Microbial biotechnologies for production of industrially-relevant molecules

Collaborators:

Giulia Barbieri (Postdoctoral fellow)

Paolo Gabrieli (Postdoctoral fellow)

Elisabetta Andreoli (technician)

Giuliano Gasperi (adjunct professor)

Research Lines

REGULATION OF GENE EXPRESSION IN THE GRAM + MODEL ORGANISM *Bacillus subtilis*

A. Albertini; in collaboration with A.L. Sonenshein and B.R. Belitsky (Department of Molecular Biology and Microbiology Tufts University of Boston MA, USA)

One of our main research interests is the study of regulation of gene expression in *Bacillus subtilis*. Bacteria can use different strategies to cope with difficult environmental conditions and with scarcity of nutrients. These include the ability to move, the synthesis of degradative enzymes and transporters able to import the products of degradation, and the production of antibiotics. At the onset of stationary phase, *Bacillus subtilis* is also able to differentiate into competent cells capable of taking up DNA from the environment, or into metabolically dormant spores, highly resistant to external stresses. Our group is interested in the study of the regulatory pathways that control these differentiation processes at the beginning of stationary phase. In collaboration with professors A.L. Sonenshein and B. Belitsky, we recently demonstrated that the global transcriptional regulator CodY, one of the main regulators of the nutritional stress response, is involved in the regulation of four extracellular proteases in *B. subtilis*.

Related papers

1. Barbieri G, Albertini AM, Ferrari E, Sonenshein AL, Belitsky BR. Interplay of CodY and ScoC in the Regulation of Major Extracellular Protease Genes of *Bacillus subtilis*. J Bacteriol. Jan 4;198(6):907-20. doi: 10.1128/JB.00894-15, 2016
2. Belitsky BR, Barbieri G, Albertini AM, Ferrari E, Strauch MA, Sonenshein AL. Interactive regulation by the *Bacillus subtilis* global regulators CodY and ScoC. Mol Microbiol., Aug;97(4):698-716. doi: 10.1111/mmi.13056 4.419, 2015
3. Barbieri G, Voigt B, Albrecht D, Hecker M, Albertini AM, Sonenshein AL, Ferrari E, Belitsky BR. CodY regulates expression of the *Bacillus subtilis* extracellular proteases Vpr and Mpr. J Bacteriol. Apr;197(8):1423-32. doi: 10.1128/JB.02588-14. 2.808, 2015

IDENTIFICATION OF NEW BIOLOGICAL INSECTICIDES BY BACTERIA FOR THE CONTROL OF *Aedes albopictus* (ASIAN TIGER MOSQUITO)

A. Albertini, G. Gasperi

The project, funded by the Bussolera-Branca Foundation, interests two laboratories with complementary skills of the Department of Biology and Biotechnology, and aims to the identification of new bacterial strains able to produce molecules characterized by specific insecticidal activity for the control of *Aedes albopictus*. For this purpose, bacteria with larvicidal activity are sought in nature (soil, standing water, etc.) tested and validated on standard tiger mosquito strain larvae of Italian origin, available in the Insects Genomics and Biotechnology Laboratory. Subsequently will be assayed the bacterial strains and the compounds endowed with bio-insecticide activities isolated from soil, for toxicity against other natural populations of *Aedes albopictus,* as well as for the safety for vertebrates, insects and other animal and plants species of agronomic interest, in view of the validation of a possible field use.

Genomic and metagenomic approaches are employed to identify genes involved in the production of these bacterial secondary metabolites; to improve the level of expression in a bacterium suitable for the production on a commercial scale, such as *B. subtilis*, will be set up cloning and "genome shuffling" methods.

BACTERIAL CELL FACTORIES FOR CATALYSTS PRODUCTION AND MUTAGENESIS.

A. Albertini and C. Calvio; in collaboration with D. Ubiali and T. Bavaro (Department of Drug Science, University of Pavia) and C. Morelli and G. Speranza (Department of Chemistry, Statale University of Milano)

E. coli is well suited for the expression of foreign genes, due to the vast knowledge of its metabolism and the availability of a large collection of different vectors and strains.
The group of Microbial Genetics and Biotechnology collaborated with the Biocatalysis Laboratory of the Department of Drug Sciences for the production of new biocatalysts, such as PGA (Penicillin G-acylase, catalyst for the production of β lactam nuclei, to be used for the synthesis of semi-synthetic antibiotics), or purine and pyrimidine phosphorylases, catalysts for the transglycosilation reactions between a nucleoside (natural or modified in the sugar moiety) and a natural or modified purine or pyrimidine base.
Aims of these projects are the production of new immobilized catalysts trough the search of new genes for purine and pyrimidine phosphorylases from different bacterial sources, and new strategies coupling in vitro site directed mutagenesis and immobilization for the rational design of more efficient biocatalysts.
Recently, in collaboration with C. Morelli (Department of Chemistry, Statale University of Milano), a CARIPLO research grant is funding studies on the trans-peptidation reactions catalyzed by γ-glutamyl transpeptidases (GGTs) of microbial origin to improve through mutagenesis the catalytic properties of the enzymes to produce new γ–glutamyl derivatives of interest in the food and pharma industry. The mutagenesis and selection of recombinant GGTs will allow the immobilization for the development of large scales processes.

Related papers

1. Biagiotti M, Borghese G, Francescato P, Morelli C F, Albertini AM, Bavaro T, Ubiali D, Mendichi R and Speranza G. Esterification of poly(γ-glutamic acid) (γ-PGA) mediated by its tetrabutylammonium salt. RSC Adv., 6, 43954-43958. doi: 10.1039/C6RA08567A, 2016
2. Ubiali D., Morelli C.F., Rabuffetti M., Cattaneo G., Serra I., Bavaro T., Albertini A.M. and Speranza G. Substrate Specificity of a Purine Nucleoside Phosphorylase from *Aeromonas hydrophila* Toward 6-

Substituted Purines and its Use as a Biocatalyst in the Synthesis of the Corresponding Ribonucleosides, Curr. Org. Chem. 19 (22): 2220 – 2225,. 2.157, 2015

3. Serra I., Ubiali D., Cecchini D.A., Calleri E., Albertini A.M., Terreni M., Temporini C. Assessment of immobilized PGA orientation via the LC-MS analysis of tryptic digests of the wild type and its 3K-PGA mutant assists in the rational design of a high-performance biocatalyst. Anal Bioanal Chem. Jan; 405(2-3):745-53. doi: 10.1007/s00216-012-6143-z , 2013
4. Serra I., Bavaro T., Cecchini D. A., Daly S., Albertini A.M., Terreni M., Ubiali D., A Comparison between Immobilized Pyrimidine Nucleoside Phosphorylase from *Bacillus subtilis* and Thymidine Phosphorylase from *Escherichia col*i in the Synthesis of 5-Substituted Pyrimidine 2'-Deoxyribonucleosides. Journal of Molecular Catalysis B: Enzymatic, 95, 16-22, DOI information: 10.1016/j.molcatb.2013.05.007, 2013
5. Serra I., Ubiali D., Piskur J., Christoffersen S., Lewkowicz E., Iribarren A. M.,. Albertini A. M, Terreni M., Developing a Collection of Immobilized Nucleoside Phosphorylases for the preparation of Nucleoside Analogues: Enzymatic Synthesis of Arabinosyladenine and 2',3'-Dideoxyinosine, Chem Plus Chem, 78 (2), 157-165. DOI: 10.1002/cplu.201200278, 2013
6. Serra I., Ubiali D., Cecchini D.A., Calleri E., Albertini A.M., Terreni M., Temporini C. Assessment of immobilized PGA orientation via the LC-MS analysis of tryptic digests of the wild type and its 3K-PGA mutant assists in the rational design of a high-performance biocatalyst. Anal Bioanal Chem., 405(2-3):745-53, DOI: 10.1007/s00216-012-6143-z, 2012
7. Ubiali D., Serra C.D., Serra I., Morelli C.F., Terreni M., Albertini A.M., Manitto P. and Speranza G. Production, characterization and synthetic applicatio of a purine nucleoside phosphorylase from *Aereomonas hydrophyla*. Adv Synth. Catal. 354, 96-1041, 2012
8. Serra I, Cecchini DA, Ubiali D, Manazza EM, Albertini AM, Terreni M. Coupling of site-directed mutagenesis and immobilization for the rational design of more efficient biocatalysts. The case of immobilized 3G3K PGA from *E. coli.* Eur. J. Org. Chem., 9:1384 -1389, 2009
9. Cecchini DA, Serra I, Ubiali D, Terreni M and Albertini AM. New active site oriented glyoxyl-agarose derivatives of *Escherichia coli* penicillin G acylase. BMC Biotechnol.7: 54, 2007.