Bacillus subtilis STRAIN IMPROVEMENT FOR γ-PGA PRODUCTION

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. However, for its full industrial exploitation it is mandatory to reduce production costs, both increasing bacterial productivity and reducing fermentation costs either increasing its yield or decreasing fermentation costs. Our Lab has obtained a producer strain derived from the *B. subtilis* lab strain, 168, fully characterized. The availability of a well defined strain, which is 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 (RIVARIO). 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 valorization of the rice and biodiesel production chains and reinforce the development of new bio-economy sectors. This research line is currently funded by two CARIPLO FOUNDATION grants and is carried out in collaboration with Profs. P. Mustarelli (Chemistry Dept., Pavia University) and P. Magni (Dept. Electrical, Computer & Biomedical Engineering, Pavia University) 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. Currently, we are finely characterizing, 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. These activities are carried out in collaboration with Proff. A. Pastore and G. Pietrocola, (Dept. of Molecular Medicine, Pavia University) and C. Morelli (Dept. of Chemistry, Milan University) and Dr M. Fabbi (Istituto Zooprofilattico della Lombardia e dell'Emilia-Romagna, in Pavia).

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 now would like to extend our analyses to other genetic pathways DegU-regulated, 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.

BACTERIAL γ-GLUTAMYL TRANSPEPTIDASES AS BIOTECHNOLOGICAL ENZYMES

We recently characterized the activities of *B. subtilis* e *E. coli* GGTs and started the construction of mutant 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. 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), T. Bavaro (Dip. Drug Science, Pavia University) and A. Albertini (Dept.Biology and Biotechnology, Pavia University).