MICROBIOLOGY AND MOLECULAR BIOLOGY

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ABSTRACTS

S-Adenosylmethionine as a Biochemical Correlate in the Dimorphism of Mucor racemosus. ROBERTO GARCIA, Department of Biology, 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 or anaerobic conversion of yeasts to hyphae. The data also suggested that the increase might be a morphological correlate since it closely paralleled the time course of yeast-hyphae morphogenesis. The present report deals with an extension of those studies, utilizing a morphological mutant (COY) which requires high levels of methionine to undergo the shift in vegetative cell type and cycloleucine which is an inhibitor of the enzyme responsibile for the synthesis of SAM, to further examine the role of SAM in morphogenesis. When the COY mutant is grown in the presence of high levels of methionine it can undergo the normal shift in vegetative cell type displayed by the wild type. The magnitude and time course of the increase in intracellular SAM is similar to that seen in the wild type. However, if the mutant is grown in the absence of methionine it fails to undergo the shift in cell type. An examination of intracellular SAM revealed that the mutant experienced a tenfold decrease in this metabolite immediately after the shift to an aerobic environment which was used to induce the morphological change. In experiments with cycloleucine, an inhibitor of SAM Synthease activity, the morphological transition of the wild type was inhibited and the intracellular concentration of SAM dramatically decreased. This data coupled with that obtained from previous studies further strengthens the notion that the reported increase in SAM, observed during the conversion of yeasts to hyphae, is necessary for morphogensis. An examination of the specific activity of SAM Synthetase (in wild type *Mucor racemosus* and COY), under the experimental conditions previously described, was also part of these studies.

The Effect of Different Membrane Vesicle Preparation Techniques on Membrane Bound Enzymes of *Paracoccus denitrificans*. DANIEL P. KELLAR, WILLIAM W. BALDWIN and W. MARSHALL ANDERSON, Indiana University School of Medicine, Northwest Center for Medical Education, Gary, Indiana 46408.——*Paracoccus denitrificans* is the organism of choice in studing energy linked reactions in bacteria because of the similarities between the electron transport chain of *P. denitrificans* and mammalian mitochondria. Bacterial membrane vesicles that contain the enzymes of these energy linked reactions can be prepared by numerous methods. We studied several of these methods for vesicle preparation. Membrane vesicles were prepared in four ways. The organisms were converted into vesicles by means of sonic disruption, physical disruption by glass beads, enzymatic disruption using lysozyme and by treatment with a french pressure cell. Each preparation

exhibited some degree of inside-out orientation with respect to the physical make up of the vesicles. Transhydrogenase, NADH-oxidase, ATPase, and succinate dehydrogenase enzymatic activities were determined and were found to be dependent upon the manner in which these vesicles had been prepared. The results indicate that the final use to be made of the vesicles should indicate the method of preparation.

The Effect of the Host Genome on the Precise Excision of Tn*10*. DAVID P. KREPS, Department of Biology, Manchester College, North Manchester, Indiana 46962.——The effects of various mutations involving aspects of DNA repair, replication, and recombination upon the frequency of precise excision of the transposable genetic element Tn*10* were studied. *Escherichia coli* K-12 strains with a common *hisG*::Tn*10* insertion were constructed from *his*$^+$ parents via transduction; selected strains also received the mutagenesis-enhancing plasmid pKM101 via conjugation. The frequency of spontaneous precise excision of Tn*10* was quantitated and found to vary considerably among strains of different genotypes, relevant to the precise excision frequency of a wild type. Profound increases in the precise excision frequency from this insertion site were found to occur in strains possessing mutations in the *uvr D156*, *lig-4*, *mut LI3*, and *mut H34* genes. The frequency of induced precise excision of Tn*10* was also quantitated following exposure to ultraviolet irradiation. A slight enhancing effect on this frequency over that of the wild type was found among those strains listed above. The effects of the chemical mutagens N-methyl-N′-nitro-N-nitroso guanidine and B-propiolactone on the precise excision frequency were also examined using spot-plate tests and were not found to influence this frequency.

Prostaglandin E_2 Synthesis by Adherent and Non-adherent Tumor Cells. COLLEEN MAY and M. RITA YOUNG, Department of Biology/Center for Medical Education, Ball State University, Muncie, Indiana 47306.——Tumor cells produce more prostaglandin E_2 (PGE_2) and are less adherent to artificial substrates than normal cells. Since reduced adherence contributes to metastasis of the tumor, this study proposes that there is a relationship between the amount of PGE_2 secreted by a tumor cell and the reduced adherence of more virulent tumors.

P815 mastocytoma cells were cultured *in vitro* under conditions which selected for adherent and non-adherent variants. The amount of PGE_2 in the culture supernatants of these cells of common origin but different degrees of adherence was quantitated by a radioimmunoassay for PGE_2.

In this study, tumor cells which were less adherent *in vitro* were shown to secrete more PGE_2 into the culture medium than more adherent tumor cell variants. These results suggest a correlation between secretion of PGE_2 and reduced adherence which is associated with metastasis of tumor cells.

Detection of Prostaglandin E_2 in the Sera of Tumor Bearing Mice. MICHAEL MCTAGUE and M. RITA YOUNG, Department of Biology/Center for Medical Education, Ball State University, Muncie, Indiana 47306.——Tumors may protect themselves from destruction by secreting products which suppress the immune responses of their hosts. Homogenates of tumor cells contain elevated levels of prostaglandin E_2 (PGE_2) which inhibits macrophage tumoricidal and lymphocyte immunoregulatory activities *in vitro*.

This study proposes that if PGE_2 synthesis by tumor cells occurs early in the course of tumor development, then serum levels of PGE_2 may be elevated at this time. Supernatants of cultured Lewis lung carcinoma (LLC) were examined by a

radioimmunoassay for PGE_2 to determine if tumor cells secrete PGE_2. Furthermore, the PGE_2 concentrations in sera of normal and LLC-bearing mice were compared and found to be elevated in tumor bearing mice.

The results of this study suggest that presence of tumor is associated with elevated serum PGE_2 levels. Thus, monitoring serum PGE_2 concentration could become a method for early tumor detection.

Characteristics of a More Efficient Glycerol Utilizing Mutant of *Rhodopseudomonas capsulata*. LOY PIKE, Department of Biological Sciences, Indiana University at South Bend, South Bend, Indiana 46615.——The purple nonsulfur photosynthetic bacterium *Rhodopseudomonas capsulata* is noted for its inability to grow with glycerol as the sole source of carbon. However, occasional strains are isolated from nature which are capable of utilizing glycerol as a nutrient. One such strain, designated SCJ, was capable of metabolizing glycerol under aerobic-dark or anaerobic-light (photosynthetic) conditions. This organism possessed a soluble glycerokinase and a particulate pyridine nucleotide independent glycereophosphate dehydrogenase. When strain SCJ was grown on glycerol agar plates two colony sizes were consistently seen, with the larger being the least numerous. This large colony type was isolated in pure culture, but all cultures cloned from a small colony produced some large colonies when plated on glycerol agar. The large colony type had a much faster growth rate on glycerol than did the small colony type. While the specific activities of the glycerophosphate dehydrogenases from the two colony types were virtually identical, the specific activity of the glycerokinase from the large colony type was markedly reduced from that of the small colony type. Indications are that the large colony type has a mutant glycerokinase which is no longer susceptible to feedback inhibition.

The Etiology of Wilt in *Helianthus annuus*. MARY LEE RICHESON, Department of Biological Sciences, Indiana University-Purdue University at Fort Wayne, Fort Wayne, Indiana 46805.——Two types of bacteria and a filamentous fungus were isolated from lesions inside the stem of *Helianthus annuus* showing wilt symptoms. Biochemical, morphological, and cultural tests indicated that one bacterial isolate was *Erwinia carotovora* var. *carotovora* and the other *Xanthomonas campestris*. The morphology of the fungus was similar to *Cephalosporium*. Suspensions of the bacterial cells or fungal spores were inoculated into the growing plants by scratch, spray, or injection at two week intervals until the plants blossomed. Results indicated that healthy young plants are resistant to infection by these organisms. As the plants matured, 20% of the plants inoculated by injection with *E. carotovora* collapsed at the infection site and the tissue above the injection site died. *E. carotovora* was isolated in large number from the diseased stem areas. Plants similarly inoculated at two week intervals with spores of *Cephalosporium* remained healthy until head set at which time necrotic areas covered with mycelium appeared on the stems. *X. campestris* inoculation caused the stem to weaken and bend but the plants did not die. These results indicate that both *E. carotovora* and *Cephalosporium* may be a cause of late season wilt syndrome in sunflower.