

## Final project report

*Project ID:* 2002/1.05  
*Title:* Bacteria from the Antarctic marine environment: biodiversity and biotechnological potential

*Principal investigator:* Prof. Viviana Bruni  
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*Duration:* two years  
*Assigned funding:* € 161.129,44

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### Activities and results

Research activities were carried out both in Antarctica and in Italy. Superficial seawater and marine sediments for microbiological analyses were collected during the XVIII Italian Antarctic Expedition (Austral Summer 2002/2003). The preliminary treatment of samples, needed to preserve them during transportation to Italy, was performed at the "Mario Zucchelli" Research Base. Sample aliquots were spread on both Marine Agar and selective media. In addition, for the isolation of hydrocarbon-degrading bacteria, enrichment cultures were carried out by adding diesel oil, tetradecan or naphthalene to the basal medium ONR7a.

Until in Italy, samples were processed for: 1) taxonomic characterization of bacterial isolates by using biomolecular techniques; 2) screening of the isolates for the production of cold-adapted enzymes (lipases and esterases) and antibiotics, which were biochemically characterized after extraction; 3) screening of the isolates for the degradation of hydrocarbons and the estimation of their biodegradative potential; 4) test for heavy metal tolerance. It could be noted that investigations were also carried out on bacteria belonging to the Italian Collection of Antarctic Bacteria (CIBAN) of the Antarctic National Museum.

Bacterial isolates mainly belonged to the gamma-Proteobacteria, followed by the *Bacteroidetes*, the Actinobacteria and at a less extent by the alpha-Proteobacteria and *Firmicutes*.

The majority of screened isolates resulted lipolytic and most of them hydrolyzed all the substrates tested, suggesting the potential production of lipases and esterase with a different degree of specificity. In addition, data obtained suggest the predominance of bacteria with lipases active on short-chain triglycerides. The influence of different environmental factor was also investigated for bacteria belonging to the genera *Psychrobacter*, *Vibrio* and *Pseudoalteromonas*. Finally, further biochemical studies were performed on the previously isolated lipolytic bacterium *Psychrobacter* sp. TAD1.

Antimicrobial assays were carried out against bacteria both of terrestrial origin (and pathogenic for man) and retrieved from Antarctic marine environment. A low percentage of Antarctic isolates (4.9 %) inhibited the growth of at least one pathogen used as a target, whereas 16 % of them resulted active against autochthonous bacteria. Active bacteria mainly belonged to the *Actinomycetales* and the  $\gamma$ -*Proteobacteria*.

Diesel oil biodegradation was deeply investigated for *Rhodococcus*, *Arthrobacter* and *Alcaligenes* isolates, by performing gas-chromatographic analysis and searching for *alkB* genes.

All isolates were sensitive to mercury (range 10-10000 ppm), whereas most of them (mainly affiliated to  $\gamma$ -Proteobacteria) resulted generally tolerant to cadmium, copper and zinc until the concentration of 1000 ppm. Any correlation seemed to exist between the occurrence of plasmid molecules and the heavy metal tolerance.

## Programma Nazionale di Ricerche in Antartide (PNRA)

### Products

#### A – papers in scientific magazines

1. DE DOMENICO M., LO GIUDICE A., MICHAUD L., SAITTA M., BRUNI V. (2004). Diesel oil and PCB-degrading bacteria isolated from Antarctic seawaters (Terra Nova Bay, Ross Sea). *Polar Research*, 23 (2): 141-146. IF 2007: 0.778.
2. MICHAUD L., LO GIUDICE A., SAITTA M., DE DOMENICO M., BRUNI V. (2004). The biodegradation efficiency on diesel oil by two psychrotrophic Antarctic marine bacteria during a two-month-long experiment. *Marine Pollution Bulletin*, 49 (5-6): 405-409. IF 2007: 2.334.
3. MICHAUD L., DI CELLO F., BRILLI M., FANI R., LO GIUDICE A., BRUNI V. (2004). Biodiversity of cultivable Antarctic psychrotrophic marine bacteria isolated from Terra Nova Bay (Ross Sea). *FEMS Microbiology Letters*, 230: 63-71. IF 2007: 2.274.
4. YAKIMOV M.M., GENTILE G., BRUNI V., CAPPELLO S., D'AURIA G., GOLYSHIN P.N., GIULIANO L. (2004). Crude oil-induced structural shift of coastal bacterial communities of rod bay (Terra Nova Bay, Ross Sea, Antarctica) and characterization of cultured cold-adapted hydrocarbonoclastic bacteria. *FEMS Microbiology Ecology*, 49: 419-432. IF 2007: 3.039.
5. FERRER M., LÜNSDORF H., CHERNIKOVA T.N., YAKIMOV M., TIMMIS K.N., GOLYSHIN P.N. (2004). Functional consequences of single:double ring transitions in chaperonins: life in the cold. *Molecular Microbiology*, 53: 167-182. IF 2007: 5.462.
6. GENTILE G., GIULIANO L., D'AURIA G., SMEDILE F., AZZARO M., DE DOMENICO M., YAKIMOV M.M. (2006). Study of bacterial communities in Antarctic coastal waters by a combination of 16S rRNA and 16S rDNA sequencing. *Environmental Microbiology*, 8: 2150-2116. IF 2007: 4.929
7. LO GIUDICE A., MICHAUD L., de PASCALE D., DE DOMENICO M., di PRISCO G., FANI R., BRUNI V. (2006). Lipolytic activity of Antarctic cold-adapted marine bacteria (Terra Nova Bay, Ross Sea). *Journal of Applied Microbiology*, 101 (5): 1039-1048. IF 2007: 2.501.
8. PEPI M., CESÀRO A., LIUT G., BALDI F. (2005). An Antarctic psychrotrophic bacterium *Halomonas* sp. ANT-3b, growing on n-hexadecane, produces a new emulsifying glycolipid. *FEMS Microbiology Ecology*, 53: 157-166. IF 2007: 3.039.
9. PINI F., GROSSI C., NEREO S., MICHAUD L., LO GIUDICE A., BRUNI V., BALDI F., FANI R. (2007). Molecular and physiological characterisation of psychrotrophic hydrocarbon-degrading bacteria isolated from Terra Nova Bay (Antarctica). *European Journal of Soil Biology*, 43: 368-379. IF 2007: 0.500

#### B – book chapters

None

#### C - proceedings of international conferences

1. LO GIUDICE A., MICHAUD L., DE DOMENICO M., BRUNI V. (2005). Antagonism among Antarctic marine bacteria (Terra Nova Bay, Ross Sea). 9TH Symposium on Aquatic Microbial Ecology (Same-9), Helsinki (Finland).
2. GENTILE G., GIULIANO L., SMEDILE F., BRUNI V., YAKIMOV M.M. (2005). Study of bacterial communities in Antarctic marine areas by a combination of 16s rRNA and 16s rDNA gene sequencing. 8th Symposium on Bacterial Genetics and Ecology (BAGECO-8), Lyon (France).
3. PINI F., RICCI S., BRUNI V., BALDI F., FANI R. (2005). Molecular and genetic characterization of psychrotrophic hydrocarbon-degrading bacteria isolated from terra Nova bay (Antarctica). 3rd European Bioremediation Conference, Chania (Crete).
4. de PASCALE D., LO GIUDICE A., MICHAUD L., di PRISCO G., BRUNI V. (2004). Antarctic bacteria as source of novel enzymatic activities: screening for lipolytic enzymes. XXVIII SCAR, Bremen (Germany).
5. PINI F., RICCI S., LO GIUDICE A., BRUNI V., BALDI F., FANI R. (2005). Molecular and genetic characterization of psychrotrophic hydrocarbon-degrading bacteria isolated from terra Nova bay (Antarctica). 3rd International Conference on the Oceanography of the Ross Sea Antarctica, Venezia (Italy).
6. MICHAUD L., LO GIUDICE A., DI MARCO G., DE DOMENICO M., FANI R., BRUNI V. (2006). Psychrotolerant polychlorobiphenyl-degrading bacteria isolated from a coastal station in the Terra Nova Bay (Ross Sea, Antarctica). International Conference on Alpine and Polar Microbiology, Innsbruck (Austria).

#### D – proceedings of national meetings and conferences

1. GROSSI C., NEREO S., BRILLI M., MICHAUD L., LO GIUDICE A., BRUNI V., FANI R. (2005). Isolation and phenotypic and molecular characterization of hydrocarbon-degrading bacteria isolated from Terra Nova Bay (Antarctica). *Polarnet Technical Report*, PTR-1: 157-165.
2. de PASCALE D., MEOLI L., di PRISCO G. (2005) Cold-active lipasi from the Antarctic psychrotolerant bacterium *Psychrobacter* sp TAD1. *Polarnet Technical Report*, PTR-1: 153-156.

## **Programma Nazionale di Ricerche in Antartide (PNRA)**

3. DE DOMENICO M., GENTILE G., MICHAUD L., LO GIUDICE A., BRUNI V. (2005). Characterization of hydrocarbon-degrading cold-adapted bacteria isolated from Antarctic superficial seawaters in the Road Bay (Terra Nova Bay). Polarnet Technical Report, PTR-1: 23-29.
4. LO GIUDICE A., MICHAUD L., PRESTINENZI M., BRUNI V. (2005). Influenza di temperatura, pH e NaCl sull'attività lipolitica di batteri marini antartici psicrotrofi. *Biologia Marina Mediterranea*, 12 (1): 695-697.
5. LO GIUDICE A., MICHAUD L., GENTILE G., DE DOMENICO M., BRUNI V. (2005). Ecophysiological characterization of cultivable Antarctic psychrotolerant marine bacteria able to degrade hydrocarbons. *Atti dell'Accademia Peloritana dei Pericolanti*. LXXXIII: 1-12.
6. MICHAUD L., LO GIUDICE A., BRUNI V. (2004). Characterization of hydrocarbon degrading psychrotrophic bacteria isolated from the sea surface micro-layer. Incontro Congiunto CoNISMa (IV) e AIOL (XVI), Terrasini (PA).
7. LO GIUDICE A., MICHAUD L., PRESTINENZI M., BRUNI V. (2004). Influenza di temperatura, pH e NaCl sull'attività lipolitica di batteri marini antartici psicrotrofi. XXXV Congresso della Società Italiana di Biologia Marina (SIBM-35).
8. LO GIUDICE A., MICHAUD L., BRUNI V. (2004). Bacterium-bacterium inhibitory interactions among Antarctic marine isolates and their antibiotic activity against human pathogens. Incontro Congiunto CoNISMa (IV) e AIOL (XVI), Terrasini (PA).

### **E – thematic maps**

None

### **F – patents, prototypes and data bases**

None

### **G – exhibits, organization of conferences, editing and similar**

1. Organization of the 5th Meeting on Antarctic Biology, Messina, 29-30 april 2004.

### **H - formation (PhD thesis, research fellowships, etc.)**

1. PINI F.: Analisi della biodiversità di microrganismi antartici. Degree in Biological Sciences, University of Firenze, AA 2004-05. Supervisor: Prof. Renato Fani.
2. SCIARRONE R.: Caratterizzazione fisiologica e fenotipica di batteri psicrotrofi isolati da un piccolo sistema lacustre antartico (lago n° 14 – Edmonson Point). Degree in Biological Sciences, University of Messina, AA 2004-05. Supervisor: Prof.ssa M. De Francesco.
3. PRESTINENZI M.: Influenza congiunta di alcuni fattori abiotici (pH, temperatura, NaCl) sull'attività lipolitica di batteri marini antartici psicrotrofi. Degree in Biological Sciences, University of Messina, AA 2004-05. Supervisor: Prof.ssa V. Bruni.
4. MANGANO S.: Caratterizzazione fenotipica e fisiologica di alcuni ceppi batterici isolati in un piccolo lago presso Edmonson Point (Antartide). Degree in Marine Biology and Ecology (BEM), University of Messina, AA 2003-2004. Supervisor: Prof.ssa M. De Francesco, Co-Tutor: Dr. G. Gentile.
5. NEREO S.: Analisi di comunità microbiche idrocarburo-degradanti isolate dal Mare di Ross (Antartide). Degree in Biological Sciences, University of Firenze, AA 2003-04. Supervisor: Prof. Renato Fani.
6. GROSSI C.: Caratterizzazione fisiologico-molecolare di batteri antartici idrocarburo degradanti. Degree in Biological Sciences, University of Firenze, AA 2003-04. Supervisor: Prof. Renato Fani.

## Programma Nazionale di Ricerche in Antartide (PNRA)

### Research units

#### UO-1: University of Messina – Dept. Animal Biology and Marine Ecology (DBAEM)

Name	Role	Institution
Maria De Francesco	Associate Professor	Dept. Animal Biology and Marine Ecology
Angelina Lo Giudice	Researcher	Dept. Animal Biology and Marine Ecology
Luigi Michaud	Ph.D. Student	Dept. Animal Biology and Marine Ecology
Simona Scarfi	Researcher	Dept. Animal Biology and Marine Ecology
Gaetano Di Marco	Researcher	IPCF-CNR

#### UO-2: University of Florence – Dept. Animal biology and Genetics

Name	Role	Institution
Renato Fani	Associate Professor	Dept. Animal biology and Genetics
Matteo Brilli	Ph.D. Student	Dept. Animal biology and Genetics
Marco Fondi	Ph.D. Student	Dept. Animal biology and Genetics

#### UO-3: University of Venice – Dept. Environmental Sciences

Name	Role	Institution
Franco Baldi	Full Professor	Dept. Environmental Sciences
Francesco Pini	Researcher	Dept. Environmental Sciences
Davide Marchetto	Researcher	Dept. Environmental Sciences

#### UO-4: CNR, Section of Messina – Institute for the Coastal Marine Environment (IAMC)

Name	Role	Institution
Laura Giuliano	Researcher	IAMC
Mikhail Yakimov	Researcher	IAMC
Gabriella Gentile	Post-doc fellow	IAMC

#### UO-5: CNR, Section of Naples – Institute for Protein Biochemistry (IBP)

Name	Role	Institution
Donatella de Pascale	Researcher	IAMC
Guido di Prisco	Research Manager	IAMC
Vito Carratore	Technician	IAMC

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**Date: 11/11/2008**

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