MICROBIOLOGY AND MOLECULAR BIOLOGY

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ABSTRACTS

Separation and Characterization of Chitinase and Chitobiase from CDC Group *EF4a*. Sheila M. Bailey and Carl E. Warnes, Department of Biology, Ball State University, Muncie, Indiana 47306.——The separation of the bacterial chitinase/chitobiase enzyme complexes poses problems due to their similar molecular weights and cooperative mode of action. However, application of affinity chromatography and temperature treatments have allowed for effective separation of these two enzymes. Further treatment of concentrated cell-free supernates by column chromatography and SDS polyacrylamide gel electrophoresis (PAGE) indicates that the organism secretes chitinase/chitobiase enzymes of similar molecular weight as that reported in the literature for *Vibrio*, *Streptomyces*, and *Serratia* species but may differ significantly from that of *Aeromonas* species.

Isolation of Mevinolin Resistant Strains in Yeast. M. Bard, A.S. Burnett, L.S. Burrows, Department of Biochemistry and R.A. Parker, Department of Biology, Indiana University School of Medicine, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46223.——The drug mevinolin will be used as an anti-hypercholesterolemic drug since it is a competitive inhibitor of the rate limiting enzyme in cholesterol biosynthesis, HMG-CoA reductase. Mevinolin treatment concomitantly results in increased enzyme synthesis; mammalian cells selected for resistance to this drug show greatly increased levels of enzyme activity as well as gene amplification. The ergosterol biosynthetic pathway in the yeast *Saccharomyces cervisiae* is very similar to mammalian cholesterol biosynthesis at least through the formation of lanosterol. We have isolated a number of mevinolin resistant strains and find that our most resistant strain, MV71, does not demonstrate any alteration in HMG-CoA reductase activity. All resistant strains grew better in the presence of mevinolin plus ergosterol than in mevinolin alone. Sterol analyses indicate that MV71 accumulates more total sterol than wild type when grown in the presence of mevinolin. We interpret these results as being due to differential uptake in wild type and resistant strains.

Decreased Survival of *Leishmania tropica* in Macrophages Treated with Cyclosporine A. Nancy C. Behforouz and Charlotte Wenger, Department of Biology, Ball State University, Muncie, Indiana 47306.——Normal Balb/C macrophages were treated *in vitro* with Cyclosporine A (CSA) or medium alone either before, during or after infection with *Leishmania tropica.* Those macrophages treated with CSA had significantly decreased infection rates as compared to control macrophages. A concentration of 1 ug/ml of CSA was effective in inducing a 66% to 95% decrease in numbers of intracellular leishmania depending on the length of exposure to the drug *in vitro.* The decreased survival of the amastigote form may be due either to direct drug action on the parasite or

to a drug-induced increase in leishmanial activity of the macrophages. The anti-leishmanial activity of Cyclosporine A described here may be in part responsible for the protective effect which Cyclosporine A has *in vivo* towards this parasite.

Bathocuproine Disulfonate and Interferon: Progress and Prospects. JAY G. CALVERT AND EDWARD H. SIMON, Purdue University, West Lafayette, Indiana 47907.——Bathocuproine disulfonate (BCS) is a potent and specific chelator of copper ions. The BCS_2Cu (II) complex has a higher redox potential than Cu(II) alone, which facilitates its reduction to the stable $BCS_2Cu(I)$ complex with the concomitant oxidation of a suitable substate. Several mouse interferons are particularly susceptible to this oxidative damage, since addition of millimolar concentrations of BCS to the growth medium of interferon treated cells results in rapid inactivation of residual or cell-bound interferon. Addition of micromolar amounts of $CuSO_4$ potentiates the inactivation of interferon by BCS. Treatment with BCS is not cytotoxic (although prolonged treatment inhibits cell division) and does not inhibit the growth of mengovirus. BCS is at least as effective an anti-interferon antibody at neutralizing residual or cell-bound interferon activity, and has the additional benefits of being less expensive and more readily standardized. BCS may prove to be useful probe for dissecting early events in the course of interferon action.

Location of Sites on Calmodulin that React with a Phenothiazine. FREDERICK FAUST AND HARRY W. JARRETT, Department of Biology, Indiana University-Purdue University at Indianapolis, Indiana 46223.——Calmodulin is a Ca^{2+}-dependent regulatory protein that activates several enzymes including phosphodiesterase, Ca^{2+}-transport AT-Pase, and NAD kinase. Calmodulin also binds hydrophobic drugs including the phenothiazine antidepressants. Subsequent to drug binding calmodulin is unable to activate enzymes; it has been suggested that the drug-binding and enzyme-binding sites must be near each other in the tertiary structure of calmodulin. A chemically reactive phenothiazine, 10-(3-propionyloxysuccinimide)-2-(trifluormethyl) phenothiazine, reacts 2:1 with calmodulin. Studies of the drug-labelled tryptic peptides have shown that the drug reacts first with Lys_{148} and then subsequently with lysines 21, 75, and 77.

S-Adenosylmethionine Synthetase and Spore Germination in *Mucor racemosus*. ROBERT GARCIA, Ball State University, Muncie, Indiana 47306.——Previous studies, with this dimorphic fungus, have shown that the intracellular concentration of S-Adenosylmethionine (SAM) increases approximately threefold during the aerobic conversion of yeasts to hyphae. The specific activity of SAM Synchetase, the enzyme responsible for the synthesis of SAM, also increased during the conversion in cell type. Subsequent work has shown that this observed increase in SAM is at least, in part, necessary because of an increase in protein methylation.

This study examined the specific activity of the synthetase during a different phase of the life cycle; the germination of the spore under aerobic and anaerobic conditions. Sporangiospores were placed in a semi-defined medium and samples were taken throughout the time period required for aerobic and anaerobic germination. Additionally, experiments were performed with a SAM Synthetase activity inhibitor in order to determine whether methylation is central to the germination process.

Isolation and Identification of *Azospirillum* and *Herbaspirillum* Strains from Soil of Several Eastern States. EDWIN M. GOEBEL, JENNY I. JOCKEL AND JAMES K. MATCHUNY. Department of Biological Sciences, Indiana University-Purdue University at Fort Wayne, Fort Wayne, Indiana 46805.——Soil samples were collected from nine sites in the eastern United States. Those sites were rest areas along the interstate highway system in Ken-

tucky and Ohio. One sample was obtained in a national park in Tennessee. Soil was inoculated into an enrichment medium containing malic acid and lacking organic nitrogen at either pH 6.0 or pH 6.8. Further isolation utilized Congo Red agar at either pH. Presumptive isolates were gram stained and speciated via the results from carbohydrate or malic acid utilization in either nitrogen-fixing or fixed-nitrogen conditions. *Azospirillum* and/or *Herbaspirillum* species were recovered from eight of the nine sites.

The Effect of *Azospirillum* and *Herbaspirillum* Inoculation upon the Yield of Winter Wheat (*Triticum aestivum*). Edwin M. Goebel, James J. Tobolski and Dennis A. Friedle. Department of Biological Sciences, Indiana University-Purdue University at Fort Wayne, Fort Wayne, Indiana 46805.——The soil of field-grown winter wheat was inoculated with *Azospirillum lipoferum* (strain Sp59b), *Herbaspirillum seropedicae* (strain Z-67) or an autoclaved culture of strain Z-67 (control). The experimental design consisted of seven blocks in which each of the three treatments was completely randomized. Each plot was 2.25 meters square (1.5 m on a side) in size and wsa inoculated with ca. 10^8 microbial cells diluted in 4 liters of water. The inoculations were made on 14 May 1986 just prior to the emergence of spikes. One square meter areas were harvested by hand from the center of each plot on 12-14 July 1986. Seeds were removed from the spikelet by hand, dried and plot seed weights were determined. Approximately a 13% increase in total dry weight was found between control plots and plots inoculated with *H. serapedicae*. This difference was significant at the 0.20 level as determined by a paired T-test. No differences were found between control plots and plots inoculated with *A. lipoferum*. These results are in agreement with other studies conducted in Israel.

Characterization of SPR6, a Sporulation Regulated Gene in Yeast. Stanley N. Grove, Richard F. Iannacone, Carmelo A. Milano and Mary J. Clancy, Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana 46556.——Sporulation in the yeast, *Saccharomyces cerevisiae*, is a model for understanding developmental control of gene expression in higher cells. We have cloned 27 distinct SPR (Sporulation regulated) sequences which can be grouped into early, middle and late transcription classes. We have constructed a plasmid, pRI1, containing the yeast sequence which includes SPR6, a late gene. An extensive restriction map has been developed. The approximate origin of the SPR6 transcript was determined via Northern blot analysis of selected restriction fragments using sporulation poly A mRNA's as probes. The direction of transcription was determined by cloning an internal restriction fragment into M13 cloning vectors to see which form yielded ssDNA complementary to sporulation RNA. This work provides the background for sequencing and gene disruption experiments to determine how expression is regulated.

Dietary Effects of Tannic Acid on the Gut Microflora of Sprague-Dawley Rats. Kathleen Hardy, Carol Branka, Elizabeth Watson and Kara Eberly, Saint Mary's College, Notre Dame, Indiana 46556.——Tannic acid is a naturally occurring polyphenolic compound in many herbivore diets. In vitro tannic acid is inhibitory to many bacteria, especially Gram positive organisms. This study examined the effects of feeding tannic acid (0% to 5%) in a balanced rodent ration on total fecal bacteria and populations of facultative organisms (enterics, enterococci, staphylococci, lactobacilli) in rats. Total bacterial counts, which represent mostly anaerobes, increased with dietary tannic acid, while facultative levels were generally unaffected. These results are compared to similar experiments with red squirrels, whose natural diet is high in tannic acid. (K. Eberly, E. Mould, and D. Duff 1982). Effect of Dietary Tannic Acid on Facultative Gut Microflora of the Red Squirrel. Proceedings of the Indiana Academy of Science *92*: 317-322.)

Synthesis and Characterization of Protein A-silica for High Performance Affinity Chromatography. LARRY R. MASSOM AND HARRY W. JARRETT, Department of Biology, Indiana University-Purdue University at Indianapolis, Indianapolis, Indiana 46223. ——Protein A from *Staphylococcus aureus* binds nocovalently IgG-class antibodies. This protein was reacted with 5 micron 3-glycidyloxypropyl-silica beads with 1000 angstom pores and was compared to commerical Protein A-Sepharose in the immunoprecipitation of rat liver arginase using anti-arginase rabbit serum. The silica resin was more effective than the Sepharose in this immunoprecipitation and was easier to separate from solutions. Protein A-silica also was packed into a 4.6 × 30 mm column and used for high pressure affinity chromatography (HPAC). The column was effectively used to isolate IgG from blood serum in less than 15 min. Using homogeneous IgG, peak height was found to be highly linear with sample load over a wide range of concentration. Using such a standard curve, the Protein A-HPAC method should be useful clinically in the analytical determination of serum IgG concentration.

Lyme Disease in Indiana: Isolation of *Borrelia burgdorferi* from Ticks (*Dermacentor variabilis* and *Amblyomma americanum*) and White-footed Mice. MARTHA M. RITTER AND ROBERT PINGER, Ball State University, Muncie, Indiana 47306. ——Lyme disease is a human disease caused by the tick-borne spirochete, *Borrelia burgdorferi*, and is now the most commonly reported tick-borne disease in the U.S. The disease usually begins with a skin lesion (EMC) and may develop to cause cardiovascular complications or acute arthritis. Although the extent of the spirochete's distribution in the midwest is unknown, four clinical cases of the disease possibly originating in Indiana have been reported to the State Board of Health, suggesting the need for more information about the distribution of the spirochete in this region of the country.

Ticks collected from three areas in Indiana and white-footed mice from Crawford, Martin, and Lawrence counties, were examined for the presence of *Borrelia burgdorferi.* Testing procedures included culture of tick midguts and mouse spleen, kidney, and blood samples in BSK-II medium; dark-field exam of direct smears of tissue samples; and direct immunofluorescent tests with rabbit anti-*B. burgdorferi* FITC conjugate.

The Construction of Three Hybrid Cloning Vectors and the Use of Low Iron Medium to Improve Transformation Efficiency in the Cyanobacteria *Anacystis nidulans* R2 and *A. nidulans* R2 SPc. CAROLYN N. VANN, Department of Biology, Ball State University, Muncie, Indiana 47306 AND LAUREN A. PLAYL AND LOUIS A. SHERMAN, Division of Biological Sciences, University of Missouri, Columbia, Missouri 65211. ——Three hybrid cloning vectors have been constructed which are capable of transforming at a high frequency both *Escherichia coli* and *Anacystis nidulans* strains R2 and SPc. The first vector, pPGV5, contains the indigenous plasmid of *A. nidulans* ligated to an *E. coli* plasmid, pLC28, which carries the powerful leftward promoter of phage lambda. The second vector, pROAN1 is similar but has the leftward and rightward promoters of lambda in addition to sequences encoding the temperature-sensitive CI 857 repressor protein. The third vector contains multiple cloning sites, the T7 and SP6 promoters, the bacteriophage F1 origin of replication, and allows for positive selection of inserts cloned into it. An *A. nidulans* gene encoding a 36 kDa protein involved in iron metabolism was cloned into pPGV5 and transformed into *A. nidulans* R2-SPc. The gene is expressed under control of the lambda promoter when the cells are grown in low iron medium. Transformation efficiency of *A. nidulans* strains R2 and R2-SPc may be improved as much as tenfold by the use of iron deficient medium.