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Biodiversity and plant growth promoting traits of culturable endophytic actinobacteria associated with *Jatropha curcas* L. growing in Panxi dry-hot valley soil

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ABSTRACT

One of the proposed mechanisms through which plant growth-promoting endophyte (PGPE) enhances plant growth is the production of 1-aminocyclopropane-1-carboxylic acid deaminase (ACCD). However, information about the endophytic actinobacteria with ACC deaminase activity associated with native plants is still very scarce. In this study, a total of 257 endophytic actinobacterial isolates were obtained using actinobacteria-selective media from surface sterilized roots, stems, leaves and seeds of the oil-seed plant *Jatropha curcas* L. collected from dry-hot valley soil. Morphological and the 16S rRNA sequence analysis showed that most of the isolates belong to the *Streptomyces* genus and other non-*Streptomyces* strains distributed onto 13 genera, with several new species. 19 strains were found to have ACC deaminase activity and they belong to the genera *Streptomyces*, *Nonomuraea*, *Micrococcus* and *Kibdelosporangium*. The functional ability of the ACC deaminase producing isolates to produce indole-3-acetic acid (IAA), siderophores, mineral phosphate solubilization and growth on nitrogen free semi-solid medium was also determined. Seven strains were selected to inoculate the axenically grown seedlings and they resulted in a significant increase in the seedling fresh weight, the seedling length, the root length and the leaf area of the endophytes-treated seedlings compared to the control. This is the first report on the diversity and characterization of endophytic actinobacteria associated with important oil-seed plant *J. curcas* L. Our results demonstrate that some endophytic actinobacterial strains have the promising PGP attributes to be developed as biofertilizers to enhance soil fertility and promote the plant growth.

1. Introduction

Endophytes are organisms that colonize the internal tissues of higher plants without causing any symptoms and these microbes can be isolated from plant tissues using strict surface-sterilized methods (Hallmann et al., 1997; Strobel, 2003). Endophytic actinobacteria have been isolated from a variety of crop plant species, such as wheat, rice, potato, carrots, tomato (Coombs and Franco, 2003; Tian et al., 2007; Rungin et al., 2012), different kinds of medicinal plants (Li et al., 2008; Qin et al., 2009; Zhao et al., 2011) and other various type of plants (Janso and Carter, 2010; Trujillo et al., 2010), with many novel and bioactive actinobacterial species having been found from articles that have appeared in the recent literatures. Endophytic actinobacteria have attracted significant interest for their capacity to produce a vast array of secondary metabolites exhibiting a wide variety of biological activity and their diverse functions (Conn et al., 2008; Qin et al., 2011b; Li et al., 2012).

Recent works showed that many endophytic actinobacteria are also beneficial to host plants, including biological control of phytopathogens (Cao et al., 2005; Misk and Franco, 2011) and promoting plant growth and they can help their host resist biotic and abiotic stresses (Sziderics et al., 2007; Qin et al., 2014). Endophytic actinobacteria may promote plant growth directly or indirectly through a combination of mechanisms, including solubilization of nutrients, nitrogen fixation, production of growth hormones, siderophores, antibiotics and hydrolytic enzymes

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(Qin et al., 2011b; Kim et al., 2012a,b; Rungin et al., 2012). Besides these commonly studied mechanisms, PGPE are also beneficial to plant growth due to their production of the enzyme 1-amino-cyclopropane-1-carboxylic acid (ACC) deaminase, that converts ACC, the precursor of ethylene in plants, into ammonia and α-ketobutyrate, thereby lowering plant ethylene levels and enhance plant growth (Rashid et al., 2012; Glick, 2014). Inoculation of various plants with ACC deaminase-producing PGPE had been reported and these studies indicated that bacteria having ACC deaminase activity reduce the level of ethylene stress conferring resistance and resulting in better growth of plants under various stress environments (Kuffner et al., 2010; Khan et al., 2011; Jha et al., 2012). To date, however, scarce study has been made specially to screen actinobacteria for their potential to produce ACC deaminase and to enhance plant growth.

Jatropha is an important energy plant which has received more attention in recent years for its utilization in biodiesel production as well as for other beneficial use, such as antimicrobial and pesticidal activity. *J. curcas* is widely grown in Central and South America, Southern Asia, and Central-Southern Peninsular Asia. In China, this plant is mainly distributed from the west of Panzhihua prefecture in Sichuan, most of Yunnan province and the southwest of Guizhou province (Ye et al., 2009). Its peculiar features like drought tolerance, rapid growth, easy propagation, adaptation to a wide range of environmental conditions make it as a special second generation biofuel resource (Abhilash et al., 2011). Its adaptation to diverse agro-climatic condition is linked to its ecological fitness, which possibly could be in part, due to the presence of endophytes (Rodriguez et al., 2009). The Panxi plateau is in the southwest of Sichuan province. The weather in this plateau valley is characterized with abundant rainfall and sunshine and with wet and dry seasons. It is one of the major *Jatropha* production bases in China. Previous study on the endophytes of medicinal plants in the Panxi plateau showed that the medicinal plants are a reservoir of endophytic actinobacterial strains with many good candidates for finding novel antibiotics (Zhao et al., 2011).

It is expected that plants, which are endemic and unique to specific areas, are likely to yield a high diversity of endophytes. To date, only nitrogen-fixing root associated *Enterobacter* species and a small number of endophytic fungi have been isolated from *J. curcas* (Madhaiyan et al., 2013; Kumar and Kaushik, 2013). However, information about the diversity and PGP potential of endophytic actinobacteria associated with *J. curcas* and their interactions with host is scarce. Moreover, the fact that they are naturally occurring actinobacteria ensures that these are already adapted to the habitat of Panxi dry-hot valley soil. Accordingly, the aims of the present study were to isolate endophytic actinobacterial strains from the different tissues of *J. curcas* L. plants from the Panxi dry-hot valley soils of Sichuan, China and study their genetic and functional diversity. In addition, their ACC deaminase producing ability and other PGP activities were evaluated and the effects of selected actinomycete strains on seedling growth were also investigated.

2. Materials and method

2.1. Samples collection and endophytes isolation

Healthy *J. curcas* L. plant samples were collected from Panxi plateau (Panzhihua) in South-west Sichuan, China in October 2010 (26°35′54–26°41′57N, 101°48′50–101°51′10S). Plant samples were washed with tap water, and then separated into roots, stems, leaves and seeds. Plant tissue samples were then surface sterilized using the five-step procedure (Qin et al., 2009). After that, surface-sterilized dry tissues were heat treated at 80 °C for 30 min and aseptically crumbled into smaller fragments using a commercial blender. The treated plant tissues were then spread onto six different selective isolation media: (a) modified TWYE agar supplemented with plant extract; (b) trehalose–proline agar (trehalose 5.0 g, proline1.0 g, $(NH_4)_2SO_4$ 1.0 g, NaCl 1.0 g, $CaCl_2$ 2.0 g, K_2HPO_4 1.0 g, $MgSO_4{\cdot}7H_2O$ 1.0 g, agar 15.0 g); (c) sodium propionate agar (sodium propionate 1.0 g, L-asparagine 0.2 g, KH_2PO_4 0.9 g, K_2HPO_4 0.6 g, $MgSO_4{\cdot}7H_2O$ 0.1 g, $CaCl_2{\cdot}2H_2O$ 0.2 g, agar 15.0 g); (d) cellulose–arginine agar (2.5 g cellulose, 1.0 g arginine, 1.0 g $(NH_4)_2SO_4$, 2.0 g $CaCl_2$, 1.0 g K_2HPO_4, 0.2 g $MgSO_4{\cdot}7H_2O$, 10 mg $FeSO_4{\cdot}7H_2O$, 15.0 g agar); (e) ISP 5 agar; (f) starch–casein agar (Kuster and Williams, 1964). These media were supplemented with nystatin (50 mg l^{-1}) and nalidixic acid (50 mg l^{-1}) to inhibit growth of other endophytic fungi and bacteria, respectively. The plates were incubated at 28 °C for 14–60 days. To confirm that the surface sterilization process was successful, the aliquots of the sterile distilled water used in the final rinse were set on ISP 2 and LB media plates. The plates were examined for bacterial growth after incubation at 28 °C and 37 °C for 7 days. If no microbial growth occurs on the media surface, the sterilization is considered complete and the samples were used for pure culture isolation study.

2.2. Identification of culturable endophytic actinobacteria

The isolated strains were initially characterized by morphological criteria and cultural characteristics (the properties of colonies on different media agar plates and slants; the presence and color of aerial mycelium and substrate mycelium; spore mass color and spore chain morphology; distinctive reverse colony color and diffusible pigments). Then, representative strains were selected for partial and full-length 16S rRNA gene sequencing. Genomic DNA was extracted from the actinobacterial strains using the method of Qin et al. (2012). The 16S rRNA genes were amplified with forward primer 27f (5′-CAGAGTTTGATCCTGGCT-3′) and reverse primer 1492r (5′-AGGAGGTGATCCAGCCGCA-3′) as described previously (Frank et al., 2008). The PCR program used was an initial denaturation (95 °C for 10 min), 35 cycles of denaturation (96 °C for 1 min), annealing (55 °C for 1 min) and extension (72 °C for 2 min), and a final extension (72 °C for 10 min). Then the 16S rRNA gene sequence of strains was compared against a database via BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi/; Altschul et al., 1990) and the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012a) of type strains to retrieve most similar sequences of recognized species. A phylogenetic tree was constructed with the neighbour-joining (Saitou and Nei, 1987) methods using MEGA version 5.0 (Tamura et al., 2011). Bootstrap analysis was performed based on 1000 replicates.

2.3. Plant growth promoting traits

2.3.1. Qualitative and quantitative determination of ACC deaminase activity

ACC-deaminase activity of the isolated strains was detected on plates with DF minimal medium containing 1-aminocyclopropane-1-carboxylate (ACC) as the sole source of nitrogen (Penrose and Glick, 2003). The plates were incubated at 28 °C in the dark for 5 days. Growth of the isolates on DF agar medium amended with ACC was taken as a qualitative indicator of the efficiency of selected isolates to utilize ACC and to produce ACC deaminase. For the detailed quantitative determination of ACC deaminase activity, the protocol described by El-Tarabily (2008) was carried out. After determining the amount of protein and α-ketobutyrate (α-KB), the enzyme activity was expressed as micromoles of α-KB per milligram of protein per hour of the active isolates.