

2007 INTERMOUNTAIN SYSTEMS BIOLOGY SYMPOSIUM POSTER ABSTRACTS

(POSTERS ELIGIBLE FOR STUDENT COMPETITION)

1. BIODEGRADATION OF PENTACHLOROPHENOL IN CONTINUOUS FLOW BIOREACTORS

Keith Albretsen, and Jeremy Neff, Utah State University, Logan, UT, USA

Pentachlorophenol (PCP) is a commonly used wood preservative that is toxic and was a common contaminant used at industrial wood preservative sites before its use became regulated in the U.S. PCP is often found associated with the carcinogenic polycyclic aromatic hydrocarbon (PAHs) in mixtures of PCP/PAHs. The object of current research is to isolate the microorganism(s) responsible for the biodegradation of PCP from a Superfund Site in Libby, Montana contaminated with a creosote mixture of PCP and PAHs. Site-specific microorganisms are being used in continuous flow bioreactors to treat contaminated groundwater. Laboratory-scale tests were conducted to determine the degradability of PCP by specific microorganisms isolated from contaminated soil at the same Superfund site. These isolates were found to contain the genomic-based enzymatic pathways necessary to degrade PAHs, and also to contain the first enzymatic step for PCP degradation. Mineralization tests were conducted with the isolated microorganisms, *Mycobacterium* JLS, *Mycobacterium* KMS, and *Mycobacterium* MCS, and with samples taken from a bioreactor designed to remove PCP from contaminated ground water. The tests were performed in liquid-media microcosms spiked with ¹⁴C-PCP and inoculated with one of the isolated microorganisms or an aqueous sample from the bioreactor. Radiolabeled carbon dioxide was measured to determine the amount of PCP mineralized by the microcosms. Triplicates of each sample were evaluated under aerobic and anaerobic conditions. Results showed that microcosms inoculated with any of the isolated microorganisms under aerobic or anaerobic conditions were unable to mineralize the ¹⁴C-PCP. Microcosms inoculated with bioreactor samples were shown to mineralize approximately 25% and 22% of the ¹⁴C-PCP two weeks after inoculation under aerobic and anaerobic conditions, respectively.

While the isolated microorganisms were not capable of mineralizing PCP under aerobic or anaerobic conditions, they may be capable of initializing the first step in PCP degradation. However samples from the bioreactor showed immediate mineralization of PCP. Current research is being implemented to isolate and characterize the microorganism(s) responsible for the degradation of the PCP in the bioreactors in order to optimize the PCP biodegradation rate and extent within the continuous flow bioreactor.

2. MICROBIAL DIVERSITY AND ZINC TOXICITY TO PSEUDOMONAS SP. FROM LAKE COEUR d'ALENE SEDIMENT

Sutapa Barua¹, David E. Cummings², Yvette M. Piceno³, James G. Moberly¹, Rajesh K. Sani⁴ and Brent M. Peyton¹, ¹Montana State University, Bozeman, MT, ² Point Loma Nazarene University, San Diego, CA, ³Lawrence Berkeley National Laboratory, CA, ⁴South Dakota School of Mines and Technology, Rapid City, SD, USA

Lake Coeur d'Alene (LCDA), the second largest Idaho's lake, is one of the metal contaminated lakes in US. The sediments of the lake are enriched with Fe, As, Pb, Zn and Cu which are toxic, heavy metals to humans and animals. It is hypothesized that the microorganisms live in this lake sediments can remove the metals and thus detoxify their environment. The objective of this work is to investigate the microbial communities existing in LCDA sediment using 16S ribosomal RNA (rRNA) gene sequencing, phylogenetic analyses and 16S rRNA gene-based microarray assay technology. According to our phylogenetic analysis, the LCDA clones fell into 13 distinct phylogenetic classes including 2 environmental samples, 1 uncultured bacterium and an unclassified *Chloroflexi*. Ninety one sequences belonged to the class of β -*Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Flavobacteria*, α -*Proteobacteria*, *Bacteroidetes*, δ -*Proteobacteria* *Chloroflexi*, γ -*Proteobacteria*, *Sphingobacteria*, *Chlorobi*, *Cyanobacteria* and *Clostridia*. The major representative genera found in the sediment were *Thiobacillus* (7/91 i.e., 7 out

of 91), *Azoarcus* (7/91), *Acidobacterium* (6/91), *Janthinobacterium* and *Flavobacterium* (5/91). The remaining clones formed significant genetic diversity. Microarray analysis of PCR amplicons of the same sediment sample showed the presence of 1551 operational taxonomic units (OTUs). 97% of the clone library sequences matched at various taxonomic levels with the microarray results. The results from the clone library and PhyloChip have provided the broad picture of a variety of microorganisms inhabiting in the metal polluted LCDA sediment. Identification of the iron oxidizers, iron reducer, sulfate reducer, nitrifying bacteria, and several other heterotrophs in the LCDA clone library indicate the presence of microbiological interactions on the transformation and toxicity of metals and metalloids in the sediment. To better understand the metal toxicity kinetics of an isolate from the LCDA sediment, cell growth and aqueous Zn removal rate of *Pseudomonas* sp. strain JM001 was observed in different batch kinetic experiments. The minimum inhibitory concentration (MIC) of Zn to the cells was 24 μ M and the 50% inhibition in specific growth rate was found at 11 μ M Zn. The results are significant to understand the metal-microbe interaction in a complex environment such as LCDA.

3. THE STABILITY OF THE RNA DEPENDENT RNA POLYMERASE GENE L1 OF BLUETONGUE VIRUS IN THE PRESENCE OF 6-AZAUROIDINE

Chad W. Dallan*, Ryan N. Jackson, Margaret Buccambuso, Daniel F. Child, Janette Starks, Uyen T. Lam, and Joseph K. K. Li. Department of Biology, Utah State University, 5305 Old Main Hill, Logan, Utah 84322-5305

Blue Tongue Virus (BTV), of the genus *Orbivirus* and family *Reoviridae*, has a ten segment double-stranded RNA (ds-RNA) genome. It causes many symptoms, including death, in sheep and cattle, but is not infectious to humans. Researching BTV is important due to its potential use as an agricultural bioterrorist weapon and the \$125 million worth of lost trade in the U.S. each year. A quantitative Real Time Polymerase Chain Reaction (qRT-PCR) assay was used to detect the stability of the viral messenger RNA (mRNA) for the L1 gene in the presence of the nucleoside analog 6-azauridine at different concentrations and post infection times. The L1 gene codes for Viral Protein 1 (VP1) which acts as BTV's RNA Dependent RNA Polymerase (RDRP) and is essential for successful replication of infectious virions. The decrease in the L1 mRNA stability in the presence of 6-azauridine will decrease the ability of BTV to replicate and be infectious. Three different primers corresponding to the 3', middle, and 5' sections of the L1 mRNA were used to determine the stability of the gene at different locations. 6-Azauridine significantly reduced the stability of the viral L1 mRNA in the BTV infected cells at concentrations between 4 and 32 μ g/ml with a concentration of 16 μ g/ml being the most effective.

4. ANTIMICROBIAL ACTIVITY AND TRANSCRIPTIONAL PROFILE OF LISTERIA MONOCYTOGENES IN RESPONSE TO SYRINGOPEPTIN 25A AND RHAMNOLIPIDS.

Prerak T. Desai^{1,2,3}, Patricia J. Champine³ AND Bart C. Weimer^{1,2,3,1} Department of Nutrition and Food Sciences; ²Center for Microbe Detection and Physiology; ³Center for Integrated BioSystems; Utah State University, Logan 84322-4700.

We investigated the antimicrobial properties of two pore-forming compounds: syringopeptins (SPs); cyclic cationic lipodepsipeptides produced by a plant pathogen *Pseudomonas syringae* pv. *syringae* and a rhamnolipid mixture (RLs) produced by *Pseudomonas aeruginosa*. SP 25A, one type of lipodepsipeptides and RLs, inhibited growth in all the Gram-positive organisms tested. SP 25A was bactericidal to *Mycobacterium smegmatis*, bacillus and clostridial spores, and multiple drug resistant strains of staphylococci and enterococci, while RLs failed to inhibit the growth of multiple drug resistant organisms. Further, no toxicity to mouse enteroendocrine, human embryonic kidney, and human lung fibroblasts was observed during exposure to SP 25A and RLs *in vitro*. Subsequently we determined the gene expression changes of *Listeria monocytogenes* EGDe when challenged with sublethal doses of SP 25A and RLs to compare the transcriptional response elicited against the two different compounds. Cell growth, membrane permeabilization, and gene expression profiles were determined immediately prior to treating the cells with both compounds, 30 min after exposure and 120 min after exposure. SP 25A

increased membrane permeabilization by ~ 2% which led to a 100 % reduction in growth. RLs on the contrary increased membrane permeabilization by ~53% which lead to 47% reduction in growth as compared to control. The reduction in cell density as compared to control after treatment with SP 25A was associated with repression of key genes in cell division, genome replication, transcription, and translation, cellular energy, central metabolism, and virulence. Conversely, RLs affected relatively few genes the phenotypic effects of which couldn't be assessed. Taken together, these data led us to conclude that both the compounds, even-though acting on the cell membrane, inhibited *L. monocytogenes* via distinctly different mechanism with SP 25A having a multi-hit mechanism by acting on regulation of gene expression.

5. REAL TIME CAPTURE AND DETECTION OF BACTERIA USING GANGLIOSIDES AND REAL TIME PCR.

Prerak T. Desai, Marie K. Walsh, Bart C. Weimer, Department of Nutrition and Food Sciences; Western Dairy Center; Center for Microbe Detection and Physiology; Center for Integrated BioSystems. Utah State University, Logan 84322–4700.

The aim of this work is to develop a method to rapidly detect variety of pathogens from one sample preparation without the need for pre-enrichment within 3 hours. We demonstrate that gangliosides; a non specific host receptor that mediate pathogen attachment to the cell can be used to capture bacteria from solution onto a solid phase and subsequently detect them using real time PCR in three hours. We immobilized gangliosides (a mixture of GM1, GM2, GM3, GD3, GD1b, and asialo-GM1) purified from bovine buttermilk onto 3 mm glass beads via a polyethylene spacer and screened the ability of twenty different strains mainly comprising of *Salmonella*, *Eschereschia*, *Lactobacillus*, *Lactococcus*, *Listeria*, *Bacillus* and *Erwinia* to bind to the immobilized gangliosides. Ten (out of 10 tested) strains of *Salmonella*, two (out of two) strains of *Eschereschia*, *Lactococcus lactis* and *Bacillus globigii* spores showed positive binding while *Lactobacillus acidophilus*, *Lactobacillus gasseri*, *Lactobacillus helveticus*, *Listeria monocytogenes* and *Erwinia herbicola* showed negative binding to the immobilized ganglioside mixture. We also demonstrated the ability of the gangliobeads to capture and detect bacteria from solution with the minimum detection limit of ten CFU within three hours. *E. coli* O157:H7 could be captured from solution with gangliobeads at a recovery rate of ~97 % when exposed at a cell population of log 2 CFU or less. The captured organisms were detected using the Genevision rapid pathogen detection system for *E. coli* O157:H7 (Warnex Diagnostics, Quebec, Canada). Use of gangliobeads along with the Genevision system bypassed the need for sample pre-enrichment and yet has a detection limit of ten CFU. When coupled with Immunoflow technology a detection limit of less than 1 cfu/ml will be achieved.

6. FROM MASSES TO PATHWAYS: CIB-USU METABOLOME SEARCHER

Ranjitha Dhanasekaran, Jon L. Pearson, Balasubramanian Ganesan, and Bart C. Weimer
Affiliation: Utah State University, Logan UT, USA

Understanding the metabolism of an organism provides vital clues to which pathways are activated by key substrates, and provides biomarkers for infected and normal states of organisms. High throughput biochemical analysis by coupling liquid chromatography to mass spectrometry makes large lists of such molecules available as either monoisotopic masses (LC-MS) or identified names of compounds from spectral library searches (GC-MS and LC-MS). The challenge remains to integrate such large data sets into biochemical pathways that are of interest to the researcher. We have developed a tool that addresses this shortcoming by allowing the researcher to query metabolic databases and biochemical databanks from either a generic string query of names of biomarkers to masses of compounds, either molecular weights or monoisotopic masses from MS analysis. The queries are submitted by an HTML interface. The users can select single or multiple databases from the available listings, and set parameters relevant to their analysis. Available options for MS data include mass deviation, adducts, deducts and detection mode. Multiple queries can be submitted as a delimited text file, either created by the users or obtained from MS analysis software. The results of the queries are made available as both

an HTML results page and a downloadable text file. The matched compounds are presented along with any data supplied by the users, and the pathways in which they are present are also provided as links. The links allow the users to navigate to extensively curated metabolic databases built on PathwayTools at the Center for Integrated BioSystems, Utah State University, as well as the KEGG Pathway database. Data in the text file can further be manipulated and sorted by the user relatively easily into different pathway classes of interest that are over-represented in the samples. Near future additions will include the more extensive PubChem databank. The interface provides biological researchers an opportunity to rapidly narrow down a mass list to a limited set of known metabolites of interest, on which they can sort their data and understand the relevance of pathways and their networks in metabolism.

7. A COMPARATIVE RADIATION HYBRID MAP OF OVINE HOMOLOGS OF HUMAN CHROMOSOME 4 (HSA4)

W. Jin¹, C. H. Wu¹, K. Nomura², T. Goldammer³, T. Hadfield¹, J. E. Womack⁴ and N. E. Cockett¹

¹ Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, UT 84322-4700 USA. ² Tokyo University of Agriculture, Department of Animal Science, Laboratory of Animal Genetics and Breeding, 1737 Funako Atsugi-shi, Kanagawa 243-0034, Tokyo, Japan. ³ Research Unit for Molecular Biology, Research Institute for the Biology of Farm Animals (FBN), Dummerstorf, Germany.

⁴ Department of Veterinary Pathobiology, Texas A&M University, College Station, TX 77843-4467 USA.

A 5000-rad sheep RH panel, named USUoRH5000, was constructed recently and was successfully used for building RH map of several ovine chromosomes. In the present study, our objective was to construct the comparative radiation hybrid maps of ovine chromosomal homologs of HSA4 and take advantage of the well developed closely related or other mammalian species research results, such as, cattle and pig, or horse and dog. The ovine orthologous genome positions were identified in the human, cattle and dog, or referenced the position in horse and pig genome with the latest RH maps data. We constructed the comparative RH map of the five genomes that gave approximately 50-93% comparative coverage of the related genome with an average spacing of 1.5 Mb on the ovine homologous chromosomal regions of HSA4. Our RH results have a good corresponding with the previous chromosome painting data. Alignment of the human, cattle, pig, horse and dog chromosomes (or genome Build) provides insight into the different chromosomal rearrangement characteristics that have occurred in the related lineages, built by using markers selected from the most recent sheep linkage map and the annotated genes from NCBI UniGene database and BESs from the virtual sheep genome. The available comparative maps are not only useful for navigating and establishing the orthology to regions of the related species genome precisely, but also for providing an independent source of data to assist the sheep genome sequence assembly process towards a finished quality sequence and sharing a light on mammal chromosome evolution.

8. USE OF REAL-TIME PCR TO QUANTIFY ACTIVITY OF *TRICHODERMA* SPP.

Tae Gwan Kim and Guy R. Knudsen. University of Idaho, Moscow, Idaho, USA

Members of the fungal genus *Trichoderma* have been used as agents for bioremediation as well as for biological control of plant pathogens. For example, *Trichoderma* spp. are able to mycoparasitize sclerotia, which are the resting structures of the plant pathogenic fungus *Sclerotinia sclerotiorum*. Unfortunately, traditional methods (e.g., culture plating, microscopy) to quantify growth and colonization activity of *Trichoderma* spp. and other fungi in natural environments are labor-intensive, time-consuming, and limited in their resolution. Our objective was to use a bioinformatics approach for improved quantification of biomass and activity of *Trichoderma* spp. in natural soil habitats. A total of 180 of the Internal Transcribed Spacer (ITS) 1, 5.8S small-subunit rDNA, and ITS2 sequences of *Trichoderma* spp. were aligned, and a consensus sequence was obtained. A *Trichoderma* genus-specific primer/Taqman-probe (TGP4) set targeting the ITS region of the genus *Trichoderma* was produced for real-time PCR. The TGP4 set successfully amplified and detected DNA of *T. harzianum* and 6 other randomly selected

Trichoderma isolates. Concentrations of PCR components such as primers, probe, Taq DNA polymerase, dNTP, and Mg²⁺, as well as PCR cycling conditions including annealing/extension temperature and time, were optimized for the TGP4 set. The TGP4 set showed high precision and reproducibility over a linear range of 8 orders of magnitude, ranging from 8.5 fg to 85 ng of *T. harzianum* genomic DNA. A standard curve between the log-transformed concentrations of *T. harzianum* DNA and the calculated threshold cycle values by the TGP4 set generated a linear fit with a slope of -3.4 to -3.6, correlation coefficient of > 0.99, and PCR amplification efficiency of > 90%. The real-time PCR assay with the TGP primer/probe set accurately quantified *Trichoderma* DNA in DNA extracted from sclerotia of *S. sclerotiorum* which were recovered from soil. The results suggested that use of the real-time PCR assay with the TGP4 primer/Taqman-probe set is a sensitive and precise method to quantify activity of the genus *Trichoderma* in natural soils. When this method was used in conjunction with confocal laser scanning microscopy, the two methods together allowed precise quantification and spatial analysis of a GFP-transformed strain of *T. harzianum*.

9. KINETIC ANALYSIS OF BLUETONGUE VIRAL mRNA BY QUANTITATIVE RT-PCR

Uyen T. Lam*, Janette Starks, Maggie Buccambuso, Ryan N. Jackson, Chad Dallan, Daniel F. Child, Melissa Garner, and Joseph K. -K. Li. Department of Biology, Utah State University, 5305 Old Main Hill, Logan, Utah 84322-5305

Bluetongue Virus (BTV) transmitted by an insect vector, the biting midge, infects only sheep, cattle and other ruminants but not humans. Symptoms of Bluetongue disease include fever, blue discoloration of the tongue, fetal malformation and even death. BTV remains a disease of international and economical importance. Our research objective was to determine the kinetics, stability and degradation of BTV mRNA transcripts of L1, M3, S1, and S4, which encode for viral protein VP1, NS1, VP7, and NS3, respectively. These transcripts from BTV-infected BHK-21 and A498 cells were determined and analyzed using Polymerase Chain Reaction (PCR) and quantitative Real Time Polymerase Chain Reaction (qRT-PCR) with primers designed to analyze the 3' end, middle and 5' end of each of these four BTV transcripts. After the correct viral amplicons were obtained by these two techniques, we determined the kinetics of these four transcripts and found the differential degradation of these mRNA transcripts was initiated selectively at the 3' end, the middle, and the 5' end of the BTV mRNA. The degradation of the L1 and S1 transcripts was initiated by 3' exonuclease followed by 5' decapping. Degradation of the S4 transcript started at the middle portion potentially by some cellular endonucleases, and the M3 transcript was degraded at the 5' end but not the 3' end. These results revealed the kinetics, stability, and degradation of viral mRNA in BTV-infected cells, and can be used for the screening of anti-viral agents against BTV and other viruses.

10. INVOLVEMENT OF ATLAC15 IN LIGNIN SYNTHESIS OF ARABIDOPSIS SEEDS

Mingxiang Liang¹, Elizabeth Davis¹, Dale Gardner², Xiaoning Cai¹, Yajun Wu¹
1: Utah State University, Logan, UT 84322, USA and 2: The Poisonous Plant Research Laboratory, USDA-ARS, Logan, UT 84341, USA

Laccase, EC 1.10.3.2 or *p*-diphenol:dioxygen oxidoreductase, has been proposed to be involved in lignin synthesis in plants based on its *in vitro* enzymatic activity and a close correlation with the lignification process in plants. Despite many years of research, genetic evidence for the role of laccase in lignin synthesis is still missing. We took an advantage of the Arabidopsis genome sequence and studied the Salk T-DNA insertional mutants for all the laccase genes in the genome. Of all the screened mutants available for the annotated laccase gene family, we identified two mutants for a single laccase gene, *AtLAC15* (At5g48100) with a pale brown or yellow seed coat which resembled the *transparent testa* (*tt*) mutant phenotype. A chemical component analysis revealed that the mutant seeds had nearly a 30% decrease in extractable lignin content and a 59% increase in soluble proanthocyanidin or condensed tannin compared with wild-type seeds. In an *in vitro* enzyme assay, the developing mutant seeds showed a significant reduction in polymerization activity of coniferyl alcohol in the absence of H₂O₂. Among the

dimers formed in the *in vitro* assay using developing wild-type seeds, 23% of the linkages were β -O-4 which resembles the major linkages formed in native lignin. The evidence strongly supports that *AtLAC15* is involved in lignin synthesis in plants. To our knowledge, this is the first genetic evidence for the role of laccase in lignin synthesis. Changes in seed coat permeability, seed germination and root elongation were also observed in the mutant.

11. METABOLIC INFLUENCE INSERTING SYNTHETIC GLUTAMATE DEHYDROGENASE (GDH) GENE INTO LACTOCOCCUS LACTIS SSP. CREMORIS SK11

Sweta Rajan, Balasubramanian Ganesan, & Bart C. Weimer, Utah State University, Logan, UT, USA

The hypothesis of this work questioned the role of glutamate and α -ketoglutarate during growth and metabolism to produce flavor compounds. In spite of over 30 dehydrogenase genes in the SK11 genome, addition of another dehydrogenase led to wide-scale metabolic changes. Transformation of lactococci with a vector containing *gdh* was possible only in cells that contained the *lac*-plasmid. Upon insertion of *gdh*, the plasmid pool rearranged that resulted in *gdh* transfer to the chromosome via an unknown mechanism. Despite this event, active GDH was over-produced by ~5-fold as compared to the control. Metabolite analysis during growth in laboratory media found that amino acid metabolism significantly changed with the induction of *gdh* where more than 300 unique compounds found using metabolomic analysis with LC/MS. Comparison between the intra- and extracellular fractions allowed substrate transport to be followed during the incubation time. Induction of *gdh* allowed a shunt in the TCA cycle to form between α -ketoglutarate, glutamate, and oxaloacetate to produce aspartate, which accumulated during growth. Metabolism of other keto acids also significantly changed, suggesting that all amino acid metabolism will change with the induction of *gdh*. A certain connection between aspartate, glutamate, serine, and methionine was found that led to increased production of methionine that was exported into the medium. Serine depleted from the medium as incubation progressed. Over 1,900 (of ~2,500) genes were significantly regulated with the induction of *gdh*. Genes associated with amino acid, nucleic acid, and carbohydrate metabolism changed significantly. Induction of *gdh* also led to a reduction of all the volatile sulfur compounds in the headspace. Overall, it appears that *gdh* led to the production of methionine via at least five different pathways that involved aspartate, serine, oxaloacetate, acetate, methanethiol, hydrogen sulfide, 2-oxobutanoate, and other TCA cycle intermediates. Review of all the aminotransferases and dehydrogenases in the SK11 genome indicated broad-scale changes in expression due to *gdh* induction. In conclusion, induction of *gdh* had broad impacts on cellular metabolism. Amino acid metabolism via aspartate, serine, and methionine were important for cellular metabolism along with the utilization of small organic acids. Extensive changes were found beyond the common observations in the literature.

12. PROTEOMICS OF SALMONELLA TYPHIMURIUM LT2 IN RESPONSE TO COLD STRESS.

Jigna D. Shah, Dong Chen and Bart C. Weimer, USU, Logan, UT 84322, USA.

Salmonella, a Gram-negative bacterium, is a significant food borne pathogen throughout the world that leads to substantial illness worldwide. The genus broadly causes food borne salmonellosis, which is a zoonosis of world wide economic importance. The incidence of food borne salmonellosis every year in the USA is estimated to be between 7.4×10^5 and 5.3×10^6 cases. Worldwide there are more than 1.3 billion cases of salmonellosis annually with 3 million deaths. With over 3,000 serotypes the organism has an unusual ability to adapt to many microenvironments; as a result the largest reservoir of disease in humans is often domesticated animals. A large number of *Salmonella* species infect animals that range from cold-blooded animals to nearly all primates. The virulence strategy common to *Salmonella* species is to invade the intestinal mucosa and multiply in the gut associated lymphoid tissue (GALT). The pathogens pass from the infected intestinal tissues to the regional lymph nodes, where macrophages that line the lymphatic sinuses form a first defensive barrier to prevent further spread. As *Salmonella* encounters a variety of drastically different microenvironments of the host and host defense mechanisms during its course of infection, adaptations to these conditions involve a large number of functional genes. The

defenses used to survive these encounters can be specific or can provide cross protection to a variety of hostile or stress conditions. These organisms have developed elaborate systems for sensing stress and for responding to those stresses in a protective fashion. *Salmonella* has been reported to survive in cold storage at 5°C for 8 months. Therefore, the survival of this organism at cold temperature is of concern for persistence in ready-to-eat foods that account for a substantial proportion of the infection route.

In the present study we defined the changes in global protein profiles in response to cold temperatures found in the food chain (i.e. the proteome changes associated with cold shock (30 min at 5°C) and persistent treatment with cold temperature (5°C for 336 hrs)). The aim was to define the cellular changes associated with cold shock, persistent cold exposure and the cellular changes with pre adaptation at 10°C. It was observed that *Salmonella typhimurium* LT2 grows exponentially for ~10 h, reaching mid-log phase in about ~ 5 h at 37°C. The cell population persisted for ~200 hrs and subsequently increased in density at 5°C. Cell free extracts were prepared and subjected to protein expression profiling using LC/MS/MS. The resulting data set, normalized to added BSA and enolase, was statistically analyzed for significant proteins at an FDR of 9.76%. The significant list was further sorted on the basis of COG assignments. During *in vitro* culturing in pure culture, protein expression was dominated by highly expressed factors involved in protein biosynthesis, maturation, and folding (COG J) of proteins, which accounts for cellular metabolic shifts in survival. There was a decline in all proteins in all COGs after 240 hrs of growth at 37°C, resulting in growth limitation, compared to proteins in all COGs at 240 hrs at 5°C. The proteins associated with stress (COG K) remained high during exposure to persistent cold conditions and also after pre adapting and subsequent persistent cold conditions. Proteins in COG O (protein turnover) remained high in the treatment, but declined in the control. Of note was clpX, a chaperone associated with protein stability and folding, in the samples which were given cold shock and persistent cold exposure. This was not significantly differentially expressed in the samples which were pre adapted. Treatment with cold shock induced production of proteins associated with cell division, central metabolism, and energy metabolism. This study demonstrated that *Salmonella typhimurium* LT2 is well adapted to remain viable and active during exposure to cold shock and cold persistence. These data identify specific proteins and functional categories that are involved in growth and adaptation to the effect of temperature shifts.

(POSTERS NOT ELIGIBLE FOR STUDENT COMPETITION)

13. ALTERNATE PATHWAYS FOR METABOLISM OF N-BUTANOL to N-BUTYRIC ACID BY SACCHARPCES CEREVISIAE

Balasubramanian Ganesan, Mark Signs, Tony Sorenson, Jake Michaelson, Dong Chen & Bart C. Weimer, Affiliation: Utah State University

Yeasts metabolize n-butanol to n-butyric acid, which is a key product in the flavor and fragrance industries for synthesizing esters on an industrial scale. Fatty acids such as n-butyric acid are also suitable alternatives as biofuels. We examined the mechanisms by which *Saccharomyces cerevisiae* produces fatty acids from alcohols. The production of fatty acids was alcohol-dependent, with n-butanol being the most suitable source among short chain alcohols. The ability to metabolize alcohols was also manifest only when the cells were starved of sugar and were then shifted to an alkaline pH, the lack of either condition being detrimental to fatty acid production. Initial gene expression data and metabolite production indicated that fatty acid production occurred through a two-step pathway via an alcohol dehydrogenase and an aldehyde dehydrogenase. The deletion of ALD4, the gene for the key aldehyde dehydrogenase that was specifically induced upon addition of n-butanol did not completely abolish n-butyric acid production but only reduced the quantity by ~50%. Functional genomics of the ALD4 deletion mutant in comparison to the parent strain suggested that n-butyric acid production in the mutant is also possible at least via three alternate routes, involving 34 different genes in *Saccharomyces cerevisiae*. The first alternate pathway potentially involves metabolism of n-butanol via n-butyryl-CoA and then either by an acyltransferase or substrate level phosphorylation to produce n-butyric acid and ATP. Alternatively, n-butyryl-CoA may also be metabolized via crotonyl-CoA and n-butenoyl-CoA. A third pathway proceeds via acetyl-CoA, acetoacetyl-CoA, and α -hydroxybutyryl-CoA; which then enters fatty acid biosynthesis and is

catabolized via acyl-ACP to produce n-butyric acid. At least 34 genes involved in multiple steps of all these pathways were differentially induced concomitant with n-butanol addition in the ALD4 mutant. While the alternate pathways are 30-50% less efficient in n-butyric acid production as evidenced by the concentrations, deletion of some of those genes alone either maintained or slightly improved n-butyric acid production. This suggests that n-butyric acid production may be improved by reducing the number of routes that the reaction intermediates are diverted by. The results of this study also suggest that multiple pathways are likely to be induced upon inhibition/deletion of a highly efficient step in catabolism.

14. METABOLIC RECONSTRUCTION OF PATHWAYS AND THEIR NETWORKS IN LACTOCOCCUS LACTIS SSP. CREMORIS SK11

Balasubramanian Ganesan, Jon L. Pearson, Jake Michaelson, Dong Chen, Lan-Szu Chou, Yi Xie, & Bart C. Weimer, Affiliation: Utah State University

Recently eleven genomes of lactic acid bacteria were sequenced and compared as evolution models of a phenotypically related group at the whole genome level. One of the organisms was the industrial acid producer and fermentation model *Lactococcus lactis* ssp. *cremoris* SK11. We used PathwayTools software for reconstruction and visualization of its metabolic capabilities. In order to accelerate the curation process, we developed several scripts that matched functional curation and gene/protein ontologies from other extensively curated genomes, imported literature information for each gene, and added connections to external resources for comparative and motif analysis. The manual effort required for curation was thus reduced to less than 4 h from 40 h per bacterial genome. Enzymes with putative substrate diversities were classified into all the different reactions they potentially catalyze. Any incomplete pathways with at least two or more genes required for the pathway's function were left intact in the database so as to provide opportunity for discovering novel metabolism under previously uncharacterized physiological states. Some pathways were also not appropriately assembled, and this was overcome by developing a script for visualizing metabolic networks of substrates, denoted "Pathway Webbing". This not only facilitated ease of navigation across the database but also provided a larger perspective of substrate relevance across multiple pathways. A key example is arginine catabolism that leads to production of carbamoyl phosphate, which is also the substrate for nucleotide biosynthesis. Another example is the central amino acid glutamate that networks across 150 pathways within the organism. Over 195 metabolic pathways from 500 available in MetaCyc were found in this organism, which included known mechanisms based on literature, and contained 819 enzymes and 76 transporters, as opposed to the plasmid-free *L. lactis* IL1403 which had only 151 pathways, 797 enzymes and 49 transporters. Most pathways common across multiple organism classes such as lactose catabolism and amino acid biosynthesis were accurately identified and curated using tools available within the software suite itself, while selected pathways specific to the lactic acid bacteria such as volatile fatty acid and volatile sulfur production were not identified. Comparative analysis of the lactococcal databases highlight key differences that are attributable to metabolism encoded on lactococcal plasmid important for maintenance, survival, fermentation and metabolism. The new PathwayTools database with the implementation of these visualization capabilities is publicly available for researchers at the CIB-USU's website for navigation and suggestions for curation.

