Crop Protection 32 (2012) 129–137

Contents lists available at SciVerse ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Identification and characterization of *Chryseobacterium wanjuense* strain KJ9C8 as a biocontrol agent of Phytophthora blight of pepper

Hye-Sook Kim [a,1,2], Mee Kyung Sang [a,b,2], Ho Won Jung [c], Yong-Chull Jeun [d], Inn-Shik Myung [e], Ki Deok Kim [a,*]

[a] *Laboratory of Plant Disease and Biocontrol, Division of Biotechnology, Korea University, Seoul 136-713, South Korea*
[b] *Institute of Life Science and Natural Resources, Korea University, Seoul 136-713, South Korea*
[c] *Department of Genetic Engineering, Dong-A University, Busan 604-714, South Korea*
[d] *College of Applied Life Science, Faculty of Bioscience and Industry, The Research Institute for Subtropical Agriculture and Biotechnology, Jeju National University, Jeju 690-756, South Korea*
[e] *Crop Protection Division, National Academy of Agricultural Science, Rural Development Administration, Suwon 441-707, South Korea*

ARTICLE INFO

Article history:
Received 23 August 2011
Received in revised form
15 October 2011
Accepted 21 October 2011

Keywords:
Antagonistic rhizobacteria
Biological control
Chryseobacterium wanjuense
Phytophthora capsici
Root colonization
Swarming motility

ABSTRACT

Several *Chryseobacterium* species associated with the suppression of soilborne plant pathogens have been identified, but little is known about the properties of these antagonistic bacteria. In this study, we identified the previously isolated bacterial strain KJ9C8 as *Chryseobacterium wanjuense*, an effective biocontrol agent against Phytophthora blight of pepper caused by *Phytophthora capsici*, using Biolog, fatty acid methyl ester, phylogenetic, biochemical, and physiological analyses as well as transmission electron microscopy. Properties of *C. wanjuense* have been also investigated for its biocontrol activities, including colonization on pepper roots, microbial activity or population in bulk (pot) or rhizosphere soil, the productions of extracellular enzymes, antibiotics, biosurfactant, and hydrogen cyanide (HCN), and other related traits (swarming motility and biofilm formation). Strain KJ9C8 produced protease and HCN with swarming activity, but not antibiotics, biofilm, biosurfactant, and chitinase. In addition, *C. wanjuense* KJ9C8 effectively colonized on pepper roots, suggesting that this bacterium might be able to protect infection courts from soilborne plant pathogens. Furthermore, the survival of strain KJ9C8 in bulk or rhizosphere soil persisted as long as 4 weeks although it gradually decreased over time. Taken together, these results indicate that the colonization and survival of *C. wanjuense* strain KJ9C8, which produces protease and HCN with swarming activity, on pepper roots and in rhizosphere soil might confer effective biocontrol activities to this strain.

1. Introduction

Recently, the interest in biological control by beneficial microorganisms (Kim et al., 2008a, 2008b, 2009; An et al., 2010; Sang et al., 2011) or composts (Sang et al., 2010; Sang and Kim, 2011) has increased consistently as an alternative disease control to substitute for various hazardous chemical controls against airborne or soilborne plant pathogens (Lamour and Hausbeck, 2000; Kim et al., 2008c; Kim and Kim, 2009). In this regard, some rhizobacterial strains isolated from root surface or interior have been known as beneficial microorganisms and have been utilized as biocontrol agents. These beneficial bacteria have the ability to colonize rhizosphere or roots and/or to produce secondary metabolites including antibiotics, extracellular enzymes, hydrogen cyanide (HCN), siderophores, and phytohormones (Kamilova et al., 2005; Kim et al., 2009). For effective and reliable methods to control plant diseases using biocontrol agents under field conditions, it is important to understand the characteristics of bacterial strains that possess biocontrol activities, including their root colonization, and survival and protection of infection courts from plant pathogens (McSpadden Gardener and Weller, 2001; Kamilova et al., 2005). In addition, some beneficial bacterial strains can enhance or induce plant resistance and promote plant growth (Han et al., 2005; Sang et al., 2011).

Phytophthora blight by (*Phytophthora capsici* Leonian) causes severe yield loss on pepper plants worldwide and the disease is difficult to control due to rapid reproduction, dissemination of zoospores and dormant structures of the pathogen (Lamour and Hausbeck, 2000). Microorganisms such as *Bacillus* spp., *Paenibacillus*

* Corresponding author. Tel.: +82 2 3290 3065; fax: +82 2 925 1970.
E-mail address: kidkim@korea.ac.kr (K.D. Kim).
[1] Present address: Department of Biochemistry and Molecular Biology, The University of Chicago, Chicago, IL 60637, USA.
[2] The first two authors contributed equally to this work.

0261-2194/$ – see front matter
doi:10.1016/j.cropro.2011.10.018

illinoisensis, and *Streptomyces halstedii* are known as biocontrol agents against Phytophthora blight of pepper, but only a few strains have the potential to control the disease under field conditions (Joo, 2005; Jiang et al., 2006; Kim et al., 2008a; Sang et al., 2008). In our previous studies (Kim et al., 2008a), we selected three antagonistic bacterial strains (KJ1R5, KJ2C12, and KJ9C8) against Phytophthora blight of pepper by a sequential selection procedure using radicle, seedling, and plant assays as well as plant tests under field conditions. Among these strains, strain KJ2C12 was identified as *Bacillus luciferensis* (Kim et al., 2009) and strains KJ1R5 and KJ9C8 belonged to the genus *Chryseobacterium* based on our preliminary tests.

Chryseobacterium spp. are commonly found in soil and water and easily distinguished from other bacteria due to their distinct yellow colonies on culture media. It has been reported that some *Chryseobacterium* spp. can suppress plant diseases in compost-amendment treatments, but do not suppress the growth of beneficial rhizobacteria (Krause et al., 2001; McSpadden Gardener and Weller, 2001). However, only a few *Chryseobacterium* spp. have been identified as biocontrol agents and their antagonistic mechanisms are still unclear. As mentioned above, we found that *Chryseobacterium* strains KJ1R5 and KJ9C8 had biocontrol activity against *P. capsici* (Kim et al., 2008a, 2008b), in which the activity of strain KJ1R5 was enhanced by adding carbon sources such as L-arabinose (Kim et al., 2008b). Unlike strain KJ1R5, for strain KJ9C8, we investigated the antagonistic properties of the strain that might contribute to the suppressiveness of Phytophthora blight of pepper. In the present study, we (i) identified the bacterial strain KJ9C8 using morphological, physiological, and biochemical analyses as well as Biolog, fatty acid methyl ester (FAME), and 16S rRNA gene sequence analyses, and (ii) characterized properties of the strain in relation to its biocontrol activities against *P. capsici* on pepper.

2. Materials and methods

2.1. Bacterial strain and plant inoculation assay

The bacterial strain KJ9C8 antagonistic to *P. capsici* was isolated from the root interior of a pepper plant grown in Kwangju, Korea (Kim et al., 2008a). This strain was deposited as *Chryseobacterium wanjuense* strain KJ9C8 (KACC 13030) in the Korean Agricultural Culture Collection (KACC) of National Institute of Agricultural Biotechnology in Suwon, Korea. *Escherichia coli* DH5α was used as a control in bacterial characterization tests. Strains KJ9C8 and DH5α were grown at 28 and 37 °C, respectively, in tryptic soy agar (TSA) or broth (TSB) for bacterial identification tests and in nutrient agar (NA) or broth (NB) for plant inoculation and bacterial characterization tests, unless otherwise stated, as described by Kim et al. (2008a). Inhibitory capability tests of strain KJ9C8 against *P. capsici* were conducted on 5-week-old pepper plants (cv. Nockwang) as described by Kim et al. (2008a). Disease severity was evaluated on a 0-to-5 scale (0 = no visible disease symptoms; 1 = leaves slightly wilted and/or brownish lesions beginning to appear on the stem; 2 = stem lesions extending to cotyledons and/or defoliated first or second leaf, or 30–50% of plant diseased; 3 = stem lesions extending to second leaf, yellowing or defoliation of some upper leaves, or 50–70% of the plant diseased; 4 = long, brownish lesions on a stem extending up to at least 10 cm from the soil, all leaves except for the upper most leaf defoliated, or 70–90% of the plant diseased; 5 = plant dead) (Kim et al., 1989) 11 and 18 days after pathogen inoculation in repeated experiments.

2.2. Identification of the antagonistic bacterial strain KJ9C8

2.2.1. Biolog analysis and fatty acid methyl ester profiling

Carbon source utilization by strain KJ9C8 was tested using the Biolog GN Microplate system (Biolog, Hayward, CA, USA) with the Biolog software (Microlog 3 database release 4.01A) according to the manufacturer's instructions. In addition, total cellular FAME analysis of the strain was performed by gas chromatography using the MIDI system (Microbial Identification System, Inc., Newark, USA).

2.2.2. 16S rRNA gene sequence analysis

Chromosomal DNA of strain KJ9C8 was isolated using a QIAGEN Genomic-tip kit (Qiagen GmbhH, Hilden, Germany) according to the manufacturer's instructions. The 16S rDNA gene of strain KJ9C8 was amplified by polymerase chain reaction (PCR) using universal primers fD1 and rP2 (Table 1). The PCR amplification program consisted of 30 cycles of 95 °C for 4 min, 58 °C for 1 min, and 72 °C for 2 min, followed by a final extension step (72 °C for 8 min). The sequence of the PCR-amplified fragment was obtained using an ABI Prism Dye Primer Cycle Sequencing Kit (Perkin Elmer) with the specific primers listed in Table 1. DNA sequence analysis was performed using BLAST network services at the National Center for Biotechnology Information (NCBI) of the U.S. National Library of Medicine (Bethesda, MD, USA). A phylogenetic tree was constructed with the neighbor-joining method using Molecular Evolutionary Genetics Analysis (MEGA) version 4.1 (The Biodesign Institute, Tempe, AZ, USA) (Saitou and Nei, 1987); bootstrap analysis was conducted using the same program. The 16S rDNA sequence of strain KJ9C8 has been deposited in GenBank (accession number = AY514022).

2.2.3. Biochemical and physiological characteristics

Phenotypic characteristics of strain KJ9C8 were compared with those of other reference species (Table 4) of *Chryseobacterium* obtained from Korea Collection for Type Culture (KCTC) (Taejeon, Korea) and C. *wanjuense* from KACC (Suwon, Korea). Colony color, Gram staining, and hydrolysis of casein and starch were examined by the methods of Williams et al. (1989); urea hydrolysis, malonate utilization, and β-galactosidase, indole production, and nitrate reduction were tested as described by Gerhardt (1994).

2.2.4. Morphological observation using transmission electron microscopy

The morphological characteristics of strain KJ9C8 including shape, size of bacterial cell, and existence of flagella were examined using transmission electron microscopy (TEM) (LEO 912AB, LEO Electron Microscopy, Ltd., Cambridge, England). For TEM, cells of the strain grown on NA at 28 °C for 20 h were suspended in sterile water on a glass slide; then, grids were soaked in the bacterial suspension. The cells of strain KJ9C8 were negatively stained with 1% (w/v) uranyl acetate and then examined after the grids were air-dried.

Table 1
Oligonucleotides for 16S rRNA gene sequence analysis used in this study.

Primer	Sequence (5′ → 3′)	Amplified region	Reference
fD1	AGAGTTTGATCCTGGCTCAG	≈ 1.4 kb of 16S rRNA gene	Weisburg et al. (1991)
rP2	ACGGCTACCTTGTTACGACTT	≈ 1.4 kb of 16S rRNA gene	Weisburg et al. (1991)
SP1	GCCACACTGGAACTGAGACAC	See footnote[a]	This work
SP2	TGTAGCGGCCACACTGGAACTGAGACAC	See footnote[a]	This work
SP3	GGAGCATGTGGTTTAAGTGAAATGCGTG	See footnote[a]	This work
SP4	CTACACACGTGCTACGGTGG	See footnote[a]	This work
rSP1	TTCGCACCTGAGCGTCAGTC	See footnote[b]	This work
rSP2	TGACGACAGCCATGCAGCAC	See footnote[b]	This work

[a] Used to sequence forward strand of 16S rRNA gene amplified by the primer set of fD1 and rP2.
[b] Used to sequence reverse strand of 16S rRNA gene.