GIULIA DEGIACOMI - *CURRICULUM VITAE*

Name and Surname	**GIULIA DEGIACOMI**
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E-mail	**giulia.degiacomi@unipv.it**
Date of birth	**3rd March 1984**
Citizenship	**Italian**

EDUCATION

January 2012 — **PHD** in Genetic and Biomolecular Sciences
Department of Genetics and Microbiology, University of Pavia, Italy
Title of the thesis: "A magic target and new promising drugs against tuberculosis"
SUPERVISORS: Prof. Giovanna Riccardi and Dr. Maria Rosalia Pasca

• **July 2008** — **MASTER DEGREE** in Industrial Biotecnology
University of Pavia, Italy
Title of the thesis: "Preparazione e studi *in vitro* di nanotubi funzionalizzati per il rilascio controllato di farmaci (Preparation and *in vitro* characterization of functionalized nanotubes for controlled release of drugs)"
Final mark:110/110 *cum laude*
SUPERVISOR: Prof. Pier Carlo Mustarelli

• **November 2006** — **FIRST LEVEL DEGREE** in Biotecnology
University of Pavia, Italy
Title of the thesis: "Purificazione e preliminare caratterizzazione della FHIT bovina (Purification and preliminary characterization of bovine FHIT)"
Finalmark: 110/110 *cum laude*
SUPERVISOR: Prof. Giovanna Valentini

CURRENT POSITION

1st December 2021- present RESEARCHER IN MICROBIOLOGY (Contract RTD – B, SSD: 05-I2, Microbiology BIO/19)

NATIONAL SCIENTIFIC QUALIFICATION

13th September 2018 National Scientific Qualification as Associate Professor in Microbiology (SSD: BIO/19; 05/I2).

AFFILIATION TO ACADEMIC SOCIETIES

Since 2019 Member of the Italian Society of General Microbiology and Microbial Biotechnology (SIMGBM).

WORKING EXPERIENCE

• **October 2020-November 2021** — ***POST-DOCTORAL FELLOWSHIP*** **(ASSEGNO DI RICERCA DI TIPO B)**
Department of Biology and Biotechnology, University of Pavia, Via Ferrata, 9, 27100, Pavia
SUPERVISOR: Prof. Maria Rosalia Pasca

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- **Characterization of new antitubercular drugs**

The 'European Regimen Accelerator For Tuberculosis' (ERA4TB) project involves public and private entities and is funded by the European Commission. The aim of the project is to develop new multi-drug therapies for the treatment of tuberculosis (TB), in particular antibiotic-resistant TB. The consortium plans to develop at least one or more new drug combination regimens ready for phase II clinical development. The Mycobacteriology group is involved in the *in vitro* preclinical studies of new anti-TB compounds.

In this context, I am responsible for supervising, coordinating, and performing the experiments necessary to characterise the mechanisms of action of new compounds in order to evaluate their entry into phase I clinical development (study of mechanism of action and drug resistance; characterisation of new drug combinations; etc.).

• October 2018-September 2020

POST-DOCTORAL FELLOWSHIP **(ASSEGNO DI RICERCA DI TIPO A)**

Department of Biology and Biotechnology, University of Pavia, Via Ferrata, 9, 27100, Pavia

SUPERVISOR: Prof. Giovanna Riccardi

E-MAIL: giovanna.riccardi@unipv.it

• Research topics

- **A molecular and microbiological approach to characterize a new antitubercular drug and to detect the bedaquiline resistance mechanism**

In this project, I focused my research activity on the compound 11726172, a new benzothiazolthiazolidine derivative endowed with potent antitubercular activity (MIC of 0.25 µg/ml). We also investigated the mechanism of action of 11726172 by applying a multidisciplinary approach, including transcriptomic, labelled metabolomic, biochemical, and microbiological procedures (Salina, *et al.*, 2022)

I also validated CanB, a beta-carbonic anhydrase (AC) as a cellular target of *M. tuberculosis*. ACs are metalloenzymes that catalyse the reversible hydration reaction of CO_2 to form HCO_3^- and H^+; in *M. tuberculosis*, CanB is the β-AC that shows greater catalytic activity for CO_2 hydration than the other two mycobacterial ACs. To validate CanB as a therapeutic target, I constructed knock-down conditional mutants (TetR-PipOFF and PipON systems), demonstrating the essentiality of CanB for pathogen survival *in vitro*. Furthermore, the conditional CanB mutants obtained with the Pip-ON system were used to find possible CanB inhibitors, through a target-based screening by resazurin assay (REMA), in collaboration with Prof. Fabrizio Manetti (Degiacomi, *et al.*, 2023).

A second part of the project was to study the mechanisms of resistance to bedaquiline (BDQ), approved in 2012 by the FDA for the treatment of multidrug-resistant (MDR) TB (WHO, 2018). Unfortunately, circulating BDQ-resistant strains of *M. tuberculosis* are already known. To understand the spread of resistance to this drug, I generated *in vitro* BDQ-resistant *M. tuberculosis* mutants from MDR clinical isolates as parental cultures, as this drug is used to treat MDR TB patients (Degiacomi G[§], *et al.*, 2020).

• January 2016–October 2018

COLLABORATION WITH PROF. GIOVANNA RICCARDI AND PROF. MARIA ROSALIA PASCA

• Research topics

- **Target identification of new antitubercular drugs: 7947882, 7904688 and TP53**

This collaboration was possible in the framework of “More Medicines for tuberculosis” project funded by European Commission. This project had as objective the discovery of novel antituberculars and the validation of new targets.

Two leads active against *Mycobacterium tuberculosis* H37Rv were discovered by a phenotypic screening. As demonstrated, they were two prodrugs (7947882 and 7904688), activated by the monooxygenase EthA, targeting the CTP synthetase PyrG (Mori, *et al.*, 2015). Recently, by microbiological, biochemical, and *in silico* methodologies, a second target, the pantothenate kinase PanK, was identified for

7947882 and 7904688 (Chiarelli *et al.*, 2018). Moreover, the validated drug target PyrG was exploited to assess a target-based approach of commercially available, but untargeted, antimycobacterial compounds (Esposito *et al.*, 2017).
Another research line of our collaboration was the identification of the mechanism of action of TP53 thienopyrimidine antitubercular drug: this was related to nitric oxide release mainly targeting protein synthesis (Chiarelli, *et al.*, 2020; Mori, *et al.*, 2020).

• **January 2013–December 2015** ***POST-DOCTORAL FELLOWSHIP***

Department of Molecular Medicine, University of Padova, Via Gabelli, 63 35121, Padova
SUPERVISOR: Prof. Riccardo Manganelli
E-MAIL: riccardo.manganelli@unipd.it

• Research topics

1) Target identification of new antitubercular compounds

This research was part of the already mentioned "More Medicine for Tuberculosis" project. My activity was to select and isolate *Mycobacterium tuberculosis* H37Rv mutants resistant to candidate compounds to finally find their target(s). Moreover, for target validation purpose we used inducible/repressible expression systems. The most important findings were:
-Identification of MmpL3 as target of spiropiperidines (Tantry *et al.*, 2015).
-Validation of PyrG as target of two prodrugs by using the TetR-PipOFF system to construct a *pyrG* conditional knock-down mutant (Mori *et al.*, 2015).
-Characterization of the thiopeptide micrococcin P1 as an antitubercular agent and identification of RplK as its target by recombineering approach (Degiacomi *et al.*, 2016).

2) Optimization of TetR-PipOFF system

A regulated gene expression system was developed for mycobacteria to facilitate the study of essential genes (Boldrin *et al.*, 2010). The TetR/Pip-OFF repressible promoter system was successfully used in the last few years, but, in the first version of the system, the repressible promoter was P_{ptr}, a strong Pip-repressible promoter of *Streptomyces pristinaespiralis*, that might hamper effective downregulation of genes with a low basal expression level.
We improved the system allowing more effective control of genes expressed at low level. To this end, we subjected P_{ptr} to targeted mutagenesis and produced 16 different promoters with different strength. Three of them were selected and characterized to improve the performances of TetR/Pip-OFF repressible system. Finally, we used these promoters to construct a series of bacterial biosensors with different sensitivity to DprE1 inhibitors and developed a whole-cell screening assay to identify inhibitors of this enzyme (Boldrin *et al.*, 2018).

3) Characterization of MmpL3 transporter in *Mycobacterium tuberculosis*

MmpL3 membrane-transporter has emerged as a target for antimycobacterial therapy. By construction of a knock-down mutant (by TetR-PipOFF system), I confirmed the essentiality of this protein *in vitro* and *ex vivo* and I studied the physiological role of MmpL3 and its interacting partners in *M. tuberculosis* H37Rv, in collaboration with Prof. Katarína Mikušová (Comenius University, Slovak Republic) and Dr. Claudia Sala (EPFL, Switzerland) (Degiacomi *et al.*, 2017).

4) GarA, an important metabolic regulator of *Mycobaterium tuberculosis*

In a framework of a collaboration with Dr. Helen O'Hare, University of Leicester (UK), and Prof. Pedro Alzari, Pasteur Institute (France), we analyzed the role of GarA enzyme and its requirement *in vitro* and in macrophages. We were able to highlight the importance of this enzyme in regulation of tricarboxylic acid cycle and glutamate synthesis through the binding to three different enzyme targets (Ventura *et al.*, 2013). Then, we tried to understand the stimuli that lead to phosphorylation of GarA and its role, together with other regulatory enzymes. We found out that GarA is a cellular target of

PknG and the metabolomics data demonstrated that the function of this signaling system is in metabolic regulation (Rieck *et al*., 2016).

• October 2011 - October 2012

POST-DOCTORAL FELLOWSHIP

Department of Biochemistry Mlynska Dolina, Comenius University, 842 15 Bratislava, Slovak Republic

SUPERVISOR: Prof. Katarína Mikušová

E-MAIL: mikusova@fns.uniba.sk

• Research topic

- Study of an ABC-transporter and of PimA enzyme, both involved in cell-wall biosynthesis in *Mycobacterium tuberculosis*

The aim of my work was the analysis of the ABC transporter $(Rv3781)_2/(Rv3783)_2$. This transporter $(Rv3781)_2/(Rv3783)_2$, encoded by two genes from "arabinogalactan biosynthetic cluster", is the only ABC transporter suggested to be involved in the export of polysaccharides to the cell surface in *M. tuberculosis* H37Rv (Centárová *et al*., 2012, poster at EMBO Conference, 2012).

Moreover, in collaboration with Prof. Manganelli, we studied the phosphatidyl-myo-inositol mannoside biosynthetic pathway in *M. tuberculosis* and confirmed that PimA is a novel target for future drug discovery programs (Boldrin *et al.*, 2014).

• March 2011

RESEARCH STAGE DURING THE DOCTORATE

Department of Biochemistry Mlynska Dolina, Comenius University, 842 15 Bratislava, Slovak Republic

SUPERVISOR: Prof. Katarína Mikušová

E-MAIL: mikusova@fns.uniba.sk

- Research topic

During my PhD, I spent a month in the laboratory of Prof. K. Mikušová to learn biochemical techniques to analyze the biosynthesis of arabinogalactan in *M. tuberculosis*.

• November 2008- October 2011

PHD STUDENT

Laboratory of Molecular Microbiology, Department of Genetics and Microbiology, University of Pavia – Via Ferrata, 1, 27100 Pavia, Italy

SUPERVISORS: Prof. Giovanna Riccardi and Prof. Maria Rosalia Pasca

E-MAILS: giovanna.riccardi@unipv.it; mariarosalia.pasca@unipv.it

• Research topic

- **Identification of target of new antitubercular drugs using *Mycobacterium smegmatis* mc²155 as model organism**

This research was part of a "New medicines for tuberculosis" project funded by EC-VI framework (2006-2011). Two different classes of chemical series, the benzothiazinones (BTZ) and the dinitrobenzamide (DNB) derivatives were found to be highly active against *M. tuberculosis*.

DprE1, coding an enzyme essential for the construction of cell wall components, was previously identified as the cellular target of BTZ, we monitored the possible diffusion among *M. tuberculosis* circulating clinical isolates of mutations in the *dprE1* gene and for BTZ susceptibility (Pasca *et al.*, 2010).

Another mechanism of resistance to BTZ by NfnB nitroreductase was identified and characterized (Manina *et al*., 2010).

Moreover, I isolated several *M. smegmatis* spontaneous mutants resistant to DNB, harbouring a mutation in *dprE1*. Finally, I demonstrated that both DNB and BTZ share common mechanisms of resistance and action in *M. smegmatis* (Ribeiro *et al.*, 2011).

• November 2007 – July 2008

THESIS INTERNSHIP

Laboratory of Thin Films, Department of Chemistry, University of Pavia – Viale Taramelli, 16 27100 Pavia, Italy

SUPERVISOR: Prof. Pier Carlo Mustarelli

November 2005–November 2006 **THESIS INTERNSHIP**
Laboratory of Functional and Structural Biochemistry of Proteins, Department of Biochemistry "A. Castellani", University of Pavia – Viale Taramelli, 3/b 27100 Pavia, Italy
SUPERVISOR: Prof. Giovanna Valentini

SCIENTIFIC RESPONSIBILITY IN FUNDED PROJECTS

- Responsible of the Research Unit in the project "An ANTIbody drug conjugate approach to face multidrug Resistant TUBerculosis (AntiReTub)" – PRIN (Progetti di ricerca di rilevante interesse nazionale) 2022 (Prot. 2022JTPP53).
- Partner in the one-year project "Resolving *Mycobacterium abscessus* infections with a phages-inspired therapy" funded by the Italian Cystic Fibrosis Research Foundation (FFC#11/2023).
- Principal investigator in the project associated with the funding of a type A grant for 2 years from the University of Pavia entitled: "A molecular and microbiological approach to characterize a new antitubercular drug and to detect the bedaquiline resistance mechanism" (from 1 October 2018 to 30 September 2020).

PARTICIPATION IN NATIONAL AND INTERNATIONAL RESEARCH PROJECTS

- Component of the Project funded by the European Commission ("Innovative Medicines Initiative 2" - Horizon 2020): "European Regimen Accelerator For Tuberculosis" (ERA4TB; 1/01/2020-31/12/2026; https://era4tb.org/).
- Component of the project funded by the Italian Cystic Fibrosis Research Foundation (FFC#9/2023, FFC#18/2021, FFC#14/2020; FFC#19/2018).
- Component of the European Commission-funded Project (FP7-HEALTH-2010-single-stage): More Medicines for Tuberculosis (MM4TB; Duration: 60 months; 1 February 2011 to 30 June 2016).
- Component of the European Commission funded Project (FP6-2004-LIFESCIHEALTH-5): New medicines for tuberculosis (NM4TB; Duration: 60 months; 1 January 2006 to 31 December 2010).

TEACHING ACTIVITIES

Basic Microbiology (6 CFU) – Master in Molecular Biology and Genetics, curriculum Molecular and Digital Biology, University of Pavia
Analisi Microbiologiche (3 CFU) – Master in Experimental and Applied Biology, curriculum Bioanalysis, University of Pavia

LANGUAGES

English 10th June 2017 Fist Level certificate, grade A (Level C1).

PATENT

Pasca MR, Degiacomi G, Riabova O, Makarov V. 2022. "Pyridine-2-thiol 1-oxide derivatives and their use for the treatment of infections in mammals caused by *Mycobacterium* or fungi". Italian patent (P2245IT).

AWARD

- The importance of the following article was underlined by a 'Focus' in the same issue (Cook GM, Heikal A. Bridging the gap between a TB drug and its target. Sci Transl Med. 4(150):150fs33):
Neres J, Pojer F, Molteni E, Chiarelli L, Dhar N, Boy-Röttger S, Buroni S, Fullam E, Degiacomi G, Lucarelli AP, Read RJ, Zanoni G, De Rossi E, Pasca MR, Riccardi G, Mattevi A, Dyson PJ, Cole ST, Binda C. 2012. Structural basis for benzothiazinone-mediated killing of *Mycobacterium tuberculosis*. Science Translational medicine. 4: 150ra121.
- Best oral presentation at MM4TB meeting, Lille, 2014. Presentation title: Improvement of TetR-PipOFF system and mutants update.

Il sottoscritto, consapevole che – ai sensi dell'art. 76 del D.P.R. 445/2000 – le dichiarazioni mendaci, la falsità negli atti e l'uso di atti falsi sono puniti ai sensi del codice penale e delle leggi speciali, dichiara che le informazioni rispondono a verità.
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