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Meeting Report

Microbial stress: from molecules to systems (Belgirate, May 2012)

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The meeting "Microbial Stress: from Molecules to Systems" held in Belgirate (Italy) in May 2012 focussed on how organisms cope with intracellular and environmental stresses, how signal detection is turned into a response; the effects of stress on energy metabolism; and the effect of diverse stress responses on industrial biotechnology. Key conclusions included that stress responses are complex and far from being completely understood even for model organisms. Examples were given of how a combination of tools developed for systems and synthetic biology can be exploited to close knowledge gaps. Microbes must be able to balance energy demand and supply in order to survive. Mechanism exploited to achieve this vary depending on the lifestyle of the microbe and the physiological conditions encountered, especially stresses imposed by the artificial conditions in industrial fermenters. Obvious examples include solvent tolerance that must be developed by organisms used for biofuel production; the potential of using high hydrostatic pressure as a bioprocessing parameter; and opportunities to exploit natural diversity gleaned from metagenomic sequencing to find organisms with natural traits suitable for the development of new biotechnologies. The meeting is now part of a biennial series that will be advertised on the EFB website: www.efb-central.org.

The meeting "Microbial Stress: from Molecules to Systems" held in Belgirate (Italy) from 10 to 13 May 2012 was organised by an international team led by Paola Branduardi (University of Milano-Bicocca, Italy), Michael Sauer (BOKU Univ. Natural Resources and Life Sciences Vienna, Austria) and Peter Lund (University of Birmingham, UK). The Conference attracted a total of 140 delegates, both from Academia and from Industry with 30 different countries represented, including participants from the USA, Japan, Australia, Brazil and Saudi Arabia. It was organized on behalf of the Microbial Physiology Section of the European Federation of Biotechnology. FEMS supported the Conference providing grants for keynote speakers and young scientists. Sponsorship from DASGIP and SARTORIUS provided sponsorship including cash prizes for the best posters by young scientists, and support from Instrumentation Technology (Beckman Coulter), Purac, ACS Dobfar and Bicocca Biotechnicum Center is gratefully acknowledged.

Keeping diversity together, to find common outcomes and aims

The meeting title reflects the aim of examining various stresses at diverse levels, from detailed molecular biology through whole organism systems biology to the level of populations. In contrast to organising the programme into sessions based on different stresses or organisms, three broad research themes covered different experimental approaches and model organisms grouped logically into each thematic session. The first session focused on how organisms cope with intracellular and environmental stresses, and how signal detection is turned into a response. This was followed by a session focused on how stress and responses are related to energy metabolism. The final session was dedicated to how industrial production by microorganisms is influenced by these stress responses. To ensure coverage of both fundamental and applied research, the programme was designed by the joint work of both a scientific and an industrial committee. The concept was fully embraced by the speakers, so that in all the three sections there was a continuous cross-fertilization between these two branches of research. Moreover, a round table entitled "What does industry really need from science?", which closed the conference, provided an opportunity to compare how Research Institutes, Universities and Centres of Excellence are organized, how they face and support the challenges of an upcoming bio-based society and, last but not least, how they recruit new personnel.

Intracellular and environmental stresses: how cells copy with them

The first session on how cells cope with intracellular and environmental stresses was designed to investigate the links between stress detection and cellular response(s) and defence(s). An opening plenary lecture by Michael Hecker (Institut für Mikrobiologie Universität Greifswald, Germany) illustrated how

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a combination of gel-based and gel-free proteomic technologies has allowed the detection and the quantification of 70–80% of the proteins expressed by the model organisms *Bacillus subtilis* and *Staphylococcus aureus*. This has provided proteomic maps and libraries that can be consulted for many different physiological applications, from industrial exploitation to clinical related conditions.

The following presentations covering diverse prokaryotic and eukaryotic microorganisms revealed that the stress responses and the acquisition of cell tolerance can occur differently in different strains of the same species. They even vary at micro-scale level in cells coming from the same colony. John Morrissey (Microbiology Department, University of Cork, Ireland) compared *Kluyveromyces marxianus* strains obtained from European culture collections and found that they varied widely in their responses to thermo, osmotic, cell wall and organic acid stress. These data underline the need to develop strategies for understanding the molecular basis of these traits in species for which only limited genetic and molecular tools are available. This is essential to facilitate selection of improved strains for industrial applications. Claude Saint Ruf (INSERM, Universitè Descartes, Paris, France) showed the dynamic heterogeneity in metabolic activity, morphology and gene expression patterns, including stress response induction, among *Escherichia coli* cells inside single colonies. In aged colonies, oxidative stress is the major cause of death. She showed that it is possible to identify cells that are still metabolically active, surrounded by metabolically inactive or dead cells. They develop structured environments called "islands" in which the phenotypic variants are more robust and adapted to stress. Sequencing revealed that some of these variants carry mutations in the cold shock-like protein CspC, suggesting that its role in mRNA stabilization could correlate with the acquired phenotypes. The conclusion is that genetic modifications of general classes of regulatory molecules can be identified amongst the extreme diversities of phenotypes observed within a single colony.

Small non-coding RNAs, common both to prokaryotes and eukaryotes, which are expressed under stressful conditions, were described by Joseph Shiloach (Biotechnology Core Laboratory NIH Bethseda, USA). They act by altering the expression of specific genes through interaction with preformed messenger RNA. This mechanism is opening new possibilities for controlling microbial growth and productivity, especially when the organism is exposed to stress conditions. Controlling glucose assimilation by over-expression of the small RNA SgrS was given as an example. This illustrates how regulatory macromolecules belonging to different classes can be utilized by the cells in parallel or in combination and with a wide array of interactions to organize a specific stress response. An example of a highly specific defence mechanism was given by Jeannette Winter (Technische Universität Munchen, Germany) who illustrated how reversible methionine oxidation is responsible for protecting cells against the consequences of hypochlorite stress by activating the *E. coli* transcription factor YjiE. Remarkably, YjiE utilizes the usually damaging and inactivating methionine oxidation to directly trigger the stress response. A specific class of molecules can also undergo intraspecific interactions to address cellular needs, as in the case of cyanobacterial chaperones. Hitoshi Nakamoto (Saitama University, Japan) described the defence role of the cyanobacterial DnaK2 protein, which, in association with DnaJ2 and GrpE, forms the chaperone machinery assisting protein refolding in stressed cells, and controls the composition and physical state of the cellular membranes.

A common conclusion in all of the talks was that stress responses are complex and far from being completely elucidated even for model organisms. Connor O'Byrne (Microbiology, NUI, Galway, Ireland) reviewed the role and the regulation of the stress-inducible sigma factor SigB in the Gram positive human pathogen *Listeria monocytogenes*. He presented an elegant system that allows the real-time activity of SigB to be monitored through a fluorescent reporter fusion to a strongly σ^B-dependent promoter. SigB turned out to be not only central to the osmotic stress response, but also linked to the PrfA regulon, a transcription regulator that controls virulence gene expression. This underlines the cross-talk between the stress detection system and the virulence cascade, and, more generally, the complexity of cell responses to external stimuli. Daniela De Biase (Università La Sapienza Roma, Italy) reported that the *E. coli* GadX transcriptional regulator of the *gad* (glutamate dependent) system involved in acid resistance of the "pathotypes" also affects the expression of approximately 100 genes, many more than previously expected. Some of these genes are known to play major roles in important physiological processes apparently unrelated to the response to acid stress.

The need to adapt to particular environmental fluctuations represents one of the driving forces for the evolution of specific mechanisms of cell defence. Laurent Beney (Universitè de Bourgogne/Agrosup Dijon, France) traced the origin of the ergosterol biosynthetic pathway (EBP), which is peculiar to fungi, to their experience of life under frequent fluctuations in water availability. Survival of *Saccharomyces cerevisiae* wild type and EBP-mutants strains (accumulating different sterol intermediates) after transition from aquatic to aerial media was compared. Increased ergosterol synthesis correlated with an increase in the resistance to air drying, being maximal for the wild type strain. This suggests that the survival to continuous drying and wetting, in a context of solid-aerial interfacial habitat, is facilitated specifically by ergosterol. Loss of stress resistance by EBP mutants supported suggestions that ergosterol might play a protective role against mechanical and oxidative stress. Finally, several talks illustrated how coping with stress is linked to energy requirements. For example, Aurelio Serrano (CSIC-Universidad de Sevilla, Spain) showed how from bacteria and archea to protists and higher plants, pyrophosphate is used as an alternative to ATP in bioenergetics. Ion-pumping membrane pyrophosphatases, proteins that couple pyrophosphate hydrolysis to the generation of transmembrane electrochemical ion gradients, may have a key role in conferring a major adaptative advantage under conditions of severe and chronic stress. Serrano's talk thus constituted the ideal *trait d'union* between the first and the second section, which was completely dedicated to bioenergetics.

Cell stress and energy metabolism

All living cells have developed mechanisms to balance energy demand and supply under physiological conditions. Stress perception and the subsequent response are active processes that require additional or alternative energy to survive under adverse conditions. In this context, Vassily Hatzimanikatis (Ecole Polytechnique Federal of Losanna, Switzerland) opened the second session under the slogan "Keep in mind the Energy". His lecture discussed how metabolic network-based models can shed light on how cells couple energetic requirements with metabolism. He presented a consistently reduced core *E. coli* model, and, through a thermodynamic approach, he used it to analyze how perturbations and reactions can link wet and *in silico* data to study the complexity of stress management during growth at low pH. The same topic was also discussed by Kaspar

Valgepea (Competence Centre of Food and Fermentation Technologies, Tallinn, Estonia) who, by applying a systems biology approach and using quantitative transcriptome, proteome and metabolome analyses coupled to steady-state cultivation and metabolic flux analysis, focused attention on the cellular energy costs for regulating specific reactions and pathways. He showed the importance of post-transcriptional modifications in regulating flux rates compared with regulation at the transcription level. Cofactor metabolism can also dramatically influence cell physiology. Luigi Palmieri (University of Bari, Italy) illustrated how global cellular bioenergetics can be changed by varying mitochondrial NAD^+ levels, for example through the altered expression of the two isoforms of the specific mitochondrial carrier for NAD^+ of *S. cerevisiae*. Mike Merrick (John Innes Centre, Norwich, UK) discussed P_{II} signal transduction proteins that bind ATP and have been suggested to respond to the cellular energy charge. Based on a study of *E. coli* P_{II} protein GlnK, he proposed that P_{II} proteins actually have a 2-oxoglutarate-dependent ATPase activity that allows them to behave as molecular switches that sense the cellular nitrogen status. Vasili Hauryliuk (Uppsala University, Sweden) presented a novel single-molecule tracking methodology to characterize the intracellular catalytic cycle of RelA, the integrating-molecule that plays a central role in bacterial adaptation to starvation and stress and is responsible for ppGpp synthesis. Having reminded the audience that "cells have evolved biological responses to different stress and other signals", Tim Donohue (University of Wisconsin, USA) described the convergence of transcriptional responses to reactive oxygen species, in particular to singlet oxygen, and heat shock stresses in the α-proteobacterium *Rhodobacter sphaeroides*. It was demonstrated, by transcriptional profiling, chip-chip and reporter gene analyses, that the sigma factor $RpoH_{II}$, which is homologue to $RpoH_I$ for heat stress, is specifically active when stress is induced by reactive oxygen species, with the possibility that there is crosstalk between the systems. Anne Francez-Charlot (ETH Zurich, Switzerland) illustrated how reprogramming gene expression is an essential component of adaptation to changing environmental conditions with a study on the general stress response in two different Alphaproteobacteria.

The ability of cyanobacteria to perform oxygenic photosynthesis is *per se* stressful but also constitutes the most powerful mechanism that has evolved to capture solar radiation to drive biosynthesis. Eva Mari Aro (University of Turku, Finland) illustrated how one specific system of flavodiiron proteins (FDPs) is important as ROS scavenger especially under condition of fluctuating light, and the other one is essential for the escape of electrons and keeping the Photosystem II linked to phycobilisomes under low Ci/hight light stress. Taina Tyystjärvi (University of Turku, Finland) showed that the SigB sigma factor is essential for the acclimation of the cyanobacterium *Synechocystis* sp. PCC 6803 to salt stress. Pascale Daran-Lapujade (Delft University of Technology, The Netherlands) explained the difference between extreme calorie restriction and carbon starvation in *S. cerevisiae*, suggesting important consequences when considering these microorganisms as cell factories.

Cell stress and bio-based microbial production

The effect of diverse stresses on microbial based industrial production was a recurring theme of the third and final session. The message from this section was that for successful commercial exploitation, it is essential to study the entire production process, from stress at the single cell level to the development of robust strains and production processes, considering such factors as microbial population, raw materials and process conditions. The last keynote speaker, Jens Nielsen (Chalmers University of Technology, Gothenburg, Sweden) gave a comprehensive lecture on modelling and engineering of protein secretion by yeasts. Starting from the question "Is protein production and release into the medium a stress response?" he demonstrated that the production of each individual recombinant protein presents unique challenges. A different perspective on recombinant protein production in bacteria was given by Antonio Villaverde (Universitat Autònoma de Barcelona, Spain), who reported an unusual application of bacterial inclusion bodies, which are typically regarded as undesired aggregates of unfolded proteins. Surprisingly, the mechanical stability of inclusion bodies makes them attractive as nanoparticulate materials and, in the case of those formed by therapeutic proteins, as agents for slow drug release in protein replacement cell therapies.

In contrast to recombinant proteins, many of which are high added value products, commodities and biofuels represent low added-value products. Development of cheap processes for their production is more than necessary, it is mandatory, starting with a low cost substrate. Lignocellulosic biomasses are certainly cheap, but they represent a real challenge for the microbial cells because of both complexity and for the high level of toxic compounds. Eckhard Boles (Goethe-University Frankfurt, Germany) showed how industrial yeasts have been developed that co-metabolize a mixture of C5 and C6 sugars. These strains are also able to cope with the constraints caused by pre-treatment of biomass. Maurizio Bettiga (Chalmers University of Technology, Gothenburg, Sweden) reported the identification of *S. cerevisiae* molecular targets for different classes of fermentation inhibitors present in lignocellulosic hydrolysates, with the final goal of developing robust strains exhibiting higher tolerance, and therefore higher productivity. An evolutionary engineering approach that combined improved thermotolerance and higher robustness against lignocellulose-derived inhibitors was described by Valeria Wallace-Salinas (Lund University, Sweden). This resulted in the development of a yeast strain capable of growing and fermenting under combined stresses. The study of the genetic basis of yeast acetic acid tolerance was the focus of the talk by Jean-Paul Meijnen (KU Leuven, Belgium). Acetic acid is one of the most important inhibitors released by lignocellulose pretreatments, and the development of robust industrial strains requires multiple genetic modifications. A new method was described that combined protocols to enrich causative mutations (pooled segregants) with next-generation sequencing to identify multiple quantitative trait loci (QTLs) possibly responsible for the phenotype of interest.

When eventually a microorganism is developed that is efficient at producing a biofuel, for instance ethanol or butanol, the product also turns out to be toxic for the cell. The development of tools suitable to investigate the mechanisms involved in solvent tolerance and their cross-interactions was reported by Eugene Fletcher (School of Biological Sciences, University of Edinburgh, UK). A synthetic biology approach was adopted to generate a library of standardised BioBrick parts involved in organic solvent tolerance, thus obtaining a set of tolerance modules that can be combined with genetic modules encoding substrate breakdown and product formation pathways. "Playing with DNA" was also the central point of the presentation by Ryan Gill (University of Colorado, USA). He illustrated how easy it is nowadays to combine different pieces of genes, but how difficult it remains to predict the results of efforts "to re-write" a genetic trait to obtain the optimal organism performance. Another way of addressing the question of tolerance to stress is

to look for the most suitable organisms that are naturally adapted to the stressful condition of interest. Biodiversity exploitation and bioprospecting were exemplified by the talk of Michael Sauer (BOKU – VIBT University of Natural Resources and Life Sciences, Vienna, Austria), who reminded to the audience that "a glimpse into nature is generally helpful because nature provides some solutions to problems which modern technologies pose". He "fished" *Megasphaera elsdenii* from animal rumen as a favourable microbial cell factory for organic acid production, due to its high tolerance of volatile fatty acids. After genome sequencing, the annotation is now helping in supporting the metabolic data, with a special eye for hexanoic acid production and tolerance. The theme of organic acid bio-based production, a fast-expanding market in white biotechnology, was further examined by Zheng Zhao (DSM Biotechnology Center, Delft, The Netherlands), who described a process for succinic acid production in metabolically engineered yeast.

The question of process constraints was central in this last section of the programme. The last speaker, Abram Aertsen (KU Leuven, Belgium), added another element to the picture: the potential of using high hydrostatic pressure as a processing parameter. This parameter can obviously change the thermodynamics of the reactions, the conformation or stability of macromolecules and their proximity, all having a clear impact on production. Aertsen showed that *E. coli* strains can significantly extend their native boundaries for growth or survival under high pressure. The take-home message is that any genome contains unexploited solutions.

Future plans

The great support for this and the previous conference in the series, combined with the high number of people working in this area, convinced the Board of the EFB Microbial Physiology Section that this Conference should become a regular series as the European Meeting on Microbial Stress. Moreover, considering the great potential for applications in fields such as biotechnology and microbial pathogenesis and the fast-moving level of research, it was proposed to organize the next event in the autumn of 2014 rather than to wait until 2015. Details will be posted on the EFB website: www.efb-central.org.

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