

## 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 <sup>32</sup>P labeled and used to probe a Southern blot (15) of the genomic DNA. A 4.4-kb *Pst*I-*Bgl*III 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-*Hind*III 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'-<sup>32</sup>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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CACTGCCATATAGGAACGATGAT

AGTATCTCTTCATGAGTATCGATAAATACTCTGTAAATATTAATCATGCGCAATTAGCACCTTCTCATTGAATATAGTAATATTAACATTA  
ATAACAGATTATGATTAATATAATATGAACATACCTAAGTGTCCCTCCGTTCAAGCACTTAGACGGTAAATTACAACATTCGGTTACAAC  
TCTATTAAATCGAAAAGTTAATATTTCATATATAAACAGTAATTAGTTATATTGATCCACAAAGCCCTAGTTTCCGCAATAAATCCGA  
TCGCCCTTTTTATACACATTCTCATTATGTCTCAGTTGCAACATCCCTTTTTCACTTTAAAGTAACGGTTACCTTTACTTATACGTC  
GGCGTTTGTCCCATTAATTTTTAGTTCTATTTTTTATTCTTTAAAGAAAATAAGGATGATCCGCCCTGATTAATAATCAATAATGGTT  
[ TATACT ] AGATTATGATTTCAAGTTGAATTCGGTTACATACATAGTGTGT [ TGTAAT ] TACGGATTTACATATTTTT AGGAGGTTTTATG

0 M S S S L K I A S T Q E A R Q Y F D T D R V V V D A V G S D  
ATGAGTCTTCTCTTAAATTTGCTTCGACTCAAGAAGCGCGTCAATATTTTCGATACTGACCGCGTTGTTGTCGATGCTGTAGGCTCTGAT  
30 F T D V G A V I A M D Y E T D V I D A A D A T K F G I P V F  
TTTCTGATGTCGGTGTGTTATCGCAATGGATTACGAAACAGATGTCATCGACGCTGCTGATGCAACTAAGTTTGGTATTCTGTGTTTT  
60 A V T K D A Q A I S A D E L K K I F H I I D L E N K F D A T  
GCCGTAACTAAGGATGCCAAGCTATCAGTGTGATGAGCTGAAGAAGATTTCCACATCATTGATTGGAAAACAATTTGATGCTACT  
90 V N A R E I E T A V N N Y E D S I L P P F F K S L K E Y V S  
GTTAACGCTCGTAAATCGAACTGCTGTTAAACAACACGAAGCAGCATTACCACCATTCTCAAGTCAATTGAAAGAATACGTTAGC  
120 R G L I Q F D C P G H Q G G Q Y Y R K H P A G R E F Y D F F  
CGTGGTTTTAATCCAATTCGACTGCCAGGTCACCAAGGTGGTCAATACTACAGAAAGCACCCAGCTGGTGGTGAATTCACGACTTCTC  
150 G E T V F R A D L C N A D V A L G D L L I H E G P A V A A E  
GGCGAACTGCTTCCGTCAGACTTATGTAACGCTGACGTTGCCTGGGTGACTTGGTGTGATCCACGAGGCTCTGCTGTGCTGCTGAA  
180 K H A A R V Y N A D K T Y F V L G G S S N A N N T V T S A L  
AAGCATGCTGCAGTGTGTTACACGCTGACAAGACTTACTTCGTTTTAGTGGTCTTCCAACGCTAACAACTGTAACATGCTGTTA  
210 V S N G D L V L F D R N N H K S V Y N S A L A M A G G R P V  
GTTTCTAACGGCGACTTGGTATTGTTCCGACCGAACAACCACAAGTCCGTTTACAACCTAGCTTTAGCTATGGCTGGTGGCCGCTCTGTT  
240 Y L Q T N R N P Y G F I G G I Y D S D F D E K K I R E L A A  
TACCTCAAACAACCTAACCCATACGGCTTCATCGGTGATCTACAGCAGCGACTTCGATGAAAAGAAGATCCGTGAATGGCAGCT  
270 K V D P E R A K W K P F R L A V I Q L G T Y D I Y N A  
AAGTTGACCCAGAAGCTGCTAAGTGAAGCGTCCATTCCGTCCTGGCTGTTATCCAATTAGGTACTTACGATGGTACTATCTACAACGCA  
300 H E V V K R I G H L C D Y I E F D S A W V G Y E Q F I P M M  
CACGAAGTTGTAAGCGTATCGGTACCTTTGTGATTACATCGAATTCCGACTCTGCTGGGTAGGTTACGAACAATTCATTCCCTATGATG  
330 R N S S P L L I D D L G P E D P G I I V V Q S V H K\* Q Q A G  
CGTAACCTCTACCATTTATGATTGATGACCTTGGTCCAGAAGATCCCTGGTATCATTGTTGTTCAATCAGTTCACAAGCAACAGCCGGC  
360 F S Q T S Q I H K K D S H I K G Q L R Y C D H K H F N N S F  
TTCTCAAACCTCACAAATCCACAAGAAGGATAGCCACATCAAGGGTCAATTACGTTACTGTGACCACAAGCACTTTAAACAACCTCTC  
390 N L F M S T S P F Y P M Y A A L D V N A A M Q E G E A G R K  
AACTTGTTCATGCTTACCATTTACCATTTACCAATGTATGAGCATTAGACGTTAACGGTGTATGCAAGAAGCGCAAGCAGTCCGCAAG  
420 L W H D L L I T T I E A R K K L I K A G S M F R P F V P P V  
TTATGGCATGACCTTCTGATTACTACCATTTGAAGCTCGTAAGAAGTTGATCAAGGCTGGCTCAATGTTCCGCTCCATTGCTCCACCTGTT  
450 V N G K K W E D G D T E D M A N N I D Y W R F E K G A K W H  
GTTAACGGCAAGAAGTGGGAAGATGGCGACACTGAAGATATGGTAAACAACATTTGACTACTGGCGCTTTGAAAAGGGTGTAAAGTGGCAT  
480 A Y E G Y G D N Q Y Y V D P N K F M L T T P G I N P E T G D  
GCTTACGAAGGCTACGGGACAACTAATACGTTGATCCAAACAAGTTCATGTTAACTACACCTGGTATCAACCCAGAAACTGGTGAC  
510 Y E D F G V P A T I V A N Y L R D H G I I P E K S D I L N S I  
TACGAAGACTTCGGTGTCCAGCTACTATCGTTGCTAACTACTTACGTGACCACGGTATCATCCCTGAAAAGTCTGACTTGAACCTATC  
540 L F L M T P A E T P A K M N N L I T Q L L Q L Q R L I E E D  
TTGTTCTTGATGCTCCAGCTGAACTCCAGCTAAGTGAACAACCTGATCACTCAACTCTTCAATTACAACGCTTGATCGAAGAAGAT  
570 A P L K Q V L P S I Y A A N E E R Y N G Y T I R E L C Q E L  
GCTCCATTGAAGCAAGTCTTCCCTCAATCTACGCTGCTAAGCAAGCAAGTTCATAATGGCTACACTATCCGTTGAACCTGGCAAGAATG  
600 H D F Y K N N N T F T Y Q K R L F L R E F F P E Q G M L P Y  
CAGGACTTCTACAAGAACAACAACACGTTACATACCAGAAGCGTCTCTTCTTACGTGAATTCCTCCAGAACAAGGTATGCTTCCATAC  
630 E A R Q E F I R N H N K L V P L N K I E G E I A L E G A L P  
GAAGCTCGTCAAGAATTCATCCGCAACCACAAGCTTGTTCATTGAACAAGATCGAAGGGCAAAATCGCCCTCGAAGGTGCTCTTCCA  
660 Y P P G V A F C V A P G E K W S E T T A V K Y F T I L Q D G I N  
TACCTCCAGGAGTATTCTGTGTAGCACCAGGTGAAAAGTGGTCAGAACTGCTGTTAAGTACTTCACTATCTACAGATGGTATCAAC  
690 N F P G F A P E I Q G V Y F K Q E G D K V V A Y G E V Y D A  
AACTTCCCTGATTCGCTCCAGAAATCCAAGGTGTACTTCAAGCAAGAAGCGCAAGGTTGTTGCTTACGGTGAAGTTTACGATGCA  
720 E V A K N D D R Y N N \* \*  
GAAGTTGCTAAGAACGATGATCGTTACAACAATAAATGCTTGGCTGATTAACAGGCAGGAAAAACCGCGTTATGCCGGTTTTT  
TGTTGCACAATTTTTCTTTTTAACTTGATTGCAATAAGTAAGTTTTATCCATAGTAGTCCATAGCTATTGAACATATAAACCAAGAC  
CAATATCCGTGCAATTTTTAAGGACCTGGCAGAAGTTAACGAGTTAGATACTCTTGTCTTACACACTACGGAGCTCACTATTGTGG  
AGATTAAGGTTCTTACATAGGTAGTACACCTCCGATCACTAAATCTAACAGGACGAGGATATATGCAGATTCTCGAATCCGGTTCCA

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



This work was supported by the National Institutes of Health (GM 30105) and the Foundation for Research.

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