As. J. Food Ag-Ind. **2009**, *Special Issue*, S93-S98

Asian Journal of Food and Agro-Industry
ISSN 1906-3040
Available online at www.ajofai.info

The potential of endophytic actinomycetes, (*Streptomyces* sp.) for the biocontrol of powdery mildew disease in sweet pea (*Pisum sativum*)

Podjanee Sangmanee[1]*, Ampan Bhromsiri[2] and Angsana Akarapisan[3]

[1]Faculty of Agriculture, Uttaradit Rajabhat University, Uttaradit, Thailand.

[2]Tangerine farmer competitiveness project, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand.

[3]Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand.

*Author to whom correspondence should be addressed, email: u4508191@hotmail.com

This article was originally presented at the International Symposium "GoOrganic2009", Bangkok, Thailand, August 2009.

Abstract

The effectiveness of a selected isolate of endophytic actinomycetes (*Streptomyces* sp.) to control powdery mildew in sweet pea (*Pisum sativum*) was evaluated in the field. The trial was conducted at the Maeya Noi experimental unit of Inthanon Royal Project Research Station, northern Thailand with an elevation of about 1600 meters ASL during the wet season of 2008. The experimental design was a split plot with 4 replications using disease control treatments as the 2 main plots including with and without inoculation of a P4 isolate of endophytic *Streptomyces* sp. and three varieties of sweet pea, namely snap pea, sweet pea and top green pea consisted of the sub plot treatments. Inoculation of the P4 isolate was done by seed inoculation with a mycelial suspension and a foliar spraying with spore suspension, three times at 15, 34 and 49 DAT. It was found that when the pea plants were seriously infected by *Oidium* sp. powdery mildew pathogenic fungus at 67 DAT the P4 inoculation resulted in significant reduction of percentage of leaf damaged by powdery mildew in the upper and middle parts plants. The percentage of leaf area in the upper part which showed powdery mildew disease symptoms from P4 inoculated treatment were reduced about 45% for snap pea, 48% for sugar pea and 82% for top green pea compared to those of uninoculated control treatments. The severity symptoms of powdery mildew infection was affected significantly by sweet pea

varieties. The effect of spraying of P4 spore suspension on the inhibition of appressorial formation of Odium fungal spores was also observed.

Keywords: on endophytic actinomycetes: *Streptomyces* sp.: biocontrol: powdery mildew: sweet pea

---

Introduction

Sweet pea (*Pisum sativum*) is one of the vegetable crops which grows well on the highlands of northern Thailand. This leguminous cash crop is sensitive to powdery mildew disease caused by Odium sp., an obligate pathogenic fungus. To get satisfactory yield of sweet peas, alternating foliar spraying of Benlate and Caratan is recommended. However, the use of alternative disease controls by biological means is becoming more desirable for highland agriculture due to the increased awareness of the environmental pollution caused by agrochemicals. The use of endophytic actinomycetes for plant disease control is currently an active subject of research. In the case of sweet pea, Thapanapongworakul (2003) found out that a P4 isolate of endophytic actinomycetes obtained from sweet pea roots was antagonistic against various plant diseases. This endophytic actinomycete was identified as *Streptomyces* sp. Not only was it effective for disease control, but the P4 isolate was also compatible with several leguminous hosts and root nodule bacteria. (Thapanapongworakul, 2003). Furthermore, seed inoculation with mycelial suspensions of the P4-isolate showed a trend to reduce powdery mildew symptoms in sweet pea under pot trials in an open field on a highland area (Akarapisan et al., 2007). This experiment was therefore conducted to evaluate the potential of a P4 streptomyces isolate for powdery mildew disease control on sweet pea varieties commonly grown in the northern highland area of Thailand.

Materials and Methods

A field experiment was conducted in the wet season of 2008 at Maeya Noi experimental unit, Inthanon Research Station of Royal Project. The experimental site was located at about 1600 meters ASL. The experiment had a nested split plot design with 4 replications. There were two main plot treatments: with and without a P4-isolate of *Streptomyces* sp. The P4-isolate was determined previously by Thapanapongwarakul (2003), to be an effective endophytic actinomycetes (*Streptomyces* sp.) for sweet pea. Three sweet pea varieties namely, Sugar Pea with round shape pods, Snap Pea with flat pods and Top Green pea were assigned as subplots. Inoculation of P4 was done firstly by applying a mycelial suspension of P4 in the broth of IM medium on the surface sterilized seeds of each sweet pea variety germinated in seedling tray using a sterile peat moss pot mix. The rate of inoculation was 106 cfu/seed. One week after seed germination, seedlings from all treatments were transplanted in the field. All plots were sprinkle irrigated as needed. The standard fertilizer application rates used at the Royal Project Experiment Station were used as follow: 8 ton/rai of chicken manure before seedling transplanting, the mixture of 46-0-0 and 15-15-15 (1:1 ratio) at the rate of 16.5 kg/rai at 10 days after transplanting (DAT) and 16.5 kg/rai of 13-13-21 at 40 DAT [HV note- if possible could use rates of Kg/Ha]. The percentage of leaf area showing symptoms of powdery mildew disease was evaluated at 38, 52 and 67 DAT from the lower, middle and upper parts of the plants using 5 randomly selected plants per plot.

Results and Discussion

At the early growth stage, powdery mildew disease symptoms were not observed in all sweet pea plants. The occurrence of this disease was firstl observed at 49 DAT and the symptom was severe by 67 DAT. When the pea plants were severely infected by powdery mildew (67 DAT), the leaves from the lower parts of the pea plants (1^{st} – 6^{th} node) from all varieties, both with and without P4 inoculation, were seriously damaged (Figure 1).

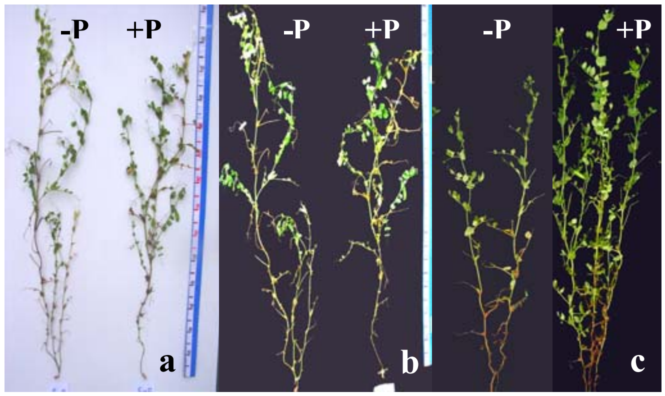

Figure 1.: Appearance of different sweet pea varieties with and without P4 inoculation at 67 DAT.

(a) snap pea,

(b) sugar pea,

(c) top green pea

However, the percentage of leaf area showing powdery mildew symptoms in the middle (7^{th} – 12^{th} node) and the upper parts (14^{th} – 20^{th} node) of the pea plants were significantly affected by P4 inoculation and the severity of disease damage in the upper plant parts varied significantly by pea variety (Table 1).

Table 1. Analysis of variance of percentage of leaf area infected by powdery mildew disease at 67 DAT.

SOV	df	plant part		
		lower part	middle part	upper part
disease control	1	NS[1/]	*	**
(I)	3	NS	NS	NS
block	3			
error A	2	NS	NS	**
pea varieties	2	NS	NS	NS
(V)	12			
I x V				
error B				

[1/] NS = non significant, * = significant at P< 0.05, ** = significant at P< 0.01

Interaction effects between P4 inoculation and sweet pea variety on powdery mildew disease symptoms on all plant parts were not significant. Based on an average of all sweet pea varieties, inoculation with the P4 isolate resulted in a significant reduction of percentage of leaf area damaged by powdery mildew in the middle plant parts by about 39% while that uninoculated control treatment were reduced by about 64% (Table 2).

Table 2. Percentage of pea leaves from the middle part of the plants infected by powdery mildew disease at 67 DAT.

varieties	disease control		means of varieties
	-P	+P	
snap pea	82.5	40.0	61.3
sugar pea	65.0	40.0	52.5
top green pea	45.0	37.5	41.3
means of disease control	64.2 a	39.2 b	

The percentage of leaves showing disease symptoms in the upper part of plants was significantly affected by P4 inoculation. The leaf area percentage in the upper parts which showed powdery mildew disease symptoms on the P4 inoculated treatments were reduced by about 45% for snap pea, 48% for sugar pea and 82% for top green pea compare to the uninoculated controls (Table 3). These results indicated that snap peas had a greater disease severity damage than sugar pea and top green pea, respectively ($P<0.01$).

Table 3. Percentage of pea leaves from the upper part of the plants infected by powdery mildew disease at 67 DAT.

varieties	Disease control		Means of varieties
	-P	+P	
Snap pea	50.0	27.5	38.75 A
	(100)	(45)*	
Sugar pea	32.5	17.0	12.38 B
	(100)	(48)	
Top green pea	27.5	5.0	16.25 C
	(100)	(82)	
Means of disease control	35.7 a	16.5 b	

* The reduction of percentage of pea leaves infected by powdery mildew compared with –P.

Among the three tested pea varieties, top green pea was the most tolerant to powdery mildew followed by sugar pea while snap pea was the most sensitive one ($P < 0.05$). Akarapisan et al. (2007) also observed a positive trend of P4 seed inoculation for control of powdery mildew in these same three sweet pea varieties under pot experiments grown in open fields on the highland area at Nong Hoi Royal Project Experimental Station, during the wet growing season. Our experimental observations supported their results.

According to Samac et al. (2003), alfalfa leaves, roots and nodules were colonized by endophytic actinomyces following inoculation of the planting mixture. The population densities of inoculated *Streptomyces* on the plant surface were affected by inoculation concentration. They suggested that the host plant should be able to provide sufficient nutrients to support development of selective high population densities of *Streptomyces* on the rhizosphere soil without sacrificing plant biomass. In this experiment even though a positive effect of P4 inoculation on shoot biomass in all tested sweet pea varieties was not significant, a better growth was observed in P4 inoculated plants at all studied periods (data not shown).

Thus, the tested pea varieties, particularly the top green pea and sugar pea varieties might provide sufficient nutrients to inoculated P4 isolates for successful establishment in all plant parts resulting in less severity of powdery mildew infection in the middle and upper plant parts.

Nevertheless, ineffective inoculations of the P4 isolate may occur under windy conditions and without the use of effective surfactant/adhesive materials when spraying the P4 isolate spore suspension, resulting in poor stomatal penetration and incomplete protection of the plant against powdery mildew infection. it is plausible that the density of the P4 inocolum in the lower leaves might have been too low resulting in less effective powdery mildew control in the lower plant parts. Additional experiments are needed to determine the population densities of inoculated endophytes required to produce sufficient antifungal compounds on the leaf surface in order to better understand the mechanisms of disease control by the P4 isolate.

Throughout this experiment, we did not confirm the existance of inoculated endophytes in the tissue of sweet pea plants but we did observ interesting an phenomenon of P4 isolates under microscopic investigation. A majority of the powdery mildew spores placed on onion tissue could not form appressoria after spraying of P4 isolate spore suspensions of on the fungal spores (Figure 2).

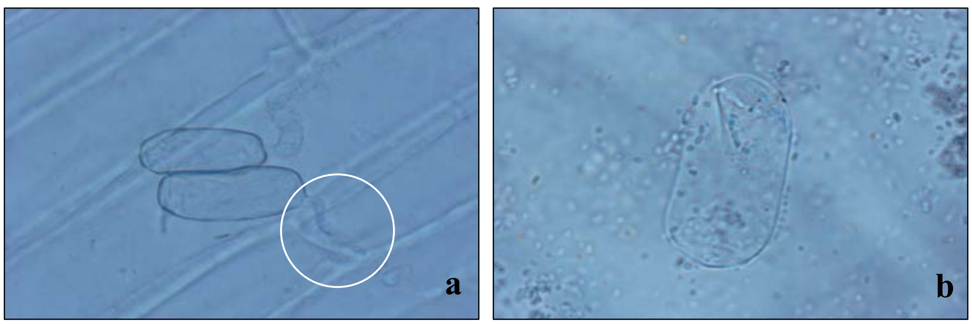

Figure 2. Appressorial formation of Odium fungal spore treated without (a) and with P4 (b) spore suspension.

The potential use of P4 isolates of *Streptomyces* sp. for the cultivation of sweet pea and other legumes calls for further investigation, not only because of its ability to control powdery mildew in important legumes, but also because the P4 isolate has shown positive synergistic effects on rhizobia nodulation, N2 fixation and on the growth of leguminous host plants (sweet pea, Akarapisan et al., 2008; and soybean, Soe, 2009). These positive interactions were observed under the proper environmental conditions, and when the proper varieties, and proper nodule bacteria were used with this isolate.. Our experimental results and those reported by Akarapisan et al. (2008) and Soe (2009) suggest that the P4 isolate is one of potential value as a microbial biocontrol agent with potential application for the organic production of leguminous crops of economic importance in Thailand.

Conclusions

Seed inoculation with mycelial suspensions of the *Streptomyces* sp., P4 isolate used at the rate of 10^6 cfu/seed in three foliar sprays with spore suspensions of P4 (106 cfu/mL.) were effective to significantly reduce the leaf area percentage infected by powdery mildew disease in sweet peas under field conditions in northern Thailand. The severity of powdery mildew disease damage varied significantly between the sweet pea varieties.

Acknowledgements

This research received financial support from the Highland Research and Development Institute and benefited from the generous collaboration provided by the Inthanon Royal Research station, Chiang Mai province, Thailand.

References

Akarapisan, A., Bhromsiri, A. and Thapanapongworakul, N. (2007). Effectiveness testing of endophytic actinomycete *Streptomyces* sp. isolate P4 for N2 fixing ability and controlling of sugar pea diseases. **Final Report for Highland Research and Development Institute**, 58 p.

Akarapisan, A., Bhromsiri, A. and Sangmanee, P. (2008). Selection of suitable isolates of endophyitc actinomycetes and rhizobia for improvement of N_2 fixation and disease control of various *Pisum sativum* on the highland area. **Final Report for Highland Research and Development Institute**, 63 p.

Samac, D.A., Willert, A.M., McBride, M.J. and Kinkel, L.L. (2003). Effects of antibiotic-producing *Streptomyces* on nodulation and leaf spot in alfalfa. **Appl. Soil Ecol.**, Vol. 22, pp. 55-56.

Soe, K.M. (2009). Effects of endophytic actinomycetes and bradyrhizobia on nodulation and nitrogen fixation of different soybean varieties. MS. Thesis. Chiang Mai Univ. Thailand. 99 p.

Thapanapongworakul, P. (2003). Characterization of endophytic actinomycetes capable of controlling sweet pea root rot diseases and effects on root nodule bacteria. MS. Thesis. Chiang Mai Univ. Thailand. 109 p.

Tokala, R.K., Strap, J.L., Jung, C.M., Crawford, D.L., Salove, M.H., Deobald, L.A., Bailey, J.F. and Morra, M.J. (2002). Novel plant-microbe rhizosphere interaction involving *Streptomyces lydicus* WYEC 108 and the pea plant (*Pisum sativum*). **Appl. Environ. Microbio.**, Vol. 68, pp. 2161-2171.