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Promoter and Nucleotide Sequences of the *Zymomonas mobilis* Pyruvate Decarboxylase†

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DNA sequence analysis showed that pyruvate decarboxylase (one of the most abundant proteins in *Zymomonas mobilis*) contains 559 amino acids. The promoter for the gene encoding pyruvate decarboxylase was not recognized by *Escherichia coli*, although the cloned gene was expressed at relatively high levels under the control of alternative promoters. The promoter region did not contain sequences which could be identified as being homologous to the generalized promoter structure for *E. coli*. Hydropathy plots for the amino acid sequence indicated that pyruvate decarboxylase contains a large number of hydrophobic domains which may contribute to the thermal stability of this enzyme.

The regeneration of NAD^+ in the obligately fermentative bacterium *Zymomonas mobilis* is carried out with two enzymes, pyruvate decarboxylase (EC 4.1.1.1) and alcohol dehydrogenase (EC 1.1.1.1) (23, 30). Although this simple regeneration system is widely utilized by eucaryotes such as the yeasts, fungi, and higher plants (31), it is relatively rare among the bacteria (10, 29). Very few bacterial genera have been shown to contain pyruvate decarboxylase, the key enzyme catalyzing the nonoxidative decarboxylation of pyruvate to produce acetaldehyde plus CO_2 In *Z. mobilis*, this enzyme is reported to be a tetramer of identical subunits with a molecular weight of approximately 55,000 ± 5,000 (13). Recent studies by Brau and Sahm (5) have described the cloning of pyruvate decarboxylase from *Z. mobilis*. These studies indicated that the gene encoding pyruvate decarboxylase is contained on a 4.6-kilobase-pair (kb) *Sph*I fragment.

The pyruvate decarboxylase gene is normally expressed at high levels in *Z. mobilis* and constitutes up to 5% of the total soluble protein (2). Brau and Sahm (5) have also reported a high level of expression of this *Z. mobilis* gene in *Escherichia coli*. Previous studies in our laboratory (6; T. Conway and L. O. Ingram, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, H62, p. 137; T. Conway and L. O. Ingram, submitted for publication) defined sequences of DNA fragments from *Z. mobilis* which exhibited promoter activity in both *E. coli* and *Z. mobilis*. These studies indicated that similar regions of DNA were recognized as promoters by both organisms, although the dominant sites of transcriptional initiation were usually different. In this study, we examined the sequence of the pyruvate decarboxylase gene from *Z. mobilis* and its promoter and identified the sites of transcriptional initiation in both *Z. mobilis* and *E. coli*. Our studies indicate that the high level of expression of this gene in *E. coli* is not due to the recognition of the native *Z. mobilis* promoter.

MATERIAL AND METHODS

Bacterial strains, plasmids, and growth conditions. The strains and plasmids used in this study are summarized in Table 1. *Z. mobilis* CP4 was grown at 30°C in complex medium (24). Strains of *E. coli* were grown in Luria broth (21) at 37°C containing no added carbohydrate unless indicated otherwise. Ampicillin (50 mg/liter) was used to select transformants of *E. coli*, and 5-bromo-4-chloro-3-indolyl-β-D-galactoside (20 mg/liter) was used to detect β-galactosidase activity.

Purification of pyruvate decarboxylase from *Z. mobilis* and preparation of antibodies. Six-liter batches of *Z. mobilis* CP4 were harvested at the end of exponential growth, suspended in 0.05 M sodium phosphate buffer (pH 6.5), and disrupted by three passages through a French pressure cell (20,000 lb/in^2). Cell debris was removed by centrifugation for 30 min at 20,000 × *g* at 4°C. Pyruvate decarboxylase (120 mg) was purified from the resulting soluble protein fraction essentially as described by Hoppner and Doelle (13). The final yields of electrophoretically pure pyruvate decarboxylase ranged between 10 and 20 mg/25 g (wet weight) of cell pellet. Antibodies were prepared in a female goat by the injection of 2 mg of pure pyruvate decarboxylase in Freund complete adjuvent (Difco Laboratories, Detroit, Mich.) at multiple subcutaneous sites. On day 5 and again on day 17, the animal was injected with 2 mg of pyruvate decarboxylase in Freund incomplete adjuvent. After an additional 2 weeks, antibody-containing serum was prepared from 100-ml samples of clotted blood drawn twice weekly. The serum formed a single precipitin band on Ouchterlony plates when tested against lysates of *Z. mobilis* which exhibited identity with purified pyruvate decarboxylase. No cross-reactivity was observed with lysates of *E. coli* JM101, TC4, or HB101 containing the plasmid pUC18 (lacking *Z. mobilis* DNA inserts).

Cloning of the pyruvate decarboxylase gene. Chromosomal DNA was isolated from *Z. mobilis* CP4 as previously described (6). *Sph*I fragments (4 to 6 kb) were purified from a complete digestion of chromosomal DNA by agarose gel electrophoresis and ligated into the *Sph*I site of pUC18, and the resulting plasmids were used to transform *E. coli* JM101 (22). Recombinant plasmids were identified as lacking β-galactosidase activity and were grown in 3-ml cultures. Cell pellets from 1 ml of these overnight cultures were suspended in 0.1 ml of 0.05 M Tris hydrochloride (pH 8.0) and lysed by repeated freezing and thawing. These were initially screened as mixtures of 10 clones and subsequently as individual clones for the presence of pyruvate decarboxylase by using Ouchterlony diffusion plates. The presence of pyruvate

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TABLE 1. Plasmids and strains used in this study

Strain or plasmid	Relevant genotype	Source or reference
Zymomonas mobilis CP4	Prototroph	24
Escherichia coli		
HB101	*recA lacYl recA*	23
TC4	*recA lacZY*	This paper[a]
JM101	*lacI*^{q} *lacZ*	BRL[b]
Plasmids		
pUC18	*bla lacI'Z'*	BRL[b]
pLOI275	*bla pdc*$^+$	This paper
pLOI276	*bla pdc*$^+$	This paper

[a] Spontaneous mutant of strain S17-1 (6).
[b] BRL, Bethesda Research Laboratories.

decarboxylase was confirmed by direct spectrophotometric assay.

Pyruvate decarboxylase was assayed in *Z. mobilis* as described by Hoppner and Doelle (13) by monitoring the pyruvic acid-dependent reduction of NAD^+ with alcohol dehydrogenase as a coupling enzyme. Small-scale enzyme extracts of *E. coli* and of *Z. mobilis* were prepared from 1-ml samples of cultures. Cells were harvested at room temperature by centrifugation for 2 min at 10,000 × *g*. Each cell pellet was washed and suspended in 0.1 ml of 0.05 M sodium phosphate buffer (pH 6.5). Cells were permeabilized by mixing vigorously for 15 s with 2 drops of chloroform. Extracts of *E. coli* contained enzymes such as lactate dehydrogenase which directly oxidized pyruvate and reduced NAD^+, interfering with this type of assay. The relative heat stability of pyruvate decarboxylase allowed the complete elimination of confounding activities in *E. coli* by heat treatment (60°C, 30 min) after the addition of thiamine pyrophosphate and magnesium chloride (1 mM each). Extracts from *Z. mobilis* CP4 which received analogous treatment retained 80% of the original pyruvate decarboxylase activity. Pyruvate decarboxylase activities are reported as international units per milligram of total cell protein. Protein was measured by the method of Lowry et al. (20) with bovine serum albumin as a standard.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cultures were grown to an optical density (550 nm) of 0.8, and washed whole cells were suspended in distilled water to an approximate protein concentration of 2 mg/ml. Samples were mixed with treatment buffer and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described by Laemmli (19). Samples containing 10 to 15 μg of cell protein were applied to each lane and separated with a 6-cm slab gel (Idea Scientific, Corvallis, Oreg.). Protein bands were visualized with Coomassie blue.

DNA sequence analysis. The *Dra*I fragment of pLOI276 containing the gene encoding pyruvate decarboxylase was sequenced in both directions by the dideoxy method (28) with M13mp18 and M13mp19 with the sequencing kit from Bethesda Research Laboratories, Inc. (Gaithersburg, Md.). A short segment of sequence was also determined in one direction upstream from the *Dra*I fragment of pLOI275. The ratios of dideoxynucleotide/deoxynucleotide were modified (32) to allow the use of [α-^{35}S]dATP (New England Nuclear Corp., Boston, Mass.). Additional M13 sequencing primer (17-mer) was obtained from New England BioLabs, Inc. (Beverly, Mass.). Sequencing reactions were separated on an International Biotechnologies, Inc. (New Haven, Conn.) gel-sequencing apparatus (8% acrylamide) with multiple loadings. Bands were visualized by overnight exposure to Kodak XAR 5 film at room temperature.

The resulting sequences were analyzed by the programs described by Pustell and Kafatos (26), obtained from International Biotechnologies. Hydropathy was computed as described by Kyte and Doolittle (18), using a window of nine amino acids.

Analysis of transcriptional initiation sites. The 5′ termini of transcripts were determined by primer extension analysis (25). RNA was isolated as described by Bialkowska-Hobrazanska et al. (4). Contaminating DNA was removed by treatment with RNase-free DNase (22). A 19-base primer (3′-CGAACAGGTCTAACCAGAG-5′) which was complementary to the noncoding strand of the pyruvate decarboxylase gene was synthesized with an Autogen 500 oligonucleotide synthesizer obtained from Millipore/Genetic Design (Bedford, Mass.). This primer was labeled on the 5′ end with T4 polynucleotide kinase (International Biotechnologies) and [γ-^{32}P]ATP (New England Nuclear Corp.) as described by Maniatis et al. (22). Labeled primer (0.1 pmol) was hybridized to 50 μg of RNA in a total volume of 10 μl of hybridization buffer (9 mM Tris hydrochloride, 0.35 mM EDTA; pH 8.3) by denaturing at 94°C for 5 min followed by chilling to −70°C for 2 min and incubation at 43°C for 3 h. Deoxynucleotide triphosphates (1 mM final concentration), 10× buffer (500 mM Tris hydrochloride, 100 mM $MgCl_2$, 40 mM dithiothreitol; pH 8.3), 30 U of avian myeloblastosis virus reverse transcriptase (Pharmacia, Inc., Piscataway, N.J.), and distilled water were added to a final volume of 25 μl and incubated for 30 min at 43°C to allow cDNA synthesis. RNA was degraded with DNase-free RNase A (100 μg/ml, 1 h, 37°C). The cDNA was extracted with phenol, precipitated with ethanol, dissolved in 4 μl of distilled water–4 μl of formamide dye stop buffer, and electrophoresed adjacent to a sequence ladder (generated by using the same primer and DNA from appropriate M13 clones).

RESULTS

Isolation of the *pdc* gene. We screened 500 clones from our *Sph*I library of 4- to 6-kb *Z. mobilis* chromosomal fragments in pUC18 for the presence of the pyruvate decarboxylase gene (*pdc*). Four positive clones were identified. Three of these produced pyruvate decarboxylase as determined by Ouchterlony testing and appeared to contain identical DNA based on size (4.7 kb) and restriction pattern. One of these, designated pLOI275, was chosen for further investigation.

The insert in pLOI275 was mapped with a variety of restriction enzymes and appeared similar to that described by Brau and Sahm (5) with the exception that our insert contained two additional *Pvu*II sites (Fig. 1). Subcloning of fragments from this insert into appropriate sites in pUC18 indicated that the gene encoding pyruvate decarboxylase was located on the largest (1.8 kb) internal *Dra*I fragment. Ten pyruvate decarboxylase-positive subclones (*Dra*I fragments cloned into the *Sma*I site of pUC18) were examined for orientation by digestion with *Hin*dIII, and all exhibited an orientation opposite to that of the original *Sph*I clone (Fig. 1). One of these, designated pLOI276, was selected for further study.

Expression of the pyruvate decarboxylase gene from *Z. mobilis* in *E. coli*. Figure 2 shows a sodium dodecyl sulfate-polyacrylamide gel electrophoresis profile of purified pyruvate decarboxylase, extracts of *Z. mobilis* CP4, and extracts from *E. coli* TC4 containing pLOI276. The abundant new

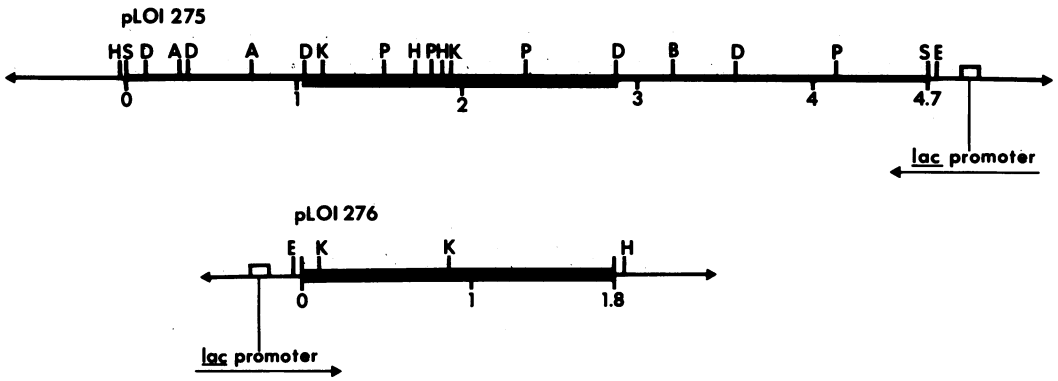

FIG. 1. Restriction endonuclease map of the 4.7 kb *Sph*I fragment from *Z. mobilis* in the *Sph*I site of pUC18 (pLOI275) and the 1.8-kb *Dra*I fragment (subclone) contained in the *Sma*I site of pUC18 (pLOI276). The thin line denotes the cloning vector, pUC18, and the bold line in pLOI275 denotes the 1.8-kb *Dra*I fragment on which *pdc* is located (the region subcloned to make pLOI276). The complete gene encoding pyruvate decarboxylase is present in both plasmids. The terminal restriction sites in the polylinker regions and the *lac* promoter are marked on both constructions to facilitate orientation. Abbreviations are (sites for endonucleases): H, *Hin*dIII; S, *Sph*I; D, *Dra*I; A, *Ava*I; K, *Kpn*I; P, *Pvu*II; B, *Bst*EII; E, *Eco*RI.

protein contained in strains harboring pLOI276 appeared identical in size to that of purified pyruvate decarboxylase from *Z. mobilis*. A band of similar apparent molecular weight was also evident in extracts from *Z. mobilis*. The size of the pyruvate decarboxylase monomer unit was estimated to be 60,000 daltons. Extracts of *E. coli* containing pLOI275 contained a prominent protein of identical size (not shown).

The *pdc*-containing plasmid, pLOI276, was transferred to *E. coli* HB101 by transformation for further study. The strong orientational preference in *pdc*-positive clones containing the *Dra*I fragment suggested that adjacent portions of plasmid DNA are important for expression. To examine the possibility that the expression of *pdc* on pLOI276 in *E. coli* was due in part to the *lac* promoter, we determined the specific activity of pyruvate decarboxylase in cells grown with medium under inducing conditions with added isopropyl-β-D-thiogalactopyranoside (1 mM) and under repressing conditions with added glucose (5 g/liter). Growth in the presence of glucose resulted in a 75% decline in specific activity (0.3 IU/mg of cell protein as compared with 1.2 IU/mg of cell protein). The addition of isopropyl-β-D-thiogalactopyranoside caused an increase in specific activity to 1.5 IU/mg of cell protein. Assuming a maximal specific activity for pure enzyme of 100 IU/mg of protein, a minimum of 1.5% of the protein in these isopropyl-β-D-thiogalactopyranoside-induced cells is pyruvate decarboxylase. No activity was detected in the extracts from strain HB101 containing pUC18 lacking the *pdc* gene.

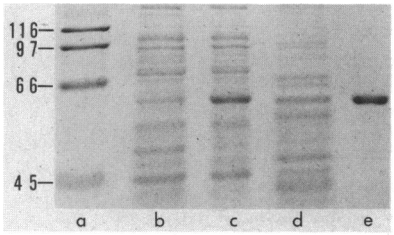

FIG. 2. Sodium dodecyl sulfate-polyacrylamide gel showing expression of pyruvate decarboxylase gene in *E. coli* TC4. Lanes: a, molecular weight markers with molecular weight ($\times 10^3$) indicated to the left; b, strain TC4 containing pUC18; c, strain TC4 containing pLOI276; d, strain CP4; e, purified pyruvate decarboxylase from *Z. mobilis*. The gel has been stained with Coomassie blue.

Sequence of *pdc*. Figure 3 shows a summary of the sequence for the gene encoding pyruvate decarboxylase. A single open reading frame was identified of sufficient length to encode this protein which contained an ATG start located 10 bases downstream from GGAG, a potential ribosome-binding site (17). This fragment encoded 559 amino acids (including the amino-terminal methionine) with an aggregate molecular weight of 59,998. Although the region upstream from the translational start was very A+T-rich, no sequences were evident which were strongly homologous to the consensus −10 and −35 region proposed for *E. coli* (9, 12, 27). This upstream region did, however, contain poly(A) (three with five bases; one with six) and poly(T) (three with four bases; one with five bases) in addition to a seven-base repeat (TTCGGCA). These features appear to be unique to this upstream region. The reading frame terminated with a stop codon (TAA) followed by a second in-frame stop codon (TAA) and an out-of-frame stop codon (TAG).

Figure 4 shows a hydropathy plot for pyruvate decarboxylase. These data reveal the relatively hydrophobic nature of this protein with at least 15 hydrophobic domains. Hydrophobic interactions are strengthened by elevated temperature (3), and it is likely that these multiple hydrophobic domains contribute to thermal stability. Indeed, this enzyme is denatured by freezing at neutral pH. Denaturation was accompanied by aggregation into an insoluble form. The denatured enzyme was not readily solubilized by the addition of base or acid but appeared to remain immunogenic.

Transcriptional initiation in *Z. mobilis* and *E. coli*. The site of transcriptional initiation for *pdc* in *Z. mobilis* was identified by primer extension analysis (Fig. 5). In these experiments, we hybridized a synthetic 19-mer which was complementary to the noncoding strand spanning base pair 79 (3′) to base pair 97 (5′) to RNA from *Z. mobilis* and elongated this primer to the end of the transcript with reverse transcriptase. A single major site of transcriptional initiation was observed corresponding to a guanine residue. This base was assigned as base pair 1 in Fig. 3 and was 37 base pairs upstream from the first in-frame start codon (ATG).

Analogous primer extension experiments with RNA from *E. coli* TC4 containing pLOI276 (1.8-kb *Dra*I fragment) revealed that the *Z. mobilis* initiation site was not recognized

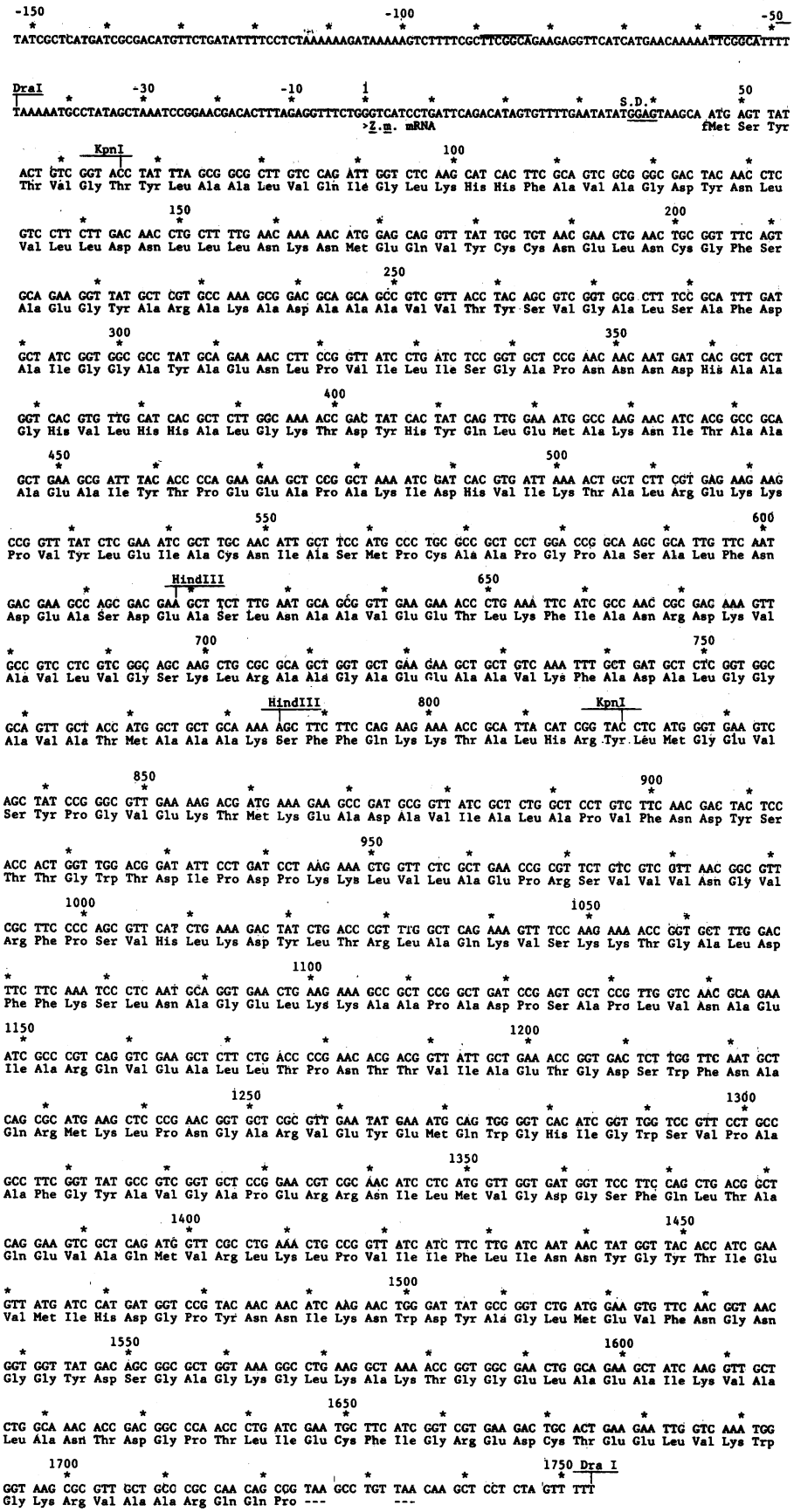

FIG. 3. Sequence and translation of the pyruvate decarboxylase gene from *Z. mobilis* (Z.m.). The repeated sequence upstream from *pdc* is overlined. The initiation of transcription for *Z. mobilis* is marked and assigned base pair position 1. A potential ribosome-binding region is labeled S.D. and underlined. Translation was begun at the first in-frame methionine codon. Restriction endonuclease sites are marked at the position of the cut (┬┴).

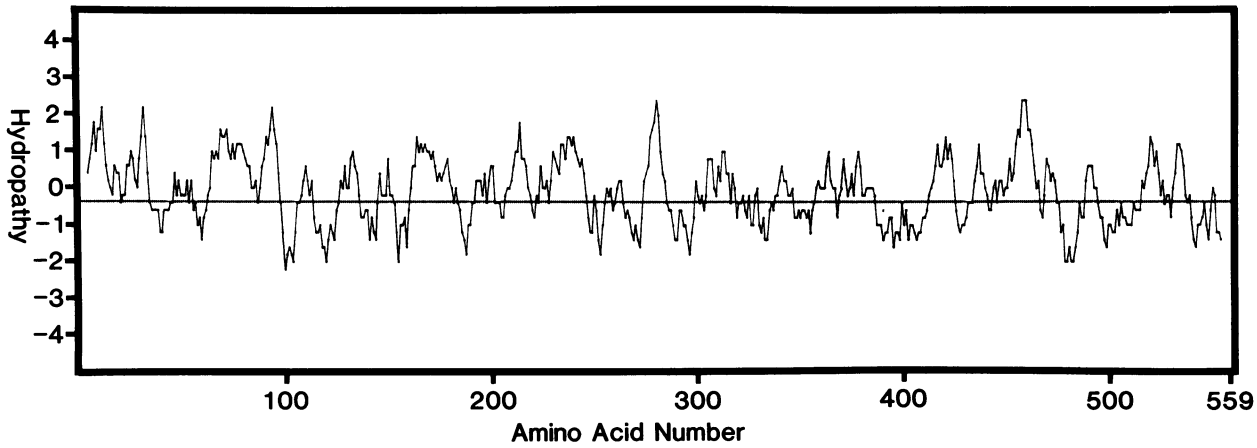

FIG. 4. Hydropathy plot of the pyruvate decarboxylase gene.

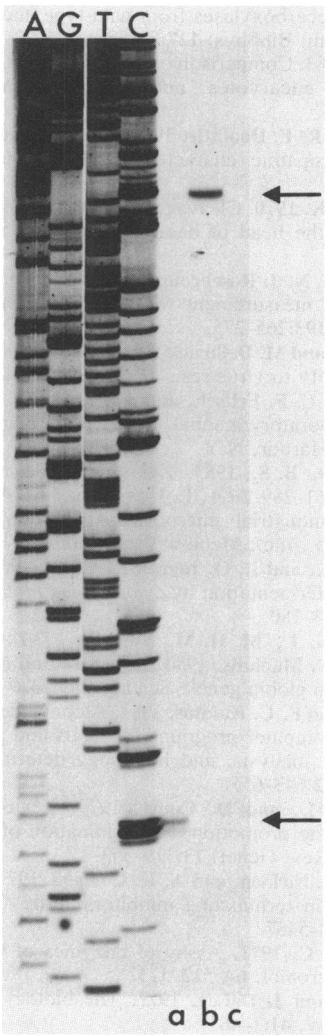

FIG. 5. Primer extension analysis of transcriptional initiation. Lanes: a, *Z. mobilis* CP4; b, *E. coli* TC4 containing pLOI276; c, control RNA from strain TC4. A, G, T, C (left) represent a sequencing ladder of the coding strand. The positions of transcript initiation are indicated by arrows.

by *E. coli* (Fig. 5). Transcriptional initiation mapped within the enteric *lac* promoter (8), 71 bases upstream from the *Sma*I-*Dra*I junction. Similar experiments (data not shown) were performed with *E. coli* TC4 carrying pLOI275 (4.7-kb *Sph*I fragment). Only a weak initiation of transcription was observed, and this appeared well above the site used by *Z. mobilis* in an unsequenced area. The weak band observed coupled with the strong expression of *pdc* in strains containing pLOI275 make it unlikely that this band represents the dominant site for transcriptional initiation. It is possible that the major transcriptional initiation site is even further upstream, beyond the region adequately evaluated by the elongation of our primer.

DISCUSSION

Our results indicate that the *Z. mobilis* promoter for pyruvate decarboxylase is not recognized by *E. coli*. This gene was expressed in *E. coli* under the control of the *lac* promoter in pLOI276 and under the control of an unidentified promoter far upstream from the sequences recognized by *Z. mobilis* in pLOI275. During previous studies of promoter structure in *Z. mobilis*, we examined DNA fragments with promoter activity as components of functional β-galactosidase fusion genes (6). These fragments of partially digested chromosomal DNA from *Z. mobilis* were fused to a truncated *E. coli lacZ* gene and contained many of the features of *E. coli* consensus promoter regions (9, 12, 27). Although our earlier results suggested a general similarity of promoter structure between the two organisms, this may not be the case. Pyruvate decarboxylase is one of the most abundant proteins in *Z. mobilis*, and its promoter does not contain sequences resembling those of the *E. coli* consensus sequence. The enzymes of glycolysis and NAD^+ regeneration (pyruvate decarboxylase and alcohol dehydrogenase) are present in very high levels in *Z. mobilis*, representing over half of the soluble protein (2). High levels of expression require efficient transcription and translation. Transcription may be facilitated by specialized sequences for promoter recognition. Further studies are under way to identify the promoter regions of other glycolytic enzymes from *Z. mobilis* and to establish common features which may be involved in their efficient transcription.

The efficiency of translation can be influenced by a number of factors. In *E. coli*, translation is promoted by adenine and thymidine residues between the ribosome-binding site

and the translational start (7). The occurrence of TAA immediately downstream from the ribosome-binding region in *pdc* from *Z. mobilis* may contribute to its high level of expression in *Z. mobilis*. Codon usage and tRNA abundance (11, 14, 15) may also contribute to the high level of *pdc* expression. The most abundant codons for each amino acid in pyruvate decarboxylase from *Z. mobilis* (data not shown) were the same as the most abundant codons used by *E. coli* (1) with the exception of tyrosine (TAC in *E. coli*) and alanine (GCG in *E. coli*). TCC and AGC were used to encode serine with near equal frequency in *E. coli* and in *pdc*. Ten codons were not present in *pdc*, seven of which corresponded to uncommon codons in *E. coli* for which tRNA species are in low abundance (1, 11, 14, 15). Thus, the utilization of codons for abundant tRNA species may also contribute to the high level of pyruvate decarboxylase synthesized in *Z. mobilis* and in *E. coli*.

The translated amino acid composition for pyruvate decarboxylase from *Z. mobilis* is similar in many respects to the reported amino acid composition of pyruvate decarboxylase from *Saccharomyces cerevisiae* (31). The mol% levels of lysine, arginine, histidine, proline, and glycine are very similar. The sum of aspartic acid plus asparagine and the sum of glutamic acid plus glutamine from the *Z. mobilis* pyruvate decarboxylase are very similar to the values reported for aspartic acid (includes asparagine) and glutamic acid (includes glutamine) in the yeast enzyme (31). The pyruvate decarboxylases from both *Z. mobilis* and *S. cerevisiae* (16) are relatively heat stable.

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LITERATURE CITED

1. **Alff-Steinberger, C.** 1984. Evidence for coding pattern on the non-coding strand of the *E. coli* genome. Nucleic Acids Res. **12**:2235–2241.
2. **Algar, E. M., and R. K. Scopes.** 1985. Studies on cell-free metabolism: ethanol production by extracts of *Zymomonas mobilis*. J. Biotechnol. **2**:275–287.
3. **Ben-Naim, A., and M. Yaacobi.** 1974. Effects of solutes on the strength of hydrophobic interactions and its temperature dependence. J. Phys. Chem. **78**:170–175.
4. **Bialkowska-Hobrazanska, H., C. A. Gilchrist, and D. T. Denhardt.** 1985. *Escherichia coli rep* gene: identification of the promoter and N terminus of the Rep protein. J. Bacteriol. **164**:1004–1010.
5. **Brau, B., and H. Sahm.** 1986. Cloning and expression of the structural gene for pyruvate decarboxylase of *Zymomonas mobilis*. Arch. Microbiol. **144**:296–301.
6. **Byun, M. O.-K., J. B. Kaper, and L. O. Ingram.** 1986. Construction of a new vector for the expression of foreign genes in *Zymomonas mobilis*. J. Ind. Microbiol. **1**:9–15.
7. **De Boer, H. A., L. J. Comstock, A. Hui, E. Wong, and M. Vasser.** 1983. A hybrid promoter and portable Shine-Dalgarno regions of *Escherichia coli*. Biochem. Soc. Symp. **48**:233–244.
8. **Dickson, R. C., J. Abelson, W. M. Barnes, and W. S. Reznikoff.** 1975. Genetic regulation: the *lac* control region. Science **187**:27–35.
9. **Doi, R. H., and L.-F. Wang.** 1986. Multiple procaryotic ribonucleic acid polymerase sigma factors. Microbiol. Rev. **50**:227–243.
10. **Finn, R. K., S. Bringer, and H. Sahm.** 1984. Fermentation of arabinose to ethanol by *Sarcina ventriculi*. Appl. Microbiol. Biotechnol. **19**:161–166.
11. **Grosjean, H., and W. Fiers.** 1982. Preferential codon usage in prokaryotic genes: the optimal codon-anticodon interaction energy and the selective codon usage in efficiently expressed genes. Gene **18**:199–209.
12. **Hawley, D. K., and W. R. McClure.** 1983. Compilation and analysis of *Escherichia coli* promoter DNA sequences. Nucleic Acids Res. **11**:2237–2255.
13. **Hoppner, T. C., and H. W. Doelle.** 1983. Purification and kinetic characteristics of pyruvate decarboxylase and ethanol dehydrogenase from *Zymomonas mobilis* in relation to ethanol production. Eur. J. Appl. Microbiol. Biotechnol. **17**:152–157.
14. **Ikemura, T.** 1981. Correlation between the abundance of *Escherichia coli* transfer RNAs and the occurrence of the respective codons in its protein genes: a proposal for a synonymous codon choice that is optimal for the *E. coli* translational system. J. Mol. Biol. **151**:389–409.
15. **Ikemura, T., and H. Ozeki.** 1982. Codon usage and transfer RNA contents: organism-specific codon-choice patterns in reference to the isoacceptor contents. Cold Spring Harbor Symp. Quant. Biol. **47**:1087–1097.
16. **Juni, E., and G. A. Heym.** 1968. Properties of yeast pyruvate decarboxylase and their modification of proteolytic enzymes. I. Stability of decarboxylases from wild-type and mutant strains. Arch. Biochem. Biophys. **127**:79–88.
17. **Kozak, M.** 1983. Comparison of initiation of protein synthesis in procaryotes, eucaryotes, and organelles. Microbiol. Rev. **47**:1–45.
18. **Kyte, J., and R. F. Doolittle.** 1982. A simple method of displaying the hydropathic character of a protein. J. Mol. Biol. **157**:105–132.
19. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
20. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
21. **Luria, S. E., and M. Delbruck.** 1943. Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28**:491–511.
22. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **Montenecourt, B. S.** 1985. *Zymomonas*, a unique genus of bacteria, p. 261–289. *In* A. L. Demain and N. A. Solomon (ed.), Biology of industrial microorganisms. Benjamin/Cummings Publishing Co., Inc., Menlow Park, Calif.
24. **Osman, Y. A., and L. O. Ingram.** 1985. Mechanism of ethanol inhibition of fermentation in *Zymomonas mobilis* CP4. J. Bacteriol. **164**:173–180.
25. **Proudfoot, N. J., M. H. M. Shander, J. L. Manley, M. L. Gefter, and T. Maniatis.** 1980. Structure and *in vitro* transcription of human globin genes. Science **209**:1329–1336.
26. **Pustell, J., and F. C. Kafatos.** 1984. A convenient and adaptable package of computer programs for DNA and protein sequence management, analysis, and homology determination. Nucleic Acids Res. **12**:643–655.
27. **Rosenberg, M., and D. Court.** 1979. Regulatory sequences involved in the promotion and termination of RNA transcription. Annu. Rev. Genet. **13**:319–353.
28. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
29. **Scrutton, M. C.** 1971. Assay of enzymes of CO_2 metabolism. Methods Microbiol. **6A**:512–513.
30. **Swings, J., and J. DeLey.** 1977. The biology of *Zymomonas*. Bacteriol. Rev. **41**:1–46.
31. **Ullrich, J.** 1982. Structure-function relationships in pyruvate decarboxylase of yeast and wheat germ. Ann. N.Y. Acad. Sci. **378**:287–305.
32. **Williams, S. A., B. E. Slatko, L. S. Moran, and S. M. DeSimone.** 1986. Sequencing in the fast lane: a rapid protocol for [α-^{35}S]ATP dideoxy DNA sequencing. Biotechnology **4**:138–147.