3-kb *Sal*I fragment of pTGL114 in orientation II relative to the adjoining 1.5-kb *ilvA*-containing *Sal*I fragment common to pTGL114 and pTGL116. The 2.5-kb *ilvA* transcript is depicted under the pTGL116 map.

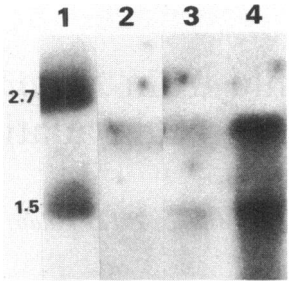

FIG. 3. Identification of *ilvA* transcripts by Northern blot hybridization. Preparations of glyoxylated RNA (12.5 μg) or DNA (0.1 μg) were resolved electrophoretically on a 1.2% agarose gel (5), transferred to a Gene Screen Plus nylon membrane (New England Nuclear), and hybridized as recommended by the supplier with a preparation of nick-translated DNA (10^8 cpm/μg) containing only the 1.3- and 1.5-kb *SalI* fragments of pTGL114. Lane 1, which contained denatured *SalI* fragments of pTGL116, shows the 1.5- and 2.7-kb fragments of this plasmid as reference markers. Lane 2 contained RNA from *P. cepacia* 249. Lanes 3 and 4 contained, respectively, RNA from derivatives of *E. coli* CU406 containing pTGL114 and pTGL116.

Our results showing IS2-dependent expression of the *P. cepacia ilvA* gene in *E. coli* are similar to those of other investigators who reported that, when inserted in orientation II relative to the activated gene, this element increased expression of a *trp* gene from *Saccharomyces cerevisiae* (4, 18) and of *arg* genes from *Methanococcus voltae* (20) and *Aspergillis nidulans* (7). These observations indicate that IS2 can promote the expression in *E. coli* of foreign genes of diverse origin and suggest that such cases of insertional activation are relatively common. This points out the potential danger of using complementation as the sole criterion to test whether genes from one organism are expressed normally in another.

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