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Highlight

New molecular tools for enhancing methane production, explaining thermodynamically limited lifestyles and other important biotechnological issues

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In this special issue a series of articles appear detailing methods of energy generation under different conditions; processes which are related to the future vision of microbes as a source of electric power (Lovley, 2009). Methanogenesis is identified as an important part of the biogeochemical carbon cycle in diverse anaerobic environments. Methane is an important fuel that can be generated from several wastes in these anoxic environments and therefore, understanding how it is produced, which organisms produce it and the reactions required to achieve high yields is of major biotechnological interest. Alves and colleagues (2009) present a mini-review on the use of lipids as substrates for methane production. Conversion of lipids into methane is achieved by the syntrophic consortia of acetogenic bacteria and methanogenic archeae. The microbes involved are influenced by the nature of the incoming lipids and examples of different microbial communities when feeding reactors with oleate or palmitate are reviewed. Alves and colleagues (2009) point out that the number of acetogenic microorganisms that degrade butyrate or higher fatty acids is very low and includes microbes of the *Syntrophaceae* and *Syntrophomonadaceae* families. These microbes live together with hydrogen-consuming methanogenic archeae and sulfate-reducing bacteria. The syntrophic cooperation seems to be optimal when microbes are organized as microcolonies, which is considered to favour interspecies hydrogen transfer. As in many biotechnological processes, lipid conversion into methane requires both the appropriate microbes and their performance optimization in the reactors, each of which still require some improvement. The authors note that previous failures to deal with high load lipids were related mainly to sludge flotation and biomass washout. To overcome these limitations the authors describe a new reactor concept which involves primary biomass retention through flotation and secondary biomass retention via settling. These reactors are being tested in scale and if, as expected, they solve the problem may soon come into industrial use.

The seminal review of Bernhard Schinck (Schink, 1997) reveals that catabolic reactions mediated by syntrophic bacteria are thermodynamically unfavourable and only feasible when at least a two-member consortium is in operation. In this issue of *Microbial Biotechnology*, Kato and colleagues (2009) present an outstanding study on propionate utilization by *Pelomaculum thermopropionicum* under syntrophic conditions when associated with the archeae *Methanobacterim thermautotrophus*. To understand how archeae affect the metabolism of the bacterium Kato *et al.* exploit previous group results (Kato *et al*., 2008; Kosaka *et al*., 2006) and construct a microarray for global transcriptional responses to chemicals that are used only under syntrophic conditions and chemicals, i.e. fumarate, that can be used in mono-cultures. One of the beauties of the work is that expression of the bacterium's central pathways is influenced by the substrate, but the sheer presence of the archeon leads to the induction of a number of central metabolism genes including those for amino acids and cofactors. The authors indicate that this is most likely due to cross-feeding of the bacterium by the hydrogenotrophic *Methanothermobacter*, and supports why *Pelomaculum* as mono-culture only grows if yeast extract is supplemented. Microarray analysis identified Fe-hydrogenase III as the major hydrogenase in all co-cultures, as well as the pyruvate: formate lyase which catalyses the conversion between pyruvate and acetyl-CoA (pyruvate + CoA → acetyl-CoA + formate). In all, they found that in co-cultures, regardless of the substrate used, almost 70 genes were upregulated and about

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50 were downregulated. This represents an excellent example of microbial interactions within the crystal ball concept presented by Handelsman (2009), which includes the term *metagenetics* and will allow us in the near future to achieve in-depth knowledge of the way microbes interact to survive in a given niche. This is an excellent piece of scientific work that will open new research avenues beyond methanogenesis.

On the topic of thermodynamic limits; is it possible to convert polycyclic aromatic hydrocarbons (PAHs) into methane? This issue is analysed by Dolfing and colleagues (2009) for two- to four-ring aromatic compounds using a theoretical basis and via wet analysis with naphthalene. The thermodynamic calculations for three potential pathways show that the thermodynamic properties are not independent to the biodegradation of PAHs under methanogenic conditions, because the process is exergonic. The authors speculate that some PAH degraders would simultaneously act as PAH degraders and acetogens. Degradation of PAHs has experienced an outburst in the last decade (Atlas and Bragg, 2009; Gieg *et al*., 2009; Parisi *et al*., 2009) and a thorough review on the main advances in the field will appear in one of the future issue of *Microbial Biotechnology* (Kanaly and Harayama, 2009).

In this issue of *Microbial Biotechnology* several other interesting aspects regarding the biology of microbes and their potential exploitation in biotechnology are considered. Jeon and colleagues (2009) show how modern genomic and proteomic information can be used to design strategies to combat *Campylobacter*, a major food-borne pathogen of animal origin and a leading cause of human gastroenteritis. Among the species being the main cause of infections 92% of the reported cases correspond to *Campylobacter jejuni*. The authors analysed in detail the main metabolic pathways used by this microorganism in the chicken gut, as well as adaptation variability, protein glycosylation systems, mechanisms of adherence and antibiotic resistance. This analysis revealed key features of the biology of these microorganisms that are, in turn, used to identify potential targets to prevent and control *Campylobacter* colonization in animal reservoirs.

One of the emerging features of the genomic analysis of *C. jejuni* is that the main source of acetyl-CoA generation and therefore electron transfer to the respiratory chains are amino acids, in particular L-aspartate, L-glutamate, L-serine and L-proline which are preferentially used by this microorganism. A characteristic of *Campylobacter* is that they have a highly branched electron transport system that allows them to derive energy through respiration using a wide variety of electron acceptors, which include oxygen under aerobic conditions or hydrogen peroxide and sulfite under oxygen-depleted conditions. Of particular interest is the ability to respire sulfite, which is currently used as a food preservative.

Colonization of the animal gut requires multiple functions and is a complex process that involves attachment, motility and tolerance to stressing agents such as bile salts. *Campylobacter* adherence to epithelial cells in the intestine is required to resist intestinal peristalsis, and flagella, CadF, PEB1 and PEB4 are critical surface proteins involved in adherence. Resistance to bile is mainly mediated by an RND family efflux pump called CmeABC that is regulated by the CmeR regulator. The regulator prevents expression of the efflux pump genes, but in the presence of bile salts is inactivated and transcription takes place. In addition, this efflux pump removes antibiotics and other drugs that may be used to prevent growth. Moreover *Campylobacter* also exhibit an arsenal of tools to resist different stress conditions, such as acid stress, heat changes and exposure to oxidative conditions.

Fluoroquinolones are often used to combat *Campylobacter* infections; however, it has been found that FQ resistance appears in this microorganism with a high frequency. This could be related to the fact that a number of enzymes needed for error-prone correction, such as UvrR, MutL, MutH are not present in the genome of *C. jejuni*, which in turn seems the basis of a high genetic and phenotypic variability in this species. This review also explores different alternatives to decrease infection by *Campylobacter*, including the use of bacteriophage.

In fact, the topic of bacteriophage biocontrol is covered in an extremely insightful mini-review by R.J. Atterbury (Atterbury, 2009). The author reflects on the early promising results from bacteriophage application in so called 'Phage Therapy' which were performed in the 1930s and 1940s while lamenting the paucity of rigorous well-controlled studies in these early trials. The lack of well-controlled and standardized methods for bacteriophage testing and the discovery and use of antibiotics led to a rapid decline in the use of bacteriophage as a therapeutic agent; particularly in the West. However, recently there has been a revival in the use of bacteriophage in the treatment of disease in animals, and surface decontamination (biotic and abiotic). Bacteriophage being ubiquitous in the environment and therefore already a part of the human food chain suggests that they can be regularly consumed without ill effects on human health. The recent approval of phage treatments for foods by the US Food and Drug Administration has opened the door for a preponderance of new applications for bacteriophage in the reduction of zoonotic infections or elimination of food spoilage bacteria. The author points out that careful screening of the bacteriophage is of great importance to ensure that only virulent self-limiting phage that cannot transfer bacterial DNA are used. It appears that a new era is dawning for what is essentially an 80-year-old biotechnology; although bacteriophage are unlikely to be a universal remedy for disease treatment and food

preservation they will almost certainly be a valuable addition to our current arsenal.

Yang and colleagues present a thorough and interesting review of virulence factor expression in the enteric model bacteria *Citrobacter rodentium* (Yang *et al*., 2009). When moving from the environment to the host, enteric pathogens need to modify their gene expression, often upregulating the expression of virulence genes and downregulating housekeeping genes. The authors describe how *C. rodentium* is able to modulate gene expression during the infection process. *Citrobacter rodentium* carries the locus for enterocyte effacement (LEE), which is a pathogenicity island that is highly conserved among diverse enteric pathogens and is required for the development of lesions in the intestinal epithelium. By adapting pre-existing regulatory mechanisms and newly acquired regulatory genes *C. rodentium* is able to ensure its survival; the intricate regulatory networks allowing specific control of virulence gene expression in response to the different environments. Yang and colleagues detail how the bacterium utilizes a positive regulatory loop involving LEE-encoded regulatory proteins (Ler, GrlA and GrlR) to control LEE expression along with several transcription factors not encoded by LEE; some of which respond to specific environmental signals. One of these is a recently described AraC-like regulatory protein, RegA, which is key to the ability of *C. rodentium* to colonize the intestine. RegA activates the transcription of numerous operons that encode virulence factors, while at the same time repressing transcription of a number of housekeeping genes. Of great significance is that RegA is the only known AraC-like regulator shown to respond directly to a gut-specific environmental signal, bicarbonate, in order to exert its regulatory effects (Yang *et al*., 2008). Information gained regarding regulatory proteins, particularly the important and diverse AraC-like regulators will undoubtedly lead to future advances in the use of these proteins in biotechnology.

In the upcoming edition of *Microbial Biotechnology* there is an article by Linares and colleagues (2009) that describes the first report of transcriptional regulation of tyramine biosynthesis in lactic acid bacteria (LAB). Tyramine is a low-molecular-weight biogenic amine that is formed by the enzymatic decarboxylation of tyrosine; such molecules are of importance because they are formed from the natural action of bacteria on foods such as cheese and can be toxic in high concentrations. Tyramine formation and secretion in LAB requires the products of two genes *tdcA* (tyrosine decarboxylase) and *tyrP* (tyramine/tyrosine antiporter). In this article the authors describe the genetic regulation of tyramine biosynthesis in *Enterococcus durans*; showing that *tdcA* and *tyrP* form an operon that is transcribed from a single promoter P_{tdcA}. They also consolidate previous studies that noted that decarboxylase gene expression requires acidic pH and high amino acid concentrations. Their quantitative gene expression data confirmed that both high concentrations of tyrosine and acidic pH conditions during log phase growth led to increased transcription from the P_{tdcA} promoter. These results together with those previously reported indicate the importance of the decarboxylation pathway in the regulation of internal pH in LAB when placed in an acidic environment; knowledge which could prove beneficial for both food production and prevention of food spoilage.

Saccharomyces cerevisiae is an important microorganism from a biotechnological point of view because of its use in industrial processes such as bread making and fermented beverage production. Different compounds including volatile esters are responsible, even at trace amounts, for highly desired flavours for example fruity, mellow, etc. There are two main categories of flavour-active esters in fermented beverages: acetate esters (produced from acetate + ethanol or a complex alcohol derived from amino acid metabolism) and medium-chain fatty acid (MCFA) ethyl esters (formed from MCFA and ethanol). The review by Saerens and colleagues (2009) deals with the different tactics that can be used to modify the production of these esters. The final concentration of these products is influenced by the overall rates of synthesis (influenced in turn by substrate availability and enzyme activity) and degradation. The authors summarize data on acetate ester synthesis, which is mainly performed by alcohol acetyl transferases I and II (encoded by *ATF1* and *ATF2*), although double null mutants still produce other esters indicating the existence of other synthases. *ATF1* is the most important enzyme in terms of activity and is subject to a complex regulation; responding to unsaturated fatty acids, oxygen, nitrogen, phosphate, ethanol and heat stress. All of these factors provide manufacturers with several options for external influence.

The participation of enzymes such as Eht1 or Eeb1 in ethyl ester synthesis is also discussed and the corresponding null mutants present lower levels of particular MCFA ethyl esters; however, yet again they do not account for all MCFA derivative synthesis. The regulation of Eht1 is complex because the enzyme participates in both the synthesis and the hydrolysis of the esters. The authors suggest that its activity is influenced by nutrients, oxygen and inositol, and also depends on the genetic background.

Phosphorus (P) is an essential element classified as a macronutrient because of the relatively large amounts required by plants. Although P is widely distributed in nature, it is recognized that the availability of phosphate in soils is a major factor limiting the productivity of many ecosystems. The main biological mechanism for mineral phosphate solubilisation is the production of organic

acids; for example gluconate and 2-keto gluconate. In Sashidhar and Podile (2009), they describe the construction of recombinant genetically modified organisms (GMOs) from the free-living N_2 fixer and common soil habitant *Azotobacter vinelandii* which allow improved mineral phosphate fertilization of sorghum seeds. Membrane-bound Gcd (quinoprotein glucose dehydrogenase) from *Escherichia coli* converted glucose to gluconic acid which was released directly into the periplasmic space and subsequently released to the cell's environment. The *gcd* was introduced into *A. vinelandii* broad-host-range plasmids in which the gene was under the control of *A. vinelandii glnA* (glutamine synthetase) or *pts* (phosphate transport system) promoters. Sorghum seeds were inoculated with the GMOs and gene expression, production of gluconic acid, nitrogenase activity, inorganic phosphate release and plant growth were monitored. The recombinant genes responded to the expected environmental/chemical signal and were affected by nitrogen availability in the constructions containing the *glnA* promoter or by available inorganic phosphate in those with *pts* promoters. The GMOs presented differences in morphology and exopolysaccharide content and a slight decrease in their nitrogen fixation ability, while at the same time improving biofertilizer potential and plant growth-promoting activity. Interestingly, significant growth enhancement was seen 3–4 weeks after inoculation of the plants with the GMOs indicating potential future use of these types of organisms may provide long-term benefits to crop plants.

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