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Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. การนำผลงานวิจัยไปใช้ประโยชน์

มีการนำผลของงานวิจัยไปเผยแพร่และเสนอแนะอย่างไม่เป็นทางการกับผู้ประกอบการผลิตผักไฮโดรโพนิกส์ รวมทั้งได้มีการรับฟังความคิดเห็นและคำวิจารณ์ ถึงความเป็นไปได้ในการนำไปใช้ประโยชน์

ในด้านวิชาการ คณะผู้วิจัยได้นำผลงานวิจัยนี้ไปเป็นองค์ความรู้ใหม่ใช้ประกอบการเรียนการสอนให้กับนักศึกษาระดับปริญญาตรีและปริญญาโท ที่คณะเทคโนโลยีการเกษตร สถาบันเทคโนโลยีพระจอมเกล้าเจ้าคุณทหารลาดกระบัง และ คณะเกษตร มหาวิทยาลัยเกษตรศาสตร์ และยังสามารถใช้เป็นข้อมูลเพื่อใช้ในการวิจัยและพัฒนาต่อยอดต่อไป ให้แก่นักศึกษาระดับปริญญาเอก

2. การเสนอผลงานในที่ประชุมวิชาการ

Jaenaksorn, T., P. Koohakan and S. Pratuangwong. 2010. Bacterial antagonists increase growth promotion and natural defense response of lettuce (*Lactuca sativa* L.) under hydroponics. Proceedings of the 12th International Conference on Plant Pathogenic Bacteria. Parc des Expositions et des Congres de Saint-Denis, Ile de la Reunion, France. June 7-11, 2010. (p 140).

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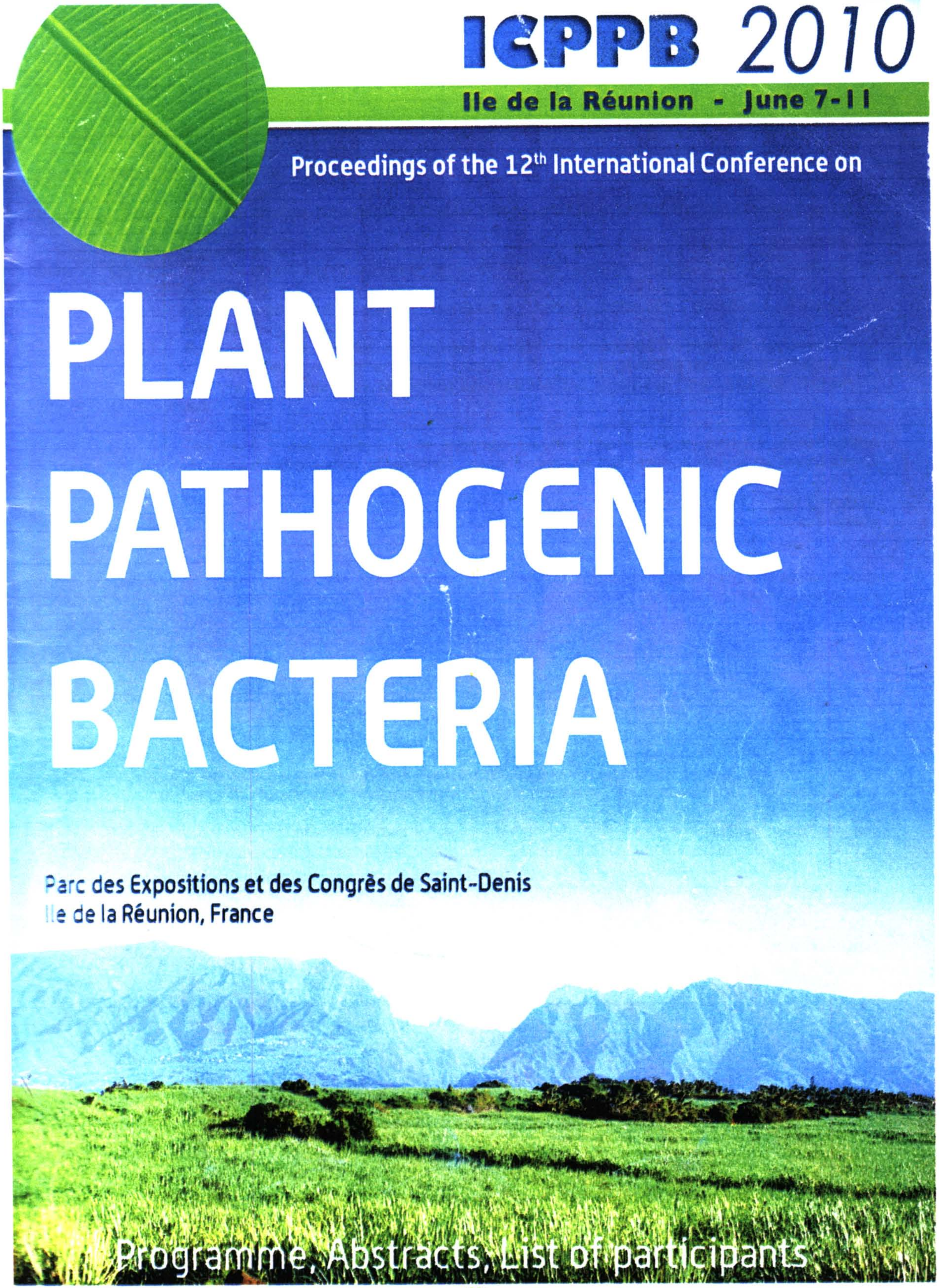
ภาคผนวก



ICPPB 2010

Ile de la Réunion - June 7-11

Proceedings of the 12th International Conference on

PLANT PATHOGENIC BACTERIA

Parc des Expositions et des Congrès de Saint-Denis
Ile de la Réunion, France

Programme, Abstracts, List of participants

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Thursday, June 10, Session 3 : Molecular Plant Pathogen Interactions & Plant Resistance

Chair : ***Subhadeep Chatterjee & Mari-Ann Newman***

Keynote talk

9h00 K3-05 Bacterial Microbe Associated Molecular Patterns (MAMPs): elicitors of plant innate immunity
Newman M.-A.

Session talks

9h45 O3-22 The *Xanthomonas campestris* type III effector XopAC triggers vascular immunity in *Arabidopsis*
Guy E., Chabannes M., Hajri A., Genissel A., David P., Messad N., Boureau T., Poussier S., Arlat M. and L. D. Noël

10h05 O3-23 Hpa2, as a T3S Effector, is regulated by HrpG and required by HrpF for pathogenicity of *Xanthomonas oryzae* pv. *oryzicola* in rice, but not for hypersensitive response in tobacco
Yu-Rong Li, Yi-Zhou Che, Hua-Song Zou, Li-Fang Zou, Gong-You Chen, and Alan Collmer

10h25 ***Coffee break***

10h45 O3-24 The PecS global regulator is an important player in the regulatory network governing the coordinated expression of virulence genes during the interaction between *Dickeya dadantii* and *Arabidopsis*
Mehdbi N., Malfatti P., Gaubert S., Alunni B., Chapelle E., Reverchon S. and F. Van Gijsegem

11h05 O3-25 The dicarboxylate transport protein (KgtP) is required as a T3S effector for dicarboxylate utilization and full virulence by rice pathogen *Xanthomonas oryzae* pv. *oryzae*
Liu X. L., Zou H. S., Zou L. F., Li Y. R. and G. Y. Chen

11h25 O3-26 *In planta* comparative gene expression profiling of *Ralstonia solanacearum* reveals primary physiology and new regulatory model for type three secretion during bacterial wilt pathogenesis
Jacobs J. M., Meng F., Babujee L., Milling A. and C. Allen

11h45 ***Posters***

P4-65 The effect of plant extracts as seed treatment on the control of bacterial leaf spot of tomato caused by xanthomonads pathogens
Mbega E. R., Mortensen C. N., Mabagala R. B. and E. G. Wulff

P4-66 Antagonistic activity of the rhizobacterial isolates and plant secondary metabolites towards plant pathogens from *Dickeya*, *Pectobacterium* and *Pseudomonas* genera
Jafra S., Potrykus M., Krzyzanowska D., Golanowska M., Taraszkiewicz A., Lojkowska E. and A. Krolicka

P4-67 Endophytic *Methylobacterium* influences potato and pine disease resistance in cultivar- and treatment-specific manner by inducing changes in plant resident mirobiota
Ardanov P., Sessitsch A., Häggman H., Kozyrovska N. and A. M. Pirttilä

P4-68 David versus Goliath: understanding how plant pathogenic *Pseudomonas* resist invertebrate predators
Dorati F., Sanchez-Contreras M., Arnold D. L., Waterfield N. R. and R. W. Jackson

P4-69 Characterization of bacteria isolated from rotten potato tissue exposing antagonistic activity towards *Dickeya solani*
Czajkowski R., Van Veen J. A. and J. M. Van der Wolf

P4-70 Endophytic bacteria for suppressing banana blood disease bacterium
Hadiba N., Wibowo A., Widada J. and S. Subandiyah

P4-71 *Pantoea agglomerans* biosafety for use in plant protection: genotypic and phenotypic comparison of biocontrol and clinical isolates
Rezzonico F., Bonaterra A., Badosa E., Smits T. H. M, Frey J. E., Montesinos E. and B. Duffy

P4-72 Characterization of the antibacterial peptide herbicolin I biosynthetic operon in the fire blight biocontrol agent *Pantoea vagans* C9-1
Kamber T., Lansdell T. A., Smits T. H. M., Stockwell V. O., Ishimaru C. and B. Duffy

P4-73 Pantocin A antibiotic produced by *Pantoea vagans* C9-1: Chemical and genetic characterization
Ishimaru C. A., Lansdell T. A., Gross J., Clardy J., Smits T. H. M. and B. Duffy

P4-74 Bacterial antagonists increase growth promotion and natural defense response of lettuce (*Lactuca sativa* L.) under hydroponics
Jaenaksorn T., Koohakan P. and S. Pratuangwong

P4-75 Bacterial canker of mango in Japan caused by yellow-pigmented strain of *Xanthomonas campestris* pv. *mangiferaeindicae*
Furuya N., Kinoshita K., Nishi N., Kurose D. and K. Tsuchiya

P4-76 Bacterial shoot blight of mango (*Mangifera indica* L.) caused by *Erwinia chrysanthemi*
Tsuchiya K, Miyahira N., Takushi T., Kawano S. and N. Furuya

Bacterial antagonists increase growth promotion and natural defense response of lettuce (*Lactuca sativa* L.) under hydroponics

Jaenaksorn T.[1], Koohakan P.[1] and S. Pratuangwong[2]

[1]Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Thailand; [2]Faculty of Agriculture, Kasetsart University, Thailand.

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The 468 strains of endophytic and epiphytic bacteria were isolated from recirculating hydroponic system. Of which, some strains were shown to be effective in inhibiting the *in vitro* growth of *Pythium* sp., a cause of root rot of lettuce. Percent inhibition was between 87.9 and 65.8 which strain KUKL205 showed the best potential equivalent to commercial strain *Pseudomonas fluorescens* SP007s in suppressing the target pathogen. The efficiency of seed treatment with tested antagonist bacteria significantly gave better results in terms of seed germination, shoot and root length compared to non-treated control. Seed treatment and mixed nutrient solution with each antagonist exhibited good protection against disease (86.2% reduction). The accumulation of β-1,3 glucanase, a defense-related enzyme was also greater in bacterial antagonist-treated plants compared to control. Based on biochemical and 16S rRNA-PCR characterization, strain KUKL205 obtained in this study was identified as *Bacillus* sp. To our knowledge, this is the first report of induced systemic resistance under hydroponics with plant growth promoting rhizobacteria.

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1 Chen *et al.*, 2000. *Physiological and Molecular Plant Pathology* 56, 13-23.
2 Pan *et al.*, 1991. *Physiol Mol Plant Pathol* 39, 25–39.
Prathuangwong S. 2007. Acta Phytopathol et Entomol Hungarica 42: 321-330.

Keywords:
bacterial antagonist, endophytic and epiphytic bacteria, PGPR, lettuce, hydroponics

Bacterial antagonists increase growth promotion and natural defense response of lettuce (*Lactuca sativa* L.) under hydroponics

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Keywords: bacterial antagonist, endophytic and epiphytic bacteria, PGPR, lettuce, hydroponics

Introduction

Presently, *Pythium* causing root rot is a real problem in recirculating hydroponic systems. Biological-based approach to hydroponic cultivation may probably provide a helping hand in managing the root disease and maintaining a more sustainable and healthier hydroponic crop ecosystem. Therefore the objective of this study was first to evaluate the four reported-beneficial rhizosphere bacteria, having high antagonistic potential in suppressing important pathogens, for their biological control of *Pythium* root rot of lettuce (*Lactuca sativa* L.) in hydroponics compared with commercial product. Second, isolation, screening and evaluating the *in vitro* antagonistic ability of new indigenous endophytic and epiphytic bacteria from recirculating hydroponic system was conducted. Third, the most beneficial indigenous isolate was further evaluated in lab-scale hydroponics. Besides, β-1,3 glucanase, a defense-related enzyme was checked accordingly.

Materials and Methods

Evaluation of reported-rhizosphere bacteria for controlling Pythium root rot of lettuce in hydroponics

1. **Evaluation of four reported-rhizosphere bacteria for controlling Pythium root rot of Cos grown in NFT**

This experiment was aimed to determine whether the beneficial rhizosphere bacteria earlier reported to be the potential antagonists against several pathogens of soil-grown crops (1, 2) could retard the disease of hydroponic crops. Therefore, the ability of *Bacillus amyloliquefaciens* (KPS46), *Paenibacillus pabuli* (SW01/4), *Pseudomonas fluorescens* (SP007s) and *B. lichiniformis* (SP009s) for controlling Pythium root rot of Cos grown in outdoor NFT was investigated. Treatments were arranged in CRD with three replications of 9 plants. The pH and EC of NS was monitored daily. Antagonists (OD 0.1, 120 ml) were applied directly into the NFT-nutrient solution at 2 days before inoculation with 5×10^5 sporangium / ml of *Pythium* sp. Disease severity and crop growth was weekly assessed. Besides, pathogen and BCAs survival as well as potential of antagonists was weekly monitored in NS while last check was made on root at harvest.

Results and Discussions

Regarding the NFT experiment, the pathogen-inoculated control significantly showed mushy and sad root system compared to the non inoculated control. Besides, the soft to slimy rotted portion of the root was easily separated from the inner core. Unexpectedly, diseases severity of lettuce in the treatments added with either the tested beneficial rhizosphere bacteria or bacterial bioproduct were not different from that in the pathogen-inoculated control (Table 1). Figure 1 revealed that pathogen survivals in NS of all tested treatments decreased sharply in the 1st-2nd wk. and continued to decrease till harvest. Surprisingly, the BCAs survival could hardly be detected either from nutrient solution or root. To sum up, the all tested BCAs did not improve disease control in NFT although the *in vitro* bi-culture was positive (Figure 2). In terms of crop growth, the tested treatments did not significantly influence shoot height, leaf number, leaf size, root length, shoot or root fresh weight compared to those of inoculated control (Table 2, Figure 3). This might probably due to the incidence of having nil BCAs survival in the cropping system. This finding was not in line with the other reports (1, 2, 3, 4) of which achieved good result of using some rhizosphere bacteria for controlling damping-off caused by *Pythium aphanidermatum.*

Table 1 Disease severity of Cos grown in NFT applied with 4 beneficial rhizosphere bacteria and one commercial biocontrol product.

Treatment	Disease severity[1/]		
	1st week	2nd week	3rd week
Non-inoculated control	0	0	0
Inoculated control	1.05	3.50	2.56
T1	0.94	2.72	3.17
T2	0.80	2.89	3.00
T3	1.06	2.89	3.39
T4	1.11	2.83	2.67
T5	1.22	3.22	3.22

[1/] Based on pathogenicity test, disease severity was assessed from incidence of brown root tips and percent total roots with brown discoloration using increment scale of 0-5.

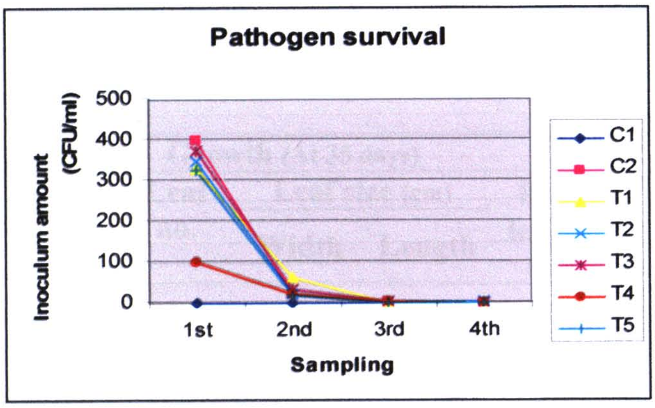

Figure 1 Pathogen survival in NFT-nutrient solution during cropping.

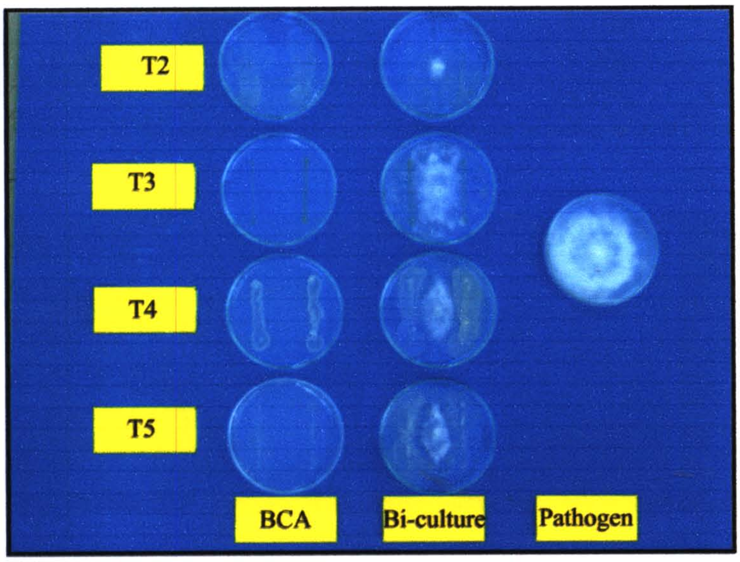

Figure 2 Bi-culture for rechecking the potential of BCAs (beneficial rhizobacteria) after being added into the nutrient solution.

Table 2 Growth and yield of Cos grown in NFT-nutrient solution applied with beneficial rhizosphere bacteria and one commercial biocontrol product for controlling Pythium root rot.

Treatment	Growth (At 35 days)					Yield (g)	
	Shoot height (cm)	Leaf no.	Leaf size (cm) Width	Leaf size (cm) Length	Root length (cm)	Shoot fresh wt.	Root fresh wt.
Non-inoculated control	15.81[1/]a	19.3a	8.48a	11.45a	42.50a	93.82a	23.45a
Inoculated control	10.69bc	11.9b	4.91b	7.17b	6.43b	10.03b	5.06b
T1	9.54bc	11.7b	4.50b	5.83bc	4.68b	7.51b	4.61b
T2	10.61bc	12.1b	4.87b	6.40bc	4.90b	10.17b	5.04b
T3	9.93bc	11.8b	4.79b	6.08bc	4.82b	9.50b	4.05b
T4	9.08c	10.3b	3.89b	5.24c	5.79b	6.43b	2.51b
T5	10.90b	12.0b	4.97b	6.32bc	5.90b	11.51b	4.93b
CV	16.72%	25.37%	24.37%	21.30%	55.92%	130.92%	81.70%

[1/] Means within the column followed by the same letter are not significantly different according to DMRT (P<0.01)

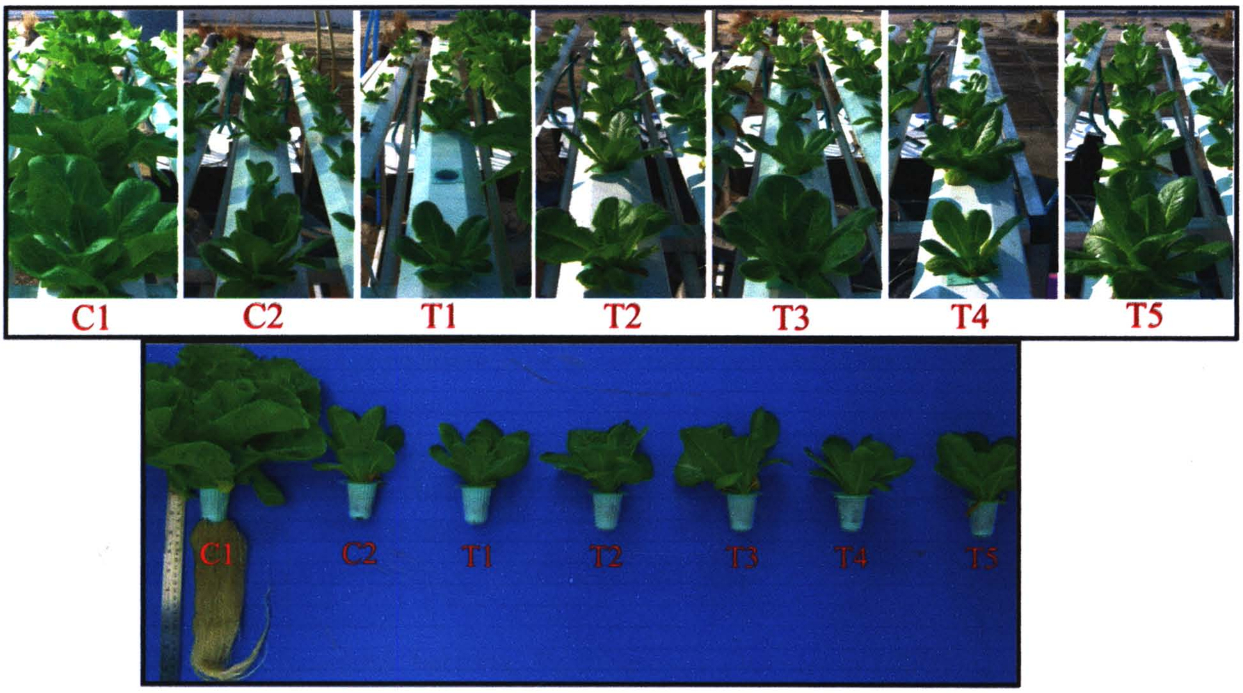

Figure 3 Growth at harvest of Cos in NFT-nutrient solution applied with 4 kinds of four beneficial rhizosphere bacteria (T1, T2, T3 and T4) and one commercial product (T5) for controlling Pythium root rot.

2. Evaluation of reported-rhizosphere bacteria for controlling Pythium root rot of green oak grown in DRFT

2.1 *Testing their ability for germination stimulation and growth promoting of lettuce (green oak) seedling*

Four reported-rhizosphere bacteria were tested for promoting the seed germination seedling growth. Green oak seeds were disinfested with 10% clorox for 5 min. then soaked with distilled water 3 min. twice before soaking in antagonist suspension of 10^6 cfu/ml for 10 min. and let them air-dried. Treated seeds were separately checked for germination by blotter method. Rate of germination, root length, stem height of seedlings were noted comparing with non-treated control.

Result

The efficiency of seed treatment with tested antagonistic bacteria significantly gave better results under laboratory condition in terms of seed germination, shoot and root length compared to that of the control (Table 3). Regarding this, seed treatment with *Pseudomonas fluorescens* (SP007s) was the best.

Table 3 Efficiency of seed treatment with four antagonistic bacteria as PGPR on the germination and growth of green oak seedling under laboratory condition.[1/]

Treatment	Laboratory condition		
	Seed germination (%)	**Shoot length (cm)**	**Root length (cm)**
T1 (KPS46)	98b	4.43b	4.10b
T2 (SW01/4)	100a	4.43b	4.95a
T3 (SP007s)	100a	5.47a	5.26a
T4 (SP009s)	98b	4.87b	4.98a
Control[2/]	92c	3.48c	3.86c

[1/] Means of 10 replications. Means in a column followed by the same letter are not significantly different according to DMRT.

[2/] Without seed bacterization.

2.2 *Testing their ability against Pythium sp.*

Four rhizosphere bacteria were tested for their ability against *Pythium sp* using dual culture and mixed culture.

Result

Among the tested rhizosphere bacteria, *Pseudomonas fluorescens* (SP007s) was the best in retarding the growth of *Pythium sp.* under dual culture and mixed culture (Table 4, Figure 4).

Table 4 Effect of antagonistic bacteria on growth of *Pythium* sp.

Treatment	Growth inhibition of *Pythium* sp.(%)
T1 (KPS46)	55.56c
T2 (SW01/4)	66.67b
T3 (SP007s)	75.00a
T4 (SP009s)	62.00b
Control	0c

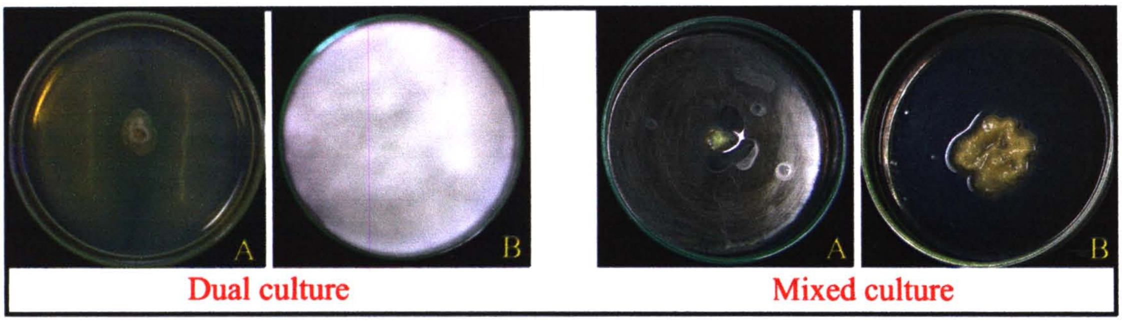

Figure 4 Effect of *Pseudomonas fluorescens* (SP007s) on growth of *Pythium* sp.:1. Dual culture (A), Control (B) 2. Mixed culture (A), Control (B)

2.3 *Testing their viability in nutrient solution*

Rhizosphere bacterium showing the highest ability from 1.1 and 1.2 was selected. Then its viability (using 10^{6} and 10^{9} cfu / ml) was tested in flask containing 500 ml of hydroponic nutrient solution. During incubation (shaking condition for 1 month), its viability was checked weekly.

Result

Regarding the viability of *Pseudomonas fluorescens* (SP007s) in nutrient solution, it could survive up to 21 days, after that the cell amount decreased (Table 5).

Table 5 Viability of *Pseudomonas fluorescens* (SP007s) in nutrient solution.

O.D.	Cell amount (cfu/ml)				
	0 day	7 days	14 days	21 days	28 days
0.1	2.3x10^{4}	3.1x10^{6}	1.1x10^{8}	2.3x10^{6}	1.2x10^{3}
0.2	3.2x10^{6}	4.3x10^{8}	3.2x10^{9}	6.1x10^{7}	2.1x10^{4}

2.4 *Evaluation of the selected rhizosphere bacteria for controlling Pythium root rot of lettuce (green oak) grown in DRFT*

Based on the result of the 2.1, 2.2, 2.3 experiment, *Pseudomonas fluorescens* (SP007s) was chosen to evaluate the appropriate application for controlling Pythium root rot of lettuce (green oak) in DRFT (Figure 4). Treatments [seed treatment (T1), application into nutrient solution (T2), seed trt + application into nutrient solution (T3) and control] were arranged in CRD with three replications of 20 plants. All tested treatments were inoculated with 5x10^5 sporangium / ml of *Pythium* sp. The pH and EC of NS was monitored daily. Parameters measured were disease incidence and crop growth.

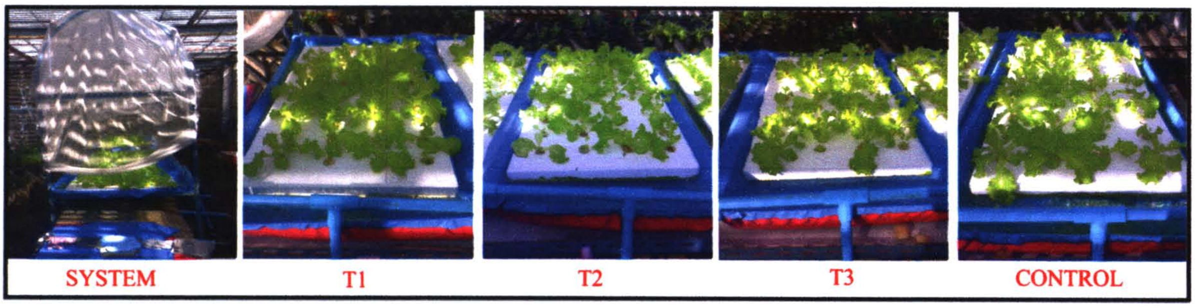

Figure 5 DRFT system for evaluating the beneficial rhizosphere bacteria for controlling Pythium root rot of lettuce (green oak).

Result

All tested methods of applying the *Pseudomonas fluorescens* (SP007s) into the DRFT system could significantly reduce the disease incidence compared with that of control (Table 6). Besides, combined method [seed trt + application into nutrient solution (T3)] was the best on this regard. Crop growth result was in line with the disease incidence, that is combined application gave the best result in terms of shoot and root length as well as fresh weight (Figure 6).

Table 6 Effect of *Pseudomonas fluorescens* (SP007s) on Pythium root rot of lettuce (green oak) grown in DRFT.

Treatment	Disease incidence (%)
Seed treatment (T1)	35c
Application into nutrient solution (T2)	25b
Seed trt + Application into nutrient solution (T3)	15a
Control	85d

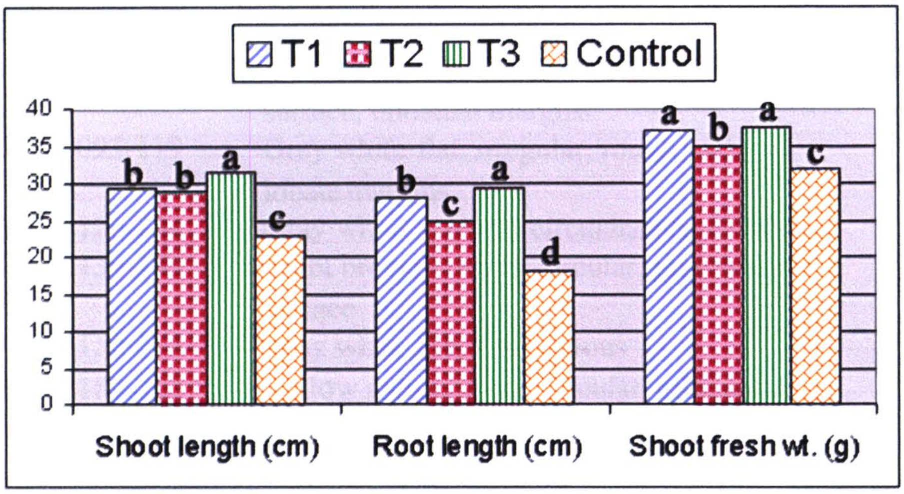

Figure 6 Growth and yield of lettuce (green oak) grown in DRFT added with *Pseudomonas fluorescens* (SP007s).

Based on both experiments, it should be pointed out that the method of applying the beneficial rhizosphere bacteria into hydroponic system plays the crucial role for achieving their satisfying antagonist potential.

Isolation, screening and evaluating the *in vitro*-ability of new indigenous endophytic and epiphytic bacteria from recirculating hydroponic systems

Indigenous rhizosphere bacteria were isolated, using serial dilutions and spread on selective media in Petri dishes, from nutrient solution and root of hydroponic crops collected from commercial hydroponics located in central region of Thailand. Morphologically distinct bacteria were isolated and grown in pure culture on NA.

Result

468 isolates of miscellaneous endophytic and epiphytic bacteria are obtained (Table 1, Figure 1) and some isolates are shown to be effective in inhibiting the *in vitro* growth of

Pythium sp. (Table 2, Figure 2). Percent inhibition is between 87.9 and 65.8 while isolate KUKL 205 is the best potential to produce secondary metabolites for suppressing the target pathogen.

Table 1 Bacterial morphotypes on NA isolated from nutrient solution and plant parts (root, stem, and leaf) grown in hydroponics.

Group	Bacterial isolate	Morphotypes[1/]	Number of isolate[2/]
1	KUKL001-057	Gray white, flat, circular, glint slimy	57
2	KUKL058-080	Yellow, convex, glint slimy	23
3	KUKL081-092	Gray white, convex, circular, glint smooth surface, undulate margins	12
4	KUKL093-119	Gray white, flat, irregular, rough surface, lobate margins	27
5	KUKL120-138	Gray white, convex, circular, lobate margins	19
6	KUKL139-149	Light brown, convex, circular, glint smooth surface	11
7	KUKL150-188	Gray white, flat, filamentous	39
8	KUKL189-201	Yellow pale, convex, circular, glint smooth surface, lobate margins	13
9	KUKL202-210	Yellow pale, irregular, glint smooth surface	9
10	KUKL211-225	Light white, oval, entire margins	15
11	KUKL226-253	Gray white, flat	28
12	KUKL254-274	Gray white, flat, circular, undulate margins	21
13	KUKL275-284	Gray white, circular, glint smooth surface	10
14	KUKL285-291	Gray white, convex, irregular, glint smooth surface	7
15	KUKL292-313	Brown yellow (soluble on medium), irregular	22
16	KUKL314-533	Gray white, convex, glint, smooth surface	20
17	KUKL534-346	Gray white, convex, circular, glint smooth surface, spread colony	13
18	KUKL347-374	Gray white, irregular, smooth surface	28
19	KUKL375-400	White, flat, rough surface, undulate margins	26
20	KUKL401-415	Dark yellow, irregular, flat, smooth surface, entire margins	15
21	KUKL416-447	Dark yellow, circular, undulate margins	32
22	KUKL448-454	Gray white, circular, umbilicate, rough surface, undulate margins	7
23	KUKL456-468	Whitish cream, circular, convex, entire margins	14

[1/] Comparison of bacterial morphotypes based on color and colony characteristics.

[2/] Total collected bacteria are 468 isolates.

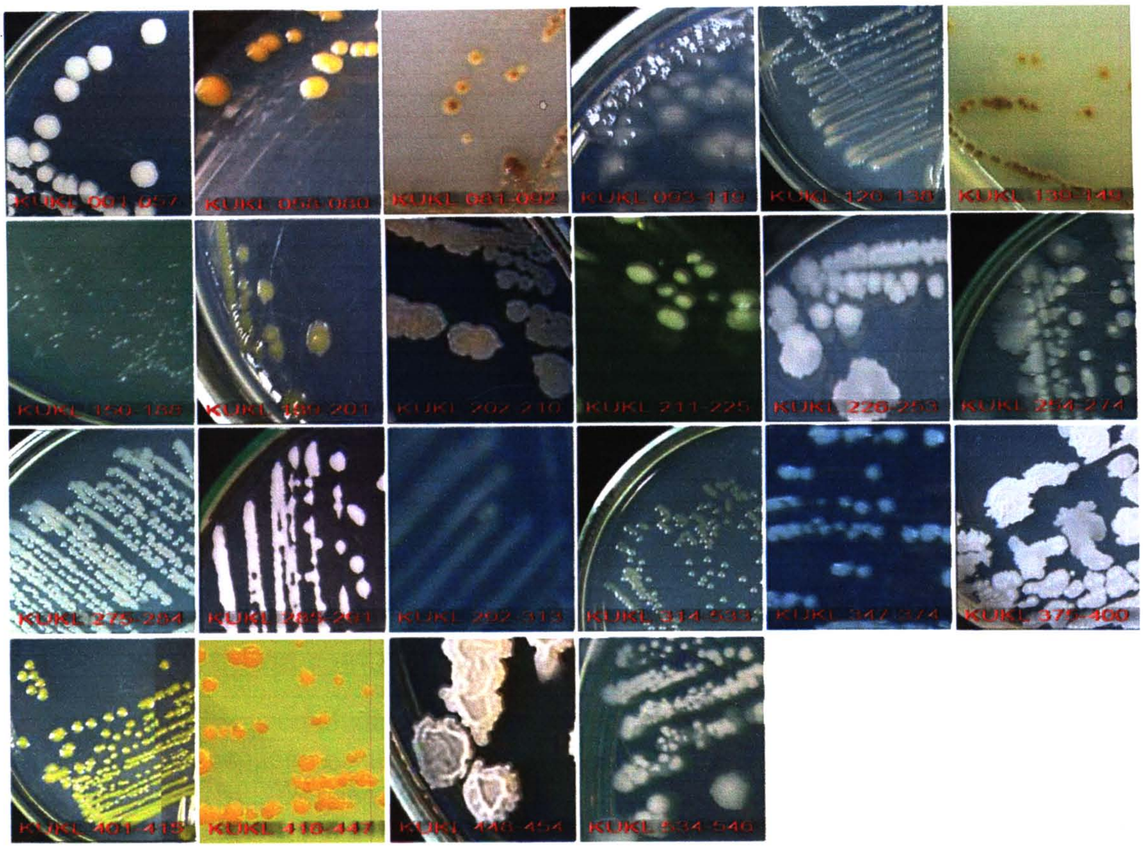

Figure 1 Bacterial morphotypes on NA isolated from nutrient solution and plant parts (root, stem, and leaf) grown in hydroponics

Table 2 Inhibition of *Pythium* sp. by different antagonistic bacteria with dual culture method under laboratory condition.

Bacterial isolate	Source	Inhibition diameter[1/] (mm)	% Inhibition
KUKL357	Root of pak-khom-kaew	30.8	65.8^{c}
KUKL432	Root of green spinach	30.8	65.8^{c}
KUKL209	Root of butter head lettuce	30.8	65.8^{c}
KUKL319	Leaf of pak-khom-kaew	20.5	77.3^{b}
KUKL210	Leaf of green oak	30.5	66.2^{c}
KUKL205	Leaf of peppermint	10.9	87.9^{a}
KUKL077	Leaf of butter head	30.6	66.0^{c}
KUKL189	Nutrient solution in green spinach culture	30.8	65.8^{c}
KUKL344	Nutrient solution in butter head culture	40.0	55.6^{d}
KUKL371	Nutrient solution in mint culture	30.6	66.0^{c}
Control	ddH_2O	90	0^{e}

[1/] Means calculated from 4 replications.

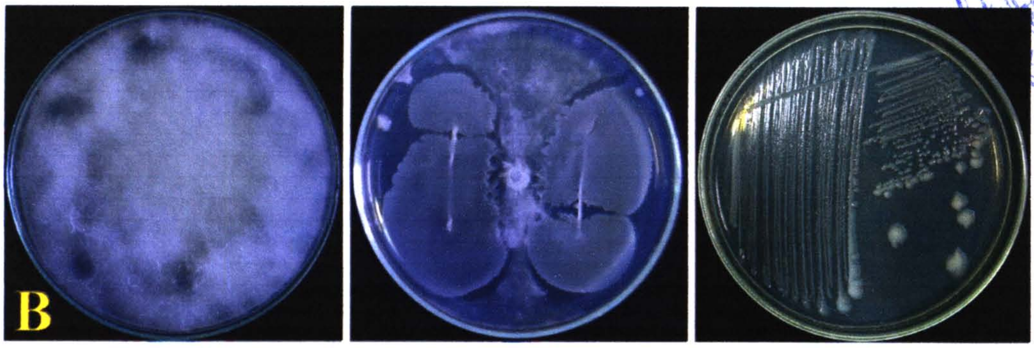

Figure 2 Bi culture test of bacterial isolate (KUKL 205) shown to be effective in inhibiting the *in vitro* growth of *Pythium* sp.

Evaluation of the new indigenous rhizosphere bacterium (KUKL 205) for controlling Pythium root rot of lettuce in hydroponics

This experiment was conducted to determine the effect of KUKL205, the newly-obtained indigenous rhizosphere bacterium, on root rot of lettuce in DRFT compared to the reported PGPR (SP007s) and the elicitor (salicylic acid). Besides, the accumulation of β-1, 3-glucanase, the defense-related enzyme, in the treated plants was assayed by the laminarin dinitrosalicylic acid method (Pan *et al.,* 1991; Buensanteai *et al.,* 2007) during 6 days after pathogen-inoculation.

Result

All the bacterial treated-plants showed no symptom of root rot compared to the non-treated plants (Figure 1). However, all the treatments had the same leaf SPAD value. Regarding the defense-related enzyme, it was found that the accumulation of β-1, 3-glucanase in bacterial antagonist-treated plants which were inoculated with *Pythium* sp. were significantly greater than those in non-treated control (Figure 2). β-1, 3-glucanase activity of the KUKL205 treatment seemed to be in line with the SA and SP007s treatments. However, the accumulation of this enzyme in the SA and SP007s treatments were significantly higher than that in KUKL205 treatment on the 2nd and 3rd day after pathogen-inoculation (Figure 2).

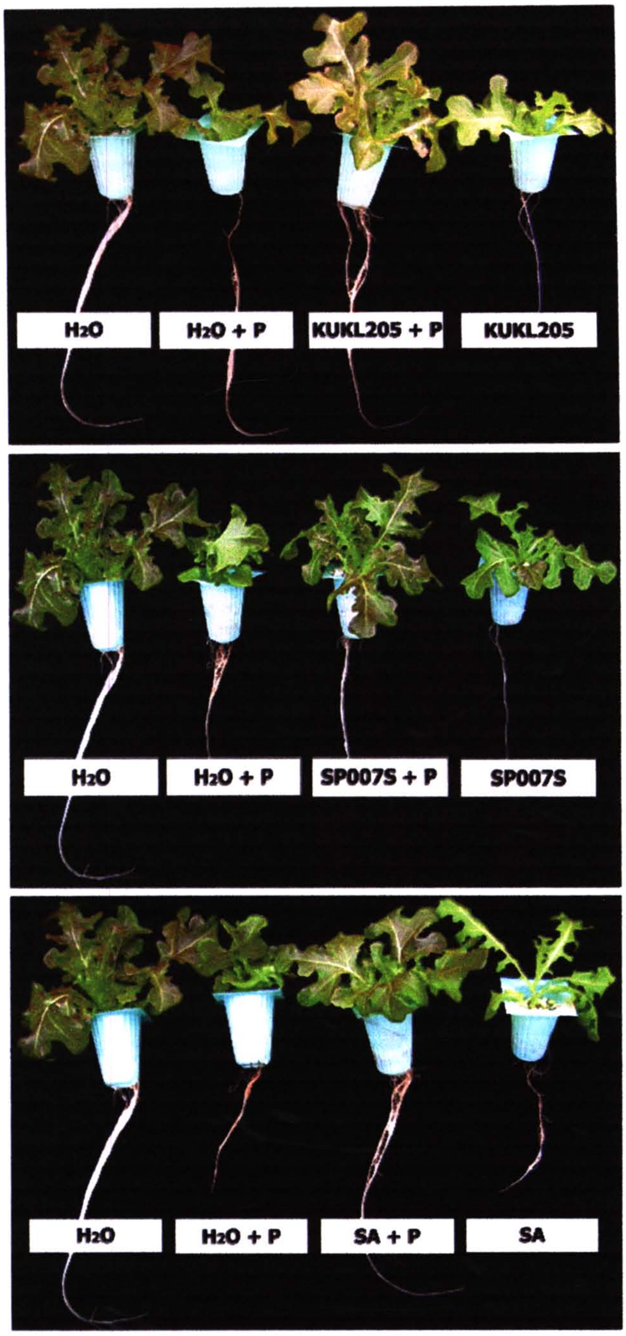

Figure 1 Effect of bacterial antagonists (including an indigenous PGPR) as seed treatment on Pythium root rot of lettuce grown in DRFT.

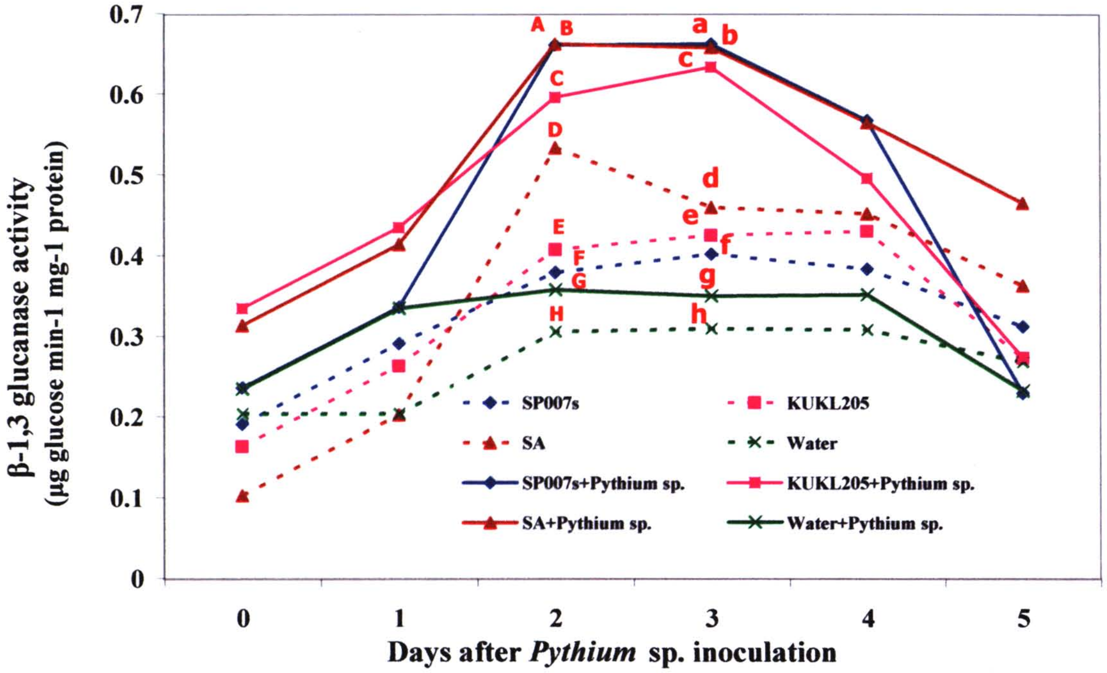

Figure 2 β-1, 3-glucanase activity of the antagonists-treated plants and non-treated plants during 6 days after *Pythium* sp.-inoculation.

Conclusion

1. Directly applying the four beneficial rhizoshere bacteria and bioproduct into the hydroponic nutrient solution gave unsatisfactory result in controlling Pythium root rot of lettuce since their survival in the system was hardly detected.
2. Among the four tested rhizosphere bacteria, seed treatment with *Pseudomonas fluorescens* (SP007s) gave the best result in terms of percent seed germination and growth of green oak seedling under laboratory condition. Seed treatment + directly added into nutrient solution gave the best result in decreasing the disease incidence and increasing the crop growth.
3. Based on biochemical and 16S rRNA-PCR characterization, KUKL205, the newly-obtained indigenous rhizosphere bacterium, was identified as *Bacillus* sp. being effective in inhibiting the *in vitro* growth of *Pythium* sp.
4. The accumulation of β-1,3 glucanase, a defense-related enzyme was also greater in bacterial antagonists-treated plants compared to control which was in line with disease incidence.

Acknowledgement

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P6-12

Test of Four Commercial Bioproducts on Pythium Root Rot of Hydroponically-Grown Cos Lettuce (*Lactuca sativa* L.)

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Abstract

Hydroponics originally came about to eliminate many of the variables that contribute to disease in the field, but other variables may have been created in the process. At present, *Pythium* causing root rot is a real problem in recirculating hydroponic systems as they provide ideal condition for rapid growth and spread of infectious propagules. Besides, it is an opportunistic disease which means that it is looking for weak or injured plants. To manage this disease, biological-based approach would be implemented for maintaining a more sustainable and healthier hydroponic crop ecosystem. Therefore, the research was aimed to determine if bioproducts presently marketed for conventional crops could retard the disease in hydroponics. First, the *Pythium* pathogenicity test on lettuce (*Lactuca sativa* L.) was performed in deep flow technique for obtaining the most aggressive species to be used in the following experiment. Second, four commercial bioproducts (*Trichoderma*) for soil-grown crops were evaluated for their biological control of *Pythium* root rot of lettuce grown in nutrient film technique. Their effect on crop growth and yield was determined while the survival of pathogen and biological control agents were weekly monitored from nutrient solution and from plant root at harvest. Also, the advantages and limitations of the measures will be discussed.

Keywords: *Pythium* spp., root rot, Cos lettuce, bioproducts, *Trichoderma,* NFT, DFT

Introduction

Hydroponics originally came about to eliminate many of the variables that contribute to disease in the field, but other variables may have been created in the process. Presently, *Pythium* causing root rot is a real problem in recirculating hydroponic systems as they provide ideal conditions for rapid growth and spread of infectious spores. Besides, it is an opportunistic disease which means that it is looking for weak or injured plants. To manage this disease, biological-based approach would be implemented for maintaining a more sustainable and healthier hydroponic crop ecosystem. The objectives of this research were: (i) to study the *Pythium* pathogenicity test on lettuce (*Lactuca sativa* L.) in deep flow technique (ii) to determine if commercial bioproduct for conventional crops have potential in controlling root rot disease of hydroponic lettuce.

Materials and Methods

Pythium pathogenicity test with lettuce (Lactuca sativa L.) in DFT

Survey on Pythium root rot was conducted at commercial hydroponic farms in Bangkok, Samutprakarn, Nakornrajsima. Then five isolates of *Pythium* sp. obtained from the survey were tested for their pathogenicity with 4 kinds of lettuce in DFT (data not shown). The most aggressive species of *Pythium*, the most susceptible lettuce (Cos) and disease index obtained from this experiment are employed in the following trials.

Evaluation of commercial bioproducts for controlling Pythium root rot of Cos grown in NFT

This experiment was aimed to determine if bioproducts presently marketed for conventional crops could retard the disease of hydroponic crops. Hence the activity of 4 formulations of commercial products of *Trichoderma harzianum* [starter (T1), wettable powder (T2, T3) and spore suspension (T4)] for controlling Pythium root rot of Cos grown in outdoor NFT was investigated. Three replications of 9 plants per treatment were arranged in CRD. The pH and EC of nutrient solution (NS) was monitored daily. Each product was applied at the recommended rate to the NFT-nutrient solution at 2 days before inoculation with 5×10^6 zoospores/mL of *Pythium* sp. Disease severity and crop growth were weekly assessed until harvest. Besides, pathogen and BCAs survival was weekly monitored in NS and last check was made on root at harvest.

Results and Discussion

Evaluation of commercial bioproducts for controlling Pythium root rot of Cos grown in NFT

No root discoloration developed in the uninoculated control. Application of the 4 products did not give any control of root rot compared to that in inoculated control (Table 1). Figure 1 revealed that pathogen survivals in NS of all tested treatments decreased sharply in the 1st-2nd wk. and continued decreasing till harvest. Unlike, the BCAs survival tended to fluctuate from week to week (Figure 1). It should be pointed out that BCAs in T2, T3, T4 were hardly detected at 1, 2, 3 days after application into NS. At harvest, there were significantly more cfu of BCAs and pathogen recovered from root than from NS (Figure 1). The detected buildup of BCAs in the harvested root although it was applied to the nutrient solution suggests that application into recirculation nutrient solution would allow association of the agent with the roots. However, whether the buildup density is sufficient to effectively control *Pythium* sp. or not would be considered.

Responses of plant growth components to the tested products were in line with those in the disease control. ANOVA indicated that treatments did not significantly influence shoot height, leaf number, leaf size, root length, shoot fresh or root fresh weight compared to inoculated control (Table 2, Figure 2). This finding does not agree with preliminary observations with other vegetables crops grown in DFT (Hatairat and Tanimnun, 2007).

Collectively, findings that the tested products were ineffective against Pythium root rot disease in NFT which are not in line with recent observation with other DFT crops probably suggest that the agent might be host-specific in controlling. Besides, it is possible that NFT would not provide the ideal condition to maintain the agents in the system sufficient to control the disease compared to that in DFT. The unsuited formulation to hydroponics as well as quality of the products would possibly be attributed to the experiment.

Table 1 Disease severity of Cos grown in NFT applied with commercial bioproducts.

Treatment	Disease severity[1/]		
	1st Week	2nd Week	3rd Week
Non-inoculated control	0	0	0
Inoculated control	1	1.5	2.5
T1	1	1.72	2.72
T2	1	1.4	2.3
T3	1	1.1	2.6
T4	1	1.0	2.4

[1/] Based on pathogenicity test, disease severity was assessed from incidence of brown root tips and percent total roots with brown discoloration using increment scale of 0-5.

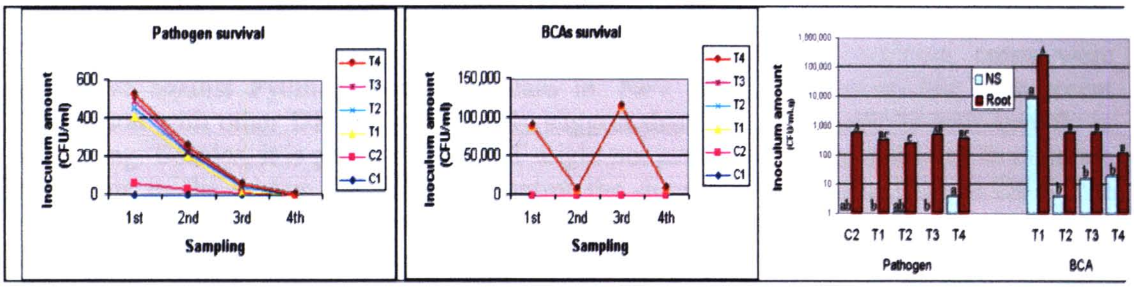

Fig. 1 Pathogen and BCAs survival in NFT-nutrient solution during cropping, their recovery from NFT-nutrient solution and root of Cos at harvest. [1/]Bars in each group labeled by the same letter are not significantly different according to DMRT (P<0.01).

Table 2 Growth at harvest and yield of Cos grown in NFT-nutrient solution applied with 4 kinds of commercial bioproducts for controlling Pythium root rot.

Treatment	Growth					Yield (g)	
	Shoot height (cm)	Leaf no.	Leaf size (cm)		Root length (cm)	Shoot fresh wt.	Root fresh wt.
			Width	Length			
Non-inoculated control	19.04[1/]a	18a	10.15a	14.3a	48.45a	118.62a	20.48a
Inoculated control	12.11b	12b	6.41bc	8.9b	10.31b	19.3bc	8.11b
T1	11.95b	13b	4.92de	6.44cd	8.77b	14.12bc	6.2b
T2	12.26b	13b	5.47cd	7.31c	5.79b	17.46bc	7.78b
T3	9.6c	13b	4.22de	5.27d	4.2b	8.56c	5.51b
T4	13.16b	14b	6.55b	9.76b	6.8b	23.02b	7.6b
CV	4.9%	5.7%	4.1%	4.8%	18.7%	10.1%	15.6%

[1/]Means within the column followed by the same letter are not significantly different according to DMRT (P<0.01)

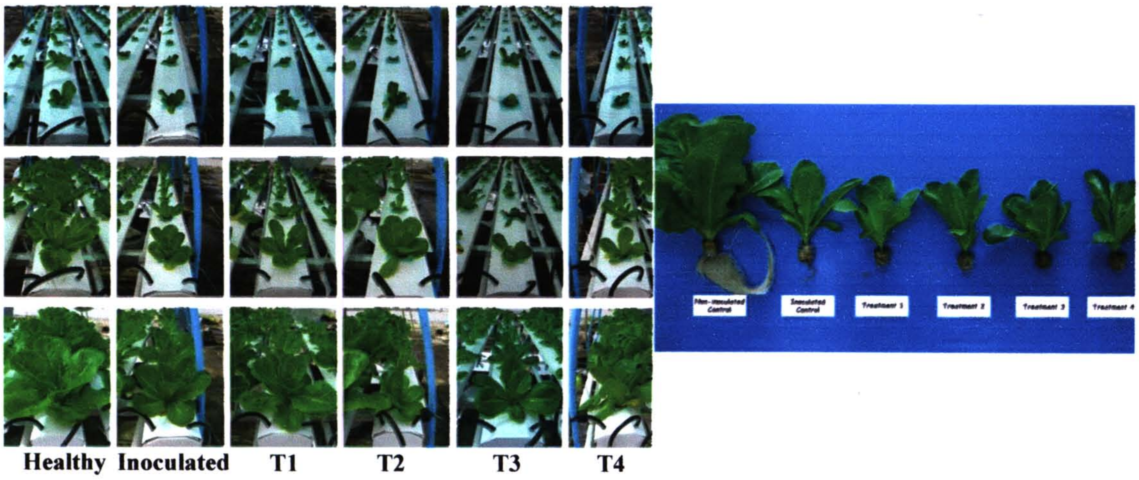

Fig. 2 Growth at 1st wk, 2nd wk and harvest of Cos in NFT-nutrient solution applied with 4 kinds of commercial bioproducts for controlling Pythium root rot (A). Inoculated control (C2), T1, T2, T3 and T4 showed the mushy and sad root (B).

Conclusion

The four formulations of commercial bioproducts for soil-grown crops were ineffective against Pythium root rot disease in. NFT which are not in line with recent observation with other DFT crops probably suggest that the agent might be host-specific in controlling. Besides, it is possible that NFT might not provide the ideal condition to maintain the agents in the system sufficient to control the disease compared to that in DFT. The unsuited formulation to hydroponics as well as quality of the products would possibly be attributed to the experiment.

Acknowledgement

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P6-13

Efficacy of Indigenous Trichoderma and PGPR for Controlling Pythium Root Rot of Lettuce (*Lactuca sativa* L.) Grown in Deep Flow Technique

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Abstract

Due to the efficacy limit of several bioproducts to be used in hydroponics, employing the indigenous microorganisms including PGPR from hydroponic system as biocontrol agents would be an alternative biological-based approach for sustainable disease management. Therefore, our research aim was to isolate, screen and evaluate the *in vitro* and *in vivo* ability of new indigenous microorganisms against Pythium root rot of lettuce. From the obtained-*in vitro* beneficial indigenous fungi, 3 isolates of *Trichoderma* spp. were determined together with PGPR for their efficacy in controlling Pythium root rot of Cos lettuce grown in deep flow technique (DFT). Indigenous PGPR (KUKL 205) was used as seed treatment while the 3 indigenous *Trichoderma* spp. were applied as root-dip solution and added into DFT-nutrient solution at the concentration of 10^8 conidia/ml (10 ml per plant). Disease incidence, disease severity, survival of pathogen and of biocontrol agents as well as plant growth, were determined accordingly. The result showed that no root discoloration or root lesion developed in the uninoculated control. The application of indigenous PGPR and the 3 isolates of *Trichoderma* spp. did provide significant control of root rot disease compared to that in inoculated control. Responses of plant growth components to the tested treatments were in line with those in the efficacy of disease control.

Keywords: *Pythium* spp., root rot of Cos, *Trichoderma* spp., PGPR, biological control products, DFT

Introduction

Pythium root rot disease is widespread and frequently destructive in lettuce (*Lactuca sativa* L.) grown in hydroponics. To manage this disease, the awareness of the importance of protecting our global environment and the efficacy limit of several bioproducts to be used in hydroponics would be taken into consideration. Therefore, employing the new indigenous microorganisms including PGPR from hydroponic system as biocontrol agent for such a disease would be an alternative biological-based approach for sustainable disease management. Our research was first conducted to isolate, screen and evaluate the *in vitro* antagonistic ability of new indigenous fungi against *Pythium* sp. Second, efficacy of indigenous *Trichoderma* and PGPR for controlling Pythium root rot of lettuce (*Lactuca sativa* L.) grown in Deep Flow Technique was determined. Besides, the synergistic effect of biological control agents on disease control efficacy and plant growth components was considered.

Materials and Methods

Isolation, screening and evaluating the in vitro antagonistic ability of new indigenous fungi against Pythium sp.

Indigenous fungi were isolated, using serial dilutions and spread on selective media in Petri dishes, from nutrient solution and root of hydroponic crops collected from commercial hydroponics located in central region of Thailand. Morphologically distinct fungi were collected and grown in pure culture on PDA for further evaluation on their antagonistic ability against *Pythium* sp. using *in vitro* bi-culture test.

Efficacy of indigenous Trichoderma and PGPR for controlling Pythium root rot of lettuce (Lactuca sativa L.) grown in Deep Flow Technique

Since KUKL205 (*Bacillus* sp.), the indigenous PGPR from hydroponics, was proved to be effective both in inhibiting the *in vitro* growth of *Pythium* sp. and in controlling Pythium root rot of lettuce in DRFT (Jaenaksorn *et al.*, 2010), therefore this experiment was conducted to determine the synergistic effect between KUKL205 and three indigenous *Trichoderma* selected from the above experiment for controlling Pythium root rot of lettuce (*Lactuca sativa* L.) grown in Deep Flow Technique. KUKL205 (OD 0.1) was used as seed treatment (Jaenaksorn *et al.*, 2010) while 3 indigenous *Trichoderma* spp. were applied as root-dip solution and added into DFT-nutrient solution at the concentration of 10^8 conidia/ml (10 ml per plant). Seven treatments were arranged in CRD with five replications of 2 plants. Besides, *Pseudomonas fluorescens* (SP007s)-seed treatment (Prathuangwong, S., 2007) was included as compared treatment. The pH and EC of NS was monitored daily. Disease incidence and severity, survival of pathogen and biocontrol agents as well as plant growth were determined.

Results and Discussion

Isolation, screening and evaluating the in vitro antagonistic ability of new indigenous fungi against Pythium sp.

Regarding bi-culture test, twenty indigenous fungi obtained from hydroponic system gave percent growth inhibition of *Pythium* sp. at 3 days about 14-35 percent. A003, A018 and A019 were chosen for further test in mini DFT since their *in vitro* antagonistic ability at 20 days were quite satisfactory (Fig. 1).

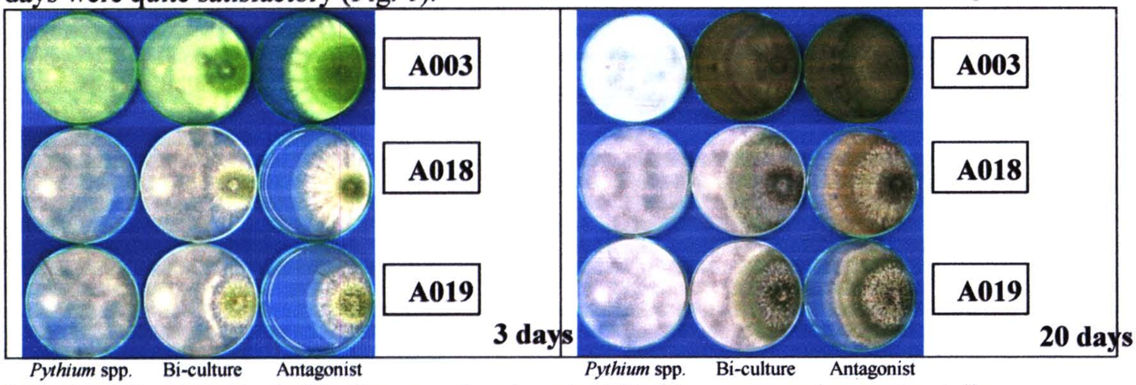

Fig. 1 Bi-culture test of indigenous fungi against *Pythium* sp. causing root rot disease.

Efficacy of indigenous Trichoderma and PGPR for controlling Pythium root rot of Cos (Lactuca sativa L.) grown in Deep Flow Technique

The result showed that no root discoloration or root lesion developed in the healthy control while the pathogen-inoculated control significantly showed mushy and sad root system on 21 dpi (Table 1). The application of indigenous KUKL205 alone (T1) and with the 3 isolates of *Trichoderma* spp.(T2, T3, T4) did provide significant control (on 21 dpi) of root rot disease compared to that in the inoculated control (Table 1, Figure 2). No synergistic effect between indigenous KUKL205 and the 3 indigenous isolates of *Trichoderma* spp. (A003, A018 and A019) was noted on disease control efficacy. Figure 3 revealed that pathogen could survive in harvested-root in all tested treatments except healthy control. Its highest survival was noted on T5 (inoculated control) while the lowest survival was found on T3 (KUKL205+A018). Regarding the *Trichoderma* treatment, their viability could be detected from harvested root which was in line with the 3 reports mentioning that *Trichoderma* spp. could colonize the root of ***Cucumis sativus*** **L.** (Yedidia *et al.*, 1999), *Brassica campestris* L. var. chinensis (Pariyaporn and Jaenaksorn, 2007), and *Brassica oleracea* L. var. acephala DC. (Jaenaksorn and Rachniyom, 2008) after applying into hydroponic system. T3 (KUKL205-seed treatment+A018 in NS) was having the lowest survival of pathogen and the highest viability of *Trichoderma.* This might result in a reduction in disease severity compared to that in inoculated control. Surprisingly, response of plant growth components (especially shoot fresh weight) to T3 was not in line with that in the disease control efficacy (Table 2). For leaf SPAD value, all treatments were not significantly different. In terms of shoot fresh weight, T1 and T2 gave significantly better result than that in inoculated control. Besides, synergistic effect on plant growth was significantly detected in T2 (KUKL205 and *Trichoderma* A003).

Table 1 Root rot disease severity of Cos grown in mini-DFT on the 1, 2, 4, 11 and 21 days post inoculation (dpi) with *Pythium* sp.

Treatment	**Disease severity**$^{1/}$				
	1 dpi	**2 dpi**	**4 dpi**	**11 dpi**	**21 dpi (at harvest)**
T1 (KUKL205-seed treatment)	0.80ab^{2}	1.90b	1.90b	2.20ab	1.30ab
T2 (KUKL205-seed treatment + A003 in NS)	1.00ab	1.80b	1.80b	1.40ab	1.10ab
T3 (KUKL205-seed treatment + A018 in NS)	0.80ab	1.40b	1.90b	2.60ab	1.90b
T4 (KUKL205-seed treatment + A019 in NS)	1.20ab	1.20b	1.40ab	2.80ab	1.80b
T5 (Inoculated control)l	1.80b	2.00b	2.50b	3.30b	3.80c
T6 (Healthy control)	0.20a	0.00a	0.00a	0.00a	0.00a
T7 (SP007s-seed treatment)	1.40ab	1.50b	1.90b	2.30ab	1.20ab
CV (%)	74.85	41.49	57.53	57.27	44.59

$^{1/}$Disease index rated as 0 = healthy root, 1 = 1-20% diseased roots, 2 = 21-40% diseased roots, 3 = 41-60% diseased roots, 4 = 61-80% diseased roots, and 5 = 100% diseased roots and rot.

$^{2/}$Means within the column followed by the same letter are not significantly different at probability level 0.05 by Duncan's Multiple Range Test.

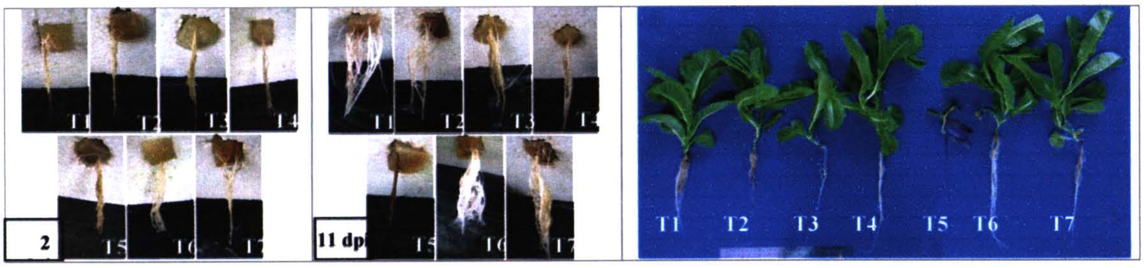

Fig. 2 Cos showing the Pythium root rot disease on 2, 11 and 21 days post inoculation.

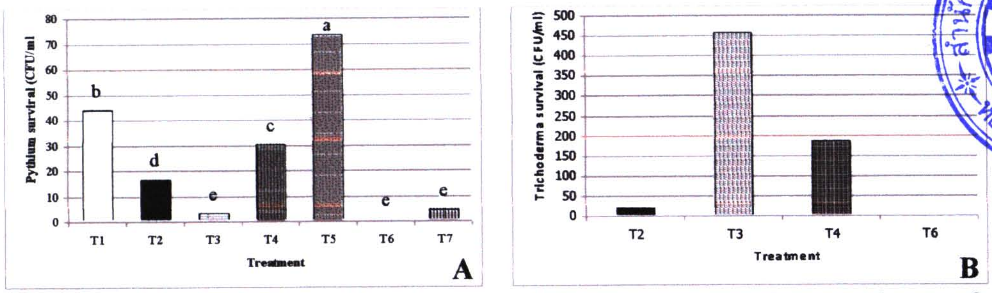

Fig. 3 Survival of *Pythium* (A) and *Trichoderma* spp. (B) from harvested root of treated Cos grown in mini-DFT.

Table 2 Growth and yield of treated Cos in mini-DFT at harvest (42 days).

Treatment	Leaf number (No.)	Leaf area[2/] (cm^2)	Leaf SPAD value	Shoot fresh wt. (g)	Root length (cm)	Root wt. (g)
T1	10.6abc[1/]	108.68a	38.52ab	32.50c	22.44b	2.17b
T2	12.0a	80.80ab	40.81a	39.50b	30.89a	3.45a
T3	8.1c	61.62ab	38.52ab	18.12e	22.65b	1.41c
T4	8.5bc	46.94b	39.15ab	22.55de	13.74c	1.52c
T5	12.0a	74.90ab	37.50ab	26.36d	16.05c	1.98bc
T6	12.5a	111.02a	37.64ab	45.75a	29.01a	3.31a
T7	11.3ab	92.35ab	36.59a	22.25de	24.85b	2.26b
CV (%)	19.10	45.85	6.79	13.00	11.61	18.11

[1/] Means within the column followed by the same letter are not significantly different at probability level 0.05 by Duncan's Multiple Range Test. [2/] Leaf area of the biggest leaf.

Conclusion

The application of indigenous KUKL205 alone (T1) and with the 3 isolates of *Trichoderma* spp.(T2, T3, T4) did provide significant control (on 21 dpi) of root rot disease compared to that in the inoculated control. No synergistic effect between indigenous KUKL205 and the 3 indigenous isolates of *Trichoderma* spp. (A003, A018 and A019) was noted on disease control efficacy. However, synergistic effect on plant growth was significantly noted in T2 (KUKL205 and *Trichoderma* A003).

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