Programma Nazionale di Ricerche in Antartide (PNRA)

Final project report

Project ID: 2003/1.04
Title: *Monitoring and screening of bioactive substances and toxins produced by Antarctic cyanobacteria*

Principal investigator: Dr. Benjamin Pushparaj
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Duration: 2 years
Assigned funding € 25.000,00

Activities and results

The present study is oriented towards the isolation of organisms able to survive under extreme low temperature condition and the analysis of their chemical constituents. The possibility to measure the bioactivity of the cyanobacterial strains isolated from remote Antarctic regions, could represent a novelty in the search new active biometabolites. Antarctic microorganisms (cyanobacteria) are able to withstand extreme environmental conditions and they are therefore to be considered as a potential source of new species and new metabolites associated with them. Studies on cyanobacterial strains isolated from Antarctic environments are limited and on a thorough investigation of their possible use in medicine and agriculture can offer multiple benefits for the protection of environment and of human health. The main objective of the proposal is focused on the study of the bioactivity (in particular antibacterial, antifungal and anticancer activity) of extracts of Antarctic cyanobacteria.
During the Antarctic Campaign XIX (October 2003-January 2004) about 150 samples were collected from different areas of Antarctica. All the isolates are stored on liquid medium at 20°C and cryopreserved at -80°C. The isolated Cyanobacteria are under investigation for their biodiversity as genetic and phenotypic variations. The isolated strains will be included in a National Collection of Cyanobacteria of Antartic origin. Studies have been conducted on physiology and biochemistry of the isolated strains, optimizing growth conditions (light, temperature, pH, nutrients) for the production of both biomass and bioactive molecules. About 30 strains have been cultivated to produce biomass which were then lyophilized for in vitro bioassays and for biochemical analysis.
Results: The cyanobacterial crude extracts were tested for bioactivity such as allelopatic, antibacterial, antifungal and specific target enzyme inhibitory activity. The lipophilic extracts of two strains, *Plectonema* sp. and *Nostoc* sp., showed inhibitory activity against a test cyanobacterial strain, *Anabaena variabilis*.
MTT cytotoxicity tests were carried out with cell lines YAC1 and WEH1. The lipophilic extract of the strain *Gloeocapsa* sp. showed a strong and significant cytotoxic activity. About 16 strains were tested for cytotoxic activity against 2 Sp2 cells. Hydrophilic extracts of two strains (*Nostoc* sp. 99S05 and *Nostoc* sp. 72EPS01) showed a strong cytotoxic activity. The cytotoxic effect of lipophilic extracts were very frequent (extracts from 4 strains showed inhibitory activity on cell tested from 47 to 60%).
Biodiversity was investigated at various levels including morphological, genetic and physiological aspects. Most of the strains belong to genera among Nostocales and Oscillatoriales. The fatty acid composition varied among the strains tested with a relatively high quantity of polyunsaturated fatty acid production. A first genetic investigation by RAPD markers was performed. Cyanobacteria strains were also identified by ITS and TrnL sequencing. Antarctic cyanobacteria diversity and their geographical distribution revealed their particular tolerance range to harsh environmental conditions. A successful classification of cyanobacterial species requires the application of multiple taxonomic criteria.

Products

A – papers in scientific magazines:

1. Antarctic microorganisms for bioactive compound production I- Isolation and characterization of cyanobacteria". Pushparaj B., Paperi R., Sili C., Faraloni C., Ena A. & Torzillo G.. In **Polarnet Technical Report** ISSN 1592-5064 p 11-16. PTR-1/2005.
2. Fatty acid composition of Antarctic cyanobacteria, 2008. Pushparaj B., Boccioni A., Paperi R., Piccardi R., Ena A., Carlozzi P., Sili C. **Phycologia 47 (4), 430–434.**
3. Geographical and microsite distribution of cytotoxicity and secondary metabolites production in terrestrial cyanobacteria.. Hrouzek P., Tomek P., Lukešová E., Urban J., Voloshko L., Pushparaj B., Lukavský J., Stys D., Kopecký J. (sent for publication in **FEMS** 2009).

B – book chapters

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C - proceedings of international conferences:

1. **Micheli C., Spinosa F., Paperi R., Buccioni A., Pushparaj B.** Proceeding 38°. Commission Internazionale pour l'Exploration Scientifique de la mer Mediterraneè (CIESM), Istanbul, Turchia, 9-13 aprile 2007.
2. **Pushparaj B., Piccardi R., Josef E. -Presentazione of poster:** The development of polar based photobioreactor for the production of bioactive compounds by indigenous micro-algae and cyanobacteria, CAREX-ESF workshop, Girona, Spagna, 3-4 dicembre 2008.

D – proceedings of national meetings and conferences

1. **Micheli C., Spinosa F., Paperi R., Pushparaj B**. Screening filogenetico di cianobatteri antartici. Congresso Società Italiana di Ecologia (SitE), Viterbo-Civitavecchia, 19-22 settembre 2006.
2. **Pushparaj B., Piccardi R., Josef E. -Presentazione of Poster: Paperi R., Piccardi R., Juttner F., Gerwick W. H., Pushparaj B.** Studio preliminare sulla bioattività dell'estratto idrofilo di *Nostochopsis lobata*, 26° congresso della Società Chimica Italiana (SCI), Firenze, 10-15 settembre 2006.

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. Dr. Raffaella Paperi, as assegnist and on contract
2. Dr. Raffaella Piccardi as research fellow (2204-2005) and on contract.

Research units

A- Research unit Firenze Responsible scientist: Dr. Benjamin Pushparaj
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List of researchers:

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B- Research unit Rome (ENEA)– Responsible scientist: Dr. Carla Micheli
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List of researchers: Semeria Diego Tech ENEA BIOTEC

C – Research unit Naples: Responsible scientist:- Dr. Guido Cimino.
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List of researchers: Dr. Angelo Fontana I° ricercatore ICB-CNR

Date: 02 April 2009

Notes

Lack of sufficient funding and the delay in distributing the same to the research units in time, most of the research activities could not be successfully completed in time. There are interesting findings with cyanobacterial extracts, which could be investigated in detail to obtain both scientific and economic advantages.

However, the responsible of the research project thanks the PNRA for having given him the opportunity to take up this study and conclude it. This could not be achieved without the help of other operating units at Rome and Naples, the Coordinator of the Project Prof. Dr. Piero Luporini, University of Camerino and his collaborators, Dott.ssa Luana Testa, PNRA-ENEA unit at Rome, Italian Antarctic Base logistic unit, CNR and finally Prof. Carlo Alberto Ricci, President of CSNA.
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Dr. Benjamin Pushparaj