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Characterization of a Gene Encoding Diaminopimelate Decarboxylase from Rice

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Abstract: Diaminopimelate decarboxylase (DAPDC, EC 4.1.1.20) catalyzes the conversion of diaminopimelate into lysine (Lys), which is the last step in Lys biosynthetic pathway. The genes for DAPDC have been reported in many bacteria, and more recently in Arabidopsis. Here we report characterization of a gene for DAPDC from rice (Os*DAPDC*). Sequence analysis of a cDNA clone revealed a full-length open reading frame for Os*DAPDC* that encoded 490 amino acids, approximately 53.2 kDa protein. The OsDAPDC protein contains a consensus binding site for pyridoxal-5'-phosphate as a cofactor and has a sequence at the amino terminus that resembles a transit peptide for localization to plastids, similar to that of Arabidopsis. Single gene encoding DAPDC was found in chromosome II in rice. The predicted amino acid sequence of OsDAPDC is highly homologous to that of the enzymes for DAPDC encoded by *lysA* of many bacteria. Expression of OsDAPDC in *lysA* mutants of *Escherichia coli* shows that the gene is able to functionally complement the mutants. These results suggest that *OsDAPDC* encodes a protein for diaminopimelate decarboxylase in rice.

Key words: Rice, diaminopimelate decarboxylase, complementation, lysA, pyridoxal-5'-phosphate

Lysine (Lys) has important agricultural relevance because animals lack the ability to synthesize Lys and they must obtain it from their diet. It is for this reason that Lys is an essential amino acid in animals that includs humans. The biosynthetic pathway of Lys that includs methionine (Met) and threonine (Thr) is initiated from aspartate (Asp) called Asp family pathway (Azevedo et al., 1997). Through six subsequent steps, diaminopimelate (DAP) is synthesized. Finally, Lys is formed by pyridoxal-5'-phosphate (PLP)-dependent decarboxylation of DAP by diaminopimelate decarboxylase (DAPDC, EC 4.1.1.20), which catalyzes the conversion of DAP into Lys as the last step in Lys biosynthesis pathway.

The genes *lysA*, encoding DAPDC have been cloned and characterized from many bacteria (Cremer et al., 1988; Martin et al., 1988; Yamamoto et al., 1991; Mills and Flickinger, 1993), and recently in Arabidopsis (Hudson et al., 2005; Kim, 2006). DAPDC is well characterized in *Mycobacterium tuberculosis* and requires PLP for catalytic activity (Gokulan et al., 2003).

The binding motif for PLP, a cofactor of DAPDC, is well conserved; [FY]-[PA]-x-K-[SACV]-[NHCLFW]-x(4)-[LIVMF]-[LIVMTA]-x(2)-[LIVMA]-x(3)-[GTE] in bacteria (Sandmeier et al., 1994). The binding between DAPDC and the cofactor involves the formation of shiff base (Gokulan et al., 2003).

Many antibiotics or herbicides for killing microorganisms or plants, respectively, are targeted to specific enzyme in amino acid biosynthesis (Girodeau et al., 1986; Kelland et al., 1986). Several toxic analogs of DAP, which is a substrate of DAPDC, have been often used as antibiotics. The principle of the antibiotics is that a DAP analog is toxic to metabolize DAP-derivatives and finally leads to death because of Lys deprivation as a result of inhibition of DAPDC (Kelland et al., 1986).

Major cereal crops, such as corn and rice, are low in Lys whereas leguminous crops such as soybean are low in Met. To improve nutritional qualities of crops, it is necessary to investigate Lys biosynthetic pathway in crop plants. Here we report characterization of a gene for OsDAPDC, a key step of Lys pathway in rice.

MATERIALS AND METHODS

Sequencing analysis

An EST clone (GenBank Accession Number AK067100, clone ID J013098O07) was obtained from the Rice Genome Resource Center (RGRC). The clone was derived

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from rice cDNA library (Osato et al., 2002) from shoot plants prepared in pFLC vector. DNA sequencing was performed by an automatic sequencer with synthetic oligonucleotide primers. Nucleotide sequences and amino acid sequences were compared with sequences present in the GenBank and EMBL databases and analyzed using BLAST (Wheeler et al., 2003) and Clustal W multiple sequence alignment program (Tompson et al., 1994) or Biology WorkBench 3.2 (http://workbench.sdsc.edu; San Diego Supercomputer Center; University of California San Diego). Comparison of sequences was performed at the nucleotide and amino acid level. Motifs were searched by GenomeNet Computation Service at Kyoto University (http://www.genome.ad.jp).

Polymerase chain reaction (PCR) and construct for expression

PCR analysis (Sambrook and Russell, 2001) was performed as follows. After a plasmid was purified from a pellet harvested from a liquid culture containing ampicillin (Amp), the cDNA was amplified by using designed primers from Os*DAPDC* sequence: DAPDC-F (5'-AAAGCTTACCCTA AACCCTAGCCATG-3) and DAPDC-R (5'-AAAGCTTC GAGGAAAACGAGTTAGGCA-3). The polymerase chain reaction was performed using MyCycler™ PCR system (Bio-Rad, U.S.A) for 35 cycles with 95°C for 1 min, 55°C for 1 min, and 72°C for 2 min, with 10 μM primers. PCR products were analyzed on 1% (w/v) agarose gel.

The 1.5 kb PCR fragment was subcloned into a pXcm TA cloning vector and digested with *Bam*HI and inserted into the same site of pQE30NST plasmid to construct pQE::*OsDAPDC*. Restriction analysis was performed to confirm the construct.

Functional complementation

Three *lysA* mutant strains come from *E. coli* Genetic Resource Center (CGSC, Yale University). The genotype of the strains is as follows, ET505 (*λ⁻, lysA::Tn10, IN(rrnD-rrnE)1*), KL334 (*lacZ118(Oc), lacI22, λ⁻, lysA23*), and POP458 (*proC32, pgm-67, λ⁻, lysA23, rpsL109(strR), metE70*). These strains were transformed with pQE::*OsDAPDC* by electroporation (ECM399, BTX, USA) after producing competent cells by washing with water and glycerol (Kim and Leustek, 1996) using a cuvette with 0.1 cm electrode gap. Amp-resistant colonies were then replica plated onto M9 minimal medium supplemented with IPTG and 19 amino acids excluding Lys, each at a concentration of 40 mg/ml (Sambrook and Russell, 2001). The cultures were incubated at 30°C for 2 days. Growing colonies were retested for growth on Lys-free medium (Kim and Leustek, 1996).

RESULTS

The EST clone (clone ID J013098O07) was determined for nucleotide sequence using designed primers. The sequence of *OsDAPDC* cDNA contained a full-length open reading frame of 1473 bp and encoded a protein of approximately 53.2 kDa. The expected isoelectric point of the protein was 6.4. The predicted amino acid sequence of OsDAPDC was highly homologous to two DAPDC sequences from Arabidopsis that showed identities of 71.4% and 70.2% with AtDAPDC1 (GenBank Accession Number AF227913; Kim, 2006), and AtDAPDC2 (GenBank Accession Number NP_568252), respectively. The amino acid sequence of OsDAPDC was also homologous to the DAPDC from many bacteria, showing similarities ranging from 48% to 61%.

Analysis of the amino acid sequence of OsDAPDC revealed a signature binding motif for PLP in the N-terminal region (127-145) (Fig. 1). The motif sequence (YAVKANNNLR VLQLLRELG) was highly homologous to the consensus [FY]-[PA]-x-K-[SACV]-[NHCLFW]-x(4)-[LIVMF]-[LIV MTA]-x(2)-[LIVMA]-x(3)-[GTE], where the in which underlined amino acids are well conserved. The binding motif for PLP is present and well conserved in bacterial DAPDC and Orn/Arg decarboxylases family that uses PLP as a cofactor. The exact PLP binding site seemed to be K-130, identified by comparison with the binding site of bacterial LysA as shown in Fig. 1 (Gokulan et al., 2003). The finding suggests that the OsDAPDC product uses PLP as a cofactor to synthesize Lys. Another signature binding motif for substrate exists in the middle region (298-311) of the OsDAPDC (Fig. 1). The motif sequence (GFELEYLN IGGGLG) for DAP is highly homologous to the consensus [GSA]-x(2,6)-[LIVMSCP]-x(2)-[LIVMF]-[DNS]-[LIVM CA]-G-G-G-[LIVMFY]-[GSTPCEQ] in which the underlined amino acids are well conserved (Hofmann et al., 1999). The binding motif for an Orn/DAP/Arg is present and well conserved in bacterial DAP/Orn/Arg decarboxylases family. Phylogenetic analysis of the related sequences revealed further that OsDAPDC is divergent and evolved from ancestral bacterial DAPDCs (Fig. 2).

The size of OsDAPDC consisted of 490 amino acids, which was slightly larger than that of bacterial DAPDCs. About 60 amino acids at the amino terminus of the OsDAPDC product are not similar to any part of the bacterial enzymes (Fig. 1). This region contains a high concentration of alanine and hydroxyl amino acids such as serine, which are the features of a transit or signal sequences for protein transport into plastids (Nakai and Kanehisa, 1992). Organellar transit peptides are usually cleaved from the precursor protein and are not necessary for attainment of catalytic activity of the mature protein. In this respect,

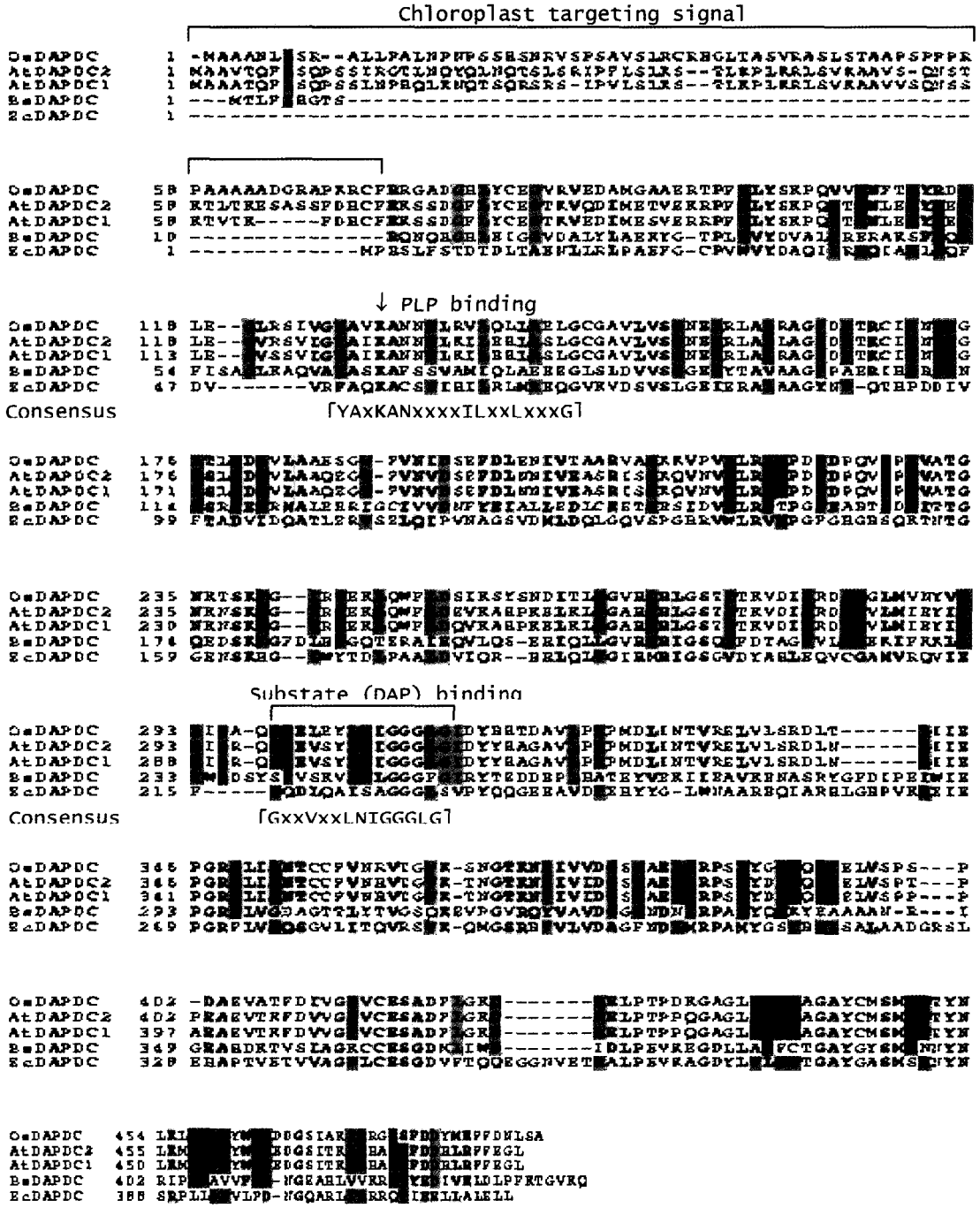

Fig. 1. Amino acid sequence alignment of DAPDCs using Boxshade program after Clustal W alignment. Completely conserved, identical, and similar residues are visually shown as yellow, green, and cyan, respectively. Accession numbers are as follows: AK067100 (OsDAPDC from *Oryza sativa*, this study), AF227913 (AtDAPDC1 from *Arabidopsis thaliana*), NP_568252 (AtDAPDC2 from *Arabidopsis thaliana*), P23630 (BsDAPDC from *Bacillus subtilis*), and P00861 (EcDAPDC from *Escherichia coli*. The binding motif for PLP and DAP is indicated including consensus sequences.

OsDAPDC may be synthesized in cytosols and transported into chloroplasts.

Analysis of nucleotide homology in rice genome database with OsDAPDC suggested that there is a single gene for DAPDC in rice. *OsDAPDC* sequence is identical to a genomic region located in chromosome II in rice (Os02g0440000, Ohyanagi et al., 2006). Through combined analysis of the cDNA and genomic sequences it was determined that the *OsDAPDC* consists of eight exons.

Expression of OsDAPDC in *lysA* mutants of *E. coli* shows that the OsDAPDC is able to functionally complement the mutants. The ability to grow on minimal medium lacking

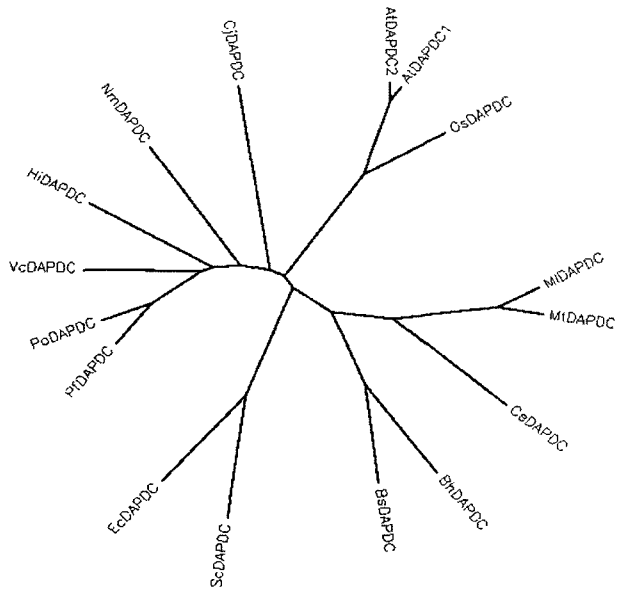

Fig. 2. Phylogenetic tree. Phylogenetic analysis of DAPDC related proteins using BLOSUM matrix in Clustal W. Accession numbers are as follows: AK067100 (OsDAPDC from *Oryza sativa*, this study), AF227913 (AtDAPDC1 from *Arabidopsis thaliana*), NP_568252 (AtDAPDC2 from *Arabidopsis thaliana*), Q9PII5 (CjDAPDC from *Campylobacter jejuni*), CAB83764 (NmDAPDC from *Neisseria meningitidis* serogroup A), P44316 (HiDAPDC from *Haemophilus influenzae*), Q9KVL7 (VcDAPDC from *Vibrio cholerae*), P19572 (PaDAPDC from *Pseudomonas aeruginosa*), AAY95201 (PfDAPDC from *Pseudomonas fluorescens*), P00861 (EcDAPDC from *Escherichia coli*), Q9ZBH5 (ScDAPDC from *Streptomyces coelicolor*), P23630 (BsDAPDC from *Bacillus subtilis*), Q9KCM5 (BhDAPDC from *Bacillus halodurans*), Q8RQM6 (CeDAPDC from *Corynebacterium efficiens*), P31848 (MtDAPDC from *Mycobacterium tuberculosis*), and Q50140 (MlDAPDC from *Mycobacterium leprae*).

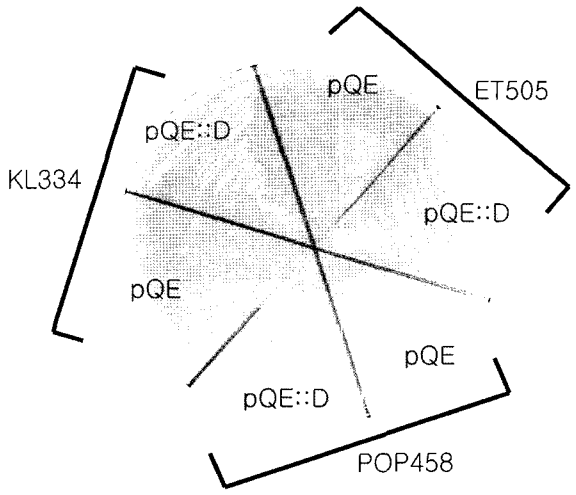

Fig. 3. Complementation assay. *E. coli* strains ET505, KL334 and POP458 were transformed with a vector only (pQE) or an *OsDAPDC*-expression construct (pQE::*OsDAPDC*, pQE::D) and then streaked on M9 medium supplemented 19 amino acids without Lys.

Lys was tested in the culture expressing OsDAPDC. *E. coli* strains, ET505, KL334, and POP458, were transformed with an *OsDAPDC*-expression construct and then streaked on the M9 medium supplemented with amino acids without Lys. The *lysA* mutants with the construct could grow without Lys whereas the mutants without the construct could not grow (Fig. 3). These results suggest that *OsDAPDC* encodes an enzyme for diaminopimelate decarboxylase in rice.

DISCUSSION

The functional characterization of DAPDC encoded by *lysA* gene has been reported in many bacteria. Here we report characterization of a gene encoding the rice DAPDC (*OsDAPDC*). Following determination of the nucleotide sequences, whether the encoded protein might be novel enzyme for DAPDC in rice was analyzed. The full-length cDNA encodes a protein that is highly homologous to *lysA* of bacterial DAPDCs. There is a single gene for *OsDAPDC* in chromosome II of rice. The OsDAPDC protein from rice consists of 490 amino acids that is slightly larger in size than that from bacteria, which suggests that there is a transit peptide to plastids in the amino terminal of the protein.

We are investigating to get some important clues about substrate specificity of the enzyme by purifying recombinant OsDAPDC in *E. coli* and physiological function of this novel enzyme for Lys metabolism by screening T-DNA insertion mutants in which the expression of the *OsDAPDC* gene is knocked out in rice.

It is still unknown why major cereal crops, such as corn and rice, are low in Lys whereas leguminous crops such as soybean are low in Met. Lys and Met including Thr biosynthesis as classified as the Asp pathway. The metabolites would be distributed in the action of several key enzymes by an unknown mechanism in the Asp pathway.

To improve nutritional qualities of crops, it is necessary to investigate Lys biosynthetic pathway in crop plants. The report about characterization of a gene encoding OsDAPDC, the last step of the Lys pathway, would be a starting point at to investigate the Lys biosynthesis in rice molecular level, toward modification of nutritional composition by metabolic engineering and overexpression in such an important crop (Galili et al., 2005).

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