APPENDICES

APPENDIX A

MEDIA

Various media were used in this study as isolation medium, storage medium or test medium and prepared as followed formula (per liter).

Carboxymethylcellulose (CMC) agar or Cellulose agar (Kasana *et al.* 2008)

Sodium nitrate	2	g
Dipotassium hydrogen phosphate	1	g
Magnesium sulfate	0.5	g
Potassium chloride	0.5	g
CMC sodium salt	2	g
Peptone	0.2	g
Agar	15	g

pH 7.0

The media was sterilized by autoclaved at 121˚C for 15 min.

Chrome azurol S agar (Milagres *et al.* 1999)

Potato dextrose agar (PDA)

Potato	200	g
Glucose	20	g
Agar	15	g
Distilled water	900	ml

pH 7.0

Dispensed into containers and sterilized by autoclaved at 121˚C for 15 min. Let it cool to 55-60˚C. Added 100 ml of CAS sterile solution and mixed well.

CAS solution

CAS	60.5	mg
Hexadecyltrimethylammonium	72.9	mg

CAS was dissolved in 50 ml deionized water, and mixed with 10 ml iron (III) solution (1 mM $FeCl.6H_2O$, 10 mM HCl). Under stirring, this solution was slowly mixed with HDTMA dissolved in 40 ml water. The resultant dark blue solution was autoclaved at 121 ˚C for 15 min.

Czapek Dox modified agar (Yamato *et al.* 2005)

Sodium nitrate	0.33	g
Potassium chloride	1	g
Potassium phosphate	0.2	g
Magnesium sulphate	0.2	g
Sucrose	0.5	g
Yeast extract	0.1	g
Agar	15	g

pH 6.8-7.0

The media was sterilized by autoclaved at 121˚C for 15 min.

Oatmeal agar (Stewart and Kane, 2006)

Pulverized rolled oat	3	g
Agar	15	g
pH 6.0		

The rolled oat was dissolved with 200 ml of distilled water and heat at 60˚C in water bath for 4 hours. Then, the suspension and agar was dissolved in 800 ml of distilled water and sterilized by autoclaved at 121˚C for 15 min.

Potato dextrose agar (PDA)

Potato	200	g
Glucose	20	g
Agar	15	g
pH 7.0		

Potato dextrose broth (PDB)

Potato	200	g
Glucose	20	g
pH 7.0		

The pieces of potato (200 g) were boiled in 500 ml distilled water for 15 – 20 min. Discarded the boiled potato and (added agar, PDA) adjusted volume to 1,000 ml with distilled water (PDB). The media was sterilized by autoclaved at 121˚C for 15 min.

Vacin and Went (VW) agar (Vacin and Went, 1949)

Tricalcium phosphate	0.2	g
Potassium nitrate	0.525	g
Monopotassium acid phosphate	0.25	g
Magnesium sulphate	0.25	g
Ammonium sulphate	0.5	g
Ferric tartrate	0.028	g
Maganese sulphate	0.75	mg
Sucrose	20	g
Agar	16	g

pH 7.0

The media was sterilized by autoclaved at 121˚C for 15 min.

APPENDIX B

CHEMICAL REAGENTS AND BUFFER

10% Cetyltrimethylammonium bromide (CTAB)

CTAB	10	g
0.7 M sodium chloride	100	ml

Added CTAB slowly to NaCl solution at 65 ˚C and sterilized by autoclaved at 121˚C for 15 min.

0.5 M Ethylenediaminetetraacetic acid (EDTA)

$Na_2EDTA.2H_2O$	18.6	g
Sodium hydroxide	2	g
Distilled water	88	ml

pH 8.0

10X TE buffer

1 M Tris-HCl	10	ml
0.5 M EDTA	2	ml
Distilled water	88	ml

pH 8.0

1 M Tris-(hydroxymethyl) aminomethane (Tris-HCl)

Tris-base	121	g
Conc HCl (37% v/v)	48.3	g
Distilled water	845	ml

pH 8.0

0.05% Trypan blue solution

Trypan blue	5	g
Lactoglycerol	100	ml

Lactoglycerol; mixed lactic acid: distilled water: glycerol, 1: 1: 1 (v/v/v).

APPENDIX C

GRAIN AND POTTING MEDIA

Grain media (black bean, corn, kidney bean and sorghum)

Each grain was soaked with water for 4 – 6 hours before transferred into a container (test tube 16 × 150 mm) and sterilized by autoclaved at 121˚C for 15 min.

Potting media

Coconut coir

Coconut coir were soaked with water for 12 hours before transferred into a container (test tube 16 × 150 mm or flask 250 ml) and sterilized by autoclaved at 121˚C for 15 min.

Coconut coir with PDB

Coconut husk were soaked with PDB for 12 hours before transferred into a container (test tube 16 × 150 mm or flask 250 ml) and sterilized by autoclaved at 121˚C for 15 min.

Coconut husk

Coconut husk were soaked with water for 12 hours before transferred into a container (test tube 16 × 150 mm or flask 250 ml) and sterilized by autoclaved at 121˚C for 15 min.

Coconut husk with PDB

Coconut husk were soaked with PDB for 12 hours before transferred into a container (test tube 16 × 150 mm or flask 250 ml) and sterilized by autoclaved at 121˚C for 15 min.

APPENDIX D

STANDARD CRUVE OF INDOLE ACETIC ACID (IAA) PREPARATION

(Gordon and Weber, 1951)

Reagent

Salkowski reagent: added 1 ml 0.5 M $FeCl_3$ into 50 ml of 35% $HClO_4$ and mixed well.

Procedure

1. Mixed 1 ml of standard IAA with 2 ml of Salkowski reagent and incubated at room temperature for 30 min in the dark. The appearance of pink color indicated IAA production.

2. The absorbance of the pink pigmentation that developed was measured at 530 nm, uninoculated media mixed with reagent used as a blank.

Table D Absorbance at 530 nm of various concentration of standard IAA

IAA concentration (µmol/ml)	Absorbance 530 nm
0	0.000
10	0.032
20	0.083
50	0.273
100	0.598
150	0.810
200	0.998

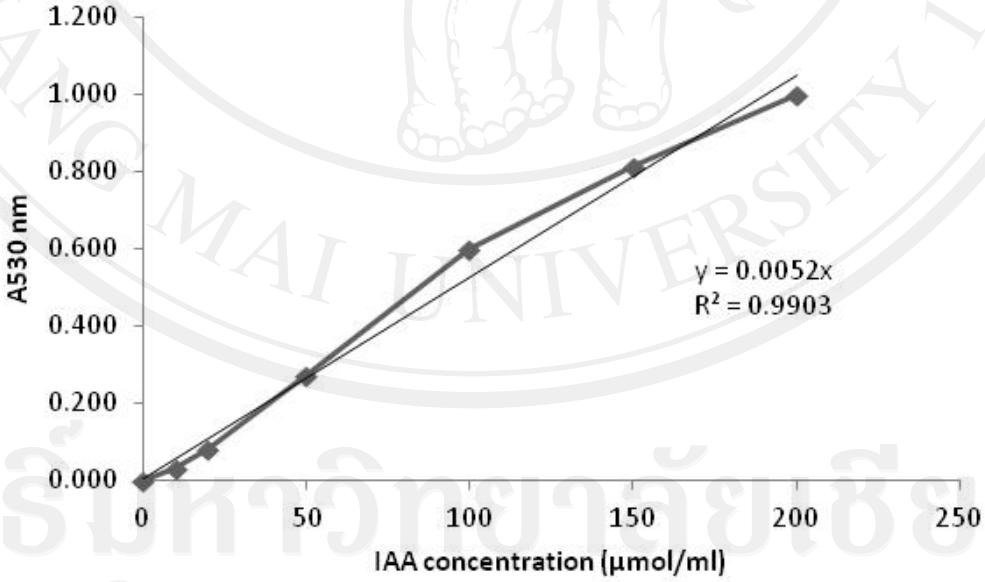

Figure D Standard cruve of IAA concentration against absorbance at 530 nm

APPENDIX E

DETERMINATION OF HYDROXAMATE-TYPE SIDERPHORE CONCENTRATION BY IRON-PERCHLORATE ASSAY (Atkin *et al.* 1970)

The iron-perchlorate assay is a colorimetric assay used for detection and estimation of hydroxamate-type siderophores under acidic condition.

Reagent

Ferric perchlorate solution: containing 5 mM $Fe(ClO_4)_3$ in 0.1 M $HClO_4$

Procedure

1. Added 0.5 ml of culture supernatant sample to 2.5 ml ferric perchlorate solution and incubated at room temperature for approximately five minutes. The presence of a hydroxamate-type siderophore is shown by the development of an orange-red color.

2. Recorded the absorbance at 480 nm using uninoculated media mixed with reagent as a blank.

Table E Absorbance at 480 nm of various concentration of deferoxamine mesyilate by iron-perchlorate assay

Deferoxamine mesylate concentration (µmol/ml)	Absorbance 480 nm
0	0.000
50	0.158
100	0.344
150	0.473
200	0.667
250	0.827
300	0.976
350	1.132

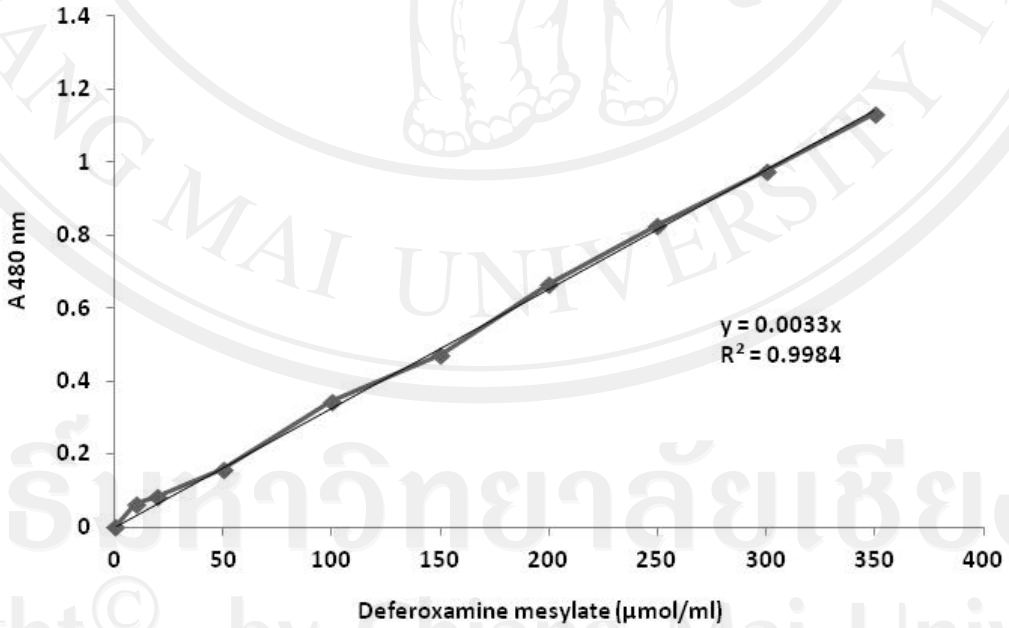

Figure E Standard curve for hydroxamate concentration determination using deferoxamine mesylate as standard

CURRICULUM VITAE

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Plublication

1. Chutima R, Dell B, Vessabutr S, Lumyong S (2011) Endophytic fungi from *Pecteilis susannae* (L.) Rafin (Orchidaceae), a threatened terrestrial orchid in Thailand. *Mycorrhiza* 21: 221-229.

2. Chutima R, Dell B, Lumyong S (2011) Effects of mycorrhizal fungi on symbiotic seed germination of *Pecteilis susannae* (L.) Rafin (Orchidaceae), a terrestrial orchid in Thailand. *Symbiosis* 53: 149-156.