Society for General Microbiology
Irish Branch - Spring Meeting
Institute of Technology, Carlow

Metals, Microbes & the Environment
March 26th and 27th, 2002

Dear Colleague,

On behalf of the organisers, welcome to the Institute of Technology Carlow.

The campus is located on the banks of the River Barrow in Carlow, one of Irelands largest inland towns.

IT Carlow is one of Irelands leading higher education colleges, providing a broad range of Undergraduate programmes and Postgraduate research in the fields of Science, Engineering, Technology and Business Management. The Institute has recently completed a major development program which has been tailored to ensure that its graduates are at the forefront of technological developments both nationally and worldwide. The focus of this new development is the learning resource centre which has transformed the learning experience at IT Carlow through easy access to the worlds resources for learning and research.

During this SGM meeting, oral presentations will take place in theatre A102. Poster presentations will be located in the main reception area, close to the registration desk. If you require any further assistance, please contact Niamh or Jill at the registration desk. The organisers would like to thank Mary Bates, Caroline Dermody and Breda Fagin for their help in preparation for this SGM meeting.

Yours Sincerely,

David Dowling
Catherine O Reilly
Dina Brazil
David Ryan

Scientific Programme

Scientific Programme 26th March 2002

Chairperson – Dr DN Dowling

14.00-14.45 "A Bacterial View of the Periodic Table: Genes for Bad Ions."
Professor Simon Silver, Microbiology & Immunology, University of Illinois at Chicago USA.

14.45- 15.30 "Bacterial mercury uptake and mercury resistance - complex genotypes, simple phenotypes."
Professor Nigel Brown, University of Birmingham, UK

15.30 – 16.00 Coffee and poster viewing

Chairperson – Prof Simon Silver

16.00- 16.45 "Novel approaches to metal bioremediation and nanocatalysis"
Dr Lynne Macaskie, University of Birmingham, UK

Offered Papers

16.45- 17.05 " Detection systems for genetically modified microorganisms **"**
Orla Sherlock, Institute of Technology Carlow, Kilkenny Road, Carlow, Ireland

17.05 – 17.25 "Use of 16srDNA sequencing and T-RFLP analysis to monitor the microbial community structure and dynamics of low temperature anaerobic digesters"
Gavin Collins, Microbial Ecology Laboratory, Dept. of Microbiology, National University of Ireland, Galway

17.25 – 17.45 "Anerobic treatment of phenol and related compounds"
Aoife Thornton, Environmental Microbiology Research Unit, Dept. of Microbiology, National University of Ireland, Galway

17.45 - 18.05 "PHA production by *Pseudomonas putida* CA-3"
Patrick Ward, Dept of Industrial Microbiology, University College Dublin

19.00 - Buffet reception in the Dolmen Hotel

Scientific Programme 27th March 2002

Chairperson Dr C.O'Reilly

9.15-10.00 "Metal-specificity in transcriptional-regulators, transporters and metallochaperones"
Professor Nigel Robinson, University of Newcastle, UK

10.00 – 10.45 "The multiple repressor mechanisms of the Fur protein of *E. coli*"
Dr Victor de Lorenzo, CSIC Madrid, Spain

10.45-11.15 Coffee and poster viewing

Chairperson Prof Victor De Lorenzo

11.15-12.00 "Iron Uptake in Rhizobium - do these Symbiotic Bacteria think they are Pathogens?"
Professor Andrew Johnston, University of East Anglia, UK

Offered Papers

12.00-12.20 "Optimization of a chemostat based system for production and characterization of bioaerosols"
Kevina O'Donohoe, Dept. of Microbiology, National University of Ireland, Galway

12.20-12.40 "Strain typing of *Mycobacterium bovis"*
Louise Bailey, Dept of Zoology, University College Dublin

12.40-13.00 "Environmental influences on organotin-yeast interactions"
Jane White, School of Biotechnology, Dublin City University, Dublin

END OF MEETING

Oral Presentations

1. A BACTERIAL VIEW OF THE PERIODIC TABLE: GENES FOR BAD IONS

Simon Silver

University of Illinois, Chicago USA; e-mail simon@uic.edu

Bacteria have plasmid and chromosomal genes for resistances to metalions including Ag+, AsO2-, AsO43-, Cd2+, Co2+, CrO42-, Cu2+, Hg2+, Ni2+, Pb2+, Sb3+, TeO32-, Tl+ and Zn2+. Those for arsenic resistance and silver resistance will be discussed. The widely-found plasmid arsenic resistance (ars) systems includes the enzyme arsenate reductase that converts intracellular arsenate [As(V)] to arsenite [As(III)], the substrate of the efflux system encoded by additional gene(s) in the system. With multiple sequences from new genomic analysis and x-ray crystal structures of three arsenate reductases, it has become apparent that arsenate reductase arose more than once in evolution (three times in fact), with differing solutions the same problem - a bit like the unrelated wings of birds and butterflies. The ArsB membrane efflux pump proteins fall in to two independent clades. Arsenite oxidase converts arsenite to arsenate in a few known bacteria. We have the gene sequence for arsenite oxidase (aso) for the first time. Arsenite oxidase contains two subunits, and the asoB gene which encodes the Rieske 2Fe-2S subunit gene lies 12 nte upstream from the asoA gene for the large HiPIP 3Fe-4S molybdopterin-containing subunit. The asoB gene product contains a Tat (twin arginine translocator) signal peptide sequence which is predicted to transport the assembled AsoBAsoA heterodimeric protein with bound cofactors in to the periplasm. By Southern hybridization and PCR amplification, homologs of the arsenite oxidase gene were found in additional arsenite oxidizing isolates of Gram-negative bacteria, including the A. faecalis strain of Osborne and Ehrlich (1976). Silver compounds are used widely as biocides, including for treatment of animal and human water sources, and in household plastic products and human clothing. Silver resistance determinants, involving up to nine
genes, have been found and sequenced on plasmids of the IncH incompatibility groups and on the chromosome of E. coli and related bacteria. The product of one gene, the SilE protein is a unique Ag+-specific periplasmic binding protein.

- S. Silver and L.T. Phung. 1996. Bacterial heavy metal resistance: new surprises. Annu. Rev. Microbiol. 50, 753789.
- Gupta, K. Matsui, J.F. Lo, and S. Silver. 1999. Molecular basis for resistance to silver cations in Salmonella. Nature Medicine 5, 183-188.
- A. Gupta, L. T. Phung, D. E. Taylor and S. Silver. 2001. Silver resistance genes in plasmids of the IncH incompatibility group and on the Escherichia coli chromosome. Microbiol. 147: 3393-3402. http://mic.sgmjournals.org
- S. Silver, L.T. Phung, and B. P. Rosen. 2001. Arsenic metabolism: resistance, reduction and oxidation. In: "Environmental Chemistry of Arsenic" (W. T. Frankenberger, Jr., Ed.), Marcel Dekker, Inc., New York, pp. 247-272.

2. Bacterial mercury uptake and resistance - complex genotypes, simple phenotypes.

- N.L. Brown, Y.-C. Shih, C. Leang, J.R. Wilson and J.L. Hobman, School of Biosciences, The University of Birmingham, Edgbaston, Birmingham B15 2TT, U.K.

Mercury resistance in Gram-negative bacteria is encoded on non-chromosomal *mer* determinants. Resistance is due to reduction of Hg(II) to elemental mercury, Hg(0), by the cytoplasmic flavoenzyme, mercuric reductase, and requires transport of Hg(II) across the cytoplasmic membrane by an integral membrane protein. Transport has long been known to be rate-limiting for mercury reduction. We have shown that there are at least 3 genes, *merT, merC* and *merF*, encoding integral mercury uptake transporters, which occur in different combinations in different determinants.

The topologies of mercury transport proteins, MerT, MerC and MerF, were predicted from their amino acid sequences and confirmed by gene fusion experiments. Although MerT, MerC and MerF have different topologies in the membrane, with 3-, 4- and 2-transmembrane regions, respectively, mutagenesis studies will be described which indicate that they have similar mechanisms for transport of Hg(II) across the membrane. MerC and MerF appear to be later acquisitions in the evolution of *mer* determinants and their exact roles in addition to MerT are not yet clear.

3. Novel approaches to metal bioremediation and nanocatalysis

Dr. Lynn Macaskie, University of Birmingham, UK

Heavy metals are usually toxic and an environmental burden if released. Biotechnological approaches to metal remediation have traditionally concentrated on heavy metal cations but anionic forms of high-valence metals, such as Cr(VI) and Tc(VII), pose special environmental problems due to their ability to mimic sulphate, and also because of their high chemical- or radiotoxicity. The use of microbial reductase activities has been harnessed to the bioremediation of these metals via their reduction to lower valence species, which are insoluble. These reductions are mediated via hydrogenase activity, which can also be coupled to the reduction of soluble palladium (recovered from spent automobile catalysts), to form biomass-bound base metal. The recovered Pd^0 has a very high catalytic activity which can be used as a bioinorganic catalyst to treat other classes of waste which are recalcitrant to attack using microbial or chemical catalytic activity alone.

4. DETECTION SYSTEMS FOR GENETICALLY MODIFIED MICROORGANISMS.

Orla Sherlock, David Ryan, Clare Whelan and David Dowling.

Institute of Technology Carlow, Kilkenny Road, Carlow, Ireland.

A genetically modified microorganism *Pseudomonas fluorescens* F113rifpcb (F113PCB) was developed with improved ecological traits for in situ biodegradation of biphenyl and polychlorinated biphenyls. This strain contains a random insertion of the bph operon (coding PCB catabolism) that has been chromosomally integrated into F113PCB using a plasmid based delivery system (pDDPCB). A requirement for the use of this GMO for the degradation of organic contaminants in soil, is the provision of a safe, secure and rapid method for the detection and monitoring of this strain. While the strain could be enumerated on selective plates a non-culture based environmental detection method was desirable. A Real-Time PCR protocol for the specific detection and quantification of this GMO was developed using SYBR Green 1 chemistry and the Roche lightcycler. Detection is based on three characteristics of F113PCB.

PCR amplification of a DNA sequence named vector*/bph* junction region, has enabled detection of strains that have been manipulated with pDDPCB. This junction region is unique to strains constructed with the pDDPCB *bph* operon delivery system, including F113PCB.

The insertion site of the *bph* cassette in the F113 chromosome has been sequenced and bioinformatic data generated. PCR amplification of the terminal end of the *bph* operon, a putative gene *bphO*, and the F113 chromosome insertion site enables the unique detection of this GM strain.

Quantification of this GM in the environment is based on amplification of the *bphC* gene using Real-Time PCR technology. The entire detection process can be accomplished in less than 90 minutes, is strain specific and can quantify from 10^7-10^2 cells per gram of soil.

5. USE OF 16SrDNA SEQUENCING AND T-RFLP ANALYSIS TO MONITOR THE MICROBIAL COMMUNITY STRUCTURE AND DYNAMICS OF LOW TEMPERATURE ANAEROBIC DIGESTERS.

Gavin Collins, Micheal Carton and Vincent O'Flaherty
Microbial Ecology Laboratory, Department of Microbiology, National University of Ireland, Galway, Ireland

Psychrophilic (<20°C) wastewater treatment technologies offer an attractive potential alternative to established mesophilic (20-45°C) systems, as maintenance of the latter incurs considerable financial costs. Despite the economic importance and widespread harnessing of anaerobic digestion in engineered systems, and the development of *de novo* reactor types, little knowledge has been acquired with regard to the structure and function of the microbial populations within the process. Recently, however, nucleic acid based approaches have proven useful to describe the microbial ecology of such communities. In the current study, an anaerobic granular sludge, obtained from a full-scale digester, was characterised with respect to bacterial diversity. A total DNA recovery procedure and PCR protocols for the amplification of 16SrDNA using archaea and eubacteria-specific oligonucleotide primers were initially optimised. *E. coli* cells, cloned with PCR products, were subsequently used in the identification of community fingerprints by Amplified rDNA Restriction Analysis (ARDRA). Operational Taxonomic Units (OTUs) were constructed, sequencing of the inserts from clones representing the OTUs was conducted and a phylogenetic tree prepared. In addition to this, flourescently tagged PCR products were generated for use in terminal restriction fragment length polymorphism (T-RFLP) analysis. T-RFLP is a novel and high throughput technology, providing rapid analysis of microbial population dynamics. Two EGSB bioreactors were seeded with the sludge and used to treat a high strength (10 g COD l^{-1}) industrial wastewater at low temperatures. This paper details successful reactor operation, with methane yields and COD removal efficiencies of 60-75 % and 65-85 %, respectively being recorded. DNA was isolated from sludge samples during the test period and shifts in population structure, in response to environmental conditions, elucidated. The study also confirms the efficacy of T-RFLP as a useful tool for molecular biomonitoring of engineered ecosystems.

6. ANAEROBIC TREATMENT OF PHENOL AND RELATED COMPOUNDS.

Aoife Thornton and Emer Colleran
Environmental Microbiology Research Unit,
Department of Microbiology,
National University of Ireland, Galway.

Anaerobic digestion has traditionally been confined to the treatment of agricultural and food-processing wastewaters. Industrial and pharmaceutical companies have conventionally relied on processes such as aerobic digestion to treat recalcitrant xenobiotic aromatic compounds due to fears of toxicity towards anaerobic microbes. However, it has been demonstrated that anaerobic digestion may be successfully applied to the amelioration of toxic wastewaters provided adequate provision is made for bacterial acclimation. Although many industrial chemicals are amenable to anaerobic metabolism, wastewaters usually consist of complex intermediates that may hinder effective treatment. The degradation of industrial organics following exposure of sludge to a related compound has obvious advantages due to the batch nature of industrial processes. Development of a microbial population capable of cross-acclimation is vital to the success of a facility treating problematic wastewaters. The toxic aromatic phenol, commonly found in resin wastewaters, has been successfully treated anaerobically at fullscale. The purpose of this study was to assess the ability of a mesophilic (37°C) Upflow Anaerobic Sludge Blanket (UASB) reactor, treating a phenolic wastewater, to degrade related aromatic compounds. A 5L UASB reactor was seeded with phenol-unacclimated sludge and started up at a hydraulic retention time of 2 days on an artificial wastewater with a total chemical oxygen demand (COD) of 12 g l^{-1} consisting of phenol (0.5 g l^{-1} COD), ethanol, propionic acid and butyric acid (1:1:1 COD ratio). After 24 days, the COD removal efficiency was >95%. Subsequent increases in influent phenol (up to 7.15 g l^{-1} COD) did not result in any significant increase in effluent COD, phenol or VFA concentrations. Biodegradability tests indicated that the phenol-acclimated sludge degraded phenol, p-hydroxybenzoate and p-hydroxyphenylacetate but was unable to degrade p-cresol and catechol.

7. PHA PRODUCTION BY PSEUDOMONAS PUTIDA CA-3

Patrick G. Ward, Guy De Roo and Kevin E. O'Connor
Department of Industrial Microbiology, University College Dublin, Ireland. and Institute of Biotechnology, ETH-Honggerberg, CH8093 Zurich,. Switzerland.

Polyhydroxyalkanoates (PHA) are polyesters produced by bacteria as intracellular storage materials in response to a variety of nutritional and environmental conditions. PHAs offer a biodegradable alternative to the existing environmentally unfriendly petrochemical-based plastics currently in use.

The monomer composition of PHA determines the physical and chemical properties of the plastic. Properties such as stiffness, brittleness, melting point and resistance to organic solvents can change dramatically as a result of monomer composition, and thus the basis for bioplastic diversity lies in the variation of monomer composition. Monomer composition in turn, varies according to the carbon and energy source fed to the microorganism.

While many substrates have been used to produce PHA in *Pseudomonas* species, to date only 3 strains have been shown to accumulate PHA from aromatic carbon sources.

We have shown the formation of aromatic monomers in PHA produced by *P. putida* CA-3 grown on novel aromatic substrates such as phenylvaleric acid. We have also shown PHA formation from a range of aromatic acids. These abilities demonstrate the potential of *P. putida* CA-3 for novel bioplastic production.

In addition *P. putida* CA-3 is capable of producing PHA from a range of aliphatic fatty acids. We have investigated the relationship between aromatic and aliphatic carbon sources and the monomer composition of PHA in *P. putida* CA-3.

8. METAL-SPECIFICITY IN TRANSCRIPTIONAL REGULATORS, TRANSPORTERS AND METALLOCHAPERONES

ROBINSON, Nigel, TOTTEY, Stephen, BORRELLY, Gilles, RONDET, Sabine, MENG, Wenmao, CAVET, Jennifer

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Cells must acquire and distribute a sufficient number of atoms of metals that are needed to where they are needed, while sequestering expelling or excluding those that are not. This requires multiple sensors, multiple transporters and possibly multiple metallochaperones with differing metal specificities.

The first part of this talk will introduce a family of related metal-transporters (PacS, CtaA, ZiaA, CoaT) that select different metal ions from a common cytosol. The recent characterisation of a new bacterial copper metallochaperone (bacterial Atx1) that interacts with amino-terminal domains of two of these transporters will be then described. Finally, a new member with a novel metal specificity (NmtR) of a family (that includes SmtB, ZiaR) of DNA-binding metal-sensors will be reported along with experiments identifying factors that determine which inducers alleviate transcriptional repression by these proteins.

Tottey, S., Rondet, S.A.M., Borrelly, G.P.M., Rich, P.R., Robinson, N.J. (2002) A copper metallochaperone for photosynthesis and respiration reveals metal specific targets, interaction with an importer and alternative sites for copper acquisition *Journal of Biological Chemistry* (in press).

Tottey, S., Rich, P.R., Rondet, S.A.M., Robinson, N.J. (2001) Two Menkes-type ATPases supply copper for photosynthesis in *Synechocystis* PCC 6803. *Journal of Biological Chemistry* 276: 19999-20004.

Rutherford, J.C., Cavet, J.S., Robinson, N.J. (1999) Cobalt-dependent transcriptional switching by a dual-effector MerR-like protein regulates a cobalt-exporting variant CPx-type ATPase. *Journal of Biological Chemistry* **274**: 25827-25832.

Thelwell, C., Robinson, N.J., Turner-Cavet, J.S. (1998) An SmtB-like repressor from *Synechocystis* PCC 6803 regulates a zinc exporter. *Proceedings of the National Academy of Sciences U.SA*. **95**: 10728-10733.

9. The multiple mechanisms of repression of the Fur protein of *Escherichia coli*

Lucía Escolar[1], Giovanni Bertoni[2], Víctor de Lorenzo* *Department of Microbial Biotechnology, Centro Nacional de Biotecnología CSIC, 28049 Madrid, Spain.* [1]Institut für Genetik, Biozentrum, 06120 Halle (Saale), Germany. [2] Dip. di Genetica e Biologia dei Microorganismi, Universitá degli Studi di Milano, 20133 Milano, Italy.

Fur is a DNA binding protein which interacts reversibly with its target sequences depending on the presence of intracellular Fe^{2+}. The cation binds directly to the Fur protein which, in turn, acquires a configuration capable of binding DNA. Since most known Fur binding sites overlap the –10/-35 hexamers of cognate promoters, this simple scheme allows the metallo-regulation of a large number of genes through a classic transcriptional repression mechanism Apart from the Fur protein itself, its target sites are also very conserved in many bacteria. The so called *Fur box* consist of a 19 bp inverted repeat (5' GATAATGATAATCATTATC 3'), an addition assembly of three hexameric repeats of the sequence $^{G}/_{C}AT^{A}/_{T}AT$. It has been know for a number of years that this 19 bp minimal *Fur box*, placed overlapping or downstream of a heterologous promoter, ensures that its transcriptional activity is regulated by iron. In this seemingly clear context, it comes as a surprise that some genes are regulated positively, rather than negatively, by Fur-Fe^{2+}. Since transcriptional regulators with double functions as activators or repressors are not uncommon, we sought to ascertain whether this was the case of the Fur protein. In some studied cases, it is the position of the *operator box* with respect to the promoter that defines the role of such regulators as activators or repressors. It could therefore be possible that the binding of Fur to target sites upstream of the promoter, instead of overlapping the sequence motifs at -10 or –35, and in positions similar as those occupied by activators, promotes transcription. Should Fur have such activation capacity, then an optimal distance and relative geometry for the regulator and polymerase ought to give a positive outcome *in vitro* and *in vivo.* To this end, a synthetic promoter construct was manufactured in which Fur binding sites could be conveniently located at various distances and in different helical phases with respect to a weak promoter. Work *in vitro* and *in vivo* will be presented which provides *in vitro* and *in vivo* evidence that the consensus sequence for the Fur repressor of *Escherichia coli* can increase *per se* transcription initiation when located upstream of suboptimal –10/-35 hexamers within a specific length and phase of the DNA helix. This was shown by monitoring the effect of fusing a Fur Box

(5'GATAATGATAAT CATTATC3') at various 5' distances from the tandem *P1/P2* promoter *Pr* of the TOL plasmid. With the activity of the *P2* downstream promoter as an internal control, we detected a positive effect of the Fur operator sequence on *P1* when the full operator was placed at the –45 upstream position. On the contrary, the presence of Fur-Mn^{2+} specifically downregulated the *P1* promoter of the same construct. The reflection *in vivo* of this phenomenon, which is in part reminiscent of that caused by UP sequences in many promoters, was demonstrated by following the induction of a *Pr-lacZ* fusion added with or without an upstream Fur operator. These results support the notion that Fur operators can act as somewhat distant repressor sites. We have made a survey in the genome of *E. coli* which indicated that such an arrangement of functional sites is not unusual and has a functional significance.

Escolar, L., J. Pérez-Martín and V. de Lorenzo (2000) Evidence of an unusually long operator for the Fur (ferric uptake) repressor in the aerobactin promoter of *Escherichia coli*. J. Biol. Chem. 275: 24709-24714.

Escolar, L., Pérez-Martín, J. and de Lorenzo, V. (1999) Opening the iron box: transcriptional metalloregulation by the Fur protein. J. Bacteriol. 181: 6223-6229.

Escolar, L., Pérez-Martín, J. and de Lorenzo, V. (1998) Binding of the Fur (ferric uptake regulator) repressor of *Escherichia coli* to arrays of the GATAAT sequence. J. Mol. Biol. 283: 537-547.

10. DOES *RHIZOBIUM* ADOPT THE STRATEGIES OF PATHOGENS FOR ACQUIRING IRON?

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Bacteria known collectively as "rhizobia" induce N_2-fixing nodules on the roots of their host legumes. In addition to engaging in this complex interaction, these bacteria have the wherewithal to prosper in the oligotrophic conditions of the soil. Recent studies have shown that *Rhizobium leguminosarum* has remarkable flexibility in its ability to import the important, but sparingly soluble metal, iron. In addition to the synthesis and uptake of siderophores, these bacteria also use haem as sole Fe source and possess at least three sets of *fbp*-like genes that may be involved in importing Fe^{3+}, independently of siderophores. Interestingly, haem uptake and the possession of these Fbp-like transporters have, hitherto, been found only in bacterial pathogens. Genomic sequences of two other rhizobia, *Sinorhizobium* and *Mesorhizobium* (nodulating Alfalfa and *Lotus* respectively) , indicate that these species, too, have multiple pathways of Fe import.

We identified the *vbs* genes involved in the synthesis of the siderophore Vicibactin, which is characteristic of *R. leguminosarum* and propose a novel biosynthetic pathway for the synthesis of this "hybrid" molecule that resembles both polyketides and non-ribosomal proteins. We also identified three separate *fbp*-like operons and one operon for the uptake of haem in this species.

Not surprisingly, there is tight regulation of the genes involved in Fe uptake, their expression being greatly enhanced in Fe-deficient growth media. Although *R. leguminosarum* contains a homologue of the *fur* transcriptional regulatory gene, Fur^- mutants are not affected in transcription of any of the Fe-responsive genes identified so far. (An unexpected bonus, though, is that Fur protein of *R. leguminosarum* could be crystallized, allowing a structure of a member of this wide-ranging family of transcriptional regulators to be determined).
However, there does appear to be at least one "global regulator" in *R. leguminosarum* Mutations in a gene termed *girA* result in high level, constitutive expression of <u>*all*</u> the known structural genes involved in Fe uptake. The mechanisms involved in this GirA-mediated, Fe-dependent gene regulation remain to be established.

11. OPTIMIZATION OF A CHEMOSTAT-BASED SYSTEM FOR PRODUCTION AND CHARACTERIZATION OF BIOAEROSOLS.

Kevina O'Donoghue, Gerard Fleming, Anthony P. Moran, Gerard Jennings,, and Cyril Carroll.
Department of Microbiology, NUI, Galway.

Aerosols are colloidal suspensions of liquid droplets or solid particles in air. Bioaerosols are aerosols of biotic nature and may contain viruses, bacteria, fungi, or parts or products of organisms. Bioaerosols are ubiquitous and can constitute an important route of bacterial transmission. They are found in the workplace, medical facilities, residential housing, manufacturing processing plants, animal houses, sanitary landfills and sewage plants and are known to cause various health effects including infectious disease, hypersensitivity, toxic reactions, irritations and inflammatory responses.

The aim of this study was to optimize a system for the production and characterization of bioaerosols. Firstly, a novel chemostat system was designed for this purpose. This system allowed the cells to grow in a constant environment, e.g., nutrient concentration, temperature and aeration. Also, the system was optimized for different media (Nutrient broth, Minimal salts) and bacteria (*Escherischia coli, Staphylococcus aureus, Pseudomonas aeruginosa*). Secondly, the physical properties of the chemostat system influencing the production of Film and Jet drops, was investigated in culture-free media and with planktonic culture-based cells. Total particle counts and particle sizes were obtained at different heights above the liquid surface and across various lengths of capture tubing to determine the optimal conditions for aerosol sampling.

The results showed that such a chemostat system could be optimized for aerosol generation. It was shown that capture tube length rather than capture height was more critical for aerosol characterization, and aerosolized bacterial suspensions increased particle counts. The results were robust and reproducible, illustrating a defined system, and will be applied in further bioaerosol investigations, including, the formation of biofilms from aerosolized bacteria on varied biomaterials (glass, silicone, and polyutherane).

12. STRAIN TYPING OF *MYCOBACTERIUM BOVIS*

Louise Bailey [1,2,3], Orla Flynn [4], Eamon Costello [4], Don O' Grady [4] Rory O'Brien [1,2,3] and Dr. Mark Rogers [1,2,3,]

Conway Institute for Biomolecular Science [1] and Department of Zoology, 2 University College Dublin, Belfield, Dublin 4, and Tuberculosis Investigation Unit, Faculty of Veterinary Medicine, University College Dublin, Ballsbridge, [3] Dublin 4, and Central Veterinary Research Laboratory, Abbotstown, Dublin 15, [4] Ireland.

Abstract

A novel DNA probe (pUCD) for the strain typing of *Mycobacterium bovis* (*M. bovis*) was characterized. Four tandem repeats (pUCD 1, pUCD 2, pUCD 3, pUCD 4) were derived from the full-length pUCD probe. Restriction Fragment Length Polymorphism (RFLP) analysis was carried out on 21 *M. bovis* isolates using pUCD 1, 2, 3 and 4 as oligonucleotide probes. A 36bp probe found in the Direct Repeat (DR) region of the *Mycobacteria* tuberculosis genome was combined with the oligonucleotides in a mixed hybridization. A Variable Number Tandem Repeat (VNTR) typing system was developed using the pUCD repeats as individual PCR targets. A comparison was made of twenty isolates typed by VNTR-PCR and by the pUCD oligonucleotide probes. It was found that the VNTR-PCR typing system compared favourably with pUCD in terms of its ability to discriminate between isolates. The VNTR-PCR typing system was found to be a more desirable typing system because typing could be completed in a day as opposed to three days with RFLP. This study was extended to ascertain whether the polymorphic properties of pUCD were due to mutations in *Alu I* restriction sites or changes in the number of tandem repeats. The initial study of 21 *M. bovis* isolates was extended to 100 *M. bovis* isolates chosen at random from various sources.

13. ENVIONMENTAL INFLUENCES ON ORGANOTIN-YEAST INTERACTIONS

Jane S. White and John M. Tobin
School of biotechnology, Dublin City University, Dublin 9, Ireland.

Solution pH and ionic composition influence the chemical speciation and toxicity of organotins in the aquatic environment. Organotin compounds undergo pH-dependent hydrolysis when introduced into water, with the formation of hydrated, cationic species and neutral hydroxides. In the presence of NaCl, formation of chloride species occurs. As organotins are found in both freshwater and marine ecosystems, the influence of pH and salinity on uptake and toxicity of organotins by microorganisms is key in assessing the effects of organotin pollution.

Uptake by microorganisms has largely been overlooked when considering the fate and effect of organotins. It is important to understand such interactions because microorganisms are at the base of the food web and mediate a number of important environmental processes, including degradation of many toxic compounds. Despite the fact that accumulation is a prerequisite for any subsequent toxic effects, few studies have focused on the uptake of organotins. The uptake and toxicity of the triorganotins, tributyltin (TBT) and triphenyltin (TPT) by *Candida maltosa* was investigated between pH 3.5 and 7.5 and in concentrations of up to 500 mM NaCl. A theoretical model was used to predict the speciation and overall octanol-water distribution ratios (D_{ow}). TBT and TPT toxicity was correlated with D_{ow} values, corresponding to increasing pH and NaCl concentration and implicating compound lipophilicity as a toxicity determinant. As interactions with *C. maltosa* were dependent on the organotin species present in solution, variation in TBT and TPT toxicity in different ecosystems would be expected.

Poster Presentations

Poster 1

CHARACTERISATION OF INTERACTIONS BETWEEN THE TRANSCRIPTIONAL REPRESSOR PHLF AND ITS BINDING SITE AT THE *PHLA* PROMOTER IN *PSEUDOMONAS FLUORESCENS* F113

Abdelhamid Abbas, Pilar Carnicero Marquez, Michel Sheehan, John Morrissey, Isabel Delany and Fergal O'Gara.
Biomerit Research centre, Department of Microbiology, National University of Ireland, Cork, Ireland

Abstract
The *phlACBD* genes responsible for the biosynthesis of the antifungal metabolite 2,4-diacetylphloroglucinol (PHL) in the biocontrol *strain Pseudomonas fluorescens* F113 are regulated at the transcriptional level by the pathway specific repressor, PhlF. Strong evidence suggest that this regulation occurs mainly in early logarithmic phase of growth. First, the *phlF* gene is relatively highly expressed between 3 and 13 hours of growth and relatively low thereafter with the *phlA* gene following an opposite expression profile; second, the kinetics of PHL biosynthesis are specifically altered at this stage of growth in an F113-*phlF*$^-$ mutant (Delany *et al*, 2000). The *phlA*-*phlF* intergenic region presents a complex organisation in that *phlA* is transcribed from a ^{70} RNA polymerase dependant promoter that is likely to overlap that of the divergently transcribed *phlF* gene. The repression by PhlF is due to its interaction with a specific sequence, *phO,* located downstream of the *phlA* promoter. This strongly suggests that the transcriptional repression of *phlA* occurs by inhibition of promoter clearance. We have mapped the *phO* site and find that PhlF binds as a dimer to an inverted repeat sequence. Furthermore, we find that certain regulators of PHL synthesis act via modulation of PhlF binding to *phO*. PHL, which has been demonstrated to be an autoinducer of PHL biosynthesis (Schnider-Keel *et al*. 2000), interacts with PhlF to destabilise the PhlF-*phO* complex. Conversely, the PhlF-*phO* complex is further stabilised by the presence of salicylate, which has been shown to be a strong inhibitor of *phlA* expression.

Poster 2

Characterisation of Adhesion Properties of *Ralstonia pickettii*

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Ralstonia pickettii is an environmental organism with a worldwide distribution. It has been isolated from water, soil and plants, both fruits and vegetables. Members of this genus are recognised as phytopathogens. They are not considered to be of major clinical importance, however they have been implicated in nosocomial infections and to be associated with permanent indwelling devices.

The *Ralstonia pickettii* under study in our laboratory has been isolated from water tubing and clinical cystic fibrosis patients.
Bacterial cell surface hydrophobicity is one of the most important factors that influence bacterial adhesion. The hydrophobicity of isolates were measured by their ability to adhere to p-xylene has been carried out. *In vitro* adhesion studies in microtitre plates and measured quantitatively by Elisa reader has been performed.

Initial antimicrobial susceptibility testing showed multiresistant patterns and susceptible to Sulphamethoxazole/trimethoprim (Sxt-23.75/1.25 μg/ml). Resistance to the Cepham Cefotaxime (Ctx -30 μg/ml) was observed, as was resistance to the fluoroquinolone Ciprofloxacine (Cip-5 μg/ml). This is cause for concern as the fluoroquinoles are used as a first line of defence against this organism, as are Cefotaxine and the aminoglycoside Gentamicin. The contribution to fitness/adhesion by antibiotic resistance determinants is being compared.

Poster 3

COMPARATIVE ANALYSIS OF GRAM-POSITIVE AND GRAM-NEGATIVE PLANKTONIC AND BIOFILM BACTERIA.

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Biofilm growth is well recognised as being dominant for bacteria growing within many diverse natural habitats. Surface molecules of bacterial cells are important for the formation of biofilms and are involved in initial attachment to the surface and to each other. Thereby they provide an anchor by which the biofilm is attached. The aim of this study was to set up and optimise a system for the growth of Gram-positive and Gram-negative biofilms and to analyse their surface molecules, enzymes and adherence properties.

A chemostat, allowing defined growth conditions, was coupled to a flow-through system in which glass and polyurethane-coated glass surfaces were used for biofilm development. Sampling and removal of biofilm material were also optimised.

Biofilm formation by *Staphylococcus aureus* NCIB 9518 and *S. aureus* 4118 (clinical isolate) as well as *Escherichia coli* ATCC 25922 and *E. coli* 4119 (clinical isolate) were examined. Electrophoretic analyses of total cellular proteins and lipopolysaccharide (LPS) were performed, and enzyme production assayed in biofilm and planktonic forms. Also, adherence of planktonic forms to extracellular matrix (ECM) proteins was performed.

For *S. aureus* NCIB 9518, three high (95.2, 92.5 and 87.1 kDa) and one low-molecular weight (29.9 kDa) proteins were present in the planktonic but not in the biofilm form. *E. coli* clinical isolate, but not the culture collection strain, had a different core LPS structure. Moreover, a greater variety of enzymes were expressed in biofilm than in planktonic *E. coli*. Furthermore, bacterial strains exhibited differences in ability to adhere to ECM protein-coated surfaces.

This study shows differences in antigen expression, adherence, and enzyme production as a result of biofilm growth on a surface.

Poster 4

Analyses of the metabolic capabilities of *Rhodococcus erythropolis*, enabled due to the strains novel enzymatic pathways.

By John Doran, Orla Cahill & Dr. Catherine O'Reilly,

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Nitrile degradation can occur by two distinct pathways. The one step nitrilase (EC 3.5.1.4)[1] system, where with the addition of 2 moles of water the nitrile moiety can be transformed directly to the corresponding carboxylic acid, with the production of ammonia. The two step Nitrile Hydratase (EC 4.2.1.84)[1] (NHase)/amidase (EC 3.5.1.4) system also adds two mole of water to the nitrile. The NHase adds one mole of water to transform the nitrile group to an amide group. In the second step the amide group has an additional mole of water added to it by the second enzyme amidase resulting in the corresponding carboxylic acid and ammonia. NHase the first enzyme in the transformation is a soluble metalloenzyme, which contains either a non-Heme iron or a non-corrinoid cobalt atom. To see what level of nitrile metabolism could occur if the organism was deprived either iron or cobalt and whether either of the two atoms could be substituted with one another (it has been demonstrated that cobalt can be substituted for iron in Fe-type NHase expressed in *Escherichia coli*).

To investigate this the culture *Rhodococcus erythropolis* was grown up in a minimal media supplemented with either iron or cobalt, the nitrile (butyronitrile) was supplied as the sole source of carbon and nitrogen. The rates of growth of the cultures on the different medias were compared, and samples taken so that the activity of the nitrile metabolising system could be assayed by monitoring ammonia production one of the final products. The initial results indicated that substantially more growth occurred when the culture was incubated in a media containing iron and no cobalt, when compared to the culture growing on a media containing cobalt and no iron, suggesting the presence of the Fe-type Nitrile Hydratase.

To further analyse the growth diversity of the strain a range of nitrile/amide substrates were utilised. Growth assays were carried out supplementing each respective substrate, at different concentrations into the culture media. Growth was monitored and initial results concluded that the substrate specificity of the strain was much broader than other Rhodococcal strains. Subsequently, from these results, substrates that indicated an elevated growth pattern were chosen to assess the enzymatic activity. Results suggested that in these optimum substrates the enzyme was expressed at a very high level e.g. 12.4 μmol NH_3 /min @ 30°C in the presence of Valeronitrile.

Poster 5

IDENTIFICATION AND MOLECULAR ANALYSIS OF NOVEL AHL SYNTHASES IN *Pseudomonas fluorescens*

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Many bacterial species use chemicals to signal each other and co-ordinate their activities. N-acyl homoserine lactone (AHL) mediated quorum sensing is one type of cell-cell signalling found in Gram negative bacteria. This method of signalling co-ordinates the production of a plethora of virulence factors and secondary metabolites in many bacteria. Our research encompasses AHL-mediated quorum sensing in both *Pseudomonas aeruginosa* PAO1 and *Pseudomonas fluorescens* F113.

Many root-colonising fluorescent Pseudomonads, including *P. fluorescens* F113, can control diseases caused by soil-borne pathogens and so there is interest in exploiting their potential as crop-protectants. The biocontrol ability of *P. fluorescens* F113 is dependant on the production of the secondary metabolite 2,4-diacetlyphloroglucinol (Phl). Phl is produced in stationary phase cultures and may be under quorum sensing control. We are investigating the importance of quorum sensing gene regulation for production of secondary metabolites and other phenotypes in *P. fluorescens* F113.

P. fluorescens F113 has been shown to produce a number of AHL molecules. Three of these have been structurally characterised and identified as N-hexanoyl homoserine lactone (C6-HSL), N-decanoyl homoserine lactone (C10-HSL) and N-(3-hydroxy-7-cis-tetradecenoyl)homoserine lactone (3OH,C14:1-HSL). A further two have been provisionally identified, namely N-octanoyl homoserine lactone(C8-HSL) and N-oxo-hexanoyl homoserine lactone (3O,C6-HSL). Production of these AHL molecules is regulated by environmental signals. Two putative AHL synthase genes have been cloned and sequenced, termed *hdtS* and *pasA*. Both have been demonstrated to produce AHLs in *Escherichia coli.* Neither *pasA* nor *hdtS* share identity to any known AHL synthase families and therefore may represent novel families of AHL synthase genes. Interestingly, both genes have close homologues in other Pseudomonads including *P. aeruginosa.* Molecular analysis of these genes will shed light on their importance of AHL molecules and their cognate biosynthetic genes in both *P. fluorescens* and *P. aeruginosa.*

Poster 6

MICROTITRE-WELL GROWN *Staphylococcus epidermidis* BIOFILMS: QUANTIFICATION AND THE POTENTIAL FOR RAPID ANTIMICROBIAL SUSCEPTIBILITY TESTING.

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Bacterial biofilms on implanted medical devices, in particular on central venous catheters, pose a serious problem for modern healthcare. Treatment of catheter related infection based on conventional antimicrobial susceptibility testing is often unsuccessful if the catheter remains *in situ* due to the resistance of biofilms to antimicrobials.
We sought to establish a microtitre-tray based model of biofilm growth on round-bottomed and flat-bottomed microtitre-tray wells. Wells were inoculated with *Staphylococcus epidermidis* ATCC 35984 in Mueller Hinton broth (MHB). Following incubation for 24 or 48 hours the broth was removed, trays washed in sterile saline and wells filled with a 2% solution of crystal violet (CV) for 5 minutes. This solution was then removed and CV trapped in the biofilm eluted by adding 33% glacial acetic acid. Quantification of eluted CV, by spectrophotometry, was used as an index of the intensity of biofilm formation which was further confirmed using scanning electron microscopy, and fluorescent microscopy. Viable cell counts of the biofilms in microtitre-wells was attempted by sonication of the wells in saline followed by decimal dilution and plating of the resulting suspensions. Biofilms were exposed to concentrations of vancomycin, up to 1024 μg/ml, and linezolid, up to 256 μg/ml, for 24 hours after which the antimicrobial solution was removed and the wells washed with sterile saline. This was followed by the addition of 200 μl of MHB and the re-incubation of the plates for 24 hours. Re-growth of *S. epidermidis* indicated that viable cells had persisted in the biofilm.
The results suggested that flat-bottomed microtitre-wells produced a greater density of biofilm than round-bottomed wells. Sonication was unsatisfactory for biofilm detachment and supplementation with mechanical detachment, i.e. scraping of the wells, was required. Viable cell counts revealed the presence of, on average, 10^6 Colony Forming Units per well. Neither vancomycin (1024 g/ml) nor linezolid (256 g/ml) nor the combination of both agents had any measurable effect on the viability of the biofilm.
This work established a simple model of *S. epidermidis* biofilm formation, however, the relevance of this model to clinical infection requires further study.

Poster 7

ISOLATION AND MOLECULAR TYPING OF *CAMPYLOBACTER* SPECIES: A BROILER FARM STUDY.

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Following the discovery at the beginning of the century, Campylobacter have emerged as the most common cause of bacterial enteritis in industrialized countries. Campylobacter enteritis is considered to be a food-borne disease, the most common source of infection being poultry. Campylobacter form part of the normal intestinal tract of these birds and during processing exposure of this pathogen to other parts of the carcass may occur resulting in contamination of retail products.

The major importance of monitoring *Campylobacter* at the farm level lies in the ongoing debate as to whether vertical or horizontal transmission occurs, and the presumption that all chicks are *Campylobacter* free when hatched. However, normally between 22 and 29 days of age all chickens tend to be colonised. Therefore the route of transmission must be investigated.

A number of molecular typing methods can be used to classify bacteria based on genomic diversity into groups of closely related isolates, presumed to arrive from a common ancestor. Such methods are mainly used in epidemiology studies.

This study involves the collection of samples on a routine basis from broiler farms. Samples include claocal swabs, litter, dust, feed, drinking water and environmental samples such as puddle water, mud etc. from outside the broiler house. These samples are then analysed for the presence or absence of *Campylobacter.*

To date, over 100 isolates obtained from five different broiler houses have been studied. These isolates have been subject to PFGE which, is a DNA based typing method used to study the epidemiology of bacteria and also Flagellin gene typing by restriction fragment length polymorphism The banding patterns obtained using both methods illustrate the dominance of certain strains in these broiler houses.

Poster 8

Rhizoremediation of Heavy Metals

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Metal contaminated land is increasingly an important environmental, health, economic and planning issue in the world. Toxic pollutants such as heavy metals are persistent in the soil environment and the results of current remediation technologies are highly variable, and costly. Rhizoremediation is a relatively new approach to the problems of contamination of soil, exploiting the genetic and the physiological properties of the bacteria living in the plant rhizosphere to degrade organic contaminants and possibly protect plants from heavy metals and other toxins.

In this study, a rhizosphere bacterium, *Rhizo3*, was isolated from Glyceria fluitans in a metal contaminated site in Glendalough, Co.Wicklow.
Rhizo3 was identified by 16sRNA gene sequencing a Pseudomonas strain and was tested for its resistance to numerous metals, antibiotics as well as its ability to colonise the rhizosphere of a model plant: *Medicago sativa.*
A PCR based detection system was developed to monitor this strain in the environment and is currently being evaluated as a possible detection system for this organism. A fragment of *Rhizo3* chromosomal DNA (P6) was blasted against the databanks for comparison analysis. Primers were designed to amplify a region of the sequence which is thought to encode for a transmembrane protein called menaquinone, involved in bacterial electron transfer.

A Southern blot was carried out using both genomic DNA from *Rhizo3* and colony lifts of a collection of bacteria from different contaminated sites. Hybridisation is currently being tested to confirm the origin of P6 and the possibility of using this sequence as a probe for this organism in the environment.

Poster 9

The detection and study of the *bphK* gene involved in the biodegradation of PCBs

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Burkholderia sp. strain LB400 is one of the most potent aerobic polychlorobiphenyl (PCB) degrading microorganisms that has been characterised to date. The PCB degrading activity originates either predominantly or exclusively from the enzymes encoded in the *bph* gene cluster. One of these genes, *bphK* encodes a protein with significant sequence similarity to both prokaryotic and eukaroyotic glutathione S-transferases (GST). This gene was found at a central location within the bph gene cluster. However the exact function of this GST has not been determined but it has been suggested that this GST maybe involved in dehalogenation of PCB degradative intermediates.

In order to study this gene it has been cloned independentlyof the bph operon into *E. coli* JM109. This poster describes describes both an *in vivo* and *in vitro* detection method for this enzyme. The *in vivo* method has been used to successfully distinguish active and non active GST mutants in *E. coli*. Bioinformatics tools such as BLAST, PROSITE, PFAM and Swiss- Model have helped to identify domains of interest and can give clues to its function.

Acknowledgement: This work was funded in part by the HEA cycle I programme and EU contracts QLK3-CT2000-00164 and QLRT-2001-00101.

Poster 10

Analysis of the nitrilase activity of cyanide hydratase from *F.lateritium* and *F. solani*.

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Cyanide (CN-) is one of the most toxic poisons known. Cyanide detoxification occurs when the enzyme, cyanide hydratase, converts cyanide to the less toxic compound formamide by hydration. Certain filamentous fungi, namely *Fusarium lateritium* and *Fusarium solani* have the ability to produce this enzyme in nature. Due to it's bioremediation potential the enzyme was developed by ICI (now Astra Zeneca) as a product for the treatment of cyanide-containing waste. The cyanide hydratase gene itself has since been cloned and expressed at high levels in E. coli, under the control of the LAC operon where expression levels are five times higher than those in it's native *F. lateritium.*

The DNA sequence of the cyanide hydratase gene indicated that the predicted protein sequence , when compared to the NBRF databank, has strong homology (35% identity) to a group of enzymes known as nitrilases. Nitrilases are enzymes that directly convert nitriles to the corresponding acid and ammonia. This is unusual given the functional differences in the proteins.
To generate cyanide hydratase variants with increased substrate range for nitriles it was proposed to use site-directed mutagenesis within the conserved regions given the structural similarities between the enzymes. Such an enzyme would have great potential for herbicide detoxification as well as for treatment of industrial waste. Ammonia assays to detect any newfound nitrilase activity led to the determination that wild-type cyanide hydratase actually has nitrilase activity, also, a combination of three mutations introduced into the enzyme's DNA sequence leads to an increase in it's nitrilase activity.

Current research focuses on cyanide hydratase from both *F. lateritium* and *F. solani.* The work on the *F. lateritium* system has been broadened to include a wider range of nitriles assessing how they interact with the cyanide hydratase enzyme. The work on the *F.solani* system involves cyanide hydratase activity and how the enzyme itself acts on nitriles. Comparisons and differences between both systems, where relevant, have been made.

Poster 11

Isolation of Novel Cyanide and/or Metal Tolerant Microorganisms.

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Although it is highly toxic, cyanide occurs naturally in the environment, for example several plants including cassava, some bacteria and several species of invertebrate organisms produce natural cyanide. Large amounts are also produced by industry in the production of chemicals, synthetic fibres and in the wastes of coal gasification and coking, steel, electroplating and mining industries. Moreover, cyanide tends to react readily with many other chemical elements and molecules to form hundreds of different compounds, many of which are known to be toxic to aquatic organisms and persist in the environment for significant periods of time. An example of such is the formation of metal-cyano complexes of variable stability and toxicity.

As it is an acute poison, the release of cyanide and related compounds into the environment may be catastrophic. Therefore, cyanide containing wastes must be treated before release. At present many chemical treatment methods are available including the Alkaline-Chlorination-Oxidation Process, Electrolytic Decomposition and Ozonation, but these methods have their disadvantages. Aswell as many of these processes being very expensive and requiring special equipment, in many cases not all cyanide complexes are degraded. Biological detoxification methods for the treatment of cyanide bearing wastes offer many advantages over traditional chemical methods.
This project seeks to replace chemical methods with a more benign biological system; the aim being to isolate novel metal cyanide tolerant/degrading microorganisms. Although metal cyanide complexes constitute a quite significant fraction of cyanide related wastes their degradation by microorganisms has not generally been investigated.

Soil samples have been collected from a number of sites and microorganisms tolerant to cyanide and/or metals (such as copper, zinc, iron) are being selected on appropriate media. The level of tolerance will be assessed by standard methods. Cyanide tolerant microorganisms will be investigated for the nature of the cyanide breakdown and for the tolerance to metal complexed cyanide. In many cases resistance to metals is plasmid borne and the location of the resistance genes will be assessed by DNA isolation and gel electrophoresis. Further progress of the project will depend on the nature of the strains isolated.

Work on the cyanide tolerant soil fungus *Fusarium solani* is also being done. This involves looking at the induction of the cyanide hydratase enzyme by cyanide and metallocyanides, and to look at its ability to grow on and the biotransformation of metal-complexed (nickel, copper, zinc and iron) cyanides. This project has potential applications for the bioremediation of metal cyanides.

Poster 12

Soil Metabolic Fingerprinting

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It is estimated that only 1 – 5% of soil microbes have been classified taxonomically. This has been due to the lack of culturability of most soil bacteria. The development of Genetic engineering technologies, has led to the increased use of Genetically Modified Organisms (GMO). It is important that we develop a baseline for the diversity and activities of the natural microbial soil flora, so that the use of GMOs can be monitored for any impact they may have on the environment. Various methods have been developed to measure the diversity of the soil micro flora, including genetic fingerprinting such as Denaturing Gradient Gel Electrophoresis (DGGE), Randomly Amplified Polymorphism DNA (RAPD) and metabolic fingerprinting etc. This work investigates metabolic fingerprinting, using 96 well GN2 Biolog® plates. Different soils have different fingerprints, indicating that they harbour different microorganisms, and/or express different metabolic activities. Such data can be analysed by using Principal Components Analysis (PCA), which shows the pattern of relationships among the variables. The research site for this project is located at Teagasc, Oak Park, Carlow, and makes use of a long term experiment that is investigating the effects of high and low inputs on a sustainable crop production system. Initial analysis revealed seasonal variations, with respect to the metabolic profile, possibly due to the presence or absence of actively growing crops. Ultimately it is planned to establish a database of fingerprints, which will be used to observe differences in soil microbial flora, in response to environmental disturbances.

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Poster 13

Regulation of bioluminescence in *Photorhabdus luminescens* (Enterobacteriaceae*)*

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The nematode *Heterorhabditis* is pathogenic to a wide variety of insect larvae and the pathogenic effectiveness of this nematode is dependent on symbiotic bacteria, *Photorhabdus luminescens,* which are selectively packaged and retained by the infective juvenile (IJ) stage of the nematode. Following infection of an insect larva, the IJ releases *P. luminescens* into the insect haemolymph where the bacteria proliferate and, on reaching stationary phase, bioconvert the insect into a food source for the nematodes. A remarkable feature of the post-exponential life of *P. luminescens* is that these bacteria can bioluminesce. In this study we investigate the role and regulation of bioluminescence in the tripartite association between the bacteria, the nematode and the insect. Although light production *in P. luminescens* is associated with high-cell density we show that, unlike other bioluminescing bacteria, *P. luminescens* does not regulate bioluminescence by quorum sensing and does not appear to produce any homoserine lactone-like molecules. Moreover, bioluminescence is not regulated by nutrient limitation, as invoking the stringent response had no effect on light production. However, luminescence can be induced to high levels in exponentially growing cultures that are specifically limited for iron. Moreover, we show that membrane protein profiles from iron starved bacteria are similar to profiles from bacteria isolated from infected insects suggesting that iron availability may regulate the *in vivo* expression of bioluminescence in *P. luminescens*.

Poster 14

THE EFFECTS OF ORGANIC MATERIAL ON TRIBUTYLTIN RESISTANCE IN COASTAL BACTERIOPLANKTON FROM GALWAY BAY

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Abstract
Exposure of mixed populations of organisms, such as a coastal bacterioplankton community, to an inhibitor would be expected to favour the dominance of more resistant organisms in that population. Studies on sites with and without Tributyltin (TBT) contamination however have failed to establish a link between contamination levels and the resistance spectrum exhibited by the indigenous bacterial population. Measures of resistance have also been seen to vary according to the method used. It has been suggested that these effects could be due to the influence of organic matter. Large molecules may sequester organotins making them inaccessible to bacteria. In addition, metabolisable organic compounds, while increasing cell nutrition may also increase resistance. The objective was to investigate the effects of an inert (peat) and a bioactive (glucose and yeast extract mix) organic compound on TBT resistance in water column bacterial populations using microcosms containing seawater from Clarinbridge, Galway Bay. Tributyltin was added to the flasks to a concentration of 8.4 M. Enumeration of resistant bacteria was via plate counts on Marine Broth agar (MBA) and total counts using epifluorescent microscopy. Percentage resistance of these isolates was determined using replica plating on TBT containing MBA. The unculturable portion of the community was analyzed using nucleic acid-based techniques, including PCR and Denaturing Gradient Gel Electrophoresis (DGGE). The culturing results revealed that incubation with TBT selected for a higher percentage of TBT resistant culturable bacteria. The addition of inert and bioactive compounds had a positive effect on resistance to TBT in solid media. DGGE analysis revealed differences in community structure between the flasks over time.

Submitted for a poster presentation.

Poster 16

PSEUDOMONAS FLUORESCENS M114 ALKALINE PROTEASE, APRA, IS REGULATED TRANSCRIPTIONALLY BY THE IRON STARVATION ECF SIGMA FACTOR, PBRA AND POST-TRANSCRIPTIONALLY BY THE PRPA/PRRB REGULATORY SYSTEM.

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Extracellular proteolytic activity in the soil isolate *Pseudomonas fluorescens* M114 is negatively regulated by iron. In previous studies, an iron-starvation extracytoplasmic function (ECF) sigma factor, PbrA, required for the transcription of siderophore biosynthetic genes, was implicated in *P. fluorescens* M114 protease regulation. In addition to being siderophore negative, a *P. fluorescens* M114 (pbrA mutant was found to be protease negative on skimmed milk agar. An alkaline protease gene, *aprA*, was cloned from the *P. fluorescens* M114 genome using PCR with primers designed from the alkaline protease genes of other Pseudomonas species. Transcriptional *lacZ* fusions revealed that expression of aprA is reduced in the wild type strain under high iron conditions. *aprA* transcription is also reduced in *P. fluorescens* M114 (*pbrA* mutant. Thus, PbrA is required for full transcription of *aprA*.
Previous research undertaken in this and another laboratory suggested that protease activity in *P. fluorescens* species F113 and CHA0 was regulated post-transcriptionally by a regulatory system involving a mRNA binding repressor protein, PrpA (RsmA) and a regulatory RNA molecule PrrB, (RsmB). *prpA* and *prrB* genes from *P. fluorescens* M114 were cloned and sequenced. Analysis of the P. fluorescens M114 *aprA* promoter revealed a sequence previously implicated this post-transcriptional gene regulation *in P. fluorescens* CHA0. The possibility that *P. fluorescens* M114 *aprA* is also regulated by this system was investigated.

Poster 17

DEVELOPMENT OF A RAPID MOLECULAR METHOD TO CARRY OUT ANTIBIOTIC RESISTANCE PROFILE OF THE BACTERIAL POPULATIONS OF THE RIVER BARROW .

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Since their discovery there has been a dramatic increase in use of antibiotics in medicine, industry and agriculture, the residues of which ultimately end up in our rivers, streams, lakes and seas. The presence of these antibiotics in the environment has led to an increase in the occurrence of antibiotic resistant bacterial strains, and the need has arisen to monitor the levels of resistant strains.

The river Barrow is one of Irelands major navigable waterways and therefore is of economical importance to its local tourist industry for cruising, fishing, swimming and other recreational activities. This research project involved compiling an antibiotic resistance profile of target bacteria, both indigenous and introduced from the river Barrow between Maganey and Milford incorporating Carlow town. Samples were taken from four points along the river one up stream and two down stream of Carlow town. Bacterial resistances to a range of veterinary antibiotics were screened and high levels of single and multidrug resistances were found, including widespread resistance to kanamycin, spectinomycin and streptomycin. A complementary study was carried out in Mortarstown wastewater treatment plant to determine the plants input of antibiotic resistant bacteria to the river Barrow.

To complement this study, a range of PCR primers have been developed to detect antibiotic resistance genes from aquatic environmental samples. Protocols are being developed to carry out PCR on environmental samples with minimal pretreatment for both single and multiplex PCR. This will allow screening of large volumes of river water sample within a short space of time.

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Supported by HEA Programme for Research in Third Level Institutions Cycle 1

Poster 18

The Optimisation of Recombinant Cyanide Hydratase(s)

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Early work showed that the filamentous fungus, *Fusarium lateritium* had the ability to tolerate the toxic chemical cyanide. This was due to the ability of the fungus to produce an enzyme cyanide hydratase (formamide hydrolyase) under induction from cyanide. In the 1980s ICI Biological Products set out to produce the fungus with the enzyme present as a means of detoxifying cyanide. Mining processes and other metallurgic processes like metal plating readily produce cyanide-containing wastes. The enzyme has been successfully cloned into a number of microorganisms, namely, *Escherichia coli, Saccharomyces cerevisiae* and *Pichia pastoris.*

Growth of the recombinant *E. coli.* with a high expression of the cyanide hydratase enzyme was achieved. The expression has been measured at approximately eight times that of the native host. It was then possible to carry out enzymatic kinetic studies and to investigate the stability of the enzyme. The half-lives between the recombinant host and the native host have been compared. Biodegradation of cyanide gives many advantages over chemical methods most commonly used. Such advantages include higher treatment performance, low sludge production and metal removal and recovery to name just some.

In terms of biotechnological applications, however, the use of *Fusarium* and *E. coli* is not ideal. Production of the enzyme in *Fusarium* requires continual induction with cyanide, which has obvious disadvantages for large-scale production, as well as growing *F. lateritium* on a large scale being technically difficult. The use of *E. coli* as a host is also non-ideal, as many of the biotechnological applications of the enzyme would require the use of whole cells and not pure enzyme, and so, the host would have to be classified as GRAS (generally considered as safe). It was proposed to transfer the cyanide hydratase gene to the GRAS yeast, *S. cerevisiae* using the intracellular vector pYES 2 and the secretory vector YEpFLAG-1, as well as into the yeast *Pichia pastoris* using the intracellular pPIC3.5 vector and the secretory pPIC 9 vector. Using a process of screening(assays, SDS gels, western blots) as well as RNA analysis and expression induction experiments the enzyme's stability and activity will be analysed in both yeast systems with a view to assessing their potential usefulness in cyanide biotransformation to formamide in comparison to the *Fusarium* and *E. coli* systems.

Poster 19

Characterisation of a regulator gene in *Pseudomonas fluorescens* F113, which is important for growth, iron acquisition, and biocontrol.

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Pseudomonas fluorescens F113 is a beneficial bacterium that can efficiently colonise the rhizosphere of many plants and protect them against a range of phytopathogenic fungi, the most important of these being the phytopathogenic *Pythium ultimum*, which causes "damping off" of sugarbeet seedlings and extensive economic loss. Both these organisms occupy the same ecological niche, and hence compete with each other in order to suceed and become established in the rhizosphere. The biocontrol ability of *P. fluorescens* F113 is well studied and understood, and is primarily attributed to the production of the antifungal metabolite 2,4-diacetylphloroglucinol (Phl)(1).

In order to investigate the molecular interactions between *P. fluorescens* F113 and *P. ultimum*, a screening system was developed to detect differential expression of bacterial genes in the presence of *P. ultimum*. Generation of a bank of *P. fluorescens* F113 mutant derivatives, constructed by random insertion of Tn5-LacZ into the F113 genome, led to the identification of five classes of mutated genes that were downregulated in the presence of *P. ultimum*. This is the first reported case of a fungus negatively affecting bacterial expression (2).

One mutant strain was taken from each class for further study (SF2, SF3, SF5, SF9, SF10). The ecological fitness of all reporter mutants is significantly compromised. This presentation describes the ecological, physiological and molecular characterization of these five reporter mutants. Two of the mutants (SF3,SF5) were identified as ribosomal operon mutants. *Pseudomonas fluorescens* F113 contains five rRNA operons and it was found that the SF3 and SF5 mutants carried insertions in the tRNAIle and the16S rRNA genes respectively. The SF10 mutant was affected in the ability to assimilate nitrogen via the GS-GOGAT pathway. The *gltB* gene of the glutamate synthase operon was disrupted in this mutant. A putative transcriptional regulator gene designated *espA* was disrupted in the SF2 mutant. This mutant displays a number of phenotypes, consistent with its poor persistence in the rhizosphere.

This work investigates signalling between fungi and bacteria. In particular, we explore how the *espA* regulatory gene contributes to the long term rhizosphere persistence, and ultimately, the biocontrol activity of *Pseudomonas fluorescens* F113, through the regulation of genes that are essential for long term survival within the rhizosphere.

Poster 20

BIOSYNTHESIS OF FLUOROACETATE AND 4-FLUOROTHREONINE IN *STREPTOMYCES CATTLEYA*

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[b] University College Dublin, Department of Industrial Microbiology, Belfield, Dublin.

The actinomycete *Streptomyces cattleya* is unusual in that it biosynthesises the fluorinated natural products fluoroacetate and 4-fluorothreonine as secondary metabolites. Fluorinated organic compounds are rare in Nature and consequently the biosynthetic pathways involved in their production are poorly understood.
Extensive isotope-labelling experiments have indicated that there is only one fluorinating enzyme in *S. cattleya*, and that fluoroacetate and 4-fluorothreonine are derived from a common precursor. Experiments with resting cells and cell-free extracts have established that fluoroacetaldehyde is this common precursor and here we report on the recent identification of two enzymes in *S. cattleya* that transform this compound to fluoroacetate and 4-fluorothreonine. One is an NAD^+-dependent aldehyde dehydrogenase that oxidises fluoroacetaldehyde to fluoroacetate and the other is a novel pyridoxal phosphate-dependent 'transaldolase' enzyme, which converts threonine and fluoroacetaldehyde to 4-fluorothreonine and acetaldehyde.

Poster 21

MOLECULAR AND MICRO-ANALYTICAL CHARACTERISATION OF THE MICROBIAL DIVESRITY AND POPULATION DYNAMICS IN A WEST OF IRELAND PEAT BOG

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The recent application of molecular techniques to the study of natural microbial populations has revealed the true diversity of microorganisms in the biosphere been recognised. These techniques have circumvented the necessity to culture bacteria in the laboratory before identification can take place. The objective of the present study is to carry out a culture independent survey of the archaeal and eubacterial diversity present in a peat bog. Following DNA extraction, the communities were analysed using Amplified rDNA restriction analysis (ARDRA) of clone libraries. Following digestion with *Hae III,* the resultant fragments were separated by gel electrophoresis and visualised using UV excitation. The restriction patterns were grouped into operational taxonomic units (OTUs), and a representative clone from each group was sequenced. Sequence analysis identified that an unusual and diverse group of microorganisms were present in this environment. In particular, methanotrophs, a group of proteobacteria, were identified in the upper layers of the bog. These aerobic microorganisms are involved in the oxidation of methane, and will play an important role in the regulation of methane emissions from wetlands. This is of crucial importance with respect to current concerns on the effects of global warming and climate change. Peatland communities were also examined for spatial and temporal patterns during seasonal cycles using a novel technique, terminal restriction fragment length polymorphism (T-RFLP). This method combines elements of polymerase chain reaction (PCR) technology, automated nucleic acid electrophoresis and comparative genomics. Archaeal and Eubacterial communities were analysed separately by the careful choice of domain specific primers. Results from both molecular approaches presented are compared.

Poster 22

The Role of Non-Ribosomal Peptide Synthesis in Gliotoxin Biosynthesis in *Aspergillus fumigatus*.

Claire Neville, Kevin Kavanagh and Sean Doyle.
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Aspergillus fumigatus is an opportunistic fungal pathogen, which colonises the lung in immunocompromised hosts. Culture filtrates of *A. fumigatus* contain many secondary metabolites including helvolic acid, fumagillin and gliotoxin. Gliotoxin mediates the immunosuppressive effects of *A. fumigatus* and may lead to subsequent mortality in the compromised host. Gliotoxin achieves this in many ways, including induction of apoptosis in cells of the immune system *in vivo*, inhibition of phagocytosis by activated macrophages and also inhibition of mitogen induced T- cell proliferation. It is known that phenylalanine and serine are the precursor amino acids for *in vivo* gliotoxin formation, however, the mechanism for this formation is unknown. Also, the origins of the N-methyl group and disulfide bond are unknown. We propose that gliotoxin is synthesised via a non-ribosomal peptide synthetic pathway by means of multifunctional enzymes known as peptide synthetases. We postulate that the NRP synthetase responsible for gliotxin production is bimodular, as gliotoxin is comprised of a basic dipeptide structure (Phe-Ser). As gliotoxin is N-methylated it is presumably synthetised by these NRP synthetases and another component known as S-adenosyl-L-methionine, this hybrid system is known as N-methlytransferases.

Our results have shown that spiking cultures with various concentrations of phenlyalanine and serine has increased gliotoxin production. We have also developed a radiolabelled assay; to determine thioester formation (gliotoxin-NRP synthetase activity) in gel purified fractions. We have identified a gene present in *A. fumigatus* which shows sequence homology to other NRP-synthetases, and we are currently expressing this synthetase in *Escherichia coli* to determine whether it is responsible for gliotoxin synthesis. The objective of this work is to identify the steps involved in gliotoxin biosynthesis with a view to identifying inhibitors of toxin production *in vivo.*

Poster 23

THE DEVELOPMENT OF A MOLECULAR APPROACH TO IDENTIFY ISOLATES FROM AGRICULTURAL SOIL AS MEMBERS OF THE GENUS *PSEUDOMONAS*.

Fiona Ní Mhulláin, Dominic Garvin, Bryan Mitchell *, James Burke* and Dina Brazil.
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* Teagasc Oak Park, Carlow

This project deals with the molecular approach to the identification and comparison of glyphosate resistant organisms, in particular organisms of the genus *Pseudomonas* found in an Irish agricultural soil. Organisms have been isolated and characterised from both treated and untreated soil from Teagasc, Oak Park, Carlow.

One of the methods these isolates have been used in the development of is a molecular method to identify an isolate as a member of the genus Pseudomonas. The development of this method involved the use of Bioinformatics tools such as the GenBank database, ClustalW, Blast and Primer3 to identify of a highly conserved region of the Pseudomonas 16S rDNA and subsequently design PCR primers to amplify part of this region.

The PCR product from several isolates has been sequenced and these sequences show a 99% identity to each other demonstrating the conservational nature of the region.
Supported by HEA Programme for Research in Third Level Institutions Cycle 1.

Poster 24

Investigation of the role of the anti – neoplastic agent Adriamycin in conferring resistance to Amphotericin B, in the pathogenic yeast *C. albicans*

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Candida albicans is an opportunistic fungal pathogen of humans and is a major secondary pathogen in immunocompromised individuals suffering from human immuno deficiency virus or cancer. Emergence of anti – fungal drug resistance has hampered effective treatment in the eradication of *C. albicans*. Several resistance mechanisms have been elucidated; including decreased cellular accumulation of drug and enhanced efflux of anti – fungal agents.
We have previously shown that incubation of *C. albicans* with the anti – neoplastic agent adriamycin has no effect on growth, but increases tolerance to the anti – fungal agent, Amphotericin B by 30% relative to the control. Exposure of yeast to other anti – neoplastic agents such as hydroxyurea has not lead to an increase in tolerance to Ampothericin B. We have established that adriamycin exposure results in an increase in cellular respiration rates (up 40% relative to the control) and sterol levels (a major site for the action of Amphotericin B). Photomicrographs have shown that adriamycin is incorporated into the yeast cell and may be exuded in a non – selective manner along with Amphotericin B. Investigation of the role of the detoxifying enzyme glutathione S – transferase (GST) has indicated that there is an increase in activity in adriamycin treated cells (up 20% relative to the control).
Exposure of *Candida* cells to adriamycin increases resistance to Amphotericin B by increasing the respiration rate, sterol levels and increased GST, which detoxifies drug metabolites. Clinical relevance of this finding indicates that treatment with one type of drug (adriamycin) may confer resistance to a second structurally and functionally unrelated compound (Amphotericin B).

Poster 25

Molecular analysis of the structure and regulation of the nitrile hydratase / amidase operons of three novel *Rhodococcal* species.

Rebecca O'Mahony and Catherine O'Reilly, Waterford Institute of Technology

Nitriles (ie organo cyanides of the general formula RCN) are common constitutes of plants and occur as intermediates of microbial metabolism.Their hydrolysis is catalysed by nitrile degrading enzymes which occur in a variety of plants, mesophilic bacteria, *Rhodococcus* and fungi.

Nitrile hydrolysis occurs by two major enzymatic pathways. One pathway involves the sequential hydrolysis of the nitrile molecule to its corresponding carboxylic acid and ammonia via an amide intermediate catalysed by two different enzymes ; a hydratase and an amidase. The other is the direct hydrolysis of the nitrile by a nitrilase to the corresponding carboxylic acid and ammonia. A number of novel nitrile utilising bacterial strains have been isolated. Three of these, AJ270, AJ300 and AJ115 are currently being analysed. The nitrile utilisation in these strains is due to the presence of nitrile hydratase and amidase enzymes These strains are thought to be novel *Rhodococcal* species. Sequencing of the 16S RNA genes is being carried out to confirm this taxonomic classification. PCR is being used to amplify the 16S genes. Strains AJ 115 and AJ 270 have been amplified sucessfully and their 16S DNA sequences determined.

Another aspect of this project is to study the control region for the NHase operons in each of these strains. Downstream of the amidase gene are putative nitrile hydratase regulatory genes namely NHR1 and NHR2 . It is thought that these regions are responsible for coding for proteins which may be responsible in transport mechanisms involving the uptake of cobalt or iron .They may also play a significant role in the photoreactivityof the nitrile hydratase enzyme.NHR2 has been identified in all three strains therefore it is plausible to expect NHR1 is also present.

Poster 26

CONTROL OF SULPHATE REDUCING BACTERIA DURING ANAEROBIC DIGESTION OF WASTEWATERS.

Caroline O'Reilly & Emer Colleran
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Department of Microbiology
NUI, Galway

During anaerobic treatment of sulphate-containing wastewaters, the reduction of sulphate to sulphide often presents difficulties, leading to a reduction in the methane yield and problems of odour, toxicity, corrosion and atmospheric pollution. Sulphide production is due to the activity of sulphate reducing bacteria (SRB), which competitively interact with the other bacteria involved in the process.
Addition of sulphate analogs, such as molybdate, have been used in the past to inhibit the activity of the SRB. However, non-specific inhibitory effects have been reported.
The inhibitor selected for investigation in this study was nitrite, since it had been reported that nitrite is highly inhibitory toward SRB. The aim of this research was, therefore, to determine the nitrite toxicity thresholds of the syntrophic populations, the acetoclastic and hydrogenotrophic methanogens as well as the sulphate reducers in various anaerobic sludges. Full-scale industrial, sulphate-adapted sludge was obtained from the citric acid production plant (ADM), Ringaskiddy, Co. Cork. Lab-scale anaerobic sludges consisting of sulphate and non-sulphate adapted granular sludges were obtained from mesophilic and thermophilic Upflow Anaerobic Sludge Blanket (UASB) reactors used in a parallel study. Nitrite, in the form of sodium nitrite, was included in test vials at varying concentrations to establish approximately what concentration of the compound was inhibitory for each trophic group. Toxicity was defined in terms of the IC_{50} value, i.e. the concentration of nitrite which resulted in 50% inhibition of the control vial specific methanogenic activity (SMA) or substrate utilisation rate.
The trophic groups examined exhibited varying sensitivities to nitrite at concentrations up to 150ppm. Lag phases in biogas production and activity were frequently observed, particularly in acetoclastic SMA tests.

Poster 27

Increased metal resistance and biodetection systems in a bioremediation strain

Robert Ryan, David Ryan and David Dowling
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Pseudomonas fluorescens F113rifPCB is a genetically modified rhizospheric bacterial strain developed for the degradation and bioremediation of polychlorinated biphenyls (PCBs). Metal contamination, by zinc, lead, cadmium, copper, mercury and arsenic is common in organic polluted ecosystems and their presence can inhibit the activity of metal intolerant strains such as *P. fluorescens* F113rifPCB. The stable insertion and expression of metal resistance determinants in important bioremediaton strains would provide and obvious advantage allowing such strains to carry out their bioremediation activities in the presence of otherwise inhibitory pollutants. These determinants can also provide additional detection systems for such strains as a flawless detection system is a prerequisite for the intentional release of any genetically modified organism. This poster describes the stable insertion and expression of two metal resistance operons in the target strain *P. fluorescens* F113rifPCB producing the strains *P. fluorescens* F113rifPCB-ars which is resistant to arsenicals and *P. fluorescens* F113rifPCB-cu, a copper resistant version of the parent strain. The methods used in doing this were both conjugation and electroporation. Development of PCR detection methods based on inserted metal resistance determinants to monitor these new strains has also been initiated and results are described here.

Acknowledgements: This work is funded in part by the Technological Sector Research Strand I post-graduate R&D skills programme at Institute of Technology Carlow.

Poster 29

MOLECULAR ANALYSIS OF KEY SOIL MICROBIAL COMMUNITIES

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1Irish Sugar plc. Carlow, Ireland.

In recent years, the interest in terrestrial ecosystem functioning and plant biodiversity has increased; this is in part due to the controversial debate on whether a reduction in plant diversity results in decreasing plant productivity and overall ecosystem functioning. Microbes play a major role in biogeochemical cycles, maintaining soil fertility, and potentially contributing to the regulation of plant productivity. In order to accurately assess the impact of a reduction in plant diversity on ecosystem function, diversity of key microbial group needs to be evaluated. Evidence has indicated that crop plants select for specific microbial populations. As estimates indicate that up to 90% of soil microbes have not yet been cultured, culture-independent methods are required to permit accurate assessment of microbial diversity. A state of the art culture-independent technique called denaturing gradient gel electrophoresis (DGGE) is currently being used at the BIOMERIT Research Centre to profile indigenous key microbial communities associated with the rhizosphere of different plant genotypes. Pseudomonads are a key functional microbial group exhibiting plant growth promoting characteristics. Inhibition of damping-off in sugarbeet by pseudomonads has been shown at the BIOMERIT Research Centre. The central mechanism promoting this inhibition is the production of an anti-fungal metabolite, 2,4-diacetyphloroplucinol (Phl). Investigations are currently being conducted at BIOMERIT Research Centre to evaluate the phenotypic and genotypic diversity of Phl producing pseudomonads associated with the rhizosphere of different sugarbeet varieties, via a molecular approach. Potential shifts in the structure and function of these indigenous Pseudomonad communities will be presented.

Poster 30

ENDEGRADERS

Kieran Germaine, Elaine Keogh, Guiomar Garcia-Cabellos, Dr David Ryan, Dr David Dowling, Dr. Ulrich Karlson, Dr. Stefan Trapp, Dr. Daniel van der Lelie, Dr. Edward Moore, Dr. Jaco Vangronsveld and Gunther De Becker.

In many cases the ability of microorganisms to degrade toxic compounds is due to possession of autonomous plasmids, which carry the necessary catabolic genes. For bioremediation purposes however, the natural host may not be suitable for one reason or another and so transfer of these catabolic pathways into more suitable hosts is necessary. We describe an experiment designed to allow the natural transfer of two catabolic plasmids into a mixed culture of plant associated, endophytic bacteria for use in xenobiotic degradation/phytoremediation. These endophytic strains were isolated from poplar trees in Belgium. The plasmids chosen were pJP5 (host strain *Ralstonia eutropha*), which encodes for the degradation of the herbicide 2,4-dichlorophenoxyacetate, and pTOM (host strain *Burkholderia cepacia*), which encodes for the degradation of trichloroethylene (TCE), a common pollutant of soil and water. The strategy involved growing both the donors and the recipients together in a biolayer. Selective pressure was increased by culturing the biolayer in progressively higher concentrations of 2,4-dichlorophenoxyacetate and toluene, plus an antibiotic to select against the donors. The biolayers were sampled and colonies were tested for the presence of the catabolic plasmid. The colonies can be identified by a DNA fingerprinting technique, ERIC- RAPD (Enterobacterial Recognition Intergenic Concenous sequence-Random Amplified Polymorphic DNA). This method uses primers whose sequence is arbitrarily chosen. As a result a characteristic and representative fingerprint of the genome can be obtained. Identification is confirmed by comparing the new fingerprint to those of the original donor and recipient strains. Plasmid identification is obtained by using PCR primers detecting for one or more genes on the plasmid.

Acknowledgement: This work is funded in part by the HEA and EU Contracts QLK3-CT2000-00164 & QLRT-2001-00101

Poster 31

ISOLATION OF A NOVEL ZINC EFFLUX PROTEIN FROM *T. THERMOPHILUS*

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We have generated a genomic library of *Thermus thermophilus*, a thermophilic eubacterium, in *Escherichia coli* and, using a phage display approach, have isolated a gene providing resistance to a number of divalent cations. Genetic analysis revealed homology to proteins involved in metal transport in a variety of bacterial and mammalian species, while expression in *E. coli* provided resistance to zinc and cadmium but not cobalt. The gene could be induced by zinc and cadmium and by heat shock in the *E. coli* host, leading in each case to elevated resistance to zinc. Atomic absorption analysis of cells grown in high zinc concentrations revealed lower intracellular levels of zinc in clones containing the gene, suggesting a role in efflux of the metal. Finally, co-expression of the *Thermus* gene in *E. coli* cells producing recombinant antibody fragments led to greatly improved growth of the host cells and higher production levels of functional recombinant protein.

Poster 32

COMPLETE NUCLEOTIDE SEQUENCE OF THE 89 KB ENTERIC CONJUGATIVE TRANSPOSON-LIKE ELEMENT R391 REVEALS A MOSAIC OF PHAGE λ LIKE INTEGRATION AND INCF AND INCP PLASMID LIKE TRANSFER MODULES

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[3)] Department of Genetics, School of Biological Sciences, University of Liverpool, United Kingdom.

The conjugative, chromosomally integrating element R391 is the prototype of the IncJ group of mobile genetic elements. Originally found in a South African *Providencia rettgeri* strain, R391 carries antibiotic and mercury resistance traits, as well as genes involved in mutagenic DNA repair and an unusual UV-sensitising function. Whilst initially being described as a resistance plasmid, R391 has subsequently been shown to integrate into the bacterial chromosome in a conjugative transposon-like fashion, employing a phage λ-like integration mechanism closely related to the SXT CONSTIN element from *Vibrio cholerae* O139. Isolation of an extrachromosomal circular form of R391 from a *recA* strain of *E. coli* containing the related IncJ element R997 integrated in the chromosome, has allowed for the first major molecular characterisation of the element. The complete nucleotide sequence of R391 (89 kb) has been determined, revealing a mosaic structure of elements originating in bacteriophage, plasmids, and transposable elements. 96 ORFs were identified, encoding transfer genes, partitioning and restriction modification genes, an integrase gene, resistance genes, and 30 ORFs of unknown function. Most of the 17 transfer genes identified have homologs on the *Salmonella typhi* plasmid R27. A composite transposon, carrying the kanamycin resistance gene, and a novel IS element were identified. The mercury resistance operon, consisting of 4 structural genes (*merT*, *merP*, *merC*, *merA*) and a regulatory gene *merR*, is almost identical to that of the IncJ element pMERPH, and is highly divergent from other enterobacterial mercury detoxification systems, placed phylogenetically between Gram-positive and Gram-negative *mer* operons. Challenging the previous assumption of IncJ elements being plasmids, and despite the fact that a circular form can be isolated, no plasmid replicon was identified on R391.

Poster 33

DEVELOPMENT OF A CONSTRUCTED WETLAND MICROCOSM TO INVESTIGATE THE MICROBIOLOGY OF THE RHIZOSPHERE OF WETLAND PLANTS

Department of Applied Biology and Chemistry, Institute Of Technology, Kilkenny Road, Carlow.

G Garcia-Cabellos &D. N.Dowling

Constructed wetlands are man-made systems designed for the treatment of polluted water. They are increasingly used in Ireland for the cost effective treatment of wastewater from agriculture and leisure industry. One of the main mechanisms of detoxification is considered to be the high microbial activity in the nutrient and oxygen rich environment surrounding the roots of wetland plants. However, the microbiology of reedbeds and wetlands is poorly understood. A simple model system (microcosm) was developed to evaluate bacteria strains and plants for a range of ecological parameters. The plant species used were *Iris sp, Glyceria sp, Rannunculus sp, Typha latifolia* and *Myrophyllum aquaticum*. This microcosm was evaluated with artificial organic waste for the following parameters pH, PO_3^{4-}, NO_3^-, suspended solids, SO_4^{2-}, turbidity and its ability to remove zinc. The results to date show a decrease in the following 95% reduction in SO_4^{2-}, 79% in PO_3^{4-}, 99% in NO_3^-, 99.5% in turbidity and 98% decrease in zinc levels. Indicating that the microcosm is capable of representing a full scale example.
Acknowledgment: This work was founded in part by Enterprise of Ireland Applied Research program and EU Contract BIO-CT97-2227.

Attendees and Presenters

First Name	Last Name	Presentation	First Name	Last Name	Presentation
Hamid	Abbas	Poster 1	David	Dowling	
Claire	Adams		Sean	Doyle	
Catherine	Adley	Poster 2	Guio	Dunne	Poster 33
Louise	Baily	Speaker 12	Hanane	ElBaz	Poster 8
Angus	Bell		Michelle	Gannon	
Dina	Brazil		Pat	Geraghty	
Samantha	Broaders	Poster 3	Kieran	Germaine	Poster 30
Nigel	Brown		Niamh	Gilmartin	Poster 9
Conor	Buggy	Speaker 2	Inka	Harju	
Orla	Cahill	Poster 4	Padraigin	Harnedy	
Paul	Clarke		Audrey	Hearne	Poster 10
Gavin	Collins	Speaker 5	Jill	Hogan	Poster 12
Sean	Connaughton		Linda	Jennings	
Stephen	Cooper		Andrew	Johnston	Speaker 10
Maebh	Cullinane	Poster 5	Susan	Joyce	Poster 13
John	Curtin	Poster 6	Siobhan	Kavanagh	Poster 14
Victor	de Lorenzo	Speaker 9	Kevin	Kavanagh	Poster 15
Sabina	Delaney		Elaine	Keogh	
Imelda	Doolan	Poster 7	Lynne	Macaskie	Speaker 3
John	Doran		Louise	Mark	

First Name	Last Name	Presentation	First Name	Last Name	Presentation
Patrick	McDonagh	Poster 17	John	Quinn	
Barry	McGrath	Poster 32	Simon	Reddy	
John	McGrath		Nigel	Robinson	Speaker 8
Christopher	McSweeney	Poster 18	David	Ryan	
Susanna	Morris	Poster 19	Robert	Ryan	Poster 27
Michael	Mullan		Orla	Sherlock	Speaker 4
Cormac	Murphy	Poster 20	Simon	Silver	Speaker 1
Audrey	Murray	Poster 21	Shona	Stewart	Poster 28
Claire	Neville	Poster 22	Pat	Sweeney	Poster 29
Fiona	Ni Mhullain	Poster 23	Karen	Tambling	Poster 11
Paraic	O Cuiv		Aoife	Thornton	Speaker 6
Kevina	O' Donoghue	Speaker 11	Caroline	Viguier	
Catherine	O' Reilly		Alan	Vivian	
Michael	O'Connell		Gerard	Wall	Poster 31
Fergal	O'Gara		Patrick	Ward	Speaker 7
Joseph	O'Keeffe	Poster 24	Jane	White	Speaker 13
Rebecca	O'Mahony	Poster 25			
Caroline	O'Reilly	Poster 26			
Thomas	Quigley				