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Characterization of a novel plant growth-promoting bacteria strain *Delftia tsuruhatensis* HR4 both as a diazotroph and a potential biocontrol agent against various plant pathogens

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Abstract

A novel, plant growth-promoting bacterium *Delftia tsuruhatensis*, strain HR4, was isolated from the rhizoplane of rice (*Oryza sativa* L., cv. Yueguang) in North China. In vitro antagonistic assay showed this strain could suppress the growth of various plant pathogens effectively, especially the three main rice pathogens (*Xanthomonas oryzae* pv. *oryzae*, *Rhizoctonia solani* and *Pyricularia oryzae* Cavara). Treated with strain HR4 culture, rice blast, rice bacterial blight and rice sheath blight for cv. Yuefu and cv. Nonghu 6 were evidently controlled in the greenhouse. Strain HR4 also showed a high nitrogen-fixing activity in N-free Döbereiner culture medium. The acetylene reduction activity and $^{15}N_2$-fixing activity (N_2FA) were 13.06 C_2H_4 nmol ml^{-1} h^{-1} and 2.052 ^{15}Na.e.%, respectively. The *nif* gene was located in the chromosome of this strain. Based on phenotypic, physiological, biochemical and phylogenetic studies, strain HR4 could be classified as a member of *D. tsuruhatensis*. However, comparisons of characteristics with other known species of the genus *Delftia* suggested that strain HR4 was a novel dizotrophic PGPB strain.

Keywords: *Delftia tsuruhatensis*; Rice; Plant growth-promoting bacteria; Characterization

Introduction

Beneficial free-living soil bacteria in the rhizosphere are generally referred to as plant growth-promoting bacteria (PGPB) and are found in association with the roots of various plants [21]. The high concentration of bacteria around the roots, i.e., in the rhizosphere, presumably occurs because of the presence of high levels of nutrients exuded from the roots of most plants that can support bacterial growth and metabolism [23]. Besides rhizosphere PGPB, PGPB also include phyllosphere PGPB [4] and endophytic PGPB [13].

In recent years, much attention has been paid to natural methods of crop growing in expectation of moving toward agriculturally and environmentally sustainable development [68]. PGPB promote plant growth due to their abilities in nitrogen fixation, phytohormone production, solubilization of phosphorus and disease

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control [38], and therefore have the potential to reduce the application of agro-chemicals and maintain biotic diversity in the plant associated bio-community. This fascinating area of research has an impact on the study of a number of fundamental aspects of both plant growth and development and the strategies employed by soil microorganisms, while providing us with promising approaches to alter both agricultural and horticultural practices dramatically [21].

Numerous PGPB have been isolated from the tissues of many crop plants [20,21,46]. In addition, PGPB have also been isolated from deep-water rice [74], wild rice [7,15] and cultivated rice in the tropics [25,70]. Recently, four PGPB strains isolated from rice in California were found to possess potential for control of seedling diseases of rice and for plant growth promotion [2].

In this work, we characterized a novel diazotrophic PGPB, strain HR4, which was isolated from the rhizoplane of rice (*Oryza satica* L., cv. Yueguang). Phenotypic characterization and phylogenetic analysis indicated its affiliation to *Delftia tsuruhatensis*. Its plant-promoting roles were assayed for inhibiting various plant pathogens, biocontrol activity and nitrogen fixing activity. To the authors' knowledge, HR4 is the first identification of a plant growth-promoting bacterium which inhibits various plant pathogens and shows N_2-fixing activity in the *Delftia* genus.

Materials and methods

Bacterial strains and culture

Strain HR4 was isolated in our lab from the rhizoplane of rice (*Oryza satica* L., cv. Yueguang), a widely planted rice cultivar in the temperate climatic regions in western Beijing (China). *D. tsuruhataensis* ATCC BAA-554^T was purchased from NBRC (Japan). *Escherichia coli* K_{12} AS1.365 was provided by Professor Shuangjiang Liu, Institute of Microbiology, Chinese Academy of Sciences. *Klebsiella pneumoniae* was provided by Professor Jiudi Li, Institute of Botany, Chinese Academy of Sciences. Routinely, the strains were cultivated in Luria–Bertani (LB) medium [60], unless otherwise indicated below.

Cell and colony morphology

Gram reactions were determined according to standard microbiological procedures [19]. Spore formation was determined by malachite green staining of cells grown on LB agar. PHB formation was determined by the Sudan black B staining method. Cell morphology and size were observed by scanning electron microscope (S570, HITACHI, Japan) and transmission electron microscope (H600, HITACHI, Japan) as previously described [1].

Biochemical characterization

Basal medium used for C assimilation tests contained 0.1% $(NH_4)_2SO_4$, 0.3% KH_2PO_4, 0.7% K_2HPO_4 and 0.01% $MgSO_4 \cdot 47H_2O$. The carbon sources listed in Table 1 were sterilized by filtration (pore size 0.2 μm, Jinteng Company, China) and added aseptically to the autoclaved-based medium at final concentration of 0.2% (wt./vol.). The strain was incubated for 24 h at 30 °C on a shaker at 180 rpm and subcultured in the same medium three times. The growth was determined by measuring OD_{600}. Basal medium was used as the negative control and a basal medium containing 0.2% DL-malate was used as the positive control. All experiments were done in triplicate.

Arginine dihydrolase, catalase, oxidase, urease, lipase (Tween 80 hydrolysis) activity assays and formation of indole, production of 3-ketolactose, V.P. test, gelatin liquefaction test, and starch hydrolysis test were performed according to standard microbiological procedures [19]. Denitrification and nitrite reduction were determined by the method of Stanier et al. [66].

Determination of cellular fatty acid composition

Cellular fatty acid composition of HR4 and *D. tsuruhataensis* ATCC BAA-554^T was analyzed using the Sherolock system (Midi Company, USA) and according to the manufacturer's instructions.

DNA base composition and DNA–DNA hybridization

Genomic DNA from strain HR4 and *D. tsuruhataensis* ATCC BAA-554^T was extracted and purified according to the method of Marmur [50] except for the addition of protease K in the SDS-treatment step. DNA base compositions were determined by thermal denaturation [51] using a spectrophotometer (DU800, BECKMEN, Germany), DNAs from *E. coli* K_{12} were used as standard for the calibration of the T_m value. DNA–DNA hybridizations were carried out according to De Ley et al. [12] and Huss et al. [32].

Phylogenetic analysis

The 16S rRNA gene was amplified by PCR with primers (Pf 5′-CGGGATCCAGAGTTTGATCCTGG CTCAG-3′ and Pr 5′-CGGGATCCAAGGAGGT-GATCCAGCC-3′, corresponding to positions 8–27 and 1525–1541, respectively, of the 16S rDNA of *E. coli*) [16], as previously described [8] using Perkin-Elmer