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Pathogenesis of *Streptoverticillium albireticuli* on *Caenorhabditis elegans* and its antagonism to soil-borne fungal pathogens

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Aims: To examine the biological activity of *Streptoverticillium albireticuli*.

Methods: Isolation of *S. albireticuli* was carried out using the dry-heat technique. Nematicidal and pathogenic activity on *Caenorhabditis elegans* was measured by mortality in metabolites and colonization rate on fishmeal extract agar. Antifungal and enzymatic activities of *S. albireticuli* were measured by the agar plate method and the semidefined solid media method, respectively.

Results: *S. albireticuli* showed strong nematicidal activity against *C. elegans*. Pathogenic activity was also evident with the colonized nematode by the isolate on fishmeal extract agar. It also showed antifungal activity against certain fungal pathogens such as *Rhizoctonia solani*, *Phytophthora cinnamomi* and *Fusarium oxysporum*.

Significance and Impact of the Study: The discovery of an actinomycete showing pathogenic activity against the nematode may indicate the potential for it to be used as a biocontrol agent of parasitic nematodes, in addition to its ability to suppress fungal pathogens.

INTRODUCTION

The activity of actinomycetes as pathogens of human, animal and plant hosts has been far outweighed by the benefits they mediate in soil, such as antagonism to plant pathogens (Sutherland *et al*. 1984; Filonow and Lockwood 1985; Whipps 2001), as plant growth stimulators (Black and Beute 1985; Strzelczyk and Leniarska 1985; Whipps 2001) and as producers of biocides, including antibiotics, insecticides and herbicides (Anke 1986; Bérdy 1986). As antibiotic producers or hyperparasites of fungi, actinomycetes have played an important role in controlling soil-borne plant pathogens (You *et al*. 1996; El-Tarabily *et al*. 2000).

Actinomycetes, however, have received little attention as biological control agents of plant-parasitic nematodes. Katznelson and Henderson (1962) tested the influence of actinomycetes and fungi isolated from soil and plant roots on bacteria-feeding nematodes. They found that metabolites of a large percentage of actinomycetes obtained from shake-culture filtrates strongly attracted nematodes, although none of the isolates repelled or were toxic to the nematodes. Mishra *et al*. (1987) also screened a large number of metabolites from actinomycetes and found that 12 isolates showed nematicidal properties. Dicklow *et al*. (1993) investigated a species of *Streptomyces* for the control of plant-parasitic nematodes. They showed that the metabolites produced by the isolate of *Streptomyces* inhibited reproduction of *Caenorhabditis elegans* used as a screening model *in vitro* and consequently, this activity was also found to reduce the extent of root galling in tomato caused by *Meloidogyne incognita*.

Most of the actinomycetes commonly isolated from soil belong to the genus *Streptomyces* because they are more common in the environment and tend to have rapid growth rate and good sporulation compared to other actinomycetes

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(Williams and Vickers 1988). Many other actinomycete genera also produce biologically active secondary metabolites of medical importance and show promising biological activity including parasitism and antibiosis (Goodfellow and O'Donnell 1989). To isolate a wider spectrum of actinomycetes, the design of soil pretreatments such as dry-heating (Nonomura and Ohara 1969), the use of *Streptomyces*-specific lytic actinophage (Kurtböke *et al*. 1992; Long and Amphlett 1996) and the use of selective media amended with antibiotics (Williams *et al*. 1993) have been tried.

Among hundreds of isolates of soil streptomycete and non-streptomycete actinomycetes obtained from the cultivated soils in the Esperance District of Western Australia, as a part of state-wide survey of microorganisms, a strain of *S. albireticuli* was observed to cause mortality of *C. elegans*. The aim of this study was to examine the interaction of this strain with *C. elegans* for nematicidal and pathogenic activity and with certain common fungal pathogens for antifungal activity.

MATERIALS AND METHODS

Soil characteristics and enrichment

The strain of *S. albireticuli* was isolated from a field soil (loamy sand, pH 6·7 in 0·01 M $CaCl_2$) collected at Salmon Gums, Western Australia in April 1997. Twenty-five pregerminated chickpea seeds (*Cicer arietinum*, cv. Amethyst) were sown at 2-cm depth in a pot (40 l) in three successive sowings. The pots were kept in a growth chamber for 3 months at 17/12°C (d/night) and were watered every second day to maintain field capacity. After cycles of simulated cultivation of chickpea in the pots for 3 months, rhizosphere soils were sampled and air-dried for 4 d at 28°C to reduce the numbers of viable vegetative bacterial cells (Williams *et al*. 1972).

Isolation of non-streptomycete actinomycetes by the dry-heat technique

The strain used in the investigation was isolated using the dry heat technique of Nonomura and Ohara (1969). This method is considered to reduce the dominance of streptomycete colonies on isolation plates and to facilitate the isolation of non-streptomycete actinomycetes. The air-dried soil sample was placed in a drying oven for 1 h at 120 °C. A serial dilution of the soil sample was prepared in sterile distilled water. Surface-inoculation was made with 0·3 ml of the selected dilution onto the arginine vitamin agar containing cycloheximide (50 mg ml^{-1}) and nystatin (50 mg ml^{-1}). The plates were incubated at 28 ± 2°C in the dark for 3 weeks.

Identification of the isolate to species level was based on morphological, cultural, physiological and biochemical characteristics as described by Locci (1989).

Nematicidal activity on *C. elegans*

C. elegans maintained on *Escherichia coli* was surface-sterilized with 0·1% chlorohexidine diacetate (Hibitane®) and streptomycin sulphate (1 mg ml^{-1}). For routine culture, nematodes were reared in 250-ml flasks containing 50 ml of haemoglobin medium (Vanfleteren 1978).

The actinomycete isolate was grown on oatmeal agar (OMA) and was inoculated onto fishmeal extract agar (FMEA; El-Tarabily *et al*. 1997), 1·5% water agar (WA) and 1/5 M32 agar (Sivasithamparam *et al*. 1979) with the help of an inoculation loop and incubated for 7 d at 28°C. A suspension of *C. elegans* containing *c*. 50 individuals was transferred onto the edges of colony. Nematicidal activity was measured by counting the immotile nematodes at every hour for a period of 12 h. Three replicates were employed.

Pathogenic activity on *C. elegans*

The actinomycete isolate was inoculated on FMEA, WA and 1/5 M32 agar and incubated for 7 d at 28°C. A suspension of *C. elegans* containing *c*. 50 individuals was transferred onto the plates approximately 10 mm from the margin of a colony. Pathogenic activity was measured by counting the colonized nematodes by the isolate at every 6 h for 2 d. Three replicates were employed.

Chitinases and proteases activities

Chitinases and proteases were assayed to relate their pathogenesis of the actinomycetes on the nematodes. Protease and chitinase activities were tested by determining the ability of the isolate to grow on specific substrates in the semidefined solid media. The media used were the gelatine hydrolysis medium for proteases (Kunert *et al*. 1987) and the colloidal chitin medium for chitinases (Dackman *et al*. 1989). The actinomycete isolate was incubated on the gelatine medium for 1 week and on the colloidal chitin medium for 3 weeks, both at 28°C. The activity was evaluated by measuring the extent of clear zone (halo) around or underneath of colony. Three replicates were employed.

Antifungal activity

Antifungal activity was assayed to determine whether the actinomycete isolate also exhibited biological activities other than those related to nematodes. Antifungal activity of the isolate was tested against the test fungi: *Rhizoctonia solani*, *Phytophthora cinnamomi* and *Fusarium oxysporum*. The test fungi, grown on potato dextrose agar (PDA), were co-inoculated with the actinomycete isolate on 1/5 M32 agar

opposite each other at the periphery of a Petri dish. The plates were incubated for 3 d at 25°C and distances between margins of colonies were measured. The activity was then converted into a percentage after comparing with controls. The control was prepared as for antifungal activity, but uncolonized OMA plug was used instead of OMA colonized with the isolate. Three replicates were used for each treatment.

RESULTS

Identification

The isolate was identified as *Streptoverticillium albireticuli* Nakazawa. The microscopic examination revealed that the isolate produced stable branched yellowish brown substrate mycelium and well-developed whitish yellow aerial mycelium. Under low magnification the 'barbed wire' structure of the aerial mycelium was clear and consisted of long straight filaments showing at regular intervals, with side branches arranged in whorls. Each branch of the verticil produced a chain of spores. Electron microscopy of the spores revealed that the isolate produced cylindrical spores with more or less rounded ends with smooth spore surface. The isolate produced melanin pigment on peptone–yeast–iron agar and tyrosine agar but no diffusible pigment was noticed on FMEA or OMA.

Nematicidal activity on *C. elegans*

S. albireticuli grown on FMEA caused immobility of more than 50% of the nematodes exposed to it within 3 h and 90% in 6 h, but did not kill them when *S. albireticuli* was grown on either WA or 1/5 M32 agar.

Pathogenic activity on *C. elegans*

S. albireticuli grown on FMEA showed pathogenic activity detected by hyphal growth in the nematodes. The hyphal growth was evident in 54% (27 ± 6·1 standard error of the 50 individuals tested) of the nematodes after 6 h of incubation and reached 88% (44 ± 3·6) after 12 h. There was evidence of hyphal growth within the nematodes (Fig. 1a,b,c). However, *S. albireticuli* on WA and 1/5 M32 agar failed to show pathogenic activities even after 2 d of incubation.

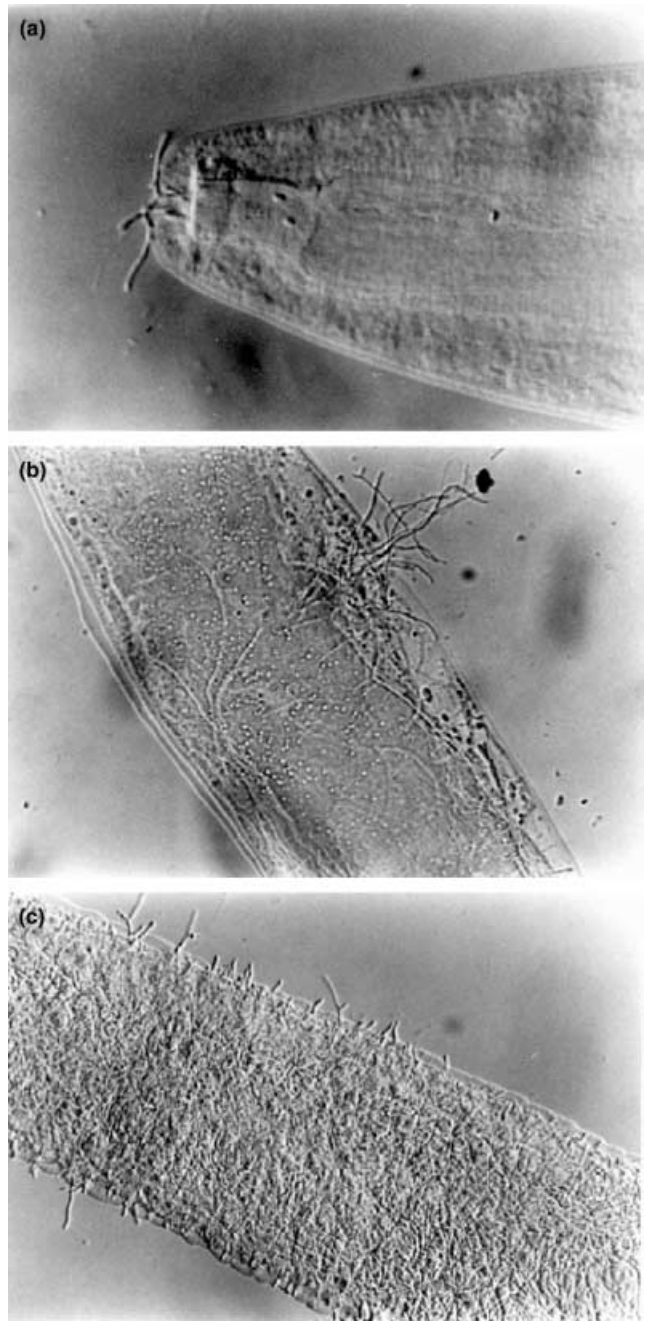

Fig. 1 Pathogenic activity observed on *Caenorhabditis elegans* by *Streptoverticillium albireticuli* on fishmeal extract agar. Colonization of nematodes by the actinomycete as a stage in the pathological process leading to the death of the host

Enzymatic activity

S. albireticuli showed chitinase activity on the colloidal chitin medium after the 2 weeks of incubation, but failed to show any protease activity on the gelatine medium.

Antifungal activity

S. albireticuli showed total or partial growth inhibition of the test fungi: *F. oxysporum* (100% inhibition), *R. solani* (41%) and *P. cinnamomi* (19%) on 1/5 M32 agar.

DISCUSSION

Nematicidal and pathogenic activities of *S. albireticuli* on *C. elegans* were demonstrated in this study. In addition, this actinomycete isolate showed noticeable antifungal activity against the fungal pathogens tested.

The discovery of the avermectins, a new class of macrocyclic lactones isolated from *S. avermitilis*, as potent anthelmintics has stimulated the search for other anthelmintic compounds from actinomycetes. Garabedian and Van Gundy (1983), in their evaluation of avermectins for the control of *Meloidogyne incognita*, concluded that avermectins provided longer protection with 10 times less chemical applied to the environment than commercial nematicides such as oxamyl or aldicarb.

Fishmeal extract agar (FMEA) was used in screening of actinomycete isolates for nematicidal activities, because it has been shown to induce production of antifungal metabolites by actinomycete species (El-Tarabily *et al.* 1997). The behaviour of nematodes on FMEA inoculated with the actinomycete during the first 48 h was different to those on WA or 1/5 M32 agar and mortality of nematodes occurred after 2 d on FMEA. Nematicidal activity was not noticed on inoculated WA or 1/5 M32 agar, suggesting that metabolites produced by the isolate on FMEA were different and probably predisposed the nematodes to pathogenic invasion by the actinomycete.

The ability of *S. albireticuli* to kill parasitic nematodes in natural soil remains to be tested. Cook and Baker (1983) also mentioned that antibiotics produced by actinomycetes are not always effective in soils; such activities often depend on soil conditions such as soil types, soil pH, nutrients and moisture.

Actinomycetes have been reported to produce a variety of enzymes such as amylases, cellulases, xylanases, chitinases, lipases and proteases (Miller and Sands 1977; Peczynska-Czoch and Mordarski 1988). These enzymes could affect nematodes. Chitinase activity has been reported to destroy the cuticle of adult nematodes and eventually kill the nematodes, although chitin is present only in nematode eggs (Miller and Sands 1977).

The pathogenic activity of the actinomycete isolate on *C. elegans* in our study was observed on FMEA, but not on WA or 1/5 M32 agar. It is proposed that metabolites produced on FMEA involved in pathogenesis of the nematode. In addition, growth of the antagonist on FMEA was superior to that on the other two media.

The identification of the exact mode of action and characterizations of the metabolites by *S. albireticuli* would aid in understanding their potential as a bionematicide and a biofungicide.

REFERENCES

Anke, T. (1986) Further secondary products of biotechnological interest. In *Biotechnology* ed. Rehm, H.J. and Reed, G. pp. 620–628. Weinheim, FL: VCH.

Bérdy, J. (1986) Further antibiotics with practical application. In *Biotechnology* ed. Pape, H. and Rehm, H.J. pp. 229–242. London, New York: Academic Press.

Black, M.C. and Beute, M.K. (1985) Soil components that affect severity of *Cylindrocladium* black rot on peanuts. *Plant Disease* **69**, 36–39.

Cook, R.J. and Baker, K.F. (1983) *The Nature and Practice of Biological Control of Plant Pathogens.* St Paul, MN: American Phytopathological Society Press.

Dackman, C., Chet, I. and Nordbring-Hertz, B. (1989) Fungal parasitism of the cyst nematode *Heterodera schachtii*: infection and enzymatic activity. *FEMS Microbiology and Ecology* **62**, 201–208.

Dicklow, M.B., Acosta, N. and Zuckerman, B.M. (1993) A novel *Streptomyces* species for controlling plant-parasitic nematodes. *Journal of Chemical Ecology* **19**, 159–173.

El-Tarabily, K.A., Soliman, M.H., Nassar, A.H., Al-Hassani, H.A., Sivasithamparam, K., McKenna, F. and Hardy, G.E.St J. (2000) Biological control of *Sclerotinia minor* using a chitinolytic bacterium and actinomycetes. *Plant Pathology* **49**, 573–583.

El-Tarabily, K.A., St. Hardy, G.E.J., Sivasithamparam, K., Hussein, A.M. and Kurtböke, D.I. (1997) The potential for the biological control of cavity-spot disease of carrots, caused by *Pythium coloratum*, by streptomycete and non-streptomycete actinomycetes. *New Phytologist* **137**, 495–507.

Filonow, A.B. and Lockwood, J.L. (1985) Evaluation of several actinomycetes and the fungus *Hyphochytrium catenoides* as biocontrol agents for *Phytophthora* root rot of soybean. *Plant Disease* **69**, 1033–1036.

Garabedian, S. and Van Gundy, S.D. (1983) Use of avermectins for the control of *Meloidogyne incognita* on tomato. *Journal of Nematology* **15**, 503–510.

Goodfellow, M. and O'Donnell, A.G. (1989) Search and discovery of industrially significant actinomycetes. In *Microbial Products – New Approaches* ed. Baumberg, S., Hunter, I.S and Rhodes P.M. pp. 343–383. Cambridge, UK: Cambridge University Press.

Katznelson, H. and Henderson, V.E. (1962) Studies on the relationships between nematodes and other soil microorganisms. I. The influence of actinomycetes and fungi on *Rhabditis* (*Cephalobodes*) *oxycerca* De Man. *Canadian Journal of Microbiology* **8**, 875–882.

Kunert, J., Zemek, J., Augustín, J. and Kuniak, L. (1987) Proteolytic activity of ovicidal soil fungi. *Biologia (Bratislava)* **42**, 695–705.

Kurtböke, D.I., Murphy, N.E. and Sivasithamparam, K. (1992) Use of bacteriophage for the selective isolation of thermophilic actinomycetes from composted eucalyptus bark. *Canadian Journal of Microbiology* **39**, 46–51.

Locci, R. (1989) Streptomycetes and related genera. In *Bergey's Manual of Systematic Bacteriology*, Vol 4. ed.Williams, S.T., Sharpe, M.E. and Holt, J.G. pp. 2493–2504. Baltimore, MD: Williams & Wilkins.

Long, P.F. and Amphlett, G.E. (1996) A super lytic actinophagous systems as a pre-treatment in the isolation of non-streptomycete actinomycetes from soil. *Letters in Applied Microbiology* **22**, 62–65.

Miller, P.M. and Sands, D.C. (1977) Effects of hydrolytic enzymes on plant-parasitic nematodes. *Journal of Nematology* **9**, 192–197.

Mishra, S.K., Keller, J.E., Miller, J.R., Heisey, R.M., Nair, M.G. and Putnam, A.R. (1987) Insecticidal and nematicidal properties of microbial metabolites. *Journal of Industrial Microbiology* **2**, 267–276.

Nonomura, H. and Ohara, Y. (1969) Distribution of actinomycetes in soil (VI). A culture method effective for both preferential isolation and enumeration of *Microbispora* and *Streptosporangium* strains in soil (part 1). *Journal of Fermentation Technology* **47**, 463–469.

Peczynska-Czoch, W. and Mordarski, M. (1988) Actinomycete enzymes. In *Actinomycetes in Biotechnology* ed. Goodfellow, M., Williams, S.T. and Mordarski, M. pp. 219–283. London, UK: Academic Press.

Sivasithamparam, K., Parker, C.A. and Edwards, C.S. (1979) Rhizosphere micro-organisms of seminal and nodal roots of wheat grown in pots. *Soil Biology and Biochemistry* **11**, 155–160.

Strzelczyk, E. and Leniarska, V. (1985) Production of B group vitamins by mycorrhizal fungi and actinomycetes isolated from the root zone of pine (*Pinus silvestris* L.). *Plant and Soil* **86**, 387–394.

Sutherland, E.D., Baker, K.K. and Lockwood, J.L. (1984) Ultrastructure of *Phytophthora megasperma* f. sp. *glycinea* oospores parasitized by *Actinoplanes missouriensis* and *Humicola fuscoatra*. *Transactions of the British Mycological Society* **82**, 726–729.

Vanfleteren, J.R. (1978) Axenic culture of free-living, plant-parasitic, and insect-parasitic nematodes. *Annual Review of Phytopathology* **16**, 131–157.

Whipps, J.M. (2001) Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany* **52**, 487–511.

Williams, S.T., Locci, R., Beswick, A. et al. (1993) Detection and identification of novel actinomycetes. *Research in Microbiology* **144**, 653–656.

Williams, S.T., Shameemullah, M., Watson, E.T. and Mayfield, C.I. (1972) Studies on the ecology of actinomycetes in soil. VI. The influence of moisture tension on growth and survival. *Soil Biology and Biochemistry* **4**, 215–225.

Williams, S.T. and Vickers, J.C. (1988) Detection of actinomycetes in the natural environment. In *Biology of the Actinomycetes,'88.* ed. Okami, Y., Beppu, T. and Ogawara, H. pp. 265–270. Tokyo, Japan: Japanese Scientific Press.

You, M.P., Sivasithamparam, K. and Kurtböke, D.I. (1996) Actinomycetes in organic mulch used in avocado plantations and their ability to suppress *Phytophthora cinnamomi*. *Biology and Fertility of Soils* **22**, 237–242.