Genetics and Molecular Biology, 23, 4, 907-913 (2000)

Thesis Abstracts

Comparative cytogenetic study of *Pseudis minuta* and *Pseudis* (sp. aff.) *minuta* (Anura, Pseudidae)

(Estudo citogenético comparativo de *Pseudis minuta* e de *P.* (sp. aff.) *minuta* (Anura, Pseudidae))

*Carmem Sílvia Busin**

The family Pseudidae includes the genera *Pseudis* and *Lysapsus*, and is widely distributed in the southern, southeastern and central regions of Brazil, as well as in Uruguay and Argentina. In the present study a cytogenetic analysis was done of two populations of *Pseudis minuta* occurring in São Jerônimo and Eldorado do Sul, Rio Grande do Sul, Brazil and a population of closely related animals, *Pseudis* (sp. aff.) *minuta*, occurring in Tainhas, RS. The latter are larger than *P. minuta* individuals and have a slightly different call. Chromosome preparations obtained from a suspension of intestinal and testis cells were stained with Giemsa solution or processed for C-banding and NOR detection. Specimens from São Jerônimo and Eldorado do Sul had the same karyotype with 2n = 24 chromosomes. The centromeric regions of all the chromosomes contained C-banded heterochromatin. Two interstitial bands were also observed in the short arms of chromosomes 2 and 4. Chromosome pair 7 had a subtelocentric band on the long arm coincident with an active NOR region. Animals from Tainhas population had 2n = 28 chromosomes, with four additional pairs of telocentric chromosomes. Heterochromatic blocks were observed in all centromeric regions. In addition, pair 1 had two distinct interstitial C-bands on the short arms and the telocentric pair 9 had one C-band. In pair 5, the heterochromatic block of the long arm was coincident with the NOR. Combining the telocentric pairs 6 + 7 and 8 + 9 of *P.* (sp. aff). *minuta* yielded a karyotype with 2n = 24 chromosomes, similar to that of *P. minuta*. Organized as such, the similarities included the same chromosome number, a similar chromosome morphology, and similar localization of some interstitial heterochromatic bands and NOR. However, there were differences in the amount of heterochromatin, which was larger in the Tainhas population. These findings suggest a common origin for these two karyotypes and that centric fission and heterochromatin addition have been involved in their differentiation. The cytogenetic results indicate that the population from Tainhas with 2n = 28 chromosomes is an undescribed species.

**2000. Mestrado Interinstitucional UNICAMP, Universidade de Passo Fundo, Passo Fundo, RS, Brasil. Master's thesis. Orienting Professor: Dr. Shirlei Maria Recco-Pimentel.*

Telomeric associations as indicators of chromosomic instability in patients with myeloid leukemias and myelodisplastic syndromes

(Associações teloméricas como indicadores de instabilidade cromossômica em pacientes com leucemias mielóides e síndromes mielodisplásicas)

*Cléber Fontoura Marcolan**

The associations between specific chromosomal abnormalities and different types of hematological neoplasias are well established. However, telomeric associations (tas), which are associations between termini regions of chromosomes (telomeres), are sporadically observed in neoplasias, leading to the question about their role as a biological indicator of occupational exposure to enviromental genotoxic agents. These associations can arise in consequence of shortening telomere length and the inhibition of telomerase activity, reflecting in chromosomal instability. The objective of this work was to determine the karyotype and to verify the occurrence of tas in myelodisplastic syndromes (MDS) and acute or chronic myeloid leukemias (AML and CML). Metaphases of bone marrow cells of 30 patients (10 with MDS, 10 with AML and 10 with CML) were analyzed. The comparison of tas frequency data in these different hematological neoplasias was realized by the Kruskal Wallis test with significance level of 5%. In the MDS patients, the clonal cytogenetic abnormality findings were: monosomy of chromosome 7 in association with monosomy of chromosomes 13 and 20, trisomy of chromosomes 15, 19, 21, and tetrasomy of chromosome 22. New structural rearrangements were observed involving the 2q37, 4q35, 9p11, 12p13, 7p21 and 12q24 regions. Non-clonal chromosomal abnormalities were observed in AML, such as the trisomy of chromosome 14 and monosomy of chromosome 1, 7 and 22, deletion of short arm of chromosome 7 and the presence of Philadelphia (Ph) chromosome. In CML, the results showed the presence of Ph chromosome as the principal clonal abnormality, as expected. An extra Ph chromosome and monosomy of chromosome Y, which are secondary chromosomal abnormalities, were also observed in some patients. Other non-clonal abnormalities such as polyploidy, hypodiploidy, hyperdiploidy, marker chromosome, chromatid break and precoce centromere division were occasionally observed. With regard to tas, our results showed the presence of this phenomenon in all patients with MDS, AML and in eight among ten patients with CML. The frequence of metaphases with tas was higher in MDS, AML showed intermediate frequency and CML, lower

frequency. The comparison of these data showed a significative difference between MDS and CML ($P < 0.01$), whereas no significative levels between AML regard to MDS ($P > 0.05$) and CML ($P > 0.05$) were detected. Considering our results, it is possible to suppose that agrochemicals can be inhibitors of telomerase activity, preferentially attaining the stem cells of bone marrow, leading to the loss of telomeric sequences and to the cytogenetic expression of tas. Thus, tas can be considered an indicator of chromosomal instability, related to environmental exposure to pesticides. However, our data are only indicative, because in the present study, there was a predominancy of individuals from rural zone, not allowing us to compare these data with occupational non-exposed individuals. Additional cytogenetical and molecular studies should be performed to investigate the relationship between telomerase activity and shortening telomere length with tas, in patients with hematological disease, exposed or non-exposed to environmental genotoxic agents such as pesticides.

Publication supported by FAPESP.

*1999. *Departamento de Biologia Celular, Instituto de Biologia da Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brasil. Master's thesis. Orienting Professor: Dr. Christine Hackel.*

Aneuploidy detection by FISH technique in interphase nuclei of bladder lesions

(Detecção de aneuploidias pela técnica FISH em núcleos interfásicos de lesões da bexiga)

*Lucimari Bizari Di Cézar**

The prognosis of bladder cancer has been related to its histological type and grade and to the clinical stage at the time of diagnosis. The predominant histological type of bladder cancer is transitional cell carcinoma (TCC). Some genetic studies have associated the occurrence of chromosome alterations in tumor suppressor genes and oncogenes, such as *CDKN2, ARF, RB, TP53, BCL-2, c-ERBB-2* and the receptor EGF-R, to the process of initiation and progression of bladder tumors. The tumor progression appears to occur jointly with the acquisition of chromosomal abnormalities as deletions of 9p, 9q and 17p and gain of 1q, 5p, 7p, 11q, and 17q. Due to the higher incidence of bladder tumors and the necessity to identify chromosome markers to help early diagnosis and to reduce the risk of recurrence, the present study was carried out. Fluorescence *in situ* hybridization (FISH) was used to investigate numerical chromosome alterations with 7, 9 and 17 centromeric and classical satellite probes in interphase nuclei of fresh tissues. We have analyzed fourteen bladder malignant tumors, two chronic inflammations and three normal urothelium biopsies from patients with TCC. Five bladder descamation cell samples and five lymphocytic specimens, both originated from health people, were used as a control. As the statistical analysis showed a significative difference between both control samples, we have used the values obtained in bladder descamation cells. The cutoff criterium was defined adding four standard deviations to the averages of the bladder cell descamation data.

FISH analysis of the bladder malignant tumors has shown trisomy 7 and/or tetrasomy 7 in 85.7% (12/14) of the samples, monosomy 9 in 28.6% (4/14) and trisomy or tetrasomy 17 in 57.1% (8/14) of the samples. The two samples of cystitis from the patient with TCC recurrence showed trisomy 7 and monosomy 9, as the most important alterations. One of three samples of the normal bladder, also originated from the patient with TCC, showed the same numerical alterations (polysomy 7, 9 and 17) of the tumoral tissue. However, the other two samples showed only trisomy 7. The comparative analysis of low grade (I) with invasive (II until IV) TCC and undifferentiated anaplasic carcinoma showed higher number of aneuploidy in higher grade tumors. These tumors showed increased frequency of the trisomy and tetrasomy 7, 9 and 17, characterizing the polysomy and genetic instability. In TCC grade I and II trisomy 7 associated with aneuploidy 9 was frequently observed, thus suggesting the existence of a relationship between the histopathology of the tumors and the numerical alterations. This study indicates the participation of the chromosomes 7, 9 and 17 in urothelial carcinogenesis: the alterations of the chromosomes 7 and 9 were related to the initiation process and the chromosome 17 with tumoral progression and recurrence. In these chromosomes are mapped the *ERBB, CDKN2* and *c-ERBB-2* genes, respectively, whose products perform important functions in the regulation of cellular cycle. Therefore, genetic evaluation using these chromosomes associated to molecular studies may be indicated for early diagnosis in patient of high risk and the follow-up of that ones with recurrence and metastasis risk.

Research supported by CNPq and CAPES. Publication supported by FAPESP.

*2000. *Departamento de Biologia, Instituto de Biociências Letras e Ciências Exatas, Universidade Estadual Paulista, UNESP, São Paulo, SP, Brasil. Master' thesis. Orienting Professor: Dr. Ana Elizabete Silva.*

Age structure in experimental populations of *Megaselia scalaris* Loew (Diptera, Phoridae)

(Estrutura etária em populações experimentais de *Megaselia scalaris* Loew (Diptera, Phoridae)

*Antonio José Manzato**

The knowledge of the age structure of the population is indispensable to understand the population dynamics in which different age groups present specific mortality and birth rates. Laboratory models have greatly contributed toward the understanding of such mechanisms. In this study, using four geographical strains of *Megaselia scalaris*, 24

experimental populations were founded and kept through serial transference technique in constant temperature room of 25 ± 1.0°C and 20 ± 1.0ºC. In order to determine age structure, the younger flies added to the adults in the population during the census were marked with a different color from those in the previous census, thus identifying their ages. The maintenance of those populations for time periods which varied from 41 to 48 weeks allowed data collection for the study of interrelated characteristics which were relevant to the determination of the population dynamics, such as population size, productivity, mortality, longevity and age structure. With the data obtained, through adjustments of linear models and statistic techniques of analysis of variance, the environmental and genetic effects were detected, respectively associated with temperature and geographic strain variations. As to the population size, independently of strain, a greater number of flies were observed at 25°C than at 20°C, and the strain with greater adaptedness, regardless of temperature, was SR, and the smaller was CP, with R4 and BG showing intermediate values. The effect presented by strains should be attributable to the genetic variability among them. Concerning productivity, it was observed that it was significantly greater at 25°C than at 20°C, with the strain effect in the overall production of flies being determined by the quantity of males produced by the different geographic strains. Birth sex ratio is favorable to males. At 25°C, ratio varied from 1.5 to 1.8 times, and at 20°C, it varied from 1.5 to 2.5 times. It was observed a growing sexual ratio with the temperature change from 25° to 20°C, with strains SR and R4 presenting the greatest variation. Through this experiment for three weekly transferences, it was possible to determine effects in birth sex ratio. Other authors working with the same species, in experiments with 1 and 2 weekly transferences, found a 1:1 sex ratio. To establish age structure, the techniques of serial transference of Buzzatti-Traverso (*Heredity 9*: 153, 1955) and insect marking of Tadei and Mourão (*Cien. Cult. 28*: 550, 1976) were relevant. From the data obtained, the genetic and environmental effects in the determination of average longevity of males and females in the populations could be shown. In the adult portion, due to the high mortality rate of males, an inversion in the values related to the birth period was observed. Concerning the average quantities of females to males, at 25°C, the variation in the four strains was from 8.5 to 17.4 times greater, and, at 20°C, the same measure varied from 1.4 to 2.7. The environmental effect due to temperature variation agreed with the studies from many authors, showing that longevity increases when the temperature is lowered. The environmental effect associated with temperature was significant in the longevity of males: at 25°C, the great majority did not survive more than three days, and at 20°C, lifetime extended to 10 days. For females, at 25°C, 98% did not survive more than 14 days, whereas at 20°C, the same survival rate occurred until the age of 21 days. The establishment of age structure allows the description of the population age profile, with interest in verifying whether or not it went through alterations, due to environmental and genetic variations. The comparison of average profiles was obtained through the females, making use of the bootstrap simulation process, and through the sample process with replacement, from the data generated by the three original experimental replicas, it was possible to enhance age profiles and to compare them using statistic techniques of multivaried analysis of variance. The effects of strong and weak selective pressures have showed that young reproduction takes its costs. With data obtained from planned experiments under strong and weak selective pressure conditions, it was possible to verify that male longevity was more affected by sexual activity than by density, whereas female longevity was more affected by density than by sexual activity. In *M. scalaris* populations, the estimations for female average longevity was obtained through the Levene theoretical model with two weekly transferences. Through the extension of this model to three weekly transferences, estimations for male and female average longevity were obtained, and, through comparison with actual average longevity ($\bar{\chi}$) of the flies, the Levene model was validated. The attempt to measure the effective size was accomplished, and during the bottle discarding the female percentual for each age group which produced progeny was counted up. Temperature as an environmental effect was significant: at 25°C, the variation was from 37% to 51%, and at 20°C, the variation was from 17% to 18% of fertilized flies. These results seem to indicate that the survival strategy of the species would be in accordance with the proposition that the sexual activity effects on males present high reproductive costs due to young reproduction. It was also observed that older females are reproductive, and that the force of natural selection acts powerfully during the young stage, which would be in accordance with Charlesworth (*Genetica 91*: 11-19, 1993) proposition that this force could be expressed as a fraction of the average life expectation, and this fraction is maximum until the age of the first reproduction, after which it would irreversibly decline until zero.

Publication supported by FAPESP.

**1999. Pós-Graduação em Genética, Instituto de Biociências, Letras e Ciências Exatas, IBILCE-UNESP, São José do Rio Preto, SP, Brasil. Ph.D. thesis. Orienting Professor: Dr. Wlademir João Tadei.*

Molecular phylogeny and evolution of Cracidae (Aves)

(Filogênese molecular e evolução de Cracidae (Aves))

*Sérgio Luiz Pereira**

The family Cracidae is one of the most threatened bird groups in the Americas, and its phylogeny at the generic level has not been established. Morever, other taxonomic

problems are related to species and subespecies definition. To answer some of these taxonomic questions we estimated the phylogenetic relationships of the eleven Cracidae genera (*Aburria, Chamaepetes, Crax, Mitu, Nothocrax, Oreophasis, Ortalis, Pauxi, Penelope, Penelopina,* and *Pipile*) based on phylogenetic analysis of 4,519 base pairs of six mitochondrial genes (12S and 16S rDNA, COI, COII, COIII and cyt *b*). Our results showed the separation of the two Cracidae subfamilies (Cracinae and Penelopinae). The relationships among the Cracinae were (((*Crax, Nothocrax*), *Pauxi*), *Mitu*) and among the Penelopinae were ((((((*Aburria, Pipile*), *Penelope*), *Chamaepetes*), *Penelopina*), *Ortalis*), *Oreophasis*). Our sequences seem to have evolved at a constant rate; thus we estimated the time of Cracidae origin as around 75.5 million years ago and genus diversification occurred in the last 33 million years. We also related Earth history events to cracid diversification. We could also establish that the family Megapodiidae, found in the Australian region nowadays, is the sister clade to Cracidae. The exclusion of the Cracidae and Megapodiidae from the Galliformes, as suggested by other molecular studies, was not supported by our sequence analysis. We also studied the first domain of the mitochondrial control region (CR-I) to verify its utility in genus phylogenetic inference. The CR-I is widely used to establish relationships of lower taxonomic levels such as species and populations, but rarely above the species level. Our results showed that the Cracidae CR-I sequences evolved at similar rates as cyt *b* and COI sequences, considered to have lower evolutionary rate than the CR-I. Cracid genus phylogeny obtained through analysis of CR-I was similar to that obtained by the analyses of other mitochondrial genes cited above, when a more realistic model of DNA evolution was used in tree reconstruction. We sequenced the complete Cracidae COII, a highly conserved gene among metazoans due to its function in the respiratory chain. Our results show that cracid COII is similar to other metazoan COII in relation to base composition, codon usage and patterns of nucleotide and amino acid substitution. Some conserved amino acid residues among the metazoan were also found in Cracidae but some residues considered being conserved in animals, presented amino acid substitutions in Cracidae COII, leading us to suggest that different taxa could choose different amino acid residues to function as a cleft for cytochrome c-binding and copper-ligand residues.

Publication supported by FAPESP.

**2000. Departamento de Biologia, Instituto de Biociências, USP, São Paulo, SP, Brasil. Doctoral thesis. Orienting Professor: Dr. Anita Wajntal.*

Chromosome instability in farm workers induced by pesticides in the region of Passo Fundo, RS

(Instabilidade cromossômica induzida por agroquímicos em trabalhadores rurais da região de Passo Fundo, RS)

*Adil de Oliveira Pacheco**

Much of grain cultivation (wheat and soybean) in Rio Grande do Sul State is carried out in the region of Passo Fundo. For crop pest control, great amounts of agrochemicals (fungicides, insecticides, herbicides) are used. To evaluate the genotoxicity of these products, the micronucleus test was applied in the study of rural workers directly exposed to these chemicals. The micronuclei are small nuclei resulting from the loss of whole chromosomes or acentric fragments during cell division, that can be identified in binucleated cells. Heparinized blood samples were drawn by venepuncture from 30 exposed workers and 30 non-exposed controls. To perform micronucleus analysis on lymphocyte cultures, Cytochalasin B was added to block cytokinesis after 44-h incubation at 37°C, resulting in the formation of multinucleated cells. Data about smoking and drinking habits, sex, age and exposure duration were also identified. Micronucleus frequency was evaluated by counting 1000 binucleated cells in both groups. Statistical analysis showed significantly higher mean number of binucleated cells with micronuclei in exposed individuals (14.4/1000) than in controls (7.2/1000). Other factors related to chromosome instability, such as smoking habits, age and exposure duration, showed no effect on the frequency of micronuclei in both groups.

Finally, we can conclude that the micronucleus test is an efficient biological assay for monitoring population exposure to mixtures of agrochemicals.

Publication supported by FAPESP.

**2000. Departamento de Biologia Celular, Instituto de Biologia, UNICAMP, Campinas, SP, Brasil. Master´s thesis. Orienting Professor: Dr. Christine Hackel.*

Genetic diversity and population structure of "cagaiteira" (*Eugenia dysenterica* DC.) in southeastern Goiás State, Brazil

(Diversidade genética e estrutura populacional de "cagaiteira" (*Eugenia dysenterica* DC.) na região sudeste de Goiás, Brasil)

*Mariana Pires de Campos Telles**

The studies about the organization of the genetic variability and population structure in plant natural populations permitted great advances in the knowledge about microevolutionary processes. More recently, efforts to understand

these patterns in natural populations of tropical species have also been used to support conservation and management programs. Among the "Cerrado" fruit tree species that possess potential economic importance in agriculture, the "Cagaiteira" (*Eugenia dysenterica* DC.), of the family Myrtaceae, deserves a special position. To obtain information about allelic dynamics in some populations of this species, genetic parameters were estimated in 10 local populations, with samples from 112 families. Six isozymes were analyzed (SKDH, 6-PGD, α-EST, MDH, PGI and PGM), in a total of 9 loci. Around 83% of the loci were polymorphic, with 3 alleles per loci, on average, and the frequency of heterozygous was lower than expected under Hardy-Weinberg equilibrium. The fixation index was 0.337 and the outcrossing rate was 0.835, suggesting a mixed mating system for the species, which seems to be preferably alogamous. The population structure was initially analyzed using genetic diversity and analysis of variance techniques, and they showed a significant and high degree of population differentiation ($\theta_P = 0.154$ and $G_{ST} = 0.164$), by comparing these values with those from other tropical species. Genetic divergence, analyzed by Nei's genetic distances (clustered with UPGMA and ordinated by non-metric multidimensional scaling) and by a maximum likelihood approach of neutral evolution (assuming divergence of allelic frequencies under Brownian motion), showed spatial patterns of clusters of local populations. Explicit spatial analyses, using autocorrelation and matrix comparison techniques (Mantel tests), confirmed these patterns and revealed a clinal pattern for most allele frequencies. Multiple total and partial matrix correlation analyses, using data from genetics, geography, soil components and phenotypic variation (defined with variables from trees, growing patterns of young plants and fruits), also confirmed the importance of spatial distribution to genetic differentiation. In general, all analyses showed that a stochastic model, such as isolation-by-distance or stepping stone, could be used to explain population differentiation. In these models, the local genetic drift is counteracted by gene flow at low geographic distances, forming clusters of relative genetic homogeneity, and allele dynamics among these clusters tend to be evolutionarily independent by increasing geographic distance. These results can also be important in future conservation, domestication, management and breeding programs for this species.

Research supported by CNPq and FUNAPE.

**2000. Escola de Agronomia, Universidade Federal de Goiás, Goiânia, GO, Brasil. Master's thesis. Orienting Professor: Dr. Lázaro José Chaves.*

Study of glutenins and gliadins in *Triticum turgidum* L. and their relationship with gluten strength

(Estudo de gluteninas e gliadinas em *Triticum turgidum* L. e sua relação com a força do glúten)

*Marcondes Maurício de Albuquerque**

The composition of prolamins was analyzed by electroforesis in gel acid A-PAGE and in two step-one dimension SDS-PAGE, of the descendants F_2 of four crosses among the varieties of durum wheat **Blatfort, Ferox, Lakota, Peñafiel and Safari** in order to study the segregation and cromosomal localization of reserve endosperm proteins. Likewise, the influence of the prolamins on the semolina quality has been studied in the grains F_3 of seven crosses among the same varieties and measured through the content in protein, degree of vitrification, gluten strength, and their correlation. The results showed that the glutenins of lower molecular weight LMW-1, LMW-3 and LMW-4 were allelic variants of the locus Glu-B3, while LMW-2 and LMW-5 were allelic variants of the locus Glu-A3. The gliadins ω-26, ω-33-35-38 and γ-42 formed a block that was allelic to the block ω-35, γ-45 and ω-39, γ-null, all belonging to the locus Gli-B1. The gliadins ω-27 and γ-47 were located in the locus Gli-A1. The gliadins β-56 and β-65 (Allelics), β-61 and β-62 (allelics) and α-73 belonged to the group of homeology 6 (locus Gli-2). Between the loci Gli-A1 and Glu-A3 a percentage of recombination of 1.7% was estimated. The glutenin of lower molecular weight LMW-1 was the main source of the gluten strength, while the allelic glutenins LMW-3 and LMW-4 produced weak gluten.The glutenins of high molecular weight HMW-6+8 had a positive influence in the strength of the gluten but lower than the LMW-1. By contrast the glutenin HMW-20 had a negative influence. The glutenin LMW-2 and gliadins seemed not to have significant effect on the gluten strength and the glutenin LMW-5 influenced positively in the origin of the best glutenin LMW-1. Significant relations among prolamin content of protein and the vitreous degree of the grains were not detected. There was no significant correlation among these three quality components in homogeneous parcels or grains with high vitreous degree. Although the production of nonvitreous grains reduced the protein content and gluten strength, it was an undesirable character in relation to the semolina quality.

Research and publication supported by EMBRAPA.

**1993. Departamento de Bioquímica y Biología Molecular, Genética, Microbiología y Fitopatología, Universidad Politécnica de Madrid, Madrid, España. Doctoral thesis. Orienting Professor: Dr. José Francisco Vázquez Muñiz.*

Phenotypic and genotypic characterization of bacteriocin-producer strains of *Staphylococcus aureus* associated with bovine mastitis

(Caracterização fenotípica e genotípica de estirpes de *Staphylococcus aureus* produtoras de substâncias antimicrobianas e associadas à mastite bovina)

*Janaína dos Santos Nascimento**

The production of bacteriocins (Bac) by strains of *Staphylococcus aureus* has been described and studied in our laboratory because of their potential use either in food industries as biopreservatives or in the prevention of some infectious diseases. Bacteriocins are proteinaceous antimicrobial substances (AMS) produced by some strains of bacteria with antimicrobial activity against other bacteria. The bacteriocin production is generally associated to plasmids, which also code for immunity. In the present study, 50 strains of *Staphylococcus* isolated from bovine mastitis cases in several herds from different Argentine provinces were screened for antimicrobial substance production. Twelve strains (24%) exhibited a high inhibitory activity against the indicator strain (*Corynebacterium fimi*) and were chosen for a more detailed characterization. These strains were identified as *S. aureus* by the amplification of the *femA* gene. They were tested for sensitivity to phages and antimicrobial agents (showing different patterns), for spectrum of action against other Gram-positive bacteria and plasmid profiles. The strains were also analyzed for the presence of genes similar to those found in the bacteriocinogenic plasmid pRJ6, detected in an *S. aureus* strain isolated from food. pRJ6 encodes a bacteriocin named aureocin A70. The spectrum of action of the 12 AMS^+ strains was narrow. Few tested bacteria were inhibited. Some AMS^+ strains were able to inhibit *Listeria monocytogenes*, an important food-borne pathogen. The plasmid profile of the 12 AMS^+ strains revealed the presence of at least one plasmid DNA. Eleven strains carried a plasmid with a size similar to that of pRJ6 (8.0 kb). DNA/DNA hybridization experiments, using the pRJ6 *Hin*dIII-A fragment as probe, have shown that the 12 strains presented a plasmid with homology to pRJ6. PCR experiments, using specific primers for amplification of the *bac* operon from pRJ6, showed that all strains, including the host strain of pRJ6, had a 525-bp fragment amplified. These results indicated that the genomic DNA from these strains carries similar sequences to those found in the *bac* operon from pRJ6, suggesting that the antimicrobial substances produced may be related to aureocin A70. The antimicrobial substances produced by strains 22 and 39 were semi-purified by ammonium sulfate precipitation, exhibiting a spectrum of inhibitory activity larger than that previously observed. The biochemical properties exhibited by the antimicrobial substances produced by strains 22 and 39 were investigated and compared with the properties exhibited by aureocin A70. As these antimicrobial substances were inactivated by proteolytic enzymes, they might be bacteriocins. These bacteriocins seem to possess a bactericidal activity, but not bacteriolytic, according to the results using *Listeria innocua* as an indicator strain. The dendrogram constructed from the analysis of the results obtained in the AP-PCR, RAPD and rep-PCR experiments showed that 8 out of the 12 AMS^+ strains have 100% of similarity, indicating that these strains may belong to the same clone. PFGE experiments showed that 8 out of the 12 AMS^+ strains isolated from bovine mastitis cases belong to the same clone; the remaining strains are either closely related or possibly related to the prevalent clone.

**2000. Departamento de Microbiologia Geral, Instituto de Microbiologia Professor Paulo de Góes, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brasil. Master's thesis. Orienting Professor: Dr. Maria do Carmo de Freire Bastos. Coorienting Professor: Dr. Selma Soares de Oliveira.*

Genetic and molecular characterization of the regulatory genes *toxR* and *toxS* and identification of other loci from the variant Amazonia of *Vibrio cholerae* in comparison with the genome of *Vibrio cholerae* biotype El Tor

(Caracterização genético-molecular dos genes regulatórios *toxR* e *toxS* e identificação de outros loci do variante Amazônia de *Vibrio cholerae* em comparação com o genoma de *Vibrio cholerae* biotipo El Tor)

*Marcelo André de Souza Baptista**

The Amazonia variant of *V. cholerae* O1 consists of clinical enteropathogenic strains isolated in 1991-92 from the far northwest region of the Brazilian Amazon. Molecular analysis has shown that Amazonia strains form a distinct group from that of the epidemic El Tor strain prevalent in the same area and time period. Amazonia strains present unusual phenotypic traits such as the production of a hemolysin/cytotoxin and the absence of cholera-producing virulence-associated factors, including the cholera toxin CT and the colonization pilus TCP. In the present work, the main regulatory genes required for the production of virulence factors in epidemic strains were studied in the Amazonia variant. It was shown that the *toxR* and *toxS* regulatory genes are present in the Amazonia strains. On the other hand, the *toxT* gene was not detected, denoting that part of the pathogenic island VPI is missing in the Amazonia variant. Restriction maps comparing the *toxRS* region from the Amazonia strain and the El Tor biotype indicate a significant genome divergence between them. The *toxR* and *toxS* genes were characterized. Two plasmids containing part of the *toxR* gene were constructed and analyzed. One of these is a suicide plasmid in *V. cholerae*. A total of 2113 nts from the *toxRS* region were sequenced. The Amazonia *toxRS* sequence was very similar to the classical and El Tor biotypes

(97% identity), presenting the same genomic organization. The conserved genomic organization included the *toxS* downstream region from which two undescribed ORFs were mapped. One of them, orf3 (369 bp), partially overlaps *toxS*. The sequence conservation and the genomic localization in relation to the *toxRS* operon support the hypothesis that orf3 may represent a third element in this operon. The Amazonia strains displayed three different profiles concerning the major outer membrane proteins OmpU and Omp35. *toxR* insertion mutants construction and analysis combined to complementation assays revealed that ToxR plays a role in the transcriptional activation of the OmpU homolog in the Amazonia variant, as it does in epidemic strains. The Omp35 protein is probably under negative regulation by ToxR. The partial sequence of a 228-nts PCR product led to the identification of a protease ORF with homology to the lon proteases class (*lonh*). It was described for the first time for *V. cholerae*. Searches in the *V. cholerae* El Tor N16961 genome database (TIGR) identified two *lonh* homologous regions (*lonh1* and *lonh2*), each one mapping to a different chromosome. The *lonh* homologous region from the larger chromosome (*lonh2*) also showed a conserved organization in the adjacent loci in a comparison with *E. coli* and *H. influenzae* genomes, suggesting that the larger chromosome of *V. cholerae* seems to have the most conserved regions related to other one-megareplicon genomes and possibly carries the most basic functions for the bacterial physiology.

Research supported by CAPES, FUJB, FAPERJ and PRONEX.

**2000. Mestrado em Genética, Centro de Ciências da Saúde, Instituto de Biologia, Departamento de Genética, Universidade Federal do Rio de Janeiro (UFRJ), RJ, Brasil. Master's thesis. Orienting Professor: Dr. Ana Coelho.*

In vitro morphogenesis and *Agrobacterium tumefaciens*-mediated transformation of eggplant (*Solanum melongena* L. cv. Embu)

(Morfogênese *in vitro* e transformação genética de berinjela (*Solanum melongena* L. cv. Embu) mediada por *Agrobacterium tumefaciens*)

*Edgard Augusto de Toledo Picoli**

Eggplant explant responses were evaluated in the present work. Cotyledon and hypocotyl from 15-day *in vitro* grown plantlets were used as explants for somatic embryogenesis and organogenesis, respectively. It was verified that MS-based medium supplemented with 0.1 mg/l IAA provided higher shoot regeneration frequencies and that 2.5 to 10 mg/l NAA had similar effects over somatic embryogenesis. An average of 0.57 shoots and 80 embryos per explant were observed for organogenesis and embryogenesis, respectively. Regarding to antibiotics it was observed that Timentin added to the medium led to better regeneration responses, either shoots or embryos, compared to cefotaxime. On the other hand, cefotaxime supplementation in combination with NAA increased callus weight. Also, a significant decrease on total embryo number and shoots were observed with the later. Kanamycin (50 mg/l) added to selective medium completely inhibited morphogenesis in hypocotyl and cotyledon explants, in spite of the high frequency of escapes in transformation experiments. Organogenic responses were suppressed by hygromicin at 7.5 mg/l, whereas some globular embryos were observed in the presence of 10 mg/l of this antibiotic. But, as the time progressed, the eventually differentiated embryos oxidized without any further development. Aiming the optimization of the transformation protocol, other factors which affect transformation efficiency were examined, including the vector harboring *nptII* or *hpt* gene and co-cultivation temperature. In transformation experiments organogenesis occurred only when kanamycin was the scorable marker, whereas somatic embryos regenerated on both kanamycin and hygromicin-supplemented medium. Although the number of explants harboring embryos was statistically nonsignificant, hygromicin was more efficient than kanamycin as shown by the higher frequencies of transformed plants among the regenerated ones. The agrobacterium strain C58C1 pRGG harboring *hpt* gene led to larger number of embryos as compared to C58C1 pRGG neo5. Shoots regenerated only when explants were transformed with the latter. Co-cultivation temperatures ranging from 22° to 28°C did not affect the number of explants with embryos, although a higher number of regenerants were obtained at 24°C. The *Sw-5* gene transfer to eggplant 'Embu' was successfully accomplished. Segregation analysis of the transgene and resistance evaluation by means of tospovirus inoculation and DAS-ELISA were carried out with the R1 generation. PCR and inoculation data indicated that two or more copies were inserted in each transformation event. The plants that possessed at least one copy of the transgene presented hypersensitivity reaction, suggesting that the *Sw-5* gene was functionally active in eggplant genome. Different types of lesions were observed in the inoculated transformed plants, this probably may be related with the number of transgene copies.

**1999. Departamento de Biologia Vegetal, Curso de Genética e Melhoramento, Universidade Federal de Viçosa, Viçosa, MG, Brasil. Master's thesis. Orienting Professor: Dr. Wagner Campos Otoni.*