MICROBIOLOGY AND MOLECULAR BIOLOGY

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ABSTRACTS

The Effects of *In Vivo* Exposure to Ozone on Hamster Tracheas. DOROTHY ADALIS, Department of Biology, Ball State University, Muncie, IN 47306.—Syrian Golden hamsters were exposed to ozone to determine the effects on the ciliated epithelium of the upper respiratory tract. A variety of times and doses were studied to include 0.25, 0.5, and 1.0 ppm for 1, 3, or 5 days. Quantitative measurements of the cilia beat frequency were determined using a stroboscope and statistically analyzed. Results of *in vivo* exposures to 0.25 ppm and 0.5 ppm ozone indicated a dose related reduction in cilia beat frequency that was statistically significant at the $P < 0.01$ level. A cytopathological assessment was made to determine if ozone alters or interferes with the structural integrity of the ciliated epithelium. The histologic assessment indicated dose-related, adverse pathological effects in the tracheas of hamsters exposed to ozone. The results indicate an altered functioning of the tracheal ciliated epithelium which would possibly interfere with the host's normal pulmonary clearance mechanism.

Construction of a Hybrid Cloning Vector with a Temperature-Regulatable Promoter for *Synechocystis* 6803. IN SO BAE, JO ANN MEUNIER, and CAROLYN VANN, Biology Department, Ball State University, Muncie, IN 47306 and Animal Science Dept., The Ohio State University, Wooster, Ohio.—Two hybrid plasmid vectors were constructed which are capable of regulating the expression of cloned genes in both *Escherichia coli* and in the cyanobacterium, *Synechocystis*. Vector pBV1 contains origins of replication for both organisms, an ampicillin resistance gene, a lambda temperature sensitive repressor gene (cI857) to regulate gene expression, and the powerful rightward promoter of bacteriophage lambda to ensure that cloned genes are transcribed at a high level. Vector pTC1 was created by inserting a gene for chloramphenicol resistance (CAT) downstream of the lambda promoter in vector pBV1. The expression of the CAT gene in *E. coli* was quantified spectrophotometrically following the addition of cm and a shift in growth temperature. A 10-fold increase was observed, indicating temperature-regulated expression.

Azole Sensitivity and Hyphal Formation of a Cytochrome P450 Mutant of *Candida albicans*. M. CHRISTINE BROUGHTON, NORMAN D. LEES, and MARTIN BARD, Indiana University-Purdue University at Indianapolis,.Dept. of Biology, 1125 E. 38 St., P.O. Box 647, Indianapolis, IN 46205.—Low concentrations of azole antibiotics inhibit the enzyme 14α-demethylase in yeast. Subsequent accumulation of 14α-methylsterols in the membrane has been reported to result in growth

inhibition and lack of hyphae formation. Other demonstrated effects noted at higher drug concentrations involve respiratory inhibition and membrane disruption. Strain D10, a cytochrome P450 demethylase mutant of *C. albicans,* has been shown to be viable on normal media and to be defective in hyphae formation. Addition of three azoles at concentrations of 10, 50, and 100 μM to actively growing cultures of D10 and a wild type revertant, D10R, resulted in immediate growth inhibition of both strains. The viability of D10 demonstrates that the substitution of 14α-methylsterols would not result in complete growth inhibition. Therefore, the fungistatic effect seen clinically may be due to a combination of factors including altered sterols, respiratory inhibition, and inability to form hyphae.

Methylation and Sporangiospore Germination in *Mucor racemosus*. KATHRYN FOSS and J.R. GARCIA, Ball State University, Muncie, In 47306.—A previous study with this dimorphic fungus showed that the specific activity of S-Adenosylmethionine (SAM) Synthetase increased significantly during the aerobic germination of sporangiospores. It was also shown that the addition of cyloleucine prevented both the increase in specific activity and the formation of germ tubes. In the present study we attempt to determine how early, in the germination process, the enzyme is active and the effect of the addition of cycloleucine at different times during germination. Sporangiospores were placed in a semi-defined medium, at 25°C, and samples were taken throughout the time period required for germination. We showed that the enzyme was active even in the dormant sporangiospores. We also showed that the addition of cycloleucine, during the first 4.5 hours, would block the formation of germ tubes. Experiments with cycloleximide showed that *de novo* protein synthesis was necessary throughout the experiment in order for germ tubes to be synthesized. These results suggest that methylation of macromolecules could play a central role in germination.

Site-Directed Mutagenesis of the Manganese Stabilising Protein (MSP) in *Anacystis nidulans* R2. KENNETH FRIMPONG and CAROLYN N. VANN, Department of Biology, Ball State University, Muncie, IN 47306.—Homology analyses and predictive algorithms have been employed to analyse the photosystem II MSP of *A. nidulans R2.* Amino acid homology searches between MSP and six other manganese binding proteins were conducted using the IBI/Pustell Sequence Analyses System (International Biotechnologies, New Haven, Conn.). The entire MSP protein was further analysed using the predictive algorithms of Chou and Fasman (1978), Argos and Palau (1982), Parker and Guo (1986), and Manavalan and Ponnuswamy (1978). The study has elucidated probable manganese-binding residues. Combinatorial, site-directed mutagenesis will be conducted to investigate the information content of the positions of the probable ligands.

Cloning of a Gene Encoding a Mosquitocidal Toxin from *Bacillus thuringiensis* var. *isrealensis*. LEAH M. HELVERING and CAROLYN N. VANN, Ball State University, Muncie, IN 47306.—*Bacillus thuringiensis* var. *isrealensis* (Bti) produces a crystalline endotoxin specific for larvae of mosquitoes that may be vectors of malaria. The gene encoding the endotoxin is being cloned into the cyanobacterium, *Anacystis nidulans* R2, to determine if its level of expression is sufficient for mosquito lethality. A 108kb plasmid from Bti 4Q2-72 has been isolated and digested with XbaI to obtain a 3.8kb fragment that encodes a protein which is part of the crystal endotoxin. This fragment has been cloned into the hybrid cloning vector, pTNTV, and the chimaera transformed into *Escherichia coli.*

Transformants are being screened and the level of toxin expression in *E. coli* and *A. nidulans* will be examined.

Transformation Efficiency and Stability of Two Hybrid Cloning Vectors in *Synechocystis* 6803. KEMP HORKEN and C.N. VANN, Ball State University, Muncie, IN 47306.—Transformation efficiencies of two newly constructed hybrid cloning vectors, pBV1 and pTC1, into the cyanobacterium *Synechocystis* 6803 will be presented. The hybrid vector pBV1 is capable of replicating in both *Escherichia coli* and in the cyanobacterium *S.* 6803. In addition, the vector contains bacteriophage Lambda sequences to allow temperature regulated expression of a cloned gene. Plasmid pTC1 is the vector pBV1 with the reporter chloramphenicol acetyl transferase gene cloned into it. Since previously constructed hybrid vectors have undergone recombination upon transformation, the plasmid pTC1 is also being transformed into a previously transformed strain to determine if this will result in enhanced stability.

New Structural Alleles for Mouse Androgen-Binding Protein (ABP) and a New Gene Capable of Modifying ABP. ROBERT C. KARN, THERESA S. REMPEL, TINA M. WALLS, and STEPHEN R. DLOUHY, Dept. of Biological Sciences, Butler University, Indianapolis, IN 46208.—Mouse salivary androgen binding protein (ABP) is a series of dimers composed of an alpha subunit disulfide-bridged to a beta or a gamma subunit. We have proposed separate genes, *Abpa, Abpb* and *Abpg,* respectively, for the subunits. Genetic data suggest that the *Abpa* and *Abpg* are closely linked on chromosome 7 (Dlouhy, *et al.,* Genetics 115: 535, 1987). ABP phenotypes may be more complex in some mouse salivas due to the presence of the dominant allele, Ssp^S, of a modifier gene, *Sex-limited saliva pattern* (Karn, *et al.,* Biochem. Genet. 20: 493, 1982) which is not linked to *Abp* (Dlouhy and Karn, Biochem. Genet. 22: 657, 1984). We have studied inbred mouse subspecies determining ABP phenotypes by detection of bound ^{14}C-testosterone and by immunostaining western transfers. Two of these subspecies, IS/CameEi and CAST/Ei, were selected for further study and crossed with C3H/St and with DBA/2J. The F_1 from the IS/CamEi x C3H/St cross, was intercrossed to obtain the F_2. The phenotypes observed in these crosses suggest the presence of a variant of one of the subunit genes in the IS/CamEi strain. Preliminary analyses of CAST/Ei salivas revealed sex-limited phenotypes. The progeny from crosses with C3H/St and with DBA/2J show similar sex-limited variation in F_1 progeny. Backcross data suggest that the CAST/Ei strain has both a unique structural variant of *Abp* and a modifier gene with action different from that of Ssp^S. We designate the new structural variants seen in CAST/Ei and IS/CamEi as c and d variants of *Abp,* respectively. Studies are underway to determine whether the modifier seen in the CAST/Ei strain is an allele of *Ssp* or a distinct gene.

Molecular Analyses of the Structure of Mouse Submaxillary Androgen-binding Protein (ABP). ROBERT C. KARN and ROSEMARY WOOD, Department of Biological Sciences, Butler University, Indianapolis, IN 46208.—Mouse saliva contains a series of androgen-binding proteins (ABPs) contributed mainly by the submaxillary glands. Molecular weight analyses of ABPs indicate that they are dimers each consisting of a larger (alpha) subunit disulfide-bridged to a smaller (beta or gamma) subunit. Genetic studies suggest that the subunits are determined by distinct genes, a concept supported by the observation of three products of *in vitro* translation. The goal of this study is to isolate cDNAs corresponding to the various ABP subunit mRNAs from a mouse submaxillary library and sequence

them in order further to explore the three-gene hypothesis. We purified the alpha subunit and obtained approximately a third of its N-terminal amino acid sequence and then determined about the same portion of the amino acid sequence of the beta subunit by sequencing the dimer and subtracting the alpha sequence. The intact dimer has two free N-termini in equimolar concentrations consistent with our dimer model for ABP. Using the genetic code we obtained a mixed oligomer probe for the alpha subunit and used it to explore sequences in a mouse submaxillary cDNA library. Three clones were selected by this method and grown up for further analysis. Electrophoresis of fragments obtained by *PstI* digestion of the plasmids suggests that the three clones contain cDNA inserts smaller than 1 kb. All three clones were positive on Southern transfer analyses probed with the end-labeled oligomer. Our future work will involve recloning the inserts into the M13 sequencing vector for analysis by the Sanger sequencing method.

Restriction Mapping of a Plasmid from *Bacillus thuringiensis* var. *isrealensis*. SARA L. LITZ and CAROLYN N. VANN, Ball State University, Muncie, IN 47306.—*Bacillus thuringiensis* var. *isrealensis* 4Q2-72 harbors a 108kb plasmid which contains genes responsible for the production of crystalline endotoxins. The plasmid has been isolated and is being randomly restricted to produce fragments of approximately 10kb. Lambda GEM-11 cloning vector will be used in making a genomic library. The endotoxin genes will be identified and the fragments hybridized for the construction of a map. Progress will be reported.

Inhibition of Antibody Formation by Cyclosporine A. ANGELA SCHILKE and NANCY BEHFOROUZ, Ball State University, Muncie, IN 47306.—Cyclosporine A (CsA) is a widely used immunosuppressive drug which has novel, clinically beneficial effects on the immune system. There is substantial evidence that CsA preferentially acts by impairing T cell lymphokine production, but there is some evidence that CsA may also affect B cells and other antigen presenting cells directly. Using an *in vitro* antibody response to SRBC, we have examined the effect CsA has on different populations of mouse lymphocytes. CsA appears to have a profound, early acting inhibitory effect in a dose dependent manner at physiologically achievable level *in vitro* on whole splenocytes from naive animals. Antibody production by B and T cell mixtures could also be inhibited by CsA in a similar manner. T cell lymphokines added to B and T cell cultures could not overcome the CsA induced inhibition. Indeed, populations of highly enriched or positively selected B cells stimulated to produce antibody by antigen and T cell lymphokines are profoundly inhibited by the early addition of CsA. These findings suggest that CsA has direct inhibitory effects on lymphocytes, including B cells, aside from, or in addition to, a reduction of lymphokine production by T cells.

Construction of a Hybrid Cloning Vector with an Iron-Sensitive Promoter. WILLIAM E. SNYDER, JR. and CAROLYN VANN, Biology Dept., Cooper Hall, Ball State Univ., Muncie, IN 47306.—A hybrid cloning vector is being constructed that possesses a promoter that responds to the iron content in the growth media. The vector, pANIC 1, will contain sequences from the resident plasmid, pANS, of *Anacystis nidulans* R2. It will also contain *E. coli* sequences from the pUC 18 vector, enabling it to replicate in either system. The promoter allows for expression of a cloned gene in low iron media. A segment of pKJ110 containing the sequences encoding the promoter were ligated into pUC 18 to form pIC 1. Sequences downstream of the promoter were cleaved out of pIC 1 to form pIC 4, which has the promoter directly upstream from the multicloning site of pUC 18. The final phase

of construction is to ligate a segment of pANS into this vector to form pANIC 1. PANIC 1 will be used to study the effects of mutated photosynthesis genes on the photosynthetic processeses in *A. nidulans* R2.

Effect of Toxicants on Chitin Decomposition. CARL E. WARNES, J.A. BALDAUF, and L.M. GARRIOTT, Department of Biology, Ball State University, Muncie, IN 47306.—Highly active chitinolytic isolates from soil and water were examined for sensitivity to varying concentrations of zinc and the pesticide diflubenzuron. Preliminary studies conducted with cell free enzyme preparations showed increased sensitivity when zinc concentrations approached 5 mg/l. Purified chitinase enzyme preparations however showed no sensitivity well beyond the range considered toxic (5 mg/l). Diflubenzuron, on the other hand, did not inhibit cell growth or chitinase production at levels approaching maximum solubility. Chitinase assays conducted in the presence of varying concentrations of diflubenzuron likewise showed no inhibitory response on enzyme activity. Therefore, this insecticide which interferes with chitin deposition through chitin synthetase showed no adverse effect on bacterial chitinase production or activity. Both processes may act cooperatively in the elimination of chitinous pests.

Isolation of Viral RNA from *Cattleya*-Alliance Orchids. PAULA D. WILLIAMS, HERBERT L. SAXON, and CAROLYN VANN, Biology Dept., Muncie, IN 47306.—The two most economicaly important viruses of orchids are Cymbidium Mosaic Virus (CyMV) and Tobacco Mosaic Virus-O Strain (TMV-O). Published research has shown that viral symptoms of both viruses can be attenuated in host plants where previous viral infections have conferred resistance. Plants expressing high copy numbers of virus coat protein genes have a similar resistance to viral infections. The genetic engineering of orchids to express these virus coat proteins presents significant challenges and opportunities. Successful isolation of viral RNA from infected orchids is a necessary early step for expressing high copy numbers of virus coat proteins in transgenic orchids. The speaker reports the results of research to isolate the viral RNA of CyMV from infected Cattleya-alliance orchids.