

Sequence comparison of plant ornithine decarboxylases reveals high homology and lack of introns

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Abstract

We have designed and constructed four oligonucleotides corresponding to the most conserved regions of ornithine decarboxylases (ODC; EC 4.1.1.17) of plant origin. These oligonucleotides were used for the amplification of homologous fragments from several plants (*Zea mays*, *Capsicum annuum*, *Sorghum bicolor*, *Phaseolus vulgaris*, *Carica papaya* and *Daucus carota*). The amplified fragments were cloned and sequenced, revealing high homology to other ODCs. Peptide sequences coded by these fragments were compared by Clustal analyses. These analyses identified the location of the conserved sequences corresponding to the binding sites of substrate and cofactor. Data demonstrated that the plant ODCs fragments lacked intron sequences and were extremely homologous (over 80 %), constituting a compact group separated from other eukaryotic ODCs.

Additional key words: *Capsicum annuum*, *Carica papaya*, *Daucus carota*, *Phaseolus vulgaris*, polyamines, *Sorghum bicolor*, *Zea mays*.

Introduction

Ornithine decarboxylase (ODC) is the enzyme involved in the first step of polyamine biosynthesis in most organisms. The enzyme catalyzes the decarboxylation of ornithine to produce putrescine. In plants and some bacteria a second mechanism exists for the synthesis of polyamines. This pathway involves the action of arginine decarboxylase (ADC) to produce agmatine (Tabor and Tabor 1984). While the genes encoding ODC and ADC are believed to be present in most plants, their contribution to putrescine production is often tissue-specific and developmentally regulated (Walden *et al.* 1997). Noticeably, no ODC-coding gene has been identified in the already sequenced genome of *Arabidopsis thaliana* (Hanfrey *et al.* 2001). Polyamines are polycations found to be essential in all organisms (Tabor and Tabor 1984), and they play different roles in DNA protection: from enzymatic degradation, X-ray irradiation, mechanical shearing and oxidative damage

(McCann *et al.* 1987). In plants, polyamines affect growth rate and are involved in regulation of light-induced growth response, somatic embryogenesis, flower and fruit development, pollen formation, root meristem function and lateral growth of leaf-homolog organs, senescence and responses to biotic and abiotic stresses (DeScenzo and Minocha 1993, Edreva 1997, Watson *et al.* 1998, Kakkar *et al.* 2000). Despite the importance of polyamines, the metabolism in plants has not received enough attention; for example, only cDNAs corresponding to four plant ODC's are known: *Nicotiana tabacum* (GenBank AB031066.1), *Nicotiana glutinosa* (GenBank AF323910), *Lycopersicon esculentum* (Kwak and Lee 2001) and *Datura stramonium* (Michael *et al.* 1996).

As a first step in our research interest on the role of ODC and ADC pathways in polyamine synthesis in plants, we have isolated conserved fragments of genes

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Abbreviations: ODC - ornithine decarboxylase; ADC - arginine decarboxylase; PCR - polymerase chain reaction.

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encoding ODCs from the following plant species: *Zea mays*, *Sorghum bicolor*, *Capsicum annuum*, *Phaseolus vulgaris*, *Carica papaya* and *Daucus carota*. In the present communication we described their isolation,

Materials and methods

The following plants and cultivars were utilized: *Zea mays* (hybrid line LPC13), *Capsicum annuum* (cv. Tampiqueño 74; serrano type), *Sorghum bicolor* (cv. Esmeralda), *Phaseolus vulgaris* (cv. Negro Queretaro), *Carica papaya* (cv. Maradol), *Arabidopsis thaliana* (ecotype Columbia C24) and *Daucus carota* (cv. Nantes). All plant materials utilized for DNA isolation were calli obtained by standard techniques (Cabrera-Ponce *et al.* 1995, Lopez 1999, O'Connor-Sanchez *et al.* 2002).

Genomic DNA was isolated as described by Shure *et al.* (1983). Oligonucleotides for PCR were designed from conserved regions corresponding to cDNAs from *Nicotiana tabacum* (GenBank AB031066.1), *Nicotiana glutinosa* (GenBank AF323910), *Lycopersicon esculentum* (Kwak and Lee 2001) and *Datura stramonium* (Michael *et al.* 1996). Four oligonucleotides were synthesized: two sense primers, OP-1 5'-GCGGCCATTCTCAGTCACAAT-3' coding for residues 14-21 from *N. tabacum* (AAILQSTI) and OP-4 5'-TACGCTGTTAAATGTAACCCCTGAACC-3' coding for residues 93-101 (YAVKCNPEP); and two anti-sense primers,

OP-2 5'-TCGATGAACTGTGTACTTACGAC-3' coding for residues 336-343 (SMNCVLYD) and OP-3 5'-TTTCCTAATATGGGTGCTTA-3' coding for residues 401-407 (FPNMGAY). Incubation mixtures (0.05 cm³) for each PCR reaction contained: 150 ng of genomic DNA, 0.2 pmol of each primer, 10-mM dNTPs, 10-mM Tris-HCl pH 8.3, 3.0 mM MgCl₂, 2.5 U Taq DNA polymerase (*Invitrogen*, Carlsbad, USA). The employed "touch down" protocol was as follows: 5 min at 94 °C, and 5 cycles each with annealing temperatures of 30, 35, 40, 45, 50 and 55 °C for 1 min and 2 min at 72 °C. Nesting rounds using different oligonucleotides were performed under the following conditions: 1 min at 94 °C, 1 min at 45 - 55 °C, and 2 min at 72 °C. After 30 cycles, extensions were continued at 72 °C for 10 min. Samples were analyzed by agarose gel electrophoresis. Fragments of the expected size were cloned in *TOPO-pCR II* (TA Cloning Kit, *Invitrogen*) and sequenced. For dendograms and alignments, we used *MegAlign* (DNASTAR, Madison, USA) and *CLUSTAL* programs (Higgins and Sharp 1988). The reported ODC sequences were obtained from the EMBL GenBank.

Results and discussion

Using the different oligonucleotides and PCR conditions for nested amplifications, it was possible to isolate fragments corresponding to the genes encoding ODCs in the analyzed species. Three different bands were amplified with oligonucleotides OP1 and OP3, when DNA from *Z. mays* (GenBank AY123224), *C. annuum* (GenBank AF521192) and *S. bicolor* (GenBank AF522351) were used. However, when the amplified fragments were subjected to a further round of PCR using nested conditions with oligonucleotide OP2, a single band was obtained in each case, 1018, 1005 and 1018 bp in length, respectively. In the case of *P. vulgaris* (Genbank AY125817), *C. papaya* (Genbank AY125815) and *D. carota* (Genbank AY125816), it was necessary to use three rounds of amplification in order to obtain the corresponding single fragment in each case. First amplification with oligonucleotides OP1 and OP3 gave rise to several bands. A second nested amplification with oligonucleotide OP2, instead of OP3, produced very weak bands. It was necessary a further round of nested amplification with oligonucleotide OP4, instead of OP1,

to amplify single fragments of 761, 761 and 763 bp in length for *P. vulgaris*, *C. papaya* and *D. carota*, respectively.

Arabidopsis thaliana (ecotype Columbia C24) genomic DNA was used to isolate the ODC gene fragment by PCR. First amplification using oligonucleotides OP1 and OP3 gave rise to four bands. Next amplification using nested conditions with oligonucleotides OP1 and OP2 produced two bands. However, in the last amplification round using oligonucleotides OP2 and OP4, no bands were detected. This result agrees with the absence of ODC sequences reported by Hanfrey *et al.* (2001) for *A. thaliana*.

Cloning and sequencing the amplified fragments revealed the corresponding amino acid sequences. As expected, according to the PCR conditions utilized for their synthesis, fragments from *P. vulgaris*, *C. papaya* and *D. carota*, were shorter than those from *Z. mays*, *C. annuum* and *S. bicolor*. Comparison of the sequences with those from the cDNAs corresponding to the five genes encoding plant ODCs isolated up to now: *Datura*

Ca1	-----AAILQSTIGGAPPSTAAAAAENGDT-TRKVVPLSKDALQDFMVSIIITQKLQGEKKPFYVLDLGEVVSIMDQWNVALP	
Zm	-----AAILQSTIGGAPPSTAAAAAENGDT-TRKVVPLSKDALQDFMVSIIITQKLQGEKKPFYVLDLGEVVSIMDQWNVALP	
Sb	-----AAILQSTNGGAPPSTAAAAAENGDT-TRKVVPLSKDALQDFMVSIIITQKFQGEKKPFYVLDLGEVVSIMDQWNVALP	
Ca2	MAGQTIVIVSGLNPAAILQSTIGGAPPSTAAAAAENGDT-TRKVVPLSKDALQDFMVSIIITQKLQGEKKPFYVLDLGEVVSIMDQWNVALP	
Cp	-----	
Pv	-----	
Dc	-----	
Le	MAGQTIVIVSGLNPAAILQSTIGGA--PVAAAENGH--TRKVVPLSKDALQDFMVSIIITQKLQDDQPFYVLDLGEVVSIMDQWNVALP	87
Ds	MAGQTIVIVSGLNPAAILQSTIGGA--TPAAPAENDH--TRKVVPLSRDALQDFMVSIIITQKLQDEKQPFYVLDLGEVVSIMDQWNAGLP	
Ng	MAGQTIVIVSGLNPAAILQSTIGGASPTAAAAA-ENG--TRKVIPLSRDALQDFMLSIIITQKLQDEKQPFYVLDLGEVVSIMDQWKSLP	
Nt	MAGQTIVIVSGLNPAAILQSTIGGASPTAAAAAENG--TRKVIPLSRDALQDFMLSIIITQKLQDEKQPFYVLDLGEVVSIMDQWKSLP	
Ca1	NVRPFYAVKCNPEPSFLSMLAAMGSNFDCASRAEIEYVLSLGISPERIVFANPRKPESDIIIFAEEKVGVLNTTYDSEDEVYKIKKHPKCE	
Zm	NVRPFYAVKCNPEPSFLSMLAAMGSNFDCASRAEIEYVLSLGISPERIVFANPCKPESDIIIFAEKVGVLNTTYDSEDEVYKIKKHPKCE	
Sb	NVRPFYAVKCNPEPSFLSMLAAMGSNFDCASRAEIEYVLSLGISPERIVFANPCKPESDIIIFAEKVGVLNTTYDSEDEVYKIKKHPKCE	
Ca2	NVRPFYAVKCNPEPSFLSMLAAMGSNFDVCASRAEIEYVLSLGISPERIVFANPCKPESDIIIFAEKVGVLNTTYDSEDEVYKIKKHPKCE	
Cp	-----YAVKCNPEPSFLSMLAAMGSNFDVCASRAEIEYVLSLGISPERIVFANPCKPESDIIIFAEKVGVLNTTYDSEDEVYKIKKHPKCE	
Pv	-----YAVKCNPEPSFLSMLAAMGSNFDVCASRAEIEYVLSLGISPERIVFANPCKPESDIIIFAEKVGVLNTTYDSEDEVYKIKKHPKCE	
Dc	-----YAVKCNPEPSFLSMLAAMGSNFDVCASRAEIEYVLSLGISPERIVFANPCKPESDIIIFAEKVGVLNTTYDSEDEVYKIKKHPKCE	
Le	NIRPFYAVKCNPEPSFLSMLSAMGSNFDCASRAEIEYVLSHGISPDRIVFANPCKPESDIIIFAEKIVGVLNTTYDSEDEVYKIRKHPKCE	177
Ds	NIRPFYAVKCNPEPSFLSMLSAMGSNFDCASRAEIEYVLSLGISPDRIVFANPCKPESDIIIFAAKVGVLNTTYDSEDEVYKIRKHPKCE	
Ng	NIRPFYAVKCNPEPSFLSMLSAMGSNFDCASRAEIEYVLALGIGPDRIVFANPCKPESDIIIFAAKVGVLNTTYDSEDEVYKIRKHPKCE	
Nt	NIRPFYAVKCNPEPSFLSMLSAMGSNFDCASRAEIEYVLSLGISPDRIVFANPCKPESDIIIFAAKVGVLNTTYDSEDEVYKIRKHPKCE	
Ca1	LLLRIKPMNDGNARCPMPGPKYGAPEEIPLLRIAQASRLTVSGVSFHISSGDADSNAYLGAIAAAKQVFKTAAKFGMSKVNVLVDVGDF	
Zm	LLLRIKPMNDGNARCPMPGPKYGAPEEIPLLRIAQASRLTVSGVSFHISSGDADSNAYLGAIAAAKQVFETAAKFGMSKVNVLIDGGF	
Sb	LLLRIKPMNDGNARCPMPGKSFQGAFPEEIPPFVRIAQASRLTVSGVSFHISSGDADSNAYLGAIAAAKQVFETAAKFGMSKVNVLIDGGF	
Ca2	LLLRIKPMNDGNARCPMPGPKYGAPEEIPLLRIAQASRLTVSGVSFHISSGDADSNAYLGAIAAAKQVFETAAKFGMSKVNVLIDGGF	
Cp	LLLRIKPMNDGNARCPMPGPKYGAPEEIPLLRIAQASRLTVSGVSFHISSGDADSNAYLGAIAAAKQVFETAAKFGMSKVNVLIDGGF	
Pv	LLLRIKPMNDGNARCPMPGPKYGAPEEIPLLRIAQASRLTVSGVSFHISSGDADSNAYLGAIAAAKQVFETADKFGMSKVNVLIDGGF	
Dc	LLLRIKPMNDGNARCPMPGPKYGAPEEVEPLLRIAQASRLTVSGVSFHISSGDADSNAYLGAIAAAKQVFETADKFGMSKVNVLIDGGF	
Le	LLLRIKPMNDGNARCPMPGPKYGAPEEVEPLLRIAQASRLTVSGVSFHISSGDADSNAYLGAIAAAKQVFETAAQLGMPKMTVLIDGGF	
Ds	LLLRIKPMNDGNARCPMPGPKYGAPEEVEPLLRIAQASRLTVSGVSFHISSGDADSKAYLGAIAAAKQVFETAAQLGMPKMTVLIDGGF	
Ng	LLLRIKPMNDGNARCPMPGPKYGAPEEVEPLLRIAQASRLTVSGVSFHISSGDADSNAYLGAIAAAKQVFETAAQLGMSKMTVLIDGGF	
Nt	LLLRIKPMNDGNARCPMPGPKYGAPEEVEPLLRIAQASRLTVSGVSFHISSGDADSNAYLGAIAAAKQVFETAAQLGMSKMTVLIDGGF	267
Ca1	TSGHQFTTAATAVKSALQQHFSNEPELTIIIAEPGRFFAETAFTLATTIIGKRVRGDLREYWINDGLYGSMNCVLYD-----	
Zm	TSGHQFTTAATAVKSALQQHFSNEPELTIIIAEPGRFFAETAFTLATTIIGKRVRGDLREYWINDGLYGSMNCVLYD-----	
Sb	TSGHQFTTAATTVKSALQQHFSNEPELTIIIAEPGRFFAETAYTLATTIIGKRVRGDLREYWINDGLYGSMNCVLYD-----	
Ca2	TSGHQFTTAATAVKSALQQHFSNEPELTIIIAEPGRFFAETAFTLATTIIGKRVRGDLREYWINDGLYGSMNCVLYD-----	
Cp	TSGHQFTTAATAVKSALQQHFSNEPELTIIIAEPGRFFAETAFTLATTIIGKRVRGDLREYWINDGLYGSMNCVLYD-----	
Pv	TSGHQFTTAATAVKSALQQHFSNEPELTIIIAEPGRFFAETAFTLATTIIGKRVRGDLREYWINDGLYGSMNCVLYD-----	
Dc	TSGHQFTTAATAVKSALQQHFSNEPELTIIIAEPGRFFAETAFTLATTIIGKRVRGDLREYWINDGLYGSMNCVLYD-----	
Le	TSGHQFTTAAPAVKSALQHDFPELTIIIAEPGRFFAETAFTLATTIIGKRVRGELKEIWINDGLYGSMNCVLYD-----	
Ds	TSGHQFTTAAVAVSALQHDFPELTIIIAEPGRFFAETAFTLATTIIGKRVRGELKEIWINDGLYGSMNCVLYD-----	
Ng	TSGHQFTTAAVAVKSALKQHDFPELTIIIAEPGRFFAETAFTLATTIIGKRVRGELREYWINDGLYGSMNCVLYD-----	
Nt	TSGHQFTTAAVAVKSALKQHDFDEPELTIIIAEPGRFFAETAFTLATTIIGKRVRGELREYWINDGLYGSMNCVLYD-----	357
Ca1	-----	
Zm	-----	
Sb	-----	
Ca2	RVNLNCGSKMFSTIFGPTCDALDTVLRDYHVPPELQVNDWVIFPNMGAYTKAAGSNFNGFNTSAIVTHLAYAPS	
Cp	-----	
Pv	-----	
Dc	-----	
Le	RNNLNCGGSKTFPSTVFGPTCDALDTVLRDYQLPPELQVNDWLIFPNMGAYTKAAGSNFNGF-----	
Ds	RSNLNCGGSKTFPSTVFGPTCDALDTVLRDYQLPPELQVNDWLIFPNMGAYTKAAGSNFNGFNTSAIVTHLAYAPS	
Ng	RTNVTCGGSKTFPTVFGPTCDALDTVLRDYQLPPELQVNDWLVPNMGAYTKAAGSNFNGFNTSTIVTHLAYTYS	
Nt	RSNVTCGGSKTFPTVFGPTCDALDTVLRDYQLPPELQVNDWLVPNMGAYTKAAGSNFNGFNTSAIVTHLAYAPS	433

Fig. 1. Multiple amino acid alignment as derived by maximal homology of ODCs from genomic DNA fragments isolated in this study and with the corresponding sequences of cDNA's encoding other plant ODC's: Zm, *Zea mays*; Ca1, *Capsicum annuum* (Tampiqueño 74); Ca2 *Capsicum annuum* (cDNA from cultivar MC11); Sb, *Sorghum bicolor*; Pv, *Phaseolus vulgaris*; Cp, *Carica papaya*; Dc, *Daucus carota*; Le, *Lycopersicon esculentum*; Ds, *Datura stramonium*; Ng, *Nicotiana glutinosa*; Nt, *Nicotiana tabacum*. Identical residues (asterisks) in the eleven polypeptides and conserved amino acid substitutions (dots) are indicated. The putative PEST regions are in italics and underlined. The double-underlined sequence corresponds to the cofactor (pyridoxal phosphate) binding site. The signature sequence of the conserved decarboxylase family 2 appears underlined. The wave-underlined motif within the second PEST region corresponds to the conserved ODC catalytic sequence. The star-underlined sequence corresponds to the region of the antizyme-binding of mouse.

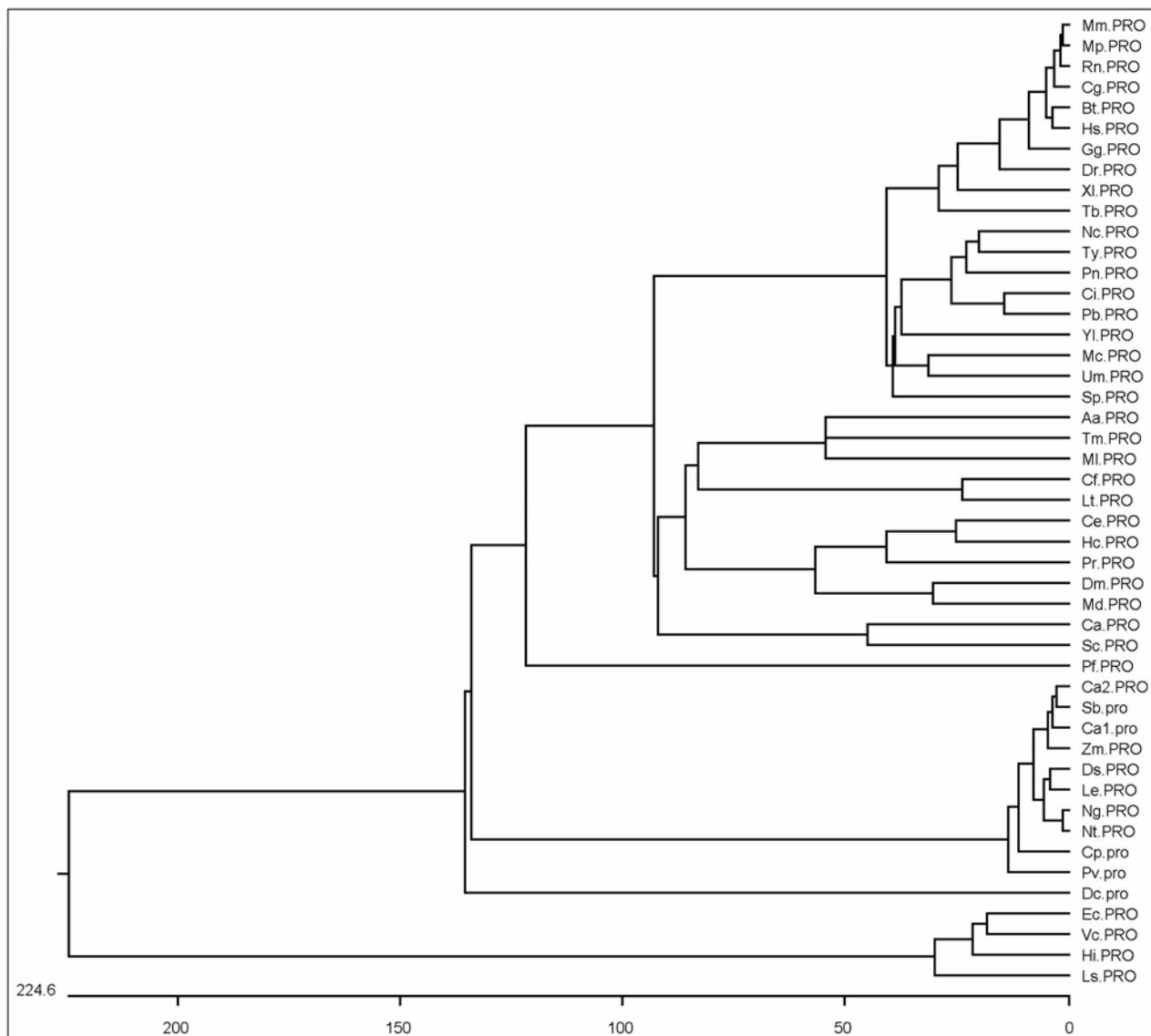


Fig. 2. Phylogenetic tree of ODC's belonging to 46 organisms representing bacteria, plants, protozoa, fungi and animals: Zm, *Zea mays*; Ca1, *Capsicum annuum* (var. Tampiqueño 74); Ca2 *Capsicum annuum* (cDNA from cultivar MC11); Sb, *Sorghum bicolor*; Pv, *Phaseolus vulgaris*; Cp, *Carica papaya*; Dc, *Daucus carota*; Le, *Lycopersicon esculentum*; Ds, *Datura stramonium*; Ng, *Nicotiana glutinosa*; Nt, *Nicotiana tabacum*; Cf, *Crithidia fasciculata*; Lt, *Leishmania tarentolae*; Ce, *Caenorhabditis elegans*; Hc, *Haemonchus contortus*; Pr, *Panagrellus redivivus*; Dm, *Drosophila melanogaster*; Md, *Musca domestica*; Tm, *Thermotoga maritima*; Mi, *Mesorhizobium loti*; Aa, *Aquifex aeolicus*; Pf, *Plasmodium falciparum*; Mm, *Mus musculus*; Mp, *Mus pahari*; Rn, *Rattus norvegicus*; Cg, *Cricetulus griseus*; Hs, *Homo sapiens*; Bt, *Bos taurus*; Gg, *Gallus gallus*; Dr, *Danio rerio*; XI, *Xenopus laevis*; Tb, *Tripanosoma brucei*; Ca, *Candida albicans*; Sc, *Saccharomyces cerevisiae*; Nc, *Neurospora crassa*; Ty, *Tapesia yallundae*; Pn, *Phaeosphaeria nodorum*; Ci, *Coccidioides immitis*; Pb, *Paracoccidioides brasiliensis*; Yl, *Yarrowia lipolytica*; Mc, *Mucor circinelloides*; Um, *Ustilago maydis*; Sp, *Schizosaccharomyces pombe*; Ec, *Escherichia coli*; Vc, *Vibrio cholerae*; Hi, *Haemophilus influenzae*; Ls, *Lactobacillus* sp.

stramonium, *Nicotiana tabacum*, *Nicotiana glutinosa*, *Lycopersicon esculentum* and the very recently described from *C. annuum* (GenBank AF480882) (Fig. 1), showed that the amplified region from all the ODC fragments contained the characteristic motifs of ODCs:

1. The binding site for the pyridoxal phosphate cofactor located between amino acids 93-111 in *N. tabacum* [YAVKCNPEPSFLSM(I)LA(S)AMG]. Only two changes

were detected in this motif, isoleucine instead of methionine in ODCs from *N. tabacum* and *N. glutinosa*, and serine instead of alanine in *N. tabacum*, *N. glutinosa*, *L. esculentum* and *D. stramonium* (Fig. 1). In the ODC from mouse, the amino acid directly involved in pyridoxal phosphate binding was identified as the lysine residue located in the sequence PFYAVKC (Poulin *et al.* 1992). In the case of the ODC from *N. glutinosa*, a

change of the corresponding residue (Lys⁹⁵) by an alanine, using site-directed mutagenesis, gave rise a substantial loss in enzyme activity (Lee and Cho 2001).

2. The conserved sequence of family 2 decarboxylases, located between amino acids 251-268 of *N. tabacum* [A(D)K(Q)FGMS(P)KM(V/I)N(T)V(I)LDIG(D)G(D)GFT] responsible for substrate binding (Moore and Boyle 1990). As noticed, several plant ODCs (*L. esculentum*, *D. stramonium*, *C. annuum*, *N. tabacum*, *N. glutinosa*) showed the conserved amino acid changes in this motif (Fig. 1). Lee and Cho (2001) found that residue Cys³³⁸ was an important residue for substrate binding.

3. The catalytic site located at the consensus sequence [IWGPTCDGL(I)D] in mouse corresponds to amino acids 373-382 in *N. tabacum* [VFGPTCDALD] and the rest of plant ODCs (Fig. 1). It is known that catalysis depends on the conserved cysteine residue of the motif (Poulin *et al.* 1992). When the cysteine residue (Cys³⁷⁷) from *N. glutinosa*, was changed by an alanine through site-directed mutagenesis, also a substantial loss in enzyme activity occurred (Lee and Cho 2001). This sequence is missing in the shortest DNA fragments amplified here.

4. A comparison of the amino acid sequences of mouse and plant ODCs in the antizyme-binding region reveals that 12 out of 24 residues are identical to those in *N. tabacum*, and correspond to amino acids 144-167 (Fig. 1). Antizyme is a spermidine-induced protein that binds and stimulates ornithine decarboxylase degradation (Li and Coffino 1992). Although an antizyme mechanism for regulation of ODC levels in plants has not been identified, these results raise the attractive possibility that it could be operative in these organisms.

5. Two putative PEST regions [sequences rich in proline (P), glutamic acid (E), serine (S) and threonine (T)

residues] characteristic of proteins with a high rate of turnover (Rechsteiner and Rogers 1996) with PEST scores of +0.56 and -8.48 were identified, respectively, at residues 287-302 and 367-386 of *N. tabacum* (Fig. 1) by use of the algorithm described by Rechsteiner and Rogers (1996). Interestingly, the second putative PEST box is located at the active site of the enzyme. Both PEST motifs had a low score (particularly one of them) in agreement with fungal ODCs (*e.g.* Jiménez-Bremont *et al.* 2001).

Interestingly, the isolated fragments of plant ODCs reported here have no conserved sequences corresponding to introns. These results are in contrast with the presence of introns in animal ODCs (Katz and Kahana 1988, Fitzgerald and Flanagan 1989), and in at least one fungal ODC (Guevara-Olvera *et al.* 2000).

A phylogenetic tree analysis of ODCs from plants, protozoa, animals, fungi and bacteria was constructed using the Clustal method (Higgins and Sharp 1988) (Fig. 2). It can be observed that plant ODCs constitute a compact group separated from the rest of the eukaryotic and prokaryotic enzymes. Within the plant ODCs, those from solanaceous species appeared as a group separated from the rest, with the exception of *Capsicum* enzymes that grouped with ODCs from monocots. It will be necessary to isolate the whole genes encoding the ODCs of the species studied in this work, and genes from plants belonging to different groups, to investigate whether relatedness of these enzymes follow the phylogenetic relationships among the corresponding plants. Finally, it will also be interesting to determine whether the differences in sequence observed between the fragment of the gene encoding the *C. annuum* ODC isolated in this work, and the cDNA described by Sajari and Zainal (GenBank AF480882) are due to the use of different cultivars.

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