

Final project report

<i>Project ID</i>	2004/1.07
<i>Title</i>	Post-genomics of <i>Pseudoalteromonas haloplanktis</i> TAC125: towards the biotechnological applications of an Antarctic micro-organism
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<i>Duration</i>	3 years
<i>Assigned funding</i>	60.000,00 Euro

Activities and results

Over the last decade, we have focused our attention on the molecular biology of the Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125, which was selected as model organism to investigate cellular strategies responsible for the adaptation to cold lifestyle. In 2004, an European consortium has embarked in the genome sequencing of this micro-organism which represents the first Antarctic eubacterium genome available. Our group has actively taken part to the genome annotation process, which was the first of the aims of the present research project. Furthermore, thanks to the above knowledge and the post-genomics tools available in our group, this project has been mainly focused on studying the regulation of gene-expression, the enzyme set involved in oxidative protein folding (also known as the Dsb family), and extra-cellular protein secretion mechanisms. The final goal was to coordinate information coming from the above research tasks to develop a new and improved generation of cold-adapted gene-expression systems, more effective and versatile in the production of difficult recombinant proteins.

Main results of our project can be schematically summarised as follow:

- 1) *P. haloplanktis* TAC125 genome annotation- our contribution to the curated manual annotation of the genome was included in the online MaGe platform. This piece of information opens the way to investigate structural/functional relationships underlying bacterial cold-adaptation at global level.
 - 2) Identification and use of the Dsb family proteins to investigate enzyme adaptation to the cold activity- Two DsbA enzymes were recombinantly expressed and structurally/functionally characterised with respect to the mesophilic counterparts, suggesting interesting novel strategies to perform catalysis at low temperatures.
 - 3) Identification and study of gene expression of regulated psychrophilic promoters by differential proteomics analyses- Improved cultivation media and fermentation strategies were developed to exploit cold-bacteria growth. In these experimental conditions, the effect of the growth on specific substrates as carbon sources was studied with advanced differential proteomics methods. A novel and L-malate inducible promoter was identified and structurally/functionally characterised, and it was used to set up the first inducible cold gene-expression system.
 - 4) Molecular and functional characterization of protein secretion machineries of *P. haloplanktis* TAC125- Beside the molecular characterisation of the canonical protein secretion machinery (the GSP or Type II secretion system), our research activity allowed us to describe for the first time the occurrence in the Antarctic bacterium of a novel protein secretion system (that we called PSS), which seems to be present in marine Antarctic bacteria only. Its presence, indeed, could be an environmental signature of psychrophilic lifestyle.
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Products

A – papers in scientific magazines

1. Corsaro M. M., Lanzetta R., Parrilli E., Parrilli M., Tutino M. L., e Ummarino S. (2004) Influence of growth temperature on lipid and phosphate contents of superficial polysaccharides from Antarctic *Pseudoalteromonas haloplanktis* TAC 125 bacterium. *J. Bacteriology*, 186: 29-34.
2. Duilio A., Madonna S., Tutino M. L., Pirozzi M., Sannia G., and Marino G. (2004) Promoters from a cold-adapted bacterium: definition of a consensus motif and molecular characterization of UP regulative elements. *Extremophiles*, 8:125-32. D.O.I.10.1007/s00792-003-0371-2.
3. Mitova M, Tutino ML, Infusini G, Marino G, and De Rosa S. (2005) Exocellular Peptides from Antarctic Psychrophile *Pseudoalteromonas haloplanktis*. *Mar Biotechnol* (NY). Sep-Oct;7(5):523-31. Epub 2005 Jun 17.
4. C. Médigue, E. Krin, G. Pascal, V. Barbe, A. Bernsel, P. N. Bertin, F. Cheung, S. Cruveiller, S. D'Amico, A. Duilio, G. Fang, G. Feller, C. Ho, S. Mangenot, G. Marino, J. Nilsson, E. Parrilli, E. P.C. Rocha, Z. Rouy, A. Sekowska, M. L. Tutino, D. Vallenet, G. von Heijne, and A. Danchin. (2005) Coping with cold: the genome of the versatile marine Antarctica bacterium *Pseudoalteromonas haloplanktis* TAC125. *Genome research* Oct;15(10):1325-35. Epub 2005 Sep 16.
5. Madonna, S., Papa, R., Birolo, L., Autore, F., Doti, N., Marino, G., Quemeneur, E., Sannia, G., Tutino, M. L. and Duilio, A. (2006) The thiol-disulphide oxidoreductase system in the cold-adapted bacterium *Pseudoalteromonas haloplanktis* TAC 125: discovery of a novel disulfide oxidoreductase enzyme. *Extremophiles*. 10(1):41-51. Epub 2005 Sep 23.
6. Cusano AM, Parrilli E, Duilio A, Sannia G, Marino G, and Tutino ML. (2006) Secretion of psychrophilic alpha-amylase deletion mutants in *Pseudoalteromonas haloplanktis* TAC125. *FEMS Microbiol Lett*. May;258(1):67-71.
7. Vigentini I, Merico A, Tutino ML, Compagno C, Marino G (2006) Optimization of recombinant Human Nerve Growth Factor production in the psychrophilic *Pseudoalteromonas haloplanktis*. *J Biotechnol* Dec 15;127(1):141-50. Epub Jun 12; [Epub ahead of print] PMID: 16859797
8. Cusano AM, Parrilli E, Marino G, and Tutino ML. (2006) A novel genetic system for recombinant protein secretion in the Antarctic *Pseudoalteromonas haloplanktis* TAC125. *Microbial Cell Factory* 5:40 doi:10.1186/1475-2859-5-40
9. Papa R, Glagla S, Danchin A, Schweder T, Marino G, Duilio A. Proteomic identification of a two-component regulatory system in *Pseudoalteromonas haloplanktis* TAC125. *Extremophiles*. 2006 Dec;10(6):483-91.
10. Papa R, Ripa V, Sannia G, Marino G, Duilio A. (2007) An effective cold inducible expression system developed in *Pseudoalteromonas haloplanktis* TAC125. *J Biotechnol*. 127:199-210.
11. D. Giordano, E. Parrilli, A. Dettai, R. Russo, G. Barbiero, G. Marino, G. Lecointre, G. di Prisco, M. L. Tutino, and C. Verde. (2007) The truncated hemoglobins in the Antarctic psychrophilic bacterium *Pseudoalteromonas haloplanktis* TAC125. *Gene*, Aug 15;398(1-2):69-77 [Epub ahead of print] PMID: 17582708
12. Kivelä HM, Madonna S, Krupovic M, Tutino ML, Bamford JK. (2008) Genetics for *Pseudoalteromonas* provides tools to manipulate marine bacterial virus PM2. *J Bacteriol*. Feb;190(4):1298-307. Epub 2007 Dec 14
13. Parrilli E., De Vizio D., Cirulli C., and Tutino M.L. (2008) Development of an improved *Pseudoalteromonas haloplanktis* TAC125 strain for recombinant protein secretion at low temperature *Microbial Cell Factories*, 7:2
14. de Pascale D, Cusano A M., Autore F, Parrilli E, di Prisco G, Marino G and Tutino ML. (2008) The cold-active Lip1 lipase from the Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125 is a member of a new bacterial lipolytic enzyme family. *Extremophiles* May;12(3):311-23. Epub 2008 Apr 24.
15. Gasser B, Saloheimo M, Rinas U, Dragosits M, Rodríguez-Carmona E, Baumann K, Giuliani M, Parrilli E, Branduardi P, Lang C, Porro D, Ferrer P, Tutino ML, Mattanovich D, Villaverde A. (2008) Protein folding and conformational stress in microbial cells producing recombinant proteins: a host comparative overview. *Microb Cell Fact*. Apr 4;7:11
16. Di Rocco G, Battistuzzi G, Borsari M, De Rienzo F, Ranieri A, Tutino ML, Sola M. (2008) Cloning, expression and physicochemical characterization of a di-heme cytochrome c (4) from the psychrophilic bacterium *Pseudoalteromonas haloplanktis* TAC 125. *J Biol Inorg Chem*. Jun;13(5):789-99. Epub 2008 Apr 2.
17. Corsaro MM, Pieretti G, Lindner B, Lanzetta R, Parrilli E, Tutino ML, Parrilli M. (2008) Highly Phosphorylated Core Oligosaccharide Structures from Cold-Adapted *Psychromonas arctica*. *Chemistry*. 14(30):9368-76. PMID: 18770509
18. Corsaro MM, Parrilli E, Lanzetta R, Naldi T, Pieretti G, Lindner B, Carpentieri A, Parrilli M, Tutino ML. (2009) The presence of OMP inclusion bodies in a *Escherichia coli* K-12 mutated strain is not related to lipopolysaccharide structure. *J Biochem*. Apr 13. [Epub ahead of print]

B – book chapters

1. Duilio A., Tutino M.L., and Marino G. (2004) Recombinant Protein Production in Antarctic Gram-Negative Bacteria, *Methods Mol Biol*. 2004; 267:225-37.
2. Parrilli E., Duilio A., and Tutino M.L. (2008) Heterologous protein expression in psychrophilic hosts. in *Psychrophiles: from Biodiversity to Biotechnology*, Margesin, R.; Schinner, F.; Marx, J.-C.; Gerday, C. (Eds.) Springer-Verlag Berlin Heidelberg, pp.365-379.

Programma Nazionale di Ricerche in Antartide (PNRA)

C - proceedings of international conferences

1. Ermenegilda Parrilli, Angela M. Cusano, and M. Luisa Tutino, Secretion in Antartic bacteria: a novel tool for recombinant protein production, EFB Recombinant Protein Production, Tavira Portugal, 11-14/11/2004.
2. M. Luisa Tutino, Stefania Madonna, Angela Duilio, Nunzia Doti, Concetta Compagno, Annamaria Merico, Ileana Vigentini, and Gennaro Marino, Recombinant protein production in the periplasm of Antartic gram negative bacteria, EFB recombinant Protein Production, Tavira Portugal, 11-14/11/2004.
3. E. Parrilli, A. M. Cusano, M. Giuliani, G. Marino and M. L. Tutino, A lesson from the cold: how to efficiently produce and secrete recombinant proteins, 2nd International Conference on Alpine and Polar Microbiology, Innsbruck, 27-31/03/2006.
4. Ermenegilda Parrilli, Angela Maria Cusano, Maria Giuliani, and Maria Luisa Tutino, Cell engineering of *Pseudoalteromonas haloplanktis* Tac125: construction of a mutant strain with reduced exo-proteolytic activity, The 13th International Symposium on Polar Sciences, KOPRI, Incheon, South Korea, 9-12/05/2006.
5. Angela M. Cusano, Donatella De Pascale, Flavia Autore, Gilda Parrilli, M. Luisa Tutino, The cold-active Lip1 lipase from the Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125 is a member of a new bacterial lipolytic enzyme family, 16th PNRA meeting on Antarctic Biology: critical issues and research priorities for the IPY and 2007-9, Follonica, 7-9/06/2007.
6. Ermenegilda Parrilli, Maria Giuliani, Gennaro Marino and Maria Luisa Tutino, Cell Engineering Of *Pseudoalteromonas Haloplanktis* Tac125: Construction Of A Mutant Strain With Reduced Exo-Proteolytic Activity, 16th PNRA meeting on Antarctic Biology: critical issues and research priorities for the IPY and 2007-9, Follonica, 7-9/06/2007.
7. Maria Luisa Tutino, Ermenegilda Parrilli, Angela Duilio, and Gennaro Marino, The Genome Of The Antarctic Marine Bacterium *Pseudoalteromonas haloplanktis* TAC125, 6th PNRA meeting on Antarctic Biology: critical issues and research priorities for the IPY and 2007-9, Follonica, 7-9/06/2007
8. Parrilli E., Cusano A.M., Giuliani M., and M.L. Tutino. (2006) The genome of marine Antarctica bacterium *Pseudoalteromonas haloplanktis* TAC125: cold adaptation and use as cell factory. Pag. 39-43 in Proceedings of The 13th International Symposium on Polar Sciences "From molecules to ecosystem in polar science: toward IPY 2007-2008", 9-12 Maggio 2006 KOPRI, Incheon Korea
9. Parrilli E., Cusano A.M., Giuliani M., and M.L. Tutino. (2006) Cell engineering of *Pseudoalteromonas haloplanktis* TAC125: construction of a mutant strain with reduced exo-proteolytic activity. Proceedings of The 4th Recombinant Protein Production Meeting: a comparative view on host physiology Barcelona, Spain. 21-23 September 2006 *Microbial Cell Factories* 2006, **5**(Suppl 1):P36 doi:10.1186/1475-2859-5-S1-P36
10. Ermenegilda Parrilli, Maria Giuliani and Maria Luisa Tutino. Protein secretion secrets: the "cold" case of α -amylase. The 3rd International Conference on Polar and Alpine Microbiology, Banff Canada, May 11/15, 2008.
11. Ermenegilda Parrilli, Maria Giuliani and Maria Luisa Tutino. Antarctic truncated haemoglobin: from gene to function. The 3rd International Conference on Polar and Alpine Microbiology, Banff Canada, May 11/15, 2008.

D – proceedings of national meetings and conferences

1. E. Parrilli, A.M. Cusano, and M.L. Tutino, A novel protein secretion pathway in the Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125: the psychrophilic answer to a key question, Congresso nazionale FISV, Riva del Garda, 30/09-03/11/2004.
2. Ermenegilda Parrilli, M. Luisa Tutino, Angela M. Cusano, and Gennaro Marino, Secretion in Antartic bacteria: a novel tool for recombinant protein production, FISV, Riva del Garda, 22-25/09/2005.
3. Vigentini, A. Merico, M.L. Tutino and C. Compagno, Production of recombinant human nerve growth factor (rhNGF) in Antarctic bacteria, FISV, Riva del Garda, 22-25/09/2005.
4. Ermenegilda Parrilli, Angela Maria Cusano, Gennaro Marino, and Maria Luisa Tutino, Cell engineering of *Pseudoalteromonas haloplanktis* Tac125: construction of a mutant strain for recombinant protein secretion, 25^o Congresso Nazionale della SIMGBM, Orvieto, 8-10/06/2006

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

1. participation to the Organising and Scientific Committee of the International workshop on The polar and alpine environments: molecular and evolutionary adaptations in prokaryotic and eukaryotic organisms, Naples, 29-30 May 2008

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

1. Rosanna Papa- Ph.D thesis in Biotechnological Sciences (2006), Regulation of gene expression in psychrophilic micro organisms: molecular aspects and biotechnological applications. University of Naples Federico II

Programma Nazionale di Ricerche in Antartide (PNRA)

2. Angela Maria Cusano- Ph.D thesis in Biotechnological Sciences (2006), Sistemi di secrezione in batteri adattati al freddo e loro applicazioni nella produzione di proteine ricombinanti. University of Naples Federico II.
 3. Maria Giuliani- Ph.D thesis in Biotechnological Sciences (2009), Novel processes and products for recombinant production of biopharmaceuticals. University of Naples Federico II
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Research unit

Research Unit at the Department of Organic Chemistry and Biochemistry

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Date: 31-05-2009

Notes