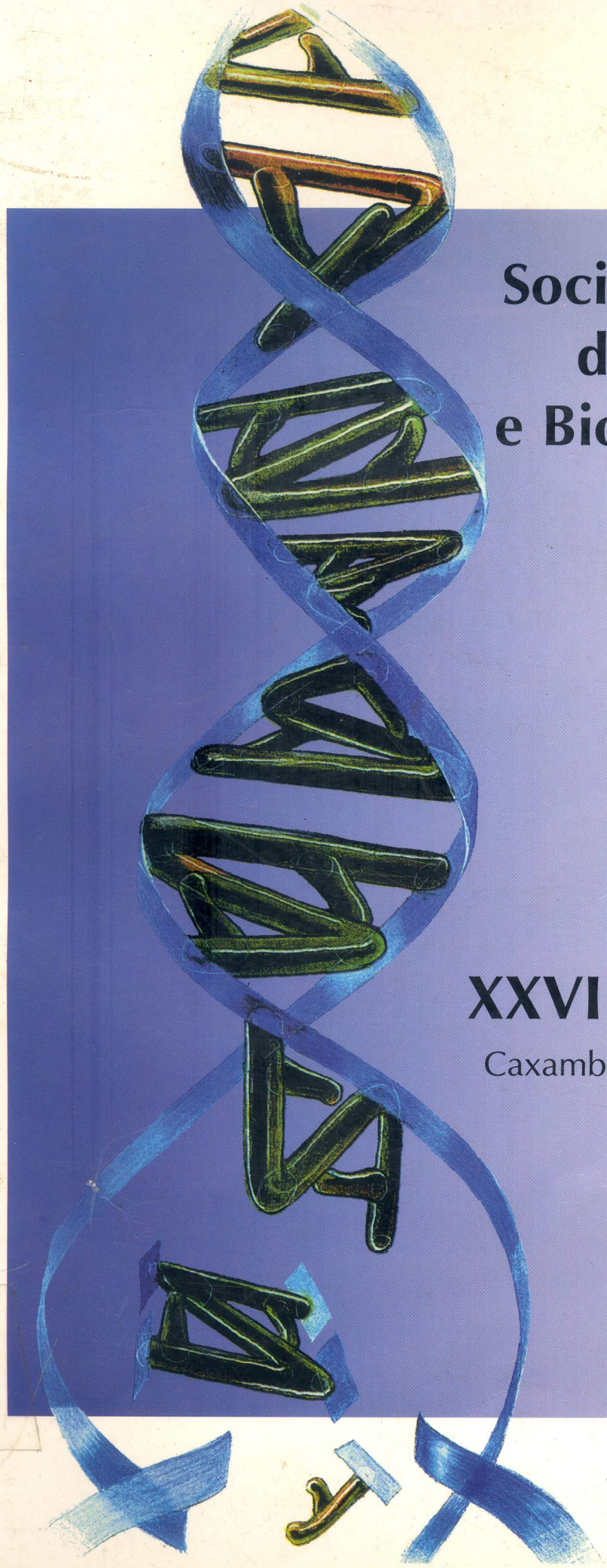

Sociedade Brasileira de Bioquímica e Biologia Molecular SBBq

XXVII^{a} Reunião Anual

Caxambu - 23 a 26 de maio de 1998

Sociedade Brasileira de Bioquímica e Biologia Molecular - SBBq

Programa e Resumos da XXVIIª Reunião Anual

Hotel Glória
Caxambu, MG
23 a 26 de maio
1998

Colegiado Diretor da SBBq

Diretoria

Presidente: Profa. Dra. Helena Maria Scofano
Vice-Presidente: Prof. Dr. Paulo Antonio de Souza Mourão
Secretário: Prof. Dr. Walter Ribeiro Terra
Tesoureira: Profa. Dra. Iolanda Midea Cuccovia
Presidente Eleito: Prof. Dr. Hector Francisco Terenzi

Membros do Conselho

Prof. Dr. Anibal Vercesi
Prof. Dr. Antonio C.M. Paiva
Profa. Dra. Bianca Zingales
Profa. Dra. Glaci Zancan
Prof. Dr. Jorge A. Guimarães
Prof. Dr. Paulo Sergio L. Beirão
Prof. Dr. Sergio T. Ferreira
Profa. Dra. Suely L. Gomes

Apoio à Secretaria

Profa. Dra. Lélia Mennucci

Representantes Regionais

Prof. Dr. Hatisaburo Masuda	Rio de Janeiro
Profa. Dra. Celia Carlini	Rio Grande do Sul
Profa. Dra. Vera Lucia Menezes de Lima	Pernambuco
Prof. Dr. Marcelo Matos Santoro	Minas Gerais
Prof. Dr. Marcelo Hermes-Lima	Brasília

Secretaria Administrativa

Cynthia Sayuri Bando
Viviane Ferreira Campos Bando
Marino Scheid

G - 79

EFFECTS OF POLYOLS ON OXIDATION OF LIGNIN WITH PHENOLASE TO IMPROVE ITS CHELATING CAPACITY

M.A. Soto and A.R. Gonçalves
Departamento de Biotecnologia - FAENQUIL - CP 116,12600-000, Lorena- SP

Chelating agents have been widely used as sequestrating agents for immobilization and separation of metals from wastewater or from sludges of various origins. Lignin is a compound with high chelating capacity due to the presence of groups with high electronic density. The chelating capacity is improved by oxidation and the objective of our work was to study the effects of polyols on the stability of phenolase during the oxidation of lignin obtained from Acetosolv pulping of sugarcane bagasse. The oxidation was performed using O_2 in a homogeneous phase and glycerol and polyethylene glycol (PEG) were the polyols. The chelating properties of original and oxidized lignins were compared by monitoring the amount of Cu^{2+} bound to lignin by gel permeation chromatography. Original Acetosolv lignin showed a chelating capacity of 354 mg Cu^{2+} / g lignin. On the other hand, the used of phenolase, O_2 and polyols improved the stability of enzyme, increasing the chelating properties of the oxidized lignins. Lignins oxidized with phenolase, O_2 and PEG showed an increase in chelating properties of 17 % in relation to the original lignin, and replacing PEG with glycerol the oxidized lignin showed an increase of 73 % in its chelating properties in relation to the original lignin. The average molecular weight (Mw) measured by gel permeation chromatography was smaller for the oxidized lignins than for the original Acetosolv lignin. The chelating properties of the oxidized lignins increase with the decreasing of Mw of the polyol used.

Supported by FAPESP

G - 80

RHIZOBIAL DIVERSITY IN AMAZON SOILS BY PCR-RFLP ANALYSIS OF 16S rRNA

Flávia V. Silva[1], Norma G. Rumjanek[1] and José Pereira[2]
[1] EMBRAPA-CNPAB, Seropédica, CP 74.505, CEP 23851-970, RJ, Brasil, [2]EMBRAPA - CPAA, Am

Rhizobia are soil bacteria which possess a nitrogenase complex capable to fix atmospheric nitrogen. These bacteria interact with leguminous plants forming root nodules.

This work aims to evaluate rhizobia richness in six different areas of Manaus: a 5 year old natural reclamation area, a 5 year old reclamation area enriched with 4 native species, a cultivation plot with 4 associate crops, a cultivation plot with just 1 crop (monoculture), a neighboring area covered by the native forest and another in a process of natural reclamation for 17 years.

Rhizobia were isolated from cowpea nodules cultivated in soil samples from the areas described above. The nodules were sterilized and isolated on yeast/manitol medium and the individual colonies were characterized according to growth rate, size, form, elevation, optics, surface, consistency of mucous and pH of media. According to these morphological characteristics, the isolates were arranged in 13 different groups.

Eleven unclassified isolates representing the different groups morphologically characterized were examined by restriction fragment lenght polymorphism (RFLP) analysis of 16S rRNA genes amplified by polymerase chain reaction (PCR).

Nine genotypes were obtained from the data of the RFLP analysis with nine endonucleases. The slow growing strains seems to be very similar among them while fast growing rhizobia showed a higher level of polymorphism profile of the 16S region of rRNA. The isolates showed a distinct pattern when compared to the restriction analysis of ten type strains also included in the experiment, suggesting that they may belong to a new group of rhizobia.

Acknowledge: CAPES and EMBRAPA/Agrobiologia.

G - 81

Endospermic gums from *Delonix regia, Parksonia aculeata* e *Schizolobium parahybum* as affinity matrices for isolation of plant lectins.

Matos[1],V.C.; Rosa[2], I.G.; Tavares[3], R.O.; Teixeira[3], D.M.A.; Braga[2] R.C., M.V.L.Milhome[2]; & R.A. Moreira[3].
[1]Centro de Ciências da Saúde, Universidade de Fortaleza, [2]Dept de Patologia, Universidade Federal do Maranhão, [3]Dept. de Biologia, [4]Dept. Bioquimica, Universidade Federal do Ceará.

Many proteins have the ability to reversibly bind specific molecules. This property can be used to purify such proteins by affinity chromatography. The ligand used in such chromatography for the isolation of a particular protein must have a high affinity for the protein. After binding the protein, the column is washed free of impurities, and the protein released by interacting to a soluble ligand. Carbohydrates constitute an important component of plants, appearing under different forms and playing different functions. A group of natural polysaccharides are the gums made, mainly, by complex heteropolysaccharides. These include gums form seed endospermic, plant exsudates and algae.

Lectins are proteins widely distributed in the plant and animal kingdom, that have the distinctive property of binding to carbohydrates of specific structure and configuration. The use of seed endospermic gums from *Delonix regia, Parkinsonia aculeata* and *Schizolobium parahybum* as matrices for affinity chromatography of lectins were investigated. These seed endosperms give high yields of galactomannans, whose Man/Gal ratio were 2:1; 3,34:1 and 3,0:1, respectively. Their structures consist of polymeric main chains of (1-4)-linked β-D-mannopyranosyl residues substituted at O6 by single-unit chains of α-D-galactopyranose.

When lectins from *Artocarpus integrifolia, Artocarpus incisa* and *Abrus precatorius*, all D-galactose binders, were applied to the columns, two fractions were obtained, the first one, eluted with the equilibration solution, showing no hemagglutinating activity, and the second, eluted with 0.1 M D-galactose, containing all the hemagglutinating activity. When, on the other hand, D-glucose-binding lectins (*Dioclea altissima, D. violacea, C. ensiformis, C. brasiliensis* and *Cratylia floribunda*) were applied to the columns, no retained material was found. These results shows the efficiency of the columns to isolate D-galactose-specific lectins.

CNPq, CAPES, FUNCAP

G - 82

PARTITIONING AND PURIFICATION OF ASCORBIC OXIDOREDUCTASE IN AQUEOUS TWO-PHASE SYSTEMS

A. L. F. Porto[1,2]; R. A. F. Dutra[1,3]; H. J. F, Melo[1]; K. A. Moreira[1]; E. B. Tambourgi[4] and J. L. Lima Filho[5].
1. Laboratório de Imunopatologia Keizo Asami – LIKA; 2. Departamento de Morfologia e Fisiologia Animal – UFRPE; 3. Departamento de Patologia ESEF/UPE; 4. FEQ/ DESQ – UNICAMP; 5. Departamento de Bioquímica – UFPE.

Liquid – liquid partitioning of proteins in aqueous two phase systems in a efficient and versatile method for the separation and purification of proteins. These systems are usually composed of aqueous solutions of polyethylene glycol (PEG) and salt solutions, commonly phosphate. This work describes the partitioning and purification of ascorbic oxidoreductase, enzyme extracted from *Cucurtiba maxima* in polyethylene glicol – salt two-phase systems. The study of partitioning behaviour at differents tie line was carried varying the weight mass PEG (550, 1000, 3350 and 8000) at pH = 5,0. Partition coefficient, k, was defined as the ratio between ascorbic oxidoreductase activity in the top and bottom phase, respectively. Aqueous two – phase system (total mass 6g) was prepared according to Sarmento, *et al.* , 1994. Total ascorbic oxidoreductase activity was assayed at 25°C in the both phases as described by Carvalho, *et al.* , 1981, used ascorbic acid 150 µM in citrate-phosphate 0,1M pH = 6,0 as substrate. The amount of total protein in both phases was determined by Bradford (1976). Results have shown the ascorbic oxidoreductase partition preferencially into high density salt phase. The best results of ascorbic oxidoreductase activity recovery (about 312%) were obtained with PEG 1000 in the concentration 19,7 w/w and 17,75 of phosphate salt, wich purification factor of the 22.

Supported by: JICA, FINEP, CNPq, FACEPE

G - 83

LIQUID-LIQUID EXTRACTION OF CITRININ FROM FERMENTATION BROTH USING AQUEOUS TWO-PHASES SYSTEMS

Araújo, A.I.C. [1]: Carrazzoni-Pereira. A.R. [1]: Porto. A.L.F. [1,2]: Pimentel. M.C.B. [1]: Tambourgi. E.B. [3]. Lima Filho. J.L. [2]: Melo. E.H.M. [1]

1. Dept. Bioquimica (UFPE). Lab. de Imunopatologia Keizo Asami (LIKA): 2. Departamento de Morfologia e Fisiologia Animal (UFRPE): 3. FEQ-DESQ-UNICAMP- Campinas-SP

Liquid-liquid extraction is a well known and extensively used unit operation in chemical and pharmaceutical industry, being particularly useful when dealing with labile substances. The extraction of citrinin from fermentation broth was investigated used polycthylene glycol (PEG) and potassium phosphate salt system. The mycotoxin, citrinin, known as an antibiotic, is a secundary metabolite of several fungal species belonging to the genera *Penicillium* and *Aspergillus* (Melouk and Akem. 1987). Citrinin was produced from *Penicillium citrinum* in fermentation medium described by Pimentel et al., 1996, during 120 hours in orbital shaking (100 rpm) at 28°C. The study of citrinin partitioning behavior at differents tie lines was carried out varying the weight mass PEG (550, 3350, 4000 and 8000) at pH 5.7. Aqueous two-phases system of total mass 6g was prepared according to Sarmento et al., 1994. Citrinin was identified by its yellow fluorescence at 360nm in both phases (light and heavy) and by TLC spot detected with UV light. The results showed recovery of the citrinin in the PEG-rich phase, with high recovery yields (up to 95%) using aqueous two-phases systems of 15% (w/w) PEG 3350 and 10% (w/w) potassium phosphate.

Supported by CNPq, JIKA, CAPES, UFPE.

G - 84

EVOLUATION OF CYANIDE, STARCH AND FIBER IN CASSAVA (*Manihot esculenta* cv. IAC 576-70), SUBMITTED OF GROWTH REGULATORS.

A.A.Fernandes[1], J.D. Rodrigues[2], M.P. Cereda[3].
1. Departarmento de Química e Bioquímica-IB-UNESP-18.618.000-Botucatu,SP.
2. Departamento de Botânica-IB-UNESP 18.600.00-Botucatu,SP
3. Centro de Raízes Tropicais (CERAT)-FCA-UNESP-18.603.970

In the selection of varieties of cassava, are appraised certain approaches, that include the contents of hydrocyanic acid, starch and fibers. Growth regulators improve the quality of the root tuber for be capable to affect several metabolic processes in the plant, mainly during the period of tuberization. In the present study it was checked the effects of the growth regulators, such as Chlormequat (2-chloroethyl-trimethylammonium chloride), gibberellins (GA_{4+7}) and cytokinin (6-fenylmethylaminopurine) on the synthesis of starch, production of cyanide and fibers in cassava storage roots. For so much, it was used twelve treatments, corresponding to different concentrations, ways and times of application of the growth regulators. The hydrolysis of cyanogenic glucosides to cyanohydrins can be achieved either by enzymic hydrolysis, in which the linamarase is usually added to the acid extract and the pH adjusted to about 6.The breakdown of cyanogenic glucosides to cyanohydrins, in which are decomposed rapidly to cyanide in alkaline solution. The total amount of cyanide in solution is then determined by a colorimetric method. The evoluation of the content of starch was accomplished by enzymatic hydrolysis (amiloglucosidase). Fiber fraction was determined according to A.A.C.C. (1975). Gibberellin and cytokinin interfered in the metabolism of the cyanogenic glucosides (linamarin), possibly for increasing the activity of the linamarase, provoking hydrolysis and liberating hydrocyanic acid. Opposite behavior was observed when the plants received the growth retardant. Cytokinin increased the starch content, probably for stimulating the activity of the starch synthetase. Similar results were obtained with application of Chlormequat. The fiber quantities was lower in the plants treated with the growth regulators. Furthermore, the cyanogenesis and mode of action hormonal, at metabolic level, are discussed. Through the obtained results, it can be concluded that the substances, with the exception of the gibberellin and cytokinin in relation to the HCN content, improve the quality of the roots tubers.