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Original Article

A halotolerant *Enterobacter* sp. displaying ACC deaminase activity promotes rice seedling growth under salt stress

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Abstract

Agricultural productivity is proven to be hampered by the synthesis of reactive oxygen species (ROS) and production of stress-induced ethylene under salinity stress. One-aminocyclopropane-1-carboxylic acid (ACC) is the direct precursor of ethylene synthesized by plants. Bacteria possessing ACC deaminase activity can use ACC as a nitrogen source preventing ethylene production. Several salt-tolerant bacterial strains displaying ACC deaminase activity were isolated from rice fields, and their plant growth-promoting (PGP) properties were determined. Among them, strain P23, identified as an *Enterobacter* sp. based on phenotypic characteristics, matrix-assisted laser desorption ionization-time of flight mass spectrometry data and the 16S rDNA sequence, was selected as the best-performing isolate for several PGP traits, including phosphate solubilization, IAA production, siderophore production, HCN production, etc. *Enterobacter* sp. P23 was shown to promote rice seedling growth under salt stress, and this effect was correlated with a decrease in antioxidant enzymes and stress-induced ethylene. Isolation of an *acdS* mutant strain enabled concluding that the reduction in stress-induced ethylene content after inoculation of strain P23 was linked to ACC deaminase activity.

Keywords: Salt stress; Halotolerance; Stress ethylene; ROS; PGPR; ACC deaminase

1. Introduction

The worldwide population is increasing rapidly; it could reach 9.1 billion globally in the year 2050 [1], and India will be the most populated country (population 1.7 billion) (U.N. report, 2016). The production of food is not significantly increasing, and must substantially increase to meet demands for food in the coming years. The area of arable land is gradually decreasing due to the population explosion and industrialization. Moreover, crop production is affected by various abiotic stresses in the agricultural system. Soil salinity is one of the major abiotic stresses responsible for reduction of plant growth and crop productivity. Worldwide, more than 800 million hectares of land (6% of the total land mass) are potentially usable for agriculture but are severely affected by salinity and thus reduce agricultural production [2]. According to the Food and Agricultural Organization (FAO), the amount of land loss due to salinity will represent 50% of total land mass worldwide in the year 2050 [3].

NaCl is the most dominant salt causing soil salinity. Devastating effects of high salinity upon plants include a

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decrease in K^+ and Ca^+ uptake by plants, enzyme inactivation, inhibition of protein synthesis, early senescence in leaves, formation of burn-like lesions, a decrease in the rate of photosynthesis and respiration, loss of cellular integrity, etc. [3]. Moreover, under salinity stress, plants synthesize more ethylene by utilizing 1-aminocyclopropane-1-carboxylic acid (ACC) as an immediate precursor. This ethylene is known as a salt stress-induced ethylene. Ethylene is a gaseous plant hormone produced by all higher plants, mediating a wide range of beneficial plant responses at low concentrations (as low as 10 μg/L). Based on many reports, however, it is established that higher levels of ethylene (as high as 25 μg/L) may inhibit elongation of roots and shoots, suppress leaf expansion and promotes epinasty [4]. This implies that if the ethylene concentration increases above the threshold level, it becomes detrimental. A check on increased ethylene production in plants may be helpful in reducing the negative effects of salt stress on plant growth and production, leading to increased agricultural yield. ACC deaminase-producing plant growth-promoting rhizobacteria (PGPR) enhance plant growth by decreasing the internal concentration of stress-induced ethylene through degradation of ACC into α-ketobutyrate and ammonia in the roots [4,5]. Thus, isolation from the plant root system of salt-tolerant bacterial strains possessing ACC deaminase activity may contribute to mitigating plant stress-induced ethylene and promoting plant growth in salt-affected soil [5]. These strains also colonize roots of plants and enhance plant growth by possessing important PGP traits, i.e. IAA production, phosphate solubilization, siderophore production and HCN production [6]. Moreover, some salt-tolerant PGPR strains secrete large amount of EPS that may bind to many cations, including Na^+ [7], and thereby decrease the amount of Na^+ available for plant uptake and reduce the salt stress of plants [8].

In addition, some PGPR can produce antioxidant enzymes like superoxide dismutase (SOD), peroxidase (POD) or catalase (CAT), which degrade detrimental reactive oxygen species (ROS) generated in plants during salt stress [9]. The increase in reactive oxygen species (ROS) under saline conditions causes cellular toxicity in plants. Thus, under high salinity, the role of the antioxidant system in protecting plants against such cellular stress is immensely important [9]. SOD is a potent scavenger of superoxide [10]; to destroy superoxide, it forms hydrogen peroxide, which is further scavenged by CAT and POD. Polyphenol oxidases (PPOs) are common oxidative and plant defense enzymes [11]. Many other redox-reducing enzymes are involved in management of ROS during stress conditions [12]. Overall, these antioxidant enzymes play a defensive role against oxidative stress developing due to salinity. Moreover, some salt-tolerant PGPR produce various compatible solutes, e.g., proline which gives osmo-protection to plant [13]. By producing these antioxidant enzymes and osmoprotectants, PGPR can induce various chemical changes, like alterations in total protein, the IAA concentration, total sugar, ethylene content in plants which may enhance tolerance to abiotic stress, termed 'induced systemic tolerance' (IST) [14]. Thus, salt-tolerant PGPR can directly promote the plant growth by enhancing the various morphological parameters, i.e. an increase in germinability, seedling vigor index, root length and root weight, shoot length and shoot weight, chlorophyll content, etc. [15].

Many bacteria are being used to mitigate salt stress as well as promoting plant growth; they include *Burkholderia* sp. [7], *Klebsiella* sp. [7], *Microbacterium* sp. [15], *Alcaligenes* sp. [15], *Enterobacter* sp. [7,16–18], *Ochrobactrum* sp. [15,19], *Bacillus* sp. [7,20], *Kocuria* sp. [21], *Arthrobacter* sp. [22], *Serratia* sp. [23,24] and *Pseudomonas* sp. [7,25,26].

The present work endeavors to isolate potent salt-tolerant ACC deaminase containing PGPR from a natural habitat like that of the rice field near the coastal belt of Odisha, India. One (P23) of the potent strains was selected on the basis of various PGP traits and identified as *Enterobacter* sp. based on phenotypic characteristics, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) of ribosomal protein and 16S rDNA sequence homology. Attempts were also made to detect the salt stress ameliorating ability of the selected strain by directly inoculating it in rice seedlings under salt stress and to evaluate it by measuring antioxidant, stress-induced ethylene production along with other parameters of plant growth promotion.

2. Materials and methods

2.1. Isolation and screening for salt-tolerant ACC deaminase-producing bacteria

Three rhizospheric soil samples were collected from rice fields near the coastal belt of Odisha (20° 59′N latitude and 78° 96′E longitude), India. ACC deaminase-containing bacteria were isolated from rhizosphere soil samples by uprooting the remaining parts of the rice plants in rice fields, and the soils attached to the roots were separated by a scalpel [27]; 1 g of soil sample was mixed in 100 mL sterile distilled water. The mixture was filtered and serially diluted (10 times). A 0.5 mL aliquot of each treatment was plated in Davis Mingioli (DM) medium [K_2HPO_4 − 7 g/L, KH_2PO_4 − 3 g/L, $(NH_4)_2SO_4$ − 1 g/L, $MgSO_4$, $7H_2O$ − 200 mg/L, $C_6H_5O_7Na_3$, $2H_2$ − 500 mg/L, $C_6H_{12}O_6$ − 10 g/L; pH: 7.0] devoid of ammonium sulfate with 5 mM ACC as nitrogen source. The medium was also supplemented with 2% (345 mM) NaCl. The plates were incubated at 30 ± 2 °C for 72 h. Based on luxuriant growth on selected media, morphologically different types of 12 bacterial colonies were selected for further studies. These isolates were subcultured for several generations in DM with ACC (instead of nitrogen source) agar plates to ensure their ability to use ACC as nitrogen source.

2.2. Characterization of the soil sample

The different physicochemical parameters of the rhizospheric soil sample, including electrical conductivity (EC), total salinity, pH etc. were determined by following the methods of Spark et al. [28].