BQ001 - **THE PRESENCE OF THE SYMBIOTIC BACTERIUM INFLUENCES THE O_2 CONSUMPTION IN ITS HOST CELL, *CRITHIDIA DEANEI.***

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Some trypanosomatids harbour a symbiotic bacterium which co-evolves with the host. This relationship constitutes an excellent model in the study of the organelle origin and the cellular evolution. The presence of this symbiont promotes morphological alterations in the host. Furthermore, protozoan metabolism is altered and an intense metabolic exchange occurs between both partners. The presence of the symbiont also inlfuences the host energetic metabolism, since the wild strain of *Crithidia deanei* shows an increased O_2 consumption when compared to the aposymbiotic strain. In order to characterize the mitochondrial energetic metabolism in *C. deanei*, we tested the effect of different inhibitors and ionophore that specifically target the protein complexes of the respiratory electron system. It was not observed a significant inhibition of oxygen consumption after addition of oligomycin to the culture medium. This result suggests a minor contribution of complex V (FoF1 ATP synthase) to the ATP synthesis in wild strain. Unexpectedly, oligomycin promoted a increase in O_2 consumption in the aposymbiotic cells. The FCCP, a proton ionophore, increased the O_2 consumption in both strains to the same value, however the stimulation was more pronounced in the aposymbiotic strain. The antimycin A and cyanide, potent inhibitors of complexes III and IV respectively, completely abolish O_2 consumption in both strains, but the aposymbiotic protozoa are more sensible to these compounds. The TTFA, a potent inihbitor of complex II, partially blocks O_2 consumption, whereas rotenone, a potent inhibitor of complex I showed little effect in complex I. After searching sequences in *C. deanei* genome we found genes that encode subunits of the complexes I and IV, but some important sequences are absent, as the subunit of complex I which is sensible to rotenone. Our next goal is to explore the genome of *C. deanei* in order to construct the protozoan metabolic network.
Supported by:CAPES, FAPERJ, CNPQ, INCTEN

BQ002 - **TRYPANOSOMA CRUZI: A MITOCHONDRIAL DELTA-1-PYRROLINE-5-CARBOXYLATE DEHYDROGENASE (TCP5CDH) IS INVOLVED IN THE ENERGETIC METABOLISM**

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In most of eukaryotes, delta-1-Pyrroline-5-Carboxylate (P5C) is an intermediate between the metabolic pathways leading to proline, ornithine and glutamate, bridging the Krebs and urea cycle. The proline-glutamate pathway in Trypanosoma cruzi is catalyzed by two oxido-reduction reactions: first, proline is oxidized to P5C which is -in turn- irreversibly converted to glutamate by TcP5CDH. The functionality of the proteic product from Tcp5cdh open reading frame was initially assessed by yeast complementation. Furthermore, the recombinant TcP5CDH-6xHis was purified and characterized, being this enzyme highly specific for P5C as substrate. Kinetic parameters for P5C and cofactors, NAD(P)+ were obtained (Km values: 144, 705 and 342 microM; Vmax values: 44, 22 and 7 nmol/min/mg, respectively). Moreover, parameters such as temperature, reaction buffer and pH optimal were also determined. Therefore, enzymatic activity of TcP5CDH was measured in crude extracts of epimastigotes. The levels of mRNA, analyzed by qRT-PCR, and protein, as verified by western blotting, were congruent with the enzymatic activity measured in all the life cycle stages. It was revealed a three-fold higher ratio in the infective stages (metacyclic and culture-derived trypomastigotes). Digitonized epimastigotes were used for sub-cellular localization of TcP5CDH, as well as immune-fluorescence microscopy in all the parasitic stages. Mitochondrial vesicles isolation, lacking of their matrix content, led us to detect TcP5CDH and its activity as a membrane-associated enzyme. In addition, T. cruzi cells, conditioned to nutritional starvation, were incubated with P5C showing a P5C-dependent ATP production, which was abolished by antimycin A. These results suggest that this process is dependent on oxidative phosphorylation. Our data strongly supports –by the first time to our knowledge– an involvement of TcP5CDH in energy management and nutritional stress resistance and, thereby, in cell maintenance.
Supported by:FAPESP, INBEQMEDI, CNPq, USP

BQ003 - **LEISHMANIA (VIANNIA) BRAZILIENSIS MULTINUCLEATION AND CYTOKINESIS INHIBITION BY MYRIOCIN**

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Sphingolipids have been related with several biological processes, such as intracellular transport, modulation of signal transduction and apoptosis. Inositolphosphorylceramide is the major sphingolipid expressed in promastigote forms of *leishmania*. In this study it was investigated the effect of myriocin (sphingolipid synthesis inhibitor) in promastigote forms of *L. (V.) braziliensis*. Myriocin, from 0.4 to 5 µM, reduced in dose-dependent manner promastigote growth rate, a complete parasite division inhibition was detected with 10 µM myriocin. Myriocin IC50 was determined by MTT assay, as 0.40 µM with 95% confidence limits of 0.23 to 0.73. When MTT assay was carried out with parasites cultivated at 1.2 x 10^7 parasites/ml (Log phase), myriocin inhibited partially the parasite metabolism. Although myriocin promoted parasites growth inhibition, no toxicity was observed by MTT assay when 1.2 x10^8 parasites/ml (stationary phase) were incubated with myriocin up to 30 µM, during 72 hours. A dramatic morphological alteration was observed by scanning electron microscopy. Parasites treated with 1 µM myriocin assume rounded morphology with a shorter flagellum in comparison with control parasites. In addition, parasites treated with 5 µM myriocin resulted in huge cell masses with short flagellum. Nucleus(N)/kinetoplasts(K) were analyzed by fluorescence microscopy using DAPI labeling. 1 µM myriocin treated-cultures showed a significant reduction of 1N1K forms and an increase of parasites presenting 1N2K, 2N2K and 4N4K, with incomplete cytokinesis. High proportion of parasites 3N2/3K, 4N2K and XNXK (u) were observed when 5 µM myriocin were added to the culture. These data suggest that sphingolipid biosynthesis is essential for *L. (V.) braziliensis* promastigote flagellum biogenesis, cell cycle and cytokinesis.
Supported by:FAPESP, CNPq, CAPES

BQ004 - **MOBILITY AND MORPHOLOGY AND EXPRESSION OF ANTIOXIDANT ENZYMES IN TRYPANOSOMA CRUZI TRYPOMASTIGOTES UPON H2O2 – TREATMENT**

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The survival of Trypanosoma cruzi and maintenance of infection is also guaranteed by the antioxidant enzymes repertoire present in the different forms of the parasite enabling it to cope with oxidative stress. Herein, the expression of cytosolic (TcCPx) and mitochondrial (TcMPx) tryparedoxin peroxidase was evaluated in trypomastigotes upon incubation with different concentrations of H2O2. In parallel, trypomastigotes and epimastigotes mobility and morphology were evaluated. Trypomastigotes are less resistant to H2O2 when compared to epimastigotes, since at 50 µM H2O2 their mobility was altered. TcCPx expression increased at low H2O2 concentrations (10 µM) compared to the control. Interestingly, a decrease in TcCPx expression was observed with higher H2O2 concentrations, until 50µM where an increase in its level was observed. Under H2O2-treatment, TcMPx expression increased (~53%), followed by a reduction (~46%) with the highest concentration. In epimastigotes, the mobility and morphology did not change upon incubation with 50-200 µM H2O2. However, for the trypomastigotes, the incubation with 50 µM H2O2 was able to markedly decrease their mobility, although morphology was not affected. Upon 200 µM H2O2-treatment, the trypomastigotes were misshapen, more elongated; some were lysed, and mobility of the entire population was severely impaired. Altogether, the results indicate that trypomastigotes are more sensitive to H2O2 than epimastigotes and are capable of altering the expression of antioxidant enzymes under oxidative stress conditions
Supported by:Fapesp, Faep-Unicamp, Capes

BQ005 - **RIBOSE-5-PHOSPHATE ISOMERASE FROM TRYPANOSOMA BRUCEI: FUNCTIONAL AND BIOCHEMICAL CHARACTERIZATION**

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Trypanosoma brucei is the parasite responsible for sleeping sickness in sub-Saharan Africa. Glycolysis is essential for the bloodstream form of this parasite, but glucose is also a substrate for the Pentose Phosphate Pathway (PPP). This pathway serves two very important purposes: it is responsible for maintenance of the redox level in the parasite, by production of NADPH, and for the synthesis of the ribose 5-phosphate that is used for nucleotides production. The enzyme ribose-5-phosphate isomerase (Rpi) catalyses the reversible isomerization between ribulose 5-phosphate and ribose 5-phosphate, at the end of oxidative branch of the PPP. Two types of Rpi exist: RpiA, that is broadly distributed among eukaryotic organisms, in metazoa as well as fungi, and in some bacteria; and RpiB that is found in most prokaryotic organisms. Previous studies showed that trypanosomatids possess the isoform RpiB that is not homologous to the RpiA found in human. We performed some kinetic and functional studies of the RpiB from T. brucei. The gene of the T. brucei RpiB (TbRpiB) has 458 bp and has been cloned in pET28a. The protein was expressed, with an N-terminal His-tag, in a soluble form and purified by affinity chromatography. The active form of the TbRpiB is a homodimer and this recombinant enzyme showed a *Km* = 1.26 ± 0.49mM, that is similar to this kinetic parameter described for the corresponding enzyme from T. cruzi and some bacteria. By site-direct mutagenesis we constructed two mutants, substituting the catalytic residue Cys69 by Ala (TbRpiB-C69A) and Pro (TbRpiB-C69P). The TbRpiB-C69A was expressed in soluble form, purified and showed no activity, demonstrating that TbRpiB has a similar catalytic behavior as the other RpiBs. In contrast, the TbRpiB-C69P could not be expressed in soluble form. The RNAi approach showed a short reduction in growth rate of both the bloodstrean and procyclic forms of the cultured parasite, under normal growth conditions.
Supported by:CNPq, FIOCRUZ, dDI

BQ006 - **INVOLVEMENT OF REDOX-STATUS IN MACROPHAGE INFECTION BY TRYPANOSOMA CRUZI**

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Trypanosoma cruzi, the causative agent of Chagas disease is an obligate intracellular pathogen being the first line of mammalian host defense against the pathogen infection represented by macrophages. During the course of the infection process, macrophages produce reactive oxygen species as defense. Thus, we investigated the association of abundant molecules that possess different status redox in the hosts with the parasite-macrophage interaction. Then we used heme, a pro-oxidant molecule present in various steps of T cruzi life cycle; urate, an antioxidant molecule present during the metacyclogenesis, and NAC, another antioxidant and potential drug. Macrophages (RAW 264.7) were pre-incubated for 30 min in DMEM containing heme, urate or NAC and then, T. cruzi trypomastigotes at a 1:10 rate were allowed to interact with the mammalian cells for 2h (early infection stage) or 70h (later infection stage). Afterwards, parasite load was accessed by cells staining with Panotico for posterior light microscopy quantification. We also performed real-time PCR using cells genomic DNA, specific T. cruzi TCZ primers and mouse GAPDH primers to normalize the relative quantification. In the early infection stage, we observed an increment of 2.4 fold in the infection of macrophages treated with NAC. In the later infection stage, when the presence of amastigotes was quantified, we observed a decrease of infection in macrophage treated with urate and NAC. Our results suggest that the reduced environment improvement the macrophage-parasite interaction. But interesting, reduced environment is harmful to amastigotes forms. Additionally, other results of our group showed that in epimastigotes, a replicative form present in invertebrate hosts, urate and NAC decrease the proliferation, but increase the differention to infective forms (trypomastigotes) and survival. Considering actual and previous results, we suggest that reduced environment is better for infective form than replicative forms.
Supported by:UERJ/PIBIC, FAPERJ and CNPq

BQ007 - **NEW ADVANCES IN TRYPANOSOMA CRUZI PROTEOMIC MAP: A BLOODSTREAM TRYPOMASTIGOTES STUDY**

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Chagas' disease caused by protozoan *Trypanosoma cruzi*, is an endemic neglected illness in Latin America, responsible for considerable human mortality and morbidity. Parasite's life cycle involves two hosts (triatomine and mammals) and three evolutive forms. The bloodstream trypomastigotes can infect all mammalian cells, disseminating the disease. In this study, we performed the proteomic analysis of bloodstream forms. The purification of the parasites from mice blood was done by differential centrifugation. All peptides identification was performed in MALDI-TOF/TOF. Proteins were extracted by freezing-thawing in a lysis solution (8M urea, 4M thiourea, 4% CHAPS, 40 mM Tris, 60 mM DTT, 1% ampholytes and protease inhibitor cocktail), and quantified using the 2D Quant kit (GE Healthcare). Two-dimensional electrophoresis (500 µg) was carried out using 4-7 and 3-10 IPG strips followed by 12% SDS-PAGE, being 1214 and 935 spots detected, respectively, in Comassie colloidal-stained gels. Interestingly, trypomastigotes' proteins were identified in 50% of analysed spots (15/30), validating the parasites' purification method employed. Glycan-specific staining (Pro-Q-Emerald, Molecular Probes) and phosphoprotein-specific staining (Pro-Q-Diamond, Molecular Probes) were also performed, being 16 and 46 detected, respectively. The proteomic data of bloodstream forms will provide important insights in *T. cruzi* infectivity and virulence, supplying additional information for the development of alternative drugs for Chagas' disease.
Supported by:FAPERJ,CNPq,FIOCRUZ

BQ008 - **BLOODSTREAM TRYPOMASTIGOTES OF TRYPANOSOMA CRUZI AND NAPHTHOIMIDAZOLES: DIFFERENTIAL GEL ELECTROPHORESIS (DIGE) INSIGHTS IN ITS MECHANISMS OF ACTION**

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The chemotherapic agents used in the treatment of Chagas' disease present variable efficacy, especially in the chronic phase, besides others disadvantages, what incites the research for new medicines. Synthetic derivatives of naphthoquinones were produced and assayed on all *T. cruzi* life stages, being three naphthoimidazoles (N1, N2 and N3) the most active. In this study, we evaluated the differential protein expression in bloodstream trypomastigotes treated with these three naphtoimidazoles by 2D-DIGE technique (two-dimensional difference gel electrophoresis). The parasites were obtained from the blood of infected mice in the peak of parasitemia (seventh day) and isolated by differential centrifugation. The protein extraction was done with lysis solution (8M urea, 2M thiourea, 4% CHAPS and 40mM Tris) associated to cycles of freezing and thawing. The samples were quantified by 2D Quant kit (GE Healthcare) and labeled with CyDye DIGE Fluor minimal dyes (GE Healthcare) before being applied in the two-dimensional electrophoresis, which was carried out using strips of 18 cm with pH range of 4-7 followed by 12% SDS-PAGE. In the total, 18 gels were made, each containing the internal pool, a control and a treated (N1, N2 or N3) of the six biological replicates. Each gel was scanned using a fluorescence scanner Typhoon (GE Healthcare) and the images were analysed by the DeCyder Differential Analysis software (GE Healthcare). The preliminary results indicated 66 differential spots among control and parasites treated with N1, N2 and N3 ($p<0.01$), which will be identified using mass spectrometry MALDI-TOF-TOF (Applied Biosystems). These strategies can contribute to elucidate action mechanism of the compounds and to supply additional information about the parasite cell biology, crucial for the development of alternative drugs for this disease.
Supported by:FAPERJ,CNPq,FIOCRUZ

BQ009 - **TRITRICHOMONAS FOETUS EXPRESS DIFFERENT ECTO-PHOSPHATASE ACTIVITIES DURING THE PSEUDOCYST FORMATION**

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Tritrichomonas foetus, a parasite of cattle and domestic cats, displays a pear-shaped form and a pseudocyst stage (endoflagellar form). The pseudocyst is reversible and its formation represents a mechanism against stress conditions. The aim of the present study was to assess whether occur a modulation of the ecto-phosphatase activity, which is present on the external surface of *T. foetus*, during the pseudocyst induction. To clarify this question, the ecto-phosphatase activity of intact living pseudocysts and pear-shaped parasites from a cultured and a freshly isolated *T. foetus* was determined spectrophotometrically by measuring the rate of p-nitrophenol (p-NP) production. This work demonstrates that, in both *T. foetus* isolates, the ecto-phosphatase activity increased during the time course of pseudocyst induction. The ecto-phosphatase activity of pseudocysts was approximately 5.0 and 1.6-fold greater than the ecto-phosphatase activity of pear-shaped parasites from the cultured and fresh isolates, respectively. In addition, pseudocysts and pear-shaped parasites from the fresh isolate exhibited a higher ecto-phosphatase activity when compared to pseudocysts and pear-shaped parasites from the cultured *T. foetus*. Moreover, during the time-course of pseudocyst reversibility, the ecto-phosphatase activity of both *T. foetus* isolates decreased and it was restored to the same level as found in the parasites before the pseudocyst induction. Experiments using inhibitors of acid phosphatases, such as ammonium molybdate and sodium fluoride, as well sodium orthovanadate, an inhibitor of phosphotyrosine phosphatase, showed that the ecto-phosphatase activity of both *T. foetus* forms decreased under different patterns of inhibition. Our results clearly indicate a positive correlation between the ecto-phosphatase activity and the pseudocyst formation of *T. foetus*.
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BQ010 - **VITELLOGENIC ENZYMES OF CULEX QUINQUEFASCIATUS**

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Despite the fact of Culex quinquefasciatus being an efficient vector of human diseases such as lymphatic filariasis, West Nile fever and diverse viral encephalitis, very limited research concerning its embryonic development has been conducted. Like all oviparous animals, the embryonic development of arthropods depends on the yolk degradation by vitellogenic hydrolases. Cathepsin-like proteases involved in yolk protein hydrolysis have been identified in several model-organism where they were characterized in lesser or greater detail.
Here we describe in Cx. quinquefasciatus egg extracts a cathepsin-like activity against benzyloxycarbonylarginyl-arginine 4-methylcoumarin-7-ylamide (Z-Arg-Arg-NHMec), with optimal temperature and pH of 27 oC and 5.0, respectively. This same activity is suppressed by trans-epoxysuccinyl-L-leucylamido-(4-guanidino)butane (E-64), a specific cysteine protease inhibitor. The enzymatic activity is intact after an 18 h incubation of the egg extract at the optimal temperature and pH. SDS-PAGE analysis of the proteolysis products reveals the disappearance of most of the extract bands and the persistence of a 50 kDa band. The E-64 addition to the extract incubation almost entirely inhibited the proteolysis, suggesting that the enzymatic activity is responsible for the egg proteins digestion. Furthermore, HPLC fractionation allowed the isolation of the same proteolytic activity as a single isolated peak.
Our preliminary data strongly suggest that, like Ae. aegypti (Cho et al., J Biol Chem 274: 13311-21, 1999), the Cx. quinquefasciatus cathepsin B-like protease plays a key role in the yolk proteins degradation.
Supported by:Fapesp

BQ011 - **LEISHMANIA (VIANNIA) BRAZILIENSIS ISOLATED FROM MUCOSAL LESIONS EXPRESS MORE THIOL-SPECIFIC ANTIOXIDANT PROTEIN THAN PARASITES ISOLATED FROM CUTANEOUS LESIONS**

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Leishmania (Viannia) braziliensis is able to cause different clinical manifestations of tegumentary leishmaniasis, ranging from simple cutaneous lesions (CL) to destructive mucosal ones (ML). The mechanism of ML development has not been established, but it depends on the host and parasite factors. To search for parasite factors, we isolated L. (V.) braziliensis from three CL and three ML patients and investigated the expression of three different proteins related to parasite´s virulence (META-2; stress-inducible protein/1-STI-1; thiol-specific antioxidant/TSA). L. (V.) braziliensis species identification was confirmed by PCR of the glucose-6-phosphate dehydrogenase gene. Protein expression in each isolate was evaluated by western blotting in stationary phase promastigotes (cultured for 8 days) or amastigotes obtained from footpad-infected interferon gamma knockout mice. Expression of META-2 and STI-1 was variable among isolates, but we were unable to observe significant differences when comparing parasites from ML and CL. On the other hand, expression of TSA was 80% higher in promastigotes from ML than in parasites from CL ($p<0.05$). Amastigotes from ML also expressed TSA in higher abundance, however the differences were not statistically significant ($p=0.30$). Since TSA is related to resistance to reactive oxygen species, our results suggest that parasites from ML can be more resistant to the microbicidal activity of activated macrophages than parasites from CL.
Supported by:CNPq, CAPES, FAPEG, FUNAPE

BQ012 - **FUNCTIONAL AND STRUCTURAL CHARACTERIZATION OF THE ACTIVATOR OF HSP90 ATPASE-1 PROTEIN (AHA1) FROM LEISHMANIA BRAZILIENSIS**

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Molecular chaperones are important proteins for protein homeostasis in cells. The Hsp90 molecular chaperone is critical for cell viability and act stabilizing proteins related to cell cycle control. Hsp90 is essential for cell adaptation, working as a thermal sensor even in protozoaires like *Leishmania major* and *Plasmodium falciparum*. Hsp90 is a flexible homodimer comprehended by an N-terminal domain that possesses low ATPase activity, a middle domain and a C-terminal domain. Since its ATPase activity can be inhibited competitively, the Hsp90 is considered a good target for therapeutics against protozoaires. The function of Hsp90 is driven by an ATPase cycle which is aided by Hsp90 cochaperones. One of them, called Aha1 (Activator of Hsp90 ATPase-1), increase the Hsp90 ATPase activity and its overexpression is described to provide resistance against Hsp90 inhibitors. The Aha1 is formed by two similar domains, N- and C-terminal, both of them involved with the interaction with the middle domain of Hsp90. Although the Aha1 significance for Hsp90 cycle has been showed it is not completely understood and studies regarding this protein of protozoaires are absent. Here, we present the expression, structural and functional characterization of the Aha1 from *Leishmania braziliensis* (LbAha1). The protein was expressed soluble in bacteria and purified by ion exchange and size exclusion chromatography. Circular dichroism (CD) and fluorescence experiments showed that the protein was obtained folded and presents a proper α+β conformation. Size exclusion chromatography and analytical ultracentrifugation experiments revealed that LbAha1 is an asymmetric monomer in solution. Chemical-induced unfolding followed by CD and fluorescence showed that LbAha1 unfolds by at least two events, which could be related to differential domains stability. Experiments to access the ability of LbAha1 in stimulate the ATPase activity of the LbHsp90 are in progress and results will be presented.
Supported by:FAPESP and CNPq.

BQ013 - **HEPARIN RECEPTOR FROM LEISHMANIA (V.) BRAZILIENSIS**

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We report the subcellular distribution of Heparin Binding Proteins (HBPs; heparin receptor) in *L. (V.) braziliensis* and their biochemical characteristics. Promastigotes were submitted to subcellular fractionation and enriched samples of membrane and flagella were applied to HiTrap-Heparin columns; the eluted proteins were named HBP M*f* and HBP F*f*, respectively. HBP F*f* presented a higher concentration of HBPs than HBP M*f* and SDS-PAGE showed two main protein bands in both fractions (65 kDa and 55 kDa). Furthermore, our results indicate gelatinolytic activity at alkaline pH in both fractions, which could be inhibited by 1, 10-phenanthroline. Additionally, the presence of HBPs on promastigotes surface was confirmed by surface plasmon resonance (SPR) assays, where sensorgram indicated higher resonance units (RU) values after interaction of fixed parasites with immobilized heparin onto a Biocap sensor chip; RU increase could be inhibited by pre-incubation of parasites with free heparin in a dose-dependent fashion. Similar SPR analysis of subcellular fractions confirmed that HBP M*f* and HBP F*f* have distinct heparin-binding capacities, presenting different RU values. Also we have evidence that both surface membrane and flagella proteins fractions have characteristics of metallo-proteinases - new function of theses proteinases in *L. (V.) braziliensis*, acting in the binding to glycosaminoglycans.
Supported by:FAPERj; CAPES; PAPES- Fiocruz/CNPq

BQ014 - **MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF AN ALANINE RACEMASE FROM TRYPANOSOMA CRUZI**

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The involvement of L-amino acids in a quantity of relevant biological processes in *Trypanosoma cruzi* was extensively demonstrated. However, little is known about the functions of D-amino acids on the biology of the parasite. The first amino acid racemase isolated from *T. cruzi* was the proline racemase, which was shown to be a potent mitogen and to be involved in the infectivity of the parasite (Reina San Martin et al., 2000, Chamond et al., 2003). In the *T. cruzi* genome database we identified a gene encoding a putative alanine racemase (Alr) (EC 5.1.1.1). Alrs are pyridoxal 5'-phosphate (PLP) dependent enzymes which catalyze the racemization of alanine (interconversion between L-and D-alanine). In the present work, the ORF encoding Alr was amplified from *T.cruzi* genomic DNA and cloned into the pET19b vector using NdeI and BamHI enzyme sites. This construction was used for protein expression in *E. coli* BL21 cells. The recombinant Alr containing a 6xHis-tag showed high levels of expression as a soluble and active protein. Subsequently, the Alr was purified by affinity chromatography, and showed racemization activity in both directions (conversion of D into L and L into D alanine). The recombinant protein was used to produce anti-TcAlar polyclonal monospecific antibodies. Western blot analysis showed that the anti-Alar antibody recognized both, the recombinant and native proteins (a single polypeptide of the 42 KDa in *T.cruzi* epimastigotes extracts). Since the Alr is an enzyme not present in the mammalian host, it constitutes a promising target for drugs. In this sense, the effects on *T. cruzi* viability of a set of Alr inhibitors are being evaluated.
Supported by:Fapesp, CNPq, INBEQMEDI, USP.

BQ015 - **UPTAKE OF INORGANIC PHOSPHATE IN LEISHMANIA CHAGASI**

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In this work, we show, for the first time, the presence of the inorganic phosphate (P_i) transporter of *Leishmania chagasi*, which causes visceral leishmaniasis. This transport increase with time and cell number, however is not modulated by pH variation. The transport shows a Michaelis-Menten kinetics with values of Km and $V_{máx}$ of 0.016 ± 0.002 mM and 9.415 ± 0.301 pmol x min^{-1} x 10^{-7} cells, respectively. These values include the P_i transporter of *L. chagasi i*n the group of high affinity transporters, as the Pho89 of *Saccharomyces cereviseae*. FCCP, a known proton ionophore, and valinomycin, a K^+ ionophore, inhibit the transport of Pi. It is known that environmental changes are perceived as signals by the cells and generate responses in the expression and activity of proteins. Based in the known genome of *Saccharomyces cerevisiae*, we found the PHO89 homolog sequence in *L. major* genome. The protein encoded by this sequence, Pho89, has high affinity to P_i and it is modulated (both the activity of the protein such as the gene expression) by environmental P_i variations. These facts led us to believe that the same occurs for *L. chagasi*. When cells are grown for 3 days in low concentration of P_i, it have higher PHO89-gene expression than cells cultured for 3 days in high P_i concentration, while cells grown for 6 days in low P_i condition have higher transport rate and gene expression than those grown for 6 days in the presence of high concentrations of P_i. In addition, cells grown for 3 days have higher PHO89-gene expression than cells grown for 6 days, both in low or high P_i concentration. These data indicate the importance of this transporter to cells growth in different environmental conditions. The findings here confirm the presence of a P_i transporter in *L. chagasi*, able to contribute to the acquisition of inorganic phosphate, growth and survival of promastigote forms of *L. chagasi.*

Supported by:CNPq/ FAPERJ

BQ016 - **HISTIDINE METABOLISM IN TRYPANOSOMA CRUZI: BIOCHEMICAL CHARACTERIZATION OF HISTIDINE AMMONIA-LYASE (TCHAL)**

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Trypanosomatids are able to catabolize both proteins and amino acids with ammonia production. Moreover, it has been reported that amino acids have different roles on the biology of T. cruzi, besides to its role in protein synthesis and energetic metabolism. It is interesting to note that despite the several biological functions demonstrated for histidine in different organisms, little is known about its metabolism in T. cruzi. Histidine can be degraded to glutamate in a non-oxidative pathway involving four enzymatic steps. In a search for the putative genes encoding these enzymes in the T. cruzi genome databases, we found four open reading frames encoding the enzymes linking histidine with glutamate. The first step in the pathway is the conversion of histidine in urocanate and ammonia, catalyzed by the enzyme histidine ammonia-lyase (HAL, EC 4.3.1.3). In the present work, we cloned the putative gene for this enzyme (TcHAL) and the recombinant protein was expressed and purified. TcHAL was recovered soluble and its histidine ammonia-lyase activity was measured spectrophotometrically at 28°C, recording the formation of urocanate at 277 nm. TcHAL presented a Michaelis constant (K_M) of 1,03 mM and a maximum velocity (V_{MAX}) of 2,54 µmoles . min^{-1}. mg^{-1}, in agreement with other characterized orthologs. As described elsewhere, HALs are dependent on divalent ions as cofactors. We observed that TcHAL is dependent on divalent cations for optimal activity. Polyclonal antibodies raised against the entire protein were obtained and a high specificity against TcHAL was detected. Western blot analysis and indirect immuno-fluorescence assays in epimastigotes showed that TcHAL is expressed in this stage of the life cycle of T. cruzi, as a cytoplasmic protein. Further experiments are being done to validate the activity, expression and localization of the native enzyme in other stages of Trypanosoma cruzi.

Supported by:FAPESP, INBEQMEDI, CNPq and USP

BQ017 - **GLYCEROL KINASE OF TRYPANOSOMA EVANSI: AMPLIFICATION, CLONING, AND SEQUENCING**

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In trypanosomatids, glycolysis occurs in a specialized organelle called glycosome. The glycolysis is an important process to obtain energy, since the trypanosomes bloodstream forms, are highly dependent on that to produce ATP. The glycerol kinase is one of the enzymes involved in the glycolytic pathway Trypanosoma evansi is the most widespread of the pathogenic salivarian trypanosomes and affects most livestock and wild animals mainly in endemic regions. The T. evansi infection is popularly known as “surra” or “mal das cadeiras” and there are no effective drugs or vaccines to cure or prevent the disease. In order to obtain a purified genomic DNA (gDNA), the blood of a Wistar rat infected with T. evansi was first purified by Percoll® gradient and ion exchange chromatography with DEAE-cellulose. The gDNA was then obtained by extraction with phenol-chloroform. The open reading frame encoding TeGK was obtained by using primers derived from T. brucei Glycerol kinase gene. A fragment of 1500 base pairs was amplified by polymerase chain reaction, extracted, purified and cloned into a commercial vector. TeGK displays a high homology with T. brucei glycerol kinase (TbGK) and low homology with the host sequence. The recombinant protein is being processed for further biochemical and structural characterizations.
Supported by:CNPq, FINEP, FAPESC

BQ018 - **RELATIONSHIP BETWEEN OSMOTIC FRAGILITY AND LIPID PEROXIDATION OF RED BLOOD CELLS IN RATS INFECTED BY TRYPANOSOMA EVANSI.**

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Trypanosoma evansi is a widely-distributed haemoflagellate of veterinary importance. It is responsible for a disease known as “Mal das Cadeiras”, and has the greatest economical impact on domestic stock. The acute form of the disease is characterized by hematological changes, progressive anaemia, anorexia, emaciation, fever, edema and paralysis of hind limbs.. The osmotic fragility expresses the ability of the membranes maintains their structural integrity when exposed to osmotic stress. The disintegration of the membranes may be one factor that contributes to lipoperoxidation caused by T. evansi. Eighteen animals were each inoculated intraperitoneally with blood containing 104 parasites/mL. Infected animals were classified according to average number of parasites in ten random homogeneous fields. Group A: control; B: 1-10 trypanosomes/field; C: 11-30 trypanosomes/field; D: 31-60 trypanosomes/field; E: more than 61. Blood samples were used to determine the osmotic fragility of red blood cells.Briefly, erythrocyte stability was measuring with increasing concentration of buffered saline ranging from 0% (distilled water) to 0.85% NaCl at pH 7.4. Hemolysis in each NaCl concentration was expressed as percentage of the absorbance in distilled water. The effective concentration of the NaCl solution inducing 50% hemolysis (OF50) of RBCs was calculated from the hemolysis curve. Packed cell volume (PCV) or erythrocyte volume fraction is percentage of blood volume that is occupied by red blood cells was calculated. Lipid peroxidation in the RBC hemolysate was determined as thiobarbituric acid reactive substances (TBARS). There was a significant decrease in the values of packed cell volume in group E when compared with control ($p<0.05$). Osmotic fragility was increase in group E when compared with the other groups. Infection results enhanced erythrocytic lipid peroxidation in group D and E when compared with control. Decrease of packed cell volume, increased level of fragility osmotic and rise progressively peroxidative damage of the red blood cells caused by the parasite, may contribute to the pathogenesis of the anemia seen in trypanosomiasis.
Supported by:CNPq, FINEP, FAPESC

BQ019 - **NEW ANTIGENS SEARCH FOR CANINE VISCERAL LEISHMANIASIS TO IMPROVEMENT OF SERODIAGNOSIS: STUDY OF LC06 GENE**

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Serological diagnosis of visceral leishmaniasis (VL) in dogs is problematic due low sensitivity and/or specificity of the available tests, interfering in disease control and consequently the interruption of its transmission to humans. This works reports the study of Lc06, an orphan Leishmania chagasi gene, and its potential to be used in the development of a serological diagnostic test for canine VL. Bioinformatic characterization of Lc06 such as presence of transmembrane domains, hydrophilic regions, linear epitopes, tandem repeat (TR) and absence of Tripanosomatidae similarity reveal interesting features that put it as putative antigen for development of serological tests. In order to confirm this in silico approach, we decided to test overlap peptides within a TR repetition 61 amino acids long and test their potential immunoreactivity against sera from dogs with VL. Within this repetition sequence of 61 amino acids, we selected a 40 sequential region from which two peptides were synthesized, with 20 and 30 sequential amino acids: EV0710 (residues 20 to 40) and EV0410 (residues 10 to 40). Therefore we used the Solid Phase Peptide Synthesis technique (Marrifield, 1963). The experimental molecular weight for EV0710 and EV0410 determined by mass spectrometry were 2,115.2 and 3,047.3 g.mol-1, respectively, and their purity, determined by HPLC analysis, was almost 95%. EV0710 and EV0410 were tested in an enzyme-linked immunosorbent assay (ELISA) for their capacity of detection of antibodies anti - L. chagasi in dogs, and both strongly reacted with serum from infected dogs. In such a way, these peptides have shown potential to be applied in a new diagnostic test for canine VL.
Merrifield, R. B.Solid phase peptide synthesis I. The synthesis of a tetrapeptide.
Supported by:Fapesp

BQ020 - **SUBSTRATE INHIBITION AND ALLOSTERIC MODULATION OF BRUCIPAIN BY HEPARAN SULPHATE**

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The cathepsin L-like peptidase (TbCATL or rhodesain) of *Trypanosoma brucei*, the causative agent of Human Sleeping Sickness, is implicated in the traversal of the blood brain barrier by the parasite. The parasite alternates between the invertebrate and the mammalian hosts, where it is exposed to rapid changes of temperature. TbCATL is used as a framework for the development of inhibitors that display anti-parasitic activity. We show that recombinant TbCATL lacking the C-terminal extension undergoes significant inhibition by the substrate Z-Phe-Arg-MCA at concentrations above the K_M, when assayed at room temperature. This characteristic was reported for the cathepsin L-like peptidase of Trypanosoma cruzi, cruzain and for mammalian cathepsin K. Substrate inhibition is not apparent when TbCATL is assayed at 37ºC, suggesting that a putative allosteric site is available for substrate binding at 25ºC, but is partially concealed at 37ºC. This behavior was not observed during the hydrolysis of a structurally similar substrate, Z-Val-Leu-Arg- MCA. Evaluation of the intrinsic tryptophan fluorescence showed that the enzyme undergoes discrete conformational changes caused by the shifts in temperature. We also found that heparan sulphate (HS) interacts with the enzyme, preventing substrate inhibition and modulating catalytic activity. We propose that the activity of TbCATL can be modulated through interactions of molecules with an allosteric site, which could be explored for the design of new inhibitors.
Supported by:CNPq/FAPERJ

BQ021 - **SODIUM-DEPENDENT UPTAKE OF INORGANIC PHOSPHATE IN TRYPANOSOMA CRUZI**

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Trypanosoma cruzi is the etiological agent of Chagas´ disease, a chronic debilitating disease highly prevalent in Latin America. Living epimastigotes cells of *T. cruzi* grown under limiting inorganic phosphate (Pi) are able to transport this anion to cytosol with high rate, through a carrier-mediated process. The dependence on Na^+ concentration revealed a Michaelis-Menten like kinetics for the uptake of Pi and the values of apparent $K_{0,5}$ and Vmax were 2.7 ± 0,5 mM and 27.5 ± 0.9 pmol × min^{-1} × $(10^7$ cells$)^{-1}$, respectively. Addition of Na^+ ionophore, monensin, reduced the Pi accumulation into the cells in the presence of NaCl to the level observed in absence of Na^+. Treatment with valinomycin, a K^+ ionophore, and nigericin, a K^+-H^+ exchanger, significantly also inhibit the Pi uptake, result consistent with the fact that K^+ and Li^+ ion stimulated Pi transport. Molecular biological data demonstrated that this parasite express *TcPho89*, a high-affinity Na^+:Pi-symporter. Epimastigotes grown in Pi-supplemented LIT medium exhibited a Pi-influx that was 46% lower than that found with those growning in low Pi LIT medium without alterations in *TcPho89* expression, showing that symporter turnover is stimulated by Pi-starvation in culture. Moreover, *T. cruzi* cell differentiation gives rise to four morphogenetic forms. This process is highly regulated and includes significant changes in the surface molecules of these parasites. Indeed, cells grown at TAU medium are inefficient to uptake Pi when compares to those grown at LIT medium. Altogether, these results suggests the presence of a Na^+/Pi cotransporter present in *T. cruzi*, contribute to inorganic phosphate acquisition by epimastigotes development and its differentiation to trypomastigotes forms.
Supported by:MCT/CNPq, CAPES, FAPERJ (INCT/INBEB).

BQ022 - **STIMULATION OF LEISHMANIA CHAGASI INFECTIVITY BY INTRINSIC PLATELET-ACTIVATING FACTOR**

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PAF, 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholine, exhibits potent biological activity and is synthesized by a wide variety of cells, including neutrophils, platelets, macrophages, and lymphocytes. Previous data from our group showed some physiological roles related to cell differentiation induced by PAF in trypanosomatids and suggested that *Trypanosoma cruzi* synthesizes a PAF-like phospholipid that modulates the parasite's differentiation and infectivity. Here we describe the purification and molecular characterization of PAF species from complex lipid mixtures of *Leishmania chagasi* promastigotes. Using solid-phase extraction (SPE) followed by electrospray ionization tandem mass-spectrometry (ESI-MS/MS), we identified three species of PAF, namely, the common C16:0-PAF, the rare C16:1-PAF, and the novel C16:2-PAF. C16:0-PAF was present in very low amounts, whereas C16:1- and C16:2-PAF species were found in much higher concentrations. We also show that a mixture of these three PAF species induced the aggregation of rabbit platelets and stimulated mouse macrophage infection by *L. chagasi*, when promastigotes were kept for 5 days in the presence of *L. chagasi*-PAF species, before interacting with the macrophages, with the involvement of the protein kinases PKA, PKC, and CK2. *L. chagasi*-PAF species induced an inhibition of NO production by these macrophages and also stimulated *L. chagasi* cysteine and metalloproteinases, as well as PKA, PKC, and CK2 activities. All *L. chagasi*-PAF species effects were abrogated by WEB 2086, a classic antagonist of PAF that binds specifically to PAF-receptor. These data suggest that *L. chagasi* contains the components of an autocrine PAF ligand-receptor system that modulates *L. chagasi* infectivity towards mouse macrophages.
Supported by:CNPq, FAPERJ, CAPES, INCTEM

BQ023 - **SEQUENCE ANALYSIS OF PROTEASES FROM LEISHMANIA (VIANNIA) BRAZILIENSIS GENOME**

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The genus Leishmania includes protozoa parasites related to several types of mammals infection: ranging from the visceral form to the tegumentary forms (cutaneous, diffuse cutaneous, mucocutaneous and post-kalazar dermal). As an array of parasite's molecules, proteases from Leishmania are involved in its survival inside the mammalian host. Their action is related to hydrolysis of peptide and protein degradation and can influence a broad range of biological functions, including the infection process. Therefore they can be classified as virulence factors, since they promote lesion formation and immune modulation in the mammalian host. In this study we used sequence analysis tools to analyze the set of proteases predicted in Leishmania (Viannia) braziliensis annotated genomes and search for proteases initially not assigned as such. As search terms we used: (1) protease, (2) peptidase, (3) proteinase, (4) aspartic protease, (4) cysteine protease, (5) metallo protease and (6) serine protease; in the following databases: Tritrypdb, Genedb and Uniprot. Afterwards, we submitted each sequence of protein found to BLAST (blastp) for the purpose of finding proteases non-described or hypothetical ones, then each of those sequences were analyzed in the web services Pfam, InterProScan and Superfamily in order to correctly classify them as proteases. We could see that L. (V.) braziliensis has at least one aspartic protease, forty-four cysteine proteases, twenty-three serine proteases and ninety-seven metalloproteinase. Furthermore, the analysis of hypothetical sequences enabled the classification of eleven proteins in Superfamily, seven in Pfam and three in InterProScan. This study indicates the potential for discovery of new proteases not identified in genome of L. (V.) braziliensis. The better understanding of this role in Leishmania biology is important since they may constitute novel targets for drug development leading to a more effective control of the disease.
Supported by:capes

BQ024 - **PHENOTYPIC CHARACTERIZATION AND GROUPING OF REFERENCE STRAINS OF TRYPANOSOMA CRUZI CHAGAS, 1909 THROUGH THEIR TOTAL PROTEIN PROFILES**

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In the present study we used SDS-PAGE to identify the protein profiles of eight reference strains of *Trypanosoma cruzi* and subsequently their affinities for the cluster analysis based on procedures of numerical taxonomy. Epimastigotes of the eight strains were grown for six days at 28°C in BHI + LIT medium with 10% fetal calf serum. The parasites were harvested and washed twice in PBS and then frozen in liquid nitrogen in a buffer containing 20 mM Tris-HCl and the protease inhibitors 2 mM 4-(2-aminoethyl)benzenesulfonyl fluoride, 1 mM ethylenediaminetetraacetic acid disodium salt dihydrate, 130 µM bestatin hydrochloride, 14 µM N-(trans-epoxysuccinyl)-L-leucine 4-guanidinobutylamide, 1 mM leupeptin hemisulfate salt, 0.3 µM aprotinin, 100 µM phenylmethylsulphonyl fluoride, and 1% (w/v) SDS, at pH 7.2. After thawing, the parasites were homogenized and centrifuged at 5000 x g for 10 min at 4°C. Aliquots of the supernatant containing the protein extract, corresponding to 60 µg protein, were separated by 10% SDS-PAGE at 250 V and 25 mA. The gels were then stained with Coomassie Brilliant Blue for visualization and analysis of the protein bands of each strain. For computer processing (software NTSYS), we constructed a data matrix, considering only the bands not shared by all samples. Then we used the coefficient of association and SM UPGM clustering algorithm to obtain final phenograms. More than 40 protein bands were observed and ~25% of them were shared by all strains (conserved proteins). We suggest the presence of heat shock proteins (HSPs) and some glycoproteins (GPs) among them. Cluster analysis revealed two main groups of strains in *T. cruzi*. The first one included a subgroup with isolates of human origin (Y and SF21, both previously typed as Tc II) and the other with strains originating from the insect vectors (including CL Brener, now classified as TcVI). The second group included only samples identified as TCI, with a subset of isolates from opossums (G, SC28, Dm28c) and another with a sample of TcIII (Colombian strain). This study confirms the potential of this approach to demonstrate the variability in *T. cruzi* and typing of strains. In addition, the present approach enables the control of the authenticity of the reference strains of the parasite, since all the peculiarities revealed in their protein profile are consistent with previous data from other authors using various techniques.
Supported by:CNPq, FAPERJ

BQ025 - **RELATIONSHIP BETWEEN GLYCERALDEHYDE-3-PHOSPHATE DESIDROGENASE ACTIVITY AND PROTEIN NITRATION IN LEISHMANIA AMAZONENSIS RESISTANT TO NITRIC OXIDE-NO**

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Leishmania spp are the agent causative of leishamaniasis disease that affects more than 2 million people worldwide. Although it is known that reactive nitrogen species, produced by host cells, are involved in the parasitism control, little is known about the mechanism by which leishmania resist to nitric oxide-NO. The deleterious effects of NO include nitration of tyrosine residues that can abolish the activity of a number of enzymes. It was demonstrated that Tyr^{311} and Tyr^{317} of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are target of nitration by NO. Moreover, this enzyme was shown to be overexpressed in isolates of *L. infantum* resistant to NO. The aim of this study was to verify the relationship among levels of GAPDH activity and total protein nitration with the resistance to NO presented by isolates of *L. amazonensis*. To this, promastigotes ($5x10^8$) of isolates resistant and susceptible to ON, in logarithmic growth phase were submitted to conditions of oxidative stress using $NaNO_2$ as nitric oxide-NO donor. GAPDH activity was determined by NADH oxidation using spectrophotometry. The levels of nitration in total proteins were assessed by Slot Blotting using monoclonal anti-3-nitrotyrosine as primary antibody. The results showed that the activity of GAPDH in NO resistant isolate was 2.2 times higher than in the sensitive one. In addition, analysis of protein nitration showed that total protein extracts of the resistant isolate present lower levels (8 %) of nitration compared to the susceptible isolate. The observed relationship between resistance to ON, a lower level of protein nitration and increased activity of enzyme GAPDH could explain, at least in part, a mechanism used by these parasites to evade of the host organism defenses.
Supported by:Fapitec-SE; Capes PROCAD/NF; PIBIC/CNPq/UFS

BQ026 - **LOCALIZATION OF INOSITOL PHOSPHORYLCERAMIDE IN PLASMA MEMBRANE INNER LEAFLET IN L. AMAZONENSIS PROMASTIGOTES**

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In eukaryotes, sphingolipids are important membrane components and powerful signaling molecules. In Leishmania, the major sphingolipid is inositol phosphorylceramide (IPC), which are absent in mammals and thus, the IPC metabolic pathway could be considered a new target for therapy. To identify the IPC cell localization we analyzed by flow cytometry promastigotes label with the monoclonal antibody LST-1 (directed to parasite IPC) and with Annexin-V (which binds specifically to phosphatidylserine expressed mainly in the inner membrane leaflet). Five preparations of L. amazonensis promastigotes were analyzed: live log-phase parasites; live old stationary-phase parasites; log-phase live 0.05% saponin permeabilized parasites; log-phase live 0.1% saponin permeabilized parasites; and log-phase formaldehyde fixed parasites. There was no detectable reactivity of LST-1 and Annexin-V with live log-phase parasites. Interestingly, in live old stationary-phase parasites (some probably apoptotic) showed almost only LST-1 labeling, while fixed cells showed high labeling for both markers. When permeabilized parasites were analyzed,LST-1 and anexin label were observed with both preparatiobn of saponin treated parasites, however a higher LST-1 and Annexin-V labeling was detected for 0.1% saponin. These results suggest that the promastigote IPC is: i) cryptic in the plasma membrane of live parasites, ii) probably localized in the inner leaflet, and iii) externalized in apoptotic parasites, as described for phosphatidylserine in several cell lines. More studies have been carring out in our lab to outsourcing IPC localization in apoptotic cells.
Supported by:Fapesp

BQ027 - **PHOSPHOLIPIDS MOVEMENT ACROSS THE PLASMA MEMBRANE ON THE LEISHMANIA GENUS.**

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The protozoan *Leishmania* alternates the two forms between insect and mammalian hosts. In the latter host it is an intracellular parasite infecting macrophages. It has been suggested that lipid organisation of the plasma membrane of the parasite can play a role in the phagocytic process as well as in the ability to survive in mammal host. Phosphatidylserine (PS) exposure at the exoplasmic leaflet of the plasma membrane could be one of the signals that promotes phagocytosis and inhibits the macrophages activation.
We compared lipid transport across the plasmatic membrane in different *Leishmania* species, covering both *L. (Leishmania)* and *L. (Viannia)* subgenus. All tested parasites species did not bind annexin V in log phase, but part of the population bind the protein when in late stationary phase. The inward transport showed no difference on the lipid specificity at 25 ^{o}C when comparing the cells in log and stationary phases and can not be accounted for the observed differential annexin V binding, even if in most cases, the transport velocity was slightly decreased on stationary phase cells. At 0 ^{o}C, the endocytosis on the cell is hampered, permiting a more precise analysis on the specific lipid transporte machinery. At this temperature the cells from the *L. (Viannia)* subgenus showed no significant phosphatidylcholine (PC) transport. This observation is correlated to an increase in the resistance to the PC analog miltefosine. Only *L. (L.) amazonensis* showed a significant phosphatidylethanolamine (PE) transport, which correlates with an increase resistance to the PE binding drug duramycin. The observed data suggests that the *L. (Viannia)* subgenus parasites, regulates their PS exposure independently of an inward transport machinery on the plasma membrane.
Supported by:Fapesp, CNPq

BQ028 - **STRUCTURAL AND MOLECULAR CHARACTERIZATION SELENOPROTEINS OF TRYPANOSOMA BRUCEI.**

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Selenium is an essential micronutrient for all forms of life. The main and most fascinating way in which selenium is incorporated into proteins is through the amino acid selenocysteine (Sec, U), being co-translationally incorporated into the nascent polypeptide at specific positions of the stop-codon UGA [1,2]. The selenoproteins, proteins that possess this amino acid, present in the active site as the catalytic residue, are involved in several metabolic processes and have unique features. To understand the functions of selenoproteins in kinetoplastids, we initiated studies with the proteins SelK, SelT, and Sel*Tryp* of *Trypanosoma brucei*. Genes selk, and selt sel*tryp* were cloned into the pET 28 and pET 29 vectors with appropriate restriction enzymes for subsequent expression in *E. Coli* BL21(DE3) cells. SelT protein is expressed in *E. Coli* BL21(DE3) soluble fraction and a purification protocol has been development. The expression processes of SelK and Sel*Tryp* is under development. The expressed and purified selenoproteins SelK, SelT, and Sel*Tryp* are been characterized and crystallization screening are under way.
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BQ029 - **CHARACTERIZATION OF HSP90 MOLECULAR CHAPERONE FROM LEISHMANIA BRAZILIENSIS: STRUCTURAL FEATURES, ATPASE ACTIVITY AND INHIBITION STUDIES**

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Protein synthesis *in vivo* is supported by molecular chaperones like Hsp90 that is critical for the proper functioning of the cells. The Hsp90 molecular chaperone is essential for life cycle of protozoaires such as *Leishmania braziliensis* and *Plasmodium falciparum*, acting as a thermal sensor for these organisms. The Hsp90 contains three conserved domains: an N-terminal that binds ATP and has ATPase activity, an intermediary domain (M-domain) and a C-terminal domain. Hsp90 works as a flexible dimer and its function is dependent on its ability to bind and hydrolyze ATP that can be inhibited competitively. The present work aims to produce, by heterologous expression, the Hsp90 from *Leishmania braziliensis* (LbHsp90) for structural and functional characterization and also inhibitory studies. The Hsp90 recombinant protein was expressed in a bacterial system and purified with affinity chromatography followed by size exclusion chromatography. Circular dichroism and fluorescence studies showed that LbHsp90 was obtained folded and analytical ultracentrifugation experiments revealed that the protein is an asymmetric dimer in solution. To access information regarding the Hsp90 affinity for ligands, fluorescence experiments were performed to determine the dissociation constant (K_D) for adenosine nucleotides and the inhibitor geldanamycin, which were of about 130 µmol/L and 3 µmol/L, respectively. The kinetics of ATP hydrolysis by LbHsp90 was also investigated and the K_M and V_{max} values were of about 400 µmol/L and 0.5 µM/min, respectively. Preliminary LbHsp90 inhibition studies showed that its ATPase activity can be inhibited by geldanamycin with an IC_{50} (half-maximal inhibition concentration) in low micromolar concentration range, comparable to the IC_{50} for human Hsp90 protein. More conclusive experiments considering LbHsp90 inhibition are in progress and these results will be presented.
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BQ030 - **ASSOCIATION OF COMPOUNDS ISOLATED FROM THE BRAZILIAN FLORA WITH DRUGS USED AGAINST AMERICAN VISCERAL LEISHMANIASIS**

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The most commonly used drugs against visceral leishmaniasis are based on pentavalent antimonial compounds that play a fundamental role in its therapy for over 70 years. However, the treatment is painful, use the intravenous pathway and have severe toxic side effects that can be fatal. In some parts of India the antimonial resistance is spreading, reaching alarming proportions. It has been shown that some compounds isolated from the Brazillian flora, such as linalool and eugenol, are able to kill *Leishmania amazonensis* and *Trypanosoma cruzi* at low doses. In the present study, we show the effects of these compounds on *L. chagasi* (visceral leishmaniasis) and compared to the effects of glucantime, which was used as a positive control. The MIC for eugenol on *L. chagasi* was 0.1 nanograms/ml and 50% inhibition of proliferation occurred between 100 nanograms/ml and 100 micrograms/ml. The MIC for linalool was 100 nanograms/ml and and 50% inhibition of proliferation occurred between 100 nanograms/ml and 100 micrograms/ml. Regarding the ability to kill *L. chagasi*, the LD 50 of linalool was between 350 and 450 micrograms/ml and eugenol was about 250 micrograms/ml. In the interaction of *L. chagasi* promastigotes with BALBc peritoneal mouse macrophages, when the macrophages were pretreated with linalool, eugenol or glucantime, there was no significant change in the profile, as compared to the untreated control group. In other set of experiments, *L. chagasi* was added to the peritoneal macrophages previous to any drug treatment and let to interact for 4 hours with the macrophages. There was a significant decrease in the number of parasites present within the macrophages when these systems were challenged with the same concentrations of eugenol, linalool or glucantime, suggesting that these drugs killed the parasites inside the macrophages. We conclude that both linalool and eugenol were able to inhibit the proliferation and to kill *L. chagasi*, but this effect was more prominent during the interaction between the parasites and peritoneal macrophages. This set of results suggests that more studies are needed in order to discover a possible mechanism of action of these compounds on the parasites.
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BQ031 - **SIMVASTATIN INDUCES REACTIVE OXYGEN SPECIES (ROS) PRODUCTION IN LEISHMANIA AMAZONENSIS**

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Simvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), the key enzyme of the cholesterol biosynthesis pathway in mammals, is used in the treatment of dyslipidemia and cardiovascular diseases. The benefic effects have been attributed not only to its cholesterol lowering properties, but also to anti-inflammatory and anti-oxidant activities. Leishmania species, similarly to fungi, do not synthesize cholesterol, but ergosterol and derivatives. This pathway is essential to the parasites and inhibition of enzymes such as HMG-CoA reductase, squalene epoxidase, C-14 demethylase and C-24 methyltransferase, by statins, alilamines, azoles and azasterols, respectively, leads Leishmania spp., fungi and Trypanosoma cruzi to death. Investigations of additional mechanisms of action of these drugs have been carried out in fungi, but similar studies in Leishmania are still lacking. Among these alternative mechanisms, the most studied is the production of reactive oxygen species (ROS). The present work aims to investigated the significance of endogenous reactive oxygen species (ROS) produced by Leishmania amazonensis treated with simvastatin. Initially, we evaluated the leishmanial activity of simvastatin in Leishmania amazonensis promastigotes, by MTT assay. The IC50 of simvastatin was 20 µM after 72 h of incubation. ROS production in Leishmania amazonensis was measured by a fluorimetric assay with 2', 7'- dichlorofluorescein diacetate (H2DCFHDA). The ROS production was increased by simvastatin in 1, 2, 3 and 4 hours of treatment in a dose-dependent manner, with an eightfold increase detected with 50 µM. This increase of ROS production was partially inhibited by N- acetyl cysteine(NAC), an antioxidant, at 50 µM. These results indicate that simvastatin induces ROS production in Leishmania amazonensis, the opposite effect to previously described in mammalian cells. This phenomenon may contribute to its selective antileishmanial activity.
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BQ032 - **SERINE 2 PHOSPHORYLATION OF TRYPANOSOMA CRUZI EIF5A REGULATES THE ASSOCIATION WITH POLYSOMES**

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">The protein known as the eukaryotic translation initiation factor 5A (eiF5A) has been proposed to participate in translation elongation and in several organisms it is proposed to act on translation under stress conditions. We have previously shown by phosphoproteomic analysis that eIF5A of exponentially growing *T. cruzi* (TceIF5A) epimastigotes is highly phosphorylated and that it is dephosphorylated at the stationary growth phase. Phosphorylation occurs at the Ser2, Tyr21 and Ser47. As TceIF5A is enriched in polysomes during the stationary growth phase, we hypothesized that phosphorylation might change the affinity of TceIF5A to polysomes. As Ser2 phosphorylation is conserved in yeast, while the two other phosphorylation sites are unique to *T. cruzi,* we decided to investigate the role of Ser2 on the association of eIF5A to *T. cruzi* polysomes. Cells overexpressing wild type TceIF5A or TceIF5A with the serine 2 substituted by alanine (S2A) were generated to prevent phosphorylation at the position 2. At the exponential growth phase the S2A and wild type TceIF5A were found equally associated with the polysomes. In contrast, the association of S2A mutant is more pronounced in stationary cells. These results suggest that the phosphorylation of Ser2 decreases eIF5A binding to polysomes and that TceIF5A dephosphorylation at stationary growth phase should allow the preferential binding to the polysomes, possible promoting translation under unfavorable conditions.
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BQ033 - **PROLYL OLIGOPEPTIDASE, AN ORTHOLOG OF S9A SERINE PROTEASE FAMILY, IS EXPRESSED IN DIFFERENT SPECIES OF LEISHMANIA.**

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Proteases play important roles in many biological processes of parasites, including nutrition and infection, being considered promising targets for new drugs to treat trypanosomatid-caused diseases. In this context, our group previously demonstrated that prolyl oligopeptidase of *Trypanosoma cruzi* (POP Tc80), a collagenolytic serine protease, is involved in active entry of trypomastigotes into non phagocytic mammalian cells. In order to study the POP in *Leishmania* species, we analyzed its expression, activity and cytolocalization in promastigotes. Specific activity was measured from soluble protein extract of *Leishmania amazonensis*, *Leishmania braziliensis* and *Leishmania chagasi* using the fluorogenic POP Tc80 substract N-Suc-Gly-Pro-Leu-Gly-Pro-AMC in a microplate assay. We detected a similar activity in all the species which was readily inhibited by a specific and selective inhibitor of POP Tc80. Immunoblotting was carried out using an anti-POP Tc80 antiserum that revealed a single band of approximately 80 kDa, in all the three *Leishmania* ssp. extracts, corresponding to the molecular mass predicted to *Leishmania* POPs. Immunofluorescence was also performed using POP Tc80 antiserum, confirming the expression of POP in these three *Leishmania* species. Cloning of the genes that codify these POPs is already in progress aiming the production of their recombinant proteins to perform the biochemistry characterization. These recombinant enzymes will be key tools to explore the potential of *Leishmania* POPs as therapeutic targets.
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BQ034 - **RELATIONSHIP BETWEEN ECTO-ENZYMES AND DRUG TRANSPORT IN RESISTANT LEISHMANIA**

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Ecto-nucleotidases are surface membrane-bound enzymes able to hydrolyze extracellular nucleotides. Some functions are suggested for these proteins: cell adhesion, purine acquisition, protection against cytotoxic effects of extracellular ATP and, recently, multidrug resistance (MDR) phenomenon. P-glycoprotein (P-gp) is involved in the removal of drugs, most of them positively charged, from the cytoplasm. P-gp is also associated with movement of ATP, an anion, from the cytoplasm to the extracellular space. The central question of this study is to establish the relationship between ecto-enzyme activities and a possible mechanism of drug transport in Leishmania amazonensis. *L. amazonensis* promastigotes were selected by gradual increasing concentrations of the vinblastine from 10 µM to 100µM, and the cells were maintained continuously under drug pressure. The cells were used to determine Mg^{+2} dependent ecto-ATPase activity by measuring ^{32}Pi release from the substrate $\gamma^{32}Pi$ ATP. In addition, ecto-phosphatase, ecto-5'- and ecto-3'-nucleotidases were assessed by colorimetric method by the complex formation of Pi (Fiske and Subbarow, 1925). Our results show that ecto-ATPase activity from resistant Leishmania presented a higher activity rate compared with wild-type Leishmania (8,15 ± 0,74 and 19,94 ± 0,6 nmolPi x h x 10-7cels respectively). This increase is progressive with increasing drug concentrations. In addition, other ecto-enzyme activities did not presented this increment with drug pressure. Moreover, our data show that extracellular ATP (5mM) increases drug efflux. This initial results suggest a possible relationship between resistance and ecto-ATPase activity and this can contribute to elucidate the mechanism of drug transport.
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