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Phylogenetic diversity and investigation of plant growth-promoting traits of actinobacteria in coastal salt marsh plant rhizospheres from Jiangsu, China

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ABSTRACT

Actinobacteria from special habitats are of interest due to their producing of bioactive compounds and diverse ecological functions. However, little is known of the diversity and functional traits of actinobacteria inhabiting coastal salt marsh soils. We assessed actinobacterial diversity from eight coastal salt marsh rhizosphere soils from Jiangsu Province, China, using culture-based and 16S rRNA gene high throughput sequencing (HTS) methods, in addition to evaluating their plant growth-promoting (PGP) traits of isolates. Actinobacterial sequences represented 2.8%–43.0% of rhizosphere bacterial communities, as determined by HTS technique. The actinobacteria community comprised 34 families and 79 genera. In addition, 196 actinobacterial isolates were obtained, of which 92 representative isolates were selected for further 16S rRNA gene sequencing and phylogenetic analysis. The 92 strains comprised seven suborders, 12 families, and 20 genera that included several potential novel species. All representative strains were tested for their ability of producing indole acetic acid (IAA), siderophores, 1-aminocyclopropane-1-carboxylate deaminase (ACCD), hydrolytic enzymes, and phosphate solubilization. Based on the presence of multiple PGP traits, two strains, *Streptomyces* sp. KLBMP S0051 and *Micromonospora* sp. KLBMP S0019 were selected for inoculation of wheat seeds grown under salt stress. Both strains promoted seed germination, and KLBMP S0019 significantly enhanced seedling growth under NaCl stress. Our study demonstrates that coastal salt marsh rhizosphere soils harbor a diverse reservoir of actinobacteria that are potential resources for the discovery of novel species and functions. Moreover, several of the isolates identified here are good candidates as PGP bacteria that may contribute to plant adaptions to saline soils.

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Introduction

Actinobacteria produce a vast diversity of secondary metabolites, many of which exhibit activity as antibiotics, enzyme and immunosuppressive agents, and as plant growth regulators, and they thus have useful applications in medicine and agriculture [7]. Actinobacteria are diverse and widely distributed among different habitats, including many extreme environments, such as hot springs, alkaline saline soils, and deep sea sediments, in addition to animal guts and plants [62,63,72,77,78]. Extremophilic microorganisms have been investigated heavily for their unique mechanisms of adaptations for extreme environments, and also because they produce unusual metabolites [46,61]. Numerous studies have investigated the ecology of actinobacteria, but compared with the common habitats, there is still little data on their diversity, abundances, and ecological functions in extreme and special environments. Coastal salt marshes are one such special environment, and are one of the most biologically productive habitats on Earth, rivaling the productivity tropical rainforests [55]. Recent, extensive studies of actinobacterial diversity in coastal sediments, mangrove forests, and other saline environments have revealed abundant actinobacterial communities, as evinced by culture-dependent and culture-independent investigations [28,54,75,88]. Despite that coastal salt marsh ecosystems are likely to contain unique and phylogenetically diverse actinobacteria, there is little isolate or diversity information of them in these environments.

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Rhizospheric plant growth-promoting bacteria (PGPB) have been demonstrated to stimulate plant growth by diverse mechanisms [15]. Direct growth-promoting mechanisms include nitrogen fixation, ammonia production, solubilization of mineral phosphate and zinc, production of plant hormones, and the production of 1-aminocyclopropane-1-carboxylate deaminase (ACCD), which reduces plant ethylene concentrations and stimulates seedling growth [50,53]. Indirect mechanisms of plant growth promotion by PGPB primarily involve antagonistic effects against phytopathogenic microorganisms via antibiotic production, siderophores, hydrolytic enzymes, competition with pathogens, induction of systemic resistance, and competition for nutrients and niches [18,24]. Research has demonstrated that actinobacteria in rhizosphere soils have positive effects on plant growth [26]. For example, some *Streptomyces* strains can promote plant growth by producing regulators such as IAA that aid root growth or through the production of siderophores that can improve nutrient uptake [3,37,52]. Actinobacteria also play an important role in rhizosphere dynamics by secreting a wide range of antimicrobial compounds that thereby prevent the growth of root pathogens [2,35]. Despite their enormous potential as plant growth-promoting rhizobacteria (PGPR), few actinobacteria from special and extreme habitats have been investigated in detail for their potential as PGPR.

Soil salinization results in serious stress on plants and represents a global environmental problem that diminishes crop productivity [68]. Plants growing in coastal salt marshes are exposed to a number of stressful conditions including high salt concentrations, unstable substrates, sand erosion, and low organic matter content [23]. Soils, and especially rhizospheric soils, are the centers for microbial activity and rhizospheric microorganisms can play an important role in governing plant development, growth, and habitat fitness. We previously demonstrated that endophytic actinobacteria associated with the halophyte *Limonium sinense* (Girard) Kuntze from coastal salt marsh soils, belonged to the genera *Arthrobacter*, *Streptomyces*, and *Isoptericola*, and could enhance plant growth under salinity stress [59,64]. Likewise, inoculation of the halophyte *Prosopis strombulifera* with *Bacillus licheniformis*, and Thai jasmine rice with ACCD-producing endophytic *Streptomyces* sp. GMKU 336, increased the plant growth of both under salt stress [34,68]. Thus, these results suggest the need for continued study of actinobacterial diversity and their corresponding plant growth-promoting traits from coastal salt marsh soils, which may be key contributors to plant fitness in these special habitats.

Thus, the aim of our study was to evaluate the diversity of actinobacteria from rhizosphere soils of eight coastal salt marsh plants collected from the Jiangsu province in eastern China using culture-dependent and cultivation-independent 16S rRNA gene high throughput sequencing methods. Plant growth-promoting potential of isolates was also assessed in order to identify strains with biofertilizer potential for future crop inoculation experiments.

Materials and methods

Soil samples collection

Soil samples were obtained from the coastal region of Lianyungang (34°34′15–34°45′61N, 119°14′04–119°37′09S) in the Jiangsu Province of east China during the autumn of 2014. The area is characterized by ocean climate with the annual average temperature of 13–14 °C. Eight rhizosphere soils were collected from the following plants: *Phragmites australis* (RS1), *Sesbania cannabina* (RS2), *Chrysanthemum indicum* (RS3), *Metaplexis japonica* (RS4), *Suaeda glauca* (RS5), *Lycium* Linn (RS6), *Spartina alterniflora* (RS7), and *Artemisia* Linn. (RS8). The soil pH measured was 6.8–8.1. Samples were placed in sterile polyethylene bags and brought to the laboratory within 24 h, followed by storage at −80 °C before the DNA extraction, and also at 4 °C for actinobacterial isolation that was conducted within a week.

Isolation of soil actinobacteria

The soil samples pretreatment was carried out as previously described method [79], and the isolation was done using the standard dilution plate method. About 1 g sample of soil was heated in a hot air oven at 80 °C for 60 min and then suspended with 9 ml 0.1% sodium pyrophosphate solution (pH 7.5) and incubated on a rotary shaker (∼180 r.p.m.) at 28 °C for 2 h. After that, the solution was diluted with sterilized water and 100 μl volumes of the suspension were spread on agar plates. Five different selective isolation media supplemented with 3% (w/v) NaCl were used in this study: (A) starch-casein agar [42]; (B) [International *Streptomyces* Project (ISP) No. 5] agar [70]; (C) trehalose-proline agar [60]; (D) sodium propionate agar (sodium propionate 1.0 g, proline 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); (E) starch-arginine agar [2.5 g soluble starch, 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]. All of them were supplemented with two antibiotics, nalidixic acid (30 mg l^{-1}) and nystatin (50 mg l^{-1}). Agar plates were incubated at 28 °C for 2–6 weeks. Individual actinobacterial isolates were then selected and sub-cultured on ISP 2 agar supplemented with 3% (w/v) NaCl.

Identification of culturable actinobacteria

The morphological characteristics of all isolates were investigated, and included colony properties on different media agar plates and slants, the presence and color of aerial and substrate mycelium, and the presence of diffusible pigments [60]. Representative strains were then selected for 16S rRNA gene sequencing and phylogenetic analyses. Total genomic DNA extraction and PCR amplification of the 16S rRNA gene of actinobacterial strains were carried out using previously described methods and primers [58,22]. Amplified PCR products were resolved on a 1% agarose gel, and PCR products were sent for sequencing at Sangon Biotech (China). 16S rRNA gene sequences were compared against the EzTaxon-e database of type strains (http://www.ezbiocloud.net/) [39] in order to identify sequences from closely-related, recognized species. A phylogenetic tree was reconstructed using the maximum-likelihood method [20]. Phylogenetic trees were generated using molecular evolutionary genetics analysis (MEGA) software version 6 [74]. The stability of the clades in the trees was appraised using a bootstrap value with 1000 repeats [21]. The 16S rRNA gene sequences of the isolates have been submitted to the NCBI, and the GenBank accession numbers are from KP972601-KP972623, KP972625-KP972633, KP972635-KP972653, and MH184623-MH184663.

Cultivation-independent DNA extraction, PCR amplification, sequencing, and analysis

For cultivation-independent analyses, eight rhizosphere soils were frozen using liquid nitrogen and then ground to a fine powder. DNA was then extracted from 0.5 g of each soil sample using the MoBio PowerSoil DNA extraction kit (MoBio Laboratories, Carlsbad, CA, USA), following the manufacturer's instructions. Bacterial 16S rRNA genes were PCR amplified using the primers 338f/806r that amplify the V3–V4 hypervariable regions. Barcodes that were unique for each sample were attached to the primers and comprised eight base pair sequences. Each PCR reaction contained 4 μl 5 × FastPfu Buffer, 0.4 μl FastPfu Polymerase, 2 μl dNTPs (2.5 mM),

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