MICROBIOLOGY AND MOLECULAR BIOLOGY

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ABSTRACTS

A Simplified Method for Storing Anaerobic Bacteria. WILLIAM W. BALDWIN and MING TSENG, Indiana University Medical School, Northwest Center for Medical Education, Gary, Indiana 46408 and MARSHALL LANDAY, St. Margaret Hospital, Hammond, Indiana 46320.——The recommended method for storage of anaerobic bacteria is at room temperature in chopped meat broth lacking carbohydrates. There are two problems associated with the use of chopped meat broth. One is its complicated preparation and the other problem is its turbidity. This turbidity necessitates gram staining to determine if growth has occured and risks oxygen and microbial contamination. We have developed an agar medium in which we have successfully stored anaerobic bacteria for ten months. The medium is composed of Blood Agar Base (Difco) 40g/ℓ, Yeast Extract (Difco) 5g/ℓ, and cysteine HCℓ 0.05 g/ℓ. The medium is prereduced and anaerobically sterilized in 13 x 100mm screw cap tubes and inoculated with a loop long enough to reach the bottom of the tube. The tube is capped and sealed with parafilm and incubated at 37° C for 2 days, then stored at room temperature.

The Effects of a Hexaflora on the Morphology of the Gerbil. KENNETH F. BARTIZAL, MARGARET H. BEAVER and BERNARD S. WOSTMANN, Lobund Laboratory, University of Notre Dame, Notre Dame, Indiana 46556.——The use of the gerbil as an animal model in biomedical research has increased dramatically. The gerbil is of interest because its serum cholesterol is quite responsive to the level of dietary cholesterol yet, when maintained in a state of hypercholesterolemia, no evidence of plaque formation is observed. The gerbil is also an excellent model to study bile acid (BA) metabolism because of the similarity of its BA pattern to that of man.

The effects of an intestinal microflora on cholesterol and BA metabolism are substantial. In order to separate microbial factors from physiological mechanisms that influence sterol metabolism, germfree (GF) and gnotobiotic (GN) gerbils were obtained.

GF gerbils were caesarian derived; but because of cecal enlargement, they failed to reproduce. A pair of GF gerbils were associated with a defined six member murine derived microflora that was known to have very limited effects on the primary BA pattern of rats. This hexaflora, consisting of *Lactobacillus brevis*, *Streptococcus faecalis*, *Staphylococcus epidermidis*, *Bacteroides fragilis*, var. *vulgatus*, *Enterobacter aerogenes*, and a *Fusobacterium sp.* allowed the GN gerbils to reproduce and a colony of hexaflora associated hexa-gerbils has been established. In order to broaden the colonies genetic base, caesarian sections were performed to introduce additional gerbils when a female hexa-gerbil was nursing a new litter.

The hexa-gerbil has a much higher serum cholesterol level than the CV gerbil when fed a (0.1%) cholesterol supplemented diet. The serum lipoprotein pattern indicates that in GN gerbils harboring a microflora with very limited secondary BA

modifying capability, the animal accumulates cholesterol in the more suspect VLDL and LDL fractions, while the HDL fraction stays the same.

Morphological data on 4 to 7 month old male hexa-gerbils indicate a sizable reduction of the cecum when compared to its GF counterpart. However, both small and large intestine weighed substantially more than in the CV gerbil. Also, the size of the thymus was significantly reduced, suggesting a certain amount of stress imparted by this murine-derived microflora.

This chronic inflammation of the mucosa is most likely related to the presence of *Enterobacter aerogenes*, *Staphylococcus aureus* or the *Fusobacterium sp.* present in this limited component hexaflora. As monoassociates these organisms have been known to induce inflammation of the intestinal mucosa.

At present, studies of tissue histology and quantitative bacteriology of the gerbil's gut are underway to determine the possible localization of the hexaflora components and their ensuing effects on the intestinal mucosa.

Isolation, Identification, and Reinfection of *Cephalosporium* spp. and Two Bacteria Associated with Wilt in *Helianthus annuus*. LARRY HERRMAN and MARY LEE RICHESON, Department of Biological Sciences, Indiana University-Purdue University at Fort Wayne, Fort Wayne, Indiana 46805.——Recent discovery of wilt in the agronomically important crop sunflower has resulted in economic loss. In the fall of 1979 the authors observed a severely lodged and diseased field of the oil producing sunflower near Auburn, Indiana. A fungus and two bacteria were isolated from lesions on the stem of diseased plants. The fungus was identified by microscopic comparisons as *Cephalosporium* spp. The isolate was grown on Sabourard and potato dextrose agar at various temperatures. Optimum growth on agar is 20-25° C.

Cephalosporium is a known plant pathogen causing wilt in several woody plants. As far as the authors know, it has not been reported before as a pathogen of sunflower. The two bacteria identified as *Erwinia carotovora* and *Xanthomonas campestris* are also known plant pathogens. Two different times of inoculation and four methods of inoculation have established that the sunflower is not susceptible to the disease produced by *Cephalosporium* in the seedling stage. Ten day old seedlings were scratched, sprayed, and injected with a suspension of *Cephalosporium*. None of the treatments indicated any symptom of disease. In addition to the actual inoculation of the organism into the seedlings, the soil in which some of the sunflowers were to be grown was treated with a suspension of *Cephalosporium*. Seeds planted in this treated soil also produced healthy plants with no sign of disease. Plants inoculated with the *Cephalosporium* suspension at the onset of senescence, however, showed rapid hyphae growth and rapid spread of the fungus over the plant. Inoculation method did not seem to be a factor in establishment of the disease. The soil treatment was not used this time. Other etiological investigations with the two bacteria isolates and the *Cephalosporium* isolate are under way.

Dissolved Oxygen Profile of an Aerobic Bio-Reactor. ROBERT H. L. HOWE, West Lafayette, Indiana 47906.——The dissolved oxygen profile of an aerobic bioreactor is discussed. Emphasis with respect to the different utilization and oxidation rates of different substrate components is explained. A mathematical model for the prediction of the dissolved oxygen concentration progression is introduced.

Search for Phosphoproteins in *Bacillus subtilis*. MARK O. OSTER and SYLVIA BREHM OSTER. Indiana State University, Terre Haute, Indiana 47809.——Several proteins

in many biological systems are post translationally modified by phosphorylation resulting in altered activity. In order to investigate the possibility that some proteins in *Bacillus subtilis* may be phosphorylated, we grew *B. subtilis* in the presence of radioactive P^{32} orthophosphate (5mC/1). Crude extracts were obtained by treatment with lysozyme, incubated with DNase and RNase and subjected to chromatography on Sephadex G-50. The excluded fraction, containing chiefly proteins, was analyzed for the incorporation of P^{32} by scintillation counting. The nature of the phosphate attachment was investigated. Neither P^{32}-labelled phosphoserine nor phosphothreonine could be detected in the protein fraction after hydrolysis in 6N HCl at 110 C for 4 hr and chromatography on Dowex-1 with authentic standards. The P^{32}-labelled material is stable at 37° C for 30 min over a wide pH range from 2 to 12 but labile at pH1 and pH13 and above. Under the same conditions, the P^{32}-labelled product is stable in 1M neutral hydroxylamine indicating that the attachment is not an acyl phosphate, phosphohistidine nor phosphyolysine. The possibility of other covalent linkages to protein and polyphosphates is still under investigation.

Rate Studies on Microbial Chitin Decomposition in the Freshwater Habitat. TONI L. POOLE and CARL E. WARNES, Department of Biology, Ball State University, Muncie, Indiana 47306.——Microbial action on particulate chitin was analyzed in an East-central Indiana borrow pit lake. Experiments were conducted to determine the effects of water depth, particle size, mesh size of nylon bag containing chitin, and season on the decomposition process. The majority of chitinolytic bacteria isolated were classified as actinomycetes and pseudomonads. Results indicate that the sediment-water interface is the most active site of chitin mineralization in the lake environment. The samples seeded during summer showed the fastest rate of decomposition with greater than 50% weight loss after two weeks and greater than 95% weight loss after 7 weeks incubation *in situ*. Samples seeded during the spring showed considerably slower activity with only a 25% weight loss after 9 weeks incubation *in situ*. Larger particle size appears to slow the mineralization process.

Isolation and Identification of Two Bacteria Associated with Wilt in *Helianthus annuus*. MARY LEE RICHESON and LARRY G. HERRMAN, Department of Biological Sciences, Indiana University-Purdue University at Fort Wayne, Fort Wayne, Indiana 46805.——Recent discovery of wilt in the oil-producing sunflower, *Helianthus annuus*, has resulted in agricultural losses. Two types of bacteria and a fungus were isolated from lesions on the stem of diseased plants. In this study, the two bacterial isolates were identified. Microscope studies of morphology revealed both isolates to be small gram-negative asporogenous motile rods. Capsules were present. Temperature optima were 20-25 C. One isolate produced large, white, domed, mucoid colonies on nutrient agar containing 5% sucrose. The other isolate produced yellow pigmented colonies. These and other observations together with data from approximately twenty biochemical tests indicate that one organism is probably *Erwinia carotovora*, the other, *Xanthomonas campestris*. Both are known plant pathogens. *Xanthomonas* has not been associated with disease in sunflower. *Erwinia* are facultative anaerobes of the family Enterobacteriaceae which produce acid and gas from glucose but are otherwise fermentatively erratic. Growth at 36 C and lack of yellow pigment establish this as a member of the Carotovora group. *Xanthomonas* are strict aerobes, never fermentative. Ecologically, they are the plant pathogen components of the Pseudomonadaceae. Preliminary etiological experiments indicate no definite symptoms appear if sunflower plants are inoculated

with *Erwinia* as seedlings. Other etiological investigations with *Erwinia*, *Xanthomonas*, and the fungal isolate are under way.

Methodology for Measuring Nitrogen Fixation by Acetylene Reduction in *Beijerinckia* and *Klebsiella* in Stream Litter Decomposition. DILIPKUMAR VYAS, Department of Biology and BRUCE STORHOFF, Department of Chemistry, Ball State University, Muncie, Indiana 47306.——The nitrogen-fixing abilities of *Beijerinckia* and *Klebsiella* have been determined by gas chromatographic measurements of acetylene to ethylene ratios in incubated cultures. Commercially available acetylene was purified and added to the cultures, and the amount of ethylene produced was measured using a Varian 1400 Chromatograph equipped with a flame ionization detector and a 1 m x 2.5 mm I.D. column packed with phenylisocyanate on Porasil. Calibration standards consisting of mixtures of acetylene, ethylene and argon were prepared using vacuum line techniques. The chromatographic and data treatment procedures will be presented in detail.

Investigation of the Role of *Beijerinckia* and *Klebsiella* as Nitrogen Fixers in Stream Litter Decomposition. DILIPKUMAR VYAS, DONALD HENDRICKSON, and CARL WARNES, Department of Biology and BRUCE STORHOFF, Department of Chemistry, Ball State University, Muncie, Indiana 47306.——The role of *Beijerinckia* spp and *Klebsiella* spp in stream litter decomposition was investigated using acetylene reduction method. *Beijerinckia* and *Klebsiella* were isolated during winter and spring of 1980, from leaves of sugar maple (*Acer saccharum*), placed in Bell Creek, Muncie, Indiana. Nitrogen free media was used to isolate *Klebsiella*. Both of these organisms were streaked separately on nitrogen free agar slant tubes, flushed with argon and acetylene added. Gas chromatographic analyses were conducted on the inoculated tubes after incubation for 24 hours at 32° C. *Beijerinckia* spp. isolated from January, February and April fixed 4.48×10^{-2} moles and *Klebsiella* spp. isolated during the same time period was between 1.47 to 8.10×10^{-8} moles. This study indicates that *Beijerinckia* is a better N_2 fixer than *Klebsiella* in stream litter decomposition.