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ASM 2009 Perth

Program and Abstracts Book

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of the Australian Society for Microbiology**

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Perth 2009
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POSTER ABSTRACTS

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P20.04

Examining the effects of elevated CO₂ and temperature on Barley yellow dwarf virus in wheat

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The Intergovernmental Panel on Climate Change (IPCC) released their fourth assessment report in 2007 which concluded global warming is clearly occurring and that changes in the global climate system will continue into the future. These changes are expected to have major impact on agricultural systems, particularly as both CO₂ and temperature are expected to increase and more frequent severe weather events, such as drought, are expected to occur. As yet there is very little empirical data about the impact of elevated CO₂ and temperature on pest and pathogen populations and crop production. Consequently, predictions on the future of our major monoculture cropping systems such as wheat remain uncertain. The Department of Primary Industries Victoria, the University of Melbourne and the Australian Greenhouse Office have established a Free-Air CO₂ Enrichment (FACE) research facility at Horsham, Victoria, to study the effects of elevated CO₂ on wheat production in Australia. This facility is being used to study the effects of projected CO₂ concentrations (550ppm) under field conditions on Barley yellow dwarf virus in wheat. In addition to the FACE experiments a second study is being established in growth rooms to gather empirical information about the fecundity of BYDV in under elevated temperature. A third study will also be done to determine the ability of the BYDV vector, *Rhopalosiphum padi*, to acquire and transmit the virus under various climatic conditions.

P20.05

Salt inducible Biopolymer Production in Biofilms by *Bacillus megaterium*

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A biofilm-forming strain of *Bacillus megaterium* (culture no.20) was previously isolated for application in the bioremediation of oil contaminated soil and water. This pure culture showed the highest degree of biofilm formation measured using the standard Microtiter Plate Experiment protocol amongst the cultures used. When grown with a high sodium chloride content, large quantities of a white floating biopolymer material was observed, which strongly adhered to the neck of the shake flask. This was in contrast to the other strain of *B. megaterium* (culture no.20), which showed comparatively poor biofilm formation and did not produce any white floating material under the same culture conditions. The white floating material obtained (culture no.20) was denatured with β-mercaptoethanol and heated at 95°C for 20 minutes before being subjected to SDS-PAGE analysis. Interestingly, a protein molecule of about 30Kd in size was observed. The biopolymer formation was highest when the culture was carried out using sodium chloride at concentrations of 0.5% and 5.0%, respectively. The tight distinct band displayed in the SDS-PAGE suggested that we are dealing

with a biopolymer of fixed length. The same band was present both of the cultures containing 0.5% and the 5.0% of sodium chloride respectively. When the cell material was spun down a the pellet loaded, the same band was observed with less intensity suggesting that the protein was released extracellularly into the culture medium. There have been reports that certain strains *Bacillus subtilis*, *Bacillus anthracis* and *B. megaterium* produce Poly-γ-glutamate in significant quantities. The structural details the polymeric protein produced in our lab are currently under investigation.

P20.06

Gene Regulation Studies of *Leptospirillum* Species Subject to Soluble Nitrogen Starvation

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Bioleaching is the solubilisation of metals from mineral ores catalysed by micro-organisms, an action that occurs naturally in many environments. This phenomenon has been capitalized on and is now a well established and incorporated technology within the mining industry. Members of the genus *Leptospirillum* are routinely found within bioleaching systems. They are chemolithoautotrophs whose sole energy source results from the oxidation of ferrous iron. Understanding the metabolic complexity of *Leptospirillum* is necessary for the improvement of bioleaching technology. Nitrogen is an essential element required for the cellular growth of all microorganisms. In this study *Leptospirillum* species were assessed for their nitrogen fixation capabilities under aerobic conditions, in the absence of soluble ammonia.

L. ferrooxidans, *L. ferriphilum* and *L. ferrodiazotrophum* were grown in liquid suspension without a soluble nitrogen source and maintained under aerobic conditions. Culture growth was assessed by cell counting and iron oxidation. Genes selected to study nitrogen fixation were the *nifHDKEN* operon required for production of nitrogenase, the enzyme essential for catalysis of atmospheric nitrogen to ammonia, and the *nifSU-hesB-hscB-h* operon for nitrogenase protein assembly. Primers were designed from sequences of previously identified diazotrophs obtained from the NCBI database, and gene expression levels were analysed using Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR).

All three strains demonstrated an initial decrease in cell proliferation after soluble nitrogen starvation but growth resumed shortly after adaptation to culture conditions. As nitrogen fixation requires a large amount of energy, an initial decrease in cell proliferation is to be expected. When compared to control experiments containing soluble ammonia, differences in gene expression levels of the *nifHDKEN* and *nifSU-hesB-hscB-h* operons were detected in all three *Leptospirillum* strains. Differences in expression levels of nitrogen fixing genes determined after soluble nitrogen starvation indicates that *Leptospirillum* regulate the genes required to fix atmospheric nitrogen and help to limit nitrogen starvation in bioleaching environments.

P20.07

Population Dynamics of a Low-Grade Chalcopyrite Bioleach Column

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Parker Centre for Integrated Hydrometallurgy Solutions.
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Mineral bioleaching has long been identified as a possible method by which low grade ores can be processed economically. However, little is known about the population dynamics of the microorganisms that play a vital role in this extraction process. The extreme conditions found in these bioleaching environments pose problems for the more traditional culture-based microbiological techniques, as well as the use of molecular biology to study the microbial populations involved in bioleaching. This study aims to characterise the microbial population dynamics of an experimental bioleaching environment, bioleaching columns.

An experimental column charged with low-grade chalcopyrite ore (approximately 0.5% copper) was inoculated with a consortium of ten known bioleaching microorganisms (seven bacterial and three archaea). The column was maintained at 50°C with drip feed and the solution recycled. The leachate was collected from the column discharge after 275 days. The column was dismantled after 424 days and particulate matter was collected from four locations in the column. Terminal Restriction Fragment Length Polymorphism (T-RFLP) was used to determine the diversity of the bacterial and archaeal populations in both the leachate and solids of the bioleaching column. Differing populations were identified in the leachate and the column solids but there was no discernible effect of location in the column.

P20.08

Pentose Utilizing *Corynebacteria* sp. Producing Industrial Valuable Amino Acids

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Microbes prefer to use glucose as a readily fermented carbohydrate source in the presence of mixtures of carbohydrates. This is achieved through a mechanism where expression of genes associated with use of other carbon sources in metabolism is repressed until glucose is used up (catabolite repression). Previously it has been demonstrated that genetically modified *Corynebacteria* are able to produce amino acids utilizing glucose. However, amino acid production from pentose sugars by *Corynebacteria* has not been demonstrated before.

The aims of this project were: 1. Isolate Actinomycetes and determine the nature of the end-products of pentose fermentation, 2. Screen strains for physiological ability to use pentoses and hexoses separately.

Results obtained: Six *Corynebacterium* sp. were isolated from 210 actinomycetes isolates by microbiological standard culture methods and Polymerase Chain Reaction (PCR). These organisms were isolated from sugar treatment ponds of Queensland, Australia. All isolates were found to have P values of 0.1 – 0.7 > 0.05, which has statistically no significant difference in utilization between pentose and hexose sugars. *Corynebacterium* sp. cultured in the presence of pentoses and hexoses as carbon sources use pentoses equally efficiently as they do glucose. In addition These *Corynebacterium* sp. can also produce valuable amino acids, such as Asx (Asn+Asp), Glx (Glu+Gln), Arg, Ala, Phe, Leu, and Lys. The production of these amino acids by *Corynebacterium* sp. was determined by high performance liquid chromatography (HPLC).

P21

Posters – Microbial Pathogenesis

Thursday 9 July 3.30pm

Exhibition Hall (Pavilion Hall 1)

P21.01

Effect of Lactoferrin and Iron on the Growth of Human Pathogenic *Candida* Species

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Lactoferrin belongs to the transferrin family of iron binding protein and is a glycoprotein. It has significant characteristics like, antibacterial, antifungal, anti-viral, antioxidant, immunomodulatory and to acts synergistically with lysozyme to potentiate the activity of proteins. Lactoferrin bind two molecules of iron with very high affinity thus making iron unavailable to pathogen which is an essential element for bacterial and fungal pathogen to survive and multiplication inside the host. Infection caused by *Candida albicans* remains considered as one of the most pathogenic human and animal *Candida* species but during recent years other species like *C. krusei*, and *C. tropicalis* also emerged as a major pathogen of human and animals including all sites. The aim of present study is to study the effect of lactoferrin, lactoferrin free milk, and added lactoferrin with iron on the growth of human pathogenic *Candida albicans*, *C. krusei* and *C. tropicalis*. Multiplication and morphological changes has been studied. Results showed that when lactoferrin added to the milk, no pseudohyphae were produced by any of the *Candida* species even after 72 hr. of incubation but when iron added with lactoferrin, *Candida* species produced pseudohyphae although after 72 h of incubation as compared to whole milk where these *Candida* species produced pseudohyphae after 48 hr of incubation. Production of pseudohyphae of *Candida* species helps in faster spreading of *Candidal* infection *in vivo*, but lactoferrin not only checks the multiplication of yeast cells but also stopped the production of pseudohyphae by *Candida* species.

P21.02

Virulence-Associated Characteristics of *Stenotrophomonas maltophilia*

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Stenotrophomonas maltophilia is a Gram-negative bacillus readily isolated from environmental sites that can cause infection, usually in compromised patients. Treatment can be difficult due to intrinsic resistance of *S. maltophilia* to most commonly used antibiotics. Various host factors have been identified that increase risk of infection but few studies have investigated the characteristics of *S. maltophilia* strains that allow for survival in and infection of a human host. The aim of this study was to investigate putative virulence factors of a collection of *S. maltophilia* isolates. Ninety-six clinical (including 34 from hospital-acquired infections) and 36 environmental *S. maltophilia* isolates were tested for the production of protease, gelatinase, elastase, lipase, esterase, DNase, mucinase and lecithinase using substrate media, and hyaluronidase by cross-streaking with *Streptococcus equi*. Isolates were also tested for complement resistance, agglutination and lysis of human, horse and sheep erythrocytes, twitching motility, and adhesion to, and biofilm formation on, polystyrene. Protease, gelatinase, lipase,