Report

Screening for eukaryotic signal transduction and *Mycobacterium isocitrate* lyase inhibitor from actinomycetes and fungi of dipterocarp rain forests at Imbak Valley, Sabah, Malaysia

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ABSTRACT. A diversity of actinomycetes and fungi was isolated from various sites during the Imbak Valley Scientific Expedition 2000. A total of 144 soil samples were collected under trees that have been identified to species or genus level. Imbak Valley is a lowland dipterocarp forest, which is interestingly dominated by *Dryobalanops beccarii*. Isolation of *Streptomyces* and non-*Streptomyces* actinomycetes on HV medium and other specific isolation media for non-*Streptomyces* yielded 203 isolates from 89 soil samples. Morphological characterisation of the isolated actinomycetes was carried out based on aerial mycelium colour, substrate mycelium colour and diffusible pigment production on oatmeal medium. Nine strains of fungi were isolated from the six soil samples plated on PDA medium. All actinomycetes isolates were grown under aerobic condition in liquid culture and extracted with acetone, and used for screening against proteins involved eukaryotic signal transduction. Yeast MAPK kinase and MAP kinase phosphatase were some of the targeted proteins used in this research. MKK1^{P386} and MKK1^{P386}-MSG5 mutant yeasts were used to screen for these inhibitors, as these yeast kinase and phosphatase have homologous proteins in the MAP kinase signal transduction pathway in human. No inhibitors in the extracts were found in these screenings. Type 1 protein serine/threonine phosphatase (GLC7) in yeast was used to screen inhibitors against PP1 inhibitors and no inhibitor was found. None of the fungal extracts showed any inhibitory activities in all the screening systems. No Ras/Raf inhibitor was found in the in vivo Ras/Raf interaction with the yeast two-hybrid screening system, which used to screen for inhibitor against Ras/Raf protein interaction inhibitor. There were 11 actinomycetes extracts that showed toxicity against yeast strain LZ (transformant of Ras/Raf). H7667, a Streptomycete toxic to yeast is further screened for inhibitors of the GSK3-beta pathway. H7763, a *Streptomyces* species that showed positive in the primary screen for inhibitor of isocitrate lyase (ICL) which is not itaconic acid (known ICL inhibitor). H7240 showed the strongest susceptibility towards the resin in which the concentration of 5g/l of resin is sufficient to produce growth inhibition of the bacteria.

Keywords: actinomycetes, fungi, eukaryotic signal transduction, *Mycobacterium*, dipterocarp rain forests

INTRODUCTION

Borneo is listed as a hot spot of biodiversity with an estimated 20,000 – 25,000 plant species. Amongst these plant species, about 30 percent are endemic to Borneo (Nais *et al*., 2000). Sabah alone has 284 species in the Dipterocarpaeae family out of the world total of about 386 species (Meijer & Wood, 1964). Imbak Valley, among other areas is a preserved lowland tropical dipterocarp rain forest.

A major advantage of the natural products approach to drug discovery is that it is capable of providing complex molecules that would not be accessible by other routes. Thus, the search for novel metabolites ultimately is dependent on the selective forces that resulted in the origin and evolution of modified biosynthetic pathways of biosynthesis of secondary metabolites as occurred in different species. The search for novel inhibitors of eukaryotic cellular functions involves screening large collection of rare and novel actinomycetes, especially *Streptomycetes*, especially from neglected habitats such as those found in tropical rainforests. Actinomycetes were isolated from soil samples collected from just beneath the leaf litter of identified trees that were used in an initial attempt to study the possible co-evolution of the metabolic pathways of secondary metabolites of actinomycetes and plant (Ho *et al*., 2003).

Actinomycetes and fungi are prolific producers of bioactive secondary metabolites. Members of the Order Actinomycetales produce many commercially important metabolites, notably antibiotics and non-antibiotics. Some actinomycetes are also a source of inhibitors of eukaryotic cell cycle, for example, trichostatin A from *Streptomyces hygroscopicus* causes arrest of the cell cycle in synchronous cultures of normal rat fibroblasts in both the G1 and G2 phases by acting as a potent inhibitor of histone deacetylase (Yoshida *et al*.,1995). Staurosporine, an antifungal agent, is produced by *Saccharothrix aerocolonigenes* and was found to inhibit PKC. Its synthetic analog, UCN01 (7-hydroxystaurosporine) also blocks cell-cycle-checkpoint-control kinase CHK1 (Cohen, 2002).

One of the crucial conditions in natural products approach to drug discovery is the effective target-directed screening system to identify novel and specific inhibitor in the crude extracts (Kuo & Garrity, 2002). One such convenient screening system is to use the molecular targeted system in yeast (Parsons *et al*., 2003). In our laboratory, we used the genetically modified yeast systems to screen for inhibitors against protein kinases and phosphatases involved in eukaryotic signal transduction. These screening systems include the mitogen-activated protein kinase pathway (MKK1 and MSG5) as these proteins have mammalian homologues. We also screened for inhibitors against serine/threonine phosphatase (PP1) in yeast system and Ras/Raf interaction using the yeast two-hybrid system.

The abilities of *Mycobacterium tuberculosis* to infect macrophages, establishing a long term, persistent infection in the host have made it one of the most wide spread diseases in the world. During persistent infection, fatty acids are used as the main carbon source and in response, the glyoxylate shunt is up-regulated. In mice, the disruption of *icl* gene, which codes for the glyoxylate enzyme isocitrate lyase (ICL), attenuates *M. tuberculosis* persistent infection (McKinney *et al*., 2000; Muñoz-Elías & McKinney, 2005). The strategy for survival during the chronic stages of infection entails a metabolic shift in the bacilli utilization of carbon source to C_2 substrates, particularly in the form of acetyl-CoA generated by ß-oxidation of fatty acids.

A rapid inhibitor screening strategy adapted from Sharma *et al*. (2000) is being conducted in our laboratory. This screening strategy essentially searches for potential inhibitors of ICL (isocitrate lyase).The objectives of the present study are: (1) To isolate a collection of diverse strains of soil actinomycetes and fungi from lowland primary dipterocarp forest of Imbak Valley, (2) To screen for potential inhibitor of protein kinase (cancer) and phosphatases (Memory- Genoux *et al*., 2002) and (3) To screen for ICL inhibitor for persistent TB.

MATERIALS AND METHODS

Sampling Sites

This study was carried out at Imbak Valley, Sabah, during the Imbak Valley Scientific Expedition 2000 (8 - 18 June and 1 - 7 July). Soil samples were collected using sterile methods under identified trees, especially from members of the Dipterocarpaceae and Anacardiaceae. They were chosen because they produce many toxic resins and latex in their bark. The soil samples were kept at room temperature and under sterile conditions to avoid contamination. Figure 1 shows the location of the soil sampling sites in Imbak Valley.

Generally, collection was based on one soil sample per tree species. However, some of the soil samples were repeatedly collected under the same tree species but from different individual tree except for four soil samples (K1-K3 and SA-144) collected from soil under the same individual tree (*Koompassia excelsa*).

Isolation of actinomycetes

a) Serial dilution of soil suspension onto isolation medium

For the isolation of actinomycetes, 0.05g of soil was suspended in sterile distilled water with 10^{-3} serial dilution. Hundred-µl of the diluted suspension was plated on HV (humic acid + B-vitamins) agar medium (Nonomura & Hayakawa, 1988), pH 7.2 or pH 4.5, which was supplemented with cycloheximide. The plates were incubated at 28°C for 1-4 weeks until single colonies grew on the plates. An exception was the centrifugation used by Sylvia Daim (Daim, 2003).

Low salt + *p*-nitrophenol (Yoon *et al*., 1999) and glucose asparagine agar were used for the attempted isolation of *Nocardioides*. The plates were incubated at 28°C for 1-4 weeks until single colonies grew on the plates (Low, 2001).

b) Classification of strains

The actinomycetes colonies were streaked onto oatmeal agar plates, pH 7.2 and 4.5, and incubated at 28°C for 14 days. Taxonomic studies of actinomycetes were based on morphology (aerial mycelium colour, substrate mycelium colour and extracellular pigmentation). A cover slip culture followed by Gram staining was also used.

c) Production of secondary metabolites

Production of microbial secondary metabolites were from actinomycetes grown in 2% mannitol + 2% peptone + 1% glucose liquid medium at 28°C for 5 days. The 10ml culture in conical flask (125ml) was shaken at 220 rpm and terminated by extraction of secondary metabolites using equal volume of acetone to culture medium (final concentration of extract in 50% acetone).

d) Culture collection and safekeeping.

Glycerol (20%) stock of spore of actinomycetes was prepared and stored at -20°C.

Isolation of microfungi

a) Isolation and purification of microfungi

Small amount of soil samples was transferred onto Potato Dextrose Agar, (PDA) with chloramphenicol (50 µg/ml) at pH 5.5 using a sterile forceps. The soil was spread onto the agar surface using a sterile spreader. The plate was then left to incubate at 28°C for about five days. Colonies of fungi would appear after that incubation period. Colonies that were distinctly distinguishable were transferred over to normal PDA plates in which were incubated for 5-7 days. The purified isolates were then transferred over to agar slants and silica gel for storage.

b) Classification of microfungi

Isolated strains grown on normal PDA plates at 28°C for 5-7 days were observed for both the

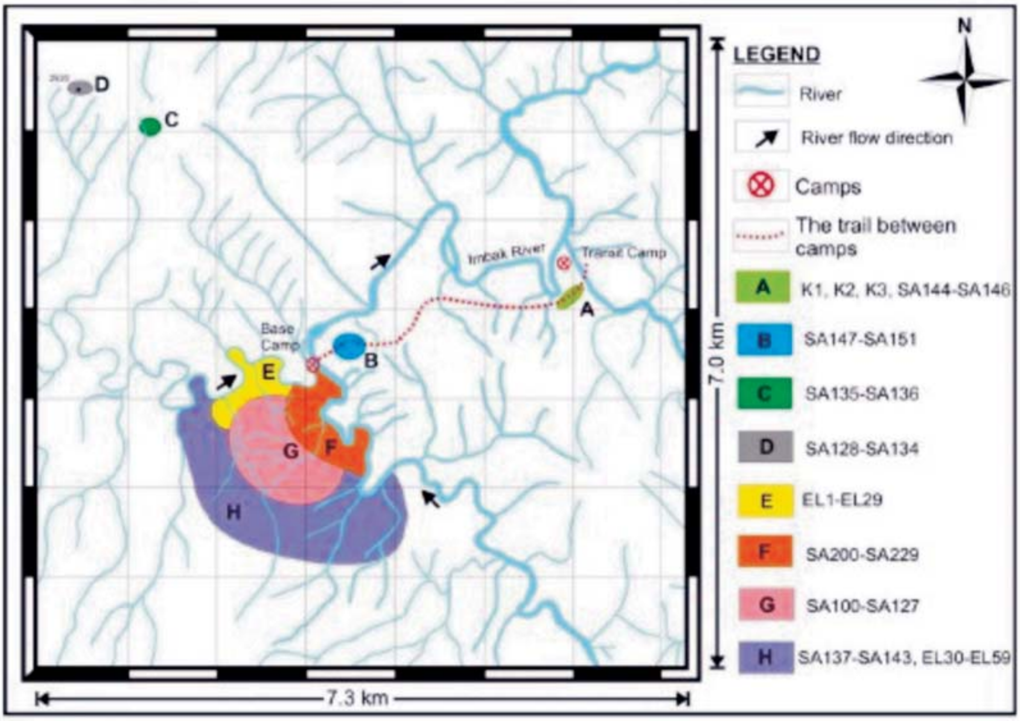

Figure 1. The location of the soil sampling sites in Imbak Valley.

colours of the aerial mycelia and spores, and the substrate mycelia. Any extracellular pigmentation observed from the fungi colony was noted. A further classification of fungi would be based on the structure of the conidiophore which is unique to certain fungi as observed under the light compound microscope.

c) Production of secondary metabolites from microfungi

Submerged aerobic growth of purified cultures were carried out in 15ml liquid medium (1% yeast extract, 1% peptone, 1% sucrose, 0.1% KH_2PO_4 and 0.03% $MgSO_4 . 7H_2O$ in Erlenmeyer flasks (125ml) for seven days at 28°C rotated 220 rpm. Resultant broths were added with equal volume of acetone to culture medium to extract secondary metabolites (final concentration of extract in 50% acetone).

Screening for specific inhibition of molecular targets in cell based yeast system

a) MAPK kinase (MKK1) and MAP kinase phosphatase (MSG5) in cell wall integrity system in yeast (Gustin *et al*., 1998;Watanabe *et al*., 1995;Yashar *et al*., 1995)

The screening for inhibitor against MKK1 and MSG5 followed the method as described in Ho *et al*. (2001). An inhibitor of Mkk1 will result in a halo of yeast growth around the paper disk containing the extract in the galactose medium while growth will cover the whole plate in the glucose medium. This behaviour is caused by the inhibitor cancelling the lethal (lysis) effect of the overexpressed mutant $MKK1^{P386}$ kinase under galactose induction through the GAL1 promoter in the cell wall integrity system.

An inhibitor of MSG5 will show a growth inhibition halo around the paper disk containing the extract in galactose medium as the protein phosphatase of MSG5 will nullify the lethal effect of overexpressed of mutant MKK1^{P386} kinase.

b) GLC7, the catalytic subunit of serine/threonine protein phosphatase type I (PP1) in yeast (Andrews & Stark, 2000; Stark, 1996)

The screening system used the temperature sensitive mutant GLC7-10 (PAY700-4) with cell cycle arrest at 37°C but the lethal effect was suppressed by high osmolarity (1M sorbitol) (Andrews & Stark, 2000). The inhibitor acting on GLC7 protein in the wild type (PAY704-1) should have the same characteristic like mutant phenotype, specifically growth inhibition at 37°C but growth recovery with the addition of 1M sorbitol.

c) Ras/Raf two-hybrid yeast system (Ki *et al*., 1998; Vojtek *et al*., 1993)

The screening utilized the yeast two-hybrid (cloned mammalian Ras and Raf) system. The LZ (H10014) is a transformant of L40 (H10006) *MATα trp1 leu2 his3 LYS::lexA-HIS 3 URA3::lex3-lacZ* with pLexA-RAS^{v12}(H10,011) and pVD16-RAF (H10,012). This transformant is able to grow on medium lacking histidine and produces ß-galactosidase since the transcription of these two reporter genes are stimulated by the formation of the complex pLex-RAS^{v12} and pVP16-RAF (Ki *et al*., 1998).

If this protein-protein interaction is interrupted by the inhibitor, the transformant can only grow in the presence of histidine but not in the absence of histidine. Expression of the *lacZ* reporter gene is also not induced.

Mycobacterium: ICL screening system

Screening for ICL inhibitors using *Mycobacterium smegmatis* H8000 (wild type) and H8012 (mutant) was done by paper disk susceptibility test method. H8012 is a *icl* deletion *M. smegmatis* complemented with *M. tuberculosis* normal *icl* gene. Both H8000 and H8012 were plated on minimal medium containing either glucose or actetate as the sole carbon source. An ICL inhibitor is expected to cause growth inhibition of *M. smegmatis* on acetate plates but not on the glucose (Sharma *et al*., 2000).

Effect of resin from *Shorea smithiana* on the growth of actinomycetes

Two-hundred-µl of actinomycetes culture was spread on the oatmeal agar. Twenty-µl of different resin concentration (3.5-5 g/l) in N,N-Dimethylformamide was applied to different sterile disk and placed onto the oatmeal agar. All plates were incubated at 40°C overnight before transferred to 28°C for 2-3 days. The result was recorded after three days (Janim, 2001).

RESULTS AND DISCUSSION

Actinomycetes

There are three distinguishing forest types in Imbak Valley, the lowland dipterocarp forest (Base camp), semi-logged forest (from Transit camp to logging road) and nutrient poor kerangas forest. The three forest types represented the soil sampling area to capture the biodiversity of microbes of Imbak Valley. The surrounding area of the Base Camp is dominated by *Dryobalanops beccarii* and other *Shorea* sp. including *S. argentifolia, S. macroptera, S. mecistopteryx* (Table 1, 2, 3 and 4). Table 5 shows the distribution of soil samples collected under identified trees (genera level) with isolates (Jagdish, 2001; Kee, 2001; Lai, 2003; Lee, 2001).

a) Numerical data of actinomycetes isolates

A total of 203 actinomycetes isolates were isolated by selective isolation media of acid (pH4.5) and neutral pH (pH7.2) to select

Table 1. List of 50 soil samples from Imbak Valley expedition I (8-18/6/2000).

Type of forest	Date of soil collected	Soil sample	Scientific name of tree	Sampling region	Location (Figure 1)	Number of actinomycete isolates (with isolation number) from HV-B vitamins, pH 7.2[a] except otherwise stated
Unlogged primary lowland dipterocarp forest, Base camp area	16/6/2000	EL 1	*Gluta sabahana*	E	Base camp	**3** (E15, E16, E108)
	16/6/2000	EL 2	*Gluta sabahana*		Base camp	**3** (E27, E28, E31)
	16/6/2000	EL 3	*Gluta sabahana*		Base camp	**3** (E40, E41, SFD2A[f])
	16/6/2000	EL 4	*Mangifera* sp.		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**9** (E49, E50, E51, E52, E55, E56, E57, E107, E109)
	16/6/2000	EL 5	*Dipterocarpus confertus*		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**1** (E65)
	16/6/2000	EL 6	*Knema* sp.		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**2** (E70, E104)
	16/6/2000	EL 7	*Elaeocarpus* sp.		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**1** (E71)
	16/6/2000	EL 8	*Ganua kingiana*		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**2** (E72, E73)
	16/6/2000	EL 9	*Lithocarpus* sp.		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**7** (E74, E75, E76, E77, E78, E79, E111)
	16/6/2000	EL 10	*Goniothalamus* sp.		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**3** (E12, E13, E14)
	16/6/2000	EL 11	*Eugenia* sp.		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**4** (SFD3A[f], SFD4A[f], SFD5A[f], SFD6A[f])
	16/6/2000	EL 13	*Durio graveolens*		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**2** (E17, SFD7A[f])
	16/6/2000	EL 14	*Dryobalanops lanceolata*		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**1** (E18)
	16/6/2000	EL 15	*Shorea ovalis*		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**3** (E19, E105, E112)
	16/6/2000	EL 16	*Mallotus* sp.		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**4** (E371,E372,E363, E364)
	16/6/2000	EL 17	*Shorea argentifolia*		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**2** (E20,E21)
	16/6/2000	EL 18	*Kopsia dasyrachis*		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**4** (E22, E80, E103, E110)
	16/6/2000	EL 19	*Shorea smithiana*		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**4** (E23, E24, E25, SFD8A[f])
	16/6/2000	EL 20	*Koilodepas longifolium*		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**1** (E26)
	16/6/2000	EL 21	*Baccaurea pulata*		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**2** (E29, E30)
	16/6/2000	EL 22	*Eusidroxylan zwageri*		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**2** (E400, E401)
	16/6/2000	EL 23	*Dacryodes* sp.		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**1** (E32)
	16/6/2000	EL 25	*Parashorea malaanonan*		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**1** (E33)
	16/6/2000	EL 26	*Dipterocarpus kerrii*		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**2** (E369,E370)

continued Table 1

Unlogged primary lowland dipterocarp forest, Base camp area	16/6/2000	EL 28	*Scaphium* sp.		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**5** (E34, E35, E36, E37, E38)
	16/6/2000	EL 29	*Shorea mecistopteryx*		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**1** (E39)
	16/6/2000	EL 30	*Dialium indum*		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m	**3** (E394, E365, E366)
	10/6/2000	SA 100	*Shorea* sp.	**G**	Hill slope, near base camp, Imbak Valley, altitude ~106.7m	**1** (LNS 30[c])
	10/6/2000	SA 101	*Shorea almon*		Hill slope, near base camp, Imbak Valley, altitude ~106.7m	**3** (L116[b], L117[b], LNS20[e])
	10/6/2000	SA 102	*Shorea macroptera*		Hill slope, near base camp, Imbak Valley.	**5** (L119[b], L120[b], L121[b], L174 , LNS 9)
	10/6/2000	SA 103	*Dryobalanops beccarii*		Hill slope, near base camp, Imbak Valley	**1** (LNS 10)
	10/6/2000	SA 104	*Mangifera* sp.		Hill slope, near base camp, Imbak Valley	**5** (L122[b], L123[b], L124[b], JAY 6, JAY 7)
	10/6/2000	SA 105	*Gluta* sp.		Slope hill down to river, near base camp, Imbak Valley. About 152.4m from the SA-100. Beside *Dryobalanops beccarii*.	**4** (L125[b], L126[b], JAY 8, JAY 9)
	10/6/2000	SA 108	*Vatica*.sp.		Hill slopes, near base camp, Imbak Valley. Latex was collected.	**2** (L127[b], L128[b])
	10/6/2000	SA 109	*Eurycoma longifolia*		Beside SA 108. Hill slopes, near base camp, Imbak Valley.	**1** (DMH 3[c])
	10/6/2000	SA 111	*Calophyllum* sp.		Near SA 110. About 300m from the base camp.	**5** (LNS2, LNS4, LNS 21[e], L129, L130)
	10/6/2000	SA 113	*Hopea pentanervia*		About 300m from the base camp. Latex was collected.	**1** (LNS11)
	10/6/2000	SA 114	*Palaquium* sp.		About 400m from the base camp	**1** (LNS 31[d])
	10/6/2000	SA 115	*Quercus* sp.		About 300m from the base camp	**1** (L131)
	10/6/2000	SA 116	*Calophyllum* sp.		About 300m from the base camp. Yellow latex.	**2** (LNS13, LNS16)
	10/6/2000	SA 117	*Shorea smithiana*		Near SA 116. Big size resin collected. About 300m from the base camp.	**2** (LNS14, LNS15)
	10/6/2000	SA 120	*Palaquium* sp.		Beside *Dryobalanops beccarii.* Tree with white latex. About 300m from the base camp.	**2** (L132, L133)
	10/6/2000	SA 121	*Dryobalanops beccarii*		About 91.4m from the base camp, beside Nyatoh Sarawak	**2** (LNS7, LNS22[e])
	10/6/2000	SA 125	*Drypetes* spp.		About 91.4m from the base camp	**5** (LBK 6, LBK 7, LBK 8, L172, L173)

continued Table 1

Unlogged kerangas forest (VGR)	13/6/2000	SA 128	*Shorea hypoleuca*	**D**	Ridge, full of rocks, two *Shorea* tree next to each other, VGR forest (left of Sg.Imbak). 360m from sea level	**1** (L134)
	13/6/2000	SA 131	*Calophyllum* sp.		453.5m from sea level, slope hill down, VGR forest (left side of Sg.Imbak)	**1** (KEE12)
	13/6/2000	SA 134	*Ficus* spp.		Bukit Imbak, associated with SA 133	**1** (KEE13)
Unlogged kerangas forest	13/6/2000	SA 136	*Calophyllum venulosum*	**C**	Down hill Bukit Imbak, tree bark with mosses growth	**1** (LBK9)

Unlogged kerangas forest	13/6/2000	SA 136	*Calophyllum venulosum*	**C**	Down hill Bukit Imbak, tree bark with mosses growth	**1** (LBK9)
Semi logged forest	10/6/2000	SA 144	*Koompassia excelsa*	**A**	Slope after Transit camp	**6** (JAY1, JAY2, JAY3, JAY4, JAY 11, JAY 1 2)
	10/6/2000	SA 145	*Eusideroxylan zwageri*		Slope after Transit camp	**2** (JAY 5, JAY10)

Part of the tree samples was field identified frequently using local name and checked with specimen voucher in Herbarium Forest Research Center, Sandakan

Abbreviation:

[a] Strains isolated on humic acid +B-vitamins agar + cycloheximide, pH 7.2, incubated at 28°c for 1-4 week.
[b] Strains isolated on humic acid +B-vitamins agar + cycloheximide, pH 4.5, incubated at 28°c for 1-4 week.
[c] Strains isolated on humic acid +B-vitamins agar + cycloheximide, pH 8, incubated at 28°c for 1-4 week.
[d] Strains isolated on glucose asparagine agar, pH 7.2, incubated at 28°c for 1-4 week
[e] Strains isolated on low salt + *p*-nitrophenol pH 7.2, incubated at 28°c for 1-4 week
[f] Strains isolated using centrifugation technique by Sylvia Daim

Table 2. List of 39 soil samples from Imbak Valley expedition II (1-7/7/2000).

Type of forest	Date of soil collected	Soil sample	Scientific name of tree	Sampling region	Location (Figure 1)	Number of actinomycete isolates (with isolation number) HV-B vitamins, pH 7.2[a] except otherwise stated
Unlogged primary lowland dipterocarp fo rest, Base camp area	3/7/2000	SA 200	*Dryobalanops beccarii*	F	Left side of Sg. Imbak, about 200 m from base camp. Near to SA-201	**1** (BEM 1)
	3/7/2000	SA 203	*Knema* sp.		Near SA202, Left side of Sg. Imbak, about 220 m from base camp. Tree with red latex.	**2** (KEE14, KEE15)
	3/7/2000	SA 206	*Baccaurea macrocarpa*		Near SA205, Left side of Sg. Imbak, about 200 m from base camp	**6** (LBK12, LBK14, LBK15, LBK16, LBK18, LBK19)
	3/7/2000	SA 207	*Scaphium* sp.		Center part near Sg. Imbak, sea level ~91.4-106.7m	**1** (KEE10)
	3/7/2000	SA 208	*Scorodocarpus borneensis*		Few meter from SA207, center part near Sg. Imbak, sea level ~91.4-106.7m	**2** (LBK10, LBK11)
	3/7/2000	SA 210	*Gluta* sp.		Near SA209, center part near Sg. Imbak, sea level ~91.4-106.7m	**4** (LBK1, LBK2, LBK3, LBK4)
	3/7/2000	SA 211	*Shorea mecistopteryx*		Center part near Sg. Imbak, sea level ~91.4-106.7m	**1** (JOE10)
	3/7/2000	SA 212	*Ptenandra coerulescens*		Near SA211, Center part near Sg. Imbak, sea level ~91.4-106.7m	**1** (LBK5)
	3/7/2000	SA 214	*Lophopetalum javanicum*		Near SA215, center part near Sg. Imbak, sea level ~91.4-106.7m	**1** (KEE11)
	3/7/2000	SA 216	*Lithocarpus* or *Quercus*		Near SA215, center part near Sg. Imbak, sea level ~91.4-106.7m	**1** (KEE1)
	3/7/2000	SA 217	*Shorea macrophylla*		Center part near Sg. Imbak, sea level ~91.4-106.7m	**2** (JOE6, JOE7)
	3/7/2000	SA 218	*Dipterocarpus confertus*		Center part near Sg. Imbak, sea level ~91.4-106.7m	**1** (JOE9)
	3/7/2000	SA 219	*Shorea smithiana*		Center part near Sg. Imbak, sea level ~91.4-106.7m	**1** (JOE8)
	3/7/2000	SA 220	*Dryobalanops lanceolata*		Center part near Sg. Imbak, sea level ~91.4-106.7m	**5** (JOE1, JOE2, JOE3, JOE4, JOE5)
	3/7/2000	SA 223	*Eusideroxylan zwageri*		Center part near Sg. Imbak, sea level ~91.4-106.7m	**2** (KEE2)
	3/7/2000	SA 224	*Parashorea malaanonan*		Center part near Sg. Imbak, sea level ~91.4-106.7m	**1** (BEM5)
	3/7/2000	SA 225	*Shorea gysbertsiana*		Center part near Sg. Imbak, sea level ~91.4-106.7m	**2** (BEM7, BEM8)
	3/7/2000	SA 226	*Shorea spp.*		Center part near Sg. Imbak, sea level ~91.4-106.7m	**1** (BEM10)
	12/7/2000	SA-229	*Koompassia excelsa*		Center part near Sg. Imbak, sea level ~91.4-106.7m	**7** (KEE3, KEE4, KEE5, KEE6, KEE7, KEE8, KEE9)

continued Table 2

Unlogged primary lowland dipterocarp forest, Base camp area	3/7/2000	EL 33	*Sarchotheca diversifolia*	**H**	Center part near Sg. Imbak, sea level ~91.4-106.7m	**2** (LBK13, LBK17)
	3/7/2000	EL 34	*Phoeba macrophylla*		Center part near Sg. Imbak, sea level ~91.4-106.7m	**2** (E42, E43)
	3/7/2000	EL 35	*Shorea macroptera*		Center part near Sg. Imbak, sea level ~91.4-106.7m	**1** (E44)
	3/7/2000	EL 36	*Shorea parvifolia*		Center part near Sg. Imbak, sea level ~91.4-106.7m	**3** (E45, E46, E47)
	3/7/2000	EL 37	*Shorea johorensis*		Center part near Sg. Imbak, sea level ~91.4-106.7m	**1** (E106)
	5/7/2000	EL 40	**Durio grandiflorus**		Left side of Sg. Imbak, sea level ~91.4-106.7m	**2** (E 48, 403)
	5/7/2000	EL 42	*Dillenia borneensis*		Left side of Sg. Imbak, sea level ~91.4-106.7m	**2** (E53, E54)
	5/7/2000	EL 45	*Eugenia syzguim*		Left side of Sg. Imbak, sea level ~91.4-106.7m	**1** (E58)
	5/7/2000	EL 46	*Teijsmanniodendron holophyllum*		Left side of Sg. Imbak, sea level ~91.4-106.7m	**3** (E59, E60, E61)
	5/7/2000	EL 47	*Gonystylus bancanus*		Left side of Sg. Imbak. sea level ~91.4-106.7m	**2** (E62, E63)
	5/7/2000	EL 48	*Antiaris toxicaria*		Left side of Sg. Imbak, near E 49, sea level ~91.4-106.7m	**1** (E271)
	5/7/2000	EL 50	*Shorea mecistopteryx*		Left side of Sg. Imbak, sea level ~91.4-106.7m	**1** (E64)
	5/7/2000	EL 51	*Archidendron jiringa*		Left side of Sg. Imbak, near E 52, sea level ~91.4-106.7m	**2** (E66, E67)
	5/7/2000	EL52	*Durio* sp.		Left side of Sg. Imbak, Near E 51, sea level ~91.4-106.7m	**1** (E395)
	5/7/2000	EL 53	*Phoeba macrophylla*		Left side of Sg. Imbak, sea level ~91.4-106.7m	**1** (E68)
	5/7/2000	EL 55	Myristicaceae		Left side of Sg. Imbak, sea level ~91.4-106.7m	**1** (E398)
	5/7/2000	EL 57	*Anisophyllea borneensis*		Left side of Sg. Imbak, sea level ~91.4-106.7m	**1** (E69)
Semi logged forest	12/7/2000	K 1	*Koompassia excelsa*	**A**	Left side from the Transit camp, ~500m, giant tree with diameter diameter ~3.1m. Height~ 15.2m.	**2** (E81, E82)
	12/7/2000	K 2	*Koompassia excelsa*		Left side from the transit camp, ~500m, giant tree with diameter diameter ~3.1m. Height~ 15.2m..	**1** (E 83)
	12/7/2000	K 3	*Koompassia excelsa*		Left side from the transit camp, ~500m, giant tree with diameter ~3.1m. Height~ 15.2m.	**1** (E102)

All soil samples collected from different tree (individual) even same species tree except soil samples SA-229, K1, K2 and K3 under the same tree. Part of the tree samples was field identified frequently using local name and checked with specimen voucher in Herbarium Forest Research Center, Sandakan.

Abbreviation:
a Strains isolated on humic acid +B-vitamins agar + cycloheximide, pH 7.2, incubated at 28^{o}c for 1-4 week.

Table 3. List of 32 soil samples from Imbak Valley without isolated actinomycetes from Imbak Valley expedition I (8-18/6/2000).

Type of forest	Date of soil collected	Soil sample	Scientific name of tree (Vernacular name)	Sampling region	Location (Figure 1)
Unlogged primary lowland dipterocarp forest, Base camp area	16/6/2000	EL 12	*Shorea* sp.	E	Right side ridge beside Sungai Imbak, sea level¬500ft
	16/6/2000	EL 24	*Dillenia borneensis*		Right side ridge beside Sungai Imbak, sea level¬500ft
	16/6/2000	EL 27	*Shorea gysbertsiana*		Right side ridge beside Sungai Imbak, sea level¬500ft
	10/6/2000	SA 106	*Shorea argentifolia*	G	About 200m from SA-105, hill slope, near base camp, Imbak Valley,
	10/6/2000	SA 107	*Dryobalanops beccarii*		Near Sa-106, hill slope, near base camp, Imbak Valley
	10/6/2000	SA 110	*Canarium* spp.		About 300m from the base camp
	10/6/2000	SA 112	*Garcinia* sp.		About 300m from the base camp, beside *Eurycoma longifolia* and *Dryobalanops beccarii*, yellow latex was collected
	10/6/2000	SA 118	(Perupok)		About 300m from the base camp
	10/6/2000	SA 119	*Durio* sp.		About 300m from the base camp
	10/6/2000	SA 122	*Scorodocarpus borneensis*		About 300m from the base camp, white latex, beside *Dryobalanops beccarii*.
	10/6/2000	SA 123	*Heritiera longipetilolata*		Near slope to the river, near base camp
	10/6/2000	SA 124	*Artocarpus odoratissimus*		Near slope to the river, near base camp, near SA-123
	12/6/2000	SA 126	(Sapindasi)		Beside Sg.Imbak, small tree, crowded with other plant like bamboo. Red-orange fruit was collected
	12/6/2000	SA 127	*Shorea* spp.		Beside Sg. Imbak, about 1-1.5m from the riverbank, a lot of mosses. Beside Fabaceae tree.
	15/6/2000	SA 137	*Goniothalamus velutimus*	H	Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m
	15/6/2000	SA 138	*Shorea argentifolia*		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m
	15/6/2000	SA 139	*Ammomum* sp.		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m
	15/6/2000	SA 140	*Eusideroxylon zwageri*		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m.
	15/6/2000	SA 141	*Dryobalanops beccarii*		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m
	15/6/2000	SA 142	*Shorea macroptera*		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m
	15/6/2000	SA 143	*Garcinia* spp.		Right side of the ridge, Sg. Imbak, altitude ~91.4-121.9m
Semi-logged forest	10/6/2000	SA 146	Scaphium sp.	A	Slope after Transit camp
Unlogged lowland forest	10/6/2000	SA 147	-	B	Slope before Sg. Imbak
	10/6/2000	SA 148	-		Slope before Sg. Imbak
	10/6/2000	SA 149	*Eusideroxylon zwageri*		Slope before Sg. Imbak
	10/6/2000	SA 150	*Dryobalanops beccarii*		Slope before Sg. Imbak
	10/6/2000	SA 151	*Dryobalanops beccarii*		Slope before Sg. Imbak

continued Table 3

Kerangas forest	13/6/2000	SA 129	*Shorea agami*	D	1200 ft from sea level, slope hill 45 °,
	13/6/2000	SA 130	*Shorea hypoleuca*		Near SA 129, slope hills 70 °, beside – rattan. Tree with white latex.
	13/6/2000	SA 132	*Syzygium* spp.		1200ft, slope hill down.
	13/6/2000	SA 133	*Dryobalanops beccarii*		Slope hill 60°
Unlogged lowland forest	13/6/2000	SA 135	(Madang tiga urat)	C	1800m, a lot of small plant, mossy, Nepenthes

Part of the tree samples was field identified frequently using local name and checked with specimen voucher in Herbarium Forest Research Center, Sandakan

Table 4. List of 23 soil samples from Imbak Valley without isolated actinomycetes from Imbak Valley expedition II (1-7/7/2000)

Type of forest	Date of soil collected	Soil sample	Scientific name of tree	Sampling region	Location (Figure 1)
Unlogged primary lowland dipterocarp forest, Base camp area	3/7/2000	EL 31	*Shorea* sp.	H	Center part near Sg. Imbak, sea level ~91.4 -106.7m
	3/7/2000	EL 32	*Sarcothera diversifolia*		Center part near Sg. Imbak, sea level ~91.4 -106.7m
	3/7/2000	EL 38	*Drybalanops beccarii*		Left side of Sg. Imbak, sea level ~91.4 -106.7m
	3/7/2000	EL 39	*Pentace adenophora*		Left side of Sg. Imbak, sea level ~ 91.4-106.7m
	5/7/2000	EL 41	*Ternstroemia aneura*		Left side of Sg. Imbak, sea level ~91.4 -106.7m
	5/7/2000	EL 43	*Artocarpus anisophyllus*		Left side of Sg. Imbak, next to Kayu malam tree, sea level ~91.4-106.7m
	5/7/2000	EL 44	Lauraceae		Left side of Sg. Imbak, sea level ~91.4 -106.7m
	5/7/2000	EL 49	*Shorea ovalis*		Left side of Sg. Imbak, near E 48, sea level ~91.4 -106.7m
	5/7/2000	EL 54	*Litsea* sp.		Left side of Sg. Imbak, sea level ~91.4 -106.7m
	5/7/2000	EL 56	*Diospyros* sp.		Left side of Sg. Imbak, sea level ~91.4 -106.7m
	5/7/2000	EL 58	*Shorea smithiana*		Left side of Sg. Imbak, sea level ~91.4 -106.7m
	5/7/2000	EL 59	*Scaphium* sp.		Left side of Sg. Imbak, sea level ~91.4 -106.7m
	3/7/2000	SA 201	*Shorea inappendiculata*	F	Left side of Sg. Imbak, sea level ~91.4-106.7m
	3/7/2000	SA 202	*Parishia insignis*		Left side of Sg. Imbak, sea level ~91.4 -106.7m
	3/7/2000	SA 204	*Shorea argentifolia*		Left side of Sg. Imbak, sea level ~91.4 -106.7m
	3/7/2000	SA 205	*Xanthophyllum ellipticum*		Left side of Sg. Imbak, sea level ~91.4 -106.7m
	3/7/2000	SA 209	*Calophyllum obliquinervium*		Left side of Sg. Imbak, sea level ~91.4 -106.7m
	3/7/2000	SA 213	*Eugenia* spp.		Left side of Sg. Imbak, sea level ~91.4 -106.7m
	3/7/2000	SA 215	*Canarium* spp.		Left side of Sg. Imbak, sea level ~91.4-106.7m
	3/7/2000	SA 221	*Shorea platyclados*		Left side of Sg. Imbak, sea level ~91.4 -106.7m
	3/7/2000	SA 222	*Shorea* sp.		Left side of Sg Sg. Imbak, sea level ~91.4 -106.7m
	3/7/2000	SA 227	*Shorea inappendiculata*		Left side of Sg. Imbak, sea level ~91.4-106.7m
	3/7/2000	SA 228	*Shorea mecistopteryx*		Left side of Sg. Imbak, sea level ~91.4 -106.7m

Part of the tree samples was field identified frequently using local name and checked with specimen voucher in Herbarium Forest Research Center, Sandakan

Table 5. Soil samples distribution based on the tree genera under which the soils were collected in Imbak Valley.

Genus	Species	No. of soil samples	Soil sample (No. of actinomycetes isolated)	No. of actino-mycetes isolated	Total no. of actino-mycetes isolated
Shorea	*S. agami*	1	SA-129(0)	0	35
	S. almon	1	SA-101(3)	3	
	S. argentifolia	4	SA-106(0), SA-138(0), EL-17(2), SA-204(0)	2	
	S. gysbertsiana	2	EL-27(0), SA-225(2)	2	
	S. hypoleuca	2	SA-128(1), SA-130(0)	1	
	S. inappendiculata	2	SA-201(0), SA-227(0)	0	
	S. johorensis	1	EL-37(1)	1	
	S. macrophylla	1	SA-217(2),	2	
	S. macroptera	3	SA-142(0), EL-35(1), SA-102(5)	6	
	S. mecistopteryx	4	EL-29(1), EL-50(1), SA-211(1), SA-228(0)	3	
	S. ovalis	2	EL-15(3), EL-49(0)	3	
	S. parvifolia	1	EL-36(3)	3	
	S. platyclados	1	SA-221(0)	0	
	S. smithiana	4	EL-19(4), EL-58(0), SA-219(1), SA-117(2)	7	
	S. sp.	6	SA-100(1), SA-127(0), SA-222(0) EL-12(0), EL-31(0), SA-226(1)	2	
Dryobalanops	*D. beccarii*	9	SA-103(1), SA-107(0), SA-133(0), SA-141(0), EL-38(0), SA-200(1), SA-121(2), SA-150(0), SA-151(0)	4	10
	D. lanceolata	2	EL-14(1), SA-220(5)	6	
Dipterocarpus	*D. confertus*	2	EL-5(1), SA-218(1)	2	4
	D. kerrii	1	EL-26(2)	2	
Hopea	*Hopea pentanervia*	1	SA-113(1)	1	1
Parashorea	*P. malaanonan*	2	EL-25(1), SA-224(1)	2	2
Vatica	*Vatica* sp.	1	SA-108(2)	2	2
Gluta	*G. sabahana*	3	EL-1(3), EL-2(3), EL-3(3)	9	17
	G. sp.	2	SA-105(4), SA-210(4)	8	
Mangifera sp.		2	SA-104(5), EL 4(9)	14	14

continued Table 5

Parishia insignis		1	SA-202(0)	0	0
Artocarpus	*A. anisophyllus*	1	EL-43(0)	0	0
	A. odoratissimus	1	SA-124(0)	0	
Ammomum sp.		1	SA-139(0)	0	0
Anisophyllea borneensis		1	EL-57(1)	1	1
Antiaris toxicaria		1	EL-48(1)	1	1
Archidendron jiringa		1	EL-51(2)	2	2
Baccaurea	*B. macrocarpa*	1	SA-206(6)	6	8
	B. pulata	1	EL-21(2)	2	
Calophyllum	*C. obliquinervium*	1	SA-209(0)	0	9
	C. venulosum	1	SA-136(1)	1	
	C. sp.	3	SA-131(1), SA-111(5), SA-116(2)	8	
Canarium sp.		2	SA-110(0), SA-215(0)	0	0
Dacryodes sp.		1	EL-23(1)	1	1
Dialium indum		1	EL-30(3)	3	3
Dillenia borneensis		2	EL-24(0), EL-42(2)	2	2
Diospyros sp.		1	EL-56(0)	0	0
Drypetes sp.		1	SA-125(5)	5	5
Durio	*D. gravoelens*	1	EL-13(2)	2	5
	D. grandiflorus	1	EL-40(2)	2	
	D. sp.	2	EL-52(1), SA-119(0)	1	
Elaeocarpus spp		1	EL-7(1)	1	1
Eugenia	*E. syzguim*	1	EL-45(1)	1	5
	E. sp.	2	EL-11(4), SA-213(0)	4	
Eurycoma longifolia		1	SA-109(1)	1	1
Eusidroxylan zwageri		3	SA-140(0), EL-22(2), SA-223(1), SA-145(2), SA-149(0)	5	5
Ficus sp.		1	SA-134(1)	1	1
Gauna kingiana		1	EL-8(2)	2	2
Garcinia sp.		2	SA-143(0), SA-112(0)	0	0
Goniothalamus sp.		2	SA-137(0), EL-10(3)	3	3
Gonystylus bancanus		1	EL-47(2)	2	2
Heritiera longipetilolata		1	SA-123(0)	0	0
Knema sp.		2	EL-6(2), SA-203(2)	4	4
Koilodepas longifolium		1	EL-20(1)	1	1
Koompassia excelsa		4	SA-229(6), K1(2), K2(1), K3(1), SA-144(6)	16	16
Kopsia dasyrachis		1	EL-18(4)	4	4
Lauraceae		1	EL-44(0)	0	0
Litsea sp.		1	EL-54(0)	0	0
Lithocarpus sp.		1	SA-216(1), EL-9(7)	8	8
Lophopetalum javanicum		1	SA-214(1)	1	1

continued Table 5

Mallotus sp.	2	EL-16(4), EL-48(1)	5	5
Myristicaceae	1	EL-55(1)	1	1
Palaquium sp.	1	SA-114(1), SA-120(2)	3	3
Pentace adenophora	1	EL-39(0)	0	0
Phoeba macrophylla	2	EL-34(2), EL-53(4)	3	3
Ptenandra coerulescens	1	SA-212(1)	1	1
Querus sp.	1	SA-115(1)	1	1
Sarcotheca diversifolia	2	EL-32(0), EL-33(2)	2	2
Scaphium sp.	4	EL-28(5), EL-59(0), SA-207(1), SA-146(4)	6	6
Scorodocarpus borneensis	2	SA-208(2), SA-122(0)	2	2
Syzygium sp.	1	SA-132(0)	0	0
Teijsmanniodendron holophyllum	1	EL-46(3)	3	3
Ternstroemia aneura	1	EL-41(0)	0	0
Xanthophyllum ellipticum	1	SA-205(0)	0	0
Unidentified tree	5	SA-118(0), SA-126(0), SA-135(0) SA-147(0), SA-148(0)	0	0
	144		**Total isolates = 203**	

acidiphilic and neutrophilic strains. The soil sample for pH determination was taken from soil under the giant tree, *Koompassia excelsa*, which was found in the site (slope) about 200-300 meter away from the transit camp. The pH of soil was pH 5.18 after two weeks of storage. Isolates were successfully obtained from 89 soil samples (61.8% of total number soil samples) (Table 1 and Table 2). Fifty-five soil samples were without isolates (38.2%) (Table 3 and Table 4) that may be due to the lower concentrations of spore present in the soil samples.

Sixty-one soil samples were collected under trees of Anacardiaceae and Dipterocarpaeae families, which covered 43.4% of the total soil sample collection (Table 5). The total actinomycetes isolates that were isolated from soil samples collected under both of these families have successfully covered 41.8% of the total actinomycetes isolates collection. A total of 54 actinomycetes isolates were isolated from 53 soil samples collected from Dipterocarpaeae family including *Shorea*, *Dryobalanops*, *Dipterocarpus*, *Hopea*, *Parashorea* and *Vatica* and they represented 26.6% of the total isolates. Eight soil samples that were collected under Anacardiaceae family, including *Gluta* sp., *Mangifera* sp. and *Parishia* sp., have produced 31 actinomycetes isolates.

Other media were also used to isolate specific type of actinomycetes such as *Nocardioides* using low salt + *p*-nitrophenol yielded 3 isolates (H7214, H7215 and H7216) and glucose asparagine agar yielded 2 isolates (H7217 and H7218).

b) Numerical data of phenotypic properties of actinomycetes

Phenotypic properties of isolated actinomycetes were recorded. Results obtained were used for classifying and characterizing the isolates. The colour of the sporulating aerial mycelium is a feature that has been used extensively for taxonomic purposes. Colour may play an important role in the classification of *Streptomyces*, providing the proper use is made of this criterion (Kutzner, 1981). Although the specific shade may vary from time to time depending upon depth of the sporulation layer, intensity of underlying pigments, age of culture, type of medium, the culture can reliably be assigned to a general colour. As description of colour is very subjective, a colour chart from Nippon 9000 (1997) was used for standard colour guide.

The isolates were grouped according to their colour of aerial mycelium and colour of diffusible pigments on oatmeal agar (Table 6). A total of 203 isolates were grouped into seven colour groups (white, grey, brown, yellow, beige, green and other series) based on the colour of the aerial mycelia on the surface of oatmeal agar, observed under normal daylight after 14 days incubation at 28°C (Table 7). Majority of the isolates was of the white colour series, which represent 34% of the total isolates. The white series include yellowish white, greyish white and brownish white. Meanwhile, there were 50 isolates and 41 isolates grouped into the grey colour group and brown colour group respectively. The grey series include pale grey, light grey, medium grey and dark grey; while brown colour group includes pale brown, greyish brown, yellowish brown and dark brown. Besides from the three main colour groups, there were 43 isolates appeared in other colour groups including beige, black, green, yellow and orange colour.

There were 28 isolates exhibited extracellular pigmentation all over the agar medium with a few colours. The colours included yellow, brown, orange, grey and green. The isolates with yellow pigmentation represented the highest percentage, 42.9% and followed by 28.6% of isolates exhibited brown pigmentation. Other pigment colour groups included orange (17.6%), grey (7.1%) and green (3.6%) (Table 8).

Table 6. List of 203 isolates actinomycetes isolated from Imbak Valley

No.	Strain number	Isolation number	Date of soil plated on HV medium pH 7.2 except other wise stated	Soil sample	Aerial mycelia on oatmeal agar (OA) pH 7.2 except other wise stated	Sporulati on OA pH 7.2 except other wise stated	Extracellular pigment colour on OA pH 7.2 except other wise stated
1.	H7116	L 116	25/11/2000 (pH4.5)	SA 101	White	+	O
2.	H7117	L 117	25/11/2000 (pH4.5)	SA 101	White	+	O
3.	H7118	L 119	25/11/2000 (pH4.5)	SA 102	Whitish grey	+	O
4.	H7119	L 120	25/11/2000 (pH4.5)	SA 102	White	+	O
5.	H7120	L 121	25/11/2000 (pH4.5)	SA 102	White	+	O
6.	H7121	L 122	25/11/2000 (pH4.5)	SA 104	White	+	O
7.	H7122	L 123	25/11/2000 (pH4.5)	SA 104	White	+	O
8.	H7123	L 124	25/11/2000 (pH4.5)	SA 104	White	+	O
9.	H7124	L 125	25/11/2000 (pH4.5)	SA 105	White	+	O
10.	H7125	L 126	25/11/2000 (pH4.5)	SA 105	Brownish grey	+	Yellow
11.	H7126	L 127	25/11/2000 (pH4.5)	SA 108	Brownish white	+	Yellow
12.	H7127	L 128	25/11/2000 (pH4.5)	SA 108	White	+	O
13.	H7128	L-129	25/11/2000 (pH4.5)	SA111	Brownish white	+	Yellow
14.	H7129	L-130	25/11/2000 (pH4.5)	SA111	n.a	n.a	n.a
15.	H7130	L-131	25/11/2000 (pH4.5)	SA115	Brownish grey	+	O
16.	H7131	L-132	25/11/2000 (pH4.5)	SA120	Dark grey	+	O
17.	H7132	L-133	25/11/2000 (pH4.5)	SA120	Light brown	+	Yellow
18.	H7133	L-134	25/11/2000 (pH4.5)	SA128	Light grey	+	O
19.	H7134	L-172	25/11/2000 (pH4.5)	SA125	Dark grey	+	Brownish
20.	H7135	L-173	25/11/2000 (pH4.5)	SA125	n.a	n.a	n.a
21.	H7136	L-174	25/11/2000 (pH4.5)	SA102	n.a	n.a	n.a
22.	H7173	JAY1	4/8/2000	SA 144	White	+	O
23.	H7174	JAY2	4/8/2000	SA 144	Whitish	+	O
24.	H7175	JAY3	4/8/2000	SA 144	Brown	+	O
25.	H7176	JAY4	4/8/2000	SA 144	Brown	+	O
26.	H7177	JAY5	4/8/2000	SA 145	Yellow	+	O
27.	H7178	JAY6	4/8/2000	SA 104	White	+	O
28.	H7179	JAY7	4/8/2000	SA 104	White	+	O
29.	H7180	JAY8	4/8/2000	SA 105	Pale yellow	+	O
30.	H7181	JAY9	4/8/2000	SA 105	Brown	+	O
31.	H7182	JAY 10	4/8/2000	SA 145	Light pale brown	+	O
32.	H7183	JAY 11	4/8/2000	SA 144	White	+	O
33.	H7184	JAY 12	4/8/2000	SA 144	Greyish brown	+	Brown
34.	H7185	LBK1	19/ 9/2000	SA 210	Yellowish grey	+	O
35.	H7186	LBK2	19/ 9/2000	SA 210	Greyish white	+	O
36.	H7187	LBK3	19/ 9/2000	SA 210	White	+	O
37.	H7188	LBK4	19/ 9/2000	SA 210	Greyish white	+	O
38.	H7189	LBK5	2/8/2000	SA 212	Yellowish grey	+	O
39.	H7190	LBK 6	25/6/2000	SA 125	Brownish white	+	O
40.	H7191	LBK 7	25/6/2000	SA 125	Greyish white	+	O
41.	H7192	LBK 8	25/6/2000	SA 125	Greyish white	+	O

continued Table 6

No.	Strain number	Isolation number	Date of soil plated on HV medium pH 7.2 except other wise stated	Soil sample	Aerial mycelia on oatmeal agar (OA) pH 7.2 except other wise stated	Sporulati on OA pH 7.2 except other wise stated	Extracellular pigment colour on OA pH 7.2 except other wise stated
42.	H7193	LBK 9	25/6/2000	SA 136	Brownish white	+	O
43.	H7194	LBK 10	14/11/2000	SA 208	Whitish grey	+	O
44.	H7195	LBK 11	14/11/2000	SA 208	Whitish grey	+	O
45.	H7196	LBK 12	14/11/2000	SA 206	Whitish grey	+	O
46.	H7197	LBK 13	14/11/2000	EL 33	Whitish brown	+	O
47.	H7198	LBK 14	14/11/2000	SA 206	Whitish grey	+	O
48.	H7199	LBK 15	14/11/2000	SA 206	Whitish grey	+	O
49.	H7200	LBK 16	14/11/2000	SA 206	Whitish grey	+	O
50.	H7201	LBK 17	14/11/2000	EL 33	Whitish grey	+	O
51.	H7202	LBK 18	14/11/2000	SA 206	Whitish grey	+	O
52.	H7203	LBK 19	14/11/2000	SA 206	Whitish grey	+	O
53.	H7204	LNS 2	14/7/2000	SA 111	White	+	O
54.	H7205	LNS 4	14/7/2000	SA 111	White	+	Brownish white
55.	H7206	LNS 7	19/7/2000	SA 121	White	+	0
56.	H7207	LNS 9	12/7/2000	SA 102	White	+	0
57.	H7208	LNS 10	12/7/2000	SA 103	White	+	Light yellow
58.	H7209	LNS 11	13/7/2000	SA 113	Brown	+	Brown yellow
59.	H7210	LNS 13	19/7/2000	SA 116	Reddish brown	+	0
60.	H7211	LNS 14	13/7/2000	SA 117	Brownish grey	+	0
61.	H7212	LNS 15	13/7/2000	SA 117	White	+	0
62.	H7213	LNS 16	19/7/2000	SA 116	Brown white	+	O
63.	H7214	LNS 20	n.a ([a])	SA 101	Greyish white	+	O
64.	H7215	LNS 21	n.a ([a])	SA 111	White	+	Brown
65.	H7216	LNS 22	n.a ([a])	SA 121	White	+	Brown
66.	H7217	LNS 30	n.a ([b])	SA 100	Greyish white	+	O
67.	H7218	LNS 31	n.a ([b])	SA 114	White	+	Brown
68.	H7219	KEE 1	3/7/2000	SA 216	Greenish brown	+	Yellow
69.	H7220	KEE 2	3/7/2000	SA 223	Greenish brown	+	O
70.	H7221	KEE 3	3/7/2000	SA 229	Greyish white	+	O
71.	H7222	KEE 4	3/7/2000	SA 229	Brownish white	+	O
72.	H7223	KEE 5	3/7/2000	SA 229	Greyish white	+	O
73.	H7224	KEE 6	3/7/2000	SA 229	Greyish white	+	O
74.	H7225	KEE 7	3/7/2000	SA 229	Greyish white	+	O
75.	H7226	KEE 8	3/7/2000	SA 229	Beige	+	O
76.	H7227	KEE 9	3/7/2000	SA 229	Brownish grey	+	O
77.	H7228	KEE 10	3/7/2000	SA 207	Brownish white	+	O
78.	H7229	KEE 11	3/7/2000	SA 214	Brownish white	+	O
79.	H7230	KEE 12	3/7/2000	SA 131	White	+	O
80.	H7231	KEE 13	3/7/2000	SA 134	Greyish white	+	O

continued Table 6

No.	Strain number	Isolation number	Date of soil plated on HV medium pH 7.2 except other wise stated	Soil sample	Aerial mycelia on oatmeal agar (OA) pH 7.2 except other wise stated	Sporulati on OA pH 7.2 except other wise stated	Extracellular pigment colour on OA pH 7.2 except other wise stated
81.	H7232	BEM 1	1/7/2000	SA 200	Brownish green	+	Dark green
82.	H7233	BEM 5	1/7/2000	SA 224	Whitish green.	+	O
83.	H7234	BEM 7	1/7/2000	SA 225	Greyish white	+	Yellow
84.	H7235	BEM 8	1/7/2000	SA 225	Whitish green	-	O
85.	H7236	BEM 10	1/7/2000	SA 226	Whitish green	+	O
86.	H7237	JOE 1	27/8/2000	SA 220	Greyish brown	+	O
87.	H7238	JOE 2	27/8/2000	SA 220	Greyish brown	+	O
88.	H7239	JOE 3	27/8/2000	SA 220	Greyish orange	+	O
89.	H7240	JOE 4	27/8/2000	SA 220	Grey	+	O
90.	H7241	JOE 5	27/8/2000	SA 220	Whitish grey	+	O
91.	H7242	JOE 6	27/8/2000	SA 217	Whitish brown	+	O
92.	H7243	JOE 7	27/8/2000	SA 217	Whitish grey	+	O
93.	H7244	JOE 8	27/8/2000	SA 219	Whitish grey	+	O
94.	H7245	JOE 9	27/8/2000	SA 218	Whitish grey	+	Brown
95.	H7246	JOE 10	27/8/2000	SA 211	Greyish brown	+	O
96.	H7247	DMH-3	28/7/2000 (pH8.0)	SA 109	White	+	O
97.	H7248	E 12	8/7/2000	EL 10	Whitish yellow	+	Yellow
98.	H7249	E 13	26/7/2000	EL 10	Brownish grey	+	O
99.	H7250	E 14	5/8/2000	EL 10	White	+	O
100.	H7251	E 15	20/7/2000	EL 1	Brownish grey	+	O
101.	H7252	E 16	5/8/2000	EL 1	Greyish brown	+	O
102.	H7253	E 17	5/8/2000	EL 13	Greyish brown	+	O
103.	H7254	E 18	5/8/2000	EL 14	Greyish brown	+	O
104.	H7255	E 19	5/8/2000	EL 15	White	-	O
105.	H7256	E 20	8/7/2000	EL 17	Brownish grey	+	O
106.	H7257	E 21	8/7/2000	EL 17	Light green	-	O
107.	H7258	E 22	12/7/2000	EL 18	Whitish beige	+	O
108.	H7259	E 23	5/8/2000	EL 19	Greyish brown	+	O
109.	H7260	E 24	26/7/2000	EL 19	Whitish red	+	O
110.	H7261	E 25	20/7/2000	EL 19	Whitish grey	+	O
111.	H7262	E 26	8/8/2000	EL 20	White	+	O
112.	H7263	E 27	12/7/2000	EL 2	Greyish black	+	Grey
113.	H7264	E 28	26/7/2000	EL 2	White	+	O
114.	H7265	E 29	11/7/2000	EL 21	Brownish grey	+	Yellow
115.	H7266	E 30	11/7/2000	EL 21	Light brown	+	Orange
116.	H7267	E 31	26/7/2000	EL 2	Yellowish white	+	O
117.	H7268	E 32	11/7/2000	EL 23	Light brown	+	O
118.	H7269	E 33	11/7/2000	EL 25	White	+	O
119.	H7270	E 34	11/7/2000	EL 28	Whitish grey	+	O

continued Table 6

No.	Strain number	Isolation number	Date of soil plated on HV medium pH 7.2 except other wise stated	Soil sample	Aerial mycelia on oatmeal agar (OA) pH 7.2 except other wise stated	Sporulati on OA pH 7.2 except other wise stated	Extracellular pigment colour on OA pH 7.2 except other wise stated
120.	H7271	E 35	11/7/2000	EL 28	Whitish green	+	O
121.	H7272	E 36	11/7/2000	EL 28	White	+	O
122.	H7273	E 37	11/7/2000	EL 28	White	+	O
123.	H7274	E 38	11/7/2000	EL 28	White	+	O
124.	H7275	E 39	11/7/2000	EL 29	Whitish brown	+	O
125.	H7276	E 40	26/7/2000	EL 3	Greyish brown	+	O
126.	H7277	E 41	26/7/2000	EL 3	Orange	-	O
127.	H7278	E 42	13/7/2000	EL 34	White	+	O
128.	H7279	E 43	13/7/2000	EL 34	White	+	O
129.	H7280	E 44	13/7/2000	EL 35	Brownish beige	+	O
130.	H7281	E 45	12/7/2000	EL 36	Light brown	+	O
131.	H7282	E 46	13/7/2000	EL 36	Whitish brown	+	O
132.	H7283	E 47	1/8/2000	EL 36	Greyish yellow	+	O
133.	H7284	E 48	13/7/2000	EL 40	Whitish grey	+	Orange
134.	H7285	E 49	8/7/2000	EL 4	Greyish black	+	O
135.	H7286	E 50	8/7/2000	EL 4	Whitish grey	+	O
136.	H7287	E 51	8/7/2000	EL 4	Brownish grey	+	O
137.	H7288	E 52	26/7/2000	EL 4	Whitish grey	+	Grey
138.	H7289	E 53	13/7/2000	EL 42	Greyish brown	+	O
139.	H7290	E 54	13/7/2000	EL 42	Brownish beige	+	O
140.	H7291	E 55	8/7/2000	EL 4	Whitish beige	+	O
141.	H7292	E 56	26/7/2000	EL 4	White	+	O
142.	H7293	E 57	8/7/2000	EL 4	Brownish grey	+	O
143.	H7294	E 58	13/7/2000	EL 45	Brownish grey	+	O
144.	H7295	E 59	13/7/2000	EL 46	Greyish brown	+	O
145.	H7296	E 60	13/7/2000	EL 46	Whitish gray	+	Brown
146.	H7297	E 61	13/7/2000	EL 46	Whitish beige	+	Brown
147.	H7298	E 62	7/8/2000	EL 47	Whitish orange	+	Orange
148.	H7299	E 63	7/8/2000	EL 47	Whitish orange	+	Orange
149.	H7300	E 64	20/7/2000	EL 50	Blackish grey	+	O
150.	H7301	E 65	26/7/2000	EL 5	Whitish brown	+	O
151.	H7302	E 66	20/7/2000	EL 51	Grey	+	O
152.	H7303	E 67	20/7/2000	EL 51	Brownish grey	+	Yellow
153.	H7304	E 68	7/8/2000	EL 53	White	+	O
154.	H7305	E 69	9/7/2000	EL 57	Whitish yellow	+	O
155.	H7306	E 70	26/7/2000	EL 6	Yellowish white	-	O
156.	H7307	E 71	8/7/2000	EL 7	White	+	O
157.	H7308	E 72	26/7/2000	EL 8	Brownish grey	+	O
158.	H7309	E 73	26/7/2000	EL 8	Brownish grey	+	O

continued Table 6

No.	Strain number	Isolation number	Date of soil plated on HV medium pH 7.2 except other wise stated	Soil sample	Aerial mycelia on oatmeal agar (OA) pH 7.2 except other wise stated	Sporulati on OA pH 7.2 except other wise stated	Extracellular pigment colour on OA pH 7.2 except other wise stated
159.	H7310	E 74	26/7/2000	EL 9	Greyish brown	+	O
160.	H7311	E 75	8/7/2000	EL 9	Whitish beige	+	Yellow
161.	H7312	E 76	8/7/2000	EL 9	Whitish beige	+	O
162.	H7313	E 77	8/7/2000	EL 9	Whitish beige	+	O
163.	H7314	E 78	8/7/2000	EL 9	Whitish beige	+	O
164.	H7315	E 79	8/7/2000	EL 9	Whitish beige	+	O
165.	H7316	E 80	12/7/2000	EL 18	Yellowish brown	+	O
166.	H7317	E 81	12/7/2000	K 1	Whitish beige	+	O
167.	H7318	E 82	12/7/2000	K 1	Whitish grey	+	O
168.	H7319	E 83	12/7/2000	K 2	Yellowish grey	+	O
169.	H7320	E 102	12/7/2000	K 3	Brownish beige	+	O
170.	H7321	E 103	12/7/2000	EL 18	Whitish beige	+	O
171.	H7322	E 104	26/7/2000	EL 6	Whitish yellow	+	O
172.	H7323	E 105	5/8/2000	EL 15	Brownish green	+	O
173.	H7324	E 106	13/7/2000	EL 37	White	+	O
174.	H7325	E 107	8/7/2000	EL 4	Blackish grey	+	O
175.	H7326	E 108	8/7/2000	EL 1	Whitish beige	+	O
176.	H7327	E 109	26/8/2000	EL 4	White	+	O
177.	H7328	E 110	12/7/2000	EL 18	Whitish beige	+	O
178.	H7329	E 111	26/7/2000	EL 9	Whitish beige	+	O
179.	H7330	E 112	7/8/2000	EL 15	White	+	O
180.	H7566	E271	2/12/2000	EL 48	Whitish grey	+	O
181.	H7658	E363	2/12/2000	EL 16	Brown	+	O
182.	H7659	E364	2/12/2000	EL 16	Greysih brown	+	O
183.	H7660	E365	2/12/2000	EL 30	Brown	+	O
184.	H7661	E366	2/12/2000	EL 30	Brown	+	O
185.	H7664	E369	2/12/2000	EL 26	Grey	+	O
186.	H7665	E370	2/12/2000	EL 26	Greyish brown	+	O
187.	H7666	E371	2/12/2000	EL 16	Whitish grey	+	O
188.	H7667	E372	2/12/2000	EL 16	Brownish grey	+	Pale orange
189.	H7689	E394	2/12/2000	EL 30	Brown	+	O
190.	H7690	E395	2/12/2000	EL 52	Greyish brown	+	O
191.	H7693	E398	2/12/2000	EL 55	White	+	O
192.	H7695	E400	2/12/2000	EL 22	Greyish grey	+	O
193.	H7696	E401	2/12/2000	EL 22	Greyish grey	+	O
194.	H7698	E403	2/12/2000	EL 40	White	+	O
195.	H7758	SFD2A	n.a ([c])	EL 3	Greyish brown	+	O
196.	H7759	SFD3A	n.a ([c])	EL 11	Yellowish brown	+	O
197.	H7760	SFD4A	n.a ([c])	EL 11	Yellowish brown	+	O

continued Table 6

No.	Strain number	Isolation number	Date of soil plated on HV medium pH 7.2 except other wise stated	Soil sample	Aerial mycelia on oatmeal agar (OA) pH 7.2 except other wise stated	Sporulati on OA pH 7.2 except other wise stated	Extracellular pigment colour on OA pH 7.2 except other wise stated
198.	H7761	SFD5A	n.a ([c])	EL 11	Yellowish brown	+	O
199.	H7762	SFD6A	n.a ([c])	EL 11	Brown	+	O
200.	H7763	SFD7A	n.a ([c])	EL 13	White	+	O
201.	H7764	SFD8A	n.a ([c])	EL 19	White	+	O
202.	H7846	KEE14	3/7/2000	SA203	Dark brown	+	O
203.	H7847	KEE15	3/7/2000	SA203	Brownish white	+	O

Abbreviation:

+ Sporulation on oatmeal agar, pH 7.2 except other wise stated
- No sporulation on oatmeal agar, pH 7.2 except other wise stated
O No extracellular pigment on oatmeal agar, pH 7.2 except other wise stated
OA Oatmeal agar, pH 7.2 except other wise stated, incubated at 28°c for 2 week
HV Humic acid agar + B-vitamins + cycloheximide, pH 7.2, incubated at 28°c for 1-4 week
([a]) Isolate was isolated on low salt + *p*-nitrophenol, pH 7.2, incubated at 28°c for 1-4 week
([b]) Isolate was isolated on glucose asparagine agar, pH 7.2, incubated at 28°c for 1-4 week
([c]) Isolates was isolated using centrifugation technique by Sylvia Daim
n.a Information not available
SA/EL/K/CV Imbak Valley, Sabah

BEM Badrul Izam Mois
DMH Daren Moh Mee Hai
E Lai Ngit Shin
JAY Jagdish Kaur Chahil
JOE Justin Janim
KEE Kee Cheng Ling
L Lo Chor Wai
LBK Lee Bee Koon
LNS Low Ai Chin
SDF Sylvia Daim

Table 7. Colour groupings of Imbak Valley actinomycetes isolates on oatmeal agar.

Colour of aerial mycelium							
Grey	**White**	**Brown**	**Beige**	**Yellow**	**Green**	**Other**[a,b]	**Total**
50	69	41	17	5	7	14	203
24.6%	34.0%	20.2%	8.37%	2.46%	3.45%	6.90%	100%

Abbreviation:

[a] Other colour includes, black, red and orange.

[b] Includes 3 isolates without any aerial mycelium colour [H7129 (L-130), H7135 (L-173) and H7136 (L-174)]

Microfungi

There were nine strains isolated from five soil samples from the Imbak Valley (Table 9). The isolation medium used for isolation was PDA with the addition of chloramphenicol (50µg/ml). Twelve soil samples were used for direct spread plate on the isolating medium. The other seven soil samples, which were plated but were overgrown with Mucor like fungi were SA144 to SA150. Five out of 12 soil samples plated on isolation medium produced one or more distinguishable fungal colonies, Therefore the percentage of successful fungal isolation with this method from this area was 41.67%.

Screening for specific inhibition of molecular targets in cell based systems

a) MAPK kinase (MKK1) screening system in yeast

A total of 119 actinomycetes extracts and nine fungal extracts (H9000-H9003, H9005-H9009) were screened for $MKK1^{P386}$ in yeast. No inhibitor against MKK1 was found. Ten isolates exhibited toxicity against yeast *Saccharomyces cerevisiae* in which the growth of the $MKK1^{P386}$ inhibited on the glucose plate without yeast growth on the galactose plate (Table 10). No inhibitors among the extracts were found against MKK1 mutant yeast in this screening system.

b) MAP kinase phosphatase (MSG5) screening system in yeast

A total of 180 actinomycetes strains and nine fungal strains (H9000-H9003, H9005-H9009) were screened for $MKK1^{P386}$-MSG5 in yeast. Nineteen strains from the isolates exhibited toxicity against yeast *Saccharomyces cerevisiae*. The strains showed toxicity to yeast when inhibitory zones appeared around the discs on both the glucose and galactose plate respectively (Table 11). No inhibitors among the strains were against MSG5 mutant yeast in this screening system.

c) GLC7, the catalytic subunit of serine/ threonine protein phosphatase type I (PP1) in yeast

A total of 98 actinomycetes strains and nine fungal strains (H9000-H9003, H9005-H9009) were screened against type 1 protein serine/ threonine phosphatase (GLC7) in yeast. Two actinomycetes strains (H7301 and H7314) were found to be toxic to yeast in the type one protein serine/threonine phosphatase (GLC7) screening system (Table 12). No inhibitors among the strains were found against mutant yeast in this screening system.

Table 8. Diffusible extracellular pigment colours of Imbak Valley actinomycetes isolates on oatmeal agar.

Diffusible extracellular pigment colour on oatmeal agar					
Yellow	**Brown**	**Orange**	**Grey**	**Green**	**Total**
12	8	5	2	1	28
42.9%	28.6%	17.6%	7.1%	3.6%	100%

Table 9. Fungal strains isolated from Imbak Valley according to soil samples.

Soil samples with successful fungal isolates	Plant associated	Sampling region (Figure 1)	No. of isolates	Strain number (s)
EL 10	*Goniothalamus sp.*	E	1	H9005
SA 101	*Shorea almon*	G	1	H9008
SA 105	*Gluta sp.*	G	1	H9007
SA 134	*Ficus sp.*	D	2	H9006, H9009
SA 151	*Dryobalanops beccarii*	B	4	H9000, H9001, H9002, H9003

Table 10. Screening for inhibitors from selected actinomycetes against Mkk1^{P386} in yeast.

Strain number	Isolation number	Mkk1^{P386}		
		Glucose	**Galactose**	**Status**
H7130	L131	+(7mm)	â,á	Toxic
H7131	L132	+(11mm)	â,á	Toxic
H7134	L172	+(7mm)	â,á	Toxic
H7287	E51	+(10mm)	â,á	Toxic
H7313	E77	+(12mm)	â,á	Toxic
H7315	E79	+(11mm)	â,á	Toxic
H7321	E103	+(7mm)	â,á	Toxic
H7329	E111	+(13mm)	â,á	Toxic
H 7666	E371	+ (10mm)	â,á	Toxic
H 7667	E372	+ (9mm)	â,á	Toxic

Abbreviation:
\+ Inhibition zone around paper disk (Diameter of paper disk = 6 mm)
â No growth of yeast on the whole galactose plate
á No growth of yeast around the disk on galactose plate

Table 11. Screening for inhibitors from selected actinomycetes against MAP kinase phosphatase (MSG 5) in yeast.

Strain numbers	Isolation number	MKK1^{P386}-MSG5		
		Glucose	Galactose	Status
H7130	L131	+ (8mm)	+(8mm)	Toxic
H7134	L172	+ (13mm)	+ (14mm)	Toxic
H7173	JAY1	+ (33mm)	+ (21mm)	Toxic
H7176	JAY4	+ (17mm)	+ (7mm)	Toxic
H7204	LNS2	+ (8mm)	+ (10mm)	Toxic
H7206	LNS7	+ (7mm)	+ (8mm)	Toxic
H7207	LNS9	+ (16mm)	+ (15mm)	Toxic
H7211	LNS14	+ (11mm)	+ (13mm)	Toxic
H7214	LNS20	+ (25mm)	+ (25mm)	Toxic
H7215	LNS21	+ (25mm)	+ (26mm)	Toxic
H7229	KEE11	+ (9mm)	+ (10mm)	Toxic
H7231	KEE13	+ (7mm)	+ (7mm)	Toxic
H7287	E51	+ (10mm)	+ (11mm)	Toxic
H7313	E77	+ (11mm)	+ (12mm)	Toxic
H7315	E79	+ (10mm)	+ (11mm)	Toxic
H7321	E103	+ (8mm)	+ (8mm)	Toxic
H7329	E111	+ (12mm)	+ (12mm)	Toxic
H 7666	E371	+ (10mm)	+ (11mm)	Toxic
H 7667	E372	+ (9mm)	+ (10mm)	Toxic

Abbreviation: +Inhibition zone (Diameter of paper disk = 6 mm)

d) Ras/Raf two-hybrid yeast system

A total of 98 actinomycetes strains and nine fungal strain (H9000-H9003, H9005-H9009) were screened for Ras/Raf interaction with the yeast two-hybrid system. Eleven actinomycetes strains were found to be toxic against Ras/Raf interaction with the yeast two-hybrid system when the cells will not grow in the presence and absence of histidine (Table 13). No inhibitor had been found against Ras/Raf interaction screening system.

Isocitrate lyase of glyoxylate pathway in Mycobacterium (ICL) screening and screening results

A total of 49 actinomycetes strains (H7758-H7764, H7116, H7118, H7122, H7126, H7129, H7175, H7176, H7177, H7180, H7182-H7186, H7190, H7192-H7194, H7196-H7203, H7220, H7222-H7228, H7237, H7239-H7242, H7846-H7847) were screened against isocitrate lyase (ICL) in *Mycobacterium*. Strain H7763, a *Streptomyces* with characteristics of repeated zigzag conidiophore leading to chains of conidia (Balingi, 2003), showed up positive in the screen, and the bioactive compound is not itaconic acid, a known ICL inhibitor (Daim, 2003). Enzymatic assay result showed that ICL is inhibited by the crude extract. While strain H7220 showed toxicity against both the wild type *M. smegmatis* (H8000) and transformed *M. smegmatis* (H8012) in acetate and glucose medium to the same amount (Table 14).

Table 12. Screening for inhibitors from selected actinomycetes against Type 1 protein serine/threonine phosphatase (GLC7) in yeast.

Sstrain number	Isolation number	Mutant PAY700 -4 (H10017)				Wild type PAY 704 -1 (H10018)				Status
		25°C		37°C		25°C		37°C		
		YPD	YPD +1M Sorbitol	YPD	YPD +1M Sorbitol	YPD	YPD +1M Sorbitol	YPD	YPD +1M Sorbitol	
H7301	E65	+(7mm)	+(7mm)	-	+(15mm)	+7(mm)	+7(mm)	0	+15(mm)	Toxic
H7314	E78	+(10mm)	+(9mm)	-	+(8mm)	+9(mm)	+9(mm)	0	+7(mm)	Toxic

Abbreviation:
+ Inhibition zone (Diameter of paper disk = 6mm)
0 No inhibition zone

Table 13. Screening for inhibitors from selected actinomycetes against Ras/Raf interaction with the yeast two-hybrid system.

Strain number	Isolation number	LZ strain (H10014) with histidine	LZ strain (H10014) without histidine	Status
H7254	E 18	+(15mm)	+(15mm)	Toxic
H7297	E 43	+(7mm)	+(7mm)	Toxic
H7298	E 62	+(7mm)	+(7mm)	Toxic
H7301	E 65	+(7mm)	+(8mm)	Toxic
H7302	E 66	+(10mm)	+(10mm)	Toxic
H7310	E 74	+(21mm)	+(21mm)	Toxic
H7311	E 75	+(13mm)	+(16mm)	Toxic
H7312	E 76	+(10mm)	+(12mm)	Toxic
H7313	E 77	+(11mm)	+(12mm)	Toxic
H7314	E 78	+(11mm)	+(12mm)	Toxic
H7315	E 79	+(11mm)	+(13mm)	Toxic

Abbreviation:
+ Inhibition zone detected (Diameter of disk = 6mm).

Screening of growth inhibition of actinomycetes against resin of *Shorea smithiana*

A total of ten actinomycetes strains were screened against *Shorea smithiana* resins at different concentration. Table 15 shows that different actinomycetes isolates have their own capability to resist *S. smithiana* resins at different concentration. Isolate H7240 showed the strongest susceptibility towards the resin in which the concentration of 5g/l is sufficient to produce inhibition of the bacteria. The next strains, which show susceptibility towards the resin were H7241 and H7242 but it requires twice the concentration of resin for this inhibition which is 10g/l. The isolates H7237, H7239, H7243, H7245 and H7246 were most resistant towards the resin; inhibited from growing when the resin concentration is at 35g/l (Table 15) (Janim, 2001).

Table 14. Screening for inhibitors against isocitrate lyase (ICL) in *Mycobacterium.*

Strain numbers	Corbon source[a]	H8000, *M. smegmatis* wild type mc^{2}155		H8012, icl-deleted *M. smegmatis* transformed with plasmids carrying *M. tuberculosis* icl gene.		Status
		Glucose	Acetate	Glucose	Acetate	
H7763	Glucose	0	+(32mm)M	0	+(43mm)M	Potential inhibitor
	Acetate	0	0	0	0	-
H7220	Glucose	+(7.5mm)	+(7mm)	+(7.5mm)	+(7mm)	Toxic

Abbreviation:
+ Inhibition zone (Diameter of paper disk = 6mm)
0 No inhibition zone
M Murky
a Strain were grown aerobically in mannitol-peptone medium containing 2%(w/v) D-mannitol, 2%(w/v) peptone and either 1%(w/v) glucose or 1%(w/v) acetate; pH 7±0.2

Strains toxic to yeast

Significantly, 29 isolates of actinomycetes from Imbak Valley showed toxicity to yeast that represented about 14.3% of the total isolates (Table 16), which the mode of action is unknown (Table 17). H7667, a *Streptomycete* toxic to yeast is further screened for inhibitors of the G5K3-beta pathway (Cheenpracha *et al.,* 2009).

CONCLUSIONS

The actinomycetes isolates were diverse based on colour grouping data and particularly on production of extracellular pigment. No inhibitor among the isolates were found with screening against MAPK kinase and MAP kinase phosphatase (MSG5), type 1 protein serine/threonine phosphatase (GLC7) in yeast screening system and Ras/Raf interaction with the yeast two-hybrid system. A high percentage of actinomycete isolates produce compounds toxic to yeast where the mode of action is unknown. H7667, a *Streptomycete* toxic to yeast is further screened for inhibitors of the GSK3-beta pathway. H7763, a presumptive ICL inhibitor, which is not itaconic acid is been currently searched for authentic ICL or MLS (malate synthase) inhibitor.

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Table 15. Effect of resin from *Shorea smithiana* on the growth of actinomycetes.

Strain number	Isolation number	Control (DMF)	*Shorea smithiana* resins concentrations (g/l) 0.1	0.2	0.3	0.4	0.5	1	1.5	2	5	8	10	15	20	25	30	35
H7237	JOE 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+
H7238	JOE 2	0	0	0	0	0	0	0	0	0	0	0	0	0	+ (8mm)	+	+	+
H7239	JOE 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+ (7mm)
H7240	JOE 4	0	0	0	0	0	0	0	0	0	+ (9mm)	+	+	+	+	+	+	+
H7241	JOE 5	0	0	0	0	0	0	0	0	0	0	0	+ (8mm)	+	+	+	+	+
H7242	JOE 6	0	0	0	0	0	0	0	0	0	0	0	+ (8mm)	+	+	+	+	+
H7243	JOE 7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+ (9mm)
H7244	JOE 8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+ (8mm)	+
H7245	JOE 9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+ (10mm)
H7246	JOE 10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+ (9mm)
H7844	JOE11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+
H7845	JOE12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+ (9mm)

Abbreviation:

0 No inhibition zone (mm) of isolates

\+ Inhibition zone (mm) of isolates

Table 16. Analysis of actinomycetes isolated from Imbak Valley.

Sampling region (Figure 1)	No. of soil samples	No. of soil samples with isolates	No. of soil samples without isolates	No. of isolates	No. of isolates which showed toxic to yeast basic-screens method only
A	7	6	1	19	2
B	5	0	5	0	0
C	2	1	1	1	0
D	7	3	4	3	1
E	30	27	3	76	13
F	29	18	11	35	1
G	28	17	11	42	9
H	36	17	19	27	3
Total	**144**	**89**	**55**	**203**	**29**

Table 17. Summary of the screening results.

Strain numbers	Isolation numbers	Yeast based-screening method			GLC	Mycobacterium ICL screening system
		MAPK kinase, ($MKK1^{P386}$)	MAP kinase phosphate ($MKK1^{P386}$-MSG5)	Ras/Raf Interaction		
H7130	L131	Toxic	Toxic	NT	NT	NT
H7131	L132	Toxic	-	NT	NT	NT
H7134	L172	NT	Toxic	NT	NT	NT
H7173	JAY1	NT	Toxic	NT	NT	NT
H7176	JAY4	NT	Toxic	NT	NT	-
H7204	LNS2	NT	Toxic	NT	NT	NT
H7206	LNS7	NT	Toxic	NT	NT	NT
H7207	LNS9	NT	Toxic	NT	NT	NT
H7211	LNS14	NT	Toxic	NT	NT	NT
H7214	LNS20	NT	Toxic	NT	NT	NT
H7215	LNS21	NT	Toxic	NT	NT	NT
H7220	KEE 2	NT	-	NT	NT	Toxic
H7229	KEE11	NT	Toxic	NT	NT	NT
H7231	KEE13	NT	Toxic	NT	NT	NT
H7287	E51	Toxic	Toxic	-	-	NT
H7254	E 18	-	-	Toxic	-	NT
H7297	E 43	-	-	Toxic	-	NT
H7298	E 62	-	-	Toxic	-	NT
H7301	E 65	-	-	Toxic	Toxic	NT
H7302	E 66	-	-	Toxic	-	NT
H7310	E 74	-	-	Toxic	-	NT
H7311	E 75	-	-	Toxic	-	NT
H7312	E 76	-	-	Toxic	-	NT
H7313	E 77	Toxic	Toxic	Toxic	-	NT
H7314	E78	-	-	Toxic	Toxic	NT
H7315	E79	Toxic	Toxic	Toxic	-	NT
H7321	E103	Toxic	Toxic	-	-	NT
H7329	E111	Toxic	Toxic	-	-	NT
H7666	E371	Toxic	Toxic	-	-	NT
H7667	E372	Toxic	Toxic	-	-	NT
H7763	SFD7A	NT	NT	NT	NT	Potential inhibitor

Abbreviation:
- No effect NT - Not tested

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