
BIOGRAPHICAL SKETCH

NAME: Giovanni Bertoni

POSITION TITLE: Associate Professor of Microbiology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Università degli Studi di Milano, Milan, Italy	M.Sc.	10/1989	Biological Sciences
Università degli Studi di Milano, Milan, Italy	PHD	10/1993	Cellular and Molecular Biology
Università degli Studi di Milano, Milan, Italy	Postdoctoral	11/1994	Molecular Microbiology
Center for Biological Investigations (CIB-CSIC), and National Center of Biotechnology (CNB-CSIC), Madrid, Spain	Postdoctoral	08/1998	Molecular Microbiology
Università degli Studi di Milano, Milan, Italy	Postdoctoral	04/2000	Molecular Microbiology

A. Personal Statement

I am an Associate Professor of Microbiology, and my current research is focused on the post-transcriptional regulation mediated by small RNAs (sRNAs) in bacteria. Specifically, I head one of the leading laboratories in the world with strong expertise on sRNAs in the opportunistic pathogen *Pseudomonas aeruginosa* (PAE). This leadership was derived from my seminal work that resulted in the first large-scale experimental survey of PAE sRNAs. Since then, my group has published the role of five sRNAs from this initial survey in several follow-up papers. As only one PAE sRNA had previously been characterized in detail, our results changed our understanding of the roles and functions of PAE sRNAs because they provided evidence that PAE sRNAs can i) respond to the type of growth, planktonic or biofilm, and culture conditions such as the presence of bacterial envelope stressors belonging to the host defense, ii) regulate biofilm formation, antibiotic resistance, and PAE anaerobic metabolism important for airways colonization in cystic fibrosis, iii) play a role in the regulatory network of PAE pathogenicity in airway infections, iv) be embedded in the PAE quorum sensing networks, and v) establish a multifaceted interplay with the RNA-binding protein Hfq, the major matchmaker between sRNAs and target mRNAs. Our results on the involvement of sRNAs in PAE virulence, biofilm formation, and antibiotic resistance allowed me to enter a frontier area of research on non-conventional antibacterials consisting of antivirulence drugs and adjuvant molecules able to resensitize bacteria to antibiotics. More recently, I have added to the study of sRNAs that act as riboregulators within PAE cells, the search for and characterization of PAE sRNAs that can be transmitted to an animal host and influence host gene regulation through RNAi. For this, I was awarded a grant by the Italian Ministry of University and Research (MUR) entitled “Trans-domain transfer of RNA signals: a novel mechanism of host-pathogen interaction (acronym TRANSMISSION – grant code 2022C5A9MT).

As PI or co-investigator of several EU and national projects, I have accumulated considerable research experience. I successfully administered the projects (e.g. staffing, research protections, budget), collaborated with other researchers, and produced several peer-reviewed publications from each project. In summary, I have the expertise, leadership, training, expertise, and motivation necessary to successfully carry out the proposed research project.

Ongoing and recently completed projects that I would like to highlight include:

MUR PRIN2022

ID: 2022C5A9MT 28/09/2023 - 28/02/2026 role: PI

Trans-domain transfer of RNA signals: a novel mechanism of host-pathogen interaction (TRANSMISSION)

Fondazione per la Ricerca sulla Fibrosi Cistica -ETS

ID: FFC#5/2024 01/09/2024 – 31/08/2025 role: PI

Targeting small RNA-mediated regulation to develop nontraditional therapeutic options *against Pseudomonas aeruginosa*

Fondazione per la Ricerca sulla Fibrosi Cistica -ETS

ID: FFC#14/2021 01/09/2021 – 31/08/2022 role: PI

Targeting small RNA-mediated regulation of virulence and antibiotic resistance to develop nontraditional therapeutic options *against Pseudomonas aeruginosa*

Fondazione per la Ricerca sulla Fibrosi Cistica -ETS

ID: FFC#10/2020 01/09/2020 – 31/08/2021 role: PI

Targeting small RNA-mediated regulation of virulence and antibiotic resistance to develop nontraditional therapeutic options *against Pseudomonas aeruginosa*

EU Commission

EU-FP7-HEALTH-2007-B ID: 223670 01/02/2009 - 31/07/2013 role: Co-investigator

Novel approaches to bacterial target identification, validation and inhibition – NABATIVI

B. Positions, Scientific Appointments

Positions and Scientific Appointments

2010-present: **Associate Professor (tenured)** of Molecular and Cellular Microbiology, Department of Biosciences, University of Milan, Italy.

May 2000–Sep 2010: **Research Associate** of Microbiology, University of Milan, Italy.

Sep 1998–Apr 2000: **Postdoctoral Fellowship** of the ICGB (International Centre for Genetic Engineering and Biotechnology, Trieste) at the Department of Genetics and Biology of Microorganisms, University of Milan, Italy.

Dec 1994–Aug 1998: **Postdoctoral Fellowship** of the Spanish Ministry of Education and Science for foreign Ph.D. visitors at the Center for Biological Investigations (CIB-CSIC) in Madrid (Spain), and the Department of Microbial Biotechnology of the National Center of Biotechnology (CNB-CSIC) in Cantoblanco-Madrid (Spain), two prestigious and internationally recognized research institutions in Spain.

Nov 1993–Nov 1994: **Postdoctoral fellowship** at the Department of Genetics and Biology of Microorganisms, University of Milan, Italy.

Jan 1991–Oct 1993: **Doctoral student** at the Department of Genetics and Biology of Microorganisms, University of Milan, Italy.

C. Contributions to Science

1. Identification and characterization of small RNAs from *P. aeruginosa* with implications for the development of antivirulence molecules and antibiotic adjuvants.

- a) Ferrara, S., Brignoli, T., **Bertoni, G.*** (2023) Little reason to call them small noncoding RNAs. *Frontiers in Microbiology* 14: 1191166.
- b) Santoro, S., Paganin, C., Gilardi, S., Brignoli, T., **Bertoni, G.***, and Ferrara, S. (2023) Multifaceted interplay between Hfq and the small RNA GssA in *Pseudomonas aeruginosa*. *mBio* 14: e02418-22.
- c) Ferrara, S., Carrubba, R., Santoro, S., **Bertoni, G.*** (2021) The small RNA ErsA impacts the anaerobic metabolism of *Pseudomonas aeruginosa* through post-transcriptional modulation of

the master regulator Anr. *Frontiers in Microbiology* 12: 691608.

- d) Ferrara, S., Rossi, A., Ranucci, S., De Fino, I., Bragonzi, A., Cigana, C., **Bertoni, G.*** (2020) The small RNA ErsA plays a role in the regulatory network of *Pseudomonas aeruginosa* pathogenicity in airway infections. *mSphere* 5: e00909-20.
- e) Falcone, M., Ferrara, S., Rossi, E., Johansen, H.K., Molin, S., and **Bertoni, G.*** (2018) The small RNA ErsA of *Pseudomonas aeruginosa* contributes to biofilm development and motility through post-transcriptional modulation of AmrZ. *Frontiers in Microbiology* 9: 238.
- f) Ferrara, S., Falcone, M., Macchi, R., Bragonzi, A., Girelli, D., Cariani, L., Cigana, C., **Bertoni, G.*** (2017) The PAPI-1 pathogenicity island-encoded small RNA PesA influences *Pseudomonas aeruginosa* virulence and modulates pyocin S3 production. *PLoS One* 12: e0180386.
- g) Carloni, S., Macchi, R., Sattin, S., Ferrara, S., **Bertoni, G.** (2017) The small RNA Real: a novel regulatory element embedded in the *Pseudomonas aeruginosa* quorum sensing networks. *Environmental Microbiology*. 19: 4220–4237.
- h) Ferrara, S., Carloni, S., Fulco, R., Falcone, M., Macchi, R. and **Bertoni, G.*** (2015) Post-transcriptional regulation of the virulence-associated enzyme AlgC by the sigma22-dependent small RNA ErsA of *Pseudomonas aeruginosa*. *Environmental Microbiology*. 17: 199-214.
- i) Ferrara, S., Brugnoli, M., De Bonis, A., Righetti, F., Delvillani, F., Dehò, G., Horner, D., Briani, F., and **Bertoni, G.*** (2012). Comparative profiling of *Pseudomonas aeruginosa* strains reveals differential expression of novel unique and conserved small RNAs. *PLoS One* 7: e36553.

2. Identification and characterization of essential functions of *P. aeruginosa* for the development of new antibacterial agents.

- a) Bollati, M., Fasola, E., Pieraccini, S., Freddi, F., Cocomazzi, P., Maspero, A., Piarulli, U., Ferrara, S., Pellegrino, S., **Bertoni, G.***, Gazzola, S (2024) Impairing protein-protein interactions in an essential tRNA modification complex: an innovative antimicrobial strategy against *Pseudomonas aeruginosa*. *Journal of Peptide Science* e3658.
- b) Fasola, E., Alboreggia, G., Pieraccini, S., Oliva, F., Agharbaoui, F.E., Bollati, M., **Bertoni, G.**, Recchia, S., Marelli, M., Piarulli, U., Pellegrino, S., Gazzola, S. (2022) Conformational switch and multiple supramolecular structures of a newly identified self-assembling protein-mimetic peptide from *Pseudomonas aeruginosa* YeaZ protein. *Frontiers in Chemistry* 10: 1038796.
- c) Uruburu, M., Mastrangelo, E., Bolognesi, M., Ferrara, S., **Bertoni, G.***, Milani M. (2019) Structural and functional characterization of TgpA, a critical protein for the viability of *Pseudomonas aeruginosa*. *Journal of Structural Biology* 205: 18-25.
- d) Vecchietti, D., Ferrara, S., Rusmini, R., Macchi, R., Milani M., **Bertoni, G.*** (2016) Crystal structure of YeaZ from *Pseudomonas aeruginosa*. *Biochemical and Biophysical Research Communications*. 470: 460-5.
- e) Rusmini, R., Vecchietti, D., Macchi, R., Vidal-Aroca, F., and **Bertoni, G.*** (2014) A shotgun antisense approach to the identification of novel essential genes in *Pseudomonas aeruginosa*. *BMC Microbiology* 14: 24.
- f) Milani, A., Vecchietti, D., Rusmini, R. and **Bertoni, G.*** (2012) TgpA, a protein with a eukaryotic-like transglutaminase domain, plays a critical role in the viability of *Pseudomonas aeruginosa*. *PLoS One* 7(11): e50323.

3. Analysis of the *P. aeruginosa* envelope proteome to identify vaccine candidates.

- a) Martínez-García, E., Fraile, S., Rodríguez Espeso, D., Vecchietti, D., **Bertoni, G.**, de Lorenzo, V. (2020) Naked bacterium: emerging properties of a surfome-streamlined *Pseudomonas putida* strain. *ACS Synthetic Biology* 9: 2477-2492.
- b) Motta, S., Vecchietti, D., Martorana, A.M., Brunetti, P., **Bertoni, G.**, Polissi, A., Mauri, P., Di Silvestre, D. (2020) The landscape of *Pseudomonas aeruginosa* membrane-associated proteins. *Cells*, 9: 2421.
- c) Vecchietti, D., Di Silvestre, D., Miriani, M., Bonomi, F., Marengo, M, Bragonzi, A., Cova, L., Franceschi, E., Mauri, P, and **Bertoni, G.*** (2012) Analysis of *Pseudomonas aeruginosa* cell envelope proteome by capture of surface-exposed proteins on activated magnetic nanoparticles. *PLoS One* 7(11): e51062.

4. Study of the interaction of *P. aeruginosa* with the airways in cystic fibrosis.
 - a) Cigana, C., Giannella, R., Colavolpe, A., Alcalá-Franco, B., Mancini, G., Colombi, F., Bigogno, C., Bastrup, U., **Bertoni, G.**, Bragonzi, A. (2023) Mutual effects of single and combined CFTR modulators and bacterial infection in cystic fibrosis. *Microbiology Spectrum* 11: e04083-22
 - b) Alcalá-Franco, B., Montanari, S., Cigana, C., **Bertoni, G.**, Oliver, A., and Bragonzi, A. (2012). Antibiotic pressure compensates the biological cost associated with *Pseudomonas aeruginosa* hypermutable phenotypes *in vitro* and in a murine model of chronic airways infection. *Journal of Antimicrobial Chemotherapy* 67:962-9.
 - c) Bianconi, I., Milani, A., Cigana, C., Paroni, M., Levesque, R.C., **Bertoni, G.** and Bragonzi, A. (2011) Positive signature-tagged mutagenesis in *Pseudomonas aeruginosa*: tracking patho-adaptive mutations promoting airways chronic infection. *PLoS Pathogens* 7(2): e1001270.
 - d) Montanari, S., Oliver, A., Salerno, P., Mena, A., **Bertoni, G.**, Tümmler, B., Cariani, L., Conese, M., Döring, G., and Bragonzi, A. (2007) Biological cost of hypermutation in *Pseudomonas aeruginosa* strains from patients with cystic fibrosis. *Microbiology* 153: 1445-1454.
5. Study of the co-regulation of sigma54-dependent expression of aromatic compound degradation in *P. putida*
 - a) Renzi, F., Rescalli, E., Galli, E., and **Bertoni, G.*** (2010) Identification of genes regulated by the MvaT-like paralogs TurA and TurB of *Pseudomonas putida* KT2440. *Environmental Microbiology* 12: 254–263.
 - b) Vitale, E., Milani, A., Renzi, F., Galli, E., Rescalli, E., de Lorenzo, V., and **Bertoni, G.*** (2008) Transcriptional wiring of the TOL plasmid regulatory network to its host involves the submission of the sigma-promoter *Pu* to the response regulator PprA. *Molecular Microbiology* 69: 698-713.
 - c) Rescalli, E., Saini, S., Bartocci, C., Rychlewski, L., de Lorenzo, V., and **Bertoni, G.*** (2004) Novel physiological modulation of the *Pu* promoter of TOL plasmid: negative regulatory role of the TurA protein of *Pseudomonas putida* in the response to sub-optimal growth temperatures. *Journal of Biological Chemistry* 279:7777-7784.
6. Study of the alternative sigma factor sigma54-dependent transcription in *P. putida*
 - a) Macchi, R., Montesissa, L., Murakami, K., Ishihama, A., de Lorenzo, V., and **Bertoni, G.*** (2003) Recruitment of σ^{54} -RNA polymerase to the *Pu* promoter of *Pseudomonas putida* through IHF-mediated positioning switch of α CTD on an UP-like element. *Journal of Biological Chemistry* 278:27695-27702.
 - b) Arengi, F. L. G., Barbieri, P., **Bertoni, G.**, and de Lorenzo, V. (2001) New insights into the activation of *o*-xylene biodegradation in *Pseudomonas stutzeri* OX1 by pathway substrates. *EMBO reports* 2: 409-414.
 - c) Carmona, M., de Lorenzo, V., and **Bertoni, G.** (1999) Recruitment of RNA polymerase is a rate-limiting step for the activation of the σ^{54} promoter *Pu* of *Pseudomonas putida*. *Journal of Biological Chemistry* 274:33790-33794.
 - d) **Bertoni, G.**, Fujita, N., Ishihama, A. and de Lorenzo, V. (1998) Active recruitment of σ^{54} -RNA polymerase to the *Pu* promoter of *Pseudomonas putida*: role of IHF and α CTD. *EMBO Journal* 17: 5120-5128.
 - e) **Bertoni, G.**, Marqués, S., and de Lorenzo V. (1998) Activation of the toluene-responsive regulator XylR causes a transcriptional switch between σ^{54} and σ^{70} promoters at the divergent Pr/PS region of the TOL plasmid. *Molecular Microbiology* 27: 651-659.
 - f) **Bertoni, G.**, Pérez-Martín, J., and de Lorenzo, V. (1997) Genetic evidence of separate repressor and activator activities of the XylR regulator of the TOL plasmid, pWW0, of *Pseudomonas putida*. *Molecular Microbiology* 23: 1221-1227.
 - g) Zurla, C., Samuely, T., **Bertoni, G.**, Valle, F., Dietler, G., Finzi, L., and Dunlap, D.D. (2007) Integration host factor alters LacI-induced DNA looping. *Biophysical Chemistry* 128: 245-252.
7. Identification and functional characterization of genes for the degradation of aromatic compounds
 - a) **Bertoni, G.**, Martino, M., Galli, E. and Barbieri, P. (1998) Analysis of the gene cluster encoding toluene/*o*-xylene-monooxygenase from *Pseudomonas stutzeri* OX1. *Applied and Environmental Microbiology* 64: 3626-3632.
 - b) **Bertoni, G.**, Bolognese, F., Galli, E., and Barbieri, P. (1996) Cloning of the genes for and

characterization of the early stages of toluene and *o*-xylene catabolism in *Pseudomonas stutzeri* OX1. *Applied and Environmental Microbiology* 62: 3704-3711.

- c) Radice, F., Orlandi, V., Massa, V., Battini, V., **Bertoni, G.**, Reineke, W., and Barbieri, P. (2007) Cloning of the *Arthrobacter* sp. FG1 dehalogenase genes and construction of hybrid pathways in *Pseudomonas putida* strains. *Applied Microbiology and Biotechnology* 75:1111-1118.
- d) Nogales, J., Macchi, R., Franchi, F., Barzaghi, D., Fernandez, C., Garcia, J. L., **Bertoni, G.** and Diaz, E. (2007) Characterization of the last step of the aerobic phenylacetic acid degradation pathway *Microbiology* 153: 357-365.
- e) Barbieri, P., Arengi F.L., **Bertoni, G.**, Bolognese, F., and Galli, E. (2001) Evolution of catabolic pathways and metabolic versatility in *Pseudomonas stutzeri* OX1. *Antonie Van Leeuwenhoek* 79:135-40.
- f) Favaro, R., Bernasconi, C., Passini, N., **Bertoni, G.**, Bestetti, G., Galli, E., and Deho', G. (1996) Organisation of the *tmb* catabolic operons of *Pseudomonas putida* TMB and evolutionary relationship with the *xyl* operons of the TOL plasmid pWW0. *Gene* 182:189-193.
- g) Polissi, A., Bestetti, G., **Bertoni, G.**, Galli, E. and Deho', G. (1990) Genetic analysis of chromosomal operons involved in degradation of aromatic hydrocarbons in *Pseudomonas putida* TMB. *Journal of Bacteriology* 172:6355-62.

Complete List of Published Work and Research Projects: ORCID [0000-0001-5761-9494](https://orcid.org/0000-0001-5761-9494)