

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.

Follow this format for each person. **DO NOT EXCEED FIVE PAGES.****NAME: Maria Luisa Tutino**

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE: Full professor of Fermentation Chemistry and Industrial Biotechnology- Dept. of Chemical Sciences- Federico II Univ. of NaplesEDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Federico II Univ. of Naples, Naples Italy	4-years master	06/92	Biological Sciences
Royal Univ. of Ghent, Ghent Belgium	Pre-doc training	12/96-03/97	Set up of transformation procedures for Antarctic bacteria
Federico II Univ. of Naples, Naples Italy	Ph.D.	10/97	Biochemistry and Molecular Biology

A. Personal Statement

Over the last 25 years, my research interests have been mainly focused on the development and pre-industrial exploitation of the Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125 (*PhTAC125*) as unconventional host for the high quality and soluble recombinant production of difficult-to-express proteins. Several eukaryotic proteins (such as the human Nerve growth factor, or human alpha galactosidase) resulted to be successfully produced in this host. Till 2016, **my research interest has been addressed towards the setup of an efficient manufacturing process for the recombinant production of the full-length TATk-hCDKL5 (isoform 1 and 2).** *PhTAC125* turned out to be the only microbial system in which the protein can be obtained in soluble and active form. A multiparametric approach -applied to either the bacterial host improvement or the genetic system makeup- allowed us to achieve a production yield of about 40 mg/liter of culture. Furthermore, a metabolic model of this production process was established, and we'll help in further optimize the production process setup.

I've also been involved in two European Union funded collaborative projects aimed at expanding our knowledge on novel strategies for enhancing quality and quantity of recombinant proteins.

Moreover, I assumed the role of group leader till 2003, when I received the first competitive funding grant for the implementation of the psychrophilic gene expression system. I therefore developed a solid experience in coordinating research teams, in keeping deliverable release time respected, and used to face any experimental issue by promptly modify the research design. I'm also active in disseminating research results by attending workshops, congresses etc.

My motivation in pursuing all the objectives of the present proposal is also due to the fact that I'm the mother to a fourteen years old girl with a CDKL5 genetic mutation.

The following are the 5 most significative publications on this topic.

- Colarusso A, Lauro C, Calvanese M, Parrilli E, Tutino ML. Active human full-length CDKL5 produced in the Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125. 2022. Microb Cell Fact 21(1):211.
- Fondi M., Gonzi S., Dziurzynski M., Turano P., Ghini V., Calvanese M., Colarusso A., Lauro C., Parrilli E., Tutino M.L. Modelling hCDKL5 heterologous expression in bacteria. 2021. Metabolites 11(8),491
- Unzueta U, Vázquez F, Accardi G, Mendoza R, Toledo-Rubio V, Giuliani M, Sannino F, Parrilli E, Abasolo I, Schwartz S Jr, Tutino ML, Villaverde A, Corchero JL, Ferrer-Miralles N. Strategies for the production of difficult-to-express full-length eukaryotic proteins using microbial cell factories: production of human alpha-galactosidase A. Appl Microbiol Biotechnol. 2015 Jul;99(14):5863-74.

4. Giuliani M, Parrilli E, Sannino F, Apuzzo GA, Marino G, Tutino ML. 2014. Recombinant production of a single-chain antibody fragment in *Pseudoalteromonas haloplanktis* TAC125. Appl Microbiol Biotechnol. 98(11):4887-95.
5. Corchero JL, Gasser B, Resina D, Smith W, Parrilli E, Vázquez F, Abasolo I, Giuliani M, Jäntti J, Ferrer P, Saloheimo M, Mattanovich D, Schwartz S Jr, Tutino ML, Villaverde A. 2013. Unconventional microbial systems for the cost-efficient production of high-quality protein therapeutics. 31(2):140-53.

B. Positions and Honors

Postdoctoral Training:

- 11/97-10/98 Post-doc position at Federico II Univ. of Naples, Italy aimed at setting up of psychrophilic cloning vectors.
- 11/98-10/99 Post-doc position at University of Liege, Liege Belgium with Charles Gerday aimed at setting up psychrophilic gene expression vectors
- 01/00-11/01 Post-doc position at Federico II Univ. of Naples, Italy aimed at genetic and genomic manipulation of Antarctic bacteria

Academic Appointments:

- 12/2001-10/2005 Research assistant in Fermentation Chemistry and Industrial Biotechnology at Federico II University of Naples.
- 11/2005- Permanent position as associate professor in Fermentation Chemistry and Industrial Biotechnology at Federico II University of Naples.
- 05/2022- Permanent position as full professor in Fermentation Chemistry and Industrial Biotechnology at Federico II University of Naples.

Awards and Honors:

- 1992 Graduate in Biological Sciences (with top marks) achieved at the Federico II Univ. of Naples
- 1998 PhD in Biochemistry and Molecular Biology at the Federico II University of Naples (with honors)
- 1997 Postdoctoral fellowship awarded by EU funded EUROCOLD project
- 1998 Postdoctoral fellowship awarded by EU funded COLDZYME project
- 1999 Postdoctoral fellowship awarded by Federico II University of Naples

Other Experience and Professional Memberships

- Reviewer activity: Journals: Applied and Environmental Microbiology, Microbial Cell Factories, Marine Drugs, FEMS Microbiological Letters.
- Guest editor for the special issue "Biotechnology of Cold-Adapted Bacteria and Marine Bacteria" of the MDPI journal "Microorganisms"
- Member of the Editorial Board for section 'Microbial Biotechnology' of the MDPI journal "Microorganisms"

C. Contributions to Science

Set up of novel gene expression systems for the recombinant protein production in psychrophilic bacteria. Most of recombinant protein productions (either for research purposes or for therapeutic uses) are successfully carried out in the mesophilic bacterium *Escherichia coli*. Unfortunately, there is still a fraction of tested recombinant proteins which fail to assume their correct folding and their production results in inclusion bodies deposition or degradation by host encoded proteases. The discovery of a low copy number endogenous plasmid (1) allowed me to set up a novel genetic system that makes use of Antarctic marine bacteria as host for the recombinant protein production at very low temperatures. Such a system turned out to be quite effective in overcoming either solubility or proteolytic issues. The bacterium *Pseudoalteromonas haloplanktis* TAC125 is now considered one of the most promising unconventional production platforms, naturally optimized for the high quality production of Eukaryotic proteins (2-4).

1. Tutino M. L., Duilio A., Parrilli E., Remaut E., Sannia G. and Marino G. 2001. A novel replication element from an Antarctic plasmid as a tool for the expression of proteins at low temperature. Extremophiles. 5, 257-264.
2. Parrilli E, Duilio A, and Tutino ML. 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.

3. Corchero JL, Gasser B, Resina D, Smith W, Parrilli E, Vázquez F, Abasolo I, Giuliani M, Jäntti J, Ferrer P, Saloheimo M, Mattanovich D, Schwartz S Jr, Tutino ML, Villaverde A. 2013. Unconventional microbial systems for the cost-efficient production of high-quality protein therapeutics. 31(2):140-53.
4. Giuliani M, Parrilli E, Sannino F, Apuzzo GA, Marino G, Tutino ML. 2014. Recombinant production of a single-chain antibody fragment in *Pseudoalteromonas haloplanktis* TAC125. Appl Microbiol Biotechnol. 98(11):4887-95.

Genomics and Genetics of Antarctic bacteria, in particular of *Pseudoalteromonas haloplanktis* TAC125 (PhTAC125). Due to its promising features as proficient bacterial host for protein production, a European Consortium headed by Antoine Danchin at the Pasteur Institute (Paris) selected in 2004 PhTAC125 for the whole genome sequencing and annotation. I've been deeply involved in the annotation process of a large genome region and in the in vivo study of some physiological aspects of the bacterium resulting in the publication in 2005 of the first genome sequence from an Antarctic eubacterium (5). During this period a gene insertion/replacement scheme to obtain directed genomic mutants of the PhTAC125 was also optimized, allowing us to construct novel mutant strains with improved features for the recombinant protein production and secretion (6-8). The PhTAC125 genome annotation is still one of the four reference organisms to study molecular, cellular and physiological adaptation to subzero lifestyle.

5. Médigue C, et al. Tutino ML, Vallenet D, von Heijne G, and Danchin A. 2005. Coping with cold: the genome of the versatile marine Antarctica bacterium *Pseudoalteromonas haloplanktis* TAC125. Genome research. 15(10):1325-35. Epub 2005 Sep 16.
6. Parrilli E, Cusano AM, Giuliani M and Tutino ML. 2006. Cell engineering of *Pseudoalteromonas haloplanktis* TAC125: construction of a mutant strain with reduced exo-proteolytic activity. Microb Cell Fact. 5(Suppl 1):P36
7. Parrilli E, De Vizio D, Cirulli C, and Tutino ML. 2008. Development of an improved *Pseudoalteromonas haloplanktis* TAC125 strain for recombinant protein secretion at low temperature Microbial Cell Factories, 7:2
8. Giuliani M, Parrilli E, Pezzella C, Rippa V, Duilio A, Marino G, Tutino ML. 2012. A novel strategy for the construction of genomic mutants of the Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125. Methods Mol Biol. 824:219-33.

Development of novel bacterial hosts and novel fermentation strategies for the large scale and quality production of protein drugs either in mesophilic *E. coli* strains or in Antarctic marine bacterium *P. haloplanktis* TAC125. I invested a part of my research efforts in upscaling the recombinant protein production processes carried out in PhTAC125 at pre-industrial level, by using lab scale automatic fermenters. Great attention has been focused on medium formulation, physiologic response to temperature fluctuations and genetic modifications makeup. I've been also active in developing pre-industrial fermentation processes for improving *E. coli* performances in recombinant production of several proteins (9-12).

9. 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. 127(1):141-50.
10. Parrilli E, Giuliani M, Marino G, Tutino ML. 2010. Influence of production process design on inclusion bodies protein: the case of an Antarctic flavohemoglobin. Microb Cell Fact. 9:19
11. Giuliani M, Parrilli E, Ferrer P, Baumann K, Marino G, Tutino ML. 2011. Process optimization for recombinant protein production in the psychrophilic bacterium *Pseudoalteromonas haloplanktis*. Process Biochemistry, 46(4), 953-959
12. Leone S, Sannino F, Tutino ML, Parrilli E, Picone D. Acetate: friend or foe? Efficient production of a sweet protein in *Escherichia coli* BL21 using acetate as a carbon source. 2015. Microb Cell Fact. Jul 25;14:106.

**A full list of my publications is available at <http://orcid.org/0000-0002-4978-6839>
My Scopus Author ID: 6701493307**

D. Additional Information: Research Support

Ongoing Research Support

MUR-PRIN 2022J5X82R Tutino (PI) € 250.000,00 10% personal effort 10/12/2023- 10/12/2025 –
Two years National grant entitled: **“Optimization of human CDKL5 recombinant production in the Antarctic bacterium *Pseudoalteromonas haloplanktis* TAC125: a multiparametric approach towards the exploitation of the enzyme replacement therapy for the CDKL5 Deficiency Disorder (ERT4CDD)”**

MUR CN00000033 Tutino (research unit leader) € 300,000.00 25% personal effort 09/2022-08/2025
Three years National grant (PNRR) entitled: “National Biodiversity Future Center- Spoke 1. Mapping and monitoring actions to preserve marine ecosystem biodiversity and functioning” aimed at collecting information about microbial diversity in marine protected and nonprotected area, to suggest intervention to restore healthy biodiversity and community functioning.

Orphan Disease Centre Pilot Grant CDKL5- 24-102-01. Tutino (co-PI) \$ 75.000,00 15% personal effort 05/01/2024- 04/30/2025

Looking for differences: *in vitro* isolation of hCDKL5-specific antibody fragments and set-up of a method to quantify hCDKL5 and possibly distinguish between its isoforms.

Orphan Disease Centre Pilot Grant CDKL5-23-103-1. Tutino (PI) \$ 150.000,00 15% personal effort 05/01/2023- 04/30/2025

Assessment of the therapeutic potential of TATk-hCDKL5 isoform 1 and 2 produced in recombinant Antarctic *Pseudoalteromonas haloplanktis* TAC125.

Completed Research Support

PNRA PdR 2018/00335 Tutino (PI) € 87,000.00 10% personal effort 03/2020-03/2023
Three years National grant (Programma Nazionale di Ricerca in Antartide 2018) entitled: “Transcriptional Regulatory Networks in Antarctic bacteria as a proxy for global warming effects on microbial life in the Polar Oceans” aimed at understanding the global transcriptional networks which allow the Antarctic bacterium to coordinate its response to environmental warming.

Orphan Disease Centre Pilot Grant CDKL5-20-101-08. Tutino (PI) \$ 150.000,00 15% personal effort 06/01/2020- 12/31/2021

Structural/functional characterization of full-length hCDKL5 isoform 1 produced in recombinant marine bacteria and use of pharmacological chaperones to stabilize hCDKL5 missense mutants.

Private company funded research project Tutino (PI) € 200.000,00 15% personal effort 04/2018- 11/2019
Development of an efficient system for recombinant production of full-length human TATk28-CDKL5_1 in the Antarctic bacterium *P. haloplanktis* TAC125 cells.

PNRA PdR 2013/B1.04 Tutino (PI) € 100,000.00 06/2014-03/2017
National grant (Programma Nazionale di Ricerca in Antartide 2013) entitled: “Antarctic marine bacteria biofilms: eco/physiology and biomedical applications” aimed at the study of microbial biofilm formation to envisage effective anti-biofilm strategies.

PNRA PdR 2013/ AZ1.04 Tutino (Research unit leader) € 40,000.00 06/2014-03/2017
National grant (Programma Nazionale di Ricerca in Antartide 2013) entitled: “New drugs against opportunistic pathogens in Cystic Fibrosis patients from Antarctic microbiota” aimed at the identification of study of novel secondary metabolites from Antarctic bacteria effective against pathogens colonising Cystic Fibrosis patients.

Azioni Integrate Italia-Spagna Tutino (PI) € 35,000.00 12/2010-12/2012
Two years mobility grant entitled: “Solubility, conformational stability and biologic activity of proteins difficult-to-produce and of relevant pharmacological interest: a comparative study amongst different cell factories”, with Prof. T. Villaverde (Barcelona- Spagna).

EU funded ERA-IB project Tutino (Research unit leader) €40,000.00 12/2009-12/2012

Three years international grant (ERA-IB project) entitled: “**IMAPPROT- Integrated, multi-host approach for the improved microbial production of high quality therapeutic enzymes and proteins**” aimed at developing effective strategies to the recombinant production of high value proteins of pharma interest.

ESF funded project Tutino (Research unit leader) €35,000.00 12/2007-12/2010

Three years international grant (European Science Foundation) in the EUROSCOPE program, entitled: “**GENOPHYS- Genome-Wide Comparison of Physiological Bottlenecks in Multi-Subunit Protein Production in Prokaryotic and Eukaryotic Microbial Hosts**” aimed at developing effective strategies to the recombinant production of high value proteins of pharma interest.