BACTERIAL γ-GLUTAMYL TRANSPEPTIDASES AS BIOTECHNOLOGICAL ENZYMES

We recently characterized the activities of *B. subtilis* e *E. coli* GGTs and constructed recombinant enzymes with altered enzymatic activity (Morelli et al., 2015; Calvio et al., 2018). With the support of the Cariplo Foundation we are now tailoring these enzymes for the synthesis of γ-glutamyl derivatives of naturally occurring and/or modified amino acids starting from bulk chemicals of biotechnological origin (Massone et al., 2019). We are also investigating the immobilization of GGTs to solid phase, in order to obtain robust and recyclable catalyst (Serra et al., 2019). The task of our unit is to obtain mutant GGT enzymes with enhanced activity and tailored for selected acceptor compounds. This work is in collaboration with Profs. C. Morelli (Dept. of Chemistry, Milan University) and T. Bavaro (Dip. Drug Science, UNIPV)

Bacillus subtilis STRAIN IMPROVEMENT FOR γ-PGA PRODUCTION FROM AGRO-INDUSTRIAL BY-PRODUCTS

The need of safer raw materials derived from renewable sources is driving an increasing interest towards natural biopolymers. γ-PGA is an anionic polymer produced by *Bacilli*, composed of thousands of glutamic acid units. Thanks to its non-toxicity, water solubility and biodegradability it finds application in several biotechnological fields as: flocculant for heavy metal removal, cryoprotectant, humectant, thickening additive in cosmetics and food industries, bioplastics, biological glue, drug or vaccine carrier or scaffold for biomedical engineering. Our Lab has obtained a hyper-producer strain derived from a *B. subtilis* lab strain. The availability of a well-defined strain, genetically amendable, offers the opportunity to apply genetic engineering to improve productivity and rationalize metabolic pathways for lowering fermentation costs. By introducing specific mutations, we already obtained strains that show higher product yield.
Currently our aim is to obtain a producer able to ferment organic components contained in some agro-industrial by-products. We took advantage of both rice straw, an abundant biomass currently under-exploited, and raw glycerol, a co-product in the biodiesel industry as bacterial feedstock. The meeting of the above objectives will not only lead to cheaper γ-PGA but will also contribute to the valorisation of abundant by-products in production chains and reinforce the development of new bio-economy sectors (Massaiu et al., 2019; Pasottiet al., 2020). This research line was currently funded by two past CARIPLO FOUNDATION grants, in collaboration with Profs. P. Mustarelli (formerly at the Chemistry Dept., UNIPV), and P. Magni (Dept. Electrical, Computer & Biomedical Engineering, UNIPV) and G. Mazzini (IGM-CNR, Pavia), and is now founded by a CARIPLO FOUNDATION grant, in collaboration with Dr D. Buonocore (DBB, UNIPV), and by the Executive Programme for Scientific and Technological Cooperation between the Italian Republic and the Republic of Poland, funded by the Italian Ministero degli Affari Esteri, in collaboration with Prof. M. Lukaszewicz (University of Wroclaw, PL).

THE ROLE OF SwrA, A RECENTLY DICOVERED *Bacillus subtilis* REGULATORY FACTOR

In *Bacillus subtilis* the two-component system DegS-DegU controls the expression of one hundred of genes involved in the exponential-to stationary phase of growth transition, coordinates single cells differentiation in multicellular communities and in pathogenic species, as *Listeria monocytogenes* or *Bacillus anthracis*, is involved in virulence. It has been shown that DegU regulates *B. subtilis* motility in a complex way. SwrA, a protein which has no similarity to previously characterized proteins, is also involved in such complex regulation. We have shown that there is a functional and molecular interaction between the two proteins, DegU and SwrA, in motility and we are now extending our analyses to other DegU-regulated genetic pathways, such as protease production. The aim is to identify the molecular signal that mediates DegSU two-component system activations and the to characterize DegU-SwrA interaction at the molecular level. To analyse the relationships among these proteins at the single cell level, the state-of-the-art technology represented by the Amnis ImageStream flow cytometer is applied. This research line is carried out in collaboration with Drs L. Pasotti (Dept. Electrical, Computer & Biomedical Engineering, UNIPV) and G. Mazzini (IGM-CNR, Pavia).

γ-PGA-HYDROLASES AS ANTIBACTERIAL TOOLS

Recently we characterized some *Bacillus* genes as encoding efficient and specific γ-PGA degrading enzymes. We determined that those gene are phage-derived and spread across bacteria through horizontal gene transfer. We also identified γ-PGA coding capacity in several microbial species, among which several pathogens (Mamberti et al., 2015). We finely characterized, both enzymatically and structurally, those hydrolases with the aim of exploring their potential as therapeutics for treatment of persistent infections caused by γ-PGA-producing pathogenic bacteria, in which the polymer acts as fundamental virulence factor (Ramaswamy et al., 2018). These activities were carried out in collaboration with Proff. A. Pastore and G. Pietrocola, (Dept. of Molecular Medicine, UNIPV) and C. Morelli (Dept. of Chemistry, Milan University) and Dr M. Fabbi (Istituto Zooprofilattico della Lombardia e dell'Emilia-Romagna, in Pavia).