Crop Protection 84 (2016) 8–13

Contents lists available at ScienceDirect

Crop Protection

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

Evaluation of bacterial biological control agents for control of root-knot nematode disease on tomato

Lihong Zhou [a, d], Gary Yuen [b], Yang Wang [a], Lanfang Wei [c], Guanghai Ji [a, *]

[a] *Key Laboratory of Agricultural Biodiversity for Plant Disease Management Under the Ministry of Education, Yunnan Agricultural University, Kunming 650201, PR China*
[b] *Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583-0722, USA*
[c] *Agricultural-based Experimental Center, YAU, Kunming, Yunnan 650201, PR China*
[d] *College of Bioengineering, Sichuan University of Science and Engineering, Zigong, Sichuan 643100, PR China*

ARTICLE INFO

Article history:
Received 24 March 2015
Received in revised form
23 November 2015
Accepted 17 December 2015
Available online xxx

Keywords:
Biological control
Bacteria
Root-knot nematode
Bacillus methylotrophicus
Lysobacter antibioticus
Meloidogyne incognita
Tomato

ABSTRACT

Among 19 bacterial strains isolated in Yunnan from rhizosphere soils and plant tissues, *Bacillus methylotrophicus* strain R2-2 and *Lysobacter antibioticus* strain 13-6 exhibited the highest antagonistic activity against the tomato root-knot nematode *Meloidogyne incognita* in plate and greenhouse pot experiments. The two strains, when applied as soil drenches or seed treatments in greenhouse experiments, reduced root-knot severity and incidence on tomato compared to no-bacteria controls. In tomato field trials conducted in separate years, soil drench treatment with either strain reduced root-knot disease levels and increased yields compared to the control. Levels of disease control and yield enhancement provided by the strains were higher than those using the chemicals abamectin and carbofuran. This is the first report of *B. methylotrophicus* being used as a biocontrol agent against a plant parasitic nematode and the first demonstration that *B. methylotrophicus* and *L. antibioticus* can suppress disease caused by root-knot nematodes in the field.

© 2016 Published by Elsevier Ltd.

1. Introduction

Tomato (*Lycopersicon esculentum* Miller) is one of the most popular vegetables in the world. Among the many biotic stresses that tomato suffers during its growing season, root-knot caused by *Meloidogyne* spp. is one of the most devastating and widespread (Hunt and Handoo, 2009). In Yunnan province, which is a center of organic vegetable production in China and which has a large area devoted to tomato production for foreign sales, the tomato root knot nematode has caused yield reductions of 30%–50%, making root-knot an important factor restricting Yunnan tomato production (Yang et al., 2011). Root-knot nematodes, as with plant parasitic nematodes in general, not only affect crop yield directly, but also cause plants to be more susceptible to fungal and bacterial diseases (Ashraf and Khan, 2010; Greco and Di Vito, 2009; Taylor and Sasser, 1978). The most common management measures for root-knot nematodes in tomato include the use of resistant cultivars, crop rotation, and soil treatment with chemical nematicides. Resistance to root-knot nematodes, however, is unstable and often associated with decreased yields (Williamson and Robert, 2009), while crop rotation is not always practical because the *Meloidogyne* spp. that feed on tomato also parasitize a wide range of economic plants. Furthermore, the overuse of chemical nematicides may lead to environmental contamination, deleterious effects on beneficial organisms, and selection for nematicide-insensitive strains of the pathogen. Therefore, non-chemical and eco-friendly alternatives such as biological control are being sought (Collange et al., 2011; Hallman et al., 2009; Huang et al., 2009; Siddiqui et al., 2009).

Plant growth-promoting rhizo bacteria produce bioactive substances in the rhizosphere to promote plant growth and protect against pathogens. They also have been investigated explored widely as biological control agents for plant parasitic nematodes (Khan et al., 2008; Siddiqui and Mahmood, 1999; Tian et al., 2007). Because they exist as facultative parasites of nematodes, rhizo bacteria can establish in the rhizosphere independently of nematode populations; this attribute confers them a distinct advantage over the obligate nematode parasite *Pasteuria penetrans* as

* Corresponding author.
E-mail addresses: zhuzhu.15983446361@aliyun.com (L. Zhou), gyuen1@unl.edu (G. Yuen), wangyang626@sina.com (Y. Wang), 1121942876@qq.com (L. Wei), jghai001@aliyun.com (G. Ji).

http://dx.doi.org/10.1016/j.cropro.2015.12.009
0261-2194/© 2016 Published by Elsevier Ltd.

potential biocontrol agents. Root colonization and biocontrol activities of rhizobacteria, nevertheless, are dependent upon soil edaphic and plant host factors (Weller, 1998). Therefore, it is important to evaluate candidate organisms against the target nematode on the specific crop grown in local soil conditions in order to identify the most effective biocontrol agents. There is considerable precedence for using rhizobacteria to suppress *Meloidogyne* spp. on tomato, with *Bacillus* and *Pseudomonas* traditionally being the most commonly tested bacterial genera (Burkett-Cadena et al., 2008; Siddiqui and Mahmood, 1999; Tian et al., 2007) and novel genera such as *Cedacea*, *Lysobacter* and *Pantoea* being revealed in more recent research (Lee et al., 2013; Munif et al., 2013). Most of the studies, however, were limited to pot experiments. The few that were conducted in the field were in locations very distant from Yunnan province. As examples, Ali et al. (2002) evaluated strains of *Pseudomonas* spp. in Pakistan and Wei et al. (2014) tested a strain of *Bacillus subtilis* in Henan province.

The goal of our research is to develop a biological control strategy to manage root-knot nematode in tomato production in Yunnan province. Because previously reported biocontrol agents of *M. incognita* were found in soils that differ from that of Yunnan, we set out to find unique biocontrol agents effective under Yunnan conditions. Our specific objectives in this study, therefore, were to identify the best candidates from among bacteria isolated from Yunnan soils and to evaluate the best strains for root-knot control under field conditions.

2. Materials and methods

2.1. Organisms and general methods

All 19 bacterial strains used in this study, including the seven listed in Table 1, were isolated in Yunnan from rhizosphere soils or plant tissue. They previously had been identified to species based on analysis of 16S rDNA gene sequence and physiological traits (Wang, 2011; Zhang, 2011). *Bacillus methylotrophicus* strain R2-2 and *Lysobacter antibioticus* 13-6 were used in all experiments in this study. Cultures were obtained from culture collections housed at Yunnan Agricultural University. Nutrient agar medium was used for routine culturing. For inoculum preparation, the bacterial strains were cultured in nutrient broth medium at 28 °C with constant shaking at 180 rpm. After three days, cell concentrations were adjusted to 3×10^8 CFU/mL using water to dilute the cultures. The whole broth culture was used as the standard treatment for each strain in all experiments, while cell-free culture fluid also was used in testing strains for in vitro nematicidal activity. To prepare cell-free culture fluid, broth cultures were centrifuged at 12,000 rpm for 5 min, and the supernatants were filtered through 0.22-um Millipore filters.

The root-knot nematode used in this study was *M. incognita* collected from naturally-infected tomato grown in Yuanmou County, Yunnan. The nematode was identified as *M. incognita* on the basis of the perineal pattern of mature females according to Taylor et al. (1955) and Seinhorst (1966). An isolate of the nematode generated from a single egg mass was increased by successive transfer to roots of non inoculated tomato plants. Three-day-old second-stage juveniles (J2) used for inoculation of tomato plants were obtained from roots of tomato plants by sieving and decantation techniques (Southey, 1986) and then incubating the egg masses in tap water at 27 °C for 2–3 days. The J2 were concentrated into small volumes of sterilized water by first filtering the water in which the nematodes had hatched through 5um filters (Whatman type) and then collecting the J2 after repeated washes.

Table 1
Effects of soil drenches with seven bacterial strains on tomato root-knot caused by *Meloidogyne incognita* under greenhouse conditions.[a]

Strain	Incidence (%)	Index (0–100)
Bacillus amyloliquefaciens M4	64 b[b]	57 b
Bacillus methylotrophicus R2-2	30 de	33 f
Bacillus subtilis M3	32 d	38 e
Lysobacter antibioticus 13-1	42 c	45 c
Lysobacter antibioticus 13-6	25 f	31 f
Lysobacter antibioticus HY	42 c	41 d
Pantoea agglomerans 1-7	33 d	38 e
No bacteria control	78 a	84 a
P	<0.0001	<0.0001

[a] Results presented are means from two experiments each with3 replications.
[b] Means in a column followed by the same letter are not significantly different at $\alpha = 0.05$based on Fisher's LSD test.

2.2. Laboratory experiment for nematicidal activity

Nineteen bacterial strains were evaluated for in vitro nematicidal activity in a single experiment. A suspension containing 100 J2 nematodes in 1 mL sterilized water was applied to each 9-cm petri dish. Then, 1 mL of whole broth culture or cell-free culture fluid of a bacterial strain was added to each of three petri dishes containing nematodes. The controls were sterile nutrient broth and sterile water added to the petri dishes containing nematodes. The number of dead nematodes, i.e., those exhibiting no movement, was recorded after 24 h.

2.3. Greenhouse experimental procedures

Greenhouse experiments were conducted in Yunnan Agricultural University. Tomato cv. Rutgers, the most widely planted cultivar in Yunnan and highly susceptible to root-knot nematodes, was used in all experiments. Seeds were sown in a potting mix contained in plastic pots (10 cm diameter, 20 cm depth). The potting mix (pH 5.8–6.2) consisted of peat and a small amount of soil and was amended with 1% (w/v) composite fertilizer. Pots were arranged in complete block arrays on a greenhouse bench. Plants were maintained at 25 to 30 °C and relative humidity between 70 and 90% for 4–8 weeks. The soil in each pot was kept highly moist by daily addition of tap water. Root knot incidence and severity were assessed after the growth period as described below. There were three replicate pots for each treatment.

2.3.1. Evaluation of bacterial isolates

Based on the results of the vitro nematicidal activity experiment, seven strains were selected for further evaluation in two sets of greenhouse experiments to identify the most effective for field testing. In one experiment, bacterial strains were applied as soil drenches and, in the other, as seed treatments. Both experiments included two control treatments: one was treated only with nematodes and the other was not treated with nematodes or bacteria. Each experiment was conducted twice.

For the soil-drench application, the whole broth culture of each bacterial strain was applied to the potting mix in three replicate pots at 50 mL per pot. Each pot contained three plants at the five leaf stage. The bacterial isolates were applied one day prior to inoculation with *M. incognita* to allow earlier establishment of the bacterial isolates. All pots, except those of the no-nematode control, were inoculated with 3000 J2 nematodes per pot.

For the seed-treatment application, tomato seeds were washed three times with sterile water for seed-surface cleaning, soaked in a whole broth culture of a bacterial strain for 1 day in the dark, and finally air dried for 1 h prior to planting (3 seeds per pot). All pots, except those of the no-nematode control, were inoculated with

Download English Version:

https://daneshyari.com/en/article/6373214

Download Persian Version:

https://daneshyari.com/article/6373214

Daneshyari.com