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A New Method for Isolating and Selecting Agents with High Antagonistic Ability Against Plant Parasitic Nematodes

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ABSTRACT

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The conventional baiting, sprinkled plate method, modified baiting bags and a novel baiting tape method were compared for their efficacies of recovering different antagonists including fungi, bacteria, nematodes and mites. The baiting bag method had the highest isolation rates of nematode-trapping fungi and nematode parasitic bacterium *Pasteuria penetrans*, and was the only method capable of recovering predatory nematodes and mites. The novel baiting tape method had the lowest isolation rates of all the antagonists except actinomycetes. However, *Arthrobotrys* sp., *Dactylaria* sp. and *Dactylella* sp. baited by the baiting tape method had the best nematode-trapping activity and *Streptomyces saraceticus*, the highest chitinolytic activity in the *in vitro* assays. Isolates of the three nematode trapping fungi and *S. saraceticus* with the best antagonistic activities *in vitro*, along with *Pasteuria penetrans* and a predatory nematode *Mononchus* sp. were tested in the greenhouse experiments. All of these antagonists, except *Mononchus* sp., greatly reduced the number of J_2 root-knot nematodes in soil and the egg masses on roots. *P. penetrans* and *S. saraceticus* had better controlling ability than the three nematode trapping fungi. The new baiting method has the ability to baite and select antagonists with high biocontrol efficacy.

Key words: Antagonists, *Arthrobotrys* sp., baiting methods, biological control, *Dactylaria* sp., *Dactylella* sp., *Mononchus* sp., *Pasteuria penetrans*, plant parasitic nematodes, *Streptomyces saraceticus*

INTRODUCTION

Antagonistic isolates from the same taxon differed markedly in general appearance, reflecting genetic differences which are invariably manifest as differences in their antagonistic abilities. This suggested that selection of the most virulent isolate from the material available must be one of the first steps in any biological control program [22]. Most early biological control researches were empirical and mainly concerning nematode-trapping fungi. Seventy six percents of researches on the natural enemies of plant parasitic nematodes were related to fungi, 5 % to bacteria and only 2 % to predatory mites. Few studies considered the fate of the organisms after it entered the soil, or the choice of target pests [10]. Many antagonists isolated were,

therefore, not effective when introduced to the field [13, 22].

Over 200 species from 6 different classes of fungi were reported to parasitize on nematode eggs, juveniles, adults and cysts [18]. The first report on plant parasitic nematode trapping fungi in Taiwan was published in 1964 [6] and six nematode trapping fungi, including *Arthrobotrys cladodes*, *A. conoides*, *A. dactyloides*, *A. oligospora*, *Dactylella ellipsospora* and *Dactylaria* sp., were identified [5]. In 1975, three more species-*Dactylaria candidas*, D. *haptotyla*, *Dacylella cionopaga*-were found [24]. A thorough sample collection from all geographic area in Taiwan was conducted in 1993 and a total of 27 species of nematode trapping fungi were identified, of which 23 species belong to the genera *Arthrobotrys*, *Dactylaria*, *Monacrosporium* and *Nematoctonus*, and four of them, including *Dactylaria haptospora*, *Monacrosporium coelobrocha*, *Nematoctonus robustus*, and a new species *Dactylella formosana*, were first reported in Taiwan [26]. Lin and Tsay [13] conducted a survey in 2003 and found *Monacrosporium eudermata*, *Arthrobotrys cladodes*, *M. ellipsospoum* had the comparable high occurrence rates among 11 species recovered. This is different from the survey done in Ireland that *Dactylella bemicodes*, *D. mammillata* and *M. ellipsosporum* had the higher occurrence rates. This indicated that nematode trapping fungi were ubiquitous but the distribution pattern would be slightly different from place to place.

Baited, sprinkled plate method is the most common method employed to isolate potential nematode antagonists. A conventional method for direct observation and isolation was done by slide-adhered agar discs (1 cm diameter, 0.3 cm thickness) that were buried in soil-containing Petri dishes for a period of time to attract antagonists [7]. Although such method have proved invaluable, they tend to favor aggressive predacious fungi, which were fast-growing, heavily sporulating and probably were over represented compared with slow-growing, host-specific endoparasitic species [20]. Up to date, none of the methods were able to isolate all antagonistic organisms from natural environments, because the factors required for optimal growth and predatory activities were different for every individual species [15].

The questions of how to effectively isolate and select virulent antagonists are critical in developing biological control agents. In light of this consideration, a novel method following the three principles: (1) providing minimum artificial nutrients to select strong competitors; (2) creating a baiting environment similar to natural habitats to ensure the antagonists would function when applied in fields; and (3) observing the collected sample directly to understand the mechanism of the antagonist--was described in this manuscript. In this paper, the isolation efficacies targeting at different antagonists and the antagonistic abilities of the baited antagonists by different methods were studied.

MATERIALS AND METHODS

Isolation of organisms antagonistic to plant parasitic nematodes

A nationwide survey was launched to locate fields that had either nematode trapping fungi, *Pasteuria* spp., actinomycetes, or predatory nematodes. Six fields were selected each for a unique target antagonist, and three baiting methods were used independently in these six fields (Table 1) to compare their baiting efficacies for antagonists in different taxon. Sampling methods were slightly different according to crop planted in the surveying field. A total of 120 zigzag spots was chosen in the vegetable crop fields and 60 spots in the fruit tree orchards (Table 1) to collect soil for the conventional baited, sprinkled plate method and to place the baiting units for the baiting bag and baiting tape methods (see text below for preparation of the baiting units). All experiments have been performed three times dating from 1995 to 1998 and approximately 3,800 samples were coleected for analysis. The data collected from the three independent experiments were pulled together and the efficacy of each method was calculated by the proportion of samples containing the target antagonist.

Conventional baited, sprinkled plate method:

The method was modified from the previous procedure [3]. Soil from parasitic nematode-infested fields was collected and air-dried till the water content was less than 10%. A 0.5 g of the soil sample was added into 1% corn meal agar plate containing 1,500 *Aphelenchoides besseyi*. The plates were incubated at room temperature (approximately 23℃) in the dark for one week, and then observed under a dissecting microscope (Olympus SZ60) to identify nematodes that were infected by fungi or other microorganisms. Each infected nematode was transferred to a fresh 0.7% PDA plate, and the plates were kept under the same condition for another five days. Culturable microorganisms on these plates were isolated again using a spread-plate technique to obtain pure cultures. After identification, the pure cultures of putative antagonists were maintained on 0.7% PDA slants for future experiments.

Modified baiting bag method:

A 20 g mixture of perlit: corn: coarsely chopped

Table 1. Information of six fields used for isolating specific antagonists

Antagonism	Field locality	Crop associated	Soil type
Arthrobotrys spp.	Tai Nan county	Sponge guard	Sandy clay loam
Dactylella spp.	Chang Hua county	Eggplant	Sandy clay
Dactylaria spp.	Chang Hua county	Snow pea	Loamy sand
Pasteuria penetrans	Ping Tung county	Banana	Sandy loam
actinomycetes	Hsing Chu county	Citrus	Sandy loam
Mononchus sp.	Nan Tow county	Banana	Sandy loam

peanut = 2:1:1 (w/w) was wrapped in a 50-mesh nylon cloth (Finnpeat®, Kekkila, Netherlands) as a baiting bag. For baiting chitinolytic actinomycetes, the mixture was replaced with 20 g crab and shrimp shell pieces. The baiting bags were buried into soil at the depth of 5-20 cm near the nematode-infested rhizoshpere. The baits in annual crop fields were retrieved two weeks later, and in perennial plant fields one month later. The baiting bags were opened under a dissecting microscope for inspection. Mites, insects or predatory nematodes that prey on nematodes were located mainly on the periphery of the bag. Therefore, 0.5 g content from the center was taken and the putative antagonistic microorganisms were isolated using the conventional baited, sprinkled plate method.

The novel baiting tape method:

The baiting tape was made by brushing a 0.4 g mixture of corn meal and peanut powder (volume ratio = 1:1) evenly onto the sticky side of a clear tape (4.8 cm width x 10 cm length, Four Pillar company, Taiwan) using a clean paintbrush. The baiting tape was stapled with a cloth labeled with date, location, and crop species on it and buried vertically at the depth of 5-20 cm near infested rhizosphere with the baiting mixture facing roots. One week later, the baits were retrieved and placed individually in a clean plastic bag. After removing soil particles from the non-sticky side, the baiting tapes were observed directly, with the non-sticky side facing up, under an upright microscope to look for deformed nematodes that were potentially infected by antagonists. The deformed nematodes were picked for the isolation of antagonists using the spread-plate technique. The corn meal and peanut powder mixture were substituted by crab and shrimp shell powders to bait for chitinolytic actinomycetes in the field at Hsing Chu County.

In vitro evaluation of the antagonistic activities of baited antagonists

Nematode trapping fungi:

Isolated nematode trapping fungi were cultured on 0.7% PDA for a week. Five discs with younger hyphae were punched out using a 0.8 cm-diameter cork borer, and each agar disc was transferred to the middle of a fresh 1% corn meal agar plate. When the new colony reached 4 cm in diameter, the original disc was removed and a 0.2 ml suspension of 200 *Aphelenchoides besseyi* was added in the middle. The plates were incubated at room temperature for 48 hours and the nematode trapping rates were determined. Data were obtained from five replicates and the experiment was repeated three times.

Chitinolytic actinomycetes:

Collodial chitin medium [14] was used to evaluate the chitinolytic ability of actinomycetes. Each isolated actinomycetes strain was cultured on the Collodial chitin medium and incubated at 28℃ for five days. Chitinolytic activity was calculated by the width of the clear zone.

Greenhouse experiments for the biological control efficacy of six isolated antagonists

Preparation of antagonists for inoculation:

Three nematode-trapping fungi, *Arthrobotrys* sp., *Dactylella* sp., and *Dactylaria* sp.; that produce adhesive net, constricting ring, and adhesive knob, respectively, were selected as biological control agents (Table 1). The fungi were cultured on 0.7% PDA for a week and their conidia were washed out and adjusted to a concentration of $1x10^4$ spores / ml for inoculation.

Pasteuria penetrans that was found to parasitize *Meloidogyne incognita* on banana roots (Table 1) was collected. The infected root systems were air-dried, ground into powder, and re-suspended in distilled water to the final concentration of 20% (w/v). The number of endospores in the suspension were counted using the Hausser hemocytometer and adjusted to 80 endospores / ml for inoculation.

Streptomyces saraceticus that has the highest chitinolytic activity among the baited chitinolytic actinomycetes was selected as a biocontrol agent in the greenhouse experiments. *Streptomyces saraceticus* was

grown in liquid collodial chitin medium with shaking (85 rpm) at room temperature for three days. The bacterial filaments were removed by passing the culture through double cheese cloth and the spores were collected in a clean beaker. 0.05% of Tween 20 was added to help the spores suspending in the solution. The spores were counted using hemacytometer (Hausser Scientific, Horsham, PA, U.S.A.), for inoculation, the concentration was adjusted to 1 x 10^7 spores / ml by adding desterilized water with 0.05% Tween 20.

To culture the predatory *Mononchus* sp., free-living nematodes (*Rhabditis* sp.) were provided as the food source. *Rhabditis* sp. was first grown in a mixture containing equal volumes of perlit, peanut powder, and soybean powder to get large quantity. Two hundreds *Mononchus* sp. nematodes and 2,000 *Rhabditis* sp. were put into a 250 ml flask containing 50 g moist coarsely chopped peanuts and incubated at room temperature for two weeks. *Mononchus* sp. was then collected by modified Baermann's funnel technique and separated from *Rhabditis* sp. by sucrose gradient centrifugation and floatation-sieving technique[2, 4].

Experimental design:

To provide a testing environment similar to the field conditions, the potting soil used in greenhouse tests was obtained from a grape orchard (Chang Hua county, Taiwan) and autoclaved (121℃, 15 lb) for one hour to eliminate biological factors. The sterile soil has a pH value of 5.9 and contains 0.8% of organic matter. Water spinach (*Ipomea reptans* cv. 931, Known-You Co., Kaohsiung, Taiwan) was chosen as the experimental host because of its high susceptibility to *Meloidogyne incognita* and apparent gall formation[25]. Seeds were sterilized by immersing in 1.05% bleach (NaOCl) for 30 minutes and rinsed with water. Sterile seeds were sown in moist peat moss till five-leaf stage and transplanted to plastic pots (14 cm in diameter, 12 cm in height) that contained 1 L soil in each pot for the following experiments.

Meloidogyne incognita as the pathogen inoculums was collected from banana rhizosphere in Kaohsiung County, Taiwan. It was identified by the perineal patterns and juvenile morphomatric data[9]. The second-staged juveniles (J_2) hatched from a single egg mass were used to inoculate water spinach roots, and the egg masses collected from a single plant were used for the inoculation.

Inoculations and plant bioassays:

In the first part of the bioassay, *Meloidogyne incognita* was inoculated at the concentration of one J_2 per gram soil when the water spinach was transplanted to the plastic pot. After one month, 100 ml of *Arthrobotrys* spp., *Dactylella* spp., *Dactylaria* spp., *P. penetrans*, *S. saraceticus* at the described ratio, or 500 *Mononchus* sp. were added into the pots. A treatment without any antagonists was also included as the control treatment. For the second part of the bioassay, *M. incognita* and the six antagonists were inoculated together at the time of transplanting. The disease severity was evaluated by the number of *M. incognita* egg masses on the root system and the number of J_2 per 200 g randomly sampled pot soil. In the first part of the bioassay, the soil samples were obtained a month post inoculation for *M. incognita*, and were 20 days after the co-inoculation in the second part of the bioassay. The fresh weight (in grams) of the water spinach aerial parts was recorded as the plant yield.

The experiments were conducted in a greenhouse with the temperature ranging from 18℃ to 36℃, a photoperiod of 12 hours and relative humidity of 78%-95%. Plants were fertilized with 10 ml Hyponex® soluble fertilizer (N: P: K= 20: 20: 20, 1 g / L) every week. All treatments had five replicates and the experiment was conducted twice. The data were analyzed by Duncan's Multiple Range test (P = 0.05).

RESULTS

Comparison of different baiting methods

In a preliminary survey, we found that the presence of antagonistic microorganisms was regional specific. Only one antagonist species was dominant and had the highest recovery rate at each site, and the rest existed in a minute amount. The isolation rate for each antagonist shown in Table 2 was the percentage of the samples pooled from the three experiments conducted in the same field. For instance, using the baiting bag method *Arthrobotrys* spp. weredetected in 85.6% samples collected from Tai Nan County, and *Dactylaria* spp. were found in 74.8% samples from Chang Hua County. Among the antagonists, actinomycetes had the highest isolation rates regardless of the isolating methods, whereas the baiting bag method was the only method that isolated predatory nematodes and mites. Among the three baiting methods, the modified baiting bag method had 74.8%-85.6% isolation rates of the three nematode trapping fungi, while the baiting tape method had only 17.3%-24.5% (Table 2). The baiting tape coated with corn and peanut powder had only 3.5% isolation rate for *Pasteuria penetrans*, which was very low compared to the 82% for the baited, sprinkle plate method and 85.5% for the baiting bag methods. All three methods had high isolation rates (89.5%-95.2%) for chitinolytic actinomycetes (Table 2).

Antagonistic ability of organisms baited by different methods

The three baiting methods resulted in thousands of putative antagonists identified based on morphological characteristics. To compare the antagonistic ability of the same taxon baited by different methods, 34 commonly found isolates of *Arthrobotrys* spp., 37 of *Dactylaria* spp., 42 of *Dactylella* spp. and 30 of chitinolytic actinomycetes isolates were studied. The *Arthrobotrys* spp., *Dactylaria* spp. and *Dactylella* spp. isolates baited by the baiting tape method had the average nematode-trapping rates of 62.3%, 58.9% and 47.6%, respectively (Table 3), which were the highest among the three methods. The strains of chitinolytic actinomycetes isolated by the baiting tape method were capable of utilizing chitin and produced clear zones with an average radius of 0.8 cm on the collodial chitin media, which was significantly larger than the radius produced by the strains of the baiting bag method (Table 3). The results indicated that the baiting tape method was able to bait for microorganisms that had high antagonistic abilities, even though the isolation rates were low (Table 2). Based on the *in vitro* antagonistic activity assays, one isolate from each antagonist taxon that showed the highest antagonistic activity was selected for the greenhouse experiments.

The efficacy of the six antagonists as biological control agents under greenhouse conditions

Streptomyces saraceticus, a chitinolytic actinomycete, was chosen because it was found in all three baiting methods and showed the highest chitinolytic activity. When antagonists were applied after the establishment of root knot nematodes, all but *Mononchus* spp. significantly decreased the number of J_2 root knot nematodes in soil and egg masses on roots (P = 0.05) (Table 4). Among the five microorganisms, *P. penetrans* and *S. saraceticus* had the best antagonistic abilities, which reduced egg masses number from 207.8 to 90.1 and 83.6, respectively, and J_2 nematodes in 200 g soil from 425 to 136.5 and 122.3, respectively. For the growth of the host plants, the fresh top weights of water spinach treated with *P. penetrans* and *S. saraceticus* were 98.5 g and 99.2 g respectively, heavier than these treated with other antagonists. Similarly, when the antagonists and *M. incognita* were inoculated at the same time, all antagonists except *Mononchus* spp. significantly reduced the *M. incognita* population and

Table 2. Isolation rates of sprinkled plate, baiting bag and baiting tape methods on recovering specific organisms antagonistic to nematodes

Antagonistic organism	Isolation rate (%)[1]		
	Sprinkle plate	Baiting bag	Baiting tape
Arthrobotrys spp.	86.2 a[2]	85.6 a	24.5 b
Dactylaria spp.	74.8 b	81.1 a	17.3 c
Dactylella spp.	69.3 a	74.8 a	20.1 b
Pasteuria penetrans	82.0 a	85.5 a	3.5 b
actinomycetes	89.5 a	92.3 a	95.2 a
Mononchus sp.	NA[3]	93.8	NA
Mites	NA	24.6	NA

[1] Percentage of valid baiting units containing the target antagonist baited from the field soil.

[2] Means in the same row followed by the same letter are not significantly different (P = 0.05) according to Duncan's multiple range test.

[3] NA: data not anailable.

Table 3. Comparison of antagonistic activity of three nematode trapping fungi and a chytinolytic actinomycete isolated by sprinkle plate, baiting bag and baiting tape methods

Antagonistic organisms	Antagonistic activity		
	Sprinkle plate	Baiting bag	Baiting tape
Arthrobotrys sp.[1]	4.5 b[3]	42.0 b	62.3 a
Dactylaria sp.[1]	42.6 b	38.7 b	58.9 a
Dactylella sp.[1]	38.4 b	34.2 b	47.6 a
Streptomyces saraceticus[2]	0.6 ab	0.5 b	0.8 a

[1] Activity of predatory fungi was estimated by the percentage of nematodes trapped out of 200 nematodes, data were means of 5 replicates.

[2] Activity of *Streptomyces saraceticus* was determined by the radius of the clear zone (cm) on collodial medium. Data were means of 50 replicates.

[3] Means in the same row followed by the same letter were not significantly different (P = 0.05) according to Duncan's multiple range test.

increased the top weight of the water spinach (P = 0.05) (Table 5). Therefore, the three nematode trapping fungi, *P. penetrans*, and *S. saraceticus* that showed high *in vitro* antagonistic activities were proved to be effective biological control agents in the greenhouse experiments. These five antagonists tested were able to reduce the population of *M. incognita* either before or after they invaded the host.

DISCUSSION

Three principles proposed for designing a novel baiting method are minimum artificial nutrient provided, baiting environment similar to natural habitats, and direct observation for picking the active microorganisms. Results of this study show that the novel baiting tape method with these principles did elevate the opportunity for isolating antagonists that have better qualities as biological control agents.

The conventional method baited antagonists by taking the field soil away and then providing artificial nutrient to microorganisms under controlled environments, This would favor the fast-growing and heavily sporulating microorganisms [20]. Fungistatic factors that prevent fungal spores from germination are prevailing in the soil [11, 15]. Nutrients in the agar plate might not be sufficient to revive dormant spores which had good antagonistic ability.

Due to the climate changes, the recovery rates of the baiting units were not 100%. Some baiting units were lost due to heavy rains, flooding, or animal feeding activities. Another environmental factor that might jeopardize the baiting efficiency in this study was the dynamic interactions among soil microorganisms, which may lead to mis-presentation of data. These problems were overcome by starting with many baiting units at the beginning of the experiments.

One of the questions this study tried to answer was whether the antagonists baited by different methods would have different antagonistic abilities [21]. Our results showed that antagonists belonging to the same taxon baited by the novel baiting tape method had significantly higher antagonistic efficacies compared to those baited by other two methods (Table 3). Baiting bags containing rich substance were buried near the rhizosphere, such design would allow primary colonizers to compete and interact. Prolonging the baiting time by increasing the amount of baiting substance would also help to select antagonists with better competition ability. Those microorganisms isolated would have a better chance to survive and perform the desired antagonistic ability. The baiting tape method provided very limited nutrient, only microorganisms that had fast colonizing and utilizing ability would be able to compete for the substrate. This might explain the high antagonistic ability of those baited with this method. Presumably, microorganisms that were able to colonize the substance in the baiting bag or on the baiting tape would

Table 4. Effect of six antagonistic organisms to nematodes as biological control agents on the growth of water spinach and the population of *Meloidogyne incognita*

Antagonism	Plant top weight (g)	# of egg mass	# of J_2/200g soil
Arthrobotrys sp.	60.7 b[1]	199.0 a	364.5 b
Dactylaria sp.	63.4 b	180.2 a	328.4 b
Dactylella sp.	62.0 b	174.2 a	333.0 b
Pasteuria penetrans	98.5 a	90.1 b	136.5 c
Streptomyces saraceticus	99.2 a	83.6 b	122.3 c
Mononchus sp.	48.0 c	203.5 a	443.6 a
Check	47.5 c	207.8 a	425.0 a

[1] Means in the same column followed by the same letter were not significantly different (P = 0.05) according to Duncan's multiple range test.

Table 5. Effect of antagonistic organisms to nematodes as a protecting agent on the growth of water spinach and the population of *Meloidogyne incognita*

Antagonism	Plant top weight (g)	# of egg mass	# of J_2/200g soil
Arthrobotrys sp.	27.0 b[1]	65.3 b	28.9 bc
Dactylaria sp.	28.8 b	63.4 b	30.4 b
Dactylella sp.	30.5 b	60.1 b	27.5 bc
Pasteuria penetrans	37.5 a	37.0 c	21.6 c
Streptomyces saraceticus	36.5 a	35.2 c	22.2 c
Mononchus sp.	20.0 c	89.0 a	40.0 a
Check	19.0 c	88.5 a	40.5 a

[1] Means in the same column followed by the same letter were not significantly different (P = 0.05) according to Duncan's multiple range test.

be pre-selected by competition and reached the population equilibrium. Over all, the novel baiting tape method described in this study was more efficient than the conventional baited, sprinkled plate method in isolating the microorganisms with high antagonistic abilities.The conventional method did not start selection until the antagonists were collected.

Greenhouse experiments were crucial before developing potential antagonists into biological control tactics. Many researchers have spent enormous time and efforts to isolate potential antagonists from soil and found their antagonistic ability were not as expected when applied to the fields. The *in vitro* assays for nematode-trapping ability may not reflect their true behavior in nature for the population density of fungi or the nutrients provided on the agar plates were relatively high [8, 23]. The results showed that both *Pasteuria penetrans* and *Streptomyces saraceticus* significantly reduced the pre-existing *Meloidogyne incognita* population (Table 5), suggesting that these two antagonists had great application potential to control *M. incognita* on annual vegetable crops and perennial fruit trees. Three nematode trapping fungi, *Arthrobotrys* sp., *Dactylaria* sp., and *Dactylella* sp. did not show significant antagonistic results in the greenhouse experiments, probably due to the lack of certain nutrients in the potting soil that are required for the expression of fungal trapping capabilities [16]. *Mononchus* sp. had low feeding activity. One adult *Mononchus* sp. feeds on a maximum of only four nematodes per day on cultured plates (Tsay, unpublished data), reducing its quality as an ideal antagonist in the field. Results from greenhouse experiments clearly showed that *Mononchus* sp. was not an effective antagonist. *Pasteuria penetrans* though had a high antagonistic efficacy in the greenhouse experiment, it is difficult to produce in large quantity for field applications due to its host specificity and obligatory dependence on host for growth [19].

Plant root system is the primary target attacked by soil-borne pathogens. Therefore, introducing antagonists that originated from the rhizosphere habitat would greatly increase the chance of successful colonization and protection. In the rhizosphere habitat, actinomycetes are present in large quantity, amounting 10^4-10^7 cells / g of soil, and most of them are *Streptomyces* spp. [1]. A total of 351 actinomycetes was isolated by the three baiting methods. Most of the isolates were *Streptomyces* spp. and some belonged to the genera *Streptosporangium*, *Micromonospora*, *Kitasatoa* and *Micropolyspora*. The result is similar to the previous study [12]. *Streptomyces* spp. inhabiting near citrus rhizosphere could parasitize *Tylenchulus semipenetrans* Cobb., due to a secreted chitinase that dissolves the chitin layer of the plant parasitic nematode egg shell [12, 17]. Greenhouse experiments revealed that *Streptomyces saraceticus* also had good antagonistic ability (Table 4 and 5). Since *S. saraceticus* was commonly found in nematode-infested fields in Taiwan, this microorganism had a promising potential for developing into a biological control agent.

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摘 要

蔡東纂[1,3]、陳珮臻[1]、吳文希[2]. 2006. 分離有效植物寄生性線蟲拮抗微生物之新方法. 植病會刊 15：9-16. ([1] 台中市 國立中興大學植物病理系；[2] 台北市 國立台灣大學植物病理與微生物系；[3] 聯絡作者，電子郵件：tttsay@mail.nchu.edu.tw; Fax: +886-4-22876712)

本試驗比較傳統平板灑佈誘釣法、改良誘釣包及新型膠帶誘釣法分離拮抗微生物之效率，以及所得拮抗菌之拮抗能力優劣。針對三種線蟲捕捉性眞菌 (*Arthrobotrys* spp., *Dactylaria* spp., 及 *Dactylella* spp.)、一種線蟲寄生性細菌 (*Pasteuria penetrans*)、獵食性線蟲 (*Monochus* sp.)、捕植蟎及放線菌 (actinomycetes)爲誘釣之對象。首先比較三種方法對不同物種之誘釣能力，結果顯示只有誘釣包方式可以成功分離獵食性線蟲和蟎類，而新型膠帶誘釣法除了針對放線菌外，其分離率爲三種方法中最低者，但是膠帶誘釣法所得之三種捕捉性眞菌和放線菌，卻在培養皿中表現出最佳之拮抗能力。培養皿中表現最好的拮抗菌株在溫室試驗中，除了獵食性線蟲外，所有的拮抗微生物皆可有效降低土壤中根瘤線蟲的二齡幼蟲數目和根系上的線蟲卵塊數，而其中以線蟲寄生性細菌 (*Pasteuria penetrans*) 和放線菌 (*Streptomyces saraceticus*) 的拮抗能力較線蟲捕捉性眞菌爲佳。本試驗中依照新思維所發明的新型膠帶誘釣法，經試驗證明所誘釣出的拮抗微生物較其他誘釣法分離出之拮抗菌確實有較佳之拮抗能力。

關鍵詞： 拮抗菌、平板灑佈誘釣法、誘釣包、新型膠帶誘釣法、線蟲捕捉性眞菌、線蟲寄生性細菌、放線菌、獵食性線蟲、捕植蟎