Cloning and nucleotide sequencing of phenylalanine dehydrogenase gene of *Bacillus sphaericus*

(Activity staining; recombinant DNA; restriction map; Southern blot analysis; translational initiation site; promoter; terminator)

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**SUMMARY**

The gene coding for phenylalanine dehydrogenase [PDH; L-phenylalanine: NAD+ oxidoreductase (deaminating); EC 1.4.1.-] from *Bacillus sphaericus* SCRC-79a was cloned onto plasmid pUC9, and the nucleotide sequence of the 2-kb DNA region of the insert was determined. A 1143-bp open reading frame consisting of 381 codons was identified as a *pdh* gene coding for PDH.

**INTRODUCTION**

NAD+ -dependent PDH (EC 1.4.1.-) catalyzes the reversible oxidative deamination of L-phenylalanine. The enzyme appears to be useful as an industrial catalyst in the asymmetric synthesis of L-phenylalanine and related L-amino acids from their oxo-analogs. PDH was first discovered by Hummel et al. (1984) in *Brevibacterium* sp. Recently, Asano et al. (1987) found the enzyme activity in other bacterial species, *B. sphaericus* and *Sporosarcina ureae*, and extensively investigated the physical, catalytic, and immunological properties of the enzymes from these organisms. While the *Sporosarcina* enzyme showed high substrate specificity toward L-phenylalanine, the enzyme from *B. sphaericus* acted on L-phenylalanine and L-tyrosine. Here we report the cloning and sequencing of the *pdh* gene from *B. sphaericus*. 

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Abbreviations: aa, amino acid(s); bp, base pair(s); bla, gene coding for β-lactamase; HPLC, high-performance liquid chromatography; INT, 2(p-iodophenyl)-3( p-nitrophenyl)-5-phenyltetrazolium chloride; kb, kilobase(s) or 1000 bp; lacZpo, lac promoter-operator; nt, nucleotide(s); ORF, open reading frame; PDH, phenylalanine dehydrogenase; *pdh*, gene coding for PDH; PMS, phenazine methosulfate; SD, Shine–Dalgarno; SDS, sodium dodecyl sulfate.
EXPERIMENTAL AND DISCUSSION

(a) Cloning of the $pdh$ gene

Chromosomal DNA from $B. sphaericus$ was partially digested with restriction endonuclease HindIII, ligated into the HindIII site of pUC9 plasmid (Vieira and Messing, 1982), and the mixture was used to transform Escherichia coli JM103. The transformants were screened for the expression of ampicillin resistance and the PDH enzyme activity by colony staining as described below (Inagaki et al., 1986). Briefly, the ampicillin-resistant colonies grown on a nitrocellulose filter were lysed, frozen at $-70^\circ$C, thawed at room temperature, and then the filter was incubated at $55^\circ$C for 10 min. The filter was transferred to a dish containing 0.5 ml of a reaction mixture for activity staining (10 mM L-phenylalanine, 0.2 M Tris · HCl pH 8.5, 1.5 mM NAD$^+$, 0.32 mM PMS, and 0.8 mM INT) and incubated at room temperature for a few minutes. A colony producing PDH became dark red. Of about 5000 colonies screened, a single positive colony was identified. The plasmid DNA isolated from the colony was 14 kb in size and designated pBPDH1. Its restriction map is shown in Fig. 1a and b.

The 5'-terminal region of the coding sequence of the $pdh$ gene was localized by using a synthetic $^{32}$P-end-labeled mixed probe [5'-dGC(A/G/T)-AT(T/C)T(T/C)T(T/C)TG(A/G)AA-3'] consisting
Fig. 2. Nucleotide sequence of the pdh gene. An ORF of 1143 bp (381 aa) is shown with the deduced amino acid sequence. The first letter of the putative translation start codon is designated as + 1. An arrow and asterisk indicate the start and stop codons, respectively. Underlined are those amino acid residues which are found in the N-terminal sequence determined with purified PDH. In the 5'-flanking region, a solid underline indicates a potential ribosome-binding site, and the dashes underline the '-35' and '-10' regions of the putative pdh promoter.
of twenty-four 14-mers. This probe was synthesized according to the amino acid sequence, Phe-16-Gln-Lys-Ile-Ala-20, which was found in the N-terminal amino acid sequence of the purified \( B. \) \( sphaericus \) PDH (Y.A., R. Matsumoto and M.O., unpublished results). Southern-blot analysis of the restriction fragments of \( pBPDH1 \) showed that the \( ^{32}P \)-end-labeled probe mixture hybridized to the 0.5-kb \( HaeIII \) fragment indicated in Fig. 1b (not shown).

(b) Nucleotide sequence of the \( pdh \) gene

Denatured \( pUC9 \) plasmid DNAs carrying restriction fragments of \( pBPDH1 \) were used as templates (Hattori et al., 1985) for nucleotide sequencing by the dideoxy chain termination method (Sanger et al., 1977). The sequencing strategy is shown in Fig. 1c. The nucleotide sequence of the 1.3-kb \( DraI-BalI \) fragment and its flanking region (total 1400 bp) is shown in Fig. 2.

An ORF of 1143 bp (381 aa) was identified (Fig. 2). The 25 aa residues (underlined in Fig. 2) coincided with those identified by the N-terminal amino acid sequencing of the purified enzyme (not shown). The \( M_r \) of the encoded protein monomer of PDH estimated from the deduced amino acid sequence, excluding methionine, was 41435, which seems to agree with the \( M_r \) of 39000 determined by SDS–polyacrylamide gel electrophoresis of the purified enzyme (Asano et al., 1987). From these results, we concluded that this ORF coded for the \( B. \) \( sphaericus \) PDH. The codon usage of the \( pdh \) gene was found unbiased like other \( B. \) \( subtilis \) genes (Piggot et al., 1985).

(c) Flanking nucleotide sequence of the \( pdh \) gene

A putative ribosome-binding site was found starting from nt position -17, where the sequence GAATGGAGG exhibits strong complementarity to the 3' end of \( B. \) \( subtilis \) 16S rRNA, 3'–CUUUCUCC–5' (Moran et al., 1982). No ORF of more than 50 aa was found up to 750 bp upstream from the start codon (not shown). The hexanucleotide sequences TTGAAT starting from nt position -67 and ATTAAT from nt position -46 closely resemble the consensus promoter sequences (TTGACA and TATAAT) in the -35' and -10' regions for major vegetative promoters of Gram-positive bacteria, respectively (Graves and Rabinowitz, 1986). But the spacing of 15 bp between the two regions differs from the consensus 17 bp, although it is not entirely exceptional (Johnson et al., 1983; Graves and Rabinowitz, 1986).

A \( G + C \)-rich stem-and-loop structure, located about 70 bp downstream from the stop codon (nt positions 1213–1239), is unlikely to be a terminator, because it lacks a row of T's (Fujita et al., 1986, and references cited therein). Rather, the \( pdh \) gene appears to be polycistronic, because the initiation codon starting at nt position 1184 is followed by an ORF consisting of more than 100 codons (not shown), with an SD sequence starting at nt position 1171.

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REFERENCES


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