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Bacteria Population in the Schirmacher Oasis

Clemente Michael Wong Vui Ling, Yong Hui Yee and Tam Heng Kiat

Biotechnology Research Institute, Universiti Malaysia Sabah,
Locked Bag 2073, 88999 Kota Kinabalu Sabah, Malaysia

ABSTRACT

Bacteria were isolated from the soil and water samples collected within the Schirmacher Oasis during the austral summer of 2005. Purified bacteria were analyzed by Gram-staining, light microscopy and molecular techniques. The 16S rDNA of the isolates were sequenced and analyzed using the basic local alignment search tool (BLAST) to resolve the identities of the bacterial isolates. Bacteria encountered were *Arthrobacter* spp. *A. oxydans, Bacillus* spp., *B. cereus, B. polyfermenticus, B. pumilus, Pseudomonas fluoresceus, P. putida, P. syringae, Nocardioides, Frigoribacterium, Rubrobacter, Rhodococcus, Flavobacterium* sp., *Burkholderia* sp. and several unidentified strains. One isolate, *Burkholderia* sp. was found to produce anti-microbial compounds against tropical pathogens, *Salmonella typhii* and *Escherichia coli* O157.

INTRODUCTION

Antarctica harbors a diverse population of unique microorganisms and is home to many unique species which are not found elsewhere. The diversities of bacteria of various parts of Antarctica are well-documented, but only a few reports are available from the Schirmacher Oasis. Schirmacher Oasis is an area of relatively ice-free, low lying-hills, in the Eastern Dronning Maud land, (70º46’04"-70º44’21"S; 11º49’54"-11º26’03"E) East Antarctica, about 70 km away from Prince Astrid coast. The Schirmacher Oasis has many fresh water lakes that harbor a wide variety of microbes (Velecar et al., 2002). Hence, the objective of this project was to analyze the bacterial population in the permanent lake of the Schirmacher Oasis. This project was carried out in collaboration with the National Centre for Antarctica and Ocean Research, India. Samples were collected from the permanent Antarctic lake water and soils within the Schirmacher Oasis for bacterial isolation and characterization. Isolated bacteria were screened for their abilities to produce anti-microbial compounds.

MATERIALS AND METHODS

Sampling Locations

The locations from where the samples were collected were generally divided into (i) west of Maitri, (ii) around Maitri and (iii) east of Maitri (circled in **Fig. 1**). The sampling locations were recorded using the Global Positioning System (GPS) detector (Garmin). Soil and water samples were collected using sterilized bottles and stored at -20ºC. About two hundred grams of soil were collected in triplicates from each site.

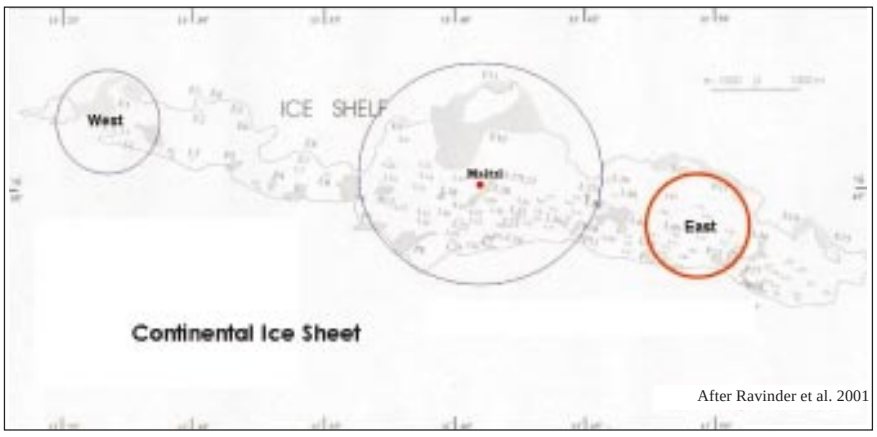

Fig. 1 : The Schirmacher Oasis

Growth Media

Bacteria were grown in two different media, viz. Luria-Bertani (LB) and Nutrient media. The Luria Bertani medium (10 g/l tryptone, 5 g/l yeast extract and 5 g/l sodium chloride), and Nutrient medium (Peptone from meat 3.45 g/l, sodium chloride 5.1 g/l and peptone from casein 3.45 g/l) were diluted to 1/10 of its original concentration, and sterilized by autoclaving at 121ºC at 15lb for 15 mins.

Isolation of Bacteria

Bacteria from liquid culture were diluted and plated onto Luria-Bertani and Nutrient Media containing 1.5% Bacto-Agar (Difco). Single colony was picked and streaked onto fresh medium and the process was repeated until a pure culture was obtained.

Extraction of Genomic DNA

Two ml of bacteria in the liquid culture were used for the extraction of genomic DNA. DNA extraction was carried out using the DNAeasy Kit (Qiagen) according to the instructions from the manufacturer.

Sequencing of the 16S rDNA

Universal primers 25f, 518r, 530f, 907r, 926f, 1114f, 1392r and 1525r were used in various combinations to amplify the 16S rDNA of bacteria. Amplified 16S rDNA genes were sequenced and analyzed using the basic local alignment search tool (BLAST), a web-based software at http://www.ncbi.nlm.nih.gov/BLAST/.

Detection of Antimicrobial Production

Antimicrobial production was determined using the modified deferred antagonism procedure of Kekessy and Piguet (1970). Replica plates were prepared using the same agar as that used for isolating the organisms on the master plates. The replica plates were overlaid with soft Nutrient agar containing one of the indicator organisms. Zones of clearance surrounding the producer colony following incubation at 20°C indicated the presence of an antagonistic agent (O'Brien *et al.*, 2004). Assay bacteria, *Escherichia coli* 0157:H7, and *Salmonella typhii* were maintained on Luria-Bertani broth and LBA or nutrient broth and agar (NB/NA).

RESULTS AND DISCUSSIONS

LB and nutrient agar media supported the growth of a substantial number of bacteria from the soil and water samples. Bacterial isolates that grew at temperatures lower than 15ºC were purified and identified based on their 16S rDNA sequences. Strains identified were, *Arthrobacter* spp. *A. oxydans, Bacillus* spp., *B. cereus, B. polyfermenticus, B. pumilus, Pseudomonas fluoresceus, P. putida, P. syringae, Nocardioides, Frigoribacterium, Rubrobacter, Rhodococcus, Flavobacterium* and *Burkholderia* sp. and several unknown species **(Table 1)**.

Among them, *Arthrobacter* sp., *A. oxydans, Bacillus* sp., *B. cereus, B. polyfermenticus, B. pumilus, Frigoribacterium* sp. and *Burkholderia* sp. were found in the east of Maitri. In contrast, *Nocardioides* sp., *Rubrobacter* sp., *Flavobacterium* sp., and *Rhodococcus* sp. found in the east of Maitri, were also found in either west or around Maitri. This indicates that not all the bacteria were distributed evenly within the Schirmacher Oasis.

Table 1—The distributions of bacteria around Maitri, East and West of Maitri within the Schirmacher Oasis

Notes: * = Bacteria found only at the east of Maitri. () = number of repeating isolates from a particular region

Around Maitri	West of Maitri	East of Maitri
Pseudomonas fluorescens(1) *P. putida (2)* *P. syringae (3)* *Rubrobacter* sp. *(3)* *Rhodococcus* sp. *(2)* *Flavobacterium* sp. *(2)* *Three (3) unidentified strains*	*Nocardioides* sp.*(3)* *Frigoribacterium* sp.*(2)* *Rhodococcus* sp. *(2)* *Flavobacterium* sp. *(5)* *Six (6) unidentified strains*	**Arthrobacter* sp. *(3)* **A. oxydans (3)* **Bacillus* sp. *(2)* **B. cereus (1)* **B. polyfermenticus (2)* **B. pumilus (2)* *Pseudomonas fluoresceus (2)* *P. putida (2)* *P. syringae (1)* *Nocardioides* sp. *(2)* **Frigoribacterium* sp. *(1)* *Rubrobacter* sp. *(1)* *Rhodococcus* sp. *(1)* *Flavobacterium* sp. *(1)* *Burkholderia* sp.*(1)* *Three(3) unidentified strains*

Some of the bacteria isolated from the Schirmacher Oasis were also found in other temperate countries, namely *Arthrobacter* sp. *A. oxydans*, *Bacillus* sp., *B. cereus*, *B. polyfermenticus, B. pumilus*.

There were several strains that have novel 16S rDNA sequences with little homologies to bacteria in the gene bank, and therefore could be new species. When all the isolates were tested for their ability to inhibit pathogens, only *Burkholderia* sp. gave positive results against *Salmonella typhii* and *Escherichia coli* O157, **(Fig. 2)**. These findings were interesting, and warrant further investigation. Hence, effort is being made to determine whether the few strains which have not been identified are novel. Additionally, the process of identifying the compound produced by *Burkholderia* sp. is currently underway.

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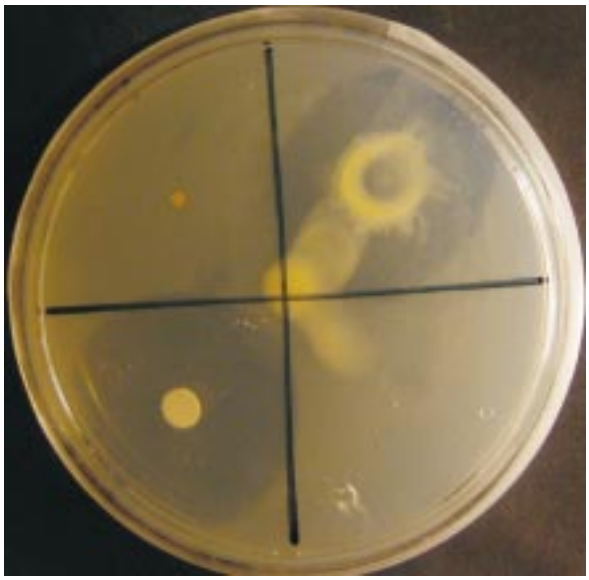

Fig. 2 : E.coli O157 was used overlaid the bacteria colony to be tested. Colony A, is a control strain did not inhibit the growth of E.coli O157; Colony B, Burkholderia sp. inhibited the growth of E.coli O157; Ampicillin disc (C) inhibited the growth of E.coli O157

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