

YPASM fellowship report 2013/2014

Title of project: Isolation and Characterization of Antarctic Actinobacteria and Bacteria with Antimicrobial Activities.

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Date and duration:

Activities	From Date	To Date
Preparation for Antarctica expedition	16 Jan 2014	21 Jan 2014
Antarctica expedition	22 Jan 2014	14 Feb 2014
Lab attachment at INACH, Punta Arenas	15 Feb 2014	14 Apr 2014

Objectives of project:

- To provide an opportunity to the PhD student to gain new knowledge and research skills from Antarctic experts;
- To isolate and characterize Antarctic Actinobacteria and bacteria from protected areas in South Shetland islands;
- To screen these microbes for antimicrobial compounds and toxin and antitoxin system.

Methodology:

Sample collection

Soil samples were collected from Deception island, Snow island, Punta Hannah, Coppermine Peninsula, Dee island, Greenwich island, Nelson island, Litchfield island and Glacier Union. The samples were stored at -20°C and transported back to Instituto Antártico Chileno.

Pretreatment

All soil samples were pooled by the location (Deception island, South Shetland islands and Glacier Union) and pretreated with 5mM phosphate buffer with 6% yeast extract and 0.05% sodium dodecyl sulphate (SDS) at 40°C for 20 mins (Hayakawa and Nonomura, 1989).

Optimization

In order to save cost and time, the soil suspensions were tested at different combinations of dilutions (10^{-1} , 10^{-2} , 10^{-3} and 10^{-4}) and aliquots (10µL, 50µL and 100µL) on oatmeal agar (Shirling and Gottlieb, 1966) (supplemented with 50mg/L cycloheximide) to determine the optimum dilution and aliquot of soil suspension for isolation. The plates were incubated at 4°C, 12°C and 20°C.

Isolation

The isolation was repeated with humic acid (HA) agar (Hayakawa, 2008; Istianto *et al.*, 2012), raffinose-histidine (RH) agar (Hong *et al.*, 2009) and Zhang's Soil Starch Extract (ZSSE) (Zhang and Zhang, 2011) agar using the optimum dilution and aliquot of each soil suspension. All media were supplemented with 50mg/L cycloheximide. The plates were incubated at 4°C, 12°C and 20°C. Purified cultures were maintained on agar slants at the respective temperatures.

Antimicrobial susceptibility test

The isolates were spot inoculated on Mueller-Hinton (MH) agar and incubated at the respective temperatures for 3-4 days. *Staphylococcus aureus* ATCC6538P and *Escherichia coli* TOP10 were grown in 1.3% MH broth at 37°C for 6 hours. The bacterial suspension was added to 10mL 0.7% molten agar to an optical density of 0.001 at 600 nm (OD₆₀₀). The molten agar was poured onto the surface of the MH agar and incubated at 37°C (adapted from Wong *et al.*, 2011). Presence of inhibition zone was checked visually after 24 hours.

DNA extraction

Isolates which showed positive results to both *S. aureus* ATCC6538P and *E. coli* TOP10 were grown in 10mL of MH broth for 3 days. Each broth culture was centrifuged at maximum speed for 10 mins and the supernatant was discarded. 500µL of Laemmli lysis-buffer was added to suspend the pellet. The suspension was transferred to a microcentrifuge tube and incubated at 100°C for 10 mins. 500µL of phenol:chloroform:isoamyl alcohol (25:24:1, v/v) was added to the tube. The mixture was vortexed for 1 min and centrifuged at maximum speed for 10 min. The upper phase was transferred to a new microcentrifuge tube and 500µL of chloroform: isoamyl alcohol (24:1, v/v) was added. The mixture was vortexed for 1 min and centrifuged at maximum speed for 10 min. The upper phase was transferred to a new microcentrifuge tube. 100µL of sodium acetate and 500µL of absolute ethanol were added into the tube. The mixture was vortexed and incubated at -20°C for 2 hours. The mixture was centrifuged at maximum speed for 10 min. The supernatant was carefully removed without touching the DNA pellet. The DNA pellet was resuspended in 500µL of 70% ethanol. The suspension was vortexed and incubated at -80°C for 1 hour. The suspension was centrifuged at maximum speed for 10 min. The supernatant was carefully removed without touching the DNA pellet. The pellet was air-dried at room temperature and resuspended in 50µL of sterile MilliQ water (adapted from Ausubel *et al.*, 1995). The DNA samples were kept at -20°C for further analysis.

Results:

10^{-3} dilution and 50 μ L aliquot of soil suspension was found to be the optimum concentration which gave the highest number of individual colonies for Deception island soil sample, while that of South Shetland islands was 10^{-3} dilution and 100 μ L aliquot of soil suspension (Table 1). The distribution of colonies on oatmeal agar spread with optimum dilution and aliquot were shown in Figure 1.

Table 1. Number of individual colonies obtained from oatmeal agar at the respective temperatures after 6-8 incubation days.

Dilution and aliquot of soil suspension	Deception island			South Shetland islands		
	4	12	20	4	12	20
10^{-1} dilution & 10 μ L	-	-	3	25	6	13
10^{-1} dilution & 50 μ L	-	-	1	-	-	-
10^{-1} dilution & 100 μ L	-	-	1	2	-	-
10^{-2} dilution & 10 μ L	29	11	3	13	9	17
10^{-2} dilution & 50 μ L	29	6	-	2	4	5
10^{-2} dilution & 100 μ L	-	5	-	20	4	1
10^{-3} dilution & 10 μ L	-	11	5	3	3	7
10^{-3} dilution & 50 μ L	38	20	15	17	11	19
10^{-3} dilution & 100 μ L	29	16	10	27	11	38
10^{-4} dilution & 10 μ L	-	-	5	-	-	-
10^{-4} dilution & 50 μ L	25	12	11	-	5	6
10^{-4} dilution & 100 μ L	35	29	10	6	6	9

No colonies were observed on the oatmeal agar spread with treated Glacier Union soil sample.

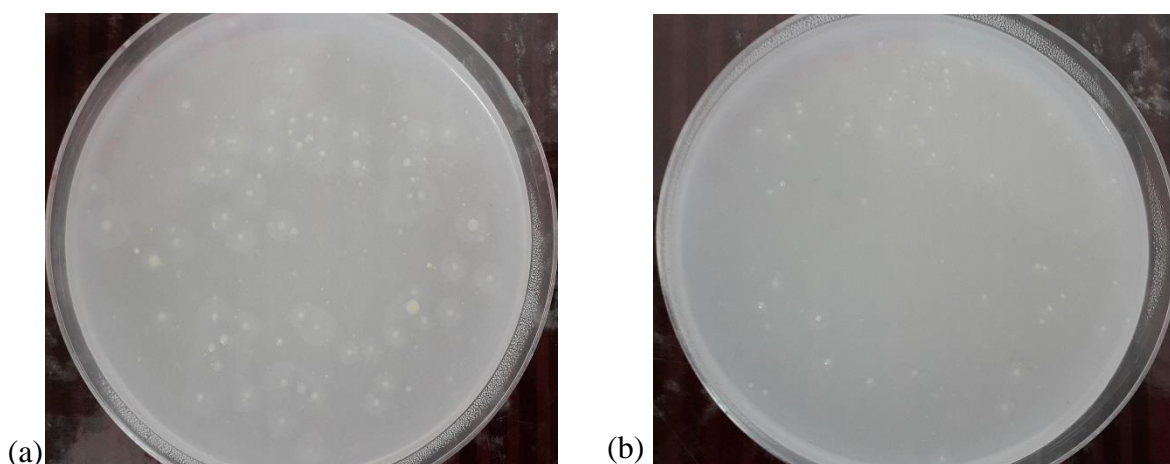


Figure 1. Distribution of colonies on oatmeal agar spread with optimum dilution and aliquot of soil suspension. *a*, Deception island soil sample, *b*, South Shetland island soil sample.

A total of 1733 isolates were obtained from three soil samples: 765 from Deception island, 968 from South Shetland islands and none from Glacier Union (Table 2).

Table 2. Number of isolates obtained from each soil sample at the respective temperature.

Soil sample Incubation temperature (°C)	Deception island (treated)	South Shetland islands (treated)	Glacier Union (untreated)
4	306	391	0
12	247	266	0
20	212	311	0
Total	765	968	0

Due to time constraints, only 648 isolates were subjected to antimicrobial susceptibility testing against *S. aureus* ATCC6538P and *E. coli* TOP10. 110 isolates (17%) exhibited inhibitory effect against *S. aureus* ATCC6538P only while 161 isolates (25%) inhibited *E. coli* TOP10 only. 77 isolates (12%) showed antagonistic activity against both *S. aureus* ATCC6538P and *E. coli* TOP10 (Table 3). 300 out of 648 isolates (46%) did not possess any antimicrobial activity against both indicator bacteria. Antimicrobial activity of the isolates against *S. aureus* ATCC6538P and *E. coli* TOP10 was shown in Figure 2.

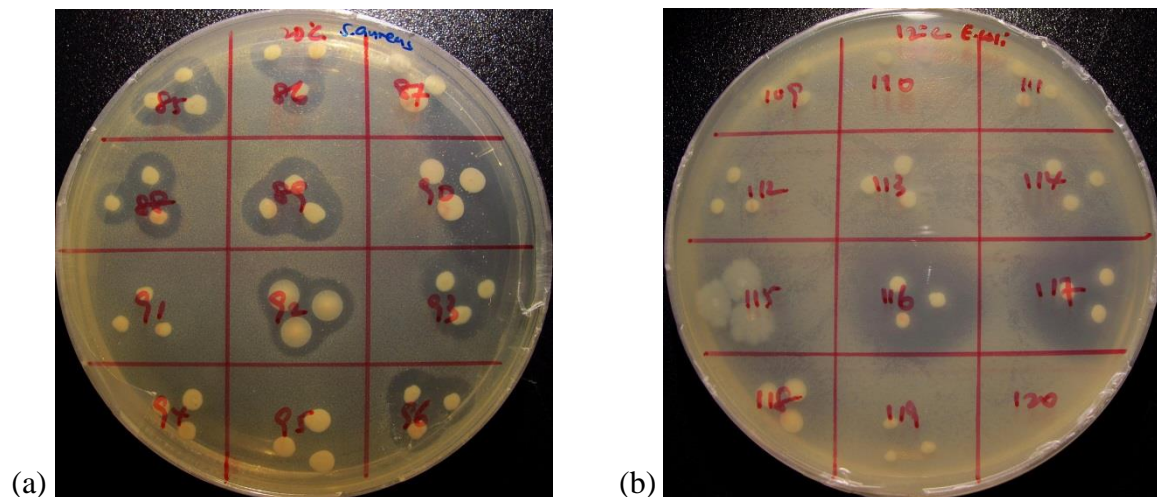


Figure 2. Antimicrobial activity of the isolates against (a) *S. aureus* ATCC6538P and (b) *E. coli* TOP10.

DNA was extracted from the 77 isolates which showed positive results to both *S. aureus* ATCC6538P and *E. coli* TOP10.

Future and on-going work:

Future work will be focused on the antimicrobial susceptibility testing on the remaining 1085 isolates. Isolates which have antimicrobial effects against both *S. aureus* ATCC6538P and *E. coli* TOP10 will be proceeded to determine the antimicrobial activity against prokaryotic and eukaryotic pathogens. Isolates of interest will be subjected to 16S rDNA sequencing, antibiotic sensitivity test, purification of the antimicrobial compounds and so on.

There will be a collaboration with the Chilean researchers in INACH to investigate the antimicrobial properties, biodiversity and other aspects of the isolates obtained from this project.

Financial

i. To specify how much YPASM funds were allocated.

Expenses Category	Total (RM)
Trip from Kota Kinabalu - Punta Arenas	14,735
Accommodation and food allowance	18,009
Other related expenses (e.g. transportation fee in Punta Arenas, chemicals & consumables, expedition equipment etc)	12,000
Total Direct	44,744

ii. To provide details on how were the funds utilized (itemized).

No.	Date	Particular	Total funding approved (RM)			
			Air tickets	Accommodation and food allowance	Other related expenses (e.g. transportation fee in Punta Arenas, chemicals & consumables, expedition equipment etc)	Total
Original funding			14,735.00	18,009.00	12,000.00	44,744.00
1	30.11.2013	Medical check up for Antarctic expedition			60.00	
2	05.12.2013	Air ticket from Kuala Lumpur to Paris and return	3,025.00			
3	05.12.2013	Air ticket from Paris to Santiago and return ^a	7,589.11			
4	05.12.2013	Air ticket from Santiago to Punta Arenas and return ^b	2,187.35			
5	13.12.2013	Travel and medical insurance for expedition			625.00	
6	13.12.2013	Borneo Outdoor Gear Sdn Bhd (hiking boots and waterproof backpack for sample collection)			418.00	
7	20.12.2013	Air ticket from Kota Kinabalu to Kuala Lumpur and return	560.00			
8	09.01.2014	Air France - additional luggage fee from Paris to Santiago ^c	267.27			
9	21.01.2014	Balfer Limitada - waterproof gloves ^d			59.33	
10	21.01.2014	Balfer Limitada - thick socks ^d			43.56	
11	21.01.2014	Comercial Balthazar S.A. - waterproof gloves and cap ^d			282.48	
12	15.02.2014	Accommodation in Punta Arenas (15/02/2014 – 16/04/2014) ^e		3,426.92		
13	26.02.2014	Merck S.A. - cardboard boxes ^e			605.59	
14	27.02.2014	Graciela González Paredes E.I.R.L - travel adapter ^e		63.14		
15	27.02.2014	GrupoBios - 2mL microtubes ^e			492.39	
16	03.03.2014	Ferreteria "El Aguila" Ltda - soil ^e			33.57	
17	03.03.2014	Merck S.A. - raffinose pentahydrate ^e			494.45	
18	05.03.2014	Merck S.A. - potassium nitrate ^e			135.62	
19	06.03.2014	Moreno Asociados Ltda. - petri plates ^e			1,018.38	
20	10.03.2014	InduLab Ltda - Mueller-hinton agar and broth, bacto agar ^e			916.05	
21	11.03.2014	Merck S.A. - calcium carbonate and L-histidine ^e			797.8	
22	20.03.2014	Merck S.A. - filter paper and absolute ethanol ^e			271.06	
23	26.03.2014	Winkler Ltda - phenol chloroform and chloroform:isoamyl alcohol ^e			421.40	
24	14.04.2014	Food allowance (3 months) (1,586,000 Chilean peso) ^e		10,065.00		
25	14.04.2014	Air France - additional luggage fee from Santiago to Paris ^d	330.00			
26	15.04.2014	Taxis G7 - transportation from Paris airport to Hotel Saint Christophe ^f			624.00	
27	15.04.2014	Hotel Saint Christophe ^f		609.60		
28	16.04.2014	Sarl Nice Shuttle - transportation from Hotel Saint Christophe to Paris CDG airport ^f			124.80	
29	23.05.2014	Transportation fee of samples from INACH to UMS ^e			307.29	
30	02.09.2014	Transportation fee of samples from INACH to UMS ^e			450.70	
31	05.09.2014	Transportation fee of samples from INACH to UMS ^e			356.46	
Total expenses			13,958.73	14,164.66	8,537.93	36,661.32
Remaining			776.27	3,844.34	3,462.07	8,082.68

^a1 USD = 3.2957155, ^b1 USD = RM 3.2735, ^c1 USD = 3.3409, ^d500 Chilean peso = 1 USD = RM 3.30, ^e520 Chilean peso = 1 USD = RM 3.30, ^f1 euro = RM 4.80

Outputs:

Since it is still an ongoing project, there are no publications and paper presentations yet.

Acknowledgments

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