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Highlight

A broad range of themes in *Microbial Biotechnology*

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In the November issue of *Microbial Biotechnology* a series of relevant articles were published regarding energy generation, thermostable enzymes, influence of transgenic plants on microbes that live in the rhizosphere of plants, degradation of hormones, identification of pathogens, and the use of phage to control bacterial populations. This abundant selection of articles reflects that *Microbial Biotechnology* is attracting interest from a wide range of fields with the aim of placing science within the prospect of future industrial developments.

One of the themes that has attracted a good number of articles in 2008 has been energy generation. The November issue brought three relevant articles. One of them dealt with microbial fuel cells (MFCs), which are based on the use of microorganisms as biocatalysts to convert chemical energy contained in electron donors to electrical energy. Microbial fuel cells are considered to have potential application in the domains of power supply, biological oxygen demand sensors and especially in sustainable wastewater treatment. However, a number of limitations appear in the system and one of the approaches to overcome a number of these limitations was described by Pham and colleagues (2008) in the previous issue of *Microbial Biotechnology*. These authors show that the application of high shear rates enriched an anodophilic microbial consortium in MFC that resulted in higher performance. Enrichment at a shear rate of about 120 s^{-1} resulted in the production of a current and power output two to three times higher than those in the case of low shear rates. A series of taxonomical analyses revealed substantial differences in the microbial biofilm covering the anode at the high shear rate with respect to the thinner biofilm present in the low shear rate bioreactor. The authors suggested that differences in microbial communities may explain the superior performance of the high shear MFCs. This article is related to an excellent overview from the same lab on the use of sediment microbial fuel cells (SMFCs), which allow the production of energy in the form of readily usable electrical power that serves, for instance, to operate a small electrical apparatus (De Schamphelaire *et al*., 2008). The authors envisage that SMFCs could be used to power sensors and data transmitters at remote sites.

Lignocellulosic biomass such as straw or waste wood is an abundant low-cost source for production of biofuels such as ethanol that does not compete for human nutritional needs. Despite these advantages, turning plant biomass into ethanol remains primarily an economic challenge. For fiscal reasons it is preferable that all sugars present in the polymers hemicellulose and cellulose are converted to ethanol. To make the monomeric sugars contained in hemicellulose accessible for fermentation by microorganisms, biomass is pretreated under rough conditions. This pretreatment leaves the cellulose intact, this is subsequently hydrolysed enzymatically. Pretreatment generates a wealth of compounds, many of which inhibit fermentation by *Saccharomyces cerevisiae* and other microorganisms. Chemical and toxicological analysis of several different lignocellulosic hydrolysates by Heer and Sauer (2008) allowed them to identify furfural as the main toxic agent for yeasts, which influences yeast performance for ethanol production. The authors designed a long-term evolution series of assays where significantly increased tolerance of a semi-industrial yeast strain to toxic hydrolysates was achieved by selection for growth in furfural-containing medium. The increased tolerance resulted in three improved parameters: ability to grow in the presence of higher concentrations of hydrolysates, significantly reduced lag phases and reduced process times in process-relevant fermentation set-up.

Evolution of enzymes for better industrial performance is one of the main targets in biotechnology. Glucoamylase (GA) from *Aspergillus niger* (EC3.2.1.3) is used as an industrial enzyme to hydrolyse maize starch into glucose. The first step in this process is the liquefaction of starch into starch dextrin in the α-amylase reaction which is performed at 105°C for 5 min and then at 95°C for 1 h. Glucoamylase is used in the second step (saccharification) to convert the dextrin into glucose monomers. This step involves cooling the dextrin to 60°C, the operational temperature of GA. The Clark laboratory presents a series of elegant experiments directed to increase thermostability of GA without a decrease in catalytic efficiency (McDaniel *et al*., 2008). To this end, the authors used the combined techniques of directed evolution (phenotype screening following random

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mutagenesis and random recombination) and site-directed mutagenesis to isolate several multiply-mutated GA variants with enhanced thermostability compared with wild-type GA. The so-called CR2-1 variant is the most thermostable *A. niger* GA enzyme described to date.

Martin Filion (2008) has approached the very relevant issue of the effect of genetically modified plants (GMPs) on rhizobacteria. His minireview is a compendium of approaches used for analysis and some specific case studies. One of the most striking conclusions concerning risk assessment studies is that in spite of the large number of parameters studied, confusion still exists as to which information is really relevant in this context. To date, most studies investigating the impact of GMPs or transgenic plants have shown at least minor effects on the abundance and/or the diversity of rhizobacteria. Clear rhizobacterial community changes have been shown in the rhizosphere of a variety of plants using different transgenes. However, based on the limited information presently available in the literature, it seems that the effects of transgenic plants, although not negligible, can be considered 'minor' when compared with other 'normal' sources of variation, such as field site, soil type, seasonal variation, plant growth stage, etc. Agricultural practices, such as irrigation, crop rotation, tillage and use of herbicides and pesticides, have been shown to influence rhizobacteria populations with similar impact and in many cases more significantly than plant genetic transformation. Martin Filion proposed a series of steps and analyses for further risk assessment.

Trace pollutants in drinking water with undesirable hormonal effects on animals and humans are of increasing concern. In the November 2008 issue of *Microbial Biotechnology* Sabirova *et al.* (2008) investigate the biological treatment of very low concentrations of recalcitrant 17α-ethinylestradiol (EE2) using manganese-oxidizing microorganisms. They proposed an efficient strategy for the removal of trace levels of xenoestrogens from environmental samples through the production of highly oxidized Mn oxides by manganese-oxidizing bacteria that, in turn, drive the indirect oxidation and cleavage of EE2. The authors discuss the development of a more general strategy for the removal not only of estrogens but other trace-level organic contaminants from waste and drinking water.

Fast identification of pathogens is critical for adequate treatment of patients. The Kauffmann–White scheme is widely used as a typing method for classification of *Salmonella* into serovars on the basis of antigenic variability in the outer membrane lipopolysaccharide (O antigen), flagellar proteins (H1 and H2 antigens) and capsular polysaccharide (Vi antigen). Tankouo-Sandjong and colleagues (2008) report in *Microbial Biotechnology* that the conventional microbiological method for serotyping *Salmonella* takes several days and proposed that a microarray-based *Salmonella* serotyping method can address hundreds of different serovars in a single reaction and deliver results within 24 h, avoiding the need for strain isolation. They report a microarray, based on two housekeeping and two virulence marker genes (*atpD*, *gyrB*, *fliC* and *fliB*) for the detection and identification of the two species of *Salmonella* (*S. enterica* and *S. bongori*), the five subspecies of *S. enterica* (II, IIIa, IIIb, IV, VI) and 43 *S. enterica* ssp. *enterica* serovars. Results demonstrated a strong discriminatory ability of the microarray among *Salmonella* serovars and the array was highly sensitive being able to detect one colony-forming unit (cfu) per 25 g of food sample following overnight enrichment. This biotechnological advancement in detection of a major food-borne bacterium will undoubtedly aid in the fast and efficient treatment of infected individuals.

Lactic acid bacteria (LAB) are widely used in the making of fermented dairy products such as cheeses and yogurts; the global market for these good is huge and naturally research into these essential bacteria is of significant importance. In the November issue of *Microbial Biotechnology*, Siezen and Bachmann (2008) presented an up-to-date overview of research into LAB. The article concentrates on the current state of genomics in this field in relation to mining and analysis of the greater than 20 genomes that are presently available. Genome mining is being used extensively to annotate the metabolic potential of these bacteria and this has allowed the construction of genome-scale metabolic models which allow energy flows to be predicted and then experimentally tested. One of the more important angles is the analysis of genomes for enzymes that are involved in proteolysis and amino acid conversions. In fact mining performed on LAB strains found that typical dairy LAB such as *Lactococcus lactis*, *Streptococcus thermophilus* and *Lactobacillus casei* contained the largest sets of enzymes involved in metabolism of sulfur-containing amino acids, which are known precursors of dairy flavours (Liu *et al.*, 2008). *In situ* transcriptome analysis of LAB has mostly been obtained from *in vitro* experiments because the high protein and fat content of dairy products makes it very difficult to isolate bacterial RNA. The authors detail some of the recent advances towards solving these problems such as DNA microarray time series and new methodologies for extraction of RNA directly from cheese or separation of bacteria from cheese prior to RNA extraction (De Jong *et al.*, 2008; Monnet *et al.*, 2008). The authors later discuss recent findings that LAB suffer extensive gene loss and horizontal gene transfer during habitat adaptation (Makarova and Koonin, 2007). Obviously, this evolutionary adaptation is of major importance because it can potentially affect the reproducibility of the production processes. Bacteriophage is an important mechanism of horizontal gene transfer and phage-induced bacterial lysis can impact heavily

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on the dairy industry. Resistance to phage infection in LAB is sometimes conferred by clustered regularly interspaced short palindromic repeats. These sequences have been shown to be responsible for increased phage resistance during successive phage challenge (Deveau *et al.*, 2008), a process which could be used to directly evolve multiple phage-resistant strains. Undoubtedly, extensive analysis of the currently available LAB genomes and confirmation of the findings with high-quality experimental techniques such as those discussed in this article will greatly aid the dairy industry in the future.

Enterobacter sakazakii is an opportunistic pathogen, which can cause rare but life-threatening infections in neonates and infants. The group referred to as *E. sakazakii* group 1 strains have a high mortality rate, and severe neurological sequelae are often observed in surviving patients. Several studies have implicated rehydrated powdered infant formula and its preparation in hospital settings with disease transmission in spite that the contamination level with *Enterobacteriaceae* is in general very low: less than 1 cfu per 100 g of formula. However, outgrowth of even very low bacterial contamination may occur if the reconstituted product is kept at room temperature or in an incubator for long periods. Zuber and colleagues (2008) present a challenging article in *Microbial Biotechnology* dealing with the potential use of bacteriophage to control these microorganisms. This follows in line with the Food and Drug Administration approval of a cocktail of phage against *Listeria* in food production. The authors emphasize the need to isolate specific phage, a process for which they consider it is critical to establish the niche of the target bacterium. Clearly then there is the question of whether the isolated set of phage is safe for a food application, as temperate phage from *Enterobacteriaceae* may carry bacterial virulence determinants. Another practical question is whether the isolated phage infects a sufficient percentage of the target. In this report they isolated and characterized five phages and showed that when they infected a low pathogen number with low phage titres, the cells grew out – apparently undisturbed by the phage – until they reached a titre of 10^4–10^5 cfu ml^{-1}. Only after crossing this threshold, did they observe phage replication and the lysis of *E. sakazakii* cells. The authors consider that product safety can only be reached with high phage concentrations added to the finished product, which raises the questions of phage safety for infants, product availability and phage production costs.

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