BQ001 - Inhibition of *Plasmodium falciparum* cysteine proteases by sugarcane cystatins

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Malaria is a disease caused by Plasmodium parasites and affects hundreds of millions of people. Plasmodium proteases are involved in invasion, erythrocytes egress and degradation of host proteins. Among the most studied plasmodial proteases are the falcipains, which are cysteine peptidases located in the parasite's food vacuole. Cystatins are natural cysteine protease inhibitors that are implicated in a wide range of regulatory processes. The phytocystatins (PhyCys) are plant cystatins whose primary sequences have high homology with the members of the cystatin family. The cDNA characterization and recombinant expression of four cystatins from sugarcane was previously reported. These proteins, named canecystatins, can inhibit cysteine cathepsins B, L, V and S and kill pathological microorganism of sugarcane. Here we report that recombinant canecystatins CPI-1, CPI-2, CPI-3, CPI-4 and CPI-4 TAT are able to inhibit the proteolytic activity of P. falciparum parasites and of the recombinant falcipains-2 and falcipain-3 with IC50 in nanomolar range. The binding of the canecystatins to the falcipains were evaluated by mass spectrometry (MALDI/TOF), which confirmed the linkage between the two proteins. In addition, Western blotting analysis was employed to verify if the parasites can degrade the canecystatins. Our results indicated that the parasites can slightly process CPI-1, CPI-2, and CPI-3, but not CPI-4 and –CPI-4 TAT. Furthermore, using FITC-labeled CPIs, we saw that canecystatins can reach the parasites host cells, suggesting that they can exert the inhibitory function inside the parasites erythrocytes. Only infected erythrocytes show fluorescence demonstrating the selectivity of the assay.
In conclusion, the sugarcanes cystatins here presented are promising inhibitors to halt the parasite's development. However, further studies are necessary to better evaluate the inhibition of malaria parasites proteases by canecystatins in vivo. **Supported by:**:CNPq, FAPESP

BQ002 - Intraspecies Variation in *Trypanosoma cruzi* GPI-Mucins: Biological Roles and Differential Expression of alpha-Galactosyl Residues

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GPI-anchored mucins of *T. cruzi* trypomastigotes play an important immunomodulatory role during the course of Chagas' disease. Biological activities of tGPI-mucins from four *T. cruzi* isolates, including benznidazole-susceptible (BZS-Y), benznidazole-resistant (BZR-Y), CL, and Colombiana, were evaluated. GPI-mucins were able to differentially trigger the production of IL-12 and NO in BALB/c macrophages and modulate LLC-MK2 cell invasion. The significance of these variations was assessed after analysis of the terminal a-GAL residues. Enzymatic treatment with a-galactosidase indicated a differential expression of O-linked a-GAL residues among the strains, with higher expression of this sugar in BZS-Y and BZR-Y *T. cruzi* populations followed by Colombiana and CL. Unweighted pair group method analysis (UPGMA) of the carbohydrate anchor profile and biological parameters allowed the clustering of two groups. One group includes Y and CL strains (*T. cruzi* IIandVI), and the other group is represented by Colombiana strain (*T. cruzi* I). **Supported by:**:CNPq FAPESP

BQ003 - Heme stimulates Na+/K+ ATPase activity trough hydrogen peroxide generation in *Leishmania amazonensis*

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Leishmania amazonensis is a protozoan that occurs in many areas of Brazil and causes cutaneous lesions. A recent work of our group has shown the activation of a Na^+/K^+ ATPase in *L. amazonensis*, through a signal transduction cascade involving the presence of heme and PKC activity. Heme is an important biomolecule with a pro-oxidant and signaling capacity. Recently, hydrogen peroxide (H_2O_2) has been considered an important second messenger, being able to stimulate PKC activity in several models. Our goal in this work is to investigate the role of heme-dependent hydrogen peroxide generation on Na^+/K^+ ATPase activity of *L. amazonensis*. Our results shows that increased concentrations of heme, stimulated H_2O_2 generation in a dose dependent manner, reaching its maximum at 2, 5 µM. At this concentration, the stimulatory effect of heme was shown to be around 30 times higher than control, being linear for at least 1 h of reaction. We also tested the effect of protoporphyrin IX, a precursor of heme, Co_protoporphyrin, Sn_protoporphyrin, and the products of heme degradation, bilirubin, and biliverdin on H_2O_2 generation, and none of them caused any effect. H_2O_2 generation seems not to be mitochondrial, since no effect of FCCP or oligomycin was observed. On the other hand, inhibitors of NADPH oxidase activity, diphenylene iodonium (DPI) and apocynin, abolished the effect of heme on H_2O_2 generation. The inhibition of H_2O_2 generation by its inhinitors suggests the participation of this enzyme on heme stimulatory effect. To confirm that heme effect on Na^+/K^+ ATPase was through H_2O_2 generation, we evaluated Na^+/K^+ ATPase activity using increasing concentration of this reactant. As expected, Na^+/K^+ ATPase was stimulated by increasing concentrations of H_2O_2 and reached its maximum at 0.1 µM. We are now investigating the presence of a NADPH oxidase in these cells, and also if this H2O2-dependent mechanism occurs via PKC activity. **Supported by:**:CNPQ, FAPERJ, CAPES

BQ004 - Apigenin induces death in promastigote and amastigote forms of *Leishmania amazonensis* by reactive oxygen species production

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*Leishmania*sis, a parasitic disease caused by protozoa of the genus *Leishmania*, affects more than 12 million people worldwide. Apigenin is a flavonoid present in common fruits and vegetables and is believed to have several biological functions. However, its mechanism of protozoan action has not been studied yet. In this present study, we report the mechanism for the anti*Leishmania*l activity of apigenin against both forms of *Leishmania amazonensis*. Apigenin inhibited promastigote and amastigote growth in a dose-dependent manner - with 24h of treatment reaching 74% of inhibition at the concentration of 96µM (IC50 = 23.68 µM) and with 72h of treatment reaching 83.8% of inhibition at the concentration of 12µM (IC50 = 4.77 µM) - respectively. We observed the activity of apigenin on amastigotes at nontoxic concentrations for macrophages (IC50 for macrophages = 78.72 µM and the Selectivity Index = 16.50). Reactive oxygen species (ROS) production was increased in a dose-dependent manner in *L. amazonensis* promastigotes treated with increase concentration of apigenin (3 – 96 µM) and in infected macrophages at the concentration of 12µM. Pre-incubation of both forms of *L. amazonensis* with reduced glutathione or N-Acetyl-cysteine significantly reduced apigenin-induced cell death. In addition, apigenin caused mitochondrial dysfunction due to collapse of mitochondrial membrane potential in *L. amazonensis* promastigotes. The effects of several drugs that interfere directly with mitochondrial physiology in parasites such as *Leishmania* have been described. Apigenin has been described as a pro-oxidant, generating ROS which are responsible for cell death in some cancer cells. Mitochondrial membrane potential loss can be brought about by ROS added directly in vitro or induced by chemical agents. Taken together, our results demonstrate that apigenin exerts its anti*Leishmania*l effect on *L. amazonensis* due to the generation of ROS and disrupted parasite mitochondrial function. **Supported by:**:FAPERJ, IOC/FIOCRUZ

BQ005 - Differential Gel Electrophoresis (DIGE) Analysis of the Activity of Naphthoimidazoles in *Trypanosoma cruzi* Bloodstream Trypomastigotes

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Chagas' disease is an endemic illness in Latin America, caused by the protozoan *Trypanosoma cruzi*. Effects of available drugs are not satisfactory, therefore we are studying the activity of naphthoimidazoles (N1, N2 and N3) on all *T. cruzi* life stages. The action mechanisms of these naphthoimidazoles have been previously analyzed in epimastigotes by proteomic approaches, confiming the mitochondrion as the main target of the treatment. Here, we are showing the effect of these compounds on bloodstream trypomastigotes using 2D-DIGE (two-dimensional difference gel electrophoresis). For 2D-DIGE assay, the blood parasites were obtained from infected mice in the peak of parasitemia and incubated with the naphthoimidazoles. The protein extraction and quantification, the samples were labeled with CyDye, followed by two-dimensional electrophoresis (pH 4-7, 18 cm, 12% SDS-PAGE). Gel images were analyzed and the gels showed 66 differential spots between untreated and treated samples with the three compounds (44, 16 and 9 for N1, N2 and N3 respectively) ($p<0.01$), which were identified by mass spectrometry. Up to now, the identification of the differential spots was 44, 16 and 9 for N1, N2 and N3, respectively. Functional analysis showed that 21% are associated with protein biosynthesis and 41% are mitochondrial, reinforcing our previous morphological and biochemical data. Those proteins that were not identified will be analyzed in the Orbitrap LTQ-XL mass spectrometer. Such additional information about the parasite cell biology could be crucial for the development of alternative drugs for this neglected and tropical disease. **Supported by:**::CNPq, FAPERJ e FIOCRUZ

BQ006 - Transport of inorganic phosphate in *Leishmania* infantum and compensatory regulation at low Pi concentration

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Background: Proliferation of *Leishmania infantum* depends on exogenous inorganic phosphate (P_i) but little is known about energy metabolism and transport of P_i across the plasma membrane in *Leishmania spp.*
Methods: We investigated the kinetics of $^{32}P_i$ transport, the influence of H^+ and K^+ ionophores and inhibitors, and expression of the genes for the Na^+:P_i and H^+:P_i cotransporters.
Results: The proton ionophore FCCP, bafilomycin A_1 (vacuolar ATPase inhibitor), nigericin (K^+ ionophore) and SCH28080 (an inhibitor of H^+, K^+-ATPase) all inhibited the transport of P_i. This transport showed Michaelis-Menten kinetics with $K_{0.5}$ and V_{max} values of 0.016 ± 0.002 mM and 9.415 ± 0.301 pmol × min^{-1} × 10^{-7} cells, respectively. These values classify the P_i transporter of *L. infantum* among the high-affinity transporters, a group that includes Pho84 of *Saccharomyces cerevisiae*. Two sequences were identified in the *L. infantum* genome that code for phosphate transporters. However, transcription of the PHO84 transporter was 10-fold higher than the PHO89 transporter in this parasite. Accordingly, P_i transport and *LiPho84* gene expression were modulated by environmental P_i variations.
Conclusions: These findings confirm the presence of a P_i transporter in *L. infantum*, similar to PHO84 in *S. cerevisia*e, that contributes to the acquisition of inorganic phosphate and could be involved in growth and survival of the promastigote forms of *L. infantum*.
General significance: This work provides the first description of a PHO84-like P_i transporter in a Trypanosomatide parasite of the genus *Leishmania*, responsible for many infections worldwide.
Supported by:::CNPq, CAPES e FAPERJ

BQ007 - BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF GLUTAMINE SYNTHETASE FROM *Trypanosoma cruzi*

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In *Trypanosoma cruzi*, is well established that glutamate participates in many biological processes such as energy production and metacyclogenesis. Additionally to glutamate, glutamine participates in the ammonium detoxification process by acting as the nitrogen source and furthermore as a link to the pyrimidine synthesis in Trypanosomatids. The conversion of glutamate to glutamine is catalyzed by the glutamine synthetase in *T. cruzi* (*Tc*GS) which accepts glutamate, NH_3 and ATP as substrates. We identify two open reading frames within the genome database of *T. cruzi* (*Tc*GSa and *Tc*GSb) which were amplified, cloned in pET28a$_{(+)}$ vector for recombinant expression and sequenced. Moreover, we determined the specific activity of trypanosomal GS in crude extracts using epimastigotes and a coupled assay, recording, at 340nm, the final oxidation of NADH to NAD^+. The K_m and V_{max} were established for glutamate (0,259 mM and 5,306 µmoles.min^{-1}.mg^{-1} respectively), NH_3 (0,698 mM and 0,154 µmoles.min^{-1}.mg^{-1}) and ATP (0,2032 mM and 0,28754 µmoles.min^{-1}.mg^{-1}). The enzymatic reaction revealed a pH optimum of 8 and is dependent on the divalent cations Mg^{+2} and Mn^{+2} while Ca^{+2} and Zn^{+2} inhibit the enzyme activity. After utilizing differential fractioning to epimastigotes, the *Tc*GS was found in the mitochondrial fraction emphasizing the localization within the respective organelle. Moreover, the *Tc*GS activity was determined in different stages of the parasite, showing the major specific activity in epimastigote form which was subsequently confirmed in real time PCR experiments using total RNA. Using structures of GS already resolved, we infer a homology model of *Tc*GSa and *Tc*GSb predicting binding sites for substrates, cofactors and intersubunit stability. Currently this model is exploited to evaluate the mode of action of inhibitors of the *Tc*GS and we are testing the optimal conditions of recombinant expression. **Supported by:**:FAPESP, CNPq and CAPES

BQ008 - *Leishmania* (L.) amazonensis metabolic profile after arginine starvation.

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Arginine is an essential amino acid to *Leishmania* survival providing polyamines for parasite growth as well as its role on the antioxidant trypanothione synthesis. On the other hand, the amino acid is used by macrophages to produce nitric oxide (NO) to control the infection. Previously we showed that arginase activity, as well as arginine uptake, are important for *Leishmania* infection (da Silva et al 2012; Castilho-Martins et al. 2011). To further verify the metabolic importance of arginine to the parasite, we used metabolic fingerprinting analysis by capillary electrophoresis coupled to mass-spectrometry to evaluate metabolic changes produced by arginine starvation on wild type (WT) or arginase knockout (KO) *L. (L.) amazonensis.* Axenically cultivated parasites at mid log phase (1-2.10^7 cells.mL-1) were or not starved from arginine for 4h. In parallel parasites were cultivated for 14h on media supplemented with ^{13}C-L-arginine to identified compounds on mass spectrometer. Cells (4.10^7) were used to extract metabolites with methanol 50% on tissuelyzer, and submitted to capillary electrophoresis. After data filtering, the compounds that change in response to starvation were identified in a database for *Leishmania*'s pathway. As expected, we found that citruline and ornithine, two arginine derivates, decreased with starvation of WT. Besides, arginine and citrulline concentration were increased on KO cells, as expected by deletion of arginase and consequently increase in the availability of arginine to produce citrulline. However, arginine starvation on those parasites did not reduce ornithine or putrescine. Interestingly, the amount of proline was diminished in arginine starved parasites as well as in KO parasites compared to WT. This observation can be an indicative of a pathway present on mammalian but not described for *Leishmania* that leads the synthesis of ornithine via proline. **Supported by:**:Airbus Military; FAPESP; CNPq

BQ009 - THE ROLE OF MEMBRANE LIPID MICRODOMAINS ON LDL UPTAKE BY *Leishmania amazonensis*.

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Leishmania amazonensis is the causative agent of *Leishmania*sis, a serious tropical disease. Trypanosomatids show incomplete lipid de novo biosynthesis pathways therefore, they take up lipids from their hosts to provide the requirements for growth and differentiation. In this work, we studied the LDL uptake process by *L. amazonensis*, as well as the membrane lipid microdomains (DRMs) involvement. To verify the LDL uptake, parasites were incubated with ^{125}I-LDL for different times. The results showed that *L. amazonensis* was able to capture LDL in a dose-dependent manner. To observe the intracellular fate of LDL lipids, parasites were incubated in the presence of fluorescently labeled LDL. After 3h, the Texas Red-PE was localized at the anterior region of the cell, close to the flagellar pocket while Bodipy-Cholesteryl was distributed in the whole cell. To observe the capacity of the parasites to synthesize cholesteryl-ester, cells were incubated with ^{3}H-palmitic acid and LDL. After 24h, the parasites were subjected to lipid extraction and thin-layer chromatography. We observe that the parasites were able to insert fatty acid into cholesterol. The presence of a LDL receptor was analyzed. Parasite membranes were incubated in the presence of ^{125}I-LDL and 70-fold excess of unlabeled proteins: LDL, HDL and the insect lipoprotein, lipophorin (Lp). The binding of LDL to the membranes was blocked in the presence of an excess of nonradioactive LDL but not in the presence of Lp or HDL. The result indicated the presence of a specific receptor for LDL in *L. amazonensis* membranes. Finally, we investigated the DRMs involvement on LDL endocytosis. Cells were treated with increasing concentrations of methyl-β-cyclodextrin (MBCD), a cholesterol depleting agent that disrupt DRMs. After treatment, parasites were incubated with radioactively ^{125}I-LDL for 24h. The LDL uptake was severely inhibited after DRMs disruption in a dose-dependent manner. **Supported by:**:CNPq, FAPERJ, IFS

BQ010 - BIOCHEMICAL CHARACTERIZATION OF HISTIDINE UPTAKE IN *Trypanosoma cruzi*

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Different roles have been reported for several amino acids beyond their well-known participation in protein synthesis and energy metabolism in the biology of trypanosomatids. It is interesting to note that despite the different biological functions demonstrated for histidine in different organisms (such as antioxidant and anti-inflammatory), little is known about its utilization in *Trypanosoma cruzi*. The uptake of amino acids could be considered as the first step in their metabolism, and this process determine the substrate availability inside the cell. In *T. cruzi* were characterized some of the amino acids transporters, such as proline, glutamate, aspartate and arginine systems. In the present work we describe the transport of L-histidine in *T. cruzi*. Histidine is one of the most abundant amino acid both in the excreted fluids and in the hemolymph of insects, including those that vectorialize this parasite. Therefore, *T. cruzi* could be adapted to consume L-His as energy or carbon source during the insect stages. Initially, was determined the time-course of the histidine transport. The incorporation was proportional to incubation time up to 20 minutes. Then, initial rates of histidine transport (V_0) were measured as a function of extracellular histidine concentration. The data were adjusted to a classical Michaelis-Menten hyperbolic function. Values for V_{max} (0,44 nmoles/min per 20 x 10^6 cells) and K_M (0,25 mM) were obtained. The uptake was not dependent of extracellular ions as Na^+ and K^+, but it was shown to be slightly dependant on pH. When the assay was performed in the presence of oligomycin, the uptake was diminished by approximately in 50%, supporting a dependence on ATP levels. As perspectives, we will test the optimal temperature, the specificity of the transport system, as well as the potential inhibition by histidine analogs. **Supported by:** FAPESP, INBEQMEDI, CNPq and USP.

BQ011 - The interaction among Glyceraldehyde 3-phosphate dehydrogenase protein and telomeric DNA is controlled by the steady-state internal redox (NAD+/NADH) in *Trypanosoma cruzi*

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Several studies show that the intracellular redox state is fundamentally important in maintaining the cellular metabolism and function. Among these redox balances one of the most important is the redox pairs NAD+/NADH, which regulates the function of many molecules. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a multifunctional protein with defined functions in numerous subcellular processes. Among these functions it should be stressed the protection of chromosomes ends in mammalian cells against rapid degradation through its ability to bind telomeric DNA. In this study, we demonstrated that recombinant GAPDH from *Trypanosoma cruzi* (rTcGAPDH) is able to interact with telomeric DNA. Using gel-shift assays, we have showed that rTcGAPDH binds directly a single-stranded oligonucleotide bearing at least one telomeric repeat. Using ChIP assay, we could demonstrate that GAPDH is in fact bound to telomeric DNA in vivo in the proliferative epimastigote cells. Interestingly, [NADH] is higher than [NAD+] in these cells, but exogenous NAD+ was able to block GAPDH-telomere interaction. Corroborating with this hypothesis that NAD+/NADH balance determines GAPDH-telomere interaction, we verified that the non-proliferative/infective trypomastigote present higher intracellular concentration of NADH compared to NAD+ and in these cells GAPDH is not able to bind telomeric DNA. Moreover, exogenous NADH is able to rescue GAPDH-telomere interaction in this stage. Our data show the importace of NAD+ /NADH balance in the interaction of telomeric DNA/GAPDH in trypanosomes, strongly suggesting that the protection of parasite chromosomes ends might be regulated by the metabolic state of the cells. **Supported by:**:FAPESP

BQ012 - Study of the phospholipase A2 in the midgut and crop of Rhodnius prolixus

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Rhodnius prolixus is a strictly hematophagous triatomine of the order Hemiptera, vector of *Trypanosoma cruzi*, the causative agent of Chagas disease. *T. cruzi* has a complex life cycle, involving insect and mammalian hosts and four distinct developmental stages: epimastigotes, metacyclic trypomastigotes, amastigotes and bloodstream trypomastigotes. Metacyclogenesis is the process by which *T. cruzi* epimastigotes differentiate into metacyclic trypomastigotes and acquire infectivity by taking up nutrients present in midgut. Our group is interested in understanding the mechanisms
by which phospholipase A_2 (PLA_2) can act in *T. cruzi* proliferation and differentiation. PLA_2 catalyzes the hydrolysis of the sn-2 fatty acyl bond of phospholipids to liberate free fatty acids and lysophospholipds. In insects, phospholipases are related to
poison, digestion, immunity and reproduction. A gene encoding a group XIIA PLA_2 was identified in the genome of *R. prolixus*. The predicted protein have medium to high levels of similarity and identity in comparison with proteins from other organisms and demonstrated structural features similar to their mammalian counterparts. As evaluated by Reverse Transcriptase-PCR (RT-PCR) using specific primers, transcription of that gene was detected in crop and midgut of fasting fifth stage *R. prolixus*. In addition, to find out the presence of this enzyme, midgut and crop from *R. prolixus* were dissected, homogenized and subjected to a Western blotting analysis, using a human PLA_2 group XIIA antibody. That preliminary result has shown the presence of PLA_2 in the midgut. In next steps of our study, we would like to analyze the influence of the PLA_2 in the intestine *T. cruzi* infection and differentiation.
Supported by::CNPq e FAPERJ

BQ013 - USING SDS-PAGE AND PROTEASE EXPRESSION FOR GROUPING REFERENCE STRAINS OF *TRYPANOSOMA CRUZI* CHAGAS, 1909

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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 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 at pH 7.2. Aliquots of the supernatant containing the protein extract, corresponding to 60 µg protein, were separated by 10% SDS-PAGE. 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 SM, Jaccard, and Dice and UPGM clustering algorithm to obtain final phenograms. For the SDS-PAGE, 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 Tc VI). The second group included only samples identified as Tc I, with a subset of isolates from opossums (G, SC28, Dm28c) and another with a sample of biodeme III (Colombian strain). Also, we demonstrated differences in protease activity profiles of some *T. cruzi* strains described above. This study confirms the potential of this approach to demonstrate the variability within *T. cruzi* species and typing of strains. The present approach enables the control of the authenticity of the reference strains of the parasite, since all the peculiarities revealed in their protein profiles are consistent with previous data from other authors using various techniques. **Supported by:**:CNPq, FAPERJ, CAPES, INCT-Entomologia Molecular

BQ014 - Activity of Acyl-CoA: Cholesterol Acyltransferase in *Leishmania amazonensis*

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Leishmania amazonensis is a flagellate protozoan that causes a serious tropical disease, the cutaneous leishmaniosis. Due to the incomplete lipid synthesis *L. amazonensis* relies on the incorporation of some molecules such as cholesterol from the external environment. Consequently, these parasites acquire exogenous cholesterol directly from the host. Excess cellular cholesterol is stored as cholesteryl esters (CHOE). The conversion of cholesterol in CHOE is catalyzed by the enzyme Acyl-Coenzyme A: Cholesterol Acyltransferase (ACAT). Our group demonstrated that *L. amazonensis* utilizes human Low Density Lipoprotein (LDL) as cholesterol source. Intracellular cholesterol is accumulated in a dose-dependent manner, indicating the activity of ACAT. The aim of this work is to analyze the activity of ACAT in *L. amazonensis*. In order to verify the enzyme activity, parasites were incubated at 4 °C and 28 °C in presence of LDL for 30 minutes and then with ^{3}H-Free Fatty Acid associated with bovine serum albumin. Lipids were extracted at different times and characterized by TLC. The spots corresponding to CHOE were re-extracted from silica and the associated radioactivity was measured by liquid scintillation. Results show that the amount of CHOE increases in a time-dependent manner up to 3 hours indicating the synthesis of these lipids and the action of the ACAT. This result was confirmed in other assay. Parasites were incubated in the same conditions for 1h, 12h, and 24h. At 4 °C, the ACAT activity was severely inhibited. After 24h at 28 °C, the amount of CHOE was up to 50% when compared with 12h of assay. For future investigations, we will purify, characterize and to localize enzyme. **Supported by:**:CNPq, FAPERJ and IFS

BQ015 - Substrate and Immucillin Inhibitor Specificity of Nucleoside Hydrolase from L. donovani

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Protozoan parasites are purine auxotrophs and use only host-derived nucleosides and nucleotides as precursors for DNA and RNA synthesis. Nucleoside hydrolase is involved in the purine salvage pathway and is a target for the development of anti-*Leishmania*l drugs. Nucleoside hydrolases are not found in mammal cells. The L. donovani nucleoside hydrolase accepts a wide range of substrates, including inosine, guanosine, adenosine, uridine and cytidine with a slight preference for adenosine and inosine. Guanosine is not a good substrate. The catalytic specificities differ substantially from those reported for *L. major* nucleoside hydrolase, with relative values of Kcat for the L. donovani enzyme with inosine, adenosine, guanosine, uridine and cytidine as substrate being 32, 53, 1.2, 16 and 9 , respectively, while those described for the enzyme of *L. major* are 100, 0.5, 0.5, 27 and 0.3 respectively.
We have analyzed potential nucleoside hydrolase inhibitors, as an approach to block purine salvage.
Immucillin-H and Immucillin-A gave Ki values of 19 and 80 nanomolar respectively. Immucillin-H (Forodesine) is currently in human clinical trials against lymphatic cancers. Our results may be useful as leads for anti-*Leishmania* agents. The existing drugs to treat *Leishmania*sis were developed for other indications and the treatment suffers from severe side effects. **Supported by:**:CNPQ –FAPERJ-PABMB/ASBMB PROLAB

BQ016 - Platelet-activating factor (PAF) stimulate *Leishmania* chagasi infectivity

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Trypanosomatids resemble higher eukaryonts in several aspects, including the fact that their cellular functions are mediated by signaling pathways involving protein kinases and phosphatases, and second messengers. In the New World, the visceral form of *Leishmania*sis is caused by *Leishmania chagasi*. Platelet-activating factor (PAF) is a potent phospholipid mediator of several cellular functions in diverse biological and pathophysiological processes, such as cell differentiation, inflammation and allergy. In earlier studies, we have demonstrated that PAF triggers the cell differentiation of *Herpetomonas muscarum muscarum* and *Trypanosoma cruzi*. Also, studies from our group showed that PAF modulates the interaction of *Leishmania amazonensis* with peritoneal mouse macrophages. PAF effects in trypanosomatids seem to occur through membrane receptor and intracellular signaling. Also, our group demonstrated a platelet activating factor-like activity isolated from lipid extract of *T. cruzi* epimastigotes. In this study, we demonstrated that intrinsic *L. chagasi* PAF (Lc-PAF) stimulated mouse macrophage infection, when promastigotes were kept for 5 days in the presence of this phospholipid, before interacting with the macrophages. Lc-PAF also modulated *L. chagasi* proliferation and cell signaling. All Lc-PAF effects were abrogated by WEB 2086, a classic antagonist of PAF that binds specifically to PAF-receptor. This set of results suggests that Lc-PAF triggers signal transduction pathways in *L. chagasi* and this may lead to an increase of parasite infectivity. **Supported by:**:CNPq, FAPERJ, CAPES, INCT-Entomologia Molecular

BQ017 - NUCLEOSIDE ANALOGS IN THE INHIBITION OF IN VITRO GROWTH OF *LEISHMANIA* CHAGASI

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Visceral *Leishmania*sis (VL) is a parasitic disease caused by a protozoan from the genus *Leishmania*, which is considered one of the major neglected diseases.
There is a general lack of effective and inexpensive chemotherapeutic for treating protozoan diseases that occur mainly in the developing world. Pentavalent antimonial drugs are a first-line treatment in most affected areas, while amphotericin B and pentamidine are used as alternative drugs. These drugs are expensive, not orally active, exhibit severe side effects and have led to resistance in the parasite population. Research for new drugs is a priority for this disease.
Nucleoside hydrolase is involved in the purine salvage pathway and represents a target for chemotherapy since it is not found in mammals. We tested the effect of eight inhibitors of purine salvage on the in vitro growth of promastigotes of *Leishmania* chagasi.
An inhibitor of adenosine kinase gave an IC50 value on L. chagasi growth of 5 nM for 72 hr growth, but did not completely inhibit growth at 50 nM. Two others gave IC50 values of 5 µM and 9.6 µM, at 72 hr but did not completely inhibit growth at 250 µM. Another gave an IC50 of 27 µM, and also showed incomplete growth inhibition at 250 µM. The others did not reach an IC50 even at a 250 µM. Animal studies will be required to test in vivo effects. The characterization the specific inhibitory effect of the immucilins on the NH of *Leishmania* donovani is in progress using the recombinant enzyme (NH36), an important antigen for vaccination against *Leishmania*sis.**Supported by:**:CNPQ

BQ018 - Characterization of phytol in intra-erythrocytic stages in P. falciparum

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Isoprenic compounds are the most diverse natural products class. There are two pathways for the synthesis of isoprenoids in plants, the 2C-methyl-D-erythritol 4-phosphate (MEP) and the mevalonate. Our group has worked with the characterization of products of isoprenoid biosynthesis in *Plasmodium falciparum* from the MEP pathway. This pathway is not shared by the human host making it an excellent target for the development of antimalarial drugs. Our group has been characterized the presence of tocopherol, therefore, we speculate that the degradation of the product of *P. falciparum* can generate free phytol as has been shown in *Arabidopsis*. In this context, we proposed characterize presence of the recycling the phytol and a salvage pathway biosynthesis for tocopherol from the recycling of phytol in *P. falciparum*. Asynchronic cultures were metabolically labeled with [3H] geranylgeranyl pyrophosphate, direct precursor for the biosynthesis of tocopherol and analyzed by reverse phase - high performance liquid chromatography (RP-HPLC). We detected a radioactive compound with coincident retention time of phytol standard in the three stages of the parasite (trophozoite young, trophozoite and schizont) suggesting that the recycle of phytol occurs in *P. falciparum*. We speculate that the degradation of tocopherol in *P. falciparum* can generate free phytol, which may be acetylated resulting in fatty acid phytyl esters or be phosphorylated, generating tocopherol as a salvage pathway of this compound as well as in *Arabidopsis*. **Supported by:**:Fapesp, CNPq e Capes

BQ019 - Metallopeptidase inhibitors impairs *Trypanosoma cruzi* adhesion to Rhodnius prolixus explanted midgut

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The most abundant surface peptidase of *Leishmania* spp., gp63, is one of the best characterized metallopeptidase in the trypanosomatidae family and homologues of this enzyme have been described in *Trypanosoma cruzi*. The etiological agent of Chagas' disease, *T. cruzi*, expresses multiple isoforms of the gp63 family. This enzyme is required for parasite infectivity in mammalian cells; however, its role in parasite-vector interaction has not been explored so far. The goal of this work is to evaluate the relevance of gp63 metallopeptidase on the adhesion rate of *T. cruzi* to *Rhodnius prolixus* explanted midgut. As a first approach, we evaluate if metallopeptidase inhibitors would influence on the interaction. In this sense, firstly, we performed viability assays of live parasites using increasing concentrations of these inhibitors. Incubation of parasites with 1,10-phenanthroline, EDTA and EGTA for 1 h with concentrations bellow 1 µM did not considerably affected parasite viability. The effects on parasite adhesion rate to *R. prolixus* posterior midgut was determined after pre-treatment of *T. cruzi* with 1,10-phenanthroline, EDTA and EGTA, for 1 hour, followed by washing and interaction with *R. prolixus* explanted guts for 15 minutes. Afterwards, the number of parasites per midgut epithelial cells was estimated by randomly counting at least 100 epithelial cells in quadruplicate. The interaction rate was reduced in the presence of 0.5 µM 1,10-phenanthroline (79 ± 5,95%), 1 µM EDTA (74 ± 12,7%) and 1 µM EGTA (68 ± 15,65%) in comparison to control parasites. Collectively, our results suggest a possible involvement of metallopeptidases in the interaction between *T. cruzi* and epithelial cells from *R. prolixus*. Other assays are being performed to verify the presence of gp63 on the parasite, aiming its future purification and characterization, as well as a more precise implication of gp63 on the interaction with the invertebrate host. **Supported by:**:MCT/CNPq, FAPERJ, CAPES and FIOCRUZ.

BQ020 - NADPH oxidase inhibition decreases macrophage infection by *Trypanosoma cruzi*

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Trypanosoma cruzi, an obligate intracellular parasite, is able to infect and replicate in macrophages, the first line of the mammalian host defense against pathogen infection. Reactive oxygen species (ROS), such as the superoxide anion (O2•-) produced by the NADPH oxidase (NOX) enzyme complex is one of the macrophage main responses to *T. cruzi* infection. Thus, we investigated the crosstalk between NOX and ROS in the *T. cruz*i-macrophage infection establishment in vitro. For this end, we used two NOX inhibitors, diphenyl iodonium (DPI) and apocynin, and the potent antioxidant, N-acetyl-cystein (NAC), and evaluated the effect of these molecules on both macrophage and metacyclic trypomastigotes survival for 3 h, 24 h, 48 h or 72 h. We observed that only the DPI treatment significantly diminished macrophage and trypomastigote survival after 72 h. Additionally, macrophages were incubated in DMEM supplemented with 10% FCS containing DPI, apocynin or NAC and trypomastigotes at a 1:10 rate, 37 °C. After 72 h, the infection was quantified by amastigote counting. We observed decrease in the infection in cells treated with DPI, apocynin and NAC. We also performed real-time PCR to quantify the parasite loads. The total genomic DNA was used as a template, and specific *T. cruzi* TCZ and mouse GAPDH primers were used to normalize the relative quantification. We also observed a decrease in the parasite loads of macrophages treated with DPI, apocynin and NAC. Our results suggest that the NOX enzyme modulates the establishment of *T. cruzi* infection. In this scenario, the oxidant environment promoted by O2•- seems to favor amastigote proliferation. Indeed, the exposure of cells to the antioxidant NAC also reduced the infection. In conclusion, we provide evidence of a new role for NOX activation in *T cruzi* infection in vitro, considering that its inhibition impairs a relevant part of the parasite life cycle. **Supported by:**:PIBIC-UERJ, FAPERJ, CNPq

BQ021 - Tellurium IV compounds as a Novel Class of *Trypanosoma brucei* Oligopeptidase B mechanism-based inhibitors

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The *Trypanosoma brucei* oligopeptidase B (OPTb) are classified as serine-peptidases that possess an important role in the pathogeny of human african trypanosomiasis (known by sleep sickness). This peptidase is involved in the disordered degradation of hormonal peptides commonly transported in the infected patient's blood, and the search for new drugs to this enzyme could be a drug target to treat the disease. It is known that OPTb possess 14 cysteine residues on its structure and that some are exposed into the solvent. Based on that, it was prepared a set of compounds that are able to irreversibly inhibit the OPTb thought reacting with the free cysteine thiol groups. The IC50 values were obtained and the inhibitions experiments were determined by an irreversible kinetic model using fluorescent substrates. We found that the tellurium IV set of compounds inactivates the OPTb in vitro showing very low Ki values (0.001 to 0.69 μM). These low Ki values are related to the structural conformation of the inhibitor, where the tellurium atom are more exposed into the solvent allowing a better interaction with the thiol groups than other compounds assayed. These preliminary results indicate that inhibition outside of the active site is a feasible approach and that compounds 10, 11 and 14 could be used for in vivo assays as a potential leads for therapeutic drugs. **Supported by:**:Capes

BQ022 - CLONING AND EXPRESION OF TWO ENZYMES INVOLVED IN BIOSYNTHESIS OF PROLINE IN *TRYPANOSOMA CRUZI*

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Trypanosomatids can use amino acids as a source of carbon and energy. These metabolites were also involved in several functions in those organisms. In *Trypanosoma cruzi*, the involvement of amino acids in osmoregulation, metacyclogenesis, cell invasion, differentiation of epimastigotes to trypomastigotes and resistance to different types of stress is well documented. As proline is mainly involved in all the previously mentioned processes, we are focusing the present work on the enzymes involved in its biosynthesis in T. cruzi. Due to the absence of a functional urea cycle in this parasite, we hipothesize that proline biosynthesis only proceeds from glutamate, involving two enzymes. A search for genes for putative enzymes of the glutamate – proline pathway was performed in the *T. cruzi* genome database. In the present work, we show that this pathway is operative in *T. cruzi* by measuring the synthesis of proline from glutamate. Both genes coding for putatives Δ1-pyrroline-5-carboxylate reductase (P5CR) (EC 1.5.1.2) and Δ1-pyrroline-5-carboxylate synthetase (P5CS) (EC: 1.2.1.41 & 2.7.2.11) were cloned and sequenced. They contain open reading frames of 810 and 2259 bp, compatible with the full length sequences. When the sequences were analyzed by using pFAM database, domains for amino acid kinase and aldehyde dehydrogenase family were found for TcP5CS and domains for NADP+ oxidoreductase coenzyme F420-dependent family were found for TcP5CR, as expected. The TcP5CR was successfully expressed and purified in its active form in Escherichia coli BL21 Codon Plus (pGro7) strain, using pET28a vector upon induction with IPTG. The recombinant protein was purified by affinity chromatography using NTA-Ni2+ resin. The apparent molecular weight for TcP5CR was 28 kDa as verified by SDS-PAGE. Kinetic analysis on parasite extracts and recombinant proteins are being performed. **Supported by:**:Cnpq, Fapesp, Inbeqmedi

BQ023 - Studies of Mitochondrion and Glycosomes of Trypanosomatid Harboring Symbiotic Bacterium, The Strigomonas culicis

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Strigomonas culicis is a trypanosomatid that harbours a symbiotic bacterium in the cytoplasm. Both maintain a mutualistic relationship, thus constituting an excellent model for studying metabolic exchanges between the partners, including, the origin of organelles and cellular evolution. The influence of the endosymbiont on the mitochondrion respiration of its host protozoan was measure. It was also studied the glycosomes distribution in host cells. The oxygen (O_2) consumption capacity of wild type and aposymbiotic strains of *S. culicis* were measured on high resolution respirometry. We utilize optical and electron microscopy to monitored the position of glycosomes. The quercetin was apply to stain glycosomes in fluorescence experiments. Results showed that the respiratory rate of wild type cells is three folds higher, when compared to data obtained with the aposymbiotic protozoa. In the presence of 3µg/mL oligomycin, the O_2 consumption of the wild type cells is reduced in 50%, while the aposymbiotic protozoa rate did not change. After titrations with FCCP, the O_2 consumption of both strains was increased, but aposymbiont uncoupled cells (FCCP) attained higher rate of consumption, compared to the aposymbiont cell respiration, without oligomycin. The complex I inhibitor, 20µM rotenone, decreased 50% wild type cell respiration. However, the effect of rotenone on aposymbiotic cells reduced 90% the O_2 consumption rate. The preliminary microscopy observation shows the distribution of glycosomes in the cytoplasm of wild type strains. Promising, the presence of endobacterum influences the glycosomes of host protozoan, once that are important energetic organelles such as mitochondrion. Plus, results of mitochondrial metabolic suggest that the bacterium can participate of energy metabolism of the trypanosomatid. **Supported by:**:CnPQ e FAPERJ

BQ024 - Na+-dependent and Na+-independent mechanisms for inorganic phosphate uptake in *Trypanosoma cruzi*

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Intact epimastigotes of *Trypanosoma cruzi* grown under limiting inorganic phosphate (P_i) are able to transport this anion to cytosol at a high rate through two different carriers, Na^+-dependent and Na^+-independent symporters. Cloning studies demonstrated that this parasite expresses TcPho89, a high-affinity Na^+:P_i-symporter and TcPho84, a high-affinity H^+:P_i-symporter. P_i influx shows a Michaelian-like dependence on P_i concentration for both mechanisms; however, the Na^+-dependent shows higher affinity, with an apparent K_m of 9.3 ± 1.2 µM (72.9 ± 10.8 µM for the Na^+-independent). Addition of the H^+ ionophore FCCP results in a decrease of P_i influx to very low levels, which is consistent with an electrical potential-driven P_i:H^+ transport. Treatment with the K^+ ionophore valinomycin, or with SCH28028, an (K^++H^+)-ATPase inhibitor, significantly inhibits P_i uptake. These results support the view that an inwardly directed H^+ gradient also contributes to energize the uphill H^+:P_i entry. Furosemide, ouabain-insensitive Na^+-ATPase inhibitor, decreased only the Na^+-dependent P_i uptake, indicating that the former is the Na^+ pump responsible for the inwardly-directed Na^+ gradient required by the symporter. Metabolic inhibitors modify the kinetic parameters of P_i uptake, indicating that both the affinity and the transport capacity do not represent only specific properties of the P_i transporters since they can be influenced by metabolism, likely through an influence in the intracellular concentration of cations and/or in the transmembrane electrical potential. Trypomastigotes forms are inefficient to uptake P_i when compared with epimastigotes forms. Altogether, the results demonstrate that P_i starvation stimulates P_i uptake allowing its acquisition via two mechanistically different and metabolism-dependent processes, during epimastigotes development and differentiation to trypomastigotes forms. **Supported by:**:MCT/CNPq, CAPES, FAPERJ, INCT/INBEB.

BQ025 - Role of the Casein Kinase 2 (CK2) in the *Leishmania* braziliensis-macrophages interaction

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When *Leishmania* parasites enter in a human host, they undergo metacyclogenesis and acquire the ability to interact with macrophages. This interaction activates signal transduction pathways inducing numerous biological activities, including protein kinase CK2. CK2 has been observed in all eukaryotic cells residing in the nucleus, the cytoplasm and on the cell surface. It appears to have an essential function and recognizes serine/threonine or tyrosine residues in target proteins for phosphorylation. In a *L. braziliensis* virulent strain, studies show CK2 activity 23-fold greater than in a non-virulent strain. Specific CK2 inhibitors can inhibit the growth of virulent promastigotes, but not those of a non-virulent strain. Initially, the ck2 gene from *L. braziliensis* was cloned into a recombinant protein expression vector. This clone, confirmed by sequencing, was used to characterize the genomic organization and to produce recombinant CK2a used for antibody production. The anti-CK2a obtained was able to recognize recombinant and native parasite CK2, both secreted and cytoplasmatic. Antibodies anti-CK2a inhibited the association index between an infective *Leishmania* strain and macrophages by 61%. Spermine was able to stimulate the native secreted CK2 (133.15%) but was not able to stimulate the recombinant CK2a activity. The recombinant CK2a specific activity was 63.11 nmoles Pi/mg.min and was inhibited in the presence of heparin (84.22%) or TBB (97.6%). Secreted CK2 was also inhibited by heparin (87.57%) and TBB (96.64%). Recombinant CK2 (0.25 mg/mL) increased the association index (11%), where the process was reversed by heparin (43.45%) and TBB (53.55%). Recombinant CK2 (1mg/mL) increased the association index (96%), with reversal by heparin (59.1%) and TBB (66.4%). These findings demonstrate the CK2 enzyme importance in the process of host - parasite interactions. In this way, studies involving this enzyme can be interesting for development of new drugs or vaccines. **Supported by:**:CNPq, FAPERJ and PIBIC-UERJ

BQ026 - Effect of aspartyl peptidase inhibitors on multiplication and aspartyl peptidase activity of *Leishmania* species, including strains obtained from HIV-positive patients

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There is a general lack of consensus about the susceptibility of distinct *Leishmania* species to the HIV aspartyl peptidase inhibitors (HIV-PIs). In this study, we performed a comparative analysis of the effect of HIV PIs on different *Leishmania* spp., including strains obtained from HIV-positive (HIV+) patients under antiretroviral treatment or not. Nelfinavir was capable of significantly reducing the multiplication of many *Leishmania* reference strains and isolates obtained from HIV+ patients either under antiretroviral treatment or not. *L. major* growth was inhibited in approximately 50%, while all other flagellates were strongly inhibited (at least 94%), except for a L. chagasi strain obtained from an HIV+ patient under treatment with Highly Active Anti-Retroviral Therapy (HAART). The cultivation of this isolate in the presence of nelfinavir induced a considerably reduction in the aspartyl peptidase activity. In addition, nelfinavir was also capable of inhibiting, in a dose-dependent manner, aspartyl peptidase activity of all *Leishmania* strains tested, which suggest that an aspartyl peptidase may be the intracellular target of the HIV-PIs. However, HIV+ patients under treatment with HIV-PIs are susceptible to develop lipodystrophy, which is characterized by lipid accumulation. In this sense, it is reasonable to consider that interference on *Leishmania* lipid metabolism may also account to HIV-PIs action. Indeed, we observed the accumulation of lipid vesicles in HIV-PI-treated *Leishmania*. Finally, it was possible to identify reactive bands to an anti-aspartyl antibody in lysates of *Leishmania*. Collectively, these data may contribute to the study of the effect of HIV-PIs on *Leishmania* infection and add new insights into the possibility of exploiting aspartyl peptidases as targets to treat *Leishmania*sis.**Supported by:**:CNPq, FAPERJ, CAPES and FIOCRUZ

BQ027 - Anti-*Leishmania* braziliensis activity of 1,10-phenanthroline and its metal-based complexes

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Peptidase inhibitors/chelating agents such as 1,10-phenanthroline and its substituted derivatives, in the metal-free state or as ligands coordinated to transition metals, interfered with the function of several biological systems. In previous works, our group described that *L. braziliensis* produced gp63 molecules sensible to 1,10-phenanthroline. Herein, we initially studied the cellular distribution of gp63 in a virulent strain of *L. braziliensis* by biochemical and immunocytochemical analyses. After that, we reported the inhibitory effects of three 1,10-phenanthroline derivative compounds, 1,10-phenanthroline-5,6-dione (phendio), [Cu(phendio)2]and [Ag(phendio)2], on both cellular and extracellular metallopeptidase activities produced by *L. braziliensis* promastigotes as well as their actions on the parasite viability and on the interaction with murine macrophage cells. The gp63 molecules were detected in several parasite compartments, including cytoplasm, membrane lining the cell body and flagellum, and flagellar pocket. The treatment of promastigotes of *L. braziliensis* for 1 hour with 1,10-phenanthroline and its derivatives resulted in a significant inhibition of cell viability and showed an irreversible mechanism of action. These metallopeptidase inhibitors induced apoptosis in *L. braziliensis* promastigotes as judged by annexin/propidium iodide staining and TUNEL assay. The pre-treatment of promastigotes with metallopeptidase inhibitors induced a diminishing on the expression of surface gp63 as well as a significant reduction on the association index with macrophages. In parallel, the treatment of *L. braziliensis*-infected macrophages with the 1,10-phenanthroline. The synergistic action on growth ability between 1,10-phenanthroline derivative compounds and glucantime, when both were used at sub-inhibitory concentrations, was also observed. Collectively, these compounds present a good perspective for studies to development of new anti-*L. braziliensis* drugs. **Supported by:**::CNPq, FAPERJ and PIBIC-UERJ

BQ028 - The use of a Cerrado soil metagenomic library in the search of new leishmanicidal drugs

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Chemotherapy of *Leishmania*sis is quite longstanding, despite the advances in recent years. The drugs used to treat this disease have high toxicity with severe side effects, reasons for looking for new treatment alternatives. Soil microorganisms have been the most valuable source of natural products, providing important active biomolecules. However, most soil microorganisms cannot be cultured using traditional cultivation techniques. Metagenomics is a promising approach that allows access to the genome of these uncultivable microrganisms and is based on environmental DNA cloning to access the genome of a given microbial population. This study shows the standardization of a soil metagenomic library screening to search for clones that produce biologically active compounds against protozoa of *Leishmania* genus. The screening was performed in a metagenomic library from Cerrado soil containing 500,000 clones in cosmid pJSS using *Escherichia coli* as host. The library was cultivated in LB-agar plates (1.500 clones/plate) for 1 day at 37 ºC and 4 days at 24 ºC and clones with leishmanicidal activity was determined using an overlay assay with *Leishmania major* promastigotes. If some metagenomic clone produces a leishmanicidal compound, the parasites will not proliferate around the colony and this clone can be identified by a halo. Our initial screening in this metagenomic library identified 85 clones that produce a proeminent halo around the colonies. After re-testing, six of them were confirmed. Two positive clones were submitted to DNA sequencing to identify the genes involved in the production of bioactive compounds against *L. major*. In conclusion, the screening of libraries derived from soil metagenome provides opportunities to explore genetic and metabolic diversity of soil microorganisms and this strategy may result in the isolation of novel bioactive molecules. **Supported by:**::FAPESP

BQ029 - Analysis of phospholipid and sphingolipid composition of L. (V.) braziliensis promastigotes by electrospray ionization-mass spectrometry (ESI-MS)

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Metabolism of phospholipids (PL) and sphingolipids (SLs) in *Leishmania* has been relatively understudied and lipidomic tools have became a powerful platform for the study of lipid metabolism in *Leishmania*. Results of our group showed using myriocin (an inhibitor of long chain base biosynthesis) that SLs for promastigote forms of *L. (V.) braziliensis* are essential for flagellum biogenesis and parasite cytokinesis. In this study it was determined the major PL and SL species using the electrospray ionization (ESI), in both positive and negative modes, in control and myriocin-treated promastigotes of *L. (V.) braziliensis*. The negative ion spectrum of lipid fraction of *L. (V.) braziliensis* promastigotes contains pseudomolecular ion peaks corresponding to phosphatidylethanolamine (at m/z 726.7 and 728.6), to inositol phosphorylceramide (IPC) (represented by the most prominent ions at m/z 778.8 and 780.6, and minor components at m/z 806.5 and 808.6), to phosphatidylinositol (represented by ion at m/z 863.7). A clear reduction in the levels of major promastigote species of IPC, the peaks at m/z 778.6 (d34:1) and 780.6 (d34:0) was confirmed for myriocin-treated promastigotes with concomitant increase of level of peak at m/z 863.7, corresponding to phosphatiylinositol (36:1). Expression of glycolipids (GLs) and IPC were also analyzed by indirect immunofluorescence using two monoclonal antibodies, LST-1 (IgM, anti-IPC) and SST-1 (IgG3 anti-GLs). While SST-1 labels GLs at parasite surface, LST-1 labels parasite internal IPC. These results may contribute to understand parasite lipid pathways and identification of new targets for chemotherapies against *Leishmania*sis. **Supported by:**:FAPESP, CNPq and CAPES

BQ030 - The role of the enzyme Alanine Racemase in *Trypanosoma cruzi*

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The enzyme Alanine Racemase (EC 5.1.1.1) is a pyridoxal-5′-phosphate (PLP) dependent enzyme which catalyzes the reversible racemization of L-alanine and D-alanine. Alanine racemase (AR) is ubiquitous prokaryotes but is mostly absent in eukaryotes (with exceptions that include some species of fungi, yeasts, mollusks and crustaceans) and is absent in mammals. In the *T. cruzi* genome database we found a gene with an open reading frame encoding a putative AR. In this work, the full-length AR ORF was cloned from *T. cruzi* genomic DNA. An anti-TcAR polyclonal antibody against the recombinant protein recognized one polypeptide of the 42 KDa in epimastigote forms. The enzyme activity was detected in different life stages of the parasite, such as epimastigote, culture-derived trypomastigote and metacyclic forms. The AR activity was diminished in intracellular forms. D-cycloserine, a structural analogue of D-alanine that inhibited the AR activity affected the growth of epimastigote. In addition, *T. cruzi* epimastigote forms, under hypotonic stress, showed lower AR activity when compared to cells incubated in isotonic medium. Further studies to determine biological role of free D-alanine in *T. cruzi* will be elicited. **Supported by:**:FAPESP AND CNPQ

BQ031 - METABOLIC NETWORK RECONSTRUCTION IN THE VARIOUS STAGES OF *Trypanosoma cruzi*

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Recently, there has been a growing interest in using the system biology approach as an alternative for analyzing and integrating large amounts of data. This data is mainly generated by technologies such as genomics, transcriptomics and proteomics. In the case of *Trypanosoma cruzi*, the etiological agent of Chagas disease, much of this information is available in the different databases. We integrated the different databases using basics programs based in perl and SQL. The prediction of links in metabolic networks was made extracting information from KEGG, BRENDA, TriTrypDB, and litereture of experimental evidence. Here, we showed a manual reconstruction of the metabolism of this parasite in each of the stages of its life cycle. Metabolic changes observed among the various stages may help to explain some of their biological differences, mainly in the use of carbone fuels, e.g. aminoacids in each stage, where apperently from our reconstruction, is the first carbone fuel in amastigotes due to topological configuration of metabolism in that stage. This complex network approach offers a new perspective for explanation of metabolic adaptation in different environmental circunstances and is posible use this information for improve the drug targeting in these kind of organisms. **Supported by:**:Fonacit

BQ032 - A mitochondrial TcP5CDH and its involvement in respiratory processes

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Trypanosoma cruzi catabolizes L-proline as a main energy source. Δ^1-pyrroline-5-carboxylate dehydrogenase (*Tc*P5CDH) is the second enzyme of the proline-breakdown pathway, catalysing the conversion of P5C into glutamate with NAD(P)+ dependence. *Tc*P5CDH is bound to mitochondrial membranes as observed in either, localisation or proteomic assays. Recombinant *Tc*P5CDH-6xHis was purified to homogeneity, and kinetic data were determined from both, recombinant form and extracts of mitochondrial-enriched vesicles. Moreover, a single putative trans-membrane domain (F198-K221), which presumably spans mitochondrial membranes, was also predicted. SAXS measurements revealed that soluble *Tc*P5CDH is multimeric, being composed by ten monomeric sub-units with a whole molecular weight of 629.7 kDa. SAXS data analysis suggested a radius of gyration of 65.21 ± 0.53 Å with a maximum diameter of 200 Å. Participation of *Tc*P5CDH in respiratory chain processes was also approached. Then, permeabilized stable mutant epimastigotes over-expressing an ectopic copy of *Tcp5cdh*, upon succinate stimulation presented higher oxygen uptake (up to 40%) when compared to wild type cells. Similar results were obtained by using L-proline or DL-P5C as respiratory substrates. Unexpectedly, in these conditions, the addition of ADP was not able to stimulate oxygen consumption, suggesting that both substrates could affect mitochondrial respiration. In addition, the effect of a reported inhibitor for ALDHs (DSF) was tested in distinct parasite life stages, showing a marked trypanocidal effect in proliferative (IC_{50} = 650 nM) and infective forms. Conversely, when cells over-expressing *Tcp5cdh* were grown in the presence of DSF, those exhibited a higher resistance than wild-type cells. Our data postulate *Tc*P5CDH as an essential enzyme involved in the bioenergetics of *T. cruzi..* **Supported by:**:FAPESP

BQ033 - Effects of sodium phosphite on the cell proliferation of different trypanosomatid parasites

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Phosphorus is an essential structural constituent of many biomolecules and plays a pivotal role in energy conservation and metabolic regulation. As a consequence, assimilation, storage, and metabolism of inorganic phosphate (Pi) are highly regulated processes that directly affect cell growth. Molecular dissection of responses to Pi starvation has provided evidence for coordinated expression of genes, including Pi transporters. Phosphite (Phi - HPO_3^{2-}), also referred to as phosphorous acid or phosphonate, is an isostere of the Pi anion in which one of the oxygens bound to the P atom is replaced by hydrogen. Phi is used extensively as a fungicide and also sold as superior source of Pi. Despite having similar structure and mobility, the published data indicate that Phi is a non-metabolizable form of Pi and plants cannot use this as the sole source of P. The observed nutritional effects of Phi are likely due to its oxidation to Pi by microbes and this biological conversion certainly makes Phi an important component of the global cycle but not a direct source of nutrient for plant cells. However, the effects of Phi in protozoa cells were never evaluated. In this context, this work will assess the nutritional and metabolic effects of sodium phosphite on the in vitro cultivation of trypanosomatid parasites. Curiously, we have encountered different effects of supplementation of Pi-starved medium with Phi when we compared two species of *Trypanosoma* (*T. cruzi* and *T. rangeli*) with two species of *Leishmania* (*L. amazonensis* and *L. chagasi*). The results showed that the cell proliferation of *T. cruzi* and *T. rangeli* was improved by the Phi addition to the Pi-starved medium while the in vitro proliferation of *L. amazonensis* and *L. chagasi* was significantly decreased. These effects on *Leishmania* occurred though the parasites were able to acquire Pi from the culture medium. **Supported by:**:FAPERJ / UEZO

BQ034 - Effects of inorganic phosphate starvation on response to osmotic stress in trypanosomatid parasites

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In general, trypanosomatid parasites pass through their digenetic life cycle, encountering many fluctuations in environmental conditions to which they must adapt in order to survive. Extreme fluctuations in osmolarity occur within the gut of the vector, and also when the infective form of the parasites pass out of the vector in the highly concentrated excreta / saliva and rapidly encounter the interstitial fluid of the mammalian host with a much lower osmolarity. Physiological adaptations to hypo-osmotic stress have been studied extensively in a wide range of mammalian cell types as well as in unicellular eukaryotes. Upon exposure to a reduction in external osmolarity, cells initially swell but soon regain nearly normal cell volume by a process that has been termed the regulatory volume decrease, which is accomplished by the efflux of various inorganic ions (such as Na^+ and K^+) and organic osmolytes to the extracellular environment. In addition, acidocalcisomes, acidic calcium-containing organelles present in a number of unicellular eukaryotes, have been postulated to be involved in osmoregulation because they change their polyphosphate and ionic content when submitted to osmotic changes. To investigate the effects of inorganic phosphate deprivation in osmoregulation of different trypanosomatid parasites, we assess the ability of these parasites in recover its normal cell volume after a hypo-osmotic stress when they were maintained at Pi-starved medium. We have observed that all the tested parasites (*Leishmania chagasi*, *L. amazonensis*, *Trypanosoma cruzi* and *T. rangeli*) were affected by the growth in Pi-starved medium, but the effects were more significantly higher in *L. chagasi* and *T. cruzi*. We are now quantifying long- and short-chain polyphosphate content for the correlation of this parameter with the defects of osmoregulation observed in trypanosomatids. **Supported by:**:UEZO / FAPERJ

BQ035 - Different effects of inorganic phosphate starvation in Na+- and (Na++K+)-ATPases in trypanosomatid parasites

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Regulation of the Na^+ concentration is important for parasite adaptation to the host environment and for parasite survival. In *Trypanosoma cruzi*, two Na^+ pumps have been described: the classic ouabain-sensitive ($Na^+ + K^+$) ATPase, and the ouabain-insensitive, furosemide-sensitive Na^+-ATPase. The latter sodium pump is localized in the plasma membrane and most probably functions as an efflux pump of Na^+, thus maintaining ion homeostasis in the parasite. The ($Na^+ + K^+$) ATPase also resides in the plasma membrane, where it catalyzes the ATP-dependent exchange of 3 Na^+ for 2 K^+ across the cell membrane, creating an electrochemical gradient. The presence of this enzyme has been shown in protozoans such as *Leishmania mexicana*, *Trypanosoma brucei* and *Trypanosoma cruzi*. In spite of evidence for the participation of these enzymes in the intracellular regulation of Na^+ and K^+ levels, nothing is known about its regulation by inorganic phosphate starvation in trypanosomatid parasites. The present work aim to study the different ways of regulation of ($Na^+ + K^+$) ATPase and Na^+-ATPase activities by the inorganic phosphate starvation in trypanosomatids. We have been shown that inorganic phosphate starvation causes an inhibition of the *Trypanosoma rangeli* and *Leishmania chagasi* ($Na^+ + K^+$) ATPase activities while stimulates the same enzyme activities from *T. cruzi* and *L. amazonensis*. On the other hands, the ouabain-insensitive, furosemide-sensitive Na^+-ATPase activities was increased by inorganic phosphate starvation in *L. amazonensis* and *T. rangeli*, inhibited in *T. cruzi* and was not different from the control cells of *L. chagasi*. We are now assessing differences in Na^+, K^+ and ATP affinities, and ouabain and furosemide sensibilities between the parasites. The results may be contributing to understanding of new possibilities for regulation of these enzymes in these so different protozoa. **Supported by:**:FAPERJ / UEZO

BQ036 - Comparative studies on the inorganic phosphate requirement for the development of trypanosomatid parasites

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Cell proliferation, growth and differentiation are examples of the cellular events regulated by the inorganic phosphate availability, a macronutrient essential to survival of many cell types. Extreme fluctuations in nutrients concentration occur within the gut of the vector, and also when the infective form of the parasites pass out of the vector in the highly concentrated excreta / saliva and rapidly encounters the intracellular environment of the mammalian host cell with a much lower nutrient availability. Physiological adaptations to starvation stress have been studied extensively in a wide range of mammalian cell types as well as in unicellular eukaryotes. For example, in *Trypanosoma rangeli*, it has been shown that parasites maintained at Pi-starved medium achieve their maximal cell number around the fourth day of the *in vitro* culture and initiates a cellular death process from this moment. In this work, we aim to study the dependence of inorganic phosphate for the cell proliferation of different trypanosomatid parasites. For this, we performed the cell culture of *Trypanosoma cruzi*, *T. rangeli*, *L. amazonensis* and *L. chagasi* in control and phosphate-starved media. As results, we encountered that all the parasites had their cell proliferation significantly arrested by inorganic phosphate starvation, with the exception of *L. amazonensis*. This latter parasite showed around twice / three times more parasites when the PBHIL culture medium was starved of inorganic phosphate. In addition, supplementation of culture media phosphate-starved with tripolyphosphate, which originates free inorganic phosphate in extracellular medium in high concentration, such as control medium, decreases the cell number of *L. amazonensis*, while improves the proliferation of other parasites. Studies with other inorganic phosphate sources and about phosphate utilization by these cells are now in course in our laboratory. **Supported by:**:FAPERJ / UEZO

BQ037 - Different binding of L-trans-epoxysuccinyl-leucyl-amido(4-guanidino)butane to cysteine proteinase of *Leishmania* (Viannia) braziliensis

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*Leishmania*sis is an infection that presents wide global distribution, especially at the tropical and subtropical regions and is caused by species of the genus *Leishmania* as the L. (V.) braziliensis in Brasil. The usual treatment of this disease has a high failure rate which itself justifies a study of new targets for therapy. In this context, enzymes as cysteines peptidases (CPs) can be potential objects for development of new drugs. This class of enzymes plays an essential role in the parasite-host interactions in *Leishmania* spp. Since the development of drugs with potential inhibitory of CPs is based in the way of the biding of their specific inhibitors, in this work we proposed to evaluate the of binding of L-trans-epoxysuccinyl-leucyl-amido(4-guanidino)butane (E-64), an inhibitor of some enzymes from CPs family, by in vitro and in silico assays. In the in vitro assays, a cytosolic fraction of amastigotes were submitted to E-64-Sepharose chromatography and bonded proteins were eluted at different NaCl. We detected that 72 kDa and 66 kDa proteins showed instable binding to E-64. After, we evaluated the binding of the E-64 to CPs in members of the family C1. The sequences of proteinases C1 were selected in GENEDB database. LBRM08.0810 and LBRM29.0850 were chosen for show the lower degree of identity. The models were generated and validated; and subsequent we perform the molecular docking and identified different geometrical of biding compared to crystalized structures deposited in PDB database. The in silico results corroborate with in vitro indicating variations of biding between E-64 and different CPs from L. (V.) braziliensis. These results are a guiding to the development of new drugs based on molecular structure of CPs, suggesting that the design of these drugs should take into account the complexity of structural complexity of these enzymes. **Supported by:**:CNPq

BQ038 - Study of *Leishmania* chagasi Lc06 gene: pH influence on ELISA tests for Canine Visceral *Leishmania*sis diagnosis

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Serological diagnosis of visceral *Leishmania*sis (VL) in dogs is problematic due low sensitivity and/or specificity of the available tests, interfering in disease control and consequently the interruption of the 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. It was synthesized a peptide 20 aminoacids long (EV0710) according to Marrifield (1963) technique. The peptide was tested with ELISA (Enzyme-Linked Immunoabsorbent Assay) using sera from positive and negative dogs for VL. For the follow tests, sera dilutions were 1/100, 1/200 and 1/400 and free peptide was coated at concentrations of 1.0 and 2.5µg/mL in 50mM sodium carbonate buffer pH 9.6. This standard coating procedure did not result in optimal ELISA assay. The obtained results showed disparity among the various tests performed (Araújo et al, 2010; Machado et al, 1997; Saravan et al, 2004). Since pH, charge and ionic strength may affect binding to the solid phase, the pH parameter was studied in more detail with this peptide. For coating of peptide buffers covering a wide pH range were used (from 2.6 to 9.6). There was a distinct optimum for coating of peptide EV0710 with a buffer of pH4.6 (50mM sodium citrate buffer). According to Geerligs et al (1998), when the peptide presents negative charge in certain pH, repulsive forces may act decreasing the interaction between the peptide and the acrylic plate, difficulting the peptide binding. Probably in this pH there was a decrease of repulsive forces, thus improving the peptide binding. The next step will be perform the same analyses with the peptide EV0410, 30 aminoacids long. **Supported by:**:Fapesp