

Antifungal Pathogen Activities and Growth Promotion of Endophytic Actinomycetes on Brassica Seedling

Kaewalin Kunasakdakul*, Prapaipit Suwitchayanon and Chatsuda Phuakjaiphae

Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand

*Corresponding author. E-mail: kaewalin.k@cmu.ac.th

ABSTRACT

Vegetable plants with disease symptoms were collected for isolation, identification and pathogenicity testing of the causing fungal pathogens. They were Alternaria brassicicola for Chinese kale alternaria leaf spot, Alternaria brassicae for leaf spot of cabbage, Sclerotium sp. for root rot of Chinese kale, Choanephora sp. for wet rot of Chinese kale and Fusarium sp. for wilt of lettuce. Eleven isolates of endophytic actinomycetes from the herbal plants as follows; KAE1, GAL1, GL11, BER1, NEE1, KMI1, FIT2, FIT3 and FIT5 isolates of Streptomyces sp, HOU1 isolate of Nocardioideis and LEM1 isolate of Nocardioopsis were used for testing of their abilities to inhibit the growth of the fungal pathogens mentioned above by dual culture technique. Their effects on root length, plant height and fresh weight of brassica seedling were also evaluated. We found that among the 11 isolates tested, KAE1, GAL1 and LEM1 showed broad spectrum to control vegetable pathogens. Under laboratory testing, these three endophytic actinomycetes had high inhibitory efficiency against Fusarium, soil borne pathogen, and two air borne pathogens, Choanephora and A. brassicae. Only one isolate, FIT5 showed fairly high inhibitory against all the tested fungal pathogens. HOU1 isolate was the only one isolate having significant improvement on root length of brassica seedling as compared to uninoculated control and this isolate was the best and significantly better than the other isolates except FIT5. HOU1 also showed the highest inhibitory percentage on Sclerotium, soil borne pathogen.

Key words: Endophytic actinomycete, Fungal inhibition, Brassica fungal pathogen, Plant growth promotion

INTRODUCTION

Vegetable production is always facing fungal diseases during planting period. The widely used diseases control methods by application of chemical pesticides are currently of concern because of their toxicity to farmers, consumers, environment and ecosystems. Combining with the consumer interest and governmental policy to achieve national goal for food safety, biological control is expected to be safe and become the basic method in IPM instead of pesticide application for disease control. The beneficial microorganism which has antagonistic activity such as endophytic actinomycete is one of the numerous effective antagonistic agents. Their effective mechanisms for plant disease control including beneficial metabolite production that can induce plant growth and development have been reported in various aspects. In this study we wanted to evaluate the antifungal activities of some selected endophytic actinomycetes and the effects of them on growth of vegetable seedlings.

MATERIALS AND METHODS

Isolation of pathogens and culture of endophytic actinomycetes

Various kinds of vegetable which showed leaf spot and wilt symptoms were collected from highland growing area in Nong Hoi village, Mae Ram sub-district, Mae Rim district, Chiang Mai

province, Thailand. Pathogens of these diseases were isolated using infected tissue transplanting method. Pure cultures of isolated disease were used for identification and pathogenicity test. Eleven isolates of endophytic actinomycete in three genera as follows; *Streptomyces*, KAE1, GAL1, GLI1, BER1, NEE1, KMI1, FIT2, FIT3 and FIT5, LEM1 isolate of *Nocardioides*, HOU1 isolate of *Nocardopsis* were used. These endophytic actinomycetes were previously isolated from Thai herbal plants (Pukclai et al., 2006). Prior to their testing, they were re-cultured on Inhibitory Mold Agar-2 (IMA-2) (Shimizu et al., 2000) using streak plate method and incubated at 30°C then microscopic observation was done to determine the morphology and mycelia growth of each endophytic actinomycete isolate (Miyadoh et al., 1997).

Testing of fungal pathogen inhibitory efficiencies of endophytic actinomycetes

Dual culture technique (Crawford et al., 1993; El-Tarabily et al., 1997) was used to determine effectiveness of each endophytic actinomycete isolate to each plant pathogenic fungus causing wet rot, damping off and leaf spot. Effectiveness of the tested isolates were evaluated by consideration of the percent inhibition of radial growth (%PIRG) performed in the dual culture trial. The inhibitory efficiency levels were indicated as low ($\leq 50\%$), medium (50-60%), high (61-74%) and very high ($\geq 75\%$) according to the criteria proposed by Soyong (1999). The experimental design for each trial was completely randomized design with 4 replications.

Evaluation of the effects of endophytic actinomycete on plant growth promotion

Eighty Chinese kale seeds were treated by soaking in spore suspension of each endophytic actinomycete isolate for 10 minutes before sowing into the sterile compost in cells of seed germinating plastic tray while control treatment were treated with sterile distilled water. After seed germination for 2 weeks, the data on fresh weight, plant height, and root length were recorded. The experimental design was completely randomized with 12 treatments and 4 replications using 20 seedlings per one replication.

RESULTS AND DISCUSSION

Identification of plant pathogenic fungi and culture of endophytic actinomycetes

The causing pathogens of the collected disease samples were identified as follows; *Alternaria brassicicola* for alternaria leaf spot of Chinese kale, *Alternaria brassicae* for leaf spot of cabbage, *Cercospora* sp. for leaf spot of Chinese kale, *Sclerotium* sp. for root rot of Chinese kale, *Choanephora* sp. for wet rot of Chinese kale and *Fusarium* sp. for wilt of lettuce. Their pathogenicity tests were proved by Koch's postulation and the pure cultures were kept in PDA slants for the subsequent experiment.

Eleven isolates of endophytic actinomycete, being kept at 4°C in IMA-2 slants and covered with mineral oil, were successfully re-cultured and well grown on the same medium. The morphology of mycelium and spore chain of each isolate was normal compared to the previous report of Pukclai et al. (2006).

Inhibitory efficiencies on fungal pathogens of the endophytic actinomycetes

The results of *in vitro* trials on dual cultures after incubation for 5-10 days revealed that some endophytic actinomycetes had the antagonistic properties against the tested pathogens (Table 1). The examples of inhibitory effect of the some endophytic actinomycete isolates on radial growth of fungal pathogens on dual cultures were shown in Figure 1. The results shown in Table 1 indicated that two soil borne pathogens, *Sclerotium* sp. was extremely inhibited by BER1, NEE1, KMI1 and HOU1 endophytic actinomycete isolates while *Fusarium* sp. was inhibited by KAE1, GAL1 LEM1 and GLI1 isolates. Two of air borne pathogens, *Choanephora* sp. and *A. brassicae* were inhibited by KAE1, GAL1 and LEM isolates. Among 11 isolates of endophytic actinomycete isolates tested at least 5 isolates showed significant antimicrobial activity on soil and air borne pathogens. Two of the tested endophytic actinomycete isolates, HOU1 and LEM1 were found to have strong acute

inhibition (%PIRG ≥ 90) to soil borne pathogen. El-Tarabily et al. (2010) found out that some streptomycete and non-streptomycete endophytic actinomycete isolates showed their very high abilities to suppress *Pythium aphanidermatum* soil borne pathogen of cucumber seedlings by producing cell-wall-degrading enzymes and diffusible inhibitory metabolites to inhibit growth of the fungi. They also conducted the disease control experiment using the mixture of these isolates which showed inhibitory effects as good as metalaxyl when it was applied in soil along with cellulose amendment. According to the results of this study, mixtures of some effective isolates of endophytic actinomycete could be used in field trials to control both soil borne and air borne diseases of brassica.

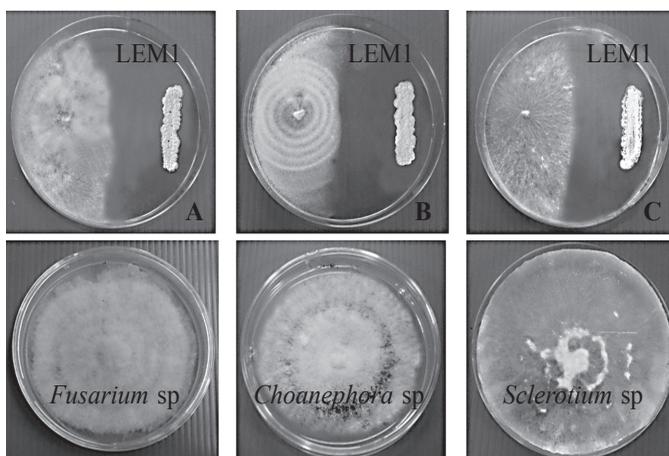


Figure 1. Inhibition of radial growth of fungal pathogens after dual cultured with endophytic actinomycete isolates for 10 days, upper row; A: LEM1 and *Fusarium* sp., B: LEM1 and *Choanephora* sp. and C: HOU1 and *Sclerotium*, compared to control treatments in the lower row.

Table 1. Efficiency of 11 endophytic actinomycete isolates in controlling fungal pathogens.

Isolates	Inhibition percentage (%) ¹					
	<i>Sclerotium</i>	<i>Fusarium</i>	<i>Choanephora</i>	<i>A. brassicae</i>	<i>A. brassicicola</i>	<i>Cercospora</i>
KAE1	72.62 ^{b*}	87.50 ^a	82.03 ^a	75.00 ^a	57.81 ^g	60.72 ^a
GAL1	64.88 ^{bc}	89.88 ^a	80.47 ^a	78.34 ^a	56.25 ^g	64.29 ^a
LEM1	55.95 ^{cde}	91.67 ^a	84.38 ^a	77.50 ^a	56.25 ^g	57.15 ^a
GLI1	49.40 ^{de}	75.00 ^b	65.63 ^b	63.34 ^{cd}	71.88 ^h	53.13 ^a
BER1	88.10 ^a	51.79 ^c	32.81 ^d	60.00 ^{cd}	52.50 ^g	67.68 ^a
NEE1	89.88 ^a	55.95 ^c	42.19 ^{cd}	60.84 ^{cd}	54.69 ^g	75.00 ^a
KMI1	89.29 ^a	57.14 ^c	43.75 ^{cd}	57.50 ^d	70.31 ^{cde}	71.43 ^a
HOU1	91.07 ^a	52.38 ^c	35.16 ^d	50.00 ^e	70.31 ^{cde}	78.57 ^a
FIT2	48.22 ^e	53.58 ^c	39.06 ^{cd}	72.50 ^{ab}	82.81 ^a	64.29 ^a
FIT3	60.71 ^{bcde}	57.14 ^c	42.19 ^{cd}	66.67 ^{bc}	75.00 ^b	67.86 ^a
FIT5	61.31 ^{bcd}	68.45 ^b	53.13 ^{bc}	74.17 ^a	67.19 ^{ef}	67.86 ^a
CV (%)	12.92	10.83	18.48	7.21	4.33	15.46

¹Values followed by the same letter within a column are not significantly different ($P > 0.05$)

Effects of endophytic actinomycete isolates on growth promotion of brassica seedlings

At 2 weeks after treating with endophytic actinomycetes (Table 2), brassica seedling from the treatment of HOU1 isolate treating had significant improvement on root length about 107% as compared to uninoculated control. Among all tested isolates HOU1 was the best and significantly better than the other isolate except FIT5. Though the following treatments, FIT5, FIT3, FIT2, BER1 and GAL1 did not differ significantly from the control for the effects on root length but they showed a trend to increase root length about 42, 39, 35 and 15 % over that of all tested endophytic actinomycete isolates on fresh weight and height of brassica seedlings were not found compared to uninoculated control. Nevertheless, some isolates such as GAL1, NEE1, FIT2, FIT3 and FIT5 showed a trend to increase bout fresh weight (11-37%) and plant height (3-24%) while BER1 and HOU1 showed a trend to improve plant height (8-16%) only. On isolate, KAE1 even showed a trend to reduce all studies growth parameters. This isolate was significantly different from GAL1, FIT2, FIT3 and FIT5 for the effects on seedling fresh weight, and from NEE1 and FIT3 for the effects on plant height. Merzaeva and Shirokikh (2010) reported that several endophytic actinobacteria isolated from winter rye produce indole-3-acetic acid. Treatment of winter rye seeds with auxin producing strains increased the germination capability and enhanced an intensive seedling growth *in vitro*. The other types of growth promoting substances, pteridic acid A and B were produced by *Streptomyces hygroscopicus* TP-A0451 endophytic actinomycete isolate obtained from a stem of bracken *Pteridium aquilinum*. At high concentration of pteridic acid (100 ppm) rice germination was inhibited but at 20 ppm pteridic acid promotes root elongation (Igarashi et al., 2002). In this study, the tendency of GAL1, NEE1, HOU1, FIT2, FIT3 and FIT5 endophytic actinomycete isolates to stimulate brassica seedling growth might be due to their abilities to produce plant growth promoting substances. Further investigation on the abilities of these tested endophytic actinomycete isolate are needed.

Table 2. Effects of endophytic actinomycete isolates on fresh weight, plant height and root length of brassica seedling at 2 weeks after seed germination.

Treatment	Fresh weight (g)	Plant height (cm)	Root length (cm)
KAE1	0.17 ^{c1} (63) ²	4.13 ^{cd} (84)	3.70 ^b (78.2)
GAL1	0.37 ^a (137)	5.03 ^{abcd} (103)	5.43 ^b (115)
LEM1	0.27 ^{abc} (100)	3.93 ^d (80.2)	4.77 ^b (101)
GLI1	0.20 ^{bc} (74)	3.8 ^d (77.6)	3.73 ^b (79)
BER1	0.27 ^{abc} (100)	5.30 ^{abcd} (108)	6.17 ^b (130)
NEE1	0.30 ^{ab} (111)	6.07 ^a (124)	4.83 ^b (102)
KMI1	0.20 ^{bc} (74)	4.3 ^{bcd} (88)	5.20 ^b (110)
HOU1	0.27 ^{abc} (74)	5.67 ^{abc} (116)	9.80 ^a (207)
FIT2	0.33 ^a (122)	5.57 ^{abc} (114)	6.40 ^b (135)
FIT3	0.33 ^a (122)	5.83 ^{ab} (119)	6.53 ^b (139)
FIT5	0.33 ^a (122)	5.33 ^{abcd} (109)	6.73 ^{ab} (142)
Control	0.27 ^{abc} (100)	4.90 ^{abcd} (100)	4.73 ^b (100)
LSD _{0.05}	0.13	1.60	3.1
CV (%)	27.69	19.07	32.48

¹Values followed by the same letter within a column are not significantly different ($P>0.05$)

²Numbers in parenthesis are related values in comparison with control

When the experimental results on the antifungal pathogen activities and the effects of the treated endophytic actinomycete isolates were considered together it was noticed that HOU1 which showed vigorous growth inhibition of *Sclerotium*, the soil borne fungal pathogen was the best isolate for improvement of root length. Thus, further testing this isolate for its effect on soil borne disease control by pot trial is needed since the information obtained from this study indicating its potential to be used as one of the biocontrol agent for soil borne disease particularly *Sclerotium*. Regarding to KAE1 and LEM1 which showed good performances on growth inhibition against *Fusarium*, soil borne disease fungi and two of air borne fungal pathogens, *Choanephora* and *A. brassicae* but these isolates did not show stimulating effects on both root and shoot development of brassica seedlings. Thus KAE1 and LEM1 may not be useful for direct application to the plants for disease controlling but it is worth to investigate the effect of its cultured filtrate on disease controlling.

CONCLUSION

Among the eleven isolates of KAE1, GAL1 and LEM1 were found to have broad spectrum to control *Fusarium*, soil borne pathogen and two air borne pathogens, *Choanephora* and *A. brassicae* with very high inhibitory efficiency at the laboratory level. Only one isolate, FIT5 showed fairly high inhibitory efficiency level against all the tested fungal pathogens. HOU1 isolate, had significant improvement on root length of brassica seedling as compared to uninoculated control and this isolate was the best and significantly better than the other isolates except FIT5. HOU1 isolate also showed the highest inhibitory percentage on *Sclerotium*, soil borne disease fungi. FIT2 and FIT3 isolates which showed the strongly inhibition against some of air borne disease fungi also had a trend to stimulate the growth of brassica seedlings.

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