08 FUNGAL MOLECULAR BIOLOGY

08.001 - THE HSP 70 GENE PRESENTS SEQUENCE DIFFERENCES AMONG ISOLATES OF PARACOC CIDIOIDES BRASILIENSIS

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Introduction and Objectives: Paracoccidioides brasiliensis is a thermophilic fungus and is the etiologic agent of Paracoccidioidomycosis. This dimorphic fungus undergoes a complex differentiation in vivo and is responsible for one of the most prevalent mycosis in Latin America. During in vitro growth, phase transitions are triggered when the incubation temperature is shifted from 26°C to 37°C or in the reverse direction. When exposed to environmental stress such as temperature elevation, all living organisms respond by rapidly producing increasing amounts of heat shock proteins (Hsps), which presumably protect cells against the effects of the stress agent. Although a number of heat shock proteins have been identified, Hsp70 is one of the most abundant. Because our group is interested in studying genes involved in the transition from mycelium to yeast, we decided to analyze sequences of the hsp70 gene in different isolates of P. brasiliensis. Methods and Results: We amplified and sequenced by 30 independent sequences of the genomic isolates, isolated the hsp70 sequences of the 21 isolates, using the program Clustal W, to verify differences or similarities between them. We found the following additional sequences within the hsp70 gene of isolate Pb01 in comparison to the homologs in other isolates: three regions with 16, 23 and 22 base pairs located in the first intron of this gene. We also confirmed that the third region (22 bp) correspond to a microsatellite (CTT) region. To verify the uniqueness of these sequences in the isolate Pb01, we designed a reverse primer based in the specific region (23 bp) located in the first intron and a forward primer based in the 5’ UTR region common to all isolates of P. brasiliensis used in this analysis. A fragment of 400 bp was amplified by PCR only in the Pb01 isolate. Conclusion: We found intrinsic differences in the hsp70 gene of P. brasiliensis which may account for heterogeneity between isolates and also it was used as a specific region for the isolate Pb01. Financial support: MCT, CNpq and FAP-DF

08.002 - TRANPOSABLE ELEMENTS IN THE EXPRESSED SEQUENCE TAGS (ESTS) OF PARACOC CIDIOIDES BRASILIENSIS: CHARACTERIZATION OF TWO MAJOR RETROTRANSPOSONS IN THE FUNGUS PARTI CULAR TRANSCRIPTOME

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Introduction and Objectives: Transposable elements (TEs) were cited among the most abundant retrotransposons in the fungus partial transcriptome. TEs are genetic units with ability to integrate into the genome at a new site within their cell of origin. They include RNA mediated LTR retrotransposon, had a conserved ribonuclease H-like domain. Two contigs, contig 400 (97 reads, ~ 50% of the retroelement related specifications: 23 contigs had significant similarity to known retrotransposons and 4 seemed 393 clones (1,5 % of the ESTs), organized in 51 contigs and 84 singlets, fulfilled the specifications: 23 contigs had significant similarity to known retrotransposons and 4 seemed to correspond to a microsatellite (CTT) region. To verify the uniqueness of these sequences in the isolate Pb01, we designed a reverse primer based in the specific region (23 bp) located in the first intron and a forward primer based in the 5’ UTR region common to all isolates of P. brasiliensis used in this analysis. A fragment of 400 bp was amplified by PCR only in the Pb01 isolate. Conclusion: We found intrinsic differences in the hsp70 gene of P. brasiliensis which may account for heterogeneity between isolates and also it was used as a specific region for the isolate Pb01. Financial support: MCT, CNpq and FAP-DF

08.003 - MOLECULAR CHARACTERIZATION OF PARACOC CIDIOIDES BRASILIEN SIS CLINICAL AND ENVIRONMENTAL ISOLATES BY RAPD, RFLP AND PFGE.

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Introduction and Objectives: Considerable genotypic variation was reported when P. brasiliensis isolates were typed by random amplification of polymorphic DNA in the polymerase chain reaction (RAPD-PCR), allowing sometimes the establishment of correlations between genetic patterns and epidemiological data. Restriction fragment length polymorphism (RFLP) analysis was scarcely used although referred as able to reveal the existence of correlations between RFLP patterns and geographic origins of the isolates. Pulsed field gel electrophoresis (PFGE) revealed chromosomal polymorphism and intraspecific genetic variation, however, no possible correlation with epidemiological data was indicated. Virtually no previous work employed multiple methods for genotyping the same set of P. brasiliensis isolates. We present data on the molecular characterization of 24 P. brasiliensis clinical and environmental isolates from different geographic areas using RAPD, RFLP and PFGE. Methods and Results: Total DNA was extracted and samples were analysed by RAPD using six primers: OPG03, OPG16, OPG18, OPG11, OPG15, OPG16. Each primer showed fingerprinting patterns that can distinguish at least two genotypic groups; two groups were distinguished with primer OPG11; 3 with OPG18. 4 groups with OPG03, OPG15 and OPG16, and 5 groups with OPG06. DNA from 8 isolates were digested with endonucleases Hinf I and BglII, and hybridized with probes corresponding to two major retrotransposons (sequences of median repetitiveness in the genome of eukaryotes, potentially useful as markers in molecular epidemiology) from P. brasiliensis transcriptome, PbRtp1 and PbRtp2. The fragment length polymorphism generated with Hinf I showed banding patterns that will be useful by itself. After hybridization of probes the presence of multiple copies of each element was shown and the profiles generated by BglII were particularly polymorphic, allowing the differentiation of stated. Karyotyping confirmed the previously stated. Conclusion: Analysis of number of (4-5) and sizes of chromosomal bands, and at least 5 clearly distinct chromosome banding profiles could be seen among 18 isolates solved by PFGE. The analysis are going to be extended to the whole set of isolates. Dendograms will be generated by computational analysis employing individual and global results on RAPD, RFLP (patterns from each endonuclease and after the hybridization with each probe), and PFGE. Results will probably permit to verify the existence of correlations between genotypic patterns generated by three different methods, the existence of correlations between particular genotypes and the epidemiological information available on each isolate, and the applicability of the results on the estimation of the genetic distance among the isolates, contributing to a better knowledge about aspects of the biology of the microorganism related to genetic variation and recombination. Financial support: CNpq

08.004 - THE CATALASE A OF PARACOC CIDIOIDES BRASILIENSIS: PROTEOMIC IDENTIFICATION, CDNA CLONING AND CHARACTERIZATION

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Introduction: The pathogenic fungus Paracoccidioides brasiliensis is the ethiologic agent of Paracoccidioidomycosis (PCM) the most prevalent systemic mycosis in Latin America. The fungus grows as mycelium at 22°C, and as yeast at 30°C in vitro and in the host tissues. Pathogens are exposed to the reactive oxygen species (ROS) derived from endogenous and exogenous sources. Those organisms possess a number of antioxidant enzymes that serve as protective mechanisms. Those enzymes include superoxide dismutase (SOD), glutathione peroxidase (GPX), peroxiredoxin and catalase. Catalases are antioxidant metalloenzymes, which protect cells against oxidative damage caused by hydrogen peroxide produced by phagocytes cells of the host. There are three families of catalases: Mn catalases, bifucatalasomes and monofucatalasomes. The last family corresponds to homotetrameric heme-containing enzymes that are composed of two clearly distinct classes, large-subunit and small-subunit catalases. In the present work we characterized the cDNA encoding a catalase A of P. brasiliensis and identified it in the fungus proteome. In addition we compared the transcriptional expression of Pb CatA in different phases of the pathogen, yeast and mycelia, as well as evaluated the expression of catalase A in the presence of H2O2. Methods and Results: A cDNA encoding a catalase A of P. brasiliensis (Pb CatA) was characterized (GenBank accession number AY494834). The complete cDNA presents 2583 nucleotides and the deduced protein presents 760 amino acids. It could be observed the active site and the presence heme-ligand signature of catalases besides also amino acids residues related to the binding to the substrate and to the correct folding of catalases had been identified. Homology search and phylogenetic analysis indicated that Pb CatA is highly similar to other catalases of large-subunits of pathogenic fungi. A protein species of 84 kDa, pI 6.12 was isolated from two-dimensional gels and submitted to microsequencing of internal peptides. The native protein revealed strong homology to catalase A from several sources and corresponds to the obtained cDNA (Pb CatA). The expression of transcripts encoding Pb CatA was assessed by Northern blot hybridizations of total RNA. These results
08.005 - PARACOCIDIOIDIES BRASILIENSIS CELL WALL: SOME GENES INVOLVED IN SYNTHESIS AND REMODELING
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Introduction: In P. brasiliensis cell wall, the yeastlike (Y) phase, but not the mycelial (M) one, contains α-1,3-glucan as the main (95%) glucose polymer (Mycopathologia 62: 77-86, 1977). It is synthesized by α-1,3-glucan synthase (PbAGS) and hydrolysed by α-1,3-glucanase (PbAGase), an autoregulatory system of synthesis and degradation. Their gene sequences are affiliated to genes from other orders of Pneumocystis and mycobacteria, the only other families of the genus, of which PbCHS5 probably directs the deposition of actin. Herein we present results on their sequencing and expression. Methods: Growth of P. brasiliensis Pbh37 (ATCC 23071) and molecular techniques were as before (Yeast 21: 211-218, 2004). In some experiments, medium was supplemented with 5% horse serum (HS). To study the PbAGase gene, heterologous Southern and colony hybridization were done using a Histoplasma capsulatum 750 bp fragment as radiolabeled probe. To analyse the upstream region of (ODC) IN, currently we are searching a genomic content. The PCR-step down technique allowed the completion of the 5’ end of PbCHS5, a task unsuccessful with other methods. A post-transcriptional regulation of PbAGase has been confirmed by RT-PCR, quantitative expression analysis, and a frameshifting of its transcript. When the levels of ODC (and PbAGase) are increased, PbAGase is the transcribed gene, to protect the genome from the oxidative stress. However, our results indicate that the higher expression at 72 h into the transition, when more than 90% of the cell phase shows yeastlike structures. This result might suggest the importance of this gene in the formation of the spherical morphology. Reverse genetics studies could help to clarify this preliminary finding and the role of the PbAGase gene in morphogenetics.

08.006 - ANTIZYME AS REGULATOR OF ORNITHINE DECARBOXYLASE (ODC) IN PARACOCIDIOIDIES BRASILIENSIS
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Introduction: In P. brasiliensis, growth of the pathogenic yeastlike (Y) phase and transition from the mycelial (M) to the Y phase are accompanied by an increase in polyamines levels and ODC activity (Arch. Microbiol. 165: 311-316, 1996; ibid 166: 411-413, 1997). Northern analysis of the PbrODC gene does not correlate with such increment in activity, in agreement with biochemical data. Subculturing of P. brasiliensis from the Y to the M phase, in absence and presence of the substrate, suggests a putative role in the round multibudding shape. There was no increment in α-glucan layer and loss of the multibudding round shape of Y cells. Addition of α-glucan to the growth medium was shown to inhibit the formation of the Y phase. The protein was induced in the presence of the substrate suggesting a putative role in the protection against ROS in the life cycle of the pathogen. Financial support: MCT/CNPq and FUNAPE/UFJ.

80.007 - PBSEP2, A MEMBERS OF THE PARACOCIDIOIDIES BRASILIENSIS SEPTIN FAMIL
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Introduction: Septins are proteins involved in a wide variety of processes from cytokinesis and cell morphology, to regulation of the cell cycle in eukaryotic cells. In fungi, they are involved in selection of the budding place, chitin deposition, and spore cell wall formation. In this work we set up to study PBSEP2, one of the genes conforming this family in P. brasiliensis. Expression of PbSEP2 was shown to be involved in the transition from the mycelial (M) to the yeastlike (Y) phase occurs. Methods: Southern analysis of P. brasiliensis DNA was done using as radiolabeled probe a PCR fragment of 660 bp, amplified with degenerated primers, designed on high homology regions for fungal septins. From Southern analysis, a new putative protein was identified, which being screened to the colony hybridization, in order to get the whole gene sequence. The polyadenylation site has been identified by the 3’ RACE system (Invitrogen, CA) using the sequence of the PCR product for the design of specific primers. Expression of the gene was followed by Northern analyses in the Y and M phases, as well as during the dimorphic switch from M to Y. As loading control, a fragment of the Pbph418S gene was used. Results: The deduced aminoacid sequence for the PCR product obtained by the use of degenerated primers, shows high identity to Emericella nidulans ascC and other fungal septin proteins, and has been designated PBSEP2. An 840 bp fragment was amplified by the 3’ RACE system. Sequencing of the amplified fragment confirmed the ending point of the gene and its 3’UTR region up to the polyadenylation site. Northern analyses show gene expression in Y and M cells, although an increasing signal was detected when transition occurred from the M to the Y phase, with higher expression at 72 h into the transition. Conclusions: Expression of the PBSEP2 gene increases during the M to Y transition, with a higher expression 72 h into the transition, when more than 90% of the cell phase shows yeastlike structures. This result might suggest the importance of this gene in the formation of the spherical morphology. Reverse genetics studies could help to clarify this preliminary finding and the role of the PBSEP2 gene in morphogenetics.
08.009 - THE RECEPTOR FOR ACTIVATED C KINASE 1 HOMOLOGUE OF PARACOCIDIOIDES BRASILIENSIS: STRUCTURAL AND PHYLOGENETIC ANALYSIS
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Introduction and Objectives: Paracoccidiodes brasiliensis is a temperature dependent dimorphic fungus, the etiological agent of Paracoccidioidomycosis (PCM). Morphogenetic transition plays a role in the fungus pathogenesis. Inhaled conidia differentiate into yeast cells in the human lungs, establishing the infection. Little is known about the signaling pathways that control the morphological changes in P. brasiliensis. Here, we focus on one cDNA PfRACK1 (GenBank: AF548359), encoding a homolog of RACK1, which has a highly conserved WD domain with homology to the b subunit of heterotrimeric G proteins. RACK1 functions on mammalian signal transduction pathways most notably as an adaptor protein for the bII protein kinase C isozyme. The interactions of RACK1 with a critical regulator of cAMP metabolism suggest that the protein may be intimately involved in the regulation of pathways activated by adenylyl cyclase. In single-cell eucaryotes, RACK1/cpc2 also regulates growth, differentiation and entry into Go stationary phase.

PfRACK1 homologs were found by bioinformatics programs revealed seven repeats in a WD motif with homology of the family of proteins. Thrombospondin-like domains described as involved in protein-protein interactions. PfRACK1 possesses conserved PKC binding sites in the WD40 repeats III and VI. The protein is 94% and 91% identical to the Aspergillus nidulans and Neurospora crassa RACKs respectively. Phylogenetic analyses among RACK homology were performed and PfRACK1 is found in a sub clade restricted to fungi. The mRNA was present in higher levels in the mycelia saprobic phase than in the subsequent yeast phase. The rack1 gene is present as a single copy in the genome of P. brasiliensis as suggested by Southern blot analysis. PfRACK1 was expressed in bacteria as a fusion protein with GST using the pGEX-4T-3 vector. The expression of the recombinant protein in Escherichia coli BL21 Star (DE) containing the plasmid was induced and the recombinant protein purification is under progress. Conclusion: Since at least some of the proteins that bind to RACKs including PKC itself regulated cell growth and differentiation the studies involving RACKs could help to elucidate the signaling pathways leading to those processes in the dimorphic pathogenic fungus P. brasiliensis. Financial support: MCT/CNPq, CAPES, FUNAPE-UFG

08.010 - A FAMILY OF BETA (1-3) GLUCANOSYTRANSFERASES OF PARACOCIDIOIDES BRASILIENSIS: IDENTIFICATION, COMPARATIVE ANALYSIS AND HETEROLOGOUS EXPRESSION
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Introduction and Objectives: Paracoccidioidomycosis is a human systemic mycosis caused by the thermal dimorphic fungus Paracoccidioides brasiliensis. The fungus molecular architecture and functional components of the yeast and mycelial forms. The role of the cell wall constituents α-1,3-glucan and β-1,3-glucan in the regulation of the dimorphic transition in P. brasiliensis has been subject of many studies and recent reviews. The β-glucan transferases are periplasmic and cell wall-associated enzymes, responsible for the elongation of β-1,3-glucan chains. They are representatives of cell wall-bound proteins with GPI linkages and function in the remodeling of newly synthesized polysaccharide polymers of the fungal cell wall. Methods and Results: Here, we describe two cDNAs PfGel1 and PfGel3 encoding beta-1,3-glucantransferases. A BLAST search analysis revealed that the cDNA PfGel1 (GenBank: AY324033) and PfGel2 (GenBank: AY340235) present homologues in fungi. Alignments of the predicted P. brasiliensis beta-1,3-GT protein sequences showed that the PfGel2 is 40% identical and 67% similar to the PfGel3. The deduced amino acid sequences of both cDNAs contain a conserved glutamine residue in two conserved catalytic motifs of this family of glycolipid hydrolases, family 76. Cysteine residues, which are conserved in the primary structure of the reported fungal glucantransferases, and a hydrophobic C terminus with a predicted GPI anchor site, were found at PfGel3 and PfGel2. A third glucantransferase sequence encoding Pfgel1 cDNA was obtained by PCR from a cDNA library of P. brasiliensis. Partial results reveal a putative signal peptide and GPI anchor sites which characterize the glucantransferases and GPI-anchored proteins in PfGel1. In order to start functional studies of those molecules, the PfGel3 cDNA was introduced into pTW11 vector to produce the recombinant protein and into pYES2 vector to functional complementation in Saccharomyces cerevisiae. Both experiments are under progress. Conclusion: The GPI-anchored proteins of microbial pathogen have been shown to be immunogenic and represent virulence factors. In addition to those aspects its localization at the cell surface and their active role in cell wall biosynthesis makes the glucantransferases interesting targets for the design of new drugs against PCM. Financial support: MCT/CNPq, CAPES, FUNAPE-UFG

08.011 - THE MITOCHLONDRIAL GENOME FROM THE THERMAL DIMORPHIC FUNGUS PARACOCIDIOIDES BRASILIENSIS
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Introduction and Objectives: Paracoccidioides brasiliensis is a thermos dimorphic fungus that causes a deep systemic infection in humans (PCM), and undergoes a complex transformation: mycelia grows at room temperature and the pathogenic yeasts form at 36°C. Mitochondrial physiology is clearly affected in the mycelial to yeast transformation (J. Bacteriology, 169:4055-4060, 1987). To deepen our understanding of mitochondrial functions in this organism we have sequenced the entire mitochondrial DNA (mtDNA) from Paracoccidioides brasiliensis, isolate 18. Methods and Results: Based on 1713 reads edited and aligned using Phred/Phrap/Consed and CAP3 programs, we could find the genes coding for the three subunits of the ATP synthase (ATP6, ATP5 and ATP7), apocytochrome b (COB), three subunits of the cytochrome c oxidase (COX1, COX2 and COX3), seven subunits of the NAD dehydrogenase (NAD1, NAD2, NAD3, NAD4, NAD5, NAD6 and NAD4L), the large (L-rRNA) and small (S-rRNA) subunits of the ribosomal RNA, two intronic maturases and a ribosomal protein (RMS-5). Twenty-five transfer RNAs were identified for all 20 amino acids. Conclusion: The circular genome comprises 71,335 bp and is characterized by an A+T content of 75%. The genomes are interrupted by polypirimidine repeats ranging from 140 to 240 bp in length. The order of genes/proteins identified appear closest to the mitochondrial genome from Aspergillus nidulans although the size of both genomes are quite different, which is due mostly to the presence of abundant extragenic DNA in P. brasiliensis and also additional introns in COX1, COB, NAD5 and S-rRNA. Another, recently published, thermo-dimorphic fungus Penicillium marneffei has also great synteny with P. brasiliensis mtDNA, with the rRNA clusters having almost the same order in both genomes. Financial support: FAPESP and CNPq

08.012 - CHARACTERIZATION AND HETEROLOGOUS EXPRESSION OF A RECOMBINANT DGFS-LIKE PROTEIN OF PARACOCIDIOIDES BRASILIENSIS
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Introduction and Objectives: Paracoccidioides brasiliensis is a dimorphic pathogenic fungus, the causal agent of paracoccidioidomycosis, a systemic disease which predominantly affects rural communities in South and Central America. The dimorphic transition in P. brasiliensis is readily induced in vitro by temperature changes, resulting in modulation of the composition of cell wall. Here, our studies focus on a DGFS-like protein, a predicted plasma membrane molecule which has been shown to be involved in cell wall biogenesis and to be essential for cell growth in organisms such as Saccharomyces cerevisiae and Candida albicans. Methods and Results: We originally identified from a cDNA library of P. brasiliensis by using the probe PhYnnt (GenBank: AF374353) encoding a mannosyltransferase, the dfg5 cDNA of P. brasiliensis. Sequences analyses revealed that DFG5 encodes a protein with a domain characteristic of the glycosyl hydrolase family 72. Cysteine residues, which are conserved catalytic motifs of this family of glycosyl hydrolases, family 76, and also presents homology to bacterial mannosidases. The translated amino acid sequence of the PhDFG5 predicted a protein of 448 amino acid residues, with a molecular mass of 49.9 Kda and pI of 5.7. A potential cleavage site characteristic of the GPI-anchored proteins was found at Ser110. The cell attachment sequence, RGD (arginine-glycine-aspartic acid), was predicted by protein sequence analysis using the PROSITE algorithm. Moreover, sequence analysis of the P. brasiliensis deduced DFG5, revealed that 22 amino acids at the N-terminus have the characteristic of a signal peptide. The cDNA encoding DFG5 was cloned into the SallNotI sites of pGEX-4T-3 to yield the dfg5-pGEX-4T-3 construct. The recombinant plasmid was used to transform Escherichia coli competent cells and the rDFG5 protein was induced with 0.4 mM of IPTG. The production of antibodies against the recombinant DFG5 is under progress. Functional studies will be performed. Conclusions: Our data suggest that PhDFG5 may be a probable GPI-anchored membrane protein predicted in cell wall biosynthesis and putatively required for cellular differentiation and growth, as described to other microorganisms. In this way, the study of the PhDFG5 may be an interesting goal for elucidating the molecular mechanisms of differentiation of P. brasiliensis and for the design of new drugs against PCM. Financial support: MCT/CNPq, CAPES, FUNAPE-UFG
08.014 - A PROLINE-RICH ANTIGEN (PRA/AG2) HOMOLOGUE OF PARACOCCIDIOIDES BRASILIENSIS
Castro, K. P.; Castro, N. S. S.; Maia, Z. A.; Felipe, M. S. S.; Pereira, M.; Soares, C. M. A.

Introduction and Objectives: The thermal dimorphic fungus Paracoccidioides brasiliensis causes paracoccidioidomycosis (PCM), the leading endemic mycosis in Latin America. The disease may develop as different forms, ranging from benign and localized to severe and disseminated forms (22-23°C). Fungal pathogenesis can be considered by conventional epidemiologic and parasitic phase in human lungs (37°C). The morphological switch from mycelia to yeast is the most important biological feature that enables P. brasiliensis to colonize, invade and survive in host tissues during infection. PRA/AG2 protein has been reported to be a surface antigen in fungi. PRA/AG2 is a component of a glycopeptide, which is probably the main T-cell-reactive component of Coccidioides immitis cell walls, for example. Also, the recombiant PRA/AG2 protein is reactive with sera from patients with active coccidioidomycosis. This protein is suggested to have an endolysoglucanase and activity for impoeration of the disease process by C. immitis. It is located in the fungal cell wall, most probably attached to the cell wall matrix. Due to those characteristics we searched for the cDNA encoding the homologue of PRA in P. brasiliensis. Methods and Results: Here, we described a complete description of a proline-rich antigen homologue of P. brasiliensis. The identified ORF codes for a putative polypeptide of 184 amino acids with a predicted molecular mass of 18.4 kDa and pI of 7.55. Analysis by bioinformatics programs suggested that PRA/AG2 contains a signal peptide with cleavage site between Ala and Glu and is probably located in cell wall. The predicted amino acid sequence also showed a region that contains 7 repeats of the tetrapeptide TXXX, where X is Ala, His, or Gln and X' is Ala, Val, or Gln. A search for protein patterns at the PROSITE Database revealed probable phosphorylation sites (Casein Kinase II and Protein Kinase C), N-myristoylation sites and one predicted CFEM domain (Gln-Glu-Glu-Ser). CFEM is a fungal specific cysteine-rich domain which is found in some proteins with proposed roles in fungal pathogenesis. A putative signal peptide and GPI anchor site (Gly-Xaa-Yaa) which characterize the GPI-anchored proteins were found in Prah49. The expression construct pra-gEX-K-F3 was introduced into Escherichia coli to produce a recombinant protein. Functional studies will be performed. Conclusions: GPI-anchored proteins of various microbial pathogens have been shown to be highly immunogenic and are required for morphogenesis, virulence and for host-fungus interactions. These reactions can be invoked to account for the importance of GPI-anchored proteins in P. brasiliensis. Financial support: MCT/CNPq, CAPES, FUNAPE-UFG

08.015 - CHARACTERIZATION OF AN ASPARTYL PROTEINASE OF PARACOCCIDIOIDES BRASILIENSIS
Tacco, R. A. A. C.; Costa, M. T.; Parente, J. A.; Felipe, M. S. S.; Castro, N. S. S.; Pereira, M.; Soares, C. M. A.

Introduction and Objectives: Paracoccidioides brasiliensis is the etiological agent of Paracoccidioidomycosis that is the most prevalent systemic mycosis in Latin America. This dimorphic fungus undergoes a complex differentiation in vivo. The mycelia produce conidia, which upon inhalation and ingestion are transformed to the yeast forms. One factor that contributes to the process of virulence seems to be the production of proteolytic enzymes. Aspartyl proteinases of fungi are secreted primarily to provide nutrients for the cell and to fulfill a number of specialized functions during the infective process. The protein allows mycelial cells to evade the host immune system, degrading the host extracellular matrix and host proteins also by promoting adhesion and degradation of host barriers during invasion. Aspartyl proteinases are synthesized as zymogens that are self-activated by proteolytic removal of a peptide segment under acidic conditions, first in the endoplasmic reticulum and then in the Golgi to generate the active, mature form of the protein. In this work we present the characterization of a 1.3 kDa cDNA (PbAP), encoding a protein homologous to related sequences of aspartyl proteinases of several organisms. Comparative analysis of the deduced protein was performed. Methods and Results: Recently we identified in P. brasiliensis a complete cDNA (GenBank-A278218) encoding an aspartyl proteinase homologous to related sequences from several microorganisms. The cDNA and the deduced protein were characterized by searches in databases. The deduced PbAP presents a molecular mass of 43.8 kDa and pI of 5.7. Conserved amino acids at the putative active site, a characteristic signal peptide and a proline-rich domain were present. An amplified 1200 bp PCR product was inserted into pGEMT Easy (Promega), in the XhoI and EcoRI sites, and the recombinant plasmid was introduced into E.coli cells. Also, the aspartyl proteinase gene was cloned by PCR of genomic DNA of P. brasiliensis. The mRNA expression profile was studied by Northern Blot Analysis. Conclusion: The characterization of the cDNA of the aspartyl proteinase from P. brasiliensis opens the possibility of analyzing the role of the protein as virulence factor in this fungus. The cloning and characterization of the cDNA, and its future heterologous expression, will provide the tools for understanding the physiological role of this protein in P. brasiliensis pathogenesis. Financial support: MCT/CNPq, CAPES and FUNAPE-UFG

08.016 - THE 14-3-3 PROTEINS OF PARACOCCIDIOIDES BRASILIENSIS: COMPARATIVE ANALYSIS OF TWO CDNAS ENCODING Pb14-3-3-I AND Pb14-3-3-2
Tacco, B. A. C. A.; Cunha-Passos, D. A.; Felipe, M. S. S.; Castro, N. S. S.; Pereira, M.; Soares, C. M. A.

Introduction and Objectives: Paracoccidioidomycosis (PCM) is a deep mycosis of high incidence in Latin America. Its etiological agent is Paracoccidioides brasiliensis, a thermally dimorphic fungus which alternates between an unicellular yeast form (37°C) and filamentous mycelia (26°C). The 14-3-3 proteins are a large family of acidic proteins with a high degree of sequence identity and conservation. A wide array of biological functions for the 14-3-3 protein family has been described, like involvement in cell cycle progression, in cytoskeleton organization, in signal transduction, in stress response, in apoptosis, in cell differentiation, among others. This wide array of cellular functions attributed to the 14-3-3 proteins especially in cellular differentiation, the latter one the main event for the establishment of infection by P. brasiliensis, has led our group to initiate studies with Pb14-3-3s. Methods and Results: Two full-length cDNAs were isolated and characterized encoding the 14-3-3 protein homologues of P. brasiliensis, 14-3-3.1 and 14-3-3.2 are 64.5% identical to Pb14-3-3.1. The deduced amino acid sequences of Pb14-3-3.1 and Pb14-3-3.2 present three regions, as following: a divergent amino acid terminus, a conserved core region and a divergent carboxy terminus. Homology search analysis and preliminary molecular modeling indicate that both Pb14-3-3s may present 9 alpha helices, that could be organized in an antiparallel manner, suggesting an L-shaped structure, as described for the 14-3-3 family. The helices H3 and H5, which contain many charged and polar amino acids and H7 and H9, which contain hydrophobic amino acids, could compose the L-shaped structure, as described for the 14-3-3 family. The characterization of 14-3-3 proteins can provide additional data for the understanding of the intracellular signaling pathways in P. brasiliensis, the mechanisms by which the microorganism may interact with the environment and with the host’s immunological system by wall metabolism, stress response, virulence, protein degradation and heat shock stress, reinforcing the role of those molecules in the temperature induced phase transition. Financial support: MCT/CTNPq
08.017 - **In Vivo-Induced Antigen Technology (IVIAT) Allows the Identification of Immunogenic of Paracoccidioides Brasiliensis**

Paracoccidioides brasiliensis, the etiological agent of paracoccidioidomycosis, is a fungal disease prevalent in Latin America. The fungus occurs as mycelium at 26°C and as yeast at 30°C. In vivo induced antigen technology (IVIAT) is a technique that identifies antigens expressed in vivo during human infection. IVIAT has been used for the identification of open reading frames (ORFs) that may play a role in virulence or pathogenesis. The objective of this work is to identify immunogenic proteins of *P. brasiliensis* expressed specifically during human infection, which might help the pathogen to adapt to the hostile in vivo environment. This technology is expected to facilitate the discovery of new targets for vaccines, for drugs, and to provide diagnostic strategies.

**Methods and Results:** The strain Pb 01 was used to infect nude mice and their infected livers were cultivated for 7 days. The fungal RNA was extracted and used to construct a cDNA expression library. Sera were collected from five patients with PCM from Cuiaíba (MT). Equal volumes of sera were pooled and adsorbed against whole yeast cells, yeast cell lysates and heat-denatured yeast cell lysates of the *P. brasiliensis* Pb 01. This adsorbed serum has been used to screen the cDNA expression library cloned into pCMV-SPORT. We identified 28 positive clones in a first screening, which encode proteins related to cell membrane (1:2-galactosyltransferase), to cellular transport (ABC-type nucleotide/sulfonate/bicarbonate), to reperistomal transport (NADH dehydrogenase, mitochondrial citochrome b and c), to cell metabolism (aldolase dehydrogenase, monoxygenase, zinc binding dehydrogenase, DOPA decarboxylase, oxidoreductase and Acyl-CoA dehydrogenase), to regulatory proteins (nitric oxide synthase, histidine protein kinase and RACK-1), to facilitative transport factors (cortin and Rho GTPases) and to 9 hypothetical proteins. Conclusions: The clones identified using the IVIAT presents limited information from the literature and databases with respect to the precise role of these genes in pathogenesis. It was clear, however, that those genes were putatively involved in a variety of functions, such as cellular metabolism, pathogenesis and adaptation of *P. brasiliensis* to the host. The genes characterized in this study will be further investigated in relation to their relevance in human infection. Financial support: CAPES, FUNAP, GO, MCT/CNPq.

08.018 - **Transcriptome Profile of Yeast Cells from Paracoccidioides Brasiliensis Within Murine Macrophages**

**Introduction and Objectives:** *P. brasiliensis* act as facultative intracellular pathogen is able to survive and replicate within the phagosome of nonactivated murine and human macrophages. This ability, as proposed for *Histoplasma capsulatum* and *Mycobacterium tuberculosis*, is crucial to the development of disease. Thus, *P. brasiliensis* may have evolved mechanisms that counteract the constraints imposed by phagocytic cells. By using cDNA microarray we evaluate the transcriptional response of this fungus to the environment of peritoneal murine macrophages in order to shed light on the mechanisms used by *P. brasiliensis* to survive within those phagocytic cells. **Methods and Results:** ex vivo peritoneal murine macrophages were infected with *P. brasiliensis* Pb 01 yeast at different time points after infection a differential lysis procedure released intracellular fungi that were collected by centrifugation and its total RNA isolated using TRIZOL reagent. Two sets of microarrays containing a total DNA deposited in each spot, membranes were used for hybridization against the amount of DNA deposited in each spot, membranes were used for hybridization against ±33P-labeled cDNA complex probes derived from reverse transcribed total RNA of intracellular yeast or intracellular yeast or 

08.019 - **Identification of New Antigens of Paracoccidioides Brasiliensis by Using the IVIAT Technology**

*Paracoccidioides brasiliensis* is a thermophilic fungus causing paracoccidioidomycosis (PCM), a mycosis that affects 10 million individuals in Latin America. The infection is acquired by inhaling airborne propagules produced by the fungal mycelial form which transforms into the pathogenic yeast form, when at the body temperature. The yeast form can either be eliminated by the cells of the immune system or disseminate to other tissues through lymphatic or hematogenous routes, occasioning several lesions, until death. *P. brasiliensis* expresses in-vivo many important virulence genes that may contribute to the overall fungus pathogenicity. We utilized in vivo-induced antigen technology (IVIAT) to identify new *P. brasiliensis* antigens that could be expressed during the infection process. IVIAT is a modified immunoscreening that circumvents the need for animal models and permits identification of antigens expressed at various stages of infection.

**Methods and Results:** We used the IVIAT strategy to identify *P. brasiliensis* genes putatively induced in vivo. Using this technique we selected immunogenic proteins which should be expressed specifically during human infection and not during growth under standard laboratory conditions. Sera from eleven patients with PCM infection obtained in Goiânia were pooled and after that were adsorbed with whole cells and lysates of the in vitro cultured yeast phase. These sera were probed to induced proteins from a cDNA expression library of the yeast phase of *P. brasiliensis* constructed in IZAPIII. Clones were obtained and characterized. Of special note is a cDNA (PbB1) encoding a 174 amino acid residues protein characterized as 6,7-dimethyl-8-ribityllumazine synthase (PbB1S) homologue *P. brasiliensis* (GenBank: DQ081183). This protein catalyzes the penultimate step in the synthesis of riboflavin in plants, fungi, and microorganisms. In order to produce antibodies against the recombinant PbB1S the expression construct plgEX-4T-3 LS was introduced into *Escherichia coli* cells and the expression and purification of the recombinant protein was obtained. Immunological activity of the recombinant protein will be investigated. **Conclusion:** Some studies reported that 6,7-dimethyl-8-ribityllumazine synthase is a strong immunogen and elicits both humoral and cellular immune responses conferring protection in mice. Those data make very interesting the study of this protein in *P. brasiliensis*. Financial support: MCT/CNPq and FUNAPE.

08.020 - **C-24 Sterol Methyltransferase (ERG6) of Paracoccidioides Brasiliensis**

**Introduction and Objectives:** *Paracoccidioides brasiliensis*, a thermomorphogenic fungus, is the etiological agent of paracoccidioidomycosis the most prevalent mycosis in Latin America. The disease is acquired when propagules of the mycelial phase (in laboratory this form is maintained at 22°C) are inhale. The infection is thought to take place primarily in the lungs and then may disseminate via the bloodstream and/or lymphatic routes to others organs and tissues. *P. brasiliensis* available antifungal agents are frequently subject to drug resistance and toxicity. The ERG6 gene has been described *P. brasiliensis* isolates resistant to antifungals, mainly in immunodepressed patients. Some of the current antifungals affect sterols of fungi in targets that are common to humans causing side effects to patients. Ergosterol is the sterol of the yeast plasma membrane, which affects its fluidity, permeability and the activity of membrane-bound enzymes. This sterol differs from cholesterol, the predominant mammalian sterol, by the presence of a 24-methyl group and D and D double bonds. The c-24 sterol methyltransferase (ERG6) is the enzyme that adds the 24-methyl group to ergosterol being absent in humans. Thus, ERG6 is considered to be a target to rational drug design avoiding possible side effects. In this work our aim is the cloning and sequencing of the ERG6 cDNA. Methods and Results: Oligonucleotides were constructed based on a partial cDNA sequence encoding to ERG6 detected in transcriptome of *P. brasiliensis* (http://www.biomol.unb.br). PCR was performed by using the mycelium cDNA library and the fragment of 500bp amplified was used to screen the cDNA library of *P. brasiliensis*. One of the clones was chosen for sequencing. The sequence was analyzed by using computational programs such as Gene Runner and BLAST, suggesting being an ERG6 cDNA. The predicted protein from cDNA presents high homology with ERG6 from fungi. Southern blot was carried out to verify the number of copies of ERG6 in *P. brasiliensis* genome, indicating a single copy. **Conclusion:** The results suggest the presence of a single copy of ERG6 in *P. brasiliensis*. The partial ERG6 cDNA sequence presents similarities with ERG6 of other fungi. Based on the results, new sequencing will be carried out to get the complete cDNA sequence. Financial support: IFS, CNPq, CAPES and FUNAPE/UPG.
Introduction and Objectives: Paracoccidioides brasiliensis is the ethiological agent of paracoccidioidomycosis, one of the most prevalent human systemic mycoses in Latin America. The pathogen is a dimorphic fungus that undergoes a complex differentiation in vivo. After entrance of acquired airborne microconidia into the mammalian host, the fungus differentiates into the parasitic yeast form. Through lytic and hemagglutinogenic differentiation routes, P. brasiliensis can reach important organs such as liver and spleen. The understanding of the complex interactions between parasites and their host must include the identification of genes expressed during infection. Although already investigated the complete repertoire of genes associated with the infective process in P. brasiliensis remain largely unexplored in the yeast form. We have utilized the strategy of cDNA differential expression analysis of differentially expressed genes, which involves rapid sets of subtraction hybridization (RDA) of cDNAs prepared from two cell populations. The RDA analysis has not been previously related to pathogenic fungi. In the present study, the strategy was applied to P. brasiliensis obtained from infected animals and to the fungus treated with plasma and blood which mimics the haemagglutinogenic events of the fungus dissemination. Sequence determination of 148 clones and comparative analysis at public databases and also at the P. brasiliensis functional genome database (http://www.bionom.unb.br/Pb) by allowed the identification of 256 clusters with known orthologues. Additionally, unknown genes have also been identified in P. brasiliensis, which has not been described in previous transcriptome analysis. Differentially expressed genes include those, which are involved in the heat stress response, protein synthesis, lipid degradation, signal transduction, osmorality response and some putative virulence factors. Some differentially expressed genes were confirmed by Northern and dot blot. Further RT-PCR experiments showed that the expression of some of those genes was also present in vivo during P. brasiliensis intravenous mouse infection.

Conclusions: The application of RDA allowed the identification of genes up-regulated during the P. brasiliensis infective process, as well as during human plasma and blood treatments. Some identified genes, such as GAPDH, serine protease and iron transporters have been described as virulence factors. Some new transcripts, not previously described in the P. brasiliensis in vitro transcriptome were present in at least one tested condition that mimics the human infection. These findings suggest the usefulness of the cDNA RDA strategy in identifying genes related to selected conditions and to the identification of new genes of this organism. Moreover the data provide an initial insight of the possible mechanisms by which P. brasiliensis ensure survival in the hostile environment of the blood and in the host tissues.

Financial support: MCT/CNPq

Introduction and Objectives: One of the most prevalent fungi in Latin America is Paracoccidioides brasiliensis, a dimorphic organism that grows either as mycelium at 22 °C or yeast at 36 °C. This pathogen apparently has its natural habitat in soil or in plants from areas where paracoccidioidomycosis (PCM) is endemic. Rural workers become infected by inhalation of airborne propagules produced by the fungal mycelium, which changes into pathogenic yeast-like cells at the host temperature and can be phagocyted by macrophages. Inside macrophages the Glyoxylate Cycle, unique pathway for glucose synthesis from lipids, can be activated by fungus. Isocitrate lyase and Malate synthase (MLS) are the key enzymes in this pathway for the production of energy. The enzyme MLS catalyzes the conversion of glyoxylate to malate, while isocitrate is transformed into oxalacetate and acetyl-CoA without side effects in the patients, once this enzyme is absent in humans. The availability of the P. brasiliensis transcriptome makes possible to get the molecules present in fungus but absent in human as new targets for the antifungal drugs. A MLS EST was identified in the transcriptome of P. brasiliensis (http://www.bionom.unb.br). The objective of this work is the cloning of the complete MLS cDNA, as well as, the characterization of the predicted protein and the transcript expression analysis in different carbon sources.

Methods and Results: Yeast and mycelium cells of P. brasiliensis, isolate P601 (ATCC MYA 826), were grown in Sabouraud Dextrose medium at 36°C and 22°C, respectively. Southern blotting was carried out to determine the number of copies of MLS in P. brasiliensis. The presence of two MLS genes named MLS1 and MLS2 was confirmed. The clones of the MLS1 and MLS2 were gotten by screening of the yeast cDNA library. The clones were sequenced and the analysis of the predicted protein showed that MLS1 has a complete cDNA but MLS2 has a partial cDNA. The high homology among MLS1, MLS2 and others MLSs from databases was observed. The expression of the MLS1 has been evaluated in different carbon sources (glucose, acetate, glycolate) by using RT-PCR. Initial results showed that MLS1 is expressed in the presence of glucose. Conclusion: The Southern blotting analysis suggests the presence of two MLS genes in P. brasiliensis. Structural analysis of the MLS shows the presence of the signature to MLS. Results obtained by RT-PCR suggest that MLS1 was expressed in the presence of glucose.

Financial support: IFS, CNPq, CAPES and FUNAPE/UFG

Introduction and Objectives: Paracoccidioidomycosis, the major human systemic mycosis in Latin America, is caused by the dimorphic fungus Paracoccidioides brasiliensis, developing in the mycelial form at 22°C and yeast at 36°C. The infection is acquired by inhalation of airborne propagules produced by the fungal mycelium, which changes into pathogenic yeast-like cells at the host temperature and can be phagocyted by macrophages. Inside macrophages the Glyoxylate Cycle, unique pathway for glucose synthesis from lipids, can be activated by fungus. Isocitrate lyase and Malate synthase (MLS) are the key enzymes in this pathway for the production of energy. The enzyme MLS catalyzes the conversion of glyoxylate to malate, while isocitrate is transformed into oxalacetate and acetyl-CoA without side effects in the patients, once this enzyme is absent in humans. The availability of the P. brasiliensis transcriptome makes possible to get the molecules present in fungus but absent in human as new targets for the antifungal drugs. A MLS EST was identified in the transcriptome of P. brasiliensis (http://www.bionom.unb.br). The objective of this work is the cloning of the complete MLS cDNA, as well as, the characterization of the predicted protein and the transcript expression analysis in different carbon sources.

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Financial support: IFS, CNPq, CAPES and FUNAPE/UFG

Introduction and Objectives: The temperature-dependent dimorphic fungus Paracoccidioides brasiliensis is responsible for paracoccidioidomycosis (PCM) in humans, an endemic disease in Latin American countries. Currently, the aim of many studies is to develop an efficient method of genetic transformation of P. brasiliensis in order to increase the knowledge of this pathogen. In this way, we showed the results of the genetic transformation of the yeast form of P. brasiliensis by electroporation using different strategies: gene replacement and RNA interference (RNAi). The target gene chosen for both strategies was the orotidine-5'-phosphate decarboxylase gene (PhbrUra3). The hygromycin B resistant gene was used as molecular marker for gene replacement and to observe the RNAi of the transcript of PhbrUra3. 5' Fluorotic acid (5'-FOA) was used to select the transformants.

Methods and Results: P. brasiliensis cells from five isolates (Pb339, Pb18, Pb1925, PbAP and Tb) were tested in presence of different concentrations of hygromycin B (25, 50, 100 and 200 µg/mL) and 5 FOA (0.5, 1.0 and 1.5 mg/mL). In all cases, no growth was observed at 50 µg/mL hygromycin B or at 0.5, 1.0 and 1.5 mg/mL 5'-FOA, except for PhbrUra3, that grew at 50 µg/mL hygromycin B. The electroporation was performed in a Gene Pulser Xcell electroporation system (Bio-RAD), with 2x10^6 viable yeast cells in 200 µL of 1 M mannitol, under constant conditions (1000 V or 750 V, 25 µF and 600 &!). After electroporation the cells were immediately spread in YPD plates and incubated at 37°C. Hygromycin B or 5' FOA were added mixed on a top agar 24h-48h after electroporation. The transformants were done in vivo in the presence of 5'-FOA and resulted in viable transformants resistant to either hygromycin B or 5'-FOA selection, which were recovered 30 days post electroporation. Transformants were maintained for about 20 passages in selective medium, then for about 30 days in medium without selection and returned to the selective medium for more 2 passages. Significant differences were observed in growth and sensitivity to hygromycin B and 5' FOA among transformants. Conclusion: The transformation of Paracoccidioides brasiliensis is feasible and the results suggest that this microorganism have a functional mechanism involved in RNAi, since transformed cells were resistant to 5'-FOA.

We are investigating the PhbrUra3 replacement in the genome. Financial support: FAPESP and CNPq.
Introduction and Objectives: *Paracoccidioides brasiliensis* is the etiological agent of paracoccidioidomycosis, a systemic life-threatening mycosis that affects the rural population of Central and South America. The fungus occurs as two morphological stages, mycelium and yeast. In this fungi, the cell wall undergoes structural change from β-glucan to α-glucan polymer is observed in *P. brasiliensis* cell wall, when compared with mycelium. The β-1,3-glucan polymer possibly gives shape to the cell and its synthesis, which occurs at the plasma membrane, may be the result of the β-1,3-glucan synthase enzyme activity. In *P. brasiliensis* just one gene homologue of β-1,3-glucan synthase (PfKFS1) has been characterized. PfKFS1 presents an important putative catalytic subunit that probably is responsible for the protein activity. The β-1,3-glucan synthetize catalytic region from *Aspergillus fumigatus* has been expressed in *Escherichia coli*. In *P. brasiliensis*, this region, PfKsc, corresponds to a hydrophile domain with 58 amino acids. Our group has been working in the studies of proteins involved on cell wall metabolism of *P. brasiliensis* to use as targets for antifungals. Here we report the heterologous expression of this conserved catalytic hydrophile domain of *P. brasiliensis* β-1,3-glucan region, PfKsc was amplified from PfKFS1 gene by PCR using primers added of the restriction enzymes sites. The heterologous expression of PfKsc was undertaken in *E. coli*, strain BL-21, by using the expression plasmid pGEX-AT3. PfKsc was cloned into the ECoRIV/NorI site of the plasmid, releasing a fusion protein of 128 kDa. A serine residue located in the active site to attack the peptide bond of the substrate. Pathogenic microorganisms use proteases to weaken and invade their hosts. In this work, we have isolated, characterized and cloned a cDNA encoding a serine protease homologue. Also the expression profile in yeast cells obtained from experimental infection in mice and the protein heterologous expression had been performed. Methods and Results: A 0.5 Kb expressed sequence tag (EST) encoding a probable serine protease homologue in *P. brasiliensis* was obtained in the Transcriptome Project database (http://www.biomol.unb.br/Pb) and used as a probe in the screening of a yeast cDNA library constructed with RNAs from yeast cells of *P. brasiliensis* recovered from liver of infected mice. The complete cDNA (PbSPs) with 1.5 Kb was obtained, sequenced and deposited in GenBank (accession number BC023910). The search for similarity was conducted using the BLASTx program (http://www.ncbi.nlm.nih.gov/). Domains and predicted active sites were screened using the ProfileScan (http://hits.isb-sib.ch/cgi-bin/PPSCAN). The complete cDNA of 1.5 Kb encodes a predicted protein of 53 kDa (PbSPs) and pf of 6.12, a probable signal peptide was found between aminocids 1-17, with possible cleavage site between aminocids 16 and 17. This cDNA was cloned into an expression vector, which was used to transform *Escherichia coli* cells for heterologous expression of the recombinant protein. The expression profile of PbSPs was obtained by Northern blot analyzes using RNAs obtained from in vitro cultured Pb3060 yeast cells recovered from infected mice. The cDNA transcription was substantially increased in the second condition. Conclusions: The analysis of the cDNA sequence demonstrated homology with serine proteases of other fungi. The predicted protein sequence shows the presence of a catalytic triad (DHS), well conserved in the subfamily serine proteases. The high expression of the cDNA during the experimental infective process in B10A mice suggests the importance of this protease to the fungus infective pathway. Financial support: MCT/CNPq and CAPES.

08.08 - HETEROLOGOUS EXPRESSION OF A RECOMBINANT PEROXISOMAL CATALASE OF *PARACOCCIDIIOIDES BRASILIENSIS*

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Background: *P. brasiliensis* is the etiological agent of paracoccidioidomycosis (PCM), a systemic disease with high prevalence in Latin America. A fungal catalase present in the fungus grows in a mycelial phase at 23—26 °C and as yeast at 35-37 °C. The conversion from mycelium to yeast constitutes the main step for the disease establishment. An important aspect in the disease establishment is the fungus potential in modulating the host immune system. Catalases (EC. 1. 11.1.6) are antioxidant proteins among aerobic organisms and protect cells against oxidative damage caused by hydrogen peroxide produced by the phagocytes cells of the host. Catalases which are responsible for the conversion of hydrogen peroxide to *H₂O* and *O₂* are immunomodulatory and putative virulence factors in several pathogens. The production of catalases is one of the strategies used by microorganisms, including *P. brasiliensis*, to react against the toxicity of oxidants, which are produced in response to the infection. The main objective of this project is to produce the recombinant catalase of *P. brasiliensis*.

**Materials and Methods:** A cDNA encoding the chitinase (PbCTS1) was cloned by screening a cDNA library from yeast cells of *P. brasiliensis*. The cDNA consists of 1,888 bp and encodes an ORF of 1218 bp corresponding to a protein of 45 kDa with 406 amino acid residues. PbCTST belongs to family 18 and to fungal/bacterial class. Southern blot analysis indicated that PbCTST is a single copy. Phylogenetic analysis with PfCTST and other chitinases point to the possibility of several chitinases to be grouped based in specialized functions, which may reflect the multiple and diverse roles played by fungi chitinases. PbCTST presents a complex structure of domains that could imply in multi-functionalities. Although PbCTST does not seem to present chitin-binding domains and serine/threonine/proline-rich regions, the immunodominant C-Type domain could promote interaction with the chitin chain during catalysis. Glycolysis hydrolysis activity was evaluated and the results demonstrated that *P. brasiliensis* is able to produce and secrete these enzymes mainly during transition from mycelium to yeast cells. Immunological analysis of chitinase found in the medium. PbCTST presents an Aamy domain indicating the possibility of alpha amylose activity. The presence of an endocytic signal in the deduced protein suggests that it could be secreted by a vesicular nonclassical export pathway. The PbCTST expression in yeast, and the degree differentiation from mycelium to yeast and in yeast cells obtained from infected mice suggests the relevance of this molecule in *P. brasiliensis* electing PbCTST as an attractive drug target. Conclusion: Here we report the molecular cloning, structural and phylogenetic analysis, as well as the expression and activity analysis of PbCTST, which is expressed in yeast cells, during differentiation from mycelium to yeast cells obtained from infected mice. In addition PbCTST presents motifs that indicate its probable bifunctionality and secretion into the medium. Financial support: CAPES, CNPq and FAPERJ.
Introduction and Objectives: Paracoccidioides brasiliensis is a dimorphic human pathogenic fungus, the ethiological agent of paracoccidioidomycosis (PCM), a systemic mycosis with restricted geographical distribution in Latin America. The infection is acquired by the inhalation of airborne microconidia, which reach the pulmonary alveolar epithelium and differentiate into the parasitic yeast form. Understanding of the complex interactions between the fungus and its host must include the identification of gene expression patterns during infection. The sequencing of cDNAs generating ESTs (Expressed Sequence Tags) provides information on gene expression during specific conditions. The objective of this work is to describe a transcriptome of P. brasiliensis yeast cells recovered from liver of infected mice. Methods and Results: B10.A mice were intraperitoneally inoculated with 5x10^6 yeast cells of Pb01 (ATCC MYA-826). The animals were sacrificed at the 7th day after infection. Inoculated mice were sacrificed. Infected liver was cultured for 14 days and total RNA was extracted. Poly(A) + mRNA was obtained and a unidirectional cDNA library was constructed with the Superscript Plasmid System. In this study, a total of 5.243 sequences, clustering into 1,029 contigs and 643 singlets were obtained. The predominant transcripts were identified as Heat Shock Proteins (HSPs) 70, 60, 30, 90, 88, 82, CLPA and DNA J, all probably involved in the stress response. Proteins (Aspartyl proteinase, Aspartyl aminopeptidase, Serine proteinase, Caax prenyl protease and Caax farnesyltransferase alpha subunit) were also highly redundant. Many sequences showed no matches with known protein sequences and hypothetical proteins were found. The contigs and singlets were classified into functional groups by annotation using a pipeline that includes automatic processing. Conclusion: Analyses and functional categorization of the transcripts of P. brasiliensis during the infective process will contribute for the knowledge of a number of expressed genes potentially involved in the infective process as well as for the understanding of the pathogenicity of this fungus. Financial support: Capes; CNPq / MCT.

Introduction and Objectives: Fungal genetic manipulation is a technique well described for Saccharomyces cerevisiae, Neurospora crassa and Aspergillus nidulans, and has contributed to the understanding of the biology and evolution of these organisms. This methodology has also been applied for human pathogenic fungi like Candida albicans, Cryptococcus neoformans and Histoplasma capsulatum, allowing studies of genes involved in pathogenesis and virulence in these pathogenic fungi. Our experimental work we developed a transformation system in Paracoccidioides brasiliensis that provides a means for genetic manipulation of this fungus. The success of this technique will be to enhance understanding of its biology, gene function, differentiation, and host-pathogen interaction including virulence and pathogenicity. As an application of this technology, we studied the molecular requirements for adhesion between transgenic, fluorescent P. brasiliensis and RAW cells, a murine macrophage cell line. Methods: We describe here the transformation of the yeast phase of P. brasiliensis (ATCC-MYA 826) with Agrobacterium tumefaciens carrying the vector pCB301-UGFP. The microorganisms were co-cultivated for 3 days. Transfected cells were selected by incubation on selective media containing hygromycin B and cotransformation in order to kill non-transformants and Agrobacterium. The expression of the transgenic GFP was detected by fluorescent and confocal microscopy. GFP P. brasiliensis were incubated with RAW cell monolayers in the presence of culture media, EDTA or anti-CD18 monoclonal antibodies. Inhibition of adhesion was quantified by fluorescent microscope. Results: From an initial inoculum of 10^5 P. brasiliensis yeast cells we typically obtained 20 hygromycin B resistant colonies. The expression of GFP was variable among the transfecteds, suggesting an effect of the position of insertion in P. brasiliensis genome, 50% of the transfecteds showed intense fluorescence. P. brasiliensis adhesion to RAW cells is dependent on the presence of divalent cations, and is inhibited by CD18 specific antibodies. Conclusions: Agrobacterium mediated transformation is an effective and efficient method of gene transfer in P. brasiliensis. It is suitable for the expression of heterologous transgenes, and, by random insertion, mutagenesis and gene deletion. Transgenic P. brasiliensis is a useful tool to study interaction between the pathogen and the host cells. Financial support: CNPq

Introduction and Objectives: Paracoccidioides brasiliensis causes paracoccidioidomycosis (PCM), a human systemic disease, which is confined to Latin America. The pathogen is a dimorphic fungus, which, under the influence of temperature, changes from a mycelial (M) to a yeast (Y) form. The mycelium to yeast transition has been shown to be essential for the establishment of the infection, although the precise molecular mechanisms of dimorphism in P. brasiliensis are still poorly understood. In this work we constructed a cDNA library to characterize the transcriptome of P. brasiliensis during the dimorphic transition, from mycelium to yeast. Methods and Results: Total RNA of P. brasiliensis, isolate Pb01 (ATCC-MYA-826), from cells in transition from mycelium-to-yeast after 22h of the temperature shift (22 oC and 37°C) was isolated by the Trizol method. Polyadenylated RNA was purified by using oligo(dt)-cellulose, and a unidirectional cDNA library was constructed in plasmid pCMV Sport 6 (Invitrogen, CA, USA). We obtained 1423 expressed sequence tags (ESTs) of high quality that were assembled into 183 contigs and 414 singlets. The ESTs were automatically analyzed and assembled using the programs Phred, Crossmatch and CAP3. The resulting contigs and singlets were annotated using a pipeline that included automatic processing. The detected ESTs were functionally classified by similarity to known genes. We have identified several genes potentially involved in cellular metabolism, cellular structure, stress response, proteins metabolism, RNA synthesis, among others. Some clusters show similarity to hypothetical proteins or have no significant similarity to any proteins in the databases. Computer subtraction analysis revealed some putative specific sequences of the dimorphic transition. In addition, we have analyzed the expression of genes during the mycelium-yeast transition by reverse transcription-PCR and Northern blot. Conclusion: Comparative analyses and functional categorization of the transcribed genes during mycelium to yeast transition in P. brasiliensis allowed the identification of some new genes as well as provided information and a base for future research in the morphological and biochemical characterization of P. brasiliensis. Theses studies may provide new insights into the pathogenesis, virulence and mechanisms involved during the pathogen-host interaction. Financial support: CNPq/MCT.

Introduction and Objectives: Paracoccidioides brasiliensis is the causative agent of Paracoccidioidomycosis (PCM) the most prevalent systemic mycosis in Latin America. The fungus presents thermal dimorphism and the transition from mycelium to yeast in the main event in the infection establishment. Different clinical manifestations of PCM can be related to characteristics of isolates. This study has the objective of identifying genes differentially expressed in isolates obtained from patients with different clinical manifestations of PCM. Methods and Results: P. brasiliensis isolates PhABS (collected from patients with oral lesions) and Ph561 (collected from patients with pulmonary and ganglionar lesions) have been grown in the yeast phase at 37°C, for 7 days and total RNAs were obtained. The cDNAs were synthesized using reverse transcriptase. Both, PhABS and Ph561 were used as testers and drivers, in the identification of specific genes related to each isolate. Differential were obtained by two rounds of subtraction and amplified by PCR reactions. The PCR products were cloned into pGEM-T-Easy (Promega). Positive clones were submitted to sequencing using a Mega BACE 1000 DNA sequencer (Amersham Biosciences). Sequences of 346 clones were clustered into contigs and singlets. A preliminary analysis using the BLASTX algorithm identified genes putatively involved in hydrolysis and absorption of lipids, in biosynthesis and transport of amino acids, in the stability of the cell wall, in biogenesis of peroxisomes, and others. The resulting contigs and singlets will be annotated using the programs Phred, Crossmatch and CAP3. Comparative analysis of the expressed sequence tags (ESTs) will be performed by using the PHOREST program. Conclusions: cDNA Representational difference analysis (cRDA) provides a powerful technique for the identification of differences between two mRNA populations. Both isolates of P. brasiliensis are virulent and can cause clinical manifestations of PCM. Therefore, overexpressed genes could be potentially involved in events leading to different types of clinical manifestations of PCM. Financial support: MCT/ CNPq – FUNAPE.

08.029 - ANALYSIS OF THE TRANSCRIPTOME OF PARACOCIDIOIDES BRASILIANII DURING THE INFECTIVE PROCESS
Costa, M. A. 1; Marins, W. S. 1; Meirelles, G. V. 1; Mendonça, Y. A. 1; Moreira, S. F. I. 1; Parente, J. A. 1; Balsal, A. M. 1; Borges, C. L. 1; Fiuza, R. B. 1; Faria, F. P. 1; Felipe, M. S. S. 1; Molinari-Madian, E. E. W. I. 1; Pereira, M. T. 1; Soares, C. M. A. 1

08.031 - ANALYSIS OF TRANSCRIPTS OF PARACOCIDIOIDES BRASILIANII DURING THE FUNGUS DIMORPHIC TRANSITION
Bastos, K. P. 1; Bailao, A. M. 1; Borges, C. L. 1; Moreira, S. F. I. 1; Parente, J. A. 1; Fiuza, R. B. 1; Faria, F. P. 1; Felipe, M. S. S. 1; Pereira, M. T. 1; Martins, W. S. 1; Soares, C. M. A. 1

08.032 - REPRESENTATIONAL DIFFERENCE ANALYSIS (RDA) FOR THE DETECTION OF DIFFERENTIAL GENE EXPRESSION IN ISOLATES OF PARACOCIDIOIDES BRASILIANII.
Santos, M. O. 1; Borges, C. L. 1; Bailao, A. M. 1; Fiuza, R. B. 1; Felipe, M. S. S. 1; Hahn, R. C. 1; Martins, W. S. 1; Pereira, M. T. 1; Soares, C. M. A. 1

References:
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INTRODUCTION AND OBJECTIVES: Paracoccidioides brasiliensis, the causative agent of Paracoccidioidomycosis (PCM), is a thermophilic dimorphic fungus, which manifests the yeast-like form at 36°C and mycelial at 28°C. The disease is endemic in Latin America, where it is believed that up to 10 million individuals could be infected. Crude extracts of plants and their fractions have been used in the treatment of chronic diseases or to maintain a better health condition. In the literature there are descriptions of the Brazilian savannah plants extracts rich in tannins and alkaloids with several antimicrobial activities. Tannins are water-soluble polyphenols that complex with proteins, polysaccharides and alkaloids and are present several biological activities. Eugenia uniflora is a savannah plant used in popular medicine and presents oenotein B as a major molecule in its leaves aqueous extract. Our objective is to study the action of the non-polar fraction of extracts from E. uniflora in yeast cells of P. brasiliensis, as well as the analyze the action on the transcripts of 

**METHODS AND RESULTS:** By using high performance liquid chromatography we isolated and characterized the main chemical component of E. uniflora, the oenotein B. The growth of yeast cells was analyzed in the presence of aqueous extract of leaves from E. uniflora (125 mg/ml to 1g/ml), in McVeigh-Morton (MVM) minimum medium. The minimal inhibitory concentration (MIC) was obtained by using the microdilution method described on NCCLS M27. The yeast cells were maintained for 15 days at 36°C and counted in a Neubauer chamber. The results were analyzed in the Microcal Origin 5.0. The growth of the yeast cells was monitored for 15 days at 36°C and counted in a Neubauer chamber. The results analyzed in the Microlab 5.0. The growth of the yeast cells was inhibited by 75% in the presence of the oenotein B fraction (500 µg/mL). Total RNAs were obtained from control and yeast cells grown in the presence of the non-polar fraction of E. uniflora. RT-PCR and electronic microscopy analysis were performed ten days after treatment of the cells. The results indicate that the 1,3-β-glucan synthase transcripts were inhibited by the soluble fraction containing oenotein B. Electronic microscopic analysis suggest morphological alterations on yeast cells of P. brasiliensis, such as depression on surface. 

**CONCLUSION:** Extracts from E. uniflora inhibited growth of the yeast cells of P. brasiliensis. Our results showed that the most effective dosage is 500 µg/ml. In addition, the 1,3-β-glucan synthase transcripts decreased with the treatment. Morphological alterations, such as depression on surface were observed in yeast cells of P. brasiliensis, indicating a possible action of the soluble fraction containing oenotein B on cell wall metabolism. 

**FINANCIAL SUPPORT:** IFS, CNPq and FUNAP/UFG.

08.034 - DETECTION AND SELECTION OF MICROSATELLITES IN THE GENOME OF PARACOCCIDIOIDES BRASILIENSIS AS MOLECULAR MARKERS FOR CLINICAL AND EPIDEMIOLOGICAL STUDIES

Nascimento, E.1; Martinez, R.1; Lopes, A. R.2; Sousa Bernardes, L. A.1; Barco, C.1; Goldman, M. H.1; Taylor, J. W.1; McEwen, J. G.2; Nobrega, M. P.1; Nobrega, F. G.1; Goldman, G.1

**FAPESP - USP - Parasitologia; 2UFG - Departamento de Biologia Celular.**

Introduction and Objectives: Paracoccidioides brasiliensis, a thermophiles fungus, is the causative agent of the prevalent systemic mycosis in Latin America, paracoccidioidomycosis. Here, we describe the microsatellite patterns observed in a collection of P. brasiliensis Random Sequence Tags (RSTs). Methods and Results: We identified 1,117 microsatellite patterns in about 3.8 Mb unique sequences (0.47% of the total DNA taken to the analysis). The majority of these microsatellites (87.5%) are found in noncoding sequences. We used two polymorphic microsatellites located on introns as molecular markers to discriminate the species. In this way, we are interested in targeting specific antifungal agents, since the cell wall is absent in human. The objective of this work is cloning of the P. brasiliensis genomic region encoding the beta 1,3-endoglucanase (β1,3-EG). The partial cDNA of the β1,3-endoglucanase was identified in the P. brasiliensis transcriptome (http://www.biomol.unb.br). Computational analyses suggest that it is a β1,3-endoglucanase belongs to the family 81 of glycosidases. Oligonucleotides were constructed and a partial cDNA of the β1,3-endoglucanase was amplified by PCR by using a mycelial cDNA library. The probe of 509 bp was used by in the screening of a cDNA library. Southern blot was also performed in order to define the number of genes of the β1,3-endoglucanase gene. The analysis indicates that β1,3-endoglucanase is not a single copy. Conclusion: Our results suggest that the cloning hold the β1,3-endoglucanase cDNA has been isolated and the presence of the homologous gene is suggested. Northern blot was performed and the results showed the presence of β1,3-endoglucanase in P. brasiliensis. Financial support: IFS, CNPq, CAPES and FUNAP/UFG.

08.035 - CLONING OF THE DNA ENCODING A β 1,3-ENDOGLUCANASE FROM PARACOCCIDIOIDES BRASILIENSIS

Cruz, A. H. S.1; Bonfim, S. M. R. C.1; Rodrigues Silva, C.1; Soares, C. M. A.4; Pereira, M.1

**UFG - Microbiologia; 2MG - Departamento de Bioquímica e Biofísica; 3UFG - Departamento de Bioquímica e Biofísica.**

Introduction and Objectives: Paracoccidioidomycosis (PCM), caused by Paracoccidioides brasiliensis, is an important human mycosis restricted to Latin America where it causes important morbidity and mortality, among native people. The fungus shows a thermal dimorphism, growing as mycelium at 22°C and yeast at 37°C. The change from the mycelial to yeast is crucial for the establishment of the infection in the human host. Infection typically occurs by inhalation of fungal propagules, followed by the thermally regulated transition to the parasitic yeast phase. The cell wall of P. brasiliensis is an essential structure since it is involved in vital processes of the cell, such as defense, cell division and growth. The main constituents of the cell wall of P. brasiliensis are chitin, glucans, lipids and proteins. The main polysaccharide of the yeast cell wall is β-glucan, whereas the polysaccharides of the mycelium wall are β-glucan and galactomannans. Enzymes involved in the cell wall metabolism, as chitinases and glucanses, have been associated to the pathogenicity and virulence. In this way are interesting targets to design specific antifungal agents, since the cell wall is absent in human. The objective of this work is cloning of the DNA encoding the β1,3-endoglucanase gene (β1,3-EG). The partial cDNA of the β1,3-endoglucanase was identified in the P. brasiliensis transcriptome (http://www.biomol.unb.br). Computational analyses suggest that it is a β1,3-endoglucanase belongs to the family 81 of glycosidases. Oligonucleotides were constructed and a partial cDNA of the β1,3-endoglucanase was amplified by PCR by using a mycelial cDNA library. The probe of 509 bp was used by in the screening of a cDNA library. Southern blot was also performed in order to define the number of genes of the β1,3-endoglucanase gene. The analysis indicates that β1,3-endoglucanase is not a single copy. Conclusion: Our results suggest that the cloning hold the β1,3-endoglucanase cDNA has been isolated and the presence of the homologous gene is suggested. Northern blot was performed and the results showed the presence of β1,3-endoglucanase in P. brasiliensis. Financial support: IFS, CNPq, CAPES and FUNAP/UFG.

08.036 - CHARACTERIZATION AND HETEROLOGOUS EXPRESSION OF A CDNA ENCODING A THIOREDOXIN HOMOLOGUE OF PARACOCCIDIOIDES BRASILIENSIS

Domungos, F. C.1; Lima, Y. A. R.2; Faria, F. P.1; Soares, C. M. A.3; Jesuino, R. S. A.4

**UFG - Bioquímica e Biologia Molecular; 2MG - Departamento de Bioquímica e Biologia Molecular.**

Introduction and Objectives: The temperature-dependent dimorphic fungus Paracoccidioides brasiliensis is the etiological agent of Paracoccidioidomycosis (PCM) in man, which is endemic in countries of Latin America. The cellular differentiation of P. brasiliensis from mycelium to yeast in the lungs is essential for the establishment of the infection. Thioredoxin (TRX), a cellular thiol, functions as a self-defense mechanism in response to both reductive and oxidative stress conditions. We here report the characterization of the cDNA PbTrx1, encoding a PbTRX1 and its phylogenetic analysis, structure secondary prediction and the production and purification of the recombinant protein in *Escherichia coli*. After its production and purification, we intend to realize activity assays with the recombinant PbTRX1. It is possible that PbTRX1 may enhance survival of the P. brasiliensis in the host protecting the fungus against the reactive oxygen species (ROS). Therefore, PbTRX1 allows the development and the progression of infection in the human host. Methods and Results: The complete cDNA (PbTrx1) (GenBank:AY376345) presented 811 nucleotides, with an open reading frame of 330 bp, encoding a protein (PbTRX1) with a predicted molecular mass of 12 kDa and pl of 5.2. The PbTRX1 presents one highly conserved motif WCGPC, described as characteristic thioredoxin active site. This protein presents identities values of 62.57 and 51% to the sequences of those from *Emericella nidulans*, *Saccharomyces cerevisiae* and *Candida albicans*, respectively. Prediction of the PbTRX1 secondary-structure contents using the expasy program (http://www.expasy.ch) folds into a pattern characteristic of the open twisted alpha/beta (5 beta-sheets associated in a parallel and anti parallel manner and surrounded by 4 helices), structure already found for human thioredoxins for which the PbTRX1 shares 46% of sequence identity. Sequence analysis of the PbTRX1 performed by PSORT, suggests a cytoplasmic localization of PbTRX1. Phylogenetic analysis of PbTRX1 shows that P. brasiliensis is enclosed in the fungi clade. The expression of the PbTRX1 was undertaken in *E. coli* host strain BL21 as a fusion protein with GST using the expression vector pGEX-4T-3. The recombinant TRX was obtained by induction with IPTG and showed a molecular mass of 35 kDa, as expected for PbTRX1 fusion with GST protein. Conclusion: This work presents for the first time the characterization of a complete cDNA encoding a TRX from *P. brasiliensis*. The PbTRX1 recombinant protein was efficiently expressed in *E. coli* and its purification is under progress. The recombinant protein will be useful for studies regarding to its role in the host fungus interaction. Financial support: MCT/CNPq and FUNAP/UFG.
Introduction: The dimorphic fungus Paracoccidioides brasiliensis is the etiological agent of paracoccidioidomycosis (PCM), a fungal disease that affects many individuals in Latin America. The mycelia found in nature constitute the infective phase that differentiates in yeast form in the mammalian host to establish the infection. The fungus promotes a cell-mediated immune and inflammatory pulmonary response. Macrophages are pivotal effector cells of the innate immune system, recognizing and eliminating invasive microbial pathogens. Initially identified as a T-cell cytokine, the Macrophage Migration Inhibitory Factor (MIF) is also a macrophage cytokine and an important mediator of inflammation. From the project “Functional and Differential Genome of *P. brasiliensis*” ESTs (Expressed Sequence tags) database we discover a putative fungal homologue to the mammalian MIF. There are no reports in the literature of MIF homologues in fungi, however the sequence described in the genome of *P. brasiliensis* has sequence similarity to genes of animal and plant origin. Objective: The aim of this work is to investigate the gene encoded by the *Pb* MIF homolog in order to characterize its coding sequence and predicted polypeptide. Methods and Results: Two MIFcDNA clones were cloned fully sequenced and predicted MIF protein is identified. The clones are identical and the mRNA expression was restricted to the yeast form, as detected by Northern Blot. Sequence analysis indicated that the predicted amino acid sequence has a similarity of 56% (32% identical) with the MIF of *Mus musculus*. The *Pb* MIF CDS (coding sequence) is preceded by a bacterial expression vector (pET21) fused to a histidine tag. This CDS will be expressed in *Escherichia coli* to produce high levels of recombinant protein. Discussion: We have detected and characterized a *Pb* MIF putative homolog. This is the first report of a MIF homolog among fungi and its role as cytokine mimic may reveal new virulent factors for *P. brasiliensis* infection. During the co-evolution with immune system, pathogens must have developed relevant strategies of evasion of host response. Incorporating cytokines homologues may be a successful strategy for *P. brasiliensis*. Conclusion: The reported putative Pb MIF homolog may be part of a previously unknown strategy for *P. brasiliensis* survival inside the mammalian host. Therefore its further characterization may lead to new therapeutic strategies for combating PCM. Financial support: CAPES and MCT.

**08.398 - TREATMENT OF PARACOCCIDIODES BRASILIENSIS WITH HYDROGEN PEROXIDE, MENADIONE AND AMINOTRIAZOL: IN VITRO EFFECTS**

Dantas, A. S.1; Felipe, M. S. S.1; Pózel, V. L. F.1; Nicolai, A. M.1; Campos, E. G.2

Introduction and Objectives: The relevance of free radical generation and oxidative stress with regard to *Paracoccidioides brasiliensis* survival in vitro is being examined by our research group. The quinone menadione (vitamin K3; 2-methyl-1,4-naphthoquinone), a synthetic derivative of vitamin K1, is activated by cytochrome P450 reductase via a one-electron reduction resulting in the formation of a semiquinone radical, which subsequently may reduce molecular oxygen to superoxide anion. Aminotriazole (3-amino-1,2,4-triazole) is also a macrophage cytokine and an important mediator of inflammation. From the project “Functional and Differential Genome of *P. brasiliensis*” ESTs (Expressed Sequence tags) database we discover a putative fungai homologue to the mammalian MIF. There are no reports in the literature of MIF homologues in fungi, however the sequence described in the genome of *P. brasiliensis* has sequence similarity to genes of animal and plant origin. Objective: The aim of this work is to investigate the gene encoded by the *Pb* MIF homolog in order to characterize its coding sequence and predicted polypeptide. Methods and Results: Two MIFcDNA clones were cloned fully sequenced and predicted MIF protein is identified. The clones are identical and the mRNA expression was restricted to the yeast form, as detected by Northern Blot. Sequence analysis indicated that the predicted amino acid sequence has a similarity of 56% (32% identical) with the MIF of *Mus musculus*. The *Pb* MIF CDS (coding sequence) is preceded by a bacterial expression vector (pET21) fused to a histidine tag. This CDS will be expressed in *Escherichia coli* to produce high levels of recombinant protein. Discussion: We have detected and characterized a *Pb* MIF putative homolog. This is the first report of a MIF homolog among fungi and its role as cytokine mimic may reveal new virulent factors for *P. brasiliensis* infection. During the co-evolution with immune system, pathogens must have developed relevant strategies of evasion of host response. Incorporating cytokines homologues may be a successful strategy for *P. brasiliensis*. Conclusion: The reported putative Pb MIF homolog may be part of a previously unknown strategy for *P. brasiliensis* survival inside the mammalian host. Therefore its further characterization may lead to new therapeutic strategies for combating PCM. Financial support: CAPES and MCT.

**08.040 - DIVERSITY POPULATION GENETICS OF THE THREE PHYLOGENETIC SPECIES OF PARACOCCIDIODES BRASILIENSIS**

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Introduction: Comparative DNA sequencing data can be used to study divergence, but the relationship between DNA sequence differences and the timing of population splitting with the processes associated with population splitting can be complex. Even under the simplest models, in which an ancestral population splits into two descendant populations with no gene exchange thereafter, the amount of divergence in DNA sequences between the two populations is a complex function of the time since the split and the relative sizes of the three populations (the two descendants and the ancestral population). Whether or not gene flow has been occurring among the three phylogenetic species of *Paracoccidioides brasiliensis* is a question of considerable interest because it might give clues of how the speciation process took place. Methods and Results: Our research group has isolated three populations of *P. brasiliensis*: S1 (found in Brazil, Argentina, Paraguay, Peru and Venezuela), PS2 (found in Brazil and Venezuela) and PS3 (found only in Colombia). Two of those *P. brasiliensis* lineages, S1 and PS2, are sympatric across their range, suggesting barriers to gene flow other than geographic isolation, PS2 and PS3 constitute a monophyletic group (group composed by the ancestral specie and its descendants). S1 is a paraphyletic group that is considered the ancestor of the other two species. The data obtained in a previous study of eight regions from 5 codifying genes were analyzed according to the model of isolation with migration. We excluded those loci that showed no variation within or between the two taxa being compared in each pairwise analysis, because they are informative for this kind of analysis. The scales of time of divergence indicate that the speciation process that gave origin to PS2 happened before the speciation process that originated PS3. The data also show that those two species have a small population size compared with the parental population. Evidence of gene flow was found in all the comparisons made for the 3 species indicating that gene exchange among the three recognized species had occurred in the recent past. Conclusions: From our analyses, S1 and PS2 appear to have been more isolated than S1 and PS3 with limited gene flow since they began to diverge. The divergence of these species appears consistent with a speciation model in which geographic isolation prevented gene flow during the separation of these species. A related riddle is why migration does not occur in a symmetric way in both the cases analyzed. Although these three populations may possess on the order of one thousand years that have passed since the two speciation processes happened, we consider the possibility that another population, that was not sampled, may play a role in explaining our results. Financial support: This research was supported by grants from Fogarty International Research Collaboration Award (AI065843) and the grant R01TW013088, Banco de la republica N°1785 and by funds of CIB and CODI, Ude. de. la Antioquia.
08.041 - MOLECULAR CHARACTERIZATION OF THE HOMOLOGS OF THE TWO SMALL GTPASES RAS1 AND RAS2 FROM THE HUMAN FUNGAL PATHOGEN PARACOCCIDIOIDES BRASILIENSES

Fernandes, L.; Andrade, R. V.; Felipe, M. S. S.

Introduction and objectives: Paracoccidioides brasiliensis is an ascomycete that displays a dimorphic temperature transition dependency, appearing as mycelium at 22 °C and at 37 °C as yeast form. It is the causative agent of a systemic mycosis of high incidence in Latin America. Although the fungus has the ability to infect and install in the host, there is an increasing need to elucidate the virulence mechanisms of this pathogen. The objective of this work is to characterize Ras1 and Ras2 homologs from P. brasiliensis for the fact that those genes codify for key regulators of signaling transduction pathways that regulate proliferation and differentiation in eukaryotes. Also, we have evaluated the expression of Ras1 and Ras2 transcripts during the dimorphic process of P. brasiliensis.

Methods and Results: In the transcriptome analysis of P. brasiliensis - P601 (Yeast: 20, 263-71, 2003, J Biol Chem. 280: 24706-14) we found the PHEAST 983 corresponding to the Ras2 5' end, with an e-value 2e-11 when compared to Aspergillus fumigatus RasB. We completely sequenced the Ras2 cDNA using primers T3 and T7 presented in the plasmid pdblastscript where the PHEASTs were originally cloned. Ras1 (AY910576) displays an ORF of 716bp, an intron of 87bp and a putative protein of 238 amino acids. The Ras1 was not presented by the transcriptome of P601 isolate. In order to verify if there was a gene encoding a putative protein which, after bioinformatics analyses, seems to be similar to the RAS motif. Prediction of its subcellular localization using PSORT II (psort.nibb.ac.jp) resulted in a cytoplasmic localization of the amino acid sequence.

Conclusion: We successfully produced recombinant his-tagged protein, which is currently in use to test the fungal chaperone which aids in the folding of particular polypeptides and stabilizes their folded conformation. It has been shown to play important roles in the physiology and pathogenesis of other fungal pathogens; therefore we decided to further study it. Methods: We obtained a phage bearing the full PHS900 cDNA from the transcriptome sequencing project. The clone was expressed in vivo and fully sequenced with vector and internal primers. Southern blotting was used to determine the number of gene copies. Northern hybridizations were used to determine the expression. In vivo sensibility microinjection tests were adapted from the international NCCLS standard M27-A2. Recombinant his-tagged HSP90 was produced using pET21a plasmid and Escherichia coli BL21 DE3 expression system. Results: The PHS900 cDNA sequence is identical to the one obtained by the transcriptome sequencing. Southern blot revealed a single copy in the genome, which means that this single gene must be constitutively expressed and stress regulated. We proved this by Northern hybridizations, which have shown that the gene is differentially expressed in the yeast phase, upregulated during the initial M to Y transition and by oxidative stress. The protein function was assessed by inhibition with geldanamycin and radicicol, which inhibited growth at 2 and 10 mM, respectively. Finally, we successfully produced recombinant his-tagged protein, which is currently in use to test its role during fungal infection. Conclusion: Our results confirm that the PHS900 cDNA encodes a stress responsive protein whose function is essential to cell viability. Financial support: CNPq.

08.042 - THE PARACOCCIDIOIDES BRASILIENSES ESTRADIOL BINDING PROTEIN (EBP) HOMOLOG IS DIFFERENTIALLY EXPRESSED IN THE YEAST PHASE

Nicola, A. M.; Andrade, R. V.; Felipe, M. S. S.

Introduction and objectives: Paracoccidioides brasiliensis is a dimorphic and human pathogenic fungus, responsible for the most prevalent systemic mycosis in Latin America. The morphologic transition from mycelium to yeast can be reproduced in vitro by raising the cultivation temperature from room temperature (~25°C) to host temperature (~37°C); such increase in temperature causes a heat shock response with the induction of several heat shock proteins (HSPs), which help to fold denatured proteins and solubilize lethal aggregates. The response to heat shock, thus, is essential to the fungal pathogenesis. HSP90 is a molecular chaperone which aids in the folding of particular polypeptides and stabilizes their folded conformation. It has been shown to play important roles in the physiology and pathogenesis of other fungal pathogens; therefore we decided to further study it. Methods: We obtained a phage bearing the full PHS900 cDNA from the transcriptome sequencing project. The clone was expressed in vivo and fully sequenced with vector and internal primers. Southern blotting was used to determine the number of gene copies. Northern hybridizations were used to determine the expression. In vivo sensibility microinjection tests were adapted from the international NCCLS standard M27-A2. Recombinant his-tagged HSP90 was produced using pET21a plasmid and Escherichia coli BL21 DE3 expression system. Results: The PHS900 cDNA sequence is identical to the one obtained by the transcriptome sequencing. Southern blot revealed a single copy in the genome, which means that this single gene must be constitutively expressed and stress regulated. We proved this by Northern hybridizations, which have shown that the gene is differentially expressed in the yeast phase, upregulated during the initial M to Y transition and by oxidative stress. The protein function was assessed by inhibition with geldanamycin and radicicol, which inhibited growth at 2 and 10 mM, respectively. Finally, we successfully produced recombinant his-tagged protein, which is currently in use to test its role during fungal infection. Conclusion: Our results confirm that the PHS900 cDNA encodes a stress responsive protein whose function is essential to cell viability. Financial support: CNPq.

Paracoccidioides brasiliensis, a facultative intracellular human pathogen, is the ethiologic agent of paracoccidioidomycosis (PCM), the most prevalent systemic mycosis in Latin America. It is assumed that this dimorphic fungus exists as a soil saprophyte, producing propagules that can infect humans and can cause the disease after the transition to the pathogenic yeast form. The establishment of infection depends on the host immunological response and the fungal virulence. The most important mechanism of host defense against P. brasiliensis infection is the cell-mediated immune response, characterized by the production of cytokines. In the absence of such cytokines, this fungus is able to survive and replicate within the phagolysosome of nonactivated murine and human macrophages. Thus, P. brasiliensis may have evolved mechanisms that made it able to survive within the phagocytic cells, considered a poor source of complex carbon. In agreement with its ability to survive in this inhospitable habitat, the analysis of P. brasiliensis transcriptome revealed several putative orthologs of virulence genes of other human facultative intracellular pathogenic fungi. In this sense, we identified orthologs of the glyoxylate cycle genes icl1 and mls1, encoding the regulatory enzymes isocitrate lyase and malate synthase, respectively. Due to the glyoxylate cycle importance in energy production during infection, this pathway occurs in both virulent bacteria and fungi. In this context, the aim of this work was the semiquantitative analysis of glyoxylate cycle genes expression by RT-PCR technique. In order to evaluate the icl1 and mls1 gene expression, the yeast form of P. brasiliensis was grown in media mimicking the phagosome milieu, in which glucose was replaced by acetate as a sole carbon source. Following different incubation times, cells were harvested and total RNA extracted. RT-PCR experiments were carried out using specific primers directed to the internal control gene (β-tubulin) and the experimental gene (icl1 or mls1). The RT-PCR products were analysed by agarose gel electrophoresis and the amplified DNA fragments corresponding to β-tubulin and one of the interest gene were quantified by densitometry employing the Scion Image software (http://www.scioncorp.com). Our results suggest a slightly increase in the icl1 expression when this fungus was grown in media with acetate as the only carbon source. Financial support: FUNDEP-DPP/UnB; FINATEC.
08.045 - GENE IDENTIFICATION DURING PARACOCIDIOIDES BRASILIENSIS (PB) CONIDIA TO YEAST TRANSITION

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Introduction: The dimorphic fungus Pb is the causative agent of Paracoccidioidomycosis (PCM). The infection is probably acquired by inhalation of the infective mycelial propagules (conidia) produced at temperatures below 25°C. When inhaled by the host, they reach the alveolus and the host’s temperature (37°C) prompts their transition to the yeast form, the first step of the infectious process. This transition is pivotal for fungal survival and as such represents a suitable target to hinder initiation of the disease process. Objectives: Our purpose was to identify genes produced during the conidia to yeast transition by comparing EST-clone libraries with the characterization of the clones by automatic sequencing and identification using Blast analysis. Methodology: Pb strain ATCC 60855, was grown in its mycelial form in petri dishes with water agar medium for 3 months. Conidia were harvested by scraping the medium surface, purified by glass-wool ultra filtration at 17°C. Yeast broth for 48 hours with shaking and the proportion of transitioned cells was counted microscopically. Total RNA was extracted using liquid nitrogen and Trizol (Invitrogen). Total RNA was purified, amplified and cDNA produced with RiboAmp (Arcturus) kit and used to perform PCR-Orestes as described by de Sousa et al. 2000. Smears of the PCR-amplified cDNA were purified by elution from the agarose gel, selecting fragments between 500 to 2000 bp. The eluted fragments were cloned using TOPO TA cloning Kit (Invitrogen), individual colonies were selected, plasmids were extracted from each colony and sequencing was made. Analysis and assembling of the EST-Orestes sequences was made using a pipeline built by the Molecular Biology group of the Faculty of Pharmaceutical Sciences, University of Sao Paulo, Ribeirão Preto, Brazil. Sequences were automatically edited for each EST-Orestes, cleaned from the vector using Phrap software and clustered using Cap3 software. Potential homologies with Pb genes were searched BlastX and BlastN algorithms against several databases. Results: 133 sequences were obtained that corresponded to 32 different sequences, 18 contigs and 14 singlets. Blast analyses with the different databases showed that 38% were unknown, 22% were hypothetical, 19% were from signal transduction, 9% corresponded to stress related proteins and 6 represent structural proteins. Eight (25%) of the sequences had not been previously described in mycelial or yeast libraries, suggesting that they are specific for the conidial transition to yeast cells. Of these 8 sequences, 4 corresponded to unknown proteins, 3 to hypothetical proteins and one to a flavoprotein ubiquinone oxidoreductase (ETT-FO). Conclusions: Getting to know these genes is the first step in understanding the host - Pb interactions as they should be involved in initiating the infectious process. Consequently, this information is pertinent for the development of strategies to hinder the first steps of the natural history of this fungal disorder.


08.046 - IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES IN PARACOCIDIOIDES BRASILIENSIS MYCELIA AND YEAST FORMS USING A HIGH DENSITY GENOMIC DNA MICROARRAY

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Introduction: Paracoccidioides brasiliensis is a dimorphic fungus in vitro and in vivo, and the etiologic agent of paracoccidioidomycosis. Mycelial (M) to yeast (Y) transition is a key step in initiation of infection. M and Y are also known to differ in nutritional requirements, cell wall composition, respiratory activity, membrane lipid composition and gene expression patterns. We report the construction of a random genomic DNA (gDNA) microarray containing some 12,300 elements and its initial application for detection of differentially expressed genes between M and Y. Methods: The Pb brasiliensis microarray was assembled with amplified inserts (ca. 1 to 2 kb) obtained from a Pb01 (ATCC/MYA-826) random shear gDNA genomic library. The amplified inserts and leaf prints from gDNA blots were purified and printed onto glass slides. M and Y cells were harvested from 3-day-old cultures grown in modified McVeigh/Morton liquid (room temperature for M, 36°C for Y, at 150 rpm). Cells were mechanically disrupted with zirconium beads and total RNA extracted using a TRIzol® reagent. cDNA probes were produced by reverse transcription and hybridizations were done in duplicate from each of two independent cultures using Cy5 labeled cDNA sample and Cy3 labeled cDNA (control) followed by in silico data extraction. Gene expression was normalized per spot to the gDNA channel, and per slide to known control genes expressed using a positive/negative system. The differentially expressed genes were scored by difference of hybridization measurements when comparing M vs. Y were sequenced. Results: Excluding the controls, a total of 98 spots showed differential expression; 70 were more highly expressed by M and 28 by Y. Thus far, sequence analysis of 89 inserts indicates that 47 encode proteins with known function, 2 were similar to ESTs or proteins of unknown function and 20 had no identifiable homologues. In Y, highly expressed genes were related to respiration and mitochondrial function, protein metabolism, cell wall biogenesis, lipid metabolism, transcription, heat shock and iron uptake. Those expressed in higher amounts by M included genes related to protein degradation, RNA processing, salicylate hydroxylase, a thermostolerance related gene and two efflux transporters. Control genes upregulated in M were hydroporph and M51, and in Y were F20, HISP04 and mannosyl transferase, confirming the reliability and specificity of the array. Conclusions: Our results confirmed previously known differences in gene expression between both forms and revealed additional candidate differentially expressed genes, adding further detail to the understanding of the biology of this organism. Financial support: Fogarty Training Grant (NIH/NERID)

08.047 - ANALYSIS OF THE PbgP43 PROMOTER REGION OF PARACOCIDIOIDES BRASILIENSIS BY DNASE I FOOTPRINTING AND ELECTROPHORETIC MOBILITY SHIFT ASSAYS

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Introduction and Objectives: Paracoccidioides brasiliensis is a dimorphic fungus known to cause paracoccidioidomycosis (PCM) in man. The gp43 of P. brasiliensis is the main diagnostic and prognostic antigen of PCM. It also elicits cellular immune response and protection against murine PCM in vaccinated mice. In vitro, gp43 bears receptor(s) for murine cells that may then express specific membrane glycoproteins. Gp43 is secreted in the culture supernatant of P. brasiliensis cultivated in vitro and can be detected as circulating antigen in the sera of infected individuals. In vitro, the yield of secretion varies with the isolate, growth phase and with regulating factors that are still unknown. We have provided the first evidences of transcriptional regulation of gp43, but we also observed that regulation at the protein and secretion levels may occur (Microbes Infect. 7:55-65, 2003). We also recorded differential gene regulation (down-regulation) upon heat shock in a fungal sample (Pb3) belonging to a phylogenetic group of isolates that have more substitutions in the promoter region than the others. Our laboratory is interested in understanding expression regulation of gp43. One approach to this problem is to investigate the control of PbgP43 gene expression by identifying transcription elements in the promoter region. Computer analysis of 306 base pairs of the known PbgP43 5' intergenic fragment identified many putative transcription elements. In a previous work, electrophoretic mobility shift assays (EMSA) using total extract of Pb339 identified at least one transcription element in the sequence tcgatttattacatagtct (BS5, 126 to 130) of the PbgP43 promoter region (Morais and Puccia, unpublished). Methods and Results: In the present work, DNAase I footprinting assays using the PbgP43 promoter fragment as probe and total extract of Pb339 revealed five protection regions between positions -261 and -146 (ET1 to ET5). EMSA was then performed to confirm the formation of binding complexes with oligonucleotides corresponding to the protected regions. We tested the probes ET1+2, ET2+3, ET4 and ET5 with total extracts of Pb339 and Pb3. We observed that ET4 and ET5 did not form binding complexes with either extract, but that the probes ET1+2 and ET2+3 formed strong binding complexes with both. The migration of the complexes varied with the extract, suggesting polymorphism in the transcription factors. Conclusions: The results presented here are promising and show that ET1, ET2 and ET3, besides BS5, are strong targets for the construction of truncated promoters to evaluate the function of these regions in the control of PbgP43 gene expression. Financial support: FAEPES and CNPq.
Introduction: Advancements in molecular biology have allowed production of reproducible and characterized antigenic proteins through cloning and sequencing. The cloning and sequencing of a 27-kDa antigenic protein from P. brasilensis (P78725) opened the possibility for the use of this protein in clinical diagnosis. It is recognized by antibodies present in sera of Paracoccidioidomycosis (PCM) patients, and appeared to be free of cross-reactivity. However, besides the sequence very few biological facts are known about this protein. Methods and results: We analyzed the characteristics of this protein using bioinformatics tools and the corresponding findings can be summarized as follows. First, sequence alignment studies and basic local alignment search tool (BLASTp) searches were performed. Based on the alignment it became apparent that although the protein has been found only in P. brasiliensis, some homologous sequences were also detected in Coccioides immitis and Histoplasma capsulatum var. capsulatum. Moreover, a comparison against PROSITE indicates that the protein is weakly related to several previously reported profiles. Several polymorphisms at the second intron of the protein from sequence analysis using the programs JUFO and SCRATCH. It was not possible to deduce its three-dimensional structure, because not significant homology was found with a protein of known structure. Third, with the DNAPG program, we determined its polymorphism among the protein belonging to the three phylogenetic species of P. brasilensis recently described by our group, using several isolates from each of one such species. For this protein, the nucleotide polymorphism was relatively low when compared with other antigenic proteins such as GP43. Fourth, the location of the protein was determined using the TMHMM program. The results indicate an extracellular location for this protein, either extruded, membranal or wall associated. No signal peptide has been associated to the protein. Finally, a search for potential epitopes was performed, finding several octamers, decamers and 15-mers, which might interact with the immune system. Conclusions: The low nuclear polymorphism might indicate that the gene encoding the 27-kDa antigenic protein from P. brasilensis is under selective constrain. Additional studies should be performed in order to confirm this hypothesis; nonetheless, we obtained new biologic information about the 27-kDa antigen of P. brasiliensis complementing the protein’s annotation. These types of tools may improve the knowledge in the evolution biology in the antigenic proteins of P. brasiliensis. Financial support: This research was supported by a grant of Banco de la republica No 1785 and by funds of CIB and CODI, U de. Antioquia.

References:

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08.051 - MICROSATELLITE EVOLUTION IN PARACOCCIDIOIDES BRASILIENSIS

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Introduction: Microsatellite loci are important markers for population genetic analyses yet their evolutionary dynamics and mutational processes are still not fully understood with their role as phylogenetic markers being opened to discussion. In some microorganisms, such as P. brasiliensis, the existence of such markers has been demonstrated only recently. To address this issue, we sequenced eight microsatellites and their flanking regions in 65 P. brasiliensis isolates obtained from several countries within the endemic area. Methods and results: To elucidate the genalogical relationships among alleles repeat number was mapped onto trees constructed from 18 nuclear regions. This allowed placement of the microsatellite mutations in the evolutionary context of the less rapidly evolving flanking regions and nuclear coding genes. In a parallel way, it was possible to determine that the flanking regions evolve slower than the coding genes. This is consistent with expectations because the non-coding regions, such as the flanking regions, are more prone to accumulate mutations than the coding regions subjected to selective constrains. The distributions of the allele repeat number, within studied isolates and the phylogenetic species described by us, were consistent with the stepwise mutational model proposed for microsatellites. These results follow the same trend of information obtained in the case of Neurospora crassa. Concomitantly, the lengths of flanking sequences were very similar among the three P. brasiliensis phylogenetic species analyzed. In a similar way, the allele repeat number distributions overlapped regularly because some alleles that were identical in state but not by descent, were shared among species. Additionally, several mutations in the tandem repeats themselves were observed with certain alleles. Moreover, the high levels of interspecific homoplasy (have the same phylogenetic origin) indicated that more microsatellite loci must be characterized before we can test their efficacy in phylogenetic reconstruction among P. brasiliensis phylogenetic species. Financial support: This research was supported by grants from Fogarty International Research Collaboration Award (FIRCA) grant R03TW01308, Banco de la republica N°1785 and by funds of CIB and CODI, U de. Antioquia.
08.052 - TRANSCRIPTOME ANALYSIS OF PARACOCCIDIOIDES BRASILIENSIS CELLS UNDERGOING THE MYCELIUM-TO-YEAST TRANSITION


Paracoccidioides brasiliensis is a thermomorphogenic fungus associated with paracoccidioidomycosis (PCM), a prevalent systemic mycosis in South America. In humans, infection starts by inhalation of fungal propagules, which reach the pulmonary epithelium and transform into the yeast parasitic form. Thus, the mycelial-to-yeast transition is of particular interest because conversion to yeast is essential for infection. We have used a P. brasiliensis biochip, carrying sequences of 4,692 genes from this fungus to monitor gene expression at several time points of the mycelium-to-yeast morphological shift (from 5 to 120 hrs). Results revealed a total of 2,583 genes that displayed statistically significant modulation in at least one experimental time point. Among the identified genes, some encoded enzymes involved in amino acid catabolism, signal transduction, protein synthesis, cell wall metabolism, genome structure, oxidative stress response, growth control and development. The expression pattern of many genes was independently verified by real-time RT-PCR, revealing a high qualitative correlation (~85%) between the data obtained with the two methodologies. One gene, encoding 4-hydroxynaphthalene-1,2-dioxygenase (HHD) was highly overexpressed during the mycelium-to-yeast differentiation process and the use of NTBc, a specific inhibitor of 4-HPPD activity (as well as a series of NTBc derivatives) was able to inhibit growth and differentiation of the pathogenic yeast phase of the fungus in vitro. These data set the stage for further studies involving NTBc and its derivatives as new chemotherapeutic agents against PCM and confirm the potential of array-based approaches to identify new targets for the development of alternative treatments against pathogenic microorganisms. Financial support: FAPESP and CNPq.

08.053 - CELL ORGANIZATION AND IONS TRANSPORTERS RELATED GENES ARE DIFFERENTIALLY EXPRESSED IN PARACOCCIDIOIDES BRASILIENSIS MYCELIUM AND YEAST CELLS

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Introduction and Objectives: The causative agent of Paracoccidioidomycosis (PCM), the thermo-regulated dimorphic fungus Paracoccidioides brasiliensis, is believed to be a free-living mycelium saprobe that undergoes transition to the yeast pathogenic form upon temperature change from the environmental 24-26°C to the mammalian body temperature of 37°C. We had previously characterized 19.718 Expressed Sequence Tags (ESTs) from both mycelium and yeast cells [Felipe et al., J. Biol. Chem. 280(26), 24706-24714, 2005]. In this work, we have extended the analysis of P. brasiliensis transcriptome by assessing differentially expressed genes in mycelium and yeast cells by two different large-scale approaches: statistical analysis of gene expression based on EST counts (in silico ESTs subtraction) and cDNA microarrays. Methods: The 19,718 EST dataset were analyzed by in silico EST subtraction. From this set we have chosen 1,152 EST to further characterize by cDNA microarray analysis. The differential feature of selected genes was confirmed by Northern-blot assays. Results and conclusion: By comparing the results of both approaches, a sub-class of 83 clones was identified as differentially expressed genes. This final set of overlapping sequences was constituted by 19 and 64 up-regulated genes in mycelium and yeast cells, respectively. We have focus on the analysis of: (i) control of cell organization and (ii) ion metabolism and transporters related genes. Hex and BGL genes encoding for hexagonal peroxisome protein and 1.3 β-glucosidase, respectively, were identified as mycelium up regulated genes; while three other genes related to cell organization were shown to be up regulated in yeast cells, such as AGS (G1,3-galactan synthase), CDA (chitin deacetylase) and VRP-verprolin. Genes involved in ion transporters, ISC and KTP, an iron-sulfur cluster and a potassium transporter respectively, were confirmed to be highly expressed in mycelium cells. Also, a putative P-type Ca2+ and choline sulfatase (CHS) were up regulated in the yeast form. Therefore the data presented here contributes to further understanding the phase transition as a key feature for the life cycle of P. brasiliensis. Financial support: MCT, CNPq and FAPESP.

08.054 - MOLECULAR STUDIES ON SULPHUR METABOLISM IN PARACOCCIDIOIDES BRASILIENSIS

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Introduction and Objectives: The dimorphic pathogenic fungus P. brasiliensis is a cysteine prototrophic in the mycelial (non-pathogenic) form, but cysteine auxotrophic in the yeast (pathogenic) form. The main objective of this study is to verify the molecular basis of this phenomenon. As a preliminary step to understand the organic sulfur auxotrophy in P. brasiliensis yeast form, we decided to characterize the positive regulator MedR and the sulfur metabolite repression ScoC homologues in this fungus. Methods and Results: We have grown 79 P. brasiliensis strains from different environments and geographical locations in minimal medium supplemented either with inorganic or organic sulfur. None of these strains was able to grow in minimal medium in the absence of organic sulfur, indicating that organic sulfur auxotrophy is a broad phenomenon in this species. Furthermore, P. brasiliensis is not able to grow in anaerobiosis and the organic sulfur auxotrophy is not related to high O2 tension. We evaluated the mRNA and protein expression during the dimorphic transition and demonstrated these genes are able to complement the corresponding A. nidulans medR and scoC3 mutants. In addition, we have performed a gene expression analysis of the genes that are transcriptionally induced during the hyphal-to-yeast transition in minimal medium supplemented with organic sulfur as single sulfur source (MM+CCM), we accomplished a large-scale analysis of gene expression in P. brasiliensis by using microarray hybridizations. Conclusion: Our results showed that both P. brasiliensis MetR and ScoC3 genes were able to complement the A. nidulans mutants. In addition, several genes previously seen as more expressed during the transition in completeYPD medium are also more expressed in MM+CCM, suggesting these genes are important for the transition independently of the culture conditions. Financial support: FAPESP and CNPq, Brazil.