Research article

Screening microbes isolated from Melalap, Crocker Range for inhibitors against both prokaryotic and eukaryotic signal transduction and isocitrate lyase in *Mycobacterium*

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ABSTRACT. In this study, 65 soil samples from underneath identified plants were collected upstream along the Melalap river. One hundred and thirty-six actinomycetes and ten microfungi were isolated using selective isolation methods. These pure isolates were cultured aerobically for secondary metabolite production. They were screened for inhibitors against three yeast-based molecular targeted screenings: protein phosphatase 1 (PP1), glycogen synthase kinase 3β (GSK-3β), Ras/ Raf-1 protein-protein interaction, and two *Mycobacterium*-based screening systems: isocitrate lyase (ICL) of the glyoxylate pathway and PhoP-PhoR two component signal transduction system. Three extracts (H11329, H11337 and H11402) were toxic to yeast in Ras/Raf-1 screening, nine extracts were toxic to yeast in PP1 screening (H11293, H11298, H11300, H11301, H11302, H11304, H11317, H11339 and H11402). One actinomycete strain H11299 showed weak inhibition to PP1. Two extracts (H11329 and H11364) showed weak inhibitory activity and three extracts (H11339, H11337, H11402) showed toxicity in the GSK-3β yeast screening. Five extracts (H11310, H11317, H11337, H11346 and H11383) showed toxic effect in the ICL screening system, and one extract (H11392) possibly showed weak inhibition to the PhoP-PhoR two component system. It is interesting that H11383 has the same inhibition characteristic as H7763, a presumptive ICL inhibitor with a wide partial inhibition zone on acetate plate (Daim, 2003).

Key words: Actinomycetes, microfungi, two component signal transduction, protein phosphatase 1,glycogen synthase kinase-3β,isocitrate lyase
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INTRODUCTION

Actinomycetes are common Gram-positive bacteria in soil, composts, river mud and lake bottom. Rich soil tends to have a high yield of actinomycetes. They are characterized by their high G+C content and cell wall compositions. Actinomycetes together with fungi are avid producers of secondary metabolites with classic examples being streptomycin and penicillin. As chemical novelty associated with natural product is high, metabolites produced by bacteria and fungi provide great structural diversity (Harvey, 2000).

Borneo is the second largest tropical island in the world, harbouring precious biodiversity resources, but their potential has yet to be fully explored. Melalap is one of the sub-stations at the southern region of the Crocker Range Park, an area under the Bornean Biodiversity and Ecosystems Conservation (BBEC) Programme. Previous studies have included areas near Mahua, Ulu Senagang, Ulu Kimanis and Gunung Emas (Lo *et al.*, 2001; Chan *et al.*, 2004), which are situated to the north-eastern part and central part of the Park. The search for biologically active compounds has led us to exploit the forest area in the hope of finding higher microbial diversity that provides better chance at obtaining useful compounds.

In this study, actinomycetes and microfungi were isolated from soil samples collected from just below the leaf litter as the degradation of the leaf litter and other dead organic material is aided by microbes of which many are fungi and actinomycetes. These microbes were selectively isolated and the acetone extracts produced by shake flask fermentation of these isolates were screened against molecular targeted screening in search for biological inhibitors. There were three yeast-based screening systems adapted in this study, the Ras/Raf-1 protein-protein interaction screening was designed to screen for inhibitors affecting mammalian Ras/Raf-1 interaction (Ki *et al.*, 1998). The GSK-3β screening detects inhibitors affecting the GSK-3β pathway (Andoh *et al.*, 2000). The protein serine/threonine phosphatases inhibitors screening was targeted at protein phosphatase 1 (Andrews & Stark, 2000). Protein kinases and protein phosphatases have been found to be important drug targets since the last decade, with potential use in the treatment of various diseases (Cohen, 2002), including cancer and neurological diseases.

Apart from these, two *Mycobacterium*-based screening systems were also employed. *M. tuberculosis* has been known to be the causative agent of the deadly tuberculosis disease. One of the important targets of persistent TB infection is the isocitrate lyase of the glyoxylate pathway (McKinney *et al.*, 2000). The PhoP-PhoR two-component signal transduction system is important for *Mycobacterium* survival in lung, which is a latent infection factor (Fontan *et al.*, 2004). *M. smegmatis* mc^2155 is used as it is a non-pathogenic surrogate host strain for *M. tuberculosis* genes with advantages of easy and efficient genetic manipulations besides being a fast grower (Jacobs, 2000).

MATERIALS AND METHODS

Location

Sampling for this study took place during the Melalap Scientific Expedition (24-31 January, 2004). Melalap is situated at the southern end of the Crocker Range in the district of Tenom, Sabah. The Expedition Base camp was not completely undisturbed as there were still some abandoned planted crops, particularly cocoa, with villagers living less than five km away. Part of the study area was once burned by fire in 1989 and wild bananas grew profusely. Soils were sampled along Melalap River, often less than one km away from the river and sometimes towards the hills area (Fig. 1). There were five sampling locations termed as follows: Site A, which was an area around the Base camp; Site B, more than one km away from the Base camp, including the area near Mesisilad waterfall and Tamburukai waterfall; Site C, 2-5 km uphill on the left side of the river, around 1000-2000 m a.s.l.; Sites D and E, situated to the north-west and west, respectively, and less than two km away from the Base camp.

Collection of soil samples

Samples were collected from below big trees, near root areas. Humus-rich soil below the leaf

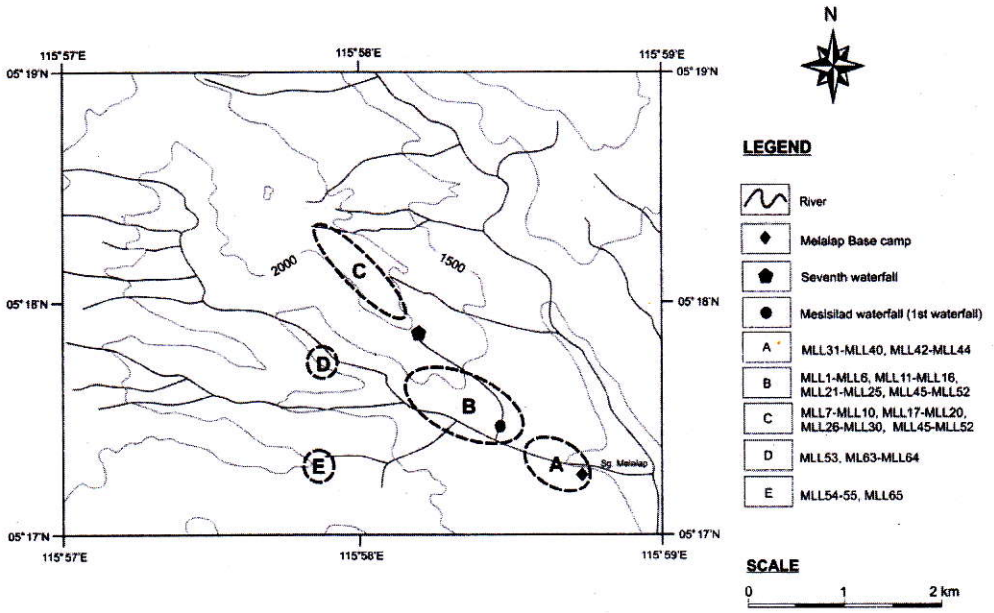

Figure 1. Topography map of the study site. Contour lines are in feet.

litters were collected aseptically into sterilized bottles with the aid of sterilized tongue depressors.

Soil pH determination

Ten grams of air-dried soil sample was mixed with 25 mL of distilled water. pH reading was taken using a calibrated WTW inoLab pH meter.

Isolation of actinomycetes

Actinomycetes were isolated by plating out diluted soil suspension onto humic acid B vitamins agar (HV), arginine vitamin agar (AV), starch casein nitrate agar (SCN) or diagnostic sensitivity test agar (DSTA). Cycloheximide was added into the media to a final concentration of 50 mg/L to prevent fungal contamination. In some cases, pre-treatment of soil samples with chloramine-T, phenol and heat treatment at 180°C for two hours was done. Distinct colonies were streaked onto oatmeal agar (OA) for observation.

Selective isolation of *Streptomycetes* (Nonomura and Hayakawa, 1988)

Soil (0.05 g) was suspended in 9.95 mL of distilled water. The soil suspension was mixed by using a vortex mixer and ten times diluted thrice. One hundred µL of the final dilution was plated onto HV agar (humic acid 0.5 g/L, Na_2HPO_4 0.5 g/L, KCl 1.7 g/L, $MgSO_4.7H_2O$ 0.05 g/L, $FeSO_4.7H_2O$ 0.01 g/L, $CaCO_3$ 0.02 g/L, thiamine-HCl, riboflavin, niacin, pyridoxin-HCl, inositol, Ca-pantothenate, p-aminobenzoic acid each at 0.5 mg/L, biotin 0.25 mg/L, cycloheximide 50 mg/L and agar 18 g/L) and incubated at 28°C for 1-2 weeks. Distinct colonies were plated on oatmeal agar (OA) (Quaker oatmeal 20 g/L and agar 12 g/L).

Selective isolation of *Streptosporangiaceae* (Hayakawa *et al.*, 1997)

Soil (0.1 g) was suspended in 9.9 mL of distilled water. The tube was vortexed and one mL of the soil suspension was transferred to nine mL of 1.0% (w/v) chloramine-T. The tube was incubated at 30°C for 30 minutes. One millilitre of the suspension was transferred to nine mL of distilled water. Two-hundred microlitres of soil suspension from this tube were plated on HV agar. Whitish, pinkish and morphologically different colonies were picked from HV plates and cultured on OA.

Selective isolation of *Micromonospora*

One g of soil was suspended into nine mL of distilled water. One mL of the mixed soil suspension was transferred into 1.5% (w/v) phenol. The treated soil suspension was further diluted by ten times twice. Two hundred microlitres of the soil suspension from the final dilution (10^{-4}) were plated onto starch-casein-nitrate (SCN) agar (starch 10 g/L, casein 0.3 g/L, KNO_3 2 g/L, NaCl 2 g/L, K_2HPO_4 2 g/L, $MgSO_4.7H_2O$ 0.05 g/L, $CaCO_3$ 0.02 g/L and agar 18 g/L; trace elements added: $CuSO_4.5H_2O$ 1.02 mg/L, $FeSO_4.7H_2O$ 176 µg/L, $MnSO_4.7H_2O$ 1.26 mg/L, $ZnSO_4.7H_2O$ 240 µg/L; 50 mg/L cycloheximide was also added). The plates were incubated at 28°C for 2-4 weeks. The colonies picked were transferred onto OA.

Selective Isolation of *Actinomadura*

The soil sample was subjected to heat treatment for one hour at 100°C. Treated soil (0.1 g) was suspended in 9.9 mL of distilled water. The suspension was diluted twice (100×) and 100 µL of final dilution (10^{-3}) was plated onto AV agar (L-Arginine 0.3 g/L, glucose 1 g/L, glycerol 1 ml/L, K_2HPO_4 0.3 g/L, $MgSO_4.7H_2O$ 0.2 g/L, NaCl 0.3 g/L, agar 15 g/L trace elements: $Fe(SO_4)_3$ 10 mg/L, $CuSO_4.5H_2O$ 1.0 mg/L, $ZnSO_4.7H_2O$ 1.0 mg/L, $MnSO_4.7H_2O$ 1.0 mg/L. Nystatin 50 mg/L, cycloheximide 50 mg/L, streptomycin 0.5 µg/L; B-vitamins as for HV were also added). Colonies were picked and transferred to OA.

Selective Isolation of *Nocardia*

Soil (0.05 g) was suspended in 9.95 mL of distilled water. The tube was vortexed and one mL of the soil suspension was transferred to nine mL of distilled water. The dilution was repeated and 100µL of the final diluted suspension was plated on Diagnostic sensitivity test agar (Sigma D8184) containing Proteose peptone 10 g/L, veal infusion solids 10 g/L, dextrose 2 g/L, sodium chloride 3 g/L, disodium phosphate 2 g/L, sodium acetate 1 g/L, adenine sulfate 0.01 g/L, guanine hydrochloride 0.01 g/L, uracil 0.01 g/L, xanthine 0.01 g/L, aneurine 0.00002 g/L and agar 15 g/L. Nystatin 50 mg/L and cycloheximide 50 mg/L were added. Plates were incubated for 2-4 weeks and colonies were transferred to OA.

Isolation of microfungi

Microfungi were isolated by plating soil suspension onto potato dextrose agar (PDA) incorporated with 7.5% (w/v) NaCl. Chloramphenicol was added as antibacterial agent. Colonies of fungi were cultured onto fresh PDA without added NaCl.

Preservation of actinomycetes

Spores of single colonies of matured, spore-producing actinomycetes were stored into 20% glycerol and kept at –20°C.

Preservation of microfungi

Conidia of single colonies of microfungi were kept in anhydrous silica gel particles at 4°C (Ogata, 1962). Cultures were preserved by

pipetting 0.5 mL conidia or mycelial fragments suspended in 4% (w/v) non-fat milk into dry heat sterilized (180°C, 2 hours) 12 x 100mm tubes half filled with anhydrous silica gel particles (6-12 mesh). The tubes were left to dry completely in a dessicator, sealed and stored.

Production of secondary metabolites

One loopful of single colonies of actinomycetes were inoculated into ten mL of mannitol-peptone broth (D-mannitol 20 g/L, peptone 20 g/L, dextrose 1 g/L, pH 7.2) in an 125 mL Erlenmeyer flask and incubated at 28°C with shaking at 220rpm for 120 hours. Acetone was added to a final concentration of 50% at the end of the incubation. For fungal cultures, the fungus was inoculated into ten mL fungi fermentation medium (yeast extract 10 g/L, peptone 10 g/L, sucrose 10 g/L, KH_2PO_4 1 g/L, $MgSO_4.7H_2O$ 0.3 g/L, pH 5.5) in an 125 mL Erlenmeyer flask and incubated at 25°C with shaking at 220 rpm for 120 hours. Acetone was added to a final concentration of 50% at the end of the incubation. Acetone was chosen as the extraction solvent for the benefits of its polar organic properties and non-toxic feature towards the screening systems.

Screenings

Three yeast-based screenings, targeting at protein kinases, protein phosphatases and protein-protein interaction and two *Mycobacterium*-based screening targeting at isocitrate lyase and PhoP-PhoR in *M. smegmatis* were carried out as described below.

Yeast Two-Hybrid Screening System: Ras/ Raf-1 protein-protein Interaction Screening

Paper disc screening: The yeast strain, H10014 (LZ) *MATa trp1 leu2 his3 LYS::lexA-HIS3 URA3::lexA-lacZ* [pLexA-RAS^{V12} + pVP16-RAF] (Ki *et al.*, 1998) was grown in minimal medium (SD-His) for 72 hours. The seed culture was inoculated into SD -His and SD +His agar and poured onto plates. To increase sensitivity, 0.001% (w/v) SDS and 1 mM 3-aminotriazole were added to the medium. Air-dried paper discs impregnated with 20 µL of acetone extracts were arranged onto the plates. Diameter of the inhibition zones were measured after three days incubation at 28°C. Extracts with significantly greater inhibition zone on SD-His plates were scored as positive as it would indicate non-expression of the *HIS3* gene. For further confirmation, freeze-dried extract after removing acetone by rotary evaporator at 40°C followed by removal of water by freeze drying was used.

β-galactosidase assay: For assay against β-galactosidase (*lacZ* reporter), LZ cells were grown in SD medium at 28°C, 220 rpm for 72 hours, OD_{600} determined, one mL of cells were dispensed into 1.5 mL microfuge tubes and treated with freeze-dried samples (final concentration 1 mg/mL) for two hours. The cells were then centrifuged at 13,000 rpm for 5 minutes, Supernatants were discarded and cells were resuspended with 0.7 mL of Z buffer, 20 µL 0.1% (w/v) SDS and 50 µL chloroform and the mixture was vortexed. A volume of 160 µL of ortho-nitrophenyl-β-galactoside (ONPG) prewarmed to 30°C was added to start the reaction and the reaction was stopped with the addition of 0.4 mL of 1M Na_2CO_3. OD_{420} of the samples was measured using a spectrophotometer. β-galactosidase activity was expressed in Miller Units (MU). Cells treated with extracts with less than half MU of the control cells are scored as positive. Miller Unit: $(OD_{420})(1000)/(OD_{600})(time)(volume)$, where time = reaction time, volume = reaction volume.

Protein Serine/Threonine Phosphatases Inhibitor Screening

Yeast strains used in this screening are as below:

H10017 (PAY700-4) *MATa ade2-1 his3-11 leu2-3,112 trp1-1 ura3-1 can1-100 ssd1-d2 glc7::LEU2 trp::glc7-10::TRP1 Gal*$^+$

H10018 (PAY704-1) *MATa ade2-1 his3-11 leu2-3,112 trp1-1 ura3-1 can1-100 ssd1-d2 glc7 :: LEU2 trp1:: GLC7 :: TRP1 Gal*$^+$

The yeast strains, PAY700-4 and PAY704-1 (Andrews & Stark, 2000) were incubated in YPD medium at 28°C for 72 hours with shaking at 220 rpm. One hundred µL of different strains of yeast cultures were added into every plate (25 mL/plate) of screening media respectively. The screening media used were similar to the cultivation media with or without 1M D-sorbitol. Extract-soaked paper discs were arranged and the plates were incubated in 25°C and 37° C for three days. Potential inhibitors would show a halo zone only against the wild type yeast at 37°C.

GSK-3β screening system

The yeast strain used in this screening is H10075 with genotype *MATα his3 leu2 ura3 trp1 ade2 mck1::TRP1 mds1::HIS3 mrk1 yol128C::leu2*[pKT10-GSK3β] (Andoh *et al.*, 2000). H10075 is grown in SC minus uracil, shaking incubation, 220 rpm, at 37°C for two days. Four hundred µL of the yeast culture is inoculated in 100 mL of SC minus uracil agar (screening medium) maintained at 50°C. Paper discs impregnated with microbial extracts are then arranged on the screening plates, and plates are then incubated at both permissive temperature (25°C) and at high temperature (37°C). Potential inhibitors would show noticeably larger halo zone at 37°C.

***Mycobacterium*-based Isocitrate lyase screening system**

This screening system was adapted from Sharma *et al.*, 2000. H8000, *Mycobacterium smegmatis* mc^{2}155 was cultured in modified M9 medium with different carbon sources, glucose or sodium acetate at 37°C for 72 hours without shaking. The bottom layer was prepared by pouring modified M9 agar into each sterile Petri dish (10 mL per plate) and left to solidify at laminar flow. Then 750µL of *M. smegmatis* grown in sodium acetate or 150µL of *M. smegmatis* grown in glucose was inoculated into 10 mL of top layer screening agar media with 0.02M sodium acetate or glucose respectively with added 10 µL of 1M thiamine chloride. The seeded agar was poured gently onto the bottom layer screening agar and paper discs were arranged. The plates were incubated at 37°C for three days. Extracts showing inhibition only on sodium acetate plate were scored as positive.

Screening of potential inhibitor against PhoP-PhoR two-component signal transduction system in *Mycobacterium smegmatis* (Groisman, 2001; Soncini *et al.*, 1996)

Screenings using wild type *M. smegmatis* mc^{2}155 (H8000) were done by the paper disc susceptibility test method, which target Mg^{2+}-dependent protein phosphorylation in PhoP-PhoR two-component system. *M. smegmatis* was cultured in M9 minimal liquid medium supplemented with trace elements, 0.5% (v/v) of glucose and 0.1% (v/v) of 1M thiamine chloride with different concentration of magnesium sulfate, 100µM and 1mM at 37°C for 72 hours without shaking. The autoclaved bottom layer M9 agar was poured into each sterile Petri dish (15 mL per plate). Two hundred and fifty µL of *M. smegmatis* seed culture grown in different concentration of magnesium were pipetted into ten mL of top

Table 1. List of soil samples collected from Melalap with reference to Figure 1.

No	Soil Sample	Plant name	Site	Location	pH
1	MLL1	*Parashorea tomentella*	B	One km upstream of Sg. Melalap	7.09
2	MLL2	*Ficus* sp.	B	Along trail made by zoology group of Sabah Parks	6.69
3	MLL3	*Syzygium* sp.	B	Along trail made by zoology group of Sabah Parks	7.43
4	MLL4	*Euphorbiaceae*	B	Along trail made by zoology group of Sabah Parks	7.44
5	MLL5	*Pitichellobium* sp.	B	10 metres away from the stream, uphill	5.56
6	MLL6	*Mangifera* sp. & *Ficus* sp.	B	Along the trail to Air Terjun Tamburukai	5.58
7	MLL7	*Shorea andulensis*	C	About 2 km uphill on the left from the 7th waterfall	3.79
8	MLL8	(Selangan kuning)	C	About 3 km uphill on the left from the 7th waterfall	3.73
9	MLL9	*Lithocarpus* sp.	C	About 3 km uphill on the left from the 7th waterfall	4.07
10	MLL10	Not available	C	About 4 km uphill on the left from the 7th waterfall. On the top of the hill.	3.99
11	MLL11	*Parashorea tomentella*	B	Along an exisiting trail, about 6 m above river. 30 ft further will be the Mesisilad waterfall.	6.47
12	MLL12	*Diospyros* sp.	B	Along the trail made by the Zoology group of the Sabah Parks	5.91
13	MLL13	Not available	B	TS survey trail, by Fred Juanis (of Sabah Parks)	6.56
14	MLL14	Not available	B	Not available	5.8
15	MLL15	*Dysoxylum* sp.	B	Not available	6.82
16	MLL16	*Lauraceae*	B	Along the trail to the waterfall	3.79

continued Table 1.

17	MLL17	*Litsea* sp.	C	About 2 km from 7th waterfall (along the trail up to Bukit Tiwung)	3.94
18	MLL18	Not available	C	About 2 km from 7th waterfall	3.92
19	MLL19	*Artocarpus* sp.	C	About 3.5 km from 7th waterfall. Northeast of MLL19 is MLL29, 12 ft apart, Southeast of MLL29 is MLL 10, also 12 ft apart	3.68
20	MLL20	*Trema* sp.	C	Along the trail descending Bukit Tiwung, along a range.	3.94
21	MLL21	*Anacardiceae*	B	Opposite MLL1	7.32
22	MLL22	*Burseraceae*	B	Along trail made by zoology group of Sabah Parks, 4 feet away from MLL2	6.12
23	MLL23	*Pitichellobium* sp.	B	Along trail made by zoology group of Sabah Parks	7.22
24	MLL24	*Dryobalanops lanceolata*	B	10 metres away from the stream, uphill	6.14
25	MLL25	*Shorea* sp.	B	Not available	6.35
26	MLL26	*Shorea inappendiculata*	C	2 km from the 7th waterfall	4.54
27	MLL27	*Artocarpus odoratissinus*	C	2 km from the 7th waterfall	3.80
28	MLL28	*Syzygium* sp.	C	About 3 km from 7th waterfall	4.56
29	MLL29	*Vitex* sp.	C	Along the trail back from primary forest, about 5 km away from waterfall. Near MLL10 and MLL19	4.62
30	MLL30	*Ficus fulva*	C	About 4 km away from waterfall.	4.52
31	MLL31	*Gigantochloa levis*	A	Base camp	7.10
32	MLL32	*Etlingera* sp.	A	Upstream of Base camp	7.39
33	MLL33	*Eupatorium*	A	On right river bank, beside rocky bank	7.74
34	MLL34	Cocoa	A	Base camp	8.93
35	MLL35	*Coscinium fenestratum* (Torlutup)	A	Base camp	6.43

continued Table 1.

36	MLL36	*Archidendron* sp.(Pelindung)	A	Base camp	8.21
37	MLL37	*Octmeles sumatrana* (Binung)	A	Base camp	6.74
38	MLL38	*Ficus septica*(Lintotobu)	A	Base camp	8.67
39	MLL39	*Musa* sp.	A	Near river, Base camp	5.47
40	MLL40	*Douax* sp.	A	Near river, Base camp	6.98
41	MLL41	Not available	C	About 5 km uphill on the left from the 7th waterfall. Began going down the hill.	4.09
42	MLL42	Not available	A	Near Base camp	5.96
43	MLL43	Not available	A	Near Base camp	6.22
44	MLL44	Not available	A	Near Base camp	6.81
45	MLL45	*Maranthes corymbosa*	B	Along Mesisilad waterfall trail, 1 hour 40 minutes walk from base camp, 2.5 m from the stream.	7.10
46	MLL46	*Prainea limpato*	B	5 minutes walk from MLL45, 2 m from the stream.	7.39
47	MLL47	*Poikilospermum* sp.	B	55 minutes walk from MLL46, 4 m from the stream.	7.74
48	MLL48	*Celtis timorensis.*	B	1 hour 5 minutes walk from MLL47, 5 m from the stream.	8.93
49	MLL49	*Artocarpus elasticus*	B	35 minutes walk along Melalap River from Base camp.	6.43
50	MLL50	*Artocarpus elasticus*	B	35 minutes walk from MLL49, 2 m from the stream.	8.21
51	MLL51	*Octomeles sumatrana*	B	50 minutes walk from MLL50.	6.74
52	MLL52	(Litak)	B	55 minutes walk from MLL51, 5 m from the stream.	8.67
53	MLL53	*Durio* sp.	D	40 minutes from MLL52.	5.47
54	MLL54	Not available	E	55 minutes walk from MLL53.	6.98
55	MLL55	*Alstonia spatulata*	E	30 minutes walk from MLL54. Near banana trees.	4.03

continued Table 1.

56	MLL56	*Parashorea tomentella*	B	824m from the Base camp and 5 m away from the river bank on the left towards upstream of river. 375 m above sea level N 05°17.523' E115°58.417'	6.55
57	MLL57	*Mallotus* sp.	B	986 m from the Base camp and 6 m away from river bank on the right towards upstream of river. 395 m above sea level N 05°17.605' E115°58.363'	7.87
58	MLL58	*Mallotus* sp.	B	838 m from the Base camp and 10 m away from river bank on the right towards upstream of river. N 05°17.534' E115°58.415'	7.33
59	MLL59	*Terminalia* sp.	B	750 m from the Base camp and 4 m away from river bank on the left towards upstream of river. N 05°17.447' E115°58.427'	8.28
60	MLL60	*Aglaia* sp.	B	986 m from Base camp and 3 m away from river bank on the right towards upstream of river. N 05°17.543' E115°58.341'	8.30
61	MLL61	(Seraya)	B	1200 m from Base camp and 5 m away from river bank on the right towards upstream of river. 415 m above sea level. N 05°17.513' E115°58.214'	7.98
62	MLL62	*Aporusa* sp.	B	10 m away from river bank on the left towards upstream of river. 465 m above sea level.	7.02
63	MLL63	*Shorea pauciflora*	D	1800 m away from Base camp and 30 m away from river bank on the left towards upstream of river. 545 m above sea level. N 05°17.721' E115°57.930'	4.76
64	MLL64	Not available	D	560 m above sea level	4.52
65	MLL65	*Teijmanniodendron* sp.	E	1800 m away from Base camp. 680 m above sea level. N 05°17.308' E115°57.862'	4.34

Local name in parenthesis

Table 2. List of actinomycetes isolated from soil samples of Melalap

H Number	Isolation method	Soil Sample No.	Characteristics on OA			
			Aerial mycelium colour	Reverse colour	Extracellular pigmentation	Sporulation
H11277	HV	MLL56	White-honey blue	Primrose	No	Not recorded
H11278	HV	MLL56	White-pinkish	Primrose	No	Not recorded
H11279	HV	MLL57	Golden sand-white	Maple	Brownish	Not recorded
H11280	HV	MLL57	Lavender grey	Primrose	No	Not recorded
H11281	HV	MLL58	White-honey blue	Primrose	Greenish	Not recorded
H11282	HV	MLL59	White	Pearl white	No	Not recorded
H11283	HV	MLL59	White	Pearl white	No	Not recorded
H11284	HV	MLL61	White	Maple	No	Not recorded
H11285	HV	MLL62	Primrose-white	Primrose	No	Not recorded
H11286	HV	MLL62	Sunflower	Sunflower	No	Not recorded
H11287	HV	MLL63	Dark grey	Dark grey	No	Not recorded
H11288	HV	MLL63	White-oak	Pearl white	No	Not recorded
H11289	HV	MLL64	Light green-oak	Primrose	No	Not recorded
H11290	HV	MLL64	Peal white-white	Ivory	No	Not recorded
H11291	DSTA	MLL58	Light purple	Red	No	Not recorded
H11292	DSTA	MLL59	Sweet dream	Marigold	No	Not recorded
H11293	DSTA	MLL59	White-maple	Jute	No	Not recorded
H11294	DSTA	MLL60	Pearl white	Ivory	Marigold	Not recorded
H11295	DSTA	MLL61	Pinkish white	Pinkish white	No	Not recorded
H11296	DSTA	MLL62	Secret	Primrose	No	Not recorded
H11297	DSTA	MLL62	Secret	Primrose	No	Not recorded
H11298	DSTA	MLL62	Marigold	Jasmine	No	Not recorded
H11299	DSTA	MLL63	White	White	No	Not recorded
H11300	DSTA	MLL63	White	White	No	Not recorded
H11301	DSTA	MLL63	White	Primrose	No	Not recorded
H11302	DSTA	MLL65	Light green	Light green	No	Not recorded
H11303	DSTA	MLL65	Greenish	Greenish	No	Not recorded
H11304	DSTA	MLL65	White	White	No	Not recorded
H11308	HV	MLL21	Whitish brown	Beige	No	Yes
H11309	HV	MLL21	Whitish metallic green blue	Orange brown	Slight orange	Yes
H11310	HV	MLL21	White	White	No	Yes
H11311	HV	MLL22	Yellowish white	Light yellow	No	Not obvious
H11312	HV	MLL22	Whitish grey	Whitish grey	No	Yes
H11313	HV	MLL22	Whitish grey	Whitish grey	No	Yes
H11314	HV	MLL23	Whitish grey	Orange Brown	No	Yes
H11315	HV	MLL23	Whitish metallic green blue	Orange brown	Slight orange	Yes
H11316	HV	MLL24	Whitish grey	Beige	No	Yes
H11317	HV	MLL25	Whitish light red	White	No	Yes

continued Table 2.

H11318	HV	MLL25	Orange white	Orange	Slight orange	Yes
H11319	HV	MLL25	Whitish light red	White	No	Yes
H11320	HV	MLL26	Whitish deep brown	Deep Brown	No	Not obvious
H11321	HV	MLL26	White	White (orange center)	No	No
H11322	HV	MLL27	Beige	Beige	No	No
H11323	HV	MLL27	Yellow grey	Orange	Yellowish	Yes
H11324	HV	MLL30	Yellow	Yellow white	No	No
H11325	HV	MLL30	Whitish green	Brown	Slight brownish	Yes
H11326	SCN	MLL21	White light grey	Light orange	No	Yes
H11327	SCN	MLL21	Whitish grey	Grey	No	Yes
H11328	SCN	MLL22	White	White	No	No
H11329	SCN	MLL23	Whitish grey	Grey	No	Yes
H11330	SCN	MLL23	Whitish deep brown	Deep brown	No	No
H11331	SCN	MLL23	White (grey centre)	White	No	No
H11332	SCN	MLL23	White	White	No	No
H11333	SCN	MLL24	Whitish grey	Brown	Slight brown	Yes
H11334	SCN	MLL25	Whitish grey	Grey	No	Yes
H11335	SCN	MLL26	White	Orange	Slight orange	Yes
H11336	SCN	MLL26	Whitish red brown	Red brown	Slight	No
H11337	SCN	MLL28	Whitish grey	Beige	No	Yes
H11338	SCN	MLL28	Whitish grey	Beige	No	Yes
H11339	SCN	MLL29	White	Beige	No	Yes
H11340	SCN	MLL23	Brownish white	Brown	Slight brown	Yes
H11341	HV	MLL1	Whitish-green	Yellowish-green	No	Yes
H11342	HV	MLL1	Whitish-peach	White	No	Yes
H11343	HV	MLL1	Whitish-grey	Light green	No	Yes
H11344	HV	MLL1	White	Brown	No	Yes
H11345	HV	MLL1	Whitish-grey	Whitish-green	No	Yes
H11346	HV	MLL2	Whitish-light brown	Yellow	No	Yes
H11347	HV	MLL2	Grey	Yellow	No	Yes
H11348	HV	MLL2	Light grey	Light grey	No	Yes
H11349	HV	MLL2	Brown	Brown	No	Yes
H11350	HV	MLL3	White	Yellow	No	Yes
H11351	HV	MLL3	Green	Brown	No	Yes
H11352	HV	MLL4	Whitish-green	Whitish-light green	No	Yes
H11353	HV	MLL4	Whitish-light brown	Light brown	No	Yes
H11354	HV	MLL4	Whitish-light grey	Whitish-light grey	No	Yes
H11355	HV	MLL5	Whitish-brown	Light green	No	Yes
H11356	HV	MLL5	Whitish-light grey	Yellow	No	Yes
H11357	HV	MLL6	Dark green	Green	No	Yes
H11358	HV	MLL6	Yellowish-white	Brown	No	Yes

continued Table 2.

H11359	HV	MLL6	Whitish-grey	Light grey	No	Yes
H11360	HV	MLL6	Whitish-light brown	Whitish-green	No	Yes
H11361	HV	MLL7	Whitish-light brown	Light yellow	No	Yes
H11362	HV	MLL7	Whitish-grey	Light grey	No	Yes
H11363	HV	MLL8	Brown	Black	No	Yes
H11364	HV	MLL9	Brownish-light green	Light brown	No	Yes
H11365	HV	MLL9	Whitish-grey	White	No	Yes
H11366	HV	MLL9	Whitish-grey	Yellow	No	Yes
H11367	HV	MLL10	Whitish-grey	Yellow	No	Yes
H11368	HV	MLL41	Whitish-light brown	White	No	Yes
H11369	AV	MLL2	White	White	No	No
H11370	AV	MLL7	White	White	No	No
H11371	AV	MLL21	White	White	No	No
H11372	AV	MLL23	White	White	No	No
H11373	HV	MLL31	Beige pink	Beige	No	Yes
H11374	HV	MLL32	Green	Beige	Light yellow	Yes
H11375	HV	MLL33	White	Brownish red	Brownish red	Yes
H11376	HV	MLL34	Brownish pink	Yellowish orange	No	Yes
H11377	HV	MLL35	Brownish pink	Light yellow	No	Yes
H11378	HV	MLL36	Brownish pink	Light yellow	No	Yes
H11379	HV	MLL37	Greenish brown	Light yellow	No	Yes
H11380	HV	MLL38	Green	Greenish brown	No	Yes
H11381	HV	MLL39	Light grey	Light yellow	No	Yes
H11382	HV	MLL40	Grey	Light grey	No	Yes
H11383	HV	MLL42	Brown	Brownish grey	No	Yes
H11384	HV	MLL43	Brownish pink	Yellowish orange	No	Yes
H11385	HV	MLL44	Grey	Yellowish brown	No	Yes
H11386	HV	MLL45	Brownish pink	Light yellow	No	Yes
H11387	HV	MLL45	Brownish pink	Light yellow	No	Yes
H11388	HV	MLL46	White	Light yellow	No	Yes
H11389	HV	MLL47	White	Light yellow	No	Yes
H11390	HV	MLL48	Green	Yellowish green	Yellowish green	Yes
H11391	HV	MLL49	Light grey	Beige	Light brownish	Yes
H11392	HV	MLL50	Dark beige	Light beige	No	Yes
H11393	HV	MLL51	Grey	Brownish grey	No	Yes
H11394	HV	MLL52	Grey	Brownish yellow	No	Yes
H11395	HV	MLL53	Beige	Light yellow	No	Yes
H11396	HV	MLL54	Beige	Light yellowish brown	No	Yes
H11397	HV	MLL11	Grey	Yellow	No	Yes
H11398	HV	MLL12	White	Light yellow	No	Yes
H11399	HV	MLL13	Brownish grey	Yellow	No	Yes
H11400	HV	MLL14	Blue-grey	Yellow	Yellow	Yes
H11401	HV	MLL15	Dark greenish brown	Dark green	No	Yes

continued Table 2.

H11402	HV	MLL16	Brown	Yellow	No	Yes
H11403	HV	MLL17	White	Light yellow	No	Yes
H11404	HV	MLL18	White	Light yellow	No	Yes
H11405	HV	MLL19	Grey	Yellowish brown	No	Yes
H11406	HV	MLL20	Light grey	Yellow	No	Yes
H11407	HVCT	MLL11	Grey	Dark yellow	Dark yellow	Yes
H11408	HVCT	MLL12	Grey	No distinctive	No	Yes
H11409	HVCT	MLL13	Grey	Light yellow	No	Yes
H11410	HVCT	MLL14	Grey	Light red	No	Yes
H11411	HVCT	MLL15	Brown	Yellowish green	No	Yes
H11412	HVCT	MLL16	Grey	No distinctive	No	Yes
H11413	HVCT	MLL17	Dark green	Dark green	No	Yes
H11414	HVCT	MLL18	Purple-grey	No distinctive	No	Yes
H11415	HVCT	MLL20	Grey	Yellow	No	Yes

Abbreviation:
HV = according to selective isolation of *Streptomycetes*
HVCT = according to selective isolation of *Streptosporangiaceae*
SCN = according to selective isolation of *Micromonospora*
AV = according to selective isolation of *Actinomadura*
DSTA = according to selective isolation of *Nocardia*

Colour description was done according to International Streptomyces Project (ISP) 7 series colour (yellow, violet, red, blue, green, grey and white) except for H11227 to H11304, which was based on Millennium Colours Edition 2 Catalogue (Nippon Paint, 2002).

Table 3. Microfungi isolated from Melalap soil sample

H Number	Soil Sample No.	Characteristic on PDA		
		Aerial mycelium	Reverse colour colour	Extracellular pigmentation
H9368	MLL56	White-pinkish	Yellow-green	Yellow
H9369	MLL59	Yellow-green	Maple-oak	None
H9370	MLL59	White-green	Primrose	None
H9371	MLL60	White-purple	Golden sand	None
H9372	MLL62	White-dark green	Barbary gold-oak	None
H9373	MLL63	White	Oak	None
H9374	MLL64	Red	Russet	None
H9375	MLL64	Jute	Woodland olive	None
H9376	MLL65	Yellow	Ivory	None
H9377	MLL65	White-Jasmine-green	Jute	None

Colour description was based on Millennium Colours Edition 2 Catalogue (Nippon Paint, 2002).

Table 4. Results for Ras/Raf-1 protein-protein interaction screening

Acetone extracts (20ml)	H10014 (LZ)	
	His+	His-
H11277-H11304	0	0
H11308-H11328	0	0
H11329	8mm (C)	8mm (C)
H11330-H11336	0	0
H11337	0	50mm (P)
H11338-H11340	0	0
H11402	9mm (W)	9mm (W)
Freeze dried extract (50mg/mL in water):		
H11337 10µL	10mm (C)	11mm (C)
H11337 20µL	14mm (C)	15mm (C)
H11337 30µL	17mm (C)	18mm (C)
H11337 40µL	20mm (C)	20mm (C)

Abbreviation:
0 No inhibition zone around paper disc
C Clear inhibition
P Partial inhibition showing little growth of yeast within inhibition zone
W Weak inhibition showing minimal yeast reduction within inhibition zone
Diameter of paper disc 6 mm

Table 5. β-galactosidase activity of LZ cells treated with freeze-dried extract of H11337.

Sample	Mean Activity (MU)
H11337	34.46
MP (control)	32.55

Abbreviation:
MP mannitol-peptone medium
MU Miller Unit

Table 6. Results for PP1 Screening

Extract	Mutant Yeast (PAY 700-4)				Wild-Type Yeast (PAY 704-1)			
	YPD		YPD +1MSorbitol		YPD		YPD +1MSorbitol	
	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C
H11277-H11292	0	-	0	0	0	0	0	0
H11293	0	-	0	11mm(P)	0	8mm (P)	0	8mm (P)
H11294-H11297	0	-	0	0	0	0	0	0
H11298	10mm (C)	-	11mm (C)	14mm (C)	12mm (C)	13mm (C)	13mm (C)	12mm (C)
H11299	0	-	0	0	0	13mm (P)	0	0
H11300	19mm (C)	-	21mm (C)	17mm (C)	15mm (C)	17mm (C)	14mm (C)	15mm (C)
H11301	9mm (C)	-	9mm (C)	8mm (C)	7mm (C)	7mm (C)	7mm (C)	7mm (C)
H11302	12mm (C)	-	13mm (C)	15mm (C)	12mm (C)	13mm (C)	12mm (C)	10mm (C)
H11303	0	-	0	0	0	0	0	0
H11304	8mm (C)		9mm (C)	8mm (C)	9mm (C)	8mm (C)	8mm (C)	9mm (C)
H11308-H11316	0	-	0	0	0	0	0	0
H11317	7mm (C)	-	7mm (C)	9mm (C)	7mm (C)	10mm (C)	9mm (C)	12mm (C)
H11318-H11336	0	-	0	0	0	0	0	0
H11339	13mm (C)	-	14mm (C)	12mm (C)	13mm (C)	11mm (C)	13mm (C)	9mm (C)
H11397-H11401	0	-	0	0	0	0	0	0
H11402	11mm (P)	-	11mm (P)	12mm (P)	11mm (P)	10mm (P)	11mm (P)	10mm (P)
H11403-H11415	0	-	0	0	0	0	0	0

Abbreviation:

0 No inhibition zone around paper disc

- no yeast growth

C Clear inhibition

P Partial inhibition showing little growth of yeast within inhibition zone

Diameter of paper disc 6 mm

40 µL of extract is applied on each disc unless otherwise stated

Table 7a. Results for GSK-3β screening.

Extract	H10075(GSK-3β) SC-ura	
	25°C	37°C
H11277-H11328	0	0
H11329	0	8 mm (W)
H11330-H11336	0	0
H11337	8 mm (W)	8 mm (W)
H11338	0	0
H11339	0	8 mm (W)
H11340-H11363	0	0
H11364	0	9 mm (W)
H11365-H11401	0	0
H11402	0	9 mm (W)
H11403-H11415	0	0

Abbreviation:

0 No inhibition zone around paper disc

W Weak inhibition showing minimal yeast reduction within inhibition zone

Diameter of paper disc 6 mm

40 µL of extract is applied on each disc unless otherwise stated

Table 7b. Results for H11329, H11337, H11339, H11364, H11402 with 100 µL of crude acetone extract.

Extract	H10075(GSK-3β) SC-Ura	
	25°C	37°C
H11329	0	9 mm (W)
H11337	23 mm	18 mm
H11339	9 mm (W)	12 mm (W)
H11364	0	15 mm (P)
H11402	9 mm (W)	11 mm (W)

Abbreviation:

0 No inhibition zone around paper disc

P Partial inhibition showing little growth of yeast within inhibition zone

W Weak inhibition showing minimal yeast reduction within inhibition zone

Diameter of paper disc 6 mm

Table 8. Results for Mycobacterium ICL screening.

Extracts	H8000, *Mycobacterium smegmatis* mc²155	
	Glucose	Acetate
H11308-H11309	0	0
H11310	8 mm (C)	8 mm (C)
H11311-H11316	0	0
H11317	8 mm (C)	8 mm (C)
H11318-H11336	0	0
H11337	14 mm (C)	12 mm (C)
H11338-H11345	0	0
H11346	30 mm (C)	30 mm (P)
H11347-H11362	0	0
H11364-H11368	0	0
H11370	0	0
H11372-H11382	0	0
H11383	9 mm (C)	9 mm (C)/42 mm (P)
H11384-H11396	0	0
H11398-H11402	0	0
H11407-H11412	0	0
H11924-H11925	0	0

Abbreviation:

0 No inhibition zone around paper disc

C Clear inhibition

P Partial inhibition showing little growth of *Mycobacterium* within inhibition zone

Diameter of paper disc 6 mm

40 µL of extract is applied on each disc unless otherwise stated

Table 9. Results for *Mycobacterium* PhoP-PhoR two -component system screening.

Extract	H8000, *Mycobacterium smegmatis* mc²155	
	100 µM $MgSO_4.7H_2O$	1 mM $MgSO_4.7H_2O$
H11308-H11337	0	N.T.
H11339-H11345	0	N.T.
H11347-H11368	0	N.T.
H11373-H11391	0	N.T.
H11392	10 mm (C)	8 mm (C)
H11397-H11415	0	N.T.
H11392 (20µL)	10 mm	8 mm
H11392 (40µL)	11 mm	9 mm
H11392 (60µL)	11 mm	10 mm
H11392 (80µL)	13 mm	12 mm
H11392 (100µL)	14 mm	13 mm

Abbreviation:

0 No inhibition zone around paper disc
C Clear inhibition
N.T. Not tested
Diameter of paper disc 6 mm
20 µL of extract is applied on each disc unless otherwise stated

Table 10. Concentration dependency of strain H11392 in 25 µM, 50 µM, 100 µM and 1 mM of Mg^{2+} media

Concentration of Mg^{2+}	Concentration of extract				
	20 µL	40 µL	60 µL	80 µL	100 µL
25 µM	7 (p)	8	9	9	10
50 µM	7 (p)	9	9	9	10
100 µM	7 (p)	9	10	10	11
1 mM	0	0	8	8	9

Abbreviation:

0 no inhibition zone around paper disc
P partial inhibition
Diameter of paper disc 6mm

layer agar media separately. The seeded agar was poured onto the bottom layer agar and paper discs pipetted with extracts were arranged onto upper layer agar. The plates were incubated at 37°C and observation was recorded after two days. Extracts showing inhibition only on low concentration magnesium are scored as positive.

RESULTS

Sixty-five soil samples were collected during the expedition, 13 samples were collected from Site A, 32 samples from Site B, 14 samples from Site C, and three each from Site D and Site E (Figure 1). The soil pH in Site A ranged from 5.47-8.93, Site B: 3.79-8.93, Site C: 3.68-4.62, Site D: 4.52-5.47, Site E: 4.03-6.98 (Table 1). The study sites showed typical characteristics of secondary forest with small trees and secondary forest species like *Octomeles* sp. , *Litsea* sp., *Mallotus* sp., with only few *Ficus* sp. and *Shorea* sp.. A number of 136 isolates of actinomycetes were obtained from the collected soil. Ninety-four of the isolates were picked from HV plates without pre-treatment. Fifteen isolates were from SCN plates with soil subjected to phenol pre-treatment, 14 isolates were from DSTA, four isolates were picked from AV in which soil samples underwent heat pre-treatment and nine were from HV with soil pre-treated with chloramine-T (Table 2) and ten microfungi were isolated from some samples (Table 3).

In the screening, three extracts (H11329, H11337 and H11402) were toxic to yeast in Ras/Raf-1 screening, where H11337 was shown not to be an authentic inhibitor of Ras-Raf interaction as the first test of no growth around the disc with extracts on plate with added histidine could not be repeated when tested in a series of concentrations (Table 4). H11337 also did not prevent the expression of the second reporter gene, *LacZ* in inhibition of β-galactosidase synthesis (Table 5). Nine extracts (Table 6) were toxic to yeast in PP1 screening (H11293, H11298, H11300, H11301, H11302, H11304, H11317, H11339, and H11402). One actinomycete strain, H11299, showed weak inhibition to PP1. In the preliminary GSK-3β screening four extracts (H11329, H11339, H11364, H11402) weakly showed inhibitory activity and one extract (H11337) showed toxicity to yeast (Table 7a). However, confirmation test at higher concentration showed H11329 and H11364 to inhibit yeast only at 37°C whereas H11339, H11337 and H11402 inhibited yeast growth at both 25°C and 37°C (Table 7b).

Five extracts (H11310, H11317, H11337, H11346, and H11383) showed toxic effect in *M. smegmatis* ICL screening system (Table 8). H11383 also showed a wide partial inhibition zone on acetate plate. In PhoP-PhoR two-component system screening, one extract (H11392) showed toxicity to *M. smegmatis* (Table 9). The growth inhibition of H11392 was less in high magnesium medium as compared to low magnesium medium (Tables 10, 11). Four extracts showed activity in more than one screening system, H11317 (toxic in PP1 and toxic in ICL), H11329 (toxic in Ras/Raf-1 and possible inhibitor in GSK-3β), H11337 (toxic in Ras/Raf-1, GSK-3β and ICL), H11339 (toxic in GSK-3β, toxic in PP1), H11402 (toxic in Ras/Raf-1, PP1 and GSK-3β).

DISCUSSION

Soil pH readings taken indicated that soil from Site C was more acidic with values ranging 3.68-4.62. H11337, H11339, and H11364, which showed biological activities as assessed from different screenings, were isolated from soils collected from Site C. As actinomycetes are commonly isolated at pH ranging above 6.5 (Holt *et al.*, 1994), the enumeration of bioactivity producing actinomycetes from acidic soil is of interest. There is, however, the possibility that the isolation media have

restricted growth of some actinomycetes at acidic condition as there are *Streptomyces* species reported to be able to grow over a broad range of pH in high organic load media (Kontro *et al.*, 2005). Most actinomycetes were isolated from soil samples collected from Site B, six of them showed activity in screening. Six out of 14 isolates selected from DSTA medium showed toxic effect towards PPI screening, fairly high as compared to colonies isolated from HV and other media. Though these *Nocardia*-like isolates produced biologically active metabolites, the specific actions of these crude extracts were not known. Soils were mostly collected under big trees. The forest ecosystem provides different organic substrates for microbes and would affect the microbial community composition (Myers *et al.*, 2001). The report of higher abundance of hydrocarbon degraders in hydrocarbon contaminated soils (Yu, 2000) and the associations of different microbial communities with different grassland community types (McCulley & Burke, 2004) are examples of the effects of different habitats on microbial abundance and diversity.

H11337 showed inhibition against *M. smegmatis* and toxicity on the yeast-based Ras/Raf-1 protein-protein interaction screen. The non-specific action was then confirmed by non-reduction of the β-galactosidase activity of cells treated with H11337. The inhibition was caused by different components as tested by using HPLC fractions (data not shown). In yeast GSK-3β screening, H11329 and H11364 only resulted in positive inhibition at high concentration (100µL). Further investigation is required to confirm the specificity of the hit.

GSK-3β inhibitors might be useful in a few therapeutic applications like nervous system disorders, type 2 diabetes, cancer, stem-cell biology and regenerative medicine (Meijer *et al.*, 2004; Strooper & Woodgett, 2003). RAS-MAPK inhibitors may also find applications in cancer therapy (Houben *et al.*, 2004; Sebolt-Leopold & Herrera, 2004) Geldanamycin and radicicol are two examples of indirect Ras/Raf inhibitors of microbial origin; both decrease Raf level by blocking the Raf-hsp90 complex (Stancato *et al.*, 1997; Soga *et al.*, 1998). A more specific Raf inhibitor, BAY 43-9006, is currently undergoing clinical trial for the development of an anti-cancer drug (Lyons *et al.*, 2001). FK506 and cyclosporin are two immunosuppresant drugs targeted at PP2B, a protein phosphatase (Cohen, 2001). Neurological disorder, metabolic disorder and cancer have been associated with protein phosphatases (McCluskey *et al.*, 2002); more potential drugs targeting at protein phosphatase have yet to be found.

Tuberculosis is still threatening life despite the availability of various drugs. Current conventional TB drugs are highly effective against actively replicating cells, but they are less potent against cells in stationary phase, when they are capable of persisting inside the human lung (McKinney, 2000). Therefore, inhibitors targeting latent persistent TB is of potential use. *Mycobacterium* ICL and PhoP-PhoR are such factors in *M. tuberculosis* infection (McKinney *et al.*, 2000; Fontan *et al.*, 2004).

The search for bioactive metabolites has changed its course tremendously over the past two decades. The applications of recombinant DNA technology in the construction of screening systems as in the Ras/Raf-1 screen and GSK-3β made possible with newer pharmacological knowledge, the expansion to other microbial sources and non-antibiotic type metabolites, has enabled simplified and cost efficient methods for novel compounds discovery (Omura, 1992). More than half of the known antibiotics are of microbial origin, like kanamycin, streptomycin, and erythromycin, whereas

daunorubicin, doxorubicin, and mitomycin are important antitumor agents (Demain, 1999). An investigation by Wang *et al.* (1999) revealed the isolation of 36 genera of actinomycetes from Singapore rainforest soils with *Streptomyces, Micromonospora, Actinoplanes, Actinomadura, Nonomuria, Nocardia*, and *Streptosporangium* as the most abundant; this gives a representation of the actinomycete diversity in the tropical rain forest. Current known microbe species are just a tiny fraction of the whole microbial diversity. They are valuable resources in biotechnology development; the conservation of microbial gene pools is essential but is often overlooked (Bull *et al.*, 2000). Sabah, being one of the world's biodiversity hotspots, offers great opportunities for the discovery of novel bioactive compounds with possible therapeutic value.

Specifically from Melalap, in the Crocker Range, preliminary results indicate the isolation of a number of strains with weak inhibition against specific targets. These include two strains of actinomycetes (H11329 and H11364) inhibiting the GSK-3b screening system, and one strain (H11299) that inhibits PP1. For mycobacterial targets, one strain of actinomycete (H11383) behaves like H7763, a presumptive inhibitor of ICL screening system (Daim, 2003). Furthermore, another actinomycete, H11392, inhibits the PhoP-PhoR system. These strains deserve further study. This study emphasizes the utilization of unseen microbial diversity of actinomycetes and fungi in the search for valuable secondary metabolites for the potential treatment of serious illnesses, cancer, neurological diseases and tuberculosis. The extensive Crocker Range Park (139,919 hectares) in Sabah, with varied topography and vegetation types, is a unique biological resource requiring investigations and conservation.

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