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Highlight

# Directed evolution, natural products for cancer chemotherapy, and micro-biosensing robots

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Directed evolution is an effective and powerful method for protein engineering. To date, several different methods have been designed to create sequence diversity; unfortunately most of them are confined to the model bacterium *Escherichia coli*, and this constitutes a challenging limitation for the improvement of many industrially relevant biocatalysts. A clear example of this restriction is the production of new secretory enzymes that are of interest in the food, textile and pharmaceutical industries. In this case, the most common procedure used involves two organisms (*E. coli* and *Bacillus subtilis*), two transformations and several intermediate DNA manipulation steps. In addition, DNA transfer into *B. subtilis* is usually highly inefficient. Zhang and Zhang (2011) present a new methodology in *Microbial Biotechnology* that overcomes this problem by addressing it from two sides simultaneously. On one hand, they have increased the receptivity of *B. subtilis* to foreign DNA by increasing expression of the competence transcription factor ComK. On the other hand, they have improved the DNA material to be transformed so that candidate sequences are cloned into multimeric plasmids, by a combination of two PCR procedures, one error-prone for the target gene and one error-free for the vector. The authors demonstrate the efficiency of the protocol obtaining mutants of endoglucanase BsCel5 with increased hydrolase activity and improved secretion properties. Finally, we would like to highlight that apart from the time saving facet of this methodology, this protocol can also avoid bias as a result of multiple steps and the limitations of cloning mutants by traditional methods (restriction enzymes and ligation); thus broadening the range of potential candidates.

Bacteria from the genus *Streptomyces* produce more than 70% of commercially available antibiotics, plus many other metabolites of therapeutic interest such as antitumour agents or immunosuppressive drugs. The production of these compounds is tightly controlled by means of γ-butyrolactones; microbial hormones that are also involved in the regulation of other processes such as morphological differentiation or pathogenesis. The article presented by D'Alia and co-workers focuses on the regulation of these hormones by the ScbA regulator, a homologue of the A-factor biosynthesis *afsA* gene from *Streptomyces coelicolor* (D'Alia *et al*., 2011). For this purpose, they studied the differences in transcript levels between the wild-type strain and a Δ*scbA* mutant using global microarray analysis, and the results were confirmed by more accurate qRT-PCR. The authors show that ScbA not only controls antibiotic production but also interferes with intracellular iron levels modulating siderophore desferrioxamine E biosynthesis. Interestingly, this study also shows that in the *scbA* mutant genes coding for enzymes related to primary metabolism are upregulated just before antibiotic production, placing butanolides upfront of general metabolic regulation in *S. coelicolor*.

When *S. coelicolor* A3(2) grows under nutrient limited conditions, it triggers a gamut of nutrient–stress responses, mediated by a number of global regulators including PhoP, GlnR, AfsR and others. The action of these regulators is also integrated at the molecular level to control secondary metabolite biosynthesis and differentiation. As a result of the potential uses of these microbes in biotechnology, nutritional control has received relevant attention in the field and, in this issue, Martin and colleagues (2011) describe how phosphate control of primary and secondary metabolism in *Streptomyces* species is mediated by the two-component regulatory system PhoR-PhoP. PhoP controls secondary metabolism by binding to the PHO box in the *afsS* promoter. They illustrate that this PHO Box overlaps with the AfsR binding site (D'Alia *et al.*,

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2011), and based on this physical organization, they suggest that the *afsS* promoter serves to integrate the PhoP-mediated response to phosphate limitation and the AfsR-mediated responses to a yet unknown stimuli. In Δ*phoP* strains it was found that some genes involved in nitrogen metabolism, i.e. *glnA*, *glnlL* and *glnK*, increased their expression. Phosphate control of these genes is exerted through binding of PhoP to the promoters of *glnR* (the global nitrogen regulator), *glnA*, *glnL* and the *amtB-glnK-glnD* operon. This regulation allows a 'metabolic homeostasis' of phosphate and nitrogen utilization pathways, preventing nutritional unbalances.

Continuing on the theme of gene regulation in *Streptomyces*, Jones and colleagues (2011) provide further evidence for the critical role of serine/threonine protein kinases in regulating metabolism (Jones *et al*., 2011). *S. coelicolor* has 34 predicted serine/threonine protein kinases (Petrickova and Petricek, 2003) of which only two have been functionally analysed (RamC and AfsK) and were demonstrated to have roles in regulating cellular development and antibiotic production (Umeyama *et al*., 2002; Kodani *et al*., 2004). In the current article Jones and colleagues concentrate on the *pknB* and two linked genes, *fhaAB*, encoding forkhead-associated (FHA) domain proteins. Their research showed that *pknB* is not essential in *S. coelicolor* while the linked genetic loss of FhaAB resulted in deregulation of central carbon metabolism; carbon flux was diverted to synthesis of the antibiotic actinorhodin. These results are very exciting because they emphasize the potential of related or linked gene manipulation in the augmentation of biomedically important processes such as antibiotic synthesis.

Still related to the Special Issue on Streptomyces, another relevant article is that of acetyltransferases such as MdmB and Asm19, which are involved in antibiotic biosynthesis and have previously been tested for their abilities to produce hybrid antibiotic products (Moss *et al*., 2002). Recently, García and colleagues (2011) concentrated on the acetyltransferase CmmA of *Streptomyces griseus* and its potential use in generating novel anti-tumour compounds. They focused on two structurally related anti-tumour compounds mithramycin and chromomycin A3 that differ in their glycosylation profiles and functional group substitutions of the backbone sugars. The acetyltransferase CmmA in *S. griseus* ssp. *griseus* incorporates two acetyl groups onto Chromomycin during biosynthesis. Using this knowledge and an engineered *S. griseus* strain the authors developed a bioconversion strategy allowing the generation of seven novel acetylated mithramycins. They then purified the newly formed compounds and characterized them by MS and NMR. The results showed that the compounds differed from the starting compounds as a result of the presence of one, two or three acetyl groups. Furthermore, the new mithramycin analogues showed anti-tumour activity when tested against glioblastoma and pancreas tumour cells. Overall the data showed that CmmA is also able to acetylate different substrates at different positions, indicating broad acceptor substrate flexibility. This highlights the potential of CmmA as a tool to create structurally diverse and novel anti-tumour compounds.

Cancer is undeniably a group of diverse pathologies characterized by an uncontrolled cell division, and anti-tumour drugs are equally heterogeneous. Luckily, millions of years of natural evolution have produced a vast number of different chemical structures, and among them, we can find thousands with anti-cancer activity. Demain and Vaishnav, 2011 present a very exhaustive revision of the already isolated natural products for cancer chemotherapy, from those produced by bacteria such as *Streptomyces* or *Myxobacteria* to those isolated from plants, fungi or marine organisms. The authors offer multiple examples of different drugs and their derivatives, most of them with complex cyclic structures, which have been modified either biologically or synthetically. Several of them have not only anti-tumour activity, but also additional pharmaceutical benefits such as antimicrobial or anti-cholesterol properties. The present and future searches are in general centred on unculturable organisms, many of which are of marine origin, thanks to new 'omics' techniques. The data presented clearly show that to achieve new pinnacles in cancer treatment the pharmaceutical industry needs to combine global screening methodologies for the selection of new metabolites together with both biological and chemical large-scale combinatorial techniques.

Live bacterial cells can be used as a framework for synthetic biology, and as such can be genetically reprogrammed to undertake novel tasks, including sensing specific chemicals, and producing drugs and biofuels (Atsumi *et al*., 2009). In the January issue of *Microbial Biotechnology*, Zhang and colleagues (2011) report on the use of genetically engineered *Acinetobacter baylyi* strains as bioreporters for the detection of salicylate, toluene, xylene and alkanes. The process involves the functionalization of the live bacteria with ~18 nm iron oxide magnetic nanoparticles (MNPs) to bring about the magnetic function. MNPs have previously been used as a tool for remote manipulation and control, and can be used to functionalize organic and inorganic microparticles and living cells such as yeast cells (Fakhrullin *et al*., 2010). The authors showed that the MNP-functionalized bioreporters were viable and functioned as well as the native non-functionalized cells in terms of their sensitivity, specificity and quantitative response. The authors went on to further demonstrate that salicylate sensing bioreporters could be applied to sediments and garden soils, and be used to semi-quantitatively detect salicylate in those

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samples. This article is essentially a proof of concept publication validating the use of micro-biosensing robots to probe the chemical/biological content of complex environments such as those found in wastewater and groundwater. The unique possibility of simply recovering the biosensors for further analysis using magnets opens the door for their use in environmental microbiology applications, such as enhancement of bioremediation and environmental monitoring and assessment.

New vectors that can be used in diverse bacterial species to express enzymes, vaccines and biotherapeutics are of great importance and Duong and colleagues (2011) emphasize this by the construction of vectors for inducible and constitutive gene expression in *Lactobacillus*. The authors exploited the available genome sequence, gene expression profiles and functional genomic data to construct a series of expression vectors and analyse their properties using a β-glucuronidase (GusA3) reporter system. The authors concentrated on a number of operons that were shown by microarray analysis to be differentially expressed in response to carbohydrate source; including operons implicated in the transport and catabolism of fructooligosaccharides (FOS), lactose (*lac*) and trehalose (*tre*). In-depth analysis of the inducible operons identified a number of putative promoter and repressor elements, which the authors then used to construct a series of expression vectors for use in lactobacilli. Tests using the newly constructed vectors showed that expression was highly inducible and dependent on the presence of the specific carbohydrate. These expression vectors provide alternative and useful tools for overexpression of specific proteins in lactobacilli. Furthermore, members of the same laboratory have shown the potential of these vectors for use in genetic complementation studies and also for expression of biotherapeutic proteins (Mohamadzadeh *et al*., 2009). Clearly, the possibilities for these vectors in the delivery of probiotics and potential use in large-scale overexpression warrants further investigation.

Using a competitive inhibition method Wu and colleagues (2011) isolated a number of soil bacteria and screened them for antimicrobial activity against *Staphylococcus aureus*. A consensus phylogenetic tree was constructed based on 16SrRNA gene sequences and a clone named F6 B70 belonging to the genus *Paenibacilus* was reserved for further characterization. Using a series of primer sets for conserved regions of PKS and NRPS the authors found that this strain has genes with low sequence identity to known PKS and NRPS, and based on this finding they hypothesized that this is an indication of the potential for the synthesis of novel secondary metabolites in the strain. After optimization of cultivation conditions they found a chemical whose molecular formula was $C_{33}H_{50}O_6$, and its chemical characterization revealed it is a new triene macrolide. This study demonstrates that penibacilli are a new group of bacteria that may be promising tools for the production of novel antibiotics.

*Fusarium oxysporum (Fox)* is a pathogen of agricultural and ornamental crops, and phytopathogenic strains are responsible for yield loss of many important crops worldwide (Cai *et al*., 2003). For example, wilt of tomato caused by *F. oxysporum* f.sp. *lycopersici* (*Fol*) and foot and root rot of tomato caused by *F. oxysporum* f.sp. *radicis-lycopersici* (*Forl*) have been reported in at least 32 countries (Jones *et al*., 2011). Besides yield decreases, many *Fusarium sp.* strains produce toxins that can accumulate in the end products and therefore may become dangerous for human and animal health (Pitt, 2000).

Monitoring of plant pathogens is crucial for disease management. Early detection, identification and quantification of the infestation level can help to choose appropriate defence measures. To approach this Validov and colleagues (2011) have developed a highly sensitive qPCR reaction for the detection of Fox DNA. The assay is based on the fact that the ribosomal operon is present in 200 copies per haploid *Fox* genome, which offers an excellent target for the qPCR reaction. The intergenic space between the 18S and 28S rRNA genes of the ribosomal operon is the specific target. The authors showed that it is possible to detect 20 fg of DNA from the three different *Fox* strains in 1 ng of tomato plant DNA. This provides exciting prospects for the future surveillance and control of these agriculturally important plant pathogens.

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