DOE/ER/15101--1

INTERFACING MICROBIOLOGY AND BIOTECHNOLOGY

CONFERENCE ABSTRACTS

DISCLAIMER

PI: Julie A. Maupin
Award Number: DE-FG02-ER15101

Description/Abstract

The 'Interfacing Microbiology and Biotechnology' Conference was held from May 17 – 19, 2001 on the University of Florida Campus in Gainesville, Florida. Over 100 faculty, post-docs, students, and research scientists from the US, Europe, and Latin America participated. The conference successfully stimulated communication and the dissemination of knowledge among scientists involved in basic and applied research. The focus of the conference was on microbial physiology and genetics and included sessions on C1 metabolism, archaeal metabolism, proteases and chaperones, gene arrays, and metabolic engineering. The meeting provided the setting for in-depth discussions between scientists who are internationally recognized for their research in these fields. The following objectives were met including:

1) The promotion of interaction and future collaborative projects among scientists involved in basic and applied research which incorporates microbial physiology, genetics, and biochemistry,
2) The facilitation of communication of new research findings through seminars, posters, and abstracts,
3) The stimulation of enthusiasm and education among participants including graduate and undergraduate students.

May 18, 2001 (Friday)

8:00 – 8:30 AM (Reitz Union - Rm 282)
Registration – Check-In / Coffee and Muffins

8:30 – 8:45 AM (Reitz Union - Rm 282)
Welcome
RICHARD JONES, Dean for Research
(University of Florida, College of Agriculture & Life Sciences)

8:45 – 10:45 AM (Reitz Union - Rm 282)
SESSION 1: C1 METABOLISM & METHANOGENS
Chair: THOMAS A. BOBIK (University of Florida, Gainesville, FL)
GERHARD GOTTSCHALK
(Inst für Mikrobiologie u Genet, Goettingen, Germany)
The Genome of *Methanosarcina mazei*, the Largest Assembly of Genetic Information in an Archaeon
ROLF THAUER
(Max-Planck Inst für Terrestr Mikro, Marburg, Germany)
Mechanism of Biological Methane Formation
KEVIN SOWERS
(University of Maryland, Baltimore, MD)
Developments in Methanosarcinal Genetics
MADELINE RASCHE
(University of Florida, Gainesville, FL)
Methanopterin Biosynthesis in Methanogens and Methyltrophic Bacteria

10:45 – 11:00 AM Coffee Break

11:00 AM – 1:00 PM (Reitz Union - Rm 282)
SESSION 2: ENVIRONMENTAL MICROBIOLOGY AND BIOREMEDIATION
Chair: JULIE MAUPIN-FURLOW (University of Florida, Gainesville, FL)
WILLIAM WHITMAN
(University of Georgia, Athens, GA)
Examination of Prokaryotic Diversity in Natural Samples
JUERGEN WIEGEL
(University of Georgia, Athens, GA)
Anaerobic Dehalogenation of PCBs: Influence of Environmental Factors on the Dehalogenation Routes
STEVE RAGSDALE
(University of Nebraska-Lincoln, Lincoln, NE)
Microbial Metabolism of Greenhouse Gases and Chlorinated Aromatics
ALFRED SPORMANN
(Stanford University, Stanford, CA)
Novel Enzymes in Anaerobic Hydrocarbon Mineralization

1:00 PM Lunch – (Reitz Union - Arredondo Room)

2:30 – 4:30 PM (Reitz Union - Rm 282)
SESSION 3: NANOCOMPARTMENTS OF PROKARYOTIC CELLS
Chair: HENRY ALDRICH (University of Florida, Gainesville, FL)
JESSUP SHIVELY (Clemson University, Clemson, SC)
The Carboxysome: A Simple Organelle that Enhances Carbon Dioxide Fixation by RuBisCO
THOMAS A. BOBIK (University of Florida, Gainesville, FL)
Nanocompartments of B12-Dependent Catabolic Processes of *Salmonella*
JOHN FLANAGAN (Brookhaven National Laboratories, Upton, NY)
Bacterial Protein Disassembly Lines: ATP-Dependent Proteases
EVERLY CONWAY DE MACARIO (Wadsworth Center, Albany, NY)
Microbial Diversity, Survival, and Stress Genes in Biotechnologically Important Ecosystems

5:30 – 9:00 PM
Reception & Poster Session (Micro Bldg - West Atrium)
Dinner (Micro Bldg – Rm 1042)

May 19, 2001

8:00 – 8:30 AM Coffee and Muffins (Reitz Union - Rm 282)

8:30 – 10:30 AM (Reitz Union Rm 282)
SESSION 4: METABOLIC ENGINEERING & REGULATION
Chair: JONG HO LEE (Sung Kyun Kwan University, Suwon, Korea)
TONY ROMEO (University of North Texas, Fort Worth, TX)
Regulation of Bacterial Metabolism and Behavior by the RNA-binding protein CsrA and the Non-coding RNA, CsrB.
JON STEWART (University of Florida, Gainesville, FL)
Engineering Whole Microbial Cells for Chiral Organic Synthesis
LONNIE O. INGRAM (University of Florida, Gainesville, FL)
Engineering Bacteria for Ethanol Production
JAMES PRESTON (University of Florida, Gainesville, FL)
Microbial Strategies for the Depolymerization of Glucuronoxylan

10:30 – 11:00 AM Coffee Break

11:00 AM – 12:30 PM (Reitz Union - Rm 282)
SESSION 5: BIOINFORMATICS AND GLOBAL GENE REGULATION
Chair: AMY GRUNDEN (North Carolina State University, Raleigh, NC)
K. T. SHANMUGAM (University of Florida, Gainesville, FL)
E. coli Gene Arrays – Analysis of Gene Regulation
STEVE BENNER (University of Florida, Gainesville, FL)
Bioinformatics Approaches to Understanding Protein Function
MICHAEL ADAMS (University of Georgia, Athens, GA)
Insights into the Metabolism of Hydrogen and Sulfur by the Hyperthermophile *Pyrococcus furiosus*

12:30 PM Lunch – (Reitz Union - Arredondo Room)

SEMINARS, MAY 17, 2001 (in order of presentation)

Biotransformation of furfural by ethanologenic bacteria: Complete conversion to furfuryl alcohol, effect on ethanol production, and the influence of selected organics on this reduction

TONY GUTIÉRREZ, MARIAN L. BUSZKO, LONNIE O. INGRAM, AND JAMES F. PRESTON. University of Florida, Institute of Food and Agricultural Science, Department of Microbiology and Cell Science, P.O. Box 110700, Gainesville, Florida, 32611-0700, USA.

The ethanologenic bacteria *Escherichia coli* strains KO11 and LYO1, and *Klebsiella oxytoca* strain P2, were investigated for their ability to degrade furfural. Using high performance liquid chromatography, and ^{13}C nuclear magnetic resonance spectroscopy, furfural was found to be completely biotransformed into furfuryl alcohol by each of the three strains in LB medium. The nature of this reduction was found to be constitutive with NAD(P)H acting as effective e⁻ donors, and glucose as an effective source of reducing power. Succinate inhibited furfural reduction, indicating that flavins are unlikely to participate in this process. During growth of these *E. coli* strains in LB medium supplemented with xylose, the effects of furfural on ethanol production were significant, with concentrations of ~ 10 mM retarding the lag (by 1 h) and decreasing the rate in ethanol production. These inhibitory effects on ethanol production suggest that furfural may act as a redox sink in redirecting reducing power away from the ethanol pathway for its own reduction to furfuryl alcohol. Since the final ethanol yields, however, were not decreased by the reduction of the furfural, this pool of redirected reducing power must have thus been replenished by some components(s) in the supplemented (LB) medium used for growing these cultures. The engineering of ethanologenic strains of bacteria to produce higher levels of furfural reductase may allow for a decrease in the time required to convert sugars to ethanol, and increase the tolerance of these strains to concentrations of furfural higher than 10 mM.

PduA is a Shell Protein of Polyhedral Inclusion Bodies Associated with the B_{12}-Dependent Degradation of 1,2-Propanediol in *Salmonella enterica* serovar Typhimurium LT2

G.D. HAVEMANN, E.M. SAMPSON, AND T.A. BOBIK. University of Florida, Institute of Food and Agricultural Science, Department of Microbiology and Cell Science, P.O. Box 110700, Gainesville, Florida, 32611-0700, USA.

The *pdu* locus of *S. enterica* encodes the genes necessary for the degradation of 1,2-propanediol. The locus has been sequenced, and a total of 23 hypothetical genes were identified. This is many more genes than required to encode the proteins for the proposed degradation scheme of 1,2-propanediol in *S. enterica*. BLASTp analyses revealed seven *pdu* genes encode proteins related to those involved in the formation of the carboxysome, an organelle found in cyanobacteria and chemoautotrophs which is thought to function in CO_2-fixation. Carboxysomes are composed of a proteinaceous shell surrounding an interior filled with the major Calvin cycle enzyme, RuBisCO. Observation of *S. enterica* cells grown aerobically or anaerobically on 1,2-propanediol by electron microscopy revealed that polyhedral inclusion bodies were synthesized, and immunolabeling demonstrated that propanediol dehydratase co-localized with these structures. As of yet, no known function has been found for these polyhedral bodies. *S. enterica* is not an autotroph and does not express RuBisCO. In order to elucidate the function of the polyhedra, studies of the carboxysomal shell protein homologues are underway. Here we report the initial characterization of PduA, a protein encoded by the *pdu* operon, which shares homology with carboxysomal shell proteins. A $His_{(6)}$-PduA gene fusion was generated and overexpressed in *E. coli*. After purification, the denatured protein was used for polyclonal antibody production. Western blots demonstrated specificity to PduA and immunocytochemistry revealed labeling localized to the periphery of the polyhedral inclusion bodies, suggesting that PduA is a component of the shell of these polyhedral inclusion bodies.

Asymmetric Reductions of β-Keto Esters Using Engineered *E. coli*

BRIAN G. KYTE, A. ANDREWS, AND J. D. STEWART. University of Florida, Department of Chemistry, P.O. Box 117200, Gainesville, Florida, 32611-7200, USA.

Two aldo-reductases, Yprlp and Gre2p, have been cloned from *Saccharomyces cerevisiae* and over-expressed in *Escherichia coli* to catalyze the whole-cell asymmetric reduction of β-keto esters. While we had initially expected that *E. coli* would be a host with no native competing enzymes, a 2,5-diketogluconate reductase first identified by Pan et al. competed with Gre2p. To solve this problem, a knockout strain in which the competing reductase has been eliminated is being developed for the expression of GRE2. The use of this and other engineered *E. coli* strains for the stereoselective β-keto ester reduction will be discussed.

Genetic analysis of formaldehyde metabolism in *Methylobacterium extorquens* AM1

CHRISTOPHER J. MARX AND MARY E. LIDSTROM. University of Washington, Dept. Microbiol., Box 357242, Seattle, WA 98195

Methylotrophic bacteria produce the toxic compound formaldehyde as a central metabolic intermediate during growth on methanol. This presents a special challenge for these organisms: to simultaneously generate a high flux of formaldehyde for optimal growth while preventing formaldehyde from accumulating to levels which would be toxic. We have identified a number of genes in the serine cycle methylotroph *Methylobacterium extorquens* AM1 that appear to play a role in preventing formaldehyde accumulation. Three of these genes, *fae*, *mtdB*, and *fmdC* encode enzymes that participate in the H_4MPT-dependent formaldehyde oxidation

pathway. Preliminary data suggest that the other genes with this mutant phenotype are involved in H_4WT biosynthesis. Strains bearing mutations in these genes not only fail to grow on methanol, but are extremely sensitive to the presence of methanol during growth on succinate. The methanol sensitive phenotype is not observed in identical mutants constructed in a methanol dehydrogenase mutant strain. Furthermore, the expression of a heterologous formaldehyde oxidation system from *Paracoccus denitrificans* that is glutathione-dependent rescues the sensitivity of these mutant strains. Our data suggest that the H_4MPT-dependent formaldehyde oxidation pathway plays a dual role in both dissimilatory metabolism and formaldehyde detoxification.

Metabolic Potential of *Erwinia chrysanthemi* PI to utilize xylan for fermentation.

ANURADHA RAGHUNATHAN AND JAMES F. PRESTON, III. Institute of Food and Agricultural Sciences. Department of Microbiology & Cell Science, University of Florida, Gainesville, FL 32611

An objective of this work is to determine the potential of *Erwinia chrysanthemi PI* (ECPI) to convert hemicellulosic biomass to biobased products, and identify relevant genes encoding enzymes with which to engineer other bacteria. The predominant polysaccharide in the hemicellulosic fraction of hardwoods and crop residues, and the source of xylose for fermentation, is glucuronoxylan (GAXn), a β-1,4- linked linear xylan with 4-O-methyl-D-glucoronic acid residues linked α-1,2 to some of xylose residues. *Erwinia chrysanthemi* PI, isolated from the dicot Anthurium sp., secretes an array of pectinases and cellulases that contribute to the maceration of plant tissue. We have now shown that this strain also secretes an endoxylanase, raising the possibility that it may be used for the fermentation of polymeric xylose in hemicellulose without the need for pretreatment. Fermentation of xylose by this organism produces predominantly butanediol and ethanol among various other products. ECPI shows sequential utilization of glucose and xylose in a mixed substrate environment, exhibiting diauxic growth indicative of a cAMP-dependent induction of xylose utilization. Although it exhibits constitutive endoxylanase activity, there is no growth on GAXn. Growth on glucose and GAXn results in the depolymerization of GAXn to limit products, which are not consumed during or after growth on glucose. Thus the xylanolytic activity of ECPI is unable to release products for its own metabolism. NMR studies indicate that addition of β- xylosidase, to a culture of ECPI (with constitutive xylanase activity), growing on glucose and glucuronoxylan, results in the release of a xylose residue that can be fermented. The β-xylosidase gene from ECPI was cloned and sequenced. Based upon deduced amino acid sequence, the β- xylosidase produced by ECPI showed 89% identity with that encoded by the bgxA gene in *Erwinia chrysanthemi* D1 isolated from corn. Thus xylanolytic strains of *E. chrysanthemi* may occur in association with dicots as well as monocots. The inability of these strains to utilize the xylanase generated products for growth 'suggest' a role that is confined to the colonization of the plant. While ECPI shows promise as a biocatalyst for the conversion of xylose to butanediol, the utilization of hemicellulose will require its engineering to produce a GH10 endoxylanase and α-glucuronidase. The well-defined secretory systems nevertheless support the further development of ECPI as a biocatalyst to produce butanediol from lignocellulosics.

Flux through citrate synthase limits growth of ethanologenic *Escherichia coli* KO11 during the fermentation of 100 g/L xylose.

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Hemicellulose hydrolysates contain 80-120 g/L of sugar, of which approximately 75% is xylose. Cells were grown in medium containing 10% xylose (simulating hydrolysate), commercial nutrients (1% corn steep liquor), and minerals. Aerobic cultures grew to cell densities of 2.5 g/L (dry weight), while anaerobic cultures reached less than 1 g/L. Surprisingly, addition of pyruvate (2 g/L) resulted in a 2-fold increase in cell mass. Acetaldehyde decreased the NADH/NAD+ ratio (whole cell fluorescence), and stimulated growth by 2-fold. Although addition of TCA pathway intermediates of the reductive arm (oxaloacetate, malate, fumarate, succinate) had no effect, addition of 2-ketoglutarate (2 g/L) increased growth 2-fold. During fermentation, the TCA pathway supplies carbon skeletons for the biosynthesis of over half the of cellular protein. Phosphoenolpyruvate carboxylase (PPC) controls the flow of carbon into the reductive arm (33% protein), while citrate synthase regulates the flow into the oxidative arm (22% protein). Our results suggest that flux through citrate synthase appears to be limiting. The native *E. coli* citrate synthase is allosterically inhibited by NADH, but this is not true of all citrate synthase enzymes. *Bacillus subtilis* has two citrate synthases, citrate synthase-I (*citA*) and citrate synthase-II (*citZ*). Citrate synthase-I is similar to the *E. coli* citrate synthase. However, citrate synthase-II is insensitive to NADH but is allosterically regulated by ATP. The *citZ* gene was cloned and expressed in *E. coli* KO11. During pH-controlled fermentations, expression of this gene at low levels resulted in a 2-fold increase in cell mass. These results confirm that there is not a mineral or energy limitation in this medium, but rather a limitation in metabolic flux through citrate synthase.

Identification of the human methylmalonyl-CoA racemase based on analysis of prokaryotic genomes: implications for decoding the human genome.

THOMAS A. BOBIK AND MADELINE E. RASCHE. Department of Microbiology and Cell Science. University of Florida. Gainesville, Florida 32611.

Prokaryotes frequently cluster genes of related function. Hence, the function of a prokaryotic gene can often be inferred from knowledge of the function of its chromosomal neighbors. Such analyses appear inapplicable to eukaryotes, since gene clustering is rare in these organisms. However, here we show that the functional information derived from analysis of prokaryotic neighbor-genes can be extrapolated to higher eukaryotes by homology searches. The genes investigated here were prokaryotic methylmalonyl-CoA mutase genes and their chromosomal neighbors. Two groups of homologues were found that frequently cluster with prokaryotic mutase genes, and both gene groups had homologues in the human genome. Because methylmalonyl-CoA mutases are involved in the metabolism of propionyl-CoA, we inferred that conserved neighbors of methylmalonyl-CoA mutase genes and their human homologues were also involved in this process. Subsequent biochemical studies confirmed this inference by showing that the prokaryotic gene PHO272 and its human homologue both encode DL-methylmalonyl-CoA racemases. This demonstrates that the analysis of prokaryotic neighbor genes can be extrapolated to higher eukaryotes by homology searches. This approach may also have allowed the identification of a putative human disease gene since defects in DL-methylmalonyl-CoA racemase may cause methylmalonyl aciduria. Given the large number of genomes sequences that are available, extrapolated neighbor analyses can be expected to have broad applicability and will likely make an important contribution to decoding the human genome.

POSTERS, MAY 17 - 18, 2001
(in order of presenting author*)

Gene Expression Analysis of *Escherichia coli* Growing under Aerobic and Anaerobic Conditions.

HAN TAO*, K.T. SHANMUGAM AND L. O. INGRAM. Department of Microbiology and Cell Science. University of Florida, Gainesville, FL 32611.

As a facultative anaerobe, *Escherichia coli* has a versatile metabolic lifestyle which allows aerobic growth as well as fermentation of sugars to useful products. Upon the switch from aerobic to anaerobic conditions, expression of several genes are significantly altered. Using physiological and genetic tools, several of these genes have been identified. Since the function of more than 50% of the genes in the *E. coli* genome is yet to be identified, it is possible that the expression of many more genes is also be regulated by the availability of oxygen. Recent advances in functional genomic technologies such as DNA microarray construction provide a unique way to explore the metabolic and genetic control of gene expression at the organismic level. In this study, the gene expression profile of aerobic and anaerobic cultures of *E. coli* was determined using *E. coli* gene array.

Altered Sugar Utilization Patterns by *Eshcerichia coli* Carrying Plasmid-Encoded Xylose Operons with cAMP/XylR-Independent Promoters

F. G. HEALY*, M. L. BUSZKO, H. TAO, R. GONZALEZ, K. T. SHANMUGAM. AND L. O. INGRAM. University of Florida, Institute of Food and Agricultural Science, Department of Microbiology and Cell Science, P.O. Box 110700, Gainesville, Florida, 32611-0700, USA.

When grown on sugar mixtures, *Escherichia coli* preferentially consumes sugars that support the highest growth rate. For example, the utilization of xylose in medium containing glucose and xylose proceeds only after the depletion of glucose. "Catabolite repression" and "inducer exclusion" are familiar mechanisms by which *E. coli* regulates the sequential utilization of glucose followed by other PTS or non-PTS carbohydrates when grown in mixed sugar medium. We are interested in cellular responses to alterations in carbon flux patterns and other physiological changes that may occur under conditions when transcriptional-level glucose repression is removed. The cAMP-CRP- and XylR-dependent expression of genes required for xylose transport and metabolism (*xyl* genes) has been confirmed through various genetic, biochemical, and genomics approaches. In order to advance our understanding of the regulation of carbon utilization through primary metabolic routes beyond the level of gene transcription, we are creating conditions in which cells are constitutively expressing *xyl* genes in glucose/xylose-containing medium. Under such conditions, pentose sugars should be made available for metabolism through the pentose phosphate reaction sequence at the same time that glucose is available for PTS-mediated uptake and metabolism via the Embden-Meyerhof-Parnas pathway. Using heterologous DNA fragments as cAMP-CRP- and XylR-independent promoters of plasmid-encoded *xyl* genes, we constructed constitutively expressed *xyl* operons and evaluated their ability to utilize glucose and xylose simultaneously. Clones that simultaneously utilized xylose and glucose were identified, as well as clones that preferentially utilized xylose. Although these studies show that xylose utilization is preferred over glucose in these constructs, the growth rate of *E. coli* in the presence of both sugars was the same as that of xylose alone. This lower growth rate and the availability of glucose imposed a selection for variants that utilized glucose and grew at higher rates. Mechanism(s) underlying this phenotypic reversion are under investigation.

Structure and Function of Proteasomes from a Halophilic Archaeon, *Haloferax volcanii*

STEVEN J. KACZOWKA*, HENRY C. ALDRICH AND JULIE A. MAUPIN-FURLOW. Department of Microbiology and Cell Science, University of Florida, Gainesville, FL.

The 20S proteasome, a multicatalytic proteinase composed of four stacked heptameric rings, appears to be ubiquitous to the Archaea and Eukarya. The proteasomal rings are made up of two families of subunits designated α and β. In the Eukarya these rings are composed of up to 14 different subunits; whereas, in the Archaea the majority of 20S proteasomes are composed of a single α and β subunit. Among the Archaea, *Haloferax volcanii* is a unique model system in that it contains two α subunits and a single β subunit.

The two proteasomal α subunits from *H. volcanii* were separately produced, with C-terminal histidine tags, from expression vectors in *H. volcanii* and purified by Ni^{2+}-NTA chromatography. Based on Western blot analysis it appears that the α_1 subunit co-purifies with the α_2 subunit in active peptide hydrolyzing proteasome complexes. Further evidence from both *H. volcanii* and recombinant *Escherichia coli* indicates the two α subunits can associate together as heterogeneous tetramers and heptamers. Analysis of α_1 and α_2 protein separately purified from recombinant *E. coli* indicates that each is capable of forming rings/discs. A time course of *H. volcanii* grown in batch culture indicates the two proteasomal α subunits appear to differ in the levels present during growth as determined by Western blot analysis. The levels of α_1 protein appear to be constitutive throughout the period of the time-course, whereas the α_2 protein only starts to appear at significant levels during the early through to the late stationary phases of growth.

Developments of an Expression Vector for the Methane-Producing Archaeon *Methanococcus maripaludis*.

WARREN L. GARDNER, TIFFANY A. MAJOR*, WILLIAM B. WHITMAN. Department of Microbiology, University of Georgia, Athens, GA 30602-2605

Archaeal shuttle vectors are under development in our laboratory for expression of proteins that cannot be produced in bacterial systems and for complementing mutations. Because little is known about the mechanisms of replication and copy number control in archaeal plasmids, this topic is of interest as well. In previous work, the expression shuttle vector pWLG30+lacZ was constructed from the cryptic methanococcal plasmid pURB500, the moderately strong *Methanococcus voltae* promoter PhmvA, the puromycin resistance cassette, and pUC. Tn7-based transposon mutagenesis was performed on pWLG30+lacZ to identify regions essential for replication in methanococci. Eight mutants contained transpositions within pURB500-derived open reading frames (ORFs). The two mutants with insertions in ORF1 failed to transform *M. maripaludis*, suggesting that ORF1 was necessary for replication. The other six mutants transformed at frequencies comparable to the parental shuttle vector and, therefore, did not appear to be essential. The levels of expression of ß-galactosidase in transformants of these mutants varied from 62% to 210% of the levels observed in transformants of the parental plasmid. Therefore, the copy number of the mutants was probably not greatly changed. Based upon these results, the vector pWLG40+lacZ was constructed by removal of 2.4 kb of methanococcal DNA from pWLG30+lacZ. Upon transformation into *M. maripaludis*, the expression of ß-galactosidase was identical to transformants of pWLG30+lacZ. Finally, the PhmvA promoter of pWLG40+lacZ was replaced with the strong archaeal surface layer protein promoter Psla. This new construct will be transformed into *M. maripaludis* to determine whether promoter strength affects ß-galactosidase expression.

Genetic analysis of formaldehyde metabolism in *Methylobacterium extorquens* AM1

CHRISTOPHER J. MARX* AND MARY E. LIDSTROM. University of Washington, Dept. Microbiol., Box 357242, Seattle, WA 98195. (as above for seminar)

Influence of the alpha subunit on the proteolytic activity of 20S proteasomes.

MARK S. OU, HENRY C. ALDRICH, AND JULIE A. MAUPIN-FURLOW. Department of Microbiology and Cell Science, University of Florida, Gainesville, FL.

The 20S proteasome is a 600- to 700-kDa multicatalytic protease complex ubiquitous to both the Eucarya and the Archaea which plays a major role in the turnover of proteins in the cell. The 20S proteasome is composed of α- and β-type subunits in a cylinder of four stacked, seven-membered rings in a $\alpha_7\beta_7\beta_7\alpha_7$ configuration. The proteolytically active sites responsible for peptide bond cleavage are sequestered within a central chamber formed by the β subunits; however, the role of the α-rings and the antechambers formed by the α/β-ring interface has not been well-studied.

To examine the role of the α subunit, we have purified and characterized 20S proteasome chimeras of *Methanosarcina thermophila* and *Thermoplasma acidophilum* α and β subunits (Mtα-Taß-20S and Taα-Mtβ-20S proteasomes). Electron micrographs show that both chimeric complexes form structures of four stacked rings with central channels like those of 20S proteasomes. The peptide hydrolyzing activities of the Mtα-Taß-20S and Taα-Mtβ-20S proteasomes were similar to that of 20S proteasomes from *T. acidophilum* and *M. thermophila*, respectively. Similar results were seen from hydrolysis of the 30 amino acid oxidized insulin chain B protein examined by reversed phase high-performance liquid-chromatography. However, hydrolysis of the 123 amino acid α-lactalbumin protein

revealed distinct differences in the composition of peptide products produced. Peptide products were identified by mass spectroscopy and showed distinct differences in the cleavage patterns between the different 20S proteasomes. These results reveal that the α subunits in the 20S proteasome can influence the types of products genereated during the hydrolysis of larger proteins without influencing the cleavage specificity of synthetic peptide and smaller protein substrates. These results also show that the protein domains required for assembly of 20S proteasomes are relatively conserved in the Archaea.

Three dimensional structure of *Thiobacillus neapolitanus* carboxysomes as determined by cryo-electron microscopy.

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Carboxysomes, found in certain photoautotrophs and chemoautotrophs, are polyhedral structures delimited by a proteinaceous shell and containing RuBisCO. Viewed in thin section, they exhibit hexagonal profiles. Cryo-electron microscopy of carboxysomes of *Thiobacillus neapolitanus* reveals that the three dimensional carboxysome structure is a regular polyhedron. Previous structural studies using negative stain methods have concluded that they are either icosahedral or dodecahedral. These two structures are related, and both have 5:3:2 symmetry. To obtain a more definitive structure, isolated carboxysomes were frozen in ethane and frozen-hydrated samples were photographed using a JEOL JEM 1200-EX transmission electron microscope operating at 100 kV. Images were recorded at a dosage of 5 to 10 electrons per square Angstrom at a magnification of 30,000X. Using a suite of virus reconstruction programs that search for 5:3:2 symmetry and which have been extensively tested, we analyzed the images and found that the carboxysomes are neither dodecahedral nor icosahedral. Having determined this, we decided to take a closer look at the data. Two major observations were noted from further study of the images. One, all of the images were six-sided polyhedrons, and two, the structures were somewhat heterogeneous in size. The six sided nature allowed us to assume a D6 symmetry, a polyhedron that explains the six sided projections observed in all micrographs. We processed the data assuming this symmetry and were able to reconstruct a stable structure. The structure appears to be unrelated to any conventional virus-like structure. The data are consistent with the conclusion that the carboxysome is a D6 polyhedron with a 6-fold axis of symmetry along the top view and two 2-foid axes of symmetry perpendicular to the 6-fold axis and along the side views.

Overexpression and purification of CprK, a putative transcriptional regulator in *ortho*-Chlorophenol respiration of *Desulfitobacterium dehalogenans*.

STELIAN M POP*, ALLEN W. TSANG AND STEPHEN W. RAGSDALE. University of Nebraska. Dept. Biochemistry, Lincoln, NE 68588.

Desulfitobacterium dehalogenans, like other dehalorespiring organisms, derives energy from the use of halogenated aromatic compounds as an electron acceptor. The *cprK* gene, one of the 8 genes identified in the *cpr* (chloro-phenol reductive) gene cluster, is constitutively expressed at a low level and encodes a putative transcriptional regulator of the *cpr* gene cluster during dehalorespiration from *Desulfitobacterium dehalogenans* (Hauke *et al.* 2000. *J. Bacteriol.* 182:5683-5691). In order to understand the regulatory system, we are using biochemical and genetic approaches. The *cprK gene* was cloned and overexpressed in *Escherichia coli* using the pQE60 overexpression system, which contains a 6xHis tag sequence, The purification of CprK was performed using a Ni-NTA affinity column, yielding ~90% homogeneity. The purified product was confirmed by N-terminal amino acid sequence analysis. Although CprK belongs to the CRP-FNR family, the results of metal analysis indicate that CprK is not a [Fe-S] containing protein like FNR. Therefore, the activation of CprK likely involves response to the chlorinated compound rather than redox conditions or the level of oxygen. Does CprK respond directly to the halogenated substrate? Or is there an interaction with another component(s) of the transcription regulation system, for example, CprC? In order to dissect the *in vivo* function of CprK and to answer these questions, several methods, including the gel shift mobility assay, are being exploited.

Pyruvate decarboxylase from *Zymobacter palmae*: Cloning, Sequencing and Biochemical Characterization.

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Pyruvate decarboxylase (PDC) is the key enzyme in ethanol fermentation. PDC is distributed widely in fungi and plants but rare in bacteria. To date sequence of only one bacterial *pdc* gene from *Zymomonas mobilis* has been reported. In this study, a *pdc* gene was isolated from a gram-negative bacterium *Zymobacter palmae*. The *pdc* gene (1668 bp) encodes for 556 amino acids and the deduced amino acid sequence showed identity of 62% to *Z. mobilis* PDC, 41% to *Zea may* (plant) PDC and 28% to baker's yeast PDC. A high level (30-35 U/mg) of *Z. palmae* PDC activity was expressed in recombinant *E. coli*. The PDC purified from *Z. palmae* and recombinant *E. coli* showed a molecular mass of 55 kDa on SDS-PAGE. The active PDC eluted at 228 kDa in gel filtration chromatography revealing tetrameric conformation. *Z. palmae* PDC was not activated by substrate. It exhibited a K_m of 0.23 to 0.27 mM for pyruvate and k_{cat} of 20520. The spectrophotometric titration demonstrated that modification of 4 of 10 Cys residues in *Z. palmae* PDC resulted in complete inhibition of the activity consistent with all other PDCs.

Microbial Diversity in an Acidic Refinery Sludge

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Sludge samples were obtained from a 100 year old Czechowice oil refinery near Czechowice-Dziedzice, Poland. The aged sludge is acidic (pH 2) and composed of asphaltics highly contaminated with polycyclic aromatic hydrocarbons (PAHs). Additionally, spent catalysts, heavy metals, diatomaceous earth, silica gel, and coal fly ash was deposited at this site. Approximately 120,000 tons of this waste was deposited in unlined lagoons 3 meter deep covering 3.8 hectares. A total of 45 bacteria, 68 fungi, and 7 yeast sp. were isolated from the sludge on an acidic minimum medium exposed to naphthalene vapor. A subset of the isolates were characterized by classical taxonomic criteria, BIOLOG©, and analysis of SSU rRNA genes. A number of bacteria grouped within the Proteobacteria and were related to *Ralstonia*, *Pseudomonas*, *Stenotrophomonas*, and *Achromobacter* spp. The most commonly encountered bacterial genus had high nucleotide sequence similarity (98-100%) to *Ralstonia* sp. KN1 (8 isolates). In addition several bacteria belonging to the Actinobacteria (high G+C gram positives) and Firmicutes (low G+C gram positives) were identified. Five of the *Ralstonia* spp. demonstrated dihydroxylating dioxygenase activity by their ability to able to convert indole and indole derivatives to indigo. Several of *Ralstonia* spp. and a *Bacillus* sp. grew on catechol as a sole carbon source and 2,3- catechol dioxygenase activity was most commonly observed. The naphthalene degradation products; dihydroxynaphthalene, trans-o-benzylidene pyruvate, and salicylic acid were identified with *Ralstonia*. Four bacterial isolates were able to clear phenanthrene crystals sprayed onto agar plates and of these one was also able to degrade acenaphthene, fluorene, fluoranthene, pyrene, and benzo[b]fluoranthene. Some fungal isolates that grew on naphthalene vapor represent taxa that have not been previously reported to degrade PAHs. This international project that is part of a joint effort between the US Department of Energy and the Polish Institute for Ecology and Industrial Areas to develop efficient bioremediation strategies for treatment of acidic- and petroleum sludge-impacted soils.

Molecular and Biochemical Investigations of Polycyclic Aromatic Hydrocarbon Degradation by *Sphingomonas paucimobilis* EPA505

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Catabolic genes, a regulatory mechanism, and metabolic pathways for naphthalene (NAP), phenanthrene (PHE), and fluoranthene (FLA) were previously defined in *Sphingomonas paucimobilis* EPA505. In the present study, cosmid clones containing 30-45 kb DNA fragments harboring a Tn*5* insertion from plasmid DNA of EPA505 mutants defective in NAP, PHE, and/or FLA were subcloned and sequenced to identify other catabolic genes. Approximately 2.4 kb of a total of 5.2 kb sequenced shared significant homology (85%) to plasmid pNL1 of *S. aromaticivorans* strain F199. Significant amino acid homology was found to known catabolic enzymes; dioxygenase subunits, a dehydrogenase, an aldolase-hydratase. Similarities were also found to IS-like elements, transposases, integrases, transcriptional regulators, heavy metal resistance, and a conjugative plasmid factor. Additionally, there was unexpected significant matches to to *Caulobacter* and nitrogen fixing *Rhizobium* and *Agrobacterium* spp. The substrate range of EPA505 with aromatic compounds was tested. EPA505 was able to utilize mono- and dicyclic aromatics including chlorodinitrobenzene (CDNB). One particular NAP/PHE mutant that accumulated *meta* ring fission products was also defective in glutathione-*S*-transferase activity. EPA505 grew on anthracene but was unable to utilize acenaphthene (ACE), fluorene (FLE), pyrene (PYR), chrysene (CHR), and benzo[b]fluoranthene (B[b]F) as primary substrates. ACE and CHR were not degraded to detectable catabolic intermediates. During incubation with FLE, both 9-fluorenone and *trans*-o-hydroxyindanyl oxobutenoic acid accumulated but hydroxy-indane was also produced. 10-Hydroxy-1-phenanthroic acid was the final catabolite detected with PYR, denoting *ortho* ring fission. Phenanthrene anhydride was the final B[b]F degradation product indicating *meta* ring fission.

Overexpression and Activation of Reductive Dehalogenase (CprA) from *Desulfitobacterium dehalogenans* in *Escherichia coli*.

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Certain anaerobic bacteria catalyze the dehalogenation of chemical organic compounds. The reductive dehalogenase (CprA) from *Desulfitobacterium dehalogenans* is an iron-sulfur and corrinoid containing enzyme. A key enzyme in dehalorespiration pathway, it catalyzes the reductive removal of chlorine atom from 3-chloro-4-hydroxyphenylacetate (CL-HPA) to yield hydroxyphenylacetate (HPA). Although several reductive dehalogenases have been overexpressed in *E. coli*, they are found in inclusion bodies without activity. We present evidence that the CprA protein is found in the soluble fraction when it is co-overexpressed with trigger factor (TF) and GroEL-GroES by using a pQE60 overexpression system. This result suggests that the role of GroEL-GroES in the activation of CprA occurs through facilitation of the correct folding. The product of the reaction was identified by thin layer chromatography. A gene cluster involved in *ortho*-chlorophenol respiration has been isolated from *Desulfitobacterium*

dehalogenans (Smidt et al. 2000). So far, except for *cprA*, there are no genetic or biochemical data to support any biological function for the rest of the *cpr* genes. Our findings show a connection between *cprA* and some of the undefined *cpr* genes.

Purification and characterization of the 20S proteasome and PAN from *Methanococcus maripaludis*.
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The 26S proteasome, composed of a 20S core and a 19S regulatory particle (RP), is responsible for energy-dependent degradation of ubiquitinated proteins in eucaryotic cells. 20S core subunits as well as proteins with similarity to the regulatory particle triphosphatase (Rpt) subunits of the RP are conserved in the Archaea. Previously, we reported the purification and characterization of the 20S proteasome and an Rpt-like protein from *Methanococcus jannaschii*. The Rpt-like protein stimulated 20S proteasome-mediated protein hydrolysis in the presence of nucleotide triphosphates, and was designated PAN (proteasome-activating nucleotidase). In this study, *Methanococcus maripaludis* was chosen as a model organism due to its close relationship to *M. jannaschii* as well as the genetic tools available for this mesophile. The *M. jannaschii pan* gene was used as a probe to isolate the related gene from *M. maripaludis*. The deduced amino acid sequence of the isolated ORF was 70% identical to *M. jannaschii* PAN (MjPAN), and was synthesized in recombinant *E. coli*. *M. maripaludis* PAN (MmPAN) cross-reacted with an anti-MjPAN antibody and formed a ~600-kDa complex. MmPAN was also less heat-stable than MjPAN and aggregated at 50°C. During attempts to isolate MmPAN directly from *M. maripaludis*, two complexes of ~200-kDa with NEM-inhibitable nucleotidase activity were identified, neither of which cross-reacted with anti-MjPAN. A 20S proteasome was also isolated which hydrolyzed the substrate LLVY-Amc at an optimum of ~80° C. Finally, the genes expressing His_6-tagged MjPAN and MmPAN have been placed into the expression shuttle vector pWLG40 and synthesized in recombinant *M. maripaludis* for further characterization.

Oligosaccharides Containing Glucuronoxylose as Substrates for Selecting Bacteria for Depolymerization of Hemicellulose.
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Hemicelluiose from plant biomass is an important renewable energy resource for production of ethanol and other bio-based products. The predominant polysaccharide in hardwood hemicelluiose is 4-O-methylglucuronoxylan (MeGAXn). Microbial strategies for the metabolism of MeGAXn invoke a battery of enzymes, including secreted β-xylanases and intracellular α-glucuronidases, for the conversion of MeGAXn into fermentable substrates. In those few cases for which definitive information exists, the α -glucuronidases are specific for 4-O-methyl-D-glucuronopyranosyl-α-1,2-xylopyranosyl-β-1,4-xylopyranosyl-β-1,4-xylose (MeGAX3), the product generated by one of the endoxylanases. Industrial processing of lignocellulosics for production of ethanol utilizes acid hydrolysis to release free xylose and oligosaccharides containing 4-O-methyl-D-glucuronic acid (MeGA) linked to one or more xylose residues. We have defined conditions for the selective acid hydrolysis of MeGAXn from sweetgum (Liquidambar styraciflua) to release desired amounts of MeGA, MeGAX1, MeGAX2, and MeGAX3. These have been isolated by preparative column chromatography and structurally characterized by HPLC, capillary electrophoresis (CZE), and 13C-NMR spectroscopy. These oligosaccharides have served in identifying bacteria able to convert the products generated by acid hydrolysis of lignocellulosics to constituent carbohydrate residues. Selected bacteria will then serve as a source of genes encoding α-glucuronidases and β-xylanases with which to engineer bacteria for fermentative conversion of hemicellulose fractions to bio-based products.

SEMINARS, MAY 18-19, 2001
(in order of presentation)

SESSION 1: C1 METABOLISM AND METHANOGENS

The genome of *Methanosarcina mazei*, the largest assembly of genetic information in an Archaeon
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The genome of *Methanosarcina mazei* comprises approximately 4 megabases. The information present not only includes the genes for methanogenesis from H2 + CO2 but also double- or triple-sets of genes encoding methyltransferases for methylamine utilization, genes for membrane-integrated and proton-translocating pyrophosphatases, the proton-translocating F420H2-dehydrogenase complex and for cytochrome and methanophenazine synthesis. A significant percentage of genes is of bacterial and eukaryal origin.

Mechanism of biological methane formation
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Methane is a quantitatively important end product of the anaerobic microbial decomposition of organic matter. It is formed by strictly anaerobic microorganisms all belonging to the archaea and in all methanoarchaea by the same reaction, namely the reduction of methyl-coenzyme M (CH_3-S-CoM) with coenzyme B (HS-CoB) to CH_4 and the

heterodisulfide CoM-S-S-CoB. This reaction is catalyzed by methyl-coenzyme M reductase which harbors a nickel porphinoid prosthetic group F430 which has to be in the Ni(I) oxidation state for the enzyme to be active. The crystal structure of the nickel enzyme has been elucidated and a catalytic mechanism involving a coenzyme M thiyl radical and a heterodisulfide anion radical as intermediates has been proposed (1). In the active site region of the methyl-coenzyme M reductase, close to where the coenzyme B sulfur in the enzyme product complex is located, a thiopeptide bound thioglycine was found to be conserved (2). Thiopeptides can be reduced by one electron generating a thioketyl radical at a redox potential predicted to be more positive than that of a disulfide anion radical ($E^{0'}$ = -1.6 V) but still negative enough for the reduction of Ni(II) F_{430} to Ni(I) F_{430} ($E^{0'}$ = -650 mV) considered to be the final step in the catalytic cycle. It is therefore proposed that the reduction of Ni(II) F_{430} to Ni(I) F_{430} involves a thioketyl radical as intermediate. Results supporting the hypothesis will be discussed.

(1) Ermler, U., Grabarse, W., Shima, S., Goubeaud, M. & Thauer, R.K. (1997) *Science* 278, 1457-1462.
(2) Selmer, T., Kahnt, J., Goubeaud, M., Shima, S., Grabarse, W., Ermler, U. & Thauer, R.K. (2000) *J. Biol. Chem.* 275, 3755-3780

SESSION 2: ENVIRONMENTAL MICROBIOLOGY AND BIOREMEDIATION

Examination of prokaryotic diversity in natural samples

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A compilation of direct cell counts from a variety of microbial habitats suggests that the total number of prokaryotes on earth is on the order of 4-6 x 10^{30} cells and that the prokaryotic biomass is 350-550 Pg of carbon or comparable to that found in plants. Even though most of the prokaryotic cells are found in the subsurface, prokaryotic productivity appears to be higher in the oceans and soil. Multiple simultaneous mutations in widely distributed genes in these environments must be very frequent, with up to five simultaneous mutations occurring in a single gene somewhere within the population every 20 minutes. Thus, the very large population size of prokaryotes provides at least a partial explanation for its enormous diversity, and attempts to understand this diversity are a special challenge.

The definition of a prokaryotic species depends in part on the extent of hybridization of the genomic DNA yet many organisms abundant in the environment are known only by their 16S rRNA gene sequence. The relationship of 16S rRNA sequence similarity (S) to DNA hybridization (D) was found to be well described by the equation: ln(-lnD) = 0.53[ln(-lnS)] + 2.201 when D was determined by either the S1 nuclease or membrane method. When the presence of nonultrametric sequences and differences between taxa were controlled, this relationship accounted for 78 % of the variability of D given S and could be used to estimate the distribution of D from S. Thus, D < 0.70 would be expect to occur 50, 95 and 99 % of the time when S is 0.998, 0.992, and 0.986, respectiveiy. This relationship suggests that many prokaryotic species are yet to be described. For instance, 167 out of 230 soil isolates from nonselective media possessed an S < 0.99 to a described species and are likely to represent novel species.

Microbial communities are frequently described by preparing libraries of the 16S rRNA genes by PCR amplification of DNA extracted directly from environmental samples. A method of analysis was developed to compare sequence libraries of this type. First, the libraries were described by a homologous coverage curve $C_x = 1 - (N_x/n)$ where N_x is the number of unique sequences in library X and n is the total number of sequences and a heterologous coverage curve $C_{xy} = 1 - N_{xy}/n$ where N_{xy} is the number of sequences in library X not found in library Y when both C_x and C_{xy} are defined over a range of 16S rRNA sequence similarities. If both libraries are from the same sample, the differences between the homologous and heterologous coverage curves should be small. Thus, a test for the significance of the differences in these curves is a test for whether or not the libraries are from different samples. To verify this method, random selections from the same library were not found to be different while libraries from different environmental samples where shown to be different. This method of analysis should have general utility for comparing rDNA libraries from environmental samples.

Anaerobic dehalogenation of PCBs: influence of environmental factors on the dehalogenation routes.

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Reductive dechlorination of sediment PCBs in Aroclor 1260-contaminated Woods Pond (Lenox, MA) sediment samples and in non PCB- contaminated lake sediment samples from Athens, GA were investigated. The influence of environmental factors such as pH (pH 5.0 to 8.5), temperatures (4 to 56EC) and H2-concentrations (0 and 10 %) on transformation rates and on the dehalogenation paths were studied. The T-studies included experiments were T-shifts were used to simulate seasonal changes. Interestingly the effect of those changes differed from those obtained with a constant temperature. Generally, sediment slurries were amended with 350 :M 2,3,4,6-tetrachlorobiphenyl (2346-CB) as a primer for dechlorination, since the dechlorination of sediment PCBs occurred only in non-autoclaved samples amended with 2346-CB: no substantial dechlorination of sediment PCBs was detected at any tested temperature within a year in autoclaved control samples or in non-amended samples. Dechlorination of sediment PCBs occurred mainly via loss of *meta* and *para* chlorines. Different dechlorination processes, however, were observed at the various temperature ranges as well as pH-ranges. Whereas at most temperatures a high reproducibility was observed, at temperatures around 18 to 30EC relatively high variations occurred among triplicates or repeated experiments. We speculate that the variations are due to competition among the different PCB-dechlorinating as well as other non-dechlorinating microorganisms in the microbial communities developing at these

temperatures. These above parameters were utilized to enrich samples with specific activities and yielded partly enrichment with very narrow substrate spectra, indicating that the overall reductive dehalogenation of PCB requires and involves many different dehalogenating microorganisms.

Microbial Metabolism of Greenhouse Gases and Chlorinated Aromatics

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During the last two centuries, overpopulation and chemical technology have burdened all life forms with problems like global warming and the introduction of hazardous chemicals into our environment. Biotechnology offers the promise of combating these problems to create a safer and cleaner Earth. At the Earth Summit in Kyoto in 1997, world leaders recognized the need to reduce emissions of CO_2, methane, and other greenhouse gases. This lecture will describe our efforts to understand the process of methanogenesis and to inhibit ruminant methanogenesis. Successful achievement of this objective has the potential to mitigate global warming since production of this potent greenhouse gas (21-fold more potent than CO_2) by cattle alone accounts for 11% of world methane output. Elimination of Persistent Organic Pollutants (POPs) is another important objective. The United Nations aims to finalize a global treaty to phase out and eventually eliminate the 'dirty dozen' POPs, which includes several organohalogens, such as PCBs. Bioremediation offers a possibility of combating these health and environmental risks. This lecture will describe our efforts to understand how a class of anaerobic bacteria (the Desulfitobacteria) sense, alter their patterns of gene expression, and catalyze the dehalogenation of the organohalogen compound.

SESSION 3: NANOCOMARTMENTS OF PROKARYOTIC CELLS

The Carboxysome: A Simple Organelle that Enhances Carbon Dioxide Fixation by RuBisCO.

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The carboxysomes of *Halothiobacillus neapolitanus* are composed of at least six peptides in addition to the large (CbbL) and small (CbbS) subunit peptides of ribulose bisphosphate carboxylase/oxygenase (RuBisCO). These six peptides, labeled CsoS appear to be structural shell components. CsoS1A, B, and C, the major shell peptides, are encoded by a three-gene repeat. A single gene encodes CsoS2A and B, glycosylated peptides differing in the degree of glycosylation. CsoS3 is present in minor amounts. The genes encoding all of these peptides along with those for CbbL/CbbS as well as ORF A and B, an uncharacterized gene repeat, constitute a carboxysome operon. The *cso* operon of either *Acidithiobacillus ferrooxidans* or *Thiomonas intermedia* is nearly identical to that of *H. neapolitanus*. In *Thiobacillus denitrificans*, *cbbL/cbbS* is not located in a putative *cso* operon. The CsoS1 peptides of the four thiobacilli are nearly totally conserved and ORF A and B exhibit a great deal of conservation. CsoS3 shows 38–49% identity and CsoS2 only 23-39%. However, close examination of the CsoS2 sequences reveals some unusual conservation. For example, the amino acid sequence [V, L, or I, (M)] – [S or T] – [G] aligns 21 times. The function of this repeat has not been elucidated.

The structure of carboxysomes has been reported to be either icosahedral or dodecahedral. Our electron cryomicroscopy studies contradict both of these conclusions and suggest that the carboxysomes of *H. neapolitanus* are D6 polyhedra.

The carboxysome operon of *H. neapolitanus* has been expressed in *Escherichia coli*. The peptide profile is nearly identical with that of purified carboxysomes. Electron microscopy reveals that carboxysomes are present in some cells. Experiments to optimize carboxysome assembly are in progress.

The carboxysome peptides (Ccm) of cyanobacteria are proposed to be part of a carbon concentrating mechanism. CcmK and L exhibit a great deal of homology to the CsoS1 and ORF A and B peptides of the thiobacilli, respectively. Recently, polyhedral bodies, enterosomes, have been demonstrated in several enteric bacteria. The enterosomes of *Salmonella enterica* are products of the ethanolamine and propanediol operons. Several of the peptides encoded by these operons show significant homology to either the CsoS1/CcmK or ORF A-B/CcmL groups presented above. How the polyhedra meet the metabolic needs of the organisms is under intense investigation.

Nanocompartments of B12-Dependent Catabolic Processes of *Salmonella*

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Salmonella enterica degrades ethanolamine and 1,2-propanediol as carbon and energy sources in a coenzyme B12-dependent fashion. The genes required for these process are found in the ethanolamine utilization (*eut*) operon and at propanediol utilization (*pdu*) locus, respectively. Both sets of genes include multiple homologues of genes involved in the formation of nanocompartments known as carboxysomes. Electron microscopy showed that *S. enterica* forms polyhedral bodies about 150 nm in cross-section when grown on either ethanolamine or propanediol, but not when grown on other carbon sources. Immuno-electron microscopy showed that coenzyme B12-dependent diol dehydratase

is associated with the polyhedral bodies formed by *S. enterica* during growth on 1,2-propanediol. Each of the *pdu* genes that encodes a homologue of a gene reported to be involved in carboxysome formation was deleted by linear transformation with PCR products. With respect to polyhedral body formation, these mutants fell into three classes: 1.) polyhedral body formation was abolished; 2.) polyhedral bodies were abnormally shaped; 3.) apparently normal polyhedral bodies were formed. However, even in mutants strains where polyhedral bodies formation was abolished growth on 1,2-propanediol was essentially wild type. We propose that the polyhedral bodies formed by *Salmonella* during growth on 1,2-propanediol consist of a protein shell that encases coenzyme B12-dependent diol dehydratase and perhaps other enzymes. However, the function these nanocompartments remains unknown.

Bacterial Protein Disassembly Lines: ATP-Dependent Proteases.
JOHN FLANAGAN. Brookhaven National Laboratories, Upton, NY.

Cells must be able to sense, and rapidly adapt to a wide range of environmental stresses. In many cases, survival requires the large scale remodeling of the cellular machinery that is essential for life. The protein content of a cell is regulated both transcriptionally/translationally to control the synthesis of new polypeptides and also post-translationally, through controlled degradation (protein turnover) of damaged or unneeded proteins. The mechanisms by which this latter process occurs are only now being elucidated.

In cells, the majority of cellular protein turnover requires energy, a constraint that contributes in a large part to the tight regulation of this process since uncontrolled proteolysis would have catastrophic consequences for cells. The majority of non-lysosomal intracellular proteolysis is carried out by a few, large, oligomeric, energy-requiring, proteases that share a barrel-like architecture and, by extension, a common mechanism. One key feature, unique to this class of protease, is that substrate degradation occurs processively, which means that the proteins being degraded are hydrolyzed to short peptides in a single cycle of degradation. These short peptides are recycled to amino acids by various peptidases, which do not require any energy input. This feature ensuring that appropriately targeted proteins are completely removed from the cell. In general, these ATP-dependent proteases are two component systems in which substrates are recognized and unfolded an ATPase assembly and actively translocated into their central proteolytic unit for degradation. A particular advantage of this system is that the ATPase and proteolytic activities can be uncoupled, studied independently and then reconstituted. In my laboratory, we have solved the crystal structures of the proteolytic components of Clp and HslUV, two bacterial ATP dependent protease machines and characterized their interactions with their ATPases. Based upon these results, we will present a structure-based model for the mechanism of protein turnover that accounts for the tight coupling between the ATPase and proteolytic subunits during substrate translocation.

Microbial diversity, survival, and stress genes in biotechnologically important ecosystems.
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Our earlier studies of samples and isolated microbes from methanogenic ecosystems revealed that methanogens: 1. Constituted an immunologically coherent group of organisms distinct from other known microbes, composed of antigenically related sub-groups paralleling the families identified at the time by rRNA comparisons; 2. Were considerably more diverse than it was presumed from the available range of species in culture collections; 3. Existed in numbers and species beyond those detectable by cultivation; and 4. Thrived in a wide variety of ecologic niches spanning our planet. All these findings were later confirmed by several techniques including refinements of the earlier rRNA comparative analyses.

It was clear from the start that methanogens had biotechnological potential for waste bioconversion and fuel production. Total dependency on non-renewable energy sources of limited duration and access represented a thread to security and welfare. This is as true today as it was then. Likewise, waste accumulation is a menace to the environment and public health. Therefore, we focused on the analysis of methanogens in waste-treating ecosystems such as bioreactors and landfills and developed: calibrated polyclonal antibody probes of defined specificity spectra and monoclonal probes of defined molecular specificities; the antigenic fingerprinting method; the slide immunoenzymatic assay (SIA); and a battery of complementary tests (SIA-constellation) for the direct study of pure and mixed cultures, and samples from complex ecosystems without intervening purification or cultivation steps. These advances made possible the identification, characterization, and manipulation of numerous microbes of relevance to methanogenesis.

It was also learned that these microbes are frequently subjected to the effects of cell stressors such as brusque changes in temperature, pH, and contents in toxic compounds. Since stress genes and their products, stress proteins--some of which are molecular chaperones--play a key role in cell survival during stress, they were targeted for investigation. Methanogens and other archaea proved to be diverse also in their anti-stress mechanisms, hence the work was focused on genes with recognized anti-stress roles in bacteria and eucarya and with considerable biotechnological potential. The goal was to manipulate the genes and proteins to engineer resistant cells that could withstand harsh environmental conditions and continue the methanogenic activity unabated under stress. Targeted genes were cloned and sequenced, their responses to relevant stressors were measured, and their expression patterns were defined. Current work aims at elucidating the transcription-initiation mechanisms of the stress genes that produce the molecular

chaperones Hsp70(DnaK), Hsp40(DnaJ), and GrpE, and at identifying genes involved in RNA processing in methanosarcinas.

SESSION 4: METABOLIC ENGINEERING AND REGULATION

Regulation of Bacterial Metabolism and Behavior by the RNA-binding protein CsrA and the Non-coding RNA, CsrB.

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Bacteria are among the simplest living organisms, but they possess highly sophisticated mechanisms for flourishing in varied environments. Bacteria turn specific genes on or off to maximize growth potential, survive under unfavorable conditions, compete with other microbes, and interact with human, plant, and animal hosts. Our research over the past several years has uncovered one of the "global regulatory systems" used by bacteria to regulate numerous genes and thereby control metabolism and behavior on a broad scale. A small RNA-binding protein, CsrA (for carbon storage regulator), and an RNA molecule (CsrB) that functions as an antagonist of CsrA, post-transcriptionally regulate genes involved in:

- Carbon/energy metabolism
- Flagellum synthesis and motility,
- Attachment to solid surfaces to form biofilms, and
- Dispersal of such biofilms.

Such processes are involved in bacterial colonization and infection. Studies on Csr offer the potential for medical and biotechnology applications, including the optimization of the production of commercially useful metabolites.

Engineering Whole Microbial Cells for Chiral Organic Synthesis

JON D. STEWART, Department of Chemistry, University of Florida

Ketone oxidations and reductions are very valuable transformations in organic synthesis. Enzymatic catalysis of these reactions offers both high efficiencies and stereoselectivities coupled with low environmental impacts. To simplify these reactions on both bench and large scales, we have created a set of engineered microbial strains that can be directly used as chemical reagents. The cells, either recombinant *Saccharomyces cerevisiae* or *Escherichia coli* supply the enzyme and NADPH cofactor, which eliminates the need for protein isolation and cofactor regeneration. Several examples of these engineered microbial strains will be discussed along with selected applications.

Microbial Strategies for the Depolymerization of Glucuronoxylan.

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The hemicellulose fraction constitutes as much as 20% of the lignocellulosic biomass of crop residues and hardwoods, and is second only to cellulose as a resource for conversion to alternative fuels and biobased products. The predominant carbohydrate polymer in this fraction is 4-O-methylglucuronoxylan (MeGAXn) in which 4-O-methyl-D-glucuronopyranosyl (MeGA) residues are linked α-1,2- to 5 to 15% of the xylose residues comprising a linear β-1,4-xylan backbone. The enzymatic depolymerization of this polymer will improve upon pretreatment protocols for processing hemicellulose to xylose, and attention has been directed to microbial endoxylanases, β-xylosidases and α-glucuronidases for the complete fermentation of MeGAXn. The endoxylanases of several bacteria and fungi have been assigned to glycohydrolase (GH) families, GH5, GH10 and GH11, each with unique sequences, structural motifs, and mechanisms of depolymerization. The GH11 xylanases have masses of ~ 25 kDa, a β-jelly roll structure, and generate an aldopentauronic acid as limit product, with an unsubstituted xylose residue at the nonreducing terminus. The G10 xylanases have masses of ~45 kDa, an alpha-beta barrel structure, and generate aldotetrauronic acid in which the MeGA is linked to X at the nonreducing terminus. The G5 xylanases have masses of ~40 kDa, with an unsolved structure, and in the case of a single example, i.e. XYNa from *Erwinia chrysanthemi*, generate oligosaccharides containing 2 MeGA and 4 to 15 X residues. The aldotetrauronic acid product generated by GH10 enzymes can be taken up and cleaved by α-glucuronidase to release MeGA and xylotriose. The further depolymerization of xylotriose requires intracelluiar exoxylanase and /or β-xylosidase for conversion to fermentable substrate. The larger products generated by the G5 and G11 xylanases may require secretion of β-xylosidase and/or other xylanases to form products that can be assimilated. Substrate specificities and enzyme stabilities may offer advantages to a host and dictate specific applications for members of each family. At present, the GH10 xylanases offer promise as secreted enzymes, which along with intracellular α-glucuronidase and β-xylosidase, may be used to design ethanologenic biocatalysts able to convert hemicelluiose polymers to useful products.

SESSION 5: BIOINFORMATICS AND GLOBAL GENE REGULATION

***E. coli* Gene Arrays – Analysis of Gene Regulation.**

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Growth rate and cell yield of *Escherichia coli* depend on the growth medium and especially the source of carbon and nitrogen. In this present study, we used an ethanologenic *E. coli*, strain KO11, which grows in rich medium with glucose at a specific growth rate of $0.30.h^{-1}$ and with xylose at a specific growth rate of $0.19.h^{-1}$ under our fermentation conditions. The pathways for these two sugars are quite distinct with a major difference in ATP yield (0.67 for xylose vs 2.0 for glucose). Since the difference in specific growth rate is a reflection of various metabolic processes in the cell, mRNA expression profiles of strain KO11with the different growth rates were obtained using *E. coli* DNA arrays. In order to determine the genes and pathways that are differentially expressed in strain KO11 grown in the two media, total RNA was isolated and cDNA corresponding to the 4,290 known ORFs were synthesized. The cDNA was hybridized to an *E. coli* gene array and the level of expression of each of these ORFs was determined. Results of these experiments will be presented and discussed.

Insights into the Metabolism of Hydrogen and Sulfur by the hyperthermophile Pyrococcus furiosus

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Many species of hyperthermophilic archaea are sulfur-dependent organisms that reduce elemental sulfur (S^0) to hydrogen sulfide. The autotrophic S°-reducers use hydrogen as the electron donor and appear to have a respiratory chain with S° as the terminal electron acceptor. The heterotrophic S°-reducers utilize proteinaceous materials and in some cases polysaccharides as sources of reductant for S° reduction. Most of them are obligately dependent upon S° reduction for growth, although a few, such as *Pyrococcus furiosus*, are able to grow well in the absence of S° and produce hydrogen as a means of disposing of the excess reductant that is generated during catabolism. The mechanisms by which these organisms reduce S° and evolve hydrogen, and whether these processes lead directly to energy conservation, however, are not clear. For example, *P. furiosus* contains three different hydrogenases, all of which have been purified and characterized. Two of them are cytoplasmic and are thought to evolve hydrogen during growth utilizing NADPH as the electron donor. NADP is reduced by ferredoxin:NADP oxidoreductase. This enzyme utilizes the reduced ferredoxin generated by the variety of oxidoreductases that are involved in the catabolism of both peptides and glucose. The third hydrogenase in *P. furiosus*, on the other hand, is membrane-bound and appears to be part of a proton-translocating respiratory complex. This enzyme does not utilize NAD(P)H or ferredoxin and its physiological electron donor is not known. The two cytoplasmic hydrogenases also catalyze the NADPH- and hydrogen-dependent reduction of S° to hydrogen sulfide in vitro, but the membrane-bound enzyme does not catalyze these reactions. Insight into the roles of the three hydrogenases and of associated oxidoreductases involved in S° reduction and hydrogen production is being obtained by detailed growth studies coupled with genomic-based analyses. The sequence of the genome (1.9 Mb) of *P. furiosus* is complete and is currently being annotated (http://comb5-156.umbi.umd.edu/genemate/). Proteomic analyses and DNA-microarrays are being utilized to investigate the functions of some of the more than 2,000 proteins that the genome appears to encode. The results so far indicate that a variety of enzymes are strongly regulated by the presence (or absence) of S°, peptides and/or sugar (maltose). These data, in combination with recent biochemical analyses, are providing a clearer picture of how *P. furiosus* evolves hydrogen from growth substrates, although the mechanism by which S° is reduced is still not understood.