and antibiotic resistant enteric bacteria, is generally applied to cropland as a fertilizer and waste disposal strategy. These practices may increase the diversity and occurrence of antibiotic resistance genes in agricultural soils. Soil samples were collected from several Illinois hog farms before and after effluent application, and from fields with histories of no effluent application and heavy effluent application. Bacteria from these samples were cultivated on unamended MR2A medium and MR2A media amended with the commonly used feed additives bacitracin, chlortetracycline, tetracycline and tylosin to assess the prevalence of organisms with resistant phenotypes. Further testing of particular isolates was done to identify multiple drug resistant organisms. DNA extracted from the soil samples was purified and evaluated by PCR for the presence of 20 tetracycline resistance genes, coding for ribosomal protection proteins (RRPs) and efflux pumps. Following effluent application, soils showed an increase in the occurrence of tetracycline resistance determinants, particularly the RPP genes tet (M) and tet (Q). Cultivation of bacteria revealed that soils with a history of heavy effluent application contained greater numbers and variety of resistant bacteria than soils with no history of effluent application. Isolates from manured soils exhibited resistance to the multiple antibiotics ampicillin, bacitracin, chloramphenicol, chlortetracyline, erythromycin, kanamycin, streptomycin and tetracycline. Results indicate that the pool of antibiotic resistance genes in agricultural soils increases, at least in the short term, as a result of effluent application from animals consuming antibiotics.

Q

Q-422. Cold Adaptation and Biodegradation of Aromatic Hydrocarbons by Antarctic Microorganisms

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A psychrotolerant bacterium, *Pseudomonas* sp. 30-3, isolated from oil-contaminated soil from Wright Valley, Antarctica, exhibited tolerance to temperatures ranging from 0° to 35°C. PCR amplification of a 248-bp DNA fragment in *Pseudomonas* sp. 30-3 using *capB*-gene specific primers exhibited a 98% amino acid sequence homology with CapB of *Pseudomonas fragi*. Elevated expression at cold temperatures suggested that CapB in *Pseudomonas* sp. 30-3 plays a pivotal role in survival and tolerance at cold and sub-zero temperatures. The putative promoter region of *capB* was cloned into promoter-probe vectors and the activity measured by β-galactosidase assay. The levels of β-galactosidase indicated a 10-fold increase in promoter activity after 6 h of exposure to 15°C. Thirty-six mesophilic and 13 Antarctic isolates were screened for the presence of either *cspA* or *capB* using PCR. Only *Pseudomonas* spp. isolated from Antarctic soils as well as the mesophilic *Pseudomonas putida* ATCC 17484 were positive for *capB*, suggesting that *capB* may be conserved in psychrotolerant *Pseudomonas* spp. An enzyme-linked lectinosorbent assay, was performed to quantify the amount of extracellular polysaccharide (EPS) produced at cold temperatures which allowed the *Pseudomonas* sp. 30-3 culture to agglutinate as a survival strategy. Twenty-seven strains isolated from oil-contaminated sites in Antarctica were screened for biodegradative genes *alkB*, *ndoB*, and *C23DO* using PCR. All naphthalene degraders showed the presence of 648-bp DNA fragment with 97% homology to a known *ndoB* sequence from *P. putida*. Five out of the sixteen isolates screened were positive for the 238-bp conserved region of *C23DO*. An *alkB* gene with 94% amino acid homology to that in *P. putida* P1 was detected in only one psychrotolerant bacterium, *Pseudomonas* sp. 7-156. Competitive PCR was also done to estimate the *ndoB* gene concentration using genomic DNA from pure cultures of *Pseudomonas* sp. 30-2. Tolerance to cold temperatures and ability to degrade hydrocarbons by the Antarctic isolate provides support for the application of bioremediation for petroleum hydrocarbons in Antarctic soils.

Q-423. Diversity of Perchlorate-Reducing Bacteria Isolated from Contaminated Sites in Nevada

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The influence of contaminants on microbial diversity continues to be of interest to microbiologists. Waters near Las Vegas, NV provide several useful and novel sampling sites in which to carry out investigations because natural bacteria present in soils, sediments and waters have been exposed to perchlorate contamination for over 50 years. This study centered on isolating bacteria from perchlorate-contaminated sites and further analyzing these strains using DNA sequencing of the 16S ribosomal gene. Soil samples were collected from along the Las Vegas Wash, and water and sediment samples were collected from Lake Mead. Environmental samples were serially diluted and cultured using a mineral salts medium containing acetate as the electron donor and perchlorate as the electron acceptor. Samples were incubated anaerobically at 25°C for 2 weeks on solid growth medium. After repeated subculturing, purity was determined using microscopy. DNA was extracted using a heat treatment for 5 minutes at 95°C. Samples were stored at -20°C until further analysis. Sequencing and fragment analysis were performed at the Nevada Genomics Center on an ABI Prism 3700 DNA Analyzer; over 30 samples were analyzed. The majority of the perchlorate-reducing strains were closely related to members of the genus *Aeromonas*. A few of these strains were closely related to members of the gamma proteobacteria group. Other strains were closely related to the following genera, *Rahnella* sp., *Shewanella* sp. and *Dechlorosoma* sp. These results will assist in adding to our understanding about the microbial diversity at these contaminated sites and have the potential to further the progress in bioremediation of perchlorate-contaminated sites in the U.S.

Q-424. Fecal Microorganisms Recovered from BromeliadPlants and Bird Droppings

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Fecal coliforms and *E. coli* recovered from water trapped in the central cups of bromeliad plants growing on tall trees in the rainforests of Puerto Rico have been reported as environmental sources of these bacteria. Bromeliads are not native to Hawaii but are extensively grown as ornamental plants. The first objective of this study was to determine whether *E. coli* and enterococci could be recovered from water trapped in the central cups of six species of bromeliad plants (*A. fulgens, A. fulgens var. discolor, N. compacta, V. imperialis, A. fasciata, G. lingulata)* grown in Hawaii. *E. coli* (36-42,800/100ml) and enterococci (4,800-97,200/100ml) were recovered from water samples obtained from all six species of bromeliads. These results indicate that bromeliad plants provide suitable habitats for fecal bacteria. The second objective of this study was to characterize the fecal microbial populations of bird (Java Sparrows) fecal droppings on a heavily used sidewalk at the University of Hawaii. The shoes of people, who use this sidewalk, become contaminated and transport this fecal contamination to other areas (offices, classrooms, laboratories, cafeterias, libraries) throughout the campus. Bird droppings were collected on clean plastic sheets and four pooled samples of 15 bird droppings were mixed well with 200ml of buffer and dilutions assayed for various fecal microorganisms. All four pools of fecal droppings contained elevated levels of *E. coli* (1,600-186,000 CFU/15 droppings) and enterococci (440-3,440 CFU/15 droppings). However, these four samples were negative for *C. perfringens* and FRNA phages. Somatic phages were detected in only 1 of the 4-pooled samples. Unlike the feces from humans and many other mammals, feces from this bird do not contain *C. perfringens*, which we previously reported to be the most reliable indicator of sewage contamination in Hawaii. Bird droppings in urbanized areas are common occurrences and represent an unknown health risk. Enforceable regulations are not available to address this kind of contamination.

Q-425. Growth and Sporulation of *Stachybotrys chartarum* on Different Media and under Different Light/Dark Incubation Conditions

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While its role in human health as an allergenic and/or toxinogenic fungus is not universally agreed upon, the presence of *Stachybotrys chartarum* on water-damaged cellulose-containing building materials is of concern to many. There is little information in the literature regarding optimal conditions (media, temperature and light) for its growth and sporulation *in vitro*. This study examined the growth and sporulation on some widely used fungal media and some that have been suggested for its isolation. 10 isolates were tested on 18 media under constant light, alternate light/dark and constant dark for 10 days at 23-24 C. Triplicate inoculum spots of 1 microliter containing 80-100 spores were placed on each plate of medium. Colony diameters were measured and the day that spores were first seen were recorded. No growth occurred on the medium containing cycloheximide. Only very slight growth occurred on media containing bile salts or 30% sucrose, with little or no sporulation. Fastest growth occurred on potato dextrose, inhibitory mold, corn meal, rice extract, 2% oatmeal, Czapek-Dox, and half strength Czapek-Dox agars, with sporulation by day 3, 4 or 5 with most isolates. Intermediate growth was seen on Sabouraud, 33% nutrient Sabouraud, rose bengal, and malt extract agars, with sporulation usually by day 4, 5 or 6. On non-nutrient (water) agar with filter paper, both shredded and intact paper, as a cellulose source, the hyphal growth was difficult to see and measure; sporulation typically occurred by day 5 or 6. With all the media there was little or no difference in growth under any of the three lighting conditions. Only on Sabouraud agar was a difference in sporulation with light exposure seen; sporulation was absent or delayed in constant light and absent in all 10 isolates in constant dark. For fastest growth and earliest sporulation, use of inhibitory mold, potato dextrose, corn meal, rice extract or 2% oatmeal agars are recommended.
The latter 4 of these, and non-nutrient agar with filter paper, are especially useful for environmental samples in which there are often other fungi that may grow faster and overgrow *Stachybotrys* on nutrient-richer media.

Q-426. Evaluation of PCR Interference From Dust Samples for Monitoring Indoor Environments

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Viable and non-viable biological contaminants in the indoor workplace have been reported to cause health problems ranging from allergic and infectious diseases to nonspecific "sick building symptoms". Recently, Polymerase Chain Reaction (PCR) technology has gained importance as a diagnostic tool for rapid detection and characterization of microorganisms. However, the potential interference with the PCR due to the presence of organic and inorganic compounds in the environmental samples have lately drawn much attention in this area. Although progress has been made by including DNA purifications in the conventional PCR protocol to reduce the interference, no detailed study has been reported in the literature by using a simple PCR protocol on fungal spores. The focus of this research was to investigate the practicality of applying a simple PCR method to the field with minimal manipulations to maintain the integrity of samples. A total of four real-life samples collected from a poultry farm, two hospital rooms and HVAC filters were studied in detail to investigate interference on PCR. The dust samples were spiked with known numbers of *Stachybotrys chartarum* spores, ranging from 2×10^2 to 2×10^5. The method of bead-beating was used for disruption of spores and release of DNA. Conventional PCR was used to calculate the

inhibition using previously designed PCR primers specific for the spores. The results indicate that the extent of inhibition by dust on PCR varies with the type and amount of dusts. For 2 out of 4 samples, inhibition was overcome by dilution, using 2 mg of dust samples and up to 2 x 10^4 spores. No interference was detected from 0.2 mg of all 4 of the tested dusts with a 10-fold dilution. The method was tested using replicate dust samples and was shown to be highly reproducible. The results of this research suggest the potential usefulness of using a simple PCR method for monitoring microbial aerosol in indoor environments.

Q-427. Differential Expression of Two Phenanthrene Quinone Reductases of *Mycobacterium* sp. PYR100 by the Change in pH of Media

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A pyrene/phenanthrene-utilizing Mycobacterium sp. PYR100 was isolated from a humus sample in Mt. Schwebian, Germany. The NADH:phenanthrene quinone reductase may play an important role for the reduction of cytotoxic ortho-quinones to the diphenol compounds in the degradation pathways of polycyclic aromatic hydrocarbons. Two NADH:phenanthrene quinone reductases were expressed during the pH-controlled cell cultivation in tryptic soy broth at 30°C. The two enzymes were separated from the cell-free extracts by the binding properties on a Cibacron Blue F3G-A immobilized agarose and a DEAE-Sepharose. At pH 7.5, 90% of the expressed NADH:phenanthrene quinone reductase activity bound on a Cibacron dye-immobilized agarose, but not on a DEAE-Sepharose. The Superdex™ 200 gel filtration followed by a Mono-S column chromatography gave an apparently purified enzyme protein (Mr = 20.1 K) containing a flavin cofactor, which exhibited an absorption maximum at 452 nm. The N-terminal amino acid sequence exhibited similarities with the NADPH:quinone oxidoreductase of Mesorhizobium loti (73%) and the chromate reductase of Pseudomonas putida (90%). On the contrary, most of the activity of phenanthrene quinone reductases expressed at pH 6.5 bound on a DEAE-Sepharose, but not on a Cibacron dye-immobilized agarose. The second enzyme was apparently purified as a single protein (Mr, 26.5 K) by the hydrophobic interaction chromatography, gel filtration and a Mono-Q column chromatography. This enzyme contained no flavin cofactor. The N-terminal amino acid sequence was similar to NAD(P)H dehydrogenases of Bacillus spp. (76 - 88%) and Staphylococcus aureus strains (80%). This study showed that the two NADH:phenanthrene quinone reductases was differently expressed due to the change in pH of media.

Q-428. Heavy Metal Biosorption Using Extracellular Polysaccharide Produced by a New Bacterium, *Paenibacillus velaei* sp. *nov.*

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Metal binding capacities of the extracellular polysaccharide (EPS) produced by a new bacterium, Paenibacillus velaei sp. nov., were determined using a membrane dialysis technique. The metals tested were cadmium, lead and mercury. Each metal solution was prepared at a concentration of 500 ppm in ion-free water. The EPS tested was at concentrations of 0.1, 0.5, and 1.0 %(w/v). The binding times tested were 1, 3, and 5 days. The results showed that the binding of cadmium, lead and mercury to EPS increased with increasing concentrations of EPS added to the metal solution. When using 1%(w/v) EPS in metal solutions, 99.7% of mercury was removed from the mercury solution whereas only 98.7% of lead was removed from the lead solution, and 88.4% of cadmium from the cadmium solution. The data indicate that the EPS produced by P.velaei sp. nov. has metal binding capacities and can bind strongly to mercury, lead and cadmium. Therefore, this EPS has a potential use in removing heavy metals contaminated in water and may also be used in the biodetoxication of other aqueous system contaminated by heavy metals.

Q-429. EDTA Resistance in *Vibrio harveyi* Mediated by at Least One Extra-Cellular Ligand That Is Not a Siderophore

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We are interested in studying the cycling of zinc in marine environments and are using *Vibrio harveyi*, a ubiquitous coastal organism, as a model system. We have isolated a collection of spontaneous mutants resistant to excess Zn^{2+} (Zn^R). One subset of these, designated "Class I," apparently hyperproduce a metal-binding ligand to prevent unregulated "leakage" of excess Zn^{2+} into the cell. Class I mutants are resistant not just to excess zinc but also to EDTA ($EDTA^R$), indicating that they produce at least one ligand that can outcompete EDTA for Me^{2+}. We have also shown that excess ligand can exclude cellular growth under particular metal-limited conditions, implying that *V. harveyi* cannot distinguish between ligand occupied or unoccupied by Me^{2+}. These conclusions suggest that *V. harveyi* regulates Me^{2+} ligand secretion via quorum sensing, preferentially secreting ligand at low population densities. The observation that some Class I mutants are non-luminescent is consistent with a model in which these strains behave as if they are perpetually at low density. *V. harveyi* quorum sensing is under the control of LuxO, a response regulator required for siderophore production and luminescence repression. To test whether the Zn^R and/or $EDTA^R$ phenotypes of our Class I mutants are the result of siderophore hypersecretion, a *luxO::kan* mutation was constructed in the Class I mutant RVH4. As expected, luxO disruption results in constitutive luminescence. Interestingly, the RVH4 *luxO*⁻ derivative is not Zn^R but retains its $EDTA^R$ phenotype. Siderophore-detection assays using HDTMA and CAS reveal that neither RVH4 nor the RVH4 *luxO*⁻ derivative produce greater levels of siderophores than wild-type *V. harveyi*. We conclude that (1) the Zn^R phenotype of RVH4 is under the control of LuxO; (2) RVH4 secretes at least one ligand not under the control of LuxO which can out-compete EDTA for Me^{2+}; (3) siderophore activity is not the primary cause of either the Zn^R nor the $EDTA^R$ phenotypes of RVH4; and (4) RVH4 probably has multiple alterations that act in concert to produce the array of phenotypes associated with that strain.

Q-430. Use of T-RFLP to Monitor Microbial Community Structure over Time in Industrial Metal Working Fluids

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Metal working fluids (MWF) are used in the machine industry for cutting, grinding, and drilling in metals. Many of them are oil-in-water emulsions that may contain 95-97% water and a combination of fatty acids, surfactants, synthetic detergents, antioxidants, and antifoam agents. This composition is favorable for microbial growth. The bacteria in MWF can create adverse human health effects such as hypersensitive and allergic problems as well as pulmonary diseases due to the endotoxin content of their cells. Several types of microorganisms are found in MWF, however most analyses have relied on culture-based methods. Recently, PCR based molecular techniques, such as T-RFLP and DGGE, have been used to examine the total microbial community in environmental systems. Our objective was to examine changes in the total microbial community without relying on culture methods in MWF over time in different machines by using T-RFLP analysis. Two MWF samples were collected weekly from two different types of metal-processing machines, Mori-seiki and Nigata, at a plant in Illinois. The main difference between the two machines was the size of the MWF reservoir, as the same MWF was used in both machines. Total DNA was extracted from each sample and digested for T-RFLP analysis after amplifying the 16S rDNA using a 5'-fluorescent-labeled primer with three different endonuleases, Hha I, Rsa I, and Msp I. Both the Mori-seiki and Nigata machines showed dominant terminal fragments over four months of monitoring corresponding in each digest to 207 bp by Hha I, 635 bp by Rsa I, and 492 bp by Msp I. However, the intensity of an 802 bp RsaI fragment increased over time in the Niigata machine. Using the RDP-based TAP T-RFLP program for fragment analysis the predominant fragment pattern suggested a bacterium closely related to a *Pseudomonas* species. This data suggested that the microbial community in the both systems is stable and consistent. This also infers that the composition of MWF is more important than environment in determining the community structure.

Q-431. Inorganic Carbon Sequestration by *Synechococcus* PCC Strain 8807 in a Closed Laboratory System

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Microbially initiated calcification by *Synechococcus* species as well as other cyanobacteria has been linked to a natural phenomenon known as whitings. Whitings is a large scale calcium carbonate precipitation that can occur in marine and freshwater environments. This is an effective sink for atmospheric carbon dioxide, a greenhouse gas. In an attempt to gain more understanding of microbially mediated calcification, experiments were done to facilitate its occurrence in the laboratory. *Synechococcus* PCC strain 8807, a broad salt concentration tolerant strain isolated from a lagoon water sample in Port Gentil, Gabon was used for testing. This cyanobacterium was cultured in ASN-III growth medium with 3.4mM calcium chloride and concentrations of $NaHCO_3$ varying from 0.5mM to 10 mM. Cultures were contained in sealed serum vials purged with nitrogen to remove carbon dioxide. Oxygen was added to a concentration of 20% v/v. Experiments were initiated with a target cell density of 1.0 x 10^5 cells/ml and a starting pH of 8.0. Experiments were run at ~22°C and at a light intensity of 6 $\mu E\ m^{-2}\ s^{-1}$. Each bicarbonate concentration and each control were run in triplicate. Maximum growth occurred in vials containing 2.5 mM bicarbonate where cyanobacterial numbers increased to approximately 1.0 x 10^7 cells/ml. The culture medium pH increased from the starting value of 8.0 to an average of 10.72. After 3 days of growth the calcium in solution began to drop. This coincided with the increasing pH measured in the growth medium. Within 2 weeks all the calcium in solution had precipitated and the cells became encrusted with $CaCO_3$ crystals as visualized by polarized light microscopy. This was especially evident in the culture containing 2.5 mM $NaHCO_3$. No calcium removal was noted in the cell free and bicarbonate free controls or when experiments were run using CO_2 as the inorganic carbon source. Abiotic precipitation of $CaCO_3$ was noted at HCO_3^- concentrations above 5 mM. Sequestration of inorganic carbon into $CaCO_3$ by *Synechococcus* PCC strain 8807 appeared to be optimum at HCO_3^- concentrations near 2.5 mM.

Q-432. Development of a GFP-Reporter System to Detect pJP4 Plasmid Transfer in Soil

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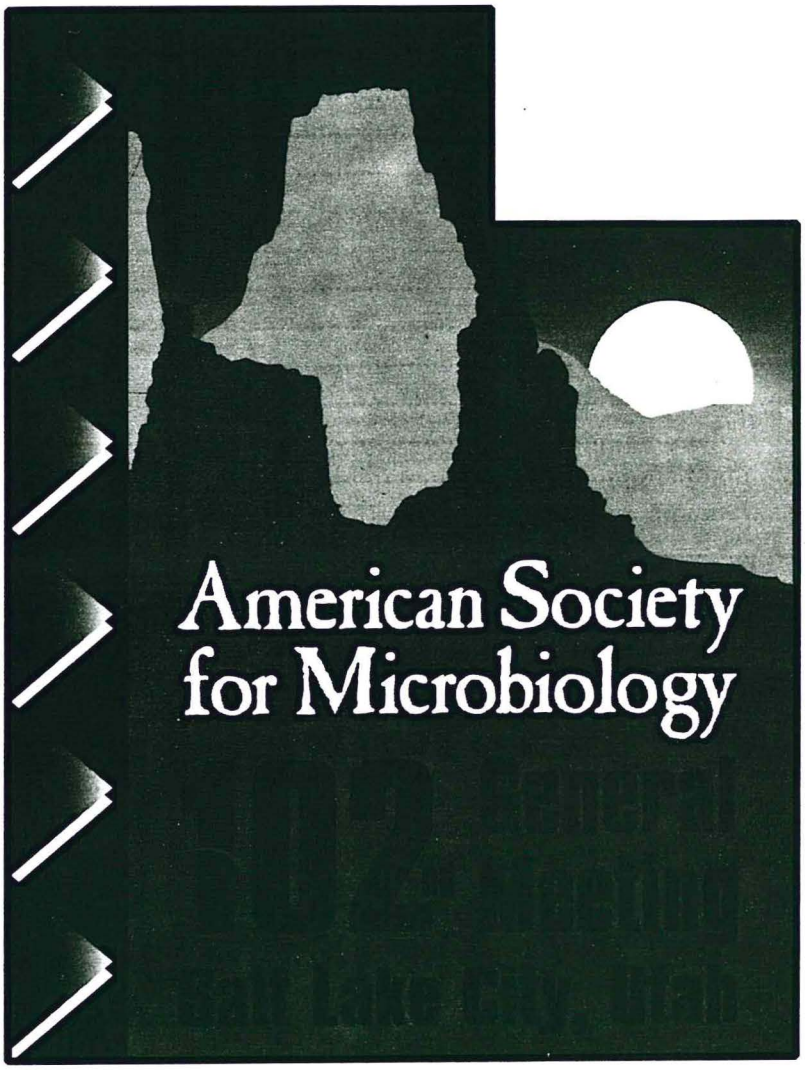
A major limitation for gene transfer studies conducted in soil is the difficulty of differentiation between donor and recipient bacteria. The screening of many organisms is often necessary to detect very low numbers of recipients. We are developing a system based on fluorescent reporter genes to enhance differentiation between donor and recipient bacteria. Two regions of the degradative plasmid pJP4 were PCR amplified for use as flanking regions for green fluorescent protein (GFP) insertion via homologous recombination. The DNA regions were specifically selected in order to insert GFP into a non-coding portion of the pJP4 plasmid. The gene for GFP was PCR-amplified and cloned, along with the gene for spectinomycin resistance, into the pLO2 suicide vector for integration into plasmid pJP4 via homologous recombination. Additionally, the genes for the red fluorescent protein

Abstracts



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