



INTERNATIONAL MEDICAL UNIVERSITY  
MALAYSIA

# Polar Research in the International Medical University





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## Acknowledgements

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We would also like to thank Mdm Lau Yoke Kuan, wife of the late Prof Chu Wan Loy, for kindly providing us with photographs from Prof Chu's collection.

Photo: Adélie penguins at Factory Cove, Signy Island, with Coronation Island in the background.

Cover Photo: Chinstrap penguin at Rock Haven, Gourlay Peninsula, Signy Island.





## Foreword

The vast, white expanse of the ice-covered Antarctic continent, seemingly devoid of life. When such images come to mind we often forget about the extraordinary abundance of life just beneath the ice, particularly microorganisms, surviving and playing a fundamental role in the functioning of the Antarctic ecosystems. The abundance and diversity of microbial life in such extreme aquatic and terrestrial environments have intrigued scientists for over a century. However, amidst the silence and solitude of the Antarctic we have begun to see dramatic changes in the environment, with climate change and the significant impacts of human activities in the region becoming ever more apparent in recent years.

The International Medical University's (IMU) foray into polar research first began with Prof Chu Wan Loy's passion for tropical and polar algae research. Scientists from the IMU have since joined in the exploration and study of these extraordinary organisms, expanding into research on bacteria, viruses, parasites and fungi. Research efforts include how these microorganisms contribute to the maintenance and delicate balance of the Antarctic ecosystem and their responses to the changing climate. In this book, we describe the IMU's endeavour in polar research, in memory of Prof Chu's vision and dedication to not only scientific exploration, but also education and a shared effort to understand the consequences of global warming.

Photo: Hiking to Tioga Hill, the highest point (279 m) on Signy Island. Dr Nur Alia Johari (right) with a British Antarctic Survey field guide.



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Photo: Factory Bluffs, Signy Island.

# Brief History of Malaysia's Involvement in Antarctica

Antarctica is the coldest and windiest continent, located at the southernmost part of the globe. Despite being approximately 10,000 km apart, Malaysia's interest in Antarctica can be traced to nearly 40 years ago. In 1983, the then Prime Minister of Malaysia, Tun Dr Mahathir Mohamad drew the attention of the world by raising the "Question of Antarctica" in the United Nations General Assembly (UNGA). It is the first official diplomatic initiative that debated the exclusivity of the Antarctic Treaty System (ATS) represented only by the developed nations. Tun Dr Mahathir called for the opening of the ATS to allow underdeveloped and developing countries to participate in the decision-making process regarding Antarctica and to share the resources of the continent.

Malaysia's position on Antarctica shifted in the 1990's, especially after the signing of the Protocol on Environmental Protection to the Antarctic Treaty (Madrid Protocol) in 1991. The agreement specifies the protection of the Antarctic environment and prohibits all activities relating to mineral resources except scientific exploration. Malaysia was active in advocating the protection of Antarctica, for instance, expressing concern over the overharvesting of krill and the illegal, unreported and unregulated (IUU) fishing off the Antarctic coast.

Photo: Adélie penguins at the Gourlay snowfield, Signy Island.

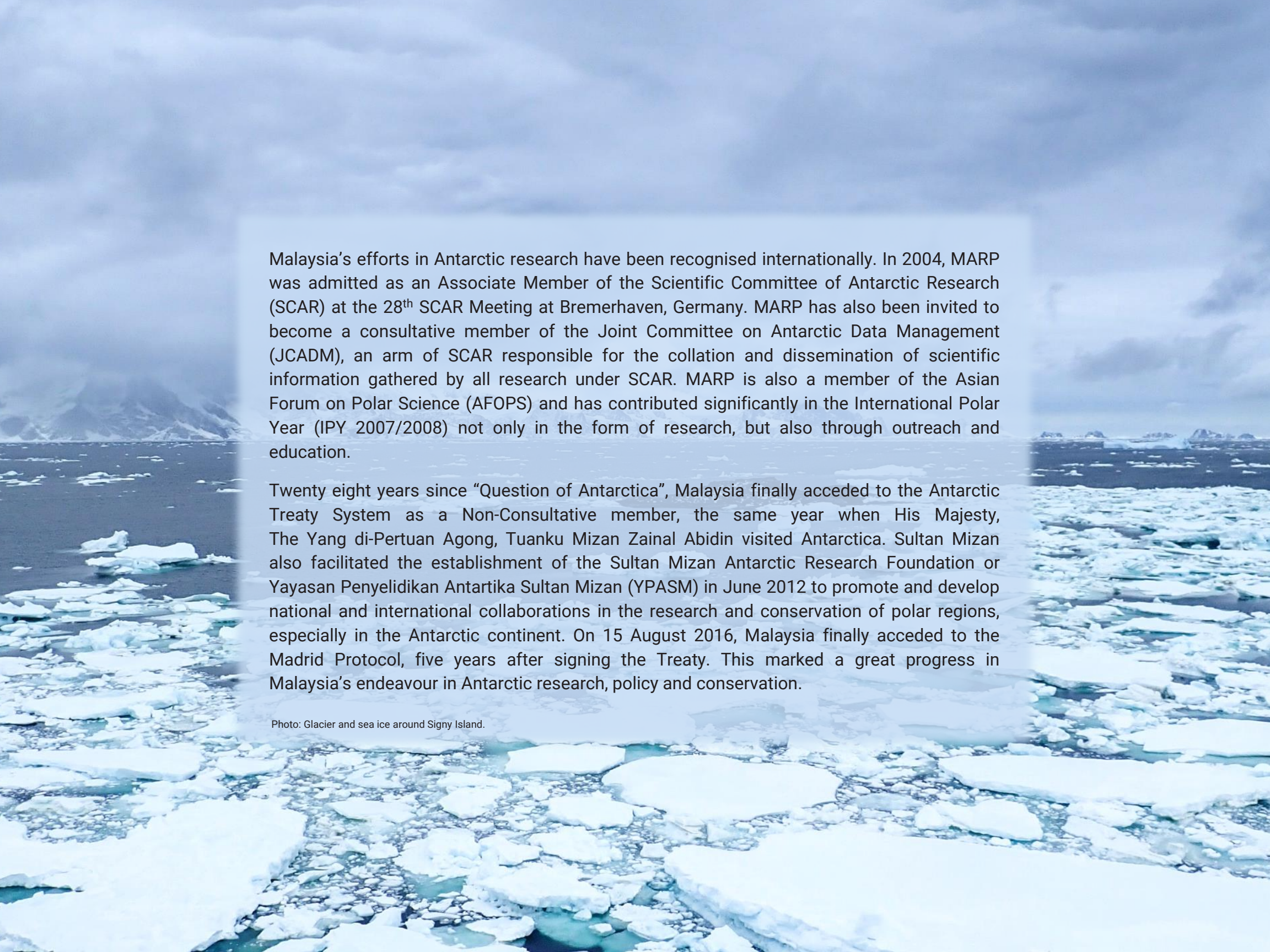




In comparison to diplomatic and political involvement, Malaysia's scientific endeavour in Antarctica started relatively late in 1997. The breakthrough came when New Zealand offered the use of Scott Base in Antarctica in 1997 for Malaysia to undertake research. This became the catalyst for the Malaysian Cabinet to commission the Academy of Sciences Malaysia (ASM) to develop the Malaysian Antarctic Research Program (MARP). MARP was established in 1998 and the young Antarctic program was given a big task to focus on climate change and biodiversity in Antarctica.

The first Malaysian scientific field research in the Antarctic was undertaken on 13 to 25 October 1999 in Scott Base, Antarctica. MARP received an injection of MYR10 million under the 8<sup>th</sup> Malaysian Plan (2000-2005) to develop resilient and innovative researchers in Antarctic Science. During this period, the MARP started extensive international collaborations with Antarctic research bodies such as Antarctica New Zealand (ANZ), Australian Antarctic Division (AAD), British Antarctic Survey (BAS), Instituto Antártico Argentino (IAA), South Africa National Antarctic Program (SANAP), Korea Polar Research Institute (KOPRI), and National Centre for Antarctic and Ocean Research (NCPOR), India. The bilateral agreements with national programmes have facilitated the logistic arrangements for field work in Antarctica and technology transfers which both benefited the development of Antarctic research in Malaysia.

Photos: (Top) BAS Twin Otter VP-FBC aircraft refuelling at Sky-Blu Field Station, Eastern Ellsworth Land; (Middle) RRS James Clark Ross (background) and the ship's cargo tender during 2019/20 summer season station opening, Signy Research Station, British Antarctic Survey; (Bottom) The Gourlay huts on Signy Island.



Malaysia's efforts in Antarctic research have been recognised internationally. In 2004, MARP was admitted as an Associate Member of the Scientific Committee of Antarctic Research (SCAR) at the 28<sup>th</sup> SCAR Meeting at Bremerhaven, Germany. MARP has also been invited to become a consultative member of the Joint Committee on Antarctic Data Management (JCADM), an arm of SCAR responsible for the collation and dissemination of scientific information gathered by all research under SCAR. MARP is also a member of the Asian Forum on Polar Science (AFOPS) and has contributed significantly in the International Polar Year (IPY 2007/2008) not only in the form of research, but also through outreach and education.

Twenty eight years since "Question of Antarctica", Malaysia finally acceded to the Antarctic Treaty System as a Non-Consultative member, the same year when His Majesty, The Yang di-Pertuan Agong, Tuanku Mizan Zainal Abidin visited Antarctica. Sultan Mizan also facilitated the establishment of the Sultan Mizan Antarctic Research Foundation or Yayasan Penyelidikan Antartika Sultan Mizan (YPASM) in June 2012 to promote and develop national and international collaborations in the research and conservation of polar regions, especially in the Antarctic continent. On 15 August 2016, Malaysia finally acceded to the Madrid Protocol, five years after signing the Treaty. This marked a great progress in Malaysia's endeavour in Antarctic research, policy and conservation.

Photo: Glacier and sea ice around Signy Island.



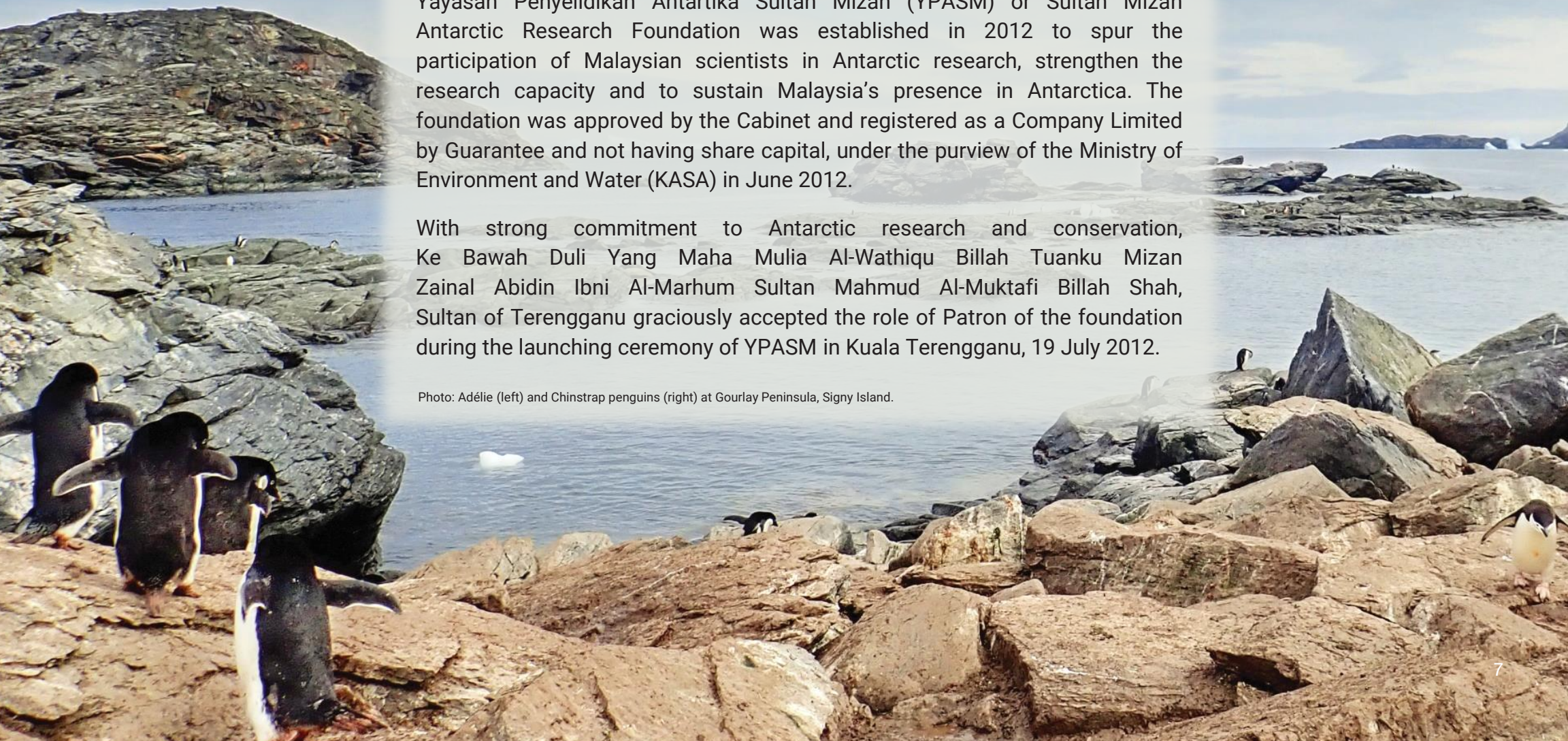


## Yayasan Penyelidikan Antartika Sultan Mizan's Smart Partnership Initiative and the IMU's Involvement

Yayasan Penyelidikan Antartika Sultan Mizan (YPASM) or Sultan Mizan Antarctic Research Foundation was established in 2012 to spur the participation of Malaysian scientists in Antarctic research, strengthen the research capacity and to sustain Malaysia's presence in Antarctica. The foundation was approved by the Cabinet and registered as a Company Limited by Guarantee and not having share capital, under the purview of the Ministry of Environment and Water (KASA) in June 2012.

With strong commitment to Antarctic research and conservation, Ke Bawah Duli Yang Maha Mulia Al-Wathiqu Billah Tuanku Mizan Zainal Abidin Ibni Al-Marhum Sultan Mahmud Al-Muktafi Billah Shah, Sultan of Terengganu graciously accepted the role of Patron of the foundation during the launching ceremony of YPASM in Kuala Terengganu, 19 July 2012.

Photo: Adélie (left) and Chinstrap penguins (right) at Gourlay Peninsula, Signy Island.



Since operation, YPASM has played an important role in connecting Malaysian researchers with scientific communities in the regions and globally. The foundation provides a fellowship scheme for Malaysian young Polar researchers to conduct short research attachment at established Polar institutions, as well as research grants to support the continuation of Polar research in Malaysia. It is noteworthy that IMU researchers are among the inauguration recipients of the YPASM Antarctic fellowship. Other than scientific research, the foundation also organises education and outreach programmes such as polar science camps, sharing sessions, contests and exhibitions for students and the public to nurture the interest in polar science.

Shortly after the setting up of YPASM, the Smart Partnership Initiative was introduced. The founding 8 members include Universiti Sains Malaysia, Universiti Malaysia Sabah, University of Malaya, Universiti Teknologi Mara, Universiti Kebangsaan Malaysia, Universiti Malaysia Terengganu, Universiti Putra Malaysia and the IMU. Each participating university is obliged to contribute MYR55,555 to YPASM annually for five years with a matching grant of MYR388,885 from YPASM offsetting more than half the overall costs.



Dr Wong Chiew Yen sharing her experiences in Antarctic research as part of the 'Malaysia's Journey to the Ice - Women in Antarctica' programme organised by YPASM.



IMU staff and students with fellow Malaysian polar researchers at the 7<sup>th</sup> SCAR Conference, Kuala Lumpur in 2016.

The IMU is proud to be the only Private University in the Smart Partnership. From 2016 to 2020, the IMU has contributed MYR277,775 to YPASM in the first cycle as a commitment for the Smart Partnership for Polar Research. In return, a total amount of MYR499,500 has been awarded to four recipients from the IMU; three from the Institute for Research, Development and Innovation (IRDI) and one from the School of Health Sciences (SOHS).

The first cycle of the Smart Partnership has ended in 2020. To continue IMU's endeavour in Antarctic Science, the IMU's Management Committee has approved the renewal of the Smart Partnership for another five years (2021-2025).

Under the new proposal by YPASM for the Smart Partnership Initiative, each participating university will contribute MYR62,500 per year for five years (amounting to MYR312,500 after five years). YPASM will match the contribution by the university on a 1:1 ratio, making the total available fund to be allocated under this initiative for a university to amount to MYR125,000 per year or MYR625,000 for five years.

#### List of IMU recipients for YPASM fellowship and research grant since 2016

Recipient	Faculty	Type and Year of Research Support	Project Title	Total Funding (MYR)
Dr Dang Nguk Ling	IRDI	Fellowship Scheme 2016	Phylogenetic study on microalgae isolated from pristine and heavy metal-impacted site on Signy Island, Antarctica.	49,500
Dr Nur Alia Johari	IRDI	Research Grant 2018	Molecular evaluation of biogeography of functional redundancy theory in Antarctic rhizosphere prokaryotic communities.	150,000
Dr Nur Alia Johari	IRDI	Berth Support to Antarctica 2019	Molecular Evaluation of Biogeography and Functional Redundancy Theory in Antarctic rhizosphere Prokaryotic Communities.	150,000
Dr Wong Chiew Yen	SOHS	Research Grant 2020	Phytoremediation Potential of Antarctic Microalgae on Diesel Hydrocarbons.	150,000
<b>Grand Total</b>				<b>499,500</b>




Dr Dang Nguk Ling.



From L-R: Dr Chong Chun Wie, Dr Mai Chun Wai, Prof Datuk Dr Lokman Hakim Sulaiman and Dr Nur Alia Johari at the 8<sup>th</sup> Malaysian International Seminar on Antarctica (MISA8) 2019, Universiti Putra Malaysia.

# The IMU's Endeavour in Polar Research





Being a medical university, the IMU's involvement in Antarctic research may have raised a few eyebrows. It is easy to forget that the health of the environment that we are living in, as well as the health of the animals that share the same space with us are important elements of overall public health. The notion that the environment can influence human health is not new. For instance, the ancient Greeks had defined health as a state of dynamic equilibrium between the internal and the external environments. Furthermore, throughout the ancient civilisation, the management of pollutants such as human waste had been the priority in the planning and establishment of human settlements (e.g. construction of bathroom, latrines, water drains). Additionally, the strong associations between disease, seasonality and climate are regularly observed. For example, water-borne diseases such as cholera, leptospirosis and typhoid fever are generally more prevalent during the rainy season.

With the recognition of the inter-relationships between climate, animals and humans in health, there is an increasing emphasis on “One Health”. Based on the WHO, “One Health is an approach to designing and implementing programmes, policies, legislation and research in which multiple sectors communicate and work together to achieve better public health outcomes”. In line with this approach, the IMU's researchers had embarked on the journey to deepen our understanding on the impact of environmental changes on the biota of Antarctica, and to search for clues of their potential link to human health. The Antarctic is a perfect natural laboratory to study the impact of environmental perturbations due to its simple ecosystems, and the fact that it is among the regions that experience a faster rate of warming.

IMU's research on Antarctica can be briefly categorised based on the studied organisms. Work has been conducted on the five groups of microorganisms, namely algae, bacteria, viruses, parasites and fungi.

Photo: An Antarctic fur seal looking out over Elephant flats, Signy Island.

Photo (previous page): View of a large glacier in South Georgia, as seen from the RRS James Clarke Ross, March 2020.

# Algae

The IMU's foray into research on Antarctic algae began with two flagship projects awarded by the Ministry of Science, Technology and Innovation (MOSTI) in 2014, under the Antarctic Flagship Programme led by Prof Dato' Dr Azizan Abu Samah, University of Malaya. Under the flagship projects, a number of IMU undergraduate and postgraduate research projects were developed and funded, including studies on Antarctic bacteria, parasites, viruses and fungi.

The first project titled 'Biomarkers of Polar and Lower Latitudes Microbes in Relation to Heavy Metal Stress' (MYR819,902) was led by Prof Chu Wan Loy, under the Flagship Programme theme 'Diversity and Adaptation of Microbes: A Cross Latitudinal Study'. The project aimed to assess the effects of heavy metals on oxidative stress response and metabolomic profiles of microalgae isolated from Signy Island, Antarctica. Although Antarctica is known to be the last pristine continent on earth, it is not free from heavy metal contamination. The major source of heavy metal pollution in Antarctica is mainly due to anthropogenic activities, especially near research stations.



Prof Chu Wan Loy on field in Antarctica for the collection of algae from sea ice and glaciers in 2001.



**Dr Chan Kok Keong**

To collect samples for the project, Dr Chan Kok Keong was a member of the project team that joined the expedition to Signy Research Station, Antarctica, organised by the British Antarctic Survey (BAS) in 2015.

Dr Chan successfully collected soil samples from various locations with biological contamination, namely those with prolonged human activities, heavy metal contamination and wildlife (penguin, seal and giant petrel) activities.

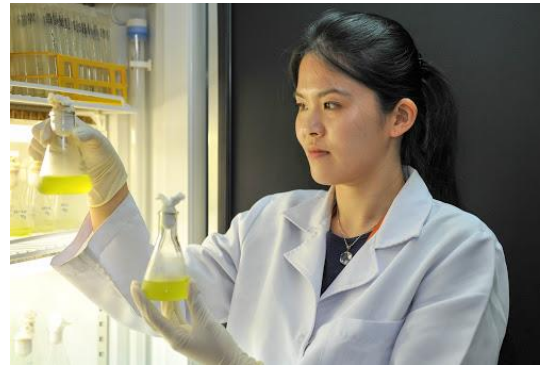
The microorganism profiles of the contaminated locations were then compared with those of pristine sites on the sub-Antarctic Signy Island. A collection of soil microalgae was subsequently established from the samples collected during the expedition. Morphological characterisation and phylogenetic studies on the microalgae have been conducted. Selected microalgae from the collection have also been used for toxicity testing of heavy metals such as copper and lead.



Dr Chan collecting samples at Three Lakes Valley, Signy Island.



Antarctic fur seals on Signy Island. A rare blonde variant surrounded by more common brown fur seals.



Dr Dang Nguk Ling completed her PhD titled 'Effects of heavy metals on oxidative stress response and metabolomics profiles of Antarctic algae' in 2020 under Prof Chu's supervision as part of Prof Chu's YPASM flagship project.



**Dr Wong Chiew Yen**

The second project was led by Dr Wong Chiew Yen, titled 'Physiological and genomic responses of microalgae to climate changes across a global gradient' (MYR827,600) under the theme 'An Integrated Approach: Microbes and Response to Climate Change'. The project was in collaboration with Prof Chu, Prof Phang Siew Moi and Prof Lim Phaik Eem (University of Malaya), Prof Pete Convey (BAS) and YPASM.

One of the seriously debated environmental issues today is the impact of climate change, particularly related to increased temperature and ultraviolet radiation (UVR). The elevated anthropogenic greenhouse gases are expected to lead to an increase in the average global surface temperatures over the next century ranging anywhere from 1.8 to 4.0°C (IPCC, 2007). Increasing levels of UVR have been reported in many parts of the world including Antarctica and the adjacent geographic regions (southern parts of South America and Australia), as well as the Arctic region.

Changes in the global environment, especially global warming and ozone depletion, which results in the increase of UVR and temperature can have far-reaching impacts on organisms especially microalgae in the Antarctic, affecting their physiological processes and productivity. Such impacts on microalgae are of particular interest as they form the basis of the food webs in the Antarctic ecosystem. Thus, the research project focused on comparing the physiological response of microalgae from different geographical regions to the effects of increased temperature and UVR.



Dr Wong in Casey Station, Antarctica (top) collecting snow algae and (bottom) conducting a UVR field experiment.



In addition, the application of transcriptomics approaches on microalgae exposed to UVR and temperature provides insight on the relevant genes and genetic responses that are involved in responding to environmental stress. This information is fundamentally important for the understanding of algal adaptation to climatic and environmental changes. The objectives of this project are closely aligned with the aims of the recently-approved Scientific Committee on Antarctic Research (SCAR) International Scientific Research Program, Antarctic Thresholds – Ecosystem Resilience and Adaptation. This project therefore provides a timely and important opportunity to place Malaysian Antarctic research firmly in the mainstream of the international research priorities identified by SCAR.

As part of the flagship project, IMU Master in Medical and Health Sciences student Aniqah Zulfa joined the YPASM Malaysian Antarctic Scientific Expedition in 2016 to Antarctica from December 2015 till February 2016 to collect samples for the isolation of algae for the study titled 'Effects of Ultraviolet Radiation on cell proliferation, oxidative stress and antioxidant enzyme activities of *Chlorella* isolates'.



Dr Wong assessing algae samples in the IMU Research Lab.



Collecting samples for isolation of Arctic microalgae in Ny-Ålesund, Norway.



Aniqah Zulfa (second from left) with the Malaysian Antarctic Scientific Expedition Team 2016 from USM, UiTM, UM and UMT.

## YPASM Research Grant 2020: Phytoremediation Potential of Antarctic Microalgae on Diesel Hydrocarbons

The latest (at time of writing) research grant for Antarctic research was awarded to Dr Wong Chiew Yen under the Smart Partnership Initiative. In collaboration with Prof Chu and Dr Kok Yih Yih (IMU), A/Prof Dr Siti Aqlima Ahmad (UPM), Prof Pete Convey (BAS), A/Prof Dr Azham Zulkharnain (Shibaura Institute Technology, Japan) and Dr Claudio Gomez-Fuentes (Universidad de Magallanes, Chile/ Centro de Investigación y Monitoreo Ambiental Antártico) (CIMAA), the project will run from 2020 and aims to identify the degradation potential of microalgae isolated from Antarctica.

Hydrocarbon pollution in Antarctica is derived from various origins such as catastrophic oil spill events, chronic fuel leakage and from local effluent, and has contaminated some of Antarctica's near-to-pristine land over the past few decades. The hydrocarbon composition of diesel is known to be toxic and harmful to terrestrial and marine ecosystems and biota, further exacerbated due to its persistence. Despite increasing knowledge about contamination in Antarctica due to increased human pressure, clean-up (remediation) of polluted sites is still in its infancy, in part owing to the extreme environment. Remediation through application of physical machinery would be laborious and costly due to the harsh conditions, as well directly damaging to the fragile terrestrial ecosystems, whilst remediation using chemical dispersants is itself a potential environmental hazard and should be critically considered.



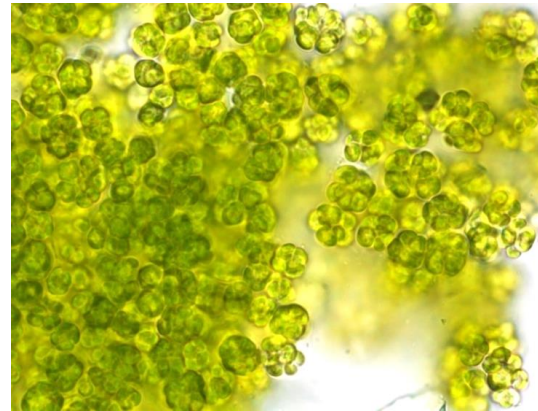
Preliminary testing for phytoremediation using Antarctic microalgae.



Dr Wong demonstrating laboratory techniques for the project to students at Universiti Putra Malaysia (UPM).

Phytoremediation is now emerging as a sustainable technique for the treatment of organic and inorganic contaminants. The ability of Antarctic freshwater algae to remediate diesel hydrocarbons in an axenic state has not been thoroughly studied, alone or in combination with characterisation of the abiotic factors that limit algal growth. Such studies have the potential to take advantage of the cold-adapted physiology of native Antarctic microbes in developing realistic and practicable remediation approaches, as well as helping to address the limitations imposed by the Antarctic Treaty System which bans the import and introduction of non-native microbes to the Antarctic environment.

The project aligns with the Integrated Science to Inform Antarctic and Southern Ocean Conservation (Ant-ICON) proposal for the next generation of SCAR programmes, which includes a large element of identifying and mitigating/providing policy advice pertinent to human impacts on Antarctic ecosystems. This project will also contribute to enhancing Malaysia's footprint in polar research, in line with the aspiration of the country to attain consultative status within the Antarctic Treaty.



*Gloeobotrys* sp., a terrestrial algae found in the soil.



Algae in Antarctic ice.



Microalgae isolated from Antarctica.





# UNIVERSITY OF MALAYA

## Effect of Temperature on the Growth and Photosynthetic Performance of Antarctic and Tropical *Chlorella* species



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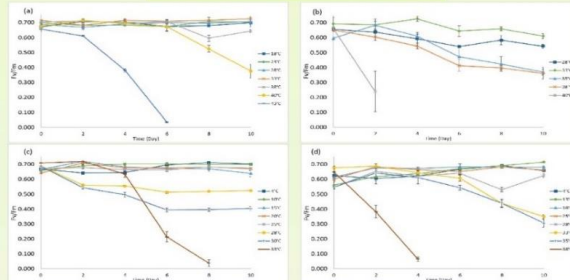
### Background



Mass coral bleaching caused by global warming coupled with El Niño. Photo: Duke University

Climate change, specifically global warming can be region dependent (IPCC, 2007). Higher average temperature and higher temperature variability are increasing the risks to species' tolerance limits. *Chlorella* species (Chlorophyta) are photoautotrophic organisms which can be found in marine and freshwater ecosystems. Photosynthesis, a thermosensitive process is playing important role in survival and distribution of *Chlorella*. However, photosynthesis can be impaired by moderate increases in temperature (Carnegie et al., 2005). Exceeding temperature above optimal by only 2-4°C may result in total culture loss (Mata et al., 2010). High temperature causes imbalance between energy supply and consumption and leads to alteration of the photosynthetic apparatus in microalgae (Ras, Steyer, & Bernard, 2013). Studies on the response of microalgae to elevated temperatures help to predict the possible ecological implications of these primary producers and their adaptability to potentially warmer ecosystems (Somero, 2010).

### Results & Discussion



### Objectives

To examine the effects of elevated temperature on the growth and photosynthesis of *Chlorella* species originating from different latitudes in terms of growth and photosynthesis.

### Methodology

Table 1: Strains used in this study

Latitudes	Strains	Habitat	Growth Medium	Inoculum Inoc. (x 10 <sup>6</sup> cells mL <sup>-1</sup> )	Incub. Time (h)
Tropical	01 <sup>†</sup> N	<i>Chlorella</i> UMACC 001	Freshwater BBM	28	18-43
	05 <sup>†</sup> N	<i>Chlorella</i> UMACC 245	Marine	Prov	28-40
Antarctic	44 <sup>†</sup> S	<i>Chlorella</i> UMACC 237	Freshwater BBM	4	4-33
	46 <sup>†</sup> S	<i>Chlorella</i> UMACC 250	Marine	Prov	4-33

- A marine and freshwater *Chlorella* strain from the tropics and the Antarctic were used for this study (Table 1).
- Illumination: ~42 μmol photons m<sup>-2</sup> s<sup>-1</sup> on 12:12 h light-dark cycles.
- Growth was monitored by measuring OD<sub>720</sub> using spectrophotometer.
- Photosynthetic parameter, Fv/Fm was obtained using a Water-Pulse Amplitude Modulated Fluorometer (Water-PAM).
- Cultures were grown in different stress-inducing temperatures (based on Figure 2 findings) until the maximum quantum yield (Fv/Fm) decreases to the threshold (~0.4 or ~0.2); the cultures were then subjected to recovery at ambient temperature.

- Temperature strongly influenced the growth and photosynthesis performance of *Chlorella* (Fig 1 and Fig 2).
- Both marine and freshwater *Chlorella* strains from the tropics recorded the highest μ at their ambient temperature.
- In both tropical strains, incubation of up to 33 °C did not affect Fv/Fm; however further increasing temperature led to photoinhibition.
- Interestingly both Antarctic strains were able to tolerate a broad range of temperatures (Figure 1 and Fig 2 (c), (d)). With the marine Antarctic strain showing a higher thermal tolerance and survived temperatures up to 38 °C.
- Antarctic strains (UMACC237 and UMACC250) seem to be of psychrotolerant rather than psychrophiles.
- High temperature may restrict the growth and the efficiency of photosystem II (PSII), suggesting the production of ROS in stressful condition (Jauzein & Erdner, 2013).
- The decreases in Fv/Fm with increasing temperature indicated the decline in the ability of *Chlorella* to utilise light (Saleh & McClain, 2011) and suggested damage to the PSII.
- All four strains were believed to experience severe photoinhibition upon exposure to higher than ambient temperatures used in this study.
- The continued decrease in Fv/Fm of both Antarctic strains on the onset of recovery indicated substantial damage to their photosynthetic machinery.
- The recovery process took longer time as it involved the *de novo* synthesis of D1 protein as compared to the much shorter exposure time to high temperatures in the work by Saleh & McClain (2011) and Wong et al. (2015).
- The study showed that the responses were clearly species-specific with different strains showing different upper temperature limit.
- The tropical strains appeared to be living at/near their upper temperature limit, as temperatures higher than their ambient temperature resulted in reduced growth and photosynthetic efficiency.

### Conclusion

The deleterious effects of increasing temperature were evident in *Chlorella*, but differences were found with respect to the extent of photoinhibition and the ability to recover from the post-exposure damage. The response of *Chlorella* to elevated temperatures vary with species and biogeographic region.

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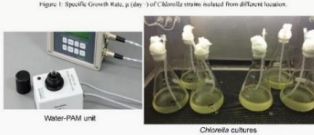
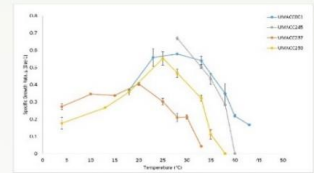
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# Response of *Chlorella* Isolates from Polar Regions and Lower Latitudes to Ultraviolet Radiation (UVR) Stress: Growth, Pigmentation and Oxidative Stress Response



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### INTRODUCTION

Increasing levels of UVR, as a result of anthropogenic ozone depletion, have been reported in many parts of the world including Antarctic (McKenzie et al., 2007), tropical and temperate regions (Blumthaler & Ambach, 1990). Such changes in the global environment can have far-reaching impacts on organisms especially microalgae, affecting their physiological processes and productivity (Wong et al., 2015, 2007; Chu et al., 2005). Impacts of these environmental changes on microalgae are of particular interest as they form the basis of the food webs in the ecosystem. The small coccoid chlorophyte, *Chlorella* with cosmopolitan occurrence is one of the best-studied phototrophic eukaryotes. Various *Chlorella* strains have potential applications in biotechnology, e.g. antioxidant compounds (Hajmahmoodi et al., 2010), waste water treatment (Chu et al., 2009) and as feedstock for biofuel production (Xu et al., 2006) as well as dietary supplements for human nutrition (Gans et al., 2010). Due to the wide occurrence and applications of *Chlorella*, this alga is chosen for the present study.

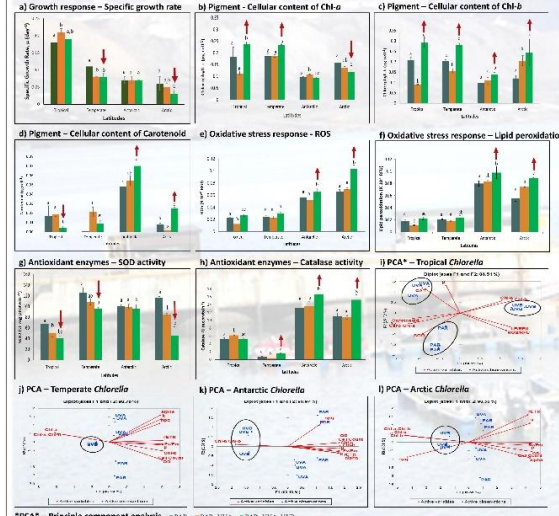
### OBJECTIVE

The aim of the present study was to assess the effects of UVR, particularly increased UVB radiation on the growth, pigmentation and oxidative stress response of *Chlorella* species isolated from different latitudes.

### METHODOLOGY



### RESULTS



### DISCUSSION

- Increased UVB caused reduction in specific growth rate (μ) in the Arctic and temperate *Chlorella*.
- Significant increase in cellular contents of chl-a and b were observed when the tropical and temperate *Chlorella* were subjected to increased UVB radiation.
- The increase in production of chlorophyll pigments is a mechanism of acclimation where algae cells utilize the extra energy supply in carboxylase activity to balance the over-produced energy caused by UVR stress (Mourain-Bertrand et al., 1998).
- However, the reversed trend was observed in cellular contents of carotenoids in tropical *Chlorella*.
- Both the Antarctic and Arctic *Chlorella* showed significant increase in ROS and lipid peroxidation, indicating that the polar *Chlorella* were under oxidative stress caused by UVB. However, both polar *Chlorella* spp. were able to survive under increased UVB condition by producing higher amounts of carotenoids and increasing their catalase activity (Dall'Osto et al., 2014; Aguilera et al., 2002; White & Jahneke, 2002).
- Principle component analysis (PCA) showed that there was correlation among lipid peroxidation, ROS, carotenoid and catalase in all *Chlorella* isolates.

### CONCLUSION

- Chlorella* isolates from different latitudes responded differently to UVB stress especially in terms of oxidative stress response.
- Both polar *Chlorella* responded to oxidative stress by increasing catalase activity and carotenoid content.

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\*PCA - Principle component analysis; PAR - PAR-UV-A; PAR-UV-B-UV-C; ROS - Reactive Oxygen Species; SOD - Superoxide Dismutase; CAT - Catalase; LPO - Lipid Peroxidation; Fv/Fm - Maximum quantum yield.

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## Antarctic algae isolates

1. A total of 22 unialgal from Signy Island (soil microalgae) successfully isolated and deposited in Research Lab, IMU.
2. A total of 6 unialgal from Antarctic Peninsular successfully isolated and deposited in Research Lab, IMU.

# Bacteria



Vegetation of Signy Island. Clockwise from top: *Deschampsia* sp., or tussock grass, amongst moss vegetation at Deschampsia Point; *Chorisodontium* sp. with dark brown patches of *Andreaea* sp. and white epiphytic lichens; *Sanionia* sp., one of the most abundant mosses in maritime Antarctica.

The cold and arid conditions of Antarctica have restricted the development of higher plants on the terrestrial ecosystems. Although sporadic prevalence of cryptogamic plants such as mosses and lichens can be detected in limited biology hotspots during summer, the vast majority of Antarctica is barren or covered by permafrost. In such a unique setting, microorganisms such as bacteria play a vital role in ensuring the functioning of the ecosystems. What drives the assembly of the bacterial community and how bacteria responds to environmental perturbations are therefore among the key fundamental questions that need to be addressed to enable ecological simulation and the design of proper conservation strategies. Indeed, these form part of the key research questions highlighted by the Scientific Committee on Antarctic Research (SCAR) Antarctic and Southern Ocean Science Horizon Scan.

In support of the SCAR Horizon Scan initiative, researchers from the IMU had initiated a research project to evaluate the distribution and interaction of microbial communities in the rhizosphere, titled 'Molecular evaluation of biogeography and functional redundancy theory in Antarctic rhizosphere prokaryotic communities' (2019-2022, MYR150,000). Rhizosphere is the area around the plant root (plant-root-soil interface) where numerous biogeochemical processes take place, pathogenic and beneficial microorganisms interact, and which constitutes a major influence on plant growth and health. Using this unit habitat as a model, the project also seeks to evaluate the validity of bacterial biogeography and functional redundancy theories by comparing bacterial community composition and rhizosphere functions across a large selection of regions in Antarctica.



A view of the landscape of Signy Island in maritime Antarctica, with a complex geology and range of terrestrial habitats providing a wide diversity of ecosystems.



Collection tubes marking bank forming mosses *Polytrichum* sp. & *Chorisodontium* sp., a drier habitat, next to *Deschampsia* sp. grass (bottom right).

The moss *Sanionia* sp. (right) lifted up to reveal the rhizosphere just beneath, next to the green algae *Prasiola* sp. (bottom left).

Antarctica also constitutes an ideal natural laboratory to test these theories, without effects of external human influences, allowing identification of major drivers of bacterial diversity, function and change. The research will be the first to assess microbial functions at a continental spatial scale, and the first to link functional diversity to the underlying chemistry through nuclear magnetic resonance (NMR) and metabolomics. Findings will serve as baseline data for future assessments of the impact of changing environmental conditions, and inform ecological forecasting; how ecological populations and ecosystems will change in the future in response to environmental factors such as climate change. These include effects on crop yield and potential risks of pathogenic bacteria in the rhizosphere to animals and humans in the future.





**Dr Nur Alia Johari**

Dr Nur Alia Johari is the principal investigator and was the field expeditioner of the project. She joined the Summer 2019/2020 expedition with the British Antarctic Survey (BAS) to Signy Research Station. Despite being one of the smaller BAS stations, Signy Island (60°43'0"S, 45°36'0"W), is one of the most isolated, requiring travel to and from aboard the RRS James Clark Ross.

The expedition lasted for 14 weeks where Dr Alia collected rhizosphere soil samples across the island. The plant species beneath which the soil samples were collected from were noted, comprising of various mosses and lichens as well as the only two flowering plants native to Antarctica, namely *Deschampsia antarctica* and *Colobanthus quitensis*. The soil samples were successfully tested and shipped to the UK and subsequently Malaysia for analysis.

The research is funded by YPASM under the Smart Partnership Programme, and is in collaboration with Prof Pete Convey (BAS), Prof David Pearce (University of Northumbria), Dr Nicole Trefault (Universidad Mayor, Chile), Dr Chong Chun Wie (Monash University, Malaysia), Dr Mai Chun Wai (IMU), Dr Ivan Yap (Sarawak Research and Development Council) and Dr Cindy Teh (University of Malaya).



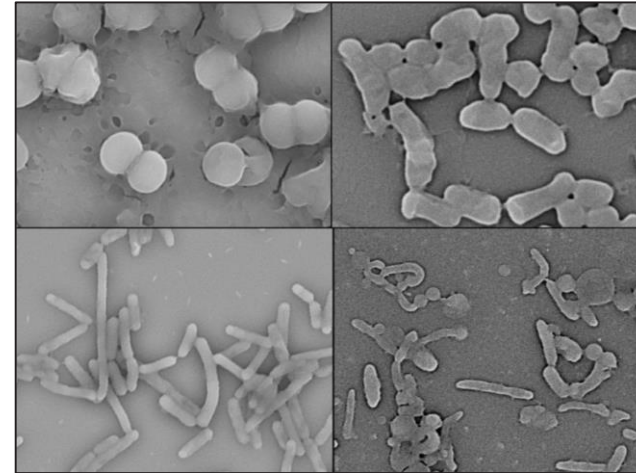
Dr Alia on Signy Island. Clockwise from top: Hiking over Knife Point with Orwell Glacier in the background; travelling on a snowmobile on the island's ice-cap; collecting soil samples at Skua Terrace on the west coast.



**Dr Fabian Davamani**

Separately, the footprint of IMU's Antarctic research is also evident in the area of bacterial biofilms, heavy metal resistance, and antibiotic resistance. Previous and existing research work under Prof Chu's flagship project were led by Dr Fabian Davamani, and have focused on the genomics of Antarctic bacteria and the production of biofilms, which provide a survival advantage to persist under harsh Antarctic conditions.

Dr Fabian developed in-house media to grow Antarctic bacteria from soil samples collected from Signy Island. His group is also currently studying both the phenomics (physical and biochemical traits) of Antarctic bacteria and creating heat maps to understand their metabolism. Advanced genomics approaches such as whole genome sequencing of Antarctic bacteria were also employed to provide further insights into the molecular evolution, antibiotic resistance, psychrotolerance and the diversity of the Antarctic bacteria. Through the annotation of the genomic sequences, Dr Fabian's research team sought to identify strains that might lead to the discovery of novel bioactive compounds with medical applications.



Samples of Antarctic biofilms grown at low temperatures (psychrophilic). Clockwise from top left: *Psychrobacter* sp, *Rhodococcus* sp, *Flavobacterium* sp, *Sporosarcina* sp.

### Antarctic bacteria isolates

Dr Fabian's work has led to the submission of Antarctic bacteria isolates to the National Center for Biotechnology Information (NCBI):

1. *Arthrobacter* sp.
2. *Rhodococcus* sp.
3. *Flavobacterium* sp.
4. *Cryobacterium* sp.
5. *Psychrobacter* sp.
6. *Sporosarcina* sp.

### Conference presentations

Davamani F, Chong CSL, Chong DHL, Mohamad NENB, Selvarajah S, Ambu S, Chu WL, Convey P. Exploiting temperature and nutritional conditions for Poly microbial biofilm formation from Antarctic soil samples. 8th Malaysian International Seminar on Antarctica - Polar regions in the Global Climate System. Kuala Lumpur, *Malaysia*, 18-19 June 2019.

# Bacteria Research Posters



## Evaluation of the growth of polymicrobial biofilms developed from soil samples of Signy Island, Antarctica

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 Fabian\_davamani@imu.edu.my

### Abstract

**Introduction:** The Antarctic continent's environment range from a well-developed and nutrients rich soil in the coastal area to oligotrophic soils in high elevation area as well as deserts. In this study 25 soil samples were used, which were collected from two different location in Signy Island, Berntsen Point and Gaurley Peninsula. Signy Island is a part of the South Orkney Islands, which is about 600km from the Antarctic Peninsula. The continent range from permanently covered ice caps to ice free coasts area during summer with a recorded maximum temperature of 19.8°C. Plants that can be found in Signy Island are mostly cryptogams (mosses, liverworts and lichens) and only two types of flowering plants can be found which are Antarctic hairgrass and Antarctic pearlwort. Algae and cyanobacteria are also present in wet areas. Animals that inhabit the island include albatross, penguins, seals and birds.

Bacteria that are found in this continent are classified as psychrophiles and they are able to survive and reproduce in cold harsh environment. Formation of biofilms can help in adapting the bacterial survival. Biofilms can grow in nutrient conditions that do not permit the growth of planktonic cells. Biofilms is a well-organized structure consisting of a community of cooperating microorganisms that attaches to a surface. It can form on any surfaces and exist as a single structure or a more complex 3-dimensional structure comprising of a single or polymicrobial origin.

**Methods:** The soil samples collected from Signy Island were collected and cultured in 6 different media consisting of 2 defined media (IMU defined media (in-house)) where all of the chemical components are known, and 4 complex media (IMU complex media (in-house)) at 10°C to estimate the growth of polymicrobial biofilms. The biofilms are allowed to form in varied media for 3 weeks at 10°C using a 24-well culture plates. The planktonic bacteria were removed and the biofilms formed adhered to the inner surface were estimated using a calorimetric methods stained with crystal violet.

### Results:

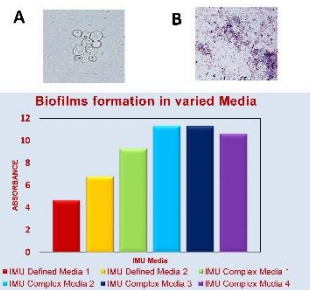


Fig. 2. A and B representative isolates from soil samples. A: Budding yeast wet mount preparation. B: a mixture of gram positive and gram negative bacilli. C: estimated amount of biofilms formed in different IMU Media at 10°C.

In defined media, total biofilms growth is approximately 45% higher in IMU Defined Media 2 compared to IMU Defined Media 1 (Graph C). Among the complex media, the biofilms formation was similar in IMU Complex Media 2, and 3 (Graph C) followed by IMU Complex Media 4 and 1, however the difference between them was quiet significant.

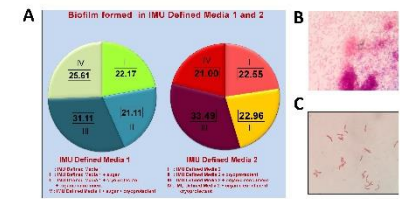


Fig. 2. A Biofilms produced in modified IMU Defined Media 1 and 2. B & C representative isolates of Bacteria from defined media with abundant growth.

Davamani F, Chong CSL, Mohamad NENB, Chong DHL, Selvarajah S, Ambu S, Chan KK and Convey P. Evaluation of the growth of polymicrobial biofilms developed from soil samples of Signy Island, Antarctica. 36<sup>th</sup> SCAR open science conference. KLCC, Kuala Lumpur, Malaysia, 22-26 August 2016.



## Isolation and biochemical characterisation of bacteria isolated from soil samples from Signy islands using defined and complex media

Nur Azmira Binti Abd Samad<sup>1</sup>, Nurliana Najihah Binti Mohd Sabri<sup>1</sup>, Che Wan Nur Asiqin Che Wan Mohd Shokri<sup>1</sup>, Norsafiana Makmur<sup>1</sup>, Chan Kok Keong<sup>1,2</sup>, Pete Convey<sup>3</sup>, \*Stephen Ambu<sup>1,2</sup> and Fabian Davamani<sup>1,2</sup>

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**Abstract:** Psychrophilic bacteria are cold-loving bacteria inhabiting the cold polar region categorised as extremophiles. Continued and coordinated efforts to understand bacterial community structure and function in Antarctic soils will be necessary to monitor and predict ecological responses in these changing environments. The primary aim of this study was to characterise the bacteria isolated from soil samples collected from Signy Island, Antarctica, as part of a bigger project to assess the toxicity of heavy metals in polar microbes.

**Methods:** A total of 57 soil samples from Signy and Rothera Islands, Antarctica were screened by inoculating them into various defined and complex media. About 10 microliter of soil suspension was seeded into different broths in 24-well micro well plates and incubated at 10°C for three weeks. After three weeks, the isolates were inoculated onto the agar medium to grow the colonies of bacteria. The biochemical characterizations were carried out on single colony of bacteria which included Gram stains, oxidase, catalase, and amino acid/sugar fermentation tests.

### Results:

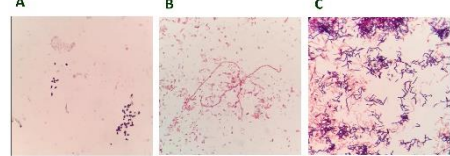


Fig. 1. A. Gram positive diplococci B. Gram negative Streptobacilli C. Gram positive and negative bacilli

The bacteria were inoculated for three weeks under temperature-controlled environment. Various types of bacteria with different morphology can be seen, isolated from broth incubated at 10°C. These bacteria were stained with Gram-stain technique and observed under 100X microscope.

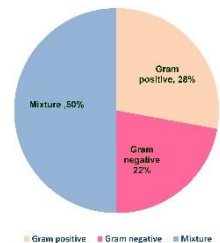


Fig. 2. Percentage of bacterial growth viewed with Gram-stain technique

Number of overall bacterial growth presented in percentage isolated from broth incubated at 10°C. From the pie chart above, it can be seen that 50% of the bacterial population consist of mixture of Gram-negative and Gram-positive bacteria.

Ambu S, Davamani F, Abd Samad NAB, Mohd Sabri NN, Che Wan Mohd Shokri CWNA, Makmur N, Chan KK and Convey P. Isolation and biochemical characterisation of bacteria isolated from soil samples from Signy Islands using defined and complex media. 36<sup>th</sup> SCAR open science conference. KLCC, Kuala Lumpur, Malaysia, 22-26 August 2016.

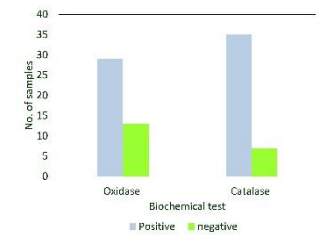


Fig. 3. Graph of number of isolates with positive or negative result for both oxidase and catalase activity.

The majority of bacteria showed positive result for both oxidase and catalase activity (45% and 55% samples respectively).

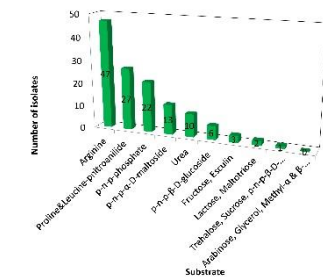


Fig. 4. Number of isolates for each substrate utilized

The majority of bacteria utilized arginine as their substrate to grow, indicated by 47 samples with positive result. None of the bacteria utilized methyl-α-β-galactoside, mannitol, arabinose, glycerol and ONPG & p-nitro-α-D-galactoside.

### Conclusions:

Antarctic bacteria from soil sample of Signy island were abundantly isolated after three weeks of inoculation in 10°C incubation temperature. Various bacterial morphology can be observed using Gram stain technique under 100X magnification. 50% of the bacteria seen comprise mixture of gram positive and gram negative bacteria. These psychrophilic bacteria utilizes arginine as the main source of sugar to support growth. Most of the bacteria showed positive results for oxidase and catalase tests.

**Acknowledgement:** Financial support provided by International Medical University Research Grant for undergraduate study for Biomedical Science (Hons) and equally from the Antarctica Flagship program, on heavy metal toxicity in polar microbes (MOSTI).

# Response of Antarctic Psychrotrophic Bacteria to Antibiotics before and after exposure to Heavy Metals

Divva Dharshini<sup>1</sup>, Azleen Binti Mohammad Abdul Aziz<sup>1</sup>, Chan Wai Yew<sup>1</sup>, Stephen Ambu<sup>1,2</sup>, Chu Wan Loy<sup>1,2</sup>, Pete Convey<sup>3</sup>, Catherine Chong Sze Ling<sup>1</sup>, Fabian Davamani<sup>1</sup>

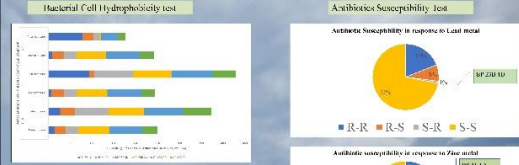
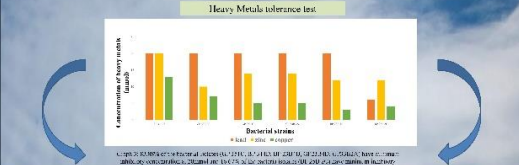
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<sup>3</sup>British Antarctic Survey, U.K



## INTRODUCTION

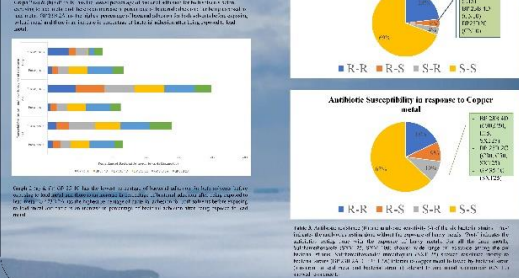
Antarctic soil with a representation of simplified structural composition harbors a wide range of cold-adapted microorganisms, psychrophiles and psychrotrophs. Disturbance of this ecosystem is mainly due to the deleterious human impacts and perturbations like global warming. Atmospheric circulation serves as a medium for transporting heavy metals, such as lead (Pb), zinc (Zn) and copper (Cu) from anthropogenic pollution site to the south polar regions. Over time, these ubiquitous microorganisms are able to integrate various levels of heavy metals in soil via bioaccumulation. Ultimately, formation of biofilms is selected by the bacteria as a survival strategy in tolerance to heavy metals. Antibiotic resistance has also been linked with biofilm formation when the antibiotics are incorporated in the matrix layer, enhancing its resistance. The mobility of antibiotic resistance genes (ARGs) between the bacterial communities are enhanced by horizontal gene transfer (HGT). This draws to the research objective which is to investigate antibiotic resistance of heavy metal tolerant psychrotrophic bacteria from Antarctic soils.

## RESULTS



## METHODOLOGY

- Site Description & Bacterial Sampling**
  - Through a flagship project 50 soil samples with replicates were sampled at Signy Island (66°43' S 45° 36' W)
  - Filtered soil samples were centrifuged and the supernatant was transferred into sterile glass plates with selective media at 3 varying temperatures (4°C, 10°C and 15°C).
- Bacterial isolation & Growth Curve**
  - 6 bacteria isolates with the most adaptive and fast-growing characteristics were cultured in broth at 15°C.
  - Bacterial growth were monitored spectrophotometrically for 2 weeks with the interval reading taken every two days.
- Biochemical Characterization of Bacteria**
  - Gram staining was performed to distinguish between gram positive and gram negative bacterial isolates. Oxidase and catalase test were studied on the utilization; pathway of bacteria before and after being treated with heavy metals.
- Biofilm Microdilution Method**
  - Heavy metals dissolved in sterile water were diluted into BHI agar plates at different concentrations ranging from 1 mg/L to 500 mg/L and the plates were left to incubate for a week at 15°C.
- Kirby-Bauer Disc Diffusion Assay**
  - Overnight bacterial broth cultures with and without heavy metals were standardized to 0.5 McFarland and were swabbed onto the BHI agar plates. 13 types of antibiotics with different concentrations were impregnated onto the plate to test the sensitivity of these heavy metal tolerant bacteria.



• The toxicity of heavy metals can be graded with the level of tolerance pattern shown by the 6 different bacterial strains. Cu metal is toxic to bacteria even in lower concentration (1 mmol), enhancing its resistance. Zinc metal shows moderate level of tolerance by the bacteria ranging from 12 mmol to 20 mmol. Bacteria tolerant to Pb metal showed greater resistance in all six strains ranging from 6 mmol to 20 mmol.

• High gradient exposure of heavy metal enables the bacteria to modify their structural characteristics by allowing hydrophobic-hydrophilic interactions with the substrates. Bacteria tolerant to Pb and Cu adopted hydrophobic properties post the metal exposure. Hydrophobic bacteria has higher tendencies to form biofilms which creates stronger resistance against the environmental stresses.

• RP231040, a gram-negative bacteria was sensitive to antibiotics prior to metal treatment but took a shift in the trend by showing multi-drug resistance in tolerance to Cu metal and single drug resistance in tolerance to Pb and Zn.

## CONCLUSION

Antarctic soil bacterial diversity was elucidated to determine the dominant bacterial strain that grows in the presence of toxic heavy metals and various antibiotics. Congregation of metal resistant bacteria conferring antibiotic resistance is well emphasized in this study. Bacteria develops several adaptation strategies to cope with the myriad of stresses in this hostile environment. Toxic metals such as lead, zinc and copper are deposited in larger amounts which exerts a selective pressure in the bacteria to thrive in this noxious ecosystem. Further studies can be expanded on the perspective of genome analysis where the bacterial genes can be assessed to locate the sequential genes that shares the same loci for metal and antibiotic resistance for the co-selection process in Antarctic soils.

## ACKNOWLEDGEMENT

Sincere gratitude to my institution, International Medical University (IMU) in funding this research project and to my research project supervisor, Dr. Fabian Davamani, in providing his invaluable guidance, suggestions and comments throughout the course of this project.



# Establishment of bacterial biofilms from Antarctic soil bacteria

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 fabian\_davamani@imu.edu.my

## Introduction

Antarctic soils are exposed to a wide range of environmental factors which can limit or promote the survival of psychrophilic/psychrotolerant microbes. Biofilms are the dominant life forms which can withstand stressful conditions while biofilm-forming ability is an important survival strategy of polymicrobial community. The physical extremes of temperature, ultraviolet (UV) radiation, salinity, low water and nutrient availability are amongst the growth-limiting factors of Antarctic bacteria. Temperature and nutrition were the growth-limiting parameters selected for the current study. The primary aim of this study was to assess the effects of temperature and nutrients on the growth of Antarctic bacterial biofilms.

**Methods:** Soil samples from Signy Island were used to extract the microbes after vortexing and centrifugation and the supernatant was plated on 24 different microbial media, including defined media and complex media and further enrichments. The cultures were incubated at 4°C, 10°C and 15°C for a period of 15-30 days and observed for the formation of biofilms estimated by crystal violet method.

**Results:** Our experimental study highlighted that soil samples showed varied degrees of biofilm formations. Simple descriptive statistics were used to describe the overall trend of biofilm growth together with the use of median absolute deviation to facilitate the identification of outliers in each data set. The data were non-normally distributed and positively skewed. Therefore, inferential statistics was used to evaluate the success in biofilm formation. Bootstrap method was used, where each data set was resampled and the mean confidence interval was determined.

Media	Media	Temperature									
		4°C		10°C		15°C					
		BP	GP	BP	GP	BP	GP				
Unfiltered	Salt-agg	0.7945	0.0931	0.1443	0.0615	0.148	0.0573	0.1477	0.0515	0.148	0.0271
Filtered	LR	0.133	0.056	0.135	0.1575	0.3895	0.2145	0.2657	0.0933	0.261	0.0448
	TSB	0.255	0.1475	0.2385	0.091	0.4445	0.13	0.2105	0.1675	0.2993	0.0468
	BHI	0.2345	0.094	0.209	0.1245	0.365	0.224	0.236	0.0633	0.35	0.0572

Table 1: Calculation of Median and median absolute deviation. Median is used as a measure of central tendency because of the non-normality distributions and hence using other parameters such as mean or mode would provide a more distorted descriptive representation for the sets of sampling data. As for the measure of dispersion, we are using the median absolute deviation (MAD) to complement with the parameter, median.

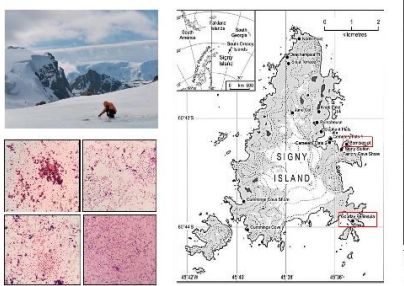


Figure 1: Sample collection from Signy island and depiction of polymicrobial bacterial associations.

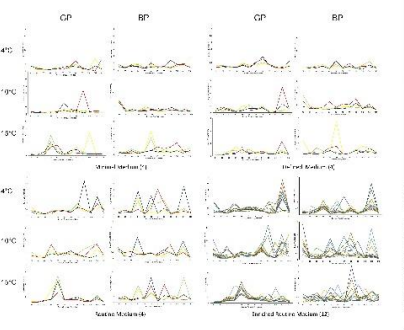


Figure 2: Polymicrobial biofilms in 24 different media for 24 soil samples (12 GP, 12 BP).

Media	Media	Media	Temperature																	
			4°C	10°C	15°C	4°C	10°C	15°C	4°C	10°C	15°C	4°C	10°C	15°C						
Unfiltered	Salt-agg	GP	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Filtered	LR	GP	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	TSB	GP	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Table 2: Significance of the difference of biofilm growth between different media, samples obtained from location at GP; temperature conditions depicted as 4°C (BP), 10°C (GP), 15°C (BP).

Media	Media	Media	Temperature																	
			4°C	10°C	15°C	4°C	10°C	15°C	4°C	10°C	15°C	4°C	10°C	15°C						
Unfiltered	Salt-agg	GP	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Filtered	LR	GP	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	TSB	GP	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Table 3: Significance of the difference of biofilm growth between different media, samples obtained from location at BP; temperature conditions depicted as 4°C (BP), 10°C (GP), 15°C (BP).

## Conclusion

Antarctic bacteria depicted biofilm formation at all conditions, however the nutritional requirements and temperature plays a vital role in establishment of the biofilm. Higher nutrition supported better biofilm growth. Different bacterial populations are observed with variation in temperature. In a poly-microbial soil environment, dominant microbes are seen in the biofilms. Biofilms are protected nodes of growth that allows survival in hostile environment. Biofilms can also be transmitted as polymicrobial clusters, these associations can benefit the microbes to establish in new environments, if dominant can undergo a succession replacing the existing microbes.

**Acknowledgement:** Financial support provided by International Medical University's Research Grant for undergraduate study for Biomedical Science (Hons) and equally from the Antarctica Flagship program, on heavy metal toxicity in polar microbes (MOSTI, Malaysia).

Dharshini D, Mohammad Abdul Aziz AB, Chan WY, Ambu S, Chu WL, Convey P, Chong CSL, Davamani F. Response of Antarctic Psychrotrophic Bacteria to Antibiotics before and after exposure to Heavy Metals. Australian Society for Microbiology Annual Scientific conference 2018. Brisbane, Australia, 1-4 July 2018.

Davamani F, Chong CSL, Chong DHL, Mohamad NENB, Selvarajah S, Ambu S, Chu WL, Convey P. Establishment of bacterial biofilms from Antarctic soil bacteria. Australian Society for Microbiology Annual Scientific conference 2018. Brisbane, Australia, 1-4 July 2018.

# Heavy metal and Antibiotic resistance in Antarctic bacteria isolated from Signy Island

Chong Sze Ling Catherine<sup>1</sup>, Stephen Ambu<sup>1</sup>, Wan Loy Chu<sup>1,2</sup>, Pete Convey<sup>3</sup>, and Fabian Davamani<sup>1</sup>  
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<sup>3</sup>British Antarctic Survey, UK  
 Corresponding author: fabian\_davamani@imu.edu.my

### Introduction

Antarctic bacteria encounter a myriad of stresses pose by their harsh environments, including anthropogenic causes. These stresses elicit various defence mechanisms that not only buttress these cold loving microbes from damaging stress, but also manifest a cascade of changes that impact bacterial recalcitrance towards antimicrobial and heavy metal activity. In particular, Antarctic bacteria are known to express antagonistic activity to eliminate competitors between microorganisms for survival [1,2]. This adaptive feature coupled with biofilm formation create a formidable defence for the bacteria to survive and disseminate antibiotic resistance genes (ARGs) through horizontal gene transfer [3]. This draws to the research aim which is to investigate antibiotic resistance of heavy metal tolerant bacteria isolated from Signy Island, Antarctica.

### Methods

Antarctic soil bacteria were isolated from two locations, Berntsen Point and Gourlay Peninsula in Signy Island. Microbial isolates that showed resistance to heavy metals like copper and zinc at various concentrations (0.03- 4 mM) were tested for antibiotic susceptibility with Phenotype Biolog MicroArray (PM) technology. The bacterial genome of resistant strains were sequenced for identification and further analysis.

### Results & Discussion

#### Microbial Genomes Atlas

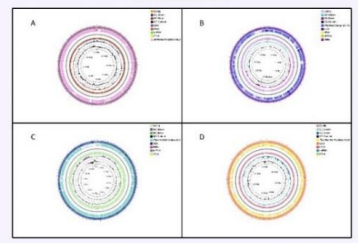


Figure 1: Genomic representation of Antarctic bacteria isolates. The representation was performed using the CGView server. The outermost rings show features calculated from the microbial genome. The inner ring shows the positions of BLAST hits detected through BLASTn comparisons of the genome against the closest genomes of the Antarctic bacteria. Darker arcs indicate high percent identity of the hit. The black circle displays the GC content, and outer circles display GC skew.

#### Metabolic Activity Rings

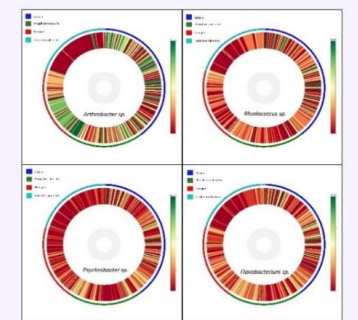


Figure 2: High-throughput metabolic activity of Antarctic isolates at 15°C. The metabolic activity is expressed as AV value and colored from red (low activity) to green (high activity) and visualized through the DuctApe Activity rings, indicating the PM substrates differentially utilized by the bacteria at 15°C. The inner ring shows the positions of BLAST hits detected through BLASTn comparisons of the genome against the closest genomes of the Antarctic bacteria. Darker arcs indicate high percent identity of the hit. The black circle displays the GC content, and outer circles display GC skew.

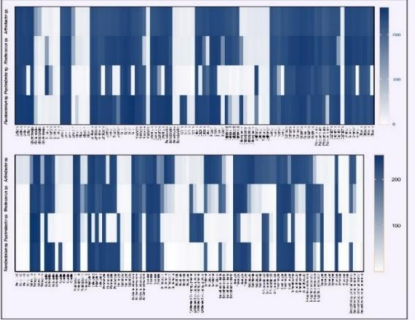


Figure 3: Heatmap of chemical substrates from phenomic analysis for PM 11 & PM 12 for four Antarctic bacteria.

PM analysis showed resistance of these strains to antibiotics belonging to the ten different chemical classes e.g., aminoglycosides,  $\beta$ -lactams, lincosamides, glycopeptides, tetracyclines, amphenicols, macrolides, sulfonamides, and rifamycins.

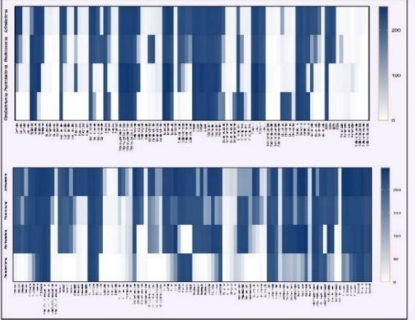


Figure 4: Heatmap of chemical substrates from phenomic analysis for PM 14 & PM 16 for four Antarctic bacteria.

PM analysis showed tolerance to a number of antibiotics and chemicals. *Arthrobacter* sp., in particular displayed the highest resistance followed by *Rhodococcus* sp., *Psychrobacter* sp. and *Flavobacterium* sp.

### Conclusions

In this study, we have identified many multiple-antibiotic resistant strains of Antarctic bacteria, suggesting that they are widespread in Antarctic soils. The ability of Antarctic bacteria to resist the inhibition of antibiotics is indeed surprising but not unexpected. It is known that most conventional antibiotics were originated from environmental microbes, which means that genes for antibiotic resistance may also emerge naturally in the environment. The results demonstrate the applicability of the microarrays to establish antibiotic susceptibility profiles of the environmental bacterial strains. Although further research is required, phenotype microarrays could be successfully used as a modern tool for identification of the multi-antibiotic resistance of bacteria and for preliminary establishing of the inhibition concentrations (ICs).

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### Acknowledgement

Financial support provided by the Antarctica Flagship program, on heavy metal toxicity in polar microbes (MOSTI, Malaysia).

Chong CSL, Ambu S, Chu WL, Convey P, and Davamani F. Heavy metal and antibiotic resistance in Antarctic bacteria isolated from Signy Island. 8<sup>th</sup> Malaysian International Seminar on Antarctica -Polar regions in the Global Climate System. Kuala Lumpur, Malaysia, 18-19 June 2019.

# Polymicrobial biofilms from Signy Island (Antarctica) and ROS production by bacterial isolates resistant to heavy metals

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<sup>1</sup>International Medical University, Bukit Jalil, Kuala Lumpur, Malaysia  
<sup>2</sup>National Antarctic Research Centre, University of Malaya, Kuala Lumpur, Malaysia  
<sup>3</sup>British Antarctic Survey, Cambridge, CB3 0ET, United Kingdom  
<sup>4</sup>Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor Darul Ehsan, Malaysia

### Introduction

Microbial biofilm formation can resist environmental fluctuations by forming a complex matrix of extracellular polymeric substances. It is a state of adaptation that is quintessential for survival, metabolism and propagation of bacteria [1]. This unique architecture serves as a vehicle for Antarctic soil bacteria to disseminate into mainstream water bodies (sea) by rain or melting of ice [2]. Climate change can further accelerate the process by ocean currents, making it permissible to reach any part of the globe [3-4]. Polymicrobial biofilms resistant to heavy metals under experimental conditions were selected and the bacteria were isolated and characterized. The isolated bacteria are able to resist the effect of ROS for its survival. Hence, this research aims to study the tolerance of Antarctic soil bacteria to heavy metals and antibiotics and the effects on biofilm formation which can facilitate bacterial resilience.

### Methods

Antarctic soil bacteria were isolated from two locations; Berntsen Point and Gourlay Peninsula in Signy Island. Microbial isolates were tested for its resistance towards heavy metals like copper and zinc at various concentrations. Isolates that showed resistance were sequenced for identification and tested for antibiotics susceptibility with Phenotype Biolog Microarray (PM) technology.

### Results & Discussions

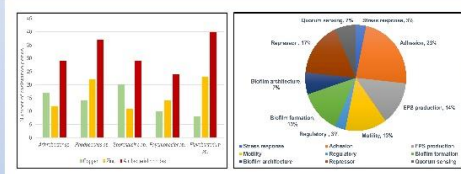


Figure 1: Heavy metal resistance and antibiotics associated genes which will help the bacteria to survive in different environment.

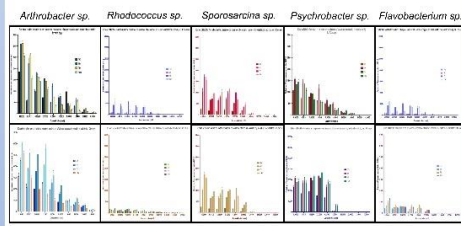


Figure 3: Isolates tolerating different concentrations of copper and zinc. *Arthrobacter* sp. displayed a higher tolerance followed by *Psychrobacter* sp. and *Sphaerobacter* sp.

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### Acknowledgement

Financial support provided by the Antarctica Flagship Programme (FP1213E036) by the Ministry of Science, Technology and Innovation (MOSTI).

Chong CSL, Ambu S, Chu WL, Convey P, Cheah YK and Davamani F. Polymicrobial biofilms from Signy Island (Antarctica) and ROS production by bacterial isolates resistant to heavy metals. XIII International Symposium on Antarctic Earth Sciences (ISAES 2019). Incheon, Republic of Korea, 22-26 July 2019.

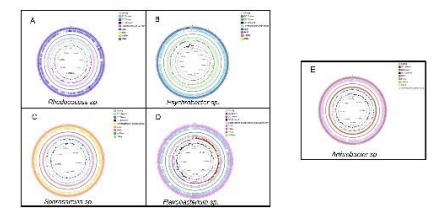


Figure 3: Genomic map of Antarctic soil bacteria. A-D represent new species based on Average Nucleotide Identity (ANI) [5]. The outermost ring represents the entire genome. The inner ring shows the positions of BLAST hits detected through BLASTn of the Antarctic bacteria. Darker region indicates high percent identity of reference genome.

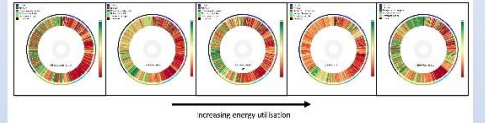


Figure 4: High-throughput metabolic activity of Antarctic isolates at 15°C. The metabolic activity is expressed as AV value and colored from red (low activity) to green (high activity) and visualized through DuctApe Activity rings, indicating the PM substrates were differentially utilized by the bacteria at 15°C.

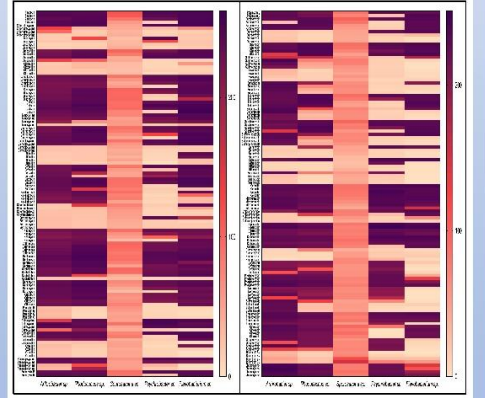


Figure 5: Heatmap of antibiotics from phenomic analysis for Antarctic isolates. PM analysis showed tolerance to a number of antibiotics and chemicals. *Arthrobacter* sp., in particular displayed the highest resistance followed by *Rhodococcus* sp., *Psychrobacter* sp., *Flavobacterium* sp. and *Sphaerobacter* sp.

### Conclusions

Climate changes in the environment can displace microbial community as biofilms from its native environment to a totally different niche and dissemination of the microbes can have both beneficial as well as harmful effect. The analysis of genome - phenome association provides insights to the capability of the bacteria to survive under less nutrients and also the ability to utilize a wide range of energy sources under nutrient-poor conditions. These Antarctic bacteria that encodes several resistance genes especially to so many different kinds of antibiotics through phenotype microarray analysis may pose serious threat to humans as they are protected by the establishment of biofilms. Hence, this draws us to questions of "are they really safe?" and "do we want these microbes out of Antarctica?"

# Virus

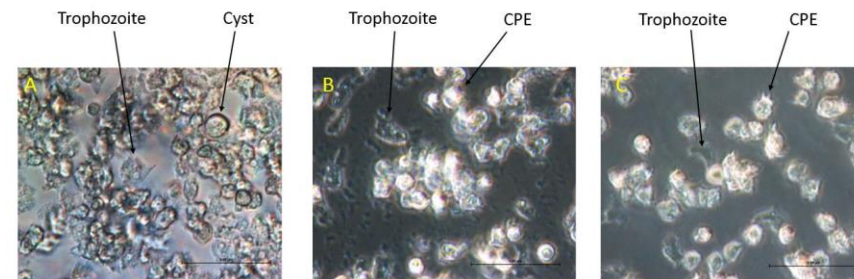


**Dr Kenny Voon**

The IMU's research work on Antarctic viruses is led by Dr Kenny Voon Gah Leong. Dr Kenny Voon's lab focuses on investigating the role of mimivirus in Antarctica, with the project titled 'Isolation and molecular characterisation of giant amoebal viruses from soils in Signy Island'. The project aims to isolate giant amoebal viruses from soils in Signy Island and perform molecular characterisation of these isolates.

He is intrigued by how the mimivirus is so ubiquitous that it could be discovered in different parts of the world, including Antarctica. With the success of isolating mimivirus from soils in Malaysia, his team used a similar approach to isolate and detect mimivirus from Antarctica, particularly soil samples from Signy Island. The genome of isolated mimivirus will be sequenced to be compared with those that were discovered from different regions. Much of the knowledge about the roles of viruses in the natural environment comes from studies of marine microbial communities.

Viruses play an important role in infecting and degrading heterotrophic microbes which later lead to increases in net respiration, the release of carbon dioxide and nutrient recycling in the world's oceans. However, the role of mimivirus in the natural environment remains to be explored.



Light micrograph of *A. castellanii* at 400X magnification; A shows the negative control; B shows the *A. castellanii* after being passaged on with samples from Berntsen Point; C shows the *A. castellanii* after being passaged on with samples from Gourlay Peninsula, Signy Island.

## Publication

Ooi ZQ, Hong KS, Thum EY, Ong SL, Voon K, Chong CW. The Discovery and Quantification of Mimivirus and Marseillevirus in Signy Island, Antarctica. *Polar Biology* (in preparation for submission).

# Parasites



Prof Patricia Lim

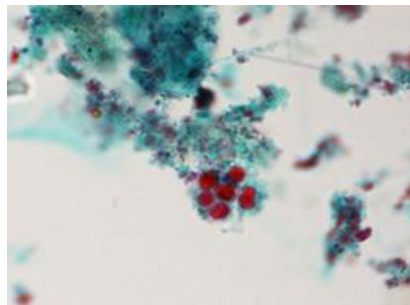
Research projects under Prof Chu's flagship grant also included work on parasites, led by Professor Patricia Lim Kim Chooi. The project titled 'Identification of parasites in soil samples collected from Signy Island, Antarctica' aimed to examine soil samples collected from various sites in Signy Island using direct smear and a concentration technique as well as identify the parasites and parasitic stages using morphological methods.

Prof Patricia's team examined a total of 135 soil samples obtained from five locations on Signy Island (South Orkney Islands, maritime Antarctic), namely North Point and Gourlay Peninsula (penguin rookeries), Pumphouse (relic coal-powered pump house), Jane Col (barren high altitude fellfield) and Berntsen Point (low altitude vegetated fellfield close to current research station).

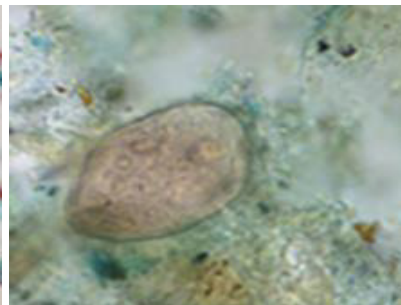
They found that approximately 10% of the soil samples (14/135) from three out of the five study sites had parasites which included Diphyllobotridae spp. eggs, *Cryptosporidium* sp., an apicomplexan protozoa (gregarine), *Toxoplasma gondii*, helminths (a cestode, *Tetrabothriussp.*, and a nematode larva) and mites. Two of the three sites with parasites are penguin rookeries (North Point and Gourlay Peninsula) while the third site (Pumphouse Lake) has human activity. Some of the parasite species found in the soil samples appear to be distinctive but there were also parasites such as *Cryptosporidium* and *Toxoplasma gondii* that have a global distribution and are potentially pathogenic.

## Publication

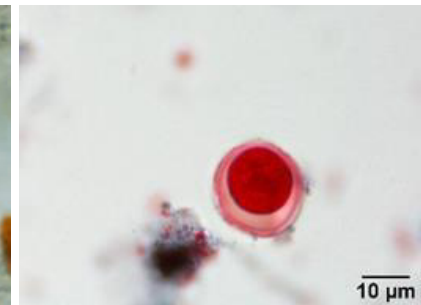
Lim PKC, Lee XC, Mohd Nazmi NMA, Tang YY, Wong SF, Mak JW, Convey P (2018). Parasites in soil samples from Signy Island, Antarctica. *Tropical Biomedicine*. 35(4): 1007-1016.



*Toxoplasma gondii* oocyst.



Gregarine.



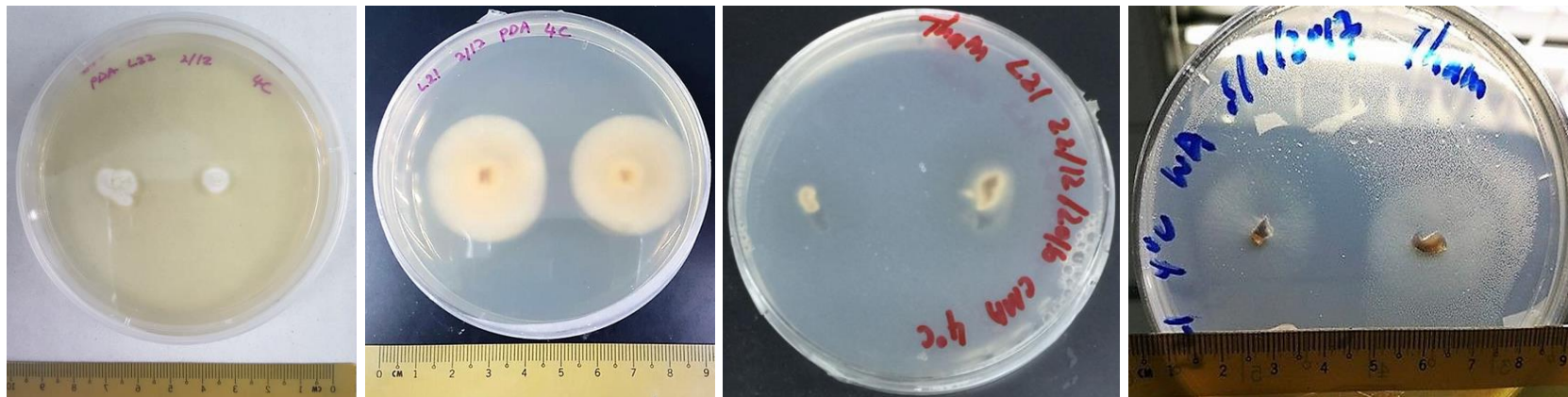
*Cryptosporidium* oocyst.

# Fungi



**Dr Carolina Santiago**

For research on fungi, Dr Carolina Santiago studied the effect of Antarctic fungal alkaloid extracts on indole production in antibiotic resistant *Escherichia coli* strains. The objective of her research was to investigate the activity of alkaloid extract on indole production in antibiotic resistant *E. coli* strains. According to her research, alkaloidal extract from a *Phoma* sp. isolate coded as L21 was found to reduce production of a bacterial signalling molecule, indole in antibiotic resistant *E. coli* strains. The compounds from this extract were deemed to have a potential application in overcoming antibiotic resistance exhibited by bacteria.



*Phoma* sp. isolate L21 (from left to right): Grown on potato dextrose agar (PDA) culture media; with colony growth at 4°C for 14 days; grown on corn meal agar at 4°C for 14 days; grown on water agar at 4°C for 14 days.





## **A Tribute to Professor Chu Wan Loy**

31 July 1964 to 15 June 2020



Prof Chu presenting his research findings on the human impacts on Antarctica at the 7<sup>th</sup> Malaysian International Seminar on Antarctica (MISA7) 2017, Universiti Malaysia Terengganu (UMT).

Professor Chu Wan Loy - A humble, kind, passionate and brilliant scholar who has made significant contributions in education and research, particularly in the field of algae research.

As an educator, Prof Chu was enthusiastic and contributed significantly to undergraduate medical education in various roles – as a lipid biochemist, as respiratory system coordinator in medicine, in curriculum review for medicine and as a programme director for medical biotechnology. For medical biotechnology, his particularly significant contribution was bringing medicinal plants into the curriculum.

Prof Chu served as the Dean, School of Postgraduate Studies (SOPG), IMU from 1 October 2013 till 15 June 2020 and was the Deputy Director, Research, Institute for Research, Development and Innovation (IRDI), IMU from 18 September 2012 till 31 December 2018.

As Dean, School of Postgraduate Studies, Prof Chu made important contributions as follows:

1. Introduction of postgraduate diplomas and certificates to SOPG, previously there were only masters programmes;
2. Introduction of Online Distance Learning (ODL) postgraduate programmes;
3. Reviewing the programme to be more flexible to adult learning and improved governance of programmes;
4. Shortening the masters programme to a minimal one year programme.

## Prof Chu's first trip to Antarctica

Prof Chu's illustrious research journey on algae began when he worked on tropical algae for his PhD under the supervision of Prof Phang Siew Moi at the University of Malaya in the 1990s. It was Prof Phang who encouraged him to continue research in this field and he was one of two successful Malaysian researchers who was offered a berth by the Australian Antarctic Division (AAD) to go on a reconnaissance voyage to Casey Station, Antarctica in 2000.

This was his first trip to Antarctica and due to several delays, Prof Chu managed to stay on Casey Station for only four days but the experience and the journey were memorable for him. He collected the first set of Antarctic polar algae samples on soil, snow and glaciers from several sites including a penguin rookery and this collection formed the basis of numerous studies on responses and adaptation of Antarctic algae to global warming and UVR stress. Such studies are important to understand the consequences of environmental changes such as global warming and the widening hole in the ozone layer and their impact on Antarctica.



The MV Polar Bird, an ice-strengthened ship chartered by the AAD for the voyage.



Prof Chu on board the MV Polar Bird.

# Sub-Antarctic and Arctic expeditions

Prof Chu subsequently made two more trips to collect samples for the isolation of sub-Antarctic and Arctic algae:

**7 April – 12 May 2005**

Expedition to Marion Island, sub-Antarctica under the South African National Antarctic Program (SANAP).

**24 July – 15 August 2007**

International Polar Year (IPY) Research Cruise to the Bering Sea (sub-Arctic) and Chukchi Sea (Arctic).



Prof Chu, with a view of penguins from the MV Polar Bird.



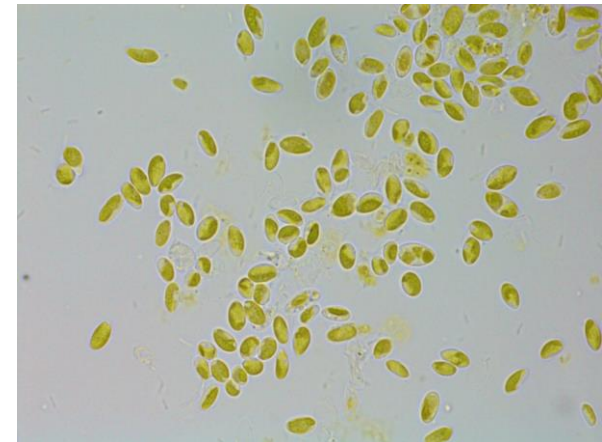
Prof Chu with the ship's cook on board the MV Polar Bird.



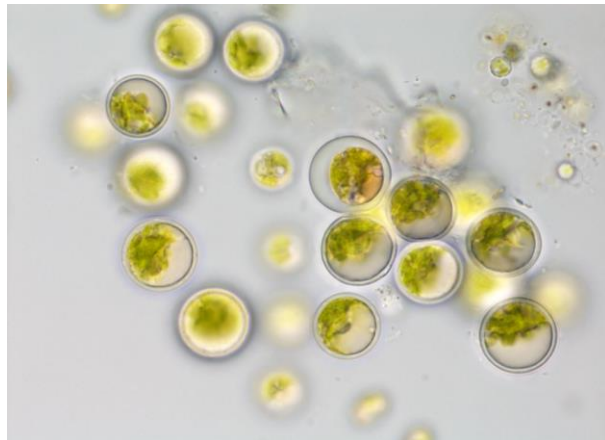
Arctic ice.

# Micro-algae cultures

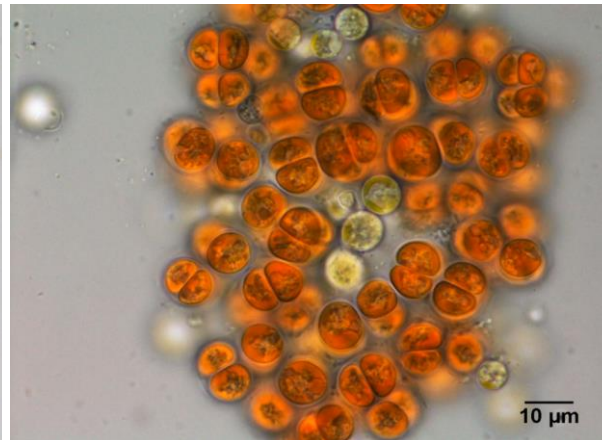
Prof Chu successfully cultured a large number of micro-algae from his Antarctic and Arctic collections at the Research Laboratory in the IMU. This is a significant contribution as his micro-algae cultures served as research materials for many research projects, which not only brought in several research grants and generated numerous publications but trained a large number of undergraduate and postgraduate students at the IMU.



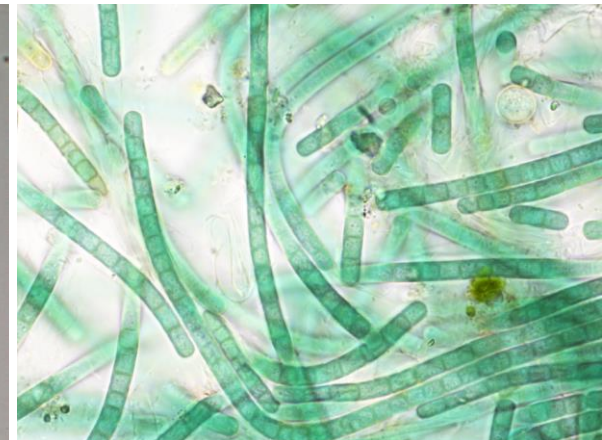
*Coccomyxa gloeobotrydiformis*.



*Chlorella* sp.



*Desmococcus olivaceus* (old cells filled with carotenoids).



*Pseudanabaena* sp.



Prof Chu representing the IMU in Antarctica.

## Prof Chu's Contributions to Research and Publications

Prof Chu's passion for algae research is indescribable and his contributions to the fields of micro-algae and polar research are reflected in his outstanding international reputation and many awards and honors.

He was a Research Associate with the Algae Research Laboratory & Institute of Ocean and Earth Sciences (IOES), University of Malaya and a member of the Malaysian Antarctic Research Taskforce. His collaborating research partners included University of Malaya, Institute for Medical Research (IMR), Cancer Research Initiatives (CARIF), Monash University, Australian Antarctic Division, University of the Western Cape (South Africa).

His research excellence can be evidenced by his success in securing 20 external and 27 IMU internal research grants on algae research. Prof Chu has published more than 50 scientific papers and book chapters, and presented over 70 papers at both local and overseas conferences.

Prof Chu was invited by PRIME College in 2002 to share highlights of his Antarctic trip with Pre-University students.

SUNDAY STAR  
May 26 2002

▶ Education 11

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# Researcher in Antarctica

**A**LAWYER, a cook, university researchers, meteorological officers, ship engineers, a naval nurse, a military psychologist, mechanics and a children's author. What do they have in common? They were among some 60 Malaysians on board cargo liner *The Polar Bird* for a month in December last year on a voyage to one of the most inhospitable places on earth: Antarctica.

Pre-university students at PRIME College's Subang Jaya campus had an insider's view of the expedition, when Dr Chu Wan Loy was invited to present a slide show at the college recently.

Dr Chu shared some of the highlights of his adventure-filled trip to the frozen continent, which included seeing a helicopter crash whilst bringing supplies to the crew.

He was one of the Malaysian contingent of researchers who made up the Malaysian Antarctic Research Programme developed by the Academy of Sciences.

Dr Chu was picked to undertake scientific research in his field of specialisation: Micro-organism Biodiversity.

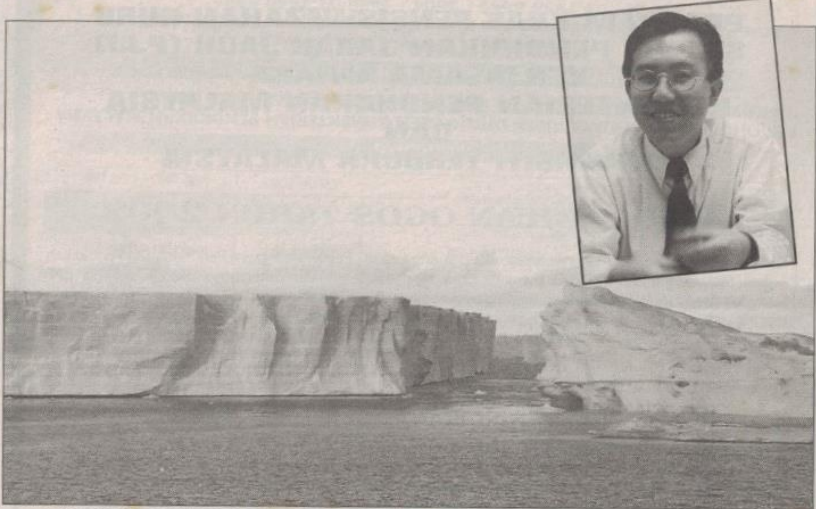
Not only did he bring home plenty of micro-algae samples for his specific study, he successfully cultured them in the laboratory at the International Medical University.

He explained that his aim during the familiarisation expedition was to examine the chain relationships of micro-organisms that are found in the coldest continent on Earth.

The expedition allowed Dr Chu to study how factors like ultraviolet radiation and the consequences of environmental changes, for example global warming and the widening hole in the ozone layer, impact on Antarctica.

He explained how the micro-algae samples which he collected and brought back were isolated in order to study their components. In this Dr Chu worked alongside his research associate from Universiti Malaya, Prof Phang Siew Moi.

Dr Chu said that although he spent only four days working at Casey Station, the permanent research centre run by the Australian Antarctic Division, his entire trip to what can be arguably called the World's End was an eye-opener.



**STILL LARGELY UNEXPLORED ... Dr Chu (inset) was part of the Malaysian contingent which recently did various scientific research in Antarctica.**

## Prof Chu - Teacher, Scientist, Mentor and Friend



Prof Chu with the Algae team at the IMU Convocation on 14 June 2015. From left to right: Dr Kok Yih Yih, Dr Dang Nguk Ling, Prof Chu Wan Loy, Ms Chin Yin Yien, Dr Tan Boon Keat.

As a teacher, Prof Chu was a walking encyclopedia for his most passionate subject - microalgae. He always wanted to do his utmost best for all his students and worked relentlessly to get his postgraduate students to complete their theses on time. So many students are indebted to Prof Chu for his guidance, advice, tutelage and mentorship. Prof Chu has supervised/ co-supervised a total of 11 PhD, 18 Master and 35 undergraduate students in their research.

Prof Chu will be remembered as an extremely creative and brilliant scientist who was able to address the biological questions he so passionately pursued with his broad background in biological principles and quantitative analysis.



Prof Chu (standing, 10<sup>th</sup> from right) with IMU colleagues during the IMU ASPIRE 1 workshop, 2010.



# Remembering Prof Chu

To his colleagues and friends, Prof Chu was much more than an outstanding scientist. He will be remembered as a dedicated mentor who always gave credit to his team and was always there to listen and advise. One of Prof Chu's favourite quotes was "The glory is for a day, but the science is forever", this is how he would like the science and its impact to be remembered.

He will be remembered as a wonderful colleague who gave freely of his time, advice and expertise. He is remembered for his love for Korean drama, good food and coffee where he shared many wonderful and happy moments with others.



Prof Chu (right) with Emeritus Professor Mak Joon Wah (middle) and Prof Leong Chee Onn, 2010.



Prof Chu with Prof Peter Pook, Deputy-Vice Chancellor (Academic), Deans and Department Heads, 2018.

And he will be remembered for serving the IMU with great distinction in many capacities, including his long tenure as the Dean of School of Postgraduate Studies.

Prof Chu's achievements and success in life are largely attributed to two important family members, his beloved mother and his wife who have served as his pillars and provided him with much love and support.

Another important pillar in his academic and research career is Emeritus Professor Mak Joon Wah, former Pro-Vice Chancellor (Research) and Director, IRDI, who nurtured and guided him throughout his career at the IMU, particularly in his roles as Dean, School of Postgraduate Studies and Deputy Director, Research in IRDI.



## Picture Credits

**Dr Carolina Santiago**

pg 30

**Dr Chan Kok Keong**

pg 13

**Dr Chong Chun Wie**

pg 5

**Dr Dang Nguk Ling**

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**Derren Fox, BAS**

pg 2

**Dr Fabian Davamani**

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**Dr Kenny Voon**

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**Martin McCallum, BAS**

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**Dr Nur Alia Johari**

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**Prof Patricia Lim Kim Chooi**

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**Dr Wong Chiew Yen**

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**YPASM**

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Photo: Equipment and fuel secured to a boulder at Khyber Pass, Signy Island, Antarctica.



