Stimulatory effects of bacterial-feeding nematodes on plant growth vary with nematode species

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Bacterial-feeding nematodes, together with protozoa, are the main grazers of soil bacteria and can account for 60-80% of the total nematode community (Griffiths, 1989; Li et al., 2001). Grazing on bacteria can accelerate bacterial turnover and increase the turnover of soil organic matter (Griffiths, 1994; Alphe et al., 1996), which releases considerable amounts of nitrogen, mainly as ammonium, and thus may enhance plant growth. Nematode grazing on soil bacteria can also alter bacterial community structure (Griffiths et al., 1999; Djigal et al., 2004), in the same way as grazing by protozoa (Griffiths et al., 1999; Rønn et al., 2002). Bonkowski and Brandt (2002) have shown that protozoan grazing stimulates the proportion and activity of bacteria that produce the plant growth hormone indole acetic acid (IAA) and so stimulate plant root growth by secretion of phytohormones (Jentschke et al., 1995; Arshad & Frankenberger, 1998; Phillips et al., 1999; Lambrecht et al., 2000; Bonkowski & Brandt, 2002; Bonkowski, 2004). A similar mechanism has also been suggested for bacterial-feeding nematodes (Mao et al., 2006, 2007). However, it is uncertain whether the identity of the nematode will affect the interaction with auxin-producing bacteria, in the same way that the degree of enhanced N mineralisation varies with nematode identity (Ingham et al., 1985). To test this, a gnotobiotic microcosm experiment was undertaken to ascertain the effects of different bacterial-feeding nematode species on the activity of auxin-producing rhizobacteria and rice root growth.

An alluvial sandy loam soil was autoclaved at 121°C for 30 min on 2 consecutive days to eliminate resident microorganisms and nematodes. The test bacterium was Burkholderia sp. (provided by MOA Key Laboratory of Microbiological Engineering of Agricultural Environment, Nanjing Agricultural University, Nanjing, P. R. China), selected for its ability to produce IAA as tested by the method of Picard and Bosco (2003). Two bacterial-feeding nematode species were used: Cephalobus sp. (N1) isolated from the experimental soil and cultured in the laboratory and Caenorhabditis elegans (N2) (supplied by Ji-hua Wu, Fudan University, Fudan, P. R. China). The experiment was set up in 50 ml beakers (height 10 cm, diam. 6.5 cm) filled with 65 g sterilised soil rewetted to 60% of its water holding capacity (24.3% water content on a dry weight basis).

Bacteria were grown in nutrient broth (10 g l⁻¹ peptone, 5 g l⁻¹ yeast extract, 10 g l⁻¹ NaCl) for 48 h at 37°C and 150 rpm, centrifuged (5000 g, 5 min), the pellets washed twice in sterile water and resuspended in sterile water to a density of 10⁷ colony forming units (cfu) ml⁻¹ and inoculated to soil at 10⁶ cfu (g dry soil)⁻¹. Nematodes were cultured in Petri dishes of nematode growth medium (NGM) (Brenner, 1974), inoculated with Escherichia coli OP50 at 24°C for 10 days, washed from the plates in sterile water and resuspended in sterile water to a density of 10⁷ colony forming units (cfu) ml⁻¹ and inoculated to soil at 10⁶ cfu (g dry soil)⁻¹. Nematodes were cultured in Petri dishes of nematode growth medium (NGM) (Brenner, 1974), inoculated with Escherichia coli OP50 at 24°C for 10 days, washed from the plates in sterile water and concentrated by centrifugation (2000 g, 5 min) (Hooper, 1986). The nematodes were surface-sterilised for 20 min in 1.0 g l⁻¹ streptomycin and 0.02 g l⁻¹ actidione and washed five times in sterile distilled water. Nematodes were then inoculated in the soil (1300 beaker⁻¹ or 20 (g dry soil)⁻¹) 2 days after the bacteria were added.

Rice seeds (Oryza sativa cv. Japonica) were surface-sterilised for 20 min in a freshly prepared 1% solution of sodium hypochlorite (NaOCl) followed by four washes in

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sterile distilled water (250 ml each wash). Subsequently, the seeds were incubated at 4°C for 2 days then at 22°C for 3 days in the dark to synchronise germination.

There were three soil treatments: i) soil inoculated with bacteria only (B); ii) soil inoculated with bacteria and nematode N1 (BN1); and iii) soil inoculated with bacteria and nematode N2 (BN2). Soil water content after inoculation of both bacteria and nematodes was maintained a soil moisture level of 24.3% (dry weight basis). For each treatment, six replicate pots were planted with three germinated rice seedlings in each pot 1 day after the nematodes were added. The day of planting was taken as day 0 of the experiment. Plants were then grown in a growth chamber maintained at 26°C, 16 h light and 22°C, 8 h dark. Sterile water was added every 1 or 2 days through a sterile tube inserted in the middle of the pot closed by a sterile film to prevent contamination. Also on day 0, another three replicates of each treatment were sampled for soil analysis. After 14 and 20 days growth, three replicates of each treatment were taken for plant sample analysis and three replicates for soil sample analysis.

Nematodes were extracted from 20 g fresh soil with a modified Baermann method using trays instead of funnels (Goodfriend et al., 2000). After 48 h at room temperature nematodes were collected and counted under a dissecting microscope. Indolyl-3-acetic acid (IAA) in soil was measured using high performance liquid chromatography (HPLC) (Hu et al., 2001). Soil basal respiration was measured, as reported by Isermeyer (1952), by alkali (1 M NaOH) absorption of the CO₂ developed over 24 h followed by titrating the residual OH⁻ with a standardised acid. Sampled plant roots were carefully washed free of soil. Images from individual washed roots were acquired by scanner (LA1600+ scanner, Canada) and root related parameters were analysed using Winrhizo2003b software (Regent Instruments, Nepean, ON, Canada). After scanning, roots and shoots were analysed to determine total plant total nitrogen content. Due to the small size of the sterile plants shoots and roots, three seedlings per pot were pooled, chopped with fine scissors and then analysed according to the method of Bremner (1965). Nematode data were analysed by Student’s t-test. Soil and plant samples data were analysed with a two-way ANOVA (with variables being days and soil treatment). The significance of differences between means was determined with a least significant difference (LSD) test. All statistical analyses were carried out using the statistical package SPSS16.0.

The abundance of the nematodes N1 and N2 did not increase over the course of the experiment, although there were 519 Cephalobus sp. (N1) and 345 C. elegans (N2) per pot at day 20 (Table 1). The presence of nematodes significantly increased soil basal respiration, and soil IAA content ($F = 19.67, P < 0.01, F = 4.23, P < 0.05$). The effects varied according to both bacterial-feeding nematode species and day of sampling. Rice seedlings developed significantly greater total root length and number of root tips in the presence of nematodes. Total plant nitrogen content was significantly increased with N1 at day 14 and with N2 at day 20 (Table 1); these effects also varied with nematode species and sampling day.

Our results confirmed that rice seedlings grown in soils with bacterial-feeding nematodes developed a more highly branched and longer root system. Similar observations have been made for the effect of bacterial-feeding nematodes on tomato plants (Mao et al., 2006). At the same time, we found an increase in the soil IAA content in the presence of both species of nematodes, Cephalobus sp. and C. elegans, over the sampling time. This result agrees with other studies showing that the bacterial-feeding nematodes can affect root growth by increasing soil auxin content (Mao et al., 2007), corresponding with increased proportion of auxin-producing bacteria for grazing effect, as have been suggested for protozoa (Bonkowski, 2004; Kreuzer et al., 2006).

Moreover, the different nematode species had different effects on the parameters investigated in this study. The soil IAA content increased significantly on day 14 with N1 and on day 0 with N2 when compared to the nematode-free control microcosm. The presence of nematodes significantly increased soil respiration on day 0 (i.e., 1 day after adding the nematodes) and respiration was significantly greater in the presence of N2 than N1. The presence of N2 increased the bacterial activity and IAA content much earlier than did N1. This is consistent with the characteristic of these two nematodes. N1 in the family Cephalobidae are cp-2 ‘general opportunists’ and respond slowly to resource availability. N2 in the family Rhabditidae are cp-1 ‘enrichment opportunists’ and respond rapidly to resource enrichment (Bongers, 1990; Ferris et al., 1995, 1997; Bongers & Bongers, 1998; Blanc et al., 2006). The slight decrease of the bacterial activity at day 20 with nematodes may reflect a decline in bacterial numbers under the grazing pressure in a small and closed microcosm where resources were limited. Furthermore, the amount of nitrogen incorporated in plants with N1 at