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Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase

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Abstract

Soil microorganisms that produce the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase promote plant growth by sequestering and cleaving plant-produced ACC, and thereby lowering the level of ethylene in the plant. Decreased ethylene levels allows the plant to be more resistant to a wide variety of environmental stresses. Here, the biochemistry of ACC deaminase; the environmental distribution, regulation, evolution and expression of ACC deaminase genes; and information regarding the effect of this enzyme on different plants is documented and discussed.

Keywords: Plant growth-promoting bacteria; Ethylene; ACC deaminase

1. Ethylene

The plant hormone ethylene has played an important role in agricultural practice since antiquity. One of the oldest references to ethylene, albeit somewhat indirect, is in the Bible where one of the prophets is described as a "scorer of figs", referring to the ancient practice of scoring, or cutting, newly picked figs (and generating stress ethylene production) in an effort to hasten ripening. Nevertheless, ethylene was not recognized as a biologically active hormone until the work of Russian scientist, Dimitri Neljubov, with etiolated pea seedlings in greenhouses in the late 19th century [1].

Ethylene is one of the simplest organic molecules with biological activity, and can function as an efficient plant growth regulator at very low concentrations – biological effects attributable to ethylene are observed at concentrations as low as 0.05 μL/L [1]. It is important for normal development in plants as well as for their response to stress [2]. Many aspects of the growth of vegetative organs such as roots, stems and petioles, and all stages of development are affected by ethylene [2]. A variety of other plant processes involve ethylene including rhizobia nodulation of legumes, rooting of cuttings, and plant response to heavy metals, ozone, pathogens and flooding.

The production of ethylene is regulated by a large number of factors including temperature, light, gravity, nutrition, and other plant hormones. The term "stress ethylene" was coined [3] to describe the acceleration of ethylene biosynthesis associated with environmental and biological stresses including pathogen attack [4]. The increased level of ethylene formed in response to trauma inflicted by chemicals, temperature extremes, water stress, ultraviolet light, insect damage, disease, and mechanical wounding can be both the cause of some of the symptoms of stress, and the inducer of defense responses which help to enhance survival of the plant under adverse conditions.

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During much of a plant's growth and development ethylene levels are low (i.e., $<0.05\ \mu L/L$), but during senescence and fruit ripening large quantities ($\sim 100\ \mu L/L$) are synthesized [1]. Many of the biological responses triggered by ethylene occur as a consequence of a small localized burst of ethylene that is sometimes difficult or even impossible to measure by gas chromatography (the standard means of assessing ethylene levels).

2. Plant growth-promoting bacteria

The interaction between bacteria and plants may be beneficial, harmful or neutral for the plant, and sometimes the effect of a particular bacterium may vary as the soil conditions change [5]. Bacteria that are beneficial to plants are of two general types: those that form a symbiotic relationship, which involves formation of specialized structures or nodules on host plant roots, and those that are free-living in the soil; the latter are often found near, on or even within the roots [6]. While numerous free-living soil bacteria are considered to be plant growth-promoting bacteria, not all bacterial strains of a particular genus and species have the same repertoire of metabolic capabilities so that only some *Pseudomonas putida* strains actively promote plant growth.

Bacteria that act by directly stimulating plant growth have received much less attention than biocontrol bacteria, reflecting the generally held view that in the field it is more difficult to reproducibly demonstrate the efficacy of these bacteria. There are several ways in which plant growth-promoting bacteria can directly facilitate plant proliferation [7]. They may: fix atmospheric nitrogen; synthesize siderophores which solubilize and sequester iron; produce phytohormones; solubilize minerals such as phosphorus; and synthesize some less well characterized low molecular mass compounds or enzymes that can modulate plant growth and development [7–9]. A particular bacterium may affect plant growth using any one, or more, of these mechanisms. Moreover, a bacterium may provide different benefits at various times during the life cycle of the plant.

The mechanism most often invoked to explain the direct effects of plant growth-promoting bacteria on plants is the production of phytohormones, including auxins such as indoleacetic acid, or IAA [8,10,11]. In addition, a number of plant growth-promoting bacteria contain the enzyme ACC deaminase and this enzyme can cleave the plant ethylene precursor ACC, and thereby lower the level of ethylene in a developing or stressed plant [7,12,13]. For many plants a burst of ethylene is required to break seed dormancy [14] but, following germination, a sustained high level of ethylene may inhibit root elongation [15]. Thus, plant growth-promoting bacteria that contain the enzyme ACC deaminase, when bound to the seed coat of a developing seedling, may act as a mechanism for ensuring that the ethylene level does not become elevated to the point where root growth is impaired. By facilitating the formation of longer roots, these bacteria may enhance the survival of some seedlings, especially during the first few days after the seeds are planted. Similarly, ACC deaminase-containing bacteria bound to the roots of plants can act as a sink for ACC and protect stressed plants from some of the deleterious effects of stress ethylene.

3. The enzyme ACC deaminase

The bulk of the biochemical studies of ACC deaminase, its physical and chemical properties, and its mode of action have been performed by Honma and his co-workers [16–24] although a few other studies of the enzyme and its properties have been reported [12,25–28].

ACC deaminase is a multimeric enzyme with a monomeric subunit molecular mass of approximately 35–42 kDa (Table 1). It is a sulfhydryl enzyme that utilizes pyridoxal 5-phosphate as an essential co-factor. Pyridoxal phosphate is tightly bound to the enzyme in the amount of approximately one molecule per subunit; it displays a characteristic pyridoxaldimine visible absorbance at 418 nm. While several D-amino acids, notably D-serine and D-cysteine can act as substrates for ACC deaminase (albeit less efficiently than ACC), L-serine and L-alanine are effective competitive inhibitors of the enzyme. ACC deaminase catalyzes a cleavage of ACC that includes cyclopropane ring fragmentation, and deamination of ACC to form α-ketobutyrate and ammonia.

Despite the fact that its substrate ACC is plant-produced, in those instances where it has been examined ACC deaminase is not a secreted enzyme. Rather, it is localized within the cytoplasm of the microorganism that produces it [12]. In this case, the substrate, ACC is exuded by plant tissues [29–31] and is then taken up by the ACC deaminase-containing microbe [13].

X-ray crystallographic analysis reveals that ACC deaminase folds into two domains, each of which has an open twisted α/β structure that is similar to the β-subunit of the enzyme tryptophan synthase [32]. However, unlike other members of the β family of pyridoxal phosphate dependent enzymes, the pyridoxal phosphate co-factor of ACC deaminase is buried deep within the protein molecule. Two key amino acid residues have been located in the enzyme: a reactive thiol group at cysteine 162, located in the internal gap between the two domains of the molecule, and the pyridoxal phosphate binding site at lysine 51.