Final project report

Project ID	2004/1.06
Title	Antarctic bacteria and cyanobacteria: biodiversity and production of biotechnologically exploitable compounds
Principal investigator	Prof. Vivia Bruni
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Duration	3 years
Assigned funding	300.000,00 Euro

Activities and results

Bacteria and cyanobacteria were studied in order to assess **1)** their phylogenetic affiliation by targeting both the whole community and the cultivable fraction and **2)** their potentialities in biotechnology as *2a*) producers of substances to be industrially exploited and *2b*) detoxification agents in contaminated environments.
Research activities were carried out both in Antarctica and in Italy. Samples were collected during three Antarctic Expeditions as follows: Austral Summers 2004-2005 from Terra Nova Bay, 2008-2009 from Byers Peninsula (South Shetland Islands) in collaboration with Spanish colleagues involved in the IPY Project "Limnopolar" and, finally, 2009-2010 from Taylor Valley (Marr Ponds) in collaboration with American colleagues involved in the "Long Term Ecological Research (LTER)" Project. In Italy, part of the research activities targeted bacterial strains belonging to the Italian Collection of Antarctic Bacteria (CIBAN-MNA), in addition to newly isolates.
The main achievements of the Project 2004/1.06 are summarized below.

1) Phylogenetic characterization.
- Marine water column.
Samples: seawater samples from six stations at Terra Nova Bay (Ross Sea).
Main results: Isolates (total 606) were predominantly *Gammaproteobacteria*, whereas the FISH analysis revealed that *Bacteroidetes* were equally dominant with *Actinobacteria* and *Gammaproteobacteria*.
- Northern Victoria land lakes.
Samples: water samples from lakes at Crater Cirque, Inexpressible Island and Luther Peak.
Main results: Overall, isolates (total 478) were predominantly *Gammaproteobacteria* and *Bacteroidetes*, whereas the culture-independent methods generally revealed the predominance of *Bacteroidetes* (Inexp. Island) or *Actinobacteria* (other two lakes).
- Marine invertebrates.
Samples: specimens of *L. nobilis*, *P. glaberrima*, *M. hanitschi*, *H. dancoi*, *H. pilosa* and *T. charcoti* from Terra Nova Bay.
Main results: predominance of *Gammaproteobacteria* among the 585 isolates; sponge species-specificity of the bacterial community was revealed by DGGE.
- Marine sediments.
Samples: coastal sediment from Terra Nova Bay.
Main results: simultaneous occurrence of anaerobic and aerobic bacterial species was determined by cloning and sequencing. Clones were mainly affiliated to *Alpha-* and *Deltaproteobacteria*, *Bacteroidetes* and Acidobacteria.
- Cyanobacteria.
Samples: algal mats, water and sediment from Terra Nova Bay.
Main results: predominance of *Nostoc* sp. and *Leptolyngbya* sp., followed by the genera *Plectonema*, *Oscillatoria*, *Lyngbya*, *Phormidium*, *Hormoscilla* and *Cyanobacterium*.

2) Biotechnological potential.

- *Exopolysaccharides (EPS)*.

EPS production was deepen investigated for 5 isolates (out of 2426) by varying cultivation conditions. EPS production was generally enhanced by higher C/N ratios, low temperature, pH around 7 and 3% NaCl. EPS amounts varied between 34 and 130 mg/l. Chemical composition of EPS was analyzed. Emulsification and cryoprotective actions, as well as the interaction with heavy metal were investigated.

- *Antibiotic and cytotoxic activities.*

Antibiotic activity against pathogens was assayed for strains from different Antarctic matrices. The highest percentage of active isolates (12.8%) was recorded among those associated with sponges. Active isolates were mainly affiliated to the *Actinobacteria* and *Gammaproteobacteria*. Among *Cyanobacteria* significative cytotoxic activities were observed for *Nostoc* sp.

- *Enzymes*.

Particular regard was paid to the enzymes HSL of *Psychrobacter* sp. TA144 and PhTACLip1 of *P. haloplanktis*, involved in the metabolism of lipids. In order to better understand molecular bases of adaptation to low temperatures, structural and functional features were analyzed by enzyme cloning and expression.

- *Biosurfactants*.

A total of 42 (out of 280) isolates from enrichment cultures on hydrocarbons were selected as they showed emulsification properties. The thin layer chromatography put on evidence their glycolipidic nature.

Products

A – papers in scientific magazines

1. BALDI F., MARCHETTO D., PINI F., FANI R., MICHAUD L., LO GIUDICE A., BERTO D., GIANI M. (2010). Biochemical and microbial features of shallow marine sediments along the Terra Nova Bay (Ross Sea, Antarctica). *Continental Shelf Research*, 30: 1614-1625.
2. HROUZEK P., TOMEK P. LUKESOVA A., URBAN J., VOLOSHKO L. PUSHPARAJ B., VENTURA S., LUKAVSKY J., TYS D., KOPECKY J. (in stampa). Cytotoxicity and secondary metabolites production in terrestrial *Nostoc* strains, originating from different climatic/geographic regions and habitats: is their cytotoxicity environmentally dependent? Environmental Toxicology. DOI: 10.1002/tox.20561
3. DE PASCALE D., GIULIANI M., DE SANTI C., BERGAMASCO N., AMORESANO A., CARPENTIERI A., PARRILLI E., TUTINO M.L. (2010). PhAP protease from *Pseudoalteromonas haloplanktis* TAC125: gene cloning, recombinant production in E. coli and enzyme characterization. *Polar Science*.
4. DE SANTI C., TUTINO M.L., MANDRICH .L, GIULIANI M., PARRILLI E., DEL VECCHIO P., DE PASCALE D. (2010). The hormone sensitive lipase from *Psychrobacter* TA144: new insight in the structural/functional characterisation. *Biochimie*, Apr 9. PMID: 20382198
5. LO GIUDICE A., CASELLA P., CARUSO C., MANGANO S., BRUNI V., DE DOMENICO M., MICHAUD L. (2010). Occurrence and characterization of psychrotolerant hydrocarbon–oxidizing bacteria from surface seawater along the Victoria Land coast (Antarctica). *Polar Biology*, 33: 929-943.
6. TUTINO M.L., PARRILLI E., DE SANTI C., GIULIANI M., MARINO G., DE PASCALE D. (2010). Cold-Adapted Esterases and Lipases: A Biodiversity Still Under-Exploited. *Current Chemical Biology*, 4: 74-83.
7. MANGANO S., MICHAUD L., CARUSO C., BRILLI M., BRUNI V., FANI R., LO GIUDICE A. (2009). Antagonistic interactions among psychrotrophic cultivable bacteria isolated from Antarctic sponges: a preliminary analysis. *Research in Microbiology*, 160: 27-37.
8. TUTINO M.L., DI PRISCO G., MARINO G., DE PASCALE D. (2009). Cold-adapted esterases and lipases: from fundamentals to application. *Protein Pept Lett.* 16(10):1172-1180.
9. DE PASCALE D., CUSANO A.M., AUTORE F., PARRILLI E., DI PRISCO G., MARINO G., TUTINO M.L. (2008). The cold-active Lip1 lipase from the Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125 is a member of a new bacterial lipolytic enzyme family. *Extremophiles*, 12(3):311-23.
10. PUSHPARAJ B., BUCCIONI A., PAPERI R., PICCARDI R., ENA E., CARLOZZI P., SILI C. (2008). Fatty acid composition of Antarctic cyanobacteria. *Phycologia*, 47: 430–435.
11. LO GIUDICE A., BRUNI V., MICHAUD L. (2007a). Characterization of Antarctic psychrotrophic bacteria with antibacterial activities against terrestrial microorganisms. *Journal of Basic Microbiology*, 47: 496-505.
12. LO GIUDICE A., BRILLI M., BRUNI V., DE DOMENICO M., FANI R., MICHAUD L. (2007b). Bacterium-bacterium inhibitory interactions among psychrotrophic bacteria isolated from Antarctic seawaters (Terra Nova Bay, Ross Sea). *FEMS Microbiology Ecology*, 60: 383-396.

B – book chapters

1. DE PASCALE D., DE SANTI C. (2010) Antarctic marine bacteria as sources of new cold-adapted lipolytic enzymes with wide range of biotechnological applications. In Volume Mare, le attività di ricerca del CNR (Ed. CNR-DTA), Roma, Italia, *in press*.

2. LO GIUDICE A., BRUNI V. MICHAUD L. (2010). Potential for microbial biodegradation of polychlorinated biphenyls in Polar environments. In: *Polar Microbiology: the Ecology, Diversity and Bioremediation Potential of Microorganisms in Extremely Cold Environments*. Bej A.K., Aislabie J., Atlas R.M. (Eds). CRC Press, Taylor and Francis Group, Chapter 11, pp. 255-275.
3. LO GIUDICE A., BRUNI V., DE DOMENICO M., MICHAUD L. (2010). Psychrophiles-Cold-adapted hydrocarbon-degrading microorganisms. In: *Handbook of Hydrocarbon and Lipid Microbiology*, Timmis K.N. (Ed), Springer Heidelberg, Berlin, Vol 3, pp. 1897-1922.
4. MANDRICH L., DE PASCALE D. (2010) Microbial wetland community as source of hydrolytic enzymes. Study on thermal adaptation of esterases and lipases from marine and non-marine micro-organisms. In "The Marine Environment: Ecology, Management and Conservation". Novapublisher, Hauppauge, NY, U.S.A. *in press*.

C - proceedings of international conferences

1. MICHAUD L. CARUSO C., LO GIUDICE A., BRUNI V., DE DOMENICO M. Diversity of planktonic bacteria of three Antarctic lakes (Northern Victoria Land – Ross Sea). 8th International Congress on Extremophiles, Ponta Delgada (Isole Azzorre, Portogallo), 12-16 settembre 2010.
2. MALAVENDA R., RIZZO C., LO GIUDICE A., MICHAUD L., DE DOMENICO M., BRUNI V. Biosurfactant-producers within the Italian Collection of Antarctic Bacteria (CIBAN). 8th International Congress on Extremophiles, Ponta Delgada (Isole Azzorre, Portogallo), 12-16 settembre 2010.
3. TUTINO ML, DE SANTI C, PARRILLI E, MANDRICH L, GIULIANI M, DE PASCALE D. The HSL from Psychrobacter sp. TAA144: a novel catalyst for fine chemicals production. 14th International Biotechnology Symposium of Human Society. 14-18 September 2010, Rimini, Italy.
4. TUTINO ML, DE SANTI C, PARRILLI E, MANDRICH L, GIULIANI M, DE PASCALE D. The HSL from Psychrobacter sp. TAA144: new insights into molecular cold-adaptation. International Polar Year. Oslo Science Conference, 2010, 8-12 June 2010.
5. LO GIUDICE A., CASELLA P., MALAVENDA R., BRUNI V., MICHAUD L. Characterization of Antarctic marine cold-adapted bacteria able to utilize hydrocarbons. International Symposium "Monitoring Effects of Aquatic Oil Pollution", Leipzig (Germania), 20-22 gennaio 2009.
6. MANGANO S., VENTURI V., LO GIUDICE A., CARUSO C., MICHAUD L., LO PARO G., BRUNI V. Production of acylated homoserine lactones by psychrotrophic bacteria isolated from Antarctic marine sponges. 11TH SYMPOSIUM on Aquatic Microbial Ecology (SAME-11), Piran (Slovenia), 30 agosto - 4 settembre 2009.
7. TUTINO ML, DE SANTI C, GIULIANO M, PARRILLI E, DE PASCALE D. The first homologue of "Hormone Sensitive Lipase" from psychrophiles: cloning, expression in E. coli and characterization of Lip2 from Psychrobacter sp. TAA144. Xth SCAR International Biology Symposium. 26-31 July 2009 Sapporo, Japan.
8. DE PASCALE D, GIULIANO M, DE SANTI C, PARRILLI E, ML TUTINO. The PSHAa2915 protease from Pseudoalteromonas haloplanktis TAC125: gene cloning, recombinant production in E. coli and enzyme characterization. Xth SCAR International Biology Symposium. 26-31 July 2009 Sapporo, Japan.
9. MICHAUD L., LO GIUDICE A., CASELLA P., DE DOMENICO M., BRUNI V. Hydrocarbon- and polychlorinated biphenyl-degrading Antarctic bacteria isolated from coastal environments in the Ross Sea. 14th International Biodeterioration and Biodegradation Symposium (IBBS-14), Sant'Alessio Siculo (Messina), 6-11 ottobre 2008.
10. CARUSO C., LO GIUDICE A., MANGANO S., FANI R., BRUNI V., MICHAUD L. Screening for exopolysaccharide-producing bacteria from Antarctic marine environments. Meeting of the three divisions of the International Union of Microbiological Societies (IUMS). Istanbul (Turchia), 5-15 agosto 2008.
11. MANGANO S., MICHAUD L., BRILLI M., CARUSO C., BRUNI V., FANI R., LO GIUDICE A. Antagonistic activity among marine bacteria associated with Antarctic sponges and application of the network theory to data obtained. Meeting of the three divisions of the International Union of Microbiological Societies (IUMS). Istanbul (Turchia), 5-15 agosto 2008.
12. MANGANO S., CARUSO C., MICHAUD L., LO GIUDICE A., BRUNI V. Assessment of phenotypic features of bacteria isolated from different Antarctic environments. Florence Conference on Phenotype MicroArray Analysis of Microrganisms. Firenze, 19-21 marzo 2008.
13. LO GIUDICE A., CARUSO C., MANGANO S., MAIMONE G., BRUNI V., MICHAUD L. Cultivable bacterial populations along the water column at Terra Nova Bay (Ross Sea, Antarctica). 10th Symposium on Aquatic Microbial Ecology (SAME-10), Faro, Algarve (Portogallo) 2-7 settembre 2007.
14. CARUSO C., MANGANO S., MICHAUD L., BRUNI V., LO GIUDICE A. Characterisation and antimicrobial activity of marine bacteria isolated from the Antarctic sponge Tedania spp. (Porifera, Demospongiae). 10th Symposium on Aquatic Microbial Ecology (SAME-10), Faro, Algarve (Portogallo) 2-7 settembre 2007.
15. MANGANO S., CARUSO C., MICHAUD L., BRUNI V., LO GIUDICE A. Antagonism among marine bacteria isolated from the Antarctic sponges Lissodendoryx nobilis (Porifera, Demospongiae) and Scolymastra joubini (Porifera, Hexactinellida). 10th Symposium on Aquatic Microbial Ecology (SAME-10), Faro, Algarve (Portogallo) 2-7 settembre 2007.
16. MICHAUD L., CARUSO C., MANGANO S., BRUNI V., LO GIUDICE A. Study on the bacterial diversity in Antarctic freshwater environments by culture-dependent and culture-independent approaches. 10th Symposium on Aquatic Microbial Ecology (SAME-10), Faro, Algarve (Portogallo) 2-7 settembre 2007.
17. MICHAUD L., CARUSO C., MANGANO S., BRUNI V., LO GIUDICE A. Phylogenetic characterization of the culturable bacterial community inhabiting three Antarctic lakes. 5th Symposium for European Freshwater Sciences (SEFS-5), Palermo 8- 13 luglio 2007.

18. BRILLI M., LO GIUDICE A., MICHAUD L., BRUNI V., FANI R. EcoNetwork: visualizing interrelationships in microbial communities. 9th Symposium on Bacterial Genetics and Ecology (BAGECO-9), Wernigerode (Germania) 23-27 giugno 2007.
19. MANGANO S., LO GIUDICE A., CARUSO C., MICHAUD L., FANI R., BRUNI V. Characterization and antagonistic activity of marine bacteria associated with Antarctic sponges. 9th Symposium on Bacterial Genetics and Ecology (BAGECO-9), Wernigerode (Germania) 23-27 giugno 2007.
20. MICHAUD L., LO GIUDICE A., CASELLA P., FANI R., BRUNI V. Characterization of hydrocarbon-degrading marine bacteria from coastal environments in the Ross Sea (Antarctica). 9th Symposium on Bacterial Genetics and Ecology (BAGECO-9), Wernigerode (Germania) 23-27 giugno 2007.
21. LO GIUDICE A., MICHAUD L., DI MARCO G., DE DOMENICO M., FANI R., BRUNI V. Exopolysaccharide-producing marine bacteria from Antartica (Terra Nova Bay, Ross Sea). International Conference on Alpine and Polar Microbiology, Innsbruck (Austria) 27-30 marzo 2006.

D – proceedings of national meetings and conferences

1. CARUSO C., PARISI T., MICHAUD L., BRUNI V., MANGANO S., LO GIUDICE A. (2009). Ricerca delle condizioni ottimali di crescita per la sintesi di esopolisaccaridi ad opera di batteri marini antartici psicrotrofi. *Atti della Società Italiana di Ecologia* (S.It.E), XXXI: 31-40.
2. CARUSO C., MANGANO S., MICHAUD L., BRUNI V., LO GIUDICE A. (2009). Diversità e attività antimicrobica di batteri marini coltivabili associati alla spugna antartica *Tedania* spp. (Porifera, Demospongiae). *Atti dell'Associazione Italiana di Oceanologia e Limnologia* Congresso AIOL-SItE,161-164.
3. LO GIUDICE A., BRUNI V., CARUSO C., MANGANO S., MAIMONE G., DE DOMENICO M., MICHAUD L. (2008). Caratterizzazione della comunità microbica coltivabile presente lungo la colonna d'acqua a Baia Terra Nova (Mare di Ross, Antartide). *Atti dell'Associazione Italiana di Oceanologia e Limnologia*, 19 (2): 273-283.
4. MICHAUD L., LO GIUDICE A., CARUSO C, MANGANO S., BRUNI V. (2008). Ricerche sulla biodiversità delle comunità batteriche in tre laghi antartici. *Atti dell'Accademia Peloritana dei Pericolanti,_Classe di Scienze Fisiche, Matematiche e Naturali*, LXXXVI: 1-13.
5. MICHAUD L., LO GIUDICE A., CARUSO C, MANGANO S., BRUNI V. (2008). Caratterizzazione filogenetica della comunità batterica di tre laghi antartici. In: La qualità ambientale del sistema fluviale - Quaderni di Idronomia Montana 28/2: 89-101.
6. MANGANO S., CARUSO C., MICHAUD L., BRUNI V., LO GIUDICE A. (2007). Caratterizzazione e attività antagonistica di batteri marini associati a poriferi antartici. *Biologia Marina Mediterranea*, 14 (2): 314-315.
7. LO GIUDICE A., MICHAUD L., DE DOMENICO M., BRUNI V. (2006). Quantificazione di esopolisaccaridi (EPS) rilasciati nel mezzo colturale (RPS) da batteri marini antartici. *Biologia Marina Mediterranea*, 13 (1): 1015-1019.

E – thematic maps

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F – patents, prototypes and data bases

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G – exhibits, organization of conferences, editing and similar

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

1. LO GIUDICE A. (2006): *Potenzialità applicative di batteri marini antartici in biotecnologia*. Ph D Course in Environmental Sciences: The marine environment and its resources, University of Messina. Supervisor: Prof.ssa V. Bruni.
2. CARUSO C. (2010): *Batteri marini antartici produttori di esopolisaccaridi: caratterizzazione e aspetti biotecnologici*. Ph D Course in Environmental Sciences: The marine environment and its resources, University of Messina. Supervisor: Prof.ssa V. Bruni.
3. MANGANO S. (2010): *Meccanismi di interazione tra batteri coltivabili associati a poriferi antartici (phylum Porifera)*. P
4. Ph course in Cellular Biology and Biotechnology, Dottorato in Biologia e Biotecnologie Cellulari, University of Messina. Supervisor: : Prof. G. Lo Paro; Co-Supervisor: Prof. V. Bruni.
5. PARISI T.: *Isolamento di batteri psicrotrofi associati a Demospongie (phylum Porifera) marine antartiche (Baia Terra Nova, Mare di Ross).* Degree in Marine Biology and Ecology, University of Messina. Tutor: Prof. V. Bruni.
6. CASELLA P.: *Batteri psicrotrofi idrocarburo-degradanti isolati da acque costiere antartiche (Baia Terra Nova, Mare di Ross).* Degree in Marine Biology and Ecology, University of Messina. Tutor: Prof. V. Bruni.
7. MANGANO S.: *Batteri associati a Poriferi antartici: isolamento, caratterizzazione e potenzialità antibiotica.* Degree in Biology and Ecology of the Coastal Marine Environment. Tutor: Prof. V. Bruni.
8. LA GRECA A.: *Applicazione della Fluorescent in situ Hybridization per lo studio della comunità batterioplanctonica in acque costiere antartiche*. Degree in Marine Biology and Ecology, University of Messina. Tutor: Prof. V. Bruni.
9. PARISI T.: *Ricerca delle condizioni ottimali di crescita per la sintesi di esopolisaccaridi ad opera di batteri marini antartici psicrotrofi.* Degree in Biology and Ecology of the Coastal Marine Environment. Tutor: Prof. V. Bruni.

10. CASELLA P.: *Degradazione di policlorobifenili e tolleranza ai metalli pesanti in batteri psicrotrofi isolati da sedimenti marini antartici.* Degree in Biology and Ecology of the Coastal Marine Environment. Tutor: Prof. V. Bruni.
11. MASCIALE V.: *Valutazione dell'attività antimicrobica di ceppi batterici antartici contro patogeni appartenenti al Burkholderia cepacia complex*. Degree in Biology. Tutor:: Prof. Renato Fani
12. MARRA A.: *Batteri psicrotolleranti capaci di crescere su policlorobifenili*. Degree in Biology. Tutor: Prof. Prof. Amalia Lania, Co-tutor: Dott. Angelina Lo Giudice.

Research units

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

Name	Role	Institution
Maria De Francesco	Associate Professor	University of Messina
Angelina Lo Giudice	Researcher	University of Messina
Luigi Michaud	Researcher	University of Messina
Consolazione Caruso	Ph D Student	University of Messina
Santina Mangano	Ph D Student	University of Messina
Filippo Interdonato	Ph D Student	University of Messina
Roberta Malavenda	Ph D Student	University of Messina
Gaetano Di Marco	Researcher	IPCF-CNR

2: University of Florence – Dept. Evolutionary Biology

Name	Role	Institution
Renato Fani	Associate Professor	University of Florence
Matteo Brilli	Ph.D. Student	University of Florence
Marco Fondi	Ph.D. Student	University of Florence
Alessio Mengoni	Researcher	University of Florence
Maria Cristiana Papaleo	Ph.D. Student	University of Florence
Giovanni Emiliani	Researcher	University of Florence
Isabel Maida	Biotechnologist	University of Florence
Elena Perrin	Biologist	University of Florence
Barbara Tarabella	Biologist	University of Florence

3: CNR, Section of Naple – Institure for Protein Biochemistry (IBP)

Name	Role	Institution
Donatella de Pascale	Researcher	IBP-CNR
Guido di Prisco	Research Manager	IBP-CNR
Concetta de Santi	Technician	IBP-CNR

4: CNR, Section of Sesto Fiorentino (FI) – Institute for the Ecosystem Study (ISE-CNR)

Name	Role	Institution
Benjamin Pushparaj	Researcher	ISE-CNR
Giuseppe Torzillo	Researcher	ISE-CNR
Stefano Ventura	Researcher	ISE-CNR
Ena Alba	Researcher	ISE-CNR
Piccardi Raffaella	Researcher	ISE-CNR
Carlozzi Pietro	Technologist	ISE-CNR
Edoardo Pinzani	Technician	ISE-CNR

5: University of Venice – Dept. Environmental Sciences

Name	Role	Institution
Franco Baldi	Full Professor	University of Venice
Michele Gallo	Technician	University of Venice
Davide Marchetto	Researcher	University of Venice

Date: October 29th, 2010