



Original Article

Screening of chitinolytic actinomycetes for biological control of *Sclerotium rolfsii* stem rot disease of chilli

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Abstract

Two hundred and eighty three strains were isolated from rhizosphere-associated soils, from Ubon Ratchathani and Srisaket province, using Enrichment Media for isolation of Chitinase-producing Actinomycetes agar (EMCA agar). All strains were screened for chitinolytic activity and sixty eight strains gave significant clear zone on EMCA agar plates. The selected chitinolytic strains were assayed for *in vitro* antagonism against *Sclerotium rolfsii* using cornmeal agar (CMA agar) assay procedure and the result showed that thirteen isolates have remarkable inhibiting the growth of the fungus and the top five antagonistic actinomycetes were PACCH 277, PACCH129, PACCH225, PACCH24 and PACCH246, respectively. The result indicated that these actinomycetes produce chitinase which catalyze the degradation of chitin, resulting in inhibition of *S. rolfsii* growth. Their abilities to control the disease development were tested for *in vivo* biocontrol assay on chilli seedlings. Two out of thirteen candidate, PACCH24 and PACCH225, antagonists reduced the disease development at 90%. It was suggested that the ability to inhibit the growth of pathogen *in vitro* was not related to the disease reduction *in vivo*. The strain PACCH24 was further identified as *Streptomyces hygroscopicus* according to morphological characteristic, cell wall and cellular sugar analysis and 16S rDNA sequencing. The study implies a novel chitinolytic actinomycete which could be developed to be a biological agent which would be included as a complement with organic fertilizers in order to control stem rot disease and promote growth of chilli.

Keywords: chitinase-producing actinomycetes, biocontrol, stem rot, chilli, *Sclerotium rolfsii*, *Streptomyces hygroscopicus*

1. Introduction

Sclerotium rolfsii is a soilborne phytopathogenic fungus affecting a large number of cultivated plants (Punja, 1985). Control of *Sclerotium* stem rot, may be achieved by applying tremendous volume of fungicides every other day during the growing season. However, problems regarding the efficacy of the chemicals and fungicide residues are of increasing concern and need to be solved because of the direct effects on human health and the environment. Biologi-

cal control of phytopathogens by microorganisms has been considered to be more natural and an environmentally acceptable alternative to the existing chemical treatment methods (Baker and Paulitz, 1996). Several applications have been reported using plant-beneficial fungi and bacteria strains to protect plants against soil-borne pathogens (Ordentlich *et al.*, 1988; Gal, 1992; Tweddell *et al.*, 1994; Mao *et al.*, 1998; Tsahouridou and Thanassouloupoulos, 2002). The main mechanisms involved in the antagonism of biocontrol agents are mycoparasitism, competition for space and nutrients, stimulation of the plant's defensive capacity, and secretion of bioactive compounds such as antibiotics and cell wall degrading enzymes (Madi *et al.*, 1997; Harman *et al.*, 2004; Bakker *et al.*, 2007; Jung *et al.*, 2003; Van der Ent *et al.*, 2008).

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Chitinolytic enzymes have been considered for bio-control of pathogenic fungi due to their ability to degrade chitin, which is a major structural component of most fungal cell wall (Bartnicki-Garcia, 1968; Chet, 1987). Several chitinolytic bacteria and fungi have been reported to be potential biological agents. For example, *Serratia mercescens* is used in control of *S. rolfsii* (Ordentlich *et al.*, 1988), *Paenibacillus illinoisensis* is for against *Rhizoctonia solani* Kuhn (Jung *et al.*, 2003), and *Trichoderma harzianum* is used in control of *Botrytis cinerea* (Tronsmo, 1991). Actinomycetes are a group of Gram-positive filamentous bacteria with high G+C ratio. They play an important role in decomposition of organic materials, such as cellulose, lignocellulose, starch and chitin in soil (Srinivasan *et al.*, 1991). Furthermore, actinomycetes are a promising group of root-colonizing microbes where they may influence plant by promoting growth and protecting against disease (Broadbent *et al.*, 1971; Filnow and Lockwood, 1985). The objectives of this study were to isolate and screen actinomycetes exhibiting a chitinolytic activity, and determine their *in vitro* and *in vivo* antagonism towards a fungal stem rot pathogen of chilli, *Sclerotium rolfsii*.

2. Materials and Methods

2.1 Isolation of chitinolytic actinomycetes

Chitinolytic actinomycetes were isolated from rhizosphere soils using Enrichment Media for isolation of Chitinase-producing Actinomycetes (EMCA agar) (Wiwat *et al.*, 1999) and incubated at 30°C for 5 to 7 days. Colonies showing zones of clearance against the creamy background were regarded as chitinase-producing and restreaked until pure cultures were obtained. The stock culture was kept in Casamino acids-yeast extract-glucose medium (CYD) (Crawford *et al.*, 1993) with 15% glycerol at -70°C for further uses.

2.2 Screening of chitinolytic activity assay using agar diffusion method

Each actinomycete strain was inoculated onto Yeast Malt Extract (ISP-2) agar plate (Shirling and Gottlieb, 1966) and incubated at 30°C for 5 days. Five agar plugs of the strain was transferred into ISP-2 broth and incubated with shaking at 150 rpm for 7 days. The cells were removed from the culture medium by centrifugation at 19,000 g for 10 minutes and the supernatant (20 µl) was dropped on EMCA agar plate. After 10 days incubation, zone of clearance was determined and a diameter greater than 5.0 mm was regarded as significant chitinase-activity.

2.3 Isolation of *Sclerotium rolfsii*, a stem rot pathogen

A chilli plants which showed a damping-off disease were collected from an agricultural field in Banrat district and Bansrichi district, Amphor Warinchamrap, Ubon Ratchathani.

The stem segments were submerged in 2% sodium hypochlorite for 5 minutes and then placed onto Potato Dextrose Agar (PDA) plus 0.5% streptomycin. The plate was incubated at 28°C for 7 days. Each colony was restreaked until pure culture was obtained. Morphology and conidia characteristics were studied under microscopy and identified as described by Barnett and Hunter (1972). Pathogenicity was proved by inoculating an agar plug of *S. rolfsii* on stem of healthy chilli seedlings and incubated at 28°C for 2 weeks. The symptom was recorded and the isolation technique was repeated as described above.

2.4 *In vitro* evaluation of microbial antagonism

Each chitinolytic actinomycete strain was streaked onto an EMCA agar plate. The culture was incubated at 30°C for 5 days. A (0.5 cm²) agar plug with actinomycete mycelia was aseptically placed onto one half of a corn meal agar plate (CMA plate) and incubated at 30°C for 8 days. A (0.5 cm²) agar plug containing *S. rolfsii* was then placed near the front of the actinomycete growth. Following 4 days incubation, antagonism was determined as the distance between actinomycete and fungal growth. Growth of fungi at a distance greater than 20.0 mm from the actinomycete colonies was considered strong antagonism.

2.5 *In vivo* biocontrol assay for stem rot disease of chilli

Chilli seeds were surface-sterilized in 2% NaClO for 3 min and 70% ethanol for 2 min and then rinsed 3 times with sterile water. The seeds were then pre-germinated in the dark on sterile wet filter paper in Petri dish at 25°C for 48 h. The pre-germinated seeds were planted in plastic pots (10 cm height and 6 cm diameter pots) filled with sterilized soil. The pots were then moved to a chamber maintained at 28°C under light at least 12 h and watered daily to maintain 70% moisture holding capacity of soil. After thirty days of incubation, spore suspension (10⁸ spores/ml) of chitinolytic actinomycete was inoculated into each pot (1:5 g of sterilized soil) every six days for three times (except for uninoculated control). After that an agar plug of *S. rolfsii* was placed close to a stem and pressed in 1 cm depth into soil (except for uninoculated control). Incubation was continued for further 21 days. Five pots were used for each treatment and repeated three times. The number of stem rot plants was visually recorded at 7-day intervals until 21 days after inoculation.

2.6 Identification of chitinolytic actinomycetes

Actinomycete strain was cross-hatched streaked onto Yeast Malt Extract (ISP-2) agar plates and incubated at 30°C for 5 days. The mature hyphae with spores in the angles of the streaks were observed under a microscope (x25 and x40). Form of mycelium (aerial or substrate mycelium) was investigated by slide culture method (Lechevalier and Lechevalier,

1967). Cell wall diaminopimelic acid (DAP) and cellular sugars (xylose, madurose, arabinose, rhamnose, galactose and mannose) were investigated using thin layer chromatography (Staneck and Roberts, 1974; Lechevalier *et al.*, 1971).

For PCR amplification a small amount of a actinomycete colony was suspended in 100 μ l of sterile deionised water (SDW), mixed and lysed at 70°C (60 min). Crude lysate (0.2 μ l) was added to 19.8 μ l SDW and used as a PCR template. Universal 16S rRNA gene primers pA (5'-AGA GTT TGA TCC TGG CTC AG-3') and pH' (5'-AAG GAG GTG ATC CAG CCG CA-3') were used to amplify the *ca* 1.5 kb 16S rRNA gene fragment (Edwards *et al.*, 1989). To each PCR template was added 20 pmol of each primer, 50 μ mol of each deoxynucleoside triphosphate, 2.5 U of *Taq* DNA polymerase (Bioline) and 10 μ l of 10x *Taq* DNA polymerase buffer (Bioline); reaction volumes were made up to 100 μ l with sterile deionised water. Lysed *E. coli* cells and 20 μ l of sterile deionised water were used as positive and negative controls, respectively. Temperature cycling comprised 35 cycles of 94°C for 40 sec, 55°C for 1 min, and 72°C for 2 min, followed by an additional 10 min at 72°C. An aliquot (10 μ l) of the PCR product was mixed with 2 μ l loading buffer (15% (w/v) Ficoll, 50 μ M disodium EDTA and 0.05% (w/v) bromophenol blue) analysed by electrophoresis (15 V/cm; 60 min) on 0.7% horizontal agarose gels in Tris-borate-EDTA buffer (45 mM Tris-borate, 1 mM EDTA, pH 8.3) containing 0.5 μ g/ml ethidium bromide, then visualised on an UV transilluminator. PCR products were cleaned using a QIA quick spin column (Qiagen Qiaquick PCR purification kits) according to the manufacturers' instructions. Purified PCR products were sequenced by Biomolecule Analysis Service Unit (Faculty of Medicine, Khon Khen University, Thailand) using 16S sequencing primer 943 reverse (Lane *et al.*, 1985). The 16S rRNA gene sequences were compared with known sequences in the EMBL database using ADVANCED BLAST to identify the most similar sequence alignment, and were analysed against sequences in the Ribosomal Database Project.

3. Results

3.1 Isolation and screening of chitinolytic actinomycetes

Enrichment of chitinolytic actinomycete was performed with rhizosphere-associated soil samples derived from chilli cultural field in Ubon Ratchathani and Srisaket province. The sample was inoculated to enrichment medium containing colloidal chitin as sole carbon and energy source. Two hundred and eighty three strains were obtained. All strains were screened for chitinolytic activity using agar diffusion method. The result showed that forty-five strains gave significant clear zone (> 5.0 mm of diameter), seventy strains were moderate (2.5-5.0 mm of diameter) one hundred and forty-seven strains were negligible (0-2.5 mm of diameter) and twenty-one strains could not grow on the medium. Fifty strains were then selected for further study.

3.2 Isolation of *Sclerotium rolfsii*, a stem rot pathogen

An infected chilli was collected from an agricultural field for isolation of stem rot pathogen, and one isolate was obtained. The pathogen was proved for pathogenicity and was identified as *S. rolfsii*. The infected plant showed a dark-brown lesion on the stem at or just beneath the soil level. The chilli seedlings were very susceptible and died quickly once they become infected.

3.3 *In vitro* evaluation of microbial antagonism

The selected strains were evaluated for their *in vitro* antagonism using dual culture assay (Table 1). After 8 days of incubation, strain PACCH 277 showed very strong antagonism toward *S. rolfsii* with the largest inhibition zones (up to 25 mm) (Figure 1). Twelve strains (PACCH 24, PACCH 42, PACCH 91, PACCH 101, PACCH 129, PACCH 133, PACCH 137, PACCH 140, PACCH 224, PACCH 225, PACCH 246, and PACCH 247) showed strong antagonism with 10.0-19.9 mm of inhibition zone, eleven strains showed weak antagonism with 0.5-9.9 mm of inhibition zone, and the remaining twenty-six strains showed no antifungal activity under the conditions employed. Thirteen strains possessing significant antifungal activity were studied further.

3.4 *In vivo* biocontrol assay for stem rot disease of chilli

Thirteen strains were further screened for their antifungal ability in a pot experiment. The incidence of stem rot disease chilli seedlings in the AS_{PACCH 24} and AS_{PACCH 225} treatments was significantly lower than in the other treatment (except the untreated, and infested control) (Table 2). No significant difference was detected among the four other treatments (AS_{PACCH 42}, AS_{PACCH 129}, AS_{PACCH 224} and AS_{PACCH 246} treatments). However, strain PACCH 277 showed very strong antagonism toward *S. rolfsii* *in vitro* assay (Figure 2). The statistic value of significant was 95%.

3.5 Identification of chitinolytic actinomycete

Chitinolytic actinomycete strain PACCH24 was identified according to its morphological and molecular charac-

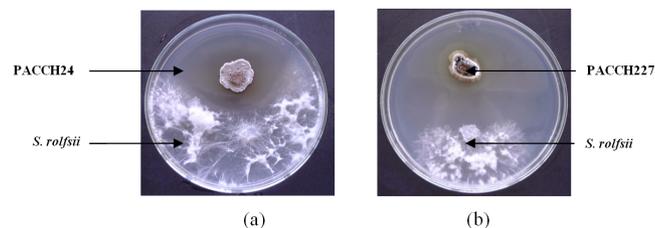


Figure 1. *In vitro* evaluation of antifungal activity assay. Chitinolytic actinomycetes were tested in a dual culture assay against pathogenic fungi on CMA agar plates; (a) PACCH24 against *S. rolfsii*, (b) PACCH277 against *S. rolfsii*.

Table 1. *In vitro* inhibitory activity of actinomycete isolates against *Sclerotium rolfsii*

Isolate	Distance of inhibition zone (mm)	Isolate	Distance of inhibition zone (mm)
PACCH24	12.0±0.96	PACCH175	9.5±1.02
PACCH28	0.0±0	PACCH185	0.0±0
PACCH35	0.0±0	PACCH186	2.0±0.43
PACCH40	2.0±.35	PACCH197	0.0±0
PACCH42	10.0±0.62	PACCH198	9.0±0.85
PACCH50	0.0±0	PACCH210	0.0±0
PACCH51	0.0±0	PACCH214	0.0±0
PACCH55	0.0±0	PACCH216	0.0±0
PACCH57	9.25±1.34	PACCH219	2.5±0.37
PACCH58	9.5±0.97	PACCH220	0.0±0
PACCH60	0.0±0	PACCH224	10.5±0.69
PACCH86	0.0±0	PACCH225	13.0±0.95
PACCH91	10.0±0.98	PACCH226	9.0±0.78
PACCH101	12.0±0.74	PACCH232	5.0±0.53
PACCH104	9.5±1.00	PACCH233	0.0±0
PACCH119	5.5±0.82	PACCH245	2.5±0.24
PACCH128	5.0±0.69	PACCH246	10.0±1.20
PACCH129	15.0±0.94	PACCH247	10.5±0.91
PACCH132	0.0±0	PACCH251	0.0±0
PACCH133	10.0±1.00	PACCH265	0.0±0
PACCH137	11.0±0.72	PACCH270	0.0±0
PACCH138	0.0±0	PACCH271	0.0±0
PACCH140	11.5±0.91	PACCH272	6.0±0.53
PACCH173	0.0±0	PACCH277	25.0±1.04
PACCH174	0.0±0	PACCH233	0.0±0

Table 2. Treatments and percentage of diseased plants.

Treatments ^a	Diseased plants (%)	Treatments ^a	Diseased plants (%)
CT	0±0	SA _{PACCH 137}	50±4
CS	98±4	SA _{PACCH 140}	20±5
SA _{PACCH 24}	10±6	SA _{PACCH 224}	30±5
SA _{PACCH 42}	30±7	SA _{PACCH 225}	10±6
SA _{PACCH 91}	70±5	SA _{PACCH 246}	30±4
SA _{PACCH 101}	50±6	SA _{PACCH 247}	60±4
SA _{PACCH 129}	30±5	SA _{PACCH 277}	50±6
SA _{PACCH 133}	60±7		

^a CT, uninoculated control (not inoculated with any microbe); CS, pathogen control (only inoculated with *S. rolfsii*); SA (inoculated with *S. rolfsii* and actinomycetes PACCH_n).

ters. This strain showed grey aerial mycelium, which formed a pale brownish diffusible pigment. This strain showed straight to primitive spiral spore chains with open loops; mature spore chains contained generally 10-50 spores per chain; the spore surface was rugose. Since the types of cell classified as

type I-VI. The BLAST search results (Atschul *et al.*, 1990) indicated that the 16S rDNA sequence of PACCH24 strain is closely related to *Streptomyces hygroscopicus* strain 3088 with 96% identity.



Figure 2. *In vivo* assay for biocontrol of stem rot on young chili seedling; (a) An infected plant and control plant, (b) Chili seedling was inoculated with PACCH24 strain and *S. rolfsii*.

4. Discussion

A high correlation between chitinolysis and antifungal properties has been reported (Chen *et al.*, 1991; Pisano *et al.*, 1992; Pleban *et al.*, 1997; Jung *et al.*, 2003; Hoster *et al.*, 2005). In this study, we isolated 283 different chitinolytic actinomycete strains from rhizosphere soils using enrichment method. Forty-five strains exhibited strong chitinolytic activity as determined by degradation of colloidal chitin on EMCA agar. All selected strains were then screened for antagonistic properties against stem rot fungus *S. rolfsii* *in vitro*. The result showed that strain PACCH277 and four other strains (PACCH129, PACCH225, PACCH24 and PACCH246) were capable of markedly inhibiting the growth of the fungal pathogen on CMA plates. The pattern of antagonism, by the most active chitinolytic actinomycete strains, indicated the production of water-soluble antifungal metabolites, since large zones of inhibition were evident on the CMA plates. However, it is possible that the mechanism of antagonism may have involved neither in the production and excretion of chitinolytic enzymes to inhibit the growth of pathogen nor the production of secondary metabolites. Thus, further study needs to be addressed regarding the mechanism of action. There are a wealth of data which support the important role of chitinolytic enzymes in antifungal activity. Lee *et al.* (1997) reported that purified chitinase of *Pseudomonas* sp. YHS-A2 inhibited the growth of some phytopathogenic fungi: *Fusarium oxysporum*, *Botrytis cinerea*, and *Mucor rouxii*. Berg *et al.* (1999) reported that *Serratia plymuthica* C48 produced and excreted a set of various chitinases. This bacterium significantly suppresses the growth of *Verticillium longisporum*, while a chemically constructed chitinase-deficient mutant C48/3Rif^r chi⁻ did not exhibit antifungal activity. Chitinase and laminarinase, when acting alone or synergistically, were shown to inhibit the growth of pathogenic fungi by degradation or lysis of the fungal cell wall (Mauch *et al.*, 1988; Wang *et al.*, 1999). Jung *et al.* (2003) reported that the suppression of damping-off incidence in cucumber seedlings caused by *R. solani* was associated with chitinases secreted by *Paenibacillus illinoisensis* KJA-424. They also suggested that chitinases play a role in hyphal swelling and lysis of

fungal cell walls of phytopathogenic fungi, resulting in inhibition of the growth of fungal hyphae. Hoster *et al.* (2005) reported that the chitinase of *Streptomyces griseus* MG3 possessed antifungal activity against the following fungi; *Aspergillus*, *Botrytis cinerea*, *Fusarium culmorum*, *Guignardia bidwellii*, and *Sclerotia sclerotiorum*. The present study showed that strains PACCH101 and PACCH227 had significant antagonism of fungi *in vitro* assay but not quite active in soil. It is possible that the condition in pot experiments may not promote the growth of these strains or these strains probably could not establish antagonistic mechanisms in the soil. The results indicated that antibiosis *in vitro* was not related to the disease reduction *in vivo*. Liu *et al.* (1996) concluded that the ability to suppress the growth of pathogen was not related to the reduction of tomato scab disease in field study by *Streptomyces* spp. Anees *et al.* (2010) also suggested that different antagonistic mechanisms were evident for different strains and the ability to produce water-soluble inhibitors or coil around the hyphae of the pathogen *in vitro* was not related to the disease reduction *in vivo*. In the present study, the most efficient strain overall was strain PACCH24, which was able to reduce the growth of the pathogen and suppress the disease both *in vitro* and *in vivo*. This strain was identified as *Streptomyces hygroscopicus*. As a whole, our data suggest that chitinolytic *S. hygroscopicus* PACCH24 might be an effective antagonist applicable to the control of the stem rot caused by *S. rolfsii*.

5. Conclusion

This study implies that the presence of chitinolytic *S. hygroscopicus* PACCH24 may play a major role in the control of stem rot caused by *S. rolfsii*. Results of this experiment could be considered in improving the strategy to use *S. hygroscopicus* PACCH24 as a biocontrol agent.

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References

- Adandonon A., Aveling, T. A. S., Labuschagne, N. and Tamo, M. 2006. Biocontrol agents in combination with *Moringa oleifera* extract for integrated control of *Sclerotium*-caused cowpea damping-off and stem rot. European Journal of Plant Pathology. 115, 409-418.
- Anees, M., Tronsmo, A., Edel-Hermann, V., Hjeljord, L. G., Heraud, C. and Steinberg, C. 2010. Characterization of field isolates of *Trichoderma* antagonistic against

- Rhizoctonia solani*. Fungal Biology. 114, 691-701.
- Altschul, S. F., Gish, W. Miller, W., Myers, E. W. and Lipman, D. J. 1990. Basic local alignment search tool. Journal of Molecular Biology. 215, 403-410.
- Baker, R., and Paulitz, T. C. 1996. Theoretical basis for microbial interactions leading to biological control of soil-borne plant pathogens In: Principles and Practice of Managing Soilborne Plant Pathogens. Hall, R. (ed.). The American Phytopathology Society. St. Paul, MN. pp. 50-79.
- Bakker, P. A. H. M., Pieterse, C. M. J. and Van Loon, L. C. 2007. Induced systemic resistance by fluorescent *Pseudomonas* spp. Phytopathology. 97, 239-243.
- Barnett, H. L., and Hunter, B. B. 1972. Illustrated Genera of Imperfect Fungi. Burgess Publishing. New York. 241 pp.
- Bartnicki-Garcia, S. 1968. Cell wall chemistry, morphogenesis and taxonomy of fungi. Annual Review of Microbiology. 22, 87-108.
- Berg, G., Frankowski, J., and Bahl, H. 1999. Biocontrol of verticillium wilt in oilseed rape by chitinolytic *Serratia plymuthica*. Proceeding of the 10th international rapeseed congress, Cabberra, Australia, September 26-29, 1999, 369-375.
- Broadbent, P. K., Baker, F., and Waterworth, Y. 1971. Bacteria and actinomycetes antagonistic to fungal root pathogens in Australian soils. Australian Journal of Biological Sciences. 24, 925-944.
- Chen, H. C., Huand, M. Y., Moody, M. W. and Jiang, S. T. 1991. Distribution and hydrolytic enzyme activities of aerobic, heterotrophic bacteria isolated from grass prawn, *Penaeus monodon*. Journal of the Fisheries Society of Taiwan. 18, 301-310.
- Chet, I. 1987. *Trichoderma*-application, mode of action, and potential as a biocontrol agent of soilborne plant pathogenic fungi. In: Chet, I., (Ed.), Innovative Approaches to Plant Disease Control, Wiley, New York, pp. 137-160.
- Chet, I., Ordentlich, A., Shapira, R. and Oppenheim, A. 1990. Mechanisms of biocontrol of soil-borne plant pathogens by rhizobacteria. Plant and Soil. 129, 85-92.
- Crawford, D.L., Lynch, J. M., Whipps, J. M. and Ousley, M. A. 1993. Isolation and characterization of actinomycete antagonists of a fungal root pathogen. Applied and Environmental Microbiology. 59, 3899-3905.
- Edward, U., Rogall, T., Bloecker, H., Emde, M. and Boettger, E. 1989. Isolation and direct complete nucleotide determination of entire genes. Characterisation of a gene coding for 16S ribosomal RNA. Nucleic Acids Research. 17, 7843-7853.
- Errakhi, R., Bouteau, F., Lebrihi, A. and Barakate, M. 2007. Evidences of biological control capacities of *Streptomyces* spp. Against *Sclectotium rolfisii* responsible for damping-off disease in sugar beet (*Beta vulgaris* L.). World Journal of Microbiology and Biotechnology. 23, 1503-1509.
- Errakhi, R., Lebrihi, A. and Barakate, M. 2009. *In vitro* and *in vivo* antagonism of actinomycetes isolated from Moroccan rhizospheric soils against *Sclerotium rolfisii*; a causal agent of root rot on sugar beet (*Beta vulgaris* L.). Journal of Applied Microbiology. 107, 672-681.
- Filnow, A. B., and Lockwood, J. L. 1985. Evaluation of several actinomycetes and the fungus *Hypochoytrium catenoids* as biocontrol agents of *Phytophthora* root rot of soybean. Plant Disease. 69, 1033-1036.
- Gal, S. W. 1992. Purification and characteristization of chitinase isozymes and cloning of a gene for 58 kD from *Serratia marcesens* KCTC2172. Doctor's thesis. Gyeongsang National University.
- Harman, G. E., Howell, C. R., Viterbo, A., Chet, I., and Lorito, M. 2004. *Trichoderma* species-opportunistic, avirulent plant symbionts. Nature Reviews Microbiology. 2, 43-56.
- Hooster, F., Schmitz, J. E. and Daniel, R. 2005. Enrichment of chitinolytic microorganisms: isolation and characterization of a chitinase exhibiting antifungal activity against phytopathogenic fungi from a novel *Streptomyces* strain. Applied Microbiology and Biotechnology. 66, 434-442.
- Jung, W. J., An, K. N., Jin, Y. L., Park, R. D., Lim, K. T., Kim, K. Y., and Tim, T. H. 2003. Biological control of damping-off caused by *Rhizoctonia solani* using chitinase-producing *Paenibacillus illinoisensis* KJA-424. Soil Biology & Biochemistry. 35, 1261-1264.
- Lane, D. J., Pace, B., Olsen, G. J., Stahl, D. A., Sogin, M. L. and Pace, N. R. 1985. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analysis. Proceedings of the National Academy of Sciences of the United States of America. USA 82, 6955-6959
- Lechevalier, H. A. and Lechevalier, M. P. 1967. Biology of actinomycetes. Annual Review of Microbiology. 21, 71-100.
- Lechevalier, H. A., Lechevalier, M. P. and Gerber, N. N. 1971. Chemical composition as criterion on the classification of actinomycetes. Advances in Applied Microbiology. 14, 47-72.
- Lee, H. S., Choi, H. J., Her, S. W. and Oh, D. H. 1997. Purification and characterization of antifungal chitinase from *Pseudomonas* sp. YHS-A2. Journal of Microbiology and Biotechnology. 7, 107-113.
- Liu, D., N. A. Anderson, and L. L. Kinkel. 1996. Selection and characterization of strains of *Streptomyces* suppressive to the tomato scab pathogen. Canadian Journal of Microbiology. 42, 487-502.
- Madi, L., Katan, T., Katan, J. and Henis, Y. 1997. Biological control of *Sclerotium rolfisii* and *Verticillium dahlia* by *Talaromyces flavus* is mediated by different mechanisms. Journal of Phytopathology. 87, 1054-1060.
- Mao, W., Lewis, J. A., Lumsden, R. D. and Hebban, K. P. 1998. Biocontrol of selected soilborne disease of tomato and pepper plants. Crop Protection. 17, 535-542.

- Mauch, F., Mauch-Mani, B., and Boller, T. 1988. Antifungal hydrolases in pea tissue II inhibition of fungal growth by combination of chitinase and β -1,3-glucanase. *Plant Physiology*. 88, 936-942.
- Ordentlich, A., Elad, Y. and Chet, I. 1988. The role of chitinase of *Serratia mercesens* in biocontrol of *Sclerotium rolfsii*. *Journal of Phytopathology*. 78, 84-88.
- Pisano, M. A., Sommer, M. J. and Tars, L. 1992. Bioactivity of chitinolytic actinomycetes from marine origin. *Applied Microbiology and Biotechnology*. 36, 553-555.
- Pleban, S. Chernin, L. and Chet, I. 1997. Chitinolytic activity of an endophytic strain of *Bacillus cereus*. *Letters in Applied Microbiology*. 25, 284-288.
- Punja, Z. K. 1985. The biology, ecology and control of *Sclerotium rolfsii*. *Annual Review of Phytopathology*. 23, 97-127.
- Shirling, E. B. and D. Gottlieb. 1966. Methods for characterization of *Streptomyces* species. *International Journal of Systematic Bacteriology*. 16, 313-340.
- Srinivasan, M. C., Laxman, R. S. and Despharde, M. V. 1991. Physiology and nutritional aspects of actinomycetes: an overview. *World Journal of Microbiology and Biotechnology*. 7, 171-184.
- Staneck, J. L. and Roberts, G. D. 1974. Simplified approach to identification of aerobic actinomycetes by thin layer chromatography. *Journal of Applied Microbiology*. 28, 226-231.
- Tronsmo, A. 1991. Biological and integrated controls of *Botrytis cinerea* on apple with *Trichoderma harzianum*. *Biological Control*. 1, 59-62.
- Tsahouridou, P. C. and Thanassouloupoulos, C. C. 2002. Proliferation of *Trichoderma koningii* in the tomato rhizosphere and the suppression of damping-off by *Sclerotium rolfsii*. *Soil Biology & Biochemistry*. 34, 767-776.
- Tweddell, R. J., Jabaji-Hare, S. H., and Charest, P. M. 1994. Production of chitinase and β -1,3-glucanase by *Stachybotrys elegans*, a mycoparasite of *Rhizoctonia solani*. *Applied and Environmental Microbiology*. 60, 489-495.
- Van der Ent, S., Verhagen, B. W. M., Van Doorn, R., Bakker, D., Verlaan, M. G., Pel, M.J.C., Joosten, R. G., Proveniers, M. C. G, van Loon, L. C., Ton, J. and Pieterse, C. M. J. 2008. MYB72 is required in early signaling steps of rhizobacteria-induced systemic resistance in *Arabidopsis*. *Journal of Plant Physiology*. 146, 1293-1304.
- Wang, S. L., Yieh, T. C. and Shih, I. L. 1999. Production of antifungal compounds by *Pseudomonas aeruginosa* K-187 using shrimp and crab shell powder as a carbon source. *Enzyme and Microbial Technology*. 25, 42-148.
- Wiwat, C., Siwayaprahm, P. and Bhumiratana, A. 1999. Purification and characterization of chitinase from *Bacillus circulans* No. 4.1. *Current Microbiology*. 39, 134-140.