already a function (i.e., cathepsin D, PPT1, and TPP1), others proteins (CLN3, CLN5, CLN6, CLN7, and CLN8) still wait to be defined. Mutations of CLN8 cause two major clinical phenotypes: the progressive epilepsy with mental retardation (EPMR or Northern Epilepsy), a juvenile-onset phenotypic variant, and a more severe form with a late-infantile onset (LINCL). A CLN8 mutation spontaneously occurring in mice results in the motor neuron degeneration (mnd) phenotype. The CLN8 is a ubiquitous membrane protein of 286 a.a., containing an ER-retrieval signal (KKRP) and primary located at the ER. Probable functions of CLN8 implies an involvement in the lipid synthesis and/or proteolipid trafficking or as a lipid sensing. In this study, to elucidate CLN8 protein function we better characterized the subcellular localization and verified its post-translational modification. Endogenous and recombinant protein expression were examined in differentiated and undifferentiated human neuroblastoma cells and in a human epithelial cell line, that were subjected to treatments with specific drugs activating phosphorylation and/or inhibiting phosphatase. Protein localization was also assessed in fractionated lysates of cerebella from wild type and mnd mice. Data obtained indicate a possible CLN8 threonine phosphorylation site which lacks in the sequence of CLN8 mutated in the EPMR disease, thus suggesting its important functional role. Also, hints regarding CLN8 role could be uncovered by a different subcellular localization of various forms of CLN8 under physiological and pathological conditions.

Microrganismi nelle Biotecnologie (MB)

MB1
Role of the two component system Dbv6/Dbv22 in regulating A40926 biosynthesis in *Nonomuraea sp.* ATCC 39727

L. Lo Grasso[1], A. Sabella[1], A. Pisciotta[1], F. Armetta[1], D. F. Chillura Martino[1], M. Massimi[2], S. Donadio[2], M. Sosio[2], R. Alduina[1]

1. Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Viale delle Scienze, 90128 Palermo, IT; 2. KtedoGen Srl; Naicons Srl, Via Ortles 22/24, 20139 Milan, Italy.

The actinomycete *Nonomuraea* sp. ATCC 39727 produces the glycopeptide A40926, precursor of dalbavancin, recently approved by FDA for treatment of severe Gram positive bacterial skin infections. Biosynthesis of A40926 is encoded by the *dbv* gene cluster, which contains 37 protein coding sequences that participate in antibiotic biosynthesis, regulation, immunity, and export. Specifically, the *dbv* cluster contains three regulatory genes, *dbv3*, *dbv4* and *dbv6*. The positive regulatory role of Dbv3 and Dbv4 on A40926 biosynthesis was already demonstrated, while the role of Dbv6 has not yet been elucidated. Dbv6 is a putative response regulator being part of the two component system Dbv6-Dbv22 in which Dbv22 should act as the sensor kinase. Two independent mutants in *dbv6* and *dbv22* were generated. Analysis of the Δ*dbv6* and Δ*dbv22* mutant strains demonstrated that Dbv6 and Dbv22 do not affect the bacterial growth, while both of them negatively control the antibiotic production. In fact, bioassays and LC-MS analyses showed that the Δ*dbv6* and Δ*dbv22* mutant strains produce 2- and 3-fold more antibiotic, respectively, than the parental strain. Quantitative RT-PCR analysis confirmed that some biosynthetic *dbv* genes are more transcribed in both mutant strains and mobility shift assays, conducted with a His-tagged Dbv6 showed that these genes are under the direct control of Dbv6. These results strongly suggest the Dbv6 and Dbv22 work together. Combining these data with previous knowledge, we propose a complex model of antibiotic regulation with three different regulatory mechanisms governing A40926 production.

MB2
Bioactive molecules from soil and marine bacteria: new potential applications

F. Ardizzone[1], S. Vogiatzis[1], C. Catania[1], T. Faddetta[1], G. Gallo[1], V. Cavalieri[1], F. Palla[1], A. M. Puglia[1]

1. STEBICEF Department, University of Palermo, Viale delle Scienze, ed. 16 - Via Archirafi 38, Palermo.

Microorganisms produce a great amount of enzymes that degrade a wide variety of organic materials such as oils, proteins, and esters. These enzymes are of great importance in the development of industrial

bioprocesses and could be used in several biotechnological fields: bioremediation, feed industry, food processing, cosmetic products and in this study have been tested for enzymatic bio-cleaning of works of art surfaces. Recently, bioactive molecules secreted by soil and marine bacteria showed encouraging results in degrading different substrates. We are analysing different hydrolases secreted by Streptomyces coelicolor, a model actinomycete, and by marine bacteria from Mediterranean Sea. The enzymatic activity of these hydrolases was tested through zymography using as substrate: gelatin and casein for proteases, 4-Nitrophenyl myristate for esterases and olive oil for lipases, respectively. Noteworthy, these novel enzymes show a good hydrolase activity at different temperatures, including temperatures below 30°C, unlike the commercial enzymes that usually need higher temperatures ($\geq$ 37 °C). Since these enzymes operate with high selectivity and at temperature < 30°C, they could be utilized in different bioprocess such as the enzymatic bio-cleaning of art surfaces, where a crucial point is represented by the temperature of application.

MB3
Isolation and partial characterization of methanogenic bacteria from a digester fed with vegetable biomass and swine manure

A. Artale[1], G. Spinelli[1], G. Sapienza[1], S. Balletta[1], G. Biddeci[1], G. Pantaleo[2], G. Ruggieri[2], F. Di Blasi[1]

1. Institute of Biomedicine and Molecular Immunology "Alberto Monroy", National Research Council, Via Ugo La Malfa 153, 90146 Palermo, Italy; 2. Institute for the Study of Nanostructured Materials, National Research Council, Via Ugo La Malfa, 153 – 90146 – Palermo, Italy

The use of renewable energy sources is becoming increasingly essential, in order to reduce emissions from fossil fuel sources that have impact on global warming. Therefore, biomass presents one of the most common form of renewable energy source for feasible utilization; it is widely available, and the energy produced can widely reduce carbon dioxide emissions. Biomass can biologically be converted to biogas (CH4, CO2). The anaerobic digestion is one of the most economic way to produce biogas from various biomass substrates; during this process different consortia of microorganisms are needed. The methanogenic archea play main role in the process of methane gas production. The aim of our project is the isolation and characterization of methanogens species present in samples from a biodigester containing vegetable biomass and swine manure. Some enrichment cultures of methanogens, through the use of anaerobic techniques, were obtained in our laboratories. Analysis of methane production, carried out using the ABB A2020 Advance Optima process gas analyzer, showed that the formate served as a substrate for the methanogenesis. We also performed PCR analysis of 16S rRNA partial sequence and mcrA gene to confirm the presence of various methanogenic bacteria. The next step is to characterize the individual micro-organisms present in the enrichment cultures. The final purpose is to better understand the metabolism of these bacteria and also to improve the efficiency of biogas production.

MB4
The seed microbiota of *Anadenanthera colubrina*

P. Alibrandi[1], M. Cardinale[2], F. Strati [3], M. D. Mahafizur Rhaman[2], P.Cinà[1], M.de Viana[4], G. Gallo[1], D. Cavalieri,[3] M.Ciaccio[5], A. M. Puglia[1]

1. Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche, STEBICEF, Palermo; 2. Institute of Applied Microbiology, Justus-Liebig-University Giessen (Germany); 3. Department of Computational Biology, Research and Innovation Centre, Fondazione E. Mach, San Michele all'Adige; 4. Banco de Germoplasma de Especies Nativas, Instituto de Ecología y Ambiente Humano, UNSA, Argentina; 5. Istituto di Biomedicina e Immunologia Molecolare "A.Monroy". CNR, Palermo.

Although the importance of plant-associated microorganisms for plant growth and health was getting more recognition recently, the role of seed-associated microorganisms, and especially seed endophytic bacteria, still is underestimated. Nevertheless, these associations could be beneficial for germination and seedling establishment as seed endophytic bacteria are already present in these very early plant growth stages. In this