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Highlight

The heat, drugs and knockout systems of *Microbial Biotechnology*

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The current issue of *Microbial Biotechnology* completes the second volume of this journal that aims to collect the best fundamental science in the field of microbiology related to biotechnological applications. The journal has maintained a regular flow of articles and has published many extremely relevant articles in the field, some of which are being extensively cited; an acknowledgement of their high scientific value.

In the upcoming issue of *Microbial Biotechnology* Martín and colleagues (2009) provide a comprehensive review of β-lactam production by deuteromycetes and fungi in their mini-review entitled 'Regulation and compartmentalization of β-lactam biosynthesis'. The authors discuss in depth the biosynthetic steps involved in production of penicillins, cephalosporins, cephamycins and cephabacins. The processes of penicillin and cephalosporin C biosynthesis are compartmentalized; beginning with the formation of a linear amino acid precursor (ACV), and the cyclization of this to isopenicillin N in the cytosol and proceeding to either penicillin G in peroxisomes or cephalosporin C via penicillin N in peroxisomes and the cytosol. Emphasis is placed on the importance of peroxisomes (single membrane bound microbodies) as many of the significant biosynthetic steps take place in these organelles. The compartmentalization of the biosynthetic process allows division of precursors and enzymes and aids in the regulation of the reactions involved. Naturally, this also brings into play a number of important transport systems that are required to localize the enzymes and reaction intermediates during the biosynthetic process; many of the players forming these transport systems are still undefined. Recent exciting research on the transport mechanisms has led to the construction of strains able to produce increased amounts of β-lactam (Nijland *et al.*, 2008). The current discovery of the CefM efflux pump protein and its inactivation in an *A. chrysogenum* strain showed the importance of precursor penicillin N transportation from the peroxisome during cephalosporin C biosynthesis (Teijeira *et al.*, 2009). The authors conclude that future experimentation on the methodology of biosynthetic intermediate and enzyme transport between the cytosol and peroxisome, and transport of the final β-lactam products out of the cells will allow manufacturers to manipulate the output of the final drug products.

Also in this upcoming edition the membrane fatty acid adaptation of anaerobic microorganisms is tackled in the primary research article presented by Duldhardt and colleagues (2009). They investigated the adaptation of bacteria of the genus *Thaurea*, *Geobacter* and *Desulfococcus* to the presence of benzene, toluene, ethylbenzene, xylenes, chlorinated phenols and aliphatic alcohols. They report that both *T. aromatica* and *G. sulfurreducens* have predominantly palmitic and palmitoleic acids in their membranes but show an increase in the level of fatty acid saturation in the presence of the tested compounds. While *D. multivorans* membranes were dominated by palmitic and *anteiso*-branched fatty acids and the bacteria responded to treatment by increasing the ratio of straight-chain saturated fatty acids to *anteiso*-branched fatty acids. The authors also showed that the adaptive responses are reliant on *de novo* synthesis of fatty acids and are therefore strictly correlated with cellular growth. So, although the anaerobic bacteria respond in a similar manner to their aerobic counterparts (See Bernal *et al.*, 2007) in the presence of toxic organic solvents the generally reduced growth rate of anaerobes results in a much delayed adaptive response. These results and further research will undoubtedly be of great importance for the future use of anaerobic bacteria in the clean-up of oxygen starved subsurface environments that have been contaminated with toxic organic pollutants.

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Microcalorimetric techniques have an increasing impact in the fields of fundamental and applied biochemistry and microbiology. The use of different calorimetric approaches to study molecular interactions and protein unfolding in the context of biotechnology was recently reviewed in this journal (Krell, 2008). Using these approaches, typically solutions of purified biomolecules are analysed. The review by Maskow and colleagues (2009) illustrates the possibilities, advantages and limitations of a calorimetric analysis of far more complex systems such as cultures of active microorganisms. The signal recorded in the latter approach is thus not that of binding or thermal unfolding of pure samples of macromolecules but the heat generated by the metabolic activity of microbial cultures.

To this end a large variety of calorimetric instruments were developed, ranging from nanocalorimeters, able to follow calorimetrically the metabolism of a single cell, to megacalorimeters, which are microbial culture vessels in the m^3 volume range. The heat production rate as monitored by calorimetry can provide information on the bioconversion stoichiometry and kinetics which are crucial parameters in the optimization of biotechnological processes. Several examples are presented which illustrate the gain in value of the information achieved by the combination of respirometric data with calorimetric measurements. The biotechnological relevance of such measurements consists primarily in the real-time control of biotransformations. This is illustrated by the possibility to detect in real time the switch between oxidative and fermentative metabolism of *S. cerevisae* or the energetically much smaller transition between *meta* and *ortho* phenol assimilation pathways in bacteria. Such information can ultimately be used to control bioprocesses in a way that maximizes the quantity of carbon flowing into the desired product.

Heap bioleaching is currently the most successful technology for the extraction of base metals from low-grade sulfide ores and scientific and commercial interest has emerged to study the microbial ecology of industrial bioleaching processes (Diaby *et al.*, 2007; Rawlings and Johnson, 2007; Garrido *et al.*, 2008; Siezen and Wilson, 2009). Remonsellez and colleagues (2009) describe the dynamics of an active microbial community in an industrial heap using molecular ecology/molecular biology tools. They propose that the chemical and physical conditions (such as pH and the Fe^{3+}/Fe^{2+} ratio) are of the utmost importance to determine which bacteria dominate commercial bioleaching processes. The authors reported remarkable differences in the microbial communities depending on the strip age. They identified a wide variety of microorganisms that correspond to different phylogenetic groups, although they are mainly bacterial species that can reach up to 10^7 cfu ml^{-1} based on 16S rRNA quantification analysis. They were able to identify among the 'active' microbes *A. thiooxidans*, *Leptospirillum ferriphilum*, the Gram-positives *Sulfobacillus* spp. and *Alicyclobacillus disulfidooxidans*, and the archaeon *Ferroplasma acidiphilum*.

An initial step in the industrial production of proteins is the search for a suitable expression system. Microorganism-based methods are usually preferred due to their ease in handling and their lower costs. The promoter-activator pair Pm/XylS combines a strong increase in transcription after induction, with very low basal levels of expression, essential characteristics for first-class systems. Additionally, this duo provides several other desirable characteristics: (i) the inducer does not need a specific uptake mechanism, (ii) the intensity of activation can be easily modulated, and (iii) there are many mutants available with altered specificities. In this issue, Aune and collaborators present a method to construct mutant Pm/XylS expression systems with improved yields (Aune *et al.*, 2009). The authors used a systematic approach that involved directed evolution with error-prone polymerases on the *xylS* coding region together with a fusion of Pm to the β-lactamase gene; alterations in ampicillin resistance were detected in the presence of *m*-toluic acid. Using this methodology they obtained mutants with a phenotype of increased resistance to the antibiotic under inducing conditions, all with changes located in the NTD of the XylS protein, in agreement with several previous reports that identified this region as responsible for inducer binding among other functions (Ramos *et al.*, 1990; Michán *et al.*, 1992; Ruiz and Ramos, 2002). Combinations of mutations (identified in this paper or from other authors) proved that incremental increases in transcription could be additional. Furthermore, the authors looked for positive combinations by staggered extension process (StEp), randomly combining *xylS* variants to obtain new chimeral proteins that could increase expression under induced conditions almost 10-fold compared with the original system and still maintaining low uninduced expression levels. Additionally, the results presented offer useful information in the understanding of how XylS recognizes effectors and in the prediction of its 3-D structure. The novel use of direct evolution followed by combination of the identified mutations provides an easy tool to manipulate the induction characteristics of regulators, particularly when their active sites are not precisely confirmed.

Knowledge regarding genetic manipulation of non-model organisms should be improved in order to develop new fields in microbial biotechnology. Traditional approaches were based on the construction of knockout mutants, but this tactic is difficult to perform in several organisms including many filamentous fungi due to their almost zero rates of homologous recombination. To overcome this difficulty, Kemppainen and Pardo (2009)

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report the construction of vectors (pSILBAs) for use in gene silencing in the mycorrhizal fungus *Laccaria bicolour*; the system could potentially be used in other basidiomycetes. The authors constructed three different silencing cassettes with high stability, due to the use of introns as spacer sequences between the inverted repeats in hpRNA. The plasmids obtained were fused to the pHg *Agrobacterium* binary vector in order to incorporate the ability for transgene integration. Constructions were tested by silencing the *Laccaria* nitrate reductase gene and monitoring growth on nitrate; the strongest inhibition was obtained with pSILBAγ that was designed to avoid inverted repeated promoter structures in the silencing triggering cassette. Additionally, silencing strength variations were observed among different strains carrying the same vector, a phenomenon frequently observed in RNA silencing studies. The authors demonstrated that, in this case, the inhibition levels obtained were biased by the transcriptional activity of the integration sites and not by the integration copy number. The new tools for gene disruption studies presented in this work will certainly contribute to the knowledge of gene functions in filamentous fungi, and therefore have many future possibilities in biotechnological applications.

All of these exciting primary research articles and their potential applications form part of the relevant scientific literature that is being published in *Microbial Biotechnology*.

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