

## MICROBIOLOGY AND MOLECULAR BIOLOGY

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### ABSTRACTS

**Prophylactic Treatment of Balb/c Mice with Two Cyclosporines Enhances Resistance to *L. tropica*.** NANCY BEHFOROZ AND CHARLOTTE WENGER. Department of Biology, Ball State University, Muncie, Indiana 47306.——The effect of Cyclosporine A (Cs A) and its analogue B-5-49 on *L. tropica in vitro* and *in vivo* in the highly susceptible Balb/c mouse strain has been investigated. *In vitro*, both of the two drugs showed significant toxicity toward *L. tropica* only at relatively high levels ( $>25 \mu\text{g/ml}$ ). However, at 5 and 10  $\mu\text{g/ml}$ , levels which correspond more closely to physiologically achievable concentrations, no growth inhibitory effect *in vitro* was observed. Upon administration of the drugs to animals with established lesions, no beneficial effect was observed and, in fact, some exacerbation of lesion development and disease progression was noted. Surprisingly, a majority of the mice treated prophylactically with Cs A for a period of seven consecutive days beginning one day before infection with *L. tropica* did not develop ulcerated cutaneous lesions although some footpad swelling was observed 10 days to 2 weeks following infection. These resistant animals displayed a sustained DTH following infection and were resistant to further challenge with virulent *L. tropica*. Prophylactic treatment with the B-5-49 analogue of Cs A was also effective in enhancing resistance to *L. tropica* infection in Balb/c mice although to a somewhat lesser degree. As the Cyclosporines tested do not appear to be directly toxic nor inhibitory *in vivo* for established *L. tropica* infections, it appears that these drugs may be effective in modulating the induction stage of the immune response toward the parasites in the Balb/c mouse in such a way as to allow a protective immunity to develop.

**Interferon-induced Inhibition of Cell Transformation by RNA Tumor Virus.** YOUNG C. CHEN AND JAMES A. EVANSON, Department of Biological Sciences, Indiana University-Purdue University at Fort Wayne, Fort Wayne, Indiana 46805.——Normal rat kidney cells (NRK) infected with temperature-sensitive (ts) mutant of avian sarcoma virus (ASV) at the permissive temperature (33°C) exhibit the morphological and growth characteristics of the transformed state, whereas at the nonpermissive temperature (39°C), they exhibit the normal characteristics. By the use of this system, we have shown that the addition of rat fibroblast interferon to infected cells grown at nonpermissive temperature prevents the retransformation when they are shifted down to permissive temperature. Merely treating cells at permissive temperature with rat fibroblast interferon causes them to change from transformed phenotype to the normal phenotype. These effects require the continuous presence of interferon and are quite species-specific since mouse fibroblast interferon and human alpha interferon were not effective in our experiments. These results suggest that interferon might restore the growth control of transformed cells and this function of interferon could in part account for its anti-tumor effect.

**A Polyacrylamide Gel Electrophoretic Assay for Chitinase Using a Substrate-included System.** THOMAS A. COLE, ROSS E. MARBURGER AND BARRY P. BONE, Department of Biology, Wabash College, Crawfordsville, Indiana 47933.—Substrate-included electrophoretic gel assays for several hydrolases for biopolymers have been developed by us and others. Here, we report a chitin-included system which utilizes decalcified, deproteinized colloidal chitin prepared from crab shells. Chitinases from *Serratia marcescens*, *Streptomyces griseus* and *Streptomyces antibioticus* have been migrated into chitin-included polyacrylamide gels in a CAPS-urea system at pH 10.5. Incubation of the gels after electrophoresis at pH 4.5 in acetate buffer allows enzymatic activity to occur. Incubation if followed by staining the undigested chitin with a variety of reagents or observing cleared areas directly in dense chitin-included gels. Staining procedures, sensitivity, pH optima and other assay parameters will be reported and discussed.

**Enzyme Characterization and Product Analysis of a Chitinase System of a Freshwater Bacterial Isolate.** J.B. ELLIS, P. EICHMAN AND C.E. WARNES, Department of Biology, Ball State University, Muncie, Indiana 47306.—The search for highly active chitinases of bacterial origin has intensified as their application to the fields of medicine and agriculture and environmental concern have been identified. A highly chitinolytic freshwater bacterium was selected for study. Extracellular chitinases from broth cultures were isolated and concentrated by  $(\text{NH}_4)_2\text{SO}_4$  fractionation, size exclusion chromatography and ultrafiltration. A tritiated chitin solubilization assay indicated major activity in the 29,000 molecular weight fraction although activity was observed in other fractions as well. Specific oligosaccharides of the enzyme substrate mixture were identified and quantified by HPLC analysis using a Beckman reverse phase, absorption ultrasil amino column and monitored at 214nm. GlcNAc, chitobiose, and chitotriose were the major products identified. Chromatographic evidence also suggested the presence of  $\alpha$  and  $\beta$  anomers of the oligomers. The results correlated in general with data on *Serratia*, *Vibrio*, and *Streptomyces* chitinases.

**S-Adenosylmethionine Synthetase and the Morphogenesis of *Mucor racemosus*.** J. R. GARCIA, Department of Biology, Ball State University, Muncie, Indiana 47306.—Earlier work with this dimorphic fungus strongly suggested that S-Adenosylmethionine (SAM) is a biochemical correlate of morphogenesis. Those studies showed that the intracellular SAM concentration increased during the conversion of yeasts to hyphae and that the increase closely paralleled the emergence of germ tubes. In addition, the specific activity of S-Adenosylmethionine Synthetase increased during the conversion in cell type and decreased only after the increase in SAM had peaked. In order to gain a better understanding of these initial observations, cycloleucine (an inhibitor of SAM Synthetase activity) was added to yeast phase cultures. In the presence of 1 mg/ml of cycloleucine, the culture experienced a twenty-five-fold decrease in intracellular SAM. Interestingly, previous work had shown that the culture failed to shift in the presence of the cycloleucine. In order to more closely examine the effect of methionine on the synthesis of SAM Synthetase, cycloleucine and methionine were added to a yeast phase culture. The addition of methionine repressed the synthesis of the enzyme suggesting that methionine and SAM are co-repressors of enzyme synthesis. Experiments with cycloheximide demonstrated that the previously reported increase in specific activity involved de novo protein synthesis while data generated utilizing an adenine-requiring mutant suggests that RNA synthesis may also be required.

**Isolation of *Azospirillum* Species from Indiana and Michigan Soils.** DEBORAH A. McMAHAN AND EDWIN M. GOEBEL. Department of Biological Sciences, Indiana

University-Purdue University at Fort Wayne, Fort Wayne, Indiana, 46805.—Members of the genus *Azospirillum* have been shown to fix nitrogen while growing under microaerophilic conditions. They have been isolated from both tropical and temperate regions and have been found to be associated with non-legume agricultural crops and grasses. We have attempted to isolate members of this genus from Indiana and Michigan soils. Samples were obtained from the soil surrounding various plants, including corn, alfalfa, soybean, as well as pasture grasses. These samples were first inoculated into a selective semi-solid medium to determine the presence of nitrogen-fixing bacteria. Various biochemical tests were used to further characterize and identify the isolates. Our results indicate that members of this genus exist in Indiana and Michigan soils.

**Comparison of Capsid Gene Products of *Ustilago maydis* Virus by *in vitro* Translation.** G.K. PODILA, W.H. FLURKEY AND R.F. BOZARTH, Indiana State University, Terre Haute, Indiana 47809.—The *Ustilago maydis* virus (UmV) is a unique mycovirus. There are three different strains of UmV(P1,P4,P6) which secrete a killer toxin that kills the sensitive strains of the same fungus. These viruses have segmented double stranded RNA(dsRNA) genomes. These dsRNAs are classified into three major classes, namely Heavy(H), Medium(M), and Light(L). The dsRNA segments were purified and tested in a reticulocyte lysate translation system. The dsRNAs were denatured in 90% dimethyl-sulfoxide and 1mM methyl mercuric hydroxide prior to addition to an *in vitro* translation system. *In vitro* translation of denatured dsRNA resulted in incorporation of 35S-Methionine into TCA precipitable products, whereas there was no incorporation for undenatured dsRNA. The major *in vitro* translation products of H2 segments of P1 and P4 and that of the H segment of P6 resulted in a 73 kd peptide that co migrates with native capsid peptides of these three strains of viruses. It was also found that this 73 kd peptide from *in vitro* translation can be immunoprecipitated with respective coat protein antisera. Cross immunoprecipitation and peptide mapping of the 73 kd peptides from these three strains indicate that all these three strains code for the similar coat protein. These results establish that the H2 segment of P1 and P4 and the H segment of P6 code for the capsid and thereby functions as helper genome by providing capsid to encapsidate other dsRNA segments of the genome.

**A Functional Comparison of Elicited Murine Peritoneal Cells and Peripheral Blood Neutrophils.** JAMES L. SHELLHAAS, Butler University, Indianapolis, Indiana 46208.—Utilizing a model system of discontinuous density gradient centrifugation and dextran sedimentation, polymorphonuclear neutrophils (PMNs) were isolated from the peripheral blood of BALB/c mice. Isolated and purified neutrophils were subsequently compared to 4 hr thioglycollate elicited murine peritoneal neutrophil populations. The responsiveness of both cell populations to the tumor promoter phorbol myristic acetate (PMA), the chemotactic peptide N-formylmethionyl-leucyl-phenylalanine (FMLP) and opsonized zymosan were ascertained employing assays of luminol dependent chemiluminescence, chemotaxis, and the generation of superoxide anion. A number of notable differences in these two cell populations will be described.

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**The Isolation of the Genes Encoding the Second Largest Subunit of Human RNA Polymerase II.** MARK WASSERMAN, SCOTT WATKINS, JOHN CUNNIFF, K. DISSER AND S. SURZYCKI. Department of Biology, Indiana University, Bloomington, Indiana 47405.—A polyclonal antibody to human placental RNA polymerase II has been used to screen a human cDNA expression library in lambda gt11. Initially, 126 positive clones were isolated from this library after screening approximately one million pla-

ques at high density. Secondary and tertiary platings have been performed and 96 positive clones remain. These positive clones will be screened by heterologous hybridization to a yeast clone that encodes the second largest subunit of RNA polymerase II. This yeast gene will be cloned into the polylinker site of a new T7 promoter vector (pST 54) which facilitates the transcription of RNA from DNA inserts. The single stranded probe that is produced by this technique will allow us to perform heterologous hybridizations with much higher stringency than is permitted with double stranded probes produced by nick translation or T4 DNA polymerase chewback. Once clones for the second largest subunit of RNA polymerase II have been isolated by heterologous hybridization to this RNA probe they will be characterized by various techniques such as DNA sequencing. This will allow for a greater understanding of the structure and function of RNA polymerase II in eucaryotes.