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APPENDICES

APPENDIX A

Media

Various media were used in this study including isolation media and test media, and they were prepared as the following formula (per liter).

1. Bile esculin agar (Fluka[®], Switzerland)

Meat extract	3	g
Meat peptone	5	g
Ox-bile	40	g
Ferric citrate	0.5	g
Esculin	1	g
Agar	14.5	g
pH 7		



Dispensed into containers and autoclaved at 121°C for 15 minutes.

2. Chrome azurol S-modified Gaus No.1 (CAS-MGs-1) medium

MGs-1 agar

D(+)-glucose	20	g
Potassium nitrate	1	g
Sodium chloride	0.1	g
di-Potassium hydrogen phosphate	0.5	g
Magnesium sulfate	0.1	g

Agar	15	g
Deionized water	900	ml
pH 7		

Dispensed into containers and autoclaved at 121°C for 15 minutes. Let it cool to 50°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 of deionized water and mixed with 10 ml of iron (III) solution (1 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 10 mM HCl). Under stirring, this solution was slowly mixed with HDTMA dissolved in 40 ml. The resultant dark blue solution was autoclaved at 121°C for 15 minutes.

3. Carbohydrate fermentation medium

Ammonium phosphate	1	g
Potassium chloride	0.2	g
Magnesium sulfate	0.2	g
Bromocresol purple	0.03	g
pH 7		

Dispensed into containers and autoclaved at 121°C for 15 minutes.

4. Decarboxylase medium

Peptone	5	g
Yeast extract	3	g

D(+)-glucose	1	g
Bromocresol purple	0.02	g
pH 6.8		

Dispensed into containers and autoclaved at 121°C for 15 minutes.

5. King's B medium

Proteose peptone No. 3	20	g
di-Potassium phosphate	1.5	g
Magnesium sulfate	1.5	g
Glycerol	10	ml
pH 7		

Dispensed into containers and autoclaved at 121°C for 15 minutes.

6. Methyl red/Voges-Proskauer medium (MRVP) (Merck®, Germany)

Peptone from meat	7	g
D(+)-glucose	5	g
Phosphate buffer	5	g
pH 6.9		

Dispensed into containers and autoclaved at 121°C for 15 minutes.

7. Motility test medium

Peptone	5	g
Meat extract	3	g
agar	5	g

pH 7.0

Dispensed into containers and autoclaved at 121°C for 15 minutes.

8. Nitrate medium

Potassium nitrate	1	g
Beef extract	10	g
Peptone	10	g
Sodium chloride	5	g

pH 7.2

Dispensed into containers and autoclaved at 121°C for 15 minutes.

9. Nutrient medium

Peptone	5	g
Meat extract	3	g

pH 7

Dispensed into containers and autoclaved at 121°C for 15 minutes.

10. Phenylalanine deaminase medium

Sodium chloride	5	g
Yeast extract	3	g
di-Sodium hydrogen phosphate	1	g
DL-Phenylalanine	2	g
Bromocresol purple	0.02	g

pH 7.3

Dispensed into containers and autoclaved at 121°C for 15 minutes.

11. Starch agar

Potato starch	10	g
Pancreatic digest of gelatin	5	g
Yeast extract	3	g
Agar	15	g
pH 7		

Dispensed into containers and autoclaved at 121°C for 15 minutes.

12. Simmons citrate agar (Merck[®], Germany)

Ammonium di-hydrogen phosphate	1	g
di-Potassium phosphate	1	g
Sodium chloride	5	g
Sodium citrate	2	g
Magnesium sulfate	0.2	g
Bromothymol blue	0.08	g
Agar	15	g
pH 6.9		

Dispensed into containers and autoclaved at 121°C for 15 minutes.

13. Triple sugar iron agar (TSI) (Merck[®], Germany)

Peptone from casein	15	g
Peptone from meat	5	g

Meat extract	3	g
Yeast extract	3	g
Sodium chloride	5	g
Lactose	10	g
Sucrose	10	g
D(+)-glucose	1	g
Ammonium iron (III) citrate	0.5	g
Sodium thiosulfate	0.5	g
Phenol red	0.024	g
Agar	12	g
pH 7.4		

Dispensed into containers and autoclaved at 121°C for 15 minutes.

14. Triphenyltetrazoliumchloride (TZC) medium

Peptone	1	g
D(+)-glucose	0.5	g
Pancreatic digest of casein	0.1	g

Dispensed into containers and autoclaved at 121°C for 15 minutes. Let it cool to 50°C. Added 10 ml of 0.5% (w/v) 2,3,5-triphenyltetrazoliumchloride sterile solution.

15. Tryptic soy broth (TSB) (Merck[®], Germany)

Peptone from casein	17	g
Peptone from soymeal	3	g
D(+)-glucose	2.5	g

Sodium chloride	5	g
di-Potassium hydrogen phosphate	2.5	g
pH 7		

Dispensed into containers and autoclaved at 121°C for 15 minutes.

16. Tryptone medium

Tryptone	10	g
pH 6.8		

Dispensed into containers and autoclaved at 121°C for 15 minutes.

17. Urease test medium

di-Sodium hydrogen phosphate	9.5	g
Potassium di-hydrogen phosphate	9.1	g
Yeast extract	0.1	g
Phenol red	0.01	g
pH 7		

Dispensed into containers and autoclaved at 121°C for 15 minutes. Aseptically added sterile urea solution (filter sterilize). Mix thoroughly and aseptically distribute into sterile tubes.

APPENDIX B

Chemical reagents for agarose gel electrophoresis

1. 0.8% Agarose

Agarose	0.8	g
Distilled water	100	ml

2. 1% Agarose

Agarose	1	g
Distilled water	100	ml

3. 0.5 M EDTA

EDTA	18.6	g
Sodium hydroxide	2	g
Distilled water	88	ml

4. Ethidium bromide (1 mg/ml)

Ethidium bromide	1	mg
Distilled water	1	ml

5. Loading dye (5X)

5X TAE

Bromophenol blue	0.05	%
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Glycerol	50	%
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6. 1M Tris-HCl

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

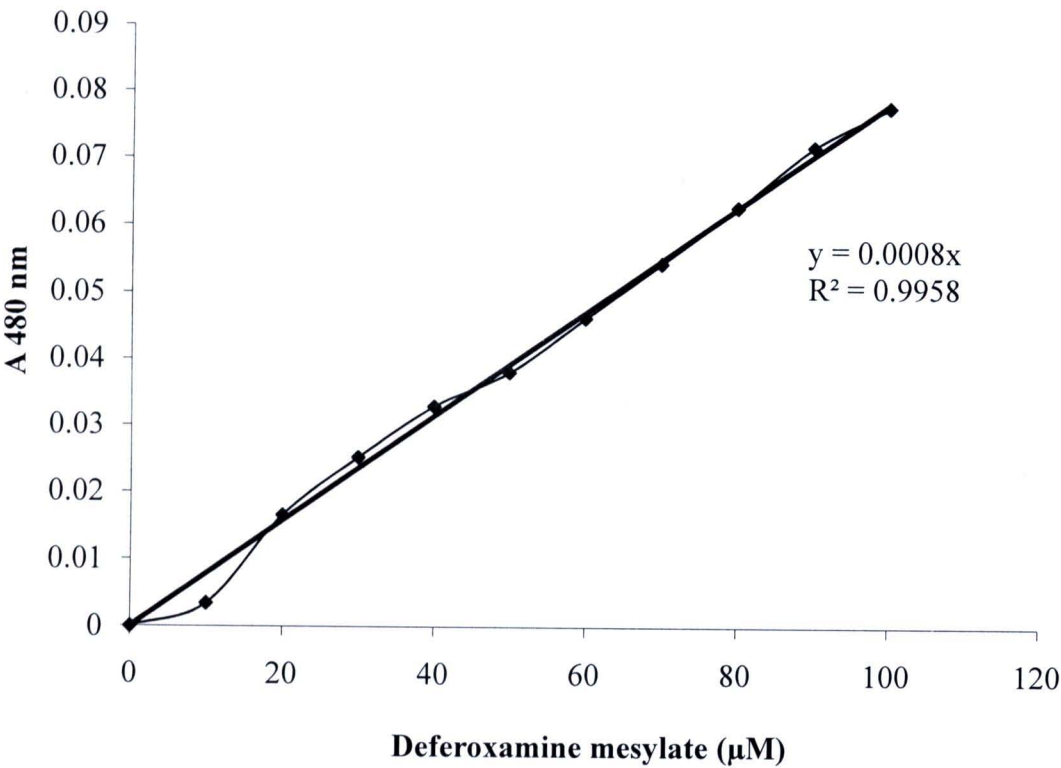
7. TAE buffer (50X)

Tris base	242	g
Glacial acetic acid	57.1	ml
0.5 M EDTA pH8	100	ml
Added distilled water to 1000 ml		

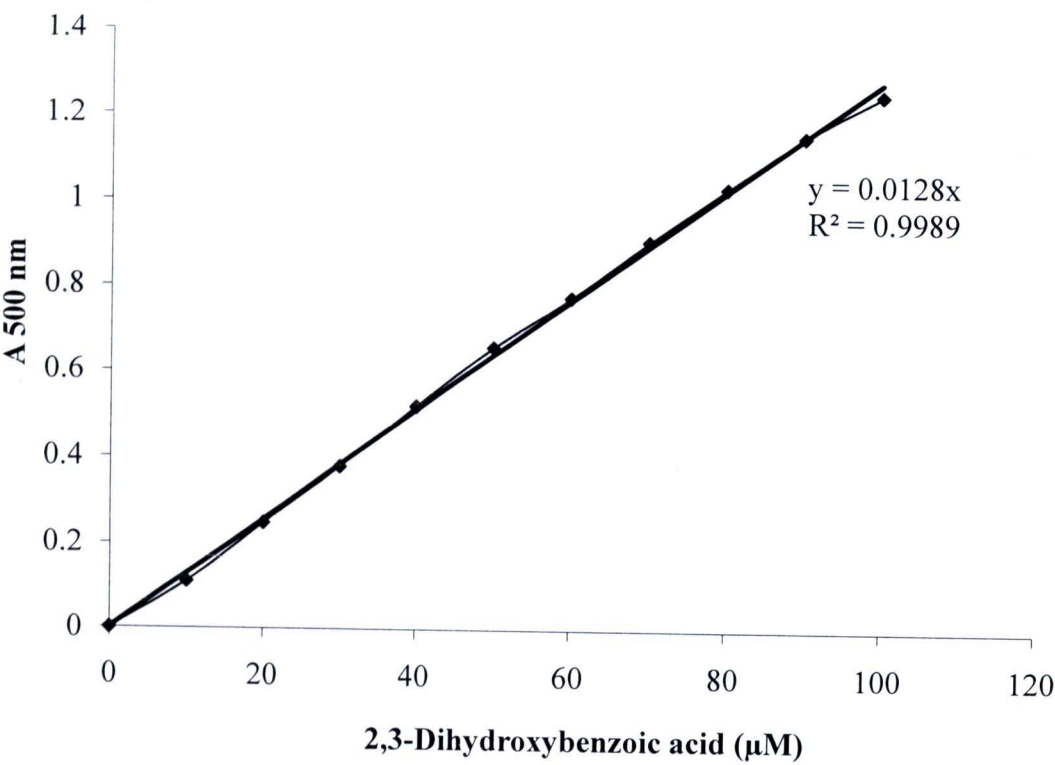
APPENDIX C

Standard curves of siderophore production

1. Standard curve of hydroxamate determination using deferoxamine mesylate as standard



2. Standard curve of catecholate determination using 2,3-dihydroxybenzoic acid as standard



CURRICULUM VITAE

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Academic activities

1. Poster presentation in the topic “Isolation and Screening of Antagonistic Bacteria for *Ralstonia solanacearum* Control in *Curcuma alismatifolia* Gagnep.” The

Commission on higher education congress I: University staff development consortium, Chonburi, Thailand. September 5-7, 2008

2. Poster presentation in the topic “Control of *Ralstonia solanacearum* in *Curcuma alismatifolia* Gagnep. by Antagonistic Bacteria” The Xth Meeting of the working group: Biological control of fungal and bacterial plant pathogens. Interlaken, Switzerland. September 9-12, 2008

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