Acetyl-CoA synthetase from *Pseudomonas putida* U is the only acyl-CoA activating enzyme induced by acetate in this bacterium

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Abstract

The gene (*acs*) encoding the acetyl-CoA synthetase (*Acs*) in *Pseudomonas putida* U has been cloned, sequenced and expressed in different microbes. The protein has been purified and characterized from a biochemical, structural and evolutionary point of view. Disruption or deletion of *acs* handicapped the bacterium for growth in a chemically defined medium containing acetate; this ability was regained when *P. putida* U was transformed with a plasmid carrying this gene. By contrast, all the *acs* knock-out mutants could assimilate *n*-alkanoic acids having a carbon length greater than C2, suggesting that other acyl-CoA activating enzymes (different from *Acs*) are involved in the catabolism of these compounds. However, these enzymes that can replace the function played by *Acs* in *vivo* are not induced by acetate.

Introduction

The assimilation of acetate in *Pseudomonas* species requires uptake from the medium, its activation to acetyl-CoA and the transformation of this thioester into general metabolites (Starai & Escalante-Semerena, 2004; Wolfe, 2005). Three different enzymes (acetate kinase, EC 2.7.2.1; acetyl-CoA synthetase-ADP-forming, EC 6.2.1.1.3; and acetyl-CoA synthetase-AMP-forming, EC 6.2.1.1) are usually involved in the synthesis of acetyl-CoA throughout fairly specific reactions that are dependent on the acetate concentration present in the medium (Martínez-Blanco *et al.*, 1990, 1992; Fernández-Valverde *et al.*, 1993; Görisch, 2003; Starai & Escalante-Semerena, 2004; Wolfe, 2005).

Although acetate catabolism has been widely studied, the molecular mechanisms involved in the regulation of acetyl-CoA synthetase (*Acs*) activity and gene (*acs*) expression, have only begun to be understood recently (Black *et al.*, 1992; Hisanaga *et al.*, 2004; Starai & Escalante-Semerena, 2004). Thus, at the present moment it is not still clear which of the different acyl-CoA ligases present in the cells are really involved in the *in vivo* activation of acetate (Martínez-Blanco *et al.*, 1990, 1992; Fernández-Valverde *et al.*, 1993; Miñambres *et al.*, 1996).

In order to clarify this point, we have studied the assimilation of acetate in *Pseudomonas putida* U. We describe the isolation and the characterization of different mutants unable to catabolize acetate in this bacterium as well as the function played by these proteins. Furthermore, structural, biochemical and evolutionary analyses about a specific *Acs* are described and discussed.

Materials and methods

Materials

Biochemicals and reagents were supplied by different commercial firms (Sigma, Merck, Lancaster, Amersham Pharmacia Biotech Gmbh, Stratagene and Promega).

Microorganisms and vectors

The *P. putida* U (Martínez-Blanco *et al.*, 1990), *Escherichia coli* HB101 (Selvaraj & Iyer, 1983), *E. coli* (pRK600), *E. coli*
DH10B and E. coli strain NM538 (Herrero et al., 1990) were
from our collection. pGEM-T Easy (Promega) was used for
subcloning genomic fragments, and the pK18::mob (Schäfer
et al., 1994) and the pJQ200KS (Quandt & Hynes, 1993)
were used to induce specific gene disruption. pBBR1MCS-3
(Tc·) (Kovach et al., 1995) was used to analyse the expres-
sion of different genes in P. putida U.

All the sequences reported in this paper correspond to the
accession numbers AY168852–AAO12523 and AF150671
(GenBank).

Culture media and growth conditions

Pseudomonas putida U was maintained in slants and cul-
tured in Erlenmeyer flasks as reported (Martinez-Blanco
et al., 1990). Each 500 mL Erlenmeyer flasks containing
100 mL of MM (Martinez-Blanco et al., 1990) was inocu-
lated with 2 mL of a bacterial suspension (A540 nm = 0.5).
Incubations were carried out in a rotary shaker (250 r.p.m.)
at 30°C for the time required. The carbon sources used for
culture were acetic acid (Ac, 30 mM) or succinic
cid (42 mM) or combinations thereof. When required
phenylacetic acid or octanoic acid (10 mM), rifampicin,
(25 μg mL−1); ampicillin (100 μg mL−1); gentamicin
(30 μg mL−1); kanamycin (25 μg mL−1) and/or tetracycline
(35 μg mL−1) were added to the media.

Escherichia coli strains were maintained on Luria–Bertani
(LB) agar plates and cultured in the required medium at
37°C (Davis & Mingioli, 1950).

When solid media were used, 25 g L−1 Difco agar was
added.

Isolation of mutants handicapped in the
degradation of acetate

The isolation of mutants unable to catabolize acetate was
carried out by mutagenesis with the transposon Tn5 (Sel-

For gene disruption, an internal fragment (919 bp) of the
gene to be knocked-out was cloned in the poly linker of
pK18::mob or pJQ200KS (two mobilizable plasmids that do
not replicate in Pseudomonas) (Quandt & Hynes, 1993;
Schäfer et al., 1994), and the resulting construct was
introduced into P. putida U by triparental filter mating
(Felsenstein, 1989).

Deletion of a selected gene was accomplished using a
methodology which involves a double-recombination event
and the selection of the required mutant by expression of the
lethal sacB gene (Quant & Hynes, 1983; Donnenberg &
Kaper, 1991). All mutants were analysed by PCR to define
the position of the disrupting element or to confirm the
extent of the deletion.

Deletions are indicated as ΔacsA, genetic disruptions,
causd by the insertion of the transposon Tn5 or of

the plasmids pK18::mob and pJQ200KS in a particular gene,
are summarized as Δgene::Tn5, Δgene::pK18::mob and
Δgene::pJQ200KS, respectively.

Enzymatic assay

Acs activity was evaluated colorimetrically as has been
reported previously (Martinez-Blanco et al., 1990, 1992;
Fernández-Valverde et al., 1993). When alkanoic acids with
lengths longer than C6 were tested as substrates, the acyl-
CoA generated was evaluated by HPLC.

Purification of Acs from P. putida U

Bacteria grown for 20 h in 5 L of MM containing sodium
acetate (30 mM) were harvested by centrifugation (10 000 g,
10 min at 2°C), washed with sterile saline solution, resus-
pended in 0.5 M K2PO4 buffer (pH 7.1) containing mercap-
toethanol (5 mM), EDTA (3 mM) and phenylmethysulfonyl
fluoride (PMSF) (1 mM), and disrupted as described pre-
viously (Fernández-Valverde et al., 1993). Cell debris was
disrupted by centrifugation (17 000 g, 10 min at 2°C) and
the supernatant fluid (200 mL) was precipitated with ammno-
sulfate. The fraction precipitating between 20% and
45% was collected, dissolved in 80 mL of phosphate buffer (see
above) and reprecipitated with ammonium sulfate (38%).
The precipitate was dissolved in the same buffer (30 mL)
containing ammonium sulfate (15%) and ultracentrifuged (250 000 g,
45 min). The supernatant, containing more than 80% of the
activity, was precipitated with ammonium sulfate (by add-
ing the quantity of salt required to reach 27%).
The supernatant was ultracentrifuged again (250 000 g, 30 min) and
the liquid containing the Acs activity was reprecipitated by adding
ammonium sulfate to reach a concentration of 45%. The
precipitate was dissolved in the same buffer and 1 mL of this
enzymatic solution was applied to a Sephadex G-200 column
(2.5 × 28 cm). Elution was carried out with the same buffer at
a rate of 25 mL h−1 (1 mL aliquots). Acs was found between
the fractions 58 to 64 showing a peak in the fraction 61.

Electrophoresis

Polyacrylamide gel electrophoresis (PAGE) under denat-
urating conditions [sodium dodecyl sulfate-PAGE (SDS-
PAGE)] was performed in 10% slab gels as reported
(Fernández-Valverde et al., 1993).

Determination of molecular mass of Acs from
P. putida U

The molecular mass of Acs was calculated using a calibrated
Sephadex G-200 column (Pharmacia) with known proteins
(Alonso et al., 1988). Kav = (Vv − V0)/(Vt − V0) where Vv is the
molecular mass of the enzyme (g mol−1), Vv is the
molecular mass of the standard (g mol−1), Vt is the
column volume and V0 is zero volume.
elution volume, $V_0$ the bed volume (36 mL), $V_t$ the total volume (94 mL) and $K_w$ the partition coefficient.

HPLC equipment and chromatographic procedure

The analysis of the reaction products (acyl-CoA) were performed by HPLC as reported elsewhere (Martin-Villacorta et al., 1991).

Bioinformatic analysis

Multiple alignments were obtained with the ClustaW program (Thompson et al., 1994). Phylogenetic trees were computed using the neighbor-joining method (Saitou, 1996) with the PHYLIP program (bootstrap scores for 500 iterations) (Felsenstein, 1989). Alternatively, results were compared using the maximum parsimony method (bootstrap scores for 500 iterations) (Fitch, 1971).

The putative structure for the *P. putida* U Acs was predicted as a function of the sequence homology between this protein and the data obtained from the structures of other Acs previously crystallized (Acs from *Salmonella enterica* – PDB accession 1PG4 and from Saccharomyces cerevisiae – PDB accession 1RY2). The predicted structure has been generated using the SWISS-MODEL (Guex & Peitsch, 1997) and 3D-PSSM (Kelley et al., 2000) and visualized using Swiss-Pdb Viewer (Guex & Peitsch, 1997).

Results and discussion

Isolation and identification of *P. putida* U mutants unable to catabolize acetate

The catabolism of acetic acid (Ac) in microorganisms involves the condensation of this compound with CoA to form acetyl-CoA (Ac-CoA) in the presence of ATP and Mg$^{2+}$ (Kameda & Nunn, 1981; Starai & Escalante-Semerena, 2004; Wolfe, 2005). Although several enzymes are able to catalyse this reaction in vitro, it is not clear which is (are) the enzyme(s) responsible for the physiological activation of acetate in vivo (Martinez-Blanco et al., 1990, 1992; Görisch, 2003; Hisanaga et al., 2004). In *Pseudomonas* species, it has been reported that different acyl-CoA ligases, acyl-CoA synthetases or acid-thiol ligases (E C. 6.2.1.), could be involved in the activation of acetate to acetyl-CoA (Fernández-Valverde et al., 1993). However, most of them have a broad substrate specificity, being able to activate aliphatic and aromatic acids ranging between C2 and C18 (Fernández-Valverde et al., 1993). In order to clarify which of these enzymes, if any, is the protein responsible for the activation of acetate in vivo, we focussed our research on isolating *Pseudomonas putida* U mutants unable to assimilate acetate when cultured in a chemically defined medium containing acetate as the sole carbon source (Fernández-Valverde et al., 1993).

Using mutagenesis with the transposon Tn5, two different mutants unable to catabolize acetate were isolated (ace’1 and ace’2). ace’1 is a mutant unable to metabolize acetate and all those compounds that lead to acetyl-CoA or acetoacetyl-CoA. By contrast, the other mutant (ace’2) is a strain specifically affected in the degradation of acetate. The location in the genome of the insertion site of Tn5 allow us to conclude that in ace’1 the transposon had disrupted the gene encoding the isocitrate lyase (aceA, Fig. 1a) and, therefore, the glyoxyl acid lyase could not be functional in this strain. However, in the ace’2 mutant the transposon was inserted in an ORF that shows a high homology with AMP-forming Acs (Fig. 2).

General characteristics of the Acs from *P. putida* (PpAcs)

In order to analyze the characteristics of Acs, the enzyme was purified (see Materials and methods). The determination of the amino terminus (MSAALYP) revealed that it corresponds to the enzyme encoded by the gene mutated in the ace’2 mutant. Acs runs in SDS-10% PAGE as a single band corresponding to a mass of 70 kDa. However, gel filtration throughout a Sephadex G-200 column indicated that the native protein had a Mr of 141 kDa, suggesting that this enzyme is a homodimer (*α*2).

Acs activity was maximal at pH 7.5 and at 38 °C and it recognizes acetic acid (100%), propionic acid (55%), butyric acid (26%) and slightly valeric acid (8%) as substrates. Other *n*-alkanoic acids whose carbon length were longer than C5 and phenylacetic acid were not activated by this enzyme.

Functional analyses of ace’2 gene and their encoding protein (Acs)

To confirm the exclusive involvement of the Acs in the specific degradation of acetate in *P. putida* U, we constructed different strains in which the *acs* gene had been either disrupted (*P. putida* U Δacs::pK18mob) or deleted (*P. putida* U Δacs) (Fig. 1b). We observed that none of these mutants were able to grow in MM containing acetate (Fig. 3a). However, both grew well in MM containing propionate, butyrate, pentanoate as well as other alkanoates with a longer acyl chain (Figs 3b–d). These results indicate that this Acs is the only enzyme involved in the synthesis of acetyl-CoA from acetate in this bacterium, and that, even though other acyl-CoA ligases can activate acetate in vitro (Martinez-Blanco et al., 1990; Fernández-Valverde et al., 1993), they do not play this function in vivo. However, it could be speculated that these other acyl-CoA ligases could activate acetate in vivo if they were induced. If acetate is not
the true inducer, then these enzymes would not be synthesized and hence the ace' 2 mutant would be unable to grow in MM containing acetate as the sole carbon source. To test this hypothesis, this mutant was cultured in MM containing acetate (30 mM) and small amounts (1 mM) of other carbon sources (alkanoic acids whose carbon length range between C3 and C12 or other compounds). We anticipated that in all cases the growth of the ace' 2 mutant would be very poor, showing that despite addition of the inducer, the acetate could not be assimilated. Surprisingly, the experimental data revealed that as long as an additional carbon source was added to the culture (even if was not a fatty acid) acetate was efficiently degraded (Fig. 4). These results indicated that: (i) among all the enzymes required for general metabolism, there are proteins (other than that the Acs mutated in the ace' 2 mutant) that catalyse the activation of acetate to acetyl-CoA (via acetyl-phosphate or acetyl-AMP), and (ii) these enzymes can not be induced by acetate.

Furthermore, the transformation of P. putida U Δacs with a genetic construction carrying in the plasmid pBBR1MCS-3 a copy of the ace gene (strain P. putida UΔacs pBBR1MCS-3 ace), restored the ability to grow in MM containing acetate as the sole carbon source (Fig. 4c).

To summarize, it may be concluded that when P. putida U was cultured in media containing acetate as the sole carbon source Acs is the only acyl-CoA activating enzyme specifically induced by acetate, and that, in contrast with other closely related microbes (Görisch, 2003), the presence of

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**Fig. 1.** Schematic representation of the transposon Tn5 insertion in the isocitrate lyase gene (aceA gene) of the Pseudomonas putida U ace' 1 mutant (a). Genetic organization of the acs gen in the genome of P. putida U and in the genome of the mutants P. putida U Δacs::pK18::mob and P. putida U Δacs (b).
high concentrations of acetate in the medium (30 mM) does not imply the use of acetate kinase (EC 2.7.2.1)/phospho-transacylase (EC 2.3.1.8) system to activate this compound (Brown et al., 1977).

**Phylogenetic studies**

A comparative study between the amino acid sequences of the AMP-forming Acs from *P. putida* U (PpAcs) and of 66
well-characterized Acs belonging to the domains eukarya, archaea and bacteria was performed. Figure 2 shows the alignment of the sequence of PpAcs with four additional sequences of Acs belonging to the three domains above indicated. PpAcs shows a high degree of identity with all them and contains the conserved sequence features (indicated as A1–A10 in the Fig. 2) that characterize the acyl-adenylate/thioester forming enzymes, a superfamily of enzymes (Babbitt et al., 1992; Chang et al., 1997; Gulick et al., 2003; Starai & Escalante-Semerena, 2004) that includes the AMP-forming Acs, the aliphatic acyl-CoA ligases (Suzuki et al., 1990; Black et al., 1992; Horswill & Escalante-Semerena, 2002), the aryl-CoA ligases (Martínez-Blanco et al., 1990; Babbitt et al., 1992; Schuhle et al., 2003), the adenylation domains of the nonribosomal peptide synthetases (Dieckmann et al., 1997) and the firefly luciferases (Conti et al., 1996; Branchini et al., 2000). One of the most highly conserved regions of these enzymes is the glycine-rich loop (YTSG(S/T)TG/2PKG) (Fig. 2), which seems to be involved in positioning the β- and γ-phosphates of ATP (Saraste et al., 1990; Chang et al., 1997).

Furthermore, sequence analysis revealed that PpAcs has a high degree of identity with the AMP-forming Acs of Salmonella enterica (SeAcs). Thus, most of the residues required in SeAcs for binding the substrate to the active site, as well as those involved in posttranslational acetylation/deacetylation of this enzyme, are also conserved in PpAcs (Figs 5 and 6) (Gulick et al., 2003). From the alignment of the PpAcs sequence with the other 66 sequences, a phylogenetic unrooted tree was constructed using the neighbor-joining method (Thompson et al., 1994; Saitou, 1996). All these sequences clustered in three main groups (Fig. 5): (i) eukaryotic Acs; (ii) Acs from Alpha-, Delta- and Gammaproteobacteria; and (iii) other Acs.

Fig. 3. (a) Bacterial growth of Pseudomonas putida U (●), P. putida U Aacs::pK18::mob (■) and P. putida U Δacsc (◆) cultured in MM+acetate 30 mM; (b) Bacterial growth of P. putida U (●, ○), P. putida U Δacsc::pK18::mob (■, □) and P. putida U Δacsc (◆, ◆) cultured in MM+propionate 24.3 mM (●, ■, ◆) or in MM+butyrate 20.4 mM (○, □, ◆); (c) bacterial growth of P. putida U (●, ○), P. putida U Δacsc::pK18::mob (■, □) and P. putida U Δacsc (◆, ◆) cultured in MM+pentanoate 17.6 mM (●, ■, ◆) or in MM+hexanoate 15.5 mM (○, □, ◆); and (d) bacterial growth of P. putida U (●, ○), P. putida U Δacsc::pK18::mob (■, □) and P. putida U Δacsc (◆, ◆) cultured in MM+pentanoate 13.8 mM (●, ■, ◆) or in MM+octanoate 12.5 mM (○, □, ◆).
belonging to Archaea, Actinobacteria, Beta- and Epsilonproteobacteria, Cyanobacteria and related groups. Similar results were obtained when the maximum parsimony method was used (Fitch, 1971).

The main group, including eukaryotic sequences, exhibits an ancient divergence into two different subgroups: one of them groups the fungal enzymes while the other includes the sequences of animals and plants Acs.

The second group, corresponding to Alpha-, Delta- and Gammaproteobacteria, is further divided into two additional clusters, one of them includes the Acs from Alpha-, and Deltaproteobacteria and the other one those belonging to Gammaproteobacteria.

In the third group, four different clusters were distinguished. The first one includes the Acs from Spirochaetes; the second cluster groups the enzymes from Beta- and Epsilonproteobacteria; the third cluster is divided into two different subgroups, one including the Acs from Actinobacteria and the other grouping the archael enzymes. Finally, the fourth cluster includes Acs from Cyanobacteria, Chlorobi, Planctomycetes and Deinococcus-Thermus.

To summarize, the overall topology of the phylogenetic tree here reported suggests, as has been reported by other authors (Karan et al., 2001), a monophyletic origin for the AMP-forming Acs.

Structural analyses

The three-dimensional putative structure of PpAcs from P. putida U is shown in Fig. 6. It has been predicted on the basis of the strong sequence homology between this protein and other Acs previously crystallized (Salmonella enterica – PDB accession 1PG4 and Saccharomyces cerevisiae, ScAcs – PDB accession 1RY2) (Gulick et al., 2003; Jogl & Tong, 2004). These three Acs (PpAcs, ScAcs and SeAcs) contain very similar sequences (about 68% of identity between PpAcs and SeAcs, and about 48% between ScAcs and PpAcs).

Taking into account the high degree of identity between all these sequences and the predicted secondary structure of the P. putida enzyme (Fig. 2), different models were constructed. A model showing very narrow identity with the crystal structure of Salmonella enterica protein was chosen (Fig. 6). This putative structure, as well as those of SeAcs,
ScAcs and other similar AMP-forming enzymes (4-chlorobenzoyl-CoA ligase from Alcaligenes sp. – PDB accessions 1TD5H and 1TD5S; firefly luciferase – PDB accession 1LCI; phenylalanine activating domain, PheA, of gramicidin synthetase S – PDB accession 1AMU; 2,3-dihydroxybenzoate activating domain, DhbE, from the Bacillus subtilis bacillibactin nonribosomal peptide synthetase system – PDB accessions 1MDB and 1MDF) (Conti et al., 1996, 1997; Starai et al., 2002; Gulick et al., 2004), comprise two domains: one large N-terminal domain (the first 517 residues of PpAcs) and another small domain formed by the C-terminal residues (from amino acid 518 to 626 of PpAcs). The structure of the large domain in the modelled PpAcs is very similar to that from SeAcs and ScAcs, and it is also very similar to that from SeAcs and ScAcs, and it is also very similar to the N-terminal domain of firefly luciferase (Conti et al., 1996), 4-chlorobenzoyl-CoA ligase (Gulick et al., 2004) and gramicidin synthetase 1 (Conti et al., 1997). However, the percentage of identity between the amino acid sequence of PpAcs and the latter three enzymes is quite low (16–21%).

As occurred in the phenylalanine activating domain of gramicidin synthetase (PheA), in firefly luciferase and in the two crystallized Acs, the N-terminal domain of PpAcs is formed by two β-sheets (sheets A and B) that are arranged parallel to each other. In SeAcs, in ScAcs and in the modelled PpAcs, sheet A contains nine strands, whereas the sheet A of PheA has only eight strands. However, in all cases studied, sheet B is formed by eight strands (Conti et al., 1997; Gulick et al., 2003, 2004).

The N-terminal domain also contains a distorted four stranded antiparallel β-sheet of five strands (sheet C), which continues to the aspartic acid residue (Asp517 in the A8 motif) that forms the hinge involved in the conformational change between the large (N-terminal) and the small (C-terminal) domains.

The C-terminal domain begins with a short loop containing two antiparallel strands at residues 520–523 and 525–527. The conserved GXR sequence of the A8 motif, an important feature of the Acs active site, is found in the short turn between these two strands. The rest of the C-terminal domain forms a three-stranded β-sheet that is surrounded on both sides by two α-helices. This structure is almost identical to that reported for SeAcs (Gulick et al., 2003).

Comparison of the modelled structure of PpAcs with the data obtained from the crystal structure of SeAcs obtained in the presence of adenosine-5′-propylphosphate and CoA, allowed the identification in the P. putida model of the two different binding sites for the adenine groups of AMP and CoA. Thus, the conserved Trp413 and Ile512 residues conform a hydrophobic pocket where the AMP moiety was placed, whereas the adenine ring is stacked against the Gly387, Glu388 and Pro389 (Figs 2 and 6). The hydroxyl groups of the AMP interact with residues Asp500, Glu415 and Arg515 whereas one phosphatidic oxygen, as has been suggested in the Salmonella enzyme, would interact with the conserved Thr416 and Arg526 residues in the P. putida enzyme. The acetyl group present in the acetyl-AMP complex would be bound to a pocket formed by Val310, Val386 and Trp414.
According to the data reported for SeAcs (Gulick et al., 2003) the nucleotide portion of the CoA moiety would be bound on the surface of the protein, fitting into a binding pocket where it is surrounded by Arg191, Gly164 and Gly165. Phe163 and Ile196 also participate in the formation of this hydrophobic pocket. The 5'-diphosphate of CoA will interact with the side chain of Arg191, while the 3'-phosphate interacts with the Arg584 and Arg194. Moreover, the phosphophasphate moiety of the CoA will be channelled between the N- and the C-terminal domains of the protein to the AMP binding site, making few direct interactions with the protein. The two amines of the pantetheinyl group would interact with Ser523 and with Gly524. In conclusion, all the residues that configure the CoA binding site in SeAcs are conserved in the sequence and in the three-dimensional architecture of the model proposed for PpAcs, indicating that both bacterial acetyl are similar enzymes (Figs 2 and 6).

To summarize all the above data and comparisons allow us to conclude that: (i) Acs is the only acyl-CoA activating enzyme induced by acetate in P. putida U when this compound is the only carbon source present in the medium; (ii) there are not constitutive acetate kinase/phosphotransacylases or analogous enzymes that can replace Acs in this bacterium (the ace2 mutant, lacking Acs activity, does not grow in MM containing acetate); (iii) the activation of acetate to acetyl-CoA observed in vitro when other acid-thiol ligases were studied revealed that in certain physiological conditions (i.e. the absence of the Acs here reported) any of these enzymes, once induced, could catalyse the synthesis of acetyl-CoA; (iv) PpAcs has a high degree of identity with the Salmonella enterica AMP-forming acetyl, such that the structure of both enzymes, composed by two domains (the large N-terminal domain and the small C-terminal domain) is similar, and that most of the residues involved in substrate binding and in posttranslational acetylation/deacetylation in SeAcs are also conserved in PpAcs; and (v) the phylogenetic tree reported here suggests a monophyletic origin for all the AMP-forming acetyl-CoA synthetases.

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