Research article

# Screening for bioactive compounds from microorganisms isolated from Trus Madi Mountain, Sabah

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***ABSTRACT.*** Sixty-four soil samples and fluid samples from seven *Nepenthes* pitchers were obtained from the Trus Madi Mountain, Sabah (2,642 m a.s.l). A total of 45 strains of actinomycetes and 55 strains of fungi were isolated from soil under identified plants, especially *Rhododendron*, *Nepenthes* and conifers growing at Trus Madi. The acetone extracts of aerobically grown cultures were tested for activity against molecular (protein) targets of the eukaryotic signal transduction pathways in yeast (*Saccharomyces cerevisiae*) and also against isocitrate lyase in *Mycobacterium*. For actinomycetes, two strains (H8535 and H8538) inhibited Mkk1 (MAPK Kinase) in the *in vivo* screening system and one strain (H8543) was found to be toxic to $Mkk1^{P386}$ yeast. One strain (H8543) was found to be toxic to Msg5 strain. No strain was found to inhibit protein phosphatases (Msg5, Glc7). One strain (H8602) showed weak inhibition against Ras-Raf protein-protein interaction. No strain was inhibitory to isocitrate lyase in *Mycobacterium smegmatis*. For filamentous fungi, two strains (H9647, H9654) also inhibited Mkk1 in yeast. Mount Trus Madi provides a microbial source-pool for the discovery of promising bioactive compounds.

*Key words*: Actinomycetes, filamentous fungi, molecular targeted screening, Mitogen Activated Protein Kinases, isocitrate lyase

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## INTRODUCTION

This paper concerns the search for soil actinomycetes and filamentous fungi producing novel bioactive compounds inhibitory against proteins involved in eukaryotic signal transduction pathways and isocitrate lyase, of the glyoxylate pathway in *Mycobacterium*. These inhibitors are of interest as biochemical reagents in molecular cell biology and as potential drugs for treatment of cancer and tuberculosis. A field exploration was carried out from 23 October 2001 to 27 November 2001 to the Trus Madi Mountain (2,642 m a.s.l), located in the middle of the Trus Madi Range (Fig. 1).

Trus Madi was chosen for its extremely nutrient-poor, highly acidic, cold and wet soils with its unique vegetation particularly at the unlogged upper montane zone (2,000-2,500 m a.s.l), which is rich in *Nepenthes* (carnivorous pitcher plants, rhododendrons and conifers (*Agathis, Dacrydium, Phyllocladus*). There are several high peaks above 2,000 m a.s.l, lined up from the southwest to the northeast, and steep ridges with slopes generally ranging from 15° to 30°. There is no ultrabasic rock, which is an important substrate for many endemic plant species in Sabah (Kitayama *et al.*, 1993). The soil in this mountain range is derived from mudstone, black shale, and argillite with

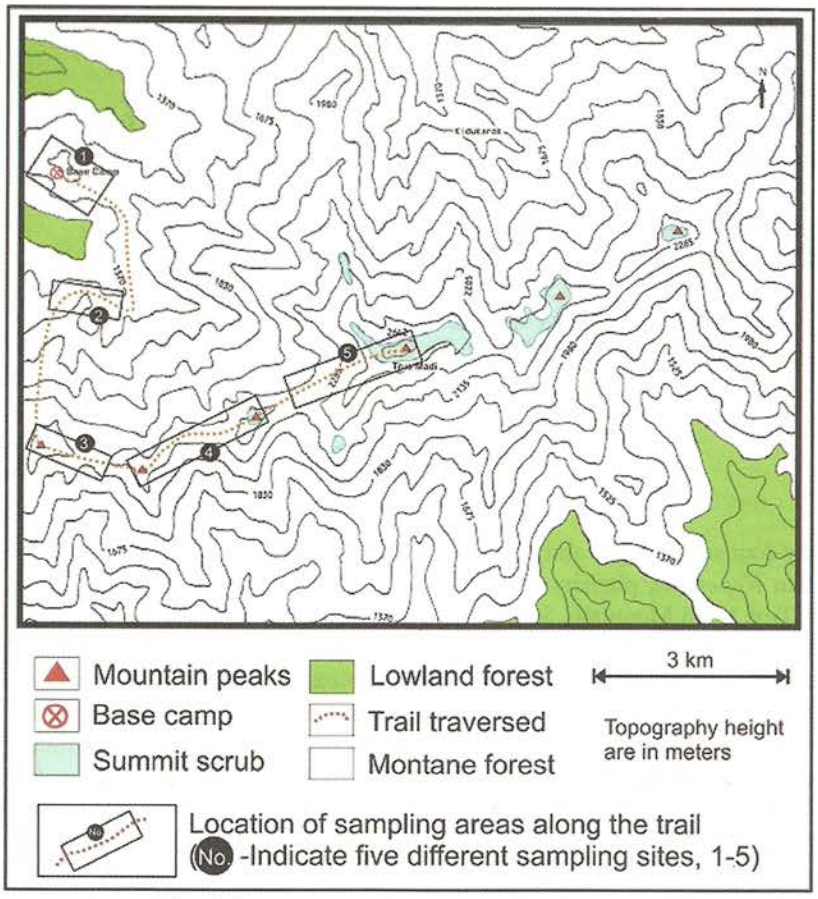


**Fig. 1.** Topography and sampling sites of Trus Madi.

subordinate beds of quartzite, sandstone, siltstone and limestone breccias (Acres, 1972). Most of the samples were collected in the upper montane zone and summit scrub zone (2,500-2,642 m a.s.l). Most of the forests below the upper montane zone had been logged. Another major sampling area was at the Base Camp (1,400 m a.s.l), consisting of regenerated logged forest with few conifer saplings (*Agathis, Phyllocladus*), geocarpic *Ficus, Duabanga moluccana, Rubus, Macaranga* and *Trema orientalis*. The open ground was frequently covered by carpets of mosses (*Campylopus*) and ferns. No rhododendron and pitcher plants were found at the Base Camp. The soils were derived from sandstone and black shale. The soil samples under identified plants were collected by aseptic methods. Fluid samples from *Nepenthes* pitchers were also collected aseptically. The lid-opened pitchers of *Nepenthes* contain, apart from the natural fluid, rainwater, larvae and soil from the surrounding area. Actinomycetes that can grow at pH 3.5 or pH 7.2 were especially isolated. The fungi were isolated at pH 5.5 on Potato dextrose agar (PDA) with 0.005% chloramphenicol and 7.5% NaCl. The pure isolates were grown aerobically and acetone extracts were made. These extracts were tested against molecular targets in yeast-based systems for screening of inhibition against components of the eukaryotic signal transduction system. These include the MAPK kinase (Mkk1), protein phosphatases (Msg5, Glc7) and Ras-Raf protein-protein interaction. The extracts were also tested for inhibition against isocitrate lyase, an enzyme in the glyoxylate pathway in *Mycobacterium*.

## MATERIALS AND METHODS

### Collection of soil and Nepenthes pitcher fluid samples

The soil samples were collected mainly in two localities (Fig. 1), from the logged forest at Base Camp (1,400 m a.s.l) and unlogged summit vegetation (2,000-2,642 m a.s.l). The top soils immediately below the fallen leaves layer under identified plants were collected by sterile spatulas into sterile bottles. Fluid from the pitchers of *Nepenthes* was collected aseptically and stored in sterile bottles. The pH values of soils and fluids from pitchers of *Nepenthes* were determined 4-8 days after collection.

### Isolation of actinomycetes and filamentous fungi

Ideally, only one single colony of actinomycete is isolated from one soil sample. However, in this study, more than one colony were taken due to a small number of actinomycetes isolated from the isolation humic acid – B vitamins medium (Nonomura & Hayakawa, 1988). Humic acid – B vitamins media were prepared at two different pH levels. Media with pH 3.5 and 7.2 were used to isolate actinomycetes from soil samples. For *Nepenthes* fluid, pH 7.2 and pH 4.5 were used. A serial dilution to $10^{-4}$ was done on all soil samples, one hundred µl of the diluted soil suspension and undiluted *Nepenthes* fluid was plated on each plate. Filamentous fungi were isolated by direct plating on Potato Dextrose Agar (PDA) with 0.005% chloramphenicol and 7.5% NaCl at pH 5.5.

### Production of microbial extracts

Actinomycetes were grown on 10 ml of cultivation medium in a 125 ml Erlenmeyer flask (D-mannitol 20g, peptone 20g, glucose 10g, adjusted to pH 7.2 or pH 3.5 and topped up with distilled water to 1L) as used for isolation media. Some isolates that failed to grow at pH 3.5 were grown at pH 4.0 or pH 4.5 and incubated with continuous shaking at 220 rpm for five days at 28°C. Fungi were grown on 10 ml of yeast cultivation medium in a 125ml Erlenmeyer flask (yeast extract 10g, peptone 10g, sucrose 10g, $KH_2PO_4$ 1g, $MgSO_4$ $7H_2O$ 0.3g, adjusted to pH 5.5 and topped up with distilled water to 1L) and incubated with continuous shaking at 220 rpm for 8 days at 28°C. After incubation, 10 ml of acetone was added to each 10 ml culture and stored at 4°C.

### Screening for MAPK kinase (Mkk1) and MAP kinase phosphatase (Msg5) inhibitor

Screening for MAPK kinase and MAPK phosphatase inhibitor was carried out according to Ho *et al.* (2001). Nobel agar (Difco) was replaced with Bacteriological agar no.1 (Oxoid), yeast nitrogen base without amino acids and ammonium sulphate was used and 0.5% ammonium sulphate were added to the screening medium as nitrogen source.

Yeast strain LZ (H10014) was cultured in SD medium (0.67% yeast nitrogen base, 0.5% ammonium sulphate, 2% glucose, 0.002% of each of: uracil, L-arginine, L-isoleucine,

Ras-Raf Interaction Inhibitors with the Yeast Two-Hybrid Screening System

| Strains | Genotype | Source of Reference |
|---|---|---|
| L40 (H10006) | *MATa trp1 leu2 his3 LYS::lexA-HIS3 URA3::lexA-lacZ* | Ki *et al.*, 1998 |
| LZ, transformant of L40 (H10014) | *MATa trp1 leu2 his3 LYS::lexA-HIS3 URA3::lexA-lacZ [pLexA-RAS $^{V12}$(H10011) + pVP16-RAF(H10012)]* | Ki *et al.*, 1998 |

L-tyrosine, L-lysine, L-phenylalanine, L-methionine, L-valine, L-threonine and 0.01% adenine) for 72 hours at 30°C. One hundred µl of this culture was inoculated into 100ml of the agar medium supplemented with ($His^+$) or without ($His^-$) 130µM histidine. To increase the drug permeability and sensitivity in the screening, 0.001% SDS and 1mM 3-aminotriazole were also added to the medium. The paper discs soaked in various microbial extracts were placed on agar plates. The plates were incubated at 30°C for 3 days and the diameter of the growth inhibition zones are measured and compared between those on $His^+$ and $His^-$.

The yeast strains, PAY700-4 and PAY704-1 were incubated in YPD medium [1% yeast extract (Sigma Y-4000), 2% peptone (Sigma P-8388), 2% D(+)glucose monohydrate (BDH 284515 V)] at 28°C for 72 hours with continuous shaking at 220rpm. One hundred µl of different strains of yeast cultures were added into every plate (25µl/plate) of the respective screening media. The screening media used were similar to the cultivation media with the addition of 1.8% bacteriological agar no.1 ($Oxoid^{L11}$) and with or without 1M D-sorbitol (BDH 302424 A). A piece of sterile aluminium foil was wiped with 70 % ethanol and sterile discs, 6 mm in diameter (Whatman No. 3) were arranged on it. Twenty µl of acetone extract (sample to be tested) was applied onto each disc. The discs were left to evaporate in the laminar flow cabinet for 5 minutes and subsequently placed on the agar assay plates with sterile forceps. Each extract was tested on four different plates. The plates were incubated in 25°C and 37° C for three days. The expected results are shown in Table 1.

Protein Serine/Threonine Protein Phosphatases Inhibitors Screening System

| Strains | Genotype | Source of Reference |
|---|---|---|
| PAY700-4, mutant strain (H10017) | *MATa ade2-1 his3-11 leu2-3,112 trp1-1 ura3-1 can1-100 ssd1-d2 glc7::LEU2 trp1::glc7-10:: TRP1 Gal+* | Andrews & Stark, 2000 |
| PAY704-1, wild-type (H10018) | *MATa ade2-1 his3-11 leu2-3,112 trp1-1 ura3-1 can1-100 ssd1-d2 glc7::LEU2 trp1:: GLC7 ::TRP1 Gal+* | Andrews & Stark, 2000 |

**Isocitrate lyase (Icl) screening system**

This screening is a safe and simple paper disc susceptibility test, using non-pathogenic *Mycobacterium smegmatis* in place of *M. tuberculosis* as the seeding bacteria. It is adapted from Sharma *et al* (2000). The method involved the impregnation of paper discs saturated with the inhibitors, onto a set of 4 agar plates. Two of the plates had glucose as the carbon source while the other two had sodium acetate. Each set of plates was divided into two subsets according to the two different strains of seeding bacteria; wild type *M. smegmatis* (H8000) and *icl*-deleted *M. smegmatis* transformed with plasmid carrying the *M. tuberculosis* ICL gene (H8012). Inhibition zones on both acetate plates are indications of inhibition of Icl activity.

For cultivation of *M. smegmatis*, modified M9 media is used (in w/v): $KH_2PO_4$ 0.7%, $K_2HPO_4$ 0.2%, $MgSO_4.7H_2O$ 0.01%, $(NH_4)_2SO_4$ 0.1%, with trace elements: $ZnSO_4.7H_2O$ 0.0002%, $CaCl_2.H_2O$ 0.0001%, $FeSO_4.7H_2O$ 0.0005%, $NaMoO_4.2H_2O$ 0.00002%, $CuSO_4.5H_2O$ 0.00002%, $MnCl_2.4H_2O$ 0.0001%, ferric ammonium citrate 0.00004%, 1M thiamine hydrochloride 0.1% and carbon sources: D(+)glucose monohydrate or sodium acetate 0.5%. For screening, 1.2% and 0.7% of bacteriological agar were added to the bottom layer and top layer, respectively.

**Table 1.** The effects of microbial acetone extracts on yeast strains, PAY700-4 and PAY704-1 for screening of PP1 inhibitors.

| Media | Growth condition | | Inhibitors condition | |
|---|---|---|---|---|
| | YPD, 25°C | YPD+1M Sorbitol, 25°C | YPD, 25°C | YPD+1M Sorbitol, 25°C |
| PAY700-4 (H10017) mutant strain | Growth | Growth | Growth | Growth |
| PAY704-1 (H10018) Wild type strain | Growth | Growth | Growth | Growth |

| Media | Growth condition | | Inhibitors condition | |
|---|---|---|---|---|
| | YPD, 37° C | YPD+1M Sorbitol, 37° C | YPD, 37° C | YPD+1M Sorbitol, 37° C |
| PAY700-4 (H10017) mutant strain | No growth | Growth | No growth | No growth (Inhibition zone around the disc) |
| PAY704-1 (H10018) Wild type strain | Growth | Growth | No growth (Inhibition zone around the disc) | No growth (Inhibition zone around the disc) |

A set of four cultivation media (15ml each in 100ml conical flasks) was autoclaved at 121°C for 15 minutes. Two of the flasks had glucose as the carbon source while the other two had sodium acetate. One full loop of H8000 was inoculated into the two flasks with different carbon sources and another two flasks were inoculated with H8012. Then, the culture was incubated at 37°C for three to four days. The bottom layer screening agar media was poured into each sterile petri dish (15ml per plate) and left to solidify in a laminar flowhood. Then 10ml of top layer screening agar media with different carbon sources and 100 ml of H8000 or H8012 was poured gently onto the bottom layer screening agar. Sterile paper discs, 6 mm in diameter (Whatman No. 3) were arranged on sterile petri dishes and 20 ml of acetone extract was applied onto each disc. The discs were left to dry in the laminar flowhood. Then, the discs were put on the screening agar plates with different carbon sources and different strains of *M. smegmatis*. The plates were incubated at 37°C and scored every 24 hours for three days.

## RESULTS

Forty-five strains of actinomycetes and 55 strains of filamentous fungi were isolated from soils collected aseptically from Trus Madi Mountain, Sabah (Tables 2 and 3). The soils at the unlogged summit under rhododendron were highly acidic (pH2.6 to pH2.9) and many samples did not yield actinomycetes even when plated at pH3.5. The fluids collected from the pitchers of *Nepenthes* were also acidic (pH4.65 to pH6.63); none of them yield actinomycetes when plated on HV media at pH4.5 and pH7.2 (Table 4). However, a few uncharacterized fungi were seen on these plates (Table 4). From the screening, it was found that two strains of actinomycetes, H8535 and H8538 and two strains of filamentous fungi, H9647 and H9654 inhibited the Mkk1. No inhibitors against MAPK phosphatase-Msg5 (Table 5) and type I protein serine/threonine phosphatase (Glc7) were found (Table 6). Strain H8543 showed a toxic effect in both the MAPK kinase and the MAPK phosphatase screening system (Table 5). H8528, H8602 and H8603 showed toxic effect in type I protein serine/threonine phosphatase

**Table 2.** List of soil samples collected under identified plants from Trus Madi Mountain, Sabah.

| Soil samples | Area (refer to Figure 1) | Plant species above soil samples | Location of soil samples | pH |
|---|---|---|---|---|
| TM1 | 4 | *Rhododendron rugosum* | Near summit camp. (First peak after the summit) (East). 2566m. 2 other species of *Rhododendron* also seen growing around the area. | 2.60 (soil) |
| TM2 | | *Rhododendron variolosum* | Near summit camp. (West). 2566m. | - |
| TM3 | | *Rhododendron cuneifolium* | Near summit camp. (North). 2566m. | - |
| TM4 | | *Rhododendron sp.* | About 500m from summit camp (North). Beside the trail heading back to Base camp. | - |
| TM5 | | *Rhododendron cuneifolium* | About 400m from TM4 (North). Beside the trail heading back to Base camp. | - |
| TM6 | | *Rhododendron sp.* | About 200m from TM5 (North). Beside the trail heading back to Base camp. | - |
| TM7 | | *Rhododendron cuneifolium* | About 200m from TM6 (North). Beside the trail heading back to Base camp. | 2.90 (soil) |
| TM8 | | *Rhododendron variolosum* | 200m from summit camp (first peak). Flat ground. | - |
| TM9 | | *Drimys piperita* | 100m from summit camp (first peak) | - |
| TM10 | 1 | *Ficus uncinata* | 500m from Base camp (behind). Non fruiting | - |
| TM11 | | *Ficus sp.* | 500m from Base camp (behind). | - |
| TM12 | | *Ficus uncinata* | 500m from Base camp (behind). | - |
| TM13 | | Unidentified (Seramon*) | Near Base camp. Trail heading to FRC camp. (Left) | - |
| TM14 | | *Elepouthopus mollis* | Near Base camp. Trail heading to FRC camp. (Right) | - |
| TM15 | | Unidentified (Torung Pipit*) | Near Base camp. Trail heading to FRC camp. (Right) | - |
| TM16 | | Rubiaceae | Trail heading to FRC camp. (1400m) | - |
| TM17 | | *Uncaria sp.* | Trail heading to FRC camp. | - |
| TM18 | | Apocynaceae | Trail heading to FRC camp. | - |
| TM19 | | *Blumea balsamifera* | Trail heading to FRC camp. | - |
| TM20 | | Asteraceae | Trail heading to FRC camp. (1400m) | - |
| TM21 | 4 | *Nepenthes lowii* | Summit scrub | - |
| TM22 | | *Nepenthes macrophylla* | Summit scrub (fluid collected) | 5.46 (fluid) |
| TM23 | | *Nepenthes tentaculata* | Upper montane (fluid collected) | 3.57 (soil) 4.92 (fluid) |
| TM24 | | *Nepenthes macrophylla* | Upper montane (fluid collected) | 6.63 (fluid) |
| TM25 | | *Nepenthes macrophylla* | Upper montane | - |
| TM26 | | *Nepenthes macrophylla* | Upper montane (fluid collected) | 4.65 (fluid) |
| TM27 | | *Nepenthes tentaculata* | Upper montane | - |
| TM28 | | *Nepenthes lowii* | Upper montane | - |
| TM29 | 1 | *Daubanga moluccana* | Base camp (1400m) | 4.90 (soil) |
| TM30 | | *Trema orientalis* | Base camp | - |
| TM31 | | *Macaranga sp.* | Base camp | - |
| TM32 | 2 | *Impatiens sp.* | Near stream, Base camp | - |
| TM 41 | 1 | *Agathis sp.* sapling | Logging trail near FRC camp (After camp, on left hand side) | - |
| TM 42 | | *Phyllocladus sp* sapling | Logging trail near FRC camp. (After camp, on left hand side) | - |
| TM 43 | | *Agathis sp.* sapling | Logging trail near FRC camp. (After camp, on left hand side) | - |
| TM 44 | 4 | *Dacrydium sp.* | Trus Madi trail. 2500m | - |
| TM 45 | | *Dacrydium sp.* | Trus Madi trail. 2500m | - |
| TM 46 | 4 | *Agathis sp.* sapling | Trus Madi trail. 2500m | - |
| TM 47 | | *Dacrydium sp.* | Trus Madi trail. >2500m | - |
| TM 48 | | *Dacrydium sp.* | Trus Madi trail. >2500m | - |
| TM 49 | 3 | *Phyllocladus sp.* Sapling | Trus Madi trail. >2000m | - |
| TM 50 | | *Phyllocladus sp.* Sapling | Trus Madi trail. >2000m | - |
| TM 51 | 1 | *Cyathea sp.* | Behind Base camp. Black soil. | 7.04 (soil) |
| TM 52 | | *Cyrtandra sp.* | Behind Base camp. Black soil. | 4.48 (soil) |
| TM 53 | | *Campylopus sp.* | Main road to Base camp. On left hand side. | 5.16 (soil) |
| TM 54 | | *Rubus fraxinifolius* | Main road to Base camp. On right hand side, opposite to TM 53. | 5.73 (soil) |
| TM 55 | | *Agathis sp.* | Track towards FRC camp, on left slope. | - |
| TM 56 | | *Agathis sp.* | Track towards FRC camp, left slope. Below TM 55. | - |
| TM 57 | | *Phyllocladus sp.* | Track towards FRC camp, left slope. Below TM 56. | - |
| TM 61 | 2 | *Campylopus umbellatus* | On track (steep climb to summit) | 5.03 (soil) |
| TM 62 | | *Dawsonia sp.* | Below TM 61 | 4.80 (soil) |
| TM 63 | | Lichen | Below TM 62 | 4.62 (soil) |
| TM 64 | | *Lygodium sp.* | Below TM 63 | 5.74 (soil) |

**continued Table 2**

| | | | | |
|---|---|---|---|---|
| TM81 | 5 | *Nepenthes macrophylla* | Below the summit (2642m-2280m) approx. (fluid collected) | 5.11 (fluid) |
| TM82 | | *Eugenia sp.* | Left trail down the summit (2642m-2280m) approx. | - |
| TM83 | | *Nepenthes lowii* | Below the summit (2642m-2280m) approx. (fluid collected) | 4.89 (fluid) |
| TM84 | | *Nepenthes tentaculata* | Below the summit (2642m-2280m) approx. (fluid collected) | 5.67 (fluid) |
| TM85 | 4 | *Xanthomyrtus sp.* | Peak before summit. 2566m. | - |
| TM86 | 1 | Dead tree trunk | Base camp. Secondary forest. | - |
| TM101 | 5 | *Drimys piperita* | Downhill from the summit. (2642m-2280m) approx. | - |
| TM102 | | Unidentified plant | Downhill from the summit (2642m-2280m) approx. | - |
| TM103 | | *Nepenthes edwardsiana.* | Downhill from the summit (2642m-2280m) approx. | - |
| TM104 | 4 | Moss | Peak before summit. 2566m. | - |
| TM105 | 3 | Moss | Third peak after the summit (rope climb area) covered with moss. 2220m | - |

* Native names

**Table 3**. Actinomycetes and fungi isolated from soils in Trus Madi Mountain.

| **Actinomycetes** | | | **Fungi** | | | |
|---|---|---|---|---|---|---|
| Stock numbers | Soil samples | pH of isolation medium | Stock numbers | Soil samples | Stock numbers | Soil samples |
| H8504 | TM2 | 7.2 | H9643 | TM22 | H9712 | TM57 |
| H8505 | TM10 | 7.2 | H9644 | TM23 | H9713 | TM57 |
| H8506 | TM10 | 7.2 | H9645 | TM24 | H9714 | TM57 |
| H8507 | TM11 | 7.2 | H9646 | TM24 | H9715 | TM61 |
| H8508 | TM12 | 7.2 | H9647 | TM25 | H9716 | TM62 |
| H8509 | TM12 | 7.2 | H9648 | TM25 | H9717 | TM62 |
| H8510 | TM12 | 7.2 | H9649 | TM26 | H9718 | TM63 |
| H8511 | TM13 | 7.2 | H9650 | TM26 | H9719 | TM63 |
| H8512 | TM13 | 7.2 | H9651 | TM26 | H9720 | TM64 |
| H8513 | TM13 | 7.2 | H9652 | TM28 | H9721 | TM64 |
| H8514 | TM16 | 7.2 | H9653 | TM28 | - | - |
| H8515 | TM16 | 7.2 | H9654 | TM29 | - | - |
| H8516 | TM10 | 3.5 | H9655 | TM30 | - | - |
| H8517 | TM10 | 3.5 | H9656 | TM31 | - | - |
| H8518 | TM11 | 3.5 | H9657 | TM31 | - | - |
| H8519 | TM11 | 3.5 | H9658 | TM31 | - | - |
| H8520 | TM11 | 3.5 | H9659 | TM32 | - | - |
| H8521 | TM16 | 3.5 | H9660 | TM32 | - | - |
| H8522 | TM16 | 3.5 | H9661 | TM32 | - | - |
| H8523 | TM16 | 3.5 | H9686 | TM41 | - | - |
| H8524 | TM2 | 7.2 | H9687 | TM41 | - | - |
| H8525 | TM16 | 7.2 | H9688 | TM42 | - | - |
| H8526 | TM10 | 3.5 | H9689 | TM43 | - | - |
| H8527 | TM19 | 7.2 | H9690 | TM43 | - | - |
| H8528 | TM29 | 7.2 | H9691 | TM44 | - | - |
| H8529 | TM29 | 7.2 | H9692 | TM45 | - | - |
| H8530 | TM29 | 7.2 | H9693 | TM45 | - | - |
| H8531 | TM30 | 7.2 | H9694 | TM46 | - | - |
| H8532 | TM30 | 7.2 | H9695 | TM47 | - | - |
| H8533 | TM30 | 7.2 | H9696 | TM47 | - | - |
| H8534 | TM30 | 7.2 | H9697 | TM48 | - | - |
| H8535 | TM31 | 7.2 | H9698 | TM49 | - | - |
| H8536 | TM31 | 7.2 | H9699 | TM49 | - | - |
| H8537 | TM30 | 3.5 | H9700 | TM50 | - | - |
| H8538 | TM30 | 3.5 | H9701 | TM50 | - | - |

**continued Table 3**

| Actinomycetes | | | Fungi | | | |
|---|---|---|---|---|---|---|
| Stock numbers | Soil samples | pH of isolation medium | Stock numbers | Soil samples | Stock numbers | Soil samples |
| H8539 | TM43 | 7.2 | H9702 | TM51 | - | - |
| H8540 | TM51 | 7.2 | H9703 | TM51 | - | - |
| H8541 | TM51 | 7.2 | H9704 | TM51 | - | - |
| H8542 | TM52 | 7.2 | H9705 | TM52 | - | - |
| H8543 | TM56 | 7.2 | H9706 | TM53 | - | - |
| H8544 | TM62 | 7.2 | H9707 | TM53 | - | - |
| H8545 | TM42 | 3.5 | H9708 | TM54 | - | - |
| H8602 | TM82 | 7.2 | H9709 | TM54 | - | - |
| H8603 | TM101 | 7.2 | H9710 | TM55 | - | - |
| H8604 | TM105 | 7.2 | H9711 | TM56 | - | - |

Note: No actinomycetes were isolated from soil TM1, TM3-TM9 using HV media at pH 3.5 and 7.2

**Table 4.** Actinomycetes and fungi isolated from *Nepenthes* pitcher fluid in Trus Madi Mountain, Sabah.

| No | Sample no | Species | Pitcher's lid | Fluid pH | No of colonies isolated Actinomycetes | Fungi |
|---|---|---|---|---|---|---|
| 1 | TM 22 | *Nepenthes macrophylla* | Open | 5.46 | 0 | + |
| 2 | TM 23 | *Nepenthes tentaculatta* | Open | 4.92 | 0 | + |
| 3 | TM 24 | *Nepenthes macrophylla* | Open | 6.63 | 0 | + |
| 4 | TM 26 | *Nepenthes macrophylla* | Closed | 4.65 | 0 | + |
| 5 | TM 81 | *Nepenthes macrophylla* | Open | 5.11 | 0 | + |
| 6 | TM 83 | *Nepenthes lowii* | Open | 4.89 | 0 | + |
| 7 | TM 84 | *Nepenthes tentaculata* | Open | 5.67 | 0 | + |

Abbreviation: + = present
0 = absent

**Table 5.** Screening for inhibitors from microbial acetone extracts against $Mkk1^{P386}$ and MAPK phosphatase Msg-5 in yeast

| H strains | MAPK Kinases $Mkk1^{P386}$ Glucose | Galactose | MAPK Kinases $Mkk1^{P386}$ MSG5 Glucose | Galactose | Remarks |
|---|---|---|---|---|---|
| H8504-H8534, H8536, H8537, H8539-H8542, H8544-H8545, H8602-H8604, H9643-H9646, H9648-H9653, H9655-H9661, H9686-H9721 | 0 | βα | 0 | 0 | No inhibitory activity |
| H8535 | 0 | βγ+(18mm) | 0 | 0 | Inhibitor in MAPK Kinase $Mkk1^{P386}$ |
| H8538 | 0 | βγ+(19mm) | 0 | 0 | |
| H9647 | 0 | βγ +(10mm) | 0 | 0 | Inhibitor in MAPK Kinase $Mkk1^{P386}$ |
| H9654 | 0 | βγ+(10mm) | 0 | 0 | |
| H8543 | +(10mm) | βα | +(19mm) | +(20mm) | Toxic |

Abbreviation: + Inhibition zone (diameter), 0 No inhibition zone, β No growth of yeast on the whole galactose plate α No growth of yeast around the disc on galactose plate, γ Growth of yeast around the disc on galactose plate (diameter in mm)

(Glc7) screening (Table 6). As for the Ras-Raf, a possible weak inhibitor, H8602 was indicated by a smaller zone of inhibition on histidine as compared to minus histidine plate (Table 7). H8602 could be inhibiting Ras-Raf protein-protein interaction directly or indirectly. No strain with specific inhibition of isocitrate lyase was found. A few extracts (H8602, H8603 and H8604) inhibited the growth of *M. smegmatis* in glucose and acetate media. One fungal strain, H9709 only inhibited *M. smegmatis* on glucose medium (Table 8).

## DISCUSSION

This preliminary investigation revealed that the soils from Trus Madi Mountain Sabah with its intact unique summit montane vegetation rich in several species of rhododendrons and pitcher plants and the regenerated forest at lower altitude (1,400 m) contained a diverse group of actinomycetes and fungi. The cell-based molecular targeted (protein) screening indicated a few of these microbial strains may have potential inhibitors affecting the protein kinases of the eukaryotic signal transduction system. In particular, the presumptive inhibitor against the MAPK kinase (Mkk1) is of interest. At present only synthetic inhibitors, namely PD98059 (Alessi *et al.*, 1995; Dudley *et al.*, 1995), U0126 (1,4-diamino-2,3-dicyano-1,4-bis[2-aminophenylthio] butadiene) (Favata *et al.*, 1998) and XK469 (Lin *et al.*, 2002) were known. Both PD98059 and U0126 were found to prevent the activation of mammalian MAPK kinase (Mkk1) and not to inhibit Mkk1 activity directly (Davies *et al.*, 2000). These inhibitors are potential anti-cancer agents and have now already being found in applications as biochemical reagents in dissecting out the signal transduction pathways in eukaryotes (Yoshida, 2000). As the MAPK kinase pathway integrity system in *Saccharomyces cerevisiae* (Gustin *et al.*, 1998) has close homology to that in the mammalian system, it will be important if the presumptive Mkk1 inhibitor found in yeast be purified and its action on the mammalian homologue determined. For bioprospecting, Trus Madi represents a high altitude, cold mountain with acidic soils with unique vegetation at the summit, such as *Rhododendron* and *Nepenthes* providing an extreme environment for unique microbes with the potential of producing important secondary metabolites of pharmaceutical interest. As such, Trus Madi deserves total conservation protection.

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**Table 6.** Screening for inhibitors from microbial acetone extracts against Type 1 protein serine/threonine phosphatase (Glc7) in yeast

| H-Series | PAY-700-4 (Mutant) | | | | PAY704-1 (Wild-type) | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|
| | YPD | | YPD + 1 M Sorbitol | | YPD | | YPD + 1 M sorbitol | | |
| | 25°C | 37°C | 25°C | 37°C | 25°C | 37°C | 25°C | 37°C | |
| H8504-H8527, H8529-H8545, H8604, H9643-H9661 | + | 0 | + | + | + | + | + | | No inhibitory activity |
| H8528 | +(10mm) | 0 | +(10mm) | +(11mm) | +(11mm) | +(10mm) | +(10mm) | +(10mm) | Toxic |
| H8602 | +(10mm) | 0 | +(11mm) | +(12mm) | +(12mm) | +(15mm) | +(11mm) | +(10mm) | Toxic |
| H8603 | +(11mm) | 0 | +(12mm) | +(12mm) | +(10mm) | +(14mm) | +(10mm) | +(11mm) | Toxic |

Abbreviation: + yeast growth on whole plate, Inhibition zone (diameter)
0 no yeast growth on whole plate
Note: H9686-H9721 were not tested

**Table 7.** Screening for inhibitors from microbial acetone extracts against Ras-Raf interaction with the yeast two-hybrid system

| H-strains | H10014 | | Remarks |
|---|---|---|---|
| | $His^+$ | $His^-$ | |
| H8504 - H8545, H8603 - H8604, H9643 - H9661, H9686 - H9721 | 0 | 0 | No inhibitory activity |
| H8602 | +(9mm) | +(12mm) | Toxic (Weak inhibitor) |

Abbreviation: + Inhibition zone (diameter)
0 No Inhibition zone

**Table 8.** Screening for inhibitors from microbial acetone extracts against Icl in *M. smegmatis*

| H-series | H8012 | | H8000 | | Remarks |
|---|---|---|---|---|---|
| | Glucose | Acetate | Glucose | Acetate | |
| H8504-H8545, H9643-H9661, H9686-H9708, H9710-H9721 | 0 | 0 | 0 | 0 | No inhibitory activity |
| H8602 | +(12mm) | +(10mm) | +(8mm) | +(8mm) | Toxic against wild type and transformant |
| H8603 | +(12mm) | +(8mm) | +(8mm) | +(8mm) | Toxic against wild type and transformant |
| H8604 | +(17mm) | +(11mm) | +(10mm) | +(9mm) | Toxic against wild type and transformant |
| | H9709 | +(10mm) | 0 | +(10mm) | 0 Toxic against wild type and transformant in glucose plates. |

**Abbreviation**: + Inhibition zone (diameter)
0 No Inhibition zone

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