

MICROBIOLOGY AND MOLECULAR BIOLOGY

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ABSTRACTS

Possible Aerobic Nitrate Reductase Activity in *Paracoccus denitrificans*. WILLIAM W. BALDWIN, W. MARSHALL ANDERSON and MING TSENG, Northwest Center for Medical Education, Indiana University School of Medicine, Gary, Indiana 46408. — *P. denitrificans* has a doubling time of 200 min. when grown aerobically on a minimal salts-nitrate-glucose medium or anaerobically on the same medium. When grown aerobically on a nutrient broth-glucose (NBG) medium the doubling time was 160 min., but decreased to 80 min. with added nitrate. Similar results were obtained if succinate was substituted for glucose. The anaerobic doubling time was 120 min. on the NBG-nitrate medium. It is generally assumed that in *P. denitrificans* O_2 inhibits nitrate reductase activity and represses its synthesis. To determine the role of nitrate reductase in aerobic growth, mutants lacking the activity (approximately 1/50 parental activity) were isolated by primary selection for chlorate resistance and tested for ability to grow anaerobically on nitrite but not grow on nitrate containing medium. One mutant selected for further study had an aerobic doubling time in NBG nitrate medium identical to the parental strain grown aerobically without nitrate. Revertants of the mutant are being isolated to define further the role of nitrate reductase in aerobic growth. It is postulated that nutrient broth may contain either a substance preferentially oxidized by nitrate reductase or a cofactor that protects the enzyme from inhibition and/or repression by O_2 . (Supported by Lake County (IN) Medical Center Development Agency).

Detection of ϵ -N-Methyllysines, in *Mucor racemosus*, by High-Performance Liquid Chromatography. J. R. GARCIA, Ball State University, Muncie, Indiana 47306 and P. S. SYPHERD, University of California at Irvine, Irvine, California. — This study was undertaken in order to test the possibility of using high-pressure liquid chromatography (HPLC) for the detection and resolution of methylated lysine residues. While HPLC has been used extensively for the detection and resolution of unmodified amino acids, it hasn't been employed in work with methylated amino acids. Dansylated derivatives of ϵ -N-mono, ϵ -N-di, and ϵ -N-trimethyllysine (MML, DML, and TML, respectively) standards were well resolved from each other and the rest of the amino acids, commonly found in proteins, through the use of a reversed-phase LDC Spherisorb column. The separation was achieved using a 0-60% concave methanol gradient and the fluorescence of the effluent monitored through the use of a fluorometer. In order to test the technique with actual biological samples, proteins known to contain methylated amino acids were acid hydrolyzed, dansylated and examined. MML, DML and TML, which had previously been tentatively demonstrated in the alpha subunit of *Mucor racemosus*

protein synthesis elongation factor-1, were all detected and well resolved. In addition, the TML in the cytochrome c of yeast was also detected. In conclusion, a rapid and rather sensitive HPLC technique has been developed which allows for the detection as well as resolution of MML, DML, and TML in hydrolyzed protein samples.

Suppression of Bacterial Endotoxin (LPS) Mitogenicity for Spleen Cells by Tumor Cell Culture Fluids. H.M. GHARPURE and K.W. BRUNSON, Northwest Center for Medical Education, Indiana University School of Medicine, Gary, Indiana 46408. — Sequential monitoring of mitogen induced proliferative responses of spleen cells from animals bearing a less metastatic RAW117-P lymphosarcoma or its highly metastatic variant RAW117-H10 lymphosarcoma tumor revealed that responses to either the B cell mitogen, lipopolysaccharide, or the T cell mitogen, concanavalin A are not affected until tumor-bearing day 13. Spleen cells from mice bearing the more metastatic tumor RAW117-H10 selectively suppressed the Con-A but not LPS induced proliferation of normal spleen cells. Direct co-culture of either of these two tumor cell lines with normal spleen cells showed that proliferation of normal spleen cells to the T cell mitogen Con-A is enhanced by the parental (less metastatic) tumor. In contrast the more metastatic tumor RAW117-H10 suppressed the Con-A response. Responses to LPS were inhibited by both RAW117-P and RAW117-H10 tumor cells. This mode of suppression was not restricted to spleen cells from syngeneic animals, but also affected spleen cell response from outbred mice. Serum free culture supernatants from both the tumor cell lines inhibited LPS induced proliferation of B cells from normal spleen. However, the supernatant enhanced Con-A induced proliferation. Increasing experimental evidence suggests a dualistic role of the culture supernatants in achieving differential effects on distinct spleen cell populations. (Supported in part by the funds from the Lake County Medical Center Development Agency).

Transfer of the Plasmid RPI into the Chemolithotrophic Bacterium, *Thiobacillus Neapolitanus*. CHARLES F. KULPA, MICHAEL T. TRAVIS, MARK T. ROSKEY, Department of Microbiology, University of Notre Dame, Notre Dame, Indiana 46556. — RPI, an incompatibility group PI plasmid specifying multiple drug resistances, has been transferred into the chemolithotroph *Thiobacillus neapolitanus*. The ability of *T. neapolitanus* to receive, express, and transmit RPI encoded antibiotic resistance is examined. We have repeatedly transferred RPI by conjugation from *Escherichia coli* ROE53I to *T. neapolitanus*, with a frequency of approximately 8.5×10^{-5} . The transfer of RPI into *Pseudomonas aeruginosa* from *E. coli* ROE53I yielded similar frequencies (4.8×10^{-5}). Reciprocal matings with strains of *T. neapolitanus* (RPI) derived from a prior cross, yielded transfer frequencies into ROE53I and PAO2 of 7.9×10^{-7} and 6.0×10^{-7} , respectively. Additionally, the transfer frequency has been shown to be dependent on the donor to recipient ratio; higher transfer frequencies occurring at decreased donor to recipient ratios. The presence of the RPI plasmid in the transconjugants was shown by isolation of RPI plasmid DNA and subsequent characterization by agarose gel electrophoresis. The presence of RPI DNA was detectable in both the *T. neapolitanus* (RPI) transconjugants and those strains derived by reciprocal mating. These data indicate that the RPI plasmid host range extends beyond heterotrophic bacteria, and RPI can be expressed in a lithotrophic bacterium totally unrelated to its previous host.

Slime Producing Bacteria Involved in Urinary Tract Infections. LYNN A. LARUE, WAYNE J. LENNINGTON and DONALD A. HENDRICKSON, Ball State University, Mun-

cie, Indiana 47306.——Urinary tract infections are one of the most commonly encountered bacterial infection found in hospital laboratories. The species, strains, and number of organisms identified at Ball Memorial Hospital, Muncie, Indiana, as the causative agent of a urinary tract infection were classified by API 20 strips or microscan plates. Special attention was shown to organisms producing a glycocalyx. Negative capsule stains were used to distinguish between capsule producing and non-capsule producing bacteria. Ten different species of organisms were identified, with *Escherischia coli* being the most abundant and most varied, having thirty-three different strains. All organisms were capsular, except for three strains of *E. coli* and one strain of *Pseudomonas aeruginosa*. The role of the glycocalyx in this infection could not be determined, but further research is being conducted to investigate this relationship. Research is continuing in order to find out if the capsule is a characteristic of this infection or if it has appeared in our data due to a new factor in this area selecting for capsular bacteria.

Fungal Culture Technique. CAROL A. WEAN and DONALD A. HENDRICKSON, Ball State University, Muncie, Indiana 47306.——A technique for the culture and microscopic examination of fungi has been devised in order to overcome inadequacies present in other methods which require a transfer from the culture plate to the microscopic slide. In this technique the fungus is cultured directly on the microscope slide, thus eliminating the transfer process.

To avoid contamination during incubation, a culture chamber is established by placing a layer of absorbant paper in the bottom of a glass Petri plate. A bent glass rod is centered in the plate, upon which a glass concavity (hanging drop) microscope slide is autoclaved.

After the chamber has cooled, sterile water is added to absorbant paper. Sterile Sabouraud dextrose broth is lightly inoculated with a fungal culture using an inoculating needle. The cover is replaced and the culture allowed to incubate undisturbed. When the culture has reached the appropriate growth stage, a cover slip is positioned over the concavity and the slide examined microscopically.

This culture technique is simple and uses materials commonly found in microbiology laboratories. The resulting slides should be beneficial for teaching purposes.

