APPENDIX A:

Figures of various location in Signy Island

Figure 1: Backslope site on Signy Island

Figure 2: Elephant Flats site on Signy Island

Figure 3: Gourlay site on Signy Island

APPENDIX B

Isolation media

One tenth strength of all ingredients for isolation media purposes were added to distilled water and mixed thoroughly. The pH was then adjusted to pH 7.0. The media was then autoclave to 121℃ at 15psi for 20 minutes. The media was subjected to autoclave for 121℃, 15psi for 20 minutes. After autoclaving, all media except SM3 was cooled to approximately 50℃ and filtered sterilized 25µg/ml of cycloheximide and nystatin was supplemented into the media.

i. Reasoner's 2A – R2A with addition of 0.4% (w/v) sodium propionate. (Ronald, 1993; Crook *et al*., 1950)

Yeast extract	-	0.5 g
Peptone	-	0.5 g
Casamino acid	-	0.5 g
Glucose	-	0.5 g
Soluble starch	-	0.5 g
Sodium pyruvate	-	0.3 g
K_2HPO_4	-	0.3 g
$MgSO_4 . 7H_2O$	-	0.05 g
Sodium propionate	-	4 g
Agar	-	15.0 g
Distilled water	-	1 liter

ii. Reasoner's 2A – R2A (Ronald, 1993)

Yeast extract	-	0.5 g
Peptone	-	0.5 g
Casamino acid	-	0.5 g
Glucose	-	0.5 g
Soluble starch	-	0.5 g
Sodium pyruvate	-	0.3 g
K_2HPO_4	-	0.3 g
$MgSO_4 . 7H_2O$	-	0.05 g
Agar	-	15.0 g
Distilled water	-	1 liter

iii. Reasoner's 2A – R2A with addition of 50 µg/ml of rose Bengal (Ronald, 1993; Ottow, 1972)

Yeast extract	-	0.5 g
Peptone	-	0.5 g
Casamino acid	-	0.5 g
Glucose	-	0.5 g
Soluble starch	-	0.5 g
Sodium pyruvate	-	0.3 g
K_2HPO_4	-	0.3 g
$MgSO_4 . 7H_2O$	-	0.05 g
Sodium propionate	-	4 g
Agar	-	15.0 g
Distilled water	-	1 liter

iv. SM3 medium (Gauze's medium 2) (Tan *et al*., 2006)

Glucose	-	10 g
Peptone	-	5 g
Tryptone	-	3 g
NaCl	-	5 g
Agar	-	15 g
Distilled water	-	1 litre

v. Starch Casein Nitrate (SCN) agar (Kuster and Williams, 1964)

Soluble Starch	-	10.0g
KNO_3	-	2.0g
NaCl	-	2.0g
Casein	-	0.3g
K_2HPO_4	-	2.0g
$MgSO_4.7H_2O$	-	0.05g
$FeSO_4.7\ H_2O$	-	0.01g
$CaCO_3$	-	0.02g
Agar	-	15.0g
Distilled water	-	1 liter

vi. Starch Casein Nitrate (SCN) agar with addition of 2% NaCL (Kuster and Williams, 1964; Mackay, 1977)

Soluble Starch	-	10.0g
KNO_3	-	2.0g
NaCl	-	2.0g
Casein	-	0.3g
K_2HPO_4	-	2.0g
$MgSO_4.7H_2O$	-	0.05g
$FeSO_4.7\ H_2O$	-	0.01g
$CaCO_3$	-	0.02g
NaCL	-	20g
Agar	-	15.0g
Distilled water	-	1 liter

vii. Tryptic soy agar with addition of 0.1% (w/v) colloidal chitin (Difco laboratories, Hsu and Lockwood, 1975)

Pancreatic digest of casein	-	15 g
Enzymatic digest of soybean meal		5g
Sodium chloride		5g
Colloidal chitin		10g
Agar		15.0g
Distilled water		1 litre

viii. Tryptic soy agar (Difco laboratories)

Pancreatic digest of casein	-	15 g
Enzymatic digest of soybean meal	-	5 g
Sodium chloride	-	5g
Agar	-	15 g
Distilled water	-	1 litre

ix. Tryptic soy agar with addition of 0.1% (w/v) starch (Difco laboratories)

Pancreatic digest of casein	-	15 g
Enzymatic digest of soybean meal	-	5 g
Sodium chloride	-	5 g
Starch	-	10 g
Agar	-	15.0g
Distilled water	-	1 litre

<u>Purification media</u>

i. Yeast Malt Extract agar (ISP2) (Shirling and Gottlieb, 1966)

Yeast extract	-	4.0g
Malt extract	-	10.0g
Dextrose	-	4.0g
Agar	-	15.0g
Distilled water	-	1 litre

All ingredients were prepared in one-tenth dilution added to distilled water and mixed thoroughly. The media was subjected to autoclave for 121 ℃, 15psi for 20 minutes.

Antimicrobial assay media and saline preparation

i. Nutrient agar (Difco laboratories)

Difco™ nutrient broth	8.0g
Agar	12.0g

ii. Saline

0.85% (w/v) NaCl was prepared by dissolving 0.85g of NaCl into 100ml of distilled water and was subjected to autoclaving for 121 ℃, 15psi for 20 minutes.

Soil suspension solution

In this study, for Quarter-strength Ringer's solution was used for the isolation of actinobacteria for all the soils. However, tryptic soy broth was used in the isolation of LV-1 on TSA.

i. Quarter-strength Ringer's solution (Oxoid)

1 tablet was dissolved in 500ml distilled water and subjected to incubation at 121 ℃ at 15psi for 20 minutes.

ii. Tryptic soy broth (Bacto)

Pancreatic digest of casein	17g
Enzymatic digest of soybean meal	3g
Dextrose	2.5g
Sodium chloride	5g
Dipotassium phosphate	2.5g
Distilled water	1 litre

All ingredients were added to distilled water and mixed thoroughly. The broth was subjected to autoclave for 121 ℃, 15psi for 20 minutes.

APPENDIX C

Thin layer chromatography

i. Mobile phase

Solutions	Ratio	Volume (ml) (small tank)	Volume (ml) (Big tank)
Methanol	80	112	224
Distilled water	26	36.4	72.8
6N HCL	4	5.6	11.2
Pyridine	10	14	28

ii. Ninhydrin (0.2% w/v)

Ninhydrin 0.2g

Acetone 100ml

APPENDIX D

Storage buffers

i. Tris HCL 1M pH 8

Tris base powder 12.114g

Deionized water 100ml

Tris HCl was adjusted to pH 8.0 with HCL and topped up to 100ml water.

ii. EDTA 0.5M pH 8

EDTA powder 18.612g

Deionized water 100ml

EDTA powder was adjusted to pH 8.0 with NaOH and topped up to 100ml water.

iii. TE buffer

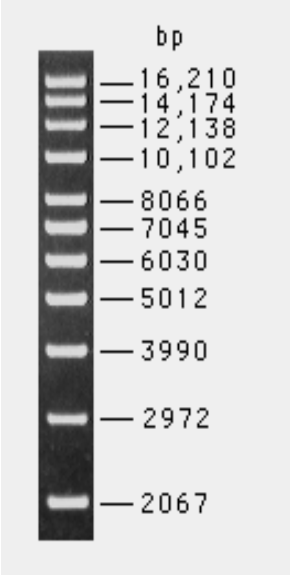
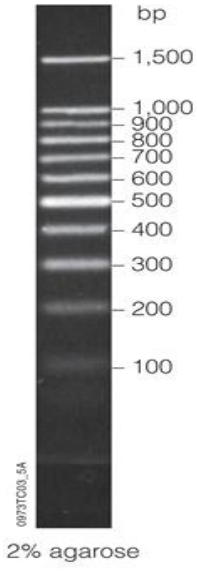
Tris 1M pH 8.0	1ml
EDTA 0.5M pH 8.0	0.2ml
Deionized water	100ml

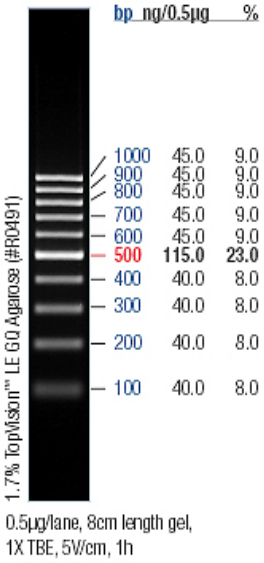
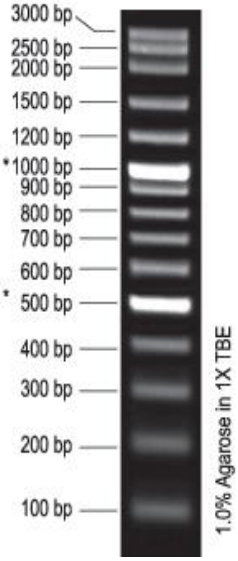
iv. 0.5X TBE buffer

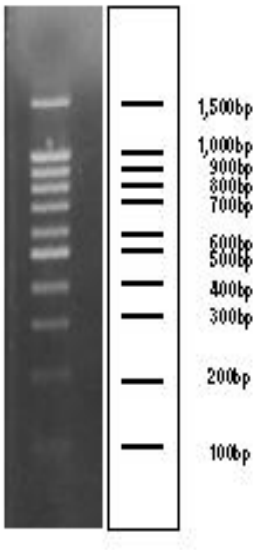
10X TBE buffer	25ml
Distilled water	475ml

Supercoiled DNA ladder and 100 bp ladder

5 µl of PCR products and NRPS gene products were mixed well with 2 µl of 1X loading dye and was then loaded into a well of agarose gel in 0.5X TBE buffer For ARDRA analysis, 13 µl of digested 16S rRNA fragments were mixed well with 2 µl of 6X loading dye was then loaded into a well of agarose gel in 0.5X TBE buffer.

Supercoiled DNA ladder (Invitrogen, USA)	100 bp DNA ladder (Promega, USA)
	
Supercoiled DNA Ladder separated by 0.9% (w/v) agarose gel stained with Ethidium Bromide.	100 bp DNA ladder separated by 2% (w/v) agarose gel in 0.5X TBE stained with Ethidium Bromide.

GeneRulerTM 100bp DNA ladder Plus (Fermentas, Lithuania)	VC 100bp Plus DNA ladder (Vivantis, Malaysia)
	
100 bp DNA ladder separated by 1.7% (w/v) agarose gel stained with Ethidium Bromide	100 bp DNA ladder separated by 1.0% (w/v) agarose gel stained with Ethidium Bromide

100 bp molecular weight DNA marker (iNtRON, Korea)

100 bp DNA ladder separated by 0.7% agarose gel stained with Ethidium Bromide