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CHARACTERIZATION OF EXOCHITINASE OF CANTALOUPE FRUIT TISSUE. M. Judkins, T. Pardue, C. Biles, B. Bruton, and J. Zhang, Biology Dept. ECU, Ada, OK 74820. USDA, ARS, Lane, OK 74555.

Exochitinase (chitobiase) is an enzyme that has bee associated with the defense against tungal invasion plant tissue. The plant produces these enzymes and they are released and synthesis enhanced in the presence of a fungal pathogen. Latent fungal pathogens invade the fruit during net development and remain dormant until fruit maturity (40 days after flowering). The induction of latency and the stimulation to continue infection at fruit maturity poorly understood. One hypothesis is that fruit defense enzymes are present early in fruit development but decrease in activity as the fruit matures, therefore rendering the fruit more susceptible to fungal pathogens. Chitobiase activity was observed in mesocarp and exocarp cantaloupe tissue, with the highest activity in fruit mesocarp. Acetone precipitated proteins were applied to an anion and cation exchange column. Two peaks were observed. The anionic peak had the most activity and the fraction was applied to a size-exclusion column with showed on peak of chitobiase activities. SDS-PAGE the chitobiase fraction indicated one protein band at 44 kD. Further chromatography of exocarp tissue showed that 10-day fruit had the highest chitobiase activity when compared to older fruit samples.

RELATIVELY INEXPENSIVE ELECTRONIC AND PHOTOGRAPHIC METHODS FOR AERIAL SURVEYS OF OKLAHOMA SEEP BOGS. John R. Wright, John Taylor, and Connie Taylor, Southeastern Oklahoma State University, Box 4181, Station A, Durant, OK 74701

Changes in the Boehler seep bog (34° 10.02' N; 95° 53.36' W) were documented photographically and by other means using a Cessna-150 aircraft. Oblique 35mm film photography from an altitude of ~300 m AGL using ~2.5 s frame intervals produced stereo pairs with a depth resolution of ~30 cm. Horizontal image resolution of about 10 cm on the ground is typical. Low overflights with a Provideo color CCD microcamera (50mm e.f.l., f/0.95, < 0.0005 s integration time) produced even higher resolution electronic images which show animal tracks and the shapes of larger leaves. An inexpensive but effective thermal infrared radiometer for recording temperature profiles was developed from a $15 Regent two element pyroelectric motion sensor, a Poly IR-2 Fresnel lens (84 mm f.l., 1/4) and a white Poly IR-7 light scattering filter (the latter selects thermal IR). The radiometer and a (recorded) near infrared CCD TV camera were mounted together on a small platform; in this arrangement the camera photographs a pointer at the center of its image (where the radiometer is aimed) along with a meter, which is located below the pointer. As the scene moves past the pointer the meter indicates temperature changes along the Earth's surface (sensitivity ~0.5 C). The survey measured thermal profiles of two bogs and detected significant movements of the vegetative mats of Hasell Bog (34° 10.64' N, 95° 53.40' W). Also, while Boehler was found dry on December 1, 1996, it retained water through the summer drought of 1998. Aerial surveys are relatively non-intrusive. Acknowledgements: Poly IR lenses and filters were donated by Fresnel Technologies, Ft. Worth, TX, and aircraft costs were supported by the first author.

HISTAMINE RELEASE ASSAY. J.A.Price and W. Coberly, Dept. of Biochemistry & Microbiology, OSU-COM, Tulsa, OK 74107. Dept of Math & Computer Science, University of Tulsa, Tulsa, OK 74104.

A microplate assay for release and measurement of histamine has been developed as reported previously. Performance questions were explored using an n=24 replicates experiment to provide a single experiment, large data population. The data is normally distributed as ascertained by descriptive analysis and the Komogorov-Smirnov test. With a coeffcient of variation of 8%, 6 replicates provide sufficient group size for analytical experiments, while groups of 4 replicates are adequate for screening tests with a coefficient of variation of 18%. Positional bias for samples on the microplate was shown to be absent. At doses of agonist showing an effect, the standard deviation of experimental groups is higher than for controls. Taken together with previous results, the data indicate that this technology is an excellent replacement for large format assays.

SEASONAL DISTRIBUTION OF THE HYPORHEOS IN FIVE OKLAHOMA STREAMS. G.W.Hunt and E.H Stanley, Department of Zoology, Oklahoma State University, Stillwater, OK 74078.

Hyporheic invertebrates were collected seasonally beginning in the summer 1996 from five Oklahoma streams (Baron Fork in Cherokee Co., Saline Creek in Delaware Co., Wild Hog Creek in Osage Co., Rock Creek in Murray Co., and the Elm Fork of the Red River in Greer Co.). Samples were collected using Bou-Rouch method at depths of 5 cm, 30cm, 60 cm and 100 cm below the stream bottom. Average annual densities ranged from 12/L or less in the Elm Fork and Rock Creek to greater than 95/L in Saline Creek and the Baron Fork. Copepods were the dominant taxa at all streams comprising from 28 to 75 percent of all organisms and 30 of the 58 taxa encountered. The most abundant copepod genera included *Diacyc!ops* and *Acanthocyclops.* Other common invertebrates included Nematoda, Oligochaeta, Chironomidac, and Ostracoda. The amphipod *Stygonectes* and the isopod *Caecidotea* often common in the northeastern streams, were the only obligatory hypogean forms collected. Total densities peaked during spring, corresponding to the seasonal maxima for cyclopoid copepods. Lowest densities occurred during the summer corresponding to decreased dissolved oxygen levels. These results indicate that the diverse assemblage of invertebrates inhabiting the hyporheic zone within Oklahoma streams is worthy of additional study.

CHARACTERIZATION OF VANCOMYCIN RESISTANT *STAPHYLOCOCCUS AUREUS* DERIVED THROUGH STEP-WISE ADAPTATION. M.D. McGuire, S. Wright, and R. S. Conrad, Department of Biochemistry and Microbiology, Oklahoma State University. College of Osteopathic Medicine. Tulsa, OK 74107.

Methicillin resistant *Staphylococcus aureus*(MRSA) is emerging as a devastating hospital acquired infection manifested in infections such as septisemia, endocarditis and pneumonia. Vancomycin, a cell wall inhibitor, is the most effective antibiotic available against this organism and is generally considered the drug of last resort. Recent reports of Vancomycin intermediate resistance by MRSA have led to concerns of inneffective therapy in the future. The mechanism of MRSA resistance to vancomycin is not known and does not appear to be a plasmid tranferred from vancomycin resistant *Enterococcus.* This mechanism was studied by collecting a clinical isolate of MRSA in cation supplemented Mueller-Hinton broth (CSMH) in the presence of vancomycin. The clinical isolate was step-wise adapted to grow in the presence of 50ug/ml of vancomycin as determined by the minimum inhibitory concentration (MIC) by tube dilution. This vancomycin resistant *S. aureus* (VRSA) demonstrated biological alterations including development of susceptibillity to methicillin and penicillin. This VRSA became coagulase and beta lactamase negative but remained catalase positive. VRSA also lost the ability to ferment lactose and trehalose. Electron microscopy of the VRSA verified the presence of thickened cell walls and aberrant cellular morphology. Although the mechanism of vancomycin resistance in this VRSA is unknown, it appears that transport through the cell wall and membrane may be affected.

***NAEGLERLA FOWLERI* ISOLATED FROM A CASE OF PRIMARY AMEBIC MENINGOENCEPHALITIS IN OKLAHOMA.** Marsha J. Howard and David T. John, Department of Biochemistry and Microbiology, Oklahoma State University College of Osteopathic Medicine, Tulsa OK 74107.

On August 9, 1998 a 3-year-old girl from Sperry Oklahoma died of primary amebic meningoencephalitis (PAM), the first confirmed case of PAM in Oklahoma. A week before, the little girl had played in the water at the edge of Fort Gibson Lake and apparently had sniffed some water containing *Naegleria fowleri*, the cause of PAM. Four days later she developed headache and fever. Over the next 2 days the symptoms worsened and she was taken to the emergency room of a Tulsa hospital. She experienced severe frontal headache, vomiting, seizures and hallucinations and died 2 days later in the pediatric intensive care unit. An autopsy was performed by the State Medical Examiner's Office in Tulsa and a diagnosis of PAM was made. We received a sample of brain tissue and confirmed the diagnosis by culturing *N. fowleri* from the specimen. The amebae were elongate and slug-like and moved in a directional manner by explosive blunt pseudopodia. The amebae did not agglutinate in the presence of concanavalin A. When amebae were suspended in distilled water, they transformed into temporary biflagellate forms. Amebae produced spherical cysts when they were grown on non-nutrient agar with bacteria. Of 3 media tested, amebae grew best in Mix medium at 37°C. Amebae produced deaths in mice when as few as 100 per mouse were inoculated intranasally.

ALTERATION IN BIOLOGICAL ACTIVITY OF BACTERIA AS A FUNCTION OF GROWTH MEDIA AND ANTIMICROBIAL SUSCEPTIBILITIES. P. E. Mooney, J. W. Bullard, and R. S. Conrad, Oklahoma State University, College of Osteopathic Medicine, Tulsa, OK 74107.

Two of the areas where lipopolysaccharides (LPS) are known to interact with and modify the human immune system response are in Complement Activation and Neutrophil Respiratory Burst. To measure the ability of LPSs of different chemical composition to activate complement we combined two clinical lab tests, Complement Fixation (CF) and CH5O test. By combining these 2 techniques we were able to detect and quantify differences in the complement activating abilities of the purified LPSs of *Pseudomonas aeruginosa* PAO 1 grown on various single source carbon media. These growth conditions have been previouslv demonstrated to alter antimicrobial susceptibility. We used the clinical lab test, Chemiluminescence to measure the effect of LPSs of different chemical composition on the Neutrophil Respiratory Burst. By exposing, "priming" the neutrophils with the various LPSs prior to using the neutrophils in the Chemilurninescence test we were able to detect and quantify differences in the LPSs ability to modulate the Neutrophil Respiratory Burst. Our preliminary results indicate that the different LPSs elicit significantly different immune responses as measured by the CF, CH5O and Chemiluminescence tests. We are in the process of a biochemical analyses of these LPSs to correlate chemical composition and biological activity.

CHARACTERIZATION OF AN ENVIRONMENTAL PATHOGENIC *ACANTHAMOEBA* ISOLATE. Heather D. Rupe, Marsha J. Howard, and David T. John, Department of Biochemistry and Microbiology, Oklahoma State University, College of Osteopathic Medicine, Tulsa, 74107.

Several species of free-living amebae belonging to the genus *Acanthamoeba* are able to cause disease in humans including a chronic infection of the central nervous system (CNS) and a serious eye infection. During a previous environmental survey in the Tulsa area, we obtained 12 pathogenic *Acanthamoeba* isolates from water and swab samples. The ameba isolates were able to produce a fatal CNS infection in mice after intranasal inoculation. One isolate, EPA-861, however, after the original recovery from brain tissue, was not able to produce deaths in mice following intranasal inoculation, although amebae could be recovered from brain tissue after intravenous inoculation. The purpose of this study was to further characterize the isolate and identify the species using an indirect immunofluorescence (IIF) test. The morphological features of the isolate were typically *Acanthamoeba.* Amebae were large and possessed tapering spike-like pseudopodia and moved on a broad front without direction. Cysts were spherical and had an outer wrinkled ectocyst and an inner smooth endocyst. Of 3 media tested amebae grew best in Mix medium at 300°C. Amebae were agglutinated by concanavalin A. After serial passage in mice, amebae we again recovered from brain tissue following intranasal inoculation . The IIF test identified isolate EPA-861 as *A. castellanii.*

AERATION AND LENGTH OF EXPOSURE TO LIGHT AFFECT DEVELOPMENT OF FUSARIUM SOLANI FORMATION OF PROTEINS. Melissa Juarez, David Juarez, Vinee Russo, and C. Biles, Eastern Oklahoma State College, Wilburton, OK 74563; Agriculture Research Service, U.S. Department of Agriculture, Lane, OK 74555; and Department of Biology, East Central University, Ada, OK 74820.

Fusarium solani was exposed to combinations of filtered air at 0, 0.1, 0.5, and 1.0 liters per minute (LPM) and multispectrum fluorescent light for 0, 12, or 24 hr in Czapek-Dox broth. After incubation for 72 h, fungal dry weight and total protein weight, as well as numbers of protein bands in SDS-PAGE gels and their weights, were determined. As exposure to light increased and aeration flow-rate was higher, the fungal dry weight increased. Total protein was not affected by exposure to light (Avg. 0.0025 ug/g), but was highest at 0 LPM (0.0052 ug/g). There was no length of exposures light by flow-rate of aeration interaction affecting fungal dry weight or total protein weight. Rate of aeration and exposure to light affected the number and weights of protein bands produced. The range of numbers of bands formed was 2 to 14, with the greatest number of bands being formed under 24 hr light. The lowest band weight was ca. 50 kD and the highest was ca. 180 kD. Fungal dry weight was not correlated with total protein weight. This suggests that increases in fungal dry weights are due to other components of the fungal biomass. Although total protein weight appears to be inhibited by aeration, these proteins are likely not important in the production of fungal dry weight which did not decrease as aeration flow-rate increased.

PHYSIOLOGICAL AND MOLECULAR CHARACTERIZATION OF PIGMENTED AND NON-PIGMENTED ISOLATES OF *MONOSPORAS-CUS CANNONBALLUS*. David Juarez, Eastern Oklahoma State College, Wilburton, OK 74563; J. Zhang and B. Bruton, USDA, ARS, Lane, OK 74555; and C. Biles, Biology Dept, ECU, Ada, OK 74820.

Monosporascus cannonballus (MC), a soilborn fungus, causes a vine-decline that significantly reduces muskmelon yields in hot, dry regions. Double stranded RNA (dsRNA) and pigmentation (PIG) are associated with some MC isolates. Pigmented isolates are often associated with reduced spore production and growth rate, and fungal degeneration. Enzyme production, dsRNA, and protein profiles from shake culture media, potato dextrose broth (PDB) and modified Fusarium nutrient solution (MFNS), were compared in PIG and non-pigmented (NP) isolates. NP isolates did not produce pigment in either media. SDS- and IEF-PAGE of proteins extracted from fungus grown in PDB showed different protein profiles between PIG and NP isolates. The protein, with a pI of 4.0, was associated only with the PIG isolates in PDB. dsRNA bands, observed in all isolates varied in number and size. Cellulase activity also varied among isolates. A protein with pI 4.0 appears to be associated with pigment production, but is only synthesized in PDB. Pigment production was not associated with the presence of dsRNA.