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Highlight

Explorative probes and biomarkers, chronic *Salmonella* infections and future vaccines

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Biosensors, probes and microbial communities

The crucial first step in tackling pollution is probably the preclusion of the toxic spread, and therefore, early contaminant detection is crucial (van Dillewijn *et al.*, 2009). Biological detectors are usually cheaper and more sensitive than chemical detection methodologies, but they may present additional problems because contaminants can prejudice growth and/or be scarce and not very accessible to cells. Both the positive and the negative sides of bioreporters are particularly true when we are dealing with marine oil spills, where clean up plus total recovery of the affected zones are enduring, difficult and multifactorial processes. For this purpose, Kumari and colleagues (2011) present in *Environmental Microbiology* a novel bioreporter assay for the detection of long-chain alkanes. The reporter construction is based on the *lux* system and the *Alcanivorax borkumensis alkSB1GHJ* genes, an operon naturally induced by *n*-tetradecane and other components of crude oil, a combination that allows reliable and simple measurements even under non-laboratory conditions. Exposing *Escherichia coli* or *A. borkumensis* bearing the reporter construct to individual alkanes gave low levels of luminescence in both free or agarose-embedded cells; however, increasing incubation times of *A. borkumensis* in the presence of crude oil dramatically increased the luminous response, probably due to the adherence of cells to oil droplets. As a final proof of concept the authors assayed the effect of adding a commercial oleophilic fertilizer on alkane degradation; the results were very striking, and showed how this supplement can have either positive or negative effects depending on the dosage. These results are very important and point out that we must be very cautious when proposing the use of potentially beneficial additives in the environment. This research article represents a valuable addition to articles previously published in this journal involving the construction of robust biosensors for detection of pollutants in the environment (Yagur-Kroll *et al.*, 2010; Zhang *et al.*, 2010).

Novel biomarkers are also proposed by Lu and colleagues (2011) for measuring the denitrification activity in wastewater cleaning. To activate the elimination of nitrate by anaerobic bacteria in sewage disposals, methanol is generally added in order to increase the C/N ratio, but this chemical is toxic, expensive, volatile and flammable, thus glycerol is the most common alternative. Lu and colleagues (2011) dealt with the need to find simple markers to predict carbon metabolism in a denitrifying microbial community. The authors proposed the use of mRNA coding for key enzymes of methanol and glycerol catabolism pathways as markers to elucidate the contribution of these carbon sources to nitrate removal in water-treatment sludge. Their analysis showed a strong correlation between the transcripts of *Methyloversatilis* spp. *mdh2* and *Hyphomicrobium* spp. *mxaF* for methanol, and *Citrobacter* spp. *dhaD* for glycerol, and their associated denitrification rates. These biomarkers could be very useful in analysing and maximizing the effectiveness of specific denitrification communities, although they do not target all the microorganisms involved. It should also be noted that in some cases transcript levels cannot be directly correlated with 'metabolism' because of post-transcriptional, post-translational and metabolic feedback control (Molina-Henares *et al.*, 2010). Regardless of this point, the study by Lu and colleagues (2011) precisely identifies the members of the bacterial population in this sludge and how their abundance varies over time in a batch reactor fed sequentially with methanol and glycerol. Other important highlights from this study are the identification of *Citrobacter* spp. as an important species in

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glycerol-based denitrification, and the author's findings on the speed of adaptation of the bacterial community during the switch from methanol to glycerol as carbon sources.

Single cell genomics (Woyke *et al*., 2009) and genome assembly from deeply sequenced metagenomes (Hallam *et al*., 2006) are two promising approaches for obtaining genomic insights into ecologically significant taxa; this information can also be relevant for the development of probes. The group of Dr Moran has dealt with this issue by studying the *Roseobacter* lineage, which represents numerically up to 20% of coastal and 15% of mixed-layer ocean communities from tropical to Polar Regions (Selje *et al*., 2004). *Roseobacter* lineage includes microbes that are aerobic, carry out anoxygenic photosynthesis and oxidize carbon monoxide (King, 2003; Cunliffe, 2010) and hydrogen sulfide (Moran *et al*., 2004), in addition to fixing carbon dioxide. Luo and colleagues (2011) computed patterns of non-synonymous (amino acid-altering) nucleotide diversity in the marine metagenome of *Roseobacter* from uncultivated members of the *Roseobacter* clade. The analysis of metagenomic DNA from *Roseobacter* revealed horizontal gene transfer from other marine bacterioplankton taxa or viruses, including pyrophosphatases and glycosylation proteins. *Roseobacter*-specific genes from metagenomic data sets are potentially useful for development of specific probes to follow *in situ* perturbations.

Microbial communities

Massive sequencing based on next-generation sequencing technologies and microarrays are currently the most promising and complementary approaches to address the complexity of microbial communities (Claesson *et al*., 2009; Roh *et al*., 2010; van den Bogert *et al*., 2011; Dugat-Bony *et al*., 2011). Two specific strategies can be applied: metagenomics, which refers to the study of the collective genomes in a given environmental community and the 16S rDNA amplicon sequencing approach. In principle, these methods enable: (i) access to the wide diversity of microbial communities, (ii) identification of unknown microorganisms and (iii) the potential to link microbial structure to function (Simon and Daniel, 2009). Some limitations of metagenomics, however, have been demonstrated: for example, the difficulty of managing large amounts of sequence data, or the short sequence read length which complicates the assembling of contigs, or sequencing errors. Comprehensive tools have been developed, such as the high-density PhyloChip, with nearly 500 000 oligonucleotide probes to almost 9000 operational taxonomic units (Brodie *et al*., 2006), and the GeoChip 3.0 with near 28 000 probes covering approximately 57 000 gene variants from 292 functional gene families (He *et al*., 2010). Microarrays are sufficiently sensitive, with detection of sequences representing genomic material from 0.05% to 5% of the total environmental community. *In silico* probe design is one of the most critical steps for microarray experiments because the selected oligonucleotide probe set will have to combine: (i) sensitivity (e.g. probes should detect low abundance targets in complex mixtures), (ii) specificity (e.g. probes should not cross-hybridize with non-target sequences), and (iii) uniformity (e.g. probes should display similar hybridization behaviour). Research in this area is advancing our knowledge of which bacteria are in a given niche and is open research to 'scavenge' further information regarding new functions from metagenomes.

Vaccines

In the latest issue of *Environmental Microbiology Reports*, Bäumler and colleagues (2011) present a comprehensive minireview of *Salmonella* virulence strategies in relation to their growth and survival in host niches that represent reservoirs for transmission. The review looks at both non-typhoidal and typhoidal strains of *Salmonella* and highlights their different strategies, virulence factors and preferred niches. *Salmonella enterica* subspecies *enterica* includes most of the serovars which cause human disease and they are reported to cause more than 100 million clinical cases of Salmonellosis per year. The authors review the three general classes of *Salmonella* infection found in immune-compromised individuals: gastroenteritis, typhoid fever and chronic carriage. The authors highlight that in gastroenteritis infections, the main virulence strategy employed by *Salmonella typhimurium* is to induce acute intestinal inflammation by employing Type III secreted virulence factors for the invasion of the intestinal epithelium. A novel luminal niche is created by the consequent inflammatory response; reactive oxygen species (ROS) produced by phagocytes oxidize endogenous sulfur compounds, which allow *S. typhimurium* to outgrow competing microbes using tetrathionate respiration. The resulting amplification in the relative abundance of *S. typhimurium* in the lumen promotes its transmission via the faecal – oral route to the next susceptible host. This however is only one facet of *Salmonella* infection; the typhoid serovars are able to cause systemic infections in immunocompromised individuals and disseminate using an entirely different strategy. The causative agent of typhoid fever, *Salmonella typhi* establishes a chronic carrier state in infected individuals by colonizing the gall bladder. Human chronic carriers of *S. typhi* are an important reservoir for transmission due to the ability to intermittently release the pathogen into the intestine. *Salmonella* serovars us a diverse array of techniques to colonize the gall bladder; a fraction of the *Salmonella* population will invade the epithelium while

another will multiply in the gall bladder lumen by either forming biofilms on gallstones or activating multiple bile defence responses. The description of these various processes by Bäumler and co-workers emphasizes the versatility and vigour of this pathogens adaptive strategy to cope with bile. Clearly the adoption of multiple lifestyles by *Salmonella* in both the intestine and the gall bladder explains the prolific presence of this pathogen in our world today.

Ennio De Gregorio and Rino Rappuoli recently presented a fantastic review in relation to the rational design of vaccines in the Early View online edition of *Microbial Biotechnology* (De Gregorio and Rappuoli, 2011). Although vaccination has been one of the most effective measures in the control of human disease there are still many infections that are not preventable using this methodology. These include diseases caused by viruses [hepatitis C virus (HCV) and human immunodeficiency virus (HIV)]; parasites (*Plasmodium, Leishmania, Schistosoma* and *Trypanosoma*) and bacteria [*Mycobacterium tuberculosis* (TB), Group A and Group B streptococcus, *Staphylococcus aureus*, *Shigella* and pathogenic *E. coli*]. Most traditional vaccines are based on inactivated or attenuated pathogens or on purified pathogen subunits, such as toxins or polysaccharides. These vaccines are quite efficient in preventing infections of pathogens with a low degree of antigen variability because they work by eliciting functional antibodies that can (i) counteract viral invasion, (ii) neutralize bacterial toxins and (iii) induce complement-mediated killing of bacteria (Germain, 2010). Vaccines containing multiple antigens (multi-valent) have also been produced to cope with the viruses and bacteria that are capable of more moderate degrees of antigen variability (Pace *et al*., 2009; Duggan, 2010; Pomfret *et al*., 2011). However, to date vaccines that can protect against infections by pathogens with a high antigen variability rate do not exist and it is with this in mind that the authors review the current state of play. They focus on the strategies that have been recently employed to manage bacterial and viral diversity and on the technologies that can be applied to the vaccines of the future to prevent infection by highly variable pathogens. These include the use of Reverse Vaccinology, and Analytical and Structural Vaccinology to cope with either bacterial or viral diversity. They later describe new technologies including novel adjuvants, delivery systems, new viral vectors and prime-boost strategies that can be used to elicit multifunctional adaptive responses during the vaccination protocol. The authors then finish off with a review of new Systems Vaccinology approaches which are being used to better understand human correlates of vaccine efficacy and evaluate in detail the quality of humoral and cellular responses to vaccination. The hope is that a more rational design of vaccines utilizing the reviewed cutting-edge advances will lead to the prevention of diseases that we never thought could be conquered.

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