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Effects of two strains of *Streptomyces* on root-zone microbes and nematodes for biocontrol of root-knot nematode disease in tomato

Yuan-yuan Ma[1], Yu-long Li, Hang-xian Lai*, Qiao Guo, Quan-hong Xue*

College of Resources & Environment, Northwest A&F University, Shaanxi, China

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ABSTRACT

Root knot nematodes (RKN) cause major losses in tomato. Some strains of *Streptomyces* are known to prevent RKN disease in plants, but little attention has been given to their biocontrol effects in crops such as tomato. In this study, pot experiments were conducted to assess the effects of a *Streptomyces* agent (1:1 mixture of *S. pactum* and *S. Rochei*) for biocontrol of RKN disease in tomato. We quantified endophytes in tomato roots, as well as microbes and nematodes in root zone soil with or without inoculation of *Streptomyces*. Additionally, we measured the plant biomass and physiobiochemical traits of tomato at different growth stages. The results showed that after inoculation of *Streptomyces*: (i) The RKN disease index of tomato plants decreased by 37%. The quantity of bacterial-feeding nematodes decreased by 14% in root zone soil, while the quantity of fungal-feeding and plant parasitic nematodes increased by 10% and 137%, respectively. (ii) The fresh weight of shoot and root increased by 14% and 35%, respectively. Meanwhile, the polyphenol oxidase activity of leaves increased by 33% at the seedling stage. (iii) The total number of culturable bacteria decreased by 49% in root knots. Regarding the total number of culturable microbes in root zone soil, bacteria and actinomycetes also decreased by 18% and 10%, respectively, whereas fungi increased by 20%. Moreover, the quantity of both plant growth-promoting bacteria and nematicidal bacteria increased, while the quantity of plant-pathogenic bacteria decreased. In conclusion, the *Streptomyces* agent could reduce RKN disease in tomato by activating the systemic resistance and defensive mechanism against nematode infection. This agent triggered complex interactions between plants, microbes, and nematodes in the micro-ecosystem of root zone soil, resulting in decreased RKN infection ability and/or enhanced tomato disease resistance.

1. Introduction

Nematodes, an important component of the soil ecosystem, are closely associated with plants grown in soil. Plant parasitic nematodes seriously affect the growth of crops, of which root knot nematodes (RKN) can lead to a large-scale invasion of crop roots, causing an estimated global loss of $100 billion per year (Oka et al., 2000). Current dissatisfaction with chemical nematicides due to safety issues and environmental concern has stimulated research for alternative control strategies that are pollution-free. Developing alternatives to hazardous chemical nematicides is one of the top priorities for the future of nematology (Javed et al., 2012).

With a wide variety and huge quantity, soil microbes are closely associated with soil nematodes and crop roots. Of these, there are many beneficial microbes that can suppress nematodes and promote the growth of plants and other beneficial microbes (Brinkman et al., 2012). Screening microbial antagonists to RKN and exploring the role of these microbes in RKN disease form the basis for the prevention and control of the same. So far, research on the resistance to RKN mainly concentrated on bacteria (Tian et al., 2007) and fungi (Chen et al., 2004). Actinomycetes can synthesize various active antibacterial substances. The insecticidal substances in actinomycetes are mainly reported in *Streptomyces* (Ruanpanun et al., 2010).

With significant effects to prevent disease development, *Streptomyces pactum* and *Streptomyces rochei* can simultaneously promote root growth of *Amorphophallus konjac* K. Koch and change the microflora in root zone soil (He et al., 2015). Recently, Duan et al., (2015) found that RKN disease was significantly reduced in a traditional Chinese herb, *Salvia miltiorrhiza* Bge. after inoculation of *S. pactum*. However, there has been no systematic and specific

* Corresponding authors at: College of Resources & Environment, Northwest A&F University, 3 Taicheng Road, Yangling, Shaanxi 712100, China.
E-mail addresses: 13363921675@163.com (Y.-y. Ma), laihangxian@163.com (H.-x. Lai), xuequanhong@163.com (Q.-h. Xue).
[1] Postal address: College of Resources & Environment, Northwest A&F University, 3 Taicheng Road, Yangling, Shaanxi 712100, China.

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study of these *Streptomyces* species elucidating their effects on plant resistance against RKN disease. Tomato is a major vegetable crop in China that is seriously affected by RKN (Yu et al., 2006). It remains unknown whether *Streptomyces* can influence RKN infection and plant growth in tomato.

In the present study, we investigated the effects of a *Streptomyces* agent on RKN infection and plant growth in tomato. We examined the quantitative changes of nematodes and culturable microbes in root zone soil, as well as culturable microbes in root system and root knots, with and without inoculation of *Streptomyces*. The objective of the study was to clarify the control effect of *Streptomyces* on RKN disease in tomato from the perspectives of RKN infection ability and plant disease resistance. The results will providing evidence for the biocontrol of RKN disease in tomato using *Streptomyces*.

2. Materials and methods

2.1. Pot experiments

Pot experiments were carried out in a greenhouse on the South Campus of Northwest A&F University during October 2014 to May 2015. The experimental soil, used as the nematode inoculum, was collected from root zones of tomato under continuous cropping in a solar greenhouse in Mengjiazhai Village in Yangling. During the full fruit period (September 2014), tomatoes heavily infected with RKN were sampled by removing *c.a.* 5 cm surface debris and litter, after which both galling roots and soil from root area (10–20 cm from taproot) were collected separately using a shovel. Soil samples were mixed thoroughly and transported in plastic bags to the laboratory. The RKN was identified to be *Meloidogyne javanica* (Accession number: KX094970) by 28S rRNA confirmed by perineal pattern examined in light microscopy, and the number of RKN in the soil was 800 thousand per kilogram. The same type of soil from a non-continuous cropping plot, applied as healthy potting soil, was collected by the same methods as described before and passed through a 10-mm sieve after air drying and crushing. The *Streptomyces* agent, a 1:1 mixture of *S. pactum* and *S. rochei* in solid-state fermentation, was developed by the Resource Microbiology Laboratory, Northwest A&F University (Yangling, China). The viable count of the *Streptomyces* agent was 2.33×10^9 cfu g^{-1}.

Two treatments were included: (i) N: 4 kg potting soil and 2 kg nematode inoculum were added to each pot with sufficient mixing and (ii) N + S: 4 kg potting soil, 2 kg nematode inoculum, and 9 g *Streptomyces* agent were added to each pot with sufficient mixing. Thirty seeds were sown in each pot, and 10 seedlings were retained after emergence. Seeds of tomato (*Lycopersicon esculentum* Mill.) cultivar 'Baiguo Qiangfeng' were purchased from an agricultural market in Yangling, Shaanxi Province, China. During the experiment, the compound fertilizer ($N\text{-}P_2O_5\text{-}K_2O$ = 15-15-15) was applied every two moths using 1 g/pot each time. Forty days later, six plants were uprooted from each pot for determining the biological traits of seedlings, biochemical indicators of leaves, and number of root knots. Soil samples shaken off from tomato roots were collected for counting the number of soil nematodes at the same time. Four well-developed plants were retained until the fruiting period for determining the biological traits of mature plants and infection of RKN.

2.2. Determination of plant biomass

At the seedling stage (November 20, 2014), an appropriate amount of water was poured to loosen the soil in the pots. Six plants were uprooted and the soil adhering on tomato roots was shaken off. The roots were rinsed carefully until there was no soil on it, and the root surface was then dried with absorbent paper. This was followed by counting the number of fibrous roots and root knots per plant and measuring the stem diameter using a vernier caliper (accuracy, 0.01 mm) and fresh weight of shoots and roots using an electronic balance (accuracy, 0.01 g). The number of root knots per 100 fibrous roots was defined as the density of root knots.

At the maturity stage (May 20, 2015), the over-ground part was clipped with scissors at the rhizome boundary. The entire soil in each pot was poured out onto plastic sheets to scoop out roots carefully. The adhering soil was then shaken off and collected for analysis of microbes and nematodes in the root zone soil. The roots and stems were numbered and the biomass (stem diameter; fresh weight of shoots, roots, and fruits; and number of fruits) was subsequently determined.

2.3. Determination of physiological and biochemical traits

Physiological and biochemical traits were measured in mature leaves of the largest area on the 3rd, 4th, and 5th lateral branches from the top of each plant. Photosynthetic parameters, including net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, and transpiration rate were determined using the LF-6400XTR portable photosynthesis system (LI-COR Biosciences, Lincoln, NE, USA). Phenylalanine ammonia lyase (PAL) activity was determined following the method of Havir (1981). Peroxidase activity (POD) was determined following the method of Nakano and Asada (1981) with slight modifications (Zhao et al., 2011). Polyphenol oxidase (PPO) activity was measured following the method of Fei et al. (2007) with slight modifications (Zhao et al., 2011). Malondialdehyde (MDA) content was detected with thiobarbituric acid (Zhang, 2001).

2.4. Determination of RKN disease index

To account for the number of total and infected lateral roots, five intact roots were selected for each treatment from the mature roots acquired. Infection rate, reflecting the severity of root galling of each plant, was calculated according to formula (1) and ranked in four grades (0: I = 0%, 1: I ∈ 1–25%, 2: I ∈ 26–50%, 3: I ∈ 51–75%, 4: I ∈ 76–100%). The disease index was calculated from the disease grade using formula (2).

$$Infection rate(\%) = \frac{Number\ of\ lateral\ roots\ infected\ by\ root\ knots}{Total\ number\ of\ lateral\ roots} \times 100 \quad (1)$$

$$Disease index(\%) = \frac{\sum(Disease\ grade \times Number\ of\ plants\ of\ corresponding\ grade)}{4 \times Total\ number\ of\ investigated\ plants} \times 100 \quad (2)$$

2.5. Enumeration and identification of soil microbes and nematodes

2.5.1. Sample collection

The root zone soil samples were collected by shaking off adhering soil on roots and then bagging it into valve bags at the time of harvesting mature roots. The root zone soil comes from the area where roots are densely distributed, so the quantity and species of microbes and nematodes in it are closely related to the biological, physiological, and biochemical traits of root systems; it