

## Conservation and characterization of Polar Cyanobacteria by the BCCM/ULC collection

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2. InBios-Centre for Protein Engineering, University of Liège, Liège, Belgium
3. BCCM/MUCL, Université catholique de Louvain, Louvain-la-Neuve, Belgium





- 1) History and objectives of BCCM/ULC
- 2) Biodiversity, genomics and exploration of the bioactivity potential

1) History and objectives of BCCM/ULC

2) Biodiversity, genomics and exploration of the bioactivity potential



Since 2006

**From** in-house research collection



**To** public collection

research-based

providing services

open for legitimate users

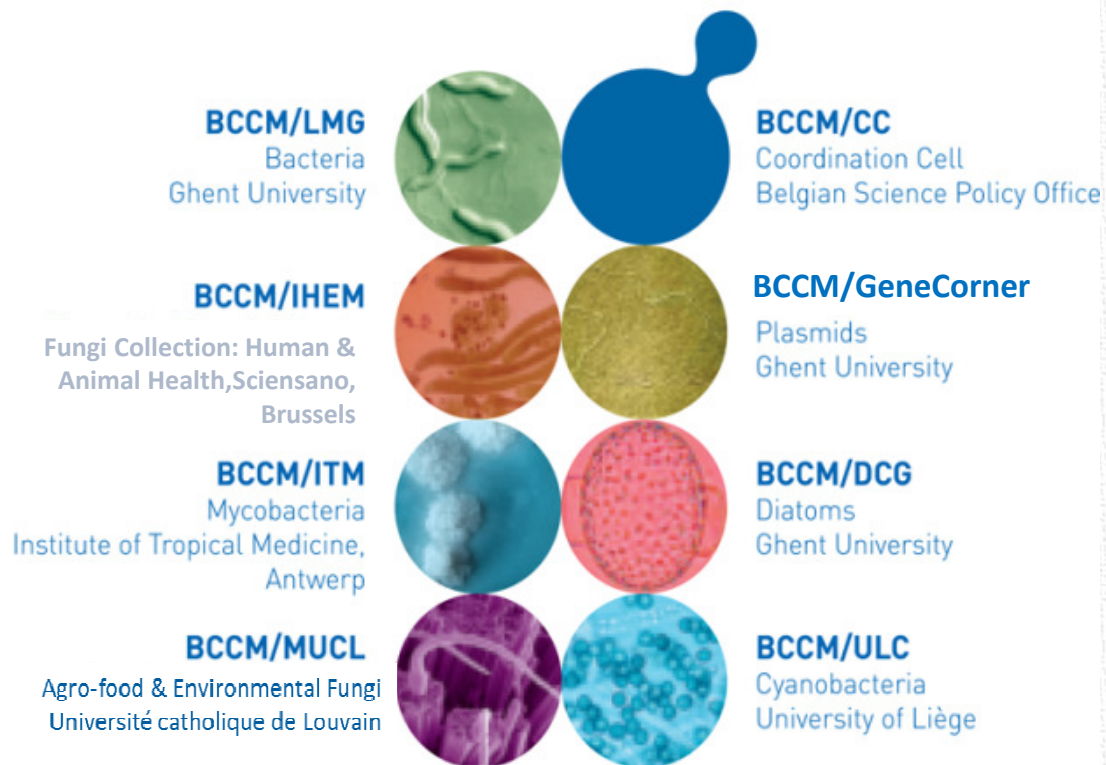
operating within a legal framework

linked to the Belgian Coordinated Cultures of Microorganisms  
(BCCM)



# The BCCM Consortium

7 decentralised culture collections, coordinated by a Central team at the Belgian Science Policy Office.



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BC

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**Culture collections are important** because  
« we publish and then, we move labs, we change jobs, .....  
or .... we perish »

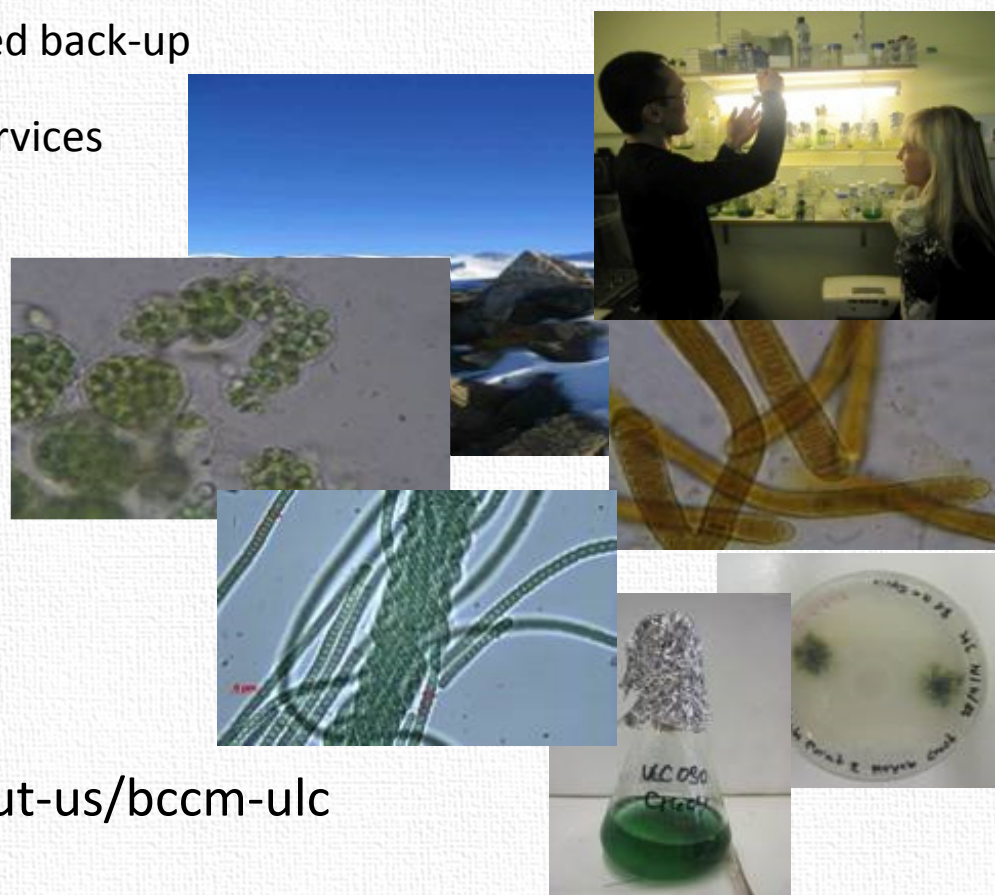
Therefore, **important biological material and related information might be lost forever.**

Hi Annick,  
Unfortunately I don't have them either in the move to \_\_\_\_\_ h about  
five years ago I think they did not move over.  
Sorry about that...



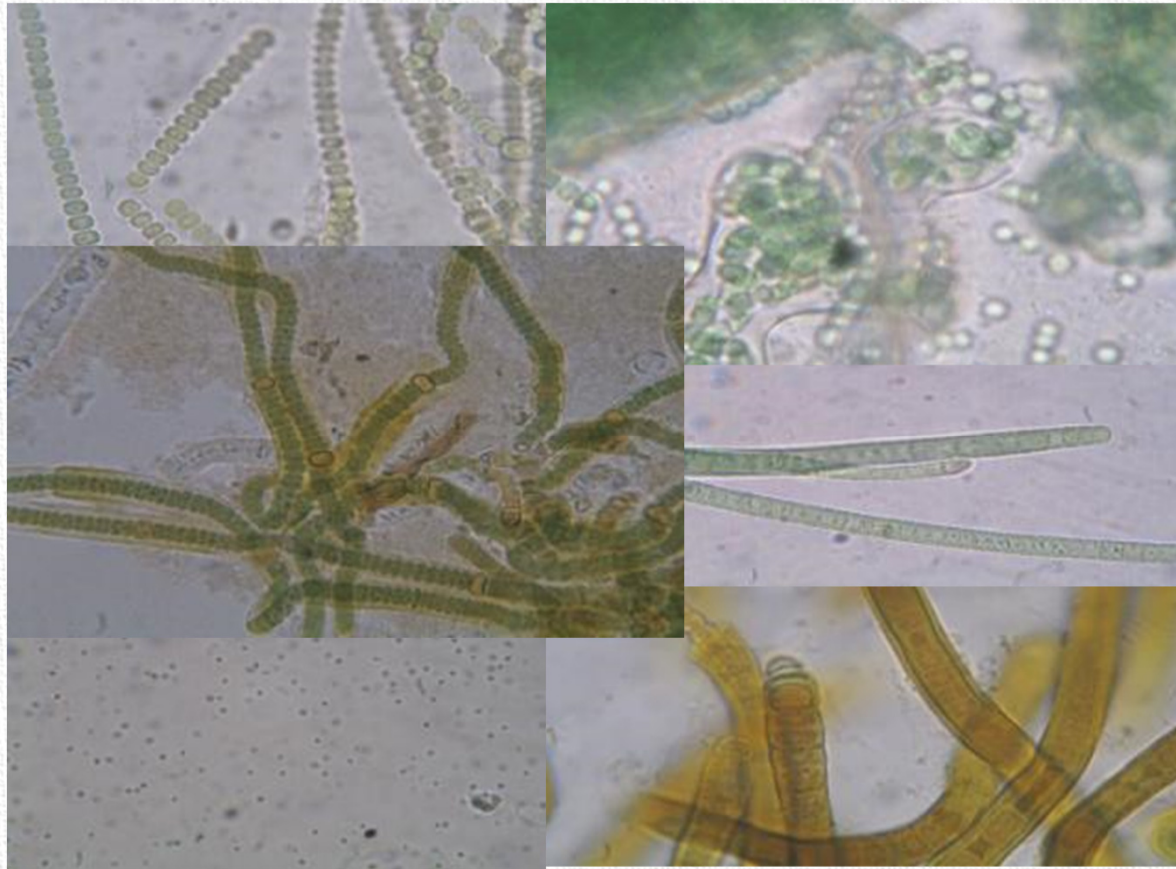
## BCCM/ULC public collection of cyanobacteria

- 246 unicyanobacterial strains, of which 120 of polar origin
- Maintenance as living cultures, with a cryopreserved back-up
- ISO 9001 certificate for deposit and distribution services
- New:** possibility of safe deposit!
- Geographic focus on cyanobacteria from the Polar Regions
- Taxonomic focus to obtain representatives of most abundant orders (Chroococcales, Chroococciopsidales, Nostocales, Oscillatoriales, Pleurocapsales, and Synechococcales)





## Quality Control of purity and authenticity: microscopic observation, molecular markers (16S rRNA, ITS sequences)





## History and objectives

Biodiversity, genomics and exploration of  
the bioactivity potential





## Large diversity in the BCCM/ULC collection

16S rRNA gene of 206 ULC strains : **82 OTUs** (99% similarity threshold), of which **45 are of Polar origin**

Maximum Likelihood tree with Kimura 2-parameter and G+I model, 500 bootstraps, built with 992 positions of the 16S rRNA gene sequence

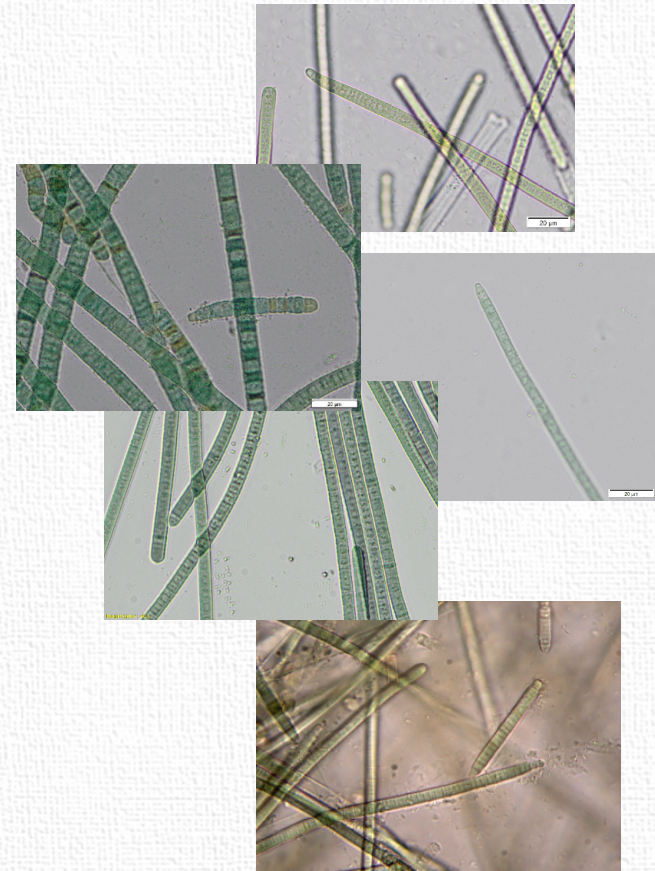


5 'Microcoleus' strains of **Arctic/Canadian origin** and 20 Antarctic strains (McMurdo Ice Shelf, James Ross Island, Antarctic Peninsula, Sør Rondane Mts, Transantarctic Mts

- 92 ULC370 *Microcoleus attenuatus* KOVACIK ANT 2003/11b
- ULC371 *Microcoleus attenuatus* KOVACIK ANT 2003/43
- ULC128 *Microcoleus favosus* JR29
- ULC307 *Phormidium autumnale* P4
- ULC061 *Phormidium insigne* E6a
- ULC076 *Phormidium autumnale* O-151
- ULC086 *Phormidium autumnale* Elster 1991/05
- 93 ULC097 *Phormidium autumnale* ANT-LUNCH
- ULC130 *Phormidium murrayi* TM2FOS130
- ULC120 *Microcoleus favosus* JR20
- ULC105 *Phormidium autumnale* ANT-ORANGE
- ULC108 *Microcoleus favosus* JR2
- ULC373 *Microcoleus favosus* KOVACIK ANT 2004/50
- ULC094 *Phormidium autumnale* Ant-R-5\*2
- ULC095 *Phormidium autumnale* ANT-PINNACLE\*4
- ULC107 *Microcoleus favosus* JR1
- 98 ULC166 *Phormidium autumnale* OTC control
- ULC176 *Phormidium* sp. OTC3
- 100 ULC172 *Phormidium* sp. OTC7
- 93 ULC174 *Phormidium lumbricale* OTC8
- ULC109 *Microcoleus vaginatus* JR3
- ULC110 *Microcoleus* sp. JR5
- ULC114 *Phormidium autumnale* JR9
- 99 ULC425 *Phormidium favosum* HK2
- ULC426 *Phormidium ambiguum* HK2

Canada Arctic  
Canada Arctic  
Canada Arctic

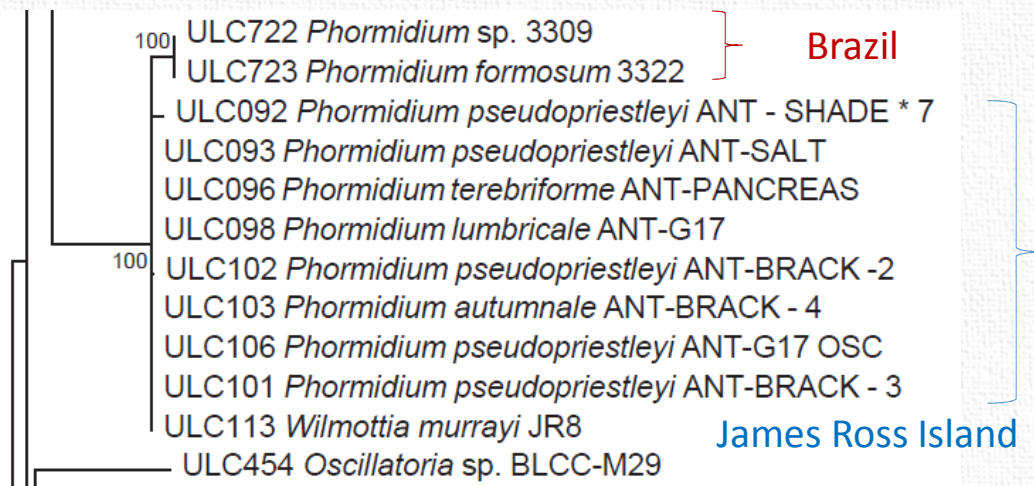
Canada Manitoba  
Canada Manitoba



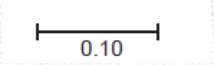
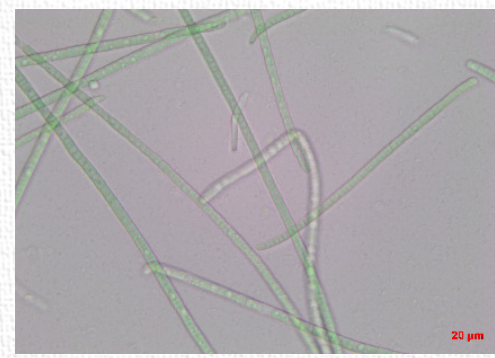
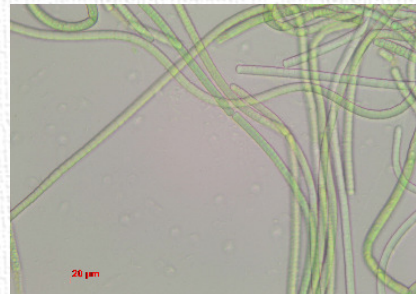
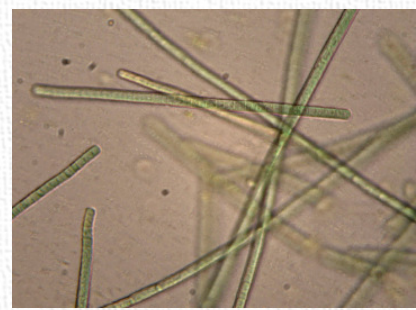


## 9 Antarctic 'Phormidium autumnale' strains

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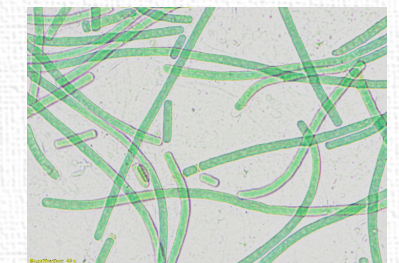
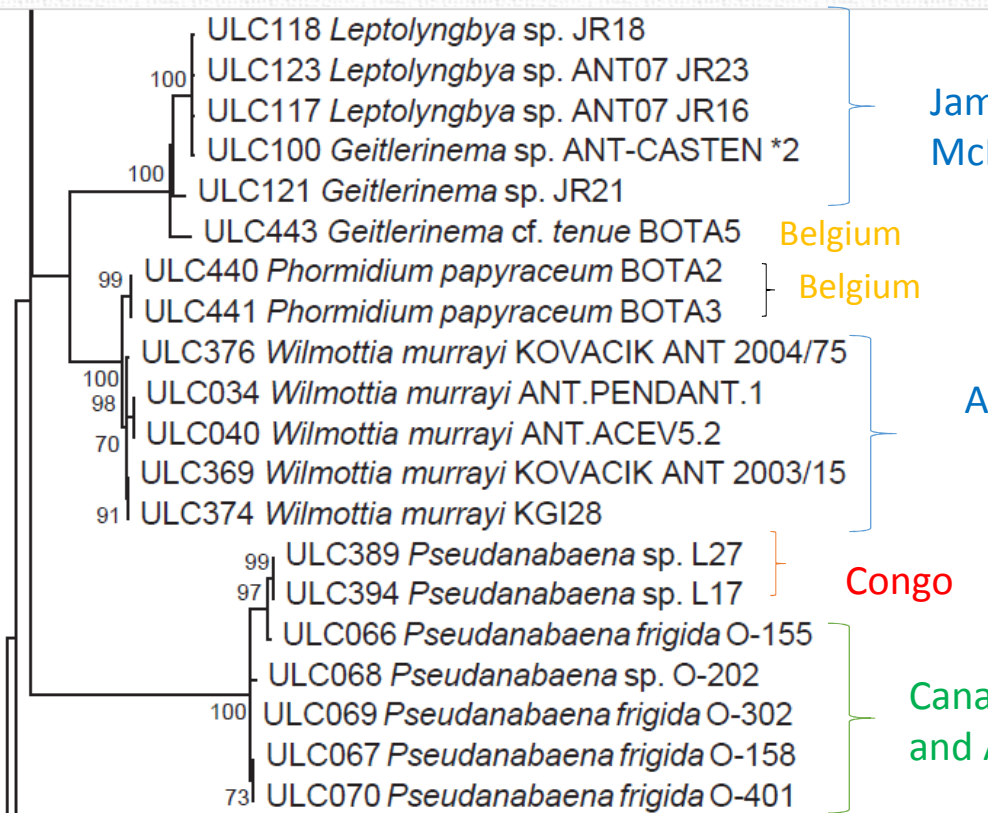
McMurdo Ice Shelf





Filamentous genera: 10 Antarctic and 5 Canadian (2 Arctic) strains

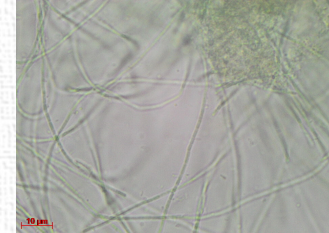
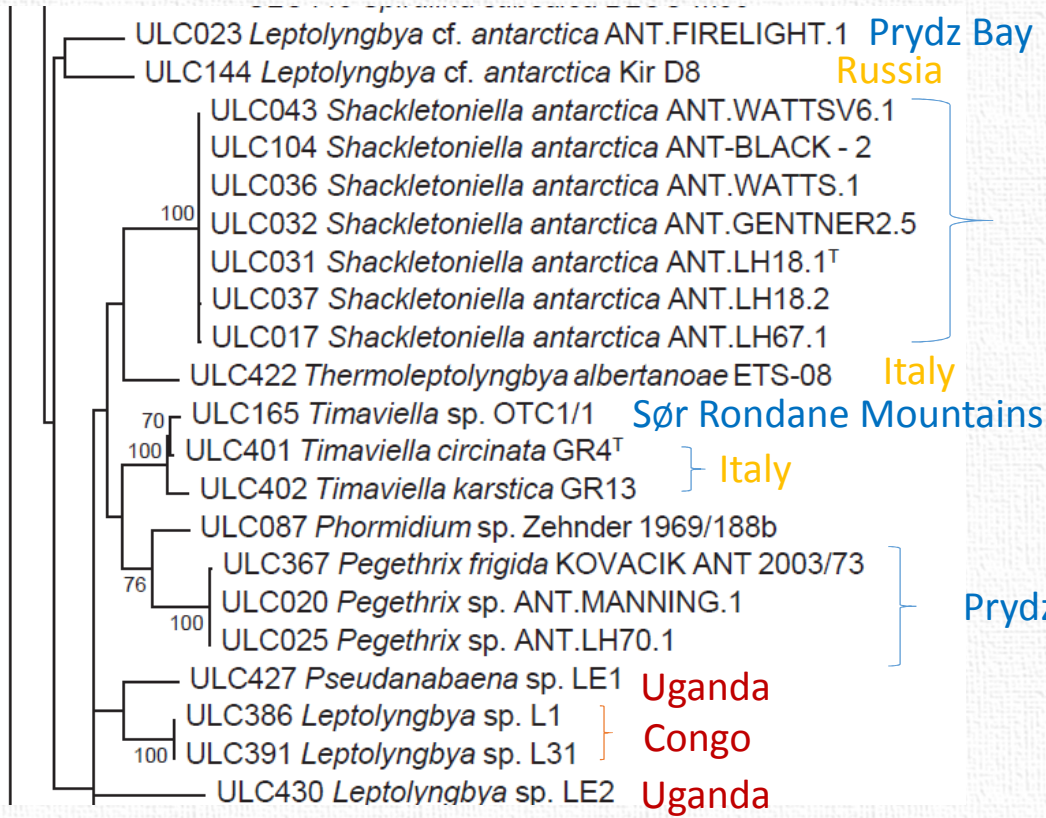
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Filamentous genera: 12 Antarctic strains

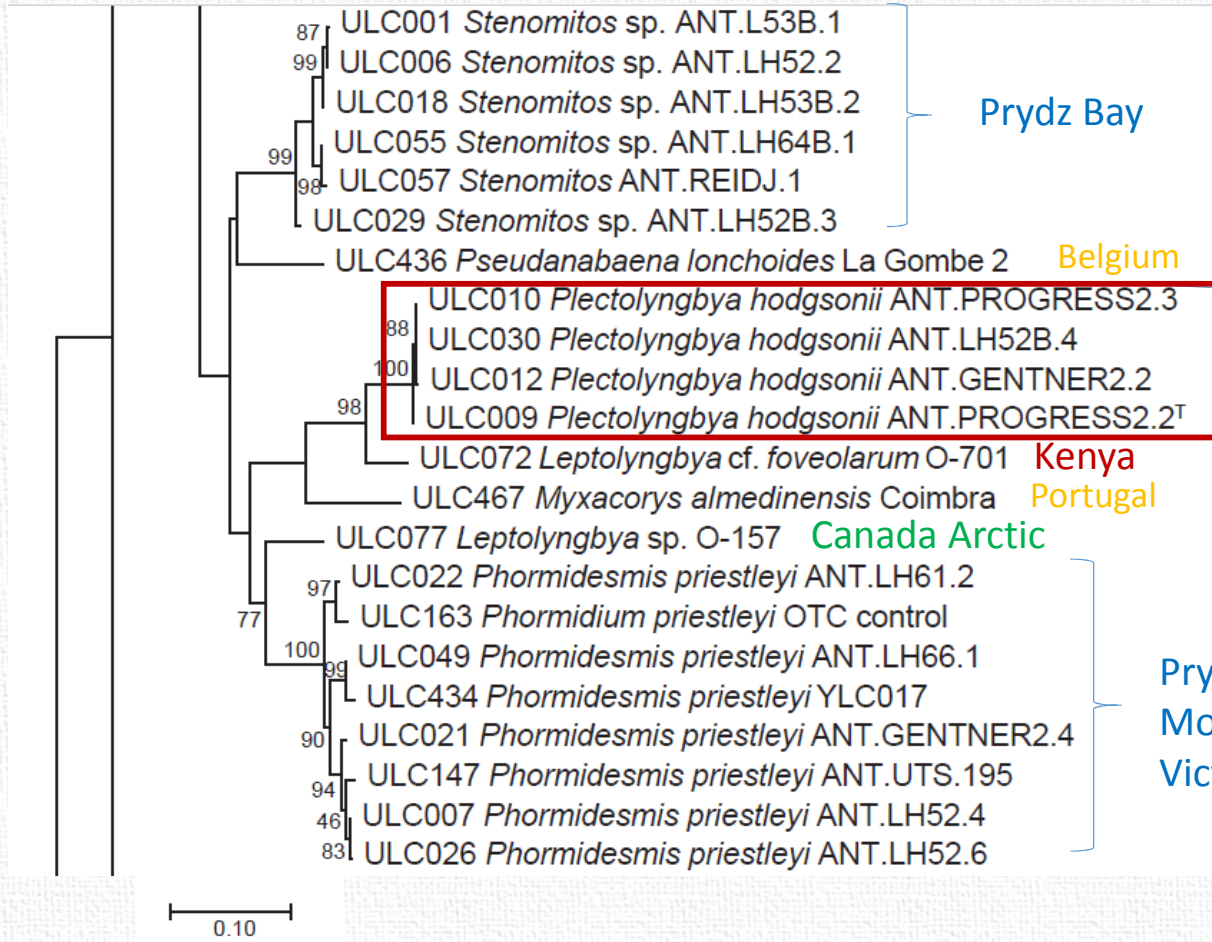
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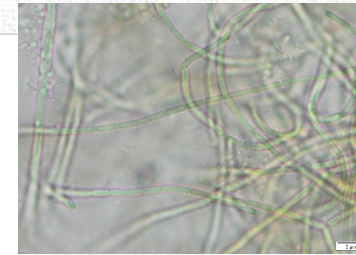
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Filamentous genera: 18 Antarctic and 1 Arctic strain



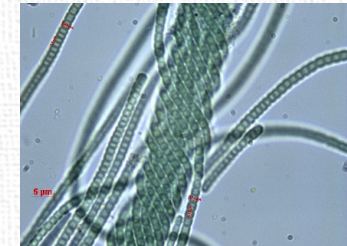
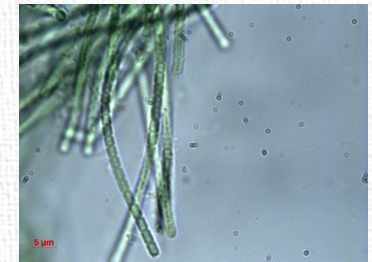
Prydz Bay



Prydz Bay



Prydz Bay, Sør Rondane Mountains and North Victoria Land

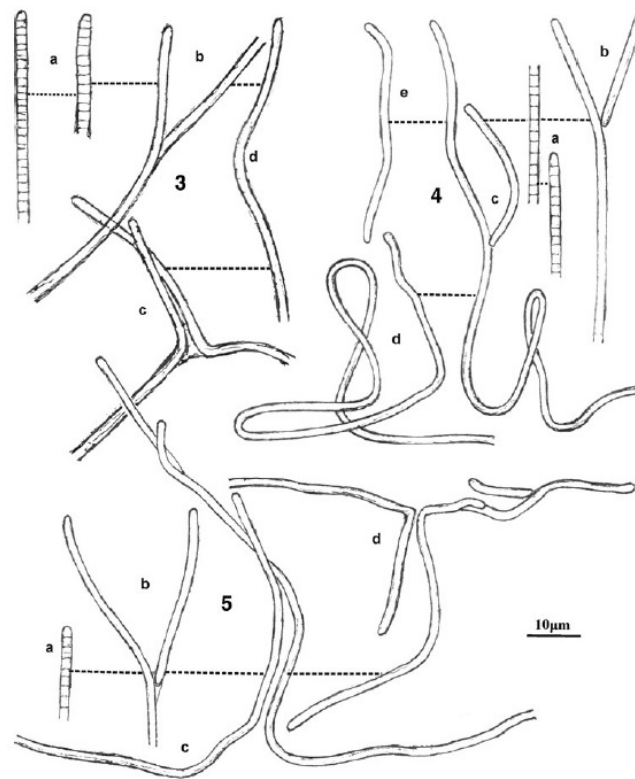




*Plectolyngbya hodgsonii*: a novel filamentous cyanobacterium from Antarctic lakes

A. Taton · A. Wilmotte · J. Šmarda · J. Elster · J. Komárek

Fig. 3–5 *Plectolyngbya hodgsonii*: 3 = drawing of type material from the strain ANT.LPR3 (a detail of trichomes; b–c false branching of filaments; d simple filament); 4–5 = two different populations from Monolith Lake, James Ross Island (a detail of trichomes; b–d filaments, mostly with false branching; e germinating hormogonium). (Orig.)

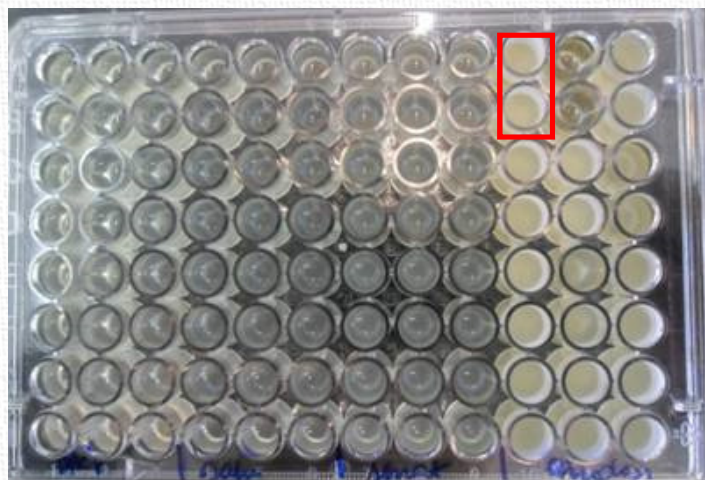


Potentially endemic genus, only found in Antarctica (Pridz Bay and Mc Murdo Dry Valleys)



## Bioassay of ULC009 against *Candida kruzeii*

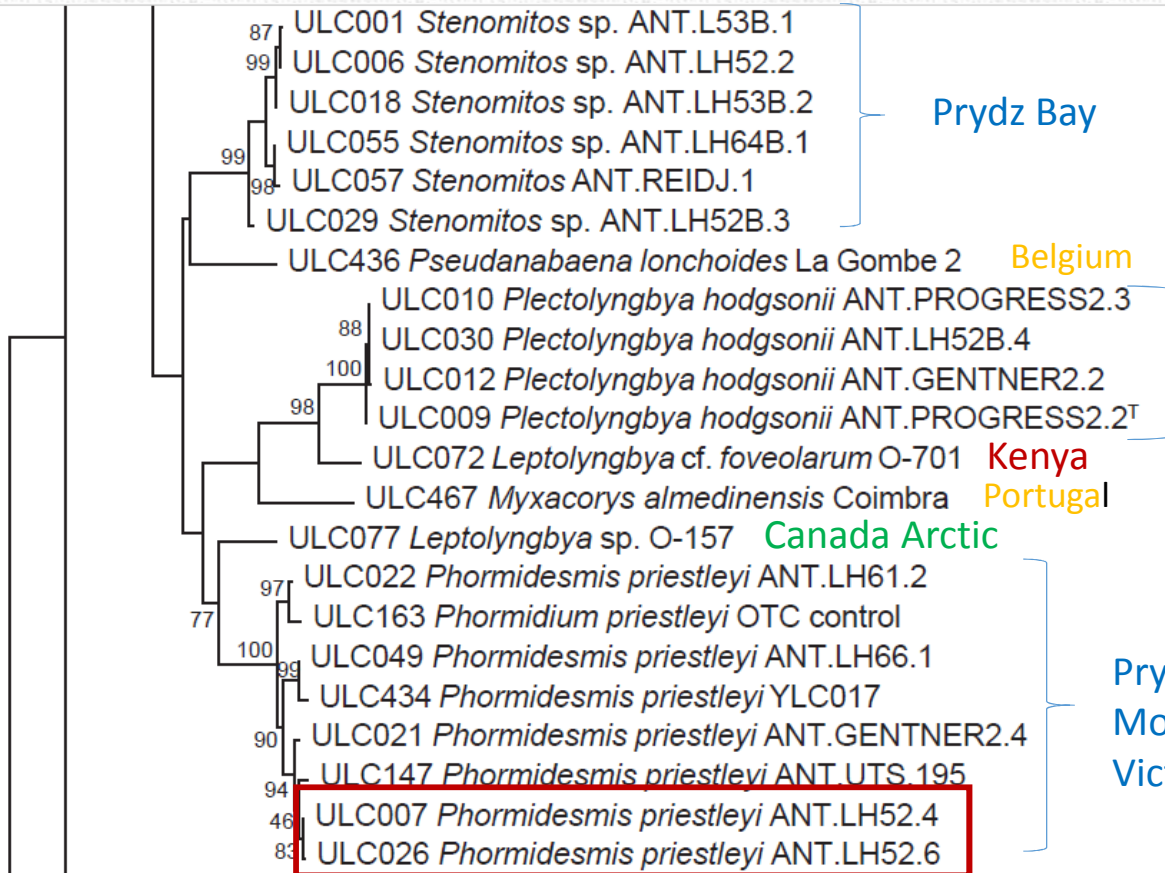
200  $\mu$ l of the diluted fungal cultures plus 20  $\mu$ l of extracts in multiwell plate



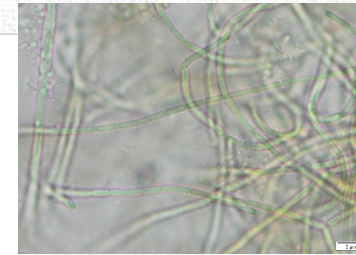
**Growth inhibition of *Candida kruzeii* by the secondary metabolites in the purified extract from ULC009 (right upper corner).**



Filamentous genera: 18 Antarctic and 1 Arctic strains



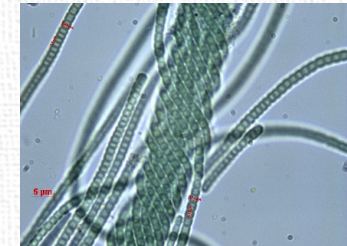
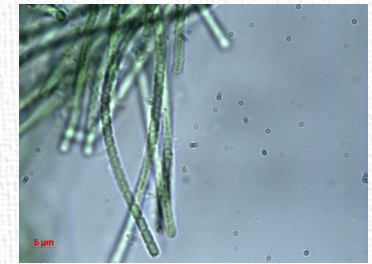
Prydz Bay



Prydz Bay




Prydz Bay, Sør Rondane Mountains and North Victoria Land





# *Phormidesmis priestleyi* BCCM/ULC007: first genome sequence of Antarctic cyanobacterial strain

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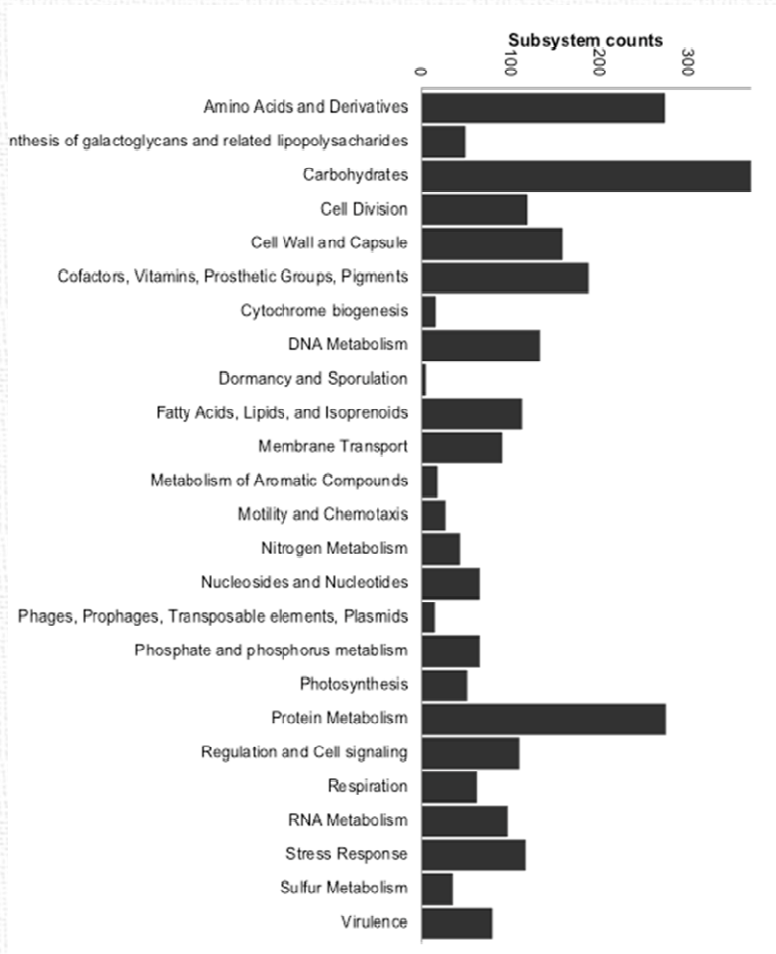


**Draft Genome Sequence of the Axenic Strain *Phormidesmis priestleyi* ULC007, a Cyanobacterium Isolated from Lake Bruehwiler (Larsemann Hills, Antarctica)**

Yannick Lara,<sup>a</sup> Benoit Duriou,<sup>a</sup> Luc Cornet,<sup>b,c</sup> Olivier Verlatne,<sup>a</sup> Rosmarie Rippka,<sup>d</sup> Igor S. Pessi,<sup>a</sup> Agnieszka Misztak,<sup>b</sup> Bernard Joris,<sup>a</sup> Emmanuelle J. Javaux,<sup>c</sup> Denis Baurain,<sup>b</sup> Annick Wilmotte<sup>a,\*</sup>

Total length : 5 684 389 bp

Annotation:  
5,604 PEGs (protein encoding genes)  
of which 4,785 PEGs already known

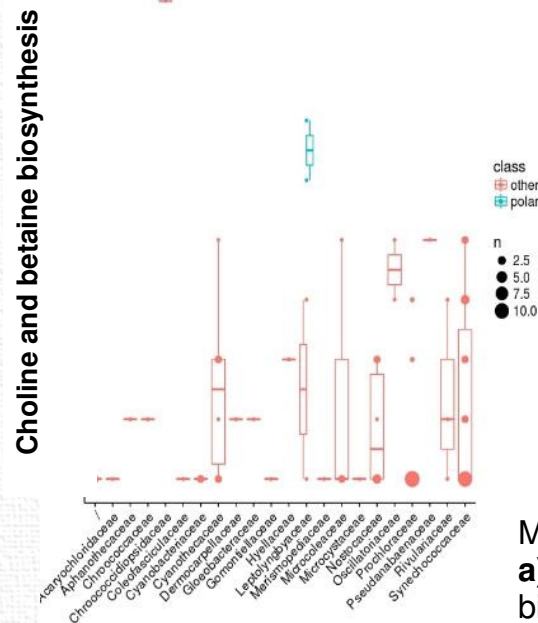
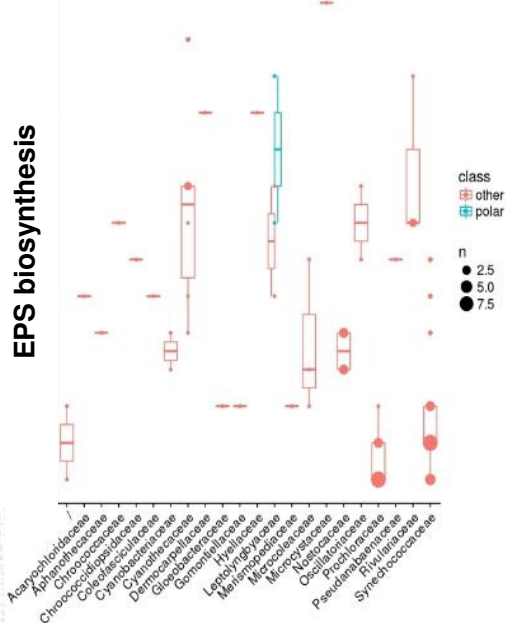
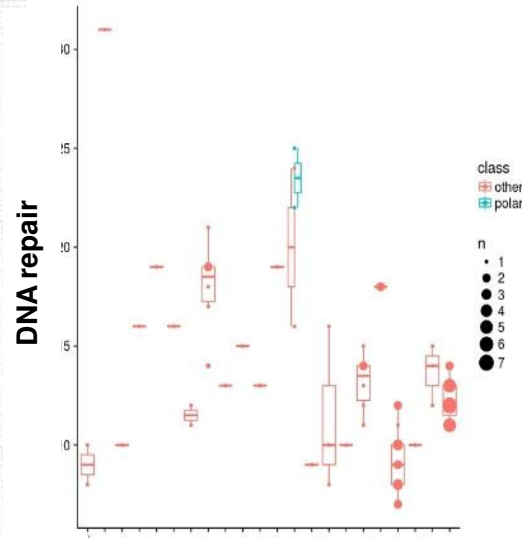
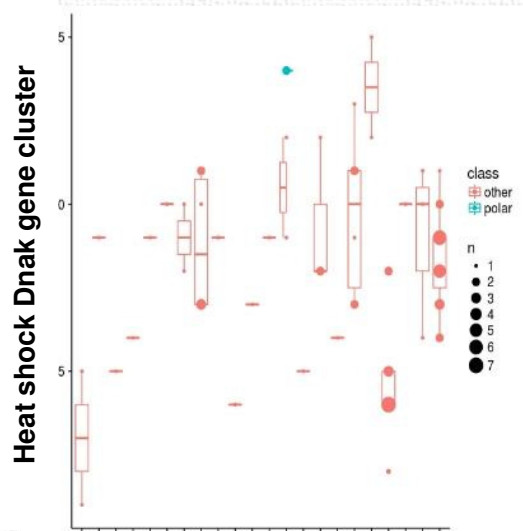


## RAST: Rapid Annotations using Subsystems Technology

Aziz et al., BMC Genomics, 2008

Number of ULC007 genes in each subsystem





Taxonomy (families sensu Komarek et al. 2014)

**69 complete and representative genomes**  
+ *Leptolyngbya boryana* PCC 6306  
+ *P. priestleyi* BC 1401 (Arctic)

Even within the Leptolyngbyaceae family, the 2 polar genomes (blue) have **highest number of genes** in the 4 functional categories involved in response to cold stress

Median and quartile of occurrence for 4 subsystems :  
**a)** DnaK gene cluster, **b)** DNA repair, **c)** EPS biosynthesis, **d)** Choline and betaine biosynthesis.



In Polar regions, low temperatures induce **molecular and cellular disturbances** (e.g. rigidity of membranes, reduction of enzyme-catalyzed reactions, inefficiency of solute transports). Therefore, these harsh conditions select organisms that present particular adaptations.

Our results underline the presence in its genome of functional categories of genes involved in the **production of key molecules** (e.g. exopolysaccharides, fatty acids and the chaperone). These molecules limit cellular and biochemical damages (e.g. EPS protect against freezing by buffering temperature fluctuations around 0°C, fatty acids decrease membranes rigidity).

The success of *P. priestleyi* ULC007 could be explained by different **functional processes** such as exopolysaccharide (EPS) biosynthesis, DNA repair, osmoprotection, etc.



## Bioactivity of ULC026

Antibiotic effect of strain ULC026 against the fungi *Phoma*



Fig. 3. *Phoma*

Antibiotic effect of strain ULC026 against the fungi *Cercospora*

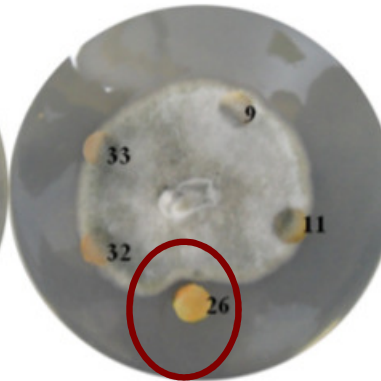


Fig. 4. *Cercospora*

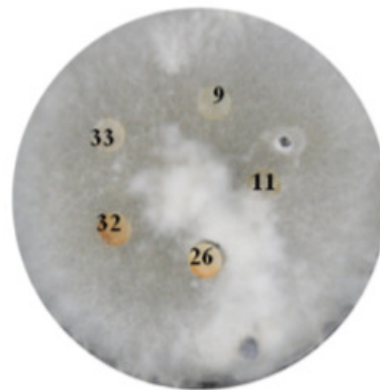


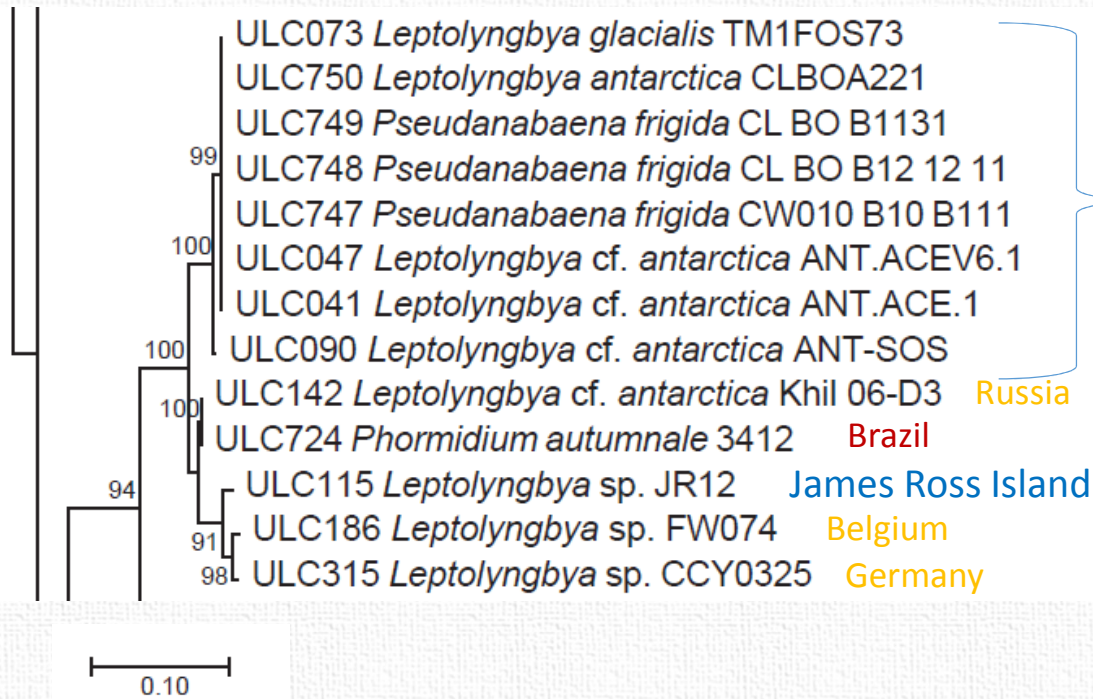
Fig. 5. *Sclerotinia*

Bilateral cooperation (FNRS/CNPq) with Estella Stenico and Marli de Fatima Fiore (CENA, Piracicaba)



Filamentous genera: 9 Antarctic strains

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Prydz Bay, Enderby Land,  
 McMurdo Ice Shelf,  
 Transantarctic Mountains

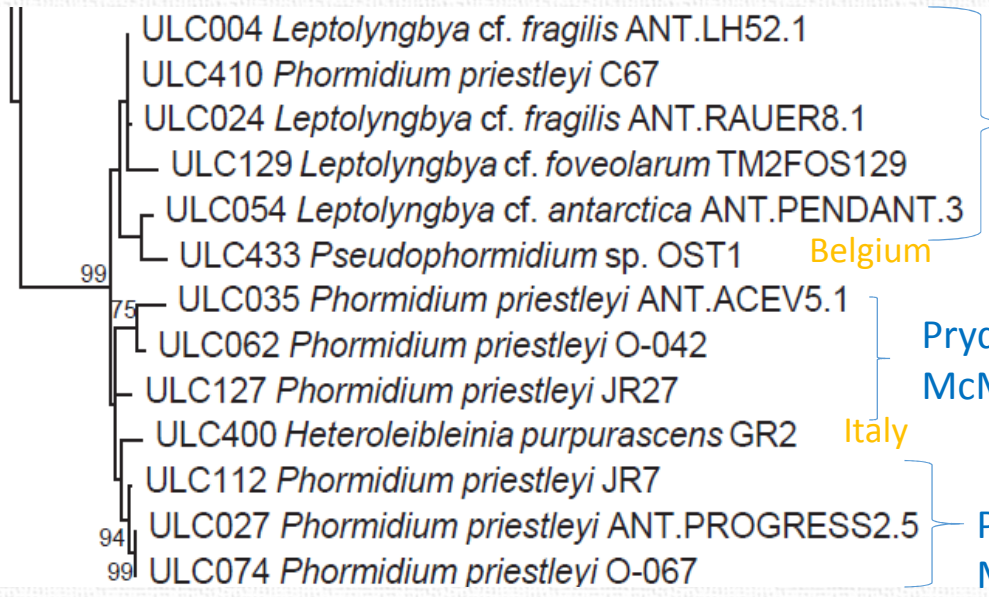






# Filamentous genera: 11 Antarctic strains

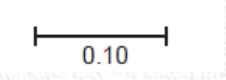
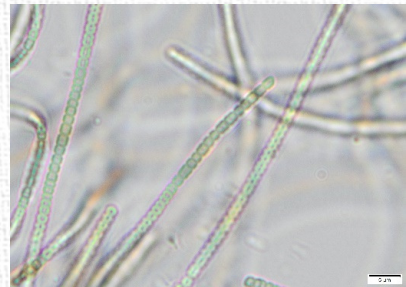
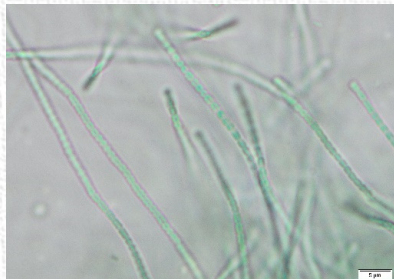
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Prydz Bay and Transantarctic Mountains

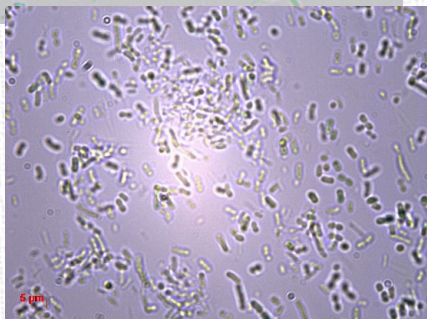
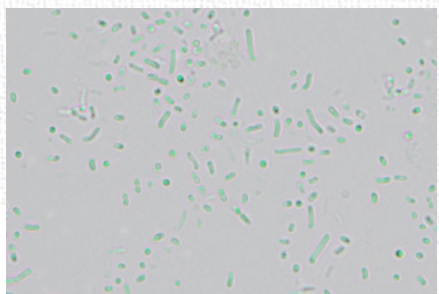
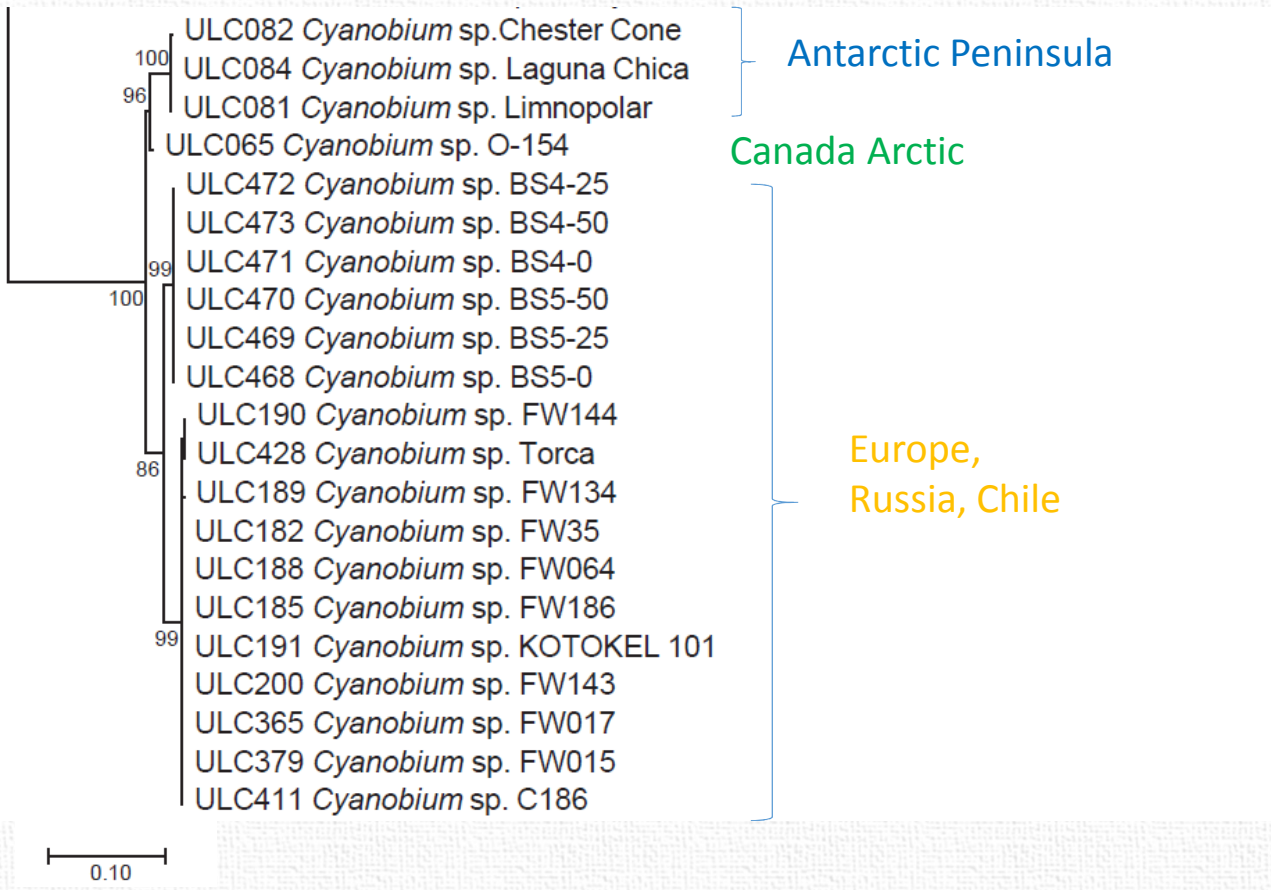
Prydz Bay, James Ross Island and McMurdo Ice Shelf

Prydz Bay, James Ross Island and McMurdo Ice Shelf





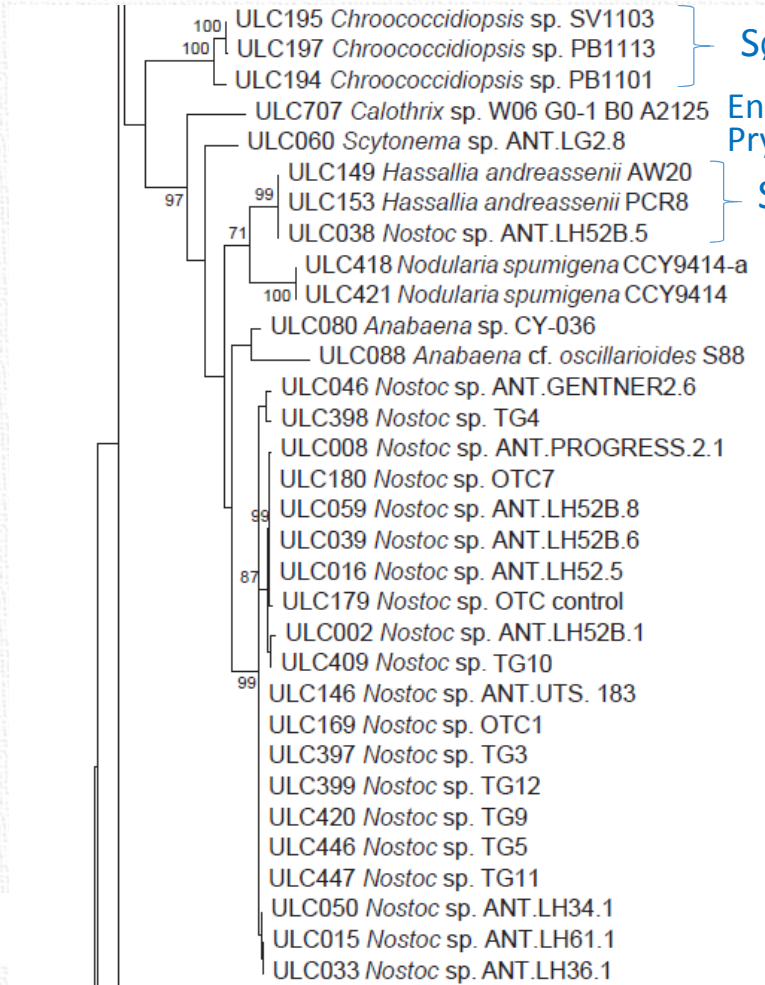
Synechococcales genera: **10 Antarctic** and **1 Arctic** strain



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# Chroococciopsidales + Nostocales genera: 28 Antarctic strains



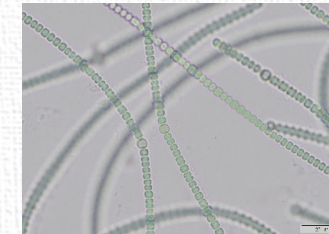
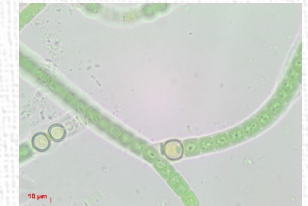
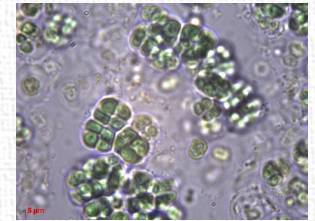
Sør Rondane Mountains

Enderby Land  
Prydz Bay

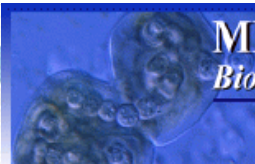
Sør Rondane Mountains and Prydz Bay

Baltic Sea

Sør Rondane Mountains  
and Prydz Bay







# MICROMAT

*Biodiversity of Microbial Mats in Antarctica*

## Cyanobacterial hits

16rRNA OTU	Morphotype	Identification	Hits	Activity on <i>S.aureus</i>	Activity on <i>A.fumigatus</i>	Activity on <i>C.neoformans</i>	Cytotoxicity
16ST03 New	OS-II	<i>Phormidium pristleyi</i>	ANT. LULC007	0	512	1024	0
16ST03 New	OS-II	<i>Phormidium pristleyi</i>	ANT. LULC026	8	512	512	160
16ST01 New	OS-I	<i>Pseudo phormidium</i> sp.	ANT. PROGRESS 2.2 LULC009	64	0	512	640
16ST13 Ant	OS-V	<i>Leptolyngbya antarctica</i>	ANT. GENTNER 2.3	64	0	0	640
16ST19	NO-I	<i>Nostoc</i> sp.	ANT. LH34.1 LULC050	8	0	0	0

Microbiological activity: the highest dilution which inhibits 80% of the pathogen growth

Cytotoxicity: the highest dilution which inhibits 40% of HeLa cell thymidine uptake

Biondi et al. 2008





→ Renewed interest for **biotechnological exploitation** of cyanobacteria, with a steadily increasing number of Antarctic cyanobacteria found to produce a variety of biologically active compounds





## Policy view on bioprospecting



→ Current discussion at ATCM level concerning the exploitation of Antarctic genetic resources and how this fits with the Antarctic Treaty and the Madrid Protocol. Belgium has been active on this topics from 2007 to 2014:

WP11 at ATCM31

WP01, WP26 and IP70 at ATCM32

WP01 at ATCM33

IP22 and IP63 at ATCM35

WP48 and IP22 at ATCM36

WP12 at ATCM37







BCCM · ULC

In 2009, Belgium (Federal Ministry of Environment) and the United Nations Environment Programme (UNEP) decided to create a database on bioprospecting activities in Antarctica: **Bioprospector**. It has been developed by the United Nations University Institute of Advanced Studies (UNU-IAS) on basis of public information, and was freely accessible on the Internet. It was discontinued due to lack of financing.

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Belgian  
Science  
Policy  
Office

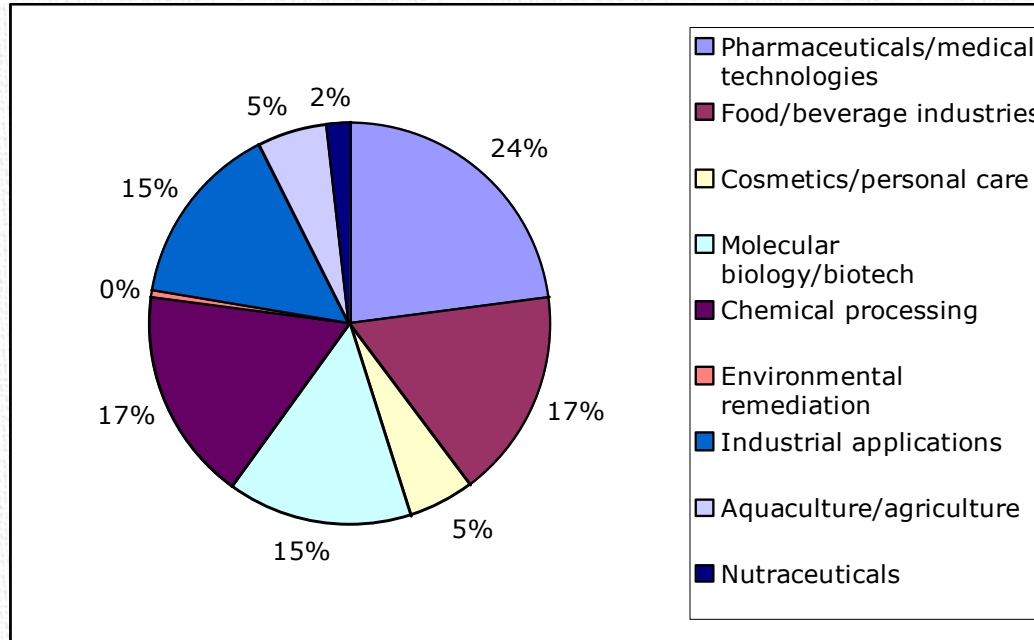
The screenshot shows a web browser window with the URL <http://www.bioprospector.org/bioprospector/antarctica/home.action>. The browser's address bar also shows 'Netscape.com', 'News Sites', and 'New to Flock?'. The website header features the text 'BIOPROSPECTING INFORMATION RESOURCE' and 'BETA'. Logos for 'UNITED NATIONS UNIVERSITY', 'UNU-IAS Institute of Advanced Studies', and 'UNEP federal public service HEALTH, FOOD CHAIN SAFETY AND ENVIRONMENT' are visible. A navigation menu on the left includes 'HOME', 'ABOUT', 'CONTACT US', 'RESOURCES', and 'PARTNERSHIPS'. The main content area is titled 'ANTARCTIC' and contains the following text: 'ANTARCTIC BIOPROSPECTOR provides details of research and commercialised products arising from biological samples that were sourced from the Antarctic region. Details of all activities registered with the database are collected and collated by UNU-IAS staff and through collaborative research. Records are accessible through the **search database** facility below. Each search will return all relevant information, such as: the title of the record; a short description; the country sponsoring the original collection mission; the organisation commercialising the product; taxonomy of the biological sample; and reference information.' Below this text is a 'BROWSE ALL' button. A 'Quick Search' section includes a search input field and a 'Filters' section with three dropdown menus: 'Area of research', 'Organisation name', and 'Government'. A 'SEARCH' button is located at the bottom right of the search section. At the bottom of the page, the text '187 records in 2009' is displayed.





## UNU/IAS database on bioprospecting in Antarctica

[www.bioprospector.org/bioprospector/Antarctica/home.action](http://www.bioprospector.org/bioprospector/Antarctica/home.action)



Users of Antarctic genetic resources

*Source: UNU & Federal public service Health, Food chain safety and Environment (Belgium), as contribution to the discussion of the Committee of Environmental Protection of the Antarctic Treaty on bioprospecting*







XXXIII Antarctic Treaty Consultative Meeting  
3<sup>rd</sup> to 14<sup>th</sup> May, 2010

Punta del Este - Uruguay

IP 96

Agenda Item: ATCM 17

Presented by: Belgium, UNEP

Original: English

## The Role of *Ex-Situ* Collections in Antarctic Bioprospecting

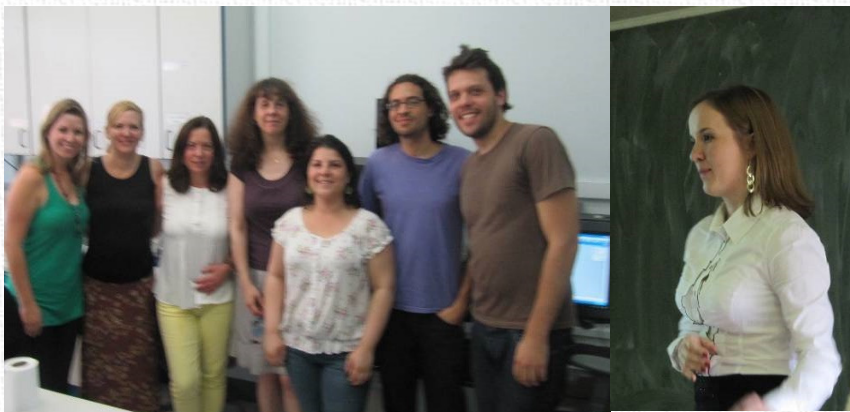
Antarctic bioprospecting is a reality and the current 'status quo' cannot be prolonged indefinitely in view of what is happening in other international fora like the Convention on Biological Diversity.





Many thanks to RW Castenholz, W. Vincent, J. Elster, L. Kovacik, H.D. Laughinghouse, Z. Namsaraev, Y. Lara, R. Fernandez-Carazo, P. De Carvalho Maalouf, MJ Mano, G. Tahon for deposits

### Bioassays for bioactive compounds



Bilateral cooperation (FNRS/CNPq) with Estella Stenico and Marli de Fatima Fiore (CENA, Piracicaba). Thanks to Magda Calusinska

