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SHORT COMMUNICATIONS

# 1-Aminocyclopropane-1-Carboxylate Deaminase of the Aerobic Facultative Methylotrophic Actinomycete *Amycolatopsis methanolica* 239

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One of the key mechanisms of the effect of bacteria on plant growth and development is their ability to reduce the level of ethylene due to the activity of 1-aminocyclopropane-1-carboxylate deaminase (ACCD) (EC 3.5.99.7) [1]. This enzyme catalyzes the hydrolysis of 1-aminocyclopropane-1-carboxylate (ACC), which is an immediate precursor in ethylene biosynthesis, to α-ketobutyrate and ammonium ions. Ethylene is one of the main phytohormones; it regulates the aging process, induces fruit ripening and flower withering, and plays a key role in stress signal transduction [2]. Increased ethylene concentration in plant roots as a part of stress response inhibits root elongation, nodulation and transport of auxins, and accelerates tissue aging and exfoliation [3, 4]. It has been shown that ACCD-possessing bacteria contribute to the enhancement of plant resistance to such negative impacts as drought, soil salinity, heavy metal pollution, and the presence of phytopathogens [1].

The facultative methylotrophic actinomycete *Amycolatopsis methanolica* 239 is able to use methanol as a growth substrate via the ribulose monophosphate pathway [5, 6]. Actinomycetes are typical soil bacteria; some of them stimulate plant development by means of ACC deaminase activity and auxin synthesis; however, the molecular and biochemical basis of their symbiosis has not yet been studied [7]. Our analysis of genomes of methylotrophic bacteria using the Protein BLAST software package (http://blast.ncbi.nlm.nih.gov/Blast.cgi) revealed the structural gene of ACCD (*acdS*) in the genome of *A. methanolica* (GenBank: CP009110). Although ACCD activity was detected in many bacteria, only four enzymes were purified and characterized: from *Pseudomonas putida* UW4, *Methylobacterium nodulans* ORS2060, *Methylobacterium radiotolerans* JCM2831, and from the yeast *Cyberlindera saturnus* (table). Comparative study of the enzymes isolated from other bacteria will make it possible to reveal the patterns in their structure and properties.

The goal of this work was to purify ACCD from *Amycolatopsis methanolica* 239 and to investigate its main biochemical properties.

The open reading frame of the *acdS* gene (locus NCBI AMETH_2787, Gene ID: 672357001) of *A. methanolica* 239 was cloned in expression vector pHUE at the *Cfr*42I and *Hin*dIII restriction sites. A strain overproducing the recombinant protein AcdS fused to ubiquitin, and a polyhistidine tag at the N-end was obtained on the basis of *E. coli* Rosetta. The protein was purified to electrophoretic homogeneity by metal chelate affinity chromatography. The protein preparation obtained by proteolysis had the amino acid sequence corresponding exactly to that encoded in the *acdS* gene of *A. methanolica* 239. The molecular mass of a subunit (36 kDa) corresponded to the theoretical value. The methods of native electrophoresis and gel filtration showed that the mass of ACCD was 144 kDa, indicating that the protein had a homotetrameric structure.

The ACC deaminase activity was detected by the following three methods: α-ketobutyrate production in the coupled reaction with lactate dehydrogenase and NADH [8], α-ketobutyrate production by the colorimetric method, and production of ammonium ions in the coupled reaction with glutamate dehydrogenase.

The enzyme from *A. methanolica* was shown to obey the Michaelis–Menten kinetics during ACC deamination. The $K_m$ of the enzyme was 1.7 ± 0.  mM, which is very close to the value for ACCD from *M. radiotolerans* JCM2831 ($K_m$ = 1.8 ± 0.3 mM) (table). ACCD from *M. nodulans* had the lowest value ($K_m$= 0.80 ± 0.04 mM); consequently, this enzyme demonstrated the highest substrate specificity to ACC among the characterized analogs. The catalytic constant of the enzyme from *A. methanolica* ($k_{cat}$ = 5.1 $min^{-1}$) was much lower than that of all previously

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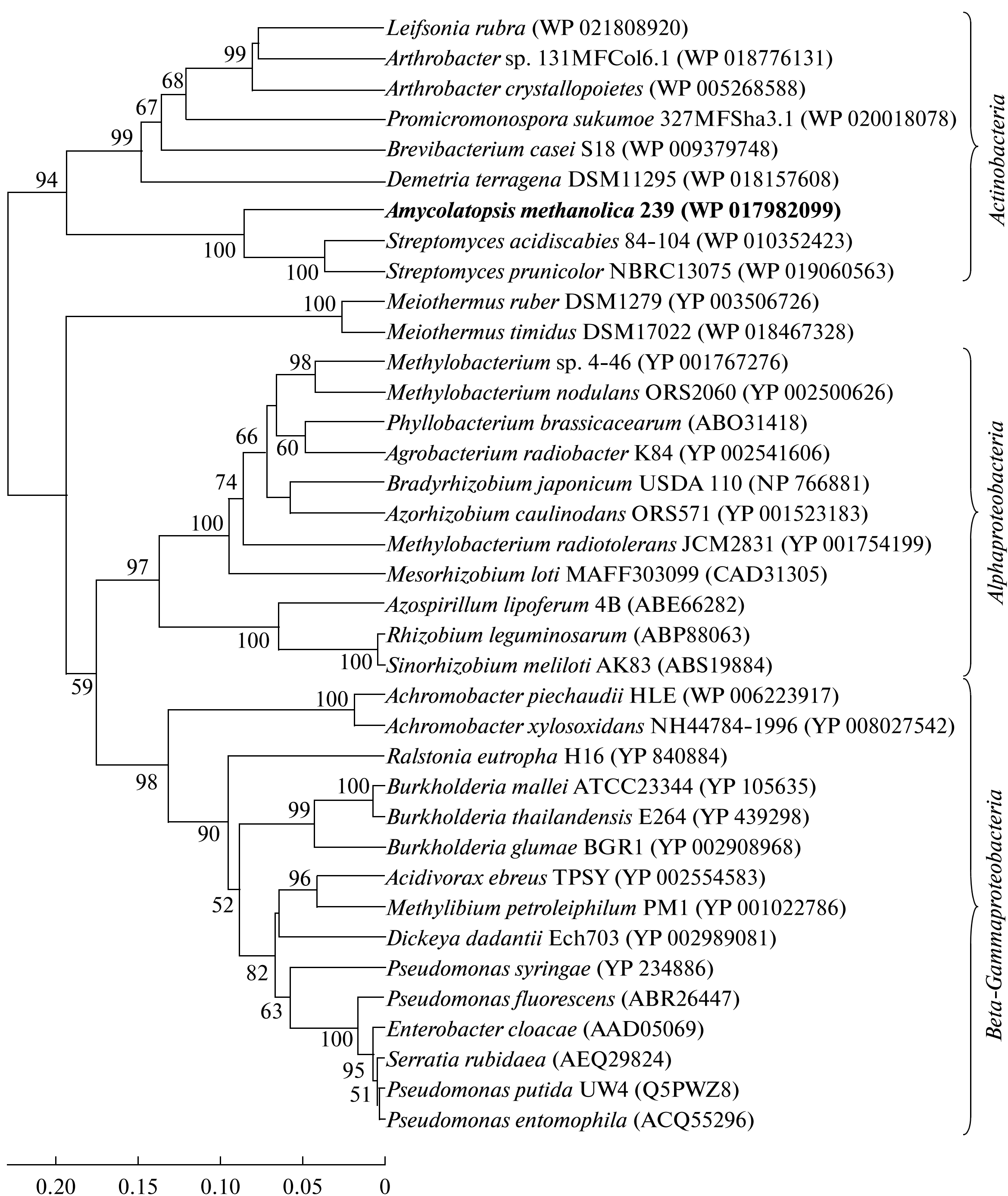


Phylogenetic tree based on the translated amino acid sequences of the AcdS protein

characterized enzymes (table). Such differences in the kinetic characteristics may be due to different habitats of these bacteria: *M. nodulans* is a root nodule phytosymbiont having high access to ACC, while *M. radiotolerans* is a phyllospheric bacterium and *A. methanolica* is a free-living soil bacterium, apparently not directly associated with plant surface.

The temperature optimum of the enzyme was shifted to the region of elevated temperature, which was also typical of the enzyme from *M. nodulans* with maximal activity at 60°C. On the contrary, the temperature optimum of homologous enzymes from pseudomonads was within a range of 30–37°C (table). ACCD from *A. methanolica* showed the highest activity in 50 mM Tris-HCl, pH 8.5.

Comparative analysis of amino acid sequences revealed that ACCDs of the members of the phylum *Actinobacteria* formed a separate phylogenetic cluster.

Comparative characteristics of 1-aminocyclopropane-1-carboxylate deaminases

| Parameter | *A. methanolica* 239 | *M. nodulans* ORS2060 | *M. radiotolerans* JCM2831 | *Pseudomonas putida* UW4 | *Cyberlindera saturnus* |
|---|---|---|---|---|---|
| $K_m$, mM | 1.7 ± 0.2 | 0.80 ± 0.04 | 1.8 ± 0.3 | 3.4 ± 0.2 | 2.6 |
| $k_{cat}$, min$^{-1}$ | 5.1 ± 0.2 | 111.8 ± 0.2 | 65.8 ± 2.8 | 146 ± 5 | – |
| pH optimum | 8.5 | 8.0 | 8.0 | 8.0 | 9.0 |
| Temperature optimum, °C | 60 | 50 | 45 | 37 | – |
| Molecular mass | Homotetramer 144 kDa | Homotetramer 144 kDa | Homotetramer 144 kDa | Homotetramer 168 kDa | Monomer 69 kDa |
| Cofactor | Pyridoxal phosphate | | | | |
| Reference | This work | [9] | [9] | [10] | [8] |

AcdS from *Amycolatopsis methanolica* 239 showed the highest similarity (83% identity) to AcdS from *Streptomyces acidiscabies* 84-104 (figure). Another cluster is formed by ACC deaminases of *Alphaproteobacteria*, in particular, of the plant-associated members of the genera *Rhizobium* and *Methylobacterium*. A separate cluster is also formed by ACCDs of the *Beta*- and *Gammaproteobacteria*, including the well-studied enzyme from *Pseudomonas putida* UW4 (figure). In general, the phylogeny of ACCDs correlates with the phylogeny of the 16S rRNA genes; nevertheless, there are data on the essential role of horizontal transfer in the distribution and evolution of the *acdS* gene, inter alia, between kingdoms [11].

Thus, in this work we have purified and studied for the first time the basic biochemical properties of recombinant ACCD from the member of the phylum *Actinobacteria*, a facultative methylotrophic actinomycete *A. methanolica* 239.

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