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Sequence of Ornithine Decarboxylase from *Lactobacillus* sp. Strain 30a

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A gene encoding biodegradative ornithine decarboxylase from *Lactobacillus* sp. strain 30a was isolated from a genomic DNA library and sequenced. Primer extension analysis revealed two transcription initiation sites. The deduced amino acid sequence is compared with the amino acid sequences of five previously reported bacterial decarboxylases, and conserved pyridoxal phosphate motif residues are identified.

Ornithine decarboxylase (ODC) (ornithine carboxyl-lyase; EC 4.1.1.17) catalyzes the initial step in the synthesis of polyamines and is a potential target in drug therapy. Bacterial ODCs are known in two forms: biosynthetic (or constitutive) ODC is produced when bacteria are grown at neutral pH in minimal medium, and biodegradative (or induced) ODC, which can be induced to high levels, is produced when cells are grown in an acidic, enriched medium containing ornithine (19).

Lactobacillus sp. strain 30a ODC is a dodecamer of approximately one million daltons (6) and requires pyridoxal-5′-phosphate (PLP) as a cofactor. As part of a study of PLP-dependent decarboxylases, we have determined the X-ray structure of the induced ODC from *Lactobacillus* sp. strain 30a. In this study, we report the nucleotide sequence and the transcription initiation sites for the *Lactobacillus* sp. strain 30a *odc* gene.

Lactobacillus sp. strain 30a cells grown anaerobically in medium containing ornithine at pH 5.4 (3) were harvested, lysed, and used to obtain purified ODC (6, 14) or the genomic DNA (1). Peptides obtained from ArgC or CNBr digests of pure ODC were sequenced and aligned with the induced ODC from *Escherichia coli* (9). Two mixed oligonucleotide primers were designed to generate a PCR fragment of the *odc* gene with genomic DNA as a template. The 1.1-kb PCR fragment was cloned into M13 and sequenced with a Sequenase 2.0 kit. The 1.1-kb PCR fragment was ^{32}P labeled and used to probe a Southern blot (15) of the genomic DNA. A 4.4-kb *Pst*I-*Bgl*II fragment was found positive, cloned (as HJ12), and sequenced. This fragment contains the 5′ end of the *Lactobacillus* sp. strain 30a *odc* gene which codes for the first 632 amino acid residues.

To obtain the whole gene, a *Lactobacillus* sp. strain 30a genomic library was made from fragments of a *Mbo*I digest, cloned into λEMBL3 which had been digested with *Bam*HI, and transfected into *E. coli* NM539. This library was screened with a digoxigenin-labeled (Boehringer Mannheim) 0.8-kb *Eco*RI-*Hin*dIII fragment of HJ12 (5). One of the positive clones was found to contain the full *odc* open reading frame (Fig. 1), corresponding to 730 amino acids (82,551 versus 85,000 Da determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis) (6). The deduced amino acid sequence agrees with the protein sequences obtained from the amino-terminal, several internal, and carboxyl-terminal peptides (Fig. 1). The estimated pI based on the amino acid composition is 5.0, and the experimental pI determined by isoelectric focusing is 4.5. A gene responsible for product transport across the membrane was found adjacent to genes encoding lysine decarboxylase and biodegradative ODC in *E. coli* (9, 11). The nucleotides upstream and downstream from the *Lactobacillus* sp. strain 30a *odc* gene were searched for a similar transport protein gene, but none was found over the range sequenced (571 bases 5′ of *odc* and 321 bases 3′ of *odc*). Two tandem ATG codons are present at the 5′ end of the coding region, with the second ATG assumed to serve as the initiating codon on the basis of its proximity to a prototypical Shine-Dalgarno sequence AGGAGGT centered at −10. There is a possible stem-loop structure which begins 25 nucleotides downstream from the second stop codon which has an 11-base stem and a 5-base loop. This structure may serve as a terminator for transcription.

A primer extension experiment (8) was performed in order to identify the transcription start site(s) and the promoters for the *odc* gene. The reverse transcription reaction was carried out by using *Lactobacillus* sp. strain 30a total RNA as the template with a 22-nucleotide, 5′-^{32}P-labeled primer complementary to nucleotides 26 to 47 (relative to the ATG) in the *odc* gene. Two transcription initiation sites were identified (Fig. 1). The first site appears at −23 and the second appears at −77 from the second ATG. Both transcription start sites have a Pribnow box sequence at −10 and a consensus sequence at the −35 region.

The deduced protein sequence of the *Lactobacillus* sp. strain 30a ODC was compared with those of other bacterial decarboxylase enzymes. Protein sequence alignment (MACAW) (17) of the three ODC enzymes (Fig. 2) shows 53 and 51% amino acid identities with the induced and constitutive *E. coli* ODCs, respectively (2, 9). There was less sequence identity found for the other *E. coli* decarboxylases, induced arginine decarboxylase (26%) (18) and lysine decarboxylase (28%) (11), or the *Hafnia* lysine decarboxylase (28%) (4).

The *Lactobacillus* sp. strain 30a ODC sequence does not align well with eukaryotic ODC enzymes. The MACAW program used to compare the prokaryotic ODC protein sequences was unable to align the prokaryotic ODCs with the eukaryotic ODCs. The eukaryotic ODCs are considerably smaller, and the PLP cofactors tend to be placed closer to the N terminus.

The amino acid sequences of aminotransferases (10) and decarboxylases (16) have recently been analyzed by (N-1)

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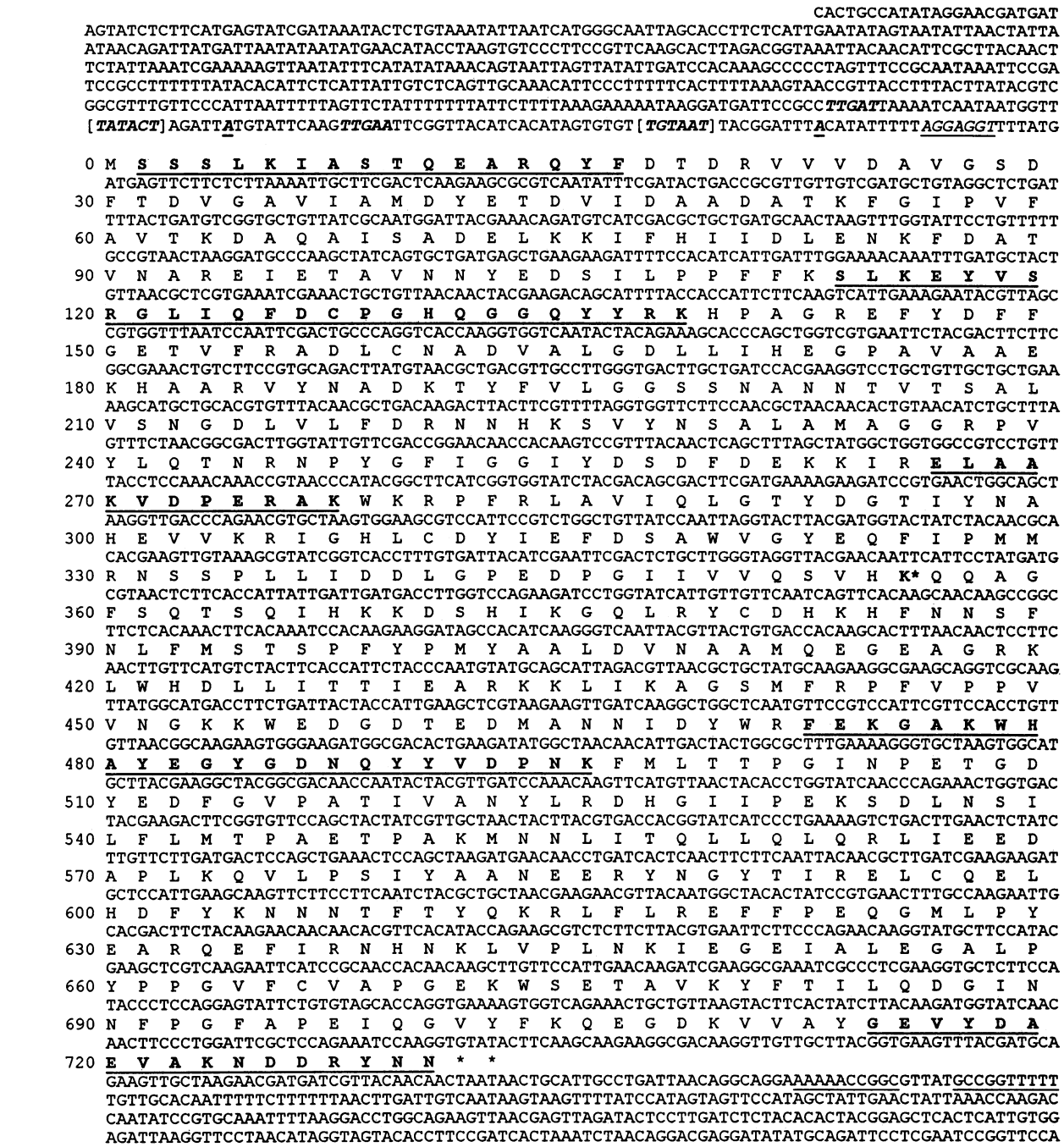

FIG. 1. Gene and deduced amino acid sequences of *Lactobacillus* sp. strain 30a ODC. The deduced amino acid sequence is shown above the nucleotide sequence, with Ser at position 1 per the N-terminal amino acid sequence data. The numbers on the left refer to the amino acid number. The Shine-Dalgarno sequence is italicized and underlined. Transcription start sites are in bold italic type and underlined. The −10 regions (Pribnow boxes) are in brackets and in bold italic type. The −35 regions of the promoter are in bold italic type. The possible stem-loop structure just beyond the second translation stop codon is underlined. Amino acids confirmed by protein sequencing are in bold type and underlined, and the PLP lysine is designated as **K***.

profile analysis. The aminotransferases are the most thoroughly studied class of PLP-dependent enzymes. A comparison of 51 aminotransferase sequences identified only four residues that remained invariant (10). Three of the four residues are involved in defining the PLP-binding pocket, and the fourth participates in substrate binding. A similar analysis divided the decarboxylases into four subgroups (16). It was concluded on the basis of the (N-1) profile search of amino acid sequences that the four subgroups of decarboxylases were not evolutionarily related to each other.

Our sequence comparisons, guided by the X-ray structure (7, 12, 13) of the *Lactobacillus* sp. strain 30a ODC (a group III

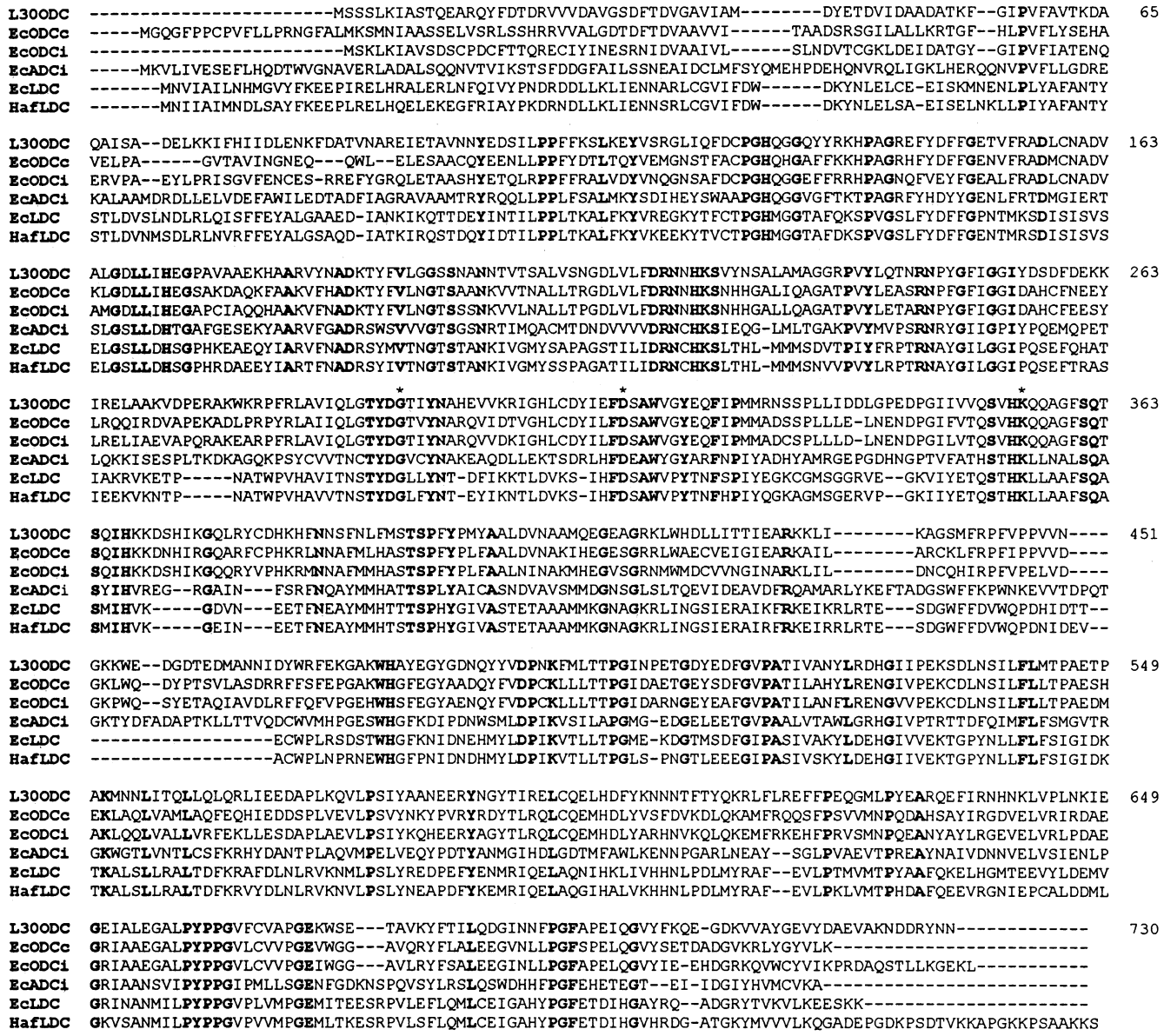

FIG. 2. Comparison of amino acid sequences of bacterial decarboxylases. Conserved amino acids are in bold type. The strictly conserved lysine that binds PLP and other residues that correspond to the structurally invariant residues found in aminotransferases are denoted with an asterisk. The numbers to the right indicate the *Lactobacillus* sp. strain 30a amino acids. Abbreviations: L30ODC, *Lactobacillus* sp. strain 30a ODC; EcODCc, *E. coli* ODC (constitutive) (2); EcODCi, *E. coli* ornithine decarboxylase (induced) (9); EcADCi, *E. coli* arginine decarboxylase (induced) (18); EcLDC, *E. coli* lysine decarboxylase (11); and HafLDC, *Hafnia* lysine decarboxylase (4).

decarboxylase), suggest that *Lactobacillus* sp. strain 30a ODC shares a PLP-binding motif with the group II decarboxylases as well as the aminotransferases. The three structural elements that are invariant in the PLP-binding site of aminotransferases (Lys-258, Asp-222, and Gly-197) also correspond well with invariant amino acid residues found in the larger, bacterial (group III) decarboxylases. These residues are denoted with an asterisk in Fig. 2. Lys-258 in porcine aspartate aminotransferase which forms the Schiff base with PLP is identified as Lys-355 for *Lactobacillus* sp. strain 30a ODC. The Asp or Glu (D-222 or E-222 in the aminotransferases) that forms a salt bridge or H bond to N-1 of the cofactor is identified as Asp-316 in *Lactobacillus* sp. strain 30a ODC (7, 13). The sequence Asp-x-Ala occurring 30 to 40 residues before the PLP-binding lysine is a common motif found in many PLP-dependent enzymes (13). The glycine that is involved in a turn at a domain interface, Gly-197 in aminotransferases, is present as Gly-294 in ODC from *Lactobacillus* sp. strain 30a. This result suggests that not only is there structural similarity between the group II and group III decarboxylases and aminotransferases but also that these enzymes are related evolutionarily. This hypothesis has now been confirmed by X-ray structural analysis (12, 13, 20).

Nucleotide sequence accession number. The nucleotide sequence of the *odc* gene in *Lactobacillus* sp. strain 30a has been deposited in GenBank and given accession number U11816.

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