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Promotion of iron nutrition and growth on peanut by *Paenibacillus illinoisensis* and *Bacillus* sp. strains in calcareous soil

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ABSTRACT

This study aimed to explore the effects of two siderophore-producing bacterial strains on iron absorption and plant growth of peanut in calcareous soil. Two siderophore-producing bacterial strains, namely, YZ29 and DZ13, isolated from the rhizosphere soil of peanut, were identified as *Paenibacillus illinoisensis* and *Bacillus* sp., respectively. In potted experiments, YZ29 and DZ13 enhanced root activity, chlorophyll and active iron content in leaves, total nitrogen, phosphorus and potassium accumulation of plants and increased the quality of peanut kernels and plant biomass over control. In the field trial, the inoculated treatments performed better than the controls, and the pod yields of the three treatments inoculated with YZ29, DZ13, and YZ29 + DZ13 (1:1) increased by 37.05%, 13.80% and 13.57%, respectively, compared with the control. Based on terminal restriction fragment length polymorphism analysis, YZ29 and DZ13 improved the bacterial community richness and species diversity of soil surrounding the peanut roots. Therefore, YZ29 and DZ13 can be used as candidate bacterial strains to relieve chlorosis of peanut and promote peanut growth. The present study is the first to explore the effect of siderophores produced by *P. illinoisensis* on iron absorption.

Introduction

Iron is an essential trace mineral for plants. Most of the iron in the soil exists as ferric iron (Fe^{3+}), which cannot be absorbed by plants. The effective iron content in the soil is very low, especially in calcareous soil; thus, plant iron deficiency is a serious global problem.[1] Peanut is one of the most important oil crops and cash crops in China. The northern region is one of the largest cultivated areas in China and is composed of sandy alkaline soil. Due to the sensitivity to iron, iron-deficiency chlorosis in peanut frequently occurs. This dis-

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ease has become an important limiting factor for peanut yield and quality.[2] Therefore, it is extremely urgent to find a safe, effective and economical approach to relieve iron-deficiency chlorosis in peanut.

A set of microbes has been discovered to be able to synthesize and secrete siderophores, a group of small molecular compounds (molecular weight: 1–2 kDa) that chelate Fe^{3+} at high specificity to absorb iron in the surrounding environment.[3] The Fe^{3+}-siderophore can be recognized and absorbed by many types of plant and is vital for iron absorption, especially in calcareous soil. In the past years, the possible implication of siderophores produced by plant growth promoting rhizobacteria (PGPR) has been considered as a potential way to alleviate iron-deficiency chlorosis and improve plant growth. Many studies focus on the role of *Pseudomonas* in promoting plant iron intake. For example, Manwar et al. have discovered that the siderophore pyoverdine produced by *Pseudomonas aeruginosa* increases chlorophyll content, percentage germination, root ramification, nodulation, height and foliage of groundnut. Siderophores produced by *Pseudomonas putida* are able to enhance the chlorophyll content of peanut in calcareous soil.[4] Siderophores produced by *P. putida* P3 have been found to enhance the chlorophyll content and the presence of ^{59}Fe in the roots of peanut in nutrient solution.[5] Furthermore, siderophores produced by some fungi have also drawn much attention. For instance, the siderophore mixture produced by *Penicillium chrysogenum* is able to increase the chlorophyll content of cucumber and maize under hydroponic conditions.[6] Rhizoferrin, siderophores secreted by *Rhizopus arrhizus*, can induce normal growth in plants with iron deficiency chlorosis.[7] However, reports on the effect of *Paenibacillus* sp. on iron absorption of plants, especially under calcareous soil conditions, are limited. In addition, all of the aforementioned experiments were conducted in the laboratory or iron-deficient indoor conditions, which were quite different from the outdoor natural conditions.

In this study, we hypothesized that siderophore-producing *Paenibacillus* sp. can promote the iron absorption of plant in calcareous soil, thus promoting plant growth. To confirm this hypothesis, potted assays and field trial were performed to investigate the effects of two siderophore-producing and spore-producing strains on iron absorption and peanut growth in calcareous soil in indoor and outdoor natural conditions. This study was expected to provide a basis for preventing iron-deficiency chlorosis of peanut using a biological method.

Materials and methods

Medium used in the assays

LB medium was used to activate and culture the isolates for the identification of strains. This medium contained 1% tryptone, 0.5% yeast extract, 1% NaCl and 1.5% agar.

Bean sprouts medium was used to prepare the inocula of the isolates and contained 1% bean sprouts, 3% saccharose and 0.8% $(NH_4)_2SO_4$.

CAS (chrome azurol S) medium was used to evaluate the siderophore production of the isolates and was carried out by the Schwyn method.[8]

Used bacterial strains and strain identification

The two strains, namely, YZ29 and DZ13, were cultivated on LB plates in a constant temperature incubator at 37 °C for 24 h. These strains were subjected to morphological observation, physiological and biochemical determination (such as fermentation of saccharides and hydrolysis of starch and gelatin)[9] and analysis of 16S rRNA gene sequences.

The DNA of the two strains was extracted using a commercially available bacteria DNA extraction kit (Bacteria DNA Kit, Tiangen, Beijing, China). Genomic DNA samples were subjected to 16S rDNA amplification using fluorescent primers 27F and 1492R. Amplified DNA was purified using a commercially available kit (Common DNA Product Purification Kit, Tiangen, Beijing, China). PCR amplified fragments were then subjected to detection in Sangon Biotech (Shanghai) Company (Shanghai, China). A phylogenetic tree based on 16S rRNA gene sequences was constructed using Molecular Evolutionary Genetics Analysis (MEGA) 4.0 software (available at: http://www.megasoftware.net).[10]

Siderophore-producing performance test

The two strains were isolated from the peanut rhizosphere soil by other researchers in our laboratory with the methods of Wang,[11] and they have been detected to produce more siderophores than other isolates, including actinomycetes. To determine the siderophore-producing ability of these two strains, they were inoculated using sterile toothpicks on the CAS plates and cultivated at a constant temperature incubator at 37 °C for 3 days to 5 days and were checked for the presence or absence of orange circles surrounding the bacteria.

Peanut cultivars

Weihua No. 8 (*Arachis hypogaea* L., vulgaris) was used in the pot assay in 2012. Baisha 1016 (*A. hypogaea* L., vulgaris) was used in the pot assay in 2013. Luhua No. 14 (*A. hypogaea* L., vulgaris) was used in the field assay. These three cultivars are all susceptible to iron deficiency.

Soil used in the assays

Soil used in the pot assay in 2012

Calcareous soil was collected from the plough layer of a farmland in Jinan, East China (36°28′N, 116°45′E). The soil was composed of 18.53 g kg^{-1} organic carbon, 100.75 mg kg^{-1} NO_3^-, 4.04 mg kg^{-1} NH_4^+, 22.89 mg kg^{-1} rapidly available P, 96.31 mg kg^{-1} rapidly available K, 2.318 mg kg^{-1} available iron (DTPA-Fe), 1.6648 mg kg^{-1} available zinc (DTPA-Zn), 0.7076 mg kg^{-1} available copper (DTPA-Cu) and 3.32 mg kg^{-1} available manganese (DTPA-Mn).

Soil used in the pot assay in 2013

The soil used in the pot assay was also obtained from the plough layer of a farmland in Jinan, East China. The soil

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