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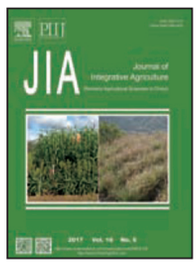

RESEARCH ARTICLE

Field evaluation of *Streptomyces rubrogriseus* HDZ-9-47 for biocontrol of *Meloidogyne incognita* on tomato

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Abstract

Streptomyces rubrogriseus HDZ-9-47, isolated from eggs of *Meloidogyne* spp., was evaluated as a potential biocontrol agent of *Meloidogyne incognita* under *in vitro* and protective field. Microscopic observations showed that HDZ-9-47 parasitized eggs of *M. incognita* within 7 days. *In vitro*, the culture filtrate of HDZ-9-47 caused 97.0% mortality of second-stage juveniles (J2s) of *M. incognita* and inhibited more than 50% egg hatching. In the field, compared with the control, the root-knot index and J2s density in the treatment of drench the broth contained 10^{12} HDZ-9-47 spores were respectively reduced by 51.1 and 80.7% at 90 days post transplantation, which were better than that in other application doses and methods. In addition, reduction rates of root-knot index and J2s density of the treatment of combined application of HDZ-9-47 with biofumigation was 87.1 and 91.0%, respectively, better than either of HDZ-9-47 or biofumigation used alone or fosthiazate treatment. And tomato yield also increased by 16.1%. Together, our results suggest that HDZ-9-47 could be an effective biocontrol agent of *M. incognita*, and that application of HDZ-9-47 combined with cabbage residue biofumigation was a promising and sustainable option for *M. incognita* control.

Keywords: *Meloidogyne incognita*, *Streptomyces rubrogriseus*, biocontrol, parasitism, nematicidal metabolites, control efficacy

1. Introduction

Root-knot nematodes (*Meloidogyne* spp.) are parasitic organisms that cause significant damage to a broad range of plants, being responsible for annual global agricultural losses of at least US$77 billion (Ding *et al.* 2000). *Meloidogyne incognita* is one of the most important species in the genus *Meloidogyne* and it causes dramatic yield losses in many crops, especially in protected agriculture (Davies and Elling 2015). The protected agriculture develops rapidly in China, increasing from 257.7 million hectare in 2003 to 386.2 million hectare in 2014 (Cao *et al.* 2014). Intensive production, suitable soil temperature and moisture, and no effective crop rotations of protected agriculture, provides highly favorable conditions for propagation of *M. incognita*. And approximately 30% of the protected field is infected by *M. incognita*, resulting in 20–40% or even up to 60% yield loss (Cao *et al.* 2014). For this reason, *M. incognita* has become promi-

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nent problems in protected agriculture in China. Chemical nematicides are the most efficient means for controlling *M. incognita*, but their use is increasingly being limited or banned due to their toxicity to humans and soil ecosystems (Yang *et al.* 2012). Therefore, safe alternative methods to control *M. incognita* are urgently needed. Biocontrol agents, which are eco-friendly and generally non-toxic to humans, provide an alternative strategy for sustainable *M. incognita* control (Chen *et al.* 1999; Ismail 2014).

Fungi, bacteria and actinomycetes are major groups of microorganisms that are abundant in soil, and some of these microbes have been characterized for biocontrol of plant parasitic nematodes. Fungi are known to reduce nematode density by parasitism, predation or antagonism. These fungi include *Paecilomyces lilacinus*, *Pochonia chlamydosporium*, *Hirsutella rhossiliensis*, *Acremonium strictum*, *Trichoderma harzianum*, *Dactylella oviparasitica*, *Arthrobotrys robusta*, *Catenaria auxiliaries*, and *Nematophthora gynophila* (Vianene and Abawi 2000; Verdejo-Lucas *et al.* 2002; Mohd and Khan 2005; Anastasiadis *et al.* 2008; Goswami *et al.* 2008; Siddiqui and Akhtar 2009; Trifonova *et al.* 2009). Bacteria are ubiquitous and destroy nematodes in virtually all soils because of their constant association with the rhizosphere. Similar to fungi, bacteria also reduce nematode density mainly by parasitism or antagonism. For example, *Pasteuria penetrans* parasitizes nematodes directly (Kumari and Sivakumar 2005; Davies 2009). In contrast, *Bacillus*, *Agrobacterium*, *Pseudomonas*, and *Clostridium* species produce toxins that kill nematodes (Lian *et al.* 2007; Vagelas *et al.* 2007; Gulnaz *et al.* 2008; Mohamed *et al.* 2009; Pak *et al.* 2009; Terefe *et al.* 2009). Actinomycetes, an intermediate group between bacteria and fungi, are widely distributed in the soil (Moosavi and Zare 2012). *Streptomyces* is the major genus of actinomycetes that is well known to be active against nematodes by antagonism or parasitism (Luo *et al.* 2006). Sun *et al.* (2006) reported that a total of 52 actinomycetes isolates were obtained from *Meloidogyne* spp. eggs and females. Most of these isolates could parasitize eggs of *Meloidogyne hapla*, inhibit egg hatch, and kill second-stage juveniles (J2s) *in vitro*. Ruanpanun *et al.* (2011) recently reported that 28 strains isolated from 20 marine samples exhibited nematicidal activity *in vitro*. Although many microbes have shown strong nematicidal activity *in vitro*, only a few have been applied in field practice. An important factor limiting large-scale utilization of biocontrol agents in the field is their inconsistent field performance (Siddiqui and Shaukat 2004). For example, *Hirsutella rhossiliensis* significantly suppressed soybean cyst nematode in laboratory and greenhouse assays, but did not reduce population densities of soybean cyst nematode in field trials (Zhang *et al.* 2008). Optimizing field application methods of biocontrol agents or combining them with other management approaches such as biofumigation will improve the field efficacy of biocontrol agents (Cayrol *et al.* 1989; Li *et al.* 2014; Luambano *et al.* 2015). Li *et al.* (2014) reported that combining biofumigation with antagonistic *Pseudomonas reinekei* SN21 in the field achieved high control efficacy against *M. incognita*. So it is necessary to find more microbes for controlling nematodes efficiently under *in vitro* and field conditions.

The aim of the study was to evaluate the efficacy of *Streptomyces rubrogriseus* HDZ-9-47, isolated from eggs of *Meloidogyne* spp., for biocontrol of *M. incognita* under *in vitro* and field conditions. The results will provide new strategies for practical management of *M. incognita*.

2. Materials and methods

2.1. *In vitro* experiments

Preparation of eggs and J2s of *M. incognita* The population of *M. incognita* was cultured from a single egg mass and routinely maintained in the greenhouse on tomato (*Solanum lycopersicum*) plants *cv*. Baiguoqiangfeng (susceptible to *M. incognita*). Egg masses were collected from heavily infected tomato roots and the eggs were extracted from the masses using the method described by Sun *et al.* (2006). J2s were obtained by incubating the egg masses on a 30-μm mesh nylon filter in a Petri dish filled with water at 25°C for 5 days. The hatched J2s were collected daily and used for *in vitro* experiments.

***S. rubrogriseus* isolate and preparation of culture filtrate** The isolate of *S. rubrogriseus* used in this study was HDZ-9-47. This isolate was obtained from the Institute of Microbiology, Chinese Academy of Science and deposited in the China General Microbiological Culture Collection Center as CGMCC 2878. The isolate was cultured on Gauze's medium (0.1% KNO_3, 2% soluble starch, 0.05% $K_2HPO_4 \cdot 3H_2O$, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% NaCl, 0.001% $FeSO_4 \cdot 7H_2O$, 2% agar, w/v) at 28°C in complete darkness (Sun *et al.* 2006). After 10 days, spores were harvested from the cultures, counted using a hemocytometer, and adjusted to a concentration of 10^6 spores mL^{-1} in a sterile water suspension. 1 mL of spore suspension was poured into 200 mL JN's liquid medium (1.65% corn flour, 1.0% bean flour, 0.15% $MgSO_4 \cdot 7H_2O$, 0.1% $K_2HPO_4 \cdot 3H_2O$, 0.1% $CaCO_3$, 0.0338% $MnSO_4$, w/v, pH 7.3) in 500-mL Erlenmeyer flasks. The Erlenmeyer flasks were incubated at 28°C for 72 h, in a gyratory shaker (HYG-B, Taicang Experimental Equipment Factory, China) at 160 r min^{-1}.

Culture filtrate of the isolate were prepared by centrifuging the cultures of HDZ-9-47 at 10 000 r min^{-1} for 20 min to remove the spores and mycelia and then filtering the supernatant through a sterile 0.22-μm polyethersulfone filter (Whatman, Clifton, NJ, USA) (Sorensen *et al.* 2009).