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Suppression of *Meloidogyne incognita* in Lettuce and Oriental Melon by *Pasteuria penetrans* KW1

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***Pasteuria penetrans* KW1 (PP), parasitic bacterium of nematode, was isolated from oriental melon greenhouse soil in Korea and evaluated for the suppression effect on the reproduction of southern root-knot nematode, *Meloidogyne incognita* (MI), in lettuce (*Lactuca sativa* L. var. Chungchima) and oriental melon (*Cucumis melo* L. var. Eunchun). Pot experiments were conducted by planting the lettuce seedlings in medium inoculated with 5,000 MI juveniles/pot (J), J + 100,000 PP endospores/1 g medium, and J + 200,000 PP endospores/1 g medium. After 11 weeks of plantation, number of root galls in J + 200,000 PP endospores/1 g medium was decreased to 92/root (38.9%, control effect), compared to the J of 150/root. In the second plantation of lettuce in the same pots, the numbers of root gall were significantly decreased in PP treated pots with 75 (77.2%, control effect) and 150/root (54.4%, control effect) in J + 200,000 and J + 100,000 PP endospores/1 g medium, respectively, compared to the J of 330/root when harvested at 10 weeks after planting. In oriental melon, root gall percentages were 32.1 (60.2%, control effect) and 52.9% (34.5%, control effect) in J + 200,000 and J + 100,000 endospores/1 g medium which were significantly lower than that of 80.7% in J.**

Keywords : biological control, *Meloidogyne* spp., *Pasteuria* spp., vegetable crops

Meloidogyne spp., a causal organism responsible for the root-knot disease, causes serious damage in greenhouse crops, especially on lettuce, oriental melon, watermelon, pepper, tomato, and cucumber in Korea. Six *Meloidogyne* species of *M. arenaria, M. incognita, M. hapla, M. javanica, M. hispanica*, and *M. cruciani* have been recorded in Korea. Among them, *M. arenaria, M. incognita*, and *M. hapla* are the major pests in greenhouse crops (Choi, 1981; Cho et al., 1993; Cho et al., 1997; Han and Kim, 1997).

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The control of *Meloidogyne* spp. is carried out largely by a combination of methods that includes nematicides and crop rotation (Dickson and Hewlett, 1988; Minton and Baujard, 1990). Economic considerations and potential environmental hazards of nematicides, however, have created a climate of uncertainty regarding their continuous use on vegetable crops. It is also documented that nematicides are often unreliable when nematode population densities are high (Dickwon and Hewlett, 1988), and some successful rotation must remain in place for more than 4 years (Dickson and Hewlett, 1989).

Recent reports on the *Pasteuria penetrans* demonstrated the potential of the bacterium for the biological control of root-knot nematodes (Chen and Dickson, 1998). A soil infested with *P. penetrans* suppressive to *M. arenaria* on peanut showed reduction of J2 penetration into the root and root galling (Minton and Sayre, 1989). In microplot test, peanut root galls and pod galls by *M. arenaria* race 1 were reduced and the yields were increased in the endospore treated plots with 100,000 endospores/1 g soil (Chen et al., 1996). Page and Bridge (1985) also observed almost complete destruction of *M. arenaria* in tomato greenhouse infected with *P. penetrans*.

This study was conducted to evaluate the biological control effect of *M. incognita* by a *P. penetrans* KW1 (PP) isolated from oriental melon greenhouse in Korea.

Pasteuria penetrans KW1 (PP) was isolated from *Meloidogyne incognita* females in the roots of greenhouse-grown oriental melon (*Cucumis melo* L.) in Kyung-buk province, Korea. Scanning electron microscopy (SEM) was used to observe the morphology of *P. penetrans* (Chen et al., 1997). The *P. penetrans* was reared in tomato (*Lycopersicon esculentum* Mill. cv. Youngkwang) by consecutively inoculating the endospore encumbered *M. incognita* J2s in the root system. The endospores were harvested from the roots by picking up the infested nematode females under dissecting microscope after the roots were treated with 10% Cytolase for 2 days (Chen et al., 1996). The female bodies were ground in microtube and

kept in refrigerator until use.

Meloidogyne incognita (MI) was collected from a greenhouse cultivating oriental melon in Kyung-buk province, Korea and maintained in tomato (*Lycopersicon esculentum* Mill. var. Youngkwang) roots. The eggs of MI were extracted from the tomato roots in 0.5% sodium hypochlorite solution and caught on a sieve with 25-μm-pore-opening (Hussey and Barker, 1973). Freshly hatched J2s, not older than 3 days after hatching, were used in the experiment.

Baroker (Seoul Agricultural Supply Company, Seoul, Korea) pot medium which consists organic matter 85%, vermiculite 8%, zeolite 5%, and fertilizers 2% with pH 5.5-6.0 was used in the experiments. Each 50 g medium was put in plastic zipper bag (15×20 cm). Similarly, 5,000 MI J2s were suspended in 20 ml distilled water and inoculated 10 ml suspension of 5,000 MI J2s into plastic bags containing medium along with 10ml of 100,000 PP endospores suspension. Treatment of endospores were increased by inoculating 200,000 PP endospores along with 5,000 MI J2s. For control, 20ml of distilled water was added to the plastic bags containing medium. Six replications were done for each treatment and treated bags were zippered and incubated at 25°C for 5 days.

The treated media were transferred in jiffy pots (5 × 5 × 5 cm) and 3-weeks-old lettuce seedlings (*Lactuca sativa* L. var. Chungchima) were transplanted in each pot. After 2 weeks, the pots were put in plastic pots (15 cm in diameter) containing the Baroker medium and the lettuces were grown in glasshouse. After 11 weeks of transplantation, the roots were removed from the pots leaving the debris in the pots. The numbers of root gall were counted and the root weight, leaves weight, and the number of total leaves per plant were recorded. As a second experiment, the 3-weeks-old lettuce seedlings were transplanted in the same pots as the above treatments with 6 replications. The lettuces were grown in the glasshouse for 10 weeks. The roots were washed free from debris and the root gall numbers were counted. Plant growth characteristic of root weight, leaves weight, and the number of total leaves per plant were recorded.

The same treatments were done for oriental melon (*Cucumis melo* L. var. Eunchun) and replicated 6 times for each treatment. The 2-weeks-old oriental melons were transplanted and were grown in the glasshouse for 10 weeks until harvest. The root gall numbers were examined. Leaves weight, root weight, and number of total leaves per plant were recorded.

Endospores of *P. penetrans* KW1 (PP) were cup-shaped and morphologically identical among isolates (Fig. 1). Spores were about 2.5 to 4.0 μm in size and adhered to the surface of the larva of a root-knot nematode. Dorsal side of endospore was smooth and round. The ventral side was wrinkled. Two distinct forms of these spores could be observed: a central endospore, that was spherical and a peripheral matrix that surrounded the central endospore. The smooth central surface of the endospore was easily distinguishable from its peripheral matrix, which formed an encircling ring with a particulate surface (Fig. 1).

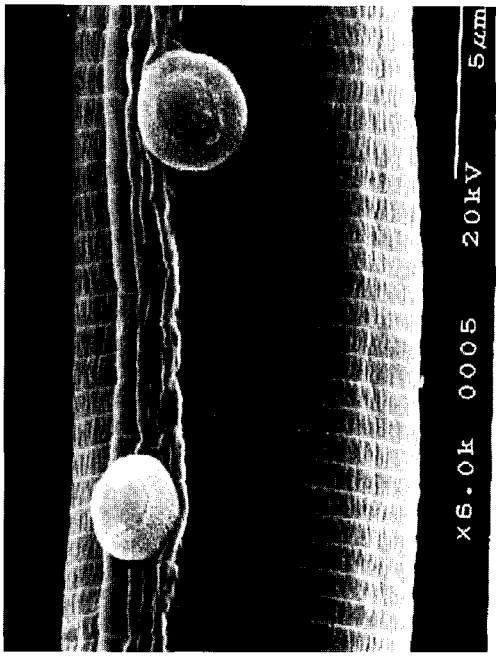

Fig. 1. *Pasteuria penetrans* KW1 (PP) attached to root-knot nematode juvenile of *Meloidogyne incognita*. Scale bar = 5 μm.

P. penetrans KW1 (PP) mixed with *Meloidogyne incognita* (MI) J2s in pot media successfully suppressed formation of root galls. After 11 weeks of lettuce transplantation, the numbers of lettuce root galling were decreased in the endospore treated pots (Table 1). The numbers of root galls in the pots treated with 200,000 and 100,000 PP endospores/g medium were 92 and 147 respectively, while the number was 150 in the pots treated with MI only.

In the second cropping of lettuces in the same pots after the first experiment was finished, the root gall numbers were lower in the endospore treated pots (Table 2). Root gall numbers were 75 and 150 in the pots treated with 200,000 and 100,000 PP endospores/g medium, respectively, while the number was 330 in MI treated pots. The control effect on the root-knot nematode was 77.2% and 54.4%, respectively, which were higher than those of the first cropping of the lettuce (Table 1). Meanwhile, production of galls was also suppressed in oriental melon roots by PP endospore treatments (Table 3). Root gall percentages were 32.1% and 52.9% in the pots treated with 200,000 and 100,000 PP endospores/g medium, respectively, while 80.7% in MI treated pots.

Table 1. Suppression effect of *Pasteuria penetrans* KW1 (PP) on reproduction of *Meloidogyne incognita* in lettuce (first experiment)[a]

Treatments	No. of root galls		Wt. of leaves harvested (g)	No. of total leaves harvested	Root wt. (g/plant)
	No. of gall/ root	Control effect (%)			
MI J2 5,000/plant (N)	150 c	-	215 a	270 a	12.6 a
N + PP 100,000 endospores/g medium	147 c	3	229 a	265 a	12.3 ab
N + PP 200,000 endospores/g medium	92 b	38.9	225 a	272 a	12.0 ab
Control	0 a	-	225 a	273 a	9.8 b

[a] Data are means of 6 replicates. Root galls, leaves weight, root weight, and number of total leaves were examined 11 weeks after planting. Means within a column followed by the same letter are not different according to Duncan's multiple-range test (P<0.05).

Table 2. Suppression effect of *Pasteuria penetrans* KW1 (PP) on reproduction of *Meloidogyne incognita* in lettuce (second experiment)[a]

Treatments	No. of root galls		Wt. of leaves harvested (g)	No. of total leaves harvested	Root wt. (g/plant)
	No. of gall/ root	Control effect (%)			
MI J2 5,000/plant (N)	330 c	-	54.6 a	40.9 a	11.9 ab
N + PP 100,000 endospores/g medium	150 b	54.4	55.5 a	38.4 a	11.6 b
N + PP 200,000 endospores/g medium	75 a	77.2	65.2 a	40.6 a	11.6 b
Control	0 a	-	57.8 a	39.5 a	14.2 a

[a] Data are means of 6 replicates. Root galls, leaves weight; root weight, and number of total leaves were examined 10 weeks after planting. Means within a column followed by the same letter are not different according to Duncan's multiple-range test (P<0.05).

Table 3. Suppression effect of *Pasteuria penetrans* KW1 (PP) on reproduction of *Meloidogyne incognita* in oriental melon[a]

Treatments	No. of root galls		Wt. of leaves harvested (g)	No. of total leaves harvested	Root wt. (g/plant)
	Root gall %	Control effect (%)			
MI J2 5,000/plant (N)	80.7 d	-	655 b	66 b	29.9 b
N + PP 100,000 endospores/g medium	52.9 c	34.5	717 a	81 a	47.1 ab
N + PP 200,000 endospores/g medium	32.1 b	60.2	756 a	88 ab	37.7 a
Control	0 a	-	732 a	76 c	11.2 ab

[a] Data are means of 6 replicates. Root galls, leaves weight, root weight, and number of total leaves were examined 10 weeks after planting. Means within a column followed by the same letter are not different according to Duncan's multiple-range test (P<0.05).

Our results showed significant reduction in root galling numbers both in lettuce and oriental melon when 100,000 and 200,000 endospores/g medium were treated. This indicated that at least 100,000 endospores/g of soil are required for significant suppression of *M. incognita*. In addition, control effect in lettuce at second experiment was higher than that of pots of the first experiment. This suggests that *P. penetrans* may lead to the soil becoming suppressive to *M. incognita* over the years. *P. penetrans* has provided a continuous suppression on a mixed population of *M. incognita* and *M. javanica* in 7 years monoculture of tobacco (Dickson et al., 1994). Therefore, it appears that endospores of *P. penetrans* are persistent in soil and result in soil becoming suppressive over time (Dickson et al., 1992; Oostendorp et al., 1991).

Meanwhile, growth characteristics of the both crops did not show significant difference between the endospore treated pots and MI only treated pots which could be due to the limited nutrient sources in the medium for their full growth. Although the experiments were conducted in small pots, the suppression effect of the *P. penetrans* KW1 isolate on *M. incognita* reproduction revealed the potentiality of the *P. penetrans* as a promising biological agent for root-knot nematode control. Since the *P. penetrans* is very resistant to various adverse environmental conditions in soil, such as high and low temperature, chemicals, and humidity changes, it has great potential to substitute chemical nematicides (Sayre and Starr, 1989). Unfortunately, there is no report on the success in artificial culture of *P. penetrans*. Mass production of the endospores is solely depending on *in vivo* cultivation of the bacterium on its host nematode in host plant (Chen, 1996). Therefore, for better economic and efficient mass production system of the endospores, practical application of the *P. penetrans* as a biological control agent in crop production should be developed.

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