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A Search and Improvement of Actinomycete Strains for Biological Control of Plant Pathogens

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ABATRACT

Two hundred isolates of actinomycetes isolated from soil samples in Sakaerat Biosphere Reserve and Suwanvajokkasikit Field Corps Research Station were tested for the ability of chitinase production and growth inhibition of three phytopathogenic fungi; *Fusarium sporotrichiodes, Rhizoctonia solani and Sclerotium rolfsii*. The result showed that SG4 and SG5 strains gave the consistent inhibitory effect against all three fungal pathogens inoculating preculture for 0, 5 and 10 days. It was found that inoculating preculture for 10 days showed the most antagonist effect and nine isolates of actinomycetes showed the highest inhibition of the tested strains, while only seven strains remained when the strain was meanwhile cultures in the plates with and without chitin. To increase the ability on antifungal activity, the selected isolates were irradiated with gamma radiation. Thirty five out of 173 isolates showed higher inhibitory effect on three phytophatogenic fungi tested than the wild type strains. Three isolates of mutant strains, SJ9I-15, SG4I-17 and SG4I-38 and two isolates of wild type strains, SJ9 and SG4, were selected for controlling phytopathogenic fungi in the greenhouse.

Key words: actinomycetes, chitin degrading enzymes, biological control, plant pathogens, strain improvement

INTRODUCTION

Actinomycetes, high DNA G+C content over 55%, gram-positive bacteria, have been recognized as sources of several secondary metabolites, antibiotics and lytic enzymes that affect fungal growth (Goodfellow and Williams, 1983). More than 90% of bioactive compounds were produced from these microorganisms which have potential applications in pharmacy, industry, agriculture and environment. Besides, their capability of growth inhibition by antibiotics, the ability on chitinase production can enhance the synergistic effect on inhibition of plant pathogenic fungi with chitin as the main component of cell wall (Goodfellow *et al.*, 1988). Streptomycetes are active in the rhizosphere and the modes of action of species tested include antibiosis, lysis of fungal

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cell walls, competition and hyperparasitism (Tapio and Pohto-Lahdenpera, 1991; Mohammadi and Lahdenpera, 1992).

The commercial product, Mycostop, based on strain K61 of *S. griseoviridis* isolate and *Streptomyces lydicus* WYEC108 can control or suppress some root rot and wilt diseases caused by *Pythium* spp., *Fusarium* spp., *Rhizoctonia* spp. and *Phytophthora* spp., if it colonizes the rhizosphere prior to the pathogens (Mahadevan and Crawford, 1997).

The objectives of this study were to screen actinomycetes for chitinase production and strong *in vitro* inhibition activity against phytopathogenic fungi from 200 strains isolated from soil samples in Sakaerat Biosphere Reserve and Suwanvajokkasikit Field Corps Research Station (Chanwit *et al.,* 2003), to mutate the strains by gamma-ray radiation and to select the mutated strains that increased the ability of chitinase production and growth inhibition of the plant pathogens for further testing as plant pathogen control agent in the greenhouse and field experiments.

MATERIALS AND METHODS

Screening of chitinase producing actinomycetes

Two-hundred strains of actinomycetes isolated from soil sample in Sakaerat Biosphere Reserve and Suwanvajokkasikit Field Crops Research Station, Nakhon Ratchasima Province, Thailand (Chanwit, 2003) were selected based on chitinase production on chitin agar medium (swollen chitin was used as a carbon sources). Actinomycetes culture grown on yeast extract-malt extract agar medium (ISP-2) at room temperature for 10 days before testing. For screening with one isolate per plate, each culture was four-point inoculated onto chitin agar plates and incubated at room temperature for 10 days. The strains which have clear zone around the colony were selected for studies on inhibition of phytopathogenic fungi.

Antifungal activity of actinomycetes

In vitro antagonism test, Dual Culture Method was used to examine the inhibition of three phytopathogenic fungi, *Fusarium sporotrichiodes, Rhizoctonia solani* and *Sclerotium rolfsii* obtained from Associate Professor Somsiri Sangchote (Department of Plant Pathology, Faculty of Agriculture, Kasetsart University), the causal organism of stem and root rot disease of backyard garden such as tomato, chilli, cucumber etc., on chitin and Glucose-meat extract-peptone (GMP) media. Each isolate of chitinase producing actinomycetes was streaked across the center of the plate. A second streak of the actinomycetes was made perpendicularly to the first and incubated at room temperature for 0, 5 and 10 days. Then the four discs of 5 mm. in diameter cut from the edge of a 7-day-old culture of phytopathogenic fungi were placed in each segment of the cross streaks of the antagonist. The distance between the two microorganisms was 2.5 cm. and the culture was incubated at room temperature for 5 days (Sadfi *et al*., 2001). Percentage of growth inhibition (GI) of fungi was calculated by the formula of Whipps (1987) : $(R1 - R2)/R1 \times 100$, where R1 = the radial distance (measured in mm) grown by phytopathogenic fungi, after 7 days of incubation, in the direction of the antagonist (a control value), R2 = the distance of fungal growth from the point of inoculation to the colony margin in the direction of the antagonist, and GI = the percentage of growth inhibition. Growth inhibition was categorized on a scale (Korsten *et al*., 1995).

Strain improvement by gamma ray induced mutation

Seven isolates of actinomycetes having antifungal activity toward the three pathogenic fungi were irradiated with 0.4-8 kGy dose of gamma ray (Office of Atom for Peace). The induced mutants were detected by observing the ability of chitinase production and the inhibition on growth of phytopathogenic fungi by method

described above while tested plant pathogenic fungi and actinomycetes were inoculated at the same time on the tested media.

RESULTS AND DISCUSSION

Screening of chitinase and antifungal substance actinomycetes producing

Among 200 isolates of actinomycetes, only 66 isolates showed clear zone around the colony on chitin agar medium, which were 33 percents of all tested strains. The strain DMKU 282 showed the most tendency to produce chitinase with the high ratio of diameter of clear zone to diameter of colony, which was 2.75. The chitinase producing actinomycetes were tested for inhibition on growth of three phytopathogenic fungi; *F. sporotrichiodes, R. solani* and *S. rolfsii* on Glucose-meat extract-peptone (GMP) and chitin media. The result showed that the growth inhibition of the phytopathogenic fungi depended on actinomycetes strains, media used and inoculation time of actinomycetes strains and the tested plant pathogen strains. *In vitro*, antagonism test to inhibit the growth of three phytopathogenic fungi precultured of actinomycetes were incubated at room temperature for 0, 5 and 10 days. It was found that incubating preculture for 10 days showed the most antagonist effect (data not shown). Ten-day preculturing of nine actinomycetes isolates showed the highest inhibition of the tested strains (growth inhibition category in level 4), while only seven strains remained when the strain was meanwhile cultured. Incubation time of actinomycetes related to their ability on growth inhibition. Longer incubation gave higher inhibitory effect than the shorter incubation since the producing of chitinase was synergistic with bioactive compound produced as secondary metabolite (Goodfellow and Williams, 1983).

Actinomycetes grown on chitin medium showed more tendency to inhibit the growth of fungi than grown on GMP medium because of the ability on bioactive compounds and chitinase production enhanced the synergistic effect on inhibition of plant pathogenic fungi where chitin was the main component of cell wall (Goodfellow *et al*., 1988; Yuan and Crawford, 1995; Mahadevan and Crawford, 1997), Chitinase are well known to lyse fungal cell walls (Gupta *et al*., 1995).

This study revealed a probable role for chitinase in the antifungal activity of seven Actinomycete strains; SA2, SA6, SB11, SG4, SG5, SJ9 and K19 when the tested plant pathogenic fungi and actinomycetes were inoculated at the same time on the tested media strains. Antifungal antibiotics produced by them also played an important role. It was found that SG4 and SG5 strains gave the consistent inhibitory effect against all three fungal pathogens (growth inhibition category were higher than level 2) as shown in Table 1. This result was the same as inoculated for 5 and 10 days.

Strain improvement for higher antifungal activity by gamma ray induced mutation.

One hundred seventy three mutants were obtained by gamma ray irradiation at 0.4-8 kGy from 7 wild type actinomycete strains (Table 2). The induced mutants were detected by observing the ability of chitinase production on chitin medium. Mutant strains showed potential to produce more chitinase than the wild type strains with the high ratio of diameter of clear zone to diameter of colony. This result also found the mutants of *Trichoderma harzianum* induced by gamma-ray radiation to increase the production of chitinase (Haggag, 2002).

The two mutant strains, SG4I-7 and SG4I-22 gave a high ratio of diameter of clear zone to diameter of colony, which was 2.00 and they showed the most tendency to produce chitinase (data not shown). Among mutant strains, it was found that 35 strains showed higher inhibition growth of the tested fungi than the wild type strain

on chitin and GMP media when tested plant pathogenic fungi and actinomycetes were inoculated at the same time on the tested media, which were 20.23 percent of all mutant strains.

The three mutant strains, SJ9I-15, SG4I-17 and SG4I-38 showed the most effective inhibition on the tested fungi as shown in Table 3. Most mutant strains grown on chitin medium also showed higher inhibitory effect on growth of the three phytopathogenic fungi than grown on GMP medium. The mutant strain, SG4I-17 gave higher inhibitory effect on growth of the three phytopathogenic fungi than the wild type, SG4 as shown in Figure 1-3.

Several studies have shown that gamma-ray radiation could change the genetic diversity of filamentous fungi and bacteria (Boominathan *et al*., 1990; Schlick *et al*., 1994), which has effect on improve the production of extracellular chitinase and antagonise the potential of biocontrol agents (Rey *et al*., 2000; Haggag and Mohamed, 2001; Haggag, 2002).

The three mutant strains were selected as biocontrol agents for further testing in greenhouse with Tomato (*Lycopersicon esculentum* Mill.) compared to the wild type strains, SG4 and SJ9.

CONCLUSIONS

Sixty-six isolates could produce chitinase enzyme. When the tested plant pathogenic fungi and actinomycetes were inoculated at the same time on the tested media strains SG4 and SG5 gave the consistent inhibitory effect against all three phytopathogenic fungi. This result was the same

Table 1 Efficiency of actinomycetes on the inhibition of growth of phytopathogenic fungi on GMP and chitin media (no incubation of actinomycetes).

Code	*Fusarium sporotrichiodes*				*Rhizoctonia solani*				*Sclerotium rolfsii*			
	% GI[1/]		GI Scale[2/]		% GI[1/]		GI Scale[2/]		% GI[1/]		GI Scale[2/]	
	GMP	Chitin medium	GMP	Chitin medium	GMP	Chitin medium	GMP	Chitin medium	GMP	Chitin medium	GMP	Chitin medium
SA2	44.00	32.50	2	2	70.42	73.84	3	3	44.53	52.58	2	3
SA6	48.40	46.25	2	2	69.49	74.42	3	3	42.60	43.10	2	2
SB11	52.67	56.82	3	3	71.88	72.16	3	3	-	72.16	0	3
SG4	60.00	60.30	3	3	74.72	74.00	3	3	53.90	51.72	3	3
SG5	56.00	59.70	3	3	65.70	76.16	3	4	62.50	51.72	3	3
SJ9	48.52	32.50	2	2	76.02	77.90	4	4	60.56	46.55	3	2
K19	48.12	55.00	2	3	53.48	51.87	3	3	20.25	63.81	3	3

1/ % GI ; the percentage of growth inhibition

2/ GI scale ; 0 = no growth inhibition, 1 = growth inhibition at 1- 25 %, 2 = growth inhibition at 26 –50%, 3 = growth inhibition 51 – 75%, 4 = growth inhibition at 76 – 100%

Table 2 A number of mutant actinomycetes obtained by gamma ray irradiation.

Code of wild type strains	A number of mutant strains
SA2	0
SA6	51
SB11	33
SG4	39
SG5	0
SJ9	29
K19	21
Total	173

as inoculated preculture for 5 and 10 days. Ten days precultured of 9 isolates of actinomycetes showed the highest inhibition on the tested strains. Actinomycetes grown on chitin medium showed more tendency to inhibit the growth of fungi than that on GMP medium. Thirty-five mutant strains induced by gamma radiation gave higher inhibition on growth of the tested fungi than wild type strains on chitin and GMP media. Three mutant strains, SJ9I-15, SG4I-17 and SG4I-38 gave the highest inhibitory effect of all the fungi tested.

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Table 3 Efficiency of two wild type and three mutant actinomycetes on the inhibition of growth of three phytopathogenic fungi on GMP and chitin media.

Code	*Fusarium sporotrichiodes*				*Rhizoctonia solani*				*Sclerotium rolfsii*			
	% GI[1/]		GI Scale[2/]		% GI[1/]		GI Scale[2/]		% GI[1/]		GI Scale[2/]	
	GMP	Chitin medium	GMP	Chitin medium	GMP	Chitin medium	GMP	Chitin medium	GMP	Chitin medium	GMP	Chitin medium
SG4	60.00	60.30	3	3	74.72	74.00	3	3	53.90	51.72	3	3
SG4I-17	79.00	80.60	4	4	75.00	81.40	3	4	78.80	88.88	4	4
SG4I-38	79.00	77.40	4	4	72.00	78.00	3	4	72.33	88.88	3	4
SJ9	48.52	32.50	2	2	76.02	77.90	4	4	60.56	46.55	3	2
SJ9I-15	78.42	80.30	4	4	75.86	77.54	3	4	66.48	85.79	3	4

1/ % GI ; the percentage of growth inhibition

2/ GI scale ; 0 = no growth inhibition, 1 = growth inhibition at 1- 25 %, 2 = growth inhibition at 26 –50%, 3 = growth inhibition 51 – 75%, 4 = growth inhibition at 76 – 100%

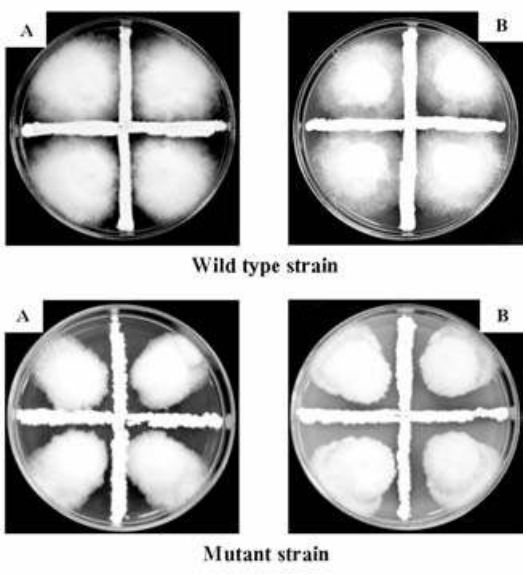

Figure 1 The efficiency of wild type SG4 and mutant SG4I-17 on growth inhibition of *Fusarium sporotrichiodes* on GMP(A) and chitin media (B).

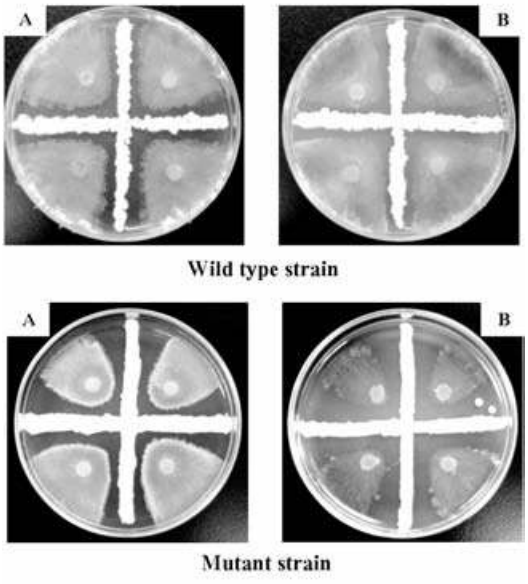

Figure 2 The efficiency of wild type SG4 and mutant SG4I-17 on growth inhibition of *Rhizoctonia solani* on GMP(A) and chitin media (B).

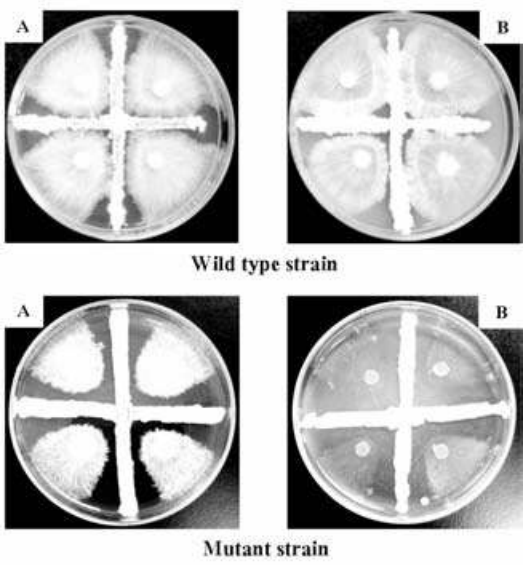

Figure 3 The efficiency of wild type SG4 and mutant SG4I-17 on growth inhibition of *Sclerotium rolfsii* on GMP(A) and chitin media (B).

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