APPENDICES

APPENDIX A
EXPERIMENTAL RESULTS

Pairwise alignment of the ITS regions of nuclear rRNA gene sequence

```
NHL-L6/6                        CCCTATGTGAACATACTATTGTTGCCTCGGCGGCGCCGCGATAGCGGCCC 50
Nodulisporium_sp._CMUUP-E34     CCCTATGTGAACATACTATTGTTGCCTCGGCGGCGCCGCGATAGCGGCCC 50
                                **************************************************

NHL-L6/6                        GCCGGTGGACCTAAACGCTAATTGTAACCACTGTATCTCTGAATGTATAA 100
Nodulisporium_sp._CMUUP-E34     GCCGGTGGACCTAAACGCTAATTGTAACCACTGTATCTCTGAATGTATAA 100
                                **************************************************

NHL-L6/6                        CTGTAATACGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGA 150
Nodulisporium_sp._CMUUP-E34     CTGTAATACGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGA 150
                                **************************************************

NHL-L6/6                        TGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCCGTG 200
Nodulisporium_sp._CMUUP-E34     TGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCCGTG 200
                                **************************************************

NHL-L6/6                        AATCATCGAATCTTTGAACGCACATTGCGCCCATTAGTATTCTAGTGGGC 250
Nodulisporium_sp._CMUUP-E34     AATCATCGAATCTTTGAACGCACATTGCGCCCATTAGTATTCTAGTGGGC 250
                                **************************************************
NHL-L6/6                        ATGCCTATTCGAGCGTCATTTCGACCCTTACGCCCTGTTGCGTAGTGTTG 300
Nodulisporium_sp._CMUUP-E34     ATGCCTATTCGAGCGTCATTTCGACCCTTACGCCCTGTTGCGTAGTGTTG 300
                                **************************************************

NHL-L6/6                        GGACTCTGCGTGTTACAGCGCAGTTCCTGAAAGCAATTGGCGGAGCTAGA 350
Nodulisporium_sp._CMUUP-E34     GGACTCTGCGTGTTACAGCGCAGTTCCTGAAAGCAATTGGCGGAGCTAGA 350
                                **************************************************

NHL-L6/6                        GCCCACTCTAGGCGTAGTAAATACCATTCTCGCTTCTGTAGTGGCTTTGG 400
Nodulisporium_sp._CMUUP-E34     GCCCACTCTAGGCGTAGTAAATACCATTCTCGCTTCTGTAGTGGCTTTGG 400
                                **************************************************

NHL-L6/6                        CGGCCAGCCAGAAAACCCCTATATTTCTAGTGGTTGACCTCGGATTAGGT 450
Nodulisporium_sp._CMUUP-E34     CGGCTAGCCAGAAAACCCCTATATTTCTAGTGGTTGACCTCGGATTAGGT 450
                                **** ***********************************************

NHL-L6/6                        AGGAATACCCGCTGAACTTAAGCATATCAATAAG 484
Nodulisporium_sp._CMUUP-E34     AGGAATACCCGCTGAACTTAAGCATATCAATAAG 484
                                *********************************
```

Length = 484 bp, % Identity = 99.79%

Figure A.1 CLUSTAL 2.1 multiple sequence alignment between internal transcribed spacer of ribosomal RNA gene of NHL-L 6/6 and *Nodulisporium* sp. CMUUP-E34 (accession no. JN558831.1). The asterisk (*) indicates the same base.

```
NHL-L6/6                    CTATGTGAACATACTATTGTTGCCTCGGCGGCGCCGCGATAGCGGCCCGC 50
Nodulisporium_sp._JP3821    CTATGTGAACATACTATTGTTGCCTCGGCGGCGCCGCGATAGCGGCCCGC 50
                            **************************************************

NHL-L6/6                    CGGTGGACCTAAACGCTAATTGTAACCACTGTATCTCTGAATGTATAACT 100
Nodulisporium_sp._JP3821    CGGTGGACCTAAACGCTAATTGTAACCACTGTATCTCTGAATGTATAACT 100
                            **************************************************

NHL-L6/6                    GTAATACGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATG 150
Nodulisporium_sp._JP3821    GTAATACGTTAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGATG 150
                            **************************************************

NHL-L6/6                    AAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCCGTGAA 200
Nodulisporium_sp._JP3821    AAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCCGTGAA 200
                            **************************************************

NHL-L6/6                    TCATCGAATCTTTGAACGCACATTGCGCCCATTAGTATTCTAGTGGGCAT 250
Nodulisporium_sp._JP3821    TCATCGAATCTTTGAACGCACATTGCGCCCATTAGTATTCTAGTGGGCAT 250
                            **************************************************

NHL-L6/6                    GCCTATTCGAGCGTCATTTCGACCCTTACGCCCTGTTGCGTAGTGTTGGG 300
Nodulisporium_sp._JP3821    GCCTATTCGAGCGTCATTTCGACCCTTACGCCCTGTTGCGTAGTGTTGGG 300
                            **************************************************


NHL-L6/6                    ACTCTGCGTGTTACAGCGCAGTTCCTGAAAGCAATTGGCGGAGCTAGAGC 350
Nodulisporium_sp._JP3821    ACTCTGCGTGTTACAGCGCAGTTCCTGAAAGCAATTGGCGGAGCTAGAGC 350
                            **************************************************

NHL-L6/6                    CCACTCTAGGCGTAGTAAATACCATTCTCGCTTCTGTAGTGGCTTTGGCG 400
Nodulisporium_sp._JP3821    CCACTCTAGGCGTAGTAAATACCATTCTCGCTTCTGTAGTGGCTTTGGCG 400
                            **************************************************

NHL-L6/6                    GCCAGCCAGAAAACCCCTATATTTCTAGTGGTTGACCTCGGATTAGGTAG 450
Nodulisporium_sp._JP3821    GCTAGCCAGAAAACCCCTATATTTCTAGTGGTTGACCTCGGATTAGGTAG 450
                            ** *************************************************

NHL-L6/6                    GAATACCCGCTGAACTTAAGCATATCA 477
Nodulisporium_sp._JP3821    GAATACCCGCTGAACTTAAGCATATCA 477
                            ***************************
```

Length = 477 bp, % Identity = 99.79%

Figure A.2 CLUSTAL 2.1 multiple sequence alignment between internal transcribed spacer of ribosomal RNA gene of NHL-L 6/6 and *Nodulisporium* sp. JP3821 (accession no. AF280627.1). The asterisk (*) indicates the same base.

```
NHL-L6/6                CCCTATGTGAACATACTATTGTTGCCTCGGCGGCGCCGCGATAGCGGCCCGCCGGTGGAC 60
Hypoxylon_sp._WHCS-8    CCCTATGTGAACATACTATTGTTGCCTCGGCGGCGCCGCGATAGCGGCCCGCCGGTGGAC 60
                        ************************************************************

NHL-L6/6                CTAAACGCTAATTGTAACCACTGTATCTCTGAATGTATAACTGTAATACGTTAAAACTTT 120
Hypoxylon_sp._WHCS-8    CTAAACGCTAATTGTAACCACTGTATCTCTGAATG-ATAACTGTAATACGTTAAAACTTT 119
                        ********************************** ***********************

NHL-L6/6                CAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT 180
Hypoxylon_sp._WHCS-8    CAACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAAT 179
                        ************************************************************

NHL-L6/6                GTGAATTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCATTAGTAT 240
Hypoxylon_sp._WHCS-8    GTGAATTGCAGAATTCCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCATTAGTAT 239
                        ************************************************************

NHL-L6/6                TCTAGTGGGCATGCCTATTCGAGCGTCATTTCGACCCTTACGCCCTGTTGCGTAGTGTTG 300
Hypoxylon_sp._WHCS-8    TCTAGTGGGCATGCCTATTCGAGCGTCATTTCGACCCTTACGCCCTGTTGCGTAGTGTTG 299
                        ************************************************************

NHL-L6/6                GGACTCTGCGTGTTACAGCGCAGTTCCTGAAAGCAATTGGCGGAGCTAGAGCCCACTCTA 360
Hypoxylon_sp._WHCS-8    GGACTCTGCGTGTTACAGCGCAGTTCCTGAAAGCAATTGGCGGAGCTAGAGCCCACTCTA 359
                        ************************************************************

NHL-L6/6                GGCGTAGTAAATACCATTCTCGCTTCTGTAGTGGCTTTGGCGGCCAGCCAGAAAACCCCT 420
Hypoxylon_sp._WHCS-8    GGCG-AGTAAATACCATTCTCGCTTCTGTAGTGGCTTTGGCGGCTAGCCAGAAAACCCCT 418
                        **** ***************************************** ***************

NHL-L6/6                ATATTTCTAGTGGTTGACCTCGGATTAGGTAGGAATACCCGCTGAACTTAAGCATATCAA 480
Hypoxylon_sp._WHCS-8    ATATTTCTAGTGGTTGACCTCGGATTAGGT-GGAATACCCGCTGAACTTAA-CATATCA- 475
                        ******************************** ******************** *******

NHL-L6/6                TA 482
Hypoxylon_sp._WHCS-8    TA 477
                        **
```

Length = 478 bp, % Identity = 98.95%, % Gap = 1.05%

Figure A.3 CLUSTAL 2.1 multiple sequence alignment between internal transcribed spacer of ribosomal RNA gene of NHL-L 6/6 and *Hypoxylon* sp. WHCS-8 (accession no. JQ362418.1). The asterisk (*) indicates the same base, horizontal line (-) represents gap.

Effect of nitrogen and carbon sources on anti-phytopathogenic compound production

Table A.1 Effect of nitrogen sources on anti-phytopathogenic compound production

Culture medium	Antimicrobial activity (AU/mL)						
	Incubation Time (Days)						
	0	2	3	4	5	6	7
Wh	NI	0.61±0.00	2.38±0.81	5.00±1.12	11.14±1.64	8.86±2.48	6.09±1.63
YE	NI	0.59±0.01	1.54±0.12	5.78±0.39	17.54±0.32	6.29±0.12	1.53±0.14
Ur	NI	NI	NI	NI	NI	NI	NI
Am	NI	NI	0.49±0.00	1.01±0.01	1.47±0.06	0.90±0.03	0.76±0.01

Note: One arbitrary unit (AU) of antimicrobial activity was defined as that gave an inhibition zone size equivalent to that produced by 250 μg/mL of mercuric chloride. NI represents no inhibitory zone, culture medium Wh, YE, Ur and Am represents sucrose (20 g/L) + whey (3 g/L), sucrose (20 g/L) + yeast extract (3 g/L), sucrose (20 g/L) + urea (3 g/L) and sucrose (20 g/L) + ammonium sulfate (3 g/L), respectively.

Table A.2 pH of culture media in preliminary screening of nitrogen sources

Cultivation Time (Days)	pH			
	Whey	Yeast extract	Urea	Ammonium sulfate
0	5.9±0.2	5.6±0.0	7.8±0.1	4.1±0.1
2	4.3±0.1	5.0±0.1	7.0±0.0	2.7±0.0
3	3.9±0.0	4.8±0.0	6.7±0.1	2.8±0.0
4	4.1±0.0	4.7±0.0	6.4±0.1	2.9±0.0
5	4.1±0.1	5.0±0.1	5.8±0.1	2.9±0.1
6	4.2±0.1	5.4±0.1	5.7±0.1	3.2±0.1
7	4.2±0.0	5.5±0.0	5.4±0.0	3.1±0.2

Note: Culture media contain 3 g/L of nitrogen sources and 20 g/L of sucrose.

Table A.3 Effect of carbon sources on anti-phytopathogenic compound production

Culture medium	Antimicrobial activity (AU/mL)						
	Incubation Time (Days)						
	0	2	3	4	5	6	7
Su	NI	0.58±0.01	1.53±0.12	5.77±0.37	17.53±0.34	6.40±0.18	1.53±0.13
Gl	NI	0.60±0.03	3.00±0.53	2.90±0.08	3.41±0.19	10.04±1.18	1.84±0.09
Mo	NI	NI	NI	NI	NI	NI	NI
Ca	NI	NI	2.69±0.36	7.33±0.58	7.75±0.56	11.22±2.75	5.58±0.40

Note: One arbitrary unit (AU) of antimicrobial activity was defined as that gave an inhibition zone size equivalent to that produced by 250 μg/mL of mercuric chloride. NI represents no inhibitory zone, culture medium Su, Gl, Mo and Ca represents sucrose (20 g/L) + yeast extract (3 g/L), glucose (20 g/L) + yeast extract (3 g/L), molasses (20 g/L) + yeast extract (3 g/L) and cassava starch (20 g/L) + yeast extract (3 g/L), respectively.

Table A.4 pH of culture media in preliminary screening of carbon sources

Cultivation Time (Days)	pH			
	Sucrose	Glucose	Molasses	Cassava Starch
0	5.6±0.0	5.5±0.0	5.6±0.0	5.5±0.0
2	5.0±0.1	4.0±0.2	5.6±0.0	3.9±0.1
3	4.8±0.0	4.7±0.3	5.5±0.0	3.3±0.0
4	4.7±0.0	5.3±0.1	7.1±0.0	4.6±0.1
5	5.0±0.1	5.6±0.2	7.6±0.0	4.5±0.0
6	5.4±0.1	6.4±0.2	7.7±0.2	4.9±0.0
7	5.5±0.0	6.6±0.2	7.8±0.1	5.3±0.0

Note: Culture media contain 3 g/L of yeast extract and 20 g/L of carbon sources.

Screening of factors affecting anti-phytopathogenic compound production by fractional factorial design

Table A.5 Corresponding responses of fractional factorial design in terms of biomass

Run No.	Biomass (g/L)				
	4 days	5 days	6 days	7 days	8 days
1	4.31	4.83	4.85	4.82	4.75
2	3.21	3.48	3.56	3.6	3.66
3	4.38	4.66	4.82	5.12	4.88
4	3.15	3.66	3.97	3.93	3.97
5	3.85	4.35	4.6	4.62	4.46
6	3.97	4.33	4.57	4.7	4.41
7	3.92	4.37	4.62	4.65	4.46
8	2.62	2.63	2.75	2.54	2.39
9	4.16	3.87	4.19	3.81	3.89
10	3.66	3.87	4.63	4.23	3.78
11	5.86	4.73	6.47	6.31	6.47
12	4.69	4.91	5.06	5.15	4.81
13	4.7	4.73	5.02	5.33	5.05
14	4.4	4.78	5.11	5.1	4.58

Table A.6 Corresponding responses of fractional factorial design in terms of antimicrobial activity

Run No.	Antimicrobial activity (AU/mL)				
	4 days	5 days	6 days	7 days	8 days
1	5.85±0.11	12.47±0.25	15.97±0.33	32.13±5.79	15.97±0.65
2	7.22±0.23	14.01±0.28	10.38±0.00	16.16±0.33	9.6±0.38
3	6.57±0.21	7.54±0.14	14.35±0.30	23.65±0.89	11.12±0.38
4	12.62±0.51	8.98±0.17	13.06±0.26	17.83±1.33	16.76±0.71
5	7.08±0.35	28.98±0.65	39.69±1.80	35.21±0.81	16.55±0.35
6	7±0.22	28.98±0.65	40.19±0.92	34.77±1.37	17.17±0.71
7	7.22±0.23	29.37±1.33	40.19±0.92	34.77±1.37	16.96±0.61
8	1.61±0.20	2.08±0.03	1.55±0.04	0.69±0.01	0.5±0.01
9	NI	NI	NI	NI	NI
10	9.93±0.51	14.18±0.00	22.23±1.28	6.5±0.12	6.7±0.12
11	3.05±0.00	5.02±0.09	14.86±0.31	3.89±0.06	4.88±0.17
12	7.46±0.24	30.51±0.68	46.61±2.15	42.42±1.70	19.63±0.72
13	7.46±0.24	30.92±1.20	46.61±2.15	42.42±1.70	19.15±0.41
14	7.38±0.14	31.31±0.71	46.59±1.10	40.75±1.63	19.88±0.84

Note: One arbitrary unit (AU) of antimicrobial activity was defined as that gave an inhibition zone size equivalent to that produced by 250 μg/mL of mercuric chloride. NI represents no inhibitory zone.

Regressive analyses 2^{4-1} FFD using antimicrobial activity at day 6 post inoculation as response.

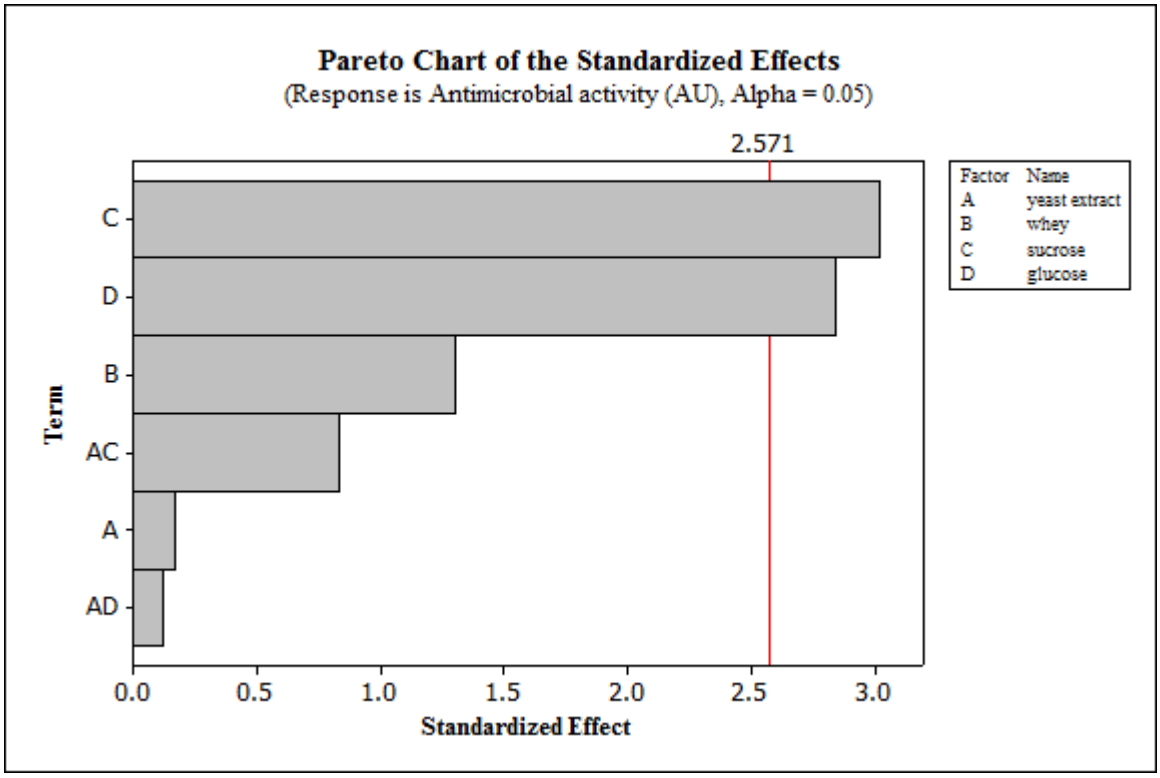

Figure A.4 Pareto chart of the standardized effect of antimicrobial activity

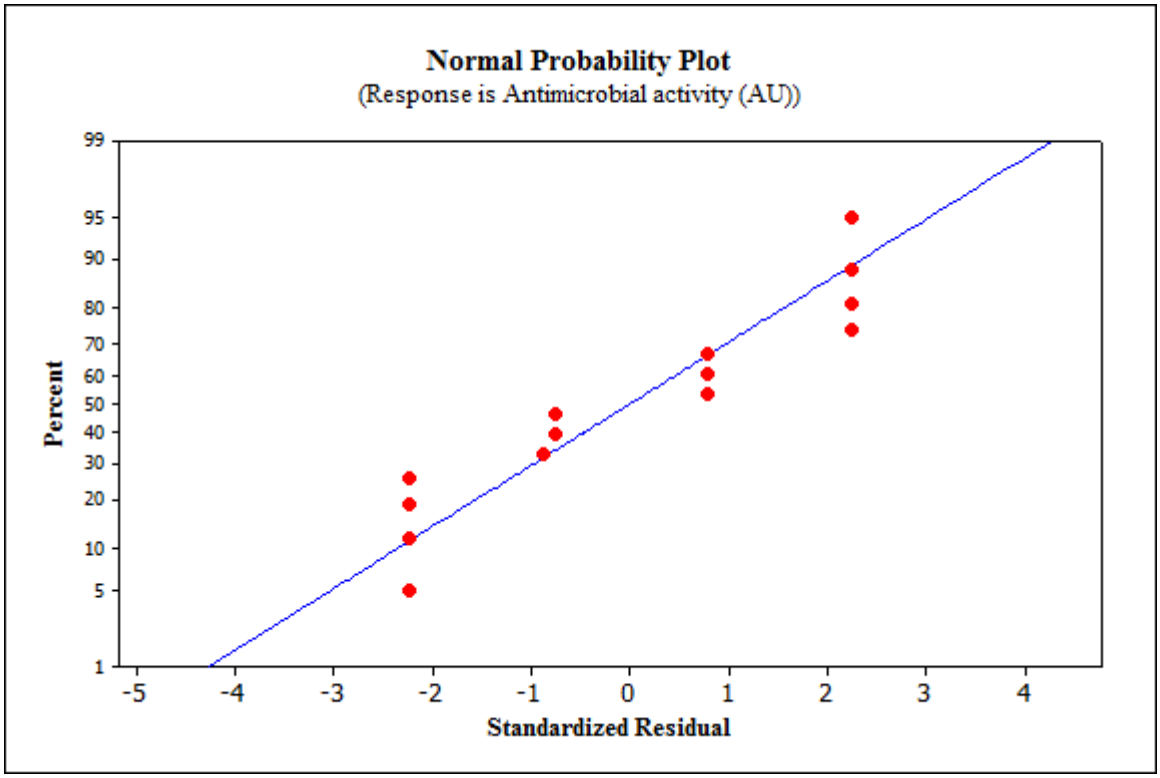

Figure A.5 Normal probability plot of the residual of antimicrobial activity

Regressive analyses 2^{4-1} FFD using biomass at day 6 post inoculation as response.

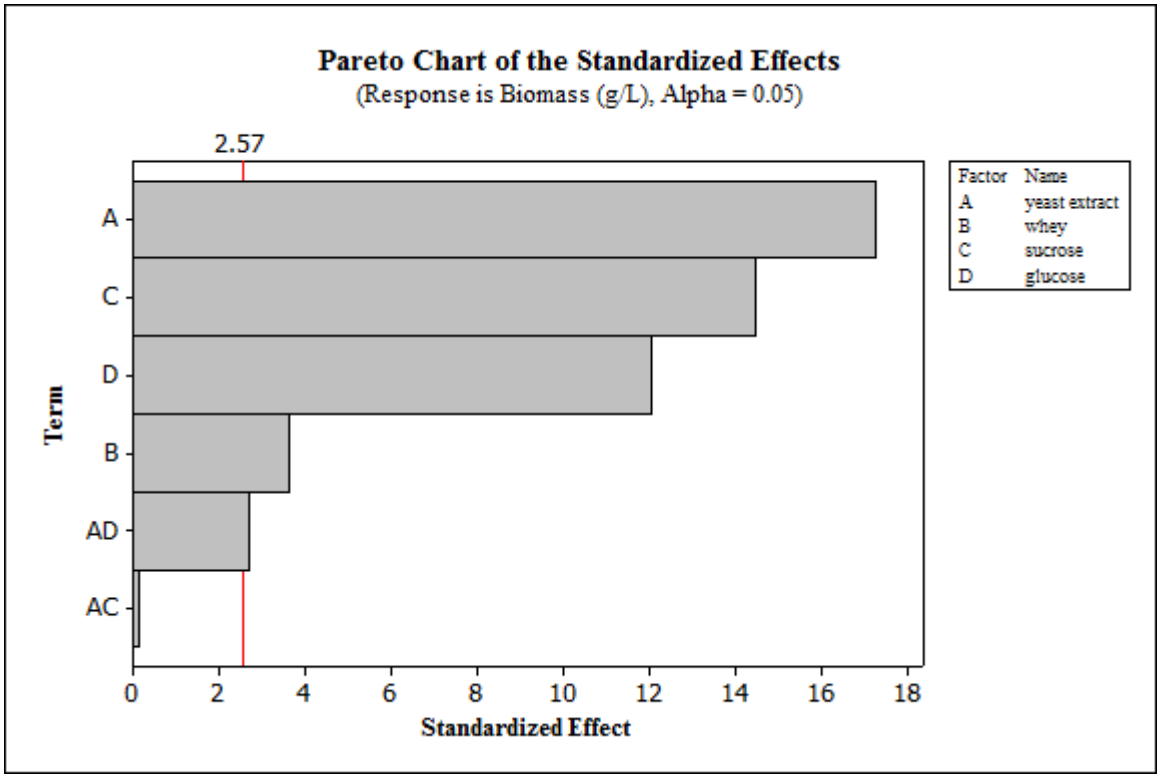

Figure A.6 Pareto chart of the standardized effect of biomass

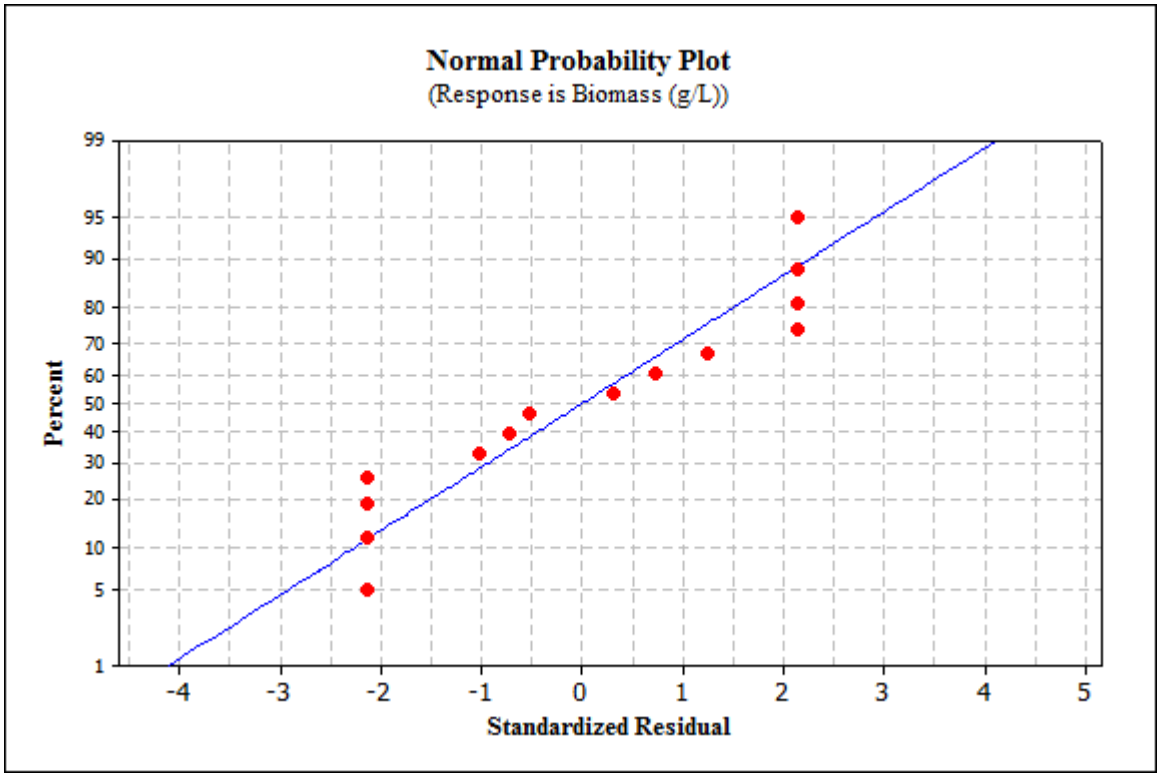

Figure A.7 Normal probability plot of the residual of biomass

Optimization of antimicrobial compound production of *Nodulisporium* sp. NHL-L 6/6 using Central Composite Design

Table A.7 The experimental design and results of CCD for antimicrobial activity

Run	Yeast extract (g/L)	Whey (mL/L)	Sucrose (g/L)	Glucose (g/L)	Antimicrobial activity (AU/mL)		
					Day 5	Day 6	Day 7
1	1.95 (-1)	8 (-1)	15 (-1)	5 (-1)	4.25±0.14	9.60±0.18	10.62±0.00
2	2.05 (+1)	8 (-1)	15 (-1)	5 (-1)	5.79±0.00	20.11±0.43	12.18±0.25
3	1.95 (-1)	12 (+1)	15 (-1)	5 (-1)	5.97±0.00	13.53±0.56	15.22±0.00
4	2.05 (+1)	12 (+1)	15 (-1)	5 (-1)	3.81±0.09	14.86±0.31	8.41±0.32
5	1.95 (-1)	8 (-1)	25 (+1)	5 (-1)	4.68±0.00	26.17±1.17	32.98±0.00
6	2.05 (+1)	8 (-1)	25 (+1)	5 (-1)	10.31±0.00	38.11±0.88	39.94±2.12
7	1.95 (-1)	12 (+1)	25 (+1)	5 (-1)	9.93±0.39	26.85±1.17	22.21±0.00
8	2.05 (+1)	12 (+1)	25 (+1)	5 (-1)	9.70±0.00	28.24±0.62	24.86±0.00
9	1.95 (-1)	8 (-1)	15 (-1)	15 (+1)	6.77±0.00	18.47±0.77	34.75±0.00
10	2.05 (+1)	8 (-1)	15 (-1)	15 (+1)	6.56±0.00	22.49±0.48	25.50±0.00
11	1.95 (-1)	12 (+1)	15 (-1)	15 (+1)	11.63±0.23	28.98±0.65	37.61±0.00
12	2.05 (+1)	12 (+1)	15 (-1)	15 (+1)	12.76±0.00	25.50±0.00	39.85±0.00
13	1.95 (-1)	8 (-1)	25 (+1)	15 (+1)	8.78±0.00	28.60±0.00	38.67±2.32
14	2.05 (+1)	8 (-1)	25 (+1)	15 (+1)	11.11±0.00	37.61±0.00	39.15±1.56
15	1.95 (-1)	12 (+1)	25 (+1)	15 (+1)	14.35±0.30	39.69±1.80	39.73±0.00
16	2.05 (+1)	12 (+1)	25 (+1)	15 (+1)	8.78±0.00	34.75±0.00	11.37±0.00
17	1.9 (-2)	10 (0)	20 (0)	10 (0)	8.41±0.32	10.38±0.00	6.92±0.00
18	2.1 (+2)	10 (0)	20 (0)	10 (0)	16.75±0.35	18.01±0.38	8.13±0.00
19	2 (0)	6 (-2)	20 (0)	10 (0)	17.58±0.00	15.97±0.33	9.05±0.30
20	2 (0)	14 (+2)	20 (0)	10 (0)	11.50±0.00	18.47±0.77	9.19±0.47
21	2 (0)	10 (0)	10 (-2)	10 (0)	5.79±0.00	10.99±0.44	6.99±0.00
22	2 (0)	10 (0)	30 (+2)	10 (0)	18.23±0.00	39.69±1.80	17.17±0.71
23	2 (0)	10 (0)	20 (0)	0 (-2)	22.77±0.00	40.73±0.00	14.46±0.00
24	2 (0)	10 (0)	20 (0)	20 (+2)	18.91±0.00	35.70±0.00	16.95±0.00
25	2 (0)	10 (0)	20 (0)	10 (0)	18.47±0.77	40.73±0.00	25.18±0.55
26	2 (0)	10 (0)	20 (0)	10 (0)	18.91±0.00	39.69±1.80	24.26±1.07
27	2 (0)	10 (0)	20 (0)	10 (0)	23.99±1.85	38.62±0.88	25.50±0.00
28	2 (0)	10 (0)	20 (0)	10 (0)	18.91±0.00	39.69±1.80	24.26±1.07
29	2 (0)	10 (0)	20 (0)	10 (0)	20.49±2.73	39.69±1.80	24.88±1.07
30	2 (0)	10 (0)	20 (0)	10 (0)	19.92±1.74	39.13±0.00	26.29±3.12
31	2 (0)	10 (0)	20 (0)	10 (0)	20.49±2.73	39.13±0.00	24.88±1.07

Note: The number in front of the parenthesis denotes the actual value and the number in the parenthesis represents the coded value: -2 for the lower level, -1 for the low level, 0 for the zero level, +1 for the high level and +2 for the higher level.

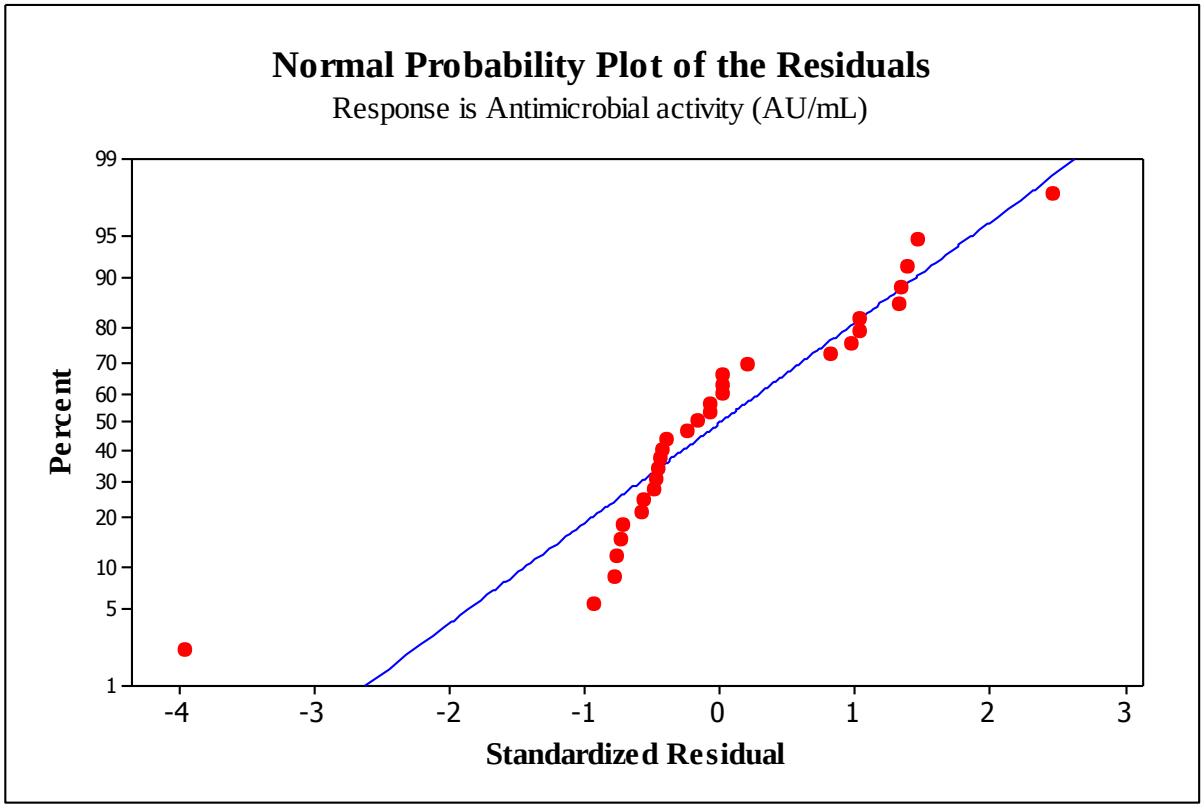

Figure A.8 Normal plot of residuals of CCD

Antimicrobial activity test

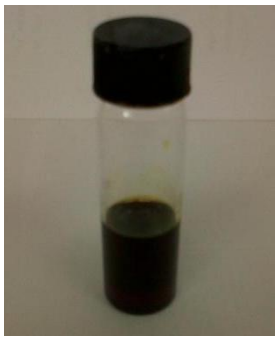

Figure A.9 A sticky brown color crude ethyl acetate extract after evaporation

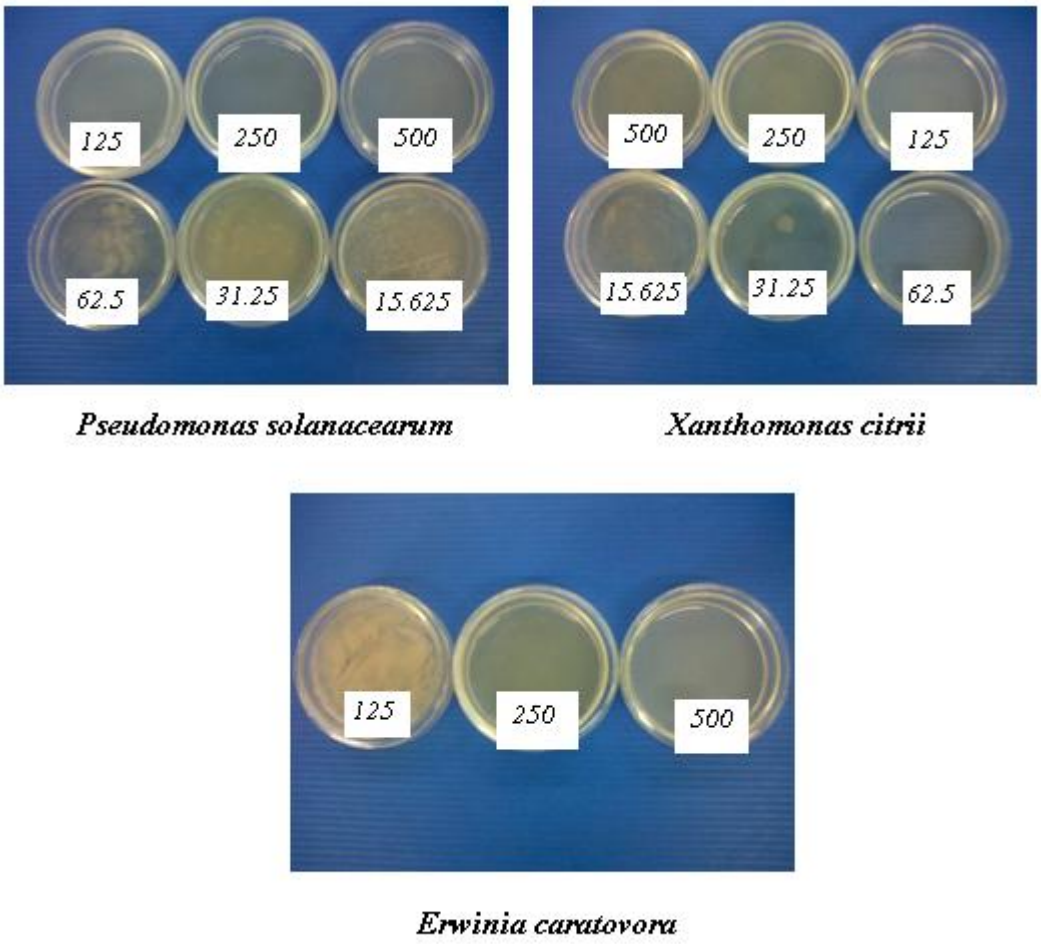

Figure A.10 MBC (µg/mL) of ethyl acetate extract of *Nodulisporium* sp. NHL-L 6/6 culture broth against plant pathogenic bacteria

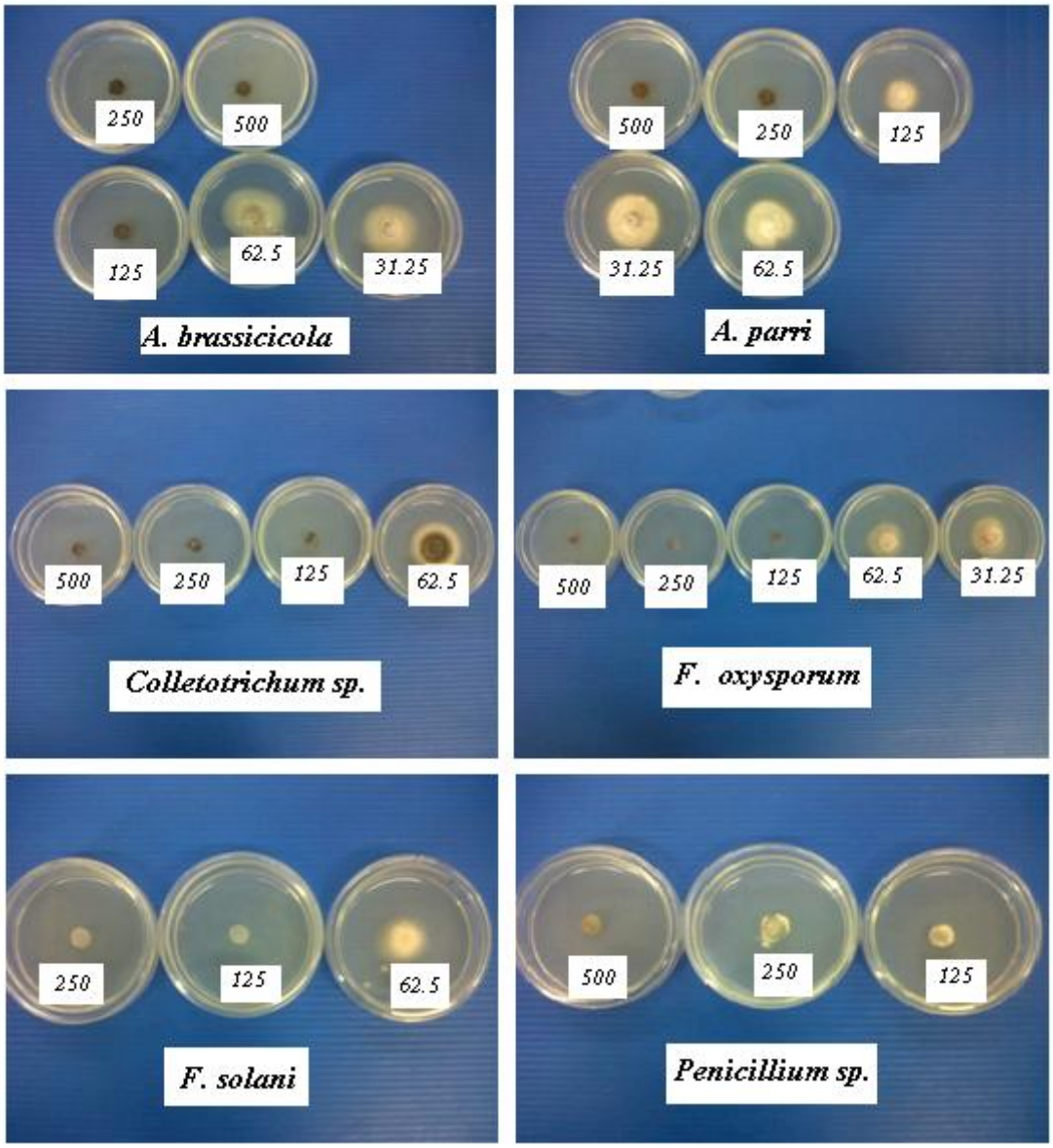

Figure A.11 MFC (µg/mL) of ethyl acetate extract of *Nodulisporium* sp. NHL-L 6/6 culture broth against plant pathogenic fungi

APPENDIX B

MEDIA and CHEMICAL PREPARATION

B.1 Media

B.11 Malt extract agar (MEA)

Malt extract	6	g
Maltose	1.8	g
Yeast extract	1.2	g
Dextrose	6	g
Agar	15	g
Distilled water	1000	ml

B.12 Modified M1D Media

$Ca(N0_3)_2$	2.8	g
$KN0_3$	0.08	g
KCl	0.06	g
$MgS0_4$	0.36	g
NaH_2P0_4	0.02	g
Sucrose	30	g
Ammonium tartrate	5	g
$FeC1_3$	2	mg
$MnS0_4$	5	mg
$ZnS0_4$	2.5	mg
H_3B0_3	1.4	mg
KI	0.7	mg
Yeast Extract*	0.25	g
Distilled water	1000	mL

Adjust pH to 5.5 with 0.1 M HCI *or omit the yeast extract and supplemented with: Stock Biotin 0.5 mg/mL or Stock Thiamine 0.5 mg/mL in 40% aq. EtOH or Stock Inositol 0.5 mg/mL (Use 2 ml/1 of broth)

B.13 Potato Dextrose Agar (PDA)

Fresh potato	300	g
Dextrose	20	g
Agar	15	g
Distilled water	1000	mL

B.14 Potato Sucrose Agar (PSA)

$Ca(N0_3)_2.4H20$	0.5	g
$Na_2HP0_4.12H_20$	2	g
Peptone	5	g
Sucrose	20	g
Agar	15	g
Fresh potatoes	300	g
Distilled water	1000	mL

Three hundred grams of fresh potatoes were cut into pieces of 1 cm^3 and boiled in 1000 mL of distilled water for 15 minutes until it became soften. Then potatoes were removed by filtering through cheesecloth. The rest of components were added and liquid volume was restored to 1 litre with distilled water.

B.15 Corn Meal Agar (CMA)

Corn meal	20	g
Agar	15	g
Distilled Water	1000	mL

All media were sterilized by autoclaving at 15 psi., 121°C for 15 minutes

B.2 Reagents

B.21 10 mg/mL Ethidium bromide solution

Ethidium bromide 1 g was dissolved in 100 mL of distilled water, and kept at room temperature in an aluminum foil container.

B.22 6X Gel loading buffer

Bromophenol blue	0.25%
Sucrose in water	40%

Keep at -20 ^{0}C

B.23 0.5 M EDTA

Add 186.1 g of EDTA (Disodium ethylenediamine tetraacetate.2H_2O) in 800 mL distilled water. Stir with magnetic stirrer and adjust the pH to 8.0 using 1M NaOH. Adjust volume to 1 liter with distilled water and sterile by autoclaving.

B.24 10% SDS

Dissolve SDS 10 g in 100 mL distilled water.

B.25 1 M HCl

HCl	86.2	mL
Distilled water	913.8	mL

B.26 1 M NaOH

NaOH	40	g
Distilled water	1000	mL

B.27 DNA extraction buffer

200 mM Tris-HCl (pH 8.5)

250 mM NaCl

25 mM EDTA

0.5% SDS

B.27 TE buffer pH 8.0

10 mM Tris-HCl pH 8.0

1 mM EDTA pH 8.0

B.28 10x TBE buffer

Tris base	108	g
Boric acid	55	g
0.5 mM EDTA pH 8.0	100	mL

Final volume was adjusted to 1 liter with distilled water and sterilize by autoclaving.

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B.29 Phenol-chloroform solution

Phenol	50%	(v/v)
Chloroform	48%	(v/v)
Isoamyl alcohol	2%	(v/v)

B.30 Mercury (II) chloride stock solution

Mercury (II) chloride 500 mg was dissolved in 10 mL of distilled water, and kept at 4 °C in an aluminum foil container.

APPENDIX C
ANALYTICAL METHOD

C.1 Agarose gel electrophoresis

Agarose gel analysis was carried out for determination of the quality of extracted genomic DNA and PCR products. Agrose gel were prepare to make a final concentration of 0.8% (w/v) by dissolving agarose in 1X Tris Boric-acid EDTA (TBE) buffer and 5 µl ethidium bromide (10 mg/ml) was filled for verification of the quality of extracted genomic DNA and amplified products. The agarose gel was melted using a microwave and then poured into a casting chamber which a comb was inserted. After the gel was solidified, a comb was removed carefully and the gel was put in an electrophoresis chamber. Sufficient volume of 1XTBE buffer was added to cover the gel to a depth about 0.5 cm. The genomic DNA or PCR products were mixed with 1 µl of 6X loading dye and was loaded to a well. Lambda DNA/ EcoRI+Hind III marker (0.04 µg/µl) was used as reference. Electrophoresis was performed at 80 volts for 40 minutes. The DNA samples were illustrated under UV trans illuminator.

C.2 Purification of PCR products

1. Transfer up to 100 µL reaction product to a microcentrifuge tube.
2. Add 5 volume of DF buffer to 1 volume of the sample and mix by vortexing.
3. Place a DF column into a collection tube.
4. Apply the sample mixture from previous step into the DF column.
5. Centrifuge at 13,000 rpm for 30 seconds.
6. Discard the flow through and place the DF column back in the collection tube.
7. Add 600 µL of Wash Buffer (ethanol added) to the DF column.
8. Centrifuge at 13,000 rpm for 30 seconds.
9. Discard the flow through and place the DF column back in the collection tube.
10. Centrifuge again at 13,000 rpm for 2 minutes to dry the column matrix.
11. Transfer dried DF column into a new microcentrifuge tube.
12. Add 20-50 µL deionized distilling water to the center of the column matrix.

13. Allow to stand for 2 minutes until deionized distilling water is absorbed by the matrix.
14. Centrifuge at 13,000 rpm for 2 minutes to elute purified DNA.
15. The purified PCR products were checked for amount by gelelectrophoresis prior to sequence.

C.3 Agar Diffusion Bioassay Method

The plant pathogen used as tested microorganism was *Erwinia caratovara*. *E. caratovara* was grown on Potato Sucrose Agar (PSA) slant at 26°C for overnight. *E. caratovara* was transfer to Potato Sucrose Broth (PSB) and incubated at 30°C on a rotary shaker at 150 rpm for 16-18 hours. Bioassay plates were sterilized by UV light exposure for overnight. The base layer was prepared by pouring 150 mL of PSA into bioassay plates. The upper layer was prepared by addition of bacterial cell suspension to 30 mL PSA to obtained OD of 0.1 at 540 nm, and then overlaid on a basal layer of solidified PSA. Wells (6 mm diameter) were made in each inoculated medium using a sterile cock borer. After fungal biomass was removed, sample (100 µL of culture broth) was added to each well. The plates were incubated at 30°C for overnight (16-18 hours) and examine for zone sizes of inhibition around the well in centimeters. Negative controls included only liquid medium. The antimicrobial activities were reported in terms of arbitrary units per milliter (AU/mL) against *Erwinia caratovara*. One arbitrary unit (AU) of antimicrobial activity was defined as the concentration of 250 µg of mercuric chloride ($HgCl_2$).

$$x^2 = \left(\frac{r - r_d}{2}\right)^2$$

Where r = radiance of inhibition zone (cm), r_d = radiance of cock borer (0.3 cm), and x = the distance between the edge of wells and the edge of inhibition zone (cm).

Table C.1 Calculation of Natural logarithm of arbitrary unit of Mercury (II) Chloride concentration and corresponding antimicrobial activity against *E.caratovora*

Concentration of $HgCl_2$ (µg/ml)	**AU**	**ln AU**	**Inhibition zone against *E.caratovora* (cm)**	***x* (cm)**	***x*² (cm²)**
50,000	200	5.3	3.36±0.00	1.38±0.00	1.90±0.00
10,000	40	3.69	3.02±0.01	1.21±0.01	1.46±0.02
5,000	20	3.00	2.86±0.00	1.13±0.00	1.28±0.00
2,000	8	2.08	2.60±0.00	1.00±0.00	1.00±0.00
1,000	4	1.39	2.40±0.01	0.90±0.00	0.81±0.01
750	3	1.10	2.22±0.00	0.81±0.00	0.66±0.00
500	2	0.69	2.06±0.01	0.73±0.00	0.53±0.01
250	1	0.00	1.72±0.00	0.56±0.00	0.31±0.00

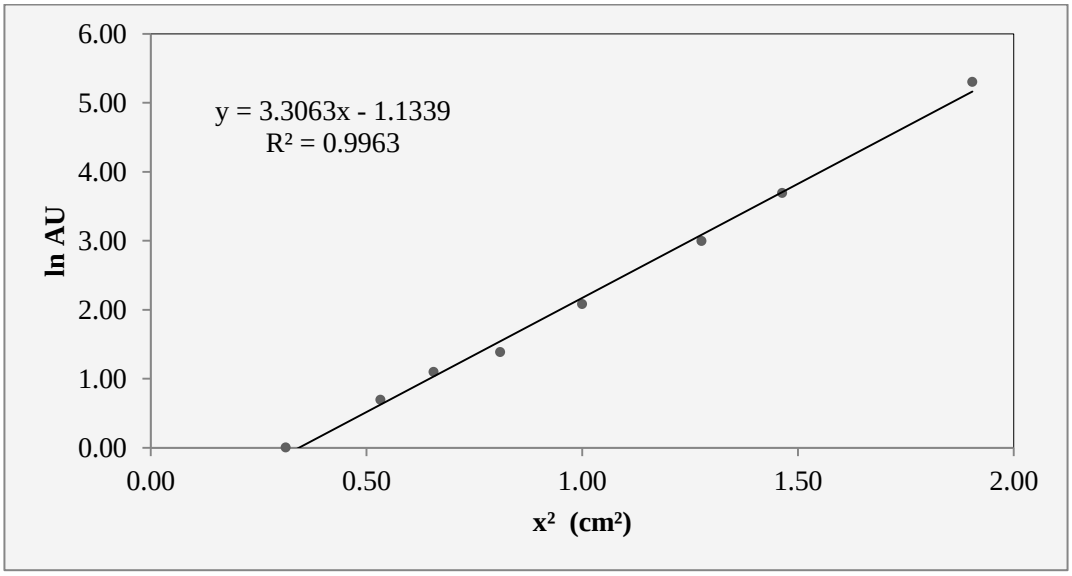

Figure C.1 Correlation between natural logarithm of arbitrary unit of mercury (II) Chloride concentration and the square of the distance between the edge of wells and the edge of inhibition zone with corresponding equation of y = 3.3063x-1.1339 (R^2 = 0.9963).

C.4 Cell Dry Weight

The mycelia were collected by filtration and washed twice with distilled water, and then transferred to weighed aluminum dishes. The fresh mycelia were dried at 80°C until a constant weight was obtained.

C.5 pH measurement

pH of culture supernatant was measured by an electric pH meter.

C.6 Total sugar

Total sugar was determinated by modified phenol-sulfuric acid method (Dobois, 1956).

1. Pipette 0.5 mL of the appropriate sugar concentration of sample into test tube.
2. Add 0.5 mL of 5% (w/v) phenol and mix.
3. Add 2.5 mL of sulfuric acid and mix.
4. Incubate at room temperature for 10 minutes.
5. Read the optical density at 490 nm and calculate sugar concentration present in sample by using standard curve of glucose concentration between 0-100 µg/mL.

Table C.2 The optical density at 490 nm of glucose concentration between 0-100 µg/mL

Glucose concentration (µg/mL)	Absorbance at 490 nm
0	0.00±0.00
25	0.17±0.02
50	0.32±0.00
75	0.46±0.02
100	0.63±0.00

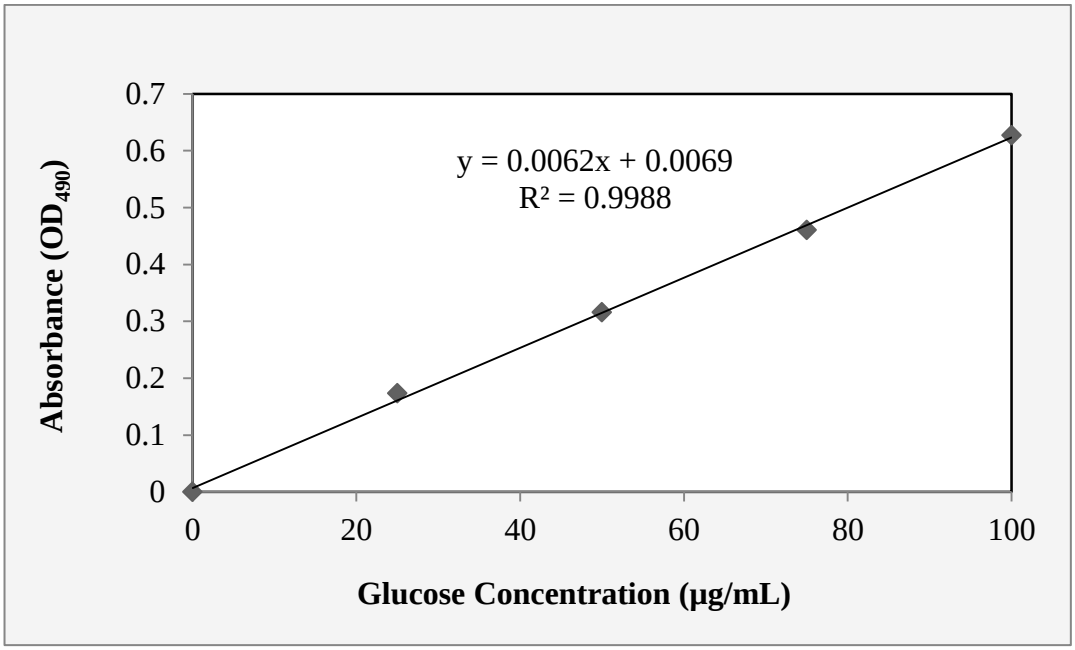

Figure C.2 Standard curve of glucose concentration (µg/mL) at optical density 490 nm for total sugar determination.