Universidade Federal de Goiás

Instituto de Ciências Biológicas

Programa de Pós-Graduação em Genética e Biologia Molecular

Igor Godinho Portis

Análise proteômica comparativa entre *Paracoccidioides brasiliensis* e *Paracoccidioides americana*

Goiânia

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Ata Nº **22** da sessão de Defesa de Tese de **Igor Godinho Portis** que confere o titulo de Doutor(a) em **Genética e Biologia Molecular**, na área de concentração em **Genômica funcional, estrutural e proteômica.**

Ao/s **dezenove dias do mês de dezembro**, a partir da(s) **14h**, no(a) **Auditório do ICB II**, realizou-se a sessão pública de Defesa de Tese intitulada "**Análise proteômica e fosfoproteômica de espécies de Paracoccidioides**". Os trabalhos foram instalados pelo(a) Orientador(a), Professor(a) Doutor(a) **Célia Maria de Almeida Soares (UFG)** com a participação dos demais membros da Banca Examinadora: Professor(a) Doutor(a) **Patrícia de Sousa Lima (UEG)**, membro titular externo; Professor(a) Doutor(a) **Juliana Santana de Curcio (UFG)** , membro titular externo, Professor(a) Doutor(a) **Kleber Santiago Freitas e Silva (UFG)**, membro titular externo; Professor(a) Doutor(a) **Juliana Alves Parente Rocha (UFG)**, membro titular interno. Durante a argüição os membros da banca **fizeram** sugestão de alteração do titulo do **trabalho.** A Banca Examinadora reuniu-se em sessão secreta a fim de concluir o julgamento da Tese tendo sido o candidato **aprovado** pelos seus membros. Proclamados os resultados pelo(a) Professor(a) Doutor(a) **Célia Maria de Almeida Soares**, Presidente da Banca Examinadora, foram encerrados os trabalhos e, para constar, lavrou-se a presente ata que é assinada pelos Membros da Banca Examinadora, ao(s) **dezenove dias de dezembro.**

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Igor Godinho Portis

Comparative proteomic analysis between *Paracoccidioides brasiliensis* and *Paracoccidioides americana*

Goiânia

2019

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“Não precisamos apagar a luz do
próximo para que a nossa brilhe”

Mahatma Gandhi

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Figure 2. Cell viability of *Paracoccidioides* species. Cell viability of *Pa* 03 and *Pb* 18 until 96 hours. Cell viability was performed with Trypan blue.

Lista de abreviaturas

PCM – Paracoccidioidomicose

Pa 03 – *Paracoccidioides americana* isolado 03

Pb 18 – *Paracoccidioides brasiliensis* isolado 18

iTRAQ – Isobariq tag for relative and absolute quantitation

mg – Miligrama

mL – Mililitro

µg – Micrograma

µL – Microlitro

µM – Micromolar

pH – Potencial hidrogeniônico

qRT-PCR – *Quantitative real-time polymerase chain reaction* - PCR em tempo real

Gp 43 – Glicoproteína 43

SNPs - *Single Nucleotide Polymorphism*

DNA – Deoxyribonucleic acid (Ácido desoxirribonucleico)

RNA – Ribonucleic acid (Ácido ribonucleico)

MALDI - mass spectrometry identification

SILAC - Stable Isotope Labeling

ICAT - Isotope-Coded Affinity Tag

TCA - Trycarboxylic acid cycle

Resumo

O gênero *Paracoccidioides* compreende fungos termo dimórficos, causadores da paracoccidioidomicose, micose sistêmica mais prevalente da América latina. Esses fungos apresentam capacidade de crescer na forma de levedura a 36°C e na forma de micélio a temperaturas inferiores a 28°C. Estudos mostraram que o fungo *Paracoccidioides*, possui diferenças moleculares que permitem a sua classificação em espécies. Inicialmente classificava-se apenas em uma única espécie, *Paracoccidioides brasiliensis*; com o avanço de algumas técnicas de biologia molecular e genômica, o gênero *Paracoccidioides* é classificado atualmente em cinco espécies, *P. lutzii, P. brasiliensis, P. americana, P. restrepiensis e P. venezuelensis*. Espécies diferentes podem apresentar proteomas e respostas celulares diferentes; em função disso, em nosso estudo, realizamos as análises proteômicas de duas espécies de *Paracoccidioides*, *P. americana* e *P. brasiliensis*, utilizando a marcação com o reagente iTRAQ e espectrometria de massas. Trezentas e oitenta e sete proteínas foram diferencialmente expressas, sendo duzentos e duas no *P. brasiliensis* e cento e onze em *P. americana*. As proteínas identificadas possuem funções diversas, como metabolismo de aminoácidos, energia, defesa celular, entre outras. Os resultados obtidos evidenciam proteínas diferenciais em processos de crescimento, profliferação e virulência.

Palavras-chave: Fungos; Proteínas; Biologia Molecular.

Abstract

The genus *Paracoccidioides* comprises termo dimorphic fungi that cause paracoccidioidomycosis, the most prevalent systemic mycosis in Latin America. These fungi can grow in yeast at 36 ° C and mycelium at temperatures below 28 ° C. Studies have shown that the fungus *Paracoccidioides* has molecular differences that allow the classification of species. Initially those fungi were classified in a single species, *Paracoccidioides brasiliensis*, but with the advancement of some molecular and genomic biology techniques, *Paracoccidioides* is currently classified into five species, *P. lutzii, P. brasiliensis, P. americana, P. restrepiensis and P. venezuelensis*. However, different species may have different proteomes and cellular responses. In this way in our study, we performed proteomic analyzes of two species of *Paracoccidioides, P. americana and P. brasiliensis*. iTRAQ labelling and mass spectrometery allowed the identification of 387 statistically significant proteins; two hundreds and two in *P. brasiliensis* and 111 proteins in *P. americana*. The identified proteins have different functions, such as amino acid metabolism, energy, cell defense, among others. The results showed differences in protein responses between *P. americana* and *P. brasiliensis* in proteins related to cell defense, mitochondria and energy metabolism, suggesting that these fungi may have different metabolism and pathways for growth, profliferation and virulence.

Keywords: Fungi; Proteins; Molecular biology.

CAPÍTULO 1

1.0. Introdução

1.1. Paracoccidioidomicose (PCM)

O fungo *Paracoccidioides* spp. é o agente etiológico da Paracoccidioidomicose (PCM), uma micose granulomatosa crônica, endêmica da América Latina, com alta incidência no Brasil, Colômbia, Venezuela e Argentina (Franco *et al*. 1993; Lacaz, 1994; McEwen *et al*., 1995; Restrepo *et al*., 2000 a; Bellisimo *et al*., 2011). O fungo *Paracoccidioides* é pertencente ao filo Ascomycota, classe Euromycetes ordem Onygenales e família Ajellomycetae (Leclerc *et al*., 1994; James et al., 1996; Bialek et al., 2000; Untereiner et al., 2004). São fungos termodimórficos, que possuem a capacidade de crescer na forma de micélio, entre temperaturas de 22°C à 28°C, no solo, e na forma de levedura nas temperaturas de 36°C à 37°C, no hospedeiro (Figura 1, A e B). Essa transição morfológica, está ligada diretamente com a capacidade de infecção e virulência (Rooney, PJ e Klein, BS, 2002).

Inicialmente acreditava-se que o fungo acometia apenas seres humanos. Porém vários trabalhos demonstraram que alguns animais podem ser infectados por *Paracoccidioides* spp., como cavalos, cachorros (Shikanai-Yasuda *et al*., 2017) e coelhos (Belitardo *et al*., 2014). Outros trabalhos demonstraram que animais silvestres também são infectados pelo fungo. Essas descobertas podem ser consideradas valiosos marcadores epidemiológicos da presença do fungo no meio ambiente (Sbeghen *et al*., 2015).

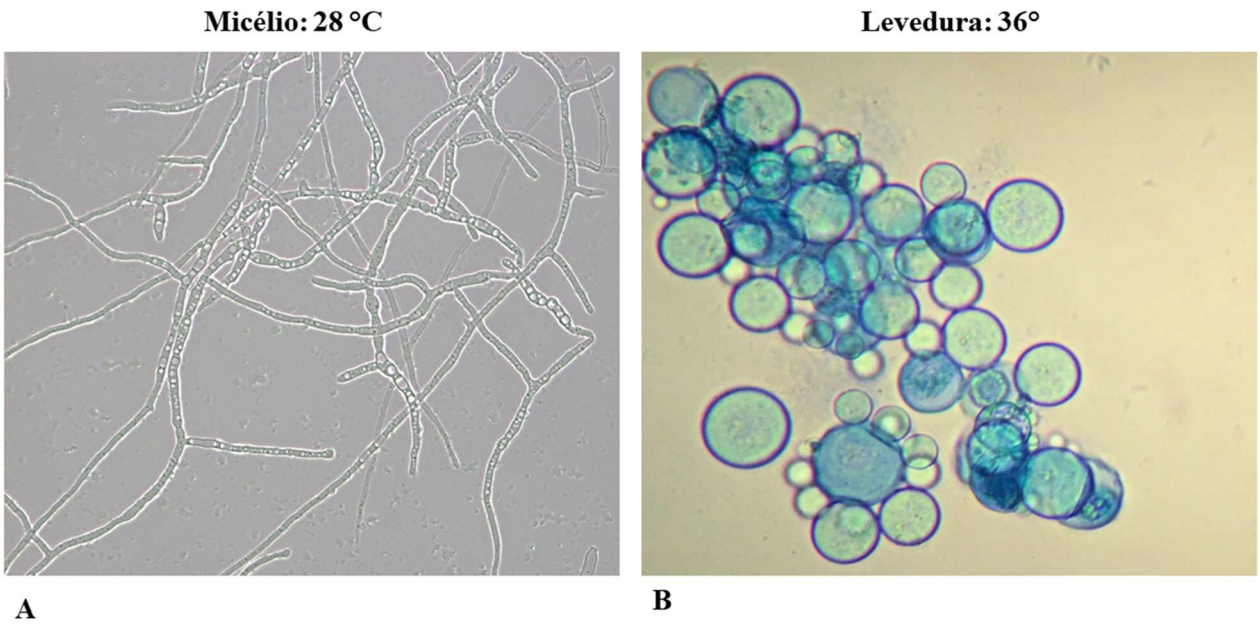

Figura 1. Fases morfológicas do fungo *Paracoccidioides*. A) Micélio, que se apresenta em temperaturas inferiores à 28°C e é encontrado mais facilmente no meio ambiente (solo) (aumento 400 X). B) Levedura, que se apresenta na temperatura de 36°C, a forma leveduriforme é encontrada mais facilmente no hospedeiro. A transição de formas está intimamente relacionada com a capacidade de infecção e virulência do fungo (aumento 400 X). Fotos: Laboratório de Biologia Molecular (UFG), autoria própria.

A infecção por *Paracoccidioides*, ocorre através da inalação de conídios ou propágulos de micélio, que ao entrarem em contato com os alvéolos pulmonares, realizam a transição morfológica, de micélio para levedura, como mostrado na Figura 2, que pode então se disseminar para outros locais do organismo utilizando as vias linfáticas e/ou hematogênica (McEwen *et al*., 1987; Shikanai-Yasuda *et al*., 2006). A Paracoccidioidomicose pode ser classificada em: PCM infecção e PCM doença, sendo a PCM doença ainda subdividida em forma aguda/subaguda e forma crônica. A PCM infecção, acontece quando o indivíduo saudável entra em contato com o fungo, sendo detectado o antígeno paracoccidioidina; no entanto não ocorrem manifestações dos sintomas da doença (Franco *et al*., 1987; Marques, 2013; Shikanai-Yasuda *et al*., 2017).

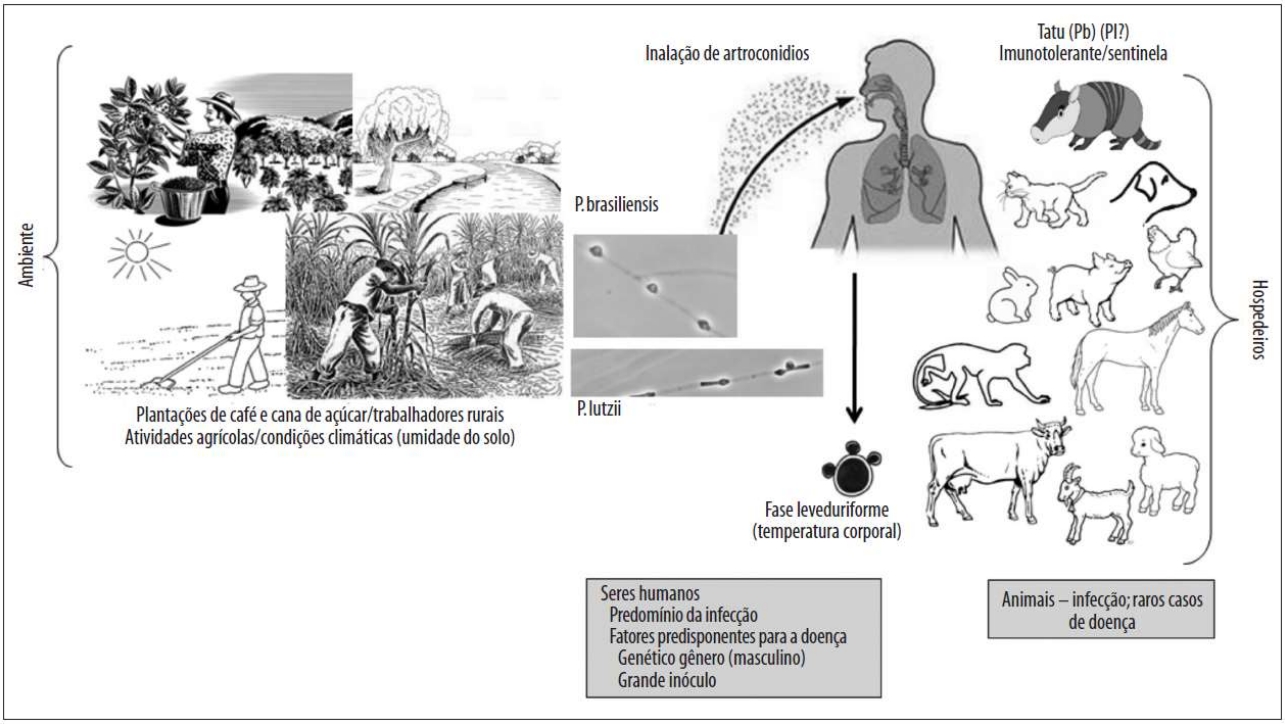

Figura 2. Formas de contágio da doença e propagação. Paracoccidioidomicose (PCM) é adquirida em contato com solo contaminado, através da inalação do fungo, ocorrendo a mudança de fases morfológicas dentro do hospedeiro. Fonte: Mendes, RP e Bagagli, E. 2017.

O grupo de risco da PCM são trabalhadores rurais, os quais têm o contato direto e continuo com o solo; mesmo após a infecção com *Paracoccidioides*, o fungo pode ser eliminado, controlado ou permanecer latente por um longo período. A evolução para uma forma crônica da doença depende de fatores como, a atividade e eficácia da resposta imune do hospedeiro, da virulência da cepa do fungo e da interação parasito-hospedeiro (Lacaz iet al., 2002). A forma crônica da doença acomete principalmente indivíduos do gênero masculino, com faixa etária entre 30 a 60 anos de idade; essa forma é responsável por 90% dos casos de PCM. A forma crônica da doença caracteriza-se como unifocal quando é restrita a apenas um órgão, geralmente o pulmão. A doença caracteriza-se como multifocal quando atinge mais de um órgão simultaneamente, como a pulmão e mucosas, os mais comuns. A forma crônica pode ser subdividida em leve, moderada e grave, dependendo das condições gerais do paciente. Os pacientes atingidos pela PCM, podem falecer ou se recuperar da infecção; muitas vezes as lesões pulmonares podem deixar sequelas, causando insuficiência respiratória (Franco *et al*., 1987; Marques, 2013; Shikanai-Yasuda *et al*., 2006 Shikanai-Yasuda *et al*., 2017).

A forma aguda/subaguda, denominada tipo juvenil, caracteriza-se por evolução rápida dos sintomas e sinais da doença; ocorre a presença de linfadenomegalia, hepatoesplenomegalia, envolvimento ósseo-articular, lesões cutâneas e manifestações digestivas. Essa forma clínica é responsável por 5 a 25% dos casos da doença, sendo mais frequente em certas regiões endêmicas. Os estados brasileiros que possuem mais relatos desta forma da micose são, Maranhão, Minas Gerais, Pará, Goiás e São Paulo. A distribuição da doença é igual entre crianças e adolescentes e apresenta diferença de gênero, acometendo mais indivíduos do sexo masculino (Franco *et al*., 1987; Shikanai-Yasuda *et al*., 2006; Shikanai-Yasuda *et al*., 2017).

A PCM não é uma doença de notificação compulsória, sendo as taxas de incidência, prevalência e morbidade da doença estimados com base em algumas analises epidemiológicas, dados de internação hospitalar e de mortalidade (Shikanai-Yasuda *et* al., 2017). No Brasil, no período de 1980 a 1995, a PCM foi considerada a oitava causa de morte entre as doenças infecciosas crônicas e parasitárias, com taxa média anual de 1,45/1,0 milhão de habitantes, sendo as maiores taxas de mortalidade nas regiões Sul (com 2,59/1,0 milhão de habitantes) e Centro-Oeste (com 2,35/1,0 milhão de habitantes (Coutinho, ZF *et al*., 2002). A taxa de letalidade desta doença é de 3% a 5% no Brasil, variando de 3360 a 5600 por ano. Análises epidemiológicas mais atuais, das 5 regiões do Brasil, apontaram as regiões Centro-Oeste e Norte do país, com as mais altas taxas de hospitalização e mortalidade, apontando essas como importantes áreas endêmicas da paracoccidioidomicose (Martinez, 2017). Em 2015 foi realizada uma avaliação sobre a morbidade da PCM no Brasil, em que foi verificado 6.732 hospitalizações, entre os anos de 1998 a 2006, representando 4,3/1,0 milhão de habitantes (Coutinho *et al*., 2015). No estado de Roraima, na região Norte do país, a prevalência da doença teve uma média anual de 9,4/100. 000 habitantes entre 1997 a 2012, mostrando uma alta taxa de incidência da PCM (Vieira *et al*., 2014). No estado de Mato Grosso do Sul, na região Centro-Oeste do Brasil, foram registrados 422 casos de PCM entre os anos de 1980 a 1999 (Paniago *et al*., 2003). Estudos epidemiológicos demonstraram que nos últimos anos, os casos de PCM aumentaram em Mato Grosso. Entre 2005 a 2011, dos 141 municípios do estado, 53 (37,58%) registraram casos de PCM (Volpato *et al*., 2016). A Figura 3 mostra os níveis de acometimento da Paracoccidioidomicose no Brasil e América Latina, que variam de baixa, moderada e alta incidência.

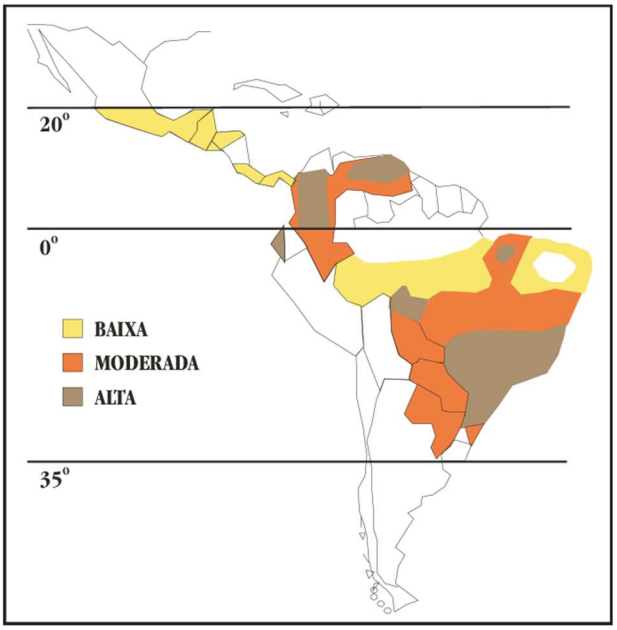

Figura 3. Distribuição geográfica da Paracoccidioidomicose (PCM). Amplamente distribuída na América Latina, desde o México até a Argentina. No mapa são demonstradas as áreas com maiores incidências da doença, variando de baixa incidência, moderada e alta. Foto: Autoria de Shikanai-Yasuda *et al*., 2006.

Foi detectado um surto de PCM, na sua forma aguda, no Rio de Janeiro, após o término da rodovia Raphael de Almeida Magalhães, com a incidência de 8,25 por milhão de pessoas. Provavelmente, essa infecção ocorreu devido ao desmatamento para a construção da rodovia; com a remoção da terra, a população foi exposta aos propágulos do fungo (Valle *et al*., 2017). A PCM possui um grande impacto de saúde e social nas áreas endêmicas da doença; alguns fatores são os números de casos, cronicidade da doença, longo período de tratamento e as sequelas, que causam complicações no trabalho e baixa qualidade de vida (Martinez, 2015).

O diagnóstico da PCM é realizado através da presença de elementos fúngicos de em amostras provenientes de escarro, aspirado de linfonodos, raspado de lesão e ou fragmentos de fungo em biopsia de órgãos. Além dessas análises, devem-se observar os principais órgãos acometidos por essa micose e a sáude geral do paciente (Shikanai-Yasuda *et al*., 2006; Bocca *et al*., 2013; Shikanai-Yasuda *et al*., 2017). O teste sorológico com o antígeno gp-43 é utilizado para diagnóstico de PCM, embora algumas espécies do gênero apresentem pouca reatividade a este exame (Capella, Machado *et al*., 2013). O tratamento para PCM é realizado com vários antifúngicos, como medicamentos do grupo

dos azólicos (cetaconazol, fluconazol, itraconazol, voriconazol), derivados de sulfonamida (cotrimoxazol, sulfadiazina, trimetopima) e anfotericina B.

1.2.Espécies de *Paracoccidioides*

A paracoccidioidomicose (PCM) foi descrita no início do século 20, na Santa Casa de São Paulo, por Adolf Lutz, a partir de pacientes que apresentavam lesões orais (Lutz, 1908). As características morfológicas foram descritas inicialmente por Splendore, que deu o nome ao fungo de *Zymonema brasiliensis* (Splendore, 1912). Alguns anos depois, a espécie foi analisada por Almeida, que comparou com o fungo *Coccidioides immitis*, observando morfologia distinta, propondo o nome da espécie de *Paracoccidioides brasiliensis,* considerada como espécie única (Floriano, 1930). A nomenclatura oficial da doença foi estabelecida em 1971, na reunião de micologistas das Américas, em Medellín (PAN AMERICAN SYMPOSIUM ON PARACOCCIDIOIDOMYCOSIS., 1971).

Análises filogenéticas classificaram o fungo *Paracoccidioides* no filo Ascomycota, ordem Onygenales, família Onygenaceae, assim como os fungos *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Lacazia loboi* e *Emmonsia parva*. Esses microrganismos se associam com hospedeiros vertebrados para o seu desenvolvimento, apresentam uma fase saprobiótica no solo, ou nas fezes, e outra fase parasitária, nos órgãos dos hospedeiros (Bagagli *et al*., 2008).

Por vários anos, *P. brasiliensis* foi considerado a única espécie como agente etiológico da Paracoccidioidomicose. Porém, estudos baseados em polimorfismo genético, realizaram a comparação de genes nucleares entre 65 isolados de *P. brasiliensis*, constatando algumas diferenças entre os isolados, propondo então, a criação de 3 diferentes clados, S1, com 38 isolados; PS2 com 6 isolados e PS3 com 21 isolados (Matute *et al*., 2006; Carrero *et al*., 2008). Análises de regiões ribossômicas entre PS2 e PS3, apontam cerca de 8,04 à 8,37 milhões de anos de tempo de divergência, apontando a especiação de PS3 como uma dispersão que levou a um isolamento genético de PS3 a partir de S1; ocorrendo também alguns eventos que levaram à especiação de PS2 (Matute *et al*, 2007). Após análises de 21 isolados pertencentes às espécies filogenéticas S1 e PS3, por análise de regiões codantes, não codantes e sequências espaçadoras internas (ITS), foi observado que um dos isolados, o *Pb* 01 se diferenciava nitidamente dos demais, podendo ser uma nova espécie. Alguns estudos também apontavam a possibilidade de espécies distintas dentro do gênero *Paracoccidioides*, baseados em variabilidade genética

entre grupos de isolados (Soares *et al.,* 1995; Montoya *et al.,* 1997, 1999; Cano *et al*., 1998; Molinari-Madlum *et al*., 1999; Niño-Veja *et al*., 2000; Hahn *et al*., 2002; 2002, 2003; Hebeler-Barbosa *et al*., 2003), possível relação entre genótipo e isolamento geográfico (Niño-Veja *et al*., 2000). Um estudo utilizando o método de GCPSR (*Genealogic concordance method of phylogenetic species recognition*), identificaram um clado com 17 isolados (incluindo o *Pb* 01) semelhantes entre si, mas que eram diferentes das 3 outras espécies filogenéticas, S1, PS2 e PS3. Assim, foram agrupados os 17 isolados em um grupo denominado "*Pb* 01", propondo também a denominação de uma nova espécie, o *Paracoccidioides lutzii*, em homenagem a Adolf Lutz, quem descreveu o *P. brasiliensis*. Estudos com 63 isolados do gênero *Paracoccidioides* pertencentes às espécies S1, PS2, PS3 e *P. lutzii*, utilizando a técnica de SNPs (*Single Nucleotide Polymorphism*), mostraram uma distribuição geográfica restrita a Colômbia, dos isolados pertencentes à espécie PS3, *P. lutzii* nas regiões centrais do Brasil e no Equador, PS2 no Brasil e Venezuela e S1 apresentou uma distribuição mais ampla, presente no Brasil, Argentina, Paraguai, Venezuela, Uruguai e Peru (Matute *et* al., 2006a; Teixeira *et al*., 20009; Theodoro *ei al*., 2012). Como mostrado na Figura 4, abaixo, a distribuição das espécies geográfica das espécies de *Paracoccidiodes*, com as espécies mais prevalente em cada região do Brasil e da América Latina.

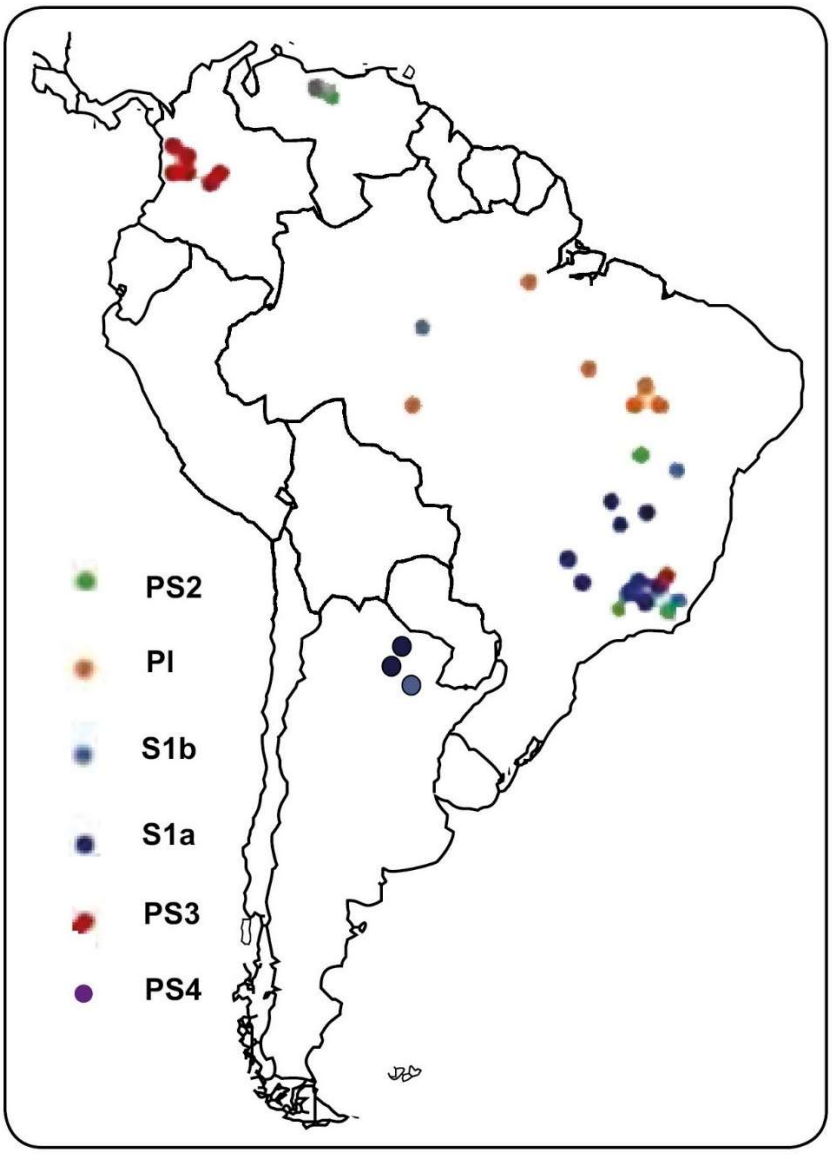

Figura 4. Mapa da distribuição geográfica das espécies de *Paracoccidioides* na América Latina, PS2 representando a espécie *P. americana*, Pl representando a espécie *P. lutzii*, S1a e S1b representando a espécie *P. brasiliensis*, PS3 representando a espécie *P. restrepiesnsis* e PS4, represnetando a espécie *P. venezuelensis*. Muñoz *et al*., 2016.

Em 2014, foi identificado uma outra espécie filogenética do complexo *Paracoccidioides brasiliensis*, a espécie PS4, assim o gênero *Paracoccidioides brasiliensis* ficou com 4 espécies filogenéticas, S1, PS2, PS3 e PS4, e a espécie *Paracoccidioides lutzii*, como espécie separada (Matute *et al*., 2006; Carrero *et al*., 2008; Teixeira *et al*., 2009; Bocca *et al*., 2013; Teixeira *et al*., 2014). No ano de 2017, após algumas análises morfológicas e moleculares, um estudo propôs a classificação das espécies filogenéticas do complexo *Paracoccidioides brasiliensis*, em espécies distintas,

classificando assim, S1 como *Paracoccidioides brasiliensis*, S2 como *Paracoccidioides americana*, PS3 como *Paracoccidioides restrepiensis*, PS4 como *Paracoccidioides venezuelensis* (Turissini DA *et al.,* 2017).

1.3. Proteômica

O termo proteoma foi utilizado inicialmente no ano de 1995, definido como análise em larga escala de proteínas expressas por uma determinada célula, tecido ou organismo em uma condição específica (Wilkins *et al*., 1997). As análises proteômicas, tornaram-se mais significantes e utilizadas após o sequenciamento do genoma humano demonstrar que os seres humanos possuíam um número muito menor de genes do que o previsto (Venter, 2001). Pode-se constatar então, que embora seja importante conhecermos a sequência de nucleotídeos do DNA, isso nem sempre reflete uma relação com os níveis de proteínas expressas e de atividade biológica (Gygi *et al*., 1999). A expressão de proteínas altera-se em situações distintas ou em diferentes tipos celulares em um mesmo organismo, respondendo a estímulos externos e internos. As proteínas, estão envolvidas em diversos processos biológicos e são detectadas nas análises proteômicas por alterações quantitativas, sendo pouco ou muito reguladas (Westermeier *et al*., 2002). O objetivo principal dos estudos proteômicos é a identificação em larga escala de todas as proteínas presentes em uma célula ou tecido, podendo ser relizado em situações específicas. São realizados análises de forma simultânea, ocorrendo a mistura complexa de proteínas, provenientes de extratos lisados de células ou extratos de tecidos, com o intuito de detectar diferenças qualitativas e quantitativas na expressão de proteínas (Westtermeier e Naven, 2002).

O proteoma pode ser definido como o produto da expressão de um conjunto de genes e das modificações pós-traducionais das proteínas produzidas em respostas a estímulos internos e externos; assim o proteoma pode abranger uma gama muito grande de áreas possíveis de estudo (Graves e Haystead, 2002). A Figura 5 mostra a ampla aplicação da proteômica, demosntrando ser uma área com muitas possibilidades de estudo e aplicações, sendo uma ferramenta para análises moleculares e biológicas poderosa.

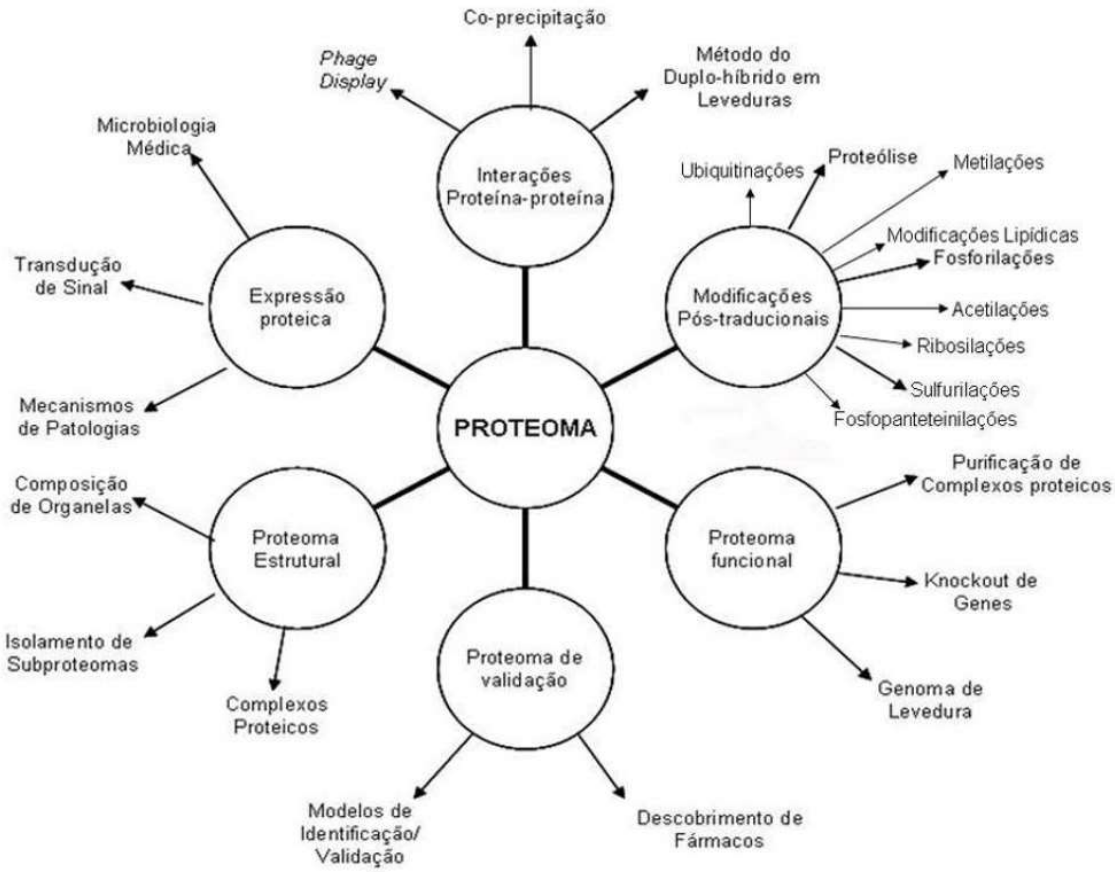

Figura 5. Mapa demonstrando as possíveis áreas de estudos das Ciências Biológicas a partir do proteoma. Fonte: Graves e Haystead, 2002.

Nas últimas décadas, a espectrometria de massas, emergiu como melhor método para a análise e identificação de proteínas em amostras biológicas, com o princípio de medir a massa/carga dos compostos ionizados. Historicamente a espectrometria de massas iniciou-se em 1899, com o físico Sir J. J. Thompson, que foi premiado com o Nobel de Física de 1906. Porém a grande utilização dessa técnica para a biologia molecular, especialmente análise de proteínas, ocorreu com o desenvolvimento de técnicas de ionização, que permitiam uma maior integridade da estrutura proteica, sendo mais utilizadas as técnicas de MALDI (Tanaka, Waki *et al*., 1988) e de ESI (Fenn, Mann *et al*., 1989). Na proteômica, a técnica mais apurada e utilizada atualmente para a separação de proteínas, são por géis 2 D ou por cromatografias (líquida e gasosa) (Salvato, F e Carvalho, MCCG, 2010). Foram desenvolvidas tecnologias que utilizam a cromatografia acoplada a espectrômetro de massas em tandem (LC-MS/MS) para a identificação de proteínas. A cromatografia baseia-se nas diferentes características das proteínas em colunas de propriedades distintas ou em uma coluna bifásica (Motoyama e

Yates, 2008). A fração de proteínas eluídas na primeira coluna é diretamente introduzida na segunda coluna que está acoplada ao espectrômetro de massas, sendo essa técnica mais sensível para identificação de proteínas menos abundantes (Washburn *et al*., 2001). O espectrômetro de massas consiste em uma fonte de íons, onde a molécula é ionizada, um analisador de massas, onde os íons são separados de acordo com sua massa/carga (m/z), e um detector de íons, onde se geram espectros de massas. Os equipamentos mais utilizados na proteômica, atualmente, são os que possuem as configurações MALDI/ESI-QToF MS ou ESI-LTQ/Orbitrap MS (Wu e Maccoss, 2002; Makarov e Scigelova, 2006).

A espectrometria de massas é uma análise apurada de proteínas, e pode ser dividida em duas grandes abordagens: a abordagem livre de marcação, denominada *Label-free* ou utilizando marcação (Neilson *et al*., 2011). Estratégias de marcação são muitas vezes consideradas mais precisas na quantificação de proteínas. Essas técnicas envolvem a marcação dos peptídeos com as chamadas "tags", ou etiquetas isobáricas, como mostra a Figura 6, para a quantificação relativa e absoluta, como no caso da marcação pelo iTRAQ (Ross *et al*., 2004). Podemos citar também a marcação das proteínas com etiquetas isotópicas – ICAT (Gygi *et al*., 1999) e as proteínas metabolicamente marcadas pela incorporação de isótopos em aminoácidos – SILAC (Ong *et al*., 2002).

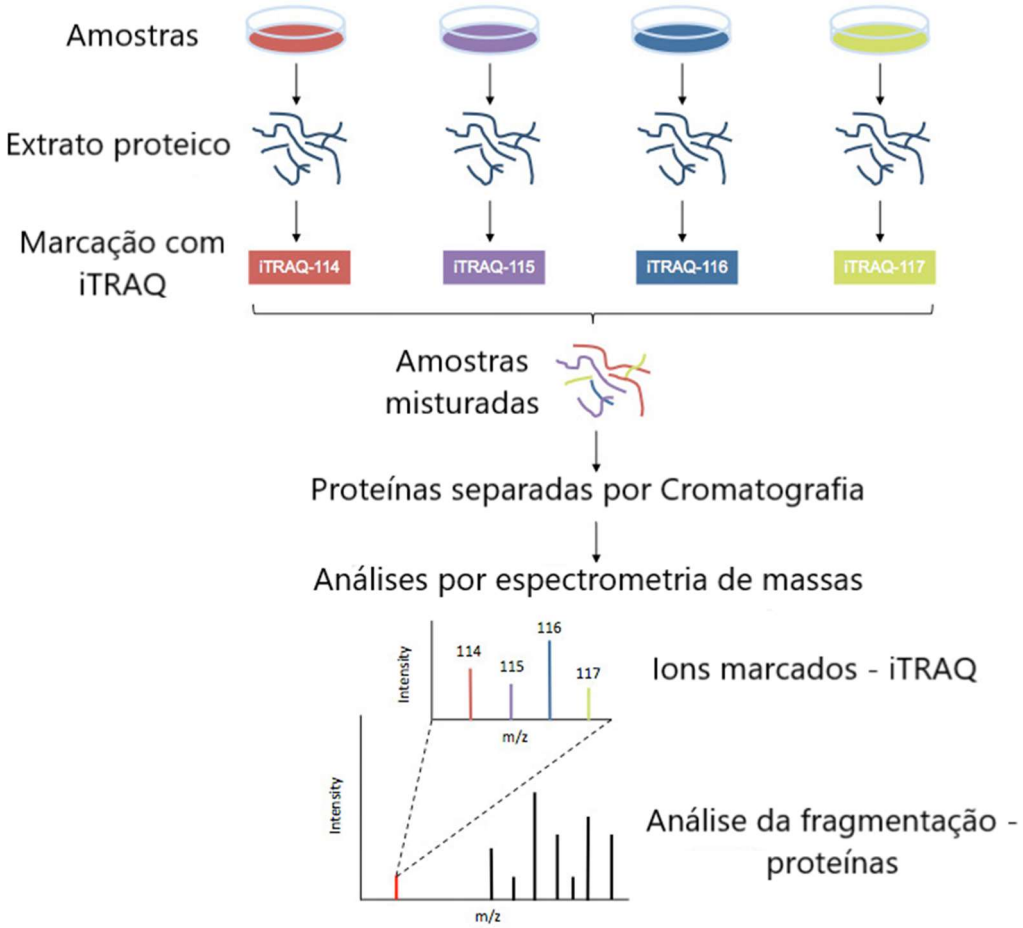

Figura 6. Marcação de amostras de proteínas com a utilização do reagente iTRAQ. Etapas da metodologia e da análise por iTRAQ. Fonte: www.creative-proteomics.com (com modificações).

2.0. Justificativa

Os estudos de proteômica têm o objetivo de analisar e identificar em larga escala, as proteínas presentes em uma célula ou tecido, analisando simultaneamente misturas complexas de proteínas provenientes de lisados de células e de tecidos, com a finalidade de detectar diferenças quantitativas e qualitativas na expressão dessas moléculas. Com as tecnologias desenvolvidas no campo da proteômica, é possível identificar proteínas relacionadas a vários processos biológicos; essas proteínas podem ser detectadas pelas alterações quantitativas pouco ou muito reguladas, por sua presença ou ausência em determinada célula ou situação e por modificações pós-traducionais (Westermeier e Naven, 2002).

Através de diversos estudos e metodologias da biologia molecular, o fungo *Paracoccidioides* spp. está atualmente descrito e classificado em 5 espécies distintas, *P. brasiliensis*, *P. americana*, *P. venezuelensis*, *P. restrepiensis* e *P. lutzii*. Dado que as espécies de *Paracoccicioides* são responsáveis pela micose sistêmica mais prevalente da América latina, torna-se importante desvendar as diferenças moleculares e biológicas entre as espécies desse fungo. Sendo assim, técnicas de proteômica, podem contribuir para a elucidação e caracterização das diferenças entre as espécies, sendo que os mapas proteômicos podem ser uma importante ferramenta para estudos de vias metabólicas, processos de virulência, sinalização celular, entre outros processos vitais, que podem influenciar nas características morfológicas e moleculares de cada espécie do *Paracoccidioides*.

Estudos anteriores tiveram como objetivo a caracterização das espécies *Pb* 01 (*P. lutzii*), *Pb* 2 (*P. americana*), *Pb* 339 (*P. brasiliensis*) e *Pb*EPM83 (*P. restrepiensis*) na presença de glicose, através da utilização de eletroforese bidimensional e identificação de proteinas por MALDI-Tof. Verificou-se nessa primeira análise que ocorrem diferenças de expressão proteica e vias metabólicas entre as espécies; algumas espécies utilizam preferencialmente as vias da glicólise e gliconeogênese, como também utilizam-se da fermentação alcoólica paara obtenção de energia; outras espécies utilizam preferencialmente a via das pentoses fosfato e ciclo do TCA. Foram verificadas também diferenças de resposta ao estresse entre essas espécies (Pigosso et al., 2013). Estudos posteriores utilizando-se as espécies *Pb* 01 (*P.* lutzii), *Pb* 03 (*P.* americana), *Pb* 339 (*P. brasiliensis*) e *Pb*EPM83 (*P. restrepiensis*) cultivadas na presença de acetato de sódio,

como única fonte de carbono, permitiram verificar que ocorreu uma diferença na expressão de proteínas relacionadas as vias da gliconeogênese, ciclo do glioxilato, reposta ao estresse e degradação de aminoácidos, indicando que as 4 espécies reorganizam seu metabolismo de maneira diferente para usar o acetato como fonte de carbono (Baeza *et al*., 2017) Em função das observações anteriores sobre as diferenças metabólicas entre diversas espécies do gênero *Paracoccidioides,* o presente projeto tem o objetivo de avaliar o metabolismo e expressão proteica entre as espécies *Paracoccidioides brasiliensis* e *Paracoccidioides americana*, revelando possíveis diferenças entre as duas espécies.

3.0. Objetivo Geral

Este estudo tem o objetivo de identificar e comparar o proteoma das espécies de Paracoccidioides: *P. brasiliensis*, *P. americana*, assim como elucidar diferenças nos perfis de expressão proteica das duas espécies do fungo.

3.1. Objetivos específicos

• Otimização do protocolo de extração e identificação de proteínas;

• Verificação dos perfis de expressão proteica entre as espécies de *Paracoccidioides*;

• Marcação com o reagente iTRAQ nos extratos de proteínas totais

• Classificação funcional das proteínas totais, como também a identificação de vias metabólicas.

CAPÍTULO 2

MANUSCRITO 1

ITRAQ Labeling: A comparative proteomic analysis between *Paracoccidioides* species

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Abstract

The genus *Paracoccidioides* comprises termodimorphic fungi present in Latin America, causing paracoccidioidomycosis, a systemic mycosis presenting high frequency in Latin America. These fungi can grow as yeast at 36 ° C and mycelium at temperatures below 28 ° C. Studies have shown that the members of this genus present molecular differences that allow the classification in distinct species. The genus which presented initially a single species, is currently classified into five, as following, *P. lutzii, P. brasiliensis, P. americana, P. restrepiensis and P. venezuelensis*. Different species may exhibit molecular peulariaties, a fact that led us to the investigation of proteomic profiles of two species of *Paracoccidioides, P. americana* and *P. brasiliensis*. iTRAQ labelling procedures were performed, the samples were analysed by LC mass spectrometry. Three hundred and eighty seven proteins were obtained and from those, 202 proteins were more abundant in *P. brasiliensis* and 111 in *P. americana*. The proteins identified have different functions, such as amino acid metabolism, energy, cell defense, among others. The results showed differences between *P. americana* and *P. brasiliensis* in proteins related to cell defense, mitochondrial and energy metabolism, suggesting that these fungi may have different metabolism and pathways for growth, profliferation and virulence.

Keywords: Fungi; Molecular biology; Molecular response; Detoxification.

1.0. Introduction

Paracoccidioidomycosis (PCM) is a chronic granulomatous mycosis, endemic in Latin America, with a high incidence in Brazil, Colombia, Venezuela and Argentina caused by the fungi *Paracoccidioides* spp. (Bellisimo, *et al*., 2011). Those fungi are members of the phylum Ascomycota, class Euromycetes order Onygenales and family Ajellomycetae (Leclerc *et al*., 1994; James *et al*., 1996; Bialek *et al*., 2000; Untereiner *et al*., 2004; Sturme *et al*., 2011). Those fungi are thermodymorphic having the capacity to grow in the form of mycelium, between temperatures of 22 ° C to 28 ° C, in the soil, and in the form of yeast at temperatures of 36 ° C to 37 ° C, in the host; this morphological transition, is directly linked to the capacity for infection and virulence (Rooney, PJ and Klein, BS, 2002).

The official nomenclature of the disease was established in 1971 at the meeting of mycologists of the Americas in Medellin (PAN AMERICAN SYMPOSIUM ON PARACOCCIDIOIDOMYCOSIS, 1971). However, the nomenclature and classification of the fungi that make up this group, continued to change over the years. Morphological characteristics were initially described by Splendore, who named the fungus *Zymonema brasiliensis* (Splendore, 1912). A few years later, the species was analyzed by Almeida, who compared it with the fungus *Coccidioides immitis*, observing distinct morphology, proposing the name of *Paracoccidioides brasiliensis*, considered as a single species (Floriano, 1930). After some analysis of many isolates of *Paracoccidioides brasiliensis*, was noted some differences among these isolates, showed polymorphism in many genes, as GP43, alfa-tubulin, adenosyl ribosylation factor, CHS2, FKS, these differences made possible the creation of clades S1, PS2 and PS3 (Matute *et al*., 2006; Carrero *et al*., 2008). Studies with 63 isolates of the genus *Paracoccidioides* belonging to species S1, PS2, PS3 and *P. lutzii*, using the SNPs (Single Nucleotide Polymorphism) technique, showed a restricted geographical distribution to Colombia of isolates belonging to species PS3, *P. lutzii* in central regions of Brazil and Ecuador, PS2 in Brazil and Venezuela and S1 presented a broader distribution, present in Brazil, Argentina, Paraguay, Venezuela, Uruguay and Peru (Matute *et al*., 2006a; Teixeira *et al*., 20009; Theodoro *et al*., 2012). In 2014, after molecular studies, another phylogenetic species was included in the *Paracoccidioides brasiliensis* complex, the PS4 species, so the genus *Paracoccidioides brasiliensis* was classified with 4 phylogenetic species, S1, PS2, PS3 and PS4, in addition

to *Paracoccidioides lutzii* (Matute *et al*., 2006; Carrero *et al*., 2008; Teixeira *et al*., 2009; Bocca *et al*., 2013; Teixeira *et al*., 2014).

In recent years, after molecular analyzes, a study proposed the classification of the *Paracoccidioides* genus in different species, as following: *Paracoccidioides brasiliensis*, *Paracoccidioides americana*, *Paracoccidioides restrepiensis*, *Paracoccidioides venezuelensis*, and *Paracoccidioides lutzii*, (Teixeira et al., 2009; Turissini DA *et al*., 2017). A study using glucose as the only carbon source, compared the proteomic profile of four isolates of , as following: *Pb* 01 (*P. lutzii*), *Pb* 2 (*P. amerciana*), *Pb* 339 (*P. brasiliensis*), *Pb*EPM83 (*P. restrepiensis*). It was observed differences among those species; *P. lutzii* used preferentially anaerobic pathways for energy generation, when compared to *P. brasiliensis,* was observed some differences in proteins related with response to reactive oxygen, indicating that the response of oxidative stress was more abundant in *P. lutzii P. Americana* and *P. brasiliensis*, the *P. restrepiensis* showed a smaller expression of oxidative stress response, these study showed many expression and metabolic differences among the *Paracoccidioides* species (Pigosso *et al*., 2013). Other study performed with acetate, as the only carbon source, compared the proteome of *Paracoccidioides* members: *P. lutzii* (*Pb* 01), *P. americana* (*Pb* 03), *P. brasiliensis* (*Pb* 339), *P,* restrepiensis (*Pb*EPM83), was observed some metabolic differences, as so the enzymes related with TCA cycle and respiratory chain more abundant in *Pb*EPM83 and *Pb* 01 (*P. restrepiensis* and *P. lutzii*, respectively), *Pb* 01 showed a increase in ethanol production, *Pb* 03 and *Pb*EPM83 utilized cell wall components for gluconeogenesis; *Pb* 01 and *Pb*EPM83 showed a major expression of proteins related with methylcitrate and β-oxidation (Baeza *et al*., 2017).

Concerning to the proteomics of *P. brasiliensis* it was observed that mycelia and yeast cells present several differences with reference to the energy metabolism. phases, showed that specie have some alterations in proteome in these morphological modifications. Was observed in yeast cells the aerobic beta-oxidation and TCA cycle was up-regulated, when compared with transition and mycelia. Was noted too, proteins of virulence in yeats cells, that assists in adaptation in the hosts. In contrast, was identified in mycelia phase, proteins related with alcoholic fermentation, when compared with the yeast cells, showing the differences in the organism when occur the morphological transition (Araujo *et* al., 2019). The protein expression and post-translational modifications, is the responsable for the morphology, biological and molecular responses

and adaptations. These events, can differentiate close organisms into distinct species, unraveling important metabolic routes. Therefore, the proteomic analysis is important to elucidate and characterize the proteome and molecular profile of the *Paracoccidioides* species. In this work, we will evaluate the differences in the protein expression profile between *P. brasiliensis* and *P. americana*.

2.0. Materials and methods

2.1. Strains and growing conditions

In all experiments the species *P. americana* (isolate 03) and *P. brasiliensis* (isolate 18) were used, which were kept in yeast form in vitro at 36 ° C in Fava-Neto medium (Fava-Neto, 1995). After growth in solid medium, the cells were inoculated in BHI (Brain Heart Infusion) medium, with glucose, 4% (w / v), at 36 ° C, under agitation of 150 rpm, for 72 hours. The experiments were carried out in biological triplicate.

2.2. Protein extraction

Yeast cells were centrifuged at 1200 xg for 10 minutes at 4°C, washed 3 times with 1X PBS (Saline Phosphate Buffer) and mechanically lysed by shaking with glass beads in lysis buffer (8 M Urea, 75 mM NaCl , 50 mM Tris, pH 8.0, 50 mM β-glycerol phosphate, 1 mM sodium orthovonadate, 10 mM pyrophosphate, 1 mM PMSF). To 300 µL of fungal cells were added 700 µL of lysis buffer at 4 ° C in microtubes containing glass beads; the material was shaked vigorously in a mini-bead beater in 4 cycles of 90 seconds, with a 1 minute interval on the ice, between each shaking. The tubes were centrifuged (10,000 x g for 15 minutes) for the separation of the cell debris. Protein concentrations of the extracts were quantified using the Qubit kit, and the profile and protein integrity of the 12% SDS-PAGE gel extracts were confirmed. The supernatants were stored at -80 ° C.

2.3.Protein Samples preparation

The protocol described by Lin et al. (2015) was used. A total of 150 µg of protein extract was used and 1% acetone was added in ice. The mixture was incubated for 16 hours at -20 ° C. Samples were centrifuged at 12,500 x g for 5 minutes and the precipitate was resuspended in lysis buffer (8 M urea and TEAB - 0.05 M Triethylammonium bicarbonate, pH 7.9).

Protein extracts (150 µg) of the *Paracoccidioides* species described in the previous section were submitted to trypsin digestion and biological triplicates were performed for each species. The sample, resuspended in the lysis buffer, was maintained at 4 ° C and subjected to a sonication step for 60 seconds. In the next step the reduction of the disulfide bridges by the addition of dithiothreitol (DTT) was carried out in a final concentration of 0.005 M and the samples were incubated for 25 minutes at 55 ° C. After incubation, the sample was allowed to stand for a few minutes and then iodoacetamide (IAA) was added at the final concentration of 0.014 M. The extract was kept at room temperature for 40 minutes, away from the light, and then was added to the sample , DTT to a final concentration of 0.005 M for discontinuation of alkylation at the cysteine residues. The mixture was diluted 1: 5 with 0.025M TEAB (triethylammonium bicarbonate buffer) (pH 7.9), adding CaCl 2 to a final concentration of 0.001 M. The protein digestion was started with the addition of trypsin at the ratio of 1:50 and incubation at 37 ° C for 16 hours. Trifluroacetic acid 10% (v / v) was added, to a final concentration of 1%. Subsequently, 50 µg of proteins from the samples were desalted in a StageTip in the low-binding P-200 tip, mounted with C18 disk, to remove all salt. The desalting eluate was concentrated in vacum and subsequently resuspended and labeled with the reagent iTRAQ. As showed in Supplementary Figure 1, the steps for protein sample preparatio for the mass spectrometry analysis.

2.4. Labeling of samples with the iTRAQ reagent

The labelling with the isobaric marker compound - iTRAQ, was performed in biological triplicates. 50 µg of each sample, which were desalted after digestion, were resuspended in 17 µl of 300 mM TEAB (triethylammonium bicarbonate). The iTRAQ reagent was resuspended in 70 µl of ethanol as described in the manufacturer's protocol

(AB Sciex^{TM}, Washington, USA). The markers (iTRAQ) were added to the samples that were incubated for 2 hours; the markers (tags) used in the labeling were 115 and 117, which are isobaric markers that bind to the N-terminus and the side chain amines of the peptides, thus inserting variable masses into the peptides, allowing the analysis of the samples simultaneously, reducing the variables that can occur from one analysis to another. All samples were labeled in the same proportion; the used tags were 115, for *P. americana*; and tag 117 for *P. brasiliensis*. The samples were again desalted with the StageTip on the low-binding P-200 tip, with C18 disk and were concentrated in vacuum, resuspended and applied to the mass spectrometer.

2.5. Mass spectrometry

Posteriorly to the labeling of samples with the iTRAQ reagent, 10 μg were resuspended in 6 μL of 1% (v / v) formic acid and applied to the Orbitrap Q-Exactive mass spectrometer (Thermo Fisher, Bremen, Germany). The total proteomes of the two species (*P. americana, P. brasiliensis*) were ionized by the nanoeletrospray (according to the protocol of Thingholm TE et al., (2010), for better ionization of the released peptides. The data obtained by the spectrometer were processed by Protein Discovery software and by PEAKS studio software (Bioinformatics solutions - http://www.bioinfor.com/peaks-studio/). Tables of global proteins and peptides were generated. Protein identification was initially selected based on an ID quality parameter, the FDR (High / medium / low), using only those proteins that showed High and Medium level. The process steps for cultivation, protein extraction and digestion, desalinization samples, iTRAQ labeling and the mass spectrometry analysis, is shown in Supplementary Figure 1.

2.6. Mitochondrial activity

Mitochondrial activity and integrity was analyzed using Rhodamine 123 (Sigma-Aldrich) and Mito-Tracker Green FM (Sigma-Aldrich) fluorescent dyes. The cells were stained according to the manufacturer's instructions. Yeast cells of *P. americana* and *P. brasiliensis* were grown for 72 hours in BHI medium at 37 C, centrifugated at 2000 g for 5 min at 4°C, diluted in 1× PBS to 2.10^6 cells/mL and stained during 30 min with 400 μM of Mito-Tracker Green FM at 36°C. After two washes with 1× PBS, the cells were

analyzed by fluorescence microscopy (Zeiss Axiocam MRc – Scope A1) using the 490-516 nm filter (FS09); the images were acquired, these step, was performed for measured the mitochondria integrity. Yeast cells of *P. amerciana* and *P. brasiliensis*, were also analyzed for mitochondria activity. Cells were centrifuged and incubated with 20 µM rhodamine for 20 min at room temperature. The cells were washed twice with 1X PBS and resuspended in 1 mL PBS for analysis in fluorescence microscopy (Zeiss Axiocam MRc – Scope A1); was used the 515-575 nm filter (FS15) for the analysis. The experiments were realized in triplicates; it were selected 30 cells for each microscope slide which were measured for the fluorescence intensity (in pixels). The Student's t-test was performed and p-values ≤ 0.05 were considered statistically significant.

2.7. Superoxide dismutase (SOD) activity determination

To detect the SOD activity in *Paracoccidioides* species, protein extraction of yeast cells of *P. americana* and *P. brasilensis* was performed with the SOD determination Kit (Sigma-Aldrich, USA). The experiment was performed in Elisa's plate, and in each plate well was added 20 µl of each sample, followed by the addition of the solutions according to the manufacturer instructions.. The absorbance of the reactions were measured at 440 nm in the Elisa equipment (SpectraMax Paradigm Microplate Reader, Molecular Devices, California, USA). The SOD activity was calculated using the following equation: SOD activity (inhibition rate %) = {[Ablank 1 - Ablank 3) - (Asample - Ablank 2)]/ (Ablank 1 - Ablank 3)} x 100. The Ablank is the sample used as blank without the addition of proteins.

2.8. Determination of the Thiol levels

After incubation in BHI media, 10^6 yeast cells were collected and resuspended in lysis buffer (50 mM Tris-Cl, 150 mM NaCl, 50 mM ethylenediamine tetraacetic acid [EDTA], pH 7,2), followed by shaking in a beadbeater equipament in 4 cycles of 30 seconds; the samples were kept on ice in the interval of each cycle. The samples were centrifuged at 10,000 x g for 15 minutes at 4 º C and the supernatant was used for the enzymatic assay for thiol levels. Posteriorly, 100 µL of supernatant was added in 100 µL of 500 mM sodium phosphate buffer, pH 7,5. The samples were added to plate wells and was added 20 µL of a solution 1 mM 5,50-dithio-bis (2-nitrobenzoic acid) (DTNB). Absrobance was determined at 412 nm in Elisa equipament (SpectraMax Paradigm

Microplate Reader, Molecular Devices, California, USA). Free thiol levels were determined with Ellman's reagent, DTNB (Sigma-Aldrich, Co).

2.9. Ethanol determination

The total of 2 g of *P. americana* and *P. brasiliensis* yeast cells were used to perform the assay. The cells were lysed using glass beads and mini beadbeater equipment in four cycles of 30 s, keeping the samples on ice in the interval of each cycle. The cell lysates were centrifuged at 10,000 X g for 15 min at 4 ºC for obtaining the supernatant used for the enzymatic assay. The concentration of ethanol was quantified by using an enzymatic detection kit (UV-test for ethanol, RBiopharm, Darmstadt, Germany). The principle of method is that ethanol is oxidized to acetaldehyde in the presence of the enzyme alcohol dehydrogenase. Subsequently, acetaldehyde is oxidized quantitatively to acetic acid in the presence of aldehyde dehydrogenase and NAD, releasing NADH, which is determined at absorbance at 340 nm. Concentrations of ethanol were determined in biological triplicates.

3. Results and discussion

3.1-Analysis of cellular viability and growth of *P. brasiliensis* and *P. americana*

Firstly, cell viability and growth of the two species were carried out. As observed in Supplementary Figure 2, the point of 72 h showed a high viability. In this way, 72 h-time-point was defined for the proteomic analysis.

3.2. Proteomic analysis of both species

A total of 1,868 proteins were obtained, wich are described in Supplementary Table 1. The total of 12,505 peptides was identified. Only proteins that presented the level of FDR (confidence in identification) high or medium remained in the table; after these analysis, remained 1313 proteins. After statistical analysis using T-test, and p-value ≤ 0,05, 357 proteins were considered.. After, it was applied a fold change of 1.2, and were obtained 240 proteins for *P. brasiliensis* and 117 proteins for *P. americana*, considered

as differentially expressed between the two species, as depicted in Supplementary Table 2 and 3. In a proteomic study with species of *Paracoccidioides* genus, as following *Pb* 01 (*P. lutzii*), *Pb* 339 (*P. brasiliensis*), *Pb* 2 (PS2 – *P. americana*) and *Pb* EPM 83 (*P. restrepiensis*), performed by 2-D gels and mass spectromety, using a label free method, were identified a total of 193 proteins and isoforms in the four *Paracoccidioides* members (Pigosso, L.L *et al*., 2013). Other study with members of *Paracoccidioides* genus, using mass spectrometry, analyzed and compared the proteome in acetate as the only carbon source, identifying in *P. lutzii* (*Pb* 01) 1160 proteins, in *Pb* 03 (*P. americana*) 1211 proteins, in *Pb* 339 (*P. brasiliensis*), 1280 proteins and in *Pb*EPM83 (*P. restrepiensis*) 1462 proteins. Applying statistical analysis, were obtained 526, 442, 744 and 569 differentially expressed proteins, respectively, comparing all the species (Baeza, LC *et al*., 2017). A proteomic comparative study with two races of *Fusarium oxysporum*, R1 and R2, identified 145 proteins with differential abundances between the two races (Li *et al*., 2015). Comparative proteomic study with *Candida albicans* and *Candida glabrata*, using the iTRAQ method, identified 500 proteins of both fungus, and after applying the fold change (1.5), and *C. albicans* presented 19 proteins dffirentially expressed and *C. glabrata* presented 12 proteins (Prasad *et al*, 2010).Those numbers are in agreement with our data.

Many proteins involved in metabolism, specifically amino acids, nucleotides carbohydrate and lipid were detected. *P. americana* presented 35% of proteins related with metabolism and *P. brasiliensis* presented 19% (Figure 1). *P. americana* expressed differentially proteins involved in glycolysis and gluconeogenesis, TCA cycle and alcohol fermentation that represented a total of 15% from the differentially expressed proteins in this species. In *P. americana* a total of 35% of proteins related with metabolism, included amino acids, carbohydrate, nitrogen, lipids. It is observed in *P. brasiliensis* 19% of the total of proteins was related to metabolism, Figure 1. Tables S2 and S3 depicts all the proteins identified as differentially expressed, when comparing *P. americana* and *P. brasiliensis*, respectively. The processes differentially regulated beteween the two species will be focused below.

A study with bacterias of the group AOB (Ammonia-oxidizing bacteria), analysed the proteome of three species, *Nitrosomonas europaea*, *Nitrosospira multiformis*, and *Nitrosomonas ureae*. The proteome showed some differences among these species, as the pyrophosphate-dependent 6-phosphofructokinase, that participates in the Calvin cycle,

this enzymes is missing in *N. multiformis* and *N. ureae*, showing differences in photosynthesis among these bacterias. Was identified some differences in proteins related with nitrogen metabolism, cell growth and stress response. These results showed that in similar species, can occur many molecular differences (Zorz *et al*., 2018).

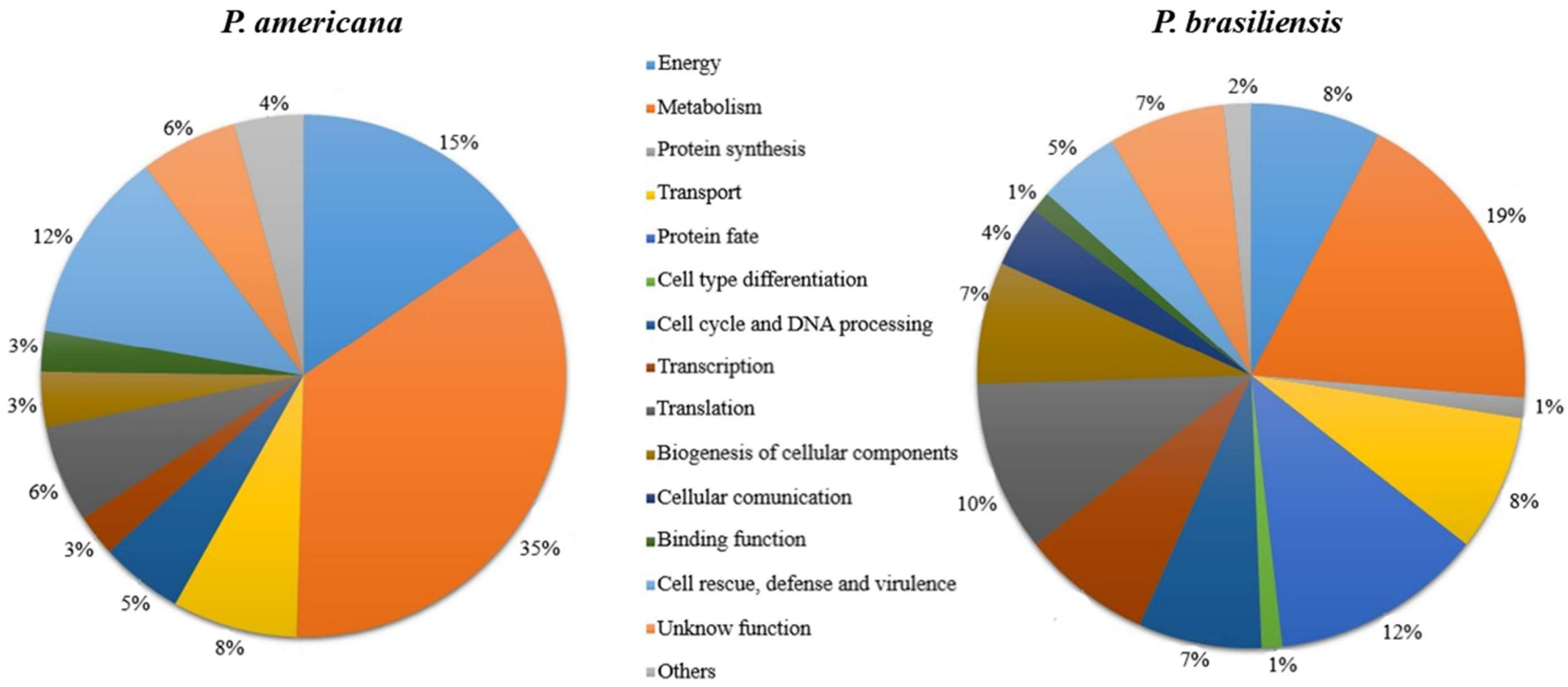


Figure 1. Functional Classification of the proteins identified in the species of *Paracoccidioides* (*P. americana* and *P. brasiliensis*). The identification was performed by software (Protein Discovery and PEAKS) and functional classification was performed through Uniprot database (http://www.uniprot.org/).

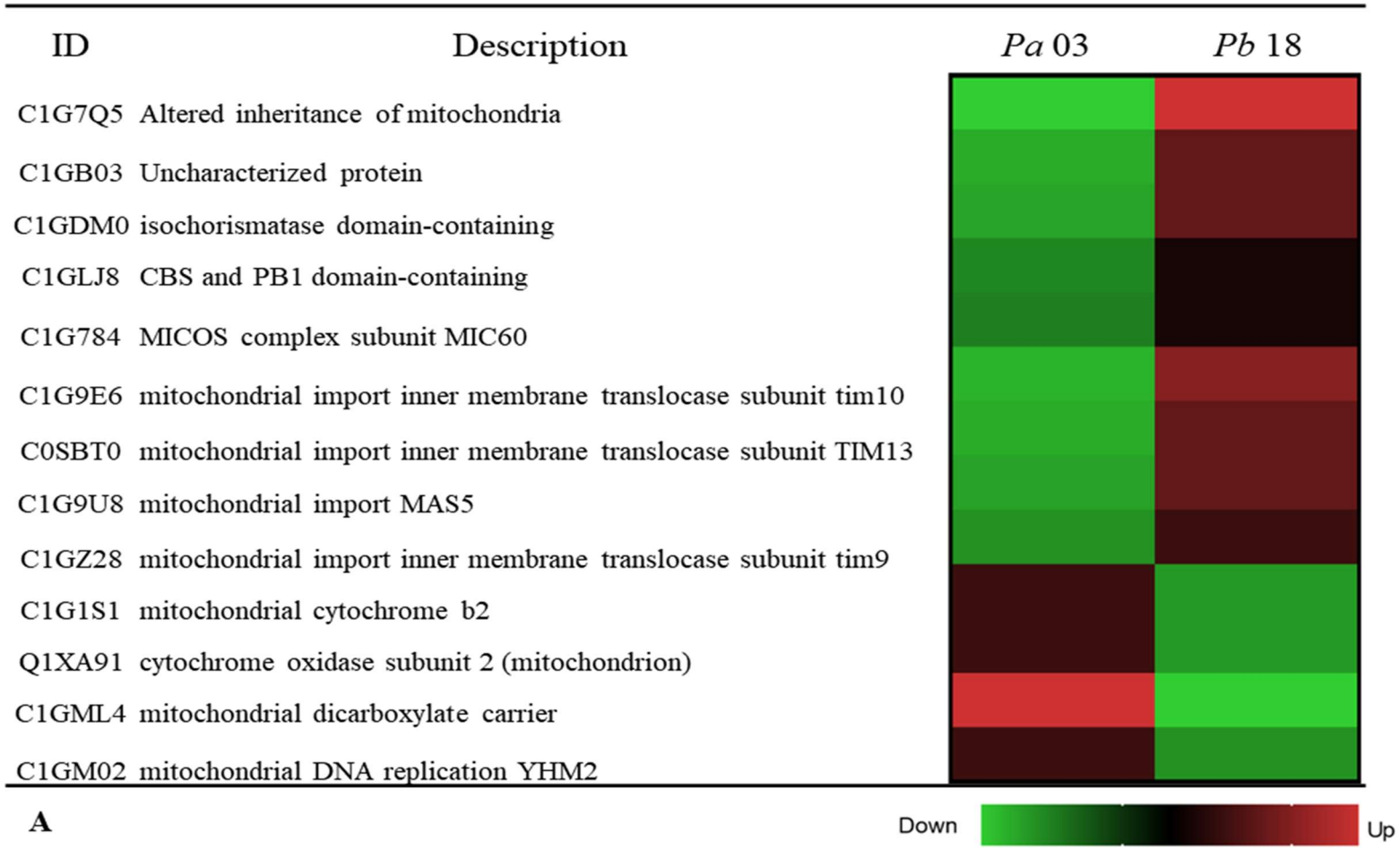
3.3. Proteins related to mitochondrial function are up regulated in *P. brasiliensis*

In the proteome *P. brasiliensis* showed more proteins of mitochondria which were up regulated when compared to *P.americana* (Supplementary Tables 2 and 3 and Figure 2. Up regulated proteins in *P. brasiliensis* presented role in the respiratory chain, eletron transport and membrane-associated energy conservation (Suplementary Table 3; Figure 2 A). In *P. brasiliensis*, the mitochondrial activity seems to be up- regulated, since it were found many proteins related to mitochondrial transport, like Tim 8, Tim 9, Tim 10 and Tim 13, in comparison to *P. americana* (Figure 2). The TIM (Trans Inner Membrane) proteins are related with the mitochondrial transport; they help the entry of proteins in mitochondria membrane, a process related to aerobic respiration and oxidative phosphorylation (Schleyer and Neupert, 1996; Perkins *et al*., 1997;Wiedemann and Pfanner., 2017).

Also, identified in proteomic analysis in this study, *P. brasiliensis* presented a higher number of proteins related to the respiratory chain, up regulated when compared to *P. americana*, as depicted in Figure 2 B. In *P. brasiliensis* were identified a group of acessory proteins of electron transport and membrane associated energy conservation, as NADH ubiquinone oxidoreductase subunit, NADH ubiquinone oxidoreductase mitochondrial 23 and 24 kDa, electron transfer flavo alpha subunit, cytochrome oxidase subunit 1 (mitochondrial) and suaprga1, all these proteins up regulated in *P. brasiliensis*, when compared to *P. americana*.

Also detected were proteins related to aminoacids metabolism that support the respiratory chain, as aspartate aminotransferase (C1G388), a protein that has a high participation in mitochondria, including the metabolism of glutamate. The branched-chain aminoacid transferase (C1GC48), NADP specific glutamate dehydrogenase (C1GBZ4) that convert glutamate in α-ketoglutamate, and can participate in respiratory chain in mitochondria and glycine cleavage system H protein (C1G4U7), related to mitochondrial activity were up regulated in *P. brasiliensis*. These proteins can provide eletrons of NADH for entry into mitochondria.

In previous studies of Pigosso et al., at our laboratory, it was demonstrated that a member of the species *brasiliensis* presented the respiratory chain more induced, when compared to other species, including *P. americana* (Pigosso *et al*., 2013).. Another study with *Paracoccidioides* members, growing in acetate as the only carbon source with *P. lutzii* (*Pb* 01), *P. restrepiensis* (*Pb* EPM83), *P. brasiliensis* (*Pb 339*) and *P. americana* (*Pb* 03), was observed some differences in the response to this carbon source. In *Pb*EPM83 (*P. restrepiensis*) were identified major proteins that participate in eletron transport and mitochondrial activity, as the suaprg 1 and cytochrome c oxidase factor 6, when compared with the other *Paracoccidioides* members (Baeza *et al*., 2017).


ID
Description
Pa 03
Pb 18
C1G7Q5 Altered inheritance of mitochondria
C1GB03 Uncharacterized protein
C1GDM0 isochorismatase domain-containing
C1GLJ8 CBS and PB1 domain-containing
C1G784 MICOS complex subunit MIC60
C1G9E6 mitochondrial import inner membrane translocase subunit tim10
C0SBT0 mitochondrial import inner membrane translocase subunit TIM13
C1G9U8 mitochondrial import MAS5
C1GZ28 mitochondrial import inner membrane translocase subunit tim9
C1G1S1 mitochondrial cytochrome b2
Q1XA91 cytochrome oxidase subunit 2 (mitochondrion)
C1GML4 mitochondrial dicarboxylate carrier
C1GM02 mitochondrial DNA replication YHM2
A
Down
Up

ID	Description
C1G256	NADH-ubiquinone oxidoreductase subunit
C0SI66	NADH-ubiquinone oxidoreductase 23 kDa mitochondrial
C1GBX9	ubiquinol-cytochrome c reductase subunit 7
C0S212	NADH-ubiquinone oxidoreductase 24 kDa mitochondrial
C1GDK7	NADH-ubiquinone oxidoreductase kDa subunit
C1G350	NADH-ubiquinone oxidoreductase 299 kDa subunit
C0RY31	NADH-ubiquinone oxidoreductase
C1GIJ5	electron transfer flavo alpha subunit
C0SC77	cytochrome c
Q1XA94	cytochrome oxidase subunit 1 (mitochondrion)
C1GGS8	suaprga1

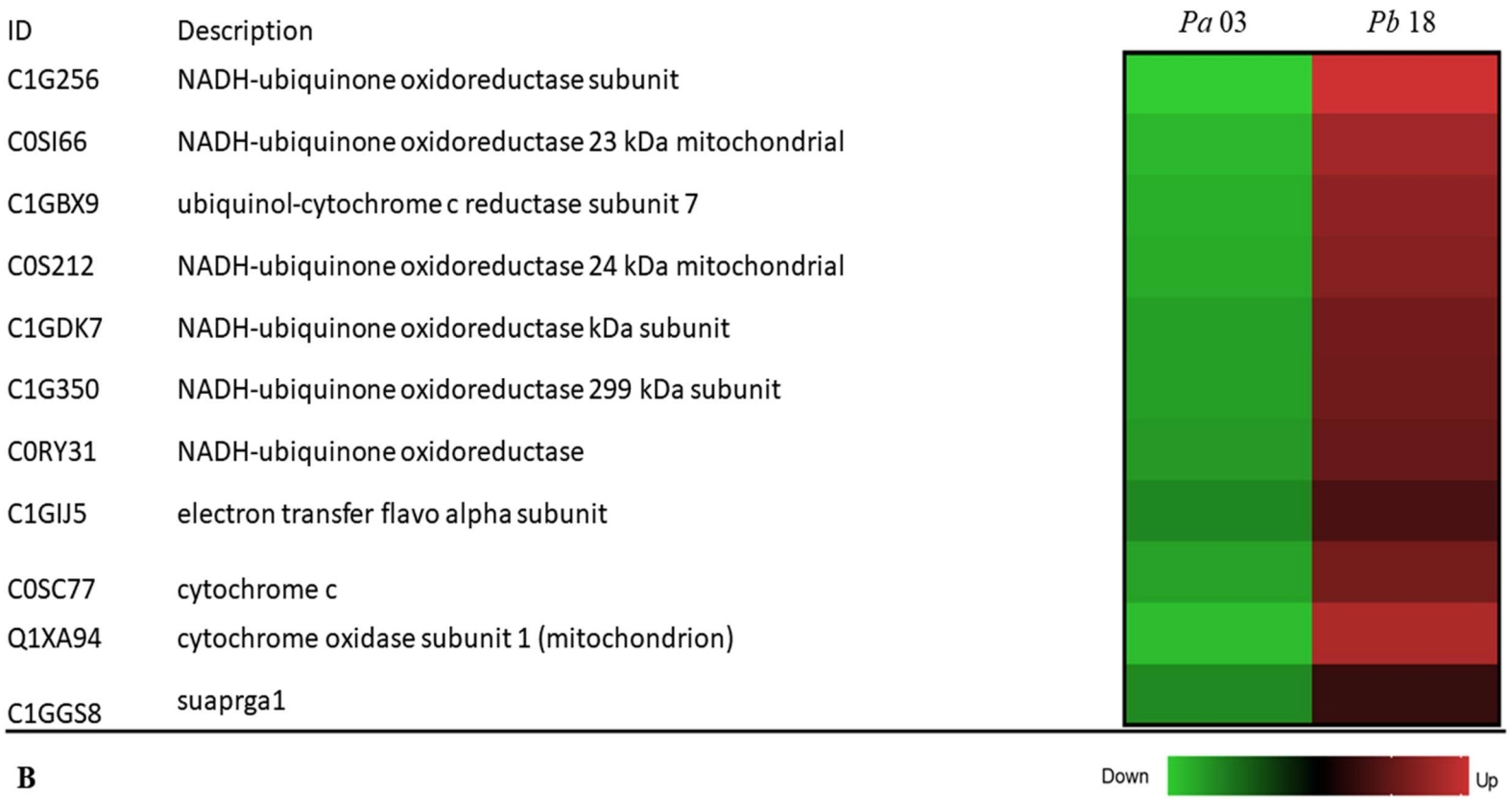
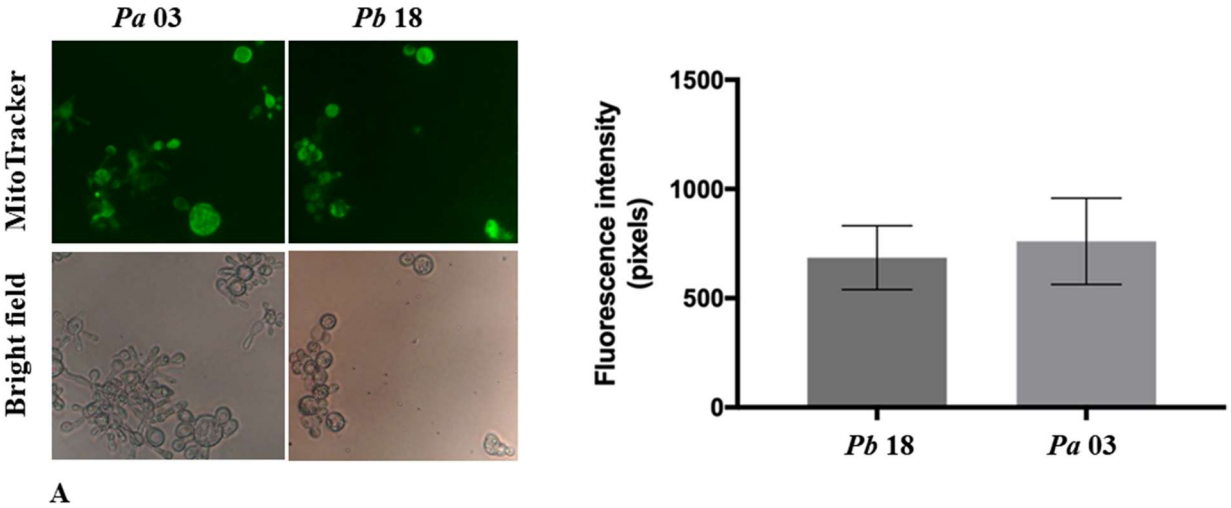


Figure 2. Heat map of proteins related to mitochondrial functions. A- Proteins identified in proteome analysis related to mitochondria structure, transport and activity in *P. americana* (*Pa* 03) and *P. brasiliensis* (*Pb* 18). Proteins regulated (up or down) in both species. B- Heat map of proteins identified by mass spectrometry, related with respiratory chain and energy aquisition functions between *Paracoccidioides* species.

Since *P. brasiliensis* presented a higher number of induced proteins related to mitochondria activity, the mitochondrial activity was analysed in both species. The integrity of mitochondria was analyzed, by using mitotracker, as depicted in Figure 3A. Both species presented mitochondria of similar size and similarly stained by mitotracker, suggesting similar integrity.

The Rhodamine assay was used in order to analyze mitochondrial activity, as depicted in Figures 3C and D. It was observed higher mitochondrial activity in *P. brasiliensis* when compared to *P. americana*, as shown in Figure 3 B. It was observed in *P.brasiliensis* a higher fluorescence when compared to *P. americana* indicating, in agreement to the proteomic data, that *P.b*rasiliensis has more active mitochondria (Figure 3 B). In previouos studies from our laboratory, using label free 2D gel proteomics it was observed that in other member of the *P. brasiliensis* species *Pb* 339, proteins related to mitochondrial electron transport were more abundant, when compared to *Pb* 01 (*P. lutzii*), *Pb* 2 (*P. americana*) and *Pb*EPM83 (*P. restrepiensis*) (Pigosso et al., 2013), corroborating more active mitochondria in *P. brasiliensis*.



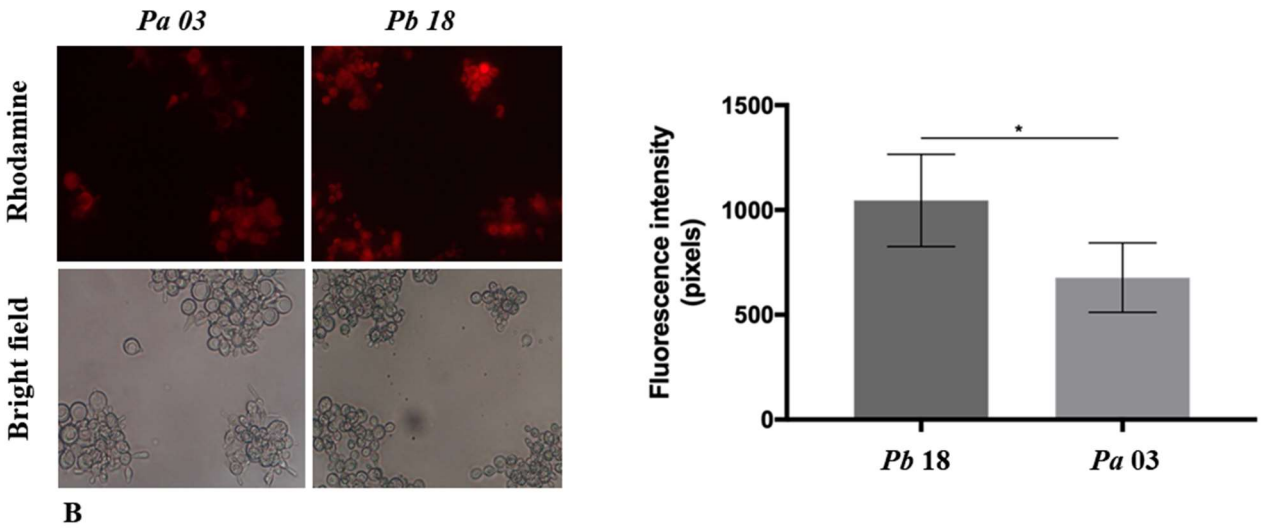


Figure 3. A) Analysis of mitochondria integrity of *P. americana* and *P. brasiliensis* with mitotracker. Yeast cells were incubated with mitotracker in order to verify mitochondria integrity; the cells were visualized by fluorescence microscopy. **B) Mitochondria activity assay.** Mitochondrial activity measured with Rhodamine. Fluorescence was quantified using the software ImageQuant. *Significantly different at $p < 0,05$.

3.4. The metabolism of carbon in *P. brasiliensis* and *P.americana*

Proteins in *P. americana* involved in gluconeogenesis were up regulated when compared to *P. brasiliensis.* Glucose-6-phosphate isomerase, fructose-1,6-phosphate isomerase that converts oxaloacetate in fructose-6-phosphate, pyruvate carboxylate and phosphoenolpyruvate carboxykinase that participate in gluconeogenesis, are increased (Figure 4 A). In *P. brasiliensis* glycolysis seems to be more active, providing the precursors for energy through TCA. Malate synthase that participates in the glyoxylate cycle, converts glyoxylate in malate (Figure 4 B), suggesting that β-oxidation provides acetyl-CoA as energy fuel and for the synthesis of carbohydrates. *Paracoccidioides* members can use different pathways for energy generation. In previous proteomic studies, *Pb* 01 (*P. lutzii*) demonstrated preference for alcoholic fermentation; *Pb* 2 (*P. americana*) for the TCA cycle and aminoacid degradation; *Pb* 339 (*P.brasiliensis*) used preferentially degradation of lipids by beta-oxidation and *Pb*EPM83 (*P. restrepiensis*) used the degradation of carbon compounds by glycolysis and TCA cycle (Pigosso *et al*., 2013). In our study *P. americana*, showed many proteins related to gluconeogenesis and alcohol fermentation. *P. brasiliensis* showed preference for aerobic metabolism, with many proteins related to the respiratory chain. In the comparative proteomic between two races of *Fusarium oxysporum* (R1 and R2), was identified many proteins related to

carbohydrate and aminoacid metabolism, in R2, more pathogenic when compared to R1 (Li *et al*., 2015). A comparative proteomic study with *C. albicans* and *C. glabrata*, described that *C. albicans* presented a higher expression of proteins related with glucose metabolism, including gluconegonegenis when compared with *C. glabrata* (Prasad *et al*., 2010).

A

ID	Description
C1GI20	triosephosphate isomerase
C1G0R1	glucose-6-phosphate isomerase
C1G440	fructose-1,6-bisphosphatase
C1GA17	pyruvate carboxylase
C1GML7	phosphoenolpyruvate carboxykinase
C1GCX3	2,3-bisphosphoglycerate-independent phosphoglycerate mutase
C1G9X3	enolase
C1G547	pyruvate dehydrogenase (acetyl-transferring) E1 alpha subunit
C1G056	pyruvate dehydrogenase E1 component subunit beta

B

ID	Description
C1GAG3	isocitrate dehydrogenase [NADP] mitochondrial
C1GLZ6	citrate synthase
C1GLB8	malate NAD-dependent
C0RXN7	fumarate reductase
C1G2X0	isocitrate dehydrogenase
C1GCI0	malate glyoxysomal

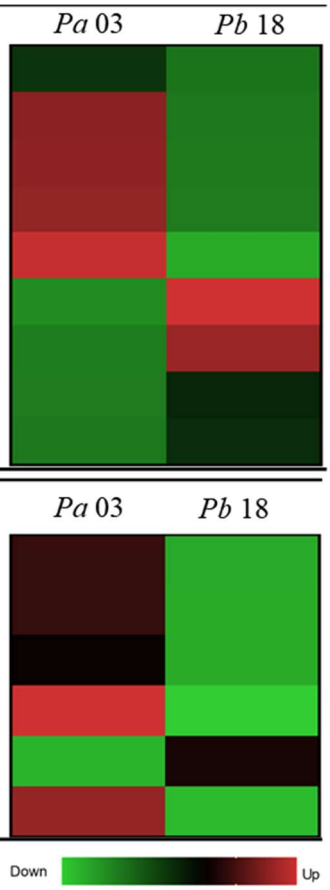
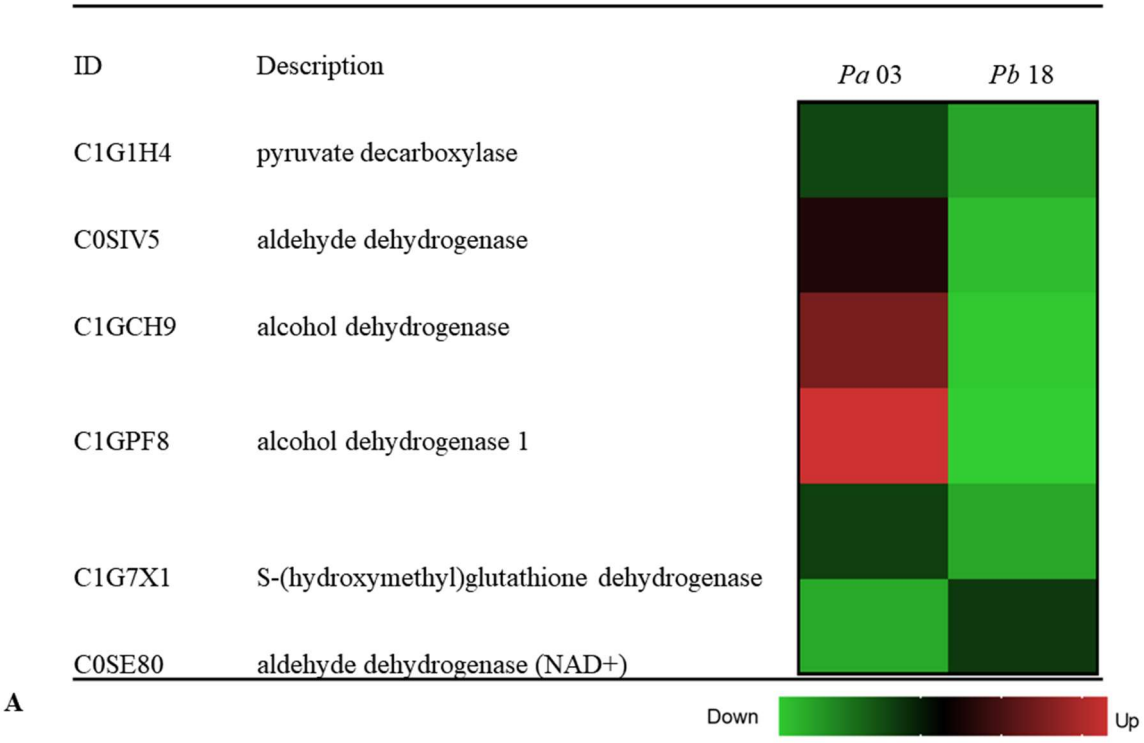


Figure 4. Proteins related to carbon metabolism in *Paracoccidioides* species. A) Glycolysis and Gluconeogenesis proteins. Proteins related to glycolysis and gluconeogenesis identified in proteomic analysis comparing the *Paracoccidioides* species. B) Proteins related to TCA and Glyoxylate cycle identified in proteomic analysis comparing the *Paracoccidioides* species.

3.5. The production of ethanol is preferential in *P. americana*

Enzymes related to the production of ethanol were increased in *P. americana*, when compared to *P. brasiliensis*, as depicted in Supplementary Table 2 and Figure 5A. The heat map in Figure 5 A, depicts proteins identified in proteomic analysis that have function in ethanol production. The proteins identified in our study, of fermentation, were alcohol dehydrogenase 1, alcohol dehydrogenase 2, pyruvate decarboxylase, aldehyde

dehydrogenase and S-(hydroxymethyl) glutathione dehydrogenase (Figure 5 A). All these proteins may be involved in the anaerobic metabolism, for energy generation in the microrganism. To confirm this data, ethanol quantification was evaluated, as shown in Figure 5B, demonstrating a higher ethanol production in *P. americana* compared to *Pb* 18 confirming and corroborating proteomic results. A study with *P. brasiliensis* phases, showed that in mycelium phase, the fungus produced more ethanol than compared to yeast and transition stage (Araujo *et al*., 2019), and in *P. lutzii*, the yeast phase have more ethanol production (Felipe, 2005; Rezende *et al*., 2011). A comparative proteomic among species of *Paracoccidioides*, demonstrated that *P. lutzii* (*Pb* 01) produced a high ethanol level than compared with other species of *Paracoccidioides*, showing that some species of this fungus, have a major anaerobic metabolism, while others prefer the aerobic metabolism (Pigosso *et al*., 2013).



A

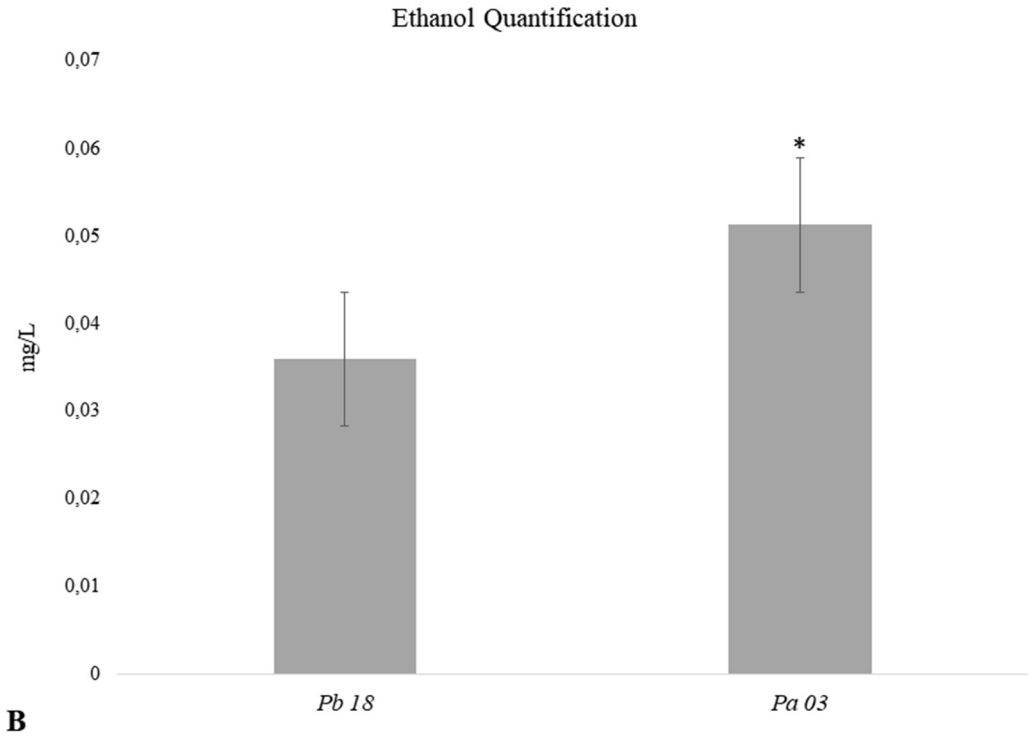


Figure 5. A) Heat map of proteins related to ethanol production in *Paracoccidioides* species. Proteins identified in proteomic analysis, which have function in ethanol generation. **B) Ethanol quantification in *Paracoccidioides* species.** Ethanol measurements in protein extracts of *Paracocidioides americana* and *Paracoccidioides brasiliensis.*

3. 5. Detoxification proteins between *Paracoccidioides*

The proteomic analysis identified some diferences in the expression of proteins related to cell rescue, defense and virulence, considering the two species studied. Proteins involved with detoxification, relevant to pathogens survival in hostile environments, presented some differential quantities considering the two species, as depicted in Figure 6. *P. americana* showed a higher expression of catalase P, Superoxide dismutase (SOD) and HSP 90, and *P. brasiliensis* (*Pb* 18) expressed preferentially heat shock STI1, Hsp 88, and thioredoxin, as shown in heat map (Figure 6).

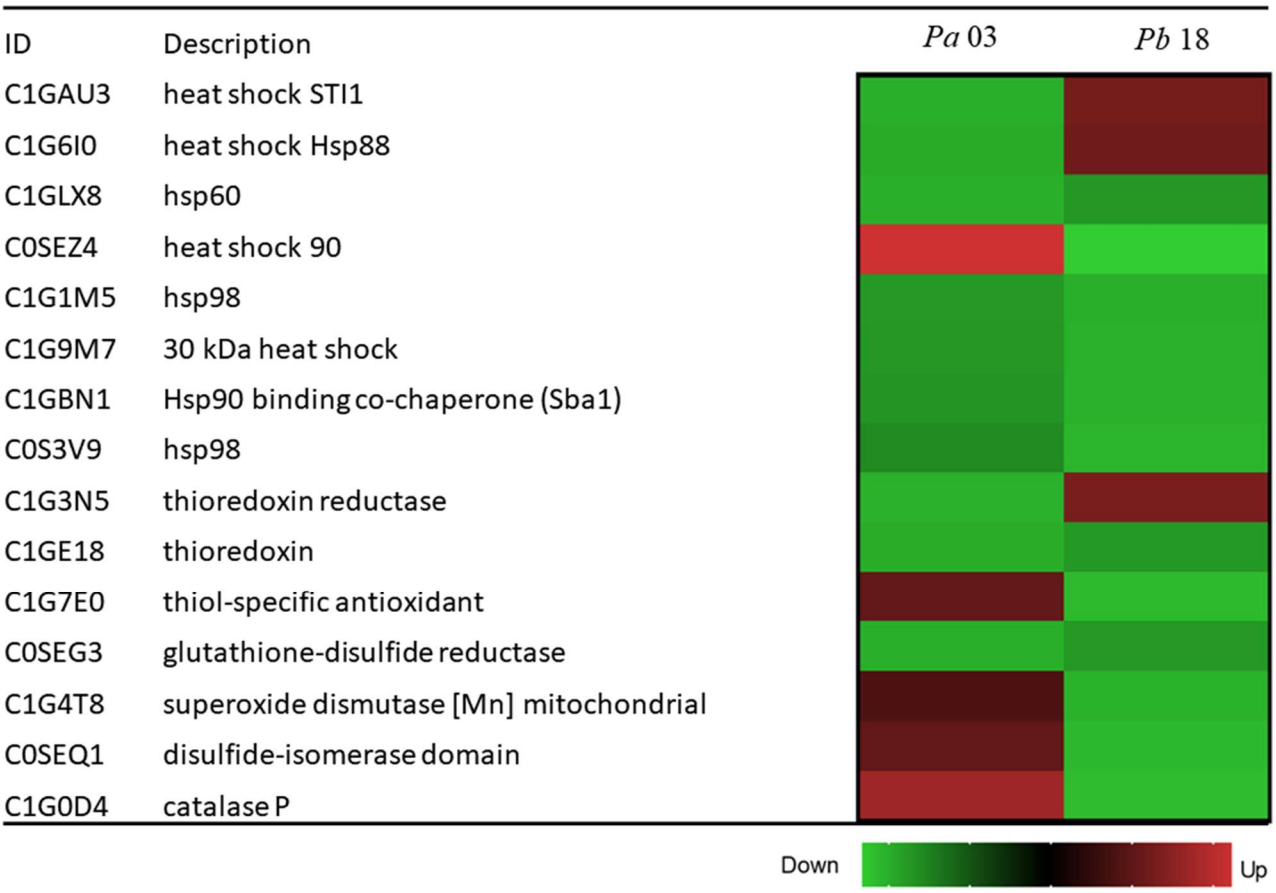


Figure 6. Proteins involved in cell rescue defense and virulence in *Paracoccidioides* species. A heat map with the proteins related to virulence and detoxification in the *Paracoccidioides* species.

For analysis of these differences in detoxification proteins, SOD activity dosage and Thiol reduced quantification were performed (Figure 7). *P. americana* showed a higher SOD activity when compared to *P.brasiliensis* (Figure 7 A). On the other hand, *P. brasiliensis* showed higher activity of thioredoxin (Figure 7 B). Other study with *Paracoccidiodies brasiliensis* phases, revealed that the mycelium phase demonstrated higher thiol levels when compared to yeast and transition phases (Araujo *et al*., 2019). In *C. albicans* and *C. glabrata*, comparative proteomics, was obseved that *C. albicans* presented the HSP 60 more abundant, but *C. glabrata* presented the HSP90 overexpressed; this is an indication for drug resistance of *C. glabrata*, which does not happen with *C. albicans* (Prasad *et al*., 2010).

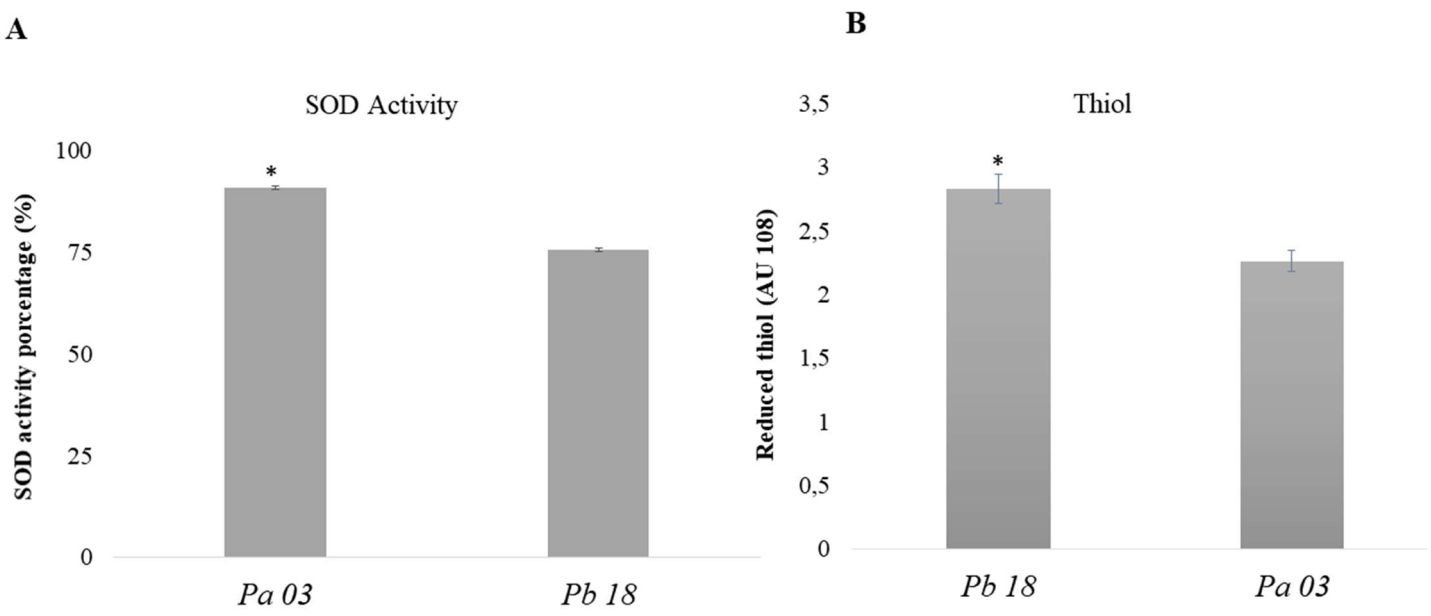


Figure 7. Detoxification analysis between *Paracoccidioides species*. SOD activity determination and Thiol reduction quantification. A) SOD activity dosage in *Paracoccidioides* species, showing the porcentage of the Superoxide Dismutase activity in the yeast cells of *Pa* 03 and *Pb* 18. **B)** Thiol dosage between *Pa* 03 and *Pb* 18, showed the activity of Trx protein.

The results obtained in our study suggest the importance of this protein group, presenting expression differences between these species of *Paracoccidioides*. Other study with *Paracoccidioides* species identified in *Pb* 2 (*P. americana*) Cu /Zn superoxide dismutase (SOD), peroxisomal catalase and 2 alkenal-reductase, more expressed than in other species. *Pb* 339 (member of the species *brasiliensis*) presented Mn superoxide dismutase (SOD) and mitochondrial peroxiredoxin PRX1, up regulated when compared to other species (*P. lutzii* and *P. restrepiensis*). The study identified some differences of these detoxification proteins in other two species, *Pb* 01 (*P. lutzii*) and *Pb* EPM83 (*P. restrepiensis*), showing differences in the detoxification process among *Paracoccidioides* species (Pigosso, L.L *et al*., 2013).

A study with 20 isolates of *Fusarium oxysporum lycopersici* (FOL), a fungus that causes disease in tomato plants, revelad that in the isolate FOL-8, 14 proteins up regulated were related to virulence, when compared to FOL-20, that is considered less virulent. The increased proteins were related to pathogenicity, sporulation and higher penetration rate on tomato root tissue (Manikandan *et al*., 2018).

4.0. Conclusion

Proteomic analysis is an important tool to provide information about the organism's biology. In our study we provided informations about two *Paracoccidioides* species, showing that *Paracoccidioides americana* (*Pa* 03) and *Paracoccidioides brasiliensis* (*Pb* 18) presented differences in the level of proteins suggesting that:

1-*P. brasiliensis* uses preferentially the electron transport pathway and oxidative phosphorylation for energy production coming from glucose through glycolysis;

2-Proteins related to signal transduction and protein fate are more expressed in *P. brasiliensis*;

3-Alcoholic fermentation and gluconeogenesis predominate in *P. americana*, with precurors coming probably form the metabolism of aminoacids;

4-Beta oxidation predominates in *P. americana*.

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Supplementary materials

Supplementary table 1. Proteins identified in proteomic analysis by mass spectrometry Orbitrap MS between *Paracoccidioides* species.

Accession	Description	Function
Metabolism		
Amino acid metabolism		
C1GG15	1,2-dihydroxy-3-keto-5-methylthiopentene dioxygenase	Amino acid biosynthesis
C1GBT4	1-pyrroline-5-carboxylate dehydrogenase	Amino acid metabolism
C1G6P2	2,4-dihydroxyhept-2-ene-1,7-dioic acid aldolase	Amino acid metabolism
C1G8C8	2-oxoisovalerate dehydrogenase E1 alpha subunit	Amino acid metabolism
C0S5A7	2-oxoisovalerate dehydrogenase E2 component (dihydrolipoyl transacylase)	Amino acid metabolism
C1G4D1	2-oxoisovalerate dehydrogenase E2 component (dihydrolipoyl transacylase)	Amino acid metabolism
C1G8P1	2-oxoisovalerate dehydrogenase subunit beta	Amino acid metabolism
C1G0J6	3-deoxy-7-phosphoheptulonate synthase	Amino acid metabolism
C1G7F9	3-deoxy-7-phosphoheptulonate synthase	Amino acid metabolism
C1GL14	3-deoxy-7-phosphoheptulonate synthase	Amino acid metabolism
C1G596	3-hydroxyisobutyrate dehydrogenase	Amino acid metabolism
C0SED7	3-isopropylmalate dehydrogenase A	Amino acid metabolism
C1GJD0	3-methylcrotonyl- carboxylase subunit alpha	Amino acid metabolism
C0SG96	4-aminobutyrate aminotransferase	Amino acid metabolism
C1G248	4-aminobutyrate aminotransferase	Amino acid metabolism
C0SIT3	5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase	Amino acid metabolism
C1GLT7	5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase	Amino acid metabolism

A0A0A0HX63	acetolactate large biosynthetic type	Amino acid metabolism
C0SIR4	acetolactate small subunit	Amino acid metabolism
C1GIE3	acetylornithine aminotransferase	Amino acid metabolism
C1GMI2	4-hydroxyphenylpyruvate dioxygenase	Tyrosine metabolism
C1G913	Acylphosphatase family	Pyruvate metabolism
C1GMM2	alanine transaminase	Amino acid metabolism
C1G765	alanine-glyoxylate transaminase	Amino acid metabolism
C1G4M0	adenosylhomocysteinase	Amino acid metabolism
C1G7J4	aminopeptidase	Amino acid metabolism
C1GC48	branched-chain amino acid aminotransferase	Amino acid metabolism
C0SF90	dihydroxy-acid dehydratase	Amino acid metabolism
C1G2Z8	family pyridoxal phosphate enzyme	Amino acid metabolism
C1GAG4	formate-tetrahydrofolate ligase	Amino acid metabolism
C1GDK1	glutamate-5-semialdehyde dehydrogenase	Amino acid metabolism
C1G215	HAD superfamily hydrolase	Amino acid metabolism
C1GHM0	histidinol dehydrogenase	Amino acid metabolism
C0S3J2	homoserine dehydrogenase	Amino acid metabolism
C1GC00	homoserine kinase	Amino acid metabolism
C1GGJ2	ketol-acid mitochondrial	Amino acid metabolism
C1G0F9	kynureninase	Amino acid metabolism
C1G2X9	kynurenine 3-monooxygenase	Amino acid metabolism
C1G4J8	Kynurenine formamidase	Amino acid metabolism
C1G3Z9	kynurenine-oxoglutarate transaminase	Amino acid metabolism
C1GLF6	L-aminoadipate-semialdehyde dehydrogenase	Amino acid metabolism
C0S1P2	l-serine dehydratase	Amino acid metabolism
C1GA28	l-serine dehydratase/l-threonine deaminase	Amino acid metabolism
C1G7L7	methylenetetrahydrofolate reductase	Amino acid metabolism
C1G3P8	methylmalonate-semialdehyde dehydrogenase (acylating)	Amino acid metabolism

C1G8D6	methylthioadenosine phosphorylase	Amino acid metabolism
C1G3Q0	methylthioribulose-1-phosphate dehydratase	Amino acid metabolism
C1GEZ1	arginine biosynthesis mitochondrial	Biosynthesis of arginine
C1FYL2	argininosuccinate synthase	Biosynthesis of arginine
C1G5I8	carbamoyl-phosphate synthase arginine-specific large chain	Biosynthesis of arginine
C1GLM1	asparagine synthetase	Biosynthesis of asparagine
C0SAC6	chorismate mutase	Biosynthesis of chorismate
C1GBW5	chorismate synthase	Biosynthesis of chorismate
C1GDG5	cystathionine beta-synthase	Biosynthesis of cysteine
C0RXA9	cystathionine gamma-lyase	Biosynthesis of cysteine
C1G3A2	cysteine dioxygenase	Biosynthesis of cysteine
A0A0A0HV38	cysteine mitochondrial	Biosynthesis of cysteine
C1G6C1	cysteine synthase	Biosynthesis of cysteine
C1GL59	imidazole glycerol phosphate synthase hisHF	Biosynthesis of histidine
C1G3U9	homocitrate mitochondrial	Biosynthesis of lysine
C1GG52	lysine decarboxylase	Biosynthesis of lysine
C1GJ38	anthranilate synthase component 2	Biosynthesis of tryptophan
C1GB53	anthranilate synthase component I	Biosynthesis of tryptophan
C1GGQ7	dihydrolipoyl dehydrogenase	Degradation of glycine
C1GJD4	methylcrotonoyl- carboxylase subunit beta	Degradation of leucine
C1G1A3	methylglutaconyl- hydratase	Degradation of leucine
C1GMH8	maleylacetoacetate isomerase	Degradation of phenylalanine
C1GMH9	fumarylacetoacetase	Degradation of tyrosine
C1G9M8	fumarylacetoacetate hydrolase	Degradation of tyrosine
C1GA56	fumarylacetoacetate hydrolase family	Degradation of tyrosine
C1GHZ0	mitochondrial 3-hydroxyisobutyryl- hydrolase	Degradation of valine
C1G7Q5	Altered inheritance of mitochondria	Glutamate metabolism
A0A0A5IGW8	altered inheritance-mitochondria mitochondrial	Glutamate metabolism

C1G3G3	gamma-glutamyltransferase	Glutathione metabolism
C1G197	arginase	Metabolism of arginine
C0S2A4	aspartate aminotransferase	Metabolism of aspartate
C1G3M0	glutamine synthetase	Metabolism of glutamate
C1G4U7	glycine cleavage system H	Metabolism of glycine
C1G6V9	glycine cleavage system T	Metabolism of glycine
C1G020	glycine dehydrogenase	Metabolism of glycine
C1GIE5	hydroxymethylglutaryl- lyase	Degradation of leucine
C0S3L1	hydroxymethylglutaryl- synthase	Degradation of leucine
C1GMI0	homogentisate 1,2-dioxygenase	Metabolism of tyrosine
A0A0A0HVA4	aminomethyl transferase	Amino acid metabolism
C0SI30	O-acetylhomoserine (thiol)-lyase	Amino acid metabolism
C1G312	ornithine aminotransferase	Amino acid metabolism
C1G9X6	peptidase family [Paracoccidioides lutzii Pb01]	Amino acid metabolism
C1GN15	phosphoglycerate dehydrogenase	Amino acid metabolism
C1GAY3	proline oxidase	Amino acid metabolism
C1GIR0	pyrroline-5-carboxylate reductase	Amino acid metabolism
C1GFV8	saccharopine dehydrogenase	Amino acid metabolism
C1G452	saccharopine dehydrogenase [NADP+ L-glutamate-forming]	Amino acid metabolism
C0S5M6	S-adenosylmethionine synthase	Amino acid metabolism
C1GCX5	serine cytosolic	Amino acid metabolism
C1GDE1	serine hydroxymethyltransferase	Amino acid metabolism
C1GBZ4	NADP-specific glutamate dehydrogenase	Degradation of glutamine
C1FYJ9	pentafunctional AROM polypeptide	Metabolism of cysteine - aromatic group
C1GCG7	N-acetyl-gamma-glutamyl-phosphate reductase	Metabolism of glutamate
C1G989	NAD-specific glutamate dehydrogenase	Metabolism of glutamate
C1GE06	serine 3-dehydrogenase	Metabolism of serine

Carbohydrate metabolism		
C1GCJ6	2-methylcitrate dehydratase	Carbohydrate metabolism
C0SF94	aldo-keto reductase	C-compoud and carbohydrate metabolism
C1G7G3	aldose 1-epimerase family	Carbohydrate metabolism
C1GKG3	minor allergen Alt a 7	Aerobic aromate catabolism
C1GA62	alpha-mannosidase	C-compoud and carbohydrate metabolism
C1G620	AMP dependent ligase	C-compoud and carbohydrate metabolism
C1GBJ2	ATP-citrate synthase subunit 1	C-compoud and carbohydrate metabolism
C0SF34	carbonic anhydrase	C-compoud and carbohydrate metabolism
C1FZY1	cytochrome b2	C-compoud and carbohydrate metabolism
C1G6C8	D-lactate dehydrogenase 2	C-compoud and carbohydrate metabolism
C0S384	dolichol-phosphate mannosyltransferase	C-compoud and carbohydrate metabolism
C1G5L7	dolichyl-phosphate-mannose- mannosyltransferase	C-compoud and carbohydrate metabolism
C1G9X3	enolase	C-compoud and carbohydrate metabolism
A0A0A0HVG0	folylpolyglutamate synthase	C-compoud and carbohydrate metabolism
C1GLI4	glucosamine 6-phosphate N-acetyltransferase	C-compoud and carbohydrate metabolism
C0S1C3	glucosamine-fructose-6-phosphate aminotransferase	C-compoud and carbohydrate metabolism
C1GAB4	glucose-6-phosphate 1-dehydrogenase	C-compoud and carbohydrate metabolism
C1G0R1	glucose-6-phosphate isomerase	C-compoud and carbohydrate metabolism
C0RZG7	inositol-3-phosphate synthase	C-compoud and carbohydrate metabolism
C1G1J5	lactam utilization lamB	C-compoud and carbohydrate metabolism
C1GF19	lactonohydrolase	C-compoud and carbohydrate metabolism
C1GJJ9	L-xylulose reductase	C-compoud and carbohydrate metabolism
C0S624	mannitol-1-phosphate 5-dehydrogenase	C-compoud and carbohydrate metabolism
C0S6R9	mannose-1-phosphate guanyltransferase	C-compoud and carbohydrate metabolism
C1G1W6	mannose-6-phosphate isomerase	C-compoud and carbohydrate metabolism

C0SCQ9	glycogen [starch] synthase	Glycogen anabolism
C1G1X9	glycogen phosphorylase	Glycogen anabolism
C0S3D9	N-acetylglucosamine-phosphate mutase	Aminosaccharide metabolism
C1GA33	Pc13g07410	C-compoud and carbohydrate metabolism
C0S189	phosphomannomutase	C-compoud and carbohydrate metabolism
C0S0L2	short chain dehydrogenase reductase	sugar, glucoside, polyol and carboxylate anabolism
C1G3H0	short chain dehydrogenase reductase family	sugar, glucoside, polyol and carboxylate anabolism
C1GCG5	short-chain dehydrogenase reductase family	sugar, glucoside, polyol and carboxylate anabolism
Lipd and Fatty acid metabolism		
C1H6E2	thioesterase family	Fatty acid
C1G2R2	3-hydroxybutyryl- dehydrogenase	Fatty acid metabolism
C1G421	3-ketoacyl- thiolase	Fatty acid metabolism
C1GD60	1-acylglycerol-3-phosphate O-acyltransferase	Lipid metabolism
C1G4X7	acetoacetate- ligase	Lipid metabolism
C1GDJ1	acetyl- carboxylase	Lipid, fatty acid metabolism
C1G6E6	acetyl- acetyltransferase	Metabolism
C1GMD1	acyl carrier	fatty acid metabolism
C1GHR9	acyl- dehydrogenase	fatty acid metabolism
C1G7S0	acyl- desaturase	fatty acid metabolism
C1GLP0	acyl- thioester hydrolase	fatty acid metabolism
C1GLP9	diphosphomevalonate decarboxylase	Isoprenoid metabolism
C0SCR2	farnesyl-pyrophosphate synthetase	Isoprenoid metabolism
C1G0X3	AMP-binding domain-containing	Lipid, fatty acid and isoprenoid metabolism
C1GBJ3	ATP citrate lyase subunit (Acl) putatibe	Lipid, fatty acid and isoprenoid metabolism
A0A0A0HW47	C-4 methyl sterol oxidase	Lipid, fatty acid and isoprenoid metabolism
C1G168	CAIB BAIF family enzyme	Lipid, fatty acid and isoprenoid metabolism

C1GID7	carnitine acetyl transferase	Lipid, fatty acid and isoprenoid metabolism
C1G347	diazepam-binding inhibitor (GABA receptor acyl- -binding)	Lipid, fatty acid and isoprenoid metabolism
C1G2P3	enoyl- hydratase	Lipid, fatty acid and isoprenoid metabolism
C1GGI8	enoyl- hydratase isomerase	Lipid, fatty acid and isoprenoid metabolism
C1G3Y3	enoyl reductase	Lipid, fatty acid and isoprenoid metabolism
C1G054	enoyl-[acyl-carrier-] reductase 1	Lipid, fatty acid and isoprenoid metabolism
C1GL27	esterase lipase	Lipid, fatty acid and isoprenoid metabolism
C1G0P4	fatty acid activator Faa4	Lipid, fatty acid and isoprenoid metabolism
C1GER5	fatty acid elongase	Lipid, fatty acid and isoprenoid metabolism
C1G065	fatty acid synthase beta subunit dehydratase	Lipid, fatty acid and isoprenoid metabolism
C1G064	fatty acid synthase subunit alpha	Lipid, fatty acid and isoprenoid metabolism
C1GAT1	fatty-acid amide hydrolase	Lipid, fatty acid and isoprenoid metabolism
C0RYV5	glycylpeptide N-tetradecanoyltransferase	Lipid, fatty acid and isoprenoid metabolism
C1G579	isopentenyl-diphosphate delta-isomerase	Lipid, fatty acid and isoprenoid metabolism
C1GJD3	isovaleryl- dehydrogenase	Lipid, fatty acid and isoprenoid metabolism
A0A0A0HVL2	long-chain-fatty-acid- ligase	Lipid, fatty acid and isoprenoid metabolism
A0A0A0HTN8	Acyl- thioesterase 1	fatty acid metabolism
C1FZN8	C-3 sterol dehydrogenase C-4 decarboxylase	Tetracyclic and pentacyclic triterpenes (cholesterin, steroids and hopanoids) metabolism
C1GJP9	hypothetical protein PADG_07485	Lip, fatty acid and isoprenoid metabolism
A0A0A0HVK7	NADPH-cytochrome P450 reductase	Lip, fatty acid and isoprenoid metabolism
C1G1D8	oxysterol binding	Lipid, fatty acid and isoprenoid metabolism
A0A0A0HUB3	oxysterol binding (Osh1)	Lipid, fatty acid and isoprenoid metabolism
C1FZ38	phospholipase D	Lipid, fatty acid and isoprenoid metabolism
C1GDU7	pyridoxamine 5 -phosphate oxidase	Lipid, fatty acid and isoprenoid metabolism
C1G7L5	serum paraoxonase arylesterase 2	Lipid, fatty acid and isoprenoid metabolism
C0S381	short-chain dehydrogenase reductase SDR	Lipid, fatty acid and isoprenoid metabolism
Nucleotide/nucleoside/nucleobase metabolism		

C1GAK7	adenine phosphoribosyltransferase	Nucleoside/nucleotide/nucleobase metabolism
C1G280	adenosine kinase	Nucleoside/nucleotide/nucleobase metabolism
C1GLG2	adenylate kinase	Nucleoside/nucleotide/nucleobase metabolism
C1GB27	adenylosuccinate lyase	Nucleoside/nucleotide/nucleobase metabolism
C0S3Z3	adenylosuccinate synthetase	Nucleoside/nucleotide/nucleobase metabolism
C1G217	ADP-ribose pyrophosphatase	Nucleoside/nucleotide/nucleobase metabolism
C1GFL8	deoxyribose-phosphate aldolase	Deoxyribonucleotide metabolism
C0SES9	deoxyuridine 5 -triphosphate nucleotidohydrolase	Deoxyribonucleotide metabolism
C1GCJ9	amidophosphoribosyltransferase	Nucleotide/nucleoside/nucleobase metabolism
C1GA13	bifunctional purine biosynthesis ADE17	Nucleotide/nucleoside/nucleobase metabolism
C1G2D3	choline-phosphate cytidylyltransferase	Nucleotide/nucleoside/nucleobase metabolism
C1GH49	GMP synthase	Nucleotide/nucleoside/nucleobase metabolism
C1G019	guanine deaminase	Nucleotide/nucleoside/nucleobase metabolism
C1G3L4	guanine nucleotide-binding subunit beta	Nucleotide/nucleoside/nucleobase metabolism
A0A0A0HYZ8	guanylate kinase	Nucleotide/nucleoside/nucleobase metabolism
C0RZD4	HD domain-containing	Nucleotide/nucleoside/nucleobase metabolism
C1GB68	HIT domain-containing	Nucleotide/nucleoside/nucleobase metabolism
C0SFQ5	homodimeric type	Nucleotide/nucleoside/nucleobase metabolism
C1GM11	Nucleotidyltransferase like protein	Nucleotide/nucleoside/nucleobase metabolism
C1G4N0	phosphoglycerate kinase	Nucleotide/nucleoside/nucleobase metabolism
C1GG60	phosphoribosylamine-glycine ligase	Nucleotide/nucleoside/nucleobase metabolism
A0A0A0HR31	phosphoribosylaminoimidazole ATPase subunit	Nucleotide/nucleoside/nucleobase metabolism
A0A0A0HWR3	phosphoribosylformylglycinamidine synthase	Nucleotide/nucleoside/nucleobase metabolism
C0SFB3	inosine-5 -monophosphate dehydrogenase	Nucleotide/nucleoside/nucleobase metabolism
Phosphate metabolism		
C1G4Q3	glycerol-3-phosphate phosphatase	Phosphate metabolism
C1GA89	inorganic pyrophosphatase	Phosphate metabolism

C1GKJ7	Phosphotransferase enzyme family domain	Phosphate metabolism
Metabolism of vitamins		
C1G8L2	3-methyl-2-oxobutanoate hydroxymethyltransferase	Metabolism of vitamins and cofactors
C1G7Q8	5-formyltetrahydrofolate cyclo-ligase	Metabolism of vitamins and cofactors
C1GMH1	biotin synthase	Biosynthesis of vitamins, cofactors and prosthetic groups
C1G0Q3	dihydropteroate synthase	Biosynthesis of vitamins, cofactors and prosthetic groups
C1GJY7	GTP cyclohydrolase I	Biosynthesis of vitamins, cofactors and prosthetic groups
C1G9P7	6,7-dimethyl-8-ribityllumazine synthase	Metabolism of vitamins and cofactors
C1GE04	molybdopterin binding domain	Metabolism of vitamins, cofactors and prosthetic groups
C1GAR9	molybdopterin synthase small subunit	Metabolism of vitamins, cofactors and prosthetic groups
C1GE78	porphobilinogen deaminase	Metabolism of vitamins, cofactors and prosthetic groups
C1GD62	pyridoxal 5 -phosphate glutaminase subunit Pdx2	Metabolism of vitamins, cofactors and prosthetic groups
C1GEY6	pyridoxine biosynthesis pyroA	Metabolism of vitamins, cofactors and prosthetic groups
C1G167	riboflavin alpha subunit	Metabolism of vitamins, cofactors and prosthetic groups
C1GFB1	nicotinate-nucleotide diphosphorylase (carboxylating)	Metabolis of vitamins, cofactors and prosthetic gorup
Nitrogen metabolism		
C1FZC4	2-nitropropane dioxygenase	Nitrogen metabolism
C1G0Q6	2-nitropropane dioxygenase	Nitrogen metabolism
C0SH73	formamidase	Nitrogen, sulfur and selenium metabolism
A0A0A0HTR8	hydrolase	Nitrogen, sulfur and selenium metabolism
C1GHS5	N-acylethanolamine amidohydrolase	Nitrogen, sulfur and selenium metabolism
C0S725	nitroreductase family [Paracoccidioides lutzii Pb01]	Nitrogen, sulfur and selenium metabolism
C1G9S7	seleno domain-containing	Nitrogen, sulfur and selenium metabolism
C1GJN5	2-nitropropane dioxygenase	Nitrogen metabolism
Secondary metabolism		
C1GGY0	FAD binding domain-containing	Secondary metabolism

C1GB98	metallo-beta-lactamase domain-containing	Secondary metabolism
Protein with binding function		
C1H2Z9	10 kda heat shock mitochondrial	ATP binding
C1G5H5	AAA family ATPase	ATP binding
C1GGX1	abc transporter	ATP binding
C0SGR5	12-oxophytodienoate reductase	FMN binding
C1G6M8	ADP-ribosylation factor	GTP binding
C1G1V7	Aflatoxin b1 aldehyde reductase	Protein binding
C1G445	Aha1 domain family	Protein binding
C1FZG2	alanyl-tRNA synthetase	RNA binding
C0S3F7	ATPase GET3	ATP binding
C1G776	COBW domain-containing 1	ATP binding
C1GIN4	annexin A7	Calcium binding
A0A0A0HUD7	apses transcription	DNA binding
C1G9D3	HMG box	DNA binding
A0A0A0HRQ4	glutaryl- dehydrogenase	FAD binging
C1GLV1	GTP-binding ypt1	GTP binding
C0RYV2	GTP-binding ypt5	GTP binding
C1G0C2	cytochrome c heme lyase	Heme binding
C1FZP5	arsenite resistance Ars2	Nucleic acid binding
A0A0A0HUZ5	ATP-dependent RNA helicase DBP2	Nucleic acid binding
A0A0A0HTT8	gag polymerase env	Nucleic acid binding
A0A0A0HXV8	glycolate subunit	Nucleotide binding
C1GLX7	cofilin	Actin binding
C0S3L9	ankyrin repeat	Protein binding
C1GDS2	CTLH domain-containing	Protein binding

C1G2E1	CUE domain-containing	Protein binding
C1G4C7	cytosolic Fe-S cluster assembly factor NAR1	Protein binding
C1GLR4	KH domain-containing	RNA binding
C1G4I0	La domain-containing	RNA binding
C1G422	DlpA domain-containing protein	Protein binding
C1G9C3	chlorophyll synthesis pathway	Zinc ion binding
C1G2C5	R3H domain-containing	Nucleic acid binding
C0RXU3	RNP domain	DNA binding
C1G741	PAB1 binding	Protein binding
C1FZS1	PKHD-type hydroxylase TPA1	Protein binding
A0A0A0HU09	Poly(rC)-binding	RNA binding
C1GCT4	RNA binding	RNA binding
C0RYT2	polyadenylate-binding 2	RNA binding
C1GBN3	RNA binding domain-containing	RNA binding
Transport		
C1GEX3	ABC metal ion transporter	Transport facilities
C0RXT4	ABC transporter ATP-binding ARB1	ABC transporters
C1G561	acyl-RNA-complex subunit	RNA transport
C0S685	ADP,ATP carrier	Transport facilities
C1G6W9	G2 M phase checkpoint control Sum2	Cellular import
C1G9U1	COPII-coated vesicle membrane Erv46	ER to Golgi transport
C1G3L2	endoplasmic reticulum vesicle 25	ER to Golgi transport
C0SGI8	iron copper transporter	Heavy metal ion transport
C1GA14	clathrin heavy chain	Intracelluar transport vesicles
C1G506	clathrin light chain	Intracelluar transport vesicles
C1GAF5	coatomer alpha subunit	Intracelluar transport vesicles
C1GBG4	Coatomer subunit	Intracelluar transport vesicles
C1G6S0	coatomer subunit beta	Intracelluar transport vesicles

C1GHB9	coatomer subunit delta	Intracelluar transport vesicles
C0S7A5	Coatomer subunit epsilon	Intracelluar transport vesicles
C1G1W8	coatomer subunit epsilon	Intracelluar transport vesicles
C0S216	coatomer subunit zeta	Intracelluar transport vesicles
C1GGE1	carnitine acyl carnitine carrier	Lipid, fatty acid transport
C1GFX9	glycolipid transfer HET-C2	Lipid/fatty acid transport
C0S5D1	mitochondrial 2-oxodicarboxylate carrier	Mitochondrial transport
C1GIA6	mitochondrial co-chaperone	Mitochondrial transport
C1GML4	mitochondrial dicarboxylate carrier	Mitochondrial transport
C0SCE1	mitochondrial GTP GDP carrier 1	Mitochondrial transport
C0SEB7	mitochondrial phosphate carrier	Mitochondrial transport
C1GAA6	mitochondrial phosphate carrier 2	Mitochondrial transport
C1GHP1	golgi membrane	Post Golgi trasnport
C0S0V1	AP-1 complex subunit beta-1	Protein transport
C1GHQ7	BAP31 domain-containing	Protein transport
A0A0A5IJI1	COPII-coated vesicle 4 Erv29	Protein transport
C0SGG8	Exportin	Protein transport
C1GF48	import inner membrane translocase subunit TIM8	Protein transport
C0SHE2	import receptor	Protein transport
C1GHE5	importin beta-1 subunit	Protein transport
C1GLJ9	importin beta-3 subunit	Protein transport
C1G8G0	importin beta-4 subunit	Protein transport
C0S4R8	importin subunit alpha-1	Protein transport
C1G5F8	inorganic phosphate transporter	Protein transport
C1GIR6	lectin family integral membrane	Protein transport
C1G784	MIC60 MICOS complex subunit	Protein transport
C1GIB2	Protein transporter SEC31	Protein transport
C1GB51	EH domain binding epsin 2	Transport routes

A0A0A0HW80	calcium transporter	Vacuolar transporter
C0SE28	BET3 family	Vesicle transport
C1GLM2	outer mitochondrial membrane porin	Anion trasnport
C0S5R1	rab GDP-dissociation inhibitor	Cellular export and secretion
C1GIL8	NADH-cytochrome b5 reductase 1	Eletron transport
C1G9D6	NADH-cytochrome b5 reductase 2	Eletron transport
C1GJ33	phenylacetate 2-hydroxylase	Eletron transport
C1GM00	plasma membrane ATPase	Eletron transport
C1GE37	quinone oxidoreductase	Eletron transport
C1G9I8	prenylated Rab acceptor 1	ER to Golgi transport
C0SF46	transport sec9 [Paracoccidioides lutzii Pb01]	Intracellular transport vesicles
C1GKL8	transporter SEC23	Intracellular transport vesicles
C1GB82	transporter SEC24	Intracellular transport vesicles
C1GEZ2	transporter SEC61 subunit alpha	Intracellular transport vesicles
A0A0A0HUL4	sideroflexin-1	Ion transport
C1GL33	presequence translocated-associated motor subunit pam17	Mithocondrial transport
C1G2M5	nuclear export Yrb2	Nuclear transport
C1G0A3	nuclear pore	Nuclear transport
C0S368	nuclear transport factor 2	Nuclear transport
C1GB05	nucleoporin Nsp1 [Blastomyces gilchristii SLH14081]	Nuclear transport
C1GBI0	nucleoporin NUP49 NSP49	Nuclear transport
C1GLM8	MSP domain-containing	Protein transport
C1FYR1	rab other	Protein transport
C0SEZ5	NTF2 and RRM domain-containing	RNA transport
C1G2H3	Poly(A)+ RNA export	RNA transport
A0A0A0HV97	Ran-specific GTPase-activating 1	RNA transport

Energy		
C1G935	acetate non-utilizing mitochondrial	Gluconeogenesis
C1GCX3	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	Glycolysis and Gluconeogenesis
C1G002	6-phosphofructokinase	Glycolysis and Gluconeogenesis
C1G411	acetyl-coenzyme A synthetase	Glycolysis and Gluconeogenesis
C1G8R5	6-phosphogluconate decarboxylating 1	Pentose phosphate pathway
C1GKI5	6-phosphogluconolactonase	Pentose phosphate pathway
C1GCI8	2-methylcitrate mitochondrial	TCA cycle
A0A0A0HUM5	aconitate mitochondrial	TCA cycle
C1GLK1	aconitate mitochondrial	TCA cycle
C1GCH9	alcohol dehydrogenase	Fermentation
A0A0A0HTP6	alcohol dehydrogenase 1	Fermentation
C1GPF8	alcohol dehydrogenase 1	Fermentation
C0SIV5	aldehyde dehydrogenase	Fermentation
C0SE80	aldehyde dehydrogenase (NAD+)	Fermentation
Q1XA94	cytochrome oxidase subunit 1 (mitochondrion)	Aerobic respiration
Q1XA91	cytochrome oxidase subunit 2 (mitochondrion)	Aerobic respiration
C1G046	glutamyl-tRNA(Gln) amidotransferase subunit mitochondrial	Aerobic respiration
C0SC21	ATP synthase delta subunit	Electron transport and membrane-associated energy conservation
C1GKM7	ATP synthase gamma subunit	Electron transport and membrane-associated energy conservation
C0SA05	ATP synthase subunit mitochondrial	Electron transport and membrane-associated energy conservation
C0SES3	ATP synthase subunit mitochondrial delta	Electron transport and membrane-associated energy conservation
C1G5V6	ATP synthase subunit mitochondrial alpha	Electron transport and membrane-associated energy conservation

C1G5X3	ATP synthase subunit mitochondrial subunit 4	Electron transport and membrane-associated energy conservation
C1GLV8	ATP synthase subunit mitochondrial subunit beta	Electron transport and membrane-associated energy conservation
C0S075	Cytochrome b5	Electron transport and membrane-associated energy conservation
C0S0G7	cytochrome c oxidase polypeptide IV	Electron transport and membrane-associated energy conservation
C1GLX6	Cytochrome c oxidase polypeptide V	Electron transport and membrane-associated energy conservation
C1GER4	cytochrome c oxidase polypeptide VI	Electron transport and membrane-associated energy conservation
C1GIA9	cytochrome c oxidase polypeptide VIb	Electron transport and membrane-associated energy conservation
C1GAW1	cytochrome c oxidase subunit mitochondrial	Electron transport and membrane-associated energy conservation
C0SHZ3	cytochrome c oxidase subunit V	Electron transport and membrane-associated energy conservation
C1GFT0	cytochrome c oxidase subunit VIa	Electron transport and membrane-associated energy conservation
C1GM66	Cytochrome c subunit VIb	Electron transport and membrane-associated energy conservation
A0A0A0HTA9	cytochrome heme mitochondrial	Electron transport and membrane-associated energy conservation
C1GIJ5	electron transfer flavo alpha subunit	Electron transport and membrane-associated energy conservation
A0A0A0HY53	electron transfer flavo beta-subunit	Electron transport and membrane-associated energy conservation
C1G137	electron transfer flavo -ubiquinone oxidoreductase	Electron transport and membrane-associated energy conservation

C1GLY2	F-type H+-transporting ATPase subunit epsilon	Electron transport and membrane-associated energy conservation
C1G1E8	F-type H+-transporting ATPase subunit H	Electron transport and membrane-associated energy conservation
C1GF69	LYR family	Electron transport and membrane-associated energy conservation
C1G440	fructose-1,6-bisphosphatase	Glycolysis and gluconeogenese
C0S3J6	fructose-bisphosphate aldolase 1	Glycolysis and gluconeogenese
C1GKU3	glucokinase	Glycolysis and gluconeogenese
C1GJT7	glucosidase II alpha subunit	Glycolysis and gluconeogenese
C0RX67	glyceraldehyde-3-phosphate dehydrogenase	Glycolysis and gluconeogenese
C1G977	hexokinase	Glycolysis and gluconeogenese
C1GCI7	methylisocitrate lyase	Glycolysis and gluconeogenese
C1GA17	pyruvate carboxylase	Glycolysis and gluconeogenese
C1G547	pyruvate dehydrogenase (acetyl-transferring) E1 alpha subunit	Glycolysis and gluconeogenese
C1GA79	pyruvate dehydrogenase complex component Pdx1	Glycolysis and gluconeogenese
C1GIX7	pyruvate dehydrogenase complex dihydrolipoamide acetyltransferase	Glycolysis and gluconeogenese
C1G056	pyruvate dehydrogenase E1 component subunit beta	Glycolysis and gluconeogenese
C1G2W2	pyruvate kinase	Glycolysis and gluconeogenese
C1G3G7	isocitrate lyase	Glyoxylate cycle
C1GML7	phosphoenolpyruvate carboxykinase	Glycolysis and gluconeogenesis
A0A0A0HX82	phosphoglucomutase	Glycolysis and gluconeogenesis
C1GCI0	malate glyoxysomal	Glyoxylate cycle
C1GLZ6	citrate mitochondrial	TCA cycle
C0SD31	dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex	TCA cycle
C1GLI3	fumarate mitochondrial	TCA cycle
C0RXN7	fumarate reductase	TCA cycle

C1G2X0	isocitrate dehydrogenase	TCA cycle
C1G6K0	isocitrate dehydrogenase [NAD] subunit mitochondrial	TCA cycle
C1GAG3	isocitrate dehydrogenase [NADP] mitochondrial	TCA cycle
C0SBN0	malate NAD-dependent	TCA cycle
C1GM03	processing enhancing	accessory proteins of electron transport and membrane-associated energy conservation
C0SI66	NADH-ubiquinone oxidoreductase 23 kDa mitochondrial	Aerobic respiration
C0S212	NADH-ubiquinone oxidoreductase 24 kDa mitochondrial	Aerobic respiration
C1G350	NADH-ubiquinone oxidoreductase 299 kDa subunit	Aerobic respiration
C1GB77	NADH-ubiquinone oxidoreductase 40 kDa mitochondrial	Aerobic respiration
C1GK68	NADH-ubiquinone oxidoreductase 49 kDa mitochondrial	Aerobic respiration
C1G413	NADH-ubiquinone oxidoreductase 51 kDa mitochondrial	Aerobic respiration
A0A0A0HWQ9	NADH-ubiquinone oxidoreductase 78 kDa mitochondrial	Aerobic respiration
A0A0A5IDQ9	NADH-ubiquinone oxidoreductase 78 kDa mitochondrial	Aerobic respiration
C1HDX3	NADH-ubiquinone oxidoreductase 78 kDa mitochondrial	Aerobic respiration
C1G8D0	NADH-ubiquinone oxidoreductase kDa mitochondrial	Aerobic respiration
C1G9E2	NADH-ubiquinone oxidoreductase kDa mitochondrial	Aerobic respiration
C0RYJ8	NADH-ubiquinone oxidoreductase kDa subunit	Aerobic respiration
C0SAL4	NADH-ubiquinone oxidoreductase kDa subunit	Aerobic respiration
C1GDK7	NADH-ubiquinone oxidoreductase kDa subunit	Aerobic respiration
C1GG42	NADH-ubiquinone oxidoreductase kDa subunit	Aerobic respiration
C1G256	NADH-ubiquinone oxidoreductase subunit	Aerobic respiration
C1G7X1	S-(hydroxymethyl)glutathione dehydrogenase	Fermentation
C1GBI8	ribose 5-phosphate isomerase A	Pentose phosphate pathway
A0A0A5IC68	ribose-phosphate pyrophosphokinase 3	Pentose phosphate pathway
A0A0A5K5P1	ribose-phosphate pyrophosphokinase 3	Pentose phosphate pathway
C1G1P0	ribose-phosphate pyrophosphokinase II	Pentose phosphate pathway
C0S018	NADH dehydrogenase (ubiquinone) 1 beta subcomplex 7	Respiration

C1G496	oxoglutarate dehydrogenase (succinyl-transferring) E1 component	TCA cycle
Cell cycle		
C0S0D2	14-3-3 family	DNA repair and proteins recombination
C1GB04	14-3-3 family like protein 2	DNA repair and proteins recombination
C1G501	calmodulin	Cell budding
C1GRP1	Cell division control protein	Cell control
C1G0Q2	arf GTPase-activating	Cell cycle
C1GI15	cell cycle control	Cell cycle
C1G6F8	cyclin-dependent kinase regulatory subunit	Cell cycle
C1G681	hob3	Cell cycle
C1G5B2	metaphase-anaphase transition	Cell cycle
C1G4D0	caffeine-induced death Cid2	Cell cycle control
C1G5M0	cytoskeleton assembly control Sla1	Cell growth and morphogenesis
C1GFT4	cytoskeleton assembly control Sla2	Cell growth and morphogenesis
C1GFG5	histone H1	Chromossome condensation
A0A0A0HUI8	histone H2A	Chromossome condensation
C1GF70	histone H2A	Chromossome condensation
C1GF71	histone H2B	Chromossome condensation
C0S430	histone H3	Chromossome condensation
C1FYJ6	histone H4	Chromossome condensation
C1GIL6	chromosome segregation sudA	Chromossome segregation/division
C1G3I3	chromatin remodelling complex ATPase chain ISW1	DNA conformation modification
C1FZ06	DNA-binding 42 kDa	DNA conformation modification
C1GIP8	histone	DNA conformation modification
C1GEM3	histone acetyltransferase type B subunit 2	DNA conformation modification
A0A0A0HV65	DNA repair	DNA repair

C1G4C9	formamidopyrimidine-DNA glycosylase	DNA repair
C1G1S8	chromosome transmission fidelity 4	DNA replication
C1G892	DNA-directed RNA polymerase	DNA synthesis and replication
C1G5B1	MCM complex subunit Mcm2	DNA synthesis and replication
C1G7P8	BAR domain-containing	Filamentous growth
C1H998	E3 ubiquitin ligase complex SCF subunit sconC	G1/S transition of mitotic cell cycle
C1GMD9	CBS domain-containing	Mitotic cell and cell cycle control
C1GII4	cell division control	Mitotic cell and cell cycle control
C1GEM8	cell division control 12	Mitotic cell and cell cycle control
C1GEJ7	cell division control 48	Mitotic cell and cell cycle control
C1G402	Cell division control Cdc31	Mitotic cell and cell cycle control
C1FZC9	condensin complex component cnd2	Mitotic cell and cell cycle control
C1GCS3	deubiquitination-protection dph1	Mitotic cell and cell cycle control
C1FYH3	nuclear segregation	Mitotic cell cycle
C1G733	mitotic control dis3	Mitotic cell cycle and cell cycle control
C1G5K0	mitotic exit network interactor 1	Mitotic cell cycle and cell cycle control
C1G0A1	RCC1 domain-containing [Paracoccidioides lutzii Pb01]	Mitotic cell cycle and cell cycle control
C1G6B9	RNA binding (Arp)	Mitotic cell cycle and cell cycle control
C1GCT8	GTP-binding nuclear GSP1 Ran	Mitotic cell and cell cycle control
C0RYS3	dynein light chain	Spindle pole body/centrosomeand microtubule cycle
C1G9X0	14-3-3 family epsilon	DNA repair and proteins recombination
C1G177	spindle pole body component alp6	Cell cycle
C1GIJ9	RSC complex subunit RSC8	DNA conformation
C1G5X0	nonribosomal peptide synthetase	DNA processing
C1GJ36	nonsense transcript	DNA processing
C0S2W4	nonsense-mediated mRNA decay	DNA processing
C0S9J0	nonspecific lipid-transfer	DNA processing
C0S9Q8	prohibitin-1	DNA synthesis and replication

C0S209	prohibitin-2	DNA synthesis and replication
A0A0A0HUX3	proliferating cell nuclear antigen (pcna)	DNA synthesis and replication
C0RYY3	nuclear movement nudC	Nuclear migration
C1G7Q4	non-repetitive nucleoporin	Nuclear pore forming protein
C0SFD3	replication factor-A [Paracoccidioides lutzii Pb01]	DNA synthesis and replication
Protein fate		
C1FYH6	class II aldolase adducin domain-containing	Assembly of protein complex
C1GFS7	complex I intermediate associated	Assembly of protein complex
C1G2I3	cytosolic iron-sulfur assembly 1	Assembly of protein complex
C1GLH3	autophagic death Aut7 IDI-	Autoproteolytic process
C1G226	autophagocytosis Aut1	Autoproteolytic process
C1GDI5	DNA RNA non-specific nuclease	Polynucleotide degradation
C1GF92	and TPR domain-containing	Protein folding and stabilization
C1G3P9	calnexin precursor	Protein folding and stabilization
C0S072	chaperone DnaK	Protein folding and stabilization
C1G7T3	chaperone dnaJ	Protein folding and stabilization
C1FZ60	chaperone dnaJ	Protein folding and stabilization
C1GD93	chaperone dnaJ 3	Protein folding and stabilization
C1G9A5	disulfide-isomerase domain	Protein folding and stabilization
C1GMV5	DnaJ domain	Protein folding and stabilization
C1G8X9	FK506-binding	Protein folding and stabilization
C1GCY8	prefoldin subunit 3	Protein folding and stabilization
C1G1L9	prefoldin subunit 4	Protein folding and stabilization
C1GCX2	prefoldin subunit 6	Protein folding and stabilization
A0A0A5K518	pre-mRNA polyadenylation factor fip1	Protein folding and stabilization
C1GMU3	FK506-binding 2	Protein folding and stabilization
C1G4I6	Hsc70 cochaperone	Protein folding and stabilization
C0SGA3	like subfamily B member 4	Protein folding and stabilization

C1G0W1	like subfamily C member 7	Protein folding and stabilization
C1GA83	ATP-dependent Clp proteolytic subunit	Protein process (proteolytic)
C1GN13	class E vacuolar -sorting machinery HSE1	Protein targeting, sorting and translocation
C0S7K2	dynamin family	Protein targeting, sorting and translocation
C0SI35	EF hand domain-containing	Protein targeting, sorting and translocation
C1G614	F-box domain-containing	Protein targeting, sorting and translocation
C1GIH8	intermembrane space import and assembly	Protein targeting, sorting and translocation
C1GI49	phosphatidylinositol 4-kinase	Modification by phosphorylation, dephosphorylation, autophosphorylation
C0S8A5	phosphatidylinositol transfer SFH5	Modification by phosphorylation, dephosphorylation, autophosphorylation
C1GJS2	phosphatidylinositol transfer SFH5	Modification by phosphorylation, dephosphorylation, autophosphorylation
C1GM90	Phosphatidylinositol transporter	Modification by phosphorylation, dephosphorylation, autophosphorylation
C1GET2	serine threonine phosphatase 2A	Modification by phosphorylation, dephosphorylation, autophosphorylation
C1GBH3	serine threonine- phosphatase PP2A catalytic subunit	Modification by phosphorylation, dephosphorylation, autophosphorylation
C1G246	serine threonine phosphatase PPT1	Modification by phosphorylation, dephosphorylation, autophosphorylation
C1GJD9	oligosaccharyl transferase complex subunit OST4	Modification with sugar residues
C1G957	oligosaccharyl transferase subunit (alpha)	Modification with sugar residues
C1GL55	oligosaccharyl transferase subunit (gamma)	Modification with sugar residues
C1G7F1	oligosaccharyltransferase subunit ribophorin II	Modification with sugar residues
C0S0R6	NADH:ubiquinone oxidoreductase subunit	Oxidative phosphorylation
C1GBD6	NADH:ubiquinone oxidoreductase subunit	Oxidative phosphorylation
C0RY31	NADH-ubiquinone oxidoreductase	Oxidative phosphorylation
C0S9X7	NADH-ubiquinone oxidoreductase 105 kDa subunit	Oxidative phosphorylation

C1G098	NADH-ubiquinone oxidoreductase 21 kDa subunit	Oxidative phosphorylation
Protein/peptide degradation		
A0A0A5IIF2	26S protease regulatory subunit 10B	Protein degradation
C0RXS9	26S protease regulatory subunit 4	Protein degradation
C1G631	26S protease regulatory subunit 4	Protein degradation
C0S3D4	26S protease regulatory subunit 6A	Protein degradation
A0A0A2V213	26S protease regulatory subunit 6B	Protein degradation
C0S7H3	26S protease regulatory subunit 7	Protein degradation
C1GSI4	26S protease regulatory subunit 8	Protein degradation
C1G6T2	26S proteasome complex ubiquitin subunit Rpn13	Protein degradation
C1G7C1	26S proteasome non-ATPase regulatory subunit 1	Protein degradation
C1GHW5	26S proteasome non-ATPase regulatory subunit 11	Protein degradation
C1GFL5	26S proteasome non-ATPase regulatory subunit 12	Protein degradation
C0S923	26S proteasome non-ATPase regulatory subunit 13	Protein degradation
A0A0A0HU84	26S proteasome non-ATPase regulatory subunit 3	Protein degradation
C1GE74	26S proteasome regulatory subunit rpn-1	Protein degradation
C1GB15	26S proteasome regulatory subunit rpn11	Protein degradation
C1GLF9	26S proteasome regulatory subunit rpn-8	Protein degradation
C1FZN1	26S proteasome regulatory subunit S5A	Protein degradation
C1GA81	aspartyl aminopeptidase	Protein/peptide degradation
C1GG77	carboxypeptidase Y	Protein/peptide degradation
C1GF86	cytosolic nonspecific dipeptidase	Protein/peptide degradation
C1GD24	dipeptidyl peptidase III	Protein/peptide degradation
C1G2I1	DUF431 domain-containing	Protein/peptide degradation
C1GGV9	glutamyl aminopeptidase	Protein/peptide degradation
C1G481	intermembrane space AAA protease IAP-1	Protein/peptide degradation

C1G2Y0	methionine type I	Protein/peptide degradation
C1GLM4	methionine type II	Protein/peptide degradation
C1GA39	mitochondrial presequence protease	Protein/peptide degradation
C1G4X6	mitochondrial processing peptidase alpha	Protein/peptide degradation
C0SE56	mitochondrial-processing peptidase subunit beta	Protein/peptide degradation
C1G9P6	Proteasome A-type subunit	Protein/peptide degradation
C1G8Z1	proteasome component	Protein/peptide degradation
C0S1A8	proteasome component C5	Protein/peptide degradation
C1G9Z0	proteasome component Pre1	Protein/peptide degradation
C1G8U4	proteasome component PRE2	Protein/peptide degradation
C0SCG0	proteasome component PRE3	Protein/peptide degradation
C1GLF1	proteasome component PRE3	Protein/peptide degradation
C1G9M9	proteasome component Pre4	Protein/peptide degradation
C0SH00	proteasome component Pre5	Protein/peptide degradation
C1G6D0	proteasome component PRE6	Protein/peptide degradation
C0RZ90	proteasome component PUP2	Protein/peptide degradation
A0A0A5IDL6	proteasome regulatory particle subunit ( )	Protein/peptide degradation
C0S0W0	proteasome regulatory particle subunit ( )	Protein/peptide degradation
C1GIV4	proteasome subunit alpha	Protein/peptide degradation
C0S3F0	proteasome subunit alpha type	Protein/peptide degradation
C1GMF6	proteasome subunit alpha type-4	Protein/peptide degradation
C1G9Y1	proteasome subunit beta type-3	Protein/peptide degradation
C1G0I9	kinase C substrate 80K-H	Regulation of protein activity
C1G3H1	OTU domain-containing 6B	Protein/peptide degradation
C1G8S4	pyroglutamyl peptidase type I	Protein/peptide degradation
C1GJI6	serine ase	Protein/peptide degradation
C1GBF1	AFG3 family	Protein/peptide degradation
C1GJM4	aspartyl aminopeptidase	Vacuolar protein degradation

Protein synthesis		
C1GIL9	arginine-tRNA ligase	Aminoacyl-tRNA-synthetases
C1GDQ2	asparagine-tRNA ligase	Aminoacyl-tRNA-synthetases
C1GBG1	aspartate-tRNA(Asn) ligase	Aminoacyl-tRNA-synthetases
C1GG12	glutaminyl-tRNA synthetase	Aminoacyl-tRNA-synthetases
C1FYE5	glutamyl-tRNA synthetase	Aminoacyl-tRNA-synthetases
C0SCX7	glycyl-tRNA synthetase	Aminoacyl-tRNA-synthetases
C1G3P2	histidyl-tRNA synthetase	Aminoacyl-tRNA-synthetases
C1FYK2	isoleucine-tRNA ligase	Aminoacyl-tRNA-synthetases
C1GB62	leucyl-tRNA synthetase	Aminoacyl-tRNA-synthetases
C1GMI6	lysyl-tRNA synthetase	Aminoacyl-tRNA-synthetases
C1GJZ6	phenylalanine-tRNA alpha subunit	Aminoacyl-tRNA-synthetase
C1G390	phenylalanine-tRNA beta subunit	Aminoacyl-tRNA-synthetase
C1GIA7	phenylalanyl-tRNA synthetase	Aminoacyl-tRNA-synthetase
C1G570	prolyl-tRNA synthetase	Aminoacyl-tRNA-synthetase
Ribosomal proteins		
C1GLR7	37S ribosomal mitochondrial	Ribosome biogenesis
C1G3I7	37S ribosomal Rsm24	Ribosome biogenesis
C1G9U4	60S acidic ribosomal P0	Ribosome biogenesis
C1G6J2	60S ribosomal L3	RNA proceesing
C1G4C2	hypothetical protein GX48_05945	Ribosomal protein
C0S6I5	mitochondrial 54S ribosomal 24 14	Ribosomal proteins
C1G3Q2	mitochondrial 54S ribosomal 36	Ribosomal proteins
C1GE87	ribosomal L11	Ribosomal proteins
C1GCK9	ribosomal L14	Ribosomal proteins
C1GD70	ribosomal L14	Ribosomal proteins
C1H7K2	ribosomal L14b L23e	Ribosomal proteins

C0S6V8	ribosomal L15	Ribosomal proteins
C1G2C4	ribosomal L17	Ribosomal proteins
C1GJQ3	ribosomal L19	Ribosomal proteins
C1GBM4	ribosomal L24	Ribosomal proteins
C1GJM0	ribosomal L27	Ribosomal proteins
C1G115	Ribosomal L28 mitochondrial	Ribosomal proteins
C1GN78	ribosomal L28e	Ribosomal proteins
C1G265	ribosomal L5	Ribosomal proteins
C0S1U8	Ribosomal mitochondrial	Ribosomal proteins
C1G6S2	ribosomal RNA processing	Ribosomal proteins
C1G1P5	ribosomal S15	Ribosomal proteins
C1GJ53	ribosomal S16	Ribosomal proteins
C1GMC7	ribosomal S2	Ribosomal proteins
C1GBD9	hypothetical protein PABG_04544	Ribosome binding
C1GF85	60S ribosomal protein	Ribosome biogenesis
C1GC24	ribosomal biogenesis Gar2	Ribosome biogenesis
C1G017	ribosome associated chaperone Zuotin	Ribosome biogenesis
C1G798	ribosome biogenesis Pescadillo	Ribosome biogenesis
C1G3G1	Ribosome biogenesis RLP24	Ribosome biogenesis
C1G6F4	ribosome recycling factor	Ribosome biogenesis
C0SDZ9	mRNA turnover MRT4	Ribosome biogenesis
C1GCV9	nucleolar NOP56	Ribosome biogenesis
Translation		
C1GL84	30S ribosomal S7	Translation
C1G548	30S ribosomal subunit S4	Translation
C1GE49	37S ribosomal S5	Translation
C1G1P4	40S ribosomal S0	Translation

C1HDW2	40S ribosomal S1	Translation
C1GB65	40S ribosomal S10-A	Translation
C1G2V1	40S ribosomal S11	Translation
C1G3B1	40S ribosomal S12	Translation
C1GK99	40S ribosomal S13	Translation
C1GSD8	40S ribosomal S14	Translation
C0RX98	40S ribosomal S15	Translation
C0S2N7	40S ribosomal S16	Translation
C1G0G4	40S ribosomal S17	Translation
C1G0G5	40S ribosomal S17	Translation
C1GG76	40S ribosomal S18	Translation
A0A0A0HQN9	40S ribosomal S19	Translation
C0SJ04	40S ribosomal S2	Translation
C1GGR5	40S ribosomal S20	Translation
C0SFB5	40S ribosomal S21	Translation
C1H3J4	40S ribosomal S22	Translation
C0SG30	40S ribosomal S23	Translation
C1GAM9	40S ribosomal S24-A	Translation
C1GH63	40S ribosomal S25	Translation
C1GLJ7	40S ribosomal S26E	Translation
C1GFL2	40S ribosomal S27	Translation
C0SJH5	40S ribosomal S28	Translation
C1GR56	40S ribosomal S29	Translation
C0S653	40S ribosomal S3	Translation
C0SFL4	40S ribosomal S30	Translation
C1G810	40S ribosomal S4	Translation
C1GHV2	40S ribosomal S5	Translation
C1GVT9	40S ribosomal S6-B	Translation

C1FYR6	40S ribosomal S7	Translation
A0A0A0HS69	40S ribosomal S8-B	Translation
C1G821	40S ribosomal S9	Translation
C1GL95	50S ribosomal 27	Translation
C1G540	50S ribosomal L12	Translation
C1GD41	50S ribosomal L3	Translation
C1GB61	50S ribosomal Mrp49	Translation
C1FYZ0	50S ribosomal subunit L30	Translation
C1GMG7	54S ribosomal L6	Translation
C1G2S6	54S ribosomal mitochondrial	Translation
C1G6M3	60S ribosomal L10a	Translation
C1G942	60S ribosomal L10-A	Translation
C1GA20	60S ribosomal L11	Translation
C1GKL7	60S ribosomal L12	Translation
C0S5P1	60S ribosomal L13	Translation
C1GR24	60S ribosomal L15	Translation
C0S349	60S ribosomal L16	Translation
C1GHJ0	60S ribosomal L17	Translation
C1GDK2	60S ribosomal L18-B	Translation
C1G283	60S ribosomal L2	Translation
A0A0A5IGQ4	60S ribosomal L20	Translation
C1G820	60S ribosomal L21-A	Translation
C0S9N3	60S ribosomal L22	Translation
C0SFL5	60S ribosomal L24	Translation
C1G7J1	60S ribosomal L25	Translation
C1GF47	60S ribosomal L25	Translation
C1GFA3	60S ribosomal L27a	Translation
C0S3E7	60S ribosomal L27-B	Translation

C1G172	60S ribosomal L27-B	Translation
A0A0A0HUJ5	60S ribosomal L3	Translation
C1G945	60S ribosomal L30	Translation
A0A0A5IBK7	60S ribosomal L31	Translation
C1FZ57	60S ribosomal L32	Translation
C1GAW6	60S ribosomal L34	Translation
C1G4P8	60S ribosomal L35	Translation
C0S1J8	60S ribosomal L36	Translation
C1GQH0	60S ribosomal L36	Translation
C0S1P6	60S ribosomal L38	Translation
C1GF00	60S ribosomal L39	Translation
C1FZ00	60S ribosomal L43	Translation
C1GEN5	60S ribosomal L4-A	Translation
A0A0A0HYV6	60S ribosomal L5	Translation
C1G6T3	60S ribosomal L6	Translation
C0S635	60S ribosomal L7	Translation
C0S900	60S ribosomal L8-B	Translation
C1GB47	60S ribosomal L8-B	Translation
A0A0A0HWY2	60S ribosomal L9	Translation
A0A0A0HT80	60S ribosomal protein L13	Translation
C1GLK3	60S acidic ribosomal P1	Translation elongation
C1G5J1	60S acidic ribosomal P2	Translation elongation
C1GLU9	KOW domain-containing domain-containing	Translation
C1G650	translation elongation factor G	Translation control
C1G4T3	translation elongation factor Tu	Translation control
C1G4T4	translation initiation factor [Blastomyces gilchristii SLH14081]	Translation control
C0S4N6	translation initiation factor 4B	Translation control
C1FZ53	translation initiation factor 4B	Translation control

C1H782	translation initiation factor 4B	Translation control
C1G0R7	translation initiation factor 4G	Translation control
C1GLA9	translation initiation factor aIF-2	Translation control
C1GBB5	translation initiation factor eIF-2B epsilon subunit	Translation control
C1G4M5	translation initiation factor RLI1	Translation control
C0SDL5	translation initiation factor SUI1	Translation control
C1G4I4	translation machinery-associated 20	Translation control
A0A0A0HV62	translational activator GCN1	Translation control
C1GL98	polyadenylate-binding cytoplasmic and nuclear	Translation initiation
C1GJS9	NSFL1 cofactor p47	Translational control
C0SJE2	cap binding	Translation
C1G1F2	elongation factor 1-alpha	Translation elongation
C1G6U1	elongation factor 1-beta	Translation elongation
C0SGK1	elongation factor 1-gamma	Translation elongation
C1GLI9	elongation factor 2	Translation elongation
C1GIZ3	elongation factor 3	Translation elongation
C1G791	elongation factor mitochondrial	Translation elongation
C1GA10	ATP-dependent RNA helicase DED1	Translation initiation
A0A0A0HV09	ATP-dependent RNA helicase eIF4A	Translation initiation
C1GEV1	ATP-dependent RNA helicase FAL1	Translation initiation
C1H4Z1	eukaryotic translation initiation factor 2 alpha subunit	Translation initiation
C1GAC4	eukaryotic translation initiation factor 2 beta subunit	Translation initiation
C1G9Z7	eukaryotic translation initiation factor 2 subunit gamma	Translation initiation
C1GFI8	eukaryotic translation initiation factor 2A	Translation initiation
C1G9T0	eukaryotic translation initiation factor 3 subunit A	Translation initiation
C0SFW7	eukaryotic translation initiation factor 3 subunit B	Translation initiation
C1G373	eukaryotic translation initiation factor 3 subunit C	Translation initiation
C1FZP0	eukaryotic translation initiation factor 3 subunit D	Translation initiation

C0S3G0	eukaryotic translation initiation factor 3 subunit E	Translation initiation
C1G2D0	eukaryotic translation initiation factor 3 subunit F	Translation initiation
C0S2X7	eukaryotic translation initiation factor 3 subunit G	Translation initiation
C0S5G5	eukaryotic translation initiation factor 3 subunit H	Translation initiation
C1G4J9	eukaryotic translation initiation factor 3 subunit H	Translation initiation
C0S0U3	eukaryotic translation initiation factor 3 subunit I	Translation initiation
C1G9X1	eukaryotic translation initiation factor 3 subunit J	Translation initiation
C0S2P5	eukaryotic translation initiation factor 3 subunit L	Translation initiation
C0S4V8	eukaryotic translation initiation factor 3 subunit M	Translation initiation
C1FZD4	eukaryotic translation initiation factor 3 subunit M	Translation initiation
C1GLA2	eukaryotic translation initiation factor 5A-2	Translation initiation
C1G686	eukaryotic translation initiation factor 6	Translation initiation
C0SFR2	eukaryotic translation initiation factor Y-chromosomal	Translation initiation
C1G8E5	eukaryotic peptide chain release factor GTP-binding subunit	Translation termination
C0SD08	eukaryotic peptide chain release factor subunit 1	Translation termination
Biogenesis of cellular components		
A0A0A0HRG7	actin	Actin cytoskeleton
A0A0A0HWK8	actin	Actin cytoskeleton
C1G1V9	actin 2	Actin cytoskeleton
C1G9A3	actin 4	Actin cytoskeleton
A0A0A0HX33	actin ARP8	Actin cytoskeleton
C1FZH1	actin binding	Actin cytoskeleton
C1GJ13	actin binding [Paracoccidioides lutzii Pb01]	Actin cytoskeleton
C1G0N2	actin cytoskeleton	Actin cytoskeleton
C1G1Z1	actin cytoskeleton-regulatory complex END3	Actin cytoskeleton
C1GI59	GYF domain-containing	Cytoskeleton/structural proteins
C1FZC6	actin cytoskeleton-regulatory complex PAN1	Actin cytoskeleton

C1G6J5	adenylyl cyclase-associated	Cytoskeleton/structural proteins
C1G9M3	ARP2 3 actin-organizing complex subunit Sop2	Actin cytoskeleton
C1GKS3	arp2 3 complex 20 kDa subunit	Actin cytoskeleton
C1GE52	Arp2 3 complex subunit	Actin cytoskeleton
C1FYR9	arp2 3 complex subunit Arc16	Actin cytoskeleton
C1GKH0	F-actin-capping subunit beta	Actin cytoskeleton
C1GDK5	fimbrin	Actin cytoskeleton
C1G8G5	F-actin-capping subunit alpha	Actin dependent transport
C1GDG7	beta-1,6-glucan boisynthesis (Knh1)	Cell wall
C1GG99	cell lysis cwl1	Cell wall
C1G6R0	cell wall biogenesis phosphatase Ssd1	Cell wall
C1GGK1	chitin synthase B	Cell wall
C1G553	glutathione S-transferase	Cell wall
C1G760	GPI anchored serine-threonine rich	Cell wall
C1GK29	immunodominant antigen Gp43	Cell wall
C1GHW6	integral membrane	Cell wall
C1GFY2	LRP16 family	Centrosome
C1GDR8	deoxyhypusine hydroxylase	Microtubule cytoskeleton
C1GMN0	emo am system B	Microtubule cytoskeleton
C1GM22	tubulin alpha-1 chain	
C1FZT8	tubulin alpha-2 chain	
C1G6U5	tubulin beta chain	
C1GDA3	tubulin-specific chaperone Rbl2	
C1FYS5	microtubule associated EB1	Microtubule cytoskeleton
C0S0V3	FAD dependent sulfhydryl oxidase Erv1	Mitochondrion
C0SCA7	mitochondria fission 1	Mitochondrion
C1GGZ6	mitochondrial	Mitochondrion
C1G8Y4	mitochondrial	Mitochondrion

C1G1S1	mitochondrial cytochrome b2	Mitochondrion
C1GM02	mitochondrial DNA replication YHM2	Mitochondrion
C1GDR6	mitochondrial F1F0 ATP synthase subunit F	Mitochondrion
A0A0A0HST0	mitochondrial F1F0-ATP synthase G subunit	Mitochondrion
C1G0S6	mitochondrial genome maintenance MGM101	Mitochondrion
C1G4S5	Mitochondrial hypoxia responsive domain	Mitochondrion
C1G9E6	mitochondrial import inner membrane translocase subunit tim10	Mitochondrion
C0SBT0	mitochondrial import inner membrane translocase subunit TIM13	Mitochondrion
C1G0H8	mitochondrial import inner membrane translocase subunit tim16	Mitochondrion
C1GB16	mitochondrial import inner membrane translocase subunit tim50	Mitochondrion
C1G4B9	mitochondrial import inner membrane translocase subunit tim54	Mitochondrion
C1GZ28	mitochondrial import inner membrane translocase subunit tim9	Mitochondrion
C1G9U8	mitochondrial import MAS5	Mitochondrion
C1G4X8	mitochondrial import receptor subunit tom22	Mitochondrion
C1GGW7	mitochondrial import receptor subunit TOM40	Mitochondrion
C1G4I7	mitochondrial inner membrane translocase subunit TIM44	Mitochondrion
C1GGL3	mitochondrial large ribosomal subunit	Mitochondrion
C1GHY8	mitochondrial large ribosomal subunit 35	Mitochondrion
C1GMR0	mitochondrial ribosomal DAP3	Mitochondrion
C1G016	mitochondrial zinc maintenance mitochondrial	Mitochondrion
C1G534	mitochondrion	Mitochondrion
C1GMJ7	heterochromatin HP1	Nucleus
C1G029	chromatin remodeling complex subunit	Organization of chromossome structure
C1GDM0	isochorismatase domain-containing	Peroxisome
C0S8P6	Profilin	Actin cytoskeleton
C1GF57	nucleosome assembly	Bud/growth tip
C1GJL8	scramblase family	Cell wall
C1GFV7	reduced viability upon starvation 167	Cytoskeleton-dependent transport

C1GEQ3	NAP family	Nucleus
C1GN88	RPEL repeat	Nucleus
C1GK84	SAP domain	Nucleus
A0A0A5IFN8	SAP domain containing	Nucleus
C0S3Q1	peroxin 19	Peroxisome
C1FZM1	peroxisomal biogenesis factor 6	Peroxisome
C1G161	peroxisomal dehydratase	Peroxisome
C0SJE6	peroxisomal hydratase-dehydrogenase-epimerase	Peroxisome
C1GMZ1	peroxisomal hydratase-dehydrogenase-epimerase	Peroxisome
C1GBX3	peroxisomal-coenzyme A synthetase	Peroxisome
Cell fate		
C1GFQ1	dsDNA-binding PDCD5	Apoptosis
C0SAZ5	glial maturation factor	Growth regulatiors/ regulation of cell size
C1G2I2	pheromone-processing carboxypeptidase KEX1	Apopitosis
C1GP72	septin 3	cytokinesis (cell division) /septum formation and hydrolysis
C0S7S2	septin-1	cytokinesis (cell division) /septum formation and hydrolysis
C1GHM7	septum formation Maf	cytokinesis (cell division) /septum formation and hydrolysis
C1GC37	progesterone binding	Growth regulators/regulation of cell size
Cellular Comunication/Signal transduction mechanisms		
C1GMA5	cAMP-dependent kinase regulatory subunit	cAMP/cGMP mediated signal transduction
C1G6P0	CORD and CS domain-containing	Cellular signaling
C1G4I3	GTP-binding	Cellular signaling
C1GK66	CAMK CAMK1 CAMK1-CMK kinase	Enzyme mediated signal transduction
C0RXV4	COP9 signalosome subunit 6	Enzyme mediated signal transduction
C1GG36	calcineurin subunit B	Phosphatase
C1GBG9	CK1 CK1 CK1-D kinase	Serine/threonine kinase

C0SJK7	CMGC GSK kinase	Serine/threonine kinase
C1FZF8	and Fes CIP4 domain-containing	Signal transductional
C1GLJ8	CBS and PB1 domain-containing	Signal transductional
C1GLU6	GTP-binding rhoA	Small GTPase mediated signal transduction
C0RZB5	myosin regulatory light chain cdc4	Cellular signaling
C1G2D4	phosphatase 2C	Cellular signaling
C1G389	phosphatase PP2A regulatory subunit A	Cellular signaling
C0SAZ3	Ras	Cellular signaling
C1HBF2	Ras GTPase	Cellular signaling
C0SB96	Ras Rab7	Cellular signaling
C1GFR7	rho GTPase activator	Cellular signaling
C1GE31	rho-gdp dissociation inhibitor	Cellular signaling
C1GG06	Ryp1	Cellular signaling
C1G8F8	Ser Thr phosphatase	Cellular signaling
C1GA74	MRS7 family	Transmembrane signal transduction
Cell rescue, defense and virulence		
C1GD03	Heat shock 70 kd protein cognate 1	Heat shock response
C0RYH7	heat shock 78	Heat shock response
C0SEZ4	heat shock 90	Heat shock response
C1GKC9	heat shock 90	Heat shock response
C0RY69	heat shock Hsp88	Heat shock response
C1G9M7	30 kDa heat shock	Stress response
C1G6I0	heat shock Hsp88	Heat shock response
C0RY45	heat shock SSB1	Heat shock response
C1GAU3	heat shock STI1	Heat shock response
C1G5F3	heat shock transcription factor	Heat shock response

C1GLX8	hsp60	Heat shock response
C1G0P0	hsp7	Heat shock response
C0S3U7	Hsp70	Heat shock response
C1GLI2	hsp72	Heat shock response
C1GBN1	Hsp90 binding co-chaperone (Sba1)	Heat shock response
C1G514	Hsp90 co-chaperone Cdc37	Heat shock response
C0S3V9	hsp98	Heat shock response
C1G0D4	peroxisomal catalase	oxidative stress respomse
C1G0Y9	glutaredoxin	oxidative stress respomse
C1GKT9	allergen Asp F3	stress response
C1G7T4	ATP-dependent protease La	stress response
C1GLZ5	clock-controlled gene-9	stress response
C0SC77	cytochrome c	stress response
C0RZ65	cytochrome c mitochondrial	stress response
C0SBB3	disulfide-isomerase domain A6	stress response
C0SEQ1	disulfide-isomerase domain MPD1	stress response
C1GEE2	disulfide-isomerase domain tigA	stress response
C1GHF4	DUF1014 domain-containing	stress response
A0A0A5IC00	Fe superoxide dismutase	stress response
C1GC65	glutathione peroxidase	stress response
C0SEG3	glutathione-disulfide reductase	stress response
C1GAY4	peptide-methionine (S)-S-oxide reductase	Stress response
C1G113	Stress response protein NST1	Stress response
C1FZT5	Grx4 family monothiol glutaredoxin	stress response
C1G5S1	DSBA family oxidoreductase	Detoxification
C1GDK8	peroxiredoxin Q BCP	Oxidative stress response
C1GSF9	peroxisomal catalase	Oxidative stress response
C1G489	superoxide dismutase	Oxidative stress response

C1GJI2	superoxide dismutase [Cu-Zn]	Oxidative stress response
C1G4T8	superoxide dismutase [Mn] mitochondrial	Oxidative stress response
C0SGG4	Stf2 like protein	Cellular response to desiccation
Regulation of metabolism and protein function		
C1GG57	HEAT repeat containing	Enzymatic activity regulation/enzyme regulator
Interact with the environment		
C1G696	ferrochelatase	Homeostasis of metal ions
C1G9B6	HIRA-interacting 5	Homeostasis of metal ions
C1G3W0	iron donor	Homeostasis of metal ions
C1GDJ9	iron sulfur cluster assembly mitochondrial	Homeostasis of metal ions
C1G0F4	iron-sulfur cluster assembly accessory	Homeostasis of metal ions
Transcription		
A0A0A0HY23	cleavage and polyadenylation specific factor 5	mRNA binding
C1GCF0	ATP-dependent RNA helicase SUB2	mRNA processing
C1FYF8	ATP-dependent RNA helicase MSS116	mRNA synthesis
C1GBD3	RNP domain-containing	RNA synthesis
C1GGW1	cap binding	mRNA synthesis
C1G9A0	heterogeneous nuclear ribonucleo HRP1	mRNA synthesis
C1G7X8	ATP-dependent RNA helicase DBP5	RNA synthesis
C0SD49	ATP-dependent RNA helicase DHH1	RNA synthesis
C1FZK1	ran GTPase activating	rRNA processing
C1GHN2	rRNA 2 -O-methyltransferase fibrillarin	rRNA processing
C1GEE6	rRNA processing	rRNA processing
C1GG78	SBDS family rRNA metabolism	rRNA processing
C1GF39	centromere microtubule-binding cbf5	rRNA processing
C1GGT3	DNA-directed RNA polymerase and III subunit RPABC5	rRNA synthesis
C1FYH2	DNA-directed RNA polymerase II polypeptide	rRNA synthesis

C1GHL1	DNA-directed RNA polymerase II subunit RPB1	rRNA synthesis
C1FZC5	DNA-directed RNA polymerase II subunit RPB11	rRNA synthesis
C1GAQ4	DNA-directed RNA polymerase II subunit RPB2	rRNA synthesis
C1GDQ7	mRNA decapping hydrolase	mRNA processing
C1G8W0	nuclear polyadenylated RNA-binding Nab2	mRNA processing
C1G5V0	nucleic acid-binding	mRNA processing
A0A0A0HTY8	nucleolar 58	mRNA processing
C1G952	polyadenylation factor subunit 64	mRNA processing
C1GIU8	pre-rRNA processing Rrp12	mRNA processing
C1G5L8	pirin	mRNA synthesis
C1GC90	H ACA ribonucleo complex subunit 2	rRNA synthesis
C0S859	H ACA ribonucleo complex subunit 3	rRNA synthesis
C1G7J2	mRNA splicing	Splicing
C1G2Y3	mRNA splicing factor	Splicing
C1GD63	nuclear cap-binding subunit 2	Splicing
C1G6U4	nuclear mRNA splicing factor-associated	Splicing
C1GDK4	pre-mRNA splicing factor	Splicing
C1G4M6	pre-mRNA-processing prp40	Splicing
C0SAR4	pre-mRNA-splicing factor cef1	Splicing
C1GDR3	pre-mRNA-splicing factor cef1	Splicing
C1GDL2	pre-mRNA-splicing factor cwc15	Splicing
A0A0A5IGD7	pre-RNA splicing factor Srp2	Splicing
C1GHD0	RNA splicing factor	SPlicing
C1GGU1	splicing factor 3b	SPlicing
C1GK92	splicing factor U2AF subunit	SPlicing
C1FYD8	branchpoint-bridging	Splicing
A0A0A0HVL0	bZIP transcription factor	Transcription
C1GKT2	C6 zinc finger domain-containing	Transcription

C1GEL8	Cullin binding	Transcriptional control
C0S6A0	KH domain RNA-binding	Transcriptional control
C1G3Y0	-like helicase 1	Transcriptional control
C1GM32	-like helicase 2	Transcriptional control
C0S3K2	rnapii degradation factor def1	RNA degradation
C1GJN3	RNase III domain-containing	RNA degradation
C1FYT8	pH-response transcription factor pacC RIM101	Transcription control
C1GD67	peptidyl-prolyl cis trans isomerase	Transcription elongation
C0S1M3	peptidyl-prolyl cis-trans isomerase B	Transcription elongation
C0SH74	peptidyl-prolyl cis-trans isomerase D	Transcription elongation
C0S7L7	peptidyl-prolyl cis-trans isomerase H	Transcription elongation
C1GKU6	peptidyl-prolyl cis-trans isomerase-like 1	Transcription elongation
C1G306	pol II transcription elongation factor subunit Cdc73	Transcription elongation
C1GF64	transcription elongation factor	Transcription elongation
C1FZJ4	transcription elongation factor S-II	Transcription elongation
C1G7B9	transcription elongation factor spt5	Transcription elongation
A0A0A0HTI2	transcription factor	Transcription elongation
C1GJI0	transcription factor	Transcription elongation
C0S802	Transcription factor (Snd1 p100)	Transcription elongation
C0S6Y3	transcription factor [Blastomyces gilchristii SLH14081]	Transcription elongation
C0S5N9	transcription factor iws1	Transcription elongation
C1GHL6	transcription initiation factor TFIID	Transcription elongation
C1G3W4	transcription regulator BDF1	Transcription elongation
C1GGW3	transcriptional repressor	Transcription elongation
C1GL30	nucleoside diphosphatase Gda1	Transcriptional
C0S8C0	nucleoside diphosphate kinase	Transcriptional
C1GAM1	mRNA binding post-transcriptional regulator	Transcriptional control
C0SIG2	NAD dependent epimerase dehydratase family	Transcriptional control

C1GCK8	nascent polypeptide-associated complex subunit alpha	Transcriptional control
C1GCD5	nascent polypeptide-associated complex subunit beta	Transcriptional control
C1G2S5	NOT2 family	Transcriptional control
C0S6U5	nuclear and cytoplasmic polyadenylated RNA-binding pub1	Transcriptional control
C1G6H8	RNA-binding La domain-containing	Transcriptional control
C0S0R4	L-PSP endoribonuclease family (Hmf1)	Transcriptional control
Cell type and differentiation		
C1GAH4	NIMA-interacting variant 2	Fungal and other eukariotic cell type differentiation
C1GLF5	cell polarity (Alp11)	Budding, cell polarity and filament formation
C1GKX2	chitin biosynthesis CHS5	Budding, cell polarity and filament formation
C1FZM0	MGS207	Uncharacterized protein
C1GJH6	DUF1479 domain-containing	Unknown protein
C0S8L9	DUF427 domain	Unknown protein
C1G7G6	DUF757 domain-containing	Unknown protein
C1GDY7	DUF775 domain-containing	Unknown protein
C1G0S3	DUF833 domain-containing	Unknown protein
C1GGS4	conserved fungal	Unknown protein
C0S9T2	dienelactone hydrolase	Unknown protein
C1GFJ7	nipsnap family	Vesicular transport
Protein folding		
C1G571	T-complex 1 subunit alpha	Protein folding
C1GL41	T-complex 1 subunit beta	Protein folding
C1H5V8	T-complex 1 subunit beta	Protein folding
C0S7H4	T-complex 1 subunit delta	Protein folding
C1GMJ8	T-complex 1 subunit epsilon	Protein folding
C1GLJ3	T-complex 1 subunit eta	Protein folding

C0S491	T-complex 1 subunit gamma	Protein folding
C1G541	T-complex 1 subunit theta	Protein folding
C0SHR4	T-complex 1 subunit zeta	Protein folding
C1GH32	TCTP family	Protein folding
Unknow function		
C1GK40	hypothetical protein ACO22_00231	Unknown protein
C1FZA2	hypothetical protein GX48_04771	Unknown protein
C1GP52	hypothetical protein PAAG_00297	Unknown protein
C0RY48	hypothetical protein PABG_00353	Unknown protein
C1GFI5	hypothetical protein PABG_05692	Unknown protein
C1G6A4	hypothetical protein PABG_11096	Unknown protein
C1G1E0	hypothetical protein PADG_00680	Unknown protein
C1FYP5	hypothetical protein PADG_00921	Unknown protein
C1G3V2	hypothetical protein PADG_01618	Unknown protein
C1G486	hypothetical protein PADG_01752	Unknown protein
C1G2H2	hypothetical protein PADG_02338	Unknown protein
C1G5K7	hypothetical protein PADG_02462	Unknown protein
C1G6F9	hypothetical protein PADG_02764	Unknown protein
C1G6Q3	hypothetical protein PADG_02858	Unknown protein
C1G7M1	hypothetical protein PADG_03176	Unknown protein
C1G8R6	hypothetical protein PADG_03652	Unknown protein
C1G8U8	hypothetical protein PADG_03684	Unknown protein
C1G9R6	hypothetical protein PADG_04002	Unknown protein
C1GFP4	hypothetical protein PADG_06080	Unknown protein
C1GID6	hypothetical protein PADG_07022	Unknown protein
C1GIY9	hypothetical protein PADG_07225	Unknown protein
C1GJ57	hypothetical protein PADG_07293	Unknown protein
C1GJX9	hypothetical protein PADG_07565	Unknown protein

C1GKB0	hypothetical protein PADG_07696	Unknown protein
C1GMI1	hypothetical protein PADG_08467	Unknown protein
C1GMJ4	hypothetical protein PADG_08480	Unknown protein
A0A0A0HT22	hypothetical protein PADG_12447	Unknown protein
C0SAR8	NADH dehydrogenase iron-sulfur 4	Unknown protein
C1FYY4	PT repeat family	Unknown protein
C1G5P6	PWWP domain-containing	Unknown protein
C0SFI8	Rhodanese domain	Unknown protein
C1GHR8	Senescence spartin-associated	Unknown protein
C1GF61	seryl-tRNA synthetase	Unknown protein
C1GJL6	SGT1 and CS domain-containing	Unknown protein
C1G0G2	SH3 domain	Unknown protein
C1G589	single-stranded DNA binding p30 subunit	Unknown protein
C1G9W2	small COPII coat GTPase sar1	Unknown protein
C0RZB0	Small nuclear ribonucleo	Unknown protein
C0S230	Small nuclear ribonucleo	Unknown protein
C1GA21	small nuclear ribonucleo	Unknown protein
C1GIS6	small nuclear ribonucleo	Unknown protein
C1FZV4	small nuclear ribonucleo LSM2	Unknown protein
C1G6M0	small nuclear ribonucleo Lsm8	Unknown protein
C0SDT1	small nuclear ribonucleo Sm D2	Unknown protein
C1GHX0	small nuclear ribonucleo Sm D2	Unknown protein
C0S6X4	Small nuclear ribonucleo Sm D3	Unknown protein
C0S8P3	SNARE Ykt6	Unknown protein
C1G050	SNF7 family Fti1 Did2	Unknown protein
C1G3N9	sorting nexin-4	Unknown protein
A0A0A0HR72	Sorting nexin-41	Unknown protein
C1GC81	spermidine synthase	Unknown protein

C0SCS9	ssDNA binding	Unknown protein
C1GEW2	ssDNA binding	Unknown protein
C0SDS9	ssDNA binding Ssb3	Unknown protein
C1G014	sterol 24-C-methyltransferase	Unknown protein
C1G4I1	stomatin family	Unknown protein
C1G0V6	structural domain	Unknown protein
C0S3I7	structure-specific recognition 1	Unknown protein
C1G1B6	structure-specific recognition 1	Unknown protein
C0SH51	suaprga1	Unknown protein
C1FZL2	succinate dehydrogenase [ubiquinone] flavo mitochondrial	Mitochondria activity
C1GL06	succinate dehydrogenase [ubiquinone] iron-sulfur mitochondrial	Mitochondria activity
C1GHM8	succinate dehydrogenase cytochrome b small subunit	Unknown protein
C1FYZ4	succinate:fumarate antiporter	Unknown protein
C1G7A3	succinate-semialdehyde dehydrogenase	Unknown protein
C1GBD8	succinyl- :3-ketoacid-coenzyme A transferase	Unknown protein
C1G294	succinyl- ligase [GDP-forming] subunit mitochondrial	Unknown protein
C1G0C7	succinyl- ligase beta-chain	Unknown protein
C1G6C3	sulfite oxidase	Unknown protein
C1GCG8	Synaptobrevin	Unknown protein
C0S2B7	Tautomerase MIF	Unknown protein
C1FZF4	UBX domain-containing	Unknown protein
C1FYN6	UDP-galactopyranose mutase	Unknown protein
C1GAM6	UDP-N-acetylglucosamine pyrophosphorylase	Unknown protein
A0A0A0HV13	Uncharacterized protein	Unknown protein
A0A0A0HV71	Uncharacterized protein	Unknown protein
A0A0A0HYI6	Uncharacterized protein	Unknown protein
C0RXC5	Uncharacterized protein	Unknown protein
C0S0D3	Uncharacterized protein	Unknown protein

C0S0L3	Uncharacterized protein	Unknown protein
C0S0W4	Uncharacterized protein	Unknown protein
C0S1R3	Uncharacterized protein	Unknown protein
C0S2Z3	Uncharacterized protein	Unknown protein
C0S6A4	Uncharacterized protein	Unknown protein
C1FYE8	NAD dependent epimerase/dehydratase family protein	Unknown protein
C1FZK6	Uncharacterized protein	Unknown protein
C1G0J1	Uncharacterized protein	Unknown protein
C1G0J8	Uncharacterized protein	Unknown protein
C1G0N9	Uncharacterized protein	Unknown protein
C1G0Z0	Uncharacterized protein	Unknown protein
C1G187	Uncharacterized protein	Unknown protein
C1G1A1	Uncharacterized protein	Unknown protein
C1G1D4	Uncharacterized protein	Unknown protein
C1G1F0	Uncharacterized protein	Unknown protein
C1G1R0	Uncharacterized protein	Unknown protein
C1G1S6	Uncharacterized protein	Unknown protein
C1G1V2	Uncharacterized protein	Unknown protein
C1G2E9	Uncharacterized protein	Unknown protein
C1G412	Uncharacterized protein	Unknown protein
C1G4A0	Uncharacterized protein	Unknown protein
C1G4Q1	Uncharacterized protein	Unknown protein
C1G5D3	Uncharacterized protein	Unknown protein
C1G5J9	NADH- ubiquinone oxidoreductase	Unknown protein
C1G6F3	Uncharacterized protein	Unknown protein
C1G712	Uncharacterized protein	Unknown protein
C1G7G0	Uncharacterized protein	Unknown protein
C1G7I0	Uncharacterized protein	Unknown protein

C1G8R8	Uncharacterized protein	Unknown protein
C1GAE3	Uncharacterized protein	Unknown protein
C1GB03	Uncharacterized protein	Unknown protein
C1GBA0	Uncharacterized protein	Unknown protein
C1GBH2	Uncharacterized protein	Unknown protein
C1GBV3	Uncharacterized protein	Unknown protein
C1GCG3	Uncharacterized protein	Unknown protein
C1GCZ3	Uncharacterized protein	Unknown protein
C1GDU6	Uncharacterized protein	Unknown protein
C1GEL7	Uncharacterized protein	Unknown protein
C1GFA4	Uncharacterized protein	Unknown protein
C1GFC3	Uncharacterized protein	Unknown protein
C1GH39	Uncharacterized protein	Unknown protein
C1GH85	Uncharacterized protein	Unknown protein
C1GH86	Uncharacterized protein	Unknown protein
C1GI30	Uncharacterized protein	Unknown protein
C1GII1	Uncharacterized protein	Unknown protein
C1GJH8	Uncharacterized protein	Unknown protein
C1GJQ2	Uncharacterized protein	Unknown protein
C1GJS0	Uncharacterized protein	Unknown protein
C1GK04	Uncharacterized protein	Unknown protein
C1GKW7	Uncharacterized protein	Unknown protein
C1GL70	Uncharacterized protein	Unknown protein
C1GMU6	Uncharacterized protein	Unknown protein
C1GN06	Uncharacterized protein	Unknown protein
C1GN32	Uncharacterized protein	Unknown protein
C0S1N7	UPF0041 domain	Unknown protein
C1G2H7	UPF0160 domain	Unknown protein

Supplementary table 2. Proteins up regulated in *Paracoccidioides americana*.

Accession	Description	Function	Fold change Pb03/Pb18	Sum PEP Score	Peptides	Unique Peptides
Energy						
Q1XA91	cytochrome oxidase subunit 2 (mitochondrion)	aerobic respiration	1,374	25,041	4	4
C1G1H4	pyruvate decarboxylase	alcohol fermenation	1,581	117,273	18	18
C0SIV5	aldehyde dehydrogenase	alcohol fermenation	2,576	11,571	3	4
C1GCH9	alcohol dehydrogenase	alcohol fermenation	3,469	67,224	8	8
C1GPF8	alcohol dehydrogenase 1	alcohol fermenation	4,322	7,052	3	5
C1G7X1	S-(hydroxymethyl)glutathione dehydrogenase	fermentation	1,643	7,713	2	2
C1GI20	triosephosphate isomerase	Glycolysis and Gluconeogenesis	1,262	147,944	16	16
C1G0R1	glucose-6-phosphate isomerase	Glycolysis and Gluconeogenesis	1,324	37,165	5	5
C1G440	fructose-1,6-bisphosphatase	Glycolysis and Gluconeogenesis	1,351	46,02	11	11
C1GA17	pyruvate carboxylase	Glycolysis and Gluconeogenesis	1,371	103,815	17	17
C1GML7	phosphoenolpyruvate carboxykinase	Glycolysis and Gluconeogenesis	2,616	147,65	20	20
C1GCI0	malate synthase	glyoxylate cycle	2,095	75,848	10	10
C0S0G7	cytochrome c oxidase polypeptide IV	respiration	1,387	4,35	1	2
C1GAG3	isocitrate dehydrogenase [NADP] mitochondrial	TCA cycle	1,232	103,737	17	17
C1GLZ6	citrate mitochondrial	TCA cycle	1,238	111,729	17	17
C1GLB8	Malate dehydrogenase NAD-dependent	TCA cycle	1,277	187,872	19	19
C1GCI8	2-methylcitrate mitochondrial	TCA cycle	1,317	221,885	25	25
C0RXN7	fumarate reductase	TCA cycle	8,251	16,595	3	5
C0SA05	ATP synthase subunit d mitochondrial	Mitochondrial transport	1,224	143,838	16	16
C1G5V6	ATP synthase subunit alpha mitochondrial	Mitochondrial transport	1,266	304,62	28	28

Amino acid metabolism						
C1G4D1	2-oxoisovalerate dehydrogenase E2 component (dihydrolipoyl transacylase)	Amino acid metabolism	1,224	119,187	16	16
C1GJD0	3-methylcrotonyl- carboxylase subunit alpha	Amino acid metabolism	1,308	106,226	16	16
C1GLT7	5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase	Amino acid metabolism	1,425	209,445	31	31
C1GJD3	isovaleryl- dehydrogenase	Amino acid metabolism	1,51	121,557	15	15
C1G3P8	methylmalonate-semialdehyde dehydrogenase (acylating)	Amino acid metabolism	1,528	110,512	18	18
C0SIT3	5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase	Amino acid metabolism	1,597	9,247	2	5
C1G248	4-aminobutyrate aminotransferase	Amino acid metabolism	2,08	191,763	22	22
C0SG96	4-aminobutyrate aminotransferase	Amino acid metabolism	2,576	4,909	2	3
C0S5A7	2-oxoisovalerate dehydrogenase E2 component (dihydrolipoyl transacylase)	Amino acid metabolism	2,617	10,495	2	3
C0S3J2	homoserine dehydrogenase	Amino acid metabolism	5,45	2,535	1	2
C1G792	threonine ammonia- biosynthetic	biosyntheis of isoleucine	1,366	5,333	1	1
A0A0A0HU75	threonine dehydratase	biosyntheis of isoleucine	4,106	12,06	2	2
C1GL59	imidazole glycerol phosphate synthase hisHF	biosynthesis of histidine	1,261	20,612	5	5
C1GCX5	serine cytosolic	degradation of glycine	1,434	107,629	16	15
C1GJD4	methylcrotonoyl- carboxylase subunit beta	degradation of leucine	1,407	108,491	16	16
C0S5M6	S-adenosylmethionine synthase	degradation of methionine	1,382	81,256	11	11
C1GAY3	proline oxidase	degradation of proline	1,315	62,044	7	7
C0S1P2	l-serine dehydratase	degradation of serine	2,308	7,173	3	3
C1GMI0	homogentisate 1,2-dioxygenase	degradation of tyrosine	2,008	107,351	15	15
C1G989	NAD-specific glutamate dehydrogenase	metabolism of glutamate	1,458	91,512	11	11
C1GMI2	4-hydroxyphenylpyruvate dioxygenase	Tyrosine metabolism	1,669	134,494	16	16

C-compoud and carbohydrate metabolism						
C1G020	glycine dehydrogenase	C-1 compound catabolism	1,329	100,837	18	18
C0S9Z4	2-methylcitrate dehydratase	Carbohydrate metabolism	3,065	7,787	2	11
C1GJJ9	L-xylulose reductase	C-compoud and carbohydrate metabolism	1,21	40,352	7	7
C0SF94	aldo-keto reductase	C-compoud and carbohydrate metabolism	1,815	4,112	2	3
C1G1J5	lactam utilization lamB	C-compoud and carbohydrate metabolism	1,964	36,949	7	7
C0SGQ8	polysaccharide deacetylase family protein	C-compoud and carbohydrate metabolism	2,651	46,862	7	7
C1GLU3	short chain dehydrogenase reductase	sugar, glucoside, polyol and carboxylate anabolism	1,4	12,563	2	2
C1G4M0	adenosylhomocysteinase	S-adenosyl-methionine - homocysteine cycle	1,497	180,338	22	22
Lipid, fatty acid and isoprenoid metabolism						
C1G421	3-ketoacyl- thiolase	Fatty acid metabolism	1,247	165,947	21	21
C1GAT1	fatty-acid amide hydrolase [Paracoccidioides lutzii Pb01]	Fatty acid metabolism	1,4	34,501	8	8
C1GMZ1	peroxisomal hydratase-dehydrogenase-epimerase	Fatty acid metabolism	1,562	163,056	27	27
C1GLP0	acyl- thioester hydrolase	lipid, fatty acid and isoprenoid metabolism	1,494	23,493	5	5
C1GGE1	carnitine acyl carnitine carrier	Fatty acid metabolism	1,504	9,553	3	3
Phenylpropanoids metabolism						
C1GJ33	phenylacetate 2-hydroxylase	metabolism of phenylpropanoids	2,513	33,586	6	6

Nitrogen, sulfur and selenium metabolism						
A0A0A0HTR8	hydrolase [Paracoccidioides lutzii Pb01]	Nitrogen metabolism	1,185	19,362	5	5
C1G0Q6	2-nitropropane dioxygenase	Nitrogen metabolism	1,579	200,725	15	15
C1GGQ3	formamidase	nitrogen, sulfur and selenium metabolism	2,128	99,024	11	11
Nucleotide, nucleoside, nucleobase metabolism						
C0SG69	adenosine kinase	Nucleotide/nucleoside/nucleobase metabolism	1,205	4,226	1	1
A0A0A0HRQ4	glutaryl- dehydrogenase	Nucleotide/nucleoside/nucleobase metabolism	1,256	75,572	12	12
A0A0A0HVK7	NADPH-cytochrome P450 reductase	Nucleotide/nucleoside/nucleobase metabolism	1,266	24,643	4	4
C1G1J4	urea carboxylase	Nucleotide/nucleoside/nucleobase metabolism	1,521	17,795	4	4
Protein synthesis						
C1G7A5	tyrosine-tRNA ligase	aminoacyl-tRNA-synthetases	1,346	2,082	1	1
Transport						
C1GLM2	outer mitochondrial membrane porin	channel / pore class transport	1,257	131,241	17	17
C1GM02	mitochondrial DNA replication YHM2	Mitochondrial transport	1,34	35,705	4	4
C1GML4	mitochondrial dicarboxylate carrier	Mitochondrial transport	1,718	13,805	2	2
C0S685	ADP,ATP carrier	Nucleotide/nucleoside/nucleobase transport	1,368	197,378	19	19
C0S3Q1	peroxin 19	Peroxisomal transport	1,822	2,577	1	1
C0S0V1	AP-1 complex subunit beta-1	Vesicular transports	1,248	4,194	2	2

Biogenesis of cellular components						
C0SF85	immunodominant antigen Gp43	Cell wall	4,578	30,54	8	8
A0A0A0HRG7	actin	Cytoskeleton	1,32	35,69	6	4
A0A0A5IGW8	altered inheritance-mitochondria mitochondrial	Mitochondrion	1,213	16,176	2	2
C1G1S1	mitochondrial cytochrome b2	mitochondrion	1,384	2,54	1	1
Cell cycle and DNA processing						
C0S0E8	peptide-methionine (S)-S-oxide reductase	DNA recombination and DNA repair	3,235	2,061	1	2
C0SCS9	ssDNA binding	DNA recombination and DNA repair	5,169	4,734	1	1
C0S0D2	14-3-3 family	DNA repair and proteins recombination	1,521	3,213	1	4
C1GB68	HIT domain-containing	mitotic cell cycle and cell cycle control	1,365	18,427	3	3
C1GEM8	cell division control 12	mitotic cell cycle and cell cycle control	1,663	50,345	9	9
C1GII4	cell division control [Paracoccidioides lutzii Pb01]	mitotic cell cycle and cell cycle control	1,959	36,912	7	7
Cell type differentiation						
C0S7S2	septin-1	fungal and other eukaryotic cell type differentiation	1,893	55,88	11	11
Transcription						
C1G7B9	transcription elongation factor spt5	general transcription activities	1,276	5,722	2	2
C1G5L8	pirin	mRNA synthesis	2,428	9,661	4	4

C0S6Y3	transcription factor [Blastomyces gilchristii SLH14081]	Transcription	1,343	5,121	2	2
Translation						
C1G3Q2	mitochondrial 54S ribosomal 36	Ribosome biogenesis	1,325	13,826	2	2
C1GMG7	54S ribosomal L6	Translation	1,209	12,753	3	3
A0A0A0HWY2	60S ribosomal L9	Translation	1,287	23,778	5	5
C1G283	60S ribosomal L2	Translation	1,497	65,802	7	7
C0S5P1	60S ribosomal L13	Translation	5,601	5,93	2	3
C1GAC4	eukaryotic translation initiation factor 2 beta subunit	translation initiation	1,236	28,25	4	4
C0S5G5	eukaryotic translation initiation factor 3 subunit H	Translation initiation	4,082	2,203	1	1
Cell rescue, defense and virulence						
C0SEZ4	heat shock 90	heat shock protein	9,276	6,774	1	6
C1G4T8	superoxide dismutase [Mn] mitochondrial	oxidative stress response	1,413	78,14	12	12
C1GKT9	allergen Asp F3	Stress response	1,203	63,554	10	10
C1G1M5	hsp98	Stress response	1,293	221,581	31	31
C1G7A3	succinate-semialdehyde dehydrogenase	Stress response	1,316	43,134	8	8
C1G9M7	30 kDa heat shock	Stress response	1,332	134,591	15	15
C1GBN1	Hsp90 binding co-chaperone (Sba1)	Stress response	1,378	37,787	6	6
C1GDK8	peroxiredoxin Q BCP	Stress response	1,467	18,022	4	4
C1GDU6	pentatricopeptide repeat protein	Stress response	1,513	4,754	1	1
C0S3V9	hsp98	Stress response	1,599	4,623	2	3
C0SEQ1	disulfide-isomerase domain	Stress response	1,837	2,228	2	2
C1G7E0	thiol-specific antioxidant	Stress response	1,9	38,37	7	7
C1G0D4	catalase P	Stress response	2,242	160,198	20	20

Protein fate						
C0S533	Ubi4p	modification by ubiquitination, deubiquitination	5,675	2,061	1	1
C1GA81	aspartyl aminopeptidase	protein/peptide degradation	1,246	87,274	13	13
Binding function						
C0RXT4	ABC transporter ATP-binding ARB1	Nucleic acid binding	1,248	17,065	4	4
C1GAR9	molybdopterin synthase small subunit	nucleotide binding	1,555	1,525	1	1
C1GCT4	RNA binding	RNA binding	2,606	20,144	3	3
Cellular comunication/Signal transduction mechanism						
C0SJK7	CMGC GSK kinase	serine/threonine kinase	1,24	8,587	3	3
Unknown function						
C1G5K7	hypothetical protein PADG_02462	Unknown function	1,127	1,684	1	1
C1GH86	Uncharacterized protein	Unknown function	1,274	9,631	1	1
C1GIY9	hypothetical protein PADG_07225	Unknown function	1,297	10,732	3	3
C1GJP9	hypothetical protein PADG_07485	Unknown function	1,483	4,439	1	1
C0RY48	hypothetical protein PABG_00353	Unknown function	2,873	3,28	2	2
C1GM11	Hypothetical protein	Unknown function	4,829	30,554	7	7
C1G215	HAD superfamily hydrolase	Unknown function	1,23	33,277	4	4

Acession: number accession of Uniprot database. **Description**: description of protein names identified by mass spectrometry and classified in ProteinLynx. **Function**: proteins function identified in mass spectrometry and classified in Uniprot database. **Fold change**: level of protein expression. **Sum PEP Score**: socre of proteins identified in mass spectrometry. **Peptides**: total number of peptides identified in protein. **Unique peptides**: uniques peptides identified in each protein.

Supplementary table 3. Proteins up regulated in *Paracoccidioides brasiliensis*.

Accession	Description	Function	Fold change Pb18/Pa03	Sum PEP Score	Peptides	Unique Peptides
Energy						
C1G256	NADH-ubiquinone oxidoreductase subunit	accessory proteins of electron transport and membrane-associated energy conservation	1,521	18,088	4	4
C0SI66	NADH-ubiquinone oxidoreductase 23 kDa mitochondrial	accessory proteins of electron transport and membrane-associated energy conservation	1,431	12,964	2	2
C1GBX9	ubiquinol-cytochrome c reductase subunit 7	accessory proteins of electron transport and membrane-associated energy conservation	1,392	60,868	5	5
C0S212	NADH-ubiquinone oxidoreductase 24 kDa mitochondrial	accessory proteins of electron transport and membrane-associated energy conservation	1,372	61,666	10	10
C1GDK7	NADH-ubiquinone oxidoreductase kDa subunit	accessory proteins of electron transport and membrane-associated energy conservation	1,33	37,436	8	8
C1G350	NADH-ubiquinone oxidoreductase 299 kDa subunit	accessory proteins of electron transport and membrane-associated energy conservation	1,324	42,425	8	8
C0RY31	NADH-ubiquinone oxidoreductase	accessory proteins of electron transport and membrane-associated energy conservation	1,304	31,234	3	3
C1GIJ5	electron transfer flavo alpha subunit	accessory proteins of electron transport and membrane-associated energy conservation	1,245	134,304	13	13
C0SC77	cytochrome c	aerobic respiration	1,335	104,083	9	9
Q1XA94	cytochrome oxidase subunit 1 (mitochondrion)	aerobic respiration	1,451	6,682	2	2

C1GGS8	suaprga1	aerobic respiration	1,451	211,406	12	12
C0SE80	aldehyde dehydrogenase (NAD+)	alcohol fermenation	1,7	65,741	10	10
C1GCX3	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	Glycolysis and Gluconeogenesis	1,659	97,439	17	17
C1G9X3	enolase	Glycolysis and Gluconeogenesis	1,407	413,159	29	29
C1G547	pyruvate dehydrogenase (acetyl-transferring) E1 alpha subunit	Glycolysis and Gluconeogenesis	1,377	163,157	22	22
C1G056	pyruvate dehydrogenase E1 component subunit beta	Glycolysis and Gluconeogenesis	1,329	145,244	15	15
C1G2X0	isocitrate dehydrogenase	TCA cycle	1,623	71,153	14	14
Amino acid metabolism						
C1GG15	1,2-dihydroxy-3-keto-5-methylthiopentene dioxygenase	Biosynthesis of L-methionine	1,364	25,506	6	6
C0SI30	O-acetylhomoserine (thiol)-lyase	Amino acid metabolism; Lyase activity	2,127	208,131	18	18
C1GDE1	serine hydroxymethyltransferase	Serine conversion glycine	1,345	101,942	15	14
C1GBT4	1-pyrroline-5-carboxylate dehydrogenase	Alanine, aspartate and glutamate metabolism	1,3	68,921	10	10
C1G388	aspartate aminotransferase	Alanine, aspartate and glutamate metabolism	1,251	76,812	15	15
C1GC48	branched-chain amino acid aminotransferase	Isolecuine, leucine and valine metabolism	1,237	76,977	8	8
C1G8D6	methylthioadenosine phosphorylase	Cysteine and methionine metabolism	1,606	38,004	6	6

C1GBZ4	NADP-specific glutamate dehydrogenase	degradation of glutamine	1,454	82,662	12	12
C1GHR9	acyl- dehydrogenase	degradation of isoleucine	1,446	123,075	13	13
C1G9M8	fumarylacetoacetate hydrolase	degradation of phenylalanine	1,317	23,037	3	3
C1GAA7	cystathionine beta-synthase	metabolism of cysteine	1,295	10,813	2	2
C1G4U7	glycine cleavage system H	metabolism of glycine	1,748	28,116	6	6
C0SAC6	chorismate mutase	metabolism of the cysteine - aromatic group	1,401	43,874	10	10
C-compoud and carbohydrate metabolism						
C1FYE8	Uncharacterized protein	C-compoud and carbohydrate metabolism	1,615	106,018	13	13
C1G7G3	aldose 1-epimerase family	C-compoud and carbohydrate metabolism	1,423	30,29	6	6
C1GF19	lactonohydrolase	C-compoud and carbohydrate metabolism	1,423	50,28	7	7
C1FYN6	UDP-galactopyranose mutase	C-compoud and carbohydrate metabolism	1,4	69,891	15	15
C0RZG7	inositol-3-phosphate synthase	C-compoud and carbohydrate metabolism	1,287	17,424	4	4
C0S189	phosphomannomutase	C-compoud and carbohydrate metabolism	1,238	52,835	8	8
C0S624	mannitol-1-phosphate 5-dehydrogenase	C-compoud and carbohydrate metabolism	1,211	81,265	12	12

Lipid, fatty acid and isoprenoid metabolism						
C1G064	fatty acid synthase subunit alpha	Fatty acid metabolism	1,459	166,691	35	36
C1G065	fatty acid synthase beta subunit dehydratase	Fatty acid metabolism	1,245	247,39	44	44
A0A0A0HTN8	Acyl- thioesterase 1	Fatty acid metabolism	1,242	48,129	8	8
C1G347	diazepam-binding inhibitor (GABA receptor acyl- -binding)	lipid, fatty acid and isoprenoid metabolism	1,427	92,118	11	11
C1G054	enoyl-[acyl-carrier-] reductase 1	lipid, fatty acid and isoprenoid metabolism	1,191	48,466	10	10
C1G2D3	choline-phosphate cytidylyltransferase	phospholipid metabolism	1,127	34,51	5	5
C1GK47	terpene synthase metal binding domain	sesquiterpenes metabolism	3,695	1,064	1	1
Biosynthesis of vitamins, cofactors and prosthetic groups						
C1G167	riboflavin alpha subunit	biosynthesis of vitamins, cofactors and prosthetic groups	1,161	11,408	2	2
C1GFB1	nicotinate-nucleotide diphosphorylase (carboxylating)	biosynthesis of vitamins, cofactors, and prosthetic groups	1,365	32,998	6	6
C1G696	ferrochelatase	biosynthesis of vitamins, cofactors, and prosthetic groups	1,259	23,541	5	5
C1GMH1	biotin synthase	biosynthesis of vitamins, cofactors, and prosthetic groups	1,124	7,547	2	2
C1G7Q8	5-formyltetrahydrofolate cyclo-ligase	Metabolism of vitamins and cofactors	1,469	2,982	1	1
C1GKG3	minor allergen Alt a 7	Metabolismo of vitamins and cofactors	1,203	182,497	15	15

Nucleotide, nucleoside, nucleobase metabolism						
C0SES9	deoxyuridine 5 -triphosphate nucleotidohydrolase	Nucleotide/nucleoside/nucleobase metabolism	1,416	1,572	1	1
C0S8C0	nucleoside diphosphate kinase	nucleotide/nucleoside/nucleobase metabolism	1,195	84,061	10	10
C1GH49	GMP synthase	purin nucleotide/nucleoside/nucleobase metabolism	1,84	13,043	4	4
C0SFB3	inosine-5 -monophosphate dehydrogenase	purin nucleotide/nucleoside/nucleobase metabolism	1,263	202,712	23	23
C1G1P0	ribose-phosphate pyrophosphokinase II	purin nucleotide/nucleoside/nucleobase metabolism	1,253	85,109	12	12
C1FZ74	uracil phosphoribosyltransferase	pyrimidine nucleotide/nucleoside/nucleobase metabolism	1,951	17,816	4	4
C1GKJ6	deoxyuridine 5 -triphosphate nucleotidohydrolase	pyrimidine nucleotide/nucleoside/nucleobase metabolism	1,656	58,22	6	6
C1G1D6	rnapii degradation factor def1	RNA degradation	1,518	191,804	16	16
Phosphate metabolism						
C1GA89	inorganic pyrophosphatase	phosphate metabolism	1,338	106,022	19	19
Secondary metabolism						
C1GB98	metallo-beta-lactamase domain-containing [Paracoccidioides lutzii Pb01]	secondary metabolism	1,671	17,985	2	2
Protein synthesis						

C1GIL9	arginine-tRNA ligase	aminoacyl-tRNA-synthetases	1,293	33,005	5	5
C1GF12	glycyl-tRNA synthetase	aminoacyl-tRNA-synthetases	1,248	79,974	12	12
C1G741	PAB1 binding	3'-end processing	1,487	48,752	9	9
Transport						
C1GKW7	Uncharacterized protein	actin dependent transport	1,84	5,484	1	1
C0SE28	BET3 family	Golgi vesicle transport	1,499	9,016	2	2
C0SGI8	iron copper transporter ATX1	heavy metal ion transport	2,138	26,225	3	3
C1G2M5	nuclear export Yrb2	intracellular transport	1,631	16,77	5	5
A0A0A0HV97	Ran-specific GTPase-activating 1	intracellular transport	1,577	139,806	17	17
C1GF48	import inner membrane translocase subunit TIM8	protein transport	1,285	36,121	5	5
C1GKV7	V-type proton ATPase subunit E	proton transport	1,544	57,558	9	9
A0A0A0HRM2	V-type proton ATPase catalytic subunit A	proton transport	1,174	78,788	12	12
C0SC96	V-type proton ATPase subunit B	proton transport	1,162	75,388	13	13
C1G2H3	Poly(A)+ RNA export	RNA transport	1,519	28,306	4	4
C1GMA2	vacuolar-sorting snf7	vacuolar/lysosomal transport	1,852	17,185	3	3
C1GN13	class E vacuolar -sorting machinery HSE1	vacuolar/lysosomal transport	1,388	17,266	5	5
C1GFJ7	nipsnap family	Vesicular transport	1,759	129,995	17	17

C0S8P3	SNARE Ykt6	vesicular transport	1,422	4,227	1	1
C1G506	clathrin light chain	vesicular transport (Golgi network, etc.)	1,341	55,472	4	4
Mitochondria						
C1G9E6	mitochondrial import inner membrane translocase subunit tim10	Mitochondrial transport	1,529	15,674	3	3
C0SBT0	mitochondrial import inner membrane translocase subunit TIM13	Mitochondrial transport	1,631	34,719	3	3
C1G9U8	mitochondrial import MAS5	Mitochondrial transport	1,408	112,111	16	16
C1GZ28	mitochondrial import inner membrane translocase subunit tim9	Mitochondrial transport	1,349	42,094	5	5
C1G7Q5	Altered inheritance of mitochondria	Mitochondrion	1,703	28,992	4	4
C1GB03	Uncharacterized protein	mitochondrion	1,479	18,124	2	2
C1GDM0	isochorismatase domain-containing	mitochondrion	1,444	68,992	9	9
C1GLJ8	CBS and PB1 domain-containing	mitochondrion	1,292	14,082	4	4
C1G784	MICOS complex subunit MIC60	Mitochondrion	1,262	115,25	15	15
C1GIA6	mitochondrial co-chaperone	Stress response	1,553	52,592	6	6
Cell fate						
C1GH32	TCTP family	anti-apoptosis	1,902	55,353	6	6
C0SCA7	mitochondria fission 1	apoptotic mitochondrial changes	1,357	30,869	6	6

Protein fate						
C1G561	acyl-RNA-complex subunit	assembly of protein complexes	1,32	80,692	9	9
C1GF86	cytosolic nonspecific dipeptidase	cytoplasmic and nuclear protein degradation	1,547	69,814	15	15
C1G2E9	Uncharacterized protein	histone modification	4,588	4,183	1	1
C1GNV9	ubiquitin-conjugating enzyme E2 35	modification by ubiquitination, deubiquitination	1,294	18,713	4	4
C0RZ90	proteasome component PUP2	Proteasomal degradation	1,494	34,256	6	6
C1GMF6	proteasome subunit alpha type-4	Proteasomal degradation	1,493	36,433	6	6
C0RXV4	COP9 signalosome subunit 6	Proteasomal degradation	1,384	2,111	1	1
C1G8Z1	proteasome component	Proteasomal degradation	1,262	4,621	1	1
C1FZN1	26S proteasome regulatory subunit S5A	Protein degradation	1,275	7,923	1	1
C0S7H3	26S protease regulatory subunit 7	Protein degradation	1,256	46,382	12	12
C1G631	26S protease regulatory subunit 4	Protein degradation	1,25	61,263	11	11
A0A0A5IIF2	26S protease regulatory subunit 10B	Protein degradation	1,234	12,216	7	7
C1G1L9	prefoldin subunit 4	protein folding and stabilization	1,653	4,54	1	1
C0SH74	peptidyl-prolyl cis-trans isomerase D	protein folding and stabilization	1,419	189,954	15	15
C1GCX2	prefoldin subunit 6	protein folding and stabilization	1,366	4,072	2	2
C1GMJ8	T-complex 1 subunit epsilon	protein folding and stabilization	1,349	38,233	8	8
C0SHR4	T-complex 1 subunit zeta	protein folding and stabilization	1,348	45,931	9	9

C1G4I6	Hsc70 cochaperone	protein folding and stabilization	1,31	112,191	13	13
C0S1M3	peptidyl-prolyl cis-trans isomerase B	protein folding and stabilization	1,218	156,729	15	15
C1G0C2	cytochrome c heme lyase	protein modification	1,617	20,56	3	3
A0A0A0HR72	Sorting nexin-41	protein modification	1,363	22,944	4	4
C1G614	F-box domain-containing	protein targeting, sorting and translocation	1,56	27,014	4	4
C1FZI7	Vacuolar protein sorting/targeting protein 10	protein targeting, sorting and translocation	1,23	6,738	2	2
C1GA83	ATP-dependent Clp proteolytic subunit	protein/peptide degradation	1,566	5,932	2	2
C1GD93	chaperone dnaJ	unfolded protein response	1,33	46,589	8	8
C1GD03	chaperone dnaK	unfolded protein response	1,311	52,05	10	10
C1GAG0	Vid27 family	vacuolar protein degradation	1,509	8,813	2	2
C1GJM4	vacuolar aminopeptidase	vacuolar protein degradation	1,422	52,943	10	10
Cell type differentiation						
C1GHM7	septum formation Maf	budding, cell polarity and filament formation	1,68	7,746	2	2
C1G4Q1	Uncharacterized protein	budding, cell polarity and filament formation	1,36	6,514	2	2
C1GFC3	Uncharacterized protein	cell growth / morphogenesis	1,203	9,873	1	1
Cell cycle and DNA processing						

C1G050	SNF7 family Fti1 Did2	cell cycle	1,547	9,959	1	1
C1FYS5	microtubule associated EB1	cell cycle	1,229	26,443	7	7
C1GF70	histone H2A	chromosome condensation	1,246	39,544	5	5
C1GE52	Arp2 3 complex subunit	cytokinesis (cell division) /septum formation and hydrolysis	1,251	51,419	6	6
C1GF57	nucleosome assembly	DNA conformation modification	1,597	128,532	18	18
C1G029	chromatin remodeling complex subunit	DNA conformation modification (e.g. chromatin)	1,254	19,205	3	3
C1GIJ9	RSC complex subunit RSC8	DNA conformation modification (e.g. chromatin)	1,224	43,703	7	7
C1GFG5	histone H1	DNA recombination	8,427	10,052	3	3
C0S9Q8	prohibitin-1	DNA synthesis and replication	1,207	97,926	13	13
C1GIL6	chromosome segregation sudA	mitotic cell cycle	1,282	16,376	3	3
C1FYH3	nuclear segregation	mitotic cell cycle	1,248	145,209	21	21
C1FZC9	condensin complex component cnd2	mitotic cell cycle and cell cycle control	1,721	5,79	1	1
C1G6F8	cyclin-dependent kinase regulatory subunit	mitotic cell cycle and cell cycle control	1,721	11,042	3	3
C1GJS9	NSFL1 cofactor p47	mitotic cell cycle and cell cycle control	1,516	35,904	3	3
C1H998	E3 ubiquitin ligase complex SCF subunit sconC	mitotic cell cycle and cell cycle control	1,472	34,212	5	5
C0RYY3	nuclear movement nudC	nuclear migration	1,415	47,985	10	10

Transcription						
A0A0A0HY23	cleavage and polyadenylation specific factor 5	mRNA modification	1,523	41,683	7	7
C1GJ49	RNA binding domain-containing	mRNA processing (splicing, 5'-, 3'-end processing)	1,384	18,964	2	2
C1G9Q2	U6 snRNA-associated Sm LSm6	mRNA synthesis	1,573	10,942	2	2
C1GN72	U6 snRNA-associated Sm LSm5	mRNA synthesis	1,324	1,133	1	1
C1GAM1	mRNA binding post-transcriptional regulator	mRNA synthesis	1,217	152,791	11	11
C1FYF8	ATP-dependent RNA helicase MSS116	rRNA synthesis	1,436	85,692	12	12
C0SDT1	small nuclear ribonucleo Sm D2	splicing	1,541	8,492	1	1
C1GAL5	u5 snrnp complex	splicing	1,395	17,065	2	2
C0S6X4	Small nuclear ribonucleo Sm D3	splicing	1,329	13,704	3	3
C1GD67	peptidyl-prolyl cis trans isomerase	Transcription elongation	1,704	43,455	6	6
C1GGW3	transcriptional repressor	Transcription repressor	1,478	146,047	17	17
C1G2I3	cytosolic iron-sulfur assembly 1	transcriptional control	1,634	25,052	3	3
C1GBN3	RNA binding domain-containing	tRNA synthesis	1,236	59,896	8	8
Translation						
C1G9U4	60S acidic ribosomal P0	Ribosome biogenesis	1,328	129,731	12	12
C1GLR7	37S ribosomal mitochondrial	Ribosome biogenesis	1,289	12,149	3	3

C0RX98	40S ribosomal S15	Translation	1,534	54,071	3	3
A0A0A0HT80	60S ribosomal protein L13	Translation	1,538	131,789	15	15
C1G1P5	ribosomal S15	Translation	1,434	23,197	3	3
C1FZD4	eukaryotic translation initiation factor 3 subunit M	Translation	1,425	37,506	8	8
C1GJ53	ribosomal S16	Translation	1,299	6,508	2	2
C1G2C4	ribosomal L17	Translation	1,282	18,543	3	3
C0S6V8	ribosomal L15	Translation	1,267	3,606	1	1
C1G820	60S ribosomal L21-A	Translation	1,262	45,701	10	10
C1FZ53	translation initiation factor 4B	Translation	1,258	105,754	11	11
C0SJH5	40S ribosomal S28	Translation	1,253	51,072	9	9
C0SDL5	translation initiation factor SUI1	Translation	1,232	51,484	6	6
C0SFR2	eukaryotic translation initiation factor Y-chromosomal	Translation	1,227	14,747	4	4
C1GKL7	60S ribosomal L12	Translation	1,213	59,101	4	4
C0S1J8	60S ribosomal L36	Translation	1,203	28,054	4	4
C1G9X1	eukaryotic translation initiation factor 3 subunit J	Translation initiation	1,65	57,58	9	9
C0S2X7	eukaryotic translation initiation factor 3 subunit G	Translation initiation	1,462	43,044	7	7
C0S0U3	eukaryotic translation initiation factor 3 subunit I	Translation initiation	1,355	41,227	7	7

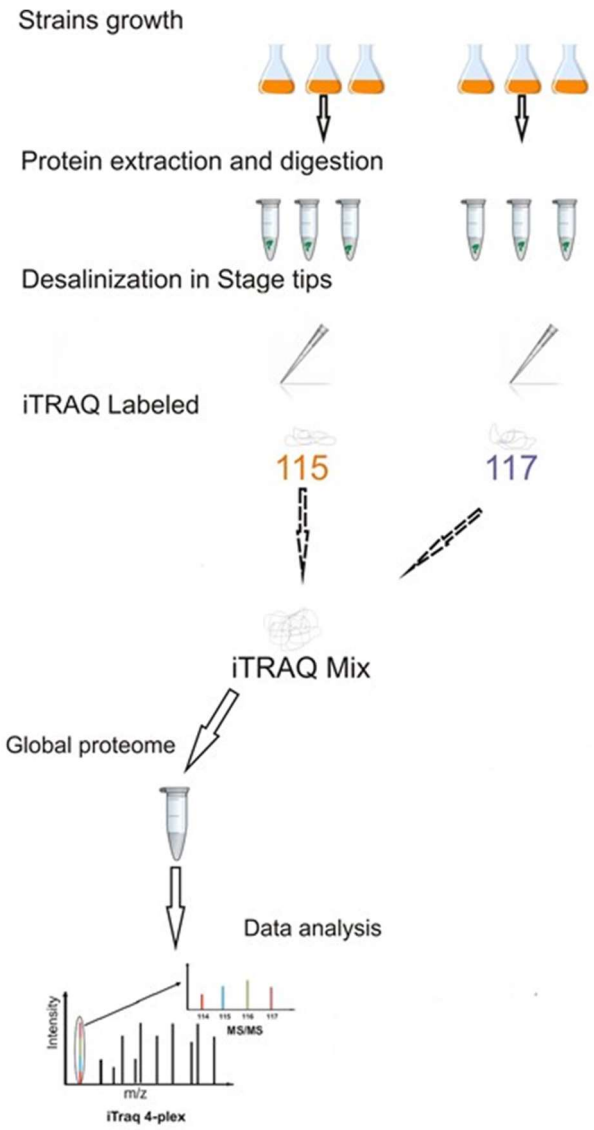
C1G4J9	eukaryotic translation initiation factor 3 subunit H	Translation initiation	1,35	66,143	8	8
C1G2D0	eukaryotic translation initiation factor 3 subunit F	Translation initiation	1,284	24,643	4	4
C1FZP0	eukaryotic translation initiation factor 3 subunit D	Translation initiation	1,243	36,08	9	9
Biogenesis of cellular components						
C1GG99	cell lysis cwl1	cell wall	1,698	36,726	6	6
C1GHW6	integral membrane	Cell wall	1,232	34,753	4	4
C1GJ13	actin binding	Cytoskeleton	1,361	79,156	12	12
C1G0N2	actin cytoskeleton	Cytoskeleton	1,36	157,192	15	15
C1G6J5	adenylyl cyclase-associated	Cytoskeleton/structural proteins	1,251	14,799	3	3
C1G177	spindle pole body component alp6	microtubule cytoskeleton	2,537	1,776	1	1
C1GBI0	nucleoporin NUP49 NSP49	nuclear membrane	1,483	44,856	5	5
C1GMJ7	heterochromatin HP1	nucleus	1,207	24,474	6	6
C1G5B2	metaphase-anaphase transition	organization of chromosome structure	1,306	17,603	2	2
C1G913	Acylphosphatase family	Regulation by modification	1,398	8,129	1	1
C1GIC8	vesicular-fusion sec17	vesicle fusion	1,215	60,267	7	7
Cellular comunication/signal transduction mechanism						

C1G6P0	CORD and CS domain-containing	cellular signalling	1,481	32,231	8	8
C1G8X9	FK506-binding	cellular signalling	1,449	59,237	11	11
C1G501	calmodulin	cellular signalling	1,406	50,687	4	4
C0RZB5	myosin regulatory light chain cdc4	cellular signalling	1,301	23,239	5	5
C1HBF2	Ras GTPase	G-protein mediated signal transduction	1,2	35,45	8	8
C1GJS0	Uncharacterized protein	signal transduction	1,543	31,926	5	5
C1GET2	serine threonine phosphatase 2A	signal transduction	1,301	9,2	1	1
Binding function						
C1G4K1	HMG box	DNA binding	1,323	83,745	12	12
A0A0A0HTT8	gag polymerase env	Nucleic acid binding	2,863	12,5	4	1
C0S2Z3	Uncharacterized protein	Nucleic acid binding	1,316	47,672	6	6
Cell rescue, defense and virulence						
C1GAU3	heat shock STI1	heat shock protein	1,294	265,291	28	28
C1G017	ribosome associated chaperone Zuotin	heat shock response	1,502	90,355	15	15
C1G3N5	thioredoxin reductase	oxidative stress response	1,384	70,999	7	7
C1GE18	thioredoxin	oxidative stress response	1,252	99,388	9	9

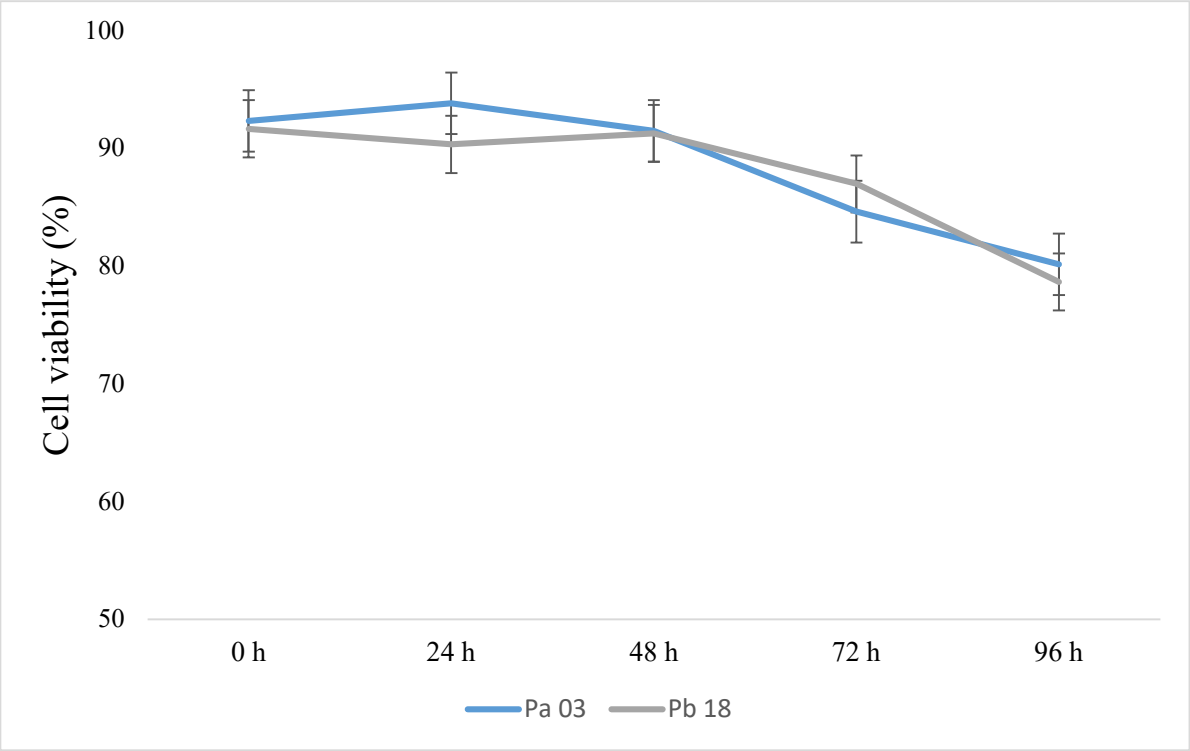
C1G4C7	cytosolic Fe-S cluster assembly factor NAR1	Stress response	1,42	2,086	1	1
C1GLX8	hsp60	Stress response	1,309	808,679	65	65
C0SEG3	glutathione-disulfide reductase	Stress response	1,286	20,257	10	10
C1G445	Aha1 domain family	Stress response	1,271	148,092	20	20
Interaction with environment						
C1FZS1	PKHD-type hydroxylase TPA1	homeostasis	1,296	6,019	2	2
C0RXC5	Uncharacterized protein	homeostasis of protons	1,73	10,384	3	3
Unknown function						
C1GN06	Uncharacterized protein	Unknown function	4,13	10,24	1	1
C1G1R0	Uncharacterized protein	Unknown function	3,313	28,849	2	2
C1GJX9	hypothetical protein PADG_07565	Unknown function	2,629	16,913	1	1
C1G1D4	Uncharacterized protein	Unknown function	2,393	13,546	2	2
C1G5D3	Uncharacterized protein	Unknown function	2,131	13,972	2	2
C1GFI5	hypothetical protein PABG_05692	Unknown function	1,618	18,203	4	4
C1GJH6	DUF1479 domain-containing	Unknown function	1,607	57,366	10	10

C1GMN0	emo am system B	Unknown function	1,588	8,916	2	2
C1G7G0	Uncharacterized protein	Unknown function	1,496	30,926	2	2
C1G1V2	Uncharacterized protein	Unknown function	1,483	7,313	1	1
C0S6A4	Uncharacterized protein	Unknown function	1,461	8,695	3	3
C1G2I1	DUF431 domain-containing	Unknown function	1,461	10,85	4	4
C0SGG4	hypothetical protein PABG_06591	Unknown function	1,437	23,276	6	6
C1G2H7	UPF0160 domain	Unknown function	1,361	26,174	6	6
C1G712	Uncharacterized protein	Unknown function	1,322	109,205	18	18
C0SIG2	Uncharacterized protein	Unknown function	1,272	20,065	5	5
A0A0A0HYI6	Uncharacterized protein	Unknown function	1,235	1,684	1	1

Acession: number accession of Uniprot database. **Description**: description of protein names identified by mass spectrometry and classified in ProteinLynx. **Function**: proteins function identified in mass spectrometry and classified in Uniprot database. **Fold change**: level of protein expression. **Sum PEP Score**: socre of proteins identified in mass spectrometry. **Peptides**: total number of peptides identified in protein. **Unique peptides**: uniques peptides identified in each protein.



Supplementary Figure 1. Workflow for proteome analysis. The fungi were grown at 36 ° C for 72 hours. Subsequently, protein extraction and tryptic digestion of the samples were performed, followed by desalination with stage tips (separation columns). After these processes were performed the iTRAQ labelling and combination of the samples for injection in the mass spectrometer, to obtain the global proteome. Data analysis was performed.



Supplementary Figure 2. Cell viability of *Paracoccidioides* species. Cell viability of *Pa* 03 and *Pb* 18 until 96 hours. Cell viability was performed with Trypan blue.

CAPÍTULO 3

Discussão

No capítulo 2, foi mostrado que na análise proteômica foram identificadas, após vários filtros e análise estatistica, 357 proteínas das espécies *P. brasiliensis* e *P. americana*. Posteriormente foi aplicado um "fold change" de 1,2 vezes, nas proteínas significativas, obtendo-se 240 proteínas para *P. brasiliensis* e 117 proteínas para *P. americana*. Em um estudo proteômico que analisou algumas espécies do gênero *Paracoccidioides*, foi utilizado espectrometria de massas, com o método "Label-free". Nesse estudo, foram identificadas, proteínas na fase de levedura de *Pb* 01 (P. *lutzii*), *Pb* 339 (*P. brasiliensis*), *Pb* 2 (PS2 - *P. americana*) e *Pb EPM* 83 (*P. restrepiensis*); a análise foi realizada com géis 2D. Inicialmente foram encontrados 714 pontos; posteriormente, 343 pontos foram selecionados e analisados considerando as quatro espécies. Após análises estatísticas foram selecionados 267 pontos, correspondendo a um total de 193 proteínas e isoformas ao comparar essas espécies (Pigosso, LL *et al*., 2013). Outro estudo com membros do gênero *Paracoccidioides*, utilizando espectrometria de massa, analisou e comparou o proteoma sob fontes de acetato, identificando 1160 proteínas em *P. lutzii*, 1211 em *Pb* 03 (*P. americana*), em *Pb* 339 (*P. brasiliensis*), 1280 proteínas e em PbEPM83 (*P. restrepiensis*), 1462 proteínas; após anaálises são 526, 442, 744 e 569, respectivamente, foram significativas e comparadas. Um estudo proteômico comparativo entre *Candida albicans* e *Candida glabrata*, utilizando os métodos iTRAQ, identificou 500 proteínas de ambos os fungos e após a aplicação de um "fold change" de 1.5, *C. albicans* apresentou 19 proteínas diferencialmente expressas e *C. glabrata* apresentou 12 proteínas (Prasad *et al*, 2010).

Um estudo com bactérias do grupo AOB (bactérias oxidantes de amônia) analisa o proteoma de três espécies, *Nitrosomonas europaea*, *Nitrosospira multiformis* e *Nitrosomonas ureae*. A análise proteômica mostrou algumas diferenças entre essas espécies, diferença está na 6-fosfofructoquinase dependente de pirofosfato, que é a enzima substituta da frutose-1,6-bisfosfatase no ciclo de Calvin, essa enzima está ausente em *N. multiformis* e *N. ureae*. Além disso, foram identificadas algumas diferenças no metabolismo do nitrogênio, crescimento celular e resposta ao estresse. Esses resultados mostraram que em espécies semelhantes, podem ocorrer muitas diferenças moleculares (Zorz *et al*., 2018).

O estudo proteômico de Pigosso *et al*. (2013), identificaram algumas diferenças no proteoma das espécies *Paracoccidioides*, os autores identificaram 59 proteínas

expressas preferencialmente em *P. lutzii* quando comparadas às demais espécies, foram encontradas muitas proteínas relacionadas à via da glicólise. Observou-se também que o *P. lutzii* apresenta alta expressão para o álcool desidrogenase, foram identificadas algumas proteínas envolvidas no metabolismo de aminoácidos e na via de desintoxicação, como a catalase peroxissômica, a Mn superóxido dismutase (SOD) e a glutationa redutase. No *Pb* 2 (*P. americana*) foram identificadas proteínas reguladas que participam da via da te pentose-fosfato. Assim como em *Pb* 01 (*P. lutzii*), o metabolismo de aminoácidos foi regulado, identificaram-se muitas enzimas da biossíntese do aminoácido de cadeia ramificada, com desintoxicação celular por ação, em *Pb* 2 (*P. americana*) a catalase peroxissômica, Superóxido dismutase de Cu e Zn e redutase de 2-alcenais. Em relação ao *Pb* 339 (*P. brasiliensis*), foi identificado neste estudo, proteínas envolvidas na β-oxidação. Observou-se que as proteínas envolvidas no transporte de elétrons mitocondriais eram mais abundantes no *Pb* 339 (*P. brasiliensis*) do que em outras espécies de *Paracoccidioides*. Para a desintoxicação celular foram identificados Mn SOD e peroxiredoxina mitocondrial. Sobre o PbEPM 83 (P. restrepiensis), foram identificadas 38 proteínas mais abundantes, relacionadas à proteína de choque térmico, glicólise e ciclo TCA (Pigosso *et al*., 2013). Este estudo demonstrou proteínas similares expressas nessas espécies de *Paracoccidioides*, porém foram verificadas algumas diferenças no perfil proteômico entre as espécies, mostrando algumas especificidades das espécies de *Paracoccidioides*, corroborando com o nosso estudo.

Em um estudo com as fases morfológicas de *Paracoccidioides brasiliensis* (*Pb* 18), foi verificado muitas modificações moleculares durante essas tranformações, de micélio para levedura, foi observado que na fase de micélio a fermentação estava mais ativada para produção de energia, enquanto as células de levedura usavam mais beta-oxidação aeróbica e ciclo TCA. Em relação ao resgate e defesa celular, foi demonstrado um aumento do citocromo c nas células de levedura, já alguns fatores de virulência, como as HSPs, foram regulados na etapa de transição, micélio para levedura, e o grupo SODs foi regulado na fase de micélio, mostrando que os fungos apresentam grandes alterações moleculares (Araújo *et al*., 2019). No estudo do proteoma comparativo entre duas raças de *Fusarium oxysporum*, foram identificadas muitas proteínas relacionadas ao metabolismo de carboidratos, metabolismo de aminoácidos e metabolismo de íons, no R2, que é a raça mais patogênica, quando comparado ao R1 (Li *et al*., 2015).

Um estudo com 20 isolados de *Fusarium oxysporum lycopersici* (FOL), um fungo causador de doenças em tomateiro, revelou que entre esses isolados há muitas diferenças moleculares, morfológicas e de virulência. No estudo observou-se que todos os isolados produziram conídios, mas com tamanhos diferentes. A análise proteômica mostrou ainda que os fatores de virulência são diferentes entre esses isolados, no isolado FOL-8, foram identificadas 14 proteínas reguladas, que estão relacionadas à virulência, quando comparadas ao FOL-20, considerado o menos virulento. As proteínas identificadas estão relacionadas à patogenicidade, esporulação e maior taxa de penetração no tecido radicular do tomate. Este estudo mostra as diferenças de resposta entre os isolados de *F. oxysporum* (Manikandan *et al*., 2018).

Em nossa análise proteômica, foram identificadas algumas proteínas relacionadas à atividade mitocondrial, incluindo algumas com funções nas membranas mitocondriais, como o MIC60 e a herança alterada das mitocôndrias. Observou-se algumas diferenças entre *Pa* 03 e *Pb* 18. No *Pb* 18, encontramos muitas proteínas relacionadas ao transporte mitocondrial, como o Tim 8, Tim 9, Tim 10 e Tim 13, em comparação ao *Pa* 03, essas proteínas, relacionadas ao transporte mitocondrial, foram menos expressas no *Pa* 03. No proteoma em geral, o *Pb* 18 mostrou mais proteínas relacionadas às mitocôndrias reguladas quando comparadas ao *Pa* 03. No estudo do proteoma global entre as espécies de *Paracoccidioides* foi observado uma maior atividade da mitocôndria no *Pb* 18, comparado ao *Pa* 03. No estudo com algumas espécies de *Paracoccidioides*, foram identificadas algumas proteínas relacionadas às mitocôndrias, especificamente no *Pb* 339 (*P. brasiliensis*), proteínas envolvidas no transporte de elétrons mitocondriais, quando comparadas com outras espécies de *Paracoccidioides*, essas proteínas foram mais abundantes. Em *Pb* 339 (Pigosso *et al*., 2013). Corroborando com nossos resultados, o *Pb* 18 apresentou uma importante proteína relacionada à função mitocondrial, sendo que o *Pb* 18 e o *Pb* 339 são classificados como espécies de *P. brasiliensis*.

Na análise proteômica, em nosso estudo, foram identificadas muitas proteínas relacionadas à energia. Observou-se algumas diferenças entre duas espécies, *P. americana* apresentou mais enzimas relacionadas à produção de etanol, como piruvato descarboxilase, aldeído desidrogenase, álcool desidrogenase e glutationa desidrogenase, foram identificadas enzimas ralizadas com glicólise e gliconeogênese, como triosefosfato isomerase, glicose-6- fosfato isomerase, futose-1,6-bifosfatase e fosfoenolpiruvato

carboxiquinase e ciclo TCA com as enzimas isocitrato desidrogenase (NADP), citrato mitocondrial, dependente de NAD do malato, 2 metilcitrato mitocondrial e fumarato redutase. Já o *P. brasiliensis*, mostrou enzimas relacionadas ao transporte de elétrons e à conservação de energia associada à membrana, como NADH-ubiquinona oxidoredutase (muitas subunidades), transferência eletrônica de flavo alfa, foram identificadas algumas proteínas da glicólise e gliconeogênese, como a enolase, 2, Mutase fosfoglicerato independente de 3-bifosfoglicerato, e mostrou algumas enzimas da respiração aeróbica, essas enzimas foram mostradas no mapa de calor. Um estudo com fases de *P. brasiliensis*, mostrou que, na fase de micélio, o fungo produzia mais etanol do que em relação ao estágio de levedura e transição (Araujo et al., 2019), e em *P. lutzii*, foi mostrado que na fase de levedura há mais etanol produção (Felipe, 2005; Rezende et al., 2011). Uma proteômica comparativa entre espécies de *Paracoccidioides*, demonstrou que *P. lutzii* (*Pb* 01) produziu um alto nível de etanol do que em comparação com outras espécies de *Paracoccidioides* (Pigosso et al., 2013).

O estudo de proteoma comparativo entre *C. albicans* e *C. glabrata* mostrou algumas diferenças entre esses fungos, sendo algumas proteínas identificadas em apenas um dos dois fungos. *C. albicans* apresentou maior expressão, como 3-fosfoglicerato quinase (Pgk1), piruvato descarboxilase, álcool imunogênico desidrogenase. Observou-se que as proteínas relacionadas ao metabolismo da glicose, incluindo a via gluconegonegenis, apresentam maior expressão em *C. albicans* quando comparadas com *C. glabrata*. Em *C. glabrata* foram identificadas proteínas que incluíam Enolase, frutose-bifosfatase, isocitrato desidrogenase mitocondrial específico da NADP e isocitrato desidrogenase específico do NADP citosólico (Prasad *et al*., 2010).

Foram identificadas na análise proteômica muitas proteínas relacionadas ao resgate, defesa e virulência das células. Principalmente proteínas envolvidas na desintoxicação. Essa classe de proteínas possui grande importância para a sobrevivência do fungo em ambientes hostis; as duas espécies de *Paracoccidioides* apresentaram um grande número dessas proteínas; apresentando algumas diferenças de expressão. *P. americana* (*Pa* 03) apresentou maior expressão para pCatalase, Superóxido dismutase (SOD) e HSP 90, e *P. brasiliensis* (*Pb* 18) apresentou maior expressão para citocromo c, citocromo c mitocondrial e tioredoxina. Um estudo com as fases *Paracoccidiodies brasiliensis*, revelou que a fase micelial demonstrou mais níveis de tióis do que a fase de levedura e a fase de transição (Araujo *et al*., 2019). Em outro estudo com

Paracoccidioides, foram identificadas na espécie *Pb* 2 (membro de *P. americana*) superóxido dismutase de Cu e Zn (SOD), catalase peroxissômica e 2 alcenal-redutase, mais expressas do que em outras espécies. No *Pb* 339 (membro de *P. brasiliensis*) foi identificado Mn superóxido dismutase (SOD) e peroxiredoxina mitocondrial PRX1, mais regulado, em comparação as com outras espécies. Ainda foi identificado algumas diferenças dessas proteínas de desintoxicação em outras duas espécies, *Pb* 01 (membro de *P. lutzii*) e *Pb* EPM83 (membro de *P. restrepiensis*), mostrando as diferenças moleculares com as espécies de *Paracoccidioides* (Pigosso, LL *et al*., 2013). Em estudo com *C. albicans* e *C. glabrata*, proteoma comparativo, observou-se que *C. albicans* apresentou o HSP 60 mais abundante, mas *C. glabrata* apresentou o HSP90 superexpresso, essa é uma indicação da resistência a medicamentos desse fungo, o que não acontece com *C. albicans* (Prasad *et al*., 2010).

Em um estudo filogenético com as espécies de *Paracoccidioides*, foram realizadas análises morfológicas, moleculares e genéticas para comparação dos fungos. Na análise morfológica, identificou-se diferenças na forma de levedura, as espécies apresentaram variação na área celular quando estão em forma de levedura, também foi compararado o número de gemas produzidas pelos fungos, observando uma heterogeneidade significativa entre as espécies de *Paracoccidioides*, que podem indicar diferenciação fenotípica nessas espécies, em ambos os casos não foram encontradas diferenças significativas entre variações intraespecíficas (nos isolados). Também foi realizada a análise molecular e genética, que utilizou algumas análises mitocondriais e nucleares, utilizando alguns marcadores, foi observado que algumas espécies são muito próximas, consideradas irmãs, como *P. restrepiensis* e *P. venezuelensis*, por outro lado, *P. americana* mostra muitas diferenças em comparação com as outras espécies, embora essas espécies tenham ancestralidade comum, esses resultados mostram as diferenças moleculares, genéticas e morfológicas entre as espécies de *Paracoccidioides* e propuseram a nova nomenclatura (Turissini, *et al*., 2017).

Conclusão

Análises proteômicas são ferramentas importantes e fornecem muitas informações sobre a biologia e repostas moleculares dos organismos. Em nosso estudo, observamos muitas informações sobre duas espécies de *Paracoccidioides*, mostrando que *Paracoccidioides americana* (*Pa* 03) e *Paracoccidioides brasiliensis* (*Pb* 18) compartilham muitas vias metabólicas, respostas moleculares e processos biológicos. Por outro lado, identificamos algumas diferenças entre as duas espécies de *Paracoccidioides*, como a seguir: diferenças nas vias preferenciais de obtenção de energia, em que *Pa* 03 mostra muitas enzimas relacionadas à gliconeogênese, ciclo do TCA, ciclo do glioxilato e fermentação, mais expressas em comparação à *P. brasiliensis*. Por outro lado, *P. brasiliensis* acumula mais proteínas relacionadas à glicólise e principalmente, respiração aeróbica, como as proteínas do transporte de elétrons e conservação de energia associada à membrana. Consequentemente foi observado em *P. brasiliensis* uma atividade mitocôndria mais alta que *P. americana.*. Para as funções de defesa e detoxificação *P. americana* apresentou um maior acúmulo de SOD e o *P. brasiliensis* apresentou maior expressão de Tioredoxina.

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CAPÍTULO 4

Produções científicas e colaborações

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ORIGINAL RESEARCH
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Metabolic Peculiarities of *Paracoccidioides brasiliensis* Dimorphism as Demonstrated by iTRAQ Labeling Proteomics

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Paracoccidioidomycosis (PCM), a systemic mycosis with a high incidence in Latin America, is caused by thermodimorphic fungi of the *Paracoccidioides* genus. The contact with host occurs by the inhalation of conidia or mycelial propagules which once reaching the pulmonary alveoli differentiate into yeast cells. This transition process is vital in the pathogenesis of PCM allowing the fungus survival in the host. Thus, the present work performed a comparative proteome analysis of mycelia, mycelia-to-yeast transition, and yeast cells of *Paracoccidioides brasiliensis*. For that, tryptic peptides were labeled with iTRAQ and identified by LC–MS/MS and computational data analysis, which allowed the identification of 312 proteins differentially expressed in different morphological stages. Data showed that *P. brasiliensis* yeast cells preferentially employ aerobic beta-oxidation and the tricarboxylic acid cycle accompanied by oxidative phosphorylation for ATP production, in comparison to mycelia and the transition from mycelia-to-yeast cells. Furthermore, yeast cells show a metabolic reprogramming in amino acid metabolism and in the induction of virulence determinants and heat shock proteins allowing adaptation to environmental conditions during the increase of the temperature. In opposite of that, the alcoholic fermentation found to *P. lutzii*, at least under laboratory conditions, is strongly favored in mycelium compared to yeast cells. Thereby, the data strongly support substantial metabolic differences among members of the *Paracoccidioides* complex, when comparing the saprobiotic mycelia and the yeast parasitic phases.

Keywords: iTRAQ, proteomics, neglected disease, paracoccidioidomycosis, dimorphism

• Artigo em preparação:

iTRAQ-based proteomic and phosphoproteomic analysis of *Paracoccidioides brasiliensis* in response to hypoxia.

Lucas Nojosa Oliveira[a,b], Danielle Silva Araújo[a,b], Igor Godinho Portis[a,c]; Agenor de Castro Moreira dos Santos Júnior[b,d]; Alexandre Siqueira Guedes Coelho[e]; Patrícia de Sousa Lima[f], Wagner Fontes[d]; Carlos André Ornelas Ricart[d]; Célia Maria de Almeida Soares[a,*]

• Artigo submetido: Copper overload in *Paracoccidioides lutzii* results in the accumulation of ergosterol and melanin

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• Phosphoproteomic comparative between *Paracoccidioides species* (artigo em preparação).

Outras contribuições:

• Protein profile of *Paracoccidioides brasiliensis* in response to chalcona (Lívia do Carmo).

• *Paracoccidioides* proteomic analysis reveals mode of action of D-123 (Lívia do Carmo).

• Phosphoproteomic analysis in *Paracoccidioides* phases (Danielle Silva Araújo).