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Highlight

Microbial Biotechnology from medicine to bacterial population dynamics

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In recent issues of *Microbial Biotechnology* there have been several exceptional articles ranging in topic from the antagonism of biofilm formation in pathogenic bacteria, and prebiotic selection of positive bacterial fermentations in the colon to the discovery of novel biotechnologically important enzymes encoded by bacteria of the deep-sea floor.

In January there appeared two stimulating articles in relation to quorum sensing and antagonism of biofilm formation (Lee *et al*., 2009; Ueda *et al*., 2009). In the initial publication Ueda and colleagues screened a transposon library of *Pseudomonas aeruginosa* PA14 in order to discover which genes impact biofilm formation. They found 137 mutants with enhanced biofilm capabilities and 88 with reduced biofilm formation. One of their unique and interesting findings was that among the mutants which showed dramatically decreased biofilm formation were seven mutants of the UMP synthetic pathway. Further biofilm analyses using compounds from the UMP synthetic pathway showed that only uracil and not UMP or UTP was able to serve as an internal signal for biofilm formation. Incredibly, transcriptome analysis of one of the UMP pathway mutants revealed that all three known quorum-sensing systems were repressed when UMP synthesis was inhibited. These findings led the authors to screen currently available uracil structural analogues for their influence on biofilm formation in *P. aeruginosa*; this led to the discovery that 5-fluorouracil (a currently utilized anticancer drug) is a potent inhibitor of biofilm formation, which is able to abolish the quorum-sensing phenotypes and reduce virulence in an infection model while remaining non-toxic to *P. aeruginosa*. The discovery of these so called 'anti-virulence molecules' is of great importance to current biotechnology endeavours as these compounds are less likely to lead to developed resistance in bacteria because they do not affect bacterial growth (Cegelski *et al*., 2008).

In the second article relating to quorum sensing and virulence of *P. aeruginosa* Lee *et al*. show that indole and 7-hydroxyindole (7HI) are able to reduce virulence. Indole is produced from L-tryptophan by many bacteria including *Escherichia coli* and in fact is used by *E. coli* as a signalling molecule; influencing biofilm formation, motility and acid resistance. Using DNA microarrays and phenotype arrays the authors showed that both indole and hydroxylated indole have global affects on gene expression in *P. aeruginosa*. The compounds decreased virulence factor production and swarming motility while also increasing antibiotic resistance. Further testing in animal models confirmed that 7HI could reduce *P. aeruginosa* colonization and increase gastric clearance of the bacteria. As reported above for 5-fluorouracil these molecules (indole and 7HI) are non-toxic to the bacteria as they do not affect growth rate; but because they extensively alter virulence gene expression they have potential pharmaceutical application in the treatment of *P. aeruginosa* infections.

In relation to the virulence topic the January issue of *Microbial Biotechnology* also contains a very pertinent review by Anna Fàbrega and colleagues (2009) in which the mechanisms of action of quinolones are examined and discussed from a clinical point of view. These drugs inhibit microbial DNA synthesis by targeting two essential topoisomerases, DNA gyrase and TopoIV; because DNA gyrase is present in prokaryotes and not in eukaryotes it is an excellent target for development of broad-spectrum antimicrobial compounds. Since the first use of nalidixic acid in 1962 four generations of more powerful quinolones have been developed and designed to overcome acquired microbial quinolone resistance. The authors emphasize that the mechanism of quinolone resistance is not solely due to mutations in the target genes of the pathogens (*gyrA*, *gyrB*, *parC* and *parE*) but also from a wide range of efflux pumps that efficiently prevent accumulation of the drug inside the cells. The authors show how up to nine RND family efflux pumps can come into operation to confer antibiotic resistance in the human opportunistic pathogen *P. aeruginosa*. While efflux pumps and target genes are often chromosomally encoded, the authors also show that some plasmid-encoded proteins

can chemically modify the aromatic rings of quinolones preventing the action of the drugs on their targets. Fàbrega and colleagues (2009) in this review analysed case-by-case the mechanism of quinolone resistance in a wide range of human pathogens including several enterobacteriaceae, the opportunistic *P. aeruginosa*, the non-fermenting *Acinetobacter braumanni*, and *Stenotrophomonas maltophilia* among the Gram-negative bacteria and a number of Gram-positive pathogens such as *Staphylococcus aureus* and *Streptococcus pneumonia*. Analysing and understanding these diverse mechanisms of quinolone resistance will undoubtedly allow the future development of broader spectrum high efficacy quinolone antibiotics.

The functional food industry including the development and use of prebiotics is an emerging field in food science. In the January issue of *Microbial Biotechnology* Sánchez and colleagues (2009) report on the effects of prebiotic oligosaccharides on the protein/carbohydrate fermentation balance and microbial population dynamics of a simulated human intestinal microbial ecosystem. Basically, prebiotics are food constituents that are not digestible in the upper gut and thus beneficially affect the host by selectively stimulating growth and/or activity of one or of a number of health-promoting bacteria in the colon. Prebiotic treatments are potentially important for colonic health in humans because they may lead to better fermentation conditions in the distal colon which is the section with the highest levels of cancer. One of the existing drawbacks of currently used prebiotic additives is that they are mostly fermented in the proximal regions of the gut and don't survive long enough to reach the later distal colon and rectal regions. The problem is believed to be mainly due to the small size and the lack of extensive side-chains of the oligosaccharides used. The authors therefore concentrate on Arabinoxylan-oligosaccharides (AXOS) which are a recently discovered class of candidate prebiotics that are likely fermented in different regions of the gastrointestinal tract due to their varied structure. Sánchez *et al.* showed that different AXOS preparations can have distinct impacts on the *in vitro* intestinal fermentation activity and microbial community structure of the digestive tract. Using AXOS with an average degree of polymerization (avDP) of 29 allowed the oligosaccharide to reach the proximal colon and sustained supplementation with this AXOS decreased the levels of the toxic proteolytic markers phenol and *p*-cresol while increasing the concentrations of beneficial short-chain fatty acids by 25–48%. Interestingly, the authors showed that the overall microbial communities were only slightly altered; an observation which suggests that the fermentation mechanisms within the colon were actually being altered. The authors conclude that AXOS of different avDP can be fermented by intestinal bacteria leading to positive human health effects and that they are therefore promising candidates to modulate the microbial metabolism in the distal colon.

Since the early days of molecular biology, protein expression has represented one of the 'golden' techniques in recombinant DNA technology. Regulated promoters are useful tools for many aspects related to recombinant gene expression in bacteria; including high-level expression of heterologous proteins and controlled expression at physiological levels in metabolic engineering applications. Svein Valla and colleagues (Brautaset *et al.*, 2009) discuss the use of prokaryotic expression systems based on the AraC-XylS family of transcriptional activators. The systems function in a wide range of microorganisms, including enterobacteria, corynebacteria, soil bacteria, lactic bacteria and streptomycetes. Prior to these applied uses, this family of regulators have attracted scientific interest for decades as novel model systems for use in basic research on bacterial gene regulation. This in turn has led to relevant information on the range of effectors recognized by these regulators so that their use to express heterologous genes is very well defined. Among the regulator/promoter pairs used are the XylS/*Pm* system of the TOL plasmid and AraC/P_{BAD}, RhaR-RhaS/*rhaBAD* promoter, of *E. coli*. The discovery, characterization and implementation of new and flexible bacterial expression systems will likely be invaluable for the future exploitation of newly discovered enzymes.

In relation to the search and discovery of new protein families in the March issue of *Microbial Biotechnology* Siezen and Wilson (2009) published a Genomics Update article which presented an extensive review of the genomics of deep-sea and sub-seafloor microbes. In the article they emphasize the importance of this vast reservoir which is purported to contain $> 10^{30}$ microbial cells (Whitman *et al.*, 1998). The conclusion is that through extensive surface sea-water samplings and metagenomic analysis such as those conducted in The Sargasso Sea (Venter *et al.*, 2004) and the Global Ocean Sampling (Yooseph *et al.*, 2007) we have been able to discover many new protein families; however, the numbers are still rising and microbes from the deep-sea have yet to be significantly exploited. Because deep-sea environments are characterized by low temperature (1–2°C), high pressure (1 MPa for every 100 m), high-salt and low-nutrient conditions microbes that live there differ greatly from those of shallow waters. For example, shallow water microbes do not require extensive chemotactic systems, flagella or pili because they live in a nutrient-rich high-oxygen environment with available sunlight; this is not the case for deep-sea microbes. The authors also illustrate the current standing of the databases and computing tools available for the cataloguing of the vast quantities of data

that are becoming available (Siezen and Wilson, 2009). The coupling of extensive sampling techniques with metagenomic strategies offers great promise for future application of marine enzyme biotechnology to novel biocatalyses using enzymes with high salt tolerance, thermostability and barophilicity.

A great complement to the genomics contribution by Siezen and Wilson (2009) is the mini-review by Ferrer and colleagues (2009) in the preceding issue of *Microbial Biotechnology*. In which several methodologies for harvesting of environmental DNA for metagenomics are presented together with some sophisticated high-throughput screening tools that have been developed by Aharoni's lab. These well-refined techniques are based on *in vitro* compartmentalization approaches, which are useful to search for novel activities or for rapid *in vitro* evolution of known enzymes. The focus of this mini-review is to demonstrate the power of small and large insert expression library construction with lambda phage, cosmid and fosmid vectors; the vectors are implemented for use in direct activity screening with the aim of discovering new enzymatic activities or novel variants of previously established enzymes. This approach is in itself an alternative to massive DNA sequencing of metagenomes that has already revealed that over 60% of environmental DNA represents novel sequences of unknown function. Ferrer and colleagues propose to go and fish for activities through the direct selection of activities based on the use of chromogenic or fluorogenic substrates that facilitate direct selection, or indirectly through the use of genetic traps as those described by de Lorenzo (Garmendia *et al*., 2008). Ferrer and colleagues detail and explain the minimal requirement for high-throughput screening and selection methodologies. A complementary approach to the *in vivo* procedures is the in vitro compartmentalization (IVC) selection mode, also described in this mini-review. The IVC is based on water-in-oil emulsion, where the water phase is dispersed in the oil to form microscopic compartments. The system has been optimized so that on average each droplet contains a single gene and serves as an artificial system for coupled transcription and translation, as well as detection of single molecules. This technology, in addition, allows the rapid evolution of the enzymes to confer new properties to the proteins, such as enhanced thermo tolerance or wider substrate specificity. The authors conclude that the rational application of high-throughput technologies in metagenome screening will unveil new enzymes that will be valuable for innovative biocatalytic processes.

An interesting continuation of this line of research is the potential use of the robust *Pseudomonas putida* organism; which is finding multiple applications in production of enzymes for biocatalytic processes. The robustness of *P. putida* is derived from their ability to react to multiple stresses such as those induced by the presence of toxic molecules in the environment including a complement of efflux pumps that prevent the accumulation of toxic molecules in the cell (Ramos *et al.*, 2009). Second generation biofuel production is searching for new molecules that can store higher energetic potential than ethanol. Among potential new biofuels are a number of aromatic alcohols that can be produced by diverting carbon flux from the biosynthesis of aromatic amino acids. Using a wide range of techniques Molina-Henares and colleagues (2009) have deciphered the biosynthetic pathways and genes for the synthesis of the three aromatic amino acids in this robust organism and have therefore established the basis for the future engineering of this microorganism for the production of new biofuels.

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