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SCIENTIFIC PROGRAM

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PLENNARY LECTURES

"Viral and cellular factors that affect HIV/AIDS in children"

Dr. Luisa Sen
Laboratorio Biología Celular y Retrovirus
Hospital de Pediatría Garrahan-Buenos Aires

"When ducks lived surrounded by dinosaurs.... The Mesozoic radiation of modern birds"

Dr. Claudia P. Tambussi
Departamento Científico Paleontología Vertebrados,
Museo de La Plata-La Plata

«New insights on an old nucleus: an overview on Edinger-Westphal».

Dr. Jackson. Bittencourt
Instituto de Ciências Biomédicas, Universidade de São Paulo –Brazil

"Role of the plants in the biodegradation of organic compounds"

Dr. Ana Maria Giulietti
Cátedra de Microbiología Industrial y Biotecnología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires

"Tucuman Biology Association (Asociación de Biología de Tucumán)" Lecture

«Regulation of ovocyte maturation in amphibians"

Dr. Liliana I. Zelarayán
Instituto de Biología INSIBIO-Universidad Nacional de Tucumán

"Cuyo Biology Society (Sociedad de Biología de Cuyo)" Lecture

«Localization and expression of Angiotensin II receptors during the rat brain development».

Dra. Gladys Ciuffo
INTEQUI-CONICET, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis

"There is life outside the laboratory, the scientific divulgement"

Dr. Diego Golombek
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"70 years of History at the Cordoba Society for Biology"
Drs. Maria Ester Celis and German Roth
Facultad de Ciencias Químicas, Universidad Nacional de Córdoba

SCIENTIFIC SYMPOSIA

I. Apoptosis and Proliferation: Die to live or live to die?

Drs. B. Caputto, V. Rivarola y C. Soñez.
Moderators: Drs. C. Casale and D. Mascó.

II. Biodiversity: a Pandora's box??

Drs. E. Bucher, I di Tada, A. Fabra, A. Martino and H. Varela.
Moderator: Dr. I di Tada.

III. Immunology: antigenic presenter cells: the skeleton of immunity.

Drs. L. Cervi, P. Iribarren, C. Motrán.
Moderators: Drs. S. Correa and A. Gruppi.

IV. CAEN-International Society for Neurochemistry Symposium "From the Molecule to the Brain"

Drs. C. Bouzat, B. Elgoyen and H. Carrer.
Moderators: Drs. V. Molina and M. Guido

V. The Communication between Plants and the Environment: a World of Signals.

Drs. J. Casal, C. Gonzalez, C. Luna and G. Racagni.
Moderators: Drs. K. Grunberg and M.E. Alvarez.

VI. Molecular Bases of Sperm Physiology and Fertilization.

Drs. L. Giojalas, P. Cuasnicú, R. Sánchez Gutiérrez (Chile).
Moderator: Dr. C. Coronel

1.

VITELLOGENESIS IN VECTORS OF CHAGAS'DISEASE (HEMIPTERA: REDUVIIDAE)

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Hematophagous insects like vectors of Chagas' disease must surpass a threshold level in terms of the amount and quality of the blood meal in order successfully to produce eggs. During this process, the fat body synthesizes the egg protein, vitellogenin (Vg), which in turn will be taken up by the ovary and stored as vitellin (Vt). Vg and Vt have been isolated from several species and their levels are indicative of the efficiency of the oogenesis process. However, information about their dynamics and regulation in Chagas' disease vectors is still scarce. In this work, therefore, we have analyzed in anautogenous and autogenous females of *Dipetalogaster maxima*: a] the kinetic of Vg synthesis in fat body and its levels in hemolymph; b] the stores of Vt in ovary. In anautogenous insects. The studies were performed between 2-7 days post-ecdysis and between 1-7 days post-blood feeding. Autogenous females were studied between 3-15 days post-ecdysis. During the period post-ecdysis anautogenous insects showed a decreased synthesis of Vg and concomitantly, low levels of Vg in hemolymph (< $1x10^{-3}$ µg/µl). After a blood meal, Vg synthesis and its levels in the hemolymph were significantly increased. Histological studies were in agreement with biochemical findings, being remarkable the development of the ovary from day 2 post-feeding. In autogenous insects the pattern for Vg and Vt was quite different, being characterized by a decreased Vg synthesis and a poor development of the ovary. However, the low levels of hemolymph Vg founded during the period post-ecdysis were enough to produce at least one batch of eggs.

2.

ACTION SPECTRUM OF METABOLITES WITH BIOCONTROL ACTIVITY PRODUCED BY NATIVE RHIZOBACTERIAS

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In commercial agriculture, crop protection against phytopathogens relies heavily on chemical pesticides. An alternative for the use of chemicals is the implementation of microorganisms able to control plant diseases caused by phytopathogens. The control of phytopathogen fungi by microbes depends on a wide variety of traits, such as the production of antifungal metabolites, among others. In this sense, we have previously isolated native strains from Córdoba soils able to promote plant growth and/or with bio control properties against *Sclerotinia* sp. In this work we characterize the action spectrum of extra cellular metabolites produced by three Gram positive native strains.

Cell-free metabolites, obtained from supernatants, showed antifungal activity against mycelia growth of four species of *Fusarium* sp. and three species of *Sclerotinia* sp. on agar PDA. Supernatants were freeze-dried and then resuspended in methanol or distilled water. The major biocontrol activity was observed in the methanolic fraction. These results are indicating that antifungal compounds secreted by these native strains could belong to the lipopeptides antibiotic group like iturin and surfactin families produced by *Bacillus* sp.

By sequencing the 16S rDNA we identified one strain as *Bacillus* sp. that excreted extra cellular metabolites resistant to high temperature (autoclaving to 121ºC for 20 min). Its profiling coincides with other reports about compounds produced by this genus.

3.

PHOTODYNAMIC ACTIVITY OF A NEW SENSITIZER DERIVED OF PORPHYRIN-C_{60} AND BIOLOGICAL CONSEQUENCES IN A HUMAN CARCINOMA CELL LINE

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Photodynamic therapy (PDT) is based on preferential accumulation of a photosensitizer in tumor tissue. Subsequent local activation of the agent by visible light induces tumor destruction. At the present, various classes of sensitizers are presently in clinical use or in stages of development in biological media. The aim of the study is to evaluate for first time the photodynamic effects of P-C_{60}, a dyad with high ability to form photoinduced charge-separated states, in a human carcinoma cell line (Hep-2). P-C_{60} is innocuous in dark until concentration 1 µM and 24 h of incubation. The sensitizer is rapidly incorporated in the cells (<4 h) and the uptake tends to a saturation value between 4 and 24 h (~1,5 nmol/10^6 cells). After irradiation, the cell viability was depending of light doses used. A high photocytotoxic effect (80%) was observed with 54 Jcm^{-2}. Moreover, the dyad keeps a high photoactivity even under argon atmosphere. Thus, two oxidative mechanisms are considered to be implicated in the photodamage of cells. The changes in the cellular morphology were analyzed using microscopy of fluorescence. Irradiation for 54 Jcm^{-2} produced chromatin nucleosomal fragmentation, characteristic of apoptosis. These results were confirmed by agarose gel electrophoresis, showing an internucleosomal fragmentation of the chromatin. These studies suggest that the dyad P-C_{60} offer a promising molecular architecture for photosensitizer agents with potential applications in cell inactivation by PDT.

Grants: CONICET, FONCYT and SECYT UNRC.

4.

FUNCTIONAL CHARACTERIZATION OF STARD7, A NOVEL GESTATIONAL TUMOR START DOMAIN CONTAINING PROTEIN

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The StAR-related lipid transfer (START) domain is defined as a motif of around 200 amino acids implicated in lipid/sterol binding. In a previous study, we identified the StarD7 transcript encoding one of the 15 family members with START domain present in the human genome. In addition, we demonstrated that StarD7 recombinant protein forms stable Gibbs and Langmuir monolayers at the air-buffer interface, showing marked surface activity and interaction with phospholipid monolayers, mainly with phosphatidylserine, cholesterol and phosphatidylglycerol (Biochem. Biophys. Res. Comm. 314: 181, 2004). To gain an insight about the role of this novel protein in the physiology of the cell, we transiently transfected Cos-7 cell line with pcDNA/TO/c-myc plasmid containing a wild type StarD7 cDNA and both NH2- and C-terminal deleted versions. Western blot and immunofluorescence assays of the transfected cells were performed with two different antibodies obtained against overexpressed StarD7 and StarD7-C-terminal amino acids, respectively. The specificity of these antibodies was verified with an antibody against c-myc epitope. The results obatined demonstrate that wild type StarD7-c-myc protein, as well as the mutant ones, are localized in the cytoplasm. These localizations were confirmed through subcellular fractionation and subsequent western blot assays. Furthermore, it was demonstrated that antibodies recognize preferentially a 40 kDa protein present in different cell lines, such as JEG-3, Caco-2 y C2BBe1. We also investigated the expression of StarD7 in JEG-3 cell line exposed to methotrexate (1µM). This drug induces differentiation of JEG-3 cells, which resulted in the enhancement of StarD7 expression. Our findings suggest that StarD7 may play a functional role in the process of trophoblast differentiation throught phospholipids uptake and transport.

5.

IDENTIFICATION OF THE VECTOR AND THE CHAGAS DISEASE IN URBANS-SUBURBANS ZONES OF SAN JUAN

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Chagas disease is one of the most common parasitosis in Latin America and it is the most frequent in the American Continent. It is very important that the inhabitants of endemic areas have basic knowledge about the disease and its prevention. The objective of the study was to evaluate the general knowledge about the vector and the Chagas disease in urban and suburban zones in San Juan province. Three zones were analyzed: two of the zones were adobe-type houses (Z1 & Z2) and the other had concrete-seismic resistant homes (Z3). The survey included questions about origin of *Triatoma infestans*, vector identification, dispersion, time of the year, illness symptoms, between others. Among the surveyed people, 76% from Z1, 42% from Z2 and 46% from Z3 had little or no knowledge. There was a significant difference in the percentage found for Z1 respect to Z2 and Z3. Nevertheless all the studied areas showed a need for education and prevention proven to be the most powerful tools to prevent the disease.

6.

REGULATION OF ZFHEP BINDING BY PKC-INDUCED PHOSPHORYLATION

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The activity of a transcription factor (TF) can be controlled, for instance by post-translational modification such as phosphorylation. Zfhep (Zinc Finger Homeodomain Enhancer-binding Protein) is a TF involved in lymphopoiesis, neurogenesis and myogenesis and it is expressed as two isoforms, Zfhep -1 and -2 which lacks the N-terminal DNA-binding domain of the larger Zfhep-1. Zfhep exists as two phosphorylated forms. Our goal was to examine the effect of phosphorylation on Zfhep binding to its promoters. Zfhep expressing cell lines were treated with 10 ng/ml phorbol esters (PMA) and 500 ng/ml ionomycin (IO) for 30 min. Nuclear extracts (NE) from untreated cells were incubated with calf intestinal phosphatase (CIP) or with CIP + phosphate. Band shift assays were performed with Jurkat/CHO-K1/COS-7 NE (CIP, CIP+phosphate or PMA/IO treated) and Zfhep-2 programmed rabbit reticulocyte lysates in the presence of [^{32}P]-labeled oligonucleotides harboring Zfhep binding sites from Zfhep, a-4integrin, CD4 and p73 promoters. Probes and NE were incubated for 1 h at 20ºC. CIP- treated samples have an increased binding capacity to all the probes assayed. Retardation complexes were competed by either anti-Zfhep antibodies or an excess of cold probe. PMA/IO- treated cells shown no band of retardation. The results show that phosphorylation changes the affinity of Zfhep for its physiologically important target genes. Zfhep activity can be regulated by a signaling pathway such as PKC. It is possible that such a modification may modulate the activity of Zfhep under different conditions of cell development.

7.

STIMULATORY ACTION OF NEUROPEPTIDE EI (NEI) ON LH RELEASE IN PRIMARY PITUITARY CELL CULTURES

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The neuropeptide EI (NEI) is derived from pro-MCH with projections to the entire central nervous system. The injection of NEI directly into the third ventricle of both female and male rats induces the release of LH. Based on these results the aim of the present investigation was to study the time-course of the effect of NEI on LH release in pituitary cells. Cellular homogenates from the pituitary of adult female rats were cultured at a density of 5 x 10^5 cells/capsule in Dulbecco's medium supplemented with serum. After 3 days of culture, the medium was replaced by an aliquot of serum-free Dulbecco's medium and the cells were incubated with 2 µg NEI for 1, 2, 3, 4, 5, 12, 24 and 36 hours. The release of LH to the medium was determined by RIA and the data was statistically analyzed with ANOVA-Tukey. When the cultures were incubated with NEI for only a short period of time (1-5 h), it was possible to observe that after only one hour of incubation LH release was induced ($p<0,05$) and that this release increased reaching the highest levels 5 hours later ($p<0,001$). In longer incubations (12, 24 and 36 h), the stimulatory effect of NEI on LH release was more noticeable at 12 and 36 hours compared to the release observed at 24 hours ($p<0,001$). In accordance to previous results as those mentioned in this summary, NEI regulates the release of LH at both the central and pituitary levels, directly on gonadotropic or neighboring cells.

8.

VEGETATIVE COMPATIBILITY IN A POPULATION OF *ASPERGILLUS PARSITICUS* FROM PEANUT AGROECOSYSTEM

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Aspergillus parasiticus is an important fungus in peanut due to their potential aflatoxigenic. Vegetative compatibility system (VCG) can be used to estimate genetic diversity. The aim of this work was to evaluate the genetic diversity of *A. parasiticus* population isolated from peanut agroecosystem using VCG analysis. Out of 185 *A. parasiticus* strains isolated from soil, debris and peanut seeds, we arbitrary selected 63 strains to perform VCG analysis. Based on complementation tests, 24 VCG_s were obtained, eleven VCG_s contained two or more isolates and 13 VCG_s contained only a single isolate. The *A. parasiticus* population evaluated in this study showed lower genetic diversity index (expressed as the number of groups divided by the total number of isolates = 0.31) compare with *A. flavus* population (0.56) isolated from the same peanut-growing area in Córdoba Province (Barros *et al*., 2005). Similar results were found by Horn and Greene (1995) who reported a VCG diversity of 0.22 for *A. parasiticus* population (76 isolates) from a peanut field in Georgia (USA). These authors proposed that the limited long-range dispersal may restrict the VCG diversity in a given area, since *A. parasiticus* is rarely detected from air samples (Zummo and Scott, 1990). On the other hand, *A. parasiticus* is more prevalent in peanuts than in other crops, so that, peanut crop could selectively impact VCG clonal densities in field soil.

9.

VITAMIN E AND PLASMATIC LIPIDS IN ACUTE AND CHRONIC STRESSED RATS

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Lipolysis, as energy homeostatic strategy of stress, is very well-known, as well as the relationship between the cardiovascular risk and the lipids plasmatic levels. The objective was to investigate the serum lipids modifications of in stressed rats and the possible preventive effects of vitamin E. Wistar males rats were divided in three groups: 1) vegetable oil (A), 2) vitamin E (V) and a third group control (Ag) without suplementation. Each group was divided in controls (C) and stressed (E) animals by immobilization (IMO) 2 hs daily during 14 days. At 1 and 14 days of stress, blood samples were obtained and total cholesterol (CT), triglycerides (TG), LDL cholesterol (LDL), HDL cholesterol (HDL) were determined and CT/HDL index was calculated. CT showed high values in AC ($p=0.0007$), VC ($p=0.0005$) and AgE ($p=0.005$) compared with AgC in acute stress; and in all chronic stressed animals ($p=0.0003$). AE and VE showed high TG regarding the AgE ($p=0.00003$ and $p=0.0005$ respectively). There were also differences among the controls animals, being the AgC values smaller than the other groups ($p=0.04$). In the chronic stress all the IMO animal presents bigger TG than their controls ($p<0.05$). HDL was smaller in all IMO animals with respect to its controls in acute stress ($p<0.05$), and bigger in chronic stress. The CT/HDL was higher in all acute IMO animals, and in no supplemented group in chronic stress ($p=0.01$). In conclusion, increases of CT and TG in the stressed animals could not be prevented with the vitamin E. Plasmatic lipids increase in A or V controls animals could be indicated that this supplementation could be a cardiovascular risk factor similar to the stress effects.

10.

THE GLUTATHIONE PLAYS AN IMPORTANT ROLE IN THE GROWTH OF *Bradyrhizobium* sp UNDER ENVIRONMENTAL STRESS CONDITIONS

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The glutathione (GSH) plays an important role in the mechanism of defense of the microorganisms against different environmental stresses. The objective of the work was to investigate the role of GSH in *Bradyrhizobium* sp SEMIA 6144 under different environmental stresses such as acid (pH 5.5), saline (0.3 M NaCl), oxidative (0.5 mM H_2O_2) and the toxic agent methylglyoxal (1.5 mM). For that, a GSH-deficient mutant (*Bradyrhizobium* sp 6144-ΔS7Z) was obtained by disruption of *gshA* gene, which encodes the enzyme γ-glutamylcysteine synthetase. Growth of the mutant strain was significantly reduced in MSM medium and the GSH content was very low, about 4% of the wild-type level. The defect caused by disruption of the *gsh*A gene in the growth of mutant strain cannot be reversed by the addition up to 100 μM GSH in MSM medium. However, the growth of the mutant strain in a rich medium (YEM) was greater suggesting that at least some of the functions of GSH could be provided by peptides and amino acids but not all. It is well known that GSH participates in the mechanism of regulation of the cytoplasmic pH increasing K^+ level. Thus, the wild-type strain increased K^+ level when cells were exposed to acid stress. In conclusion, the data presented in this study demonstrate that GSH plays an important role in the survival of *Bradyrhizobium* sp SEMIA 6144 under different stress conditions.

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11.

RELATIONSHIP BETWENN SALT, CHRONIC STRESS AND ARTERIAL BLOOD PRESSURE IN RATS

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Stress, sodium intake and genetic predisposition can chronically increase the arterial blood pressure. The objective of the present work was to investigate the influence of chronic immobilization stress and saline overload on the arterial blood pressure in rats. Four groups of male Wistar rats was considered: a- control animals with access to tap water (WC), b- animals with tap water and submitted for one hour to immobilization (IMO) over seven days (WS), c- control with access to NaCl 1.5% solution (SC) and d- animals with stress and salt intake (SS). The systolic (SBP), diastolic (DBP) and mean (MBP) arterial blood pressure, sodium plasmatic concentration, volume and urine sodium levels were measured. SAP increase 14% in the WE and a 18% in the SE compared with the control rats, but the SAP enhance a 28% en the SE in comparison with WC. Equal tendency was observed in the MAP. Plasmatic sodium levels were higher in SE than SC ($p= 0.009$). Stressed animals showed antidiuresis compared with controls rats ($p= 0.044$) and in the animals with salt in comparison with rats with access to tap water ($p= 0.0001$). The WS and SS rats show lower levels of urine sodium than the controls ($p= 0.00003$; $p= 0.00002$). His difference was maintained during chronic stress only in the WS rats ($p= 0.00006$). Thia result indicates that chronic stress and saline intake could challenge homeostatic mechanism to maintain sodium balance, enhancement the plasmatic sodium that cause grater renal water reabsorption. This change could be reflected in the higher levels of arterial blood pressure were applied when the stress and salt intake together.

12.

BIOTESTS OF ACUTE TOXICITY USING BIOLOGICAL SYSTEMS OF DIFFERENT STRUCTURAL COMPLEXITY

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Introduction: Biotests using experimental models of different structural complexity, may be used to determinate the potential bioactivity of new compounds, evaluate the acute toxicity of drugs and even to measure toxicity and possible ambient impact of effluents.
Methodology: The design and development of the acute toxicity tests is based on an experimental technique adapted by our group of research, from the tests used by U.S. Fish and Wildlife Service. Columbia National Fisheries Research Laboratory (Waynon W. 1980) to evaluate acute toxicity of diverse chemical compounds. As experimental models were used fish like *Poecilia reticulata*, mollusks like *Lymnaea sp.* and amphibious as *Xenopus laevis* and *Buffo arenarum* (larva). In order to demonstrate the potentially of those biotests and try to validate their correlation wit the bioactivity of mentioned drugs, molecules of diverse nature and origin were chosen.
Results and discussion: The highest levels of toxicity were the corresponding to compounds reported as bioactive. Likewise the drugs that presented little or none toxicity were submit to the evaluation of other bioactivities such as antifungic activity, obtaining results correlated to the ones of toxicity. This proves that the tests applied herein are good indicators of the bioactivity of the compounds.

13.

FACTORS THAT INFLUENCE ALDOSTERONE SECRETION IN RATS WITH SALINE OVERLOAD AND CHRONIC STRESS

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It is known that aldosterone (ALDO) levels increase in response to stress, although the involved mechanisms are not clearly established. The objective of this work was to evaluate the Ang II and ACTH participation on ALDO secretion induced by chronic stress. 6 groups of Wistar male rats with saline overload (NaCl 1.5%) were considered: a- control (C), b- immobilization (IMO) stress for 30' per day during 7 days (S), c- animals with dexamethasone (DEXA) 1 mg/kg i.p. (CD), d- animals with DEXA and 30' of IMO (SD), e- animals with losartan (LOSA) 10 mg/kg i.p. (CL) and f- animals with LOSA and 30' of IMO (SL). In days 1 and 7 we obtained a sampled of blood to measured plasmatic concentration of ALDO. The ALDO levels increased in the acute as in the chronic stress (C vs S $p=0.009$). ALDO levels were smaller in DEXA animals than controls (CD vs C $p = 0.00007$). Not differences in SD with respect to the CD were found. In the acute as in the chronic stress the SL group presented high ALDO values that the CL ($p <0.05$); however, chronic SL presented lower levels than acute SL ($p=0.03$). Also, the ALDO values in SL were lower than in S ($p <0.05$). Of the analysis of DEXA results we could infer that ACTH would be one of the main stimuli for the ALDO increment in normotensive rats with saline overload and subjected to stress. Moreover, the results with losartan could evidence participation of the Ang II through the AT1 receptors, although this system would not be the main involved factor.

14.

RELATIONSHIP BETWEEN THE PHOSPHOLIPIDS COMPOSITION AND THE RESISTANCE OF *P. PUTIDA* TO BACTERICIDAL ACTIVITY OF TETRADECYLTRIMETHYLAMMONIUM

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Quaternary ammonium compounds (QAC's) are commercial chemicals commonly used in a variety of products. *P. putida* A ATCC 12633 tolerate and degrade high concentrations of QAC tetradecyltrimethylammonium (TDTMA). In this work, the relationship between the membrane phospholipids (PL) composition and the resistance of *P. putida* to the bactericidal activity of TDTMA was investigated. The strain was grown on a high inorganic phosphate basal salt liquid medium with glucose and NH_4Cl until the culture reached at $OD_{660} = 1$. On this point the culture was divided and exposed to 50 mg l^{-1} of TDTMA during 15 and 30 min. *P. putida* showed an increase in total PL (143%) after 15 min of exposition to TDTMA. After 30 min, the PL level was similar to the one observed in TDTMA absence. At 30 min, the response involved alterations in the PL composition: cardiolipin decreased about 65% respect to control whereas phosphatidic acid and phosphatidylglycerol increased 220% and 313%, respectively. The increase in total of PL and specific variations of *P. putida* PL expose to TDTMA suggest a mechanism to repair damaged membranes. This, associated to that *P. putida,* as a single organism, is capable to tolerate and degrade high concentrations of TDTMA, revealed the potential of this strain for the efficient biological removal of this or similar QACs.

15.

COMPARISON OF ANTIBIOTIC RESISTANCE PROFILES FOR THE ANALYSIS OF RHIZOBIA STRAINS NODULATING PEANUT ROOTS

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Rhizobia are symbiotic bacteria that elicit on the roots of specific legume hosts the formation of new organs (nodules), within which the bacteria proliferate, differentiate into bacteroids, and subsequently fix atmospheric nitrogen into ammonia. Peanut (*Arachis hypogaea* L.) is usually nodulated by native or indigenous *Bradyrhizobium* spp. Our objectives of the present work were to evaluate the native peanut-nodulating strains of rhizobia in soils of Argentina and to study the diversity of these strains by using their intrinsic antibiotic resistance. Resistance patterns of the isolates to six antibiotics were used to group *Bradyrhizobium* strains. In general, strains analyzed were sensitive to gentamicin. Spontaneous mutants of peanut bradyrhizobia resistant to gentamicin 80 µg/ml were selected from strains used as inoculant of *Bradyrhizobium* sp., such as C-145, USDA 4438 and USDA 3180. These were used to evaluate the validity of using antibiotic-resistant mutants to make inferences about the competitiveness of inoculant strains in soil environments. In addition the effect of inoculation of peanut seed with *Bradyrhizobium* sp. C-145, USDA 4438 and USDA 3180 was determined under laboratory conditions. Results obtained showed significant differences in nodule number and plant dry biomass when peanut seeds were inoculated with the strain C-145. This strain with resistance to gentamicin (C-145 GmR) was selected to determine the competitive ability compared with native rhizobia. The use of spontaneous mutants resistant to antibiotic is a useful marker to evaluate the nodule occupancy after inoculation of peanut grown in soils with indigenous bradyrhizobial populations.

16.

CHOLINE, BETAINE AND CARNITINE UPTAKE IN *Pseudomonas aeruginosa* PAO1 MUTANTS

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Great varieties of bacteria have the ability to use choline as carbon and/or nitrogen source, or as an osmoprotectant in iso and hyperosmolar culture conditions. In our laboratory have been identified two choline transport systems in *P. aeruginosa,* a constitutive one with K_m value of 53 µM, and an inducible one formed at least by two components with K_m values of 3 and 400 µM, respectively. To identify the *P. aeruginosa* gene responsible for choline transport, we have worked with two mutants obtained from UWGC: 55818 and 18121 strains affected in PA5291 gene (probable choline transporter) and PA5370 (probable MFS transporter), respectively. Under isoosmolar culture conditions with choline, betaine and carnitine as C and N, mutants and *P. aeruginosa* PAO1 (wild type) had a similar growth. Moreover, under hyperosmolar conditions (NaCl 0,8M) these compounds were used as osmoprotectants by the three strains. However, differences were observed: a) with choline, lag phase of growth was 6-7 hours in mutants and 3-4 hs. in PAO1 strain (wt). Choline uptake rate in 55818 and 18121 was 40% and 70% minor compared with wt. b) with carnitine, the growth of 18121 and PAO1 strains was similar, while in 55818 strain was observed a 6-7 hours delayed in osmoprotectant uptake, in agreement with a lag phase of 6-7 hours. c) with betaine, a delayed in mutant lag phase of growth was found. Nevertheless, it was visualized a similar rate of betaine uptake for the three strains. These results suggest that PA5291 and PA5370 genes are involved in alkylammonium compounds uptake. However, mutants can grow under these unfavorable circumstances due to alternative route(s) for choline and other osmoprotectans uptake.

17.

EARLY ALTERATIONS OF PHENOTYPE IN RAT SUBMANDIBULAR GLAND ONCOGENESIS

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Salivary gland tumors represent a heterogeneous morphological and clinical neoplasm group. These tumors show 2.5-3.0 by 100.000 incidences per year in Occidental world, where above 80% are benign lesions, meanwhile 0.5% is malignant. This work evaluates alterations of phenotype in salivary glands during early development tumorogenesis. Submandibular glands of male rats were treated with injection with 0.5% solution 9, 10-dimetyl1, 2-benzanthracene in acetone. Gland samples were analyzed at 0, 7, 30, and 150 days post-treatment. Total proteins and protein profile were determined by Lowry and 12% SDS-PAGE, respectively. Bcl-2 and p53 immunolabeling realized by streptavidion-biotin or silver-enhancement-immunogold All treated animals developed changes similar to carcinomas at 30 and 150 days. Total protein concentration increased significantly at 7, 30 and 150 days in relation to controls ($p<0.05$). Phospholipids and cholesterol decreased at the same times. In treated animals the immunolabeling with p53 was positive at 30 and 150 days. Bcl-2 was positive at 7, 30 and 150 days. Our results suggest that early histopathological malignant changes, proteins, and lipids modifications are associated with tumorogenic process. This fact may be important to improve early diagnostic like preventive methods for human groups exposed to carcinogenic factors.

18.

***Elionurus mutycus* FROM NORTH OF ARGENTINA: EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF THREE ESSENTIAL OIL CHEMOTYPES**

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Elionurus mutycus (Aibé) is the most abundant pasture grass in the northeast region of Argentina. Its industrial applications have been described, nevertheless there are no information about the antimicrobial capacity of its essential oil (EO) that can be conditioned by its chemical composition. The objective of this work was to evaluate the *in vitro* antimicrobial activity of the 3 biotypes in *E. muticus*, against Gram+ and Gram- bacteria. The EO was obtained by hydrodistillation, analyzed by gaseous chromatography to determinate their chemotypes: Geraniol, Acorenona and Citral. The antimicrobial activity was evaluated against $Gram^+$ bateria: *S.aureus ATCC, B.cereus, and E.faecalis ATCC*, $Gram^-$: *P.mirabilis, P.aeruginosa, and E coli,* using the disk diffusion method (DD) and microdilution technique (MDT). For the DD, 100µl of microbial cultures (10^6 ufc/ml) were seeded in Petri plates containing Müller-Hinton agar. Then, disks of paper absorbed with 10µl of each EO were added. After 30 min. to ambient temperature, were incubated to 37ºC by 24 h. The inhibition halos were measured. The CIM was determined by DD and MDT for the active oils, making dilutions two fold of them in agar-agar (*Mann and Markham 1997)*. All biotypes exerted antimicrobial action. The G+ were more sensitive than G-, (100% *vs* 50%), whit 14.13 and 9.25 mm of halo inhibition respectively. The North of Argentina presents biotypes of *E.mutycus* whose EO could be applied to control microbial strains implied in alimentary contamination and human and animals pathologies. Particularly *P.aeruginosa* that was highly sensitive to EO citral chemotype with a CIM=1/64. Data reveal their importance for the possible control of this pathogen.

19.

INFLUENCE OF LANDSCAPE CONNECTIVITY ON POLIMORPHISM AND GENETIC STRUCTURE IN POPULATIONS OF ARGENTINE BOA CONSTRICTOR (*BOA CONSTRICTOR OCCIDENTALIS*)

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Many species' ecological processes are sensitive to spatial heterogeneity. How a species responds to the heterogeneous distribution of habitat and resources may strongly influence its long-term persistence. The aim of this work was to evaluate variations in polymorphism and genetic structure of Argentine boa constrictor populations and landscape composition as a whole. Therefore, we analyzed the way in which heterogeneous spatial patterns would influence the genetic process. The less landscape connectivity and integrity there are, the lower levels of gene flow and genetic variability we may expect. The study population' areas were located in the west regions of Districts of Pocho and Sobremonte in Córdoba Province. We estimated genetic variability and levels gene flow for each area using isozimes and ISSR as molecular markers. In order to characterize the landscape composition in terms of vegetation structures we analyzed satellital images Landsat 5 TM. Next, we carried out the supervised image classification and by applying majority analysis we made the patches clumper. The forest fragmentation pattern was analyzed through connectivity and isolation metrics obtained by FragStats 3.3. The genetic variability was similar in both regions, however the level of gene flow was higher in Sobremonte than Pocho. The variation of genetic parameters may be caused by the distinct features in the landscape degradation stages. In Pocho, the study area is characterized by a high fragmentation level being composed by small forest units isolated by shrublands of jarilla (*Larrea divaricata*). In contrast, the forest in Sobremonte is more continue staying under a slighter degradation process called perforation. This process leads to habitat loss but it does not reduce the landscape connectivity. This novel approach integrating and analyzing genetic and environmental data, using geographic information systems and molecular methods, will help us to understand the effects of land transformation on species' ecology and will provide critical knowledge to develop conservation strategies.

20.

***MINTHOSTACHYS VERTICILLATA* ACTIVITY ON ALLERGENIC FUNGI AND MITOGENIC EFFECTS ON B CELLS**

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Essential oil (EO) and decoction (D) from *Minthostachys verticillata* (Griseb) Epling *(Mv)* have antibacterial and antiviral activities. In cyto-morphological studies it was observed that *Mv* derivates has mitogenic properties such as PHA with CD 8 (+) lymphocytes expansion. Moreover, D and EO showed ability to inhibit ß–hexosaminidase released from allergic patient's basophils. In this study the mitogenic ability of D and EO derived from *Mv* compared to PWM. B cells by DIF were evaluated. Environmental fungi were isolated and identified and D from *Mv* anti-fungic effects were investigated. Lymphocytes from 15 allergic patients hypersensitive to environmental fungi and 10 healthy individuals were cultured. D and EO were obtained from leaves and stems. Lymphocytes isolation was done by density gradient and lymphocyte proliferation was evaluated by MTT colorimetric assay. The B cells were determined by DIF. Most fungi genus were isolated and identified from the houses patients. Different D concentrations were used to show anti-fungic activity. DMSO was used as negative control and miconazole or clotrimazole as positive controls. The lymphocyte proliferation was stimulated by D: 0,7 mg/ml and EO: 0,16 mg/ml, in similar levels to PWM (p=NS). Old PI were ≥1,30. The 54% of D stimulated cells were LB. *Penicillium sp, Aspergillus sp, Cladosporium sp* and *Alternaria sp* were the main genus isolated. D (0,625 µg/ml) and (10 µg/ml; 2,5 µg/ml y 1,25 µg/ml) inhibited respectively *Aspergillus sp* and *Alternaria sp* growth. D vs controls difference was: $p<0,05$. D and EA were mitogenic with B cells. D had antifungic activity over *Aspergillus sp*. y *Alternaria sp*.

21.

ANTIMICROBIAL ACTIVITY THE A*chirocline satureiodes* ON *Staphylococcus aureus* HUMAN ORIGIN STRAINS

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The valuable action of medicinal plants on disease organisms is due to the presence of active principle. Most of them are secondary metabolites used by the plants as defense mechanisms. Internal and/or external factors such as the season of the year in wich the collection is performed could influence quali-quantitative composition of these chemical compounds. Photochemical studies confirm flavonoids presence, with possible antimicrobial activity, on the aerial parts of *Achirocline satureioides*. Aims: To evaluate the antimicrobial activity of *A.sartureidoes*, collected in different seasons of the year, on *Staphylococcus aureus* from human origin strains. Material and methods: Decoctions were obtained from leaf and flower of *A. satureiodies*, collected in different seasons of the year. The antimicrobial activity was evaluated on 54 *S.aureus* strains by radial strike technique. The Minimal Inhibitory concentration (MIC) and Minimal Bactericide Concentration (MBC) of decoctions and Iodinepovidone disinfectant were determined.
Results: The spring decoctions obtained by the leaf (10 mg/ml) inhibited the 100% of the tested strains including S.aureus meticilline resistant with MIC=0.78 mg/ml and MBC=1.56 mg/ml. The Iodinepovidone presented MIC and MBC=3.13 mg/ml.
Discussion: The active principles with antimicrobial activity are concentrated in the leaves. The flowers presented a poor inhibitory activity on *S.aureus* strains. *A.satureioides* collected in spring presented a better antimicrobial activity that the one obtained in other seasons. The requirement of *A.satureoides* concentration to obtain a bactericide effect is lesser than the requirements of iodinepovidone concentration.

22.

TOTAL LEUCOCYTE AND LYMPHOCYTE SUBPOPULATIONS FROM MATERNAL PERIPHERAL BLOOD AND UMBILICAL CORD BLOOD DURING DELIVERY

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Parturition is a complex and dynamic process involving immune and inflammatory cells between fetal and maternal systems. The aim of the present study was investigate number and function of leukocyte and lymphocyte subpopulations from maternal peripheral blood (MPB) and umbilical cord blood (UCB) during delivery. Peripheral blood was taken from 25 healthy women at delivery concomitantly with cord blood and total leukocytes were counted. Lymphocytes were isolated and the percentage T (CD4, CD8), B (CD19) and NK (CD56) cells were determined by IFI with mAb. The proliferative response of lymphocyte was determined by [3H] T with PHA-M and Con-A. Total number of white cells was clearly increased in MPB and UBC compared with normal standard blood values. In peripheral blood, this was predominantly due to an increase in polymorphonuclear cells whereas in cord blood due to an increase in lymphocytes. In MPB and UCB no significant changes were found on the lymphocyte subpopulation percentages. Lymphocyte proliferative response to PHA-M and Con-A in MPB or in UCB were similar. This results show that the innate immunity cells are most affected and that likely they may be important in parturition process.

23.

***Elionurus mutycus´*s ESSENTIAL OIL FROM NORTH OF ARGENTINA. EVALUATION OF CYTOTOXICITY OF DIFFERENT CHEMOTYPES: PRELIMINARY STUDIES**

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Elionurus sp it's the most important genera in the Poaceae family, and *Elionurus mutycus* (Aibe) the most important specie of this genera. It has been reported that the chemical quality of its essential oil (EO) varies considerably in very next places. The chemotypes of EO: Geraniol, Acorenona and Citral have been evaluated in their antimicrobial capacity with promising results. Nevertheless their possible use for the control of microbial pathologies demands the previous determination of their cytotoxicity capacity on eucariotic cells. The aim of this work was to determine the Maximal Non Cytotoxical Concentration (MNCC) on Vero cells of two *E. mutycus´*s essential oils chemotypes collected in north of Argentina, which were obtained by hydrodistillation and analyzed by gaseous chromatography (GC) confirming their chemotypes. Toxicity assays in Vero cells were made incubating confluent cellular monolayers during 72 hs to 37ºC with different concentrations from each EO chemotype using DMSO as dissolvent. The MNCC was determined according *Andrei et al., 1985*. The cellular alterations induced by the EO at toxic concentrations were characterized: there was cells ballooned and grouping in clusters, cellular death with complete monolayer disorganization. The values of MNCC were: 0.1063 mg/ml and of 0.370 mg/ml for the biotypes Acorenona and Geraniol, respectively, surpassing widely the active concentrations to $Gram^+$ and $Gram^-$ bacteria, previously determined, limiting its application on eucariotic cells. Determination of cytotoxic capacity of EO citral biotype will be determinated.

24.

SURVIVIN EXPRESSION AND ITS CORRELATION WITH PROGNOSIS IN BREAST CARCINOMA

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Survivin is a unique member of the inhibitors of apoptosis protein (IAP) family that is involved in both control of cell division and inhibition of apoptosis. Survivin is selectively overexpressed in common human cancers but not in normal adult tissues. This expression is associated with aggressiveness of the disease, tumor progression and unfavorable outcomes. However, the biological role of survivin in breast carcinomas remains to be clarified. Here, we investigated the expression of survivin in a group of breast carcinoma (n=56), and examined the relationship of its expression with clinicopatological parameters, and its impact for tumor prognosis. Inmunostaining was performed using the ABC detection system. The peroxidase activity was detected using DAB as a chromogen. Survivin expression was assessed by counting at least 1000 cells in each case. Inmunoreactivity to survivin was observed in 93% of sample. The anti-survivin antibody stained the cytoplasm but not evidence of nuclear reactivity was observed. Survivin expression was significantly hither in tumors with high NPI values (Nottingham prognostic index), ($p<0.01$). According to Bloom and Richarson´s tumoral grade the percentage of survivin-stained cells was 14,5±5,9% in grade I, 59,1±29,5% in grade II and 63,8±26,0& in grade III. The number of survivin-positive cells was significantly higher in grade II than grade I ($p<0.03$), and higher in grade III than grade I ($p<0.01$). No association could be observed in relation to hormonal receptor status (progesterone receptor or estrogen receptor) or tumor size. In order to analyze the association between survivin expression and lymph node status we considered a high expression of survivin when over 50% of the cells were stained in each section. We found that a high expression of survivin was significantly associated with a high number of metastases ($p<0.05$). All together these results suggest that survivin overexpression may play a pivotal role in the progression of tumors and may provide an important prognostic implication for breast carcinomas.

25.

ENVIRONMENTAL CHARACTERIZATION OF THE RAINBOW BOA (BOIDAE) DISTRIBUTION AREA IN CÓRDOBA PROVINCE, BY REMOTE SENSING

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The fauna abundance and the distribution of populations change in space and in time according to the availability of the environmental components that assure the survival and reproduction. Specific distribution information, concerning to vulnerable related species, can throw new ideas on the influence of ecological, historical and contemporary factors, on the determination of the distribution pattern of the species. The aim of this work is to characterize the distribution of the rainbow boa (*Epicrates cenchria alvarezi*) analyzing the structural variables of the habitat that, at landscape level, influence the location of the populations. This boa has been included in the appendices of CITES, Convention on International Trade in Endangered Species of Wild Flora and Fauna (1997), to which Argentina adheres. Nevertheless, this subspecies continues subject to numerous factors of pressure, such as the degradation of its habitat. By applying remote sensing technology and geographic information systems the environments of the distribution zone were characterized considering environmental variables that possibly influence on the presence of the wild populations. The results indicate that in Córdoba the rainbow boa is distributed in Chaco forests of *Aspidosperma quebracho blanco*, Shrubland and Secondary Forest, of the oriental as well as the western zone of the province. In addition, its presence is registered in the Mountain Woodland, and also in peri-saline edges where an ecotone is developed between Halophitic Shrubland with Shrubland and Secondary Forest.The fragmentation and disappearance of Chaco environments, -the natural habitat of the boas-, impose the generation of scientific bases that contemplate the conservation of the populations at long-term.

26.

HUMAN PLACENTAL NITRIC OXIDE MIGTH PARTICIPATE IN THE CONTROL OF CHORIONIC VILLI *TRYPANOSOMA CRUZI* INFECTION *IN VITRO*

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The human placenta might have an active role in the control of the congenital infection of Chagas, which would partially explain the low incidence of the congenital transmission. This control could be due partly by placental nitric oxide production (NO) by the enzyme endotelial nitric oxide synthase (eNOS). Objectives: a) to quantify *T. cruzi* infection in explants from human normal placentas *in vitro*. b)to measure production of NO in co-culture media and to correlate with infeccion and viability of *T. cruzi*. c) to determine enzime expresion of eNOS in the infected placental tissue. Material and Methods: Chorionic villi explants and VERO cells (susceptible to *T.cruzi* infection) were co-cultured with 10(6) Tulahuen strain trypomastigotes of *T. cruzi* for 72hs. The area occupied by *T.cruzi* and the number of amastigotes per nest were quantified. The supernatant media of co-cultures were analysed for *T. cruzi* viability and NO level .The expression of eNOS was quantified by immuno-histochemistry staining (+: no reactive, ++: slight stained +++: intense stained). Results: *T. cruzi* infected 0.057% of the chorionic villous area with 4,5 ± 2 amastigotes per nest, while 3,32% of Vero cells area was infected, with 75 ± 20 amastigotes per nest ($p<0.05$) were found. There was no alive trypomastigotes in the culture media of chorionic villous co-culture with trypanocidal levels of NO (28,52±9,20 μM) and intense imunostaining (+++) of eNOS in the syncytiotrophoblast, in comparison with 63.33% of living parasites and low levels of NO in VERO-*T.cruzi* co-culture media ($p<0.05$). Conclusions: low sustainable *T. cruzi* infection in the chorionic villous with little or no viable *T. cruzi* in the supernatant media, which would be caused by trypanocidal concentrations of NO, coincident with an increased expression of trophoblast eNOS. The normal human placenta would control the congenital transmission of the Chagas disease.

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27.

ANTIFUNGAL AND CYTOTOXIC ACTIVITY OF ETHANOLIC EXTRACT OF *MINTHOSTACHYS VERCILLATA*

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In the last years the searching of new effective and low toxicity chemical compounds exhibiting antifungal activity has become a topic of great interest. This is based on the low number of available antifungal, the appearance of new resistant strains and the increased frequency of fungal infections. *Minthostcachys vercillata*, a characteristic specie from Cordoba province, is well known by the traditional medicine due to its antispasmodic properties. *"In vitro"* trials have proved that different fractions of this plant show antibacterial and antifungal effect. The aim of the this work was to determine the antifungal activity of the ethanolic extract (EE) of *M. vercillata* against *Microsporum canis, M.gypseum, M.nanum, Trichophyton rubrum, T terrestre, T mentagrophytes* and the cytotoxicity of EE on VERO cells. The microdilution technique recommended by the National Committee for Clinical Laboratory Standards was performed. The obtained results have shown a strong effect of the EE on *T. rubrum*, followed by *M.canis* and then *T.terrestre*. The EE maximum non-cytotoxic concentration on VERO cells was 0.88mg/ml. The active concentration against *T.rubrum* was 0.74mg/ml, showing a therapeutic index (IT) of 1.2. This parameter was lower than 1 for the other fungi. The EE obtained from *M. vercillata* has shown to have a selective toxicity, so that it would be effective for the treatment of dermatophytosis caused by *T. rubrum*, the most frequent etiological agent of skins affections.

28.

IN VITRO* ANTI- *Helicobacter pylori* ACTION OF DECOCTION OF *Minthostachys verticillata

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The human bacterial pathogen *Helicobacter pylori* (HP) has been recognized as a major causative factor in peptic ulcer diseased. It is known that HP could be eradicated by a combination of therapeutic agents such as antibiotics, bismuth subsalicylate, protom pump inhibitors and H2-blockers. However, the cure is incomplete and undesirable side effect are certain to occur. On the other hand, the anti-HP activity of the *Minthostachys verticillata* (peperina), a traditional herbal medicine, has not been studied. The vegetable decoction was obtained according to *Mongelli et al* (1995), its inhibitory action was evaluated *in vitro* against two strains of HP tox+ producer vacA toxin, both isolated of severe gastritis cases. The screening test was made by the technique of radial strikes, and the CIM determination was made by the disk diffusion method. Both strains were sensible to the inhibitory action of *M. verticillata* decoction, with 9.19 mg/ml value CIM and 15 mm ratio inhibition halos. Greater concentrations show inhibition halos of 30 mm or higher, similar to the positive control Amoxiciline. The relation of the cytotoxic concentration of the decoction on eucariotic cells evaluated on previous assays *(Escobar et al, 2004)* and the active concentration against HP, threw a therapeutic index >1. Data reveals the potential phitomedicinal application of *M. verticillata* decoction to control infection of *Helicobacter pylori.*

29.

ANTIMETASTASIC EFFECT OF DIETARY CHIA OIL (*Salvia hispanica*), RICH IN ω-3 FATTY ACIDS, ON MURINE MAMMARY GLAND TUMORS OF DIFFERENT METASTASIC CAPABILITY

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Dietary fat is thought to be one of the main risk factors on tumor growth. Many evidences indicate that high dietary intake of ω-6 polyunsaturated fatty acids (PUFAs), such as Linoleic Acid, increase tumorigenesis and metastasis, whereas ω-3 PUFAs, like α-Linolenic Acid, reduce the incidence and progression of tumors. In addition, eicosanoids derivate from PUFAs are key regulators of tumor growth. However, the role of PUFAs and their metabolites in tumorigenesis is unclear. OBJECTIVES: Study the effect of Chia oil, an ω-3 source, in tumor development, eicosanoids production and metastasis. METHODS: BALB/c mice were fed on basic diets plus 6% Chia oil, rich in ω-3 fatty acids (FA), Safflower Seed Oil (Carthamus tinctorius) rich in ω-6 FA and commercial diet (control) and were inoculated with mammary gland tumors of different metastasis capability. Survival and number of metastasis were determined. AA metabolites produced by COX and LOX pathway were detected by HPLC. RESULTS: Survival of M3 and MM3 Chia fed mice was significantly higher (54; 62 days; $p<0.05$) than Control (43; 52). The metastasis number was significantly lower in Chia M3 and MM3 (0.33; 11,44; $p<0.05$) than Controls (4; 43.67; $p<0.05$). The Chia neoplastic cells M3 and MM3 released higher quantities of 12-HHT, a lipid peroxidation marker (55,70 and 217,93 ng/10E6cells) than Controls. DISCUSSION: Diet enriched in ω-3 fatty acids, Chia oil, enhances the survival, increases 12-HHT release and reduces the metastasis.

30.

OF THE GERMINATION BEHAVIOR OF *Deyeuxia hieronymi* AND *Deyeuxia alba* UNDER DIFFERENT ENVIRONMENTAL CONDITIONS

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In the metamorphic grasslands from Córdoba mountains (Argentina) two species of the genus *Deyeuxia* (*Poaceae*) *D. hieronymi* and *D.alba* grows separately in two different and neighbouring communities. The objective of this work consisted on evaluating the effect of the light, temperature and water on the germination of both species. For this, the seeds of both species were picked up in E1 (09/02/01) and E2 (22/02/01) using a DCA in factorial arrangement with three repetitions. The treatments were: quality of light (red distant and without light), water level (–0.7, -1.0 and –1.2 and 0 MPa) and temperature (25/15 and 20/10 ºC). The seeds remained 30 days at 7ºC, then exposed to light in the distant band of the red one and finally placed in PEG. The germination was carried out on paper during 42 days and they were considered the percentage initial and final germination (PGi and PGf) and index of germination rate (IVG). The beginning of the germination took place first at 25/15ºC, with variations according to water quantity and light. In E1, the PGi of both species was influenced by the interaction temperature-light and temperature-water. In E2, the PGi, PGf and IVG of *D. alba* was influenced by the interaction temperature-water except to the PGi of *D. hieronymi.* The water stress increased the germination of *D. hieronymi* at 25/15ºC and of *D. alba at 20/10ºC* being observed bigger germination of the seeds of *D. alba* in E2. On the other hand, the roots of *D.hieronymi* presented dimorphism and smaller development. On these results we can conclude that both species have probably, different germination niches.

31.

SEASONAL STUDY OF CORTICOTROPH CELLS IN THE PARS DISTALIS OF MALE VISCACHA PITUITARY

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During the winter the viscacha lives in conditions of short photoperiod, water restriction and low temperatures. These are ambiental stressors that provoke an increase of serum glucocorticoids, who must mobilize energy and restore the homeostatic balance. ACTH acts on the cortex of the adrenal glands to stimulate the synthesis and secretion of glucocorticoids. The aim was studied the effects of the natural photoperiod and the exogenous melatonin on corticotroph cells by immunohistochemistry and image analysis in the pars distalis male viscacha pituitary. The ACTH cells were located in the dorsal and cephalic region of pars distalis. They were found in small groups or isolated, near to folicular structures and blood vassels. They were polygonal, ovales and stellate with cytoplasmatic processes. During July (winter), the ACTH cells exhibit minimum size and percentage immunopositive area. The results of control and experimental groups were similar to those found in July. However, a significant decrease of the percentage immunopositive area was observed in animals treated with melatonin. The captivity probably produces a decrease in the values of the studied parameters. They are similar to the values of July, when the stressors are present. These results suggest that the synthesis, storage and/or secretion of the pituitary ACTH undergo seasonal variations.

32.

MSP CONTENT IN HUMAN SEMINAL PLASMA AND ITS RELATIONSHIP WITH SEMINAL PARAMETERS

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Prostate ß-microseminoprotein or MSP is one of the most abundant proteins in the human seminal plasma. It is a 15 kDa cysteine-rich nonglycosylated protein. Originally, MSP was isolated from human seminal plasma but has recently been detected in several different mammalian species. In the human, the gene for MSP has three glucocorticoid response elements and one estrogen response element in the promoter region suggesting hormonal regulation of the synthesis and secretion of this protein. Although putative biological roles, such as tumor marker for prostate cancer and immunoglobulin binding factor have been suggested for MSP, its biological function in the male reproductive tract is still unknown. In previous communications we have showed that MSP binds to the acrosomal region and to the neck of the sperm cells as it was observed with seminal plasma proteins that affect the sperm physiology. The aim of this study was to quantify MSP content in human seminal plasma and analyze its relation with the seminal parameters. Semen samples from sub-fertile patients with seminal parameters lower than the reference values (WHO), were evaluated in comparison with those from fertile donors (control). To quantify MSP, a two-site binding enzyme immunoassay (ELISA) using polyclonal antibodies generated in rabbits and rats was developed. MSP concentration in the sub-fertile group was significantly higher than in the control group ($P<0.02$), reinforcing the hypothesis about its participation in physiological processes related with the sperm fertilizing capability.

33.

LIPID METABOLISM IN THE MIDGUT OF PANSTRONGYLUS MEGISTUS (HEMIPTERA: REDUVIIDAE): INTERACTION LIPOPHORIN-MEMBRANE

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In insects, the transfer of midgut lipids to the hemolymph is a remarkable event mediated by lipophorin (Lp), the main insect lipoprotein. In order to understand the regulation of this process in hematophagous insects, we have analyzed the transfer of diacylglycerol to circulation and the interaction Lp-midgut membrane using a solid-phase binding assay. The study was performed employing *P. megistus*, an important vector of Chagas' disease. This insect takes large blood meals, containing a substantial amount of lipids. Lp was isolated from hemolymph of fifth instar nymphs by a KBr gradient and the membranes were obtained by ultracentrifugation of midgut homogenates. Thereafter, the membranes were suspended by sonification, adsorbed in plates, and the amount of bound Lp was quantified by ELISA. It was analyzed, among other factors, the effect of ionic strength, the effect of pH, the requirement of divalent cations, and the effect of suramin. The saturation kinetics most likely fit a ligand-binding model for a single binding site. The interaction Lp-membrane showed a strong dependence with the pH and, in contrast with LDL receptor family, did not required Ca^{+2} / Mg^{+2}. Like other lipoprotein receptors, suramin significantly inhibited the interaction Lp-membrane. The increase of ionic strength suggested that Lp binding is optimal at low NaCl concentration. In addition, the effect of membrane treatment with protease and temperature would indicate the proteic nature of the receptor.

34.

ANTIMICROBIAL RELATIONSHIP BETWEEN TERPENES AND THE PENICILLIN OVER METICILIN RESISTANT *STAPHYLOCOCCUS AUREUS*

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Medicinal plants are able to sinthetise aromatic compounds such as essential oils (EO). These oils are complex mixtures of organic molecules like monoterpenes and sesquiterpenes. The EO as well as terpenes have antimicrobial activity. In the last years several microbial strains have adquired resistance against antibiotic, so there is a growing interest to find new drugs against bacterias such as meticillin resistant *Staphylococcus aureus* (MRSA). Aims: To evaluate terpenes antimicrobial activity and to determine the synergistic or antagonic relationship between these and penicillin. Materials and Methods: 8 terpenes were tested against *S.aureus* ATCC 25212 Meticillin sensitive (MSSA) and Meticillin resistant *S. aureus* ATCC 25923. Minimun Inhibitory Concentration (CIM) and Minimun Bactericidal Concentration (MBC) were determined using the microdilution method proposed by Mann & Markham, 1997. The relationship between terpenes and penicillin were determined using a modification of the method proposed by Didry *et al*. (1995). The Fractionary Inhibitory Concentration (FIC) was determined. Results: Carvone, eugenol, geraniol and thymol were active against these microorganisms. The most active was thymol with a CIM=7,5 µg/µl for *S. aureus* MSSA and 30,15µg/µl for MRSA and MBC=60,31µg/µl. Menthol, mentone, citronellol and mircene did not show any antibacterial activity. Carvone and penicillin were the mixture that showed a sinergistic effect, with a FIC=0,077 for MRSA, considering synergistic the values below 0,5. Discussion: Carbona can be used together with penicillin increasing its action over strain multiresistant to antibiotics as *S.aureus* MRSA.

35.

QUANTIFICATION *in vitro* OF THE BACTERIOCIN OF *Lactobacillus fermentum* ON SPECIES OF *Candida*

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The lactobacilos that produce bacteriocins keep the equilibrium of the vaginal ecosystem and protect of the vaginal infections like the vulvovaginal candidiasis (VVC). Studying the bactericidal activity of *Lactobacillus fermentum* and its quantification *in vitro* on species of isolated *Candida* of the female genital tract. 100 strains of the genus *Candida* were identified by: aerobic use of carbon hydrates and CHROMagar *Candida*. The study of the bactericidal activity was carried out by means of crossed flutes. The quantification of the bacteriocin of *L. fermentum* on *Candida* spp was carried out by the diffusion in disks. The title of the supernatant was determined in 640 AU/ml. The result of the identification was: *C. albicans* 80%, *C. glabrata* 11%, *C. krusei* 5%, *C. tropicalis* 2% and *C.guillermondii* 2%. The study of the bactericidal activity showed that the 86% of the strains of *Candida* resulted sensible to the bacteriocin. For the quantification of the bacteriocin, 64 *Candida* strains were selected which showed zones of inhibition > to 0.8 mm by crossed flutes. The supernatant inhibited the growth of 46 strains of the 64. The 46 *Candida* strains were sensible to the pure supernatant (640 AU/ml), 36 diluted to the half (320 AU/ml), 11 diluted to the quarter (160 AU/ml) and with the dilution to the eighth (80 AU/ml) 3 strains. The obtaining of a bacteriocin that present inhibitory activity on *Candida* even diluted, could by an efficient option in the VVC treatment.

36.

ANTIMICROBIAL ACTIVITY OF PLANT EXTRACTS AGAINST *Paenibacillus larvae* subsp. *larvae*, THE CAUSE OF AMERICAN FOULBROOD DISEASE

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American Foulbrood (AFB) is a serious bacterial disease that affects the brood of honeybees. The causal microorganism is *P.larvae.* The illness control is the hygienic management of hives and the use of antibiotics. The irrational use of the last ones may lead to antibiotic resistence of the microorganisms and the quimical products remains in honey, hindering its comercialization. This becomes a problem since Argentina is one of first worldwide honey exporters. Essential oils and plant extracts are another option in the control of the disease. Aims: To evaluate the antimicrobial activity of plant extracts against *P.larvae*. Materials and methods: Decoccions (D), essential oils (EO) and distillation remaining water (DRW) were obtained from different regional plants. *P.larvae* strains were isolated from hives with syntoms of AFB from Río Cuarto (CS) and INTA Balcarce (BIV, BV, BBB). The antimicrobial activity was analyzed by a method of radial strikes for D and DRW and a disk diffution technique for EO. Results: D and DRW present an enhanced antimicrobial activity as compared to EO in all the strains (91,9% for D and DRW ad 50% for EO). The plant extracts with enhanced antimicrobial activity were: *M.verticillata* (100%), *E.cinerea* (100%), *T.minuta* (91,4%) and *A.satureioides* (83,3%). BV strain was the most sensitive (88% of growth inhibition). Discussion: All the strains of *P.larvae* were inhibited by plant extracts, which were considered appropriate for "*in vivo*" tests in the control of AFB disease.

37.
ISOPEROXIDASES FROM HAIRY ROOT CULTURES INVOLVED IN REMOVAL OF PHENOL AND 2,4-DICHLOROPHENOL

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Peroxidases (Px) had been applied in the removal of phenol and 2,4-dichlorophenol (2,4-DCF), which are highly toxic compounds found in different industrial effluents. In previous studies we have used hairy root cultures (RT) from turnip (RTN) and tomato (RTT) for the removal of phenols. The aim of this study was to perform a biochemical characterization of this process through the use of Px purified from RTT and RTN. The isoenzymes were purified from crude extracts of both RT cultures by ion exchange chromatography. Fractions eluted from the columns were analyzed by isoelectric focusing. The zymograms showed the presence of different basic, neutral and acidic isoenzymes. Removal assays were performed with these isoPx, by incubation with 1 mM H_2O_2 and phenols (10 mg/l) during 1 hour. Removal efficiencies were estimated by determination of the residual phenolics as described by Klibanov *et al*. 1980. In most of the assays basic and neutral Px, from both RT cultures, were more efficient for phenol and 2,4-DCF removal than the acidic isoenzymes. These results were correlated with the higher catalytic efficiencies (E_{cat}) for these substrates found for this group of isoenzymes. Post-removal Px activities were higher for the treatments with acidic Px than those performed with neutral or basic isoenzymes for both phenolic compounds. Those results indicate that basic and neutral Px would be mainly involved in the removal, being inactivated during this action, while the acidic Px were less efficient in the oxidation but more resistant to inactivation. For this reason the acidic isoenzymes would be useful in continuous removal systems.

38.
INFLUENCE OF PORCINE EARLY PREGNANCY FACTOR (EPF) ON EMBRYO DEVELOPMENT IN RATS

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Early Pregnancy Factor is a molecule involved in pregnancy with immunossupressor and growth factor activities during first stage of embryonic development. The purpose of this work was research *in-vivo* EPF effects on embryos development by blocking its activity through porcine anti-EPF pAb in rats. Polyclonal Abs were obtained in rabbits immunizated with synthetic porcine EPF (KTYKSEIAHRDFKLDGQQLY). Mated rats were passively immunizated with 500 ug of anti-EPF pAb (TT) at 8, 16, 32 and 40 hs post mating i.p. via, we used also controls group (saline solution-SS and nonspecific IgG-(nIgG) and the effect on embryo number, weight and size and corpora lutea number (CL) were determined on d 10 of pregnancy. Average of embryo numbers in TT group decreases but this difference was not significant. In contrast, Embryos/CL ratio was significantly decreased in TT group compared with the two controls groups ($p \leq 0.05$). Embryo weights and sizes of TT group were significantly decreased ($p \leq 0.05$). These results demonstrate that the passive immunization affected the embryo growth and reproductive efficiency. Embryo weights and sizes decreased showing an important role of EPF during the early pregnancy as possible growth factor. Observations will be confirmed by analyzing histological development stage in the future.

39.
ISOLATION AND CHARACTERIZATION OF PLANT GROWTH PROMOTING MICROORGANISMS FROM ALFALFA RHIZOSPHERE

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The inoculants formulation is one of the biotechnological tools that tries to obtain greater yields from crop plants. The inoculants production includes the selection of beneficial microorganisms, which provide nutrients and protection against pathogens to the host plant. The proposed objective was to select alfalfa rhizobacteria that are nutrients solubilizers and phytopathogens antagonists.
The strains were isolated in TSA medium from alfalfa rhizospheric soil (pre and post-seeding) from Uruguay, Chile and Argentina. The characterization included: Gram reaction, nutrients solubilization (Fe, P), starch hydrolysis, exopolisaccharides production and biocontrol activity against *Macrophomina, phaseolina* and *Rhizoctonia* spp.
In the pre-seeding samples from Argentina, the strains with antifungal activity were predominant; the number of strains that are Fe and P solubilizers was highest in the pre-seeding period. In Chile the greater number of strains that solubilizated Fe and P was found in the pre-seeding samples; while antagonistic strains of *Rhizoctonia* spp. came mainly from post-seeding samples. In Uruguay, the strains with the greater antifungal capacity were the pre-seeding ones and the strains positive for siderophore production predominated in the post-seeding period. Most of the strains were Gram positive in all the samples. Future assays in alfalfa will allow us to evaluate the potential use of the studied rhizobacteria as plant growth promoters.

40.
CHARACTERIZATION OF SYMBIOTIC GENES AND PLASMID PROFILE OF PEANUT RHIZOBIA ISOLATED FROM CORDOBA SOILS, ARGENTINA

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Arachis hypogaea L. (peanut) is an important crop all over the world that provides several food products. Ninety four percent of the national peanut production is concentrated in the centre-south region of Cordoba province. To analyze the diversity of native rhizobia that nodulate this legume, a representative collection of peanut symbionts from this region was obtained. Previous studies that included the determination of the partial 16S rRNA gene sequence demonstrated that the population of native peanut symbionts of Cordoba soils is highly heterogeneous and includes slow and fast-growing rhizobia. In order to go deep in the characterization of the available collection of fast growing peanut rhizobia, we determine the *nodC* gene sequence and the plasmid profiles of two of these isolates.
Nodulation *nodC* gene of NET30 and NCHA22 isolates was amplified by using specific primers (Laguerre *et al*, 2001) and sequenced. Sequence analysis and phylogenetic trees were performed by using the BLASTN algorithm and the CLUSTALX software. Plasmid profiles were determined by Eckhardt type gels (Eckhardt, 1978; Wheatcroft *et al*., 1990).
The phylogenetic relations established for these isolates by *nodC* gene sequencing was not consistent with the data obtained from the 16S rRNA gene sequencing. The analysis of plasmid profiles revealed that both isolates share a plasmid of similar electrophoretic mobility. The results obtained suggest us that native peanut rhizobia constitute a heterogeneous group that gained the ability to nodulate this legume by horizontal transfer of genes.
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41.

CHEMOKINE *STROMAL CELL-DERIVED FACTOR* AS A POTENTIAL CHEMOATTRACTANT FOR NEURAL CREST CELLS

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In vertebrate embryos, neural crest cells (NCC) migrate toward defined sites where they differentiate in many derivatives. Several factors are involved in cell migration, but the regulation of their precise orientation is unknown. Data from our laboratory showed that directional motility of NCC may be modulated by chemotactic trophic factors. In this work we studied the optic vesicle (the area where the NCC will migrate to form the ciliary ganglion) as a possible source of the chemokine *Stromal cell-derived factor* (SDF-1). From optic vesicle and mesencephalic NCC of chick embryos (stages +10 to 12 HH), mRNA was isolated to analyze the expression pattern of SDF-1 and its receptor CXCR4. After mRNA extraction, RT-PCR and agarose-electrophoresis were performed allowing us to verify the expression of SDF-1 mRNA in optic vesicle and the corresponding receptor CXCR4 in the NCC. Present data suggest the synthesis of SDF-1 in the "target" of NCC, as well as the expression of the receptor in the NCC, supporting the idea that the chemokine segregated by the optic vesicle may guide the orientation of NCC toward the future ciliary ganglion area. These results assign a potential new function to chemokines: the modulation of oriented cell migration during normal embryogenesis.

42.

MOLECULAR CHARACTERIZATION OF *S. uberis* STRAINS ISOLATED FROM BOVINE MASTITIS

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Mastitis is the infectious disease which the greatest economical incidence in milk exploitation. The implementation of conventional strategies for disease prevention has been successful for controlling contagious pathogens. However, these measures have not shown a significant decrease in the incidence of mastitis caused by environmental microorganisms, being *Streptococcus uberis* the most frequent. The identification of predominant genotypic profiles is of epidemiological importance since, when recognizing strains particularly virulent ones, it contributes to the developing of control strategies. Among molecular methods, the DNA analysis by electrophoresis of pulse field (PFGE) is considered the gold standard by its simplicity, showing a high discriminatory power and being easy to be interpreted and reproduced. The aim of this work was to determine the genotypic profiles by PFGE of *S. uberis* strains isolated from clinical and subclinical. The virulence factors investigated were the capsule, hemolysin, plasminogen activator factor, uberis factor, protease and hialujeronidase. A fast PFGE technique fixed in our laboratory was used. From the analysis of 8 clinical strains and 16 subclinical ones, 5 and 9 different genotypic profiles were obtained respectively. The capacity of each strain to produce some of the virulence factors analyzed was considered a virulence profile. The clinical strains presented 7 different virulence profiles while the subclinical strains showed 11 different profiles. From the 24 analyzed strains only 1 genotypic profile was identified in 7 strains, 2 clinical strains and 5 subclinical ones. An association between genotypic profiles and virulence profiles or clinical or subclinical origin could not be established. The results obtained show that genotypic profiles would not allow to recognize strains with a capacity to express some of the virulence factors studied. Further investigation of clinical and subclinical strains sharing a same genotypic profile should be considered for future studies.

43.

QUORUM SENSING REGULATES TESTOSTERONE CATABOLIC GENE EXPRESSION IN *COMAMONAS TESTOSTERONI*

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Quorum sensing (QS) is a cell density-dependent signaling system used by bacteria to coordinate gene expression within a population. QS systems in Gram negative bacteria consist of transcription factors of the LuxR family and a diffusible small molecule called autoinducer. Previously, we identified a LuxR-type transcriptional factor called TeiR that positively regulates the transcription of genes involved in the initial enzymatic steps of steroid degradation in *C. testosteroni* (*J. Bacteriol*, 2004, 186:1430). In the present study, western blot assays demonstrated a TeiR density-dependent manner expression reaching peak levels at the stationary phase. The expression of a steroid-inducible transcriptional fusion (*sip48*-β*hsd::lacZ*) could be prematurely activated in cells at early to mid-exponential phase by the addition of ethyl acetate extracts obtained from the transition logaritmic-stationary-phase cell-free supernatants of *C. testosteroni* cultures grown in presence of testosterone. The gene loci *teor*, encoding acyl-coenzyme A dehydrogenase homolog, *tead* encoding enoyl-CoA dehydratase homolog and *tekt* encoding a β-ketothiolase homolog were localized upstream of the *teiR* transcriptional regulator. To prove the essential involvement of *teor*, *tead* and *tekt* genes in the catabolism of testosterone in *C. testosteroni*, these genes were inactivated separately by the insertion of omega elements. The corresponding mutant strains were not able to grow on testosterone as well as to activate the *sip48*-β*hsd::lacZ* transcriptional fusion. Interestingly, the β-galactosidase activity of *teor* mutant complemented with a plasmid encoding *teiR* gene, can be restored by a diffusible extracellular factor produced by *C. testosteroni* wt. In conclusion, the data demonstrate the necessity of a beta-oxidation cycle for testosterone metabolism that results in an intermediary compound involved in the QS signaling system.

44.

SUCEPTIBILITY OF EARLY STAGES OF *Bufo arenarum* EMBRYOS TO PHOTODYNAMIC THERAPY WITH METHYLENE BLUE (AM)

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The photodynamic therapy is an antineoplasic treatment in which a photosensitizer, oxygen and visible light are involved. It is important to investigate the toxic activity of new photosensitizer to know their reach and limitations as potential therapeutic compounds. The aim of this work was to evaluate the different susceptibility of *Bufo arenarum* embryos stages to the photosensitizer AM, standardizing an embryotoxic test. Embryos in gastrula, bud fin back and open mouth stages were used. The CL50 values and malformation in darkness and the phototoxic CL50 (irradiation 30 min.) were obtained beginning from the maximum no-toxic dose in darkness. For 24 h of exposure to AM+ in darkness, the CL50 were 2 and 1,2 mM to gastrula and open mouth stages respectively. Bud fin back couldn't be measured because the high density of the environment exceeded viability. This stage was exposed to 36 and 48 h. obtaining the CL50 of 713 and 120 µM respectively. The malformation occurred in all the tested embryonic stages. The more frequent were incurvated axis and general edema. In the light test, for 24 h. of incubation gastrula and open mouth CL50 were 760 and 50 µM; and for 36 and 48 h. in bud fin back were 60 and 20 µM respectively. The results indicate that AM+ and visible light combination is responsible for embryos death while the drug in darkness caused malformation at all developmental stages evaluated. The sensibility is related to the developing stage and the drug time exposition. This experimental model could be adapted to lethal and teratogenic tests with new photosensitizing drugs studied in PTD.

45.
CHARACTERIZATION OF GLUTAMINE SYNTHETASE ISOENZYMES IN THE SYMBIOTIC ASSOCIATION *Bradyrhizobium*-PEANUT

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Glutamine synthetase (GS) is a key enzyme in the assimilation of ammonium, catalyzing the ATP-dependent condensation of ammonium with glutamate to yield glutamine. GS in legume plants occurs as a number of isoenzymes and is encoded by a small multigene family, based on the subcellular location, GS can be broadly categorized as cytosolic (GS1), plastid (GS2) and nodule (GSn). The objective of the work was to study the molecular and physiological properties of GS in the *Bradyrhizobium*-peanut association. The experimental system consisted in the inoculation of the peanut seeds with *Bradyrhizobium* sp SEMIA 6144 (1 x 10^8 cfu/ml) and growing of the plants during forty days. The specific activity of GS was determined by Bielawski (1994). Most activity of GS was found in nodulated roots and two isoenzymes of different mobility were detected (GS1 and GSn) by electrophoresis on polyacrylamide gels. In nodules, only GSn was detected. The different electroforetic mobility of the two isoenzymes could be presumably due to a difference in the oligomeric composition of the same. The regulation of GS activity was also studied, of the effectors screened 5'AMP, 5'UMP and NADPH, were the most potent inhibitors meanwhile that 3'AMP was an activator of GS isoenzymes in roots and nodules of peanut. Our data suggest that in the *Bradyrhizobium*-peanut interaction, the activity of GS in roots increase by effect of the nodulation and the GS isoenzymes show compatible molecular properties with GS from other legumes.
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46.
COMPARATIVE STUDY OF ACUTE TOXICITY IN COMMERCIAL ANFUNGICS

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Introduction: Commercial drugs used in the treatment of pathogen fungus, whether in human or plants, present different levels of toxicity for other organisms. This fact is usually a limit for their use and application form. In this work we make a comparative study of acute toxicity of commercial antifungics used in human medicine (miconazole and griseofulvine) and against fitopatoghen fungus (carbendazim and benomil).
Methodology: The design and development of the acute toxicity tests is based on an experimental technique adapted by our group of research, from the tests used by U.S. Fish and Wildlife Service. Columbia National Fisheries Research Laboratory (Waynon W. 1980) to evaluate acute toxicity of diverse chemical compounds. Fish such as *Poecilia reticulata* and mollusks like *Lymnaea sp* were used as experimental models in this work.
Results: The drugs Miconazol and Griseofulvine turned out to be toxic in both tests (fish and mollusks) at a concentration of 20 mg/l producing a mortality of 100% of de specimens. Both, at a concentration of 2 mg/l, present different levels of toxicity. Miconazol even at a concentration of 0.2 mg/l produces a mortality of 20% in fish and 50% in mollusks at concentrations lower and equal to 20 mg/l.
Discussion: The drugs used for treatments of mycosis in humans confirm their high level of toxicity in the performed experiments. However when compared to the ones used against fitopatoghen fungus, they present significant differences what partially guarantees the use of the latest, since these may present ambient impact, regarding their application form.

47.
EFFECT OF EARLY POSTNATAL STIMULATION ON IMMUNE AND REPRODUCTIVE PARAMETERS IN PRENATALLY STRESS RATS.

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In our laboratory we have determined that prenatal immobilization stress (IMO) decreases the testicles weight and testosterone level in male rats. (Rodríguez, N. 2000). Also, it produces Hypothalamic-Pituitary-Adrenal axis (HPA) hyperactivity of adult offspring male in basal conditions, and habituation under the same acute stress. This habituation of the HPA axis could be related with an immunological alteration in response to the same stress (Mayer N., 2004). It is known that the early postnatal stimulations produce beneficial effect on the emotional reactivity at long term (Maccari and Cabbage, 1995) and HPA axis activity that can affect the immunity and the Hypothalamic-Pituitary -Gonadal (HHG) axis offspring. The objective of this work was to investigate how early postnatal stimulations in offspring male stressed prenatally affect the gonad size, the thymus and its relationship with the activity of the HPA axis. Males of three months of age were used, offsprings of IMO stressed mothers' during the pregnancy (EP) and non prenatal stressed offsprings (CP). Half of the EP animals were manipulated during the first week of life. The thymus, adrenals glands and testicles were extracted to all the groups of adult animals to obtain their respective sizes. Neither the prenatal stress nor the manipulation modified the adrenals glands and the thymus size, while the testis size increased under the manipulation effect without showing differences with the prenatal controls. In conclusion, postnatal manipulation reverts the effects of the prenatal stress on testis size.

48.
INFLUENCE OF NUTRIENTS AND WATER ACTIVITY ON SCLEROTIUM FORMATION OF *Aspergillus flavus* AND *A. parasiticus*

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The purpose of this work was to examine the effect of nutrients and water stress of different culture media, on sclerotium formation, size, number and volume, on *A. flavus* (RCM89, RCM142) and *A. parasiticus* (RCM38, RCM108) strains, isolated from stored maize. These strains grew in three culture media: Czapek Dox agar (CD, specific medium for sclerotium formation), and two maize-based media, maize meal extract agar (MMEA) and MMEA with sucrose and $NaNO_3$ (MMEA S/N), both compounds in the same concentration like in CD. Media (0.999 a_w) were modified with glycerol to 0.971, 0.955 and 0.937. Experiments were carried out at 30ºC. Sclerotia were collected after 15 days. All strains developed sclerotia on the three normal unstressed media (0.999 a_w). RCM89 strain produced small sclerotia (< 400 μm). It was the unique S strain. At 0.971 a_w RCM89 strain produced larger sclerotia (> 400 μm). None strain produced sclerotia under the driest conditions (0.955 and 0.937 a_w). Media composition tested didn't affect sclerotia size at 0.999 a_w. Although, the strains produced higher number of sclerotia on CD agar than in maize-based media. Significant difference was observed between CD medium and MMEA medium. At 0.999 and 0.971 a_w, RCM89 strain formed sclerotia of lower volume, on MMEA S/N and CD, respectively. It's important to know the impact of nutrient requirements and water stress on sclerotia formation, in spoilage fungi such as *Aspergillus* section *Flavi*, for the management of prevention strategies in storage.

49.

DISTRIBUTION OF TELOMERIC SEQUENCES IN THREE SPECIES OF *CALOMYS* (RODENTIA, SIGMODONTINAE)

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Calomys, with a wide South American distribution and at least twelve species probably represents the most primitive phyllotine genus. The genus shows a wide karyotypic diversity. We have studied the chromosome location of telomeric sequences in *C. laucha*, *C. venustus* and *C. musculinus* by means of FISH with a PNA probe $(C_3TA_2)_3$. Only a telomeric location was observed in *C. venustus* and *C. musculinus*. In *C. laucha*, in addition to the expected telomeric marks, a strong centromeric signal in two of the three biarmed autosome pairs (pairs 1 and 2) was noticed in all cells. In several metaphase plates, an interstial signal of lower intensity in an acrocentric pair was also found. The presence of these signals could correspond to the persistence of telomeric sequences after rearrangements that imply the chromosome ends. A process of decrease of chromosome number from a primitive phyllotine karyotype (2n= 70, AFN= 68), mainly by means of Robertsonian fusions, was postulated as the basic trend of karyotype evolution in the genus. However, it is noticeable the absence of interstitial telomeric signals in the supposedly more rearranged species, *C. venustus* and *C. musculinus*.

50.

PHOSPHOLIPIDS OF *Pseudomonasm aeruginosa* GROWN IN NUTRITIVE COMPLEX MEDIA SUPPLEMENTED WITH CHOLINE

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Phospholipid (PL) composition and metabolic fate of carbon chain and methyl groups of choline in *P. aeruginosa* grown in basal salt media was demonstrated in our laboratory. *P. aeruginosa* produces PLC and phosphorylcholine phosphatase activities in the presence of choline favoring this bacteria pathogenecity. With the aim to know if PL variations in nutritive complex media occurred in a situation that resemblance the environment which could be found *in vivo*, it was studied PL composition in different phases of bacterial growth in peptone 1%-NaCl 1% medium (JM), supplemented or not with choline or succinate (20mM). Cells were harvested during exponential phase (FEx) 4 hours and during stationary phase (FEs) 7 Hs. of a continuous culture. The variation of Pi concentration in the medium was in the range from 2,4 mM in starting culture to 0,35 mM in FEs. Total intracellular Pi in bacteria harvested in FEx and FEs was similar (1,1-1,2 nmol.µg $prot^{-1}$). In FEx and FEs, total PL of bacteria grown in JM, JMC and JMS media were 0,1; 0,078 and 0,062 against 0,009; 0,015 and 0,022 nmol.µg $prot^{-1}$, respectively. In FEx and FEs, PE, PG and DPG sum was equal or greater than 90% in all cases. LPE, PC and LPC were detected as minority components. In FEx, choline with respect to JMS and JM media produced a PE increase of 22% and 7%, respectively, and a decrease of PG and DPG of 30% and 40%, respectively. In FEs, similar PL composition was observed in the three culture media. Choline uptake studies during bacterial growth indicated that PL changes in *P. aeruginosa* were not due to a choline effect, because its uptake started after 15 hours of culture.

51.

APLICATION OF THE TOXICITY TEST IN *Bufo arenarum* EMBRYOS TO EVALUATE PHENOL PHYTOREMEDIATION

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The industrial effluents constitute one of the main sources of the aquatic ecosystem contamination. Therefore, is important to treat these effluents before their release to the environment. In previous works, turnip hairy roots were used in presence of H_2O_2 and polyethylene glycol (PEG) to remove phenol with high efficiency. The potential toxicity of these components and of the remaining solutions post-removal can be determined using experiments in amphibian embryos. The aim of this work was to determine the toxicology impact in *Bufo arenarum* embryos, of phenol solutions pre and post removal treatment, utilising embryos in stage 25 exposed for 96 hours to such solutions. Solutions of phenol (10-250 mg/l) and of H_2O_2 (0.1-5 mM) were analysed. Non-toxic concentrations (NOEC), lethal concentrations 50 (CL50) and 100 (CL100) were established for both components. Moreover, the minimum concentration of PEG that produces mortality was determined. The CL100, CL50 and NOEC were: 250, 200 and 25 mg/l to phenol and 1, 0.98 and 0.5 mM to H_2O_2, respectively. Phenol solutions of 100, 150, 200 and 250 mg/l treated with turnip hairy roots showed the following averages of mortality: 0, 3, 16 and 19 respectively ($p< 0.05$). The addition of PEG (100 mg/l) to these solutions reduced mortality at 0, 1, 2 and 2 respectively ($p < 0.55$). These results allow us to conclude that the proposed system is effective to reduce significantly the toxicity of phenol-contaminated solutions. PEG increases phenol removal, probably due to its protective effect on the enzymes involved in the process, which is correlated with the diminution of toxicity.

52.

PROMOTION OF ALFALFA GROWTH BY *Pseudomonas aurantiaca* IN COINOCULATION WITH *Sinorhizobium meliloti*

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Coinoculation studies with *Pseudomonas* and rhizobia have been shown to increase N_2 fixation and grain yield in various legumes such as soybean, alfalfa, chickpea, pea and white clover.
The objective of this study was to evaluate the effect of *Pseudomonas aurantiaca* on the symbiosis of *Sinorhizobium meliloti* 3DOh13 with alfalfa (*Medicago sativa* L.)
S. meliloti inhibition *in vitro* by *P. aurantiaca* was tested. Any interference was descarted by growth joint of the bacteria in YEMA and TSA medium.
Surface sterlized seeds were inoculated with broth cultures of *P. aurantiaca* and *S. meliloti* strains for 20 min. In the coinoculation experiment, seeds were treated by mixing culture broth of both the inoculant strains in a 1:1 ratio (v/v). Populations of bacteria on inoculated seeds were located in 10^6 colony-forming units (cfu) per seed inoculated separately with *S. meliloti* or *P. aurantiaca*, and 10^5 cfu per seed for each bacterium in the coinoculation. Inoculated seeds were sown in pots containing a mixture of sand and perlite (2:1). The coinoculation increased shoot and root fresh weight, shoot and root dry weight, shoot and root lenght and the rate of effective nodulation in comparison to inoculation whit *S. meliloti* alone or uninoculated controls.

53.

CHEMICAL CONTROL OF *Aspergillus* SECTION *Flavi* IN PEANUT GRAINS USING FOOD GRADE ANTIOXIDANTS

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Approximately a 98% of peanut national production is concentrated in the middle-south region of Córdoba. These crops tend to be contaminated by *Aspergillus* section *Flavi* species. These fungi produce aflatoxins, which are responsible of the rejection of grains in the exportation stage. Due to it, the purpose of this study was to control growth (UFCs g^{-1}) and aflatoxin B_1 (AFB_1) production by *Aspergillus* section *Flavi* species, using food grade antioxidant mixtures: butyl hidroxyanisol (BHA), butyl hidroxytoluene (BHT), propyl paraben (PP) at doses of 10 and 20 mmol g^{-1}, in peanut grains without inoculum and grains with 10^4 spores ml^{-1} of *A. parasiticus* CHG24 and *A. flavus* CHG46. Peanut grains were adjusted at three water activities (a_w) (0.982, 0.955, 0.937) and were incubated at 28ºC during 11 and 35 days. All antioxidant mixtures significantly reduced growth and AFB_1 production, independently of water stress and incubation period. The percentages of growth reduction at 35 days whit the mixtures: BHA-PP (10:10, 10:20, 20:10) were 9.2-100%, 87.6-100% and 90-100% at 0.982, 0.955, 0.937 a_w, respectively, with a 100% of reduction in the AFB_1 accumulation. Treatments with BHA-PP (20:20) and BHA-PP-BHT (10:20:10, 20:10:10, 20:20:10) inhibited growth and AFB_1 production. This study showed the efficacy of food grade antioxidants to control aflatoxicogenic mycoflora in peanut grains.

54.

CONTRIBUTION OF BIOFERTILIZERS TO PLANT HEALTH AND NUTRITION

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A biofertilizer is a substance which contains microorganisms which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the availability of nutrients to the host plant (Vessey, 2003).
The objective was to study the *in vitro* PGPR characteristics of bacterial strains isolated from a biofertilizer, and to determine growth promotion in tomato and pepper plants by spraying the leaves with the biological fertilizer.
The isolated strains were characterized through the following assays: phosphate solubilization, siderophore production, starch hydrolysis, exopolysaccharide production, antifungal activity and production of IAA. 20 and 35 days post application of the biofertilizer, the following variables were analyzed both in tomato and pepper plants: shoot and root system fresh and dry weight and root surface area.
20% of the ten isolates resulted positive for phosphate solubilization and one of them also produced siderophores, this property was observed in two of the total isolates. Half the isolates produced exopolysaccharides, 50% threw positive results in the hydrolysis of starch. Five isolates inhibited the growth of *Fusarium solani*. Four isolates evidenced IAA production. Growth promotion was observed in tomato but not in pepper. The microorganisms contained in the biofertilizer possess appropriate beneficial properties that potentially could exercise a plant growth stimulation effect in certain vegetables.

55.

EVALUATION OF PLANT PATHOGENS IN SOYBEAN. *GLICINE MAX* (L.). MERRILL VAR 8000

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In the present soybean constitute one of the most important crops in Salta (Argentina). In this zone the climatic conditions influence on the increase and incidence of significant pathogens. Diseases controls involved techniques that help to keep pathogens levels under UDE curve. One of the methods of control is the biological control. The aim of this work was to evaluate the incidence of plants pathogens in soybean inoculated with rhizobacterias and a commercial inoculum. This study was conducted in EEA-INTA-Salta under conventional and no-tillage systems. Four treatments with four repetitions were performed, T1: inoculated with 51B strain, T2: with D3 strain, T0: Control and T0+F: Control + Commercial inoculum. The inoculum concentration was 0,5ml/100g (10^8 cfu. ml^{-1}). The pathogens most frecuently isolated were *Peronospora sp., Alternaria sp., Cercospora sp. and Septoria sp.* There were statistically differences ($p<0,005$), between conventional and no-tillage systems. Those results allow us to conclude that rhizobacteria (D3) show a most effective action than commercial inoculum in the control of diseases in soybean.

56.

ANALYSIS OF GENETIC STRUCTURE IN POPULATIONS OF *TRIATOMA INFESTANS*

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Triatoma infestans is the main vector of *Trypanosoma cruzi* in the Southern Cone of Latin American countries between the latitudes of 10° and 46° S. With the purpose to analyze the genetic structure of *T. infestans* populations, we identified ninety-three microsatellite loci from partial genomic libraries, of which thirty were amplified by PCR and ten were selected for genotyping. These loci were used to analyze seven populations of *T. infestans* from areas treated with insecticide and three populations from areas that never received treatment. The number of alleles per locus ranged from 11 to 27. The average observed and expected heterozyosities ranged from 0.30 to 0.63 and 0.42 to 0.75, respectively. The excess of homozygotes observed in some of the loci analyzed in all the sampling localities may reflect population subdivision and/or the presence of null alleles. The significant levels of genetic differentiation detected between populations suggest that the magnitude of gene flow is not sufficiently large to mask differences eventually produced by genetic drift. The levels of genetic diversity in the populations of *T. infestans* from insecticide treated localities were similar or higher than those detected in populations from areas without treatment. In subdivided populations, the severe population reductions produced by insecticide treatments would result in several independent genetic drift effects that could randomly preserve different combinations of alleles in each subpopulation. If independent genetic drift events in the population were followed by a rapid population growth, high levels of genetic diversity could be preserved.

57.

PARTIAL SEQUENCES OF *SRY* AND *DAX*-1 IN *AKODON BOLIVIENSIS* AND *A. AZARAE*

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The sigmodontine rodents *Akodon azarae* and *A. boliviensis* belong to a group of *Akodon* species that present a proportion of fertile XY females. As a contribution to the analysis of this condition, we sequenced in the first place, a 195 bp PCR segment of the HMG box of *A. boliviensis Sry* gene. No differences between males and XY females were observed. This result, together with a similar result obtained by other authors in *A. azarae*, tends to discard a mutation in the *Sry* conserved region as the cause of sex reversion. The comparison between this partial sequence and a published partial sequence of *A. azarae* (123 nucleotides in common), revealed 12 nt substitutions (90.24% homology), which correspond to five changes in the deduced protein sequence (87.80% homology). As our previous results on *A. azarae*, as well those of others on *A. montensis*, point to a mutation in a X-chromosome gene as the possible origin of sex reversion, we studied a PCR 654 bp fragment of *Dax*-1 exon 1 from *A. boliviensis* and *A. azarae*. No differences were found between males, XY and XX females of *A. boliviensis*, nor between *A. azarae* XY and XX females, which obviously eliminates a mutation in this region of *Dax*-1 as implied in sex reversion. The comparison between both species sequence showed 15 nt substitutions (97.76% homology), corresponding to 11 in the protein sequence (93.12% homology). The completion of the sequence of *Dax*-1 and of other genes that participate in sex determination will contribute to clarify this interesting phenomenon.

58.

CHARACTERIZATION OF SYMBIOTIC PLASMIDS IN NATIVE PEANUT RHIZOBIA FROM CORDOBA

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Rhizobial species able to induce nodule formation on leguminous plants belong to the family *Rhizobiaceae.* Peanut (*Arachis hypogaea* L.) is a legume that establishes symbiotic relationship with rhizobia. In Argentina 94% of national peanut production is concentrated in the province of Cordoba, being an economically important crop for this region. Previous phenotypic studies of native peanut rhizobia isolated from this region revealed the presence of two populations which group within the slow and fast growing rhizobia. Considering these results and that there are no previous reports describing peanut fast-growing rhizobia, the aim of this study is to analyze if their symbitic genes are localized in autotransmissible plasmids as is described for other fast growing rhizobia. **Methods:** Bacteria: *Rhizobium etli* CFN42 pSym$^-$, *R.tropici* CIAT899 pSym$^-$, *Sinorhizobium fredii* 192 pSym$^-$, native peanut isolates from Cordoba soils: NCHA22 and NET30. Biparental matings (Simons *et al*., 1983). Nodulation assays (Vincent, 1970). PCR-*nodC* (Laguerre *et al*., 2001). **Results and Discussion:** Transconjugants were obtained from the mating between NET30 and *R. tropici* CIAT899 pSym$^-$, which induced efficent nodules in peanut and showed the expected 930 bp band from the *nodC*-PCR amplification. The transconjugants obtained from the mating of NCHA22 and *R. etli* CFN42 pSym$^-$ were not able to nodulate peanut but gave a 930bp product from *nodC*-PCR. These results suggest that pSym of isolates NET30 y NCHA22 are autotransmissible. Nevertheless pSym of NCHA22 would not be able to confere peanut nodulation capacity to *R. etli* CFN42.
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59.

BIOCHEMICAL AND GENETIC CHARACTERIZATION OF *OCHROBACTRUM* STRAINS ISOLATED FROM SALINE SOILS OF CÓRDOBA PROVINCE

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Soil salinity is one of the main problems affecting the productivity of agricultural lands in the Southeast of Córdoba province. As an alternative to recover these lands, we have established a collection of salt tolerant native strains, previously isolated from saline soils of this region. From this collection we selected two strains able to promote maize growth under salinity conditions. These strains were identified by sequencing the 16S rDNA as *Ochrobactrum sp. 3b* and *Ochrobactrum sp. 11a*. In order to characterize these strains, we have demonstrated some PGPR mechanisms and identified some genes associated with these traits. The presence of the *nifH* gene was tested by PCR using primers nifH1 and nifH2. Both strains were positive indicating the presence of the *nifH* gene and confirming the nitrogen fixation phenotype, previously tested by growing strains in nitrogen-free medium. In addition, we have demonstrated the presence of a megaplasmid in *Ochrobactrum sp. 11a,* which probably could be associated to some characteristics of this strain such as, heavy metal resistance and salinity tolerance among others. With the aim to identify genes associated to salinity tolerance, we generated salt-sensitive mutants from *Ochrobactrum sp. 11a* by transposition using Tn5-B21 transposon. Salt-sensitive mutants showed a decreased growth under salinity conditions, compared to the wild type strain.

60.

STUDIES OF IMMUNOLOGICAL PARAMETERS IN PATIENTS WITH SUPERFICIAL MICOSIS AND THEIR MODULATION BY DERIVATIVES OF THE *Minthostachys verticillata*

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The superficial mycosis is an infection that attacks the superficial layers of the skin and phaners nails, associated to alterations of the immunological mechanisms of the host. Different fractions from *M. verticillata* have *in vitro* antimicrobial activity and it has immunomodulating properties. The aim of this work was to demonstrate the alterations of the Immunity Cellular and Humoral in patients with superficial fungal infections by means of the count of total populations of LT, CD4+, CD8+ and LB; to detect specific Ab and to study the proliferative response of the lymphocytes stimulated with fractions of *M. verticillata*. Twenty patients with dermatophytosis and 20 healthy controls were evaluated themselves. The test of cellular lymphoprolipheration was made, stimulating the lymphocytes with essential oil, decoction and ethanolic extract derivative of the vegetable and with PWM mitogen, total LT by Spontaneous Rosettes, LTCD8+ and CD4+ by IFI and LB by IFD were quantified; they determined specific Ab by IFI against Ag anti-dermatophytes. The evaluation of the proliferation with all the vegetal fractions showed similar mitogenic effect to the produced one by PWM, the index of blastic transformation was significantly greater ($P<0.05$) as much in patients as controls. The quantification of total LT, LT-CD4+ and CD8+ p showed values diminished in the 100% of patient respect to the controls ($p<0.05$), whereas the LB were increased in all the patients ($P<0.05$) 80% of them showed specific anti-dermatophytes Ab. The superficial fungal infections altered certain immunological parameters jeopardising the humoral and cellular immunity, such alterations could be reverted by the use of the derivatives of *M. verticillata*, inferred from the results obtained *in vitro*.

61.

COMPARATIVE ANALYSIS OF PROSTATIC STROMAL REACTION INDUCED BY CASTRATION AND BY INFLAMMATION

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Introduction: Prostatic stroma is constituted by mesenchymal cells, which are key regulators of prostate growth and differentiation. In Prostatic Benign Hyperplasia and Prostate Cancer, stromal cells exhibit a high degree of plasticity in response to surrounding signals. **Objective:** Evaluation of phenotypic changes in prostate stroma induced by inflammation. It includes an ultrastructural analysis and immunocytochemistry (ICQ) for vimentin (VIM) and smooth-muscle--actin (SMA). Also, the Toll-like receptor 4 (TLR4), TNFα and TGFβ1 expression by ICQ and Western Blot was evaluated. These results were compared with a castrated model. **Experimental models:** Wistar rats (INF) were inoculated with E. coli (108 UFC) beneath the ventral prostate capsule and the glands studied at 24, 48 and 72 hours later. In control rats, the prostate was injected with PBS. Other group (OX) was orchidectomized through the scrotal via and sacrificed after 10 days. Sham operated rats served as control. **Results:** Control animals showed a thin stromal layer, made up of SMA(+) cells and few VIM (+) fibroblasts. In both INF and OX, hypertrophy and hyperplasia of stroma cells with a muscular phenotype were characterized; while in OX, this layer displayed SMA(+) and VIM(-) and smooth muscle organization, in INF the periacinar layer coexpressed VIM and SMA. We observed induction of TLR4 expression by muscular cell phenotype in both models. However, TNFα was only expressed by the epithelium. TGFβ1 expression was induced at higher levels in OX. These observations suggest that different stimuli produce transdifferentiation of stromal cells either to VIM and SMA positive-myofibroblast-like cells due to inflammation, or to SMA(+) and VIM(-) smooth muscle cells triggered by castration. TGFβ1 could be an important regulator for this transdifferentiation. The induction of the TLR4 expression in the periacinar layer shows that the prostatic stroma could function as innate immune system.

62.

EFECTS OF BENZNIDAZOLE ON PLACENTAL ALKALINE PHOSPHATASE IN *IN VITRO* INFECTION OF HUMAN TROPHOBLAST WITH *TRYPANOSOMA CRUZI*

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Introduction: Specific surface membrane components like Placental Alkaline Phosphatase (PLAP), a GPI-anchored enzyme, are involved in *T. cruzi* invasion process. It was observed that modifications of cellular lipid pattern due to *T. cruzi* infection, are reversible with Benznidazole (BNZ), a drug employed to treat acute Chagas Disease. **Objectives:** To understand the role of some plasmatic membrane components, such as lipids and PLAP in the *T. cruzi* invasion to human trophoblast. **Methods:** placental villi from non-infected patients were pretreated with BNZ in culture and then co-cultured with blood trypomastigotes (Tulahuen strain) isolated from Albino Swiss mice blood. PLAP activity was measured with biochemical methods and histochemistry; parasitic invasion was determined with microscopy and immunolabelling for *T. cruzi*. Zymmograms and Western-blotting were performed for PLAP. **Results:** Placental villi culture pre-treated with BNZ presented a significant increase in immunologically detectable PLAP and PLAP activity before infection with *T cruzi*. **Discussion:** Pretreatment with BNZ would prevent the effects of *T. cruzi* invasion on PLAP and diminish the immunologically detectable *T. cruzi*.

63.

TEMPORAL AND SPATIAL VARIATIONS IN WATER QUALITY PARAMETERS OF RIO TERCERO DAM, CORDOBA, ARGENTINA

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Considering the economic, social and ecological importance of this resort in particular, it is necessary the performing on routine monitoring in Río Tercero dam in order to know its trophyc status and water quality. The goals of this work were to evaluate seasonally the trophyc condition of the dam, asses shallow water quality for different uses by means of physico-chemical and phycological analysis, and establish the potential risks for public and animal health. Six sampling stations were considered, two seasonal samplings per chosen site, during 2003-2004. Physical, chemical and biological parameters were assessed using standard methodology. During summer 2004 general characteristics of water were those of freshwater, calcium bicarbonated, low in salts for animals and good for human consumption. According to results observed in sampled sites, the trophyc condition of the dam is mesotrophyc. Phycological counting displayed that, during summer, there was a dominance of Bacillarioficeae over Cyanophyceae, which was not a tendency along the year. Our results demonstrate that the dam is in between the limits suggested by local legislation. Some critical areas receiving effluents should be studied systematically and on bigger scope.

64.

EFFECT OF ENVIRONMENTAL FACTORS ON BIOFILM FORMATION IN *SINORHIZOBIUM MELILOTI*

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Rhizobia are soil bacteria able to fix nitrogen in symbiosis with legumes. In natural environments, bacteria are often found as sessile communities known as biofilms. To characterize the Rhizobia biofilm, an *in vitro* model was adapted where *S. meliloti* develops a biofilm on the abiotic surface of a microtiter dish wells. Biofilm was detected by staining with crystal violet and quantified by OD measurement at 560 nm. *S. meliloti* was able to form a biofilm on the abiotic surface in different media. However biofilm formation was higher in minimal medium (*Rhizobium* Defined Medium; RDM) compared with rich medium (Luria-Bertain; LB and Tryptone-Yeast extract; TY). This observation indicates that sessile growth may represent a survival strategy in a nutritionally limited environment. The presence of increasing concentrations of sucrose as carbon source in RDM increased biofilm formation. However, this effect is not related with osmolarity produced by sucrose, since bacterial adherence was decreased at high osmolarity in the presence of the osmolyte NaCl. In a 5.0-9.5 pH range, the optimal production of biofilm was obtained at pH 7.0. Therefore, these observations indicate that environmental and nutritional conditions influence *S. meliloti* biofilm formation.

65.

LYMPHOCYTE PROLIFERATION AGAINST BOVINE AND EQUINE MILK PROTEINS AND IgE LEVELS IN COW MILK ALLERGIC CHILDREN

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Cow milk (CM) allergy appears during the first years of life. Clinical symptoms can be produced by immediate type reactions (IgE), delayed (T lymphocytes) or both. In both cases there may be skin, respiratory or gastrointestinal manifestations. Diagnosis is based on Provocation-Elimination tests even when the determination of the responsible allergens can be a useful tool for diagnosis or a prediction of CM allergy. Mare milk (MM) could be used as alternative food in children with hypersensitivity to CM. Lymphocyte proliferation against CM and MM proteins and seric IgE levels against CM proteins were studied. Twenty children allergic to CM and 20 healthy controls, from 1 to 7 years old, were studied. Lymphocytes isolation was done by density gradient and lymphocyte proliferation was evaluated by MMT colorimetric assay against CM and MM proteins. IgE seric levels were measured by ELISA. There was a proliferative response against CM in 18 patients. Twelve presented digestive symptoms and 10 showed high IgE levels. Two patient's cells with high IgE showed proliferation against MM. Cells of another two children with high IgE did not proliferate against the tested allergens. There were significant differences ($p<0,05$) in allergic and controls among indexes of expanded cells stimulated with CM but not with MM. Lymphocyte proliferation in allergic to CM could confirm the hypersensitivity to the specific allergen and would agree with clinical symptoms. MM would not induce lymphocyte stimulation in CM allergic children which might be an adequate substitute formula. This factor would sum advantages to its nutritional qualities of palatability and costs.

66.

GERMINATION, EMERGENCE AND PRODUCTIVITY OF BEANS (*Phaseolus vulgaris* L.) IN REACTION TO THE INOCULATION OF SEEDS WITH FLUORESCENT *Pseudomonas* 51 B

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Seed quality is a specific request for present crop production. This research was aimed to study the effect of inoculating with fluorescent *Pseudomonas* 51 B on germination and emergence percentages in the field, and also on yield components of three bean cultivars cu differing in their qualities. For this purpose, sublots of white (Alubia) and black (Norte and Cerrillos) beans were artificially deteriorated (42°C and 100% RH) during zero day (high vigour) and one day (mean vigour); and subdivided in: i) without inoculation, ii) inoculated with *Pseudomonas* (10^8 cfu/ml^{-1}). As compared with not inoculated sublots, the 51 B strain increased significantly ($p\leq 0,05$) the germination percentages of seeds with high vigour of cv Norte and mean vigour of Cerrillos; and not significantly for white bean. On the other hand, only the lack of emergence in the field of deteriorated seeds not inoculated of the cv. Alubia was reverted as a result of treatment with *Pseudomonas* being studied. Concerning the valuated yield components-under the experimental conditions- although strain 51 B does not promote significant modifications in the productivity of the three studied bean cvs, the increase in seed number/plant does correspond with a lesser size of plants. It is concluded that inoculation with fluorescent *Pseudomonas* 51 B has some positive effects on germination and emergence of seedlings, depending on the cultivar and degree of vigour of bean seeds employed.

67.

ISOLATION, PARTIAL CHARACTERIZATION AND MODE OF ACTION OF THE BACTERIOCIN PRODUCED BY *Lactobacillus rhamnosus*

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Antagonistic activity of lactabacilli is an important factor for the protection of fertile women vagina against infection caused by some pathogens. Lactobacilli produce many antimicrobial substances including organic acids, hydrogen peroxide and bacteriocins. A total of a hundred strains of lactobacilli were isolated from sexually active women without genitourinary infection. One strain was identified as *Lactobacillus rhamnosus.* This strain was chosen for this study due to its wide spectrum of inhibitory activity and the production of hydrogen peroxide (H_2O_2). The substance with antimicrobial activity produced by *Lactobacillus rhamnosus* showed, a very wide spectrum of inhibitory activity on bacteria related or not, with the producing microorganism, a low molecular mass (< of 7.000 Da), stability during heat treatment, sensitivity to proteolytic enzymes and bacteriostatic effect. The bacteriocin maintained its activity at pH 5, which would indicate that it is active at the vaginal ecological niche pH. The viability and capacity of producing bacteriocin of *L. rhamnosus* kept at -80ºC, was maintained for a period over 36 months. The antimicrobial substance produced by *L. rhamnosus* resembles the group II bacteriocins described by Klaenhammer.

68.

STUDY OF GLYCOCONJUGATES DURING PORCINE PLACENTATION

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The glycoproteins and glycolipids, sacharide residues situated in the feto-maternal porcine interphase would allow the interactions between the fetal and maternal placental components due to the type of epitheliochorial placenta. The presence of glycosilated residues of the feto-maternal interphase belonging to porcine placenta from different gestational periods was studied. Glycosilated residues were detected through Direct Inmunofluorescence in histological slices fixed in buffered formol of the porcine placenta of 28, 55, 70 and 114 days of gestation. The following lectins conjugated with FITC were used: Vicia faba (VFA: man, glc); Concanavalin A (Con A: α-man, α-glc); Arachis hipogaea (PNA: ß-gal); Pisum sativum (PSA: α–man); Phaseolus vulgaris P (PHA-P: oligosaccharides), Phaseolus vulgaris E (PHA-E: oligosaccharides); Wisteria floribunda (WFA: galNAc) and Artocarpus integrifolia (Jacalin-AIA: α-gal). The lectins Con A, PHA P and PHA E were the most expressed over the placental villi in any gestational period, except at 28 days where only a high fluorescence with Con A was observed. PSA showed similar labeling in every period as AIA, except at 28 days of gestation when a decrease is detected. PNA is expressed at 55 days of gestation, showing high fluorescence limited to villi. In conclusion, heterogeneous labeling of glycosilated residues was detected, expressed in a differential way in the feto-maternal porcine interphase in the different gestational periods; remarking the presence of α-man, α-glc residues in any selected periods.

69.

CHRONIC STRESS IN RATS: LIPOPROTEINS PLASMATIC LEVELS AND ATHEROGENESIS

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Increased levels of LDL-cholesterol and apolipoprotein B are causally related to atherogenesis. Inverse association between total cholesterol (TC) and HDL-cholesterol has also been stablished as a risk indicator with predictive value for coronary heart disease. High corticoid levels have been related to mentioned changes. The aim of this work was to evaluate the effects of chronic stress on plasmatic levels of HDL-cholesterol, LDL-cholesterol, apolipoprotein B and atherogenic risk in rats. Wistar male rats were divided in two groups: controls (C) and stressed (S). The last group was exposed to stress by inmobilization, 2 hs. a day during 14 days. On the 1, 7 and 14 day, blood was collected to determine TC, HDL-cholesterol, LDL-cholesterol, apolipoprotein B, and corticosterone. Atherogenic index (TC/HDL cholesterol) was also calculated. Cholesterol-LDL was 62% higher in S vs C. The Apo B levels increased in S animals at day 14 (C: 47.5 mg/dl vs S: 92.2 mg/dl). TC/HDL-cholesterol index increase was observed (C: 1.76 vs S: 2.01) and LDL-cholesterol/HDL-cholesterol relation increased 39% (S vs C) at day 14. Corticosterone levels showed 162% of increase in S animals in relation with C. Since all parameters tested at day 14 depicted an important increase we can conclude that chronic stress is a main factor implicated in atherogenesis in rats.

70.

CHRONIC STRESS ADAPTATION IN RATS WITH SALT OVERLOAD

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It is well known that the stress response generally diminishes after repeated stressor presentation. It has been suggested that this lower response would be a consequence of a non associative learning processes known as adaptation. The objective of the present work was to evaluate the natremia, natriuresis and the plasmatic osmolarity and its relationship with the saline solution intake, in animals subjected to two durations of chronic immobilization stress (IMO). Wistar male rats, with access to 1.5% NaCl solution, were divided in 3 groups: Control (C), with 30 min. (E30) and with 60 min. (E60) of IMO per day, for 14 days. In the days 1, 7, 10 and 14, glycemia, corticosterone (COR), natremia, plasmatic osmolarity and natriuresis were determined. Daily saline solution intake it was also evaluated. Sodium intake was smaller than C group only in the E60 animals after 14 days of IMO. Sodium excretion was smaller in all the stressed animals in the days 1 and 7. At day 10, only E60 showed antinatriuresis and at day 14 not significant differences among the 3 groups were observed. Natremia, glycemia and plasmatic COR levels were higher in all IMO animals. The minor renal response to stress with several stressor presentation, would indicate an adaptation of this variable, that would be related with the duration of the stimulus presentation. This response would be independent of the sympathetic nervous system and cortico-adrenal activation. The sustained natremia increases without changes in the plasmatic osmolarity, and the sodium intake decreases only after several days would represent an important factor of cardiovascular risk.

71.

ESTABLISHMENT OF TRANSGENIC HAIRY ROOT CULTURES THROUGH A SUCCESSIVE TRANSFORMATION METHOD

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Hairy root (HR) cultures have received special attention in the last years because of their multiple applications. They constitute a plant system with rapid *in vitro* growth without hormones and biochemical and genetic stability. Genetic engineering has allowed to transform plants with foreign genes to give or to improve special metabolic features. The establishment of transgenic HR can be obtained through a unique step of transformation, using genetically modified *A.rhizogenes*, or by an unusual method with two steps using *A. tumefaciens* and *A. rhizogenes.* The latter technique is called successive transformation. The aim of this work was to obtain transgenic HR cultures with peroxidases genes (*tpx1, tpx2 and both)* and with a gene involved in the ascorbic acid synthesis (*GalUR*) through successive transformation, to use them for future biotechnological applications. Stable transgenic HR clones of tobacco, which expressed the foreign genes *tpx1, tpx2* and both genes and tomato HR that expressed *GalUR* gene were established. As it could be analysed by PCR, the clones contained not only the *rol C* gene, which is characteristic of HR but also the foreign genes integrated in the transgenic plants used in the first step. Growth index, enzyme activities and the isoelectric focusing zymograms of the enzymes were analysed for transgenic and wild type clones. The results showed the efficiency of the successive transformation technique to obtain stable transgenic HR clones. This method constitutes a useful tool to give or to improve HR cultures features for biotechnological applications.

72.

CHARACTERIZATION OF PEANUT RHIZOBIA WITH PGPR PROPERTIES

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Arachis hypogaea L. (peanut) is an important crop all over the world that provides food for direct human subsistence and other several food products. Argentina is one of the major peanut producers in the world, and about 94% of its production takes place in the province of Córdoba. The increase in the yield of some leguminous plants inoculated with selected rhizobia strains has been associated with the rhizobia ability to act as plant growth promoting rhizobacteria (PGPR). Like other PGPR, rhizobia are able to solubilize phosphate, produce siderophores, cyanides and show antagonistic activity against phytopathogen fungi. Because of their adaptation to the ecological conditions from a local soil, the native strains are preferentially used to inoculate a determinate area. Objetive: To select PGPR native peanut rhizobia from Córdoba soils. Methods: Phosphate solubilization (Frioni, 1999), Siderophore production (Schwyn and Neilands, 1987), Indol acetic acid production (AIA) (Glickmann y Dessaux, 1995), Antagonistic activity (March y Marinelli, 1997). Results and Discussion: It was possible to observe that in the 14 rhizobia isolates analyzed the 57% showed ability to solubilize phosphate and siderophores and 33% demonstrated to produce AIA. Antagonistic activity was observed between the isolates against the peanut pathogen fungi *Sclerotinia minor* and *S. sclerotiorum* (57 and 43%, respectivelly). The present study shows that peanut soils of the province of Cordoba harbor rhizobacteria with important PGPR properties. These bacteria represent a potential source for inoculation practices in agriculture.
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73.
PHOSPHOLIPASE C SYNTHESIS IN *PSEUDOMONAS AERUGINOSA* GROWN IN NUTRITIVE COMPLEX MEDIA SUPPLEMENTED WITH CHOLINE

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P. aeruginosa grown in a basal salt medium with choline synthesize a choline uptake system plus hemolytic phospholipase C (PLcH) and phosphorylcholine phosphatase (PChP). Both enzymes play an important role in the pathogenicity of this bacteria because through their action they breakdown host membrane phospholipds. These enzymes synthesis was repressed by the simultaneous presence of succinate and ammonium ion. With the aim to know more about this infection mechanism, bacteria were grown in the nutritive complex medium peptone 1%-NaCl 1% (JM) supplemented or not with 20 mM choline (JMC) or succinate (JMS). PLcH, PChP and alkaline phosphatase (AlkP) were determined. By measuring the Pi consumption and the choline or succinate uptake along the bacterial growth it was observed: In the three media Pi was not a limiting factor; supporting this fact was the absence of ALkP activity. Succinate uptake started at the end of the exponential phase, 4 hours, and was maintained up to 17 hours culture. Choline uptake started after 15 hours and was maintained at a constant rate up to 22 hours culture. Determination of survival of *P. aeruginosa* grown in different media shows that in JMS medium the number of viable cells increased with the succinate uptake, whereas in JMC medium the number of viable cells decreased after starting the choline uptake. Induction of PLcH activity only occurred in JMC medium and the maximal activity was found after 48 hours culture, probably as a last response to attack the host cell to maintain its survival.

74.
PROGESTERONE AT THE PICOMOLAR RANGE IS A CHEMOATTRACTANT FOR MAMMALIAN SPERMATOZOA

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Sperm chemotaxis is a cell transport mechanism that guides spermatozoa up an attractant concentration gradient in order to facilitate the gamete encounter. Sperm chemotaxis was observed towards: follicular fluid (in humans, mice and rabbits), oviductal fluid (in mice), and the egg-cumulus complex (in humans and rabbits). Progesterone (P), the main steroid of the mentioned biological sources, has been assayed for human sperm chemotaxis by different groups, giving rise to contradictory results. The aim of this work was to examine whether sperm chemotaxis is mediated by progesterone at extremely low concentrations that were not investigated before. Human and rabbit capacitated spermatozoa were confronted with several progesterone concentrations (1 pM to 1 mM) in a chemotaxis chamber, studying the sperm kinematic behavior with a video microscopy system and a computer image analysis. Relative fluorescence intensity along the egg-cumulus complex was measured as an indicator of a progesterone gradient by confocal immunocytochemistry. Only capacitated spermatozoa showed a chemotactic response towards low (pM) steroid levels with a maximum at 10 pM and 100 pM, for humans and rabbits, respectively. The percentage of hyperactivated spermatozoa significantly increased only at high hormone concentrations (10 μM to 1 mM). A progesterone gradient was detected along the cumulus mass. The results of this work show that progesterone indeed chemotactically guides mammalian spermatozoa at very low hormone concentrations, and that the cumulus oophorus could be a potential place for sperm chemotaxis mediated by progesterone *in vivo*.

75.
***Minthostachys verticillata* ESSENTIAL OIL AND THEIR MAJOR COMPONENTS: ANALYSIS OF ANTIFUNGAL ACTIVITY**

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The scarce availability of effective and low toxicity antifungal drugs, besides the increase of the fungal infections, particularly in immunoconpromised host generates the necessity to look for new chemical compounds. The medicinal plants are the most important source in new substances with different pharmacological actions. In Cordoba province *Minthostachys verticillata* specie is used by the traditional medicine as an antispasmodic, and different fractions have exhibited "*in vitro*" the antibacterial and antiviral action. The aim of this work was to determine the antifungal activity of the essential oil (EO) of *Minthostachys verticillata* and its major components against *Microsporum canis* and *Trichophyton rubrum*. The microdilution method recommended by the National Committee for Clinical Laboratory Standards was used. The values of Minimal Inhibitory Concentration (MIC) obtained by the studied fungal species were of 1.7 mg/ml, 0.9 mg/ml and 1.3mg/ml of EO, pulegone and limonene respectively. Pulegone was more effective than EO. This component represents 52% of EO suggesting that pulegone could be responsible of the antifungal capacity of the oil sample. It seems reasonable to think that an antagonist effect could be between the major components of EO. Clinical trials might be carried out in order to prove if EO is effective "*in vivo*". Nevertheless, the therapeutic properties of the active principles present in *Minthostachys verticillata*, demonstrated in this study, lead to value the potential the EO of this plant as a phytotherapeutical agent.

76.
SPERM MORPHOMETRY VARIATIONS AND ITS ROLE ON SPERM COMPETITION: THE SNAKES AS A MODEL

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Extensive variation in sperm size, even among close related species, is probably a reflection of the risk of sperm competition and the environment in which it takes place. Although the production of long sperm can be costly, an increment in sperm size may either increase its longevity if size signifies energy reserve. Conversely, this could lead to a trade off with swimming speed by increasing tail length plus its associated mitochondria in the mid-piece. Sperm competition may also select for bigger sperm because they are able to displace smaller competing sperm or serve as an indicator of male genetic quality. Snakes are excellent study subjects to test sperm competition hypotheses as they show reproductive characteristics like promiscuous mating systems, long and short-term sperm storage and unique structural spermatic features. The objective of this work was to detect variations in sperm morphometry across snake taxa and to relate it to differential sperm competition pressures. The length of the head, acrosome, midpiece and principal piece of spermatozoa of three snake taxa: Boinae, Crotalinae and Xenodontinae, was measured using phase contrast and fluorescence light microscopy. Data were controlled for normality and a parametric analysis of variance (ANAVA) was performed for each variable. Pairwise differences among means were tested a posteriori. Although head length did not varied significatively among taxa, the acrosome, principal piece and particularly midpiece length showed significant variation. The high variation degree detected in midpiece length would have an important influence in the metabolic features of these cells, as this region is the responsible for generation and maintenance of motility. The studied snake species also show differences in life histories, reproductive cycles and mating systems. Thus, it would be possible that variations in sperm length, especially in the midpiece and tail regions, were influenced by sperm competition risk derived from such differences.

77.

THE COLINERGIC STIMULATION IN SUPERIOR MESENTERIC GANGLION MODULATES THE RELEASE OF OVARIAN NITRITES IN DIOESTRUS RAT

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Besides the classical endocrine regulation, actually there are several evidences that affirm the existence of a direct neural control the gonads by the Autonomic Nervous System The Superior Mesenteric Ganglion (SMG), reaches the ovary (O) through the ovarian nervous plexus (ONP), enters for the hilium and innervates the sanguineus vassels and the theca cells. Objective of the present work is to analyze if the presence of Acetylcholine (10^{-6}M) in the Mesenteric ganglion cuvette, in an integrated *in vitro* system MG-OVP-O, modifies the discharge of NO in Dioestrus days (D1, D2) in the rats The experiment was carried out as in right and left system. Six adult virgin Holtzman rats for group and buffer Krebs-Ringer pH:7.4 in metabolic bath were used), the liberation of nitrites (soluble metabolite of the ON) in the ovary compartment, was measurement in the incubation liquid by reactive Griess Statistical Student Test, significant $p<0.05$. Results (mean values nmoles\mg ovary $\pm$SEM). Group D1 Rigt a significantly increased while for the whole time tested ($p<0.001$) while no important variations were observed in the D2. In left system only significantly increased its release in D1 in the 30min ($p<0.001$) by in D2 in 15 and 30 min ($p<0,01$) in both time. Conclusion: These results show that the neural stimulation is important in the ON release, which depends on physiologic status making evident that neural function via ONP would be directly involved at ovarian physiology. The cholinergic stimulus puts in evidence a different sensibility from the right ovary to the neural stimulus, at least for the liberation of nitrites in this stage of the estrous cycle.

78.

BEHAVIOR OF THE FRUIT FLY (*Ceratitis capitata* W.) IN RIO CUARTO AND DAMAGE ANALYSIS IN SIX PEACH CULTIVARS OF DIFFERENT RIPENING DATE

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Fruit Fly (*Ceratitis capitata* W.) populational fluctuation was analyzed in Río Cuarto (Córdoba) in the 03/04 season, as well as the damage produced in six peach cultivars of different ripening date: Spring Gold (17/11), Coronet (13/12), Chato Japonés (30/12), Royal Sogarey (6/1), Pull (13/1) and Legrand (14/1). The populational curve of the pest was inferred by means of adults capture in nutritious traps and related with the climate and the phenology of fruit tree. The ripeness in fruits to harvest was characterized with Brix degrees, diameter and weight. To determine the damage,120 fruits coming from 10 plants selected at random in each cultivar were analyzed with the Kruskal Wallis non parametric test. The results show that Fly captures happened from ends of December until ends of March with an abrupt fall in the captures at the end of January, coincident with rains occurrence and a descent of the average temperatures. The first two cultivars were harvested before the beginning of captures and they did not present damage. Of the four remaining, the Chato peach is the one that showed a significantly larger damage in spite of being the first one in being harvested. This could be related to the larger quantity of soluble solids present in the fruit, since this cultivar presents significantly bigger values of ° Brix, (16,6) and also to the heterogeneity in ripening in the same fruit, a characteristic of this cultivar. It was not possible to determine if there is a preference of the plague in relation to the morphological characteristics of the fruits.

79.

EVALUATION OF TECHNIQUE SENSIBILITY FOR THE DETERMINATION OF *Minthostachys verticillata* ESSENTIAL OIL TOXIC CAPACITY

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Of the numerous techniques used to evaluate the toxic capacity of potential herbal drugs, the cellular analysis of morphological alterations (MA) and physiological are useful. The last ones are detected by colorimetric methods. In this study those techniques were developed to evaluate the cytotoxic capacity of essential oil (EO) obtained from *Minthostachys verticillata*, whose antimicrobial capacity was verified *in vitro (Zanon et al., 1999; Demo et al., 2002; Torres et al., 2004)*. The oily sample was obtained by hydrodistillation and characterized by gaseous chromatography, verifying the chemotype of the vegetal. The cytotoxic capacity was evaluated on HEp-2 cells monolayers up to 72 h with different concentrations of EO (0.01-1.2 mg/ml). The MA was determinate using an optical microscope. The normal physiology of the cells at 24, 48 and 72 h post treatment (p.t.) was evaluated by the capacity of reduction of MTT by mitochondrial enzymes according to *Seth et al., 2004*, and the lysosomal incorporation capacity of the vital colorant neutral red (NR) according to *Gong et al., 2004.* In both cases absorbance was read at 560 nm. The MA was the less sensitive technique of the 3 developed to evaluate the citotoxicity of the EO. The results obtained by MTT and NR at 72 h were similar; nevertheless at 24 and 48 p.t. NR was more sensitive than MTT. The 50% cytotoxic concentration (CC50%) at 72 h, necessary for the determination of the Therapeutic Index of *M. verticillata* EO in its future application like a pharmacological natural product was of 0.60 mg/ml.

80.

THERAPEUTIC INDEX OF *M. verticillata* ESSENTIAL OIL AND ITS MAJOR COMPONENTS: RELATION BETWEEN ANTI-HERPETIC CAPACITY AND TOXICITY IN HEp-2 CELLS

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Minthostachys verticillata (peperina) has great application in the Cordoba folkloric medicine. The aerial parts have been used for the treatment of different diseases. Recently, many studies have been focused on the scientific evaluation of its antimicrobial properties, particulary the exhaustive analysis of its essential oil (EO) antiviral capacity. The determination of the Therapeutic Index (TI) of this vegetal sample and its majority components now deserves the attention to infer their potential applicability. It was used *Herpes suis type 1* virus propagated in HEp-2 cells monolayers in the presence of the oily samples at non toxic concentrations. The antiviral action was quantified by reduction of the number of the lysis plaques after incubation by 72 h at 37C. The same cellular support was used for the evaluation of the EO and its major components cytotoxic capacities at different concentrations (0,01-1,60 mg/ml) after 72 h of incubation at 37C. The MTT assay was performed and cytotoxic concentrations 50% (CC50%) was determined by linear regression (*Seth et al., 2004*). The CC50% of each oily sample *vs* antiviral concetrations 50% was related to obtain the TI. The main components of the EO, in decreasing order of toxicity were: pulegone, menthone and limonene, CC50%: 352, 835 and 1155 ug/ml respectively *vs* CC50% of the EO: 613 ug/ml. The antiviral action was only exerted by pulegone and the EO at concentrations 20 and 30 lower than the cytotoxic concentrations. Thus, high therapeutic index of these compounds suggests that they would have great applicability due to their selective toxicity.

81.

Staphylococcus aureus RN6390 INDUCES APOPTOSIS IN MCF7 BREAST ADENOCARCINOMA CELLS

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S. aureus has the ability to invade and persist within eukaryotic cells. It has been shown to be ingested by non-professional phagocytes. Reports have described that *S. aureus* possesses several cell surface adhesion molecules that facilitate its binding to non-professional phagocytes and also showed that intracellular bacteria are capable of inducing apoptosis. The two global gene regulatory loci *agr* and *sar* have been demonstrated to play a role in the induction of apoptosis in epithelial cells by *S. aureus* RN6390 (wild type) since mutants in the *agr* or *sar* loci internalized, but did not induce cell death. The aim of the present study was to analyze the internalization and induction of apoptosis by *S. aureus* RN6390 and its isogenic mutant *agr*$^-$, RN6911, in MCF7 breast adenocarcinoma cells.The protocol for these experiments was based on the methods described by Bayles *et al*. (1998) Infect Immun. 66(1): 336-342. Our results have demonstrated that fibronectin is essential for efficient internalization of *S. aureus* RN6390 (wild type) within these cells. Six hours after internalization, breast adenocarcinoma cells showed morphological changes compatible with apoptosis. In contrast, mutant strain RN6911 (*agr*$^-$) was internalized by the cultured cells at levels similar to those of RN6390 but failed to induce apoptosis. These results are in agreement with those reported for MAC-T cells and indicate that MCF7 cells can also be employed as a model system to study the effects on cellular death of mutants affecting virulence.

82.

PRESENCE OF ASYMMETRIC IgG ANTIBODIES IN SERUM DURING THE PORCINE GESTATION

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During the course of the humoral immune response, there is a population of IgG antibodies of relative high affinity, but which lacks the capacity to form insoluble antigen-antibody complexes, they are called asymmetric IgG antibody. They have been well described in the covering reaction of paternal and fetal aloantigens during the human and murine gestation. The objective of this study was to show the existence of asymmetric IgG in female porcine gestant serum. For this, serums from porcine females of 30 and 75 days of gestation, at term, and castrated males were used. Half of the samples were procesated with buffer solution, and the other half with Concanavalin A, to determinate the existence of symmetric IgG. The presence of total IgG and symmetric IgG were determinate by the differential ELISA technique. The percentage of asymmetric IgG was calculated as follows: % IgG bound to ConA =100– [(non-bound IgG/IgG totales) x 100]. Results: 29,67 % of asymmetric IgG in a 30 days gestation female; 34,38 ± 5,10% of asymmetric IgG in female porcine of 75 days of gestation; 54,1 ± 3,7% of asymmetric IgG in females near their delivery, and 33,32 ± 3,66% of asymmetric IgG in castrated males. Conclusion: there were not found significant differences in the percentages of asymmetric IgG between the castrated males used as witness neither in females of 35 and 75 days of gestation, but it was observed a significant increase of those in serums of porcine females near to their delivery.

83.

EVALUATION OF IMMUNOMODULATOR EFFECTS OF ACICLOVIR IN ALLERGIC CHILDREN WITH INFECTIONS BY HERPES SIMPLEX TYPE 1 (HVS-1) VIRUS

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The atopic dermatitis (AD) is a chronic disease of the skin, like manifestation of an allergic disease that produces morbidity and individual dysfunction. This disease predisposed to sobreadded infections, among them, the produced ones by HVS-1. The aim of the study was to investigate in children with AD and with infection by HVS-1 the immunomodulator effect of Aciclovir (A) on the CMI, (related samples), quantifying IFNγ, its correlation with the immunoglobulins and count of LT before and after the antiviral administration. Thirty children were studied (5-12 years old), 20 of them had AD and were infected with HVS-1 and 10 without symptoms (controls). IFNγ was quantified by ELISA in supernatants of lymphocytes stimulated with PHA, A, Betametasona (B) and different combination of those antigens. There were correlations between the concentrations of IFNγ with those of the immunoglobulins (IgG, IgM and IgA), witch were quantified by IDR. It was made the count of the subpopulation of LT by the method of spontaneous rosettes. Significant differences were demonstrated ($p<0,032$) between the concentrations of IFNγ when it was compared with the mononuclear cells stimulated with PHA+A derivated from children with active viral infections (AVI) *vs* non infection. There was positive correlation between IFNγ and IgG ($r^2=0,59$). The LT count also showed significant differences between children treated with A when they supported the viral infection *vs* control group, ($p<0,0067$). In children with AVI, it could also demonstrate that between 0 and 30 days after administration of A there were significant differences ($p<0,0179$). Aciclovir might be play like as a CMI positive inmunomodulation in allergic children associated to the infection with HVS-1, through an indirect route which alters the viability of the pathogen, stimulating the synthesis of IgG and the recovery of the number of LT.

84.

DIFFERENT MODES OF CELL DEATH INDUCED BY ZnPcOCH3-BASED PHOTODYNAMIC THERAPY IN LARYNGES CARCINOMA HUMAN CELL LINE HEP-2

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Photodynamic therapy (PDT) is a promising new treatment for malignant and non-malignant diseases. The procedure requires exposure of cell to a photosensitizing drug followed by irradiation with visible light of the appropriate wavelength in presence of molecular oxygen. The primary role of PDT is to kill unwanted photodynamic cell by two mechanisms apoptosis and necrosis.
This work describes the photophysical properties, accumulation, localization and the modes of cell death of tetra methoxy Zn II phthalocyanine on Hep-2. The first analysis showed that cell survival (MTT assay) was no affected in light alone. Both incubation time 6h and 18h with different concentrations 0.1μM and 0.5μM did no induce dark toxicity. On the contrary, when Hep-2 cells were incubated with ZnPcOCH3 and post-irradiated with different light dose it was found an efficiency cell photokilling in drug concentration, irradiation time and post-PDT time dependent manner. On the other hand, we observed that the most of sere cells present morphology apoptotic 24 h post-PDT using 0.5μM of ZnPcOCH3 and 5 min irradiation. The immunocytochemistry biotin-avidin–peroxidase (ABC) method confirmed expression of caspase *3*. On the contrary, 15 min irradiation and 24h post-PDT produced necrosis cell (Hoechst-33258, Toluidine blue and DNA fragmentation). Hep-2 cultures incubated with ZnPcOCH3 exhibited a perinuclear fluorescence suggesting that ZnPcOCH3 localizes in lysosome. Due to that i*n vitro* model ZnPcOCH3 showed a high photosensitizing efficiency, we further investigate its behaviour *in vivo* model.

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85.

Trk/p75 RECEPTORS MODULATE CHEMOTACTIC MIGRATION OF NEURAL CREST CELLS

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Neural crest cells (NCC) migrate toward specific sites of the embryo and give rise to many derivatives. Although different molecular factors contribute to cell migration, a chemotactic mechanism could modulate the precise NCC orientation. In this work, using a chemotaxis chamber, videomicroscopy and strict directional criteria, we show the chemotactic response of mesencephalic NCC migrating up to concentration gradients originated in 40 and 80 ng/ml of *Neurotrophin-3* (NT-3). In addition, we also show a repulsive response from gradients originated in 160 ng/ml of NT-3. Inhibition of Trk's receptors and the specific blocking of p75 receptor, let us to conclude that both receptors are necessary for directional responses, attraction and repulsion of NCC. If we consider that the NT-3 is expressed in the embryo at the onset of neurogenesis stage and that its receptors TrkC and p75 are present in NCC in early stages of development, our results show that -apart from the usual "trophic" functions- NT-3 participates in the modulation of oriented migration of NCC towards its target regions, providing evidences about the possible participation of its receptors. These results extend the functional scope of trophic factors by integrating new functions for the orientation of cell motility, providing interesting perspectives to investigate the molecular modulation of directional migration of embryonic cells. This paves the way for future studies about the mechanisms involved in the production of anomalies of embryonic development derivated from a wrong cell position.

86.

DIFFERENCES IN THE ACTIVATION (FOS AND FOSB) IN THE BED NUCLEUS OF THE STRIA TERMINALIS AFTER INJECTION OF KAINIC ACID

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Introduction: The concept of Extended Amygdala propose that the Bed Nucleus of the Stria Terminalis (BNST) have connection with the Amygdala in the temporal lobe. Both participate in the control of emotional and reproductive behavior.
Objective: the objective of the present work was studied the effect of a single injection of Kainic Acid (KA) in the Medial Bed Nucleus of the Stria Terminalis (MBNST) with immunocytochemistry (ICC) for c-Fos and FosB.
Methods: Five adults male rats were injected ip with KA (8 mg/kg). Control rats were injected with saline. 2, 6, 12, 24, 48 and 72 hours after injection rats were perfused by transcardiac infusion of saline followed by 4% paraformaldehyde. The brains were cutting in coronal sections with a freezing microtome and processed for ICC. Sections were mounted and observed with a microscopy. Statistical contrast were made using the Student test and results were considered statistically significant if $p<0.05$.
Results: c-Fos: 2 hours after injections was a large activation of MBNST and was maintained for 24 h. a large decrease was seen to 48 and 72 h. FosB: minimal activation was seen to 2 and 6 h, and a maximal was observed to 48 h after injections.
Discussion: we have demonstrated that the neurons of MBNST were activated for KA. We observed two patterns of activation, an early phase detected for Fos and a late phase detected with FosB.
This suggests that the neurons of MBNST participates in the epileptic mechanisms induced by injections of Kainic Acid.

AUTHOR INDEX