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Accumulation of plastic debris in the world's oceans is of increasing concern. The very properties that make plastic such an attractive material for a wide range of functions also contribute to their buildup in the environment; plastics are generally inexpensive, readily available, and physically and chemically stable. Biodegradation by bacteria is an attractive option for plastic disposal; avoiding the disadvantages associated with traditional disposal techniques. Bacteria have adapted to successfully colonize most environments on the planet and can utilize and degrade a wide variety of energy sources.

This study aimed to take advantage of these principles of bacterial nutritional adaptations in order to recover environmental bacteria capable of metabolising poly(ethylene terephthalate) (PET), a material commonly used to manufacture water and softdrink bottles. Seawater collected from St. Kilda beach, Melbourne, Australia in December 12, 2007, from an area with plastic litter present in sea water, was used for enrichment experiments. PET was provided as the sole energy and carbon source. Bacterial populations were periodically sampled to monitor individual constituent members, which were subsequently identified by 16S ribosomal DNA sequencing. Bacterial biofouling of PET surfaces was observed by confocal laser scanning microscopy (CLSM). Plastic characterization was carried out via atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS). After 9 months of the experiment representatives of two bacterial phylotypes, *Gammaproteobacteria* and *Alphaproteobacteria*, appear to be beginning to dominate the bacterial population, specifically those belonging to the genera *Alteromonas* and *Thalassospira*. Bacteria of both phylotypes exhibited specialized metabolic profiles and lack of ability to utilize various carbon sources. Evidence of plastic degradation was found in the form of alterations to surface topography and chemistry.

PP10.4

Microbial Population Dynamics of Pyrene Contaminated Soils under *Lupinus* spp. – Implications for Phytoremediation of PAH Contaminated Sites

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Hydrocarbon contamination of soils is an increasing global problem posing threats to human and environmental health. Currently many remediation strategies are not economically and environmentally feasible or efficient. Phytoremediation, the use of plants and associated rhizosphere microbes may offer a viable alternative for contaminated sites with low economic value. As part of a larger phytoremediation study, the effect of *Lupinus albus* and *Lupinus angustifolius* on pyrene degrading soil microbial populations was monitored. Total pyrene degrading microbial populations, pyrene degrading prokaryotes or eukaryotes and dioxygenase expressing soil bacteria were enumerated using four novel most probable number (MPN) assays. It was found that the presence of *L. angustifolius* and *L. albus* enhanced overall pyrene degrading microbial populations when compared with non-planted contaminated controls. Pyrene degrading prokaryotes were more abundant than eukaryotes. Dioxygenase expressing soil bacteria were more numerous when *L. albus* was present compared with *L. angustifolius*, suggesting that specific selection of degradative microbes was plant mediated. No statistically significant differences were found for pyrene degrading microbial numbers in soils with or without pyrene when lupins were present. This suggests that these lupin species select for pyrene degrading microbes independently of contaminant selective factors. This may be due to the unique ability of legumes to exude isoflavonoids, many of which are analogues of polycyclic aromatic hydrocarbons (PAHs) such as pyrene, and which may select for PAH degrading microbial communities. The data suggests that microbial degradation of pyrene in the studied system may be enhanced by the presence of *L. albus* and *L. angustifolius*, and further study of the effect of these plants species on other PAH compounds may be warranted.

PP10.5

Development of Bacterial Biosensors as tools for Risk Assessment during Remediation of Contaminated Environmental samples

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Contamination of soil, surface and subsurface water supplies by petroleum products is a serious and ongoing environmental concern. The presence of such contaminants, along with their by-products, in water supplies not only endangers human health but has a prolonged impact upon aquatic ecosystems. Currently, detection of oil contaminants in water is predominantly conducted by chemical assessment. This type of assessment is limited as it only measures the amount of a specific compound present in an aquatic sample without considering either the environmental or human risk associated with exposure to the pollutant.

During remediation of petroleum products, mineralisation occurs through a series of transformation resulting in the production of intermediate compounds which have the potential to be more toxic than the parent material. Bacteria represent ideal organisms for use as biosensors to assess the potential hazard associated with remediation. A bacterial biosensor that contains both a reporter gene (such as GFP) that generates a detectable signal coupled to a hazard sensing component (such as a DNA repair gene promoter) would permit rapid detection of mutagenic compounds in aquatic samples before, during and after the remediation process. Information is obtained relating to the potential leaching of hazardous products into proximate water supplies.

In this study, we have constructed a suite of bacterial biosensors using enteric (*Escherichia coli*) DNA bacterial promoter regions. This involved the isolation of DNA repair promoters from genomic DNA and their subsequent fusion to the coding sequence of the GFPmut3 gene. These constructs were transformed into *E. coli* and their use as bacterial biosensors validated by UVC irradiation (254nm), a known mutagen and inducer of multiple DNA repair pathways. These preliminary studies were then expanded upon to include the assessment of oil spiked freshwater samples. The biosensors capacity to detect a standard petroleum product at varying concentrations along with the rate of gene induction between the various biosensors was compared.

PP11

Microbial Ecology & Physiology

Thursday 9 July 2.20pm
Meeting Room 7, Level 2

PP11.1

An Insight Into the Activity of Chemolithotrophs During Iron / Sulfur Oxidation by Monitoring the Expression of Carbon Fixation Genes

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Acidophilic chemolithotrophs are often used in biomining, an industrial process in which low grade ores are processed for their metals of interest. The availability of organic carbon in this environment is often limited; hence a number of these microorganisms are autotrophic. During biomining there is often a range of acidophilic chemolithotrophs which contribute to the oxidation of iron / sulfur within the ore. However, the effectiveness of each microorganisms in the oxidation of iron / sulphur in these mixed populations is unknown. The aim of this study was to develop a method of monitoring the activity of biomining microorganisms. As there are many different pathways to oxidise iron / sulphur the genes of the carbon fixation pathways were targeted. Biomining micoorganisms are known to fix carbon dioxide using the Calvin-Benson-Bassham pathway and the 3-hydroxypropionate/ 4-hydroxybutyrate pathway, and possibly the reductive acetyl-CoA pathway or reductive tricarboxylic acid pathway. A SYBRGreen qRT-PCR assay was developed to monitor the expression of key carbon cycle genes in: *Acidithiobacillus ferrooxidans, Acidithiobacillus caldus, Sulfobacillus thermosulfidooxidans, Leptospirillum ferriphilum* and *Ferroplasma acidiphilum*. Growth was examined, through direct cell counting and iron oxidation rates. RNA was extracted at specified time intervals and qRT-PCR was used to investigate gene expression. A relationship between the expression of carbon cycle genes and the growth and iron oxidation was observed. This study is the first to look at the activity of biomining microorganisms using genes involved in carbon fixation, and will lay the way for future studies on the activity of biomining microorganisms in mixed communities.

PP11.2

Identification of Phenotypes Conferred by Integron-Associated Gene Cassettes in a Marine *Vibrio* sp.

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Integrons include a site-specific recombination system that is capable of integrating and rearranging mobile gene cassettes. Integrons were originally identified on mobile elements from pathogenic bacteria and are a major reservoir of antibiotic-resistance genes. Integrons are now known to be ancient structures that are phylogenetically diverse and, to date, have been found in approximately 10% of sequenced bacterial genomes. All integrons share a common structure consisting of an integrase gene (*intI*) and a neighbouring recombination site (*attI*) where gene cassettes are inserted. Associated gene cassette arrays can vary in number with some integrons containing no gene cassettes while others contain cassettes numbering in the hundreds. Vibrios in particular can contain large cassette arrays. In *Vibrio vulnificus* CMCP6 and *Vibrio cholerae* N16961 there are 217 and 179 gene cassettes respectively. Overall, gene diversity in cassettes is extraordinarily high suggesting that the integron/gene cassette system has a broad role in adaptation. However, it has been difficult to identify the adaptive role(s) that genes in cassettes provide to their host since approximately 80% of the associated genes encode proteins of unknown function. In many instances where a function can be ascribed, the function is sufficiently general (eg. acetyltransferases) that their effect is not easily inferred. To address this problem, large deletions within the cassette array of *Vibrio* sp. DAT722, which contains 116 gene cassettes, were created using homologous recombination. Four deletion mutants were isolated, encompassing deletion of more than half the gene cassettes in total. We identified numerous phenotypes encoded by cassette genes such as colony morphology changes, oxidative stress survival, changes in biofilm formation and temperature stress survival. This study is the first to look at the effect of cassette-associated genes on bacterial physiology revealing new information and insight into the importance of these mobile elements in niche specialization and adaptation.

PP11.3

Shifts in SPANC Balance Leads to Stain Variation

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It has been hypothesised that a trade-off between **S**tress **P**rotection **A**nd **N**utritional **C**ompetence (SPANC) is frequently modulated during bacterial evolution. This can be observed in outer membrane (OM) permeability as the trade-off between sensitivity to antibiotics/detergents against the ability to grow in low nutrient concentrations. For example, OM permeability is increased in low nutrient conditions, maximising the uptake of nutrients, but also increasing the intake of harmful molecules. This study used ECOR isolates to determine if natural strains conform to the SPANC hypothesis.

The levels of OM permeability in ECOR isolates can be indicated by sensitivity to 3µg/ml Cm and 0.1% SDS. Competition of selected isolates with laboratory strain MG1655 under nutrient limited conditions, affirmed permeability levels. ECOR5 and ECOR 59 were chosen as representatives of less permeable and more permeable strains respectively.

ECOR 5 grown under continued nutrient limitation produced an isolate, which showed increased sensitivity to antibiotics/detergents and increased nutritional capability. Additionally, increased permeability to the colorimetric β-lactam, Nitrocefin, was observed. This was most likely caused by an increase in expression of the larger porin, OmpF, as seen in its OM pattern on SDS-PAGE.

SPANC setting was shifted towards increased resistance and decreased nutritional capability in an isolate derived from ECOR 59 grown with 2µg/ml Cml. While this strain had decreased permeability to Nitrocefin, the OM pattern did not change. Instead, structural changes unable to be resolved with SDS-PAGE may underlie this.

We have shown that natural isolates can undergo shifts in SPANC setting under selection conditions for increased and decreased permeability. This provides an insight to adaptations which could occur in the environment leading to strain variation.

PP11.4

Promotion Of Tomato (*Lycopersicon Esculentum* Mill.) Plant Growth By Rhizosphere Competent 1-Aminocyclopropane-1-Carboxylic Acid Deaminase-Producing Streptomycete Actinomycetes

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The ability of streptomycete actinomycetes to promote growth of tomato through the production of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase was evaluated under gnotobiotic and greenhouse conditions. To achieve this, 64 isolates of *Streptomyces* spp. obtained from a tomato rhizosphere in the