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REVIEW

Prospective Use of 1-Aminocyclopropane-1-Carboxylate Deaminase-Producing Bacteria for Plant Growth Promotion and Defense against Biotic and Abiotic Stresses in Peat-Soil-Agriculture

EDI HUSEN[1], ARIS TRI WAHYUDI[1*], ANTONIUS SUWANTO[1] AND RASTI SARASWATI[2]

[1]*Department of Biology, Institut Pertanian Bogor, Darmaga Kampus, Bogor 16680, Indonesia;*
[2]*Indonesian Soil Research Institute, Jalan Ir. H. Juanda 98, Bogor 16123, Indonesia*

The 1-aminocyclopropane-1-carboxylate (ACC) deaminase (EC4.1.99.4) is an enzyme produced by some soil bacteria to degrade ACC (the immediate precursor of ethylene) to reduce ethylene biosynthesis in higher plants. Increased concentrations of ethylene in plant tissues, which are triggered by various biotic and abiotic stresses, inhibits plant growth and weakens the plant defense against the stressors. Various findings on the successful use of ACC deaminase producing bacteria for plant growth under unfavorable soil conditions are inspiring their use in tropical peat-soil-agriculture, which possesses bio-physical constraints. It has been proven that inoculation of plants with ACC deaminase producing bacteria decreased ethylene inhibition generated by unfavorable environmental conditions, such as nutrient shortage, flooding, drought, high salts, and the presence of heavy metals and organic pollutants. Understanding the mechanisms by which ACC deaminase-producing bacteria act to reduce plant stress and the fitness of bacterial traits with the properties and constraints of peat-soils becomes a key to utilize these bacteria in improving crop productivity. The bacteria may ameliorate plant stress as well as promote plant growth under seasonal bio-physical changes of peat-soils that are usually encountered in the field.

Key words: ACC deaminase, bacteria, plant growth promotion, biotic and abiotic stresses, peat-soil

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Peat-soils are important in the world to support human welfare, both locally and globally. They cover about 400 million ha area in the world (Malmer *et al*. 1994; Rubec 1996), 40 million ha of which are found in tropical regions and half of which are located in Indonesia (Driessen and Subagjo 1977; Widjaya-Adhi *et al*. 1997). Despite their potential use for agriculture, especially those of shallow peat-soils (Widjaya-Adhi *et al*. 1997), bio-physical constraints of these soils limit their capacity to produce optimal yields. Besides high acidity (pH 3.5 to 5.0), most tropical peat-soils are deficient in macro- and micro-nutrients (Driessen and Subagjo 1977; van Breemen 1995; Widjaya-Adhi *et al*. 1997). Incomplete decomposition of organic matter during peat formation also results in the accumulation of various phytotoxins, which inhibit plant growth (Salampak *et al*. 2000; Hartatik and Suriadikarta 2003). Water-logged conditions coupled with bio-physical constraints of peat-soils are considered both conducive and suppressive to soil-borne pathogens (Hoitink and Boehm 1999; Hunter *et al*. 2006). Thus, plants growing in these harsh soil conditions may be confronted with various stresses, unless a promising technology to reduce plant stress is applied.

The 1-aminocyclopropane-1-carboxylate (ACC) deaminase (EC4.1.99.4) is an enzyme produced by some soil borne bacteria to hydrolyze ACC, the immediate precursor of ethylene in higher plants, as their source of nitrogen (Jacobson *et al*. 1994; Glick 1995). The benefits of ACC deaminase-producing bacteria to diverse aspects of plant growth have been reported in various environmental conditions. These bacteria can reduce plant stress by controlling ethylene synthesis in plant tissues, which can be induced by various unfavorable environmental conditions including pathogens attack. Although this plant hormone ethylene has profound effects on plant growth and physiology, increased concentration of ethylene at an early stage of plant growth inhibits root development and weakens plant defense against various stressors (Glick 1995; Shah *et al*. 1997; Glick *et al*. 2007). A study by Wang *et al.* (2000), using ACC deaminase-producing *Pseudomonas* sp. and *Enterobacter* sp., demonstrated the effectiveness of these bacteria in enhancing growth and suppressing damping-off of cucumber root-rot diseases in tomato and potato. Subsequent studies revealed that these bacteria helped plants cope against various environmental constraints, such as heavy metals (Belimov *et al*. 2001), flooding (Grichko and Glick 2001), nutritional stress (Belimov *et al*. 2002), drought (Mayak *et al*. 2004), organic pollutants (Reed and Glick 2005) and high salts (Saravanakumar and Samiyappan 2007), which are present in most tropical peat-soils. Many more ACC deaminase-producing bacteria are still being studied to increase plant growth by using known strains or local isolates obtained from local soils. Our preliminary studies on some *Pseudomonas* sp. producing ACC deaminase isolated from the rhizosphere of soybean showed significantly increased soybean root-growth upon inoculation (Husen *et al*. 2008), so potentially this can be used as a plant helper in unfavorable soil conditions.

Based on the ability of ACC deaminase-producing bacteria to promote plant growth and provide defense against various environmental stresses, introducing these bacteria in unfavorable tropical peat-soil-agriculture may become an attractive solution to increase crop productivity.

*Corresponding author, Phone/Fax: +62-251-8622833, E-mail: atriw@ipb.ac.id

Peat-Soils, Characteristics and Constraints

Peat-soils are characterized by the accumulation of organic matter from dead and partially decaying plant materials under water-saturated conditions in wetland ecosystems. They are classified as histosols and mainly divided into fibric, hemic and sapric, based on the degree of organic material decomposition, i.e. partially, intermediately and highly decomposed materials, respectively (McKenzie 1974). The thickness of peat-soils varies from shallow (50 to 100 cm) to very deep (> 300 cm). Approximately 7.5 million ha of peat-soils in Sumatra and Kalimantan islands are suitable for agriculture (food and tree crops); □□□□□□□□□□ □□□□□ □□ ≤ 200 cm deep (Widjaya-Adhi *et al.* 1997)

Peat-soils develop either from bogs which are rain-fed and nutrient-poor or fens which are fed by surface or ground water and more nutrients-rich (Malmer *et al.* 1994). Tropical peat-soils developed from ombrogenous bogs are found in shallow depressions in the natural basins between levees (banks) of rivers traversing the coastal plain (Driessen and Subagjo 1977). Since water, organic matter and its vegetation are all interconnected, the variations of peat-soil properties are mainly determined by these three components. Therefore, a change of any one of these components will fundamentally alter the nature of peat-soils (Rubec 1996); such that they are considered as fragile soil ecosystems.

Ample reports on the constraints of peat-soils for agriculture are well documented. The extreme conditions of tropical peat-soils which prevent normal plant growth include: (i) anaerobic conditions and the presence of toxic ions in the root zone, such as Fe^{2+}, Mn^{2+}, S^{2-} (Ponnamperuma 1972; Driessen and Subagjo 1977); (ii) water-logging or high water levels that deprive oxygen from perennial plants (van Breemen 1995); (iii) spongy soil structure that weakens rooting so trees easily fall down (Malmer *et al.* 1994); (iv) scarcity of nutrients as a result of peat accumulation (by which nutrients are fixed or chelated) and leaching by peat water (Driessen and Subagjo 1977; Widjaya-Adhi *et al.* 1997); (v) acidity (pH 3.5 to 5.0) caused by organic acids and cation exchange (van Breemen 1995; Widjaya-Adhi *et al.* 1997); and (vi) the presence of toxic organic substances, such as phenolic acids, produced during anaerobic decomposition of organic materials (Salampak *et al.* 2000). Moreover, peat-soils are conducive to the development of soil-borne-pathogen diseases, such as *Pythium sylvaticum*-induced damping-off although some of them suppress disease (Hoitink and Boehm 1999; Hunter *et al.* 2006). These conditions may inhibit plant growth and prevent the proliferation of certain beneficial microbes, unless both plants and microbes develop several strategies to survive under these unfavorable or stress conditions.

Feng *et al.* (2002) reported that some root-nodule-forming bacteria developed thickened cell walls as a mechanism for long-term survival under nutrient-limited conditions of peat-soils. Involvement of certain bacteria to detoxify or transform phytotoxins generated during peat formation, which benefits both plants and microbes, has been reported (Huang and Kuhlman 1991; Blum *et al.* 1999). However, since peat-soils possess various complex biophysical constraints, various levels of plant stresses under these harsh soil conditions may not be difficult to understand. Most crop yields produced in peat-soils are less than those in mineral soils. The yields of food crops in peat-soils range from 2.7 to 4.1; 0.8 to 1.0; and 1.5 to 2.4 ton ha^{-1} for paddy field and corn; soybean and mung bean, respectively (Widjaya-Adhi *et al.* 1997). Therefore, reducing plant stress would potentially increase crop yields.

ACC Deaminase and Plant-Growth-Promotion

The ACC deaminase is a cytoplasmically localed enzyme (Jacobson *et al.* 1994) produced by soil microorganisms. It degrades cyclopropanoid amino ACC (the immediate precursor of the plant growth regulator ethylene) to form ammonia and α-ketobutyrate. After the first finding of ACC deaminase from *Pseudomonas* sp. strain ACP (Honma and Shimomura 1978), various pioneering research work conducted, such as in Jacobson *et al.* (1994), Glick (1995) and Penrose *et al.* (2001), have successfully elaborated the biochemical properties of this enzyme and its functional roles in controlling ethylene production in higher plants.

In general, the plant hormone ethylene plays important diverse roles in the growth of plants, such as root initiation and fruit ripening (Burg and Burg 1962; Arshad and Frankenberger 1991; Kende 1993). As a stress hormone, ethylene is involved in various stress responses induced by biotic (pathogenic attack) and abiotic (environmental) factors. Its biosynthesis starts with the *S*-adenosylation of methionine to *S*-adenosylmethionine (SAM) followed by the closing of a cyclopropane ring to form ACC. The ACC is then oxidatively cleaved to form ethylene. In spite of its beneficial roles in root induction, increased concentrations of plant ethylene at the vegetative stage inhibits root development, nodule formation and auxin transport and promotes senescence and abscission (Glick 1995; Shah *et al.* 1997; Wang *et al.* 1997; Ma *et al.* 2002; Glick *et al.* 2007). The antagonistic function of ethylene with IAA prevents the overgrowth (gigantism) of plants. IAA promotes rooting, but rooting is opposed by ethylene generated by IAA; thus, the promotion effects of IAA on root development can be outweighed by the inhibitory effect of ethylene (Burg 1968; Chadwick and Burg 1970; Arshad and Frankenberger 1991). The complex role of ethylene in plant-microbe interactions elicits ideas to regulate it for various purposes; it is required by both plants for resistance to pathogenic attack and by pathogens for disease susceptibility (Kunkel and Brooks 2002).

The importance of ACC deaminase-producing bacteria on plant growth is to control ethylene biosynthesis in plant tissues which is triggered by a number of biotic and abiotic factors. Inoculation of plant with ACC deaminase-producing bacteria can reduce the inhibition-effects of ethylene, and so ultimately increase plant growth. Evidence that ACC deaminase-producing bacteria increase the growth of various agricultural crops has been reported by various researchers, including those using transformed bacterial strains receiving the ACC deaminase gene (*acdS* gene) as described by Wang *et al.* (2000). Interestingly, some root-nodule-forming bacteria may produce ACC-deaminase besides producing rhizobitoxine (an ethylene inhibitor) as a strategy by these bacteria to reduce the amount of ethylene synthesized in soybeans (Ma *et al.* 2002; 2003), otherwise the nodulation process would itself be inhibited. However, since ACC deaminase is a cytoplasmic enzyme in bacteria, the substrate of ACC must be exuded by plant tissues and taken up by the bacteria in the rhizosphere and subsequently hydrolyzed into ammonia and α-ketobutyrate (Jacobson *et al.* 1994; Shah *et al.* 1997; Glick *et al.* 1998). Thus, the success of these bacteria to promote and increase plant growth will depend much on their ability to colonize the root and compete with other soil microflora.

Amelioration of Plant Stress

Plants grown in the tropical peat-soils may encounter various biotic and abiotic stresses as explained above. In general, biotic stresses could be due to pathogenic attack, either a biotroph which colonizes living plant tissues or necrotroph which rapidly kill plant cells to obtain nutrients. Meanwhile, abiotic or environmental stresses could be due to soil acidity, water-logging (osmotic pressure), nutrient shortage and phytotoxins that are generally present in tropical peat-soils. Both kinds of stressors may trigger plants to produce stress hormones as a signal to reduce the severity of the stress (Wang *et al.* 2000; Glick *et al.* 2007).

It has been known that several plant pathogenic microbes have developed the ability to modulate signaling processes mediated by plant hormones as a strategy for manipulating plant growth or host physiology (Lund *et al.* 1998). Three kinds of plant hormones, which are important in mediating plant defenses against pathogen attack, are salicylic acid (SA), jasmonic acid (JA) and ethylene (Kunkel and Brooks 2002; Glazebrook 2005). Ethylene signaling, however, plays a pivotal role in various types of stresses including those caused by various environmental factors (Glick *et al.* 2007).

Taken together, plants with a particular stress will accumulate high levels of ethylene that make them in a state of ethylene-stress and further inhibit their growth. Increasing levels of ethylene eventually exacerbates the stress which weakens plant protection against the stressors. In this condition, plants may not respond well to the availability of [illegible]

Prospective Use of ACC Deaminase-Producing Bacteria

The successful use of ACC deaminase-producing bacteria to assist plant growth under unfavorable soil conditions provides farmers an attractive method of

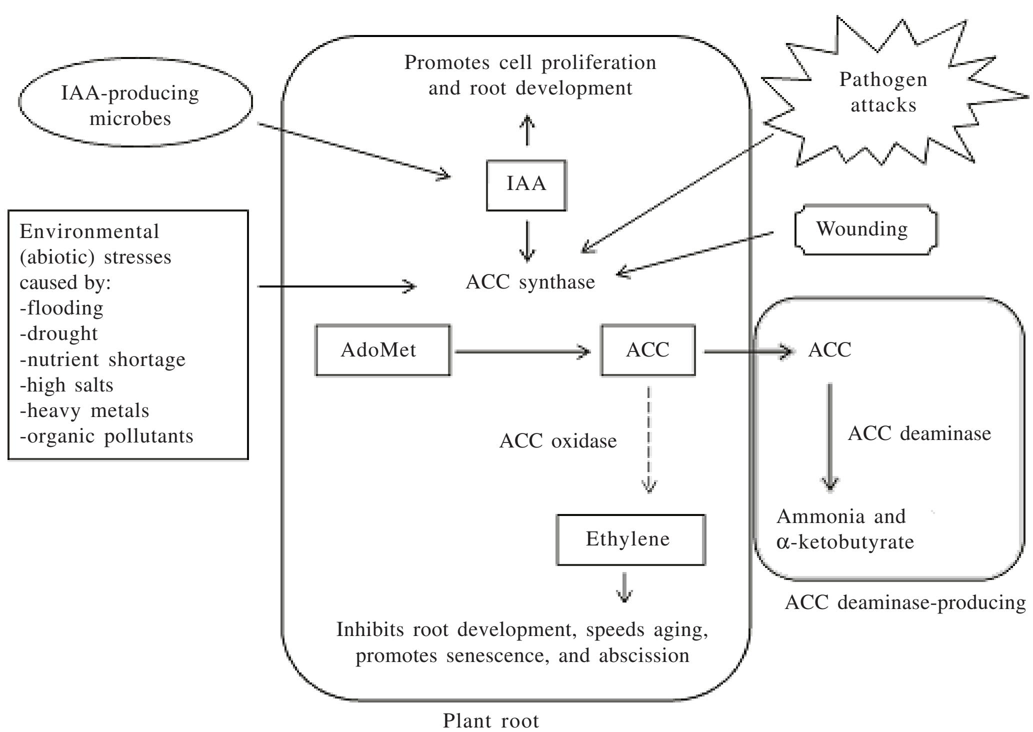

Fig 1 Schematic representation by which ACC deaminase-producing bacteria attached to plant roots lower ethylene biosynthesis. A key enzyme ACC synthase that converts AdoMet (S-adenosylmethionine) to ACC is induced by various biotic and abiotic factors including increased IAA concentrations. Bacterial uptake and hydrolysis of ACC exuded by plant roots (to maintain the equilibrium between internal and external ACC) prevents ethylene accumulation which inhibits plant growth. IAA, indoleacetic acid; ACC, 1-aminocyclopropane-1-carboxylic acid; AdoMet, S-adenosylmethionine.

increasing crop production. Their ability to promote plant growth as well as to protect the plant against biotic and abiotic stresses may reduce the level of application of fertilizers and pesticides, which currently become more expensive and also alleviate environmental problems. The challenge that remains ahead is to obtain a good candidate of ACC deaminase-producing bacteria which can live and proliferate in peat-soils and which are effective to assist plant defense against a wide range of stressors. Since none of the studies on the use of ACC deaminase-producing bacteria has been specifically conducted yet in tropical peat-soil conditions which posses a complex of constraints, a complete screening of the bacteria is required to ensure that no deleterious traits are present in the bacteria that might negate their effectiveness in this role.

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