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PEROXIDASE ACTIVITY AND ISOZYMES OF CANTALOUPE TISSUE. Ki. Kennedy, C. Biles, B. Bruton, and **J. Zhang**. Biology Dept., ECU, Ada, OK 74820, and USDA, ARS, Lane, OK 74555

Peroxidase is a plant enzyme that has been implicated in wound healing, lignification, suberization, and antifungal activity. Peroxidase activity was low in fruit harvested 10 and 15 days after anthesis (DAA), regardless of tissue type. In fruit harvested 20 and 30 DAA, peroxidase levels increased in the exocarp and peaked in fruit harvested 30-40 DAA. Peroxidase isozymes in the exocarp also increased from one faint peroxidase band (ca. pI 6.0) in fruit harvested 10 DAA to at least 3 peroxidase bands ranging from pI 3.0 to 9.0 in fruit harvested 20-30 DAA. The pI 6.0 peroxidase exocarp isozyme increased in intensity in fruit harvested 20 DAA and decreased in fruit harvested 40 and 50 DAA. Nitrocellulose tissue blots indicated that peroxidase activity was primarily located in the exocarp tissue as the fruit ripened. Peroxidase activity in the exocarp increased during net development according to total peroxidase activity, isozyme patterns, and nitrocellulose tissue blots. The role of peroxidase in cantaloupe ripening, net development, and disease resistance is unknown, but the increase of peroxidase in fruit harvested 15-20 DAA corresponds to net development and the decrease of the pI 6.0 isozyme in fruit harvested 40-50 DAA corresponds to the induction of latent fungal pathogens.

INVESTIGATION OF ANOMALOUS BEHAVIOR OF SOLUTIONS OF VARIOUS MALLOW EXTRACTS

Dwight L. Myers, and **Matthew R. Miller**, Department of Chemistry, and **Charles L. Biles**. Department of Biology, East Central University, Ada. OK, 74820, and **Vince M. Russo** and **Charles L. Webber,** United States Department of Agriculture, Agricultural Research Service, Lane, OK 74555

A previous study of fresh and weathered kenaf *(Hibiscus cannibinus)* extracts revealed anomalous behaviour during measurement of osmolality[1]. Some of the solutions proved difficult to supercool, indicating the presence of ice nucleation activity (INA). Further experimentation revealed that this effect also occurs with extracts from okra *(Hibiscus esculentum)*, but not with cotton *(Gossypium hirsutum)*. Certain species of the bacterium *Pseudomonas* also exhibit INA[2], as do some Antarctic plants[3]. SDS-PAGE experiments revealed differences in banding for okra and kenaf compared to cotton. Further experimentation is in progress to isolate the proteins unique to okra and kenaf and determine whether they exhibit INA.

1. Russo, V.M., Webber, C.L., and Myers, D.L., "Germination and Post-germination Development of Vegetable, Grass and Weed Seed Exposed to Kenaf Extracts," Accepted by *Industrial Crops and Products*, 1996.

2. Wolber, P.K., Deininger, C.A., Southworth. M.W., Vandekerckhove, J., Montagu, M.V., and Warren, G.J. "Identification and Purification of a Bacterial Ice-Nucleation Protein." *Proc. Natl. Acad. Sci. USA, 83*, Oct. 1986, pp. 7256-7260.

3. Worland, M. R.. Block, W., and Oldale, H., "Ice Nucleation Activity in Biological Materials with Examples from Antarctic Plants", *Cryo-Letters 17*, 31- 38 (1996).

SITE OF ACTION OF LUMAZINE: A SPECIFIC INHIBITOR OF: METHANOGENIC BACTERIA.

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Methanogenic archaea produce methane as their end product of metabolism, thereby influencing anaerobic biodegradation systems. Lumazine (LZ) (2,4-pteridinedione) totally inhibited growth of methanogens at sub-mM levels whereas other microorganisms were not much affected at 10 mM LZ (Nagar-Anthal *et al., Archiv. Microbiol. 166:* 136, 1996). LZ was bacteriocidal for the model organism *Methanobacterium termoautotrophicum;* addition to cultures led to rapid cessation of methanogenesis. In cell extracts LZ inhibited H_2-dependent reduction of CO_2 to methane. An initial hypothesis, that LZ inhibited reaction(s) involving methanogen coenzymes with structural resemblance to it (tetrahydromethanopterin, deazaflavin F_{420} or FAD) was rejected. i. Authentic coenzymes did not prevent LZ inhibition *in vitro;* ii. growth of archaeon *Archaeoglobus fulgidus*, which uses these in central metabolism, was not inhibited (assuming no permeability barrier); iii. methanol reduction with H_2 by *Methanosarcina*, not involving the coenzymes, was inhibited; and iv. the central enzyme of methanogenesis, Methyl Coenzyme M reductase, was inhibited. It synthesizes methane from Me-S-CoM and HS-HTP yielding CoM-SS-HTP disulfide. Kinetic analysis revealed that inhibition was mixed with respect to both substrates (K_i=50µM). LZ seems promising as a probe of this complex reaction, found only in methanogenic archaea. Ecological studies of anaerobic ecosystems may benefit from selective inhibition of methanogens by lumazine.

CRYOPRESERVATION OF OPPORTUNISTIC AMEBAE.

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Opportunistic amebae cause serious infection of the eye and the central nervous system. The purpose of this study was to develop a single procedure for freezing pathogenic *Acanthamoeba* and *Naegleria*. The amebae used in this study were *A. castellanii* (El-212), *N. australiensis* (PP-397) and *N. fowleri* (LEE). Amebae were cultivated axenically in Mix ameba medium. One-ml quantities of amebae in freezing medium were dispensed in cryogenic vials and frozen at -70ºC. Amebae were rapidly thawed by placing the cryovials in a 37°C waterbath and viability was determined by exclusion of 0.4% Congo red. The average best conditions for freezing the three species studied were: 1×10^6 exponentially growing amebae/ml of freezing medium consisting of 12% dimethylsulfoxide, 20% heat-inactivated bovine calf serum, 4% glucose, in Mix ameba medium; 30 min equilibration at 23°C, followed by 60 min at -20°C, with storage at -70°C. Viability decreased by an average of 35% over the 5 years of freezing, from 61% to 40%. Sixty-eight percent of the decrease in viability occurred in the 1st year. Pathogenicity was unaffected by cryopreservation. (Supported by EPA Grant R-818106)

IDENTITY OF PATHOGENIC *NAEGLERIA* BY INDIRECT IMMUNOFLUORESCENCE.

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Pathogenic free-living amebae cause serious human disease, including infection of the eye and central nervous system. Previously, 34 pathogenic ameba isolates were obtained from samples collected in the Tulsa area, 19 were tentatively identified as *Naegleria* spp., based on morphology, pathogenicity to mice and reactivity to Concanavalin A. An assay for indirect immunofluorescence (IIF) was developed to confirm their identities. Antisera produced in rabbits to *N. fowleri N. australiensis, N. gruberi* and *N. lovaniensis* were used in these assays. The above amebae and our environmental *Naegleria* isolates were cultured, fixed to multiwell slides and assayed in triplicate by IIF. Of the 19 suspected *Naegleria* isolates 11 were assayed. The 6 *N. fowleri* isolates all showed titers corresponding to *N. fowleri* (mean 1:1024). They also had relatively high titers to *N. lovaniensis* (mean 1:128). However, our control also showed a titer of 1:128 to *N. lovaniensis.* Assays of the *N. fowleri* isolates vs *N. australiensis* and *N. gruberi* had titers of 1:16 or less. 4 of the 5 *N. australiensis* isolates assayed were confirmed (mean titer of 1:1024) whereas the 5th exhibited its highest titer to *N. lovaniensis* (1:1024) and. only 1:128 to *N. australiensis. N. lovaniensis* is a nonpathogen; therefore, further study is needed on this particular isolate to confirm its identity. 8 *N. australiensis,* isolates remain to be confirmed.

ANTIBIOTIC SENSITIVITY OF PATHOGENIC AND NONPATHOGENIC *NAEGLERIA.*

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Naegleria is a genus of freeliving amebae which contains 3 pathogenic and 7 nonpathogenic species. *Naegleria fowleri* is responsible for a rapidly fatal infection of the central nervous system called primary amebic meningoencephalitis (PAM). A survivor of PAM in California was given amphotericin B, miconazole and rifampin. The purpose of this study was to determine the susceptibility of pathogenic and nonpathogenic *Naegleria* to the 3 antibiotics. The amebae used were *N. fowleri* (6088), the isolate from the California survivor, and *N. lovaniensis* (Aq/9/1/45/D), a thermotolerant species originally thought to be a nonpathogenic variant of *N. fowleri.* Amebae were cultivated in Mix ameba medium with various concentrations of antibiotics. Rifampin, even at concentrations of 100 µg/ml, did not inhibit the growth of either pathogenic or nonpathogenic *Naegleria*. Amphotericin B inhibited the growth of the pathogen at 0.025 µg/ml and the nonpathogen at 0.25 µg/ml. Miconazole at 0.1 µg/ml inhibited the growth of the pathogen but not the nonpathogen. It is likely that amphotericin B and miconazole were responsible for the California recovery.

DRUG SENSITIVITY TESTING OF *NAEGLERIA FOWLERI.*

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Naegleria fowleri is a pathogenic freeliving ameba and the cause of a fatal disease known as primary amebic meningoencephalitis (PAM). A survivor of PAM in California was given amphotericin B, miconazole and rifampin. The purpose of this study was to determine the susceptibility of highly virulent and weakly virulent *N. fowleri* to the 3 antibiotics. The strains of *N. fowleri* used were LEE stock (weakly virulent) and LEE m65 (highly virulent). Amebae were cultivated in Mix ameba medium with various concentrations of antibiotics. Mice were infected with *N. fowleri* and then inoculated with amphotericin B (7.5 mg/kg body weight) and miconazole (100 mg/kg) to determine if the antibiotics afforded protection. Rifampin, even at concentrations of 100 µg/ml, did not inhibit the growth of either strain of *N. fowleri*. Amphotericin B inhibited the growth of both strains at 0.25 µg/ml and miconazole inhibited both at 0.1 µg/ml. The drug combination afforded 100% protection to the *N. fowleri* - infected mice. Mortality was 40% for the untreated mice and the mean time to death was 15.6 days. (Supported by Oklahoma Partners for Biological Sciences Summer Research Award to SK)

YEASTS ISOLATED FROM OKLAHOMA WATERS AS POSSIBLE FOOD SOURCES FOR FREE-LIVING PATHOGENIC AMOEBAE.

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Free-living, pathogenic amoebae have been isolated from Oklahoma waters. *N. fowleri* has been shown to ingest *Saccharomyces cerevisiae.* The purpose of this study is to determine if other yeasts, including those isolated from these waters, are also ingested by free-living, pathogenic amoebae. Using dilution plating, several yeasts were isolated from am ornamental Pond, a golf pond and a stock pond (the latter two were found to contain free-living, pathogenic amoebae.) The isolation media included Sabouraud Dextrose Agar (with penicillin and streptomycin), Phytone Yeast Extract Agar, and Mycobiotic Agar plates. Once isolated, the yeasts were cultivated on Sabouraud Dextrose Agar or Mycologic Agar slants. A 0.2-0.3ml suspension of yeasts (McFarland Standard #5) was spread to form a "lawn" on water agar and allowed to dry overnight. *Escherichia coli, Candida albicans, Cryptococcus albidus* and *Saccharomyces cerevisiae* were used as control "lawns". *Naegleria fowleri* (1 human and 2 environmental isolates), *N. australiensis (*2 environmental isolates), or *Acanthamoeba* spp.(2 environmental isolates) were placed in one drop (30µl), containing $1X10^5$ amoebae, onto the center of each of three water agar yeast (or bacterium) coated plates. The plates were incubated at 30°C. The area cleared in the "lawn" was measured at 1 day, 3 days, and 6 days. In general, the *Naegleria* spp. cleared the bacterial but not the yeast "lawns". *Acanthamoeba* spp. cleared both bacterial and many of the yeast "lawns".

A RAPID METHOD FOR DETERMINING CARNITINE LEVELS IN SMALL SAMPLES.

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A simple, accurate and inexpensive method to assess carnitine status was developed to allow more appropriate administration of carnitine therapy. Serum was heated at 100°C for 4 min, frozen at -80ºC for 15 min and centrifuged 15 min at 20,000g to remove proteins. Supernatants were assayed in a microtiter plate for free (untreated) and total (hydrolyzed) carnitine by measuring the absorbance of reduced p-iodonitrotetrazolim (INT) at 490 nm generated in the following coupled reaction: carnitine + AcCoA E_1 => acetylcarnitine + CoASH NAD + CoASH + a-kg E_2 => sucCoA + NADH NADH + INT E_3 => INTH + NAD where E_1 = *Carnitine acetyltransferease (CAT),* E_2 = *Alpha-keto-glutarate dehydrogenase,* E_3 = *Diaphorase.* After monitoring the constant background rate for 30 min., the reaction is started with the addition of CAT (Sigma) which has been desalted to remove interfering salts and DDT. Following the rapid initial increase, the post-CAT constant background rate is monitored for 60 min. Regression analysis is used to determine the carnitine-dependent absorbance. The range for duplicate samples is typically ± .001 absorbance units. This method offers excellent reproducibility, 2 -2.5 times the sensitivity of assays monitoring NAD reduction and much greater specificity than DTNB assays detecting sulfhydryl release.

OVARIAN APOPTOSIS: POSSIBLE ROLE OF BCL_2 AND BAX PROTEINS

Tonya A. Yoney-Knox, Marva J. Volk, Colbi M. Gooden, Shawnaree L. Hamlett, **Jeffrey K. McCosh**, **Lana G. Nelson,** and **Gary H. Watson.** OSU-COM, Department of Biochemistry and Microbiology. Tulsa, OK 74107

Bcl_2 and Bax are two proteins known to be associated with apoptosis in a number of tissues. Our studies were initiated to investigate the expression of the proteins in the rat ovary during follicular athesia and to test if their expression is modulated by changing hormonal environments. These initial studies used Western blot analysis and immunohistochemistry to test for the expression of these proteins. Immunohistochemistry confirmed the expression of Bcl_2 and Bax proteins in both 9 day and 23 day old sexually immature animals. Bcl_2 expression was noted principally in the ova of primary multilaminar and vesicular secondary follicles. It also appeared to be localized in the perinuclear region. Bax protein, on the other hand, was expressed mainly in primordial and unilaminar primary follicles and appeared to be uniformly distributed throughout the cytoplasm of the cell.

Preliminary results also suggest that granulosa cells also actively express these proteins in the sexually mature ovary. Western blot analysis confirmed expression of Bax expression in this model, however, Bcl_2 antibodies cross reacted with small species suggesting active degradation of the Bcl_2 protein. These initial studies confirmed the expression of these proteins and suggest a possible role in follicular atresia. (Supported in part by a summer research grant to T.Y-K., the OPBS program, OSU).

CORRELATION BETWEEN CARBON SOURCE AND READILY EXTRACTABLE LIPIDS IN *PSEUDOMONAS AERUGINOSA.*

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Ps. aeruginosa strain PA01 was grown in basal media containing either L-leucine, L-valine, L-isoleucine or their respective acyl derivatives as the sole carbon source. Cells grown in these conditions all contained less readily extractable lipids (REL) than did cells grown in complex media (trypticase soy). All cells grown in branched-chain amino acids resulted in higher levels of REL compared to their respective branched-chain acyl derivatives. However, all acyl derivatives contained a higher percentage of phosphate in their REL compared to the branched-chain amino acids. Growth on the branched-chain acyl derivative also resulted in significant shifts from unsaturated to cyclopropyl fatty acids. Growth on carbon sources catabolized to propionyl-CoA resulted in the synthesis of odd-chain fatty acids. Growth on substrates metabolized to isobranched carbon skeletons resulted in the synthesis of branched-chain fatty acids. Branched-chain fatty acids were detected in all RELs except those cells grown in L-leucine and complex media. These results indicate carbon source has a significant impact on levels of RELs in *Ps. aeruginosa.*

EFFECT OF CARBOHYDRATES ON CHLAMYDOSPORE PRODUCTION OF *ACREMONIUM CUCURBITACEARUM*

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Acremonium cucurbitacearum is a soilborne plant pathogen that causes vine-decline of melons. Plant roots exude various carbohydrates as they grow through the soil that stimulate fungal growth, taxis, and infection. *A. cucurbitacearum* was grown on agar plates and in broth shake with different carbon sources to determine the effect on hyphal growth and chlamydospore production. Sucrose stimulated the highest level of hyphal growth. Although significant variation among isolates was observed in chlamydospore production grown on different sugars, the greatest production occurred with isolates grown on galactose, glucose and sucrose. The lowest level of hyphal growth and chlamydospore production occurred with isolates grown on fructose and mannose. Light microscopy revealed that chlamydospores produced in broth shake culture were terminal in most cases and dehiscent. Isolates grown on agar plates tended to produce intercalary chlamydospores. Chlamydospores of *A. cucurbitacearum* may play an important role in the epidemiology and overwintering of this pathogen of cantaloupe.

***β*-GALACTOSIDASE ACTIVITY OF RIPENING CANTALOUPE FRUIT AND FRUIT ROTTING FUNGI.**

C. Biles, B. Bruton, and **J. Zhang.** Biology Dept. East Central University, Ada, OK 74820; and USDA, ARS, Lane, OK 74555

β-galactosidase has been implicated in playing a role in fruit ripening in tomato, pepper, and cantaloupe. *Phomopsis cucurbitae* is a latent pathogen of cantaloupe that invades the fruit epidermis during net development causing severe postharvest decay. The purpose of this study was to compare the activity of fruit *β*-galactosidases with that of *P. cucurbitae β*-galactosidases in regard to fruit age and tissue type (i.e. inner mesocarp, middle mesocarp, outer mesocarp, and exocarp). *β*-galactosidase activity increased in exocarp tissue as the fruit ripened but began to decrease in fruit harvested at 40 days after anthesis and stored for 10 days. *P. cucurbitae* was cultured on autoclaved fruit tissue types described above for 10 days and assayed each day for *β*-galactosidase activity. *β*-galactosidase activity was highest in cultures containing the fungus using outer mesocarp or exocarp tissue as substrates, regardless of fruit age. *P. cucurbitae* also produced high levels of *β*-galactosidase in commercial pectin, but not in cellulose. Both fruit and fungal *β*-galactosidases were found to have the highest activity in the outer fruit tissue. Fruit *β*-galactosidase may play a major role in fruit softening and fungal *β*-galactosidase may play an important role in fruit decay.

LONG-TERM NORADRENERGIC ALTERATIONS FOLLOWING IN UTERO COCAINE EXPOSURE.

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The present studies examined a_2-adrenergic receptor (a_2-AR) density and [^{3}H]NE release in the hippocampus of adult rats (10 month-old) prenatally exposed to IV cocaine. At 135 days of age, female rats demonstrated increased sensitivity to the stimulatory actions of cocaine whereas and male rats exposed *in utero* to cocaine were significantly less responsive. Determination of hippocampal a_2-AR density was then performed as described (Wallace et al., Eur. J. Pharmacol., 258:67, 1994). The binding of [^{3}H]RX821002 (5 nM) displayed gender-specific alterations in the cocaine group with the density of [^{3}H]RX821002 labeled sites being decreased 18% in male rats, yet increased 11% in female rats. Potassium-stimulated (30 mM) fractional release of [^{3}H]NE in 300 µm hippocampal slices was reduced in cocaine-exposed male rats as indicated by a 18% reduction in both S1 release and S2/S1 release ratio. Release of [^{3}H]NE was slightly reduced in cocaine-exposed female rats, but the S2/S1 ratio was elevated 26% in cocaine-exposed females compared to saline rats. These data suggest that the hypoinnervation previously reported (Colson et al., Soc. Neurosci. 20:597, 1994) results in a persistent hypofunction of the noradrenergic system in the hippocampus as indicated by alterations in; 1) response to cocaine challenge, 2) receptor density and 3) [^{3}H]NE release. These changes may underlie the long-term alterations observed in offspring behavior following prenatal exposure to cocaine. (Supported by DA06638 & DA09160).

THE MORPHOLOGY OF THE EARLY INSTARS OF THE GIANT CAVE COCKROACH, *BLABERUS GIGANTEUS* (L.)[BLATTTARIA: BLABERIDAE]

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The morphology of *Blaberus giganteus* has not been studied in great detail. Piquett & Fales (JEE 46:6:1089-1090, 1953), based upon a study of 12 nymphs, concluded that *B. giganteus* had 7 or 8 nymphal instars. The results of this study using 17 cohorts containing a total of 630 individuals has produced different results. The oldest cohorts are 142 days old and in their 8th instar and are approximately one quarter of the adult size. Although the wing pads have become much larger they are not fully formed wings. The younger cohorts are following the growth curves of the older ones very closely. If these nymphs continue as projected, they should reach maturity in their 9th or more probably their 10th instar. Average durations of each instar are given, as are the average masses, lengths and widths of the cohorts while in each instar.