Antimicrobial activity of *Meloidogyne javanica* gelatinous matrix (1)

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SUMMARY

Young egg masses obtained from monoxenic cultures of the root-knot nematode *Meloidogyne javanica* were placed in a suspension of *Pseudomonas syringae* pv. *tomato* (PS), *Bacillus subtilis* (BS) or *Saccharomyces* sp. (SS). The population growth rates of BS and SS were significantly reduced in the presence of the egg masses, while PS was not affected at all. Microscope observations showed strong agglutination of both BS and SS cells in the vicinity of the gelatinous matrix. It is concluded that the gelatinous matrix has antimicrobial activity probably for the protection of the nematode eggs.

RÉSUMÉ

Activité antimicrobienne de la sécrétion gélatineuse de Meloidogyne javanica

De jeunes masses d'œufs provenant d'une culture monoxénique de *Meloidogyne javanica* sont transférées dans des suspensions de *Pseudomonas syringae* pv. *tomato* (PS), de *Bacillus subtilis* (BS) ou de *Saccharomyces* sp. (SS). En présence des masses d'œufs, la croissance des populations de BS et SS est réduite de façon significative, alors que celle de PS n'est pas affectée. L'observation microscopique révèle une forte agglutination des cellules BS et SS au voisinage de la sécrétion gélatineuse. Il en est déduit que la sécrétion gélatineuse a une activité antimicrobienne jouant probablement un rôle dans la protection des œufs du nématode.

The root-knot nematodes (*Meloidogyne* spp.) gelatinous matrix (GM) is a substance synthesized by six rectal gland cells and secreted through the anus (Maggenti & Allen, 1960). The GM dissolves the host cells, forming a cavity or a canal leading from the nematode posterior end to the gall surface (Orion, Loots & Orion, 1987; Orion & Franck, 1990) and into which the female deposits its eggs to form the egg mass. The latter, protruding from the gall, is exposed to the soil environment. In spite of the rich variety of soil microorganisms which apparently could consume the nutritious eggs, the egg mass remains intact in the soil.

One could speculate, therefore, that the GM serves as an agent protecting the root-knot nematode eggs against microflora. With this idea in mind, the antimicrobial properties of the GM were explored in the present study.

Materials and methods

The root-knot nematode *Meloidogyne javanica* was monoxenically cultured on excised tomato (*Lycopersicon esculentum* cv. E-203) roots as described previously (Orion, Wergin & Endo, 1980). Four weeks after inoculation egg masses were removed from the cultures by means of a scalpel under a stereoscopic microscope and placed separately in a suspension of three microorganisms cultured, obtained and assessed as described below.

ORGANISMS AND GROWTH MEDIA

Strains *Pseudomonas syringae* pv. *tomato* (PS), *Bacillus subtilis* (BS) and *Saccharomyces* sp. (SS) were obtained from our culture collection. The bacteria were maintained at 26 °C in the dark on slants of nutrient agar (Difco) and the fungus on potato dextrose agar (PDA; Difco).

PROCEDURES WITH INOCULA

Cultures used as inoculum were incubated for 24 h on slants : PS on King's medium B agar (agar F, Difco), BS on nutrient agar (NA, Difco) with glycerol (1 % W/V), and SS on PDA. The cells were suspended in sterile water. After centrifugation at 12 000 *g* for 10 min, the pellet was resuspended in saline (0.85 % W/V NaCl) to give 0.65 absorbance unit at 480 nm in a Beckman Model 25 spectrophotometer. This corresponded to

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Table 1

The effect of *Meloidogyne javanica* gelatinous matrix, on the growth rate during 24 h at 28 °C of *Pseudomonas syringae* pv. *tomato* (PS), *Bacillus subtilis* (BS) and *Saccharomyces* sp. (SS) in comparison with various compounds (expressed in CFU per ml).

Compounds	*PS*	*BS*	*SS*
Control	$5.06 \times 10^7 \pm 1.1 \times 10^3$	$1.27 \times 10^8 \pm 1.7 \times 10^4$	$8.12 \times 10^8 \pm 1.1 \times 10^4$
Gelatinous matrix	$5.06 \times 10^7 \pm 1.1 \times 10^3$	$1.30 \times 10^7 \pm 0.9 \times 10^4$	$1.73 \times 10^7 \pm 5.0 \times 10^3$
Gelatin (5 µg)	$5.07 \times 10^7 \pm 1.1 \times 10^3$	$1.28 \times 10^8 \pm 1.7 \times 10^4$	$8.11 \times 10^8 \pm 1.1 \times 10^4$
Pectin (5 µg)	$5.50 \times 10^7 \pm 1.2 \times 10^3$	$1.27 \times 10^8 \pm 1.6 \times 10^4$	$8.12 \times 10^8 \pm 1.1 \times 10^4$
Albumin (5 µg)	$5.11 \times 10^7 \pm 1.2 \times 10^3$	$1.32 \times 10^8 \pm 1.8 \times 10^4$	$8.10 \times 10^8 \pm 1.1 \times 10^4$
Carboxy-methyl-cellulose (5 µg)	$6.06 \times 10^7 \pm 1.1 \times 10^3$	$1.26 \times 18^8 \pm 1.7 \times 10^4$	$8.13 \times 10^8 \pm 1.2 \times 10^4$
Penicillin (500 µg/ml)	$5.06 \times 10^7 \pm 1.1 \times 10^3$	0	$8.13 \times 10^8 \pm 1.3 \times 10^4$
Penicillin (45 µg/ml)	$5.07 \times 10^7 \pm 1.1 \times 10^3$	$9.34 \times 10^7 \pm 1.6 \times 10^4$	$8.11 \times 10^8 \pm 1.1 \times 10^4$
Cycloheximide (500 µg/ml)	$5.06 \times 10^7 \pm 1.1 \times 10^7$	$1.27 \times 10^8 \pm 1.7 \times 10^4$	0
Cycloheximide (45 µg/ml)	$5.07 \times 10^7 \pm 1.3 \times 10^3$	$1.27 \times 10^8 \pm 1.7 \times 10^4$	$5.73 \times 10^8 \pm 9.2 \times 10^3$
Polymixin (50 µg/ml)	0	$1.25 \times 10^8 \pm 1.6 \times 10^4$	$7.94 \times 10^8 \pm 9.6 \times 10^3$
Polymixin (4.5 µg/ml)	$3.80 \times 10^7 \pm 1.9 \times 10^2$	$1.29 \times 10^8 \pm 1.7 \times 10^4$	$8.03 \times 10^8 \pm 1.0 \times 10^4$
Chloramphenicol (250 µg/ml)	0	0	$8.14 \times 10^8 \pm 1.3 \times 10^4$
Chloramphenicol (23 µg/ml)	$4.12 \times 10^7 \pm 2.0 \times 10^2$	$1.06 \times 10^8 \pm 1.5 \times 10^4$	$8.13 \times 10^8 \pm 1.1 \times 10^4$

approximately 10^8 colony-forming units (CFU) per ml of PS, 5×10^7 CFU of BS, and 6×10^5 CFU of SS.

Assay procedures

The effect of various substances (see Table 1) on the growth rates of the three test micro-organisms was studied in microcentrifuge tubes (1.5 ml) with attached lids.

Each of the test micro-organisms was diluted in Nutrient Broth (Difco) supplemented with 1 % glucose (W/V), to a final concentration of *ca* 500 CFU/ml. Aliquots of 0.1 ml from each diluted inoculum were delivered to sterile microcentrifuge tubes in five replications.

The microcentrifuge tubes contain all the supplements as described above and the controls, were incubated for 24 h at 28 °C. After the incubation, the contents of each microcentrifuge tube was diluted tenfold in sterile saline solution and the population was estimated using the most probable number (MPN) method, of ten tubes of inoculum from each of three successive tenfold dilutions (Meynell & Meynell, 1970; Kritzman, 1989) : King's medium B for PS; NA for BS; and PDA for SS. For microscope observations, a drop of either BS or SS suspension was placed on a microscope slide, and a young egg mass was added and observed with a compound microscope over a period of 20 min.

Results and discussion

Table 1 summarizes the effect of the various substances on the growth rates of the tested organisms. PS was not affected by the presence of the egg masses whereas BS and SS showed significantly lower growth rates as compared with the controls.

In the presence of the egg masses BS reached only 10.2 % and SS only 2.1 % of the expected population after 24 h at 28 °C. Under the experiment conditions gelatin, pectin, albumin and CMC had no significant effects on any of the tested microorganisms. The antibiotics, penicillin, cycloheximide, polymixin and chloramphenicol, inhibited the growth of bacteria or fungus.

In microscope observations clear agglutination of both BS and SS by the GM after 5-10 min of exposure (Fig. 1) was evident. The findings of the present study indicate that the GM provided protection to the root-knot nematode eggs against at least two microorganisms. It is possible, however, that this activity is efficient against a broader spectrum of microorganisms, thus explaining how root-knot nematode eggs can survive in the soil for long periods.

References

Kritzman, G. (1989). Detection, quantification and classification of soft rot Erwinias associated with potato tubers. *Phytoparasitica*, 17 : 205-219.

Maggenti, A. R. & Allen, M. W. (1960). The origin of the gelatinous matrix in *Meloidogyne*. *Proceedings of the helminthological Society of Washington*, 21 : 4-10.

Meynell, G. G. & Meynell, E. (1970). *Theory and Practice in Experimental Bacteriology. 2nd ed.* Cambridge University Press, 346 p.

Orion, D. & Franck, A. (1990). An electron microscopy study of cell wall lysis by *Meloidogyne javanica* gelatinous matrix. *Revue de Nématologie*, 13 : 105-107.

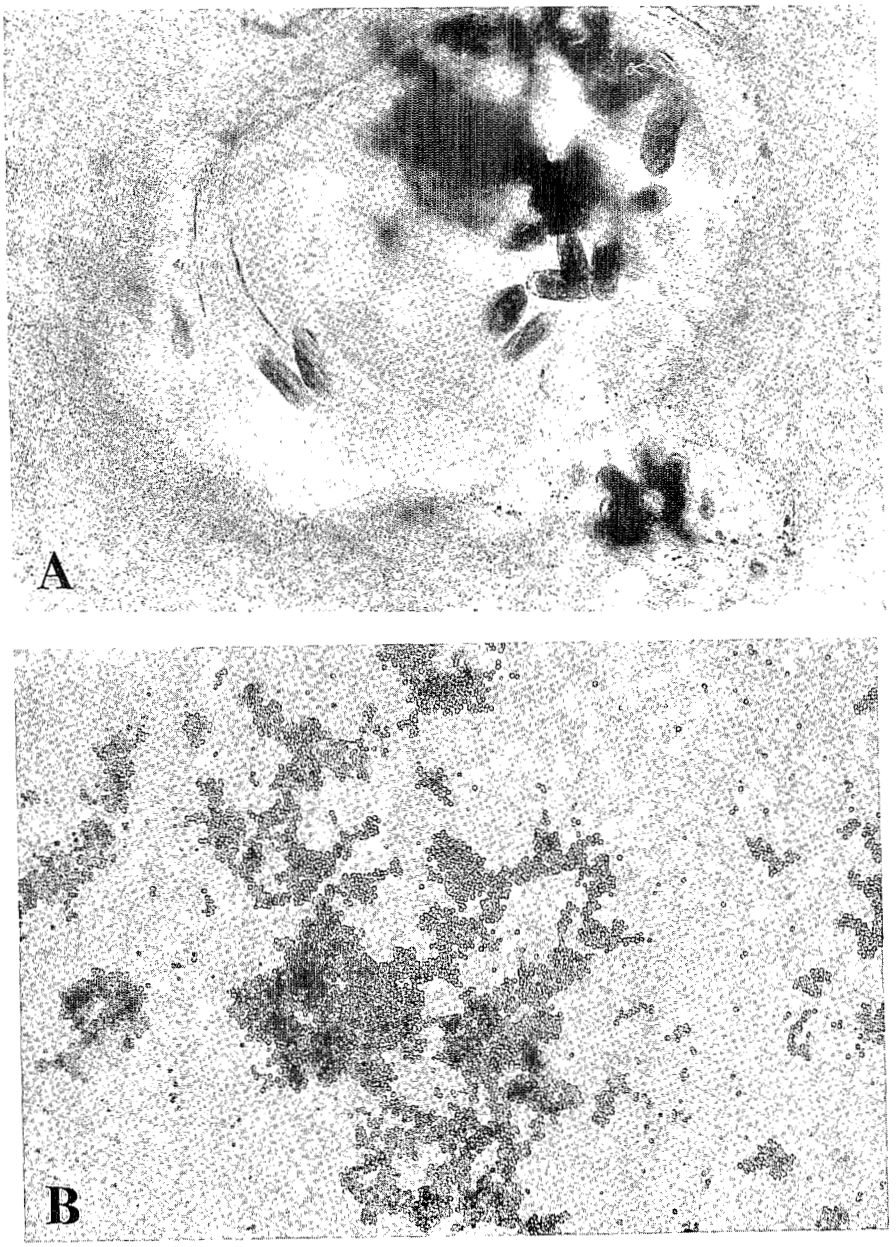

Fig. 1. Agglutination of *Bacillus subtilis* (A) and *Saccharomyces* sp. (B) in the presence of a *Meloidogyne javanica* egg mass.

ORION, D., LOOTS, G. C. & ORION, T. (1987). Cell lysis activity of *Meloidogyne* gelatinous matrix. *Revue de Nématologie*, 10 : 463-465.

ORION, D., WERGIN, W. P. & ENDO, B. Y. (1980). Inhibition of syncytia formation and root-knot nematode development in cultures of excised tomato roots. *Journal of Nematology*, 12 : 196-203.

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