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Conjunctively screening of biocontrol agents (BCAs) against fusarium root rot and fusarium head blight caused by *Fusarium graminearum*

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ABSTRACT

Background and aims: Fusarium root-rot and fusarium head blight are plant diseases caused by *Fusarium* sp. in different growth periods of wheat, bring heavy losses to crop production in China. This research is aiming to screen biocontrol agents conjunctively for controlling these two diseases at the same time, as well as evaluate our previous BCAs (Biological Control Agents) screening strategies in more complex situation, considering biocontrol is well concerned as an environmental-friendly plant disease controlling method.

Methods: Totally 966 bacterial isolates were screened from different parts of wheat tissues, of which potential biocontrol values were detected according to their abilities in antagonism inhibition and secreting extracellular hydrolytic enzyme. Biocontrol tests against fusarium root rot and fusarium head blight were carried out on 37 bacterial isolates with potential biocontrol capacity after pre-selection through ARDRA- and BOX-PCR analysis on strains with high assessment points.

Results: We acquired 10 BCAs with obvious biocontrol efficacy (more than 40%) in greenhouse and field tests. *Pseudomonas fluorescens* LY1-8 performed well in both two tests (biocontrol efficacy: 44.62% and 58.31%), respectively. Overall, correlation coefficient is 0.720 between assessment values of 37 tested BCA strains and their biocontrol efficacy in trails against fusarium root rot; correlation coefficient is 0.806 between their assessment values and biocontrol efficacy in trails against fusarium head blight.

Conclusion: We acquired 10 well-performed potential BCAs, especially *P. fluorescens* LY1-8 displayed good biocontrol capacity against two different diseases on wheat. Biocontrol efficacies results in both greenhouse and field tests showed high positive correlation with assessment values (0.720 and 0.806), suggesting that the BCAs screening and assessing strategy previously developed in our lab is also adaptable for conjunctively screening BCAs for controlling both root and shoot diseases on wheat caused by same fungal pathogen.

1. Introduction

Fusarium root rot caused by *Fusarium* sp. occurs on wheat as brown or reddish-brown lesions extending a few millimeters to one centimeter or more along the root axis in seedling period (Cook 1980), and fusarium head blight is an attack on wheat by *Fusarium graminearum* usually occur in humid climates where the primary inoculums comes from either airborne ascospores or water-splashed conidia deposited directly in or among the spikelets of heheads, usually during flowers (Sutton 1982). Chemical control and biological control are two major methods taken in controlling these two diseases (Milus and Parsons 1994; Schisler et al. 2002). The use of chemical fungicides is very costly and noxious in nature, thus, the trend towards the environmental friendly biopesticides has led to search new BCAs from various sources, along with antibiotic resistance becomes a serious problem in agriculture crop protection (Sharma et al. 2012; Tilman et al. 2002). In this

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severe situation, biological control is now been well concerned around the world in controlling fusarium root rot and fusarium head blight (Schisler et al. 2002).

Screening of BCAs was done independently against these two diseases in previous works, large amount of BCAs with biocontrol efficacy were isolated (Kim et al. 1997; Khan and Doohan 2009). However, research of screening efficient BCAs in controlling fusarium root rot and fusarium head blight at the same time was limited, although *F. graminearum* could be used as fungal pathogen to study biocontrol of these two wheat diseases. In this study, we were aiming to screen bacterial isolates with potential biocontrol capacity from different parts of wheat tissues, through efficient and valuable BCAs assessing system based on antagonism inhibition ability and activities of extracellular hydrolytic enzymes. Greenhouse and field tests were conducted to test their practical effects in controlling fusarium head blight and fusarium root rot. In addition, correlation was analyzed between biocontrol efficacies of these two diseases. Finally ponderable BCAs were identified for further industrialization to control compound wheat diseases in actual production.

2. Materials and methods

2.1. Bacteria, plants, and their culture

F. graminearum strain GF1117 was isolated from wheat plant infected of fusarium head blight from Jiangsu province of China in 2005. The strain was cultured on PDA (potato 200 g, sugar 18 g, and agar 15 g in 1 L of water) medium at 25 °C for 2–3 d.

All BCA strains were cultured on Luria–Bertani (LB) agar at 28 °C for 1–2 d.

The cultivar of wheat, namely 'Yangmai 16', used in this experiment is widely planted in Jiangsu, China. In greenhouse test, wheat seeds were sown in plastic pots (355.46 cm^3 of volume) filled with the mix of vermiculite and northeast phaeozem (a type of soil rich in humus, coming from Jilin Province, China) (1:3, vol/vol), which had been sterilized at 121 °C for 1 h three times separately on three consecutive days. Plants were grown in an insect-free greenhouse maintained at 16–20 °C with relative humidity of 60% and a 16 h/8 h day/night photoperiod (600 μmol photons/m^2/s of light supplied during the daytime).

2.2. Isolation of wheat habitant strains

Wheat samples were collected from Taicang, Jiangsu Province in China and bacterial strains were isolated from fringe, inside and outside of stem, inside/outside of blade, inside/outside of root, and rhizosphere, respectively (repeat in 3 samples). Isolation of bacteria from wheat rhizosphere, the surface of its fringe and stem, or its root system, three grams fresh weight (FW) of soil, roots, stems or leaves were placed into a sterilized Erlenmeyer flask and suspended in 27 mL of a sterile 0.85% NaCl solution. The suspension was incubated at 25 °C with shaking at 150 rpm for 30 min and then settled for 10 min; the resulting supernatant was serially diluted, plated on R_2A plate (for soil samples) or Luria–Bertani (LB) agar (for tissue samples), and incubated at 28 °C for 48 h. Colony forming units (CFU) were counted and expressed as CFU per gram (FW) soil or tissue. All colonies with different morphologies from each microenvironment were transferred to LB agar, purified, and then stored at −70 °C in LB broth containing 40% glycerol. To isolate endophytic bacteria, three gram (FW) of sampled tomato roots, stems, or leaves was first soaked in 1% sodium hypochlorite (NaClO) for 5 min and then in 70% ethanol for 2 min for surface sterilization, subsequently rinsed five times with sterile water, and finally imprinted on LB agar plates to check sterility: if no bacteria were grown on the plates after incubation at 28 °C for 48 h, the samples were assumed to be sterile. Each sterile sample was placed into a sterilized mortar containing 27 mL of a sterile 0.85% NaCl solution and homogenized with a sterilized pestle, serially diluted with the NaCl solution, plated onto LB agar, and incubated at 28 °C for 48 h.

2.3. Screen for antagonism towards F. graminearum

WA medium (5 g peptone, 10 g glucose, 3 g beef extract, 5 g NaCl in 1 L medium) plate was divided into four equal portions by two vertical lines. Single colony of bacterial strains were picked with sterile toothpick and cross-inoculated 3 cm from the center of the plate along one vertical line antagonistic and left one plate without inoculation of bacteria as a control. *F. graminearum* hyphae block (4 mm diameter circle taken on the edge of the culture) was placed at the center of a plate, incubated at 25 °C. The *in vitro* antagonistic activity was graded with 0, 1, 2, or 3 based on the diameter (in mm) of the semicircular hyaline zones after 48 h: grade 0, no antagonism; grade 1, 1–5 mm; grade 2, 5.1–10 mm; grade 3, >10 mm.

2.4. Detection of activities of extracellular hydrolytic enzymes and siderophores

All bacterial isolates were tested for *in vitro* activities of their extracellular hydrolytic enzymes (cellulase, chitinase, glucanase, protease) and siderophores, which were indicated by distinct semicircular hyaline zones around bacterial colonies on specific agar media. Cellulase activity was determined as described by Ghose (1987), chitinase activity was tested in minimal medium (Chernin et al. 2002), and glucanase activity was detected according to Fan et al. (2002). Skim milk agar (50 mL of sterilized skim milk mixed at 55 °C with 50 mL of 1/5 WA medium containing 2% agar) was used for the detection of protease activity, which was indicated by casein degradation. Siderophore's expression was determined as previously described (Shin et al. 2001).

2.5. Assessment of potential biocontrol agents for their biocontrol ability

In order to find out the relationship between potential and practical biocontrol activity, one assessment system established in our lab was used to evaluate each BCA with different values according to their antagonistic ability and enzyme producing activity (Zheng et al. 2011). The *in vitro* antagonistic activity was graded with 0, 1, 2, 3 based on the diameter (mm) of the transparent-circular zones: no antagonism (0 point); 1–5 mm (1 point); 5.1–10 mm (2 points); >10 mm (3 points). The ability of strains to produce cellulose, chitinase, glucanase was evaluated the same way. For scoring protease and siderophore production, each value was halved, as protease might play better role in biocontrol of nematode and siderophores is more important in biocontrol of bacterial pathogens as its key-position in bacteria competition (Siddiqui et al., 2005; Griffin et al., 2004) rather than controlling fugal pathogen. Grade method is the same as described in antagonism test. Summation of the 6 score became the final assessment point of each bacterial strain.

2.6. ARDRA and BOX-PCR finger-print analysis and identification of bacterial isolates

For ARDRA analysis, genomic DNA of bacterial isolates was extracted with the Mini BEST Bacterial Genomic DNA Extraction Kit (TaKaRa Biotechnology Co., Ltd, Dalien, China) and the partial nucleotide sequence of the amplified 16S rRNA gene was determined using the primers: U8-27(F) 5′-AGA GTTTGA TC(AC) TGG CTC AG-3′; L1494-1514(R) 5′-CTA CGG (AG)TACCT TGT TAC GAC-3′. 0.2 μM; 1× PCR buffer; 0.2 mM dNTPs; 3.75 mM $MgCl_2$; Taq DNA polymerase (TaKaRa Biotechnology, Dalian) enzyme 2U was added