microbial biotechnology

Microbial Biotechnology (2010) **3**(6), 624–627 doi:10.1111/j.1751-7915.2010.00225.x

Highlight

New molecular techniques for pathogen analysis, *in silico* determination of RND efflux pump substrate specificity, shotgun proteomic monitoring of bioremediation and yeast bio-applications

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Molecular techniques for pathogen analysis

In the current issue of *Microbial Biotechnology*, Kienesberger and colleagues (2010) describe in detail the recent progress in molecular approaches applicable to the study of *Campylobacter fetus*, a microorganism of mounting importance due to the continued infection of domestic herds worldwide – leading to higher abortion rates – and the rising threat in human disease. The authors highlight the pathogenic cycle of *Campylobacter* and go on to discuss genome analyses, the mobile gene pool and molecular genetics, in particular the recent availability of several plasmid vectors for use in *C. fetus*. The authors then go on to examine gene expression in the two subspecies of *C. fetus*; until recently gene expression in *C. fetus* has been restricted by the limited availability of useful vectors. The lack of the ability of exogenous promoters to function in *C. fetus* has also been a major stumbling block; however, the recent isolation of endogenous plasmids and the use of their origins of replication and the inclusion of *C. fetus*-specific promoters have opened the door to future experimental manipulation. Mutational analyses possibilities for *C. fetus* are also somewhat in their adolescence; although suitable strategies based on transposon mutagenesis and homologous recombination have been developed for *Campylobacter jejuni* these do not yet exist for *C. fetus*. However, current experiments by Gorkiewicz and colleagues (2010) have shown that specific gene knockouts can be generated in *C. fetus* and their complementation can be achieved by incorporation of a functional gene copy on a replicative plasmid. Another major block in the advancement in the knowledge of *C. fetus* biology is the lack of *in vitro* virulence assays, and mammalian cell invasion models (Colles *et al*., 2009). Current research by several research groups has indicated that a modified version of the standard gentamicin protection assay can be used to quantify the invasion efficiency of mammalian cell lines by *C. fetus* (Hiden *et al*., 2007; Gorkiewicz *et al*., 2010). Clearly the tools required for the future investigation of this important pathogen are being amassed.

In this issue of *Microbial Biotechnology* Mraheil and colleagues (2010) report on the current knowledge on small RNAs (sRNAs) in relevant Gram-positive pathogens, and summarize bioinformatics approaches for genome-wide sRNA identification and target prediction. These studies are being performed by a European consortium with *Listeria monocytogenes*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococcus faecalis* and *Clostridium difficile* as target microbes. In bacteria, sRNAs have attracted considerable attention as a new emerging class of gene regulators that influence transcription, translation or mRNA stability. These sRNAs interact by pairing with other RNAs, forming parts of RNA–protein complexes or adopting structures of other nucleic acids (Waters and Storz, 2009). sRNAs regulate processes related to stress responses, iron homeostasis, outer membrane protein biogenesis, sugar metabolism and quorum sensing, suggesting that they might also play an essential and central role in the pathogenicity of many bacteria.

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At the time Mraheil and colleagues' article was prepared the authors used the genome sequences of 39 *S. aureus*, 14 *S. pyogenes*, 23 *E. faecalis*, 12 *C. difficile* and 24 *L. monocytogenes* strains, and the information that was available in the NCBI and GOLD databases (Siezen and Wilson, 2008). Mraheil and colleagues (2010) describe that the genus of *Listeria* has a higher number of putative sRNA than other genera such as *Streptococcus*, *Staphylococcus*, *Enterococcus* and *Clostridium*, which might reflect their potential ubiquitous adaption ability in nature and mammals. Comparative analyses of the recently reported 103 regulatory RNAs of *L. monocytogenes* among the genomes of *S. aureus*, *S. pyogenes*, *E. faecalis* and *C. difficile* revealed that ribo-switches seem to be more conserved among these Gram-positive pathogens than sRNA. The authors suggest that a common ancient mechanism of *cis*-acting RNA regulation might exist in Gram-positive bacteria. The authors also suggest that the identified sRNAs are potential markers for diagnosis tests that are fast, sensitive and suitable for low-cost applications.

Godoy and colleagues (2010) report on the development of a generalized *in silico* profile that identifies members of the root-nodulation-cell-division (RND) family of efflux pumps and classifies them into four functional subfamilies (Godoy *et al*., 2010). RND efflux pumps are extremely important elements in multi-drug resistance, and their wide substrate specificity explains the cross-resistance between antibiotics, biocides, dyes and solvents in many bacterial strains; naturally their rapid identification and characterization is of great importance to both biotechnologists and medical scientists (Baquero *et al*., 2009; Aminov, 2010; Kostic *et al*., 2010). Using their new profile and the *Z*-score values Godoy and colleagues (2010) grouped the RND efflux pumps by their metabolic function, allowing, for example, the differentiation of pumps involved in antibiotic resistance (group 1) from those involved in metal resistance (group 3). The authors then validated their *in silico* data regarding the RND efflux pumps from group 1 by identifying pumps in a number of environmental microbes using ethidium bromide resistance as an isolation screen. They then reported on a re-analysis of the *Pseudomonas putida* KT2440 genome using the *in silico* profile tool and identified efflux pumps from all four of the groups and confirmed the findings by analysing a collection of mutants in the efflux pumps using a screening platform consisting of 50 different drugs. The combination of *in vivo* data with the generalized *in silico* profiles and gene annotation data allowed the functional assignment of both known and uncharacterized RND efflux pumps into subgroups. This tool and other similar innovations will be invaluable for the future classification of important bacterial elements and should provide valuable information for the initial characterization of newly isolated organisms.

Yeast biotechnology

Lipids are a chemically diverse group with the common characteristic of water insolubility. In addition to their most common functions of energy storage and temperature isolation, they are precursors of a vast variety of molecules with important biological activities (hormones, vitamins, anticoagulants, electron transporters, etc.) and expensive industrial products for the pharmaceutical and the food-manufacturing sectors among others. Sabirova and colleagues (2010) in this issue of *Microbial Biotechnology* describe the current state and the future perspectives of the use of *Yarrowia lipolytica* to produce costly lipid-derived products from cheap greasy substrates such as animal fats or vegetable oils. This yeast exhibits clear advantages for this purpose as it can grow on a wide range of substrates, it has a versatile lipid metabolism, and it is an easy host in which to express bacterial lipid modification pathways to expand its biochemical transformation potential. Moreover, recent biochemical and genomic studies have made possible the construction of strains with reduced lipid storage and oxidation rates, in order to divert their metabolism to the production of commercial derivatives. Among these by-products are wax esters for cosmetics and medical drugs (reducing the need to use highly expensive natural sources), polyhydroxyalkanoates for bioplastics of medical importance, hydroxylated fatty acids as antimicrobial agents, carotenoids for the food and pharmaceutical industries, and a very interesting group named polyenic polymers, also known as electronic plastics, that can substitute for petroleum-based electron transport devices.

Continuing on the theme of the biotechnological possibilities of yeast, Zhang and colleagues (2010) describe the construction of a *Saccharomyces cerevisiae* derivative that produces ethanol at high rates from the polysaccharide inulin. The authors propose the use of inulin-rich plants instead of starch as substrates for bioethanol, given that inulin is more water soluble and produces less dense solutions than starch, easing the exploitation process. As *S. cerevisiae* in not able to catabolize inulin, the authors have constructed a genetic derivative with the *INU1* gene from *Pichia guilliermondii* that can efficiently metabolize inulin to ethanol. The final rates presented are somewhat inferior to those obtained from starch, although the savings in the initial manipulation of substrates are not taken in consideration. Additionally, the authors suggest the use of co-cultures with *Aspergillus niger*, as this fungus produces extracellular inulinases that can improve yields. This pathway could be useful, especially in

countries with limitations of starch-rich substrates and an abundance of inulin-rich plants.

Mining enzymatic potential

At the Department of Energy (DOE) Integrated Field Research Challenge (IFRC) site in Rifle, CO, the enzymatic reduction of soluble Uranium (VI) to insoluble Uranium (IV) by stimulated indigenous *Geobacter* species has emerged as a promising bio-remediation strategy for contaminated groundwater (Scheibe *et al*., 2009; Wilkins *et al*., 2010). As bio-stimulation progresses, the subsurface microbiology shifts from this Fe(III)-reducing community to a sulfate reducing community, whose activity results in elevated sulfide production, a switch that is sometimes associated with a decrease in the efficiency of U(VI) removal from groundwater. One of the aims of the research by Wilkins and colleagues (2010) in *Microbial Biotechnology* is to establish biomarkers to track the *Geobacter* community during the remediation process, for which the authors used shotgun proteomics, a technique that offers a fast high-throughput method of analysis (VerBerkmoes *et al*., 2008; Wilkins *et al*., 2009).

The authors considered that citrate synthase (CS), which is responsible for controlling flux into the TCA cycle by catalysing the condensation of acetyl-CoA and oxaloacetate to produce citric acid, has a number of characteristics that make it a suitable candidate as a *Geobacter*-specific peptide-based biomarker, since the amino acid sequence in members of the *Geobacteraceae* is more closely related to eukaryotic CS than other prokaryotic sequences, the potential for false positive identifications is limited. Analysis of the 'global' proteomic data sets from 2 years of field research revealed that a subset of these CS conserved peptides (TIPETFEALPK, SLVTDISYLDPQEGIR and QVVPEYVYTAVR) were the most abundantly detected where *Geobacter* species were present. Experimental data supported that these biomarkers are useful as indicators of efficient U(VI) removal from groundwater.

It is also of interest to note that the study reported in *Microbial Biotechnology* showed that the initial enrichment of *Geobacter* seems to be dominated by only a few strains that couple the highest growth rates to the most efficient utilization of acetate and Fe(III) oxides. As the duration of biostimulation progresses however, strain diversity within the Fe(III)-reducing microbial community increased (Wilkins *et al*., 2009), and this was reflected in the increased diversity of unique CS peptides. Whether this was due to the emergence of slower-growing bacteria, the initial effects of sulfate reducers, or changes in the availability of different Fe(III) oxides as terminal electron acceptors is currently being analysed in the authors' lab. Therefore, shotgun proteomics is a useful approach to monitor microbial abundance and *in situ* function at least during the bio-removal of uranium.

Lignin is responsible for the mechanical strength of plant cell walls and constitutes 30% of all vegetable material. Although it is a complex polymer without a defined primary structure, polyphenolic residues are predominant in its composition. Most lignin waste is burned to generate energy for pulp mills. However, based on its interesting functionalities and properties, lignin offers perspective for higher added value manufacturing applications (Ruiz-Dueñas and Martinez, 2009). Unluckily, synthesis of these products usually includes *trans*-esterification procedures and only a few aryl esterases with activity towards phenolic acids have been described to date. Wang and colleagues (2010) have mined 11 bacterial genomes for homologous enzymes to a characterized arylesterase and found 171 potential candidates. Seventeen of these enzymes were characterized, focusing on the parameters that could increase their industrial potential: their kinetics, and their stability upon changes in temperature and in the presence of detergents, organic solvents or ionic liquids. Among the enzymes in the study, the best candidates were obtained from *Rhodopseudomonas palustris*. As a final point the authors compared their biochemical and bioinformatic analyses to disclose sequence features which could be correlated to enzymes with arylesterase activity, a revelation that will facilitate subsequent searches for novel esterases in microbial genome sequences.

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