
**PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS
(MICROBIOLOGIA APLICADA)**

**BIOCONVERSÃO MEDIANTE FERMENTAÇÃO EM ESTADO SÓLIDO
DE BAGAÇO E FOLHAS DE MANDIOCA POR FUNGOS VISANDO
MELHORIA DA QUALIDADE NUTRICIONAL**

EDUARDO MARIN MORALES

Tese apresentada ao Instituto de Biociências do Campus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutor em Ciências Biológicas – Microbiologia Aplicada.

**Rio Claro - SP
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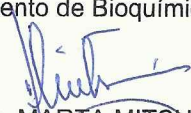
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Dedico este trabalho à minha
querida família: meus pais
Marin e Norberto, minha irmã
Mariana e, em especial, minha
querida esposa Dânia.

“A razão é o passo, o aumento
da ciência o caminho, e o
benefício da humanidade é o
fim.”

— Thomas Hobbes

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BIOGRAFIA

Eduardo Marin Morales, nascido em Limeira, interior do Estado de São Paulo, realizou sua graduação em Engenharia de Alimentos na Universidade Metodista de Piracicaba (UNIMEP), na cidade de Santa Bárbara D'Oeste, entre os anos de 2001 a 2005. Durante o 3º semestre de sua graduação iniciou estágio na área de Microbiologia, no Laboratório de Microbiologia III do Departamento de Bioquímica e Microbiologia do Instituto de Biociências da UNESP, Campus Rio Claro, sob a supervisão da Profa. Dra. Dejanira de Franceschi de Angelis, onde adquiriu conhecimento e grande afinidade pela área de microbiologia das fermentações. Após 1 ano e meio de estágio no referido laboratório, iniciou outro estágio na área de indústria de alimentos, junto a fábrica de doces RICLAN S.A., onde, posteriormente, veio a trabalhar profissionalmente. Após 4 anos, transferiu-se para a Ad'oro S.A., empresa especializada em abate e comercialização de frangos, onde permaneceu por mais 2 anos antes de ingressar na vida acadêmica. No ano de 2009, iniciou seu mestrado sob orientação da Profa. Dra. Dejanira de Franceschi de Angelis, a qual permaneceu como sua orientadora até os dias atuais. Seu mestrado foi realizado na área de fermentação em estado sólido de resíduos agroindustriais com fungo comestível, para melhoria do conteúdo proteico e da biodisponibilidade das proteínas, visando a utilização e melhor gestão de resíduos com potencial alimentício, área esta que continua se dedicando, como pode ser visto neste trabalho apresentado para a obtenção do seu título de doutor.

RESUMO

O Brasil destaca-se como um dos principais produtores e processadores de mandioca (*Manihot esculenta*) do mundo e, portanto, gera grande quantidade de produtos considerados resíduos mas, com potencial de utilização. Folhas e bagaço de mandioca são os dois principais co-produtos gerados, e juntos apresentam quantidade elevada de proteínas e carboidratos, com qualidade propícia para o processo de fermentação em estado sólido (FES). A utilização de subprodutos da agroindústria constitui uma oportunidade para aumentar e diversificar a produção de alimentos uma vez que eles apresentam composição química de carboidratos, proteínas, gorduras e minerais compatíveis com outros produtos de tradição alimentar. Além de melhorar o aproveitamento do esforço agrícola de produção, pode aumentar o rendimento financeiro e diminuir a geração de resíduos. O bagaço de mandioca é o principal subproduto resultante da extração do amido de seu tubérculo, gerando enorme quantidade de resíduos com cerca de 90 % de carboidratos com potencial de aproveitamento. As folhas de mandioca, também considerados resíduos, apresentam cerca de 40 % de proteína bruta. Estas características tornam esses resíduos ideais para serem utilizados como substrato no processo de FES. A fermentação predispõe a melhorar a biodisponibilidade de aminoácidos (incluindo os essenciais) e os ácidos graxos. Com o objetivo de verificar a influência de FES em substratos contendo folhas e bagaço de mandioca, foram realizadas avaliações quanto aos teores de aminoácidos das proteínas, ácidos graxos e cianeto em processos de FES promovidos pela ação de *Lentinula edodes*, *Pleurotus ostreatus*, *Agaricus blazei* e *Rhizopus oligosporus*. A qualidade da proteína foi determinada pelos métodos de Protein Digestibility Corrected Amino Acid Score (PDCAAS) e Relative Nutritional Value (RNV) que medem a capacidade da proteínas em fornecer aminoácidos essenciais (AAE). O conteúdo de cianeto e de carboidratos totais foi quantificado por método colorimétrico. O perfil de aminoácidos foi obtido utilizando a cromatografia líquida (HPLC) e os ácidos graxos por cromatografia gasosa (GC). Os resultados obtidos em testes realizados com o *Agaricus blazei* não apresentaram dados substanciais, mostrando-se assim, uma alternativa pouco eficiente, além de difícil controle e aplicação em um processo de FES neste substrato. Todos os experimentos de FES realizados com *Rhizopus oligosporus*, *Pleurotus ostreatus* e *Lentinula edodes* mostraram significativa diminuição na concentração de cianeto, atingindo níveis seguros de consumo após a fermentação. Os experimentos realizados com *Rhizopus oligosporus* indicam melhoras quanto a quantidade de histidina, isoleucina e leucina, com condições de fornecer mais do que 60% da necessidade diária para crianças de 2-5 anos de idade. Quantidades elevadas de ácidos graxos insaturados (AGI) foram registradas para o ácido cis-13,16-docosadienóico (22:2), ácido α -linolênico (18:3^{9,12,15}) e ácido oleico (18:1). Resultados obtidos para PDCAAS foram baixos em comparação com a caseína, contudo, os resultados do RNV revelaram grande diferença para a qualidade da proteína após a fermentação. O processo de FES realizado com *Pleurotus ostreatus* evidenciaram a formação de produto final com

melhorias significativas de AAE (valina, isoleucina, leucina e metionina) e AGI (ácido oleico - 18:1; ácido linoleico - 18:2^{9,12}; e γ -linolénico - 18: 3^{6,9,12}). Embora o PDCAAS e RNV tenham revelado valores abaixo aos encontrados na caseína, os resultados indicaram melhoria na biodisponibilidade da proteína microbiologicamente modificada. As fermentações efetuadas com *Lentinula edodes* registraram razoáveis quantidades de histidina, isoleucina, valina, metionina (com cisteína) e fenilalanina (com tirosina), quando comparado com os requisitos de crianças de 2-5 anos de idade. Alguns ácidos graxos insaturados foram estimados em quantidades consideráveis, tais como o ácido linoleico (18: 2^{9,12}); cis-13,16-docosadienóico ácido (22: 2); cis-11-eicosenóico de ácido (20: 1) e ácido α -linolénico (18: 3^{9,12,15}). Os dados obtidos permitiram concluir que houveram aumentos significativos de biodisponibilidade dos nutrientes (aminoácidos, ácidos graxos e minerais) e que a utilização de subprodutos ainda subutilizados da agroindústria poderão tornar-se fontes alternativas de alimentos não convencionais e que aumentariam a quantidade de nutrientes essenciais.

Palavras-chave: *Manihot esculenta*; *Rhizopus oligosporus*; *Pleurotus ostreatus*; *Lentinula edodes*; biodisponibilidade proteica.

ABSTRACT

Brazil stands out as one of the leading producers and processors of cassava (*Manihot esculenta*) in the world and therefore generates large quantity of products considered as waste but with potential use. Cassava leaves and bagasse are the two main byproducts generated, and together they present a high amount of proteins and carbohydrates, with quality suitable for the process of solid-state fermentation (SSF). The use of agro-industry by-products is an opportunity to increase and diversify food production since they present chemical composition of carbohydrates, proteins, fats and minerals compatible with other food tradition products. Besides improving the use of agricultural production effort may increase the financial income and reduce the generation of waste. Cassava bagasse is the main by-product of starch extraction from its tuber, generating huge amount of waste with about 90% of carbohydrate, presenting exploitation potential. Cassava leaves also regarded as waste, has about 40% crude protein. These features make these by-products suitable for use as the substrate in the SSF process. Fermentation predisposes to improve the bioavailability of amino acids (including the essential) and fatty acids. In order to check the influence of SSF on substrates containing leaves and cassava bagasse, evaluations were carried out as the amino acid content of proteins, fatty acids and cyanide in SSF processes promoted by *Lentinula edodes*, *Pleurotus ostreatus*, *Agaricus blazei* and *Rhizopus oligosporus*. The quality of the protein was determined by the methods of Protein Digestibility Corrected Amino Acid Score (PDCAAS) and Relative Nutritional Value (RNV) that measure the ability of proteins to provide essential amino acids (EAA). Cyanide content and total carbohydrates were measured by colorimetric method. The amino acid profile was obtained using liquid chromatography (HPLC) and fatty acids by gas chromatography (GC). The results obtained in tests with *A. blazei* showed no substantial data, being thus a less efficient alternative, besides presenting difficult to control and application in the process of SSF in this substrate. All FES experiments with *R. oligosporus*, *P. ostreatus* and *L. edodes* showed significant decrease in cyanide concentration reaching safe levels of consumption after fermentation. The experiments with *R. oligosporus* indicated improvements about the amount of histidine, isoleucine and leucine, with conditions to provide more than 60% of the daily requirement for children 2-5 years old. High amounts of unsaturated fatty acids (UFA) were registered for the *cis*-13,16-docosadienoic acid (22: 2), α -linolenic acid (18: 3^{9,12,15}) and oleic acid (18: 1). Results for PDCAAS were low in comparison with casein, however, the results showed a

RNV higher to the quality of protein before fermentation. The SSF process performed with *P. ostreatus* showed a final product with significant improvements of AAE (valine, isoleucine, leucine and methionine) and unsaturated fatty acids (oleic acid - 18:1, linoleic acid - 18:2^{9,12}, and γ -linolenic - 18:3^{6,9,12}). Though the PDCAAS and RNV revealed values below those found in casein, the results show improvement in the bioavailability of microbiologically modified protein. The fermentations carried out with *L. edodes* register reasonable amounts of histidine, isoleucine, valine, methionine (with cysteine) and phenylalanine (with tyrosine), when compared with the requirements of children 2-5 years old. Some unsaturated fatty acids have been estimated at considerable quantities, such as linoleic acid (18:2^{9,12}); cis-13,16-docosadienoic acid (22:2); cis-11-eicosenoic acid (20:1) and α -linolenic acid (18:3^{9,12,15}). The data obtained showed there were significant increases in bioavailability of nutrients (amino acids, fatty acids and minerals) and the use of by-products, that are still underutilized in agro-industry may become alternative sources of unconventional food and increase the amount of essential nutrients.

Keywords: *Manihot esculenta*; *Rhizopus oligosporus*; *Pleurotus ostreatus*; *Lentinula edodes*; protein bioavailability.

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LISTA DE ABREVIATURAS E SIGLAS

AA	Aminoácido/Amino acid
AAE/EAA	Aminoácido Essencial/ Essential Amino Acid
AGI/UFA	Ácidos Graxos Insaturados/Unsaturated Fatty Acids
AGM/MUFA	Ácidos Graxos Monoinsaturados/ Monounsaturated Fatty Acids
AGP/PUFA	Ácidos Graxos Poli-insaturados/ Polyunsaturated Fatty Acids
AGS/SFA	Ácidos Graxos Saturados/ Saturated Fatty Acids
Ala	Alanina/Alanine
ANOVA	Análise de Variância/ Analyzes of Variance
BCAA	Branched-Chain Amino Acids
BM/CB	Bagaço de Mandioca/Cassava Bagasse
Ca	Cálcio
Cr	Cromo
Cu	Cobre
Cys	Cisteína/Cysteine
DIAAS	Digestible Indispensable Amino Acid Score
F	Flúor
FAME	Fatt Acids Methyl Esters
FAO	Food and Agriculture Organization
Fe	Ferro
FES/SSF	Fermentação em Estado Sólido/Solid-State Fermentation
FM/CL	Folha de Mandioca/Cassava Leaves
g	Grama
GC	Gas Chromatography
GLA	Ácido Gama-Linoleico/Gamma-Linoleic Acid
Gln	Glutamina/Glutamine
H ₂ O	Água
H ₃ PO ₄	Ácido Fosfórico
HCl	Ácido Clorídrico
HCN	Cianeto de Hidrogênio
His	Histidina
HPLC	High Performance Liquid Chromatography
I	Iodo
Ile	Isoleucina/Isoleucine
ITS	Internal Transcribed Spacer
K	Potássio
Kcal	Quilo Caloria
Kg	Quilograma
LA	Ácido Linoleico/Linoleic Acid
Leu	Leucina/Leucine
Lys	Lisina/Lysine
Met	Metionina/Methionine
mg	Miligrama

Mg	Magnésio
mL	Mililitro
Na	Sódio
NaCl	Cloreto de Sódio
NaOH	Hidróxido de Sódio
NEAA	Aminoácidos Não Essenciais/Non-essential Amino Acids
OMS/WHO	Organização Mundial da Saúde/World Health Organization
PDCAAS	Protein Digestibility-Corrected Amino Acid Score
pH	Potencial Hidrogeniônico
Phe	Fenilalanina/Phenylalanine
PP	Polipropileno/Polypropylene
ppm	Parte Por Milhão
S	Enxofre
SAA	Amino Ácidos Sulfurados/Sulfur Amino Acids
Se	Selênio
TCA	Ácido Tricloroacético/Trichloroacetic Acid
Thr	Treonina/Threonine
Trp	Triptofano/Tryptophane
Tyr	Tirosina/Tyrosine
Val	Valina/Valine
VNR/ RNV	Valor Nutricional Relativo/Relative Nutricional Value
Zn	Zinco

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1. INTRODUÇÃO

O principal cultivar agrícola cianogênico é a mandioca (*Manihot esculenta* Crantz), a qual representa a sexta cultura alimentar mais importante do mundo. Este cultivar caracteriza-se pela sua fácil adaptação, principalmente em regiões tropicais. Sua importância está vinculada ao fato de ser a base alimentar de cerca de 700 milhões de pessoas. Apesar de sua intensa utilização como alimento básico, sua principal aplicação industrial ocorre pela produção de farinha e fécula (amido), gerando enorme quantidade de resíduo. Este resíduo, predominantemente sólido é gerado desde a colheita até o final do beneficiamento destacando-se o bagaço e as folhas. Quanto ao bagaço, o processamento de cerca de 300 toneladas de tubérculos gera um montante de aproximadamente 280 toneladas do resíduo (hidratado pelo processo de extração), que não apresenta interesse para a indústria. As folhas da mandioca compõem, após a colheita, um residual de cerca de 2,25 toneladas por hectare. O Brasil, sendo o quarto maior produtor de mandioca do mundo, gera enorme quantidade de resíduos, seja como bagaço, seja como folhas e seu aproveitamento para fins alimentares, embora ainda sem significância, poderia ser aperfeiçoado para aumentar a quantidade de alimentos produzidos em conjunto com a diminuição de resíduos gerados.

Para a utilização de resíduos ou sub-produtos, a aplicação de micro-organismos tem-se mostrado uma via de alto interesse comercial. A fermentação em estado sólido (FES) vem se destacando quanto à eficiência na bioconversão de resíduos ou co-produtos agroindustriais voltados para a alimentação humana, com benefícios na alimentação, refletidos na saúde. Os fatores estão relacionados ao baixo custo de processo e instalações para o desenvolvimento da FES e facilidade da aplicação de micro-organismos (principalmente os fungos filamentosos), na modificação do substrato e também das características proporcionadas pelo processo como, melhora da digestibilidade, melhor valor nutricional, detoxificação, entre outros.

O cultivo e consumo de micro-organismos é recente na dieta brasileira, contudo, o seu uso pode melhorar os aspectos nutricionais, sensoriais e funcionais após a bioconversão de resíduos agrícolas, tornando-os possíveis fontes de alimentos. Esta afirmação fica evidenciada pelo número crescente de pesquisas e processos que se utilizam de micro-organismos, principalmente fungos na indústria química e de alimentos como obtenção de xaropes de glicose e maltose, alimentos como o tempeh e o koji, além dos conhecidos cogumelos (*Pleurotus ostreatus*, conhecido como shimeji; *Lentinula edodes*, conhecido como shiitake; *Agaricus bisporus*, conhecido como cogumelo paris, etc.).

A proteína microbiana apresenta considerável digestibilidade, podendo ser utilizada como fonte adicional de proteína a ser incorporada na dieta. O consumo de proteínas está relacionado às necessidades de ingestão de aminoácidos essenciais (AAE). Esses nutrientes exercem papel determinante na eficiência do metabolismo do organismo, estando diretamente ligados à construção de proteínas, regulação de processos celulares, precursores de moléculas, entre outros. São considerados aminoácidos essenciais pois, não podem ser sintetizados pelos organismos e, conseqüentemente, devem ser supridos mediante dieta adequada. Quanto à avaliação da qualidade biológica das proteínas dos alimentos, métodos como os cálculos de aminoácidos corrigidos pela digestibilidade (PDCAAS), o de aminoácidos indispensáveis

digeríveis (DIAAS) e o valor nutricional relativo (VNR) foram desenvolvidos e reconhecidos como importantes parâmetros de avaliação. Estes parâmetros permitem visualizar a real biodisponibilidade da proteína de um determinado alimento.

Tratando-se de alimentação, a ingestão de ácidos graxos insaturados deve ser considerada pois, são essenciais dado ao fato de não serem sintetizados pelo organismo, necessitando assim, terem sua demanda metabólica suprida pela ingestão de alimentos. Os principais grupos recomendados nas dietas alimentares são os ácidos graxos monoinsaturados e poli-insaturados (que apresentam duplas ligações na cadeia carbônica), que apresentam características antioxidantes e auxiliam na prevenção de doenças cardiovasculares.

Dessa forma, o emprego de tecnologias que possam promover uma biotransformação que beneficie a biodisponibilidade de nutrientes e o uso de fungos reconhecidamente comestíveis, bem como ensaios e métodos que avaliem o potencial do uso de resíduos e co-produtos da agroindústria, podem proporcionar o melhor aproveitamento destes e, consequentemente, aumentar a disponibilidade de alimentos com valores nutricionais adequados.

2. OBJETIVOS

Pelo exposto anteriormente, a demanda de alimentos aumenta com a ampliação demográfica. Contudo, as áreas de cultivo de alimentos mantem-se semelhantes, sem expectativas de aumento, o que prejudica o aumento na produção de alimentos. No entanto, o uso de coprodutos gerados pela agroindústria para fins alimentares, possibilita promover o aumento da produção de alimentos sem a necessidade de ampliar as áreas cultiváveis, promovendo um acréscimo na disponibilidade de alimentos. O aproveitamento de bagaço e folhas de mandioca podem minimizar efeitos negativos gerados pela disposição destes em aterros, proporcionando um fim mais nobre para este material.

Assim, este trabalho teve por objetivos:

- Observar o efeito da ação dos fungos comestíveis *Lentinula edodes*, *Pleurotus ostreatus*, *Agaricus blazei* e *Rhizopus microsporus* var *oligosporus*, em processo de fermentação em estado sólido;
- Observar o efeito de cada fungo em substrato exclusivo formulado pela mistura de bagaço de mandioca e folhas de mandioca (*Manihot esculenta*);
- Analisar quimicamente os nutrientes presentes nas amostras testadas;
- Avaliar o ganho protéico derivado da fermentação;
- Avaliar as melhoras com relação aos aminoácidos essenciais;
- Comprovar a efetividade do enriquecimento do composto pela análise da biodisponibilidade das proteínas;
- Verificar as alterações na presença de ácidos graxos insaturados antes e após a fermentação;

- Verificar se a concentração de cianeto no produto final enquadra-se nas exigências para o consumo humano.

3. REVISÃO BIBLIOGRÁFICA

3.1. A Importância da nutrição adequada

A finalidade dos alimentos é fornecer a energia aos organismos pelo fornecimento de nutrientes e, assim, mediante o metabolismo promover a adequada formação e manutenção dos tecidos, regulação e funcionamento dos órgãos. Um produto, para ser considerado um alimento deve apresentar como principais componentes carbono, hidrogênio, oxigênio e nitrogênio, além de outros em menores quantidades. Tal composição é nutricionalmente descrita quanto à porcentagem de carboidratos, proteínas, gorduras, minerais e água (EVANGELISTA, 2000).

Cada organismo apresenta uma determinada exigência nutricional relacionada às suas exigências fisiológicas. O consumo adequado de alimentos visa preencher tais necessidades que são decorrentes das funções que cada nutriente apresenta sobre o sistema orgânico. Desta maneira, a diminuição ou a ausência de nutrientes necessários podem causar déficits fisiológicos e induzir a enfermidades ou afetar gravemente o desenvolvimento do organismo (MELO *et al.*, 2007a). Muitos problemas de saúde pública estão vinculados à doenças decorrentes da ingestão de quantidades inferiores desses nutrientes que não atendem as recomendações. Contudo, a carência de proteínas é o problema nutricional com maior relevância (TAVARES; KIYAN, 2002). Este quadro, considerado como desnutrição, leva os

indivíduos a uma situação preocupante, diminuindo o desempenho do organismo, em consequência da baixa disponibilidade de nutrientes essenciais.

Há, assim, elevada preocupação com relação à desnutrição infantil dadas as evidências de que uma alimentação inadequada durante a infância, além de induzir baixa taxa de crescimento, pode levar a maior mortalidade infantil, decorrente de uma maior fragilidade a doenças infecciosas, além de prejuízos para o desenvolvimento psicomotor, aproveitamento escolar e menor capacidade produtiva na idade adulta (BLACK *et al.*, 2008; VICTORA *et al.*, 2008).

A Organização Mundial de Saúde (OMS) estima que mais de 794,6 milhões de pessoas no mundo estejam sofrendo de déficits por alimentos, sendo que 779,9 milhões são habitantes de países em desenvolvimento. Considerando somente países em desenvolvimento, a desnutrição atinge 134,9 milhões de crianças menores de 5 anos (MAHAN; ESCOTT-STUMP, 1998; FAO; IFAD; WFP, 2015). Contudo, a ingestão adequada de proteínas e alimentos energéticos pode aumentar significativamente a qualidade de vida dos consumidores (RÜFENACHT *et al.*, 2010).

3.2. Produção de alimentos: novas fontes e possibilidades

A fome e o desperdício de alimentos são dois dos maiores problemas mundiais pois, estão diretamente relacionados. A quantidade de alimentos desperdiçados em países desenvolvidos é semelhante ao total produzido em toda a África Subsaariana, sendo estimado em aproximadamente 230 milhões de toneladas (CINTRA *et al.*, 2007; FAO, 2011).

Buscando aumentar a quantidade de alimentos produzidos, observa-se interesse crescente no aproveitamento de porções estruturais, tanto de animais como de vegetais que normalmente são desprezadas. Desta maneira, o aproveitamento integral de vegetais

geradores de alimentos vem sendo estimulado e estudado mediante o uso de cascas, talos, folhas, entre outros (PINTO et al., 2005; CINTRA *et al.*, 2007).

Contudo, o aproveitamento de partes até recentemente não utilizadas deve incluir boa produção, características sensoriais e nutricionais satisfatórias, além de propriedades funcionais desejáveis (COSTA; ROMANELLI; TRABUCO, 2008). Tais condições implicam em alto valor nutritivo e apresenta benefícios à saúde pela diminuição de riscos de doenças crônico-degenerativas (MORAES; COLLA, 2006).

3.2.1. O uso de co-produtos na obtenção de alimentos: bagaço de mandioca

Dentre as opções para o uso alternativo de partes descartadas de vegetais, pode-se considerar a da mandioca, uma vez que apresenta fácil adaptação, sem a necessidade de tecnologias avançadas para sua produção. Segundo a FAO, o crescimento médio da produtividade da mandioca é de 4% ao ano, sendo que o continente africano contribui com cerca de 51% do total produzido no mundo, liderado pela Nigéria e República Democrática do Congo, seguido pela Ásia que, liderada pela Tailândia e Indonésia, contribuem com 33% e a América com 15%, sendo o Brasil o principal produtor (GROXKO, 2011).

A mandioca é considerada a principal cultura cianogênica do mundo e a sexta cultura alimentar mais importante, onde 60% do total produzido enquadra-se como o alimento básico de 700 milhões de pessoas (GIRAUD, 1993; PANDEY et al., 2000; SHARIF et al., 2015). Contudo, seu processamento na indústria é realizado, principalmente, para a produção de farinha e fécula (ou amido), os quais geram resíduos líquidos e sólidos. Dentre os resíduos sólidos, a cada 250-300 toneladas de tubérculos de mandioca processadas (para a obtenção de 19,75 - 47,1 toneladas de amido) são geradas cerca de 1,6 toneladas de cascas e 280 toneladas de bagaço (com alta carga de umidade gerada pelo processo de extração) que são descartados

no ambiente (PANDEY; SOCCOL; MITCHELL, 2000; PANDEY et al., 2000; SEBRAE, 2008; NUNES; SANTOS; CRUZ, 2009; JASKO et al., 2011).

O bagaço de mandioca é um resíduo fibroso, contendo entre 5 e 10% de umidade, 0,3 a 1,6% de proteína, 0,8 a 1% de lipídeos, 15 a 50,5% de fibras, 40 a 60% de carboidratos e 0,7 a 1,1% de cinzas (SOCCOL, 1994; CEREDA, 1994; CARTA et al, 1999). Mediante esta composição há condições de se propor a utilização do mesmo como fonte alternativa de alimento, microbiologicamente modificado, a semelhança do que sugeriu Morales (2012) com farinha de mesocarpo de babaçu enriquecida com folhas de mandioca. Segundo Oliveira (2007), o uso de resíduos com potencial alimentício, além de aumentar a produção de alimentos, podem contribuir para minimizar os problemas de poluição ambiental derivado do descarte dos mesmos no ambiente. O bagaço de mandioca é utilizado em diversas aplicações como na produção de ácido cítrico, aromas, ácido fumárico, biotransformação, como também cogumelos, demonstrando a potencialidade de seu uso como substrato de processo de biotransformação em estado sólido (PANDEY et al., 2000).

Kornizejuk (2008) demonstrou que o uso de produtos regionais e produtos não convencionalmente usados na alimentação pode ser eficiente contra a desnutrição, quando comparou a mistura de ingredientes elaborada com alimentos regionais e sobras de alimentos industrializados (Multimistura) com produtos disponíveis no mercado. A multimistura apresentou resultados surpreendentes, uma vez que acrescentou grande quantidade de elementos essenciais à dieta infantil como Potássio (K), Magnésio (Mg), Cálcio (Ca), Ferro (Fe), Manganês (Mn) e Zinco (Zn), além de valor nutricional equilibrado, contendo cerca de 30% de carboidratos, 30% de fibras, 5% de lipídeos e 15% de proteínas. Esta composição representa maior quantidade de fibras e proteínas, além de quantidades inferiores de lipídeos e carboidratos que os produtos existentes no mercado. Assim, o enriquecimento de alimentos pode ser utilizado como uma estratégia de incorporação de diversos aminoácidos essenciais e

outros nutrientes e, assim, atender as necessidades orgânicas específicas de uma dada população (AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA, 1978).

3.2.2. O uso de co-produtos na obtenção de alimentos: folhas de mandioca

A inclusão das folhas de mandioca (FM), pode desempenhar importante papel, uma vez que este material apresenta altos teores de proteína, β -caroteno, vitamina C, riboflavina, niacina e tiamina, incluindo o suplemento vitamínico, de minerais e de fibras (NASSAR; MARQUES, 2006; TEO, 2007; ARO, 2008; LATIF; MÜLLER, 2015).

A adição de FM como fonte de alimentação alternativa a outros alimentos pode ser uma solução para suplementar a carência de alguns nutrientes do bagaço proveniente da industrialização das raízes da mandioca (CARVALHO *et al.*, 1989; MORALES, 2012).

Avaliações realizadas por Melo *et al.* (2008) em cinco amostras de FM moídas e fracionadas em peneiras, registraram 4,56 a 20,58 % de proteína, sendo que as amostras que apresentaram maior porcentagem de proteínas foram as constituídas, em sua maioria, por folhas, enquanto que as obtidas de talos apresentaram resultados inferiores aos das folhas.

No Brasil, a FM é usada na forma seca e pulverizada e em pequena quantidade no preparo da multimistura, utilizada no combate à desnutrição por ser uma fonte de vitaminas e sais minerais (BRANDÃO; BRANDÃO, 1989; CÂMARA; MADRUGA, 2001). Consideradas como resíduos, as FM apresentam baixo custo e não competem com o principal produto comercial, que são os tubérculos, além disto apresenta uma produção estimada de 2,25 toneladas por hectare, que é descartada quase que totalmente após a colheita (RAVINDRAN; RAJAGURU, 1988; SAGRILO, 2001).

Muitos trabalhos demonstram que as proteínas presentes nas FM são de ótimo valor biológico, que, somando aos altos teores proteicos das folhas, confere a este produto uma

ótima, aceitável e útil fonte de proteínas para a nutrição humana (TERRA, 1964; EGGUM, 1970; NASSAR; MARQUES, 2006; LATIF; MÜLLER, 2015).

Porém, como a FM apresenta algumas substâncias consideradas antinutritivas e/ou tóxicas, como o cianeto, os polifenóis, o nitrato, o ácido oxálico, as saponinas, a hemaglutinina e os inibidores de tripsina (CORRÊA, 2000; MELO et al., 2007b; WOBETO et al., 2006), existe a necessidade de uso de tecnologias que eliminem estes compostos. Dentre os procedimentos aplicáveis destaca-se a fermentação, para que os mesmos possam ser degradados com eficiência e o produto final ser aplicado na alimentação humana e sem prejuízo à saúde.

As FM, além de possuírem características interessantes para seu uso como substrato para micro-organismos, apresentam substâncias tóxicas como os glicosídeos cianogênicos, que deve ser suprimidos antes da sua incorporação aos alimentos. A eliminação destes compostos geralmente é feita pela secagem das folhas, cujo processo decompõe grande parte das toxinas (ALMEIDA; SANTANA; SOUZA, 2003), como também pela ação de micro-organismos fermentadores.

3.3. Fermentação em estado sólido como estratégia de melhora nutricional

Uma estratégia tecnológica proposta para promover o enriquecimento de alimentos, melhorando a biodisponibilidade dos nutrientes, induzindo aumento do valor econômico dos mesmos procede da aplicação de micro-organismos no processo de fermentação em estado sólido (FES) (PANDEY, 2003). No entanto, um substrato, para ser considerado ideal, deve fornecer tanto uma fonte de carbono (energia) como uma fonte de proteínas (PANDEY *et al.*, 1999).

A fermentação, utilizada desde a antiguidade, proporciona não só o aumento da vida útil e segurança dos alimentos como também os tornam mais digeríveis. No caso da

mandioca, a fermentação diminui a toxicidade do substrato, pela degradação dos compostos cianogênicos e estruturais das células, modificações consideradas positivas no melhoramento das características organolépticas do produto final (CAPLICE; FITZGERALD, 1999; KODAMA *et al.*, 2008).

Contudo, a falta de proteínas que ocorre no bagaço de mandioca impõe uma dificuldade para o desenvolvimento ideal dos fungos, necessitando, pois, de enriquecimento proteico para o fornecimento dos nutrientes necessários a um processo de FES. Nesta condição, faz-se pertinente a incorporação das folhas da mandioca (MORALES, 2012).

A obtenção de biomassa proteica microbiana produzida em substratos sólidos exige procedimentos simples e instalações de baixo custo (SERZEDELLO, 1999; COUTO; SANROMÁN, 2006). Estas condições viabilizam a implantação de projetos biotecnológicos simples em regiões de recursos econômicos restritos. Os substratos modificados constituem alimentos que podem ser consumidos diretamente e são adaptados aos hábitos regionais onde são implantados.

Assim como as bactérias, os fungos filamentosos e as leveduras podem crescer em substratos sólidos, podendo ser aplicados em processos de fermentação no estado sólido. Os fungos filamentosos são os micro-organismos mais empregados neste processo, devido às suas propriedades fisiológicas, enzimáticas e bioquímicas (SOCCOL, 1994; KRISHNA, 2005).

Os fungos podem melhorar o grau de digestibilidade de alimentos, mediante a degradação de fibras, e aumentar o valor protéico dos alimentos, relacionado ao aumento da biomassa do micro-organismo. A ação de alguns fungos pode ainda impedir a formação de produtos tóxicos típicos, como a aflatoxina B1; controlar o crescimento bacteriano, mediante a síntese de substâncias que impedem o seu desenvolvimento; produzir substâncias anticarcinogênicas; além de detoxificar os compostos cianogênicos da mandioca

(WOICIECHOWSKI, 1997; AKINFEMI, 2010). Diante dessas informações, é oportuno ressaltar as propriedades nutricionais e medicinais dos fungos comestíveis, os quais abrangem uma grande variedade de espécies como cogumelos, trufas, bolores e levedura, sejam elas cultivadas ou selvagens (SADLER, 2003).

As proteínas de micro-organismos apresentam digestibilidade em torno de 74%, considerada um índice alto, pois aproxima-se do valor da proteína padrão (caseína), que é de 93,33 % (SGARBIERI, 1987; PIRES et al., 2006). Segundo Menezes (1972), quanto maior a semelhança entre a composição de uma dada proteína com proteínas de origem animal, maior será seu valor biológico e nutricional.

Pela alta digestibilidade das proteínas de origem microbiana, elas podem ser utilizadas como fonte proteica adicional, indicadas para incorporação em alimentos (VILLAS-BOAS; ESPÓSITO, 2000).

O cultivo de micro-organismos e a utilização de proteína derivada do processo microbiano têm sido recentemente incorporados como fonte alimentar para o povo brasileiro (ANGELIS; GOES; PIÃO, 2009), não somente pelo aspecto nutricional, mas também pela capacidade de conferir propriedades sensoriais e funcionais (RODRIGUEZ-AMBRIZ *et al.*, 2005). Atualmente, cerca de 600 espécies de fungos comestíveis são conhecidas, possibilitando a aplicação na conversão de resíduos agrícolas para a alimentação humana e, conseqüentemente, de animais (HERRERA, 2001; BALDISSERA, 2007).

3.3.1. *Rhizopus oligosporus*

Enzimas amilolíticas extraídas de micro-organismos fermentadores como o *Rhizopus oligosporus* têm aplicação em biotecnologia dos processos de hidrólise do amido nas indústrias alimentícias (panificação, cervejaria, xarope de glicose ou maltose) e farmacêuticas (DOMINGOS, 2003). Além disso, o *R. oligosporus* em alimentos é utilizado no preparo do

tempeh, que é um alimento tradicional da Indonésia produzido pela fermentação de grãos de soja descascados e cozidos. Neste processo o fungo *R. oligosporus* atua como agente fermentador e figura entre as fontes alternativas que podem integrar diversos programas de alimentação e nutrição, devido ao seu alto valor proteico (HESELTIME; WANG, 1967; TAVARES; KIYAN, 2002). Morales (2012) obteve aumento significativo na quantidade de proteínas após 72 horas de fermentação de farinha de mesocarpo de babaçu (deficiente em proteínas) e FM por *Rhizopus oligosporus*.

3.3.2. *Lentinula edodes*

O fungo *Lentinula edodes*, mais conhecido como Shiitake é o segundo cogumelo mais cultivado no mundo, atingindo cerca de 7,5 milhões de toneladas produzidas no ano 2000, sendo amplamente utilizado na culinária asiática com aumento significativo do consumo mundial (PRZYBYLOWICZ; DONOGHUE, 1990; ROYSE, 2005). Algumas propriedades funcionais do fungo como antitumorais, capacidade de reduzir os níveis de colesterol, além do potencial antioxidante e antimicrobiano induzem o desenvolvimento de pesquisas ao seu respeito (HATVANI, 2001; MAU; CHAO; WU, 2001; YANG et al., 2002; SHIMADA; MORITA; SUGIYAMA, 2003). O cogumelo shiitake apresenta ainda, quantidades entre 10 e 17% de proteínas em peso seco (com todos os aminoácidos essenciais, apesar de baixos níveis de metionina e cistina), além de vitaminas e minerais, sendo pobre em lipídeos, sendo que os ácidos graxos predominantes são insaturados (BRENE, 1990; REIS et al., 2012).

3.3.3. *Agaricus blazei*

O *Agaricus blazei* é um fungo constituído por cerca de 36,7% de proteínas, 38,3% de carboidratos, 7,5% de umidade, 7,3% de cinzas, 6,8% de fibras e 3,4% de gorduras. Possui também elevada quantidade de minerais e todos os aminoácidos essenciais aos seres humanos

(BALDISSERA, 2007). Ocorre naturalmente em umidade elevada e em temperaturas entre 23 e 28°C (TEIXEIRA, 1999), muito semelhante a temperatura ambiente. O baixo teor de gordura e a elevada quantidade de proteínas são fatores importantes para suplementos destinados a alimentação uma vez que as principais doenças endêmicas estão relacionadas ao excesso de gorduras ou a subnutrição mostrando-se assim, promissor ao uso em alimentos fermentados.

3.3.4. *Pleurotus ostreatus*

Os cogumelos do gênero *Pleurotus* não apresentam exigências nutricionais elevadas, desenvolvendo-se rapidamente em substratos nutricionalmente pobres (SCHMIDT et al., 2003). Esse rápido crescimento deve-se a produção de enzimas ligninolíticas, que facilitam seu crescimento em substratos vegetais, convertendo assim compostos não convencionais em alimentos de elevado valor proteico (CAPELARI, 1996; SCHMIDT et al., 2003). Segundo Minotto et al. (2008), o crescimento do *Pleurotus ostreatus* var. *roseus* (conhecido como *Pleurotus salmon*) é estimulado em substratos com maiores teores de nitrogênio.

3.4. Aminoácidos essenciais (AAE)

Os aminoácidos são metabolitos determinantes para o funcionamento adequado de um organismo tendo, principalmente, um papel de construção de proteínas, regulação de vários processos celulares e também como precursores de outras moléculas, tais como hormônios e bases nitrogenadas (WU, 2009).

Alguns desses aminoácidos não podem ser sintetizados pelo organismo humano e, por tal motivo, considerados aminoácidos essenciais, ou seja, dependem de uma dieta com níveis adequados desses componentes para a manutenção saudável do organismo (PEREIRA et al., 2013). Assim, a qualidade da proteína e, conseqüentemente, dos aminoácidos são fatores

diretamente ligados à ingestão de alimentos, sendo que o organismo pode responder negativamente quando ocorre uma deficiência proteica oriunda da má alimentação. Contudo, existe um crescente conceito de que os aminoácidos (AA) regulam vias metabólicas importantes que são necessárias para a manutenção, crescimento, desenvolvimento, reprodução, lactação, e imunidade do organismo (WU, 2013).

Dada a importância dos aminoácidos essenciais no funcionamento adequado e, consequentemente, no desenvolvimento do organismo, algumas ferramentas foram desenvolvidas para acessar a qualidade das proteínas presentes nos alimentos e, assim, antever a real biodisponibilidade do nutriente em questão.

3.4.1. Ferramentas de avaliação da qualidade da proteína

A medida real da qualidade da proteína para o ser humano está relacionada à avaliação do equilíbrio metabólico realizado em indivíduos de uma determinada população. Contudo, por razões de custo e ética, torna-se necessário o uso de ensaios *in vitro* ou *in vivo* que apresentem correlação com um possível comportamento humano para um determinado alimento. Para assegurar a precisão e a aplicabilidade desses métodos, todos os parâmetros principais que determinam a qualidade da proteína devem ser medidos sendo: quantidade de aminoácidos essenciais, digestibilidade proteica e a biodisponibilidade desses aminoácidos (GILANI, 2012).

Neste sentido, algumas ferramentas reconhecidas permitem o acesso aos valores dos parâmetros mencionados anteriormente, sem a necessidade de experimentos com animais (que apresentam custos elevados com instalações, tempos longos de ensaios e questões éticas) (TAVANO et al., 2004).

3.4.1.1. Avaliação da qualidade proteica pelo cômputo de aminoácidos corrigido pela digestibilidade (PDCAAS - Protein Digestibility-Corrected Amino Acid Score)

Um conjunto de especialistas sobre proteína da FAO/WHO em 1989 recomendou a avaliação da qualidade da proteína pelo cálculo do cômputo de aminoácidos corrigido pela digestibilidade (PDCAAS). Neste cálculo, se estabelece um aminoácido essencial limitante (o aminoácido que supre em menor quantidade a necessidade recomendada à uma determinada população, como por exemplo: crianças entre 2-5 anos de idade), que é multiplicado pela digestibilidade da proteína avaliada e comparada com a necessidade recomendada. Este método vem sendo utilizado a mais de 20 anos com considerável êxito (HUGHES et al., 2011; FAO/WHO, 2013).

3.4.1.2. Avaliação da qualidade proteica determinada pelo cômputo de aminoácidos indispensáveis digeríveis (DIAAS - Digestible Indispensable Amino Acid Score)

A avaliação pelo PDCAAS da qualidade da proteína do alimento, mesmo utilizada por mais de 20 anos, apresenta limitações que foram reconhecidas e debatidas, possibilitando a avaliação da qualidade proteica por outros métodos. Tais limitações estabeleceram-se devido a necessidade de que os AAE devem ser tratados como nutrientes individuais, uma vez que apresentam, muitas vezes, funções particulares no metabolismo (WU, 2009; FAO/WHO, 2013). Baseado na necessidade de individualizar os cálculos para cada AAE, foi proposto o métodos DIAAS, que é definido como a porcentagem de uma determinada quantidade de um AAE em 1 g da proteína analisada dividido pela quantidade do mesmo aminoácido de uma proteína de referência (por exemplo: a necessidade deste mesmo aminoácido por crianças entre 2-5 anos de idade) (FAO/WHO, 2013).

A importância do cálculo pelo DIAAS apresenta o real alcance de cada EAA da proteína em questão para uma determinada população, possibilitando avaliar o suprimento por inteiro e, assim, verificar aonde existe a necessidade de melhoras na dieta e para os quais o consumo se encontra adequado.

3.4.1.3. Avaliação do valor nutricional relativo (RNV – Relative Nutritional Value) da proteína

Este método foi desenvolvido por Ford (1960) e consiste em avaliar a biodisponibilidade da proteína pelo crescimento da bactéria *Enterococcus zymogenes*, baseando-se nas necessidades nutricionais deste micro-organismo relacionadas aos AAE. Esse micro-organismo apresenta necessidade nutricional muito semelhante às dos seres humanos quanto aos AAE, sendo suas necessidades absolutas: valina, metionina, isoleucina, arginina, histidina, triptofano e ácido glutâmico (para os seres humanos são: histidina, isoleucina, leucina, lisina, metionina, fenilalanina, treonina, triptofano e valina) (TAVANO et al., 2004; MORALES, 2012; WU, 2013).

O método microbiológico (VNR) apresenta vantagens quanto ao uso de métodos *in vivo* pois, necessita de pequena quantidade de amostra, além de simplicidade e rapidez de resposta. Este método ainda apresenta uma correlação 81,42 % com o PDCAAS, resultado este que confere confiabilidade aos resultados (TAVANO et al., 2004).

3.5. Ácidos graxos insaturados

A recomendação diária de ingestão de gorduras está estabelecida entre 20-35 % de toda a energia ingerida, sendo que as funções principais dos ácidos graxos estão relacionadas à energia (37 kJ g^{-1} de gordura), transporte de energia (lipídios do sangue como os

triacilgliceróis nas lipoproteínas), armazenamento de energia (tecido adiposo, etc.), isolante térmico e sinais (eicosanoides, regulação gênica). Os ácidos graxos são moléculas de cadeia carbônica onde uma das extremidades da molécula possui um grupo metil (ao qual denomina-se ω o primeiro carbono ligado a este grupo) e um grupo carboxila no outro (ao qual denomina-se α o primeiro carbono ligado ao grupo). Tais ácidos graxos são divididos em 3 categorias sendo: ácidos graxos saturados (AGS); ácidos graxos monoinsaturados (AGM); e ácidos graxos poliinsaturados (AGP) (RUSTAN; DREVON, 2005; VANNICE; RASMUSSEN, 2014).

Os AGS, como o próprio nome explica, são moléculas que não apresentam insaturações na molécula. Quanto ao consumo desses AGS, recomenda-se o consumo de uma menor quantidade possível pois, a ingestão deste tipo de ácidos graxos está vinculada a problemas cardiovasculares (FARVID et al., 2014; VANNICE; RASMUSSEN, 2014).

Com relação ao consumo de AGM, o aconselhável é que se substitua as gorduras sólidas por este tipo de ácido graxo, principalmente pela adoção de uma dieta Mediterrânea (são os principais ácidos graxos encontrados no azeite de oliva), consumindo cerca de 15-20 % do total de energia diária recomendada. Os AGM apresentam apenas uma instauração na molécula, lhes conferindo melhores características nutricionais que os AGS (VANNICE; RASMUSSEN, 2014).

Os AGP são moléculas que apresentam duas ou mais duplas ligações na cadeia carbônica sendo os mais comuns os AGP das famílias ω -3 e ω -6. Pelo fato dos seres humanos não poderem sintetizá-los endogenamente são considerados nutrientes dietéticos essenciais. Os AGP mais abundantes são o ácido α -linolênico (18:3 ω -3) e ácido linoleico (18:2 ω -6), ambos com 18 carbonos na cadeia e considerados base para os AGP ω -3 e ω -6, respectivamente. A substituição de gorduras saturadas por poliinsaturadas (AGP) na dieta

auxilia na prevenção de doenças cardiovasculares (FARVID et al., 2014; VANNICE; RASMUSSEN, 2014).

4. MATERIAL E MÉTODOS

4.1. Micro-organismos

Para o desenvolvimento das fermentações, foram utilizados quatro diferentes micro-organismos de grau alimentício sendo 3 basidiomicetos e 1 zygomyceto. Os micro-organismos utilizados foram: *Rhizopus microsporus* var. *oligosporus* CCT 3762, (adquirido junto a Fundação Tropical de Pesquisa e Tecnologia “André Tosello”, Campinas – SP, fungo utilizado na culinária indonésia para a fabricação do tempeh); *Lentinula edodes* (conhecido como Shiitake); *Agaricus subrufences* (também conhecido como *Agaricus blazei* ou cogumelo do sol), *Pleurotus ostreatus* (conhecido como Shimeji). Os basidiomicetos foram adquiridos junto a Funghi & Flora, Valinhos, SP).

Os fungos foram mantidos em placas de Petri contendo meio sólido inclinado de extrato de malte 2%, a $24^{\circ}\text{C} \pm 2^{\circ}\text{C}$ por 72 horas. A inoculação foi realizada pela inserção de 10 alíquotas de 1 cm^2 de meio contendo as hifas dos fungos no material pasteurizado e umidificado.

4.2. Bagaço de mandioca (BM)

O bagaço de mandioca foi cedido pela Corn Products Industries (Conchal – SP), sendo acondicionado em embalagem de PVC e mantido à -20°C , até o momento de seu uso.

4.3. Folha de mandioca (FM)

Folhas de mandioca foram fornecidas por produtores de Rio Claro, SP. As folhas foram congeladas à -20°C em embalagens de PVC até o momento de seu uso.

4.4. Preparo das amostras

Para fins de comparação, foram preparadas amostras contendo:

- Bagaço de mandioca; Farinha de folhas de mandioca e Bagaço de mandioca + Percentuais de farinha de folhas de mandioca, de acordo com o desenvolvimento de cada fungo conforme Tabela 1.

Tabela 1 – Percentuais de co-produtos testados no preparo dos substratos para a fermentação em estado sólido

Fungo	FM:BM
<i>Agaricus blazei</i>	1:9; 2:8; 3:7; 4:6
<i>Lentinula edodes</i>	1:9; 2:8; 3:7; 4:6
<i>Pleurotus ostreatus</i>	0:10; 1:9; 2:8; 3:7
<i>Rhizopus oligosporus</i>	3:7; 4:6; 5:5; 6:4

FM: Folhas de Mandioca; BM: Bagaço de Mandioca.

As amostras, preparadas conforme descrição acima, foram utilizadas para a escolha do substrato para os fungos filamentosos. As misturas escolhidas foram preparadas e acondicionadas em sacos plásticos de polipropileno para a FES. Após a autoclavagem do substrato e inoculação dos fungos, as amostras foram incubadas em estufa a 24°C ± 2°C até a total colonização do substrato. A secagem do material para as análises foi realizada por liofilização, sendo posteriormente trituradas e armazenadas em recipientes plásticos com fechamento hermético e identificados. O experimento foi realizado em triplicata.

4.5. Análises

4.5.1. Determinação de nitrogênio e proteínas

A quantificação do nitrogênio total foi realizada pelo método Kjeldahl (AOAC, 1995), utilizando-se o fator 6,25 para conversão dos teores de nitrogênio em proteínas, para a farinha controle e fermentada (SILVA, 1998; OLIVEIRA, 2007).

4.5.2. Determinação da digestibilidade proteica

A digestibilidade das proteínas presentes no composto formulado foi determinada pelo método de análise de Digestibilidade segundo Akeson e Stahman (1964).

4.5.3. Valor nutricional relativo (VNR) da proteína

Este método indireto de avaliação do Valor Nutricional Relativo das proteínas presentes na amostra, tem sua eficiência vinculada ao uso de enzimas do trato gastrointestinal humano, aplicadas durante a hidrólise do material e comparada a uma proteína padrão (caseína).

4.5.3.1. *Enterococcus zymogenes*

A bactéria *Enterococcus zymogenes* (NCDO 592), proveniente do Agricultural Research Service (US Department of Agricultural) Northern Regional Research Center, foi gentilmente ofertada pela Profª. Dra. Aureluce Demonte, docente do Departamento de Alimentos e Nutrição, da Faculdade de Ciências Farmacêuticas - UNESP – Araraquara – SP.

4.5.3.2. Preparo do experimento

As amostras obtidas antes e após a fermentação foram desengorduradas utilizando-se n-hexano em aparelho Soxhlet. Posteriormente, o equivalente a 100 mg de proteínas de cada amostra foi pesada em um Erlenmeyer, adicionado de 40 mL de solução de β -glicerofosfato de sódio Sigma G-5422 (20g L⁻¹) ajustado o pH para 8,0. Foram acrescentados 4,0 mL de

solução de pronase Sigma P-5147 (1 mg mL^{-1}). Os Erlenmeyers foram mantidos sob agitação lenta e constante a 48°C por 3 horas.

Posteriormente à hidrólise das amostras, o pH foi ajustado para 7,2 (com NaOH ou H_3PO_4) e esterilizado por microfiltração a frio.

O ensaio foi realizado mediante o preparo de 4 tubos (3 testes e 1 branco); adicionado de 2,0 mL do meio basal esterilizado a frio + 2,0 mL da amostra hidrolisada + 7,0 mL de água destilada estéril + 100 μL do inóculo (*Enterococcus zymogenes*). No tubo “branco” foi utilizado o inóculo inativado em autoclave previamente (121°C por 15 minutos) (FORD, 1960).

Após a inoculação, o material foi incubado a 37°C por 48 horas e, ao final do período, esterilizado em autoclave (121°C por 15 minutos). As medidas de crescimento foram realizadas por acidimetria, titulando-se NaOH 0,1M ou H_3PO_4 0,0333 M até pH 7,2 em cada amostra.

Os resultados das médias dos volumes gastos na titulação, subtraído o volume do branco (com inóculo inativo), foram utilizados para expressar o valor nutricional relativo (VNR) das amostras como percentuais do valor encontrado para a análise realizada com a proteína padrão caseína (Sigma C-7078) (TAVANO *et. al*, 2004).

4.5.4. Lipídios totais

A obtenção de lipídios totais foi realizada mediante extração com n-hexano durante 8 horas ininterruptas em aparelho Soxhlet, com 2,0 g de amostra colocada em cartucho de extração, ensaiados em triplicata. O método foi adaptado de IAL (2008), no qual utiliza éter para a extração lipídica.

4.5.5. Umidade

O resíduo obtido pela diferença do material antes e após o aquecimento direto de 5 a 10 g do material a 105°C até peso constante (processo mais usual) foi considerado resíduo

seco, sendo expresso em porcentagem (IAL, 2008). Também foram utilizadas medidas obtidas em analisador de umidade em infravermelho, Sartorius MA-35 para consideração das umidades das amostras.

4.5.6. Aminoácidos essenciais (AAE)

Com o intuito de determinar a quantidade de AAE (lisina, histidina, arginina, treonina, cisteína, valina, metionina, isoleucina, leucina, tirosina e fenilalanina), foi realizada a hidrólise ácida em cada amostra antes e depois da fermentação. Aliquotas entre 200-250 mg de cada amostra foram transferidos para tubos de borossilicato (pirolisados anteriormente a 400 ° C por período de 8 h), com posterior adição de 6 mL de HCl a 6 M com 0,1% de fenol (m /v). Após homogeneização suave, os tubos foram mantidos durante 10-20 segundos em uma corrente contínua de nitrogênio gasoso para eliminar o O₂. Os tubos foram, então, transferidos para estufa à 110 °C, permanecendo com as tampas ligeiramente abertas por 1 hora. Em seguida, estes tubos foram fechados e mantidos sob a mesma temperatura durante mais 23 horas (totalizando 24 horas). A quantificação de aminoácidos foi realizada por cromatografia líquida de alta performance (HPLC) utilizando o programa ChromStar* 7.0.14a, de acordo com (MOORE; SPACKMAN; STEIN, 1958). Esse experimento foi realizado junto a Faculdade de Biologia e Química, Instituto de Química e Biotecnologia de Alimentos da Justus-Liebig Universidade de Giessen, Alemanha (Justus-Liebig Universität Giessen, Institute - Lebensmittelchemie und Lebensmittelbiotechnologie, Fachbereich – Biologie und Chemie, Gießen).

4.5.7. Cianeto

O teor total de cianeto (HCN) foi analisado com o método colorimétrico descrito por Brito et al. (2009). A medição de cianeto total foi realizada em todas as amostras para determinar a diminuição de HCN durante a fermentação e avaliar a possibilidade de utilizar o material fermentado como uma fonte de alimento. Esse experimento foi realizado junto a

Faculdade de Biologia e Química, Instituto de Química e Biotecnologia de Alimentos da Justus-Liebig Universidade de Giessen, Alemanha (Justus-Liebig Universität Giessen, Institute - Lebensmittelchemie und Lebensmittelbiotechnologie, Fachbereich – Biologie und Chemie, Gießen).

4.5.8. Ácidos Graxos

Os lipídios de cada amostra foram extraídos pelo sistema Soxtherm usando éter de petróleo como solvente, como descrito por Matthäus e Brühl (2001).

A caracterização dos ésteres metílicos de ácidos graxos (Fatty Acids Methyl Esters - FAME) foi realizada pesando-se uma fração (100-250 mg) dos lipídios extraídos de cada amostra. Após pesagem, cada amostra foi acondicionada em frasco de borosilicato firmemente vedado com tampa de teflon. Os lipídios foram saponificados com a adição de 4 mL de hidróxido de sódio metanólico a 0,5 M seguido por aquecimento a 80 °C durante 10 minutos. No passo seguinte, para promover a esterificação, 5 mL de metanol complexo de trifluoreto de boro (BF₃) foi adicionado às amostras e novamente aquecida a 80 °C por 5 min. Posteriormente, foram adicionados 5 mL de heptano, seguido por um outro aquecimento a 80 °C durante 1 minuto. As amostras foram retiradas do aquecimento e, após resfriamento, foram adicionados 3 mL de solução saturada de cloreto de sódio e os ácidos graxos foram separados após agitação de 30 segundos. O sobrenadante, contendo os ácidos graxos, foi separado em frascos viais de 4 mL para efetuar a quantificação em cromatografia gasosa (GC).

Os padrões para quantificar os perfis de ácidos graxos foram preparados de acordo Ramani, Zorn e Papenbrock (2004). Esse experimento foi realizado na Faculdade de Biologia e Química, Instituto de Química e Biotecnologia de Alimentos da Justus-Liebig Universidade de Giessen, Alemanha (Justus-Liebig Universität Giessen, Institute -

Lebensmittelchemie und Lebensmittelbiotechnologie, Fachbereich – Biologie und Chemie, Gießen).

4.5.9. Cômputo de aminoácidos corrigido pela digestibilidade (Protein Digestibility-Corrected Amino Acid Scoring – PDCAAS)

O PDCAAS foi calculado com base nos requisitos de AAE para crianças de 2-5 anos de idade, de acordo com Tirapegui e Macedo (2007), calculado pela Equação 1.

Equação 1: Cálculo para a determinação do PDCAAS.

$$PDCAAS = \frac{mg \text{ do AA limitante (1 g da amostra)} \times \text{digestibilidade (\%)}}{mg \text{ do mesmo AA em 1 g da proteína de referência}} \times 100$$

AA = Aminoácido

4.5.10. Cômputo de aminoácidos essenciais digeríveis (Digestible Indispensable Amino Acid Score – DIAAS)

O DIAAS representa uma recente medição de qualidade da proteína recomendado pela FAO/WHO (2013) para substituir PDCAAS. Esta recomendação é porque os aminoácidos dietéticos devem ser tratados como nutriente individual. Os valores de DIAAS são baseados na Equação 2.

Equação 2: Cálculo para a determinação do DIAAS.

$$DIAAS = \frac{mg \text{ do AEE digerível (1 g da proteína)}}{mg \text{ do mesmo AEE em 1 g da proteína referência}} \times 100$$

AEE = Aminoácido Essencial

4.5.11. Respirometria de Bartha e Pramer

O método respirométrico, descrito por Bartha e Pramer (1965) foi usada para avaliar o ajustamento dos fungos do substrato estudado. O respirômetro de Bartha e Pramer (1965) consiste em um sistema fechado, apresentando duas câmaras conectadas. Em um das câmaras

ocorre a biotransformação do substrato, o qual libera CO₂ no sistema. A outra câmara possui solução alcalina de KOH (de molaridade conhecida) necessária para reagir com o CO₂ produzido pela respiração microbiana. O acompanhamento da biodegradação do substrato nos respirômetros de Bartha foi realizado por uma nova técnica estabelecida por Uemura, Morales e Angelis (2013) no Laboratório de Toxicidade de Águas da Profa. Dejanira de Franceschi Angelis da UNESP – Campus Rio Claro.

A técnica permite medir a saturação de CO₂ na solução 0,2 M de KOH mediante medidas de condutividade (MONTAGNOLLI, 2015). Assim, garantiu-se eficiência e menor risco de variações nos resultados, sendo que a diminuição da alcalinidade da solução de KOH permite avaliar indiretamente o crescimento do micro-organismo, mediante titulação com solução ácida com molaridade conhecida.

4.5.12. Carboidratos totais

A quantificação de carboidratos totais dos materiais testados foi realizada segundo o método desenvolvido por Chaplin e Kennedy (1994).

4.5.13. Análise estatística

A significância estatística das amostras foi verificada por meio de análise de variância (ANOVA), utilizando Minitab expresso v. 1.4.0, a fim de comparar as diferenças entre as amostras (fermentado e não fermentado) de proteína, ácidos graxos, aminoácidos essenciais, cinzas, PDCAAS, RNV e DIAAS.

4.5.14. Confirmação por sequenciamento das espécies fúngicas utilizadas

Os fungos utilizados para a realização da FES foram identificados molecularmente pelo laboratório de sequenciamento do Centro de Pesquisa Interdisciplinar (IFZ), Instituto de Biologia Micro e Molecular, Universidade Justus-Liebig, Giessen, Alemanha. As regiões de ITS (Internal Transcribed Spacer) foram sequenciadas em um analisador genético automático

ABI Prism 310 (Applied Biosystems), a fim de comprovar a identificação dos fungos utilizados nos experimentos.

5. RESULTADOS E DISCUSSÃO

5.1. Escolha do substrato para desenvolvimento da FES

Previamente foi realizado um experimento com diferentes quantidades de cada co-produto com a finalidade de avaliar a preferência do fungo para seu melhor desenvolvimento. Desta maneira, foram testadas 4 diferentes misturas para cada fungo, conforme descrito na seção “Material e Métodos”, Tabela 1.

A escolha do substrato ideal para a realização da FES foi definida pelo substrato que apresentou crescimento mais rápido e, conseqüentemente, mais evidente. Desta maneira, o substrato escolhido e utilizado para a FES de cada micro-organismo foi:

- *Agaricus blazei*: FM:BM – 3:7
- *Lentinula edodes*: FM:BM – 3:7
- *Pleurotus ostreatus*: FM:BM – 1:9
- *Rhizopus oligosporus*: FM:BM – 5:5

5.2. Peso seco dos subprodutos da mistura para adequação da umidade

A umidade determinada para o início da FES foi de 74 %, pois de acordo com Jecu (2000), esta umidade foi a que apresentou melhores resultados em estudos desenvolvidos em FES para a obtenção de enzimas provenientes de fungos filamentosos. Para garantir umidificação adequada dos substratos foram determinadas as umidades do bagaço e das folhas da mandioca.

Os resultados obtidos estão disponibilizados na Tabela 2:

Tabela 2 – Determinação da umidade de bagaço e folhas de mandioca

	Dessecação à 105 °C (%)	Sartorius MA 35 (%)
Folhas de mandioca	79,36	81,12
Bagaço de mandioca	13,79	12,77

Mediante estes resultados, a umidade do substrato foi corrigida de acordo com a relação de folhas e bagaço de mandioca, sendo utilizado os valores obtidos em equipamento de medição de umidade em infravermelho (Sartorius MA 35). Assim, a correção da umidade foi realizada para garantir que o substrato, no início da FES, contivesse exatamente 20 g de matéria sólida e 74 % de umidade conforme descrito na Tabela 3.

Tabela 3 – Correção da umidade nos substratos para fermentação

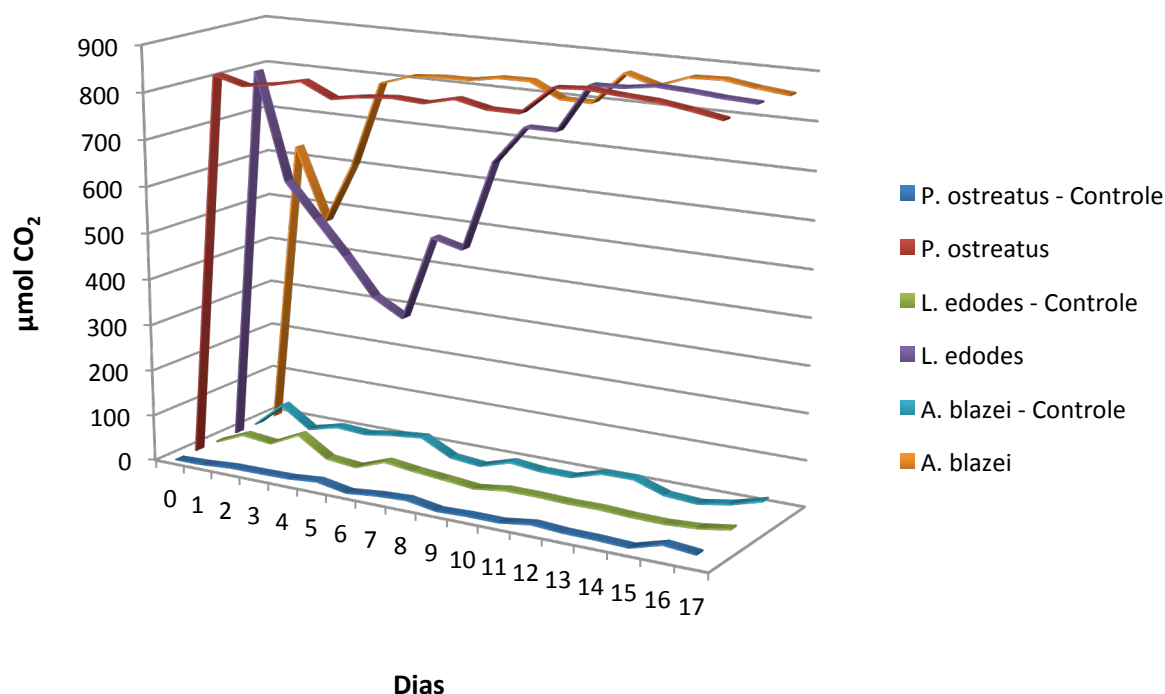
Substrato	Folhas (FM)	Bagaço (BM)	H ₂ O presente	H ₂ O necessária
<i>A. blazei</i>	31,8 g	16,0 g	27,83 g	39,35 g
<i>L. edodes</i>	31,8 g	16,0 g	27,83 g	39,35 g
<i>P. ostreatus</i>	10,6 g	20,6 g	11,23 g	55,95 g
<i>R. oligosporus</i>	53,0 g	11,5 g	44,43 g	22,75 g

FM: Folhas de Mandioca; BM: Bagaço de Mandioca.

5.3. Aplicação da respirometria de Bartha e Pramer para avaliar a adaptação e crescimento dos basidiomicetos

A respirometria de Bartha permitiu observar a adaptação de cada fungo no substrato escolhido para o desenvolvimento dos processos fermentativos em estado sólido. A Figura 1 apresenta as curvas de produção de CO₂ dos substratos colonizados e dos substratos controle, permitindo, assim, visualizar a adaptabilidade e possível desempenho.

Figura 1 – Curvas de produção de CO₂ nos processo de fermentação em estado sólido.



As informações presentes na Figura 1 mostram que o *P. ostreatus* apresentou rápida adaptação ao substrato utilizado para a FES, atingindo o máximo de crescimento imediatamente à inoculação. Com relação ao *L. edodes*, este necessitou de maior tempo para iniciar seu pleno desenvolvimento, sendo que, somente após o 6º dia foi observada a ascendência do seu crescimento. Tais resultados indicam a maior necessidade de tempo para o *L. edodes* colonizar completamente o substrato quando comparado com o *P. ostreatus*.

O *A. blazei*, apesar de mostrar melhor adaptação ao substrato que o *L. edodes*, não mostrou o mesmo desempenho no processo de FES em maior escala.

5.4. Experimentos com *Agaricus blazei*

Os experimentos realizados com o *A. blazei*, apesar de apresentarem crescimento mais acelerado que o *L. edodes*, mostraram difícil adaptação frente ao substrato testado (FM:BM – 3:7) quando aumentada a escala do respirômetro para a fermentação em sacos de PP,

dificultando o seu desenvolvimento na FES e, conseqüentemente, a obtenção de dados. Essa dificuldade na adaptação e crescimento representa maior probabilidade na ocorrência de contaminações e, conseqüentemente, menor possibilidade de emprego desse micro-organismo em procedimentos que exigem velocidade maior de crescimento para garantir custos menos elevados ao processo.

Contudo, o fungo é conhecidamente uma fonte de nutrientes importantes sendo, inclusive, utilizado em tratamentos alternativos de combate a diversas doenças, incluindo câncer (LEE, 2015). Assim, devido às dificuldades apresentadas, se faz necessário o desenvolvimento de estudos exclusivos com relação a sua adaptação, desenvolvimento, adequação de substrato, entre outros parâmetros para a obtenção de resultados mais satisfatórios que possam mostrar o real potencial deste fungo.

5.5. Confirmação por sequenciamento das espécies fúngicas utilizadas

Para a verificação das espécies de fungos utilizadas neste trabalho, foi realizado o sequenciamento genético. Os resultados dos sequenciamentos das quatro espécies de fungos utilizadas estão apresentados em anexo e mostram que os fungos utilizados neste trabalho correspondem exatamente aos propostos inicialmente, sendo possível determinar a identidade dos fungos com 98% de certeza para o *Rhizopus microsporus* var. *oligosporus*; 82% de certeza para o *Pleurotus ostreatus*; 99% de certeza para o *Lentinula edodes*; e 74% de certeza para o *Agaricus blazei*.

Como se trata apenas de confirmação de espécie, a porcentagem de assertividade relacionada as espécies sequenciadas confirma a utilização correta dos fungos. Porcentagens menores podem estar relacionadas à falhas na sequência durante a extração.

5.6. Artigos

O campo científico necessita da divulgação dos resultados de maneira rápida e efetiva e, desta maneira, os resultados desse trabalho foram divididos em três artigos, sendo cada um referente à um fungo utilizado, com exceção do *A. blazei*. O primeiro artigo trata os resultados obtidos com os experimentos relacionados ao *R. oligosporus*, o segundo apresenta os resultados obtidos com *P. ostreatus* e, conseqüentemente, o terceiro relacionado ao fungo *L. edodes*.

ARTIGO 1 - Characterization of By-Products of Cassava Fermented by *Rhizopus oligosporus*

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Resumo

O Brasil é um dos principais produtores e processadores de mandioca do mundo e, portanto, gera grande quantidade de produtos considerados resíduos mas, com potencial de utilização. Folhas e bagaço de mandioca são os dois principais coprodutos gerados, e juntos apresentam quantidade elevada de proteínas e carboidratos, com qualidade ideal para o processo de fermentação em estado sólido (SSF). Com o objetivo de verificar a influência de SSF em substrato contendo folhas e bagaço de mandioca, foram realizadas avaliações quanto à proteínas, aminoácidos, ácidos graxos e cianeto. A qualidade da proteína foi determinada pelos métodos de Índice de Aminoácido Corrigido pela Digestibilidade Proteica (PDCAAS) e Valor Nutricional Relativo (RNV) que medem a capacidade da proteínas em fornecer aminoácidos essenciais para o homem. O conteúdo de cianeto e de carboidratos totais foram medidos utilizando o método colorimétrico. O perfil de aminoácidos foi obtido utilizando a cromatografia líquida (HPLC) e os ácidos graxos por cromatografia gasosa (GC). O material fermentado mostrou decréscimo na quantidade de cianeto, permitindo classificar o material como não tóxico. O perfil de amino ácidos apresentou quantidades elevadas de histidina, isoleucina e leucina, que pode fornecer mais do que 60% da necessidade diária para crianças de 2-5 anos de idade. Quantidades elevadas de ácidos graxos foram observadas para o ácido cis-13,16-docosadienóico (22:2), ácido α -linolênico (18:3^{9,12,15}) e ácido oleico (18:1). Resultados obtidos para PDCAAS foram baixos em comparação com a caseína, contudo, os resultados do RNV revelaram uma grande diferença para a qualidade da proteína após a fermentação. Conclui-se que o material fermentado obtido mediante da utilização de subprodutos de mandioca fermentados por fungo comestível pode gerar nova fonte de alimentos com razoável quantidade de nutrientes indispensáveis.

Palavras-chave: Mandioca; aminoácidos; ácidos graxos; cianeto; PDCAAS; RNV; alimento fermentado.

Abstract

Brazil is a leading producer and processor of cassava in the world and therefore generates large amounts of waste with the potential for utilization. The leaves and bagasse of cassava are the two main by-products generated, with high amounts of protein and carbohydrates that make them ideal for the solid-state fermentation (SSF) process. With the aim of determining the influence of SSF on a substrate containing both the cassava leaves and bagasse, assessments of the proteins, amino acids, fatty acids and cyanide were performed. The quality of the protein was determined by the Protein Digestibility Corrected Amino Acid Score (PDCAAS) and Relative Nutritional Value (RNV) methods that measure the capability of proteins to provide essential amino acids for humans. The cyanide and total carbohydrate contents were measured using colorimetric methods. The amino acid profile was obtained using liquid chromatography and that of the fatty acids by gas chromatography. The fermented material showed decreases in the cyanide amount, allowing the classification of the material as nontoxic. The amino acid profile presented high amounts of histidine, isoleucine and leucine that can provide more than 60% of the daily requirements for children 2-5 years of age. High amounts of the fatty acids cis-13,16-docosadienoic acid (22:2), α -linolenic acid (18:3^{9,12,15}) and oleic acid (18:1) were observed. The PDCAAS results were lower compared to that of casein, but the RNV revealed a great difference in the protein quality after fermentation. We conclude that the fermentation of the cassava by-products of leaves and bagasse by edible fungi can be considered an important strategy to achieve foods rich in nutrients that are essential to the human diet.

Keywords: Cassava; amino acids; fatty acids; cyanide; PDCAAS; RNV; fermented food.

Introduction

The most important sector of the Brazilian economy is agriculture and livestock. Brazil is one of the largest producers of coffee, sugar cane, soybeans, cassava, fruits and other crops. However, this huge agricultural production generates a great quantity of wastes that can become an environmental problem when these residues are incorrectly disposed (PANDEY; SOCCOL, 1998; PANDEY et al., 2000; SOCCOL; VANDENBERGHE, 2003).

Cassava (*Manihot esculenta* Crantz) is the most important cyanogenic crop and is an important food source for several tropical countries (SHARIF et al., 2015). Approximately

60% of all produced cassava is intended for human consumption, 33% for the animal feed industry, and 7% for other activities such as the textile and paper industries (PANDEY et al., 2000).

Cassava is mainly used in industry to obtain flour and starch, and the industrial process generates solid and liquid waste. The processing of 250-300 tons/day of cassava tubers generates approximately 280 tons/day of solid waste known as cassava bagasse (CB), with 80-85% humidity (JASKO et al., 2011; PANDEY; SOCCOL; MITCHELL, 2000). This residue is typically discarded in landfills, which is a waste of money, as the cassava residues present 7.5-10% starch (wet basis) and high amounts of fiber (CARTA et al., 1999; JASKO et al., 2011). The protein amounts in CB are low, making its use unattractive (PANDEY et al., 2000). However, the incorporation of another material rich in protein into the CB can provide a better fermentation condition for these residues and thereby obtain a final product that may be used as food.

Cassava leaves (CL) are the main by-product of cassava because the tuber is the target of interest. The leaves are rich in protein, vitamins and minerals and have an output estimated at approximately 2.25 tons per hectare. Although cassava leaves are a cheap source of protein, tons of this material are discarded after harvesting the tubers (ARO, 2008; LATIF; MÜLLER, 2015; NASSAR; MARQUES, 2006; SAGRILO, 2001). Many countries around the world (mainly in sub-Saharan Africa and Southeast Asia) use CL as a vegetable in their diets (LATIF; MÜLLER, 2015), but, as other agro-industrial by-products, CL have high concentrations of indigestible carbohydrates (non-starch polysaccharides) and anti-nutrients such as hydrocyanic acid (HCN), tannins (polyphenols) and phytates (IYAYI; TEWE; OKI, 1997; AKPAN; IKENEBOMEH, 1995).

To modify these anti-nutritional factors, Steinkraus (1995) proposes the application of a fermentation process to detoxify the substrate, enrich it in protein, improve both the essential amino acid and essential fatty acid profiles, and enhance the taste, texture and shelf life. Solid-state fermentation (SSF) can be characterized as an efficient strategy for obtaining food with significant protein content, with costs up to 78% cheaper than those of foods produced by traditional methods (COUTO; SANROMÁN, 2006).

Filamentous fungi are considered the best microorganisms for the SSF process, as they can be grown in almost any substrate that contains carbohydrates (HÖLKER; LENZ, 2005; JIN et al., 2002). The *Rhizopus* genus is quite common in the Orient, and it is used in the production of rice wine, enzymes and the preparation of foods such as miso and tempeh (HANDOYO; MORITA, 2006; JELEN' et al., 2013; WHELAN, 2008).

According to Wronkowska et al. (2015), higher contents of unsaturated fatty acids, amino acids and protein and improved protein digestibility (more than 87%) were obtained after 24 hours of SSF with *R. oligosporus*.

The main objective of this study was to evaluate the efficiency of *R. oligosporus* in a SSF carried out to increase the nutritional profiles of essential amino acids and unsaturated fatty acids and protein digestibility. Additionally, an aim was to analyze the degradation of toxic compounds present in the agro-industrial wastes of cassava production. This research was developed to obtain a nutritionally promising food to be incorporated into the human diet, especially for low-income populations.

Material and Methods

Microorganism

The fermenter microorganism chosen for this research was *Rhizopus microsporus* var. *oligosporus* CCT 3762 (ATCC 22959), acquired from the Tropical Research and Technology Foundation "André Tosello", Campinas – SP, Brazil.

R. oligosporus was inoculated in Petri dishes containing 2% malt extract agar medium for 72 hours at $24 \pm 2^\circ\text{C}$. After this period, the plate was sectioned into portions of 1 cm^2 , and 10 aliquots were transferred to bags containing the residue used as a substrate in this study. The experiment was performed in triplicate.

Substrate

To perform the solid-state fermentation (SSF), two materials derived from cassava (*Manihot esculenta* Crantz) and considered wastes were used as a composite substrate. Cassava bagasse (CB) is considered a waste from the starch extraction, and it has no use except for in animal feed. The other material, cassava leaves (CL), is considered a waste from cassava harvesting and has few uses.

Several mixtures of CB and CL were tested for fungal growth (CB:CL - %: 70:30; 60:40; 50:50; 40:60; 30:70), and the mixture that presented the best conditions was applied in the SSF process. The SSF was performed in triplicate using polypropylene (PP) bags containing 20 g of dry matter of the substrate for 10 days at $24 \pm 2^\circ\text{C}$. The initial substrate moisture was set to 74%.

Total Nitrogen

The total nitrogen was analyzed by the Kjeldahl method, and the factor to convert to crude protein was 6.25 (AOAC 1995 - method 928.08).

Digestion

The digestion was performed in 50-mL Erlenmeyer flasks containing 1 g of the tested sample (CL; CB and CB:CL-50:50%) and 7.5 mL of pepsin solution (2 mg mL⁻¹ in HCl 0.1 M) that were incubated at 37°C for 3 hours. After the incubation, the samples were alkalized with 7.5 mL of 0.2 M NaOH, followed by the addition of 3.75 mL of pancreatin solution (2 mg mL⁻¹ in 0.1 M phosphate buffer, pH 8) and a second incubation at 37°C for 24 hours. To stop the reaction, 5 mL of 30% trichloroacetic acid (TCA) (w/v) was added, and the volume was adjusted to 25 mL by the addition of 5% TCA (w/v). The samples were centrifuged at 10,000 RPM at 4°C for 30 minutes (AKESON; STAHMANN, 1964).

The digestibility of each sample was calculated as the difference between the total protein measured in the precipitate (non-digested material) and that obtained after digestion with pepsin and pancreatin, both determined by the Kjeldahl method (AOAC 1995). The digestibility of the samples was expressed as a percentage of solubilized protein.

Ash

The total amount of ash was determined according to the method described by the AOAC (1995 - method 920.153).

Total Carbohydrates

The quantification of the total carbohydrates in the samples was performed by the colorimetric method described by Chaplin and Kennedy (1994).

Cyanide

The total cyanide content (HCN) was analyzed by the colorimetric method described by Brito et al. (2009). The total cyanide measurement was made for all samples to determine the decrease in HCN during the fermentation and evaluate the possibility of using the fermented material as a source of food.

Fatty Acids

The lipids of each sample were extracted by the Soxtherm system using petroleum ether as solvent, as described by Matthäus and Brühl (2001).

The characterization of fatty acid methyl esters (FAME) was performed by removing a fraction (100-250 mg) of the extracted lipids from each sample and placing in a vial with a tightly screwed Teflon stopper. The lipids were saponified with 4 mL of 0.5 M methanolic sodium hydroxide, followed by heating at 80°C for 10 minutes. To promote the esterification, 5 mL of boron trifluoride methanol complex was added to the samples, and they were heated again at 80°C for 5 min. For the reaction, 5 mL of heptane was added, followed by another heating at 80°C for 1 minute. After cooling the samples, 3 mL of saturated sodium chloride solution was added, and the FAME were extracted after shaking for 30 seconds. The clear supernatant from each sample was separated into 4-mL vials to perform the quantification by gas chromatography (GC).

The patterns to quantify the fatty acid profiles were developed according to Ramani et al. (2004).

Essential Amino Acids (EAAs)

With the intent of determining the amount of EAAs (lysine, histidine, arginine, threonine, cysteine, valine, methionine, isoleucine, leucine, tyrosine and phenylalanine), acid hydrolysis was performed on each sample before and after the fermentation. Aliquots of approximately 200-250 mg of each sample were transferred to borosilicate tubes (previously pyrolyzed at 400°C for 8 h) with the further addition of 6 mL of 6 M HCl with 0.1% phenol (m/v). After a smooth homogenization, the tubes were kept for 10-20 seconds under a continuous flow of nitrogen gas to eliminate the O₂. The tubes were left with the stoppers slightly open for 1 hour at 110°C, and then these tubes were closed and kept at the same temperature for an additional 23 hours. The quantification of amino acids was performed using High-Performance Liquid Chromatography (HPLC) (MOORE; SPACKMAN; STEIN, 1958).

Protein Digestibility Corrected Amino Acid Scoring (PDCAAS)

The PDCAAS was calculated on the basis of the EAA requirements for children 2-5 years of age. This score is calculated by the following formula (TIRAPEGUI; MACEDO, 2007):

$$PDCAAS = \frac{\text{mg of limiting AA (1 g of sample)} \times \text{true digestibility (\%)}}{\text{mg of the same AA in 1 g of the reference protein}} \times 100$$

Relative Nutritional Value (RNV)

The assays to calculate the RNV were performed in triplicate. The samples were previously degreased using n-hexane in a Soxhlet apparatus (AOAS, 1995). Afterwards, the samples and the casein were hydrolyzed by shaking in 4 mL of pronase Sigma P-5147 (1 mg mL⁻¹) and 40 mL of sodium glycerophosphate buffer (Sigma G-5422 – 20 g L⁻¹), adjusted to pH 8.0 (with NaOH 0.1 M or H₃PO₄ 0.033 M) at 48°C for 3 hours. After the hydrolysis, the pH and the volume of the samples were adjusted to 7.2 and 100 mL, respectively, and the samples were sterilized using a 0.22-µm filter (cold sterilization). Then, 2 mL of the basal medium, 2 mL of the hydrolyzed sample, 7 mL of sterilized water and 100 µL of *Enterococcus zymogenes* inoculum were incubated in the test tube at 37°C for 48 hours. Subsequently, the samples were sterilized by autoclaving (121°C/15 min), and the growth measurements were performed by acidimetry. The titration of each tube was performed with 0.01 M NaOH or 0.0033 M H₃PO₄. The total volume used in the test was calculated discounting the volume used for the titration of the blank (carried out with inactive inoculum). The data obtained in the test with *E. zymogenes* were compared with the value of a standard protein (casein - SIGMA C-7078), considered 100%.

The determination of RNV was taken from the growth of *E. zymogenes*, which occurs according to the quality of the protein present in the sample (TAVANO et al., 2004). The growth response is related to the absolute nutritional necessity of EAAs by the microorganism such as arginine, glutamic acid, histidine, isoleucine, leucine, methionine, tryptophan, valine and lysine. Even though lysine is not considered an EAA to *E. zymogenes*, its deficiency can impair the growth of the microorganism (FORD 1962; TAVANO et al., 2004). As the amino acid needs of this microorganism (*E. zymogenes*) are very similar to human needs (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine), this indirect method of the evaluation of the RNV presents a good match between both organisms because the enzymes used in the hydrolysis of the sample are the same enzymes present in the human gastrointestinal tract.

Statistics

The significance of the results was verified by one-way analysis of variance (ANOVA) using Minitab Express v. 1.4.0, comparing the differences between the samples (fermented and unfermented material) for protein, fatty acids, amino acids, ash, PDCAAS and RNV.

Results and Discussion

Substrate selection and moisture definition

Experiments with different quantities of cassava bagasse (CB) and cassava leaves (CL) were performed to find the ideal mixture to be used as a substrate to promote the solid-state fermentation (SSF) with the fungus *Rhizopus oligosporus*. After testing several combinations of CB and CL, it was observed that the 50:50% mixture (CB:CL – 1:1) led to the best results, showing the fastest mycelium growth.

The fermentation was performed in triplicate, with each fermentation bag containing 76.9 g of the moist substrate corresponding to 20 g of the mixture on a dry basis. To calculate the amount of water necessary to be included in the substrate, the moistures of the leaves and bagasse were assessed with an infrared moisture analyzer (Sartorius MA 35). This test showed results of 12.77% for the CB and 81.12% for the fresh CL.

Krishna (2005) related that 70-80% water is necessary in the SSF system because the great majority of viable cells contain this water content. In the present study, the fermentation was carried out with 74% moisture, considering the moisture content of the total materials used to prepare the substrate. In 20 g of the mixture (dry basis), there was determined to be a total of 44.4 mL water derived from the bagasse and leaf samples. Thus, to obtain a substrate with 74% moisture, it was necessary to include a further 12.5 mL of water. According to Jecu (2000), who carried out SSF tests with wheat bran and *Aspergillus niger*, a moisture of 74% presented the best results compared with other values (55, 60 and 80%). Suhet and Fioreze (2011) performed a SSF on pineapple residue with *R. oligosporus* using 75.2% moisture in the substrate, similar to the value used in this work.

Total carbohydrates

Normally, wastes from agriculture and food are the main substrates used to perform SSF due to their high contents of carbohydrates and other nutrients (COUTO; SANROMÁN, 2006). Hence, it is important to quantify the amount of carbohydrates present in the substrate used. The total carbohydrates had values of $72.77 \pm 6.50\%$, $58.74 \pm 8.06\%$ and $86.80 \pm 18.28\%$ for the CL:CB (1:1) unfermented substrate, CL, and CB, respectively.

According to Fakas et al. (2009), carbohydrates are the major nutrients consumed during fermentation by the fungus, and almost all of the sugar consumed by them is in the form of reducing sugars. During the process of the SSF (CL:CB – 1:1), it is possible to prove that there is a high consumption of sugar in the process, as the amount of total carbohydrates observed at the end of the fermentation period (10 days) was $57.39 \pm 0.43\%$. This result represents a decrease of 21.14% in the total amount of carbohydrates.

The degradation of toxic compounds is closely associated with the development of the fungus and its respiration and also with the consumption of reducing sugars from the substrate (SOCCOL; VANDENBERGHE, 2003).

Cyanide

Cassava is the most important cyanogenic crop in the world, due to its wide use as food in countries with a high population density, such as the tropical countries (SHARIF et al., 2015). Regarding the quantity of cyanhydric acid, cassava is classified into 3 categories: very toxic –more than 100 mg HCN/kg (ppm); moderately toxic –50-100 mg HCN/kg (ppm); and not toxic –lower than 50 mg HCN/kg (ppm) (KOBAWILA et al., 2005).

Cyanhydric acid is a very dangerous compound that can even be lethal to the exposed organism. Thus, knowing the toxicity of the cassava variety used for nutritional purposes is very important. In the present study, the analyses showed that the leaves contained 138.58 ± 4.12 mg HCN/kg (ppm) and the bagasse 7.49 ± 1.72 mg HCN/kg (ppm). Hence, it is possible to classify the leaves as very toxic and the bagasse as not toxic. The substrate used in the SSF (CL:CB) presented 73.03 ± 2.84 mg HCN/kg (ppm), showing intermediate amounts of HCN according to the classification of Kobawila et al. (2005), making it moderately toxic and dangerous to consumers.. Thus, the mixture itself (CL: CB) used to perform the SSF also contributed to the decrease in the HCN concentration of the substrate.

After the SSF process, the quantification of HCN was performed on the sample, and the result after 10 days of fermentation was 34.36 ± 9.61 mg HCN/kg (ppm). This result confirms the finding of Soccol and Vanderberghe (2003) that the consumption of carbohydrates is linked to the degradation of toxic compounds by the fungus used in the fermentation. So, the fermented substrate was reclassified from "moderately toxic" to "not toxic" (KOBAWILA et al., 2005).

Lipids and Fatty acids

Cassava bagasse, a by-product from the starch extraction industry, presents low amounts of lipids and protein, as it is mainly composed of carbohydrates and ash (TONUKARI, 2004). The total quantity of lipids found in the CB used in this study was $0.07 \pm 0.05\%$. The leaves showed $4.76 \pm 0.74\%$ total lipids content. This result corroborates the results of Morales (2012), who found approximately 5% lipids in the CL. The mixture used for the substrate (CL:CB – 1:1) before the fermentation showed a total amount of lipids of $2.42 \pm 0.28\%$. The lipid amount in the fermented substrate increased to $4.40 \pm 0.90\%$. Feng *et al.* (2014) found

similar behavior with the same fungus when rice was fermented to prepare *koji* (based on the fermentation of rice by *Aspergillus oryzae* in sake production). The authors found increases in the lipid content (16.55% to 20.22%) after 44 hours of fermentation. The results obtained by Fakas et al. (2009) showed increases in the lipid content until 212 hours of SSF of pear pomace using the fungus *Mortierella isabellina* as a fermenter agent. After this time, the authors observed a decrease in the lipid content because of the exhaustion of the reducing sugars.

The profile of fatty acids showed changes after fermentation with *R. oligosporus*. The characterizations of the fatty acids in the unfermented substrate and in that fermented by *R. oligosporus* are presented in Table 1.

The fatty acids are divided into 3 classes: 1. Saturated Fatty Acids (SFAs) –containing double bonds in the molecule that do not become liquid at temperatures above 50°C; 2. Monounsaturated Fatty Acids (MUFAs) – presenting only a single double bond in the molecule that changes the melting points to 2-4°C; and 3. Polyunsaturated Fatty Acids (PUFAs) – containing two or more double bonds in the molecule and have melting points that are inversely proportional to the number of double bonds and proportional to the carbon chain size (GERMAN, 2011; MENSINK et al., 2003; TOLEDO et al., 2013; ZIELIŃSKA; NOWAK, 2014).

Zielińska and Nowak (2014) state that SFAs, such as myristic acid, palmitic acid and arachidic acid, are commonly found in vegetables. According to Rustan and Drevon (2005), the most common saturated fatty acid in plants, animals and microorganisms is palmitic acid (16:0), which is therefore widely present in the diet (GERMAN, 2011). Table 1 shows the results according to statements previously cited in the literature, showing the presence of the most common SFAs in vegetable oils, including myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0) and arachidic acid (20:0). Although palmitic acid is an endogenously synthesized SFA, diets with a high consumption of SFAs can elevate the levels of circulating cholesterol (GERMAN, 2011). All of these SFAs, except for palmitic acid, were found at slightly higher levels in our fermented product (0.352%) when compared with the values of the unfermented product (0.319%), but the total amount of these SFAs was still below the daily recommendation by Vannice and Rasmussen (2014) of intakes lower than 7-10% or “as low as possible” in a diet with a recommended daily intake of total fat between 20 and 35%.

Table 1 also presents the variations between the MUFAs and PUFAs before and after fermentation. The results show increases in all unsaturated fatty acids (except for α -linolenic

acid - 18:3^{9,12,15}, which decreased in concentration, and linoleic acid - 18:2^{9,12}, which showed no variation in its concentration).

Iluyemi et al. (2006) found similar results upon performing the SSF of palm kernels (*Elaeis guineensis*) with many fungal strains. Their results showed improvements in oleic acid, linoleic acid and linolenic acid contents, proving that the SSF with filamentous fungi can upgrade the characteristics of unsaturated fatty acids, especially the essential fatty acids.

n-3 and n-6 PUFAs are the most important polyunsaturated fatty acids in the human diet because humans do not synthesize these molecules, making them essential fatty acids (VANNICE; RASMUSSEN, 2014). Mensink et al. (2003) concluded that the risk of coronary artery disease is reduced when there is a substitution of the consumption of trans fatty acids and saturated fatty acids with cis-unsaturated fatty acids. The major fatty acids present in the fermented substrate tested here were cis-13,16-docosadienoic acid (22:2), α -linolenic acid (18:3^{9,12,15}), oleic acid (18:1) and palmitic acid (16:0). Hence, despite the lower concentration of α -linolenic acid (compared with the unfermented substrate), the results obtained after the SSF showed a higher concentration of PUFAs, mainly related to the *cis*-unsaturated fatty acids.

Protein content and digestibility

The substrate prepared for the fungus growth presented $20.13 \pm 0.39\%$ crude protein (CL: $38.7 \pm 0.60\%$ and CB: $1.6 \pm 0.18\%$). The digestibility *in vitro* was calculated to be 25.31% for the unfermented substrate (Leaves: 19.28% and Bagasse: 31.33%).

After 10 days of fermentation, the results obtained for the total protein were $25.3 \pm 1.85\%$. However, this amount includes protein that cannot be used as a source of amino acids because fungal protein normally presents chitin, the main component of the cell wall besides glucan (FREE, 2013).

Thus, the digestible protein was analyzed in the final sample to find the total available protein content. The fermented substrate presented 79.52% digestibility (*in vitro*), which means $21.2 \pm 1.47\%$ absorbable protein. The digestible protein in the fermented substrate represents a higher initial crude protein content than in the unfermented substrate.

Comparing the digestibility between the unfermented and fermented substrates (25.31% and 79.52%, respectively), the total content of digestible protein in the fermented substrate was approximately 4 times higher than that in the unfermented substrate (21.2% and 5.09%, respectively).

Many authors working with fungi fermentation evaluated the total amounts of protein using different nitrogen conversion factors. Mattila et al. (2002) used 5 different factors (4.50, 4.55, 4.70, 4.97 and 6.25) to calculate the protein quantity in edible mushrooms. The authors used the factor 4.97 for the total protein of *Pleurotus ostreatus*, 4.55 and 4.70 for *Agaricus bisporus* (brown and white, respectively) and 4.50 for *Lentinula edodes*. The factor 6.25 (standard factor) was used to calculate the total energy (kcal/g per component) from proteins in all samples.

The digestibility *in vitro* of our substrate fermented with *R. oligosporus* (79.52%) was multiplied by the usual factor (6.25 factor, reported by AOAC, 1995) to obtain the digestible protein conversion factor, whose value was 4.97. This result corroborates the data of Mattila et al. (2002), who found the same values of protein digestibility in studies carried out with the fungus *P. ostreatus*.

According to Morales (2012), the SSF with *R. oligosporus* of a substrate containing CL and babassu mesocarp could improve the content of protein to 7.69% in only three days of fermentation. The same author found 77.4% digestibility in a fermented substrate using the same fungus. Kiers et al. (2003) observed a significant body weight gain in experiments with piglet feed with soybean fermented by *R. oligosporus*. The piglets showed masses of 1.64 kg more than that of the control in the final 28 days, indicating the improved availability and digestibility of the nutrients.

Amino acids and PDCAAS

According to Wu (2009), amino acids (AAs) are directly related to the protein synthesis, osmolytes (compounds affecting osmosis), gene expression, regulation of hormone secretion and cell signaling. Regarding the nitrogen balance in the diet, AAs are classified nutritionally as essential (EAAs) and non-essential (NEAAs) for humans and animals. EAAs are described as carbon structures not synthesized or inadequately synthesized but strictly necessary for living organisms and are only being incorporated through diet. On the other hand, NEAAs are AAs that are synthesized by the organisms in optimal requirements for their own needs (WU, 2009).

Table 2 presents the increases in EAAs after the SSF experiments. The results comparing the unfermented and fermented substrates after applying the digestibility already previously mentioned show increases in some EAAs. Lysine (Lys) was the only EAA that presented a significant decrease ($P < 0.05$). After fermentation, there were significant increases ($P < 0.05$) observed for isoleucine (Ile) and leucine (Leu), which, like valine (Val), are branched-chain

amino acids (BCAAs). According to Rogero and Tirapegui (2008), approximately 35% of the total EAAs present in the muscle protein are related to BCAAs. Ile is directly linked to the synthesis of glutamine (Gln) and alanine (Ala) (WU, 2009). Gln and Ala are NEAAs and are therefore synthesized by humans. However, the organisms require the ingestion of Ile to produce Ala and Gln. These two AAs are associated with important metabolic mechanisms. Ala is associated with the inhibition of pyruvate kinase and hepatic autophagy, gluconeogenesis, transamination and the glucose-alanine cycle. Gln is an important AA in the regulation of protein turnover, gene expression, immune function, and the synthesis of purine, pyrimidine, arginine, proline and NAD(P) (WU, 2009). Leu (EAA) is also related to the regulation of protein turnover, gene expression and activation of glutamate dehydrogenase, besides being a flavor enhancer (WU, 2009).

PDCAAS is a preferential method to measure the proteic value of the human diet. The measured PDCAAS of the fermented substrate (CL:CB – 1:1) was much lower than the casein value. The limiting EAA observed was threonine (Thr), with a PDCAAS of only 0.1270 (casein PDCAAS value is 1.0000). This result allows us to suppose that the protein of the CL:CB – 1:1 is not adequate to provide the ideal amount of EAAs, but, regarding the presence of all EAAs in the fermented substrate (CL: CB) and the daily needs of such AAs for children 2-5 years old, the fermented substrate presented appropriate amounts (≥ 0.60 mg of EAA/g of protein) of histidine (His), Ile and Val; reasonable quantities (0.30 to 0.59) of Leu, phenylalanine/tyrosine (Phe + Tyr); and low amounts (< 0.30) of Lys, methionine/cysteine (Met + Cys) and threonine (Thr) (Table 3).

Histidine is an important AA for protein methylation, hemoglobin structure and function, antioxidative dipeptides, vasodilation, central acetylcholine secretion, allergic reaction and modulation of the immune response in skin (WU, 2009). This AA was detected in a higher amount in the fermented substrate (CL:CB – 1:1).

Relative Nutritional Value (RNV)

According to Tavano et al. (2004), the microbiological method carried out with *Enterococcus zymogenes* is a significant method to assess the RNV and protein quality. The authors concluded that this method is reliable and adequate to assess the presence, quality and EAA bioavailability, as well as being responsive to obtain results.

The results observed in the fermented substrate showed a significant increase ($P < 0.01$) in the RNV relative to the unfermented substrate. The unfermented substrate showed an RNV of $7.83 \pm 1.20\%$ (after considering the digestibility), while the fermented material in the same

condition had an RNV of $69.325 \pm 7.06\%$ ($P < 0.05$). Bender et al. (2013) assessed the RVN for different sources of food (in comparison with casein) and found the following results: egg albumin – 105-125%; gelatin – 29-89%; and gluten – 15-64%. Tavano et al. (2004) evaluated the RNV in many isolated chickpea proteins: crude chickpea meal – $69.87 \pm 2.78\%$; crude chickpea albumin – $87.98 \pm 0.43\%$; cooked chickpea albumin – $98.48 \pm 0.49\%$; total crude chickpea globulin – $42.88 \pm 0.88\%$ and total cooked chickpea globulin – $85.33 \pm 3.72\%$.

The results obtained for the fermented substrate (CL:CB) with *R. oligosporus* showed an increase in the protein quality (7.830 ± 0.693 for the unfermented substrate versus $69.325 \pm 4.078\%$ for the fermented substrate). These results observed for cassava substrate indicated a nutritional value close to that of chickpea flour ($69.87 \pm 2.78\%$), which is considered a good-quality food.

Ash

The presence of minerals is associated with the content of ash. Minerals are agents in biochemical processes in the body and are therefore essential for life (GARCIA et al., 2015; GUPTA; GUPTA, 2014).

Ash includes important components present in the composition of a food and can change during the fermentation. Ash is related to the presence of minerals, and their function is associated with the regulation of metabolism, acting in important processes such as digestion and motor functions (FRANCO, 1992; SIZER; WHITNEY, 2003).

The analyses carried out on the unfermented substrate showed $4.65 \pm 0.14\%$ ash on a dry basis in CL:CB - 50:50%. When the CL and CB were singly analyzed, the results were CL: $7.30 \pm 0.30\%$ and CB: $2.00 \pm 0.09\%$. In the fermented material, the ash content was $3.89 \pm 0.15\%$. Many authors have found increases in the ash amounts after the SSF, although Silva et al. (2014) found both increases and decreases in the ash related to the material used as substrate and the time of fermentation. These authors reported an increase in the ash content for periods between 0-5 and 15-40 days of fermentation and a decrease between 5-10 days, when using *Lichtheimia ramosa* in the SSF in culture medium containing araticum (*Annona coriacea*). In the assays performed with CL:CB CB – 50:50% over the same period (5-10 days), there was a decrease in the ash. According to Afoakwa et al. (2013), the natural fermentation process in Ghanaian cocoa beans (*Theobroma cacao*), observed in periods of 0, 7, 14 and 21 days, induced a decrease in the amount of ash, especially in relation to Fe, Na, P, K and Mg.

Macrominerals mainly consist of calcium (Ca) – important for bones and teeth, muscle contraction, coagulation, blood pressure, and immune defense; phosphorus (P) – an important constituent of the genetic material, cell membranes, energy transfer and buffer systems; magnesium (Mg) – bone mineralization, protein construction, enzymatic action, transmission of nerve impulses and maintenance of teeth; sodium (Na) – critical in the transmission of nerve impulses and, together with potassium and chlorides, maintaining cellular water and the acid-base balance of body; potassium (K) – acts in the production of protein, fluid and electrolyte balance, cell integrity, transmission of nerve impulses, and muscle contraction (including the heart); chlorides – mainly linked to digestion; sulfur (S) – a component of some amino acids, vitamins (thiamine and biotin) and insulin, detoxification of harmful substances, conformation of the protein (S-S bonds) (SIZER; WHITNEY, 2003).

Trace minerals: iodine (I) – component of thyroid hormone (thyroxine) that regulates the growth, development and metabolic rate of body; iron (Fe) – component of hemoglobin and myoglobin; zinc (Zn) – component of insulin and several enzymes, immune reactions, transport of vitamin A, taste, wound healing, sperm production and proper fetal development; selenium (Se) – prevents harmful reactions to the cell; fluorine (F) – formation of bones and teeth and confers resistance to caries; chromium (Cr) – associated with insulin and necessary for the release of energy from glucose; and copper (Cu) – constituent of many enzymes and acts in the formation of hemoglobin (SIZER; WHITNEY, 2003).

These data show the importance of minerals in the daily diet.

Conclusion

The high amount of protein from the CL and the high amount of carbohydrates from the CB provided a good substrate for *R. oligosporus* growth, allowing the fast development of the mycelia of the fungus, avoiding unnecessary costs during the fermentation process.

The decreasing quantity of cyanide shows that the high amounts of carbohydrates consumed during the SSF process allowed the microorganism to detoxify the substrate, enabling the reclassification of the fermented product (CL:CB – 1:1) as “not toxic”.

The action of *R. oligosporus* during the SSF improved the amount of lipids, presenting an important enhancement of MUFAs and PUFAs that can provide access to better fats for consumers. The fungus also changed the EAA profile, providing access to good amounts of EAAs that are important in metabolism.

The PDCAAS showed a low value when compared to casein because casein is considered a complete protein. This was attributed to the fact that the limiting EAA (threonine) showed a

very low concentration. Therefore, the fermented product (CL:CB – 1:1) presented good quantities of other important EAAs such as histidine, isoleucine, valine and leucine.

Another important result in this work is connected to the RNV of the material used. The biological quality of the protein after SSF showed a significant improvement, which demonstrates that the real effect of SSF with *R. oligosporus* is to promote the biological value of protein. This means that the protein inside the fermented material is accessed more easily by the body, resulting in a better protein yield during the metabolism of the product.

The presence of minerals is related to the ash content. Thus, the fermented material can include minerals in the diet, providing more indispensable nutrients for those who consume it.

Regarding a new source of food made from agro-industrial by-products, the SSF proved to be an important tool to promote the increase in healthy nutrients (unsaturated fatty acids and essential amino acids) in vegetable substrates from agro-industrial wastes, making possible the consumption of materials previously improbable.

These results mean that agro-industrial wastes can be biotransformed to food sources that present good amounts of EAAs and unsaturated fatty acids, providing good amounts of nutrients to consumers.

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Table 1 – Characterization of fatty acids (mg/100g of Edible Portion) before and after SSF process.

		Substrate	<i>R. oligosporus</i> SSF
Myristic acid	14:0	44.378 ± 0.009	79.281 ± 0.024 *
Palmitic acid	16:0	240.877 ± 0.054	206.482 ± 0.041
Palmitoleic acid	16:1	5.111 ± 0.002	4.609 ± 0.007
Stearic acid	18:0	31.155 ± 0.005	42.136 ± 0.006 *
Oleic acid	18:1	101.437 ± 0.031	254.330 ± 0.009 *
Linoleic acid	18:2 ^{9,12}	134.257 ± 0.034	134.773 ± 0.021
γ-Linolenic acid	18:3 ^{6,9,12}	ND ^a	35.009 ± 0.003 *
α-Linolenic acid	18:3 ^{9,12,15}	689.621 ± 0.107	292.536 ± 0.041 [†]
Arachidic acid	20:0	2.373 ± 0.003	24.093 ± 0.004 *
cis-11-Eicosenoic acid	20:1	1.492 ± 0.002	25.120 ± 0.011 *
cis-11,14-Eicosadienoic acid	20:2	4.668 ± 0.001	6.053 ± 0.009
cis-8,11,14-Eicosatrienoic acid	20:3	13.343 ± 0.019	ND ^a
cis-11,14,17-Eiconatrienoic acid	20:5	ND ^a	50.553 ± 0.014 *
cis-13,16-Docosadienoic acid	22:2	252.440 ± 0.004	356.787 ± 0.018 *
Total SFAs, MUFAs and PUFAs		1521.158 ± 0.156	1511.767 ± 0.119
Total lipids		2426.667 ± 282.872	4404.069 ± 895.716

^aND – Not Detected

* Fatty Acids that significant increase was observed (P < 0.05).

[†] Fatty Acids that significant decrease was observed (P < 0.05).

Table 2 – Characterization of Essential Amino Acids (EAA mg g⁻¹ of Protein)

EAA	FAO/WHO Pattern*	Essential Amino Acids Quantity				Casein*	
		Substrate		Fermented Substrate			
		Child	EAA	%	EAA	%	EAA
HIS	1.9	0.97	51.1	1.21	63.7	3.8	112
ILE [†]	2.8	0.91	32.5	1.69	60.4	4.4	157
LEU [†]	6.6	1.32	20.0	2.07	31.4	7.6	115
LYS	5.8	1.73	29.8	1.12	19.3	6.8	117
MET + CYS	2.5	0.39	15.6	0.40	16.0	3.5	140
PHE + TYR	6.3	1.56	24.8	1.65	26.2	7.0	111
THR	3.4	0.35	10.3	0.43	12.7	2.5	131
TRP	1.1	ND ^a	-	ND ^a	-	1.0	90.9
VAL	3.5	1.32	37.7	1.67	47.7	5.5	157

* According FAO/WHO (1985);

^a ND – Not Detected[†] AA that significant increases were observed (P < 0.05);

His (histidine); Thr (threonine); Val (valine); Met (methionine); Cys (cysteine); Ile (isoleucine); Leu (leucine); Phe (phenylalanine); Tyr (tyrosine); Lys (lysine); Trp (tryptophan).

Table 3 - Protein digestibility-corrected amino acid scores (PDCAAS) of Cassava Bagasse and Cassava Leaves (1:1), casein standard and daily necessity in mg of EAA /g of protein for children of 2-5 years old

Essential Amino Acids	Fermented Substrate †	Casein †	Amino acid ratio		Standard FAO/WHO 2 – 5 years
			<i>R. oligosporus</i> SSF *	Casein	
His	15.25	18.99	0.80	<u>1.00</u>	19
Ile	21.23	46.91	0.76	1.68	28
Leu	25.99	93.05	0.39	1.41	66
Lys	14.11	78.66	0.24	1.36	58
Met + Cys	4.96	30.14	0.20	1.21	25
Phe + Tyr	20.71	109.71	0.33	1.74	63
Thr	5.43	43.22	<u>0.16</u>	1.27	34
Trp	ND	ND	-	-	11
Val	21.00	54.95	0.60	1.57	35
PDCAAS	-	-	0.1270	1.0000	-

* True protein digestibility obtained *in vitro* – 0.7952 (79.52%);

† mg EAA /g of protein;

ND – Not Detected;

Data of casein according to PIRES et al. (2006); Standard protein 2-5 years, according FAO/WHO (1985);

Underlined value represents the limiting EAA;

His (histidine); Thr (threonine); Val (valine); Met (methionine); Cys (cysteine); Ile (isoleucine); Leu (leucine);

Phe (phenylalanine); Tyr (tyrosine); Lys (lysine); Trp (tryptophan).

ARTIGO 2 - Biotransformation of cassava by-products by *Pleurotus ostreatus* to obtain fortified foods

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Resumo

O bagaço de mandioca (BM) é o principal subproduto derivado de tubérculo de mandioca, usado para a obtenção de amido. Esse resíduo, gerado em grandes quantidades nos processos de beneficiamento desta cultura, apresenta um alto potencial de aproveitamento como alimento, devido à grande quantidade de carboidratos que possui. A folha da mandioca (FM) é um outro subproduto importante desta cultura, que contém uma significativa quantidade de proteína, que pode ser utilizada como substrato para processos de fermentação em estado sólido (FES). As características destes subprodutos permitem sua utilização na obtenção de um produto final com maior biodisponibilidade e quantidade de proteínas, aminoácidos essenciais (AAE) e ácidos graxos insaturados (AGI). O objetivo deste estudo foi avaliar a eficiência da ação do fungo *Pleurotus ostreatus* sobre um substrato com elevada quantidade de carboidratos (mistura de bagaço e folhas de mandioca), para a obtenção de farinha "enriquecida" com altos teores de AAE e AGI. Os resultados revelaram que, após FES, houve melhoria no teor de proteínas, com aumentos significativos dos AAE valina, isoleucina, leucina e metionina; aumentos também de ácidos graxos importantes, como o ácido oleico (18:1), ácido linoleico (18:2^{9,12}) e γ -linolênico (18: 3^{6,9,12}); e a diminuição no teor de cianeto, para concentrações consideradas não tóxica. Embora o PDCAAS (Protein Digestibility Corrected Amino Acid Score) e o Valor Nutricional Relativo (VNR) mostraram valores abaixo dos encontrados para a caseína, os resultados indicaram uma melhoria na biodisponibilidade das proteínas presentes na mistura estudada. Esses dados comprovam a eficiência da FES realizada por *P. ostreatus* para enriquecer alimentos preparados com subprodutos agroindustriais, caracterizando-se em uma promissora estratégia para obtenção de alimentos com melhores teores proteicos.

Palavras-chave: Aminoácidos essenciais; ácidos graxos insaturados; fermentação em estado sólido; resíduos agroindustriais.

Abstract

Cassava bagasse (CB) is the main by-product from cassava tubers, which are used for obtaining starch. This waste, generated in large quantities in the processing of this material, has a high potential for use as food because of its high content of carbohydrates. Cassava leaf (CL) is another important by-product that contains a significant amount of protein that can be used as a substrate for solid-state fermentation processes (SSF). The characteristics of these byproducts allow their use in obtaining a final product with greater bioavailability and amounts of protein, essential amino acids (EAAs) and unsaturated fatty acids (UFAs). The aim of this study was to evaluate the effects of *Pleurotus ostreatus* on a substrate with a high amount of carbohydrates (mixture of bagasse and leaves of cassava) to obtain an "enriched" flour with higher levels of EAAs and AGI. The results showed that SSF improved the contents of protein, with significant increases in the EAAs valine, isoleucine, leucine and methionine; significant increases in fatty acids such as oleic acid (18:1), linoleic acid (18:2^{9,12}) and γ -linolenic acid (18:3^{6,9,12}); and a reduction of the cyanide content, with concentrations considered to be non-toxic. Although the PDCAAS (Protein Digestibility Corrected Amino Acids Score) and Relative Nutritional Value (RNV) showed values below those found for casein, the results indicated improved bioavailability of the proteins present in the mixture studied. These data demonstrate the efficacy of SSF performed by *P. ostreatus* to enrich food made with agro-industrial by-products, suggesting a promising strategy for obtaining food with better protein levels.

Keywords: Essential amino acids; unsaturated fatty acids; solid state fermentation; fortified foods; agroindustrial waste.

Introduction

Cassava bagasse is obtained from the processing of the tuber for the extraction of starch. In this process, a lot of waste is generated, which poses a major problem for the industry because these by-products have a low value for sale and a high cost of disposal in landfills,

representing a loss of profit from this crop (FIORDA et al., 2013; VILHALVA et al., 2011). Over 60% of the dried residue consists of starch that was not extracted during the process. One way to recover this co-product is by using it to produce other foods with different nutritional characteristics, transforming the original food that is exclusively energetic into a product with high added nutritional value (STRAPASSON, 2003). Fermentation in solid state (SSF) is a fermentative process that has attracted interest, according to Pandey (2003), to add value to agro-industrial waste and decrease the content of anti-nutritional compounds.

Another byproduct of interest is cassava leaves, which, although rich in protein, vitamins and minerals, exhibit anti-nutritional compounds and cyanogenic glycosides. The presence of such compounds can be minimized by processing the cassava leaves prior to human consumption (LATIF; MÜLLER, 2015).

The cultivation of the *Pleurotus* genus fungi can be carried out on various agricultural wastes such as grass, sawdust, coconut shells, sugarcane bagasse (DONINI et al., 2005). The fast growth of the edible fungus is linked to enzymes that degrade the long-chain carbohydrates found in plant substrate. The use of the substrate by the fungus enables the use of low-cost materials (such as the residues from cassava) to produce foods with higher protein content (MINOTTO et al., 2008; SCHMIDT et al., 2003).

In addition to the improvement in the protein content, digestibility, fatty acids and amino acids, another important application of SSF is the detoxification of the substrate, decreasing the cyanide glycosides and making the final product safe (STEINKRAUS, 1995).

Regarding the application of products with high levels of carbohydrates, Chavan and Kadam (1993) proposed the use of protein-rich non-wheat flour as a tool to improve the nutritional composition of bakery products. Thus, the aim of this work was to evaluate the action of *P. ostreatus* on agro-industrial by-products, the possibility of using the final product as an “enriched” food containing essential fatty acids and bioavailable essential amino acids, and the capacity of detoxification of the fungus.

The aim of this study was to evaluate the potential of the FSS process promoted by the fungus *P. ostreatus* on a substrate with a high amount of carbohydrates (CL:CB - 1:9) to obtain an energy food with better levels of protein, EAAs, and AGI, to be referred to as "enriched flour".

Material and Methods

Microorganism

The fermenter microorganism chosen in this work was *Pleurotus ostreatus*, acquired from Fungi & Flora, Valinhos – SP, Brazil.

The culture of this fungus was carried out for 72 hours at $24 \pm 2^\circ\text{C}$ in Petri dishes filled with 2% malt extract agar. After this, the inoculation proceeded, with 10 cm^2 of the medium with mycelia (10 aliquots of 1 cm^2) added to each bag containing the substrate (performed in triplicate).

Substrate

To perform the solid-state fermentation, two byproducts from cassava (*Manihot esculenta* Crantz) were used as substrates. Cassava bagasse (CB) is a waste from the starch extraction, and cassava leaves (CL) are a waste from cassava harvesting.

Different mixtures of CB and CL were tested. The tested mixtures were (in CL:CB - %) 0:100; 10:90; 20:80; 30:70; 40:60. The mixture that presented the fastest growth of the fungus was applied in the solid-state fermentation (SSF) process. The SSF was performed in triplicate using polypropylene bags containing 20 g of the substrate on a dry basis in the chosen concentration. The incubation was performed at $24 \pm 2^\circ\text{C}$ for 10 days, and the beginning of the fermentation was accomplished at 74% humidity.

Total Nitrogen

Total nitrogen was assessed by the Kjeldahl method with a protein conversion factor of 6.25 (AOAC, 1995 - method 928.08).

Digestion

The digestion assay was performed according to Akeson and Stahmann (1964) by the following steps. Erlenmeyer flasks (50 mL) containing 1 g of the sample (CL; CB and CL:CB - 10:90%) and 7.5 mL of pepsin solution (2 mg mL^{-1} in HCl 0.1 M) were incubated at 37°C for 3 hours. After the incubation, 7.5 mL of 0.2 M NaOH was added to alkalize the samples, followed by the addition of 3.75 mL of pancreatin solution (2 mg mL^{-1} in phosphate buffer 0.1 M, pH 8) and another incubation at 37°C for 24 hours.

The incubation was stopped by adding 5 mL of 30% trichloroacetic acid (TCA) (w/v), and the final volume was adjusted to 25 mL using 5% TCA (w/v). The samples were centrifuged at 10,000 RPM at 4°C for 30 minutes.

The digestibility of each sample was calculated by the difference between the total protein measured in the precipitate (non-digested material) and the total crude protein, both

determined by the Kjeldahl method (AOAC, 1995). The digestibility of the samples was expressed as the percentage of solubilized proteins.

Ash

The ash content was determined according the method described by AOAC (1995), method 920.153.

Total Carbohydrates

The colorimetric method described by Chaplin and Kennedy (1994) was used to quantify the total carbohydrates of the samples.

Cyanide

The total cyanide (HCN) was analyzed by the colorimetric method described by Brito et al. (2009).

Fatty Acids

The lipids of each sample were extracted by the Soxtherm system using petroleum ether as solvent, as described by Matthäus and Brühl (2001).

The characterization of the fatty acid methyl esters (FAME) was performed by placing 100-250 mg of the extracted lipids of each sample in a tightly screwed vial with a Teflon stopper and saponifying with 4 mL of 0.5 M methanolic sodium hydroxide, followed by heating at 80°C for 10 minutes. The esterification was achieved by the addition of 5 mL of boron trifluoride methanol complex to the samples and heating again at 80°C for 5 min. Finally, 5 mL of heptane was added, followed by another heating at 80°C for 1 minute. After cooling the samples, 3 mL of saturated sodium chloride solution was added, and the samples were shaken for 30 seconds. The supernatant from each sample was transferred to a 4-mL vial for quantification of the FAME content by GC.

The patterns to quantify the fatty acid profiles were developed according to Ramani et al. (2004).

Essential Amino Acids (EAA)

The determination of the EAAs (lysine, histidine, threonine, cysteine, valine, methionine, isoleucine, leucine, tyrosine and phenylalanine) was performed in each sample before and after the fermentation. Aliquots with approximately 200-250 mg of each sample were placed

in borosilicate tubes (previously pyrolyzed at 400°C for 8 h). To promote the acid hydrolysis of the material tested, 6 mL of 6 M HCl with 0.1% phenol (m/v) was added to the sample. The tubes with the stoppers slightly open were heated in an oven at 110°C for 1 hour. After this period, the stoppers were closed, and the tubes were heated for a further 23 hours at the same temperature (MOORE; SPACKMAN; STEIN, 1958).

Protein Digestibility Corrected Amino Acid Scoring (PDCAAS)

The PDCAAS was calculated on the basis of the EAA requirements for children (2-5 years old), according to Tirapegui and Macedo (2007), by the following equation:

$$PDCAAS = \frac{\text{mg of limiting AA (1 g of sample)} \times \text{true digestibility (\%)}}{\text{mg of the same AA in 1 g of the reference protein}} \times 100$$

Relative Nutritional Value (RNV)

The RNV tests were performed in triplicate. After a previous degreasing, carried out according to the AOAC (1995), the samples and the casein were hydrolyzed by shaking in 4 mL of pronase Sigma P-5147 (1 mg mL⁻¹) and 40 mL of sodium glycerophosphate buffer (Sigma G-5422 – 20 g L⁻¹), adjusted to pH 8.0 (with 0.1 M NaOH or 0.033 M H₃PO₄) at 48°C for 3 hours. The pH and the volume of the samples were adjusted to 7.2 and 100 mL, respectively, followed by sterilization in a 0.22-µm filter (cold sterilization). To promote the growth of microorganisms, 2 mL of the basal medium, 2 mL of the hydrolyzed sample, 7 mL of sterilized water and 100 µL of *Enterococcus zymogenes* inoculum were incubated in a test tube at 37°C for 48 hours.

The samples were sterilized by autoclaving (121°C/15 min), and the growth was measured by acidimetry. The titration of each tube was performed with 0.01 M NaOH or 0.0033 M H₃PO₄. The total volume used in the test was calculated by discounting the volume used for the titration of the blank (carried out with inactive inoculum). The data obtained in the test with *E. zymogenes* were compared with the values of standard protein (casein - SIGMA C-7078, considered 100%).

The growth of *E. zymogenes*, which occurs according to the quality of the protein present in the sample, shifts the medium pH and determines the protein RNV (TAVANO et al., 2004). The response is related to the absolute nutritional necessity of the EAAs of the microorganism, such as arginine, glutamic acid, histidine, isoleucine, leucine, methionine, tryptophan, valine and lysine (FORD, 1960). Even though lysine is not considered an EAA to

E. zymogenes, its deficiency can impair the growth of the microorganism (FORD, 1962; TAVANO et al., 2004).

This indirect method of RNV evaluation presents a good match between the microorganism and the human organism because the enzymes used in the hydrolysis of the sample are the same as those in the human gastrointestinal tract (TAVANO et al., 2004).

Statistics

The statistical significance of the samples was determined by one-way analysis of variance (ANOVA), Minitab Express v. 1.4.0, which determines the differences between samples (fermented and non-fermented) for the values of proteins, fatty acids, amino acids, ash, PDCAAS and RNV.

Results and Discussion

Substrate and moisture

Cassava bagasse (CB) and cassava leaves (CL) were tested in different ratios to evaluate the ideal concentration to promote the *P. ostreatus* growth. These tests showed that a mixture of 10% leaves and 90% bagasse (CL:CB – 1:9) promoted the fastest growth of this fungal mycelia.

The characteristics of this mixture are based on the inclusion of 10.6 g of CL and 20.6 g of CB, totaling 20.0 g of dry material and 11.2 mL of H₂O (as measured by a Sartorius MA 35 infrared moisture analyzer). According to Krishna (2005), the moisture necessary to promote the SSF is between 70-80%. The moisture used in this study was 74% because Jecu (2000) observed the best results for SSF when the substrate used in the fermentation had this moisture. Philippoussis et al. (2003) used 72.96% moisture in their experiments with the SSF process, and Suhel and Fioreze (2011) used 75.2%. The humidities used by these authors imply that a humidity of 74% is suitable for the fermentation process, which was thus used in our experiments with SSF on the substrate obtained from cassava by-products.

Total Carbohydrates

Approximately 40 to 60% of the energy used for the biomass production comes from carbon sources, so it is interesting to use alternative sources rich in carbohydrates, such as cassava bagasse (BÔAS, 2001). According to Couto and Sanromán (2006), the high amount of carbohydrates in agricultural wastes allows them to be used as a good substrate for SSF. Carbohydrates are the main compounds related to the nutritional requirements for the fungal

growth process that, according to Fakas et al. (2009), are the same compounds that are required for the growth of the fungus in the SSF process. In this study, the total amount of carbohydrates present in the mixture used as substrate (CL:CB - 1:9) was $92.91 \pm 3.75\%$. These carbohydrates are mainly related to the CB portion used to prepare the substrate, which corresponds to 90% of the total mixture. The amount of carbohydrates found in this study is similar to the data reported by Pandey et al. (2000), who observed values of 78.73 to 91.05% (dry basis).

In our study, we found a total initial amount of carbohydrates in the mixture (CL:CB - 1:9) of $90.8 \pm 0.1\%$. During the 25 days of the SSF process by *P. ostreatus*, 12.34% of the carbohydrates in the mixture were consumed, leaving a value of $81.45 \pm 0.15\%$. The results obtained by Akinfemi (2010) in an SSF using peanut husks as a substrate for SSF by *P. ostreatus* for 21 days showed a reduction in the carbohydrates. The same author, studying the decay in the amount of carbohydrates related to crude fibers promoted by SSF, observed an initial amount of 26.2% crude fiber and a final value of 16.2%. This value represented a reduction of 2.1% in the total carbohydrates in the substrate.

Our results indicate that the carbohydrates used by *P. ostreatus* during the SSF process can be related to the consumption of crude fiber in the mixture, as this fungus has lignocellulolytic enzymes that act on these fibers, providing carbohydrates that can be used as an energy source for their growth. It is important to consider the relationship between fiber and protein in a food because if there is a high fiber intake associated with a low protein intake, it can lead to a nutritional imbalance related to the nitrogen supply, which can cause nutritional harm to the individual (KORNIJEZUK, 2008). Our results indicate that the carbohydrate reduction in the mixture during the process of SSF is probably related to the consumption of fiber, suggesting a decrease in the quantity of fibers present in the tested mixture after the fermentation process, which can characterize a better ratio in the balance of fiber to nitrogen.

Whereas the substrate tested by Akinfemi (2010) had an initial amount of 78.51% total carbohydrate and our substrate had 90.8%, we can infer that the substrate from cassava by-products is more energetic than that from peanut by-products.

The carbohydrate intake observed in the Akinfemi (2010) studies was 10 times lower than that observed in our study. From these results, we can suggest that the substrate made of cassava by-products has a good energy potential for the development of *P. ostreatus*. With respect to the final content of carbohydrates, our studies showed a high value for the final product obtained from cassava (81.45% carbohydrate) than that obtained from the peanut

(76.8% carbohydrate) by the SSF process carried out with the same fungus (*P. ostreatus*), which shows that cassava by-products have a greater potential for use in human food. Although the SSF performed with cassava by-products requires a larger amount of carbohydrates (12.34%) compared to peanut husks (2.1%), the final product obtained in the SSF process with cassava presents a higher content of carbohydrates still present in the mixture.

Another important fact is the degradation of toxic compounds linked to the consumption of carbohydrates by the fungus during the SSF. The decrease in toxic compounds is directly linked to carbohydrate decay (SOCCOL; VANDENBERGHE, 2003).

Cyanide

The most toxic component of the unfermented cassava is the cyanide, and the consumption of non-detoxified cassava products is associated with diseases such as goiter, dwarfism and tropical ataxic neuropathy (HOWLETT et al., 1990; KOBAWILA et al., 2005; LATIF; MÜLLER, 2015). Thus, it is fundamental to evaluate the amount of cyanide present in the samples before and after the SSF process to ensure the safety of the product to be used as food.

The total amount of cyanide in the substrate before the SSF was 20.60 ± 1.92 ppm, which would be considered “non-toxic” according to Kobawila (2005), as the concentration is lower than 50 ppm (mg kg^{-1}). However, the FAO/WHO (1995) established a maximum concentration of 10 ppm (mg kg^{-1}) as safe for human consumption.

After the SSF process, the amount of cyanide in the substrate decreased to 5.87 ± 1.95 ppm (mg kg^{-1}), which proves that this process can be considered as an important tool for detoxifying cyanogenic substrates.

This value allows classifying the fermented product as "safe for consumption", without the necessity to apply any further treatment.

Lipids and Fatty Acids

Cassava bagasse, the main component of the substrate used in this work (90%), is known for its low lipid content. According to Pandey et al. (2000), the content of lipids in CB is 0.53-1.57% (dry basis). The substrate used in the present work (CL:CB – 1:9) showed $0.548 \pm 0.06\%$ lipids before the SSF process.

After the SSF process (25 days) performed in this work, the total lipid content increased to $1.02 \pm 0.14\%$. Many authors have observed similar increases in lipids during the SSF

process until the depletion of the carbohydrates, at which point a decrease in the amount of total lipids is observed (FAKAS et al., 2009).

Although fatty acids are the least known molecules of all of the nutrients, they are very important to the diet. Among these, the unsaturated fatty acids, especially those that are polyunsaturated, deserve the most emphasis with respect to the nutritional value (GERMAN, 2011). Fatty acids are divided into 3 classes according to the unsaturation presented in the molecule. Saturated fatty acids (SFAs) are a group of lipids that do not present unsaturation (double bonds) in the molecule, monounsaturated fatty acids (MUFAs) show only one double bond in the molecule, and polyunsaturated fatty acids (PUFAs) present two or more sites of unsaturation. The most common fatty acids have 12-22 carbon atoms in the molecule (RUSTAN; DREVON, 2005; ZIELIŃSKA; NOWAK, 2014). An SSF process performed by *P. ostreatus* induces changes in the quantitative and qualitative profiles of fatty acids of the mixture analyzed (Table 1). The most common SFA found in plants, animals and microorganisms is palmitic acid (16:0), which is synthesized endogenously by these organisms (GERMAN, 2011; RUSTAN; DREVON, 2005). In our study, a high quantity of this compound was found in the fermented substrate prepared from CB:CL. This palmitic acid present in this material is derived from both the cassava by-products and the microorganism used in the fermentation. In studies on the lipid composition from the koji fermentation process, carried out by Feng et al. (2014), it was observed that even with an increase in all SFAs during fermentation, there was still an increase in unsaturated fatty acids (MUFAs and PUFAs) in relation to the SFAs, indicating a better availability profile for the UFs.

With respect to MUFAs and PUFAs, the results of this study show significant increases ($P < 0,01$) in oleic acid (18:1 ω -9), linoleic acid (18:2^{9,12} ω -6), γ -linolenic acid (18:3^{6,9,12} ω -6) and cis-13,16-docosadienoic acid (22:2 ω -6) (Table 1). According to Carrillo, Cavia and Alonso-Torre (2012), the consumption of UFs is related to beneficial effects in combatting inflammatory diseases and consequently also beneficial effects to the human immune system.

Diets with a high content of oleic acid (a MUFA most commonly found in plants and animals) can reduce blood pressure by regulating the lipids of the cell membrane, which are controlled by G protein (guanine nucleotide-binding protein)-mediated signaling (RUSTAN; DREVON, 2005; TERÉS et al., 2008). Thus, the increase in the amount of oleic acid (approximately three times more than initial amount) observed in the final product of this study indicates that the fermented product presents a better nutritional profile post-fermentation (Table 1).

Linoleic acid (LA) is a fatty acid that belongs to the ω -6 family. This fatty acid is considered to be the most important and most consumed PUFA in the Western diet (WHELAN, 2008; ZIELIŃSKA; NOWAK, 2014). Chronic underconsumption of LA (less than 4.4 g day^{-1}) can lead to cardiovascular disease, cancer, inflammation, neurological disorders, and other pathologies (WHELAN; 2008). Our results show that the quantity of LA found in the fermented product (CL:CB – 1:9) is 16 times more (approximately $352 \text{ mg } 100\text{g}^{-1}$) than that found before the SSF process (Table 1). This amount is higher than that observed in the pork chop and white rice, as described by Whelan (2008).

Gamma-linolenic acid (GLA), also known as $18:3^{6,9,12}$, is a PUFA that belongs to the omega 6 (ω -6) fatty acid family whose members have 3 double bonds (ZIELIŃSKA; NOWAK, 2014). It is an essential fatty acid that must be provided by the diet because it is not endogenously synthesized (HORROBIN, 1992). The consumption of GLA has been proposed as a new breast cancer therapy because it promotes the modulation of steroid hormone receptors (KENNY et al., 2000). By the results observed in our work, we infer that the SSF promoted by *P. ostreatus* changes the characteristic of the linolenic acid from alpha to gamma (Table 1). The amount of α -linolenic acid was $34.543 \pm 2.519 \text{ mg } 100\text{g}^{-1}$ before the SSF process. In this study, γ -linolenic acid was not detected in the substrate. After the fermentation, γ -linolenic acid values of $32.809 \pm 3,732 \text{ mg } 100 \text{ g}^{-1}$ were found, and there was no α -linolenic acid in the substrate (Table 1). When comparing the difference between the amount of α -linolenic acid present in the unfermented substrate and the amount of γ -linolenic acid in the fermented material (Table 1), it may be observed that there is no significant difference between the values ($P < 0, 5113$). Thus, it can be inferred that there has been a complete conversion of α -linolenic acid to γ -linolenic acid during the fermentation process. The amount of GLA found after fermentation was three times more than that in raw liver (from beef) and approximately 50% higher than that in egg yolk, according to the descriptions of Horrobin (1992).

An unusual PUFA, normally found in fish fat (KOŁAKOWSKA; OLLEY; DUNSTAN, 2003), that was found in interesting amounts after our fermentation process was cis-13,16-docosadienoic acid (22:2), which belongs to the ω -6 family (JABEEN; CHAUDHRY, 2011). The Academy of Nutrition and Dietetics recommends a daily ingestion of ω -6 between 3 and 10% (VANNICE; RASMUSSEN, 2014).

Protein Content and Digestibility

Many agro-industrial wastes such as straw, grass, and sawdust, among other organic materials, are commonly used in *P. ostreatus* cultivation (DONINI et al., 2005). The substrates treated with microorganisms (by SSF or submerged fermentation) showed increases in the protein amount (BÔAS, 2001).

The substrate used to promote the SSF in our work (CL:CB – 1:9) showed an initial content of $5.3 \pm 0.1\%$ crude protein. After 25 days of treatment, the fermented material presented $6.7 \pm 0.3\%$ crude protein, an increase of 26%. This improvement becomes more evident when applying the calculated digestibility of the protein present in the substrate before and after the SSF.

The protein digestibility was calculated in vitro for the unfermented substrate. The value obtained in this analysis was 30.13%, indicating that only 30% of the protein present in the substrate is assimilable. According to Mattila et al. (2002), the protein digestibility of materials fermented by *P. ostreatus*, calculated using the conversion factor 4.97, is 79.25%. Thus, of the $5.3 \pm 0.1\%$ protein present in the unfermented substrate, only $1.6 \pm 0.1\%$ is bioavailable and is capable of being assimilated by consumers. For the fermented substrate, this ratio is $6.7 \pm 0.3\%$ protein present to $5.3 \pm 0.3\%$ protein assimilable by the consumer. Related to the digestible protein, the quantity of available protein is 3 times higher after the fermentation. Our data showed that the SSF increased the amount of available protein, and hence, the fermented material can be used as a food with a better content of protein. Mukherjee and Nandi (2004) observed the same behavior when studying the improvement of the in vitro digestibility when using two *Pleurotus* species for the SSF of water hyacinth (*Eichhonia crassipes*) biomass. According to the results obtained in studies with *Pleurotus*, materials fermented with this fungus may contain more protein that is usable by the organism consumer.

Amino Acids and PDCAAS

The traditional classification of amino acids (AA) is based upon the dietary requirements. They are classified as nutritionally essential (can not be synthesized by the body) or non-essential (when synthesized by the body) (WU, 2009). Protein quality is related to its digestibility with essential amino acids (EAA) in the protein and the bioavailability of these amino acids (TAVANO et al., 2004).

The changes in the amino acid profile that are related to the SSF process are presented in Table 2. Almost all of the EAAs presented differences between the values obtained before and after the SSF process. The amount of EAAs was low when compared to the nutritional

needs of children 2-5 years of age, but the fermented material does provide good amounts of some of the EAAs to consumers.

The amounts of valine (Val) and isoleucine (Ile) present in 100 mg of protein from the fermented product could provide approximately 60% and 45%, respectively, of the EAA requirements for children 2-5 years of age. According to Wu (2009), Val and Ile are directly related to the synthesis of alanine (Ala) and glutamine (Gln), as well as the balance among branched-chain amino acids (BCAAs). The same author describes that Ala is important to the inhibition of pyruvate kinase and hepatic autophagy, directly related to gluconeogenesis (generation of glucose from non-carbohydrate molecules), and essential to the production of pyruvate by transamination and the provision of glucose to the muscle by the glucose-alanine cycle. Gln, another amino acid, whose synthesis is related to the Val intake, is important for many purposes such as the immune function, synthesis of purine and pyrimidine, nitrogen reservoir, synthesis of NADP, and regulation of protein turnover (NAKAJO et al., 2004; WU, 2009). The recommendation for sulfur amino acids (SAAs) includes both methionine (Met) and cysteine (Cys), although only Met is considered an EAA. Among the many functions of Met is its epigenetic action as a methyl donor (COURTNEY-MARTIN; BALL; PENCHARZ, 2012; WU, 2009). Cys is mainly related to the disulfide bonds in proteins, which are important for the spatial stability of the protein molecule (WU, 2009).

The ingestion of 100 mg of the protein from fermented substrate could provide approximately 30% of the requirement of leucine (Leu) for children of preschool age. Leu is linked to the regulation of protein turnover and gene expression, insulin secretion as an activator of glutamate dehydrogenase, and the BCAA balance, besides being a flavor enhancer (HUTSON; ISLAM; ZAGANAS, 2011; WU, 2009).

Despite showing improvements in the content of bioavailable proteins, the fermented material presented low amounts of proteins and also a deficiency of threonine (Thr), generating an insignificant PDCAAS of 0.05 for CL:CB of 1:9 (Table 3), which is lower than the values reported for corn (0.37), wheat (0.40) and almonds (0.23) (HUGHES et al., 2011; PIRES et al., 2006).

This means that the product may not provide adequate amounts of proteins compared to the daily necessity, although there was an increase in the protein quality after the SSF process.

Relative Nutritional Value (RNV)

The traditional methods of protein quality assessment are carried out by *in vivo* assays, which, besides being regulated by ethical codes of animals, require long periods of treatment

and high-value infrastructure. However, according to Tavano et al. (2004), the microbiological method using the growth pattern of *Enterococcus zymogenes* produces significant relative nutritional value (RNV) results faster than the traditional methods.

RNV is an indirect method also used to investigate the bioavailability of protein. In this study, the unfermented substrate and the fermented material were evaluated to determine the changes in the protein quality and verify if the fermentation could improve the bioavailability of the protein. Before the SSF process, the substrate showed a total RNV of $7.35 \pm 0.05\%$, corresponding to a real RNV of $2.21 \pm 0.01\%$ when considering the proteic digestibility of 30.13%. On the other hand, the fermented material presented a total RNV of $71.795 \pm 5.128\%$, or $57.091 \pm 4.078\%$ (real RNV) after correcting using the digestibility rate (79.25%). This means that the bioavailability of the proteins increased by 25.8 times after the bioconversion of the substrate by *P. ostreatus*. The results obtained after the fermentation are similar to those of coconut protein concentrate and cooked and toasted soya flours (RNV = 55.1%; 57.33%; 57.57%, respectively) (HEGSTED; NEFF; WORCESTER, 1968).

The differences between the results obtained by PDCAAS and RNV may be related to the threonine (Thr) quantity because, according to Ford (1962), this AA is not an indispensable AA to *E. zymogenes*, while it is the limiting AA in the calculation of the PDCAAS.

Ash

Minerals are essential nutrients to organisms. They are substantially metals and inorganic compounds, which are classified into three categories according to the body requirements: major, secondary and trace minerals. Major minerals have requirements up to 10 g day^{-1} , secondary minerals between 400 and 1500 mg day^{-1} , and trace minerals from $45 \text{ } \mu\text{g day}^{-1}$ to 11 mg day^{-1} (GUPTA; GUPTA, 2014). Thus, the determination of the total ash content shows the richness of the mineral elements that could be used to estimate the calcium and phosphorus contents (OLIVEIRA, 2007).

The analyses carried out on the substrate (CL:CB – 1:9) showed $2.53 \pm 0.17\%$ ash (dry basis). After 25 days of the SSF process with *P. ostreatus*, the ash content was $2.70 \pm 0.02\%$, an increment of 17.39% in the mineral residue. The results obtained after the fermentation are similar to the results found by Lousada Junior et al. (2006) for the Barbados cherry (*Malpighia emarginata*) subproduct (2.68% ash), used in the production of animal feed. The results obtained by Akinfemi (2010) in the bioconversion of peanut husks with *P. ostreatus* showed an increment of 7.19% after 21 days of SSF.

Every living being requires mineral nutrients for vital processes Soetan et al. (2010) andSizer and Whitney (2003) explain the importance of many indispensable minerals including sulfur (S) – component of proteins, hormones and coenzymes; phosphorus (P) – component of nucleic acids, phospholipids, ATP and several coenzymes; potassium (K) – cofactor in protein synthesis, activation of enzymes and osmosis regulator; calcium (Ca) – maintenance of membrane structure and permeability, activation of some enzymes, and main mineral in bones and teeth; magnesium (Mg) – activation of enzymes; chloride (Cl⁻) – important in water balance and digestion; iron (Fe) – component of cytochromes, hemoglobin and myoglobin; manganese (Mn) – formation of amino acids, activation of some enzymes, and coenzyme activity; zinc (Zn) – activation of some enzymes, component of insulin, immune reactions, and transport of vitamin A; selenium (Se) – prevents harmful reactions in the cell; and copper (Cu) – constituent of many enzymes and important in the formation of hemoglobin.

These data show the importance of minerals in a balanced diet, and the results obtained in our work show that the fermented substrate can provide a good supply of minerals to the consumer.

Conclusion

The fermentation carried out by *P. ostreatus* decreased the content of cyanide to levels that are safe for consumption.

Despite being a low-protein product, the fermented product (CL:CB - 1:9) showed significant improvements in the MUFA and PUFA profiles; proteic digestibility; protein and EAA quality; and ash content.

An increase in the unsaturated fatty acids was observed mainly in the ω -6 family, which represents a healthier availability of fatty acids.

The bioconversion of the substrate could improve many EAA amounts, showing significant changes in seven of the nine EAA quantities. In addition to the improvement of EAAs, there was also a higher digestibility of the protein observed after the SSF process.

A deficiency of Thr causes a decrease in the PDCAAS value, which can mask amino acid values that are in larger quantities and that provide benefits to the consumer. On the other hand, the RNV shows that the bioavailability of the protein was improved after SSF. This means that the organism can assimilate a high amount of the total protein present in the fermented product.

The results obtained for ash (or mineral residues) are similar to those of vegetal residues used in animal feed, representing satisfactory values.

The fermented product (CL:CB – 1:9) characteristics show that it is a good carbohydrate source to prepare breads and cookies, as it is an “enriched” flour presenting higher amounts of EAAs and UFAs that are essential for nutrition.

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Table 1 – Characterization of fatty acids (mg/100g of Edible Portion) before and after SSF process.

		Substrate	<i>P.ostreatus</i> SSF
SFAs			
Myristic Acid	14:0	0.314 ± 0.306	7.291 ± 1.113 *
Pentadenoic Acid	15:0	0.747 ± 0.169	21.781 ± 1.153 **
Palmitic Acid	16:0	34.642 ± 4.857	126.178 ± 3.522 **
Stearic Acid	18:0	4.543 ± 0.668	19.013 ± 0.874 **
MUFAs			
Oleic Acid	18:1	29.644 ± 4.588	90.198 ± 0.790 *
PUFAs			
Linoleic Acid	18:2 ^{9,12}	21.818 ± 3.285	351.980 ± 11.490 **
γ-Linolenic Acid	18:3 ^{6,9,12}	ND ^a	32.809 ± 3.723 **
α-Linolenic Acid	18:3 ^{9,12,15}	34.543 ± 2.519	ND ^{a†}
cis-13,16-Docosadienoic acid	22:2	36.141 ± 3.822	84.095 ± 2.782 **
SFAs, MUFAs and PUFAs		167.540 ± 20.640	733.344 ± 1.410
Total lipids		548.0 ± 62.2	1069.6 ± 0.049

^aND – Not Detected

* Fatty Acids that significant increase was observed (P < 0.05);

** Fatty Acids that significant increase was observed (P < 0.01);

† Fatty Acids that significant decrease was observed (P < 0.05).

Table 2 – Characterization of Essential Amino Acids (EAA mg g⁻¹ of Protein)

EAA	FAO/WHO Pattern*	Essential Amino Acids Quantity				Casein*	
		Substrate		Fermented Substrate			
		Child	EAA	%	EAA	%	EAA
HIS	1.9	0.13	7.0	0.27	14.4	3.8	112
ILE ^{††}	2.8	0.10	3.7	1.24	44.3	4.4	157
LEU ^{††}	6.6	0.16	2.5	1.95	29.5	7.6	115
LYS ^{††}	5.8	0.23	3.9	0.70	12.0	6.8	117
MET + CYS ^{††}	2.5	0.06	2.3	0.78	31.0	3.5	140
PHE + TYR [†]	6.3	0.19	3.0	0.79	12.5	7.0	111
THR ^{††}	3.4	0.07	2.2	0.22	6.6	2.5	131
TRP	1.1	ND ^a	-	ND ^a	-	1.0	90.9
VAL ^{††}	3.5	0.19	5.5	2.09	59.8	5.5	157

* According FAO/WHO (1985);

^aND – Not Detected

[†] AA that significant increases were observed (P < 0.05);

^{††} AA that significant increases were observed (P < 0.01);

His (histidine); Thr (threonine); Val (valine); Met (methionine); Cys (cysteine); Ile (isoleucine);
Leu (leucine); Phe (phenylalanine); Tyr (tyrosine); Lys (lysine); Trp (tryptophan).

Table 3 - Protein digestibility-corrected amino acid scores (PDCAAS), Cassava Bran and Cassava Leaves (1:9), casein standard and daily necessity in mg of amino acid/g of protein for children of 2-5 years old

Essential Amino Acids	Fermented Substrate mg aa / g protein	Casein mg aa / g protein	Amino acid ratio		Standard FAO/WHO 2 – 5 years
			<i>P. ostreatus</i> SSF *	Casein	
His	2.73	18.99	0.14	<u>1.00</u>	19
Ile	12.41	46.91	0.44	1.68	28
Leu	19.45	93.05	0.29	1.41	66
Lys	6.97	78.66	0.12	1.36	58
Met + Cys	7.76	30.14	0.31	1.21	25
Phe + Tyr	7.89	109.71	0.13	1.74	63
Thr	2.23	43.22	<u>0.07</u>	1.27	34
Trp	ND	ND	-	-	11
Val	20.94	54.95	0.60	1.57	35
PDCAAS	-	-	0.0522	1.0000	-

* True protein digestibility obtained *in vitro* – 0.7952 (79.52%) (MATTILA et al., 2002);

ND – Not Detected;

Data of casein according to PIRES et al. (2006); Standard protein 2-5 years, according FAO/WHO (1985);

Underlined value represents the limiting EAA;

His (histidine); Thr (threonine); Val (valine); Met (methionine); Cys (cysteine); Ile (isoleucine); Leu (leucine); Phe (phenylalanine); Tyr (tyrosine); Lys (lysine); Trp (tryptophan).

ARTIGO 3 - Effects of Solid-State Fermentation and the Possibility of Use of Cassava By-Products as Fermented Food

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Resumo

A utilização de subprodutos da agroindústria pode ser uma oportunidade interessante para aumentar a produção de alimentos, reduzir gastos na produção agrícola e diminuir a geração de resíduos. Subprodutos como bagaço (BM) e folhas (FM) de mandioca são descartados, quando poderiam ser explorados para a produção de alimentos, uma vez que eles apresentam uma boa quantidade de carboidratos, proteínas, gorduras e minerais. A aplicação de Fermentação em Estado Sólido (FES) em substratos contendo esses subprodutos poderia melhorar a biodisponibilidade de nutrientes e reduzir as quantidades de anti-nutrientes como o cianeto. O processo de FES com *Lentinula edodes* foi usado com o objetivo de verificar a possibilidade do uso de subprodutos da mandioca para obter alimentação de boa qualidade nutricional, como também se esse processo fermentativo proporcionava mudanças na qualidade e quantidade dos nutrientes do material experimentado. Neste estudo foi avaliado o teor total de carboidratos e cianeto, a quantidade de ácidos graxos e aminoácidos essenciais (AAE), bem como a qualidade da proteínas, antes e após a FES. Após a fermentação, observou-se uma diminuição significativa no teor de cianeto, cujos valores finais ficaram dentro de limites considerados não tóxicos. O perfil de AAE mostrou boas quantidades de histidina, isoleucina, valina, metionina/cisteína e fenilalanina/tirosina, quando comparado com as necessidades diárias de crianças de 2-5 anos de idade. Foram estimados em boas quantidades alguns dos ácidos graxos insaturados importantes, tais como o ácido linoleico; cis-13,16-docosadienóico ácido; cis-11-eicosenóico de ácido e ácido α -linolênico. A avaliação da qualidade da proteína mostrou aumentos significativos de biodisponibilidade. Os resultados permitem concluir que a utilização de subprodutos da agroindústria poderia ser uma importante estratégia para a obtenção de alimentos de baixo custo e boa qualidade nutricional.

Palavras-chave: Biodisponibilidade proteica; *Lentinula edodes*; aminoácidos essenciais; ácidos graxos insaturados; PDCAAS, DIAAS; valor nutricional relativo.

Abstract

The use of by-products from the agro-industry can be an interesting opportunity to increase the production and financial yield of food and decrease the generation of wastes. By-products such as cassava bagasse (CB) and leaves (CL) are discarded when they could be exploited to produce food, as they present good amounts of carbohydrates, protein, fat and minerals. The application of solid-state fermentation (SSF) to substrates containing these by-products could improve the bioavailability of nutrients while reducing the amounts of anti-nutrients such as cyanide. To evaluate the possibility of using by-products from cassava to obtain food with good nutritional quality, SSF application with *Lentinula edodes* was performed to observe the changes in the nutrients of the fermented material. This study used known techniques such as colorimetric methods to evaluate the total cyanide and carbohydrate contents, gas chromatography (GC) to quantify and identify the fatty acids, and liquid chromatography (HPLC) to estimate the essential amino acids, from which the quality of the protein was calculated. After the SSF process, the cyanide content significantly decreased to safe concentrations. The profile of essential amino acids showed good amounts of histidine, isoleucine, valine, methionine (together with cysteine) and phenylalanine (together with tyrosine) compared to the requirements of 2-5-year-old children. Some important unsaturated fatty acids were estimated to be present in good quantities, such as linoleic acid (18:2^{9,12}); α -linolenic acid (18:3^{9,12,15}); *cis*-11-eicosenoic acid (20:1) and *cis*-13,16-docosadienoic acid (22:2). The evaluation of the protein quality showed significant bioavailability increases. The results support the conclusion that underused by-products from the agro-industry could become sources of food that provide important quantities of essential nutrients, thereby increasing the amount of produced food.

Keywords: Cassava by-products; *Lentinula edodes*; SSF; EAA; UFA; PDCAAS, DIAAS; RNV.

Introduction

The wastage of by-products such as cassava bagasse (CB) and leaves (CL) represents a loss of money and generates huge quantities of residues that could be exploited to add value and increase the production of food without the need to increase cultivated land (FIORDA et al., 2013; OLIVEIRA, 2007; SOCCOL; VANDENBERGHE, 2003; VILHALVA et al.,

2011). The processing of 250–300 tons of cassava tubers to starch extraction generates 280 tons of CB with high moisture (80-85%), which is normally discarded in landfills (JASKO et al., 2011; PANDEY; SOCCOL; MITCHELL, 2000). Another underused by-product, CL is a rich source of vitamins, proteins and minerals and is produced at 2.25 tons per hectare, almost all of which is discarded after harvesting (ARO, 2008; LATIF; MÜLLER, 2015; NASSAR; MARQUES, 2006; SAGRILO, 2001).

Solid-state fermentation (SSF) is an area of interest to add value to these by-products using an inexpensive process (COUTO; SANROMÁN, 2006; PANDEY, 2003). The best microorganisms for SSF are filamentous fungi that can grow very fast in any substrate that contains carbohydrates (HÖLKER; LENZ, 2005; JIN et al., 2002). This capacity is related to their enzymes that allow them to convert these by-products into products with higher levels of nutrients (MINOTTO et al., 2008; SCHMIDT et al., 2003). To enable the use of the biotransformed by-product as food, the fungus must be edible.

Lentinula edodes is an edible fungus consumed worldwide that is known as shiitake, which is the second most prevalent cultivated mushroom in the world (GAITÁN-HERNÁNDEZ et al., 2006; ROYSE, 2009). *L. edodes* additionally presents therapeutic effects such as antimicrobial and antioxidant properties and was the first medicinal mushroom used in modern biotechnology (BISEN et al., 2010; KITZBERGER et al., 2007).

The aim of this work was to evaluate the SSF carried out by the fungus *L. edodes* using by-products from the agro-industry to observe the changes in essential amino acids (EAA), unsaturated fatty acids (UFAs), cyanide, ash, digestibility, and the quality of the protein (PDCAAS, DIAAS and RNV) and thereby to determine the possibility of using the fermented material as food.

Material and Methods

Microorganism

Lentinula edodes was the microorganism chosen to serve as the fermenter agent, and it was acquired from Fungi & Flora, Valinhos – SP, Brazil. Cultures were grown in Petri plates at $24 \pm 2^\circ\text{C}$ for 72 hours in 2% malt extract agar before the transfer of 10 squares of 1 cm^2 of the mycelia to each bag (performed in triplicate) containing the substrate.

Substrate

Two by-product derivatives from cassava (*Manihot esculenta* Crantz) were used as substrates to carry out the solid-state fermentation. Cassava bran (CB), an industry waste from starch extraction, and cassava leaves (CL), a waste from cassava harvesting, were both used in the experiments at a CL:CB ratio of 3:7.

The SSF was performed in triplicate using PP bags containing 20 g of the substrate on a dry basis. The incubation was performed at $24 \pm 2^\circ\text{C}$ for 30 days.

The substrate moisture was determined to be 74% at the beginning of the fermentation.

Total Nitrogen

The total nitrogen was assessed by the Kjeldahl method (AOAC, 1995) with a protein conversion factor of 6.25 (method 928.08).

Digestion

The digestion assay was performed according to Akeson and Stahmann (1964).

The digestibility of each sample was calculated as the difference between the total protein measured in the precipitate (non-digested material) and that obtained after digestion with pepsin and pancreatin, both determined by the Kjeldahl method (AOAC, 1995). The digestibility of the samples was expressed as a percentage of solubilized proteins.

Ashes

Ash was determined according the method described by AOAC (1995), method 920.153.

Total Carbohydrates

The colorimetric method described by Chaplin and Kennedy (1994) was used to quantify the total carbohydrates in the samples.

Cyanide

The total cyanide (HCN) was analyzed with the colorimetric method described by Brito et al. (2009).

Fatty Acids

The lipids of each sample were extracted using a Soxtherm system using petroleum ether as solvent, as described by Matthäus and Brühl (2001).

The characterization of fatty acid methyl esters (FAME) was performed by the measurement of a fraction (100-250 mg) of the extracted lipids placed in a tightly screwed

vial with a Teflon stopper. The lipids were saponified by the addition of 4 mL of 0.5 M methanolic sodium hydroxide followed by heating at 80°C for 10 minutes. Next, 5 mL of boron trifluoride methanol complex was added, and the samples were heated again at 80°C for 5 min to promote the esterification. Finally, 5 mL of heptane was added with further heating at 80°C for 1 minute for reaction. The samples were cooled, and 3 mL of saturated sodium chloride solution was added to extract the FAME after shaking for 30 seconds. The supernatant from each sample was separated into 4 mL vials to perform the quantification on GC.

The patterns to quantify the fatty acid profiles were developed according to Ramani et al. (2004).

Essential Amino Acids

The determination of amino acids (lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine and phenylalanine) was performed according to Moore, Spackman and Stein (1958).

Protein Digestibility Corrected Amino Acid Scoring (PDCAAS)

The PDCAAS was calculated on the basis of the essential amino acid (EAA) requirements for children (2-5 years old), according to Tirapegui and Macedo (2007), and the following equation was used:

$$PDCAAS = \frac{\text{mg of limiting AA (1 g of sample)} \times \text{true digestibility (\%)}}{\text{mg of the same AA in 1 g of the reference protein}} \times 100$$

Digestible Indispensable Amino Acid Score (DIAAS)

A new protein quality measurement is recommended by the FAO/WHO (2013) to replace PDCAAS. This recommendation is because dietary amino acids should be treated as individual nutrient.

$$DIAAS = \frac{\text{mg of digestible dietary EAA (1 g of dietary protein)}}{\text{mg of the same EAA in 1 g of the reference protein}} \times 100$$

Relative Nutritional Value (RNV)

The RNV calculations were performed according to Ford (1960), using the growth patterns of *Enterococcus zymogenes* to reach the values by acidimetry. The data obtained in the test with *E. zymogenes* were compared with the value of a protein standard (casein - SIGMA C-7078), considered 100%.

The quality of the protein determine the growth of *E. zymogenes* defining the RNV (TAVANO et al., 2004). The response is related to the absolute nutritional necessity of essential amino acids (aa) by the microorganism, such as arginine, glutamic acid, histidine, isoleucine, leucine, methionine, tryptophan, valine and lysine (FORD, 1960). Even though the lysine is not considered an essential aa to *E. zymogenes*, its deficiency can impair the growth of the microorganism (FORD, 1962; TAVANO et al., 2004).

This method of evaluation of RNV presents a good match to human requirements because the enzymes present in the human gastrointestinal tract are the same enzymes used in the hydrolysis of the sample (TAVANO et al., 2004).

Statistics

The statistical significance of the samples was verified by one-way analysis of variance (ANOVA) using Minitab Express v. 1.4.0, comparing the differences between the samples (fermented and unfermented material) for protein, fatty acids, amino acids, ash, PDCAAS, DIAAS and RNV.

Results and Discussion

Substrate and moisture

The mixture chosen to perform the SSF was prepared with 31.8 g of CL (fresh basis) and 16.0 g of CB (fresh basis). This mixture, on a dry basis, is characterized by having 30% CL and 70% CB (CL:CB – 3:7), corresponding to 20 g of solid substrate on a dry basis.

The total water present in the mixture was 27.83 mL, totaling 58.19% moisture. According to Krishna (2005), the moisture required to perform the SSF process is between 70 and 80%, and according to Jecu (2000), a moisture level of 74% is ideal. Thus, 29.1 mL of water was added to adjust the moisture in the substrate to 74%.

Total Carbohydrates

Fakas et al. (2009) suggest that the carbohydrates are the main nutrient consumed by the fermenter agent during the SSF. Therefore, the use of agro-industrial high-carb wastes is an interesting alternative to advance the utilization of these residues adding value to them

(BÔAS, 2001; COUTO; SANROMÁN, 2006). According to Pandey (2003), SSF is a potential technology to obtain microbial products as food, feed, fuel, chemical and pharmaceutical products.

The main nutrients present in the substrate (CL:CB – 3:7) used in this work were carbohydrates. The total carbohydrate content in the substrate was $78.378 \pm 8.005\%$, mainly from the CB (corresponding to 70% of the mixture). The SSF process performed over 30 days showed the action of *L. edodes* growth in the carbohydrates. After the SSF process, the carbohydrate content was $67.73 \pm 0.28\%$, representing a decrease of 13.59%. The basidiocarp of *L. edodes* contains approximately 64.4% carbohydrates (BISEN et al., 2010), very similar to the amount found in our assays. Gaitán-Hernández et al. (2006) observed a decrease in total carbohydrates in the substrate of 13.66% in a SSF performed with barley straw as substrate and *L. edodes* as the fermenter agent over a period of 29 days.

According to Kornijezuk (2008), the reduction of crude fibers can compensate in diets with inadequate ingestion of protein. The fungus *L. edodes* is known for its ligninolytic activity, and the observed decrease implies that the fermented product could be of value in low protein intake diets.

Cyanide

Several diseases are linked to the consumption of food containing cyanide, which could affect the nervous and optical systems and cause pernicious anemia (SIMEONOVA; FISHBEIN, 2004). To decrease the toxic compound content, Soccol and Vandenberghe (2003) suggest the use of SSF because the consumption of carbohydrates is directly associated with the toxicity decay. Hence, the evaluation of the cyanide content before and after the SSF is necessary to observe the influence of the process and the microorganism in the reduction of the cyanide content.

The substrate used in this study (CL:CB – 3:7) presented an initial total cyanide concentration of 46.817 ± 1.364 ppm. The results obtained for the total carbohydrates could suggest that the amount of cyanide would decrease, as the quantity of carbohydrates diminished. Thus, the fermented material showed a total amount of cyanide of 16.483 ± 1.083 ppm, which represents a reduction of 64.79%. The results obtained by Kemdirim et al. (1995) and Kobawila et al. (2005) showed reductions in the cyanide concentration after the SSF process (52.4% and 70.67%, respectively), endorsing the results obtained in our study and proving that the SSF could decrease the total cyanide content.

Lipids and Fatty Acids

Fat in the diet should provide at least 15% of total energy for adults and 20% for women of reproductive age (MANN; TRUSWELL, 2012). Given the importance of lipids in the diet, the total amount of fat was evaluated in the substrate (CL:CB – 3:7) before and after the SSF process to observe the variation linked to the action of *L. edodes*. The initial substrate (before SSF) showed $1.48 \pm 0.19\%$ total lipids. This amount is similar to the results from studies performed by Figuerola et al. (2005) on fiber concentrates of two lemon cultivars (Eureka – $1.89 \pm 0.02\%$; and Fino 49 – $1.88 \pm 0.03\%$) and one cultivar of apple (Royal Gala – $1.57 \pm 0.08\%$). These results imply that by-products rich in fiber do not present high fat contents.

After the SSF process, the total lipid amount was quantified at $3.49 \pm 0.21\%$, showing an increase by a factor of 2.36 compared to the unfermented substrate. However, the composition of the lipids should be considered, especially that of the unsaturated fatty acids (GERMAN, 2011).

Unsaturated fatty acids (UFAs) are divided into monounsaturated fatty acids (MUFAs), which present only one double bond in the molecular chain, and polyunsaturated fatty acids (PUFAs), which are characterized by two or more double bonds in the molecule (RUSTAN; DREVON, 2005; ZIELIŃSKA; NOWAK, 2014). The most important UFAs are PUFAs, which belong to the ω -3 and ω -6 families and are considered essential fatty acids (EFAs), as they cannot be synthesized endogenously by the organism (GRESSLER et al., 2010; VANNICE; RASMUSSEN, 2014). The profile of the majority of fatty acids found before and after the SSF is available in Table 1.

Among the UFAs that showed significant increases, it is possible to highlight linoleic acid ($18:2^{9,12}$), *cis*-11-eicosenoic acid or gondoic acid ($20:1$), *cis*-11,14-eicosadienoic acid ($20:2$), and *cis*-12,16-docosadienoic acid ($22:2$).

Linoleic acid (LA) is the most important PUFA in the Western diet, found in practically all of the foods we eat, such as meats (excluding fish), dairy products, vegetables, vegetable oils, cereals, fruits, nuts, legumes, seeds and breads (VANNICE; RASMUSSEN, 2014; WHELAN, 2008). According to Farvid et al. (2014), high intakes of LA are associated with a lower risk of coronary heart disease. According to Bisen et al. (2010), LA is the major fatty acid (FA) present in the *L. edodes* fruiting body, corresponding to 68.8% of the total fatty acid amount. In this study, it was observed that LA was the most prevalent FA measured after the SSF. The results showed that the amount of LA found in the fermented product ($133.92 \pm 9.32 \text{ mg } 100\text{g}^{-1}$) is higher than that found in almonds ($12.65 \text{ mg } 100\text{g}^{-1}$); apples ($43.0 \text{ mg } 100\text{g}^{-1}$); sunflower seeds ($32.78 \text{ mg } 100\text{g}^{-1}$); soybean oil ($50.95 \text{ mg } 100\text{g}^{-1}$); corn oil (53.52

mg 100g⁻¹); potatoes (32.0 mg 100g⁻¹); green beans (23.0 mg 100g⁻¹); or peanuts (55.56 mg 100g⁻¹), according to Whelan (2008).

Gondoic acid (20:1), a MUFA that belongs to the ω -9 family, occurs mainly in animal lipids, but it is especially found in fish as herring, mackerel, capelin and cod liver oil (ANDO; SASAKI, 2011). This FA is not easily found but presented the highest antioxidant activity in the study of Henry et al. (2002) and that is the reason for the prevalent use of this FA in cosmetics (KIKUKAWA et al., 2015). The same authors reported for the first time the microbial production of gondoic acid, and the results obtained in this study prove that the action of *L. edodes* in the chosen substrate (CL:CB – 3:7) could produce this MUFA. The amount observed in the fermented substrate (86.665 ± 0.358 mg 100g⁻¹) is similar to that found in studies performed on the mollusk *Aplysia fascinate* (84.3 ± 5.86 mg 100g⁻¹) and the echinoderm *Holoturia forskali* (83.4 ± 4.04 mg 100g⁻¹) (PEREIRA et al., 2013). These results indicate that the amount of gondoic acid produced in our studies is similar to the amounts that are found in marine organisms. Another 20-carbon chain that showed a significant difference ($P < 0.01$) in fermented material was *cis*-11,14-eicosadienoic acid or homo- γ -linoleic acid (20:2 ω -6). According to Adeyeye (2013), this uncommon naturally occurring PUFA is rare in all lipid classes. These PUFAs are found in higher quantities in marine organisms (PEREIRA et al., 2013). The presence and the ingestion of eicosanoids can provide benefits to the organism by regulating blood clot formation, blood pressure, blood lipid concentration (including cholesterol), the immune response, the inflammation response to injury and infection and many other body functions (ADEYEYE, 2013).

The PUFA *cis*-13,16-docosadienoic acid (22:2 ω -6), also found in the fermented product, presents antioxidant and anti-inflammatory characteristics. The presence of this PUFA is related to neuroprotection, as it can block COX-I and COX-II enzyme activity (HENRY et al., 2002; NASARUDDIN et al., 2016). The amount observed after the final of the SSF was 257.074 ± 8.822 mg 100g⁻¹ (or 0.25 g 100g⁻¹), which is almost the same as that found in sheep milk yogurts after 7 days of storage at 5 °C in experiments performed by Serafeimidou et al. (2013). This means that the final product could have similar amounts as those found in dairy products, important in child nutrition.

The comparison between the UFAs in olive oil and in the fermented substrate (CL:CB – 3:7) allows the observation of the difference in the main FA for each product. According to Vannice and Rasmussen (2014); and Mello and Pinheiro (2012), the main FA presented in olive oil is oleic acid (18:1), constituting approximately 70.7% (or 641.96 mg g⁻¹) of all FAs, followed by linoleic acid (18:2), with 9.7% (88.076 mg g⁻¹), and linolenic acid (18:3), with

0.7% (6.356 mg g⁻¹). The most important UFA observed in our experiments was linolenic acid, constituting 13.62% (4.758 ± 0.318 mg g⁻¹) of the total lipids. The other important UFAs evaluated were α-linolenic acid (18:3^{9,12,15}), with 7.58% (2.647 ± 0.441 mg g⁻¹), and *cis*-13,16-docosadienoic acid (22:2), with 7.36% (2.571 ± 0.082 mg g⁻¹), neither of which were detected in olive oil.

Our results thereby showed that the fermented material could provide interesting amounts of these important UFAs.

Protein content and Digestibility

Novel protein sources can contribute to the future of protein nutrition by the improvement of some co-products as leaves and bagasse from the agro-industry (BOLAND et al., 2013; DONINI et al., 2005). One way to treat these co-products is the application of SSF, which, according to Bôas (2001), results in increases in the protein content.

A mixed substrate using co-products from agro-industry, was prepared with the bagasse and leaves of cassava (CL:CB – 3:7), and the initial amount of crude protein was 12.71 ± 0.08%. The SSF was performed for 30 days with the fungus *L. edodes* to promote the biotransformation of the substrate. After the period of fermentation, the total crude protein amount measured was 17.31 ± 0.06%, representing an increase of 36.19% in the amount of crude protein in the substrate. According to Alonso et al. (2000), the quantity of crude protein observed in the fermented substrate is similar to the crude protein content of peas (*Pisum sativum*), that presents 19.31 ± 0.21%.

The calculated digestibility of the proteins in the substrate before the SSF process was 27.72%. This value resulted in only 3.52% bioavailable protein content, meaning that the organism would not assimilate proteins from this material.

According Mattila et al. (2002), the digestibility of *L. edodes* was 72%, by the use of the factor 4.50 to calculate the protein. Thus, the digestible protein content in the substrate (CL:CB – 3:7) after the SSF process was determined to be 12.46 ± 0.04%. Comparing to peas, a representative legume used in human and livestock nutrition as a source of energy and protein, the content of digestible protein is similar, as it presents a 74% digestibility that results in 14.29% bioavailable protein (ALONSO et al., 2000; BOLAND et al., 2013).

The results obtained before and after the SSF with *L. edodes* imply that the fermentation process increased the bioavailability of the protein by a factor of 3.5, providing a better quality protein to consumers.

Amino Acids and PDCAAS and DIAAS

Nutritionally, amino acids are classified as essential and non-essential, with essential amino acids (EAAs) defined as those that cannot be synthesized by the organism (WU, 2009). Therefore, the quality of the protein is directly related to its digestibility (bioavailability) and the presence of EAA (TAVANO et al., 2004).

The action of the fungus in the CL:CB – 3:7 presented significant changes ($P < 0.0001$) for all EAAs (except for tryptophan). The profiles of the EAAs before and after the SSF and the DIAAS and PDCAAS are described in Table 2.

The calculated PDCAAS showed a low value compared to the casein (pattern protein). This is because the deficiency of threonine (limiting EAA) in the studied material leads to a value of 0.510 in the fermented material. However, looking at the DIAAS of the fermented substrate, it is possible to observe that the studied protein could provide enough histidine (His), isoleucine (Ile), and valine (Val), as well as good quantities of leucine (Leu); methionine + cysteine (Met + Cys); and phenylalanine + tyrosine (Phe + Tyr).

Histidine is the EAA that presents the greatest benefit to 2-5-year-old children. The studied protein could provide more than 2.5 times the total daily requirement of this EAA, with higher amounts than casein. The importance of His to the organism is related to protein methylation, hemoglobin structure and function, and antioxidative dipeptides, besides the relation with other products such as histamine and urocanic acid (WU, 2009). The amount of His found in the fermented substrate ($0.879 \text{ mg } 100\text{g}^{-1}$ of edible portion) was similar to that found in chuck short ribs ($0.836 \text{ mg } 100\text{g}^{-1}$ of edible portion), as described by Tacon and Metian (2013). This means that the quantity of His can be compared to that of meat, known as an important source of EAAs.

Valine was the second greatest EAA in terms of the benefit obtained from the fermented product, providing approximately 2 times the daily requirement. This EAA presents importance in the balance of branched chain amino acids (BCAA), besides the synthesis of alanine and glutamine (WU, 2009). The quantity of Val in chuck short ribs is $1.167 \text{ mg } 100\text{g}^{-1}$ of edible portion (TACON; METIAN, 2013). The amount of Val found in fermented CL:CB – 3:7 was $1.235 \text{ mg } 100\text{g}^{-1}$ of edible portion, a similar value to beef (chuck short ribs).

The measurement of Ile after the SSF presented an amount that could provide approximately 1.78 times more than that recommended by the FAO/WHO (1985). This quantity ($0.863 \text{ mg } 100\text{g}^{-1}$ of edible portion) resembled the amount found in pork backribs ($0.880 \text{ mg } 100\text{g}^{-1}$ of edible portion), beef brisket flat half ($0.864 \text{ mg } 100\text{g}^{-1}$ of edible portion), or and beef rib-eye steak ($0.897 \text{ mg } 100\text{g}^{-1}$ of edible portion) (TACON; METIAN, 2013). The

organic significance of Ile is directly related to the synthesis of glutamine and alanine, besides the balance of BCAAs (WU, 2009).

The sulfur amino acids (Met and Cys), which could reach 83.7% (in EAA mg g⁻¹ of protein) of the daily necessity requirements in the fermented material, are linked to homocysteine, betaine, choline, phospholipids, disulfide linkages in protein, the transport of sulfur, H₂S and taurine. Taurine is the most common free β -AA in bile, the retina of vertebrates, milk, skeletal muscle, heart, brain and many other tissues. However, human infants cannot synthesize enough taurine or cysteine due to the underdevelopment of key enzymes, necessitating its provision by nutrition (WU, 2009, 2013). The composition of the fermented substrate showed 0.361 mg 100g⁻¹ of edible portion of Met + Cys, which represents approximately 93.3% of the amount found in raw bacon (0.387 mg 100g⁻¹ of edible portion), according to Tacon and Metian (2013).

The results observed after SSF showed the possibility of providing approximately 81.7% of the daily requirements of aromatic amino acids (Phe and Tyr). This amount (0.890 mg 100g⁻¹ of edible portion) is higher than that found in raw bacon (0.823 mg 100g⁻¹ of edible portion), as described by Tacon and Metian (2013). Tyrosine (which is converted from Phe) is necessary to synthesize epinephrine, norepinephrine, thyroid hormones and melanin in animals. Phenylalanine intakes are also related to neurological development and function (WU, 2013). In addition to that, according to the same author, the consumption of all EAA can enhance the lean body mass, muscle strength, and physical function in elderly subjects.

The results obtained after the fermentation of co-products from the agro-industry with edible fungus showed that it is possible to provide good amounts of EAAs to consumers, sometimes similar to the amounts found in animal protein sources.

Relative Nutritional Value (RNV)

In addition to the PDCAAS and DIAAS, there is also another method that can be used to assess the quality of protein. The RNV, first proposed by Ford (1960), is an *in vitro* method to test the studied protein quality using a bacterium growth pattern (*Enterococcus zymogenes*). This is a significant method and faster than the traditional techniques (*in vivo*), with satisfactory results (TAVANO et al., 2004).

The fermented and unfermented material (CL:CB – 3:7) were subjected to the RNV method, and the protein showed a significant difference ($P < 0.01$). The results before the SSF process showed an RNV of $7.59 \pm 0.68\%$, which, when the digestibility (27.27%) is considered, presents a real RNV of $2.07 \pm 0.19\%$.

However, the RNV observed for the fermented CL:CB – 3:7 presented an RNV of $87.18 \pm 10.26\%$, which, when the digestibility (72.0%) is applied produces a real RNV of $69.33 \pm 8.16\%$. This means that the bioavailable total protein content improved from 2.07% to 69.33%, an increase by a factor of 33.5.

These results are similar to those obtained by Hegsted et al. (1968) for coconut flour (RNV = 63.9%) and full fat soya flour (RNV = 64.0%) and those of Tavano et al. (2004) for chickpea (*Cicer arietinum* L.) for crude chickpea (*Cicer arietinum* L.) flour and warmed glutelin (RNV = $69.87 \pm 2.78\%$ and $69.18 \pm 1.18\%$; respectively).

Ashes

Ash is defined as minerals and inorganic compounds, which can be classified nutritionally into major minerals (10 g day^{-1}), secondary minerals ($400\text{--}1500 \text{ mg day}^{-1}$), and trace minerals ($45 \text{ }\mu\text{g day}^{-1}$ to 11 mg day^{-1}) (GUPTA; GUPTA, 2014). Minerals are essential for the proper function of organisms (SOETAN; OLAIYA; OYEWOLE, 2010) and so the quantification of ash provides essential information on the nutrient content of foods.

The mixture used as a substrate for the SSF showed an initial ash content of $3.59 \pm 0.20\%$. After 30 days of fermentation, the total amount of ash increased to $3.99 \pm 0.21\%$, an improvement of 11.14%. These values are similar to those found in guava (*Psidium guajava*), a food known for its nutritional value, which has approximately 3.43% ash (DAS, 2011; LOUSADA JUNIOR et al., 2006). Our product also presents similar ash content to seeds of chia (*Salvia hispanica* L.), a functional food with approximately 4.6% ash (COELHO; SALAS-MELLADO, 2014)

Hence, the amount of ash found in the fermented substrate in this work is similar to that in known nutritive foods, making it a good source of minerals.

Conclusion

The use of *L. edodes* to carry out SSF shows many advantages in the use of by-products from the agro-industry. The fungus presents the capacity to decrease the amount of cyanide compounds in the substrate during the fermentation, significantly reducing the hazard by converting it to an edible concentration.

Carbohydrates are the main nutrient used in the SSF, as it is the only nutrient that decreased after the process, while all of the others (protein, lipids and ash) increased.

The UFAs were improved by the fungus activity during the SSF, showing important results mainly for the PUFAs, linoleic acid ($18:2^{9,12}$) and *cis*-13,16-docosadienoic acid ($22:2$).

This information indicates that the fermented material could be an important source of some PUFAs, including α -linolenic acid (18:3^{9,12,15}).

Another important finding was related to the changes in EAAs. In addition to the moderate amount of Thr (which leads to a moderate PDCAAS), the fermented substrate protein was shown to be rich in His, Ile, and Val, presenting higher amounts than casein (pattern protein) and thus making it a great source of these EAAs. Good amounts of Met + Cys and Phe + Tyr were found as well. In conclusion, despite the moderate PDCAAS, the DIAAS of many EAAs were satisfactory and could provide sufficient EAAs to the consumer.

The RNV results show that SSF with *L. edodes* could improve the bioavailability of the protein content by more than 33 times. This result also implies that the fermentation can promote an interesting conversion of the protein, facilitating the access to this nutrient by the organism.

In conclusion, the fermentation of by-products from the agro-industry (CL and CB) by *L. edodes* can become an important source of proteins, with good amounts of EAA, essential UFAs, minerals and carbohydrates, contributing to the production of food and the nutrition of humans, especially preschool-aged children.

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Table 1 – Characterization of fatty acids (mg/100g of Edible Portion) before and after SSF process.

		Substrate	<i>L. edodes</i> SSF
SFAs			
Myristic Acid	14:0	17.804 ± 1.504	15.630 ± 2.135
Pentadenoic Acid *	15:0	1.627 ± 0.355	20.236 ± 4.501
Palmitic Acid **	16:0	120.435 ± 13.300	225.236 ± 18.320
Stearic Acid **	18:0	15.662 ± 1.532	43.977 ± 3.304
Arachidic acid **	20:0	0.817 ± 0.503	45.464 ± 3.933
Lignocenic acid **	24:0	0.662 ± 0.382	44.269 ± 1.489
MUFAs			
Oleic Acid	18:1	71.049 ± 10.070	85.701 ± 11.970
<i>cis</i> -11-Eicosenoic acid **	20:1	0.548 ± 0.316	86.665 ± 0.358
PUFAs			
Linoleic Acid **	18:2 ^{9,12}	70.517 ± 8.630	475.766 ± 31.850
α -Linolenic Acid	18:3 ^{9,12,15}	257.522 ± 16.750	264.705 ± 44.140
<i>cis</i> -11,14-Eicosadienoic acid **	20:2	1.713 ± 0.205	16.195 ± 0.274
<i>cis</i> -13,16-Docosadienoic acid **	22:2	125.996 ± 4.640	257.074 ± 8.822
SFAs, MUFAs and PUFAs		684.403 ± 58.187	1563.376 ± 131.096
Total lipids		1484.00 ± 186.68	3493.80 ± 95.94

* Fatty Acids that significant increase was observed (P < 0.05);

** Fatty Acids that significant increase was observed (P < 0.01).

Table 2 – Characterization of Essential Amino Acids (EAA mg g⁻¹ of Protein)

EAA	FAO/WHO Pattern*	Essential Amino Acids Quantity				Casein**	
		Substrate		Fermented Substrate			
		Child	EAA	DIAAS ^Δ	EAA	DIAAS ^Δ	EAA
HIS [†]	19	0.73	3.9	50.82	267.5	18.99	100.0
ILE [†]	28	0.66	2.4	49.88	178.2	46.91	167.5
LEU [†]	66	0.98	1.5	64.01	97.0	93.05	141.0
LYS [†]	58	1.29	2.2	31.87	55.0	78.66	135.6
MET + CYS [†]	25	0.30	1.2	20.84	83.4	30.14	120.6
PHE + TYR [†]	63	1.14	1.8	51.44	81.7	109.71	174.1
THR [†]	34	0.30	0.9	17.32	50.9	43.22	127.1
TRP	11	ND ^a	-	ND ^a	-	ND ^a	-
VAL [†]	35	1.01	2.9	71.36	203.9	54.95	157.0
PDCAAS		0.009		0.510		1.000	

Underlined values are identifying the limiting EAA to calculate the PDCAAS;

* According FAO/WHO (1985);

** According to PIRES et al. (2006);

^Δ DIAAS express the percentage that the portion can supply of each EAA related to the FAO/WHO pattern;

^a ND – Not Detected

[†] AA that significant increases were observed (P < 0.0001);

His (histidine); Thr (threonine); Val (valine); Met (methionine); Cys (cysteine); Ile (isoleucine); Leu (leucine);

Phe (phenylalanine); Tyr (tyrosine); Lys (lysine); Trp (tryptophan).

6. Considerações Finais

Os substratos formulados pela mistura de bagaço de mandioca (BM) e de folhas de mandioca (FM), mostram-se adequados para o desenvolvimento dos fungos estudados *R. oligosporus*; *P. ostreatus*; e *L. edodes*. Contudo, o fungo *A. blazei* apresentou baixa adaptação ao substrato, impedindo a avaliação. Desta maneira, fica evidente que a utilização do fungo *A. blazei* para tratamento de resíduos e co-produtos da agroindústria necessita de estudos mais específicos. Assim, conclui-se que:

- *R. oligosporus* apresentou: rápido crescimento do fungo no substrato (BM:BF – 1:1); diminuição da concentração de cianeto para patamar considerado “não tóxico”; características nutricionais interessantes como aumento da quantidade de ácidos graxos mono e poli-insaturados (oleico; γ -linolênico; e ácidos graxos insaturados de cadeia longa) e alterações no conteúdo de aminoácidos essenciais (histidina; isoleucina; valina; e leucina); além da melhora biológica da proteína, observada pelo aumento do VNR;
- *P. ostreatus*: reduziu a quantidade de cianeto para valores insignificantes; apesar da baixa quantidade de proteínas mostrou significativo aumento em 7 de 9 AAE (isoleucina, leucina, lisina, metionina, fenilalanina, treonina e valina); maior biodisponibilidade das proteínas observada pelo VNR; significativo aumento em ácidos graxos (ácido oleico; linoleico; e γ -linolênico). Conclui-se que o material fermentado por *P. ostreatus* se caracteriza como uma interessante fonte de carbono “enriquecida” com proteína com melhores valores nutricionais referentes à AAE e AGI;
- *L. edodes*: promoveu desintoxicação significativa do substrato, atingindo concentração considerada segura de cianeto; quantidade importantes de AGI (ácido linoleico; ácido *cis*-13,16-docosadienóico; α -linolênico); importantes quantidades de histidina,

isoleucina e valina além de boa quantidade de metionina e fenilalanina; o VNR observado após a fermentação mostrou um aumento de cerca de 33 vezes na biodisponibilidade da proteína; apresenta potencial de aproveitamento como uma boa fonte de proteínas com interessantes quantidade de AAE, AGI essenciais e minerais.

A redução do cianeto para níveis seguros de consumo observada após o processo de FES mostram que a aplicação de fungos comestíveis permitem detoxificar substratos alternativos e favorecer sua aplicação na obtenção de novos alimentos. Esse importante fator, bem como o aumento da quantidade e biodisponibilidade de proteínas pode proporcionar o aumento no valor agregado de produtos produzidos a partir de materiais considerados de baixo custo, como resíduos agroindustriais. O aproveitamento desses resíduos com provável acréscimo de valor agregado pode tornar rentável materiais que, anteriormente, gerariam custos com seu manejo.

Desta maneira, a utilização de resíduos agroindustriais podem ser biotransformados a fontes de alimentos que apresentam boas quantidades de AAE e AGI trazendo boas quantidades de nutrientes para os consumidores. Assim, os resultados obtidos pelo uso de resíduos e co-produtos da agroindústria como o BM e a FM mediante a FES, mostram que o aproveitamento de outras fontes alternativas na obtenção de novos alimentos podem aumentar a disponibilidade de alimentos e, conseqüentemente, diminuir a concentração de subnutridos, principalmente pelo seu baixo custo de produção.

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8. ANEXOS

BLAST ®

Basic Local Alignment Search Tool

[NCBI/ BLAST/ blastn suite/ Formatting Results - 75B2T3HF015](#)[Formatting options](#)[Download](#)[Blast report description](#)

Nucleotide Sequence (641 letters)

RID [75B2T3HF015](#) (Expires on 12-18 19:36 pm)

Query ID lcl|Query_208063

Description None

Molecule type nucleic acid

Query Length 641

Database Name nr

Description Nucleotide collection (nt)

Program BLASTN 2.3.0+

[Graphic Summary](#)

Distribution of 100 Blast Hits on the Query Sequence



Descriptions

Sequences producing significant alignments:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Rhizopus microsporus var. rhizopodiformis strain ZJPH 1308 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KR998045.1
Rhizopus microsporus strain WM 08.191 isolate ISHAM-ITS_ID MITS2192 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KP132572.1
Rhizopus microsporus strain IHEM 21967 isolate ISHAM-ITS_ID MITS2194 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KP132570.1
Rhizopus microsporus 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KM103772.1
Rhizopus microsporus var. rhizopodiformis isolate VPCI 728/09 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KJ417564.1
Rhizopus microsporus isolate ATH23 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	1094	1094	96%	0.0	98%	KF710006.1
Rhizopus microsporus isolate ATH14 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KF710005.1
Rhizopus microsporus isolate ATH13 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KF710004.1
Rhizopus microsporus isolate ATH47 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KF710002.1
Rhizopus microsporus isolate ATH15 18S						

ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KF709999.1
Rhizopus microsporus isolate ATH59 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KF709998.1
Rhizopus microsporus isolate ATH33 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KF709997.1
Rhizopus microsporus isolate ATH10 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KF709996.1
Rhizopus microsporus isolate ATH38 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KF709995.1
Rhizopus microsporus isolate ATH66 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KF709993.1
Rhizopus microsporus isolate ATH60 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KF709991.1
Rhizopus microsporus isolate ATH58 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KF709990.1
Rhizopus microsporus isolate ATH61 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KF709989.1
Rhizopus microsporus isolate ATH41 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KF709987.1
Rhizopus microsporus isolate ATH26 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KF709986.1
Rhizopus microsporus isolate ATH27 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal						

RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KF709985.1
Rhizopus microsporus isolate ATH31 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KF709984.1
Rhizopus microsporus isolate ATH35 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KF709983.1
Rhizopus microsporus isolate ATH48 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KF709982.1
Rhizopus microsporus isolate ATH65 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KF709981.1
Rhizopus microsporus isolate ATH64 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KF709980.1
Rhizopus microsporus isolate ATH54 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KF709979.1
Rhizopus microsporus isolate ATH24 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KF709978.1
Uncultured fungus clone CMH146 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KF800237.1
Rhizopus microsporus genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, isolate: ATH63	1094	1094	96%	0.0	98%	AB894627.1
Rhizopus microsporus genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, isolate: ATH40	1094	1094	96%	0.0	98%	AB894625.1
Rhizopus microsporus genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, isolate: ATH29	1094	1094	96%	0.0	98%	AB894624.1
Rhizopus microsporus strain CBS 437.78 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KC206548.1

Rhizopus microsporus strain CBS 196.77 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	KC206547.1
Rhizopus microsporus strain 158 18S ribosomal RNA, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA, partial sequence	1094	1094	96%	0.0	98%	JX661044.1
Rhizopus microsporus strain CNM-CM-5226 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	JX120683.1
Rhizopus microsporus strain CNM-CM-5167 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	JX120682.1
Rhizopus microsporus strain CNM-CM-5165 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	JX120681.1
Rhizopus microsporus isolate F2-02 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	JN561253.1
Rhizopus microsporus strain JJ-A3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	HQ285720.1
Rhizopus microsporus strain WJ-A3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	HQ285719.1
Rhizopus microsporus strain DG-C4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	HQ285708.1
Rhizopus microsporus strain D4-1 isolate 2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	GQ502279.1
Rhizopus microsporus isolate F17 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	EF151442.1
Rhizopus microsporus var. rhizopodiformis strain CBS 343.29 18S ribosomal RNA gene,						

partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	DQ641308.1
Fungal sp. DQY-11 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	DQ658186.1
Rhizopus microsporus var. rhizopodiformis strain ATCC 200758 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	AY803934.1
Rhizopus azygosporus strain CBS 357.93 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	DQ119008.1
Rhizopus microsporus AHS-479-41 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1094	1094	96%	0.0	98%	DQ026015.1
Rhizopus rhizopodiformis isolate 31994 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	1094	1094	96%	0.0	98%	AF115720.1
Rhizopus microsporus genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, strain: AHU6540	1094	1094	96%	0.0	98%	AB097380.1
Rhizopus microsporus isolate ATH67 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1092	1092	96%	0.0	98%	KF709994.1
Rhizopus microsporus strain CNM-CM-4244 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	1092	1092	96%	0.0	99%	JX120680.1
Rhizopus microsporus strain D4-1 isolate 1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1090	1090	96%	0.0	98%	GQ502278.1
Rhizopus microsporus strain P2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1090	1090	96%	0.0	98%	FJ854337.1
Rhizopus microsporus var. oligosporus genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, strain: CBS338.62	1090	1090	96%	0.0	98%	AB097389.1
Rhizopus microsporus var. rhizopodiformis						

strain PWQ2133 isolate ISHAM-ITS_ID MITS2199 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1088	1088	96%	0.0	98%	KP132573.1
Rhizopus microsporus var. rhizopodiformis isolate VPCI 177/P/10 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1088	1088	96%	0.0	98%	KJ417571.1
Rhizopus microsporus isolate ATH50 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1088	1088	96%	0.0	98%	KF710003.1
Rhizopus microsporus var. oligosporus strain KCCM11275 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1088	1088	96%	0.0	98%	HQ285600.1
Rhizopus microsporus var. rhizopodiformis strain R-49 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1088	1088	96%	0.0	98%	DQ641306.1
Rhizopus microsporus var. chinensis isolate 31998 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	1088	1088	96%	0.0	98%	AF115732.1
Rhizopus sexualis var. americanus genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, clone: 340.62-4	1088	1088	96%	0.0	98%	AB113011.1
Rhizopus microsporus var. oligosporus strain KAS3569 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	1086	1086	96%	0.0	98%	JN942875.1
Rhizopus microsporus var. chinensis strain CBS 537.80 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1086	1086	96%	0.0	98%	AY243957.1
Rhizopus microsporus var. oligosporus strain CBS 339.62 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1086	1086	96%	0.0	98%	AY243956.1
Rhizopus rhizopodiformis isolate 31995 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	1086	1086	96%	0.0	98%	AF115721.1
Rhizopus microsporus isolate kfd internal						

transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	1083	1083	96%	0.0	98%	KR704882.1
Rhizopus microsporus var. rhizopodiformis isolate VPCI 24/p/10 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1083	1083	95%	0.0	98%	KJ417569.1
Rhizopus microsporus var. rhizopodiformis isolate VPCI 15/P/09 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1083	1083	95%	0.0	98%	KJ417563.1
Rhizopus microsporus strain CBS 220.92 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	1083	1083	95%	0.0	98%	KC206517.1
Rhizopus microsporus strain CBS 118987 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	1083	1083	95%	0.0	98%	KC206516.1
Rhizopus microsporus strain CBS 607.73 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	1083	1083	95%	0.0	98%	KC206515.1
Rhizopus microsporus var. rhizopodiformis strain CBS 196.77 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1083	1083	95%	0.0	98%	JN206361.1
Rhizopus azygosporus CBS 357.93 ITS region; from TYPE material	1083	1083	95%	0.0	98%	NR_103653.1
Uncultured zygomycete partial 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and partial 28S rRNA gene, clone 7.3	1083	1083	96%	0.0	98%	AJ876510.1
Rhizopus microsporus strain CBS 359.92 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	1079	1079	95%	0.0	98%	KC206544.1
Rhizopus microsporus strain CBS 102277 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	1079	1079	95%	0.0	98%	KC206521.1
Rhizopus microsporus var. rhizopodiformis strain CBS 102277 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1079	1079	95%	0.0	98%	JN206356.1
Rhizopus azygosporus strain CBS 359.92 internal transcribed spacer 1, partial						

sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	1079	1079	95%	0.0	98%	JN943010.1
Rhizopus microsporus strain CNM-CM-2328 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	1077	1077	95%	0.0	98%	JX120676.1
Rhizopus microsporus genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, isolate: ATH9	1075	1075	95%	0.0	98%	AB894623.1
Rhizopus microsporus genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, isolate: ATH1	1075	1075	95%	0.0	98%	AB894622.1
Rhizopus microsporus strain CBS 130968 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	1075	1075	95%	0.0	98%	KC206537.1
Rhizopus microsporus strain CBS 130966 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	1075	1075	95%	0.0	99%	KC206533.1
Rhizopus microsporus var. chinensis isolate 32006 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	1075	1075	96%	0.0	98%	AF115737.1
Rhizopus microsporus var. rhizopodiformis strain CBS 343.29 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1074	1074	95%	0.0	98%	AY243958.1
Rhizopus microsporus var. microsporus strain NRBC 32996 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1074	1074	96%	0.0	98%	AY803932.1
Rhizopus microsporus var. microsporus strain FSU10049 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	1072	1072	94%	0.0	99%	KJ408570.1
Rhizopus microsporus strain CBS 388.34 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	1072	1072	95%	0.0	98%	KC206524.1
Rhizopus microsporus var. chinensis strain CBS 388.34 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1072	1072	95%	0.0	98%	JN206354.1
Rhizopus microsporus var. chinensis genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S	1072	1072	96%	0.0	98%	AB097379.1

rRNA, partial and complete sequence, strain:
AHU6510

Rhizopus microsporus strain CNM-CM-5370
internal transcribed spacer 1, partial
sequence; 5.8S ribosomal RNA gene and
internal transcribed spacer 2, complete
sequence; and 28S ribosomal RNA gene,
partial sequence

1070

1070

94%

0.0

98%

[JX120684.1](#)

Rhizopus microsporus var. oligosporus
isolate 31996 18S ribosomal RNA gene,
partial sequence; internal transcribed spacer
1, 5.8S ribosomal RNA gene, and internal
transcribed spacer 2, complete sequence

1070

1070

96%

0.0

98%

[AF115735.1](#)

Rhizopus azygosporus strain NRBC 31989
18S ribosomal RNA gene, partial sequence;
internal transcribed spacer 1, 5.8S ribosomal
RNA gene, and internal transcribed spacer
2, complete sequence; and 28S ribosomal
RNA gene, partial sequence

1064

1064

96%

0.0

97%

[AY803937.1](#)

Rhizopus microsporus var. chinensis strain
CBS 631.82 18S ribosomal RNA gene,
partial sequence; internal transcribed spacer
1, 5.8S ribosomal RNA gene, and internal
transcribed spacer 2, complete sequence;
and 28S ribosomal RNA gene, partial
sequence

1062

1062

96%

0.0

97%

[DQ641310.1](#)

Rhizopus microsporus var. oligosporus strain
R-47 18S ribosomal RNA gene, partial
sequence; internal transcribed spacer 1,
5.8S ribosomal RNA gene, and internal
transcribed spacer 2, complete sequence;
and 28S ribosomal RNA gene, partial
sequence

1061

1061

96%

0.0

97%

[DQ641312.1](#)

Rhizopus microsporus var. rhizopodiformis
isolate HX-1 internal transcribed spacer 1,
partial sequence; 5.8S ribosomal RNA gene
and internal transcribed spacer 2, complete
sequence; and 28S ribosomal RNA gene,
partial sequence

1059

1059

95%

0.0

98%

[KM527222.1](#)

Rhizopus microsporus isolate ATH11
internal transcribed spacer 1, partial
sequence; 5.8S ribosomal RNA gene and
internal transcribed spacer 2, complete
sequence; and 28S ribosomal RNA gene,
partial sequence

1059

1059

93%

0.0

98%

[KF710000.1](#)

Rhizopus microsporus isolate ATH32
internal transcribed spacer 1, partial
sequence; 5.8S ribosomal RNA gene and
internal transcribed spacer 2, complete
sequence; and 28S ribosomal RNA gene,
partial sequence

1059

1059

93%

0.0

98%

[KF709992.1](#)

Alignments

Rhizopus microsporus var. rhizopodiformis strain ZJPH 1308 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Sequence ID: **gb|KR998045.1|** Length: 672 Number of Matches: 1

Range 1: 6 to 624

Score	Expect	Identities	Gaps	Strand	Frame
1094 bits(592)	0.0()	613/623(98%)	6/623(0%)	Plus/Plus	

Features:

Query 11 GGAAGGATCATTAACATAATGTATTGGCACTTTACTGGGATTTACTTCTCAGTATTGTTTG 70
|||||

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Sbjct  6      GGAAGGATCATTAAC TAATGTATTGGCAC TTTACTGGGATTTACTTCTCAGTATTGTTTG 65
Query  71      CTTCTATACTGTGAACCTCTGGCGATGAAGGTCGTAAC TGACCTTCGGGAGAGACTCANG 130
Sbjct  66      CTTCTATACTGTGAACCTCTGGCGATGAAGGTCGTAAC TGACCTTCGGGAGAGACTCAGG 125
Query  131     ACATATAGGCTATAATGGGTAGGCTGTTCTGGGGTTTGATCGATGCCAATCAGGATTAC 190
Sbjct  126     ACATATAGGCTATAATGGGTAGGCTGTTCTGGGGTTTGATCGATGCCAATCAGGATTAC 185
Query  191     CTTTCTTCCTTTGGGAAGGAAGGTGCCTGGTACCCTTTACCATATACCATGAATTCAGAA 250
Sbjct  186     CTTTCTTCCTTTGGGAAGGAAGGTGCCTGGTACCCTTTACCATATACCATGAATTCAGAA 245
Query  251     TTGAAAGTATAATATAATAACAAC TTTTAACAATGGATCTCTTGTTCTCGCATCGATGA 310
Sbjct  246     TTGAAAGTATAATATAATAACAAC TTTTAACAATGGATCTCTTGTTCTCGCATCGATGA 305
Query  311     AGAACGTANCAGAGTGC GATAACTAGTGTGAATTGCATATTCGTGAATCATCGAGTCTTT 370
Sbjct  306     AGAACGTAGCAAAGTGC GATAACTAGTGTGAATTGCATATTCGTGAATCATCGAGTCTTT 365
Query  371     GAACGCAGCTTGCACTCTATGGATCTTCTATAGAGTACGCTTGCTTCAGTATCATAACCA 430
Sbjct  366     GAACGCAGCTTGCACTCTATGGATCTTCTATAGAGTACGCTTGCTTCAGTATCATAACCA 425
Query  431     ACCCACACATAAAATTTATTTTATGTGGTGATGGACAAGCTCGGTTAAATTTAATTATTA 490
Sbjct  426     ACCCACACATAAAATTTATTTTATGTGGTGATGGACAAGCTCGGTTAAATTTAATTATTA 485
Query  491     TACCGATTGTCTAAAATACAGCCCTCTTTGTAATTTTCATTAAATTACGAAC TACCCGAG 550
Sbjct  486     TACCGATTGTCTAAAATACAG- CCTCTTTGTAATTTTCATTAAATTACGAAC TACCT-AG 543
Query  551     CCATCGTGCTTTTTT- GGTCCAACCCAAAAACATATAATCTAGGGGTTCTGCTAGCCAG 609
Sbjct  544     CCATCGTGCTTTTTTGGTCCAA- CCAAAAAACATATAATCTAGGGGTTCTGCTAG-CCAG 601
Query  610     CAGATATTTTAATGATCTT- AAC 631
Sbjct  602     CAGATATTTTAATGATCTTTAAC 624

```

Rhizopus microsporus strain WM 08.191 isolate ISHAM-ITS_ID MITS2192 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Sequence ID: **gb|KP132572.1|** Length: 675 Number of Matches: 1

Range 1: 18 to 636

Score	Expect	Identities	Gaps	Strand	Frame
1094 bits(592)	0.0()	613/623(98%)	6/623(0%)	Plus/Plus	

Features:

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Query  11      GGAAGGATCATTAAC TAATGTATTGGCAC TTTACTGGGATTTACTTCTCAGTATTGTTTG 70
Sbjct  18      GGAAGGATCATTAAC TAATGTATTGGCAC TTTACTGGGATTTACTTCTCAGTATTGTTTG 77
Query  71      CTTCTATACTGTGAACCTCTGGCGATGAAGGTCGTAAC TGACCTTCGGGAGAGACTCANG 130
Sbjct  78      CTTCTATACTGTGAACCTCTGGCGATGAAGGTCGTAAC TGACCTTCGGGAGAGACTCAGG 137
Query  131     ACATATAGGCTATAATGGGTAGGCTGTTCTGGGGTTTGATCGATGCCAATCAGGATTAC 190
Sbjct  138     ACATATAGGCTATAATGGGTAGGCTGTTCTGGGGTTTGATCGATGCCAATCAGGATTAC 197
Query  191     CTTTCTTCCTTTGGGAAGGAAGGTGCCTGGTACCCTTTACCATATACCATGAATTCAGAA 250
Sbjct  198     CTTTCTTCCTTTGGGAAGGAAGGTGCCTGGTACCCTTTACCATATACCATGAATTCAGAA 257
Query  251     TTGAAAGTATAATATAATAACAAC TTTTAACAATGGATCTCTTGTTCTCGCATCGATGA 310
Sbjct  258     TTGAAAGTATAATATAATAACAAC TTTTAACAATGGATCTCTTGTTCTCGCATCGATGA 317
Query  311     AGAACGTANCAGAGTGC GATAACTAGTGTGAATTGCATATTCGTGAATCATCGAGTCTTT 370
Sbjct  318     AGAACGTAGCAAAGTGC GATAACTAGTGTGAATTGCATATTCGTGAATCATCGAGTCTTT 377
Query  371     GAACGCAGCTTGCACTCTATGGATCTTCTATAGAGTACGCTTGCTTCAGTATCATAACCA 430
Sbjct  378     GAACGCAGCTTGCACTCTATGGATCTTCTATAGAGTACGCTTGCTTCAGTATCATAACCA 437
Query  431     ACCCACACATAAAATTTATTTTATGTGGTGATGGACAAGCTCGGTTAAATTTAATTATTA 490
Sbjct  438     ACCCACACATAAAATTTATTTTATGTGGTGATGGACAAGCTCGGTTAAATTTAATTATTA 497
Query  491     TACCGATTGTCTAAAATACAGCCCTCTTTGTAATTTTCATTAAATTACGAAC TACCCGAG 550
Sbjct  498     TACCGATTGTCTAAAATACAG- CCTCTTTGTAATTTTCATTAAATTACGAAC TACCT-AG 555
Query  551     CCATCGTGCTTTTTT- GGTCCAACCCAAAAACATATAATCTAGGGGTTCTGCTAGCCAG 609
Sbjct  556     CCATCGTGCTTTTTTGGTCCAA- CCAAAAAACATATAATCTAGGGGTTCTGCTAG-CCAG 613

```

Query 610 CAGATATTTTAATGATCTT-AAC 631
 Sbjct 614 CAGATATTTTAATGATCTTTAAC 636

Rhizopus microsporus strain IHEM 21967 isolate ISHAM-ITS_ID MITS2194 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Sequence ID: **gb|KP132570.1** Length: 696 Number of Matches: 1
 Range 1: 31 to 649

Score	Expect	Identities	Gaps	Strand	Frame
1094 bits(592)	0.0()	613/623(98%)	6/623(0%)	Plus/Plus	

Features:

Query	11	GG AAGGATCATTAAC TAATGTATTGG CACTTTACTGGGATTTACTTCTCAGTATTGTTTG	70
Sbjct	31	GG AAGGATCATTAAC TAATGTATTGG CACTTTACTGGGATTTACTTCTCAGTATTGTTTG	90
Query	71	CTTCTATACTGTGAACCTCTGGCGATGAAGGTCGTAAC TGACCTTCGGGAGAGACTCANG	130
Sbjct	91	CTTCTATACTGTGAACCTCTGGCGATGAAGGTCGTAAC TGACCTTCGGGAGAGACTCAGG	150
Query	131	ACATATAGGCTATAATGGGTAGGCCTGTTCTGGGGTTTGATCGATGCCAATCAGGATTAC	190
Sbjct	151	ACATATAGGCTATAATGGGTAGGCCTGTTCTGGGGTTTGATCGATGCCAATCAGGATTAC	210
Query	191	CTTTCTTCCTTTGGGAAGGAAGGTGCCTGGTACCCTTTACCATATACCATGAATTCAGAA	250
Sbjct	211	CTTTCTTCCTTTGGGAAGGAAGGTGCCTGGTACCCTTTACCATATACCATGAATTCAGAA	270
Query	251	TTGAAAGTATAATATAATAACAAC TTTTAACAATGGATCTCTTGGTTCTCGCATCGATGA	310
Sbjct	271	TTGAAAGTATAATATAATAACAAC TTTTAACAATGGATCTCTTGGTTCTCGCATCGATGA	330
Query	311	AGAACGTANCAGAGTGC GATAACTAGTGTGAATTGCATATTCGTGAATCATCGAGTCTTT	370
Sbjct	331	AGAACGTAGCAAAGTGC GATAACTAGTGTGAATTGCATATTCGTGAATCATCGAGTCTTT	390
Query	371	GAACGCAGCTTGCACTCTATGGATCTTCTATAGAGTACGCTTGCTTCAGTATCATAACCA	430
Sbjct	391	GAACGCAGCTTGCACTCTATGGATCTTCTATAGAGTACGCTTGCTTCAGTATCATAACCA	450
Query	431	ACCCACACATAAAATTTATTTTATGTGGTGATGGACAAGCTCGGTTAAATTTAATTATTA	490
Sbjct	451	ACCCACACATAAAATTTATTTTATGTGGTGATGGACAAGCTCGGTTAAATTTAATTATTA	510
Query	491	TACCGATTGTCTAAAATACAGCCCTCTTTGTAATTTTCATTAAATTACGAAC TACCCGAG	550
Sbjct	511	TACCGATTGTCTAAAATACAG-CCTCTTTGTAATTTTCATTAAATTACGAAC TACCT-AG	568
Query	551	CCATCGTGCTTTTTT-GGTCCAACCCAAAAACATATAATCTAGGGGTTCTGCTAGCCAG	609
Sbjct	569	CCATCGTGCTTTTTTGGTCCAA-CCAAAAACATATAATCTAGGGGTTCTGCTAG-CCAG	626
Query	610	CAGATATTTTAATGATCTT-AAC 631	
Sbjct	627	CAGATATTTTAATGATCTTTAAC 649	

Rhizopus microsporus 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Sequence ID: **gb|KM103772.1** Length: 699 Number of Matches: 1
 Range 1: 18 to 636

Score	Expect	Identities	Gaps	Strand	Frame
1094 bits(592)	0.0()	613/623(98%)	6/623(0%)	Plus/Plus	

Features:

Query	11	GG AAGGATCATTAAC TAATGTATTGG CACTTTACTGGGATTTACTTCTCAGTATTGTTTG	70
Sbjct	18	GG AAGGATCATTAAC TAATGTATTGG CACTTTACTGGGATTTACTTCTCAGTATTGTTTG	77
Query	71	CTTCTATACTGTGAACCTCTGGCGATGAAGGTCGTAAC TGACCTTCGGGAGAGACTCANG	130
Sbjct	78	CTTCTATACTGTGAACCTCTGGCGATGAAGGTCGTAAC TGACCTTCGGGAGAGACTCAGG	137
Query	131	ACATATAGGCTATAATGGGTAGGCCTGTTCTGGGGTTTGATCGATGCCAATCAGGATTAC	190
Sbjct	138	ACATATAGGCTATAATGGGTAGGCCTGTTCTGGGGTTTGATCGATGCCAATCAGGATTAC	197
Query	191	CTTTCTTCCTTTGGGAAGGAAGGTGCCTGGTACCCTTTACCATATACCATGAATTCAGAA	250

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Sbjct  198  CTTTCTTCCTTTGGGAAGGAAGGTGCCTGGTACCCTTTACCATATACCATGAATTCAGAA 257
Query   251  TTGAAAGTATAATATAATAACAACCTTTTAACAATGGATCTCTTGGTTCTCGCATCGATGA 310
Sbjct  258  TTGAAAGTATAATATAATAACAACCTTTTAACAATGGATCTCTTGGTTCTCGCATCGATGA 317
Query   311  AGAACGTANCAGAGTGCAGATAACTAGTGTGAATTGCATATTCGTGAATCATCGAGTCTTT 370
Sbjct  318  AGAACGTAGCAAAGTGCAGATAACTAGTGTGAATTGCATATTCGTGAATCATCGAGTCTTT 377
Query   371  GAACGCAGCTTGCACTCTATGGATCTTCTATAGAGTACGCTTGCTTCAGTATCATAACCA 430
Sbjct  378  GAACGCAGCTTGCACTCTATGGATCTTCTATAGAGTACGCTTGCTTCAGTATCATAACCA 437
Query   431  ACCCACACATAAAATTTATTTTATGTGGTGTGGACAAGCTCGGTTAAATTTAATTATTA 490
Sbjct  438  ACCCACACATAAAATTTATTTTATGTGGTGTGGACAAGCTCGGTTAAATTTAATTATTA 497
Query   491  TACCGATTGTCTAAAATACAGCCCTCTTTGTAATTTTCATTAAATTACGAAC TACCCGAG 550
Sbjct  498  TACCGATTGTCTAAAATACAG-CCTCTTTGTAATTTTCATTAAATTACGAAC TACCT-AG 555
Query   551  CCATCGTGCTTTTTT-GGTCCAACCCAAAAACATATAATCTAGGGGTTCTGCTAGCCCGAG 609
Sbjct  556  CCATCGTGCTTTTTTGGTCCAA-CCAAAAACATATAATCTAGGGGTTCTGCTAG-CCAG 613
Query   610  CAGATATTTTAATGATCTT-AAC 631
Sbjct  614  CAGATATTTTAATGATCTTTAAC 636

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Rhizopus microsporus var. rhizopodiformis isolate VPCI 728/09 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Sequence ID: **gb|KJ417564.1|** Length: 700 Number of Matches: 1

See 3 more title(s)
range 1: 18 to 636

Score	Expect	Identities	Gaps	Strand	Frame
1094 bits(592)	0.0()	613/623(98%)	6/623(0%)	Plus/Plus	
Features:					
Query 11	GGAAGGATCATTAAC TAATGTATTGGCACTTTACTGGGATT TACTTCTCAGTATTGTTTG	70			
Sbjct 18	GGAAGGATCATTAAC TAATGTATTGGCACTTTACTGGGATT TACTTCTCAGTATTGTTTG	77			
Query 71	CTTCTATACTGTGAACCTCTGGCGATGAAGGTCGTAAC TGACCTTCGGGAGAGACTCANG	130			
Sbjct 78	CTTCTATACTGTGAACCTCTGGCGATGAAGGTCGTAAC TGACCTTCGGGAGAGACTCAGG	137			
Query 131	ACATATAGGCTATAATGGGTAGGCTGTTCTGGGGTTTGATCGATGCCAATCAGGATTAC	190			
Sbjct 138	ACATATAGGCTATAATGGGTAGGCTGTTCTGGGGTTTGATCGATGCCAATCAGGATTAC	197			
Query 191	CTTTCTTCCTTTGGGAAGGAAGGTGCCTGGTACCCTTTACCATATACCATGAATTCAGAA	250			
Sbjct 198	CTTTCTTCCTTTGGGAAGGAAGGTGCCTGGTACCCTTTACCATATACCATGAATTCAGAA	257			
Query 251	TTGAAAGTATAATATAATAACAACCTTTTAACAATGGATCTCTTGGTTCTCGCATCGATGA	310			
Sbjct 258	TTGAAAGTATAATATAATAACAACCTTTTAACAATGGATCTCTTGGTTCTCGCATCGATGA	317			
Query 311	AGAACGTANCAGAGTGCAGATAACTAGTGTGAATTGCATATTCGTGAATCATCGAGTCTTT	370			
Sbjct 318	AGAACGTAGCAAAGTGCAGATAACTAGTGTGAATTGCATATTCGTGAATCATCGAGTCTTT	377			
Query 371	GAACGCAGCTTGCACTCTATGGATCTTCTATAGAGTACGCTTGCTTCAGTATCATAACCA	430			
Sbjct 378	GAACGCAGCTTGCACTCTATGGATCTTCTATAGAGTACGCTTGCTTCAGTATCATAACCA	437			
Query 431	ACCCACACATAAAATTTATTTTATGTGGTGTGGACAAGCTCGGTTAAATTTAATTATTA	490			
Sbjct 438	ACCCACACATAAAATTTATTTTATGTGGTGTGGACAAGCTCGGTTAAATTTAATTATTA	497			
Query 491	TACCGATTGTCTAAAATACAGCCCTCTTTGTAATTTTCATTAAATTACGAAC TACCCGAG	550			
Sbjct 498	TACCGATTGTCTAAAATACAG-CCTCTTTGTAATTTTCATTAAATTACGAAC TACCT-AG	555			
Query 551	CCATCGTGCTTTTTT-GGTCCAACCCAAAAACATATAATCTAGGGGTTCTGCTAGCCCGAG	609			
Sbjct 556	CCATCGTGCTTTTTTGGTCCAA-CCAAAAACATATAATCTAGGGGTTCTGCTAG-CCAG	613			
Query 610	CAGATATTTTAATGATCTT-AAC 631				
Sbjct 614	CAGATATTTTAATGATCTTTAAC 636				

BLAST ®

Basic Local Alignment Search Tool

[NCBI/ BLAST/ blastn suite/](#) Formatting Results - 75A0D61S014[Formatting options](#)[Download](#)[Blast report description](#)

Nucleotide Sequence (655 letters)

RID [75A0D61S014](#) (Expires on 12-18 19:18 pm)

Query ID lcl|Query_84065

Description None

Molecule type nucleic acid

Query Length 655

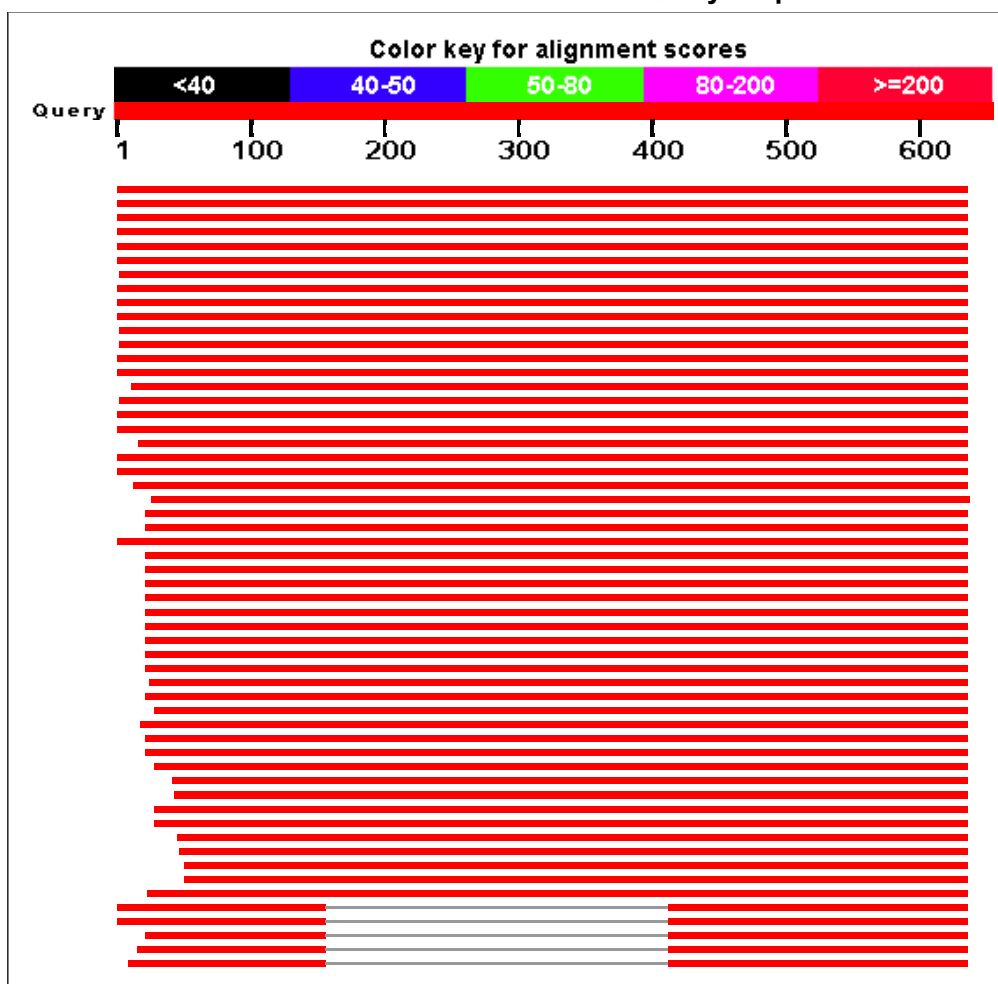
Database Name nr

Description Nucleotide collection (nt)

Program BLASTN 2.3.0+

[Graphic Summary](#)

Distribution of 146 Blast Hits on the Query Sequence



Descriptions

Sequences producing significant alignments:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Pleurotus ostreatus cultivar Yiping 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	678	678	96%	0.0	82%	FJ501550.1
Pleurotus ostreatus strain S474 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	678	678	96%	0.0	82%	AY540332.1
Pleurotus sapidus strain S047 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	678	678	96%	0.0	82%	AY540327.1
Pleurotus sapidus strain S046 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	678	678	96%	0.0	82%	AY540326.1
Pleurotus ostreatus strain S042 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	678	678	96%	0.0	82%	AY540325.1
Pleurotus ostreatus strain S041 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	678	678	96%	0.0	82%	AY540324.1
Pleurotus ostreatus genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, strain: NBRC 30160	673	673	96%	0.0	82%	AB733143.1
Pleurotus sapidus strain dd08093 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	673	673	96%	0.0	82%	FJ810181.1
Pleurotus ostreatus/Coprinus comatus fusant isolate SMCC4.11.7 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	673	673	96%	0.0	82%	FJ501571.1
Pleurotus ostreatus/Coprinus comatus fusant isolate SMCC4.11.15 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene,	673	673	96%	0.0	82%	FJ501549.1

partial sequence

Pleurotus sapidus strain ACCC50155 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	671	671	96%	0.0	82%	EU424316.2
Pleurotus cornucopiae strain P-38 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	671	671	96%	0.0	82%	FJ379271.1
Pleurotus sapidus strain hei 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	667	667	96%	0.0	81%	KP877606.1
Lepista sordida/Armillaria mellea fusant isolate SMCC4.11.6 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	667	667	96%	0.0	81%	FJ501556.1
Pleurotus ostreatus voucher HMAS 66080 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	665	665	95%	0.0	82%	EF514248.1
Pleurotus sapidus strain S2004 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	664	664	96%	0.0	81%	JQ868741.1
Lepista sordida/Armillaria mellea fusant isolate SMCC4.11.4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	662	662	96%	0.0	81%	FJ501558.1
Uncultured fungus isolate OTU 157 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	656	656	96%	0.0	81%	KT328765.1
Pleurotus ostreatus strain NAAS00508 contains 18S ribosomal RNA gene, partial sequence gene, partial sequence	656	656	94%	0.0	81%	KP004972.1
Uncultured fungus clone CMH171 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	656	656	96%	0.0	81%	KF800262.1
Poria cocos/Tremella fuciformis fusant isolate SMCC4.11.14 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	656	656	96%	0.0	81%	FJ501581.1

Pleurotus ostreatus isolate 6689 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	656	656	94%	0.0	81%	AY450345.1
Pleurotus sp. IOO-2013a isolate IOM1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	654	654	92%	0.0	82%	KF415278.1
Pleurotus ostreatus strain Pl.cy0001 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	651	651	93%	0.0	81%	HM590443.1
Pleurotus ostreatus strain CGMCC 5.37 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	651	651	93%	0.0	81%	EF514247.1
Phlebiopsis gigantea isolate UASWS0310 17S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 25S ribosomal RNA gene, partial sequence	651	651	96%	0.0	81%	EF174450.1
Pleurotus columbinus strain CBS 281.32 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA, partial sequence	651	651	93%	0.0	81%	AY265815.1
Pleurotus ostreatus strain JZB21010015 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	649	649	93%	0.0	81%	JN126336.1
Pleurotus sapidus strain NAAS04569 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	647	647	93%	0.0	81%	KP004983.1
Pleurotus spodoleucus strain ASI 2012 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA, partial sequence	647	647	93%	0.0	81%	AY265848.1
Pleurotus ostreatus strain JZB21010018 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	645	645	93%	0.0	81%	JN126338.1
Pleurotus sajor-caju 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	645	645	93%	0.0	81%	DQ077890.1
Pleurotus spodoleucus 18S ribosomal RNA gene, partial sequence; internal transcribed						

spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	645	645	93%	0.0	81%	DQ077886.1
Pleurotus sp. PHZAU15 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	645	645	93%	0.0	81%	DQ077875.1
Pleurotus ostreatus strain JZB2101001 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	641	641	93%	4e-180	81%	JN126326.1
Pleurotus ostreatus voucher HMAS 76520 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	641	641	93%	4e-180	81%	EF514242.1
Pleurotus ostreatus strain JZB21010012 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	640	640	93%	1e-179	81%	JN126335.1
Pleurotus eryngii isolate D1742 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	640	640	92%	1e-179	81%	EU233963.1
Pleurotus ostreatus isolate TFB13841 voucher TENN F-65527 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	632	632	94%	2e-177	81%	KP026251.1
Pleurotus ostreatus strain JZB2101006 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	630	630	93%	8e-177	81%	JN126330.1
Uncultured Pleurotus clone M45 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	625	625	93%	4e-175	81%	HQ875426.1
Pleurotus eryngii isolate D540 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	623	623	92%	1e-174	81%	EU233959.1
Pleurotus ostreatus strain IUM1313 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	621	621	90%	5e-174	81%	HM770884.1
Pleurotus cornucopiae strain ACCC50375 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2,	619	619	90%	2e-173	81%	EU424301.2

complete sequence; and 28S ribosomal RNA gene, partial sequence						
Pleurotus eryngii isolate D850 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	617	617	92%	7e-173	81%	EU233961.1
Pleurotus ostreatus isolate D261.7 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	617	617	92%	7e-173	81%	EU233955.1
Pleurotus ostreatus strain 32GH (H-5) internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	612	612	89%	3e-171	81%	KF932726.1
Pleurotus cornucopiae strain ACCC50234 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	610	610	89%	1e-170	81%	EU424284.2
Pleurotus ostreatus strain CCMSSC03777 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	608	608	89%	4e-170	81%	KF724528.1
Pleurotus cornucopiae strain CCMSSC03779 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	603	603	89%	2e-168	81%	KF724530.1
Pleurotus sapidus strain B10 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	560	560	93%	1e-155	79%	KM117170.1
Pleurotus sapidus strain Po1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	368	624	57%	7e-98	95%	KP189359.1
Pleurotus ostreatus isolate SMCC4.03.9 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	368	630	57%	7e-98	95%	FJ501552.1
Pleurotus eryngii var. ferulae 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	368	593	54%	7e-98	95%	DQ077888.1
Pleurotus floridanus isolate IPL/MC/PF 1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	593	55%	3e-96	95%	KT826605.1

Pleurotus floridanus strain AAU_Sap 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	609	56%	3e-96	95%	KM375930.1
Pleurotus ostreatus strain CCMSSC01336 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	545	50%	3e-96	95%	KF724525.1
Pleurotus ostreatus isolate NW444 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	602	57%	3e-96	95%	EU622254.1
Pleurotus ostreatus isolate NW424 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	607	57%	3e-96	95%	EU622250.1
Pleurotus ostreatus isolate NW423 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	363	34%	3e-96	95%	EU622249.1
Pleurotus sp. BAB-4073 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	552	50%	3e-96	95%	KJ670293.1
Pleurotus sapidus strain FPSPT 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	619	57%	3e-96	95%	JX429940.1
Pleurotus ostreatus strain L/4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	539	49%	3e-96	95%	KF932729.1
Pleurotus sp. IOO-2013b isolate IOM11 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	597	54%	3e-96	95%	KF415282.1
Pleurotus sp. IOO-2013a isolate IOM3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	576	56%	3e-96	95%	KF415280.1
Pleurotus sp. IOO-2013b isolate IOM2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	578	56%	3e-96	95%	KF415279.1
Pleurotus floridanus strain LCJ 155 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2,	363	580	55%	3e-96	95%	KF373566.1

complete sequence; and 28S ribosomal RNA gene, partial sequence						
Pleurotus ostreatus voucher ASRNA 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	619	57%	3e-96	95%	KC686866.1
Pleurotus ostreatus voucher ASDNA 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	604	56%	3e-96	95%	KC686865.1
Pleurotus floridanus isolate pau5 18S ribosomal RNA gene and internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	552	50%	3e-96	95%	JX965408.1
Pleurotus sp. MuP1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	602	55%	3e-96	95%	JX403720.1
Pleurotus ostreatus strain H-8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	541	50%	3e-96	95%	JQ837478.1
Pleurotus sp. S2007 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	611	57%	3e-96	95%	JQ868743.1
Pleurotus floridanus strain S2002 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	613	57%	3e-96	95%	JQ868739.1
Pleurotus sapidus strain FSCPS1 (PSW1) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	619	57%	3e-96	95%	JN234845.1
Pleurotus ostreatus isolate TNAUCBE1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	547	50%	3e-96	95%	JN807335.1
Pleurotus ostreatus strain JZB21010020 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	593	54%	3e-96	95%	JN126340.1
Pleurotus ostreatus strain JZB2101009 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	593	54%	3e-96	95%	JN126333.1

Pleurotus ostreatus strain pl.n0037 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	619	57%	3e-96	95%	JF758887.1
Pleurotus sapidus isolate PSW1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	619	57%	3e-96	95%	HM561980.1
Pleurotus ostreatus strain Pl.cy0007 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	593	54%	3e-96	95%	HM590444.1
Pleurotus floridanus strain PF101 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	619	57%	3e-96	95%	GU721058.1
Pleurotus eryngii isolate D1171 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	363	585	53%	3e-96	95%	EU233962.1
Pleurotus ostreatus isolate D280 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	363	587	53%	3e-96	95%	EU233952.1
Pleurotus floridanus strain dd08070 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	619	57%	3e-96	95%	FJ810170.1
Pleurotus sp. 'Florida' 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	363	604	56%	3e-96	95%	FJ608594.1
Pleurotus sp. 'Florida' internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	517	48%	3e-96	95%	EU919631.1
Laccoccephalum mylittae strain L66 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	363	34%	3e-96	95%	EU716114.1
Pleurotus eryngii isolate NW423 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	593	55%	3e-96	95%	EU520181.1
Pleurotus ostreatus isolate NW420 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	363	363	34%	3e-96	95%	EU520142.1

Pleurotus sapidus isolate NW401 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	363	363	34%	3e-96	95%	EU520136.1
Pleurotus eryngii isolate NW450 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	617	57%	3e-96	95%	EU520127.1
Pleurotus ostreatus isolate NW449 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	615	57%	3e-96	95%	EU520110.1
Pleurotus ostreatus isolate NW437 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	617	57%	3e-96	95%	EU520108.1
Laccocephalum mylittae strain L66 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	619	57%	3e-96	95%	EU157727.2
Pleurotus ostreatus strain N001 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	363	561	51%	3e-96	95%	EU162048.1
Pleurotus ostreatus strain OE-43 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	363	587	55%	3e-96	95%	AY636055.1
Pleurotus floridanus strain S040 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	363	619	57%	3e-96	95%	AY540323.1
Pleurotus floridanus strain S039 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	363	619	57%	3e-96	95%	AY540322.1
Pleurotus ostreatus isolate NW431 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	359	613	57%	4e-95	94%	EU520107.1

Alignments

Pleurotus ostreatus cultivar Yiping 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Sequence ID: **gb|FJ501550.1|** Length: 660 Number of Matches: 1

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range 1: 2 to 632

Score	Expect	Identities	Gaps	Strand	Frame
678 bits(367)	0.0()	520/636(82%)	8/636(1%)	Plus/Plus	
Features:					
Query 4	TTCCGTACGTGAACCTGCGGCAAGGAT-ATTAATGAATTC	ACTATGGAGTTGTTGCTGGC	62		
Sbjct 2	TTCCGTAGGTGAACCTGCGG-AAGGATCATTAATGAATTC	ACTATGGAGTTGTTGCTGGC	60		
Query 63	CTCTAGGGGCATGTGCACGCTTCACTAGTCTTTCAACCACCTGTGAAC	TTTTGATAGATC	122		
Sbjct 61	CTCTAGGGGCATGTGCACGCTTCACTAGTCTTTCAACCACCTGTGAAC	TTTTGATAGATC	120		
Query 123	TGTGAAGTCGTCTTTCAAGCTCGTCAGACTTGGTTNGNNGGNTATTNA	ANCTTNTNGNN	182		
Sbjct 121	TGTGAAGTCGTCTTTCAAG-TCGTCAGACTTGGTTTGCTGG-GATT	TAAACGCTCGGTG	178		
Query 183	TGNNACCCCATTCNNATTTNNTTNACACNCCCATNTGTGTGTCNACA	AAGTGTNNTTTA	242		
Sbjct 179	TGACAACGCAGTC-TATTTACTTAACACACCCCAAATGTATGCTAC	GAA-TGTCATTTA	236		
Query 243	GTGNNC NNTGNNCNTATANN CNATNANACATNTTNNACNNCNNAT	CTTNTGNCTCTCN	302		
Sbjct 237	ATGGGCCTTGTGCCTATAAACCATATAACAACCTTCAACAACGGAT	CTCTTGCTCTCGC	296		
Query 303	ATCTannaaaaaCNCCGCNANGTGTATATGTNGTGTNNNNTGCAA	ATTCNGTGNNTNA	362		
Sbjct 297	ATCGATGAAGAAGCGACGGAATGCGATAAGTAATGTGAATTGCAGA	ATTCAGTGAATCA	356		
Query 363	TCANNTNTTTNNACNCANNNTGCGCCNTNTGGTNTTCNGAGGNNC	NNGCNTGTNGNGTG	422		
Sbjct 357	TGCAATCTTTGAACGCACCTTGCGCCCTTGGTATTCCGAGGGGCAT	GCCTGTTTGAGTG	416		
Query 423	NCATTAATTTCTCACNCTCNCTTTGG-TTTTTCCAATTGTGATGTT	TGGATTGTTGGGGG	481		
Sbjct 417	TCATTAATTTCTCAAACCTCACTTGGTTTTTTCCAATTGTGATGTT	TGGATTGTTGGGGG	476		
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Sbjct 477	CTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATTAGCAGGAC	TTCTCATTGCCTCT	536		
Query 542	GCGCATGATGTGATAATTATCACTCATCAATAGCACGCATGAATAG	AGTCCAGCTCTCTA	601		
Sbjct 537	GCGCATGATGTGATAATTATCACTCATCAATAGCACGCATGAATAG	AGTCCAGCTCTCTA	596		
Query 602	ATCGTCCGCAN-GACAATTTGACAATTTGACCTCAA	636			
Sbjct 597	ATCGTCCGCAAGGACAATTTGACAATTTGACCTCAA	632			

Pleurotus ostreatus strain S474 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: **gb|AY540332.1|** Length: 678 Number of Matches: 1

Range 1: 1 to 631

Score	Expect	Identities	Gaps	Strand	Frame
678 bits(367)	0.0()	520/636(82%)	8/636(1%)	Plus/Plus	
Features:					
Query 4	TTCCGTACGTGAACCTGCGGCAAGGAT-ATTAATGAATTC	ACTATGGAGTTGTTGCTGGC	62		
Sbjct 1	TTCCGTAGGTGAACCTGCGG-AAGGATCATTAATGAATTC	ACTATGGAGTTGTTGCTGGC	59		
Query 63	CTCTAGGGGCATGTGCACGCTTCACTAGTCTTTCAACCACCTGTGAAC	TTTTGATAGATC	122		
Sbjct 60	CTCTAGGGGCATGTGCACGCTTCACTAGTCTTTCAACCACCTGTGAAC	TTTTGATAGATC	119		
Query 123	TGTGAAGTCGTCTTTCAAGCTCGTCAGACTTGGTTNGNNGGNTATTNA	ANCTTNTNGNN	182		
Sbjct 120	TGTGAAGTCGTCTTTCAAG-TCGTCAGACTTGGTTTGCTGG-GATT	TAAACGCTCGGTG	177		
Query 183	TGNNACCCCATTCNNATTTNNTTNACACNCCCATNTGTGTGTCNACA	AAGTGTNNTTTA	242		
Sbjct 178	TGACAACGCAGTC-TATTTACTTAACACACCCCAAATGTATGCTAC	GAA-TGTCATTTA	235		
Query 243	GTGNNC NNTGNNCNTATANN CNATNANACATNTTNNACNNCNNAT	CTTNTGNCTCTCN	302		
Sbjct 236	ATGGGCCTTGTGCCTATAAACCATATAACAACCTTCAACAACGGAT	CTCTTGCTCTCGC	295		
Query 303	ATCTannaaaaaCNCCGCNANGTGTATATGTNGTGTNNNNTGCAA	ATTCNGTGNNTNA	362		
Sbjct 296	ATCGATGAAGAAGCGACGGAATGCGATAAGTAATGTGAATTGCAGA	ATTCAGTGAATCA	355		
Query 363	TCANNTNTTTNNACNCANNNTGCGCCNTNTGGTNTTCNGAGGNNC	NNGCNTGTNGNGTG	422		
Sbjct 356	TGCAATCTTTGAACGCACCTTGCGCCCTTGGTATTCCGAGGGGCAT	GCCTGTTTGAGTG	415		

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Query  423  NCATTAATTTCTCACNCTCNCTTTGG-TTTTTCCAATTGTGATGTTGGATTGTTGGGGG  481
Sbjct  416  TCATTAAATTCTCAAACCTCACTTTGGTTTTTTTCCAATTGTGATGTTGGATTGTTGGGGG  475

Query  482  CTGCTGGCCTTGACAGGTCNGCTCCTCTTAAATGCATTAGCAGGACTTCTCATTGCCTCT  541
Sbjct  476  CTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATTAGCAGGACTTCTCATTGCCTCT  535

Query  542  GCGCATGATGTGATAATTATCACTCATCAATAGCACGCATGAATAGAGTCCAGCTCTCTA  601
Sbjct  536  GCGCATGATGTGATAATTATCACTCATCAATAGCACGCATGAATAGAGTCCAGCTCTCTA  595

Query  602  ATCGTCCGCAN-GACAATTTGACAATTTGACCTCAA  636
Sbjct  596  ATCGTCCGCAAGGACAATTTGACAATTTGACCTCAA  631

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Pleurotus sapidus strain S047 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: **gb|AY540327.1|** Length: 703 Number of Matches: 1

Range 1: 25 to 655

Score	Expect	Identities	Gaps	Strand	Frame
678 bits(367)	0.0()	520/636(82%)	8/636(1%)	Plus/Plus	

Features:

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Query  4    TTCCGTACGTGAACCTGCGGCAAGGAT-ATTAATGAATTCACATATGGAGTTGTTGCTGGC  62
Sbjct  25    TTCCGTAGGTGAACCTGCGG-AAGGATCATTAATGAATTCACATATGGAGTTGTTGCTGGC  83

Query  63    CTCTAGGGGCATGTGCACGCTTCACTAGTCTTTCAACCACCTGTGAACTTTGTAGATC  122
Sbjct  84    CTCTAGGGGCATGTGCACGCTTCACTAGTCTTTCAACCACCTGTGAACTTTGTAGATC  143

Query  123   TGTGAAGTCGTCTTTCAAGCTCGTCAGACTTGGTTNGNNGGNTATTNAANCTTNTNGNN  182
Sbjct  144   TGTGAAGTCGTCTTTCAAG-TCGTCAGACTTGGTTTGCTGG-GATTTAAACGCTCTCGGTG  201

Query  183   TGNNAACCCATTNNATTTNNNTNACACNCCCATNTGTGTGTCNACAAAGTGNNTTTA  242
Sbjct  202   TGACAACGCAGTC-TATTTACTTAACACACCCCAAATGTATGTCTACGAA-TGTCATTTA  259

Query  243   GTGNNCNNNTGNNCNTATANNCNATNANACATNTTNNACNNCNNATCTTNTGNCTCTCN  302
Sbjct  260   ATGGGCCCTGTGCCTATAAACCAATAATACAACCTTCAACAACGGATCTCTTGCTCTCGC  319

Query  303   ATCTannaaaaaCNC CGCNANGTGTATATGTNGTGTNNNNTGCAAATTCNGTGNNTNA  362
Sbjct  320   ATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCA  379

Query  363   TCANNNTNTTNNACNCANNNTGCGCCNTNTGGTNTTCNGAGGNNCNGCNTGTTNGNGTG  422
Sbjct  380   TCGAATCTTTGAACGCACCTTGCGCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGTG  439

Query  423   NCATTAATTTCTCACNCTCNCTTTGG-TTTTTCCAATTGTGATGTTGGATTGTTGGGGG  481
Sbjct  440   TCATTAAATTCTCAAACCTCACTTTGGTTTTTTTCCAATTGTGATGTTGGATTGTTGGGGG  499

Query  482   CTGCTGGCCTTGACAGGTCNGCTCCTCTTAAATGCATTAGCAGGACTTCTCATTGCCTCT  541
Sbjct  500   CTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATTAGCAGGACTTCTCATTGCCTCT  559

Query  542   GCGCATGATGTGATAATTATCACTCATCAATAGCACGCATGAATAGAGTCCAGCTCTCTA  601
Sbjct  560   GCGCATGATGTGATAATTATCACTCATCAATAGCACGCATGAATAGAGTCCAGCTCTCTA  619

Query  602   ATCGTCCGCAN-GACAATTTGACAATTTGACCTCAA  636
Sbjct  620   ATCGTCCGCAAGGACAATTTGACAATTTGACCTCAA  655

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Pleurotus sapidus strain S046 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: **gb|AY540326.1|** Length: 669 Number of Matches: 1

Range 1: 25 to 655

Score	Expect	Identities	Gaps	Strand	Frame
678 bits(367)	0.0()	520/636(82%)	8/636(1%)	Plus/Plus	

Features:

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Query  4    TTCCGTACGTGAACCTGCGGCAAGGAT-ATTAATGAATTCACATATGGAGTTGTTGCTGGC  62
Sbjct  25    TTCCGTAGGTGAACCTGCGG-AAGGATCATTAATGAATTCACATATGGAGTTGTTGCTGGC  83

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Query 63  CTCTAGGGGCATGTGCACGCTTCACTAGTCTTTCAACCACCTGTGAACCTTTTGATAGATC 122
Sbjct 84  CTCTAGGGGCATGTGCACGCTTCACTAGTCTTTCAACCACCTGTGAACCTTTTGATAGATC 143

Query 123  TGTGAAGTCGTCTTTCAAGCTCGTCAGACTTGGTTNGNNGGNTATTNAANCTTNTNGNN 182
Sbjct 144  TGTGAAGTCGTCTTTCAAG-TCGTCAGACTTGGTTTGCTGG-GATTAAACGCTCTCGGTG 201

Query 183  TGNNAACCCATTNNATTTNNNTTACACNCCCATNTGTGTGTCNACAAAGTGNNTTTA 242
Sbjct 202  TGACAACGCAGTC-TATTTACTTAACACACCCCAAATGTATGTCTACGAA-TGTCATTTA 259

Query 243  GTGNNCNCNNTGNNCNTATANNCNATNANACATNTTTNNACNNCNCNATCTTNTGNCTCTCN 302
Sbjct 260  ATGGGCCCTTGTGCCTATAAACCATAATAACAACCTTTCAACAACGGATCTCTTGGCTCTCGC 319

Query 303  ATCTannaaaaaCNC CGCNANGTGTATATGTNGTGTNNNNTGCAAATTCNGTGNNTNA 362
Sbjct 320  ATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCA 379

Query 363  TCANNTNTTTNNACNCANNNTGCGCCNTNTGGTNTTCNGAGGNNCNGCNTGTTNGNGTG 422
Sbjct 380  TCGAATCTTTGAACGCACCTTGCGCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGTG 439

Query 423  NCATTAATTTCTCACNCTCNCTTTGG-TTTTTCCAATTGTGATGTTGGATTGTTGGGG 481
Sbjct 440  TCATTAATTTCTCAAACCTCACTTTGGTTTTTTTCCAATTGTGATGTTGGATTGTTGGGG 499

Query 482  CTGCTGGCCTTGACAGGTCNGCTCCTCTTAAATGCATTAGCAGGACTTCTCATTGCCTCT 541
Sbjct 500  CTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATTAGCAGGACTTCTCATTGCCTCT 559

Query 542  GCGCATGATGTGATAATTATCACTCATCAATAGCACGCATGAATAGAGTCCAGCTCTCTA 601
Sbjct 560  GCGCATGATGTGATAATTATCACTCATCAATAGCACGCATGAATAGAGTCCAGCTCTCTA 619

Query 602  ATCGTCCGCAN-GACAATTTGACAATTTGACCTCAA 636
Sbjct 620  ATCGTCCGCAAGGACAATTTGACAATTTGACCTCAA 655

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Pleurotus ostreatus strain S042 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

Sequence ID: **gb|AY540325.1|** Length: 673 Number of Matches: 1

Range 1: 26 to 656

Score	Expect	Identities	Gaps	Strand	Frame
678 bits(367)	0.0()	520/636(82%)	8/636(1%)	Plus/Plus	
Features:					
Query 4	TTCCGTACGTGAACCTGCGGCAAGGAT-ATTAATGAATTC	ACTATGGAGTTGTTGCTGGC	62		
Sbjct 26	TTCCGTAGTGAACCTGCGG-AAAGGATCATTAATGAATTC	ACTATGGAGTTGTTGCTGGC	84		
Query 63	CTCTAGGGGCATGTGCACGCTTCACTAGTCTTTCAACCACCTGTGAACCTTTTGATAGATC	122			
Sbjct 85	CTCTAGGGGCATGTGCACGCTTCACTAGTCTTTCAACCACCTGTGAACCTTTTGATAGATC	144			
Query 123	TGTGAAGTCGTCTTTCAAGCTCGTCAGACTTGGTTNGNNGGNTATTNAANCTTNTNGNN	182			
Sbjct 145	TGTGAAGTCGTCTTTCAAG-TCGTCAGACTTGGTTTGCTGG-GATTAAACGCTCTCGGTG	202			
Query 183	TGNNAACCCATTNNATTTNNNTTACACNCCCATNTGTGTGTCNACAAAGTGNNTTTA	242			
Sbjct 203	TGACAACGCAGTC-TATTTACTTAACACACCCCAAATGTATGTCTACGAA-TGTCATTTA	260			
Query 243	GTGNNCNCNNTGNNCNTATANNCNATNANACATNTTTNNACNNCNCNATCTTNTGNCTCTCN	302			
Sbjct 261	ATGGGCCCTTGTGCCTATAAACCATAATAACAACCTTTCAACAACGGATCTCTTGGCTCTCGC	320			
Query 303	ATCTannaaaaaCNC CGCNANGTGTATATGTNGTGTNNNNTGCAAATTCNGTGNNTNA	362			
Sbjct 321	ATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCA	380			
Query 363	TCANNTNTTTNNACNCANNNTGCGCCNTNTGGTNTTCNGAGGNNCNGCNTGTTNGNGTG	422			
Sbjct 381	TCGAATCTTTGAACGCACCTTGCGCCCTTGGTATTCCGAGGGGCATGCCTGTTTGAGTG	440			
Query 423	NCATTAATTTCTCACNCTCNCTTTGG-TTTTTCCAATTGTGATGTTGGATTGTTGGGG	481			
Sbjct 441	TCATTAATTTCTCAAACCTCACTTTGGTTTTTTTCCAATTGTGATGTTGGATTGTTGGGG	500			
Query 482	CTGCTGGCCTTGACAGGTCNGCTCCTCTTAAATGCATTAGCAGGACTTCTCATTGCCTCT	541			
Sbjct 501	CTGCTGGCCTTGACAGGTCGGCTCCTCTTAAATGCATTAGCAGGACTTCTCATTGCCTCT	560			
Query 542	GCGCATGATGTGATAATTATCACTCATCAATAGCACGCATGAATAGAGTCCAGCTCTCTA	601			
Sbjct 561	GCGCATGATGTGATAATTATCACTCATCAATAGCACGCATGAATAGAGTCCAGCTCTCTA	620			
Query 602	ATCGTCCGCAN-GACAATTTGACAATTTGACCTCAA	636			

Sbjct 621 ATCGTCCGCAAGGACAATTTGACAATTTGACCTCAA 656

BLAST ®

Basic Local Alignment Search Tool

[NCBI/ BLAST/ blastn suite/ Formatting Results - 75A3S437014](#)[Formatting options](#)[Download](#)[Blast report description](#)

Nucleotide Sequence (654 letters)

RID [75A3S437014](#) (Expires on 12-18 19:20 pm)

Query ID lcl|Query_162717

Description None

Molecule type nucleic acid

Query Length 654

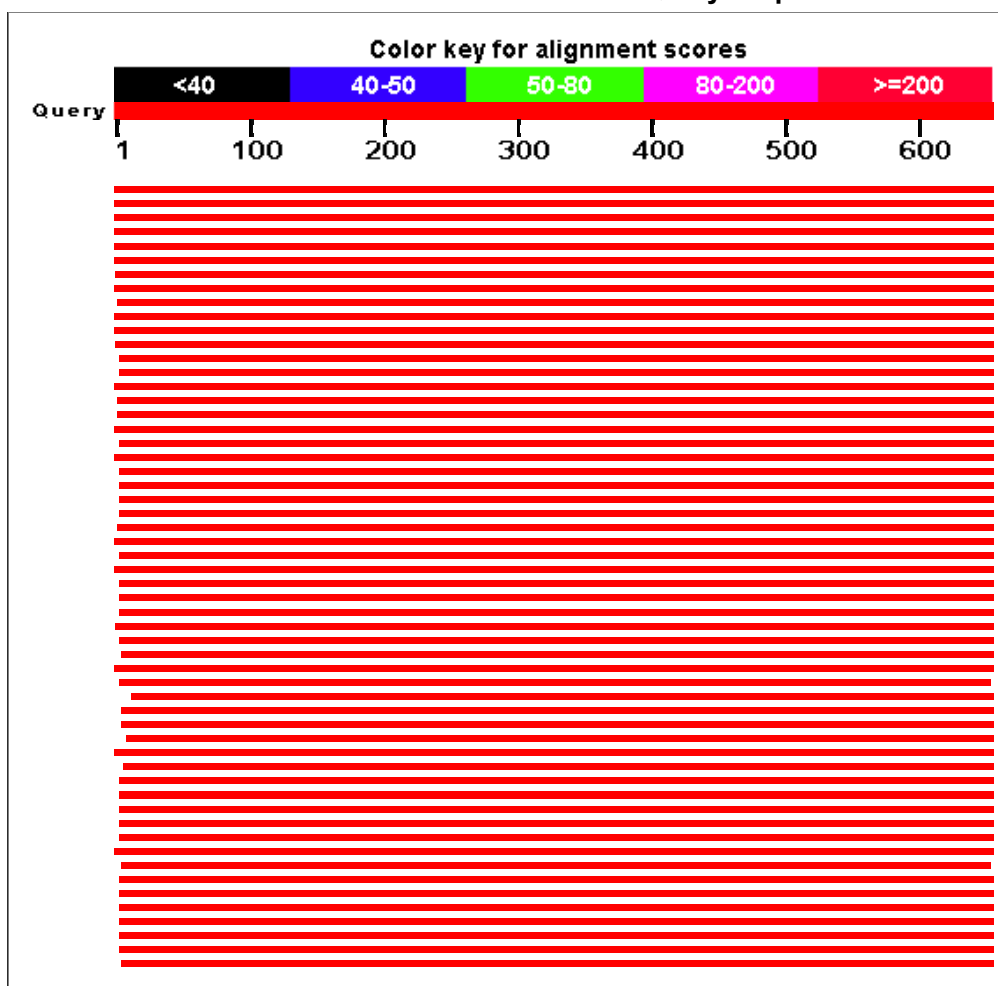
Database Name nr

Description Nucleotide collection (nt)

Program BLASTN 2.3.0+

[Graphic Summary](#)

Distribution of 100 Blast Hits on the Query Sequence



Descriptions

Sequences producing significant alignments:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Lentinula edodes genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 25S rRNA, partial and complete sequence, strain: G408PP-4	1192	1192	100%	0.0	99%	AB366150.1
Lentinula edodes genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2 and 25S rRNA, partial and complete sequence, cultivar: Chamsongi	1192	1192	100%	0.0	99%	AB286067.1
Lentinula edodes genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2 and 25S rRNA, partial and complete sequence, cultivar: 602	1192	1192	100%	0.0	99%	AB286065.1
Lentinula edodes genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2 and 25S rRNA, partial and complete sequence, cultivar: 290	1192	1192	100%	0.0	99%	AB286064.1
Lentinula edodes strain 217(LE6) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1192	1192	100%	0.0	99%	DQ467888.1
Lentinula edodes strain 271 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1192	1192	100%	0.0	99%	DQ467886.1
Lentinula edodes strain qh02 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1190	1190	99%	0.0	99%	GQ866857.1
Lentinula edodes strain LE3 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	1188	1188	100%	0.0	99%	DQ497071.1
Lentinula edodes 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1186	1186	99%	0.0	99%	JX205093.1
Lentinula edodes isolate LEB1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1186	1186	100%	0.0	99%	HM561975.1
Lentinula edodes strain LE9 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1186	1186	100%	0.0	99%	DQ676821.1
Lentinula edodes strain FFBS1 (LEB1) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2,	1184	1184	99%	0.0	99%	JN234840.1

complete sequence; and 28S ribosomal RNA gene, partial sequence						
Lentinula edodes strain GAN068-IAMHAU 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1184	1184	99%	0.0	99%	AY683145.1
Lentinula edodes BIN1024 large subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	1184	1184	99%	0.0	99%	AF356170.1
Lentinula edodes isolate TMI-941, 5.8S rRNA gene, complete sequence, 18S rRNA and 25S rRNA genes, partial sequence	1184	1184	100%	0.0	99%	U33092.1
Lentinula edodes strain H-519 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1182	1182	99%	0.0	99%	GU140095.1
Lentinula edodes strain 135 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1181	1181	99%	0.0	99%	GU001952.1
Lentinula edodes strain dd08110 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1181	1181	100%	0.0	99%	FJ810189.1
Lentinula edodes strain 26-GIM 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1181	1181	99%	0.0	99%	AY683182.1
Lentinula edodes strain 271(LE5) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1181	1181	100%	0.0	99%	DQ467887.1
Lentinula edodes strain 134-DBCUIHK 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1179	1179	99%	0.0	99%	AY683195.1
Lentinula edodes strain 006-RCMSAS 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1179	1179	99%	0.0	99%	AY683178.1
Lentinula edodes strain 75-SIECAS 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1179	1179	99%	0.0	99%	AY683175.1
Lentinula edodes strain GAN059-IAMHAU 18S ribosomal RNA gene, partial sequence;						

internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1179	1179	99%	0.0	99%	AY683144.1
Uncultured fungus clone FA1-O21 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1177	1177	99%	0.0	99%	JX984748.1
Lentinula edodes strain xsd08092 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1175	1175	100%	0.0	99%	FJ582641.1
Lentinula edodes strain SHX045-IAMHAU 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1175	1175	99%	0.0	99%	AY683174.1
Lentinula edodes isolate TMI-1148, 5.8S rRNA gene, complete sequence, 18S rRNA and 25S rRNA genes, partial sequence	1175	1175	100%	0.0	99%	U33080.1
Lentinula edodes strain 70-GIM 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1173	1173	99%	0.0	99%	AY683196.1
Lentinula edodes strain GAN057-IAMHAU 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1173	1173	99%	0.0	99%	AY683143.1
Lentinula edodes strain 922 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1173	1173	99%	0.0	99%	DQ444297.1
Lentinula sp. 507 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1171	1171	99%	0.0	99%	KF757010.1
Lentinula edodes specimen-voucher V.Bilay 361 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, and 25S ribosomal RNA gene, partial sequence	1171	1171	99%	0.0	99%	AF031189.1
Lentinula sp. R 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1170	1170	99%	0.0	99%	KF757014.1
L.edodes 5.8S rRNA gene	1170	1170	100%	0.0	99%	X63130.1
Lentinula edodes strain 83-SIECAS 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2,	1168	1168	98%	0.0	99%	AY683176.1

complete sequence; and 28S ribosomal RNA gene, partial sequence						
Lentinula edodes 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 25S ribosomal RNA gene, partial sequence	1168	1168	98%	0.0	99%	AF079572.1
Lentinula sp. N1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1166	1166	99%	0.0	99%	KF757013.1
Lentinula sp. Y3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1164	1164	99%	0.0	99%	KF757017.1
Lentinula edodes strain GL-51 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1164	1164	98%	0.0	99%	FJ379264.1
Lentinula edodes isolate UASWS0311 17S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 25S ribosomal RNA gene, partial sequence	1164	1164	100%	0.0	99%	EF174451.1
Lentinula sp. 808 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1162	1162	99%	0.0	99%	KF757011.1
Lentinula edodes strain HUN001-IAMHAU 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1162	1162	99%	0.0	99%	AY683164.1
Lentinula edodes strain HUB090-IAMHAU 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1162	1162	99%	0.0	99%	AY683163.1
Lentinula edodes strain HUB049-IAMHAU 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1162	1162	99%	0.0	99%	AY683158.1
Lentinula edodes strain HUB028-IAMHAU 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1162	1162	99%	0.0	99%	AY683156.1
Lentinula edodes strain 50786-ACCC 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA	1162	1162	99%	0.0	99%	AY683138.1

gene, partial sequence						
Lentinula edodes strain LE19 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1162	1162	100%	0.0	99%	DQ676822.1
Lentinula sp. G 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1160	1160	98%	0.0	99%	KF757012.1
Lentinula edodes strain 721(LE15) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1160	1160	99%	0.0	99%	DQ467892.1
Lentinula edodes strain 0377-EFISAAS 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1158	1158	99%	0.0	99%	AY683188.1
Lentinula edodes strain 0376-EFISAAS 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1158	1158	99%	0.0	99%	AY683187.1
Lentinula edodes strain 002-RCMSAS 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1158	1158	99%	0.0	99%	AY683177.1
Lentinula edodes strain HUN002-IAMHAU 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1158	1158	99%	0.0	99%	AY683165.1
Lentinula edodes strain STCL140 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, and 25S ribosomal RNA gene, partial sequence	1158	1158	99%	0.0	99%	AF031184.1
Lentinula sp. S8 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1157	1157	99%	0.0	99%	KF757015.1
Lentinula edodes strain L9 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1157	1157	99%	0.0	99%	FJ379266.1
Lentinula edodes strain HUB071-IAMHAU 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1157	1157	99%	0.0	99%	AY683160.1
Lentinula edodes strain HUB055-IAMHAU						

18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1157	1157	99%	0.0	99%	AY683159.1
Lentinula edodes strain 0229-EFISAAS 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1157	1157	99%	0.0	99%	AY683150.1
Lentinula edodes strain 50741-ACCC 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1157	1157	99%	0.0	99%	AY683149.1
Lentinula edodes strain 50624-ACCC 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1157	1157	99%	0.0	99%	AY683137.1
Lentinula edodes strain STCL125 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, and 25S ribosomal RNA gene, partial sequence	1157	1157	99%	0.0	99%	AF031183.1
Lentinula edodes isolate TMI-571, 5.8S rRNA gene, complete sequence, 18S rRNA and 25S rRNA genes, partial sequence	1157	1157	99%	0.0	99%	U33088.1
Lentinula edodes isolate TMI-951, 5.8S rRNA gene, complete sequence, 18S rRNA and 25S rRNA genes, partial sequence	1155	1155	99%	0.0	99%	U33093.1
Lentinula edodes strain 34-GIM 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1153	1153	99%	0.0	99%	AY683183.1
Lentinula edodes strain HUB087-IAMHAU 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1153	1153	99%	0.0	99%	AY683162.1
Lentinula edodes strain HUB084-IAMHAU 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1153	1153	99%	0.0	99%	AY683161.1
Lentinula edodes strain L42 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1151	1151	96%	0.0	99%	EU021470.1
Lentinula edodes strain 241-4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1151	1151	96%	0.0	99%	EU021464.1
Lentinula edodes strain 0375-EFISAAS 18S ribosomal RNA gene, partial sequence;						

internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1151	1151	99%	0.0	99%	AY683167.1
Lentinula edodes strain white(LE11) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1151	1151	100%	0.0	99%	DQ467891.1
Lentinula edodes isolate TMI-646, 5.8S rRNA gene, complete sequence, 18S rRNA and 25S rRNA genes, partial sequence	1151	1151	96%	0.0	99%	U33089.1
Lentinula edodes strain HNL95416 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, and 25S ribosomal RNA gene, partial sequence	1149	1149	99%	0.0	99%	AF031188.1
Lentinula edodes strain Cr62 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1147	1147	96%	0.0	99%	EU021468.1
Lentinula edodes strain Cr33 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1147	1147	96%	0.0	99%	EU005233.1
Lentinula edodes strain L26 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1146	1146	96%	0.0	99%	EU021469.1
Lentinula edodes strain 135 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1146	1146	96%	0.0	99%	EU021463.1
Lentinula edodes strain 939 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1140	1140	96%	0.0	99%	EU021465.1
Lentinula edodes strain 922(LE8) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1140	1140	99%	0.0	98%	DQ467890.1
Lentinula edodes isolate NW438 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1138	1138	99%	0.0	98%	EU520255.1
Lentinula edodes strain Minfeng No1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1136	1136	96%	0.0	99%	EU021473.1

Lentinula edodes isolate TMI-818, 5.8S rRNA gene, complete sequence, 18S rRNA and 25S rRNA genes, partial sequence	1131	1131	99%	0.0	98%	U33091.1
Lentinula edodes strain 9015 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1129	1129	96%	0.0	99%	EU021466.1
Lentinula edodes strain 921(LE7) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1122	1122	97%	0.0	99%	DQ467889.1
Lentinula edodes strain Cr02 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1120	1120	96%	0.0	99%	EU021467.1
Lentinula edodes specimen-voucher P. Arampongphan s.n. 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence, and 25S ribosomal RNA gene, partial sequence	1120	1120	99%	0.0	98%	AF031193.1
Lentinula edodes strain qh05 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1114	1114	93%	0.0	99%	GQ866859.1
Lentinula edodes strain qh01 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1114	1114	93%	0.0	99%	GQ866856.1
Lentinula edodes strain qh04 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1112	1112	93%	0.0	99%	GQ866858.1
Lentinula edodes isolate ZBH(1) internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1110	1110	93%	0.0	99%	HQ436118.1
Lentinula edodes strain qh06 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1110	1110	93%	0.0	99%	GQ866860.1
Lentinula edodes strain Shen4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	1105	1105	93%	0.0	99%	JQ797416.1
Lentinula sp. H3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial	1103	1103	99%	0.0	97%	KF757018.1

sequence

Lentinula edodes strain SHX039-IAMHAU
18S ribosomal RNA gene, partial sequence;
internal transcribed spacer 1, 5.8S ribosomal
RNA gene, and internal transcribed spacer 2,
complete sequence; and 28S ribosomal RNA
gene, partial sequence

1101

1101

99%

0.0

97%

[AY683172.1](#)

Lentinula edodes genomic DNA sequence
contains ITS1, 5.8S rRNA gene, ITS2, 28S
rRNA gene, isolate CCBAS390

1099

1099

92%

0.0

99%

[LN714563.1](#)

Lentinula edodes voucher ASIS22709 internal
transcribed spacer 1, partial sequence; 5.8S
ribosomal RNA gene, complete sequence;
and internal transcribed spacer 2, partial
sequence

1099

1099

92%

0.0

99%

[KF692084.1](#)

Lentinula edodes strain Le-217 18S ribosomal
RNA gene, internal transcribed spacer 1, 5.8S
ribosomal RNA gene, internal transcribed
spacer 2, and 28S ribosomal RNA gene,
region

1099

1099

92%

0.0

99%

[FJ379268.1](#)

Lentinula edodes strain Lv038 internal
transcribed spacer 1, partial sequence; 5.8S
ribosomal RNA gene, complete sequence;
and internal transcribed spacer 2, partial
sequence

1098

1098

92%

0.0

99%

[JN203131.1](#)

Lentinula edodes strain STCL124 18S
ribosomal RNA gene, partial sequence;
internal transcribed spacer 1, 5.8S ribosomal
RNA gene and internal transcribed spacer 2,
complete sequence, and 25S ribosomal RNA
gene, partial sequence

1098

1098

99%

0.0

97%

[AF031182.1](#)

Alignments

Lentinula edodes genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 25S rRNA, partial and complete sequence, strain: G408PP-4

Sequence ID: [dbj|AB366150.1](#) | Length: 753 Number of Matches: 1

Range 1: 14 to 666

Score	Expect	Identities	Gaps	Strand	Frame
1192 bits(645)	0.0()	651/654(99%)	1/654(0%)	Plus/Plus	

Features:

Query	1	GTTTCCGTAGGTGAACCTGCGGAAGGATCATTATTGAATTTTTTGGTGGTGGATTGTTGC	60
Sbjct	14	GTTTCCGTAGGTGAACCTGCGGAAGGATCATTATTGAATTTTTTGGTGGTGGATTGTTGC	73
Query	61	TGGCCTTTGGGTATGTGCACATCCTCCTCCGATTNTATTCATCCACCTGTGCACTTTTT	120
Sbjct	74	TGGCCTTTGGGTATGTGCACATCCTCCTCCGATTCTATTCATCCACCTGTGCACTTTTT	133
Query	121	GTAGGAGTTCTTTTCATCGGGTTTTTGAAGGTGCTCATTATGAGTTACTTGAAAAGACTAG	180
Sbjct	134	GTAGGAGTTCTTTTCATCGGGTTTTTGAAGGTGCTCATTATGAGTTACTTGAAAAGACTAG	193
Query	181	TTGACAAGGCTTCTATGTTCTTATAAACCATTGAAGTATGTTATAGAATGATCTTGTTAT	240
Sbjct	194	TTGACAAGGCTTCTATGTTCTTATAAACCATTGAAGTATGTTATAGAATGATCTTGTTAT	253
Query	241	TGGGACTTTATTGACCTTTTAACTTAATACAACCTTTCAGCAACGGATCTCTTGGCTCTC	300
Sbjct	254	TGGGACTTTATTGACCTTTTAACTTAATACAACCTTTCAGCAACGGATCTCTTGGCTCTC	313
Query	301	CCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT	360
Sbjct	314	CCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT	373
Query	361	CATCGAATCTTTGAACGCACCTTGCGCCCTCTGGTATTCGGAGGGCATGCCTGTTTGAG	420
Sbjct	374	CATCGAATCTTTGAACGCACCTTGCGCCCTCTGGTATTCGGAGGGCATGCCTGTTTGAG	433
Query	421	TGTCATTAAATTCTCAACTTTATAAGTTTTTACTTATTAAAGCTTGGATGTTGGAGGCTT	480
Sbjct	434	TGTCATTAAATTCTCAACTTTATAAGTTTTTACTTATTAAAGCTTGGATGTTGGAGGCTT	493

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Query  481  GCAGGCGTTTGTCTAGCTCCTCTTAAATTTATTAGTGGGAACCTGTTTGTAGTTCTAA  540
Sbjct  494  GCAGGCGTTTGTCTAGCTCCTCTTAAATTTATTAGTGGGAACCTGTTTGTAGTTCTAA  553

Query  541  CCTTGGTGTGATAATTATCTACATTTTGGTGGAAACCTTACAATAATAAGCTCTATTGGT  600
Sbjct  554  CCTTGGTGTGATAATTATCTACATTTTGGTGGAAACCTTACAATAATAAGCTCTATTGGT  613

Query  601  TTGGGTTGGTTCATTTAATTTGCTCAATCTGTTCTATTTCATTGGAGAAAAAGG  654
Sbjct  614  TTGGGTT-GTTGCATTTAGTTTGCTCAATCTGTTCTATTTCATTGGAGAAAAAGG  666

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Lentinula edodes genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2 and 25S rRNA, partial and complete sequence, cultivar: Chamsongi
Sequence ID: **dbj|AB286067.1**| Length: 1029 Number of Matches: 1
Range 1: 78 to 730

Score	Expect	Identities	Gaps	Strand	Frame
1192 bits(645)	0.0()	651/654(99%)	1/654(0%)	Plus/Plus	

Features:

```

Query  1    GTTTCCTAGGTGAACCTGCGGAAGGATCATTATTGAATTTTTTGGTGGTGGATTGTTGC  60
Sbjct  78    GTTTCCTAGGTGAACCTGCGGAAGGATCATTATTGAATTTTTTGGTGGTGGATTGTTGC  137

Query  61    TGGCCTTTGGGTATGTGCACATCCTCCTCCGATTTNTATTCATCCACCTGTGCACTTTTT  120
Sbjct  138   TGGCCTTTGGGTATGTGCACATCCTCCTCCGATTTCTATTCATCCACCTGTGCACTTTTT  197

Query  121   GTAGGAGTTCTTTCATCGGGTTTTTGAAGGTGCTCATTATGAGTTACTTGAAAAGACTAG  180
Sbjct  198   GTAGGAGTTCTTTCATCGGGTTTTTGAAGGTGCTCATTATGAGTTACTTGAAAAGACTAG  257

Query  181   TTGACAAGGCTTCTATGTTCTTATAAACCATTGAAGTATGTTATAGAATGATCTTGTTAT  240
Sbjct  258   TTGACAAGGCTTCTATGTTCTTATAAACCATTGAAGTATGTTATAGAATGATCTTGTTAT  317

Query  241   TGGGACTTTATTGACCTTTAAACTTAATACAACCTTCAGCAACGGATCTCTTGGCTCTC  300
Sbjct  318   TGGGACTTTATTGACCTTTAAACTTAATACAACCTTCAGCAACGGATCTCTTGGCTCTC  377

Query  301   CCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT  360
Sbjct  378   CCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT  437

Query  361   CATCGAATCTTTGAACGCACCTTGCGCCCTCTGGTATTCGGAGGGCATGCCTGTTTGAG  420
Sbjct  438   CATCGAATCTTTGAACGCACCTTGCGCCCTCTGGTATTCGGAGGGCATGCCTGTTTGAG  497

Query  421   TGTCATTAAATTCTCAACTTTATAAGTTTTTACTTATTAAAGCTTGGATGTTGGAGGCTT  480
Sbjct  498   TGTCATTAAATTCTCAACTTTATAAGTTTTTACTTATTAAAGCTTGGATGTTGGAGGCTT  557

Query  481   GCAGGCGTTTGTCTAGCTCCTCTTAAATTTATTAGTGGGAACCTGTTTGTAGTTCTAA  540
Sbjct  558   GCAGGCGTTTGTCTAGCTCCTCTTAAATTTATTAGTGGGAACCTGTTTGTAGTTCTAA  617

Query  541   CCTTGGTGTGATAATTATCTACATTTTGGTGGAAACCTTACAATAATAAGCTCTATTGGT  600
Sbjct  618   CCTTGGTGTGATAATTATCTACATTTTGGTGGAAACCTTACAATAATAAGCTCTATTGGT  677

Query  601   TTGGGTTGGTTCATTTAATTTGCTCAATCTGTTCTATTTCATTGGAGAAAAAGG  654
Sbjct  678   TTGGGTT-GTTGCATTTAGTTTGCTCAATCTGTTCTATTTCATTGGAGAAAAAGG  730

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Lentinula edodes genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2 and 25S rRNA, partial and complete sequence, cultivar: 602
Sequence ID: **dbj|AB286065.1**| Length: 1026 Number of Matches: 1
Range 1: 77 to 729

Score	Expect	Identities	Gaps	Strand	Frame
1192 bits(645)	0.0()	651/654(99%)	1/654(0%)	Plus/Plus	

Features:

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Query  1    GTTTCCTAGGTGAACCTGCGGAAGGATCATTATTGAATTTTTTGGTGGTGGATTGTTGC  60
Sbjct  77    GTTTCCTAGGTGAACCTGCGGAAGGATCATTATTGAATTTTTTGGTGGTGGATTGTTGC  136

Query  61    TGGCCTTTGGGTATGTGCACATCCTCCTCCGATTTNTATTCATCCACCTGTGCACTTTTT  120
Sbjct  137   TGGCCTTTGGGTATGTGCACATCCTCCTCCGATTTCTATTCATCCACCTGTGCACTTTTT  196

Query  121   GTAGGAGTTCTTTCATCGGGTTTTTGAAGGTGCTCATTATGAGTTACTTGAAAAGACTAG  180
Sbjct  197   GTAGGAGTTCTTTCATCGGGTTTTTGAAGGTGCTCATTATGAGTTACTTGAAAAGACTAG  256

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Query 181 TTGACAAGGCTTCTATGTTCTTATAAACCATTTGAAGTATGTTATAGAATGATCTTGTTAT 240
Sbjct 257 TTGACAAGGCTTCTATGTTCTTATAAACCATTTGAAGTATGTTATAGAATGATCTTGTTAT 316

Query 241 TGGGACTTTATTGACCCCTTTAACTTAATACAACCTTCAGCAACGGATCTCTTGGCTCTC 300
Sbjct 317 TGGGACTTTATTGACCCCTTTAACTTAATACAACCTTCAGCAACGGATCTCTTGGCTCTC 376

Query 301 CCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT 360
Sbjct 377 CCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT 436

Query 361 CATCGAATCTTTGAACGCACCTTGCGCCCTCTGGTATTCGGAGGGCATGCCTGTTTGAG 420
Sbjct 437 CATCGAATCTTTGAACGCACCTTGCGCCCTCTGGTATTCGGAGGGCATGCCTGTTTGAG 496

Query 421 TGTCATTAAATTCTCAACTTTATAAGTTTTTACTTATTAAAGCTTGGATGTTGGAGGCTT 480
Sbjct 497 TGTCATTAAATTCTCAACTTTATAAGTTTTTACTTATTAAAGCTTGGATGTTGGAGGCTT 556

Query 481 GCAGGCGTTTGTGAGCTCCTCTTAAATTTATTAGTGGGAACCTGTTTTGTTAGTTCTAA 540
Sbjct 557 GCAGGCGTTTGTGAGCTCCTCTTAAATTTATTAGTGGGAACCTGTTTTGTTAGTTCTAA 616

Query 541 CCTTGGTGTGATAATTATCTACATTTTGGTGAACCTTACAATAATAAGCTCTATTGGT 600
Sbjct 617 CCTTGGTGTGATAATTATCTACATTTTGGTGAACCTTACAATAATAAGCTCTATTGGT 676

Query 601 TTGGGTTGGTTGCATTTAATTTGCTCAATCTGTTCTATTTCATTGGAGAAAAAGG 654
Sbjct 677 TTGGGTT-GTTGCATTAGTTTGCTCAATCTGTTCTATTTCATTGGAGAAAAAGG 729

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Lentinula edodes genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2 and 25S rRNA, partial and complete sequence, cultivar: 290
Sequence ID: **dbj|AB286064.1** Length: 1024 Number of Matches: 1
Range 1: 77 to 729

Score	Expect	Identities	Gaps	Strand	Frame
1192 bits(645)	0.0()	651/654(99%)	1/654(0%)	Plus/Plus	

Features:

```

Query 1 GTTTCCGTAGGTGAACCTGCGGAAGGATCATTATTGAATTTTTTGGTGGTGGATTGTTGC 60
Sbjct 77 GTTTCCGTAGGTGAACCTGCGGAAGGATCATTATTGAATTTTTTGGTGGTGGATTGTTGC 136

Query 61 TGGCCTTTGGGTATGTGCACATCCTCCTCCGATTTNTATTCATCCACCTGTGCACTTTTT 120
Sbjct 137 TGGCCTTTGGGTATGTGCACATCCTCCTCCGATTTCTATTCATCCACCTGTGCACTTTTT 196

Query 121 GTAGGAGTTCTTTTCATCGGGTTTTTGAAGGTGCTCATTATGAGTTACTTGAAAAGACTAG 180
Sbjct 197 GTAGGAGTTCTTTTCATCGGGTTTTTGAAGGTGCTCATTATGAGTTACTTGAAAAGACTAG 256

Query 181 TTGACAAGGCTTCTATGTTCTTATAAACCATTTGAAGTATGTTATAGAATGATCTTGTTAT 240
Sbjct 257 TTGACAAGGCTTCTATGTTCTTATAAACCATTTGAAGTATGTTATAGAATGATCTTGTTAT 316

Query 241 TGGGACTTTATTGACCCCTTTAACTTAATACAACCTTCAGCAACGGATCTCTTGGCTCTC 300
Sbjct 317 TGGGACTTTATTGACCCCTTTAACTTAATACAACCTTCAGCAACGGATCTCTTGGCTCTC 376

Query 301 CCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT 360
Sbjct 377 CCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT 436

Query 361 CATCGAATCTTTGAACGCACCTTGCGCCCTCTGGTATTCGGAGGGCATGCCTGTTTGAG 420
Sbjct 437 CATCGAATCTTTGAACGCACCTTGCGCCCTCTGGTATTCGGAGGGCATGCCTGTTTGAG 496

Query 421 TGTCATTAAATTCTCAACTTTATAAGTTTTTACTTATTAAAGCTTGGATGTTGGAGGCTT 480
Sbjct 497 TGTCATTAAATTCTCAACTTTATAAGTTTTTACTTATTAAAGCTTGGATGTTGGAGGCTT 556

Query 481 GCAGGCGTTTGTGAGCTCCTCTTAAATTTATTAGTGGGAACCTGTTTTGTTAGTTCTAA 540
Sbjct 557 GCAGGCGTTTGTGAGCTCCTCTTAAATTTATTAGTGGGAACCTGTTTTGTTAGTTCTAA 616

Query 541 CCTTGGTGTGATAATTATCTACATTTTGGTGAACCTTACAATAATAAGCTCTATTGGT 600
Sbjct 617 CCTTGGTGTGATAATTATCTACATTTTGGTGAACCTTACAATAATAAGCTCTATTGGT 676

Query 601 TTGGGTTGGTTGCATTTAATTTGCTCAATCTGTTCTATTTCATTGGAGAAAAAGG 654
Sbjct 677 TTGGGTT-GTTGCATTAGTTTGCTCAATCTGTTCTATTTCATTGGAGAAAAAGG 729

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Lentinula edodes strain 217(LE6) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Sequence ID: **gb|DQ467888.1** Length: 781 Number of Matches: 1

See 1 more title(s)
range 1: 22 to 674

Score	Expect	Identities	Gaps	Strand	Frame
1192 bits(645)	0.0()	651/654(99%)	1/654(0%)	Plus/Plus	
Features:					
Query 1		GTTTCCGTAGGTGAACCTGCGGAAGGATCATTATTGAATTTTTTGGTGGTGGATTGTTGC			60
Sbjct 22		GTTTCCGTAGGTGAACCTGCGGAAGGATCATTATTGAATTTTTTGGTGGTGGATTGTTGC			81
Query 61		TGGCCTTTGGGTATGTGCACATCCTCCTCCGATTNTATTCATCCACCTGTGCACTTTTT			120
Sbjct 82		TGGCCTTTGGGTATGTGCACATCCTCCTCCGATTCTATTCATCCACCTGTGCACTTTTT			141
Query 121		GTAGGAGTTCTTTTCATCGGGTTTTTGAAGGTGCTCATTATGAGTTACTTGAAAAGACTAG			180
Sbjct 142		GTAGGAGTTCTTTTCATCGGGTTTTTGAAGGTGCTCATTATGAGTTACTTGAAAAGACTAG			201
Query 181		TTGACAAGGCTTCTATGTTCTTATAAACCATTGAAGTATGTTATAGAATGATCTTGTTAT			240
Sbjct 202		TTGACAAGGCTTCTATGTTCTTATAAACCATTGAAGTATGTTATAGAATGATCTTGTTAT			261
Query 241		TGGGACTTTATTGACCCTTTAACTTAATACAACCTTCAGCAACGGATCTCTTGGCTCTC			300
Sbjct 262		TGGGACTTTATTGACCCTTTAACTTAATACAACCTTCAGCAACGGATCTCTTGGCTCTC			321
Query 301		CCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT			360
Sbjct 322		CCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT			381
Query 361		CATCGAATCTTTGAACGCACCTTGCGCCCTCTGGTATTCGGAGGGCATGCCTGTTTGAG			420
Sbjct 382		CATCGAATCTTTGAACGCACCTTGCGCCCTCTGGTATTCGGAGGGCATGCCTGTTTGAG			441
Query 421		TGTCATTAAATTCTCAACTTTATAAGTTTTTACTTATTAAAGCTTGGATGTTGGAGGCTT			480
Sbjct 442		TGTCATTAAATTCTCAACTTTATAAGTTTTTACTTATTAAAGCTTGGATGTTGGAGGCTT			501
Query 481		GCAGGCGTTTGTGAGCTCCTCTTAAATTTATTAGTGGGAACCTGTTTTGTTAGTTCTAA			540
Sbjct 502		GCAGGCGTTTGTGAGCTCCTCTTAAATTTATTAGTGGGAACCTGTTTTGTTAGTTCTAA			561
Query 541		CCTTGGTGTGATAATTATCTACATTTTGGTGGAACTTACAATAATAAAGCTCTATTGGT			600
Sbjct 562		CCTTGGTGTGATAATTATCTACATTTTGGTGGAACTTACAATAATAAAGCTCTATTGGT			621
Query 601		TTGGGTTGGTTGCATTTAATTTGCTCAATCTGTTCTATTTCATTGGAGAAAAAGG			654
Sbjct 622		TTGGGTT-GTTGCATTTAGTTTGCCTCAATCTGTTCTATTTCATTGGAGAAAAAGG			674

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[NCBI/ BLAST/ blastn suite/ Formatting Results - 75A9RBUU014](#)[Formatting options](#)[Download](#)[Blast report description](#)

Nucleotide Sequence (632 letters)

RID [75A9RBUU014](#) (Expires on 12-18 19:23 pm)

Query ID lcl|Query_29157

Description None

Molecule type nucleic acid

Query Length 632

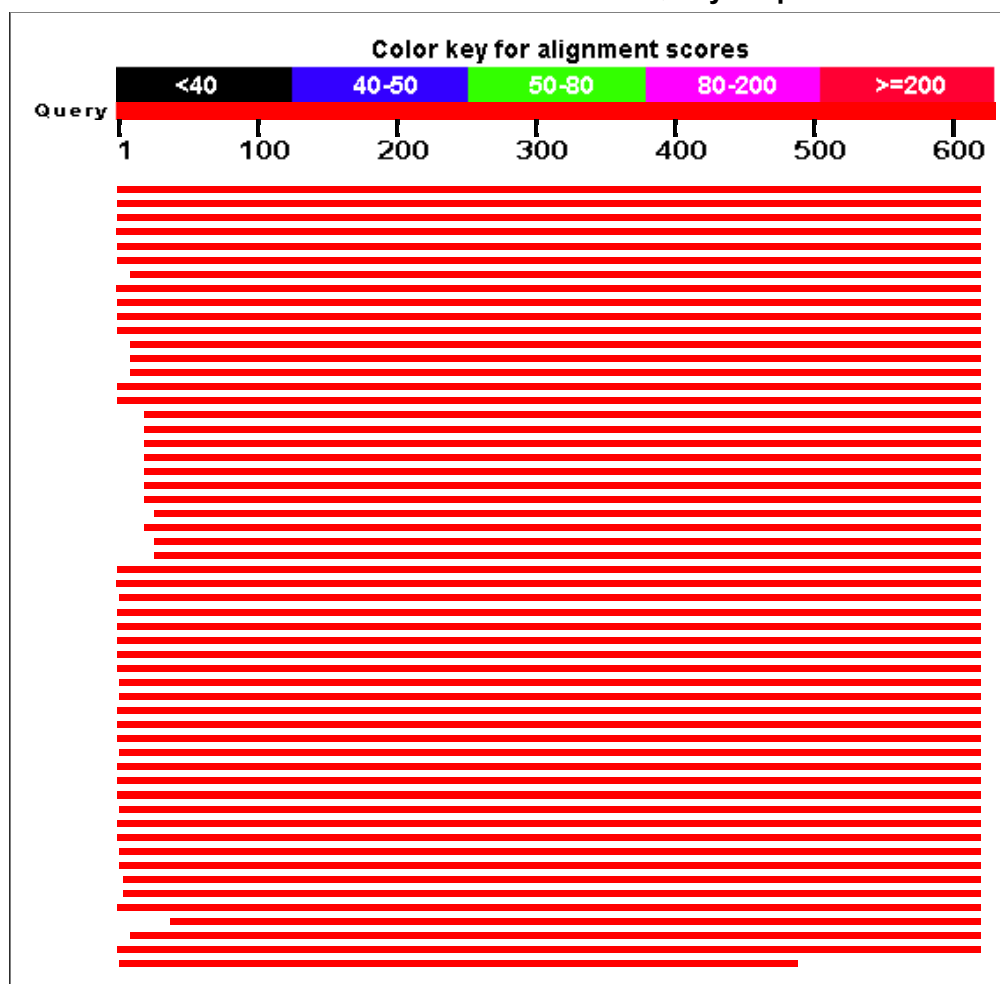
Database Name nr

Description Nucleotide collection (nt)

Program BLASTN 2.3.0+

[Graphic Summary](#)

Distribution of 100 Blast Hits on the Query Sequence



Descriptions

Sequences producing significant alignments:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Agaricus subrufescens strain CA487-S100 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	486	486	98%	2e-133	75%	KJ541799.1
Agaricus fiardii voucher F2285, LIP 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	483	483	98%	2e-132	75%	JF797201.1
Agaricus subrufescens strain WC837-S43 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	481	481	98%	9e-132	75%	KJ541797.1
Agaricus campestris voucher NVE 470 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	470	470	98%	2e-128	75%	KF937296.1
Agaricus subrufescens strain CA487-S42 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	462	462	98%	3e-126	75%	KJ541800.1
Agaricus subrufescens strain WC837-S04 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	462	462	98%	3e-126	75%	KJ541796.1
Agaricus blazei genes for ITS1, 5.8S rRNA, ITS2, partial and complete sequence, strain:SA514	462	462	96%	3e-126	75%	AB113576.1
Agaricus subrufescens isolate D116 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	453	453	98%	2e-123	74%	KT002160.1
Agaricus subrufescens voucher HKAS:81063 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	453	453	98%	2e-123	74%	KJ755633.1
Agaricus subrufescens voucher HKAS:81064 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial	451	451	98%	7e-123	74%	KJ755634.1

sequence						
Agaricus subrufescens voucher HKAS:81062 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	451	451	98%	7e-123	74%	KJ755632.1
Agaricus blazei genes for ITS1, 5.8S rRNA, ITS2, partial and complete sequence, strain:SA515	448	448	96%	9e-122	74%	AB113577.1
Agaricus blazei strain WC837 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	446	446	96%	3e-121	74%	AY484697.1
Agaricus blazei internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	446	446	96%	3e-121	74%	AF161013.1
Agaricus sp. NTF67 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	440	440	98%	1e-119	74%	JF514529.1
Agaricus subrufescens strain CA918 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	436	436	98%	2e-118	74%	KJ541798.1
Agaricus subrufescens isolate BS1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	433	433	95%	2e-117	74%	AY818654.1
Agaricus rufotegulis 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	433	433	95%	2e-117	74%	AY818649.1
Agaricus subrufescens isolate RM_Af 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	429	429	95%	3e-116	74%	AY818653.1
Agaricus subrufescens isolate RM1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	429	429	95%	3e-116	74%	AY818652.1
Agaricus subrufescens isolate RM1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	429	429	95%	3e-116	74%	AY818651.1
Agaricus brasiliensis 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete	425	425	95%	4e-115	74%	AY818650.1

sequence; and 28S ribosomal RNA gene, partial sequence						
Agaricus subrufescens isolate I_101_S1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	422	422	95%	5e-114	74%	AY818660.1
Agaricus brasiliensis ITS1, 5.8S rRNA gene and ITS2, specimen voucher HAI herbarium 0978-3	418	418	93%	7e-113	74%	AJ884653.1
Agaricus subrufescens isolate DEH513 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	416	416	95%	2e-112	74%	AY818646.1
Agaricus brasiliensis ITS1, 5.8S rRNA gene and ITS2, specimen voucher HAI herbarium 0978-1	416	416	93%	2e-112	74%	AJ884652.1
Agaricus brasiliensis ITS1, 5.8S rRNA gene and ITS2, strain 631	412	412	93%	3e-111	74%	AJ884650.1
Agaricus augustus voucher CA590 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	403	403	98%	2e-108	73%	JF797193.1
Uncultured fungus DNA sequence containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene, isolate B0740	399	399	98%	2e-107	73%	FM999675.1
Agaricus sp. GAL17755 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	394	394	97%	1e-105	73%	EF460352.1
Agaricus guizhouensis voucher HKAS:81080 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	392	392	98%	4e-105	73%	KJ755655.1
Agaricus guizhouensis voucher HKAS:81079 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	392	392	98%	4e-105	73%	KJ755654.1
Agaricus longistipes voucher HKAS:81067 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	392	392	98%	4e-105	73%	KJ755642.1
Agaricus sp. 1 YG-2014 voucher HKAS:81078 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	390	390	98%	1e-104	73%	KJ755647.1
Agaricus megalocarpus voucher HKAS:71717 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial	390	390	98%	1e-104	73%	KJ755638.1

sequence						
Agaricus sp. GAL15446 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	390	390	97%	1e-104	73%	EF460366.1
Agaricus sp. GAL9604 18S ribosomal RNA gene, partial sequence; and internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	390	390	97%	1e-104	73%	DQ232637.1
Agaricus guizhouensis voucher HKAS:81081 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	388	388	98%	5e-104	73%	KJ755658.1
Agaricus guizhouensis voucher HKAS:81084 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	388	388	98%	5e-104	73%	KJ755656.1
Agaricus abruptibulbus voucher HKAS:81074 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	388	388	98%	5e-104	73%	KJ755648.1
Agaricus sp. GAL16645 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	388	388	97%	5e-104	73%	EF460369.1
Agaricus guizhouensis voucher HKAS:81083 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	387	387	98%	2e-103	73%	KJ755661.1
Agaricus abruptibulbus voucher HKAS:81077 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	387	387	98%	2e-103	73%	KJ755649.1
Agaricus sp. 2 YG-2014 voucher HKAS:56853 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	387	387	98%	2e-103	73%	KJ755646.1
Agaricus sp. GAL9418 18S ribosomal RNA gene, partial sequence; and internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	387	387	97%	2e-103	73%	DQ232648.1
Agaricus abruptibulbus voucher HKAS:81073 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	385	385	98%	7e-103	73%	KJ755653.1
Agaricus guizhouensis voucher HKAS:54105 18S ribosomal RNA gene, partial sequence;						

internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	383	383	98%	2e-102	73%	KJ755660.1
Agaricus sp. GAL16070 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	383	383	97%	2e-102	73%	EF460363.1
Agaricus sp. GAL16056 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	383	383	97%	2e-102	73%	EF460361.1
Agaricus augustus strain WC414 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	383	383	97%	2e-102	73%	AY484672.1
Agaricus augustus strain WC908 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	383	383	97%	2e-102	73%	AY484671.1
Agaricus sp. 2 YG-2014 voucher HKAS:56209 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	381	381	98%	9e-102	72%	KJ755644.1
Agaricus blazei strain A internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	381	381	92%	9e-102	73%	EF195648.1
Endoptychum depressum internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	381	381	96%	9e-102	73%	AF482834.1
Agaricus sp. HKAS 81071 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	377	377	98%	1e-100	72%	KJ755664.1
Uncultured fungus clone D261 internal transcribed spacer 1, 5.8S ribosomal RNA gene, internal transcribed spacer 2, and 28S ribosomal RNA gene, region	377	377	76%	1e-100	74%	JX974750.1
Agaricus nivescens strain WC779 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	372	372	97%	5e-99	72%	AY484670.1
Agaricus arvensis strain AA02 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	368	368	97%	7e-98	72%	JN222407.1
Agaricus cf. fissuratus GAL16010 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	368	368	97%	7e-98	72%	EF460367.1

Agaricus sp. 0747 18S rRNA gene (partial), ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene (partial), isolate 0747	368	368	97%	7e-98	72%	AM930985.1
Agaricus augustus voucher LAPAG 233 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	364	364	95%	9e-97	72%	KJ548135.1
Agaricus crocodilinus voucher LAPAG 223 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	363	363	95%	3e-96	72%	KJ548127.1
Agaricus sp. GAL13951 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	361	361	94%	1e-95	72%	EF460373.1
Agaricus arvensis strain WC848 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	361	361	96%	1e-95	72%	AY484690.1
Agaricus cf. tenuivolvatus LAPAG 714 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	357	357	95%	2e-94	72%	KJ548134.1
Agaricus sp. TL1650 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	355	355	95%	5e-94	72%	JF495061.1
Agaricus arvensis internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	355	355	96%	5e-94	72%	AF161015.1
Agaricus sylvicola voucher LAPAG 242 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	351	351	95%	7e-93	72%	KJ548130.1
Agaricus indistinctus voucher AH-44504 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	351	351	95%	7e-93	72%	KF114475.2
Agaricus flocculosipes voucher HKAS:81070 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	350	350	98%	3e-92	72%	KJ755639.1
Agaricus flocculosipes voucher NTF15 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	350	350	98%	3e-92	72%	JN592035.1
Agaricus sp. TL-2011b voucher MURU5927 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene,	350	350	95%	3e-92	72%	JF495063.1

complete sequence; and internal transcribed spacer 2, partial sequence						
Agaricus sp. BAB-4975 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	348	348	95%	9e-92	72%	KR155065.1
Agaricus essettei voucher LAPAG 676 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	346	346	95%	3e-91	72%	KJ548131.1
Agaricus flocculosipes voucher NTT30 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	346	346	98%	3e-91	72%	JN592037.1
Agaricus flocculosipes voucher HKAS:81069 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	344	344	98%	1e-90	72%	KJ755641.1
Agaricus flocculosipes voucher HKAS:81068 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	344	344	98%	1e-90	72%	KJ755640.1
Agaricus sp. CA486 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	344	344	98%	1e-90	71%	JF797189.1
Agaricus flocculosipes voucher CA 735 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	344	344	98%	1e-90	72%	JN662352.1
Agaricus flocculosipes voucher CA 718 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	344	344	98%	1e-90	72%	JN662351.1
Agaricus flocculosipes voucher NTS67 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	344	344	98%	1e-90	72%	JN592034.1
Agaricus eburneocanus PERTH 07626789 ITS region; from TYPE material	344	344	95%	1e-90	72%	NR_119946.1
Agaricus macrolepis voucher LAPAG 409 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	342	342	95%	4e-90	72%	KJ548128.1
Agaricus flocculosipes voucher OR71 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal	342	342	98%	4e-90	72%	JN592038.1

RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence						
Agaricus subrufescens 18S rRNA gene (partial), 5.8S rRNA gene (partial) and ITS1, strain W59	294	294	54%	1e-75	75%	AM110145.1
Agaricus subrufescens 18S rRNA gene (partial), 5.8S rRNA gene (partial) and ITS1, strain W28	287	287	54%	2e-73	75%	AM110146.1
Agaricus subrufescens isolate CS4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	259	259	51%	4e-65	74%	AY818655.1
Agaricus sp. De Meijer1904 voucher De Meijer1904, BR 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	150	150	17%	3e-32	89%	JF797196.1
Agaricus aridicola voucher CA101 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	150	150	17%	3e-32	89%	JF797195.1
Agaricus pseudolutosus voucher LAPAG77 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	150	150	17%	3e-32	89%	JF727868.1
Agaricus sp. NT055 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	150	150	17%	3e-32	89%	JF727846.1
Agaricus sp. NT62 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	150	150	17%	3e-32	89%	JF727845.1
Agaricus sp. LAPAG555 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	148	148	17%	1e-31	89%	KP282690.1
Agaricus boisseletii voucher CA524 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	148	148	17%	1e-31	89%	KP282689.1
Agaricus boisseletii voucher CA863 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	148	148	17%	1e-31	89%	KP282688.1
Agaricus sp. LAP-2015 voucher KMSAG2-13 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal	148	148	17%	1e-31	89%	KP282684.1

RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence						
Agaricus sp. LAP-2015 voucher KMSAG1-13 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	148	148	17%	1e-31	89%	KP282683.1
Agaricus sp. LAP-2015 voucher EM0349-11 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	148	148	17%	1e-31	89%	KP282682.1
Agaricus sp. LAP-2015 voucher CA577 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	148	148	17%	1e-31	89%	KP282681.1
Agaricus pseudolutosus voucher AH-11488 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	147	147	17%	4e-31	88%	KF447890.1

Alignments

Agaricus subrufescens strain CA487-S100 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Sequence ID: **gb|KJ541799.1|** Length: 731 Number of Matches: 1

Range 1: 1 to 638

Score	Expect	Identities	Gaps	Strand	Frame
486 bits(263)	2e-133()	482/639(75%)	20/639(3%)	Plus/Plus	
Features:					
Query 2	TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTGAATTATGTTTCTAGATAGGTTGTAG	61			
Sbjct 1	TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTGAATTATGTTTCTAGATAGGTTGTAG	60			
Query 62	CTGGCTCTTTTAAANCANGTNCNCCCNGTTTGNNTTCATTTTCATCCACNNGTNCNC	121			
Sbjct 61	CTGGCTCTTTTAGAGCATGTGCACGCTGTTTGGACTTCATTTTCATCCACCTGTGCACC	120			
Query 122	TATNGNATNCTTNGNTNNGNCTNAGNAGGAANNANGANCATNGNNNCACNNCNGCTGGNN	181			
Sbjct 121	TATTGTAGTCTTTGGTTGGGTT-AGGAGGAAGTGATCATGTGATCAGCATCTGCTGGGAG	179			
Query 182	TNAGGACTTGCNTNGNNAACCTTTGCTGNCTTGANNTGANCANGGAANCCTTTTCTNA	241			
Sbjct 180	TGAGGACTTGCATTGTGAAAGCTTTGCTGTCTTGATGTGATCATGGAATCTTTTCTCA	239			
Query 242	NTAAANTCNATNTCANTCATNATNCTCNGNCAAANGTCATTNAANGTCTTNCNNGGCCT	301			
Sbjct 240	CTAGAGTCTATGTCACTCATTATACTCTGTGGAATGTCATTGAATGTCTTTACATGGGCT	299			
Query 302	NGTNTNCCTNTAAAAATGTANTNCANNTTTCACCACCGNATCTNTNGNNTNTCNCATCN	361			
Sbjct 300	TGTATGCCATATGAAATTTGTAATACAACCTTTCAGCAACGGATCTCTTGGCTCTCGCATCG	359			
Query 362	NTAAAAANCNCACCGAATNGCANTAAATTANNGTNAATTGCAAAATTCANTGAANCATCNA	421			
Sbjct 360	ATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATGCAGAATTCAGTGAATCATCGA	419			
Query 422	NNCTTTGANCNCNNCTNGCCCTCCTTGNNATTCCAAGGANCNNCCNGTTTAAAGTGTCAT	481			
Sbjct 420	ATCTTTGAACGCATCTTGCGCTCCTTGGTATTCGAGGAGCATGCCTGTTTGAGTGTCAT	479			
Query 482	TAAATTCCTNC-CTCTCTAA-ACCTTTTGGAAAAA-aCCTTGGACTGT-GAGNCTTGCT	537			
Sbjct 480	TAAATTCCTCAACTCTCTTATACTTTTGTAAAAGAGAGCTTGGACTGTGGAGGCTTGCT	539			
Query 538	GGCN-CTTTTGGG-TCANC-CCTCT-AAAGGC-TTACCG-AACCGTT-GCAATC-GCC-	588			

Sbjct 540 GGCCACTTTTGGGGTCAGCTCCTCTGAAATGCATTAGCGGAACCGTTTGCATCTGCCA 599
 Query 589 CA-GTGTNA-AA-TTATCTCC-C-GGCAAGGG-ATTGCT 621
 Sbjct 600 CAAGTGTGATAAGTTATCTACACTGGCGAGGGGATTGCT 638

Agaricus fiardii voucher F2285, LIP 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Sequence ID: **gb|JF797201.1|** Length: 728 Number of Matches: 1

Range 1: 1 to 638

Score	Expect	Identities	Gaps	Strand	Frame
483 bits(261)	2e-132()	481/639(75%)	20/639(3%)	Plus/Plus	

Features:

Query	2	TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTGAATTATGTTTCTAGATAGGTTGTAG	61
Sbjct	1	TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTGAATTATGTTTCTAGATAGGTTGTAG	60
Query	62	CTGGCTCTTTTAAANCANGTNCNCCCNGTTTGNNTTCATTTTCATCCACNNGTNCNC	121
Sbjct	61	CTGGCTCTTTTAGAGCATGTGCACRCCTGTTTGGACTTCATTTTCATCCACCTGTGCACC	120
Query	122	TATNGNATNCTTNGNTNGGNCCTNAGNAGGAANNANGANCATNGNNNCACNNCNGCTGGNN	181
Sbjct	121	TATTGTAGTCTTTGGTTGGGTT-AGGAGGAAGTGATCATGTATCAGCATCTGCTGGGAG	179
Query	182	TNAGGACTTGCNTNGNNAACCTTTGCTGNCCTTGANNTGANCANGGAANC'TTTTCTNA	241
Sbjct	180	TGAGGACTTGCATTGTGAAAGCTTTGCTGTCTTGATGTGATCATGGAATCTTTTCTCA	239
Query	242	NTAAANTCNATNTCANTCATNATNCTCNGNCAAANGTCATTNAANGTCTTNCNNGGCCT	301
Sbjct	240	CTAGAGTCTATGTCACTCATTATACTCTGTGCAATGTCAATTGAATGTCTTTACATGGGCT	299
Query	302	NGTNTNCCTNTAAAAATTGTANTNCANNTTTACCACCGNATCTNTNGNNTNCNCATCN	361
Sbjct	300	TGTATGCCATATGAAATTTGTAATACAACTTTTCAAGCAACGGATCTCTTGGCTCTCGCATCG	359
Query	362	NTAAAAANCNCACCGAATNGCANTAATTANNGTNAATTGCAAAATTCANTGAANCATCNA	421
Sbjct	360	ATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGA	419
Query	422	NNCTTTGANCNCNNCTNGCCCTCCTTGNNATTCCAAGGANCNNCCNGTTTAAAGTGTCAT	481
Sbjct	420	ATCTTTGAACGCATCTTGGCTCCTTGGTATTCAGGAGCATGCCTGTTTGAGTGTCAT	479
Query	482	TAAATTCCTNC-CTCTCTAA-AC'TTTTTTGAaaaaaa-aCCTTGGACTGT-GAGNCTTGCT	537
Sbjct	480	TAAATTCCTCAACTCTCTTATACTTTTTTGTAAAAGAGAGCTTGGAYGTGGAGGCTTGCT	539
Query	538	GGCN-CTTTTGGG-TCANC-CCTCT-AAAGGC-TTACCG-AACCGTT-GCAATC-GCC-	588
Sbjct	540	GGCCACTTTTGGGGTCAGCTCCTCTGAAATGCATTAGCGGAACCGTTTGCATCTGCCA	599
Query	589	CA-GTGTNA-AA-TTATCTCC-C-GGCAAGGG-ATTGCT 621	
Sbjct	600	CAAGTGTGATAAGTTATCTACACTGGCGAGGGGATTGCT 638	

Agaricus subrufescens strain WC837-S43 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Sequence ID: **gb|KJ541797.1|** Length: 731 Number of Matches: 1

Range 1: 1 to 638

Score	Expect	Identities	Gaps	Strand	Frame
481 bits(260)	9e-132()	481/639(75%)	20/639(3%)	Plus/Plus	

Features:

Query	2	TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTGAATTATGTTTCTAGATAGGTTGTAG	61
Sbjct	1	TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTGAATTATGTTTCTAGATAGGTTGTAG	60
Query	62	CTGGCTCTTTTAAANCANGTNCNCCCNGTTTGNNTTCATTTTCATCCACNNGTNCNC	121
Sbjct	61	CTGGCTCTTTTAGAGCATGTGCACGCCCTGTTTGGACTTCATTTTCATCCACCTGTGCACC	120
Query	122	TATNGNATNCTTNGNTNGGNCCTNAGNAGGAANNANGANCATNGNNNCACNNCNGCTGGNN	181
Sbjct	121	TATTGTAGTCTTTGGTTGGGTT-AGGAGGAAGTGATCATGTATCAGCATCTGCTGGGAG	179
Query	182	TNAGGACTTGCNTNGNNAACCTTTGCTGNCCTTGANNTGANCANGGAANC'TTTTCTNA	241

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Sbjct  180  TGAGGACTTGCATTGTGAAAACTTGCTGTCCTTGATGTGATTATGGAATCTTTTCTCA  239
Query  242  NTAAANTCNATNTCANTCATNATNCTCNGNCAAANGTCATTNAANGTCTTTNCNNGGCCT  301
Sbjct  240  CTAGAGTCTATGTCACTCATTATACTCTGTGCAATGTCATTGAATGTCTTTACATGGGCT  299
Query  302  NGTNTNCCNTAAAAATTGTANTNCANNTTTCACCACCGNATCTNTNGNNTNTCNCATCN  361
Sbjct  300  TGTATGCCCTATGAAAATTGTAATACAACCTTTCAGCAACGGATCTCTTGGCTCTCGCATCG  359
Query  362  NTAAAAANCNCACCGAATNGCANTAATTANNGTNAATTGCAAAATTCANTGAANCATCNA  421
Sbjct  360  ATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAATTCAGTGAATCATCGA  419
Query  422  NNCTTTGANCNCNNCTNGCCCTCCTTGNNATTCCAAGGANCNNCCNGTTTAAGTGTCAT  481
Sbjct  420  ATCTTTGAACGCATCTTGCGCTCCTTGGTATTCGAGGAGCATGCCTGTTTGAGTGTCAT  479
Query  482  TAAATTCCTNC-CTCTCTAA-ACCTTTTTGaaaaaaa-aCCTTGGACTGT-GAGNCTTGCT  537
Sbjct  480  TAAATTCCTCAACTCTCTTATACTTTTTGTAAAAGAGAGCTTGGACTGTGGAGGCTTGCT  539
Query  538  GGCN-CTTTTGGG-TCANC-CCTCT-AAAGGC-TTACCG-AACCGTT-GCAATC-GCC-  588
Sbjct  540  GGCCACTTTTGGGGTCAGCTCCTCTGAAATGCATTAGCGGAACCGTTTGCATCTGCCA  599
Query  589  CA-GTGTNA-AA-TTATCTCC-C-GGCAAGGG-ATTGCT  621
Sbjct  600  CAAGTGTGATAAGTTATCTACACTGGCGAGGGGATTGCT  638

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Agaricus campestris voucher NVE 470 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Sequence ID: **gb|KF937296.1|** Length: 749 Number of Matches: 1

Range 1: 8 to 645

Score	Expect	Identities	Gaps	Strand	Frame
470 bits(254)	2e-128()	480/640(75%)	21/640(3%)	Plus/Plus	

Features:

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Query  1  GTTTCGGTAGGTGAACCTGCGGAAGGATCATTATTGAATTATGTTTCTAGATAGGTTGTA  60
Sbjct  8  GTTTCGGTAGGTGAACCTGCGGAAGGATCATTATTGAATTATGTTTCTAGATAGGTTGTA  67
Query  61  GCTGGCTCTTTTAAANCANGTNCNCCCCNGTTGNNCTTCATTTTCATCCACNNGTNCNC  120
Sbjct  68  GCTGGCTC-TTTAGAGCATGTGCACGCCTGTTTGGACTTCATTTTCATCCACCTGTGCAC  126
Query  121  CTATNGNATNCTTNGNTNGGNCNAGNAGGAANNANGANCATNGNNNCACNNCNGCTGGNN  180
Sbjct  127  CTATTGTAGTCTTTGGTTGGGTT-AGGAGGAAGTGGTCATTGTGTCAGCATCTGCTGGAT  185
Query  181  NTNAGGACTTGCNTNGNNAACCTTTGCTGNCCTTGANNTGANCANGGAANCCTTTTCTN  240
Sbjct  186  GTGAGGACTTGCATTGTGAAAGCTTTGCTGTCTCTTGATGTGATCATGGAATCTTTTCTC  245
Query  241  ANTAAANTCNATNTCANTCATNATNCTCNGNCAAANGTCATTNAANGTCTTTNCNNGGCC  300
Sbjct  246  ACTAGAGTCTATGTCACTCATTATACTCTGTGCAATGTCATTGAATGTCTTTACATGGGC  305
Query  301  TNGTNTNCCNTAAAAATTGTANTNCANNTTTCACCACCGNATCTNTNGNNTNTCNCATC  360
Sbjct  306  TTGTATGCCCTATGAAAATTGTAATACAACCTTTCAGCAACGGATCTCTTGGCTCTCGCATC  365
Query  361  NNTAAAAANCNCACCGAATNGCANTAATTANNGTNAATTGCAAAATTCANTGAANCATCN  420
Sbjct  366  GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAATTCAGTGAATCATCG  425
Query  421  ANNCTTTGANCNCNNCTNGCCCTCCTTGNNATTCCAAGGANCNNCCNGTTTAAGTGTC  480
Sbjct  426  AATCTTTGAACGCATCTTGCGCTCCTTGGTATTCGAGGAGCATGCCTGTTTGAGTGTC  485
Query  481  TTAAATTCCTNC-CTCTCTAA-ACCTTTTTGaaaaaaa-aCCTTGGACTGT-GAGNCTTGC  536
Sbjct  486  TTAAATTCCTCAACTCTCTTATACTTTTTGTAAAAGAGAGCTTGGACTGTGGAGGCTTGC  545
Query  537  TGGCN-CTTTTGGG-TCANC-CCTCT-AAAGGC-TTACCG-AACCGTT-GCAATC-GCC  588
Sbjct  546  TGGCCACTTTTGGGGTCAGCTCCTCTGAAATGCATTAGCGGAACCGTTTGCATCTGCC  605
Query  589  -CA-GTGTNA-AA-TTATCTCC-C-GGCAAGGG-ATTGCT  621
Sbjct  606  ACAAGTGTGATAAGTTATCTACGCTGGCGAGGGGATTGCT  645

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Agaricus subrufescens strain CA487-S42 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence

Sequence ID: **gb|KJ541800.1|** Length: 730 Number of Matches: 1

Range 1: 1 to 637

Score	Expect	Identities	Gaps	Strand	Frame
462 bits(250)	3e-126()	478/639(75%)	21/639(3%)	Plus/Plus	
Features:					
Query 2	TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTGAATTATGTTTCTAGATAGGTTGTAG	61			
Sbjct 1	TTTCCGTAGGTGAACCTGCGGAAGGATCATTATTGAATTATGTTTCTAGATAGGTTGTAG	60			
Query 62	CTGGCTCTTTTAAANCANGTNCNCCCNGTTTGNNTTCATTTTCATCCACNNGTNCNCC	121			
Sbjct 61	CTGGCTC-TTTAGAGCATGTGCACGCTGTTTGGACTTCATTTTCATCCACCTGTGCACC	119			
Query 122	TATNGNATNCTTNGTNGGNCCTNAGNAGGAANNANGANCATNGNNNCACNNCNGCTGGNNN	181			
Sbjct 120	TATTGTAGTCTTTGGTTGGGTT-AGGGGGAAGTGGTCATTGTGTGAGCATCTGCTGGATG	178			
Query 182	TNAGGACTTGCNTNGNNAACCTTTGCTGNCCTTGANNTGANCANGGAANC'TTTTCTNA	241			
Sbjct 179	TGAGGACTTGCATTGTGAAAGCTTTGCTGTCTTGATGTGATCATGGAATCTCTTTCTCA	238			
Query 242	NTAAANTCNATNTCANTCATNATNCTCNGNCAAANGTCATTNAANGTCTTTNCNNGGCCT	301			
Sbjct 239	CTAGAGTCTATGTCACTCATTATACTCTGTGCAATGTCATTGAATGTCTTTACATGGGCT	298			
Query 302	NGTNTNCCTNTAAAAATTGTANTNCANNTTTCACCACCGNATCTNTNGNNTNTCNCATCN	361			
Sbjct 299	TGTATGCCATATGAAAATTGTAATAACAAC'TTCAGCAACGGATCTCTTGGCTCTCGCATCG	358			
Query 362	NTAAAAANCNCACCGAATNGCANTAATTANNGTNAATTGCAAAATTCANTGAANCATCNA	421			
Sbjct 359	ATGAAGGACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAATTCAGTGAATCATCGA	418			
Query 422	NNCTTTGANCNCNNCTNGCCCTCCTTGNNATTCCAAGGANCNNCCNGTTTAAAGTGTAT	481			
Sbjct 419	ATCTTTGAACGCATCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCGTGTTGAGTGTAT	478			
Query 482	TAAATTCNCTCTCTAA-AC'TTTTTTGaaaaaa-aCCTTGGACTGT-GAGNCTTGCT	537			
Sbjct 479	TAAATTCCTCAACTCTCTTATACTTTTGTAAAAGAGAGCTTGGACTGTGGAGGCTTGCT	538			
Query 538	GGCN-CTTTTGGG-TCANC-CCTCT-AAAGGC-TTACCG-AACCGTT-GCAATC-GCC-	588			
Sbjct 539	GGCCACTTTTGGGGTCAGCTCCTCTGAAATGCATTAGCGGAACCGTTTGCAATCTGCCA	598			
Query 589	CA-GTGTNA-AA-TTATCTCC-C-GGCAAGGG-ATTGCT	621			
Sbjct 599	CAAGTGTGATAAGTTATCTACACTGGCGAGGGGATTGCT	637			