LEMBAR PENGESAHAN

NAMA	:	Dr. Robi Binur, S.Si., M.Si
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Mengetahui,

Ketua Jurusan,

Paskalina Th. Lefaan, S.Si., M.Si

Penulis,

Dr. Robi Binur, S.Si., M.Si

Isolation, cultivation, and nutritional profiling of tropical microfungi isolated from several rivers in Indonesia for feed supplements in aquaculture

Robi Binur [1*], I Nyoman Pugeg Aryantha [2, 3], Gede Suantika [2]

[1] Departement of Biology, Faculty of Mathematics and Natural Sciences, Universitas Papua, Jl. Gunung Salju Amban, Manokwari 98314, West Papua, Indonesia
[2] Microbial Biotechnology Research Group, School of Life Sciences and Technology, Institut Teknologi Bandung, Jl. Ganesha 10, Bandung 40132, West Java, Indonesia
[3] Biosciences and Biotechnology Research Center, Institut Teknologi Bandung, Jl. Ganesha 10, Bandung 40132, West Java, Indonesia

Abstract

Microfungi that are ubiquitous and able to be cultured in vitro can be used as a source of aquaculture feed supplements due to high protein content, amino acids, essential fatty acids, vitamins, inorganic compounds, nucleic acids, and polysaccharides. Under laboratory conditions, seven isolates, i.e., *Trichoderma harzianum*, *Macrophoma theicola, Trichoderma lentiforme, Trichoderma hamatum, Mucor circinelloides, Lasiodiplodia theobromae,* and *Fusarium oxysporum* have the fastest growth rate ($p > 0.05$). Total amino acids of all isolates ranged between 11.023 - 18.881 g/100 grams consisting of EAA (5.710 - 9.539 g/100 grams) and NEAA (5.277 - 9.342 g/100 grams). Total fatty acids ranged between 1.094 - 5.253% with SAFA (0.320 - 1.415%), MUFA (0.441 - 1.968%), and PUFA (0.334 - 1.969%). Based on the percentage of EAA in the total protein of fish and shrimp requirement, it has the highest content discovered in *T. lentiforme* at 23.16% and *F. oxysporum* at 23%. The essential fatty acids contents with the highest levels were *L. theobromae* of 6.93%, *T. hamatum* of 5.93%, and *T. lentiforme* of 5.34%. The linoleic acid and linolenic acid that had fulfilled fish and shrimp requirements existed in *L. theobromae* (1.606% and 0.356%) and *T. hamatum* (1.330% and 0.455%). It could be concluded that all seven isolates can be potentially used as alternative feed supplements for aquaculture even though further deep investigation needs to be conducted to prove its effect on fish and shrimp growth and immunity.

Keywords: Microfungi, feed supplements, aquaculture, fish and shrimp, amino acids, fatty acids

1. INTRODUCTION

Aquaculture production has become a leading global industry and an essential source of revenue and food in many countries (NRC, 2011). This industry plays a significant role in supplying global protein and increasing every year to fulfill the high worldwide market demand, especially fish and shrimp. According to FAO (2018) over the past ten years, global aquaculture production had increased significantly from 44.3 million tons in 2005 to 80.03 million tons in 2016. This number increases the aquaculture production share to almost 47% of the total world fishery production (FAO, 2018).

Aquaculture industries would benefit from the cultured organisms conferred with feed supplements for optimal growth and disease resistance (Mohan et al., 2019). Therefore, new strategies are necessary to control better growth and disease-free through functional feed supplements containing various bioactive components. Many fungi are the essential source of amino acids (AA), fatty acids (FA), vitamins and minerals (Wallis, Claridge, & Trappe, 2012) as well as bioactive polysaccharides (beta-glucan, peptidoglycan, various oligosaccharides, chitin, inulin, chitosan, lentinan) (Murthy, Li, Lawrence, & Gatlin, 2009) (Ringø, Olsen, R.E., Gifstad, T.Ø., Dalmo, R.A., Amlund, H., Hemre, G.-I., Bakke, A.M., 2010) (Sang, Ky, & Fotedar, 2009) (Zhang et al., 2012). These components can be produced in the fruiting bodies, mycelium, and broth culture, which have various benefits, such as antioxidant, anti-cancer, anti-microbial, immunostimulation, and hypoglycemic activities (Cohen, Persky, & Hadar, 2002) (Wasser, 2011).

Limited food resources have encouraged the production of single-cell protein (SCP) derived from fungi, yeast, bacteria, and algae. Several advantages of SCP from fungi compared to animal and plant, i.e., (1) rapid growth rate and high protein content; (2) can be produced in large amounts in a relatively small area, using biological products as sources of the nutrient, (confectionery and distillery, vegetable and wood-processing industries); and (3) fungal cells contain carbohydrate, lipids, and nucleic acids, and a favorable balance of lysine, methionine, and tryptophan amino acids that plant-based proteins often lack (Nigam & Singh, 2014). Some microfungi, especially filamentous fungi and yeast that are commonly used for the production of SCP are *Fusarium* sp., *Aspergillus* sp., *Penicillium* sp., *Saccharomyces* sp., *Candida* sp., and *Rhodotorula* sp. SCP from microfungi has high protein content and amino acid, including various essential fatty acids (EFA), carbohydrates, vitamins, inorganic compounds, nucleic acids, and other

non-protein compounds (polysaccharides). Besides, it can add flavor to the food industry, used as a major protein source in some food additives and extenders, and improve animal feeds' protein content (Nigam & Singh, 2014). Filamentous fungi and yeast have protein content ranged between 31 - 53 %, nitrogen 5 - 8.5 %, fat 2 - 8%, nucleic acids 6 - 12%, and ash 5 - 14% on a dry weight basis. SCP is rich in essential fatty acids (EFA) such as C20 derivatives, eicosapentaenoic acid (20:5ω3), arachidonic acid (20:4ω6), linoleic acid (18:2ω6), and γ-linoleic acid(18:3ω3). In aquaculture, these fatty acids contribute to an important role in fish growth. The most common vitamins in the SCP are riboflavin, niacin, thiamine, pyridoxine, choline, pantothenic acid, folic acid, inositol, biotin, p-aminobenzoic acid, and vitamin B12 (Nalage et al., 2016). SCP contains a high quantity of nucleic acids, which is between 8 - 25 gram per 100 gram proteins whereas animals cells, fish (sardines and roe), and plant (wheat and rye flour) are 4 gram, 2.2 and 5.7 gram, 1.1 - 4.0 gram per 100 g protein, respectively (Nalage et al., 2016). Therefore, microfungi are suitable as functional feed in both fish and shrimp farming and other animals.

Polysaccharides are prebiotic substances boosting the immune responses, resistance to pathogens, and the growth performance of fish and crustaceans (Bai et al., 2015) (Mohan, Muralisankar, Uthayakumar, Chandirasekar, & Karthick Rajan, 2019) (Ringø, 2010). Several polysaccharides derived from mushrooms (Basidiomycetes) and yeast used as supplements are beta-glucan (Murthy et al., 2009), oligonucleotides (Manoppo & Sukenda, 2013) (Li, Lewis, & Gatlin, 2004), mannan oligosaccharides (MOS) (Sang et al., 2009) (Zhang et al., 2012), fructooligosaccharides (FOS), galactooligosaccharides (GOS), xylooligosaccharides (XOS), arabinoxylooligosaccharides (AXOS), isomaltooligosaccharides (IMO), and inulin (Ringø, 2010). These components can increase growth, immune response, and stress resistance both in fish and shrimp.

Ninety-three studies have used fungi and yeast as feed supplements for fish and shrimp. Of sixty-nine studies (74%) use yeast *(S. cerevisiae, S. commune),* and the remaining twenty-five studies (26%) use mushrooms (Basidiomycetes), including *P. ostreatus*, *G. lucidum*, *C. versicolor*, *P. florida*, *C. sinensis*, *L. edodes*, dan *Polystictus* spp.) (Mohan et al., 2019). These fungi can regulate growth, enhance the immune response and diversity of intestinal probiotic bacteria in shrimp and fish. According to (Jin et al., 2018) and (Xiong et al., 2018), dietary of 1% yeast or yeast hydrolysate could improve growth

performance, enhance innate immunity, and strengthen the resistance of ammonia nitrogen stress in *L. vannamei*.

Microfungi are fungi that able to develop small thallus. These fungi are also referred to as Micromycetes or microscopic fungi (Gupta & Tuohy, 2016). Research is limited regarding microfungi compared to mushrooms or yeast as a food supplement for aquaculture organisms. However, the nutritional composition may be the same and can be higher than mushrooms and yeast. At present, only mushrooms and yeast are commonly used as feed supplements for fish and shrimp, both in the form of single-cell protein (cells biomass) and hydrolyzate products. Microfungi are abundant in nature, generally cosmopolite, and ubiquitous in soil, water, plants, and animals (arthropods and mammalian). Therefore, through this research, authors strived to investigate microfungi's potential, which was isolated from tropical rivers through some examinations on the growth, proximities, amino acids, fatty acids, and beta-glucans (glucan-S1), as well as potency as feed supplements in fish and shrimp. This study's results may become a reference and considerations in developing alternative feed supplements from microfungi for aquaculture.

2. MATERIALS AND METHODS

2.1. Isolation and collection of microfungi

Samples were collected from several rivers or streams in Indonesia, i.e., Ketahun (Bengkulu), Wariori (Manokwari-West Papua), Tahura, and Sumedang (Bandung-West Java), and Pangkalan Bun (East Kalimantan). Microfungi were isolated from water foam (bubble) (Mueller, Bills, & Foster, 2004), leaf litter (Suberkropp, Arsuffi, & Anderson, 1983), wood, and debris submerged in the river (Yang, Shi, Wang, & Liu, 2016). The sample was placed in a sterile bottle and stored in a safety box. In the laboratory, samples ware stored in a refrigerator (4^{O}C) to prevent contamination (Choi, Hyde, & Ho, 1999). Samples were separated from the soil and sand and then centrifuged at 6000 rotations per minute (RPM) to obtain spore suspension. One microliter of the sample was spread onto potato dextrose agar (PDA) medium (20 % potatoes; 2 % dextrose; 2 % agar) containing antibiotic Chloramphenicol 0.1 % (w/v) and then incubated for 24 hours at room temperature (25 - 28^{O}C) until a single colony grew. The colony was sub-cultured for pure isolates. The non-single colony was purified with the following standard procedures. Once pure culture was obtained, it was then maintained on an agar slant at 4^{O}C and a few agar

pieces were stored with mycelia in cryovials with sterilized paraffin oil at 4^{O}C (Choi et al., 1999).

2.2. Molecular identification of microfungi

Isolates were identified molecularly using the Internal Transcribed Spacers (ITS) gene with primers ITS5 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS4 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) (Schoch et al., 2012). DNA was extracted using YeaStar Genomic DNA Kits (Zymo Research). Polymerase Chain Reaction (PCR) profile was for 0.2 µM, i.e. one initial denaturation cycle (95^{O}C, 10 minutes); 35 denaturation cycles (95^{O}C, 15 seconds); annealing (52^{O}C, 30 seconds); elongation (72^{O}C, 1.5 seconds); final elongation (72^{O}C, 7 minutes); and cooling (4^{O}C). DNA sequencing used the services of Macrogen, Inc. (South Korea). Microfungi identification used a database from GenBank (https://www.ncbi.nlm.nih.gov).

2.3. Radial growth test of microfungi

Radial growth was selected using rice straw medium and PDA medium (control). The rice straw media was cleaned, dried (oven) at constant temperature (50^{O}C) for 2 - 3 days, and mashed with size 0.5 mm. Afterward, the media was added with distilled water until the moisture level 60 - 70 %. Furthermore, the medium was put in a petri dish (90 mm diameter) and autoclave at 1.5 psi pressure for 1 hour. Pure microfungi cultures were previously grown on PDA media for three days at room temperature (28^{O}C). One piece of agar was placed on rice straw media (Smith & Onions, 1994). Microfungi's radial growth was measured for 12 consecutive days.

2.4. Cultivation of microfungi in liquid media

Isolates with the fastest growth for five days of culture were then cultivated using liquid media. The composition of liquid media used in one liter (1000 ml) was 30 % potatoes, 1.5 % sugar (commercial sugar), and distilled water. Then the media was sterilized (autoclave) at a pressure of 1.5 psi for 20 minutes. The pure culture was cultured as much as 100 ml in potato dextrose broth (PDB) media for three days of culture without antibiotics. Production of biomass (mycelium) using 1-liter Erlenmeyer with a pure culture ratio versus media was 0.5: 10 (50 ml of pure culture in 1 liter of media). Cultivation was carried out for five days in a dark room at room temperature (28^{O}C) and rotary shaker at 140 RPM. Biomass was harvested by filtering and then washed using

distilled water and dried (oven) at a constant temperature ($50^{O}C$) for 2 x 24 hours with water content <15%.

2.5. The nutrition profile of microfungi

The nutrition profile was analyzed through proximate, amino acid, and fatty acid content. The proximate analysis included total protein (Kjeldahl method), ash content (dry ashing method), water content (oven method), carbohydrate (by difference method), total fat (Weibull method), and total energy (calculation). Analysis of amino acid used Ultra Performance Liquid Chromatography (UPLC) and fatty acids with Gas Chromatography (GC). All analyzes used the services of PT. Saraswanti Indo Genetech (SIG), Bogor, Indonesia.

2.6. Beta-glucan test of microfungi

The content of beta-glucan (glucan-S1) was extracted using alkaline (NaOH) and acetic acid (CH_3COOH) following the method performed by (Lee et al., 2001) and (Pengkumsri et al., 2016) method. One gram of fungi was added with 5 ml of NaOH (2 %) and then incubated at $90^{O}C$ (5 hours), once being cold, then centrifuged at 3000 g (10 minutes). The supernatant was then added with 25 ml of acetic acid (2 M) and then re-incubated at $80^{O}C$ (2 hours) and centrifuged. Glucan-S1 pellets were washed with distilled water and then dried at $60^{O}C$ (12 hours).

2.7. Statistical analysis

All data were subjected to statistical analyses using Minitab version 19 for macOS. Significant differences between samples were analyzed by one-way Analysis of Variance (ANOVA) and Turkey's multiple comparisons test.

3. RESULTS

3.1. Fungal isolates from Indonesia’s tropical river habitats

From the field study, forty isolates were obtained from four rivers or streams. By using the molecular approach, 17 microfungi were identified which divided into five classes, including Sordariomycetes (12 species), Dothideomycetes (2 species), Agaricomycetes (1 species), Hyphomycetes (1 species), and Mucorales (1 species) (**Table 1**).

3.2. Fungal growth on rice straw media

From the growth test in the straw media, the fastest growth (radial growth) during five days culture period (second to fifth days) occurred in *T. harzianum* with mycelium length of 38.66 ± 5.27 mm, followed by *M. theicola* (37.39 ± 3.52 mm), *M. circinelloides* (33.53 ± 6.42 mm), *T. lentiforme* (27.82 ± 6.91 mm), and *F. oxysporum* (26.06 ± 5.87 mm) ($p > 0.05$). While the fastest radial growth of microfungi in PDA medium existed in *T. harzianum* with mycelium length of 39.03 ± 0.84 mm, followed *M. theicola* (38.66 ± 1.33 mm), *T. lentiforme* (38.44 ± 1.21 mm), *T. hamatum* (35.49 ± 6.15 mm), *L. theobromae* (34.91 ± 4.85 mm), and *M. circinelloides* (34.28 ± 5.23 mm) ($p >0.05$) (**Table 2**).

Generally, the optimum growth of microfungi happened on the fifth day of the culture period except for *T. harzianum* was obtained on the third day of the culture period. Based on the measurement on mycelium length among the isolates, after five days culture period, the highest growth of mycelium appeared in *T. harzianum* in rice straw media of 38.66 ± 5.27 mm and PDA 39.03±0.84 mm, followed by *M. theicola* (38.66 ± 1.33 mm and 34.28 ± 5.23 mm), *M. circinelloides* (33.53 ± 6.42 mm and 34.28 ± 5.23), *T. lentiforme* (27.82 ± 6.91 mm; 38.44 ± 1.21 mm), *F. oxysporum* (26.06 ± 5.87 mm; 18.43 ± 8.68), *T. hamatum* (13.04 ± 5.82 and 35.49 ± 6.15 mm), *L. theobromae* (21.98 ± 4.43 and 34.91 ± 4.85 mm). The radial growth (mycelium length) of seven isolate microfungi using rice straw media and PDA was presented in **Figure 1**.

3.3. Fungal growth on liquid media

During the five-day cultivation period, seven microfungi produced a relatively equal amount of dry weight in one liter (1000 ml) of PDB medium. The highest biomass production was found in T. harzianum (7.33 ± 1.2 grams), followed by *M. theicola* (6.50±2.1 gram), *T. lentiforme* (5.90 ± 0.6 gram), *L. theobromae* (4.67 ± 0.6 gram), *M. circinelloides* (4.20 ± 1.3 gram), *F. oxysporum* (4.13 ± 0.4gram), and *T. hamatum* (4.07 ± 1.0 gram) ($p > 0.05$). The water content in mycelium was relatively high, between 86 ± 1.2% to 97.86 ± 0.3% (**Table 3**).

3.4. Proximate composition of microfungi

The seven microfungi have crude protein ranged between 31.56 - 45.58%, crude lipid 2.22 - 6.54%, carbohydrates 32.92 - 46.42%, ash 4.66 - 8.48%, and moisture 7.18 - 13.44% (**Table 4**). Three microfungi with the highest crude protein were *M. circinelloides*

(45.58%), *F. oxysporum* (41.34%), and *M. theicola* (37.62%); crude lipid were *T. hamatum* (6.54%), *F. oxysporum* (5.20%), and *T. harzianum* (4.33%), carbohydrates were *T. lentiforme* (46.42%), *L. theobromae* (43.38%) and *M. theicola* (40.96%). At the same time, ash content was from *L. theobromae* (8.48%), *T. harzianum* (6,04%), and *F. oxysporum* (5.78%).

3.5. Composition of amino acids in microfungi

The seven microfungi had a total range of amino acids between 11.023 - 18.881 g/100 gram with Essential Amino Acids (EAA) and Non-Essential Amino Acids (NEAA) at 5.710 - 9.539 g/100 gram and 5.277 - 9.342 g/100 gram, respectively (**Table 5**). The ratio of EAA and NEAA was 51%: 49%. The five most EAAs were lysine (0.863 - 1.488 g/100 gram), leucine (0.932 - 1.451 g/100 gram), valine (0.682 - 1.211 g/100 gram), threonine (0.664 - 1.302 g/100 gram), and arginine (0.598 - 0.996 g/100 gram). While NEAAs were glutamic acid (1.525 - 2.529 g/100 gram), aspartic acid (0.870 - 2.073 g/100 gram), glycine (0.653 - 1.178 g/100 gram), alanine (0.620 - 1.037 g/100 gram), and serine (0.591 - 1.023 g/100 gram). Microfungi that have EAA and NEAA from highest to lowest were, i.e., *F. oxysporum* (9.539 and 9.342 g/100 gram), *T. lentiforme* (8.093 and 8.272 g/100 gram), *M. circinelloides* (7.925 and 8.103 g/100 gram), *M. theicola* (7.70 and 7.168 g/100 gram), *L. theobromae* (6.572 and 6.282 g/100 gram), *T. harzianum* (6.192 and 5.277 g/100 gram), and *T. hamatum* (5.710 dan 5.313 g/100 gram). The EAA composition of each microfungus was shown in **Figure 2**. Overall the total amino acids were not significantly different among microfungi (p >0.05).

3.6. Composition of fatty acids in microfungi

The fatty acids content of all seven microfungi ranged between 1.094 - 5.253% with saturated fatty acids (SAFA) (0.320 - 1.415%), monounsaturated fatty acids (MUFA) (0.441 - 1.968%), and polyunsaturated fatty acids (PUFA) (0.334 - 1.969%) (**Table 6**). The ratio of SAFA, MUFA, and PUFA was 28%: 44%: 28%. The most SAFAs were palmitic acid (C16: 0) (0.190 - 0.948%), stearic acid (C18: 0) (0.068 - 0.241%), myristic acid (C14: 0) (0.012 - 0.139%), lignoceric acid (C24: 0) (0.018 - 0.088%), and lauric acid (C12: 0) (0.006 - 0.066%). The most MUFAs were oleic acid (C18: 1ω9C) (0.420 - 1.843%), palmitoleic acid (C16: 1) (0.008 - 0.516%), and heptadecenoic acid (C17: 1) (0.009 - 0.048%). Most PUFAs were linoleic acid (C18: 2ω6) (0.269 - 0.583%) and linolenic acid (C18: 3ω3; ω6) (0.025 - 0.455%). Microfungi with the highest SAFA came

from *L. theobromae* (1.41%) and *T. hamatum* (1.40%), MUFA in *T. lentiforme* (1.97%), and *L. theobromae* (1.87%) while PUFA in *L. theobromae* (1.97%) and *T hamatum* (1.79%). Essential fatty acids (EFA) including omega 3, omega 6, omega 9, oleic acid, linoleic acid, and linolenic acid ranged between 1.51% - 6.93 %, with the highest ones were *L. theobromae* (6.93%), *T. hamatum* (5.93%), and *T. lentiforme* (5.34%). The composition of SAFA, MUFA, PUFA from each microfungus was shown in **Figure 3**, while the proportion of EFA in **Figure 4**. Overall the total fatty acids were not significantly different among microfungi (p >0.05).

3.7. Content of beta-glucan (glucan-S1) in microfungi

During the five day cultivation period, the beta-glucan (glucan-S1) content of seven microfungi ranged between 0.170 - 0.280 gram/dry weight (**Table 7**). The highest amount was found 0.280 gram/dry weight from *M. circinelloides,* followed by *T. lentiforme* (0.277 gram/dry weight), *T. hamatum* (0.270 gram/dry weight), *L. theobromae* (0.260 gram/dry weight), *T. harzianum* (0.207 gram/dry weight), *M. theicola* (0.173 gram/dry weight), and *F. oxysporum* (0.170 gram/dry weight).

3.8. Compliant nutritional factor of fungal isolated for fish and shrimp

Based on the percentage of EAA in total protein for fish and shrimp requirement, the seven microfungi have EAAs ranging between 15.16 - 23.16%. Four microfungi with the highest EAAs were *T. lentiforme* (23.16%), *F. oxysporum* (23%), *L. theobromae* (20.77%), and *M. theicola* (20.37%), consecutively. Three important EAAs for fish and shrimp are methionine ranging between 0.46 - 0.79%, arginine 1.59 - 2.42%, and lysine 2.30 - 4.17%. The highest content of these amino acids existed in *T. lentiforme* (7.08%), *F. oxysporum* (6.62%), *L. theobromae* (6.28%), *M. theicola* (5.71%), *M. circinelloides* (5.53%), *T. harzianum* (5.35%), and *T. hamatum* (4.36%). The highest methionine was in *L. theobromae* (0.79%), arginine in *M. theicola* (2.42%), and lysine in *T. lentiforme* (4.17%) (**Table 8**).

The seven microfungi contained linoleic acid between 0.292 - 1.606%, linolenic acid 0.025 - 0.455%, while Arachidonic acid (ARA), Eicosapentaenoic Acid (EPA), and Docosahexaenoic Acid (DHA) were not detected. The highest linoleic acid and linolenic acid were obtained from *L. theobromae* (1.961%), *T. hamatum* (1.785%), *T. lentiforme* (0.826%), *F. oxysporum* (0.670%), *M. circinelloides* (0.525%), *M. theicola* (0.511%), and

T. harzianum (0.334%), respectively. Based on the EFA requirement, the linoleic acid and linolenic acid in *L. theobromae* (1.606% and 0.356%) and *T. hamatum* (1.330% and 0.455%) fulfilled for fish and shrimp requirement.

All microfungi have a similar content of beta-glucan (glucan-S1) (0.170 - 0.280 gram/dry weight) from several mushrooms used in aquaculture, including *P. ostreatus* (0.24-038 g/100 gram), *L. edodes* (0.22 g/100 gram) (Manzi and Pizzoferrato, 2000). By combining the total crude protein, crude lipid, ash content, EAA, EFA, and beta-glucan (glucan-S1), the highest suitability for fish and shrimp was obtained from *M. circinelloides* (66.76%), *F. oxysporum* (66.46%), *T. hamatum* (61.44%) (**Table 9**).

4. DISCUSSION

The freshwater habitats, especially in rivers or streams and lakes, accommodate more than 600 aquatic fungi species known in the from temperate rather than in tropical regions. These include 300 Ascomycetes, 300 mitosporic fungi, and some Chytridiomycetes (Chytrids) and Oomycetes (Goh & Hyde, 1996) (Shearer et al., 2007). Three main groups are found in this habitat, i.e. (1) Ingoldian fungi which occur on decaying leaves in streams and lakes; (2) Aquatic Ascomycetes and Hyphomycetes growing on submerged woody material; and (3) Chytrids and Oomycetes, including those that cause diseases (Wong et al., 1998). Ingoldian fungi or aquatic Hyphomycetes are relatively well studied, although inventories in numerous tropical countries are lacking. Most of the freshwater fungi found in lentic habitats (lakes, ponds, swamps, pools) colonize on both wood and leaves of various plant species and are widely distributed. Besides, many freshwater fungi have been reportedly encountered from artificial habitats, such as water-cooling towers. Only one-third of the freshwater Ascomycetes have been reported from rivers and streams, mostly found in temperate regions. Many of the tropical species found are novel to science (Wong et al., 1998).

The mostly microfungi are found in the Sordariomycetes class (Ascomycota). This fungus is amongst the largest classes in Ascomycota with more than 600 known genera and 3000 unknown species, can be found anywhere (ubiquitous) and cosmopolitan as pathogens or endophytes in plants and animals (arthropod and mammalian) (Zhang et al., 2012). Many of these fungi are amphibious, suitable for both land and water (rivers), such as *Trichoderma* spp and *Mucor* spp. This fungus grows on leaf litter, fruit, twigs, wood, and

other organic materials that fall into the river's water. This fungus is commonly found because it is also influenced by collected samples (foam, leaf litter). Foam (bubble) is formed from currents and decomposed organic materials in rivers (leaves, fruit, twigs, wood). Water from the land will also enter the river during the rainy season and bring various soil materials, leaves, twigs, wood, and other organic materials. Fungal spores and other microorganisms will drift into the river and are trapped in foam; therefore, when this sample is taken, fungal spores from the land are also collected.

The difference in the number of species discovered from each location does not indicate species' diversity at that location. The low number of species was probably encountered due to the limited sample collection, the influence of place and season, and the sampling technique. Almost all microfungi found are species that highly proliferate in vitro in an agar medium. Cosmopolitan fungi, such as *Trichoderma* spp, generally grow quickly because of their ability to produce extracellular enzymes to degrade substrates. The aquatic Hyphomycetes group is typically abundant in rivers but is quite challenging to isolate and cultivate in the laboratory. Differences influence this in environmental factors from their natural habitat, especially temperature and the media's composition. Species from the temperate area can be cultivated at a temperature of 15^{O}C (Suberkropp, 1991), while those from the tropics may range from 18 - 24^{O}C according to the river water temperature. In vitro, this fungus can be cultured using 2% malt agar (Gessner & Chauvet, 1997).

Macrophoma theicola attacks through plant roots then spread to branches and shoots, making all plants die (Jeyaraman & Robert, 2018) (Nepolean P, Premkumar, & Radhakrishnan B, 2015). Spores are easily spread when exposed to rain and can survive for several weeks in dead plants. Generally, fungi that grow well on cellulose and lignin-rich substrates can survive for a long time. Based on dry weight, rice straw has high cellulose, hemicellulose, and lignin content (32.15%, 28%, and 19.64%) (Shawky, Mahmoud, Ghazy, Asker, & Ibrahim, 2011). *Trichoderma* spp, *Macrophoma* spp, and *Mucor* spp are reportedly rich in cellulase enzymes; therefore, it is easy to grow in rice straw medium. Ecological research and bioactive components of *M. theicola* are minimal, so more studies are needed, especially enzymatic activity.

Trichoderma spp. are ubiquitous colonizers of cellulosic materials and can thus be found wherever decaying plant material is available and in the plants (Kubicek, Komon-Zelazowska, & Druzhinina, 2008), they can induce systemic resistance against pathogens (Schuster & Schmoll, 2010). *Trichoderma harzianum* has high enzymatic activity, especially cellulases (1.63 FPU/mL), xylanase (23.52 IU/mL), FPase (1.63 FPU/mL), and β-glucosidase (3.55 BGU/mL) (Souza, Silva, & Bon, 2018). Cellulases and xylanase enzymes play an essential role in the degradation of plant cell walls rich in cellulose, such as rice straw. Therefore, this fungus has fast growth and optimum growth on the third day compared to others.

All isolates can be cultivated on liquid media (PDB) with varying wet weights but relatively similar dry weights. The difference between dry and wet weight is probably due to each isolate's the hypha and mycelium network each isolate to absorb water, nutrients, and food from the environment. The hypha cell wall's major constituents are chitin, beta-glucans, and proteins (Michalenko, Hohl, & Rast, 1976) (Ruiz-Herrera, 1991). The outer surface is rich in beta-glucans that serve as mucilage, and the inner layer consists of chitin microfibrils covalently cross-linked with other polysaccharides as glucans (Michalenko et al., 1976) (Ruiz-Herrera, 1991). Chitin microfibrils provide the hypha of its mechanical rigidity and strength (Islam, Tudryn, Bucinell, Schadler, & Picu, 2017). These components joined together to combat external turgor pressure with enough plasticity to deposition new material. In liquid media, the mycelium network of *Trichoderma* and *Macrophoma* is more spread; therefore, it quickly absorbs water and nutrients from the environment. Simultaneously, *Mucor circinelloides, Lasiodiplodia theobromae,* and *Fusarium oxysporum* form droplets to gain more rigidity and strength from external pressure.

Based on wet weight, *T. harzianum* and *M. theicola* produced the most mycelium biomass with rapid growth. Both these fungi like cellulosic materials (Blauth de Lima et al., 2017) (Schuster & Schmoll, 2010) and seem challenging to control its growth (Nepolean P et al., 2015). Therefore, these fungi are easy to culture in vitro using either solid or liquid media. In solid media, mycelia can proliferate in potato dextrose agar (PDA), malt extract agar (MEA), Czapek's Dox + yeast extract agar (CYA), as well as cornmeal dextrose agar (CDA), but the best growth is in the PDA and CYA media (Kim, Kwon, Lee, Ko, & Kim, 2019). *M. theicola* appeared to thrive at relatively high temperatures. The optimum

temperature for the growth was 28 to 34^{O}C. Even at 38^{O}C, the fungus was able to grow about 0.3 cm linearly in two days. However, the fungus did not grow at 12^{O}C (Chen, Thseng, & Ko, 1987).

For production-scale using liquid media, *T. harzianum* and *M. theicola* can be produced due to their potency in fast growth (optimum on the third day) and are not easily contaminated by other microbes when cultivated. To harvest maximum biomass, optimization of media composition (carbon sources, sugar, minerals) and environmental factors (temperature, shaker, volume) needs the next research. Besides, the *Trichoderma* spp generally releases several mycotoxins to inhibit the growth of bacteria and other fungi, including aflatoxins, ochratoxins, alternariols, or trichothecenes (Braun et al., 2018), which may provide supplementary effects, so they need to be purified prior and next tested.

All isolates have a total crude protein ranging between 31.56 - 45.58%. This result was the highest one if compared to Oyster mushrooms *(Pleurotus ostreatus)* with a range of 22.38 - 34.73%, *P. eryngii* 22.74 - 23.21%, *P. pulmunarius* 30.48%, and *Lentinula edodes* 15.19% per dry weight (d.w) (Manzi, Gambelli, Marconi, Vivanti, & Pizzoferrato, 1999). *Mucor circinelloides* dan *Fusarium oxysporum* have high crude protein compared to other isolates at 45.58% and 41.34%, respectively. Reported that the cell wall of *Mucor* spp *(Mucor rouxii)* are rich with the source of chitosan (28%), protein and some mannose, fucose, and galactose (10%), chitin (8%), and beta-glucan (van der Klei et al., 2011). Besides, *M. circinelloides* is also a rich source of antioxidants and secondary metabolites, especially phenolic compounds, tannins, and flavonoids (Hameed et al., 2017).

All isolates have relatively higher crude protein than the fish and shrimp feed recommendation at 25 - 60% (Molina-Poveda, 2016) (Shiau, 1998). The highest content originated in *M. circinelloides* (45.58%), *F. oxysporum* (41.34%), and *M. theicola* (37.62%). However, protein requirements in fish and shrimp vary on each species and development stages, such as in white shrimp *(L. vannamei)* is required ranged between 15 - 45% where the size 0 - 0.5 grams require 45%; size 0.5 - 3.0 grams (40%); size 3.0 - 15 grams (38%); and 15 - 40 grams (36%) (Akiyama, Dominy, & Lawrence, 1992) (Molina-Poveda, 2016). Proteins and amino acids are critical molecules because of their roles all living organisms structure and metabolism. Protein requirements will decrease in adult

(maturity), for instance catfish in sizes 14 to 100 grams requires 35% protein, while the size 114 to 500 grams requires 25% protein from the dietary needs. This requirement is also the same in salmon, common carp, and tilapia (NRC, 1993).

All isolates have a total of essential amino acids (EAA) and non-essential amino acids (NEAA) ranging between 5.710 - 9.539 g/100 grams and 5.277 - 9.342 g/100 grams. These results have relatively similar among isolates but are higher than the fruiting body of *Pleurotus ostreatus* (3.77 and 3.95 g/100 gram) (Mendez, Sandoval Castro, Belmar Casso, & Capetillo Leal, 2005) (Wang, Sakoda, & Suzuki, 2001), *Flammulina velutipes* (1.66 and 3.96 g/100 gram), and *Agrocybe chaxingu* (2.70 and 5.31 g/100 gram) (Lee et al., 2011). The most abundant EAAs from all isolates are lysine and leucine, ranging between 0.863 - 1.488 and 0.932 - 1.451 g/100 gram, while NEAA is glutamic acid and aspartic acid ranging between 1.525 - 2.529 and 0.870 - 2.073 g/100 gram, respectively. Lysine plays an essential role in helping the body absorb calcium, iron, and zinc, promoting collagen growth and producing enzymes, antibodies, and hormones, and supporting the immune system. Leucine helps regulate blood sugar levels, promotes the development and recovery of muscle and bone tissues, and stimulates the growth hormone. Generally, mushroom fruiting bodies such as *Pleurotus ostreatus*, *Agaricus* sp., *Boletus pruinatus*, *Boletinus cavipes*, and *Lactarius* sp. also contained a significant amount of leucine (Mdachi, Nkunya, Nyigo, & Urasa, 2004). *Fusarium oxysporum* has high leucine 1.451 g/100 grams than the fruit body of *Pleurotus ostreatus* (0.53 g/100 gram), *Flammulina velutipes* (0.38 g/100 g), and *Agrocybe chaxingu* (0.61 g/100 g) (Mendez et al., 2005).

Fish and shrimp can not synthesize all amino acids and acquire somel in their diet through consuming proteins or amino acids (NRC, 2011). Amino acids are essential in many other biological molecules, such as forming parts of coenzymes, precursors for the biosynthesis of structural molecules (e.g., heme, chitin, and purine bases), metabolic intermediates (e.g., acetate and pyruvate), and neurotransmitters, hormones, biogenic amines, or numerous other vital molecules (e.g., serotonin, gamma-aminobutyric acid, melamine, nitric oxide, and histamine) vital in the response of the organism to different stimuli (NRC, 2011). Fish and shrimp demand ten essential amino acids (EAA), with the three most important ones, are methionine (Molina-Poveda, 2016), arginine, and lysine (Fox, Davis, Wilson, & Lawrence, 2006). Methionine plays a role in the synthesis of creatine

and polyamines, which is a precursor of choline. Arginine plays an essential part in the urea cycle, which is necessary for disposing of toxic ammonia produced during protein and amino acid degradation. Lysine acts as a precursor for carnitine but is derived from protein catabolism (Molina-Poveda, 2016). For this reason, all analyzed microfungi analyzed can be used as feed supplements for fish and shrimp. A comparison of EAAs between isolates with fish and shrimp feed requirements was shown in **Tables 10**.

All isolates have a total crude lipid ranging between 2.22 - 6.54%. *Trichoderma hamatum* and *Fusarium oxysporum* have the highest crude lipid among other isolates at 6.54% and 5.20%. It was reported that *T. harzianum* and *T. viride* cultured on glucose-ammonium sulfate medium were able to accumulate lipids up to 17% (w/w) and 32% (w/w), respectively. In another study, *T. harzianum* cultured on sucrose-sodium nitrate could accumulate lipids up to 25% (w/w). The composition of the culture medium plays a vital role in lipid accumulation. Glucose and sucrose are the best carbon sources for lipid accumulation in *T. harzianum* (9 - 13.7% of the dry biomass weight), mainly when the nitrogen source is ammonium phosphate (Serrano-Carreon, Hathout, Bensoussan, & Belin, 1992).

A total of saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) of all isolates ranged between 0.320 - 1.415%, 0.441 - 1.968%, and 0.334 - 1.969%, respectively. These results have relatively similar among other isolates. Based on Arce Funck, Bec, Perrière, Felten, & Danger, (2015), in Hyphomycetes aquatic, abundant fatty acids are PUFA at 39.8%, followed by SAFA (33.2%) and MUFA (27%). They have five primary fatty acids, namely palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1ω9), linoleic acid (18:2ω6), and alpha-linolenic acid (18:3ω3). It was reported that fatty acid compositions of the studied terrestrial fungi were studied dominated by only four primary fatty acids, namely palmitic acid, stearic acid, oleic acid, and linoleic acid. Yet no information was given about the occurrence of alpha-linolenic acid (18:3ω3) (Stahl & Klug, 1996). The microfungi with the highest EFA were *L. theobromae* containing linoleic acid (1.601%), oleic acid (1.50%), and palmitic acid (0.948%). Based on the percentage of fatty acids, *L. theobromae* containing oleic acid (29%) and palmitic acid (18%) were higher than *P. ostreatus* (4.8%; 11%), *F. velutipes* (5.6%; 18.6%), and *A. chaxingu* (5%; 13.7%) (Lee et al., 2011).

On the other hand, all isolates have lower a lower total lipids than fish and shrimp feed recommendation at is 6.0 - 7.5% with a 10% maximum (Akiyama, Dominy, & Lawrence, 1991). However, fish and shrimp do not have precise lipid requirements (Shiau, 1998). In white shrimp, matured gonads require more than 9% (Wouters, Lavens, Nieto, & Sorgeloos, 2001). Very high lipids can inhibit the ingestion rate, causing fish and shrimp to lack nutrition (Wouters et al., 2001). Fish and crustacean species cannot synthesize essential fatty acids (EFA); therefore it is supposed to be available from the feed, especially n-3 and n-6 PUFA, i.e., linoleic acid (18: 2n6), linolenic acid (18: 3n3), Eicosapentaenoic Acid (EPA) (20: 5n3), dan Docosahexaenoic Acid (DHA) (22:6n3) (Kanazawa, Teshima, & Tokiwa, 1979) (NRC, 2011). The recommended EFAs in the feed are linoleic acid 0.4%, linolenic acid 0.3%, and arachidonic acid ranging between 0.08 - 1.5%, EPA 0.4%, and DHA 0.4% (Akiyama, Dominy, & Lawrence, 1991). In aquaculture, these fatty acids contribute to the main function in fish and shrimp growth, predominantly increasing weight and healthier (González-Félix, Gatlin III, Lawrence, & Perez-Velazquez, 2003) (Nalage et al., 2016). Dietary deficiency of these "essential fatty acids" results in various pathologies; the animal stops growing and reproducing and eventually dies (Das, 2006). A comparison of EFAs between isolates with fish and shrimp feed requirements was shown in **Tables 11**.

All isolates have a total beta-glucan ranging between 0.170 - 0.280 gram/dry weight. This result was relatively similar to other mushrooms such as *P. ostreatus* (0.24 - 0.38 g/100 gram), *P. eryngii* (0.22 - 0.38 g/100 gram), *P. pulmunarius* (0.53 g/100 gram), and *L. edodes* (0.22 g/100 gram) (Manzi and Pizzoferrato, 2000). Beta-glucan (β 1,3 glucans) is the most abundant polysaccharide in all fungi' cell walls, about 65-90% of the total glucan (Ruiz-Herrera & Ortiz-Castellanos, 2019). Glucan-S1 still contains much debris; thus, it needs to be purified further. In *M. circinelloides*, glucan was a significant component of the mycelium and spore cell walls associated with melanin, glucosamine, mannans, and proteins (Lecointe, Cornu, Leroy, Coulon, & Sendid, 2019).

To this extent, there is no clear recommended dose is available for using beta-glucan (β 1,3 glucans) in fish or shrimp feed, but from several studies revealed that it might be between 100 µg/kg to 20 g/kg (Meena et al., 2013) (Mohan et al., 2019). Other studies mentioned to have succeeded in increasing the immune system and resistance to bacterial and viral infections through oral administration, including (1) *C. carpio* by 10 g/kg (60

days) (Gopalakannan & Arul, 2010); (2) *O. niloticus* by 1 g/kg (14 days) (Barros et al., 2014); (3) *O. mykiss* by 0.5 - 2 g/kg (42 days) (Li et al., 2017); (4) *S. surata* by 1 and 10 g/kg (21 days) (Couso, Castro, Magariños, Obach, & Lamas, 2003); (5) *C. batrachus* by 1 g/kg (21 days) (Kumari & Sahoo, 2006); (6) *M. rosenbergii* by 1500 mg/kg (7 days) (Sahoo et al., 2008); (7) *P. monodon* by 2 g/kg (11 - 20 days) (Chang, Su, Chen, & Liao, 2003); (8) *F. indicus* by 0.05 - 0.4 g/100 g (21 days) (Sajeevan, Philip, & Bright Singh, 2009); dan (9) *L. vannamei* by 1 or 2 g/kg (35 days) (Bai, Gu, Zhang, Xu, & Mai, 2014) and 1 mg/kg (70 days) (Sabry Neto, Nunes, Sabry Neto, & Nunes, 2015). The use of beta-glucan from some mushrooms and yeast positively affects the growth and resistance to pathogens (bacteria and viruses) to aquaculture organisms.

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Tables:

TABLE 1. 17 species of microfungi identified from several tropical rivers or streams using a molecular approach.

No.	Species	Phylum, Class	Rivers/streams	Identity	GenBank Accession
1.	*Trichoderma lixii*	Ascomycota, Sordariomycetes	Ketahun	98.40%	KU529817
2.	*Trichoderma hamatum*	Ascomycota, Sordariomycetes	Ketahun	99.80%	KT588275
3.	*Trichoderma lentiforme*	Ascomycota, Sordariomycetes	Andai	98.20%	AF443913.1
4.	*Trichoderma harzianum*	Ascomycota, Sordariomycetes	Ketahun, Wariori	100.00%	LN846707
5.	*Fusarium oxysporum*	Ascomycota, Sordariomycetes	Sumedang	99.40%	MG661726
6.	*Colletotrichum karstii*	Ascomycota, Sordariomycetes	Tahura	100.00%	KX578788
7.	*Bionectria cf. ochroleuca*	Ascomycota, Sordariomycetes	Tahura	99.10%	EU552110
8.	*Clonostachys rosea*	Ascomycota, Sordariomycetes	Wariori	99.50%	KM460936
9.	*Eutypella scoparia*	Ascomycota, Sordariomycetes	Sumedang	99.70%	KP184334
10.	*Neopestalotiopsis* sp.	Ascomycota, Sordariomycetes	Sumedang	100.00%	MG599269
11.	*Glionectria/Gliocladiopsis* sp.	Ascomycota, Sordariomycetes	Ketahun	99.80%	KY413751
12.	*Daldinia eschscholtzii*	Ascomycota, Sordariomycetes	Ketahun	99.80%	KY792621
13.	*Macrophoma theicola*	Ascomycota, Dothideomycetes	Ketahun	99.60%	KP179222
14.	*Lasiodiplodia theobromae*	Ascomycota, Dothideomycetes	Ketahun Pangkalan Bun	100%	MG388096.1
15.	*Phellinus noxius*	Basidiomycota, Agaricomycetes	Sumedang	99.30%	KU194338
16.	*Nigrospora* sp.	Ascomycete, Hyphomycetes	Tahura	100.00%	JQ026216
17.	*Mucor circinelloides*	Mucoromycota, Mucorales	Tahura	99.70%	AM745433

TABLE 2. The mean radial growth during the five-day cultivation period (second to fifth days) of all isolates in rice straw and PDA media.

No.	Rice straw medium	N	Mean (mm)	PDA medium	N	Mean (mm)
1.	*T. harzianum*	4	38.66±5.27 [a]	*T. harzianum*	4	39.03±0.84 [a]
2.	*M. theicola*	4	37.39±3.52 [a]	*M. theicola*	4	38.66±1.33 [a]
3.	*M. circinelloides*	4	33.53±6.42 [a, b]	*T. lentiforme*	4	38.44±1.21 [a]
4.	*T. lentiforme*	4	27.82±6.91 [a]	*T. hamatum*	4	35.49±6.15 [a]
5.	*F. oxysporum*	4	26.06±5.87 [a]	*L. theobromae*	4	34.91±4.85 [a]
6.	*L. theobromae*	4	21.98±4.43 [b]	*Neopestalotiopsis sp.*	4	34.28±5.23 [a]
7.	*C. karstii*	4	21.88±12.04 [b]	*F. oxysporum*	4	25.33±6.59 [b]
8.	*D. eschscholtzii*	4	18.66±8.05 [b]	*C. karstii*	4	21.04±7.62 [c]
9.	*Neopestalotiopsis sp.*	4	16.85±6.18 [c]	*L. theobromae*	4	18.43±8.68 [c]
10.	*T. hamatum*	4	13.04±5.82 [c]	*D. eschscholtzii*	4	16.57±4.42 [c]
11.	*B. cf. ochroleuca*	4	11.37±5.23 [d]	*P. noxius*	4	16.74±4.84 [c]
12.	*P. noxius*	4	10.96±4.75 [d]	*T. lixii*	4	13.36±5.00 [c]
13.	*C. rosea*	4	9.68±4.20 [e]	*Glionectria sp.*	4	12.47±3.12 [d]
14.	*Glionectria sp.*	4	9.07±9.04 [e]	*B. cf. ochroleuca*	4	12.25±6.36 [d]
15.	*T. lixii*	4	2.92±2.01 [f]	*C. rosea*	4	8.96±4.06 [d]
16.	*E. scoparia*	4	0.92±0.94 [g]	*E. scoparia*	4	2.90±1.44 [e]
17.	*Nigrospora sp.*	4	0.00±0.00 [g]	*Nigrospora sp.*	4	0.00±0.00 [f]

PDA, potato dextrose agar
superscripts were significant at $p < 0.05$

TABLE 3. The amount of mycelium biomass during the five-day cultivation period in one liter of PDB medium.

No.	Microfungi	Cultivation period (days)	Wet weight (gram)/liter	Dry weight (gram)/liter	Water content (%)
1.	*T. harzianum*	5	357.67±112.9	7.33±1.2	97.86±0.3
2.	*M. theicola*	5	220.50±87.0	6.50±2.1	96.68±1.1
3.	*T. lentiforme*	5	42.10±1.2	5.90±0.6	86±1.2
4.	*T. hamatum*	5	41.87±9.1	4.07±1.0	90.37±0.4
5.	*M. circinelloides*	5	29.70±3.7	4.20±1.3	86.21±2.8
6.	*L. theobromae*	5	42.57±1.8	4.67±0.6	89.08±1.0
7.	*F. oxysporum*	5	33±4.1	4.13±0.4	87.44±0.5

PDB, potato dextrose broth

TABLE 4. Proximate composition of seven microfungi for a five-day cultivattion.

Proximate	Crude protein (%)	Crude lipid (%)	Carbohydrate (%)	Total energy (kcal/100g)	Moisture (%)	Energy from fat (kcal/100g)	Ash (%)
T. harzianum	35.94	4.33	40.25	343.73	13.44	38.97	6.04
M. theicola	37.62	4.12	40.96	351.40	12.02	37.08	5.28
T. lentiforme	34.82	2.22	46.42	344.94	11.88	19.98	4.66
T. hamatum	37.54	6.54	40.87	372.50	9.60	58.86	5.45
M. circinelloides	45.58	4.32	32.92	352.88	11.78	38.88	5.40
L. theobromae	31.56	4.14	43.38	337.02	12.44	37.26	8.48
F. oxysporum	41.34	5.20	35.95	355.96	11.73	46.80	5.78

TABLE 5. The amino acid composition of seven microfungi after a five-day cultivation period.

Amino Acids (g/100 gram)	***T. harzianum***	***M. theicola***	***T. lentiforme***	***T. hamatum***	***M. circinelloides***	***L. theobromae***	***F. oxysporum***
Arginine	0.797	0.910	0.799	0.598	0.830	0.668	0.996
Histidine	0.405	0.426	0.475	0.309	0.458	0.351	0.503
Isoleucine	0.561	0.768	0.779	0.558	0.764	0.632	0.935
Leucine	0.932	1.222	1.169	0.939	1.173	1.022	1.451
Lysine	0.933	1.001	1.451	0.863	1.435	1.064	1.488
Methionine	0.194	0.235	0.214	0.174	0.255	0.250	0.252
Threonine	0.732	0.959	1.036	0.664	0.900	0.717	1.302
Phenylalanine	0.571	0.819	0.713	0.539	0.737	0.633	0.913
Valine	0.682	0.964	1.027	0.744	0.993	0.835	1.211
Tryptophan	0.361	0.360	0.402	0.301	0.347	0.381	0.456
Cystine	0.023	0.036	0.029	0.020	0.034	0.019	0.032
Total EAA	**6.192**	**7.700**	**8.093**	**5.710**	**7.925**	**6.572**	**9.539**
Alanine	0.620	0.814	0.884	0.618	0.838	0.772	1.037
Aspartic acid	0.870	1.422	1.727	1.113	1.957	1.227	2.073
Glutamic acid	1.532	1.914	2.463	1.525	2.459	1.871	2.529
Glycine	0.669	0.899	1.021	0.653	0.880	0.805	1.178
Proline	0.498	0.720	0.825	0.475	0.665	0.543	0.914
Serine	0.678	0.871	0.881	0.591	0.822	0.644	1.023
Tyrosine	0.410	0.527	0.471	0.338	0.481	0.422	0.587
Total NEAA	**5.277**	**7.168**	**8.272**	**5.313**	**8.103**	**6.285**	**9.342**
Total AA	**11.468**	**14.867**	**16.365**	**11.023**	**16.027**	**12.856**	**18.881**

EAA, essential amino acids; NEAA, non-essential amino acids; AA, amino acids

TABLE 6. The fatty acids composition of seven microfungi after a five-day cultivation period.

Fatty Acids (%)	*T. harzianum*	*M. theicola*	*T. lentiforme*	*T. hamatum*	*M. circinelloides*	*L. theobromae*	*F. oxysporum*
C 4:0	n.d	n.d	0.002	0.002	0.003	n.d	0.002
C 6:0	n.d	n.d	n.d	n.d	n.d	n.d	n.d
C 8:0	n.d	n.d	0.035	n.d	0.006	n.d	n.d
C 10:0	n.d	n.d	0.023	0.010	0.018	5E-03	0.003
C 12:0	0.009	0.006	0.019	0.035	0.066	0.020	0.033
C 13:0	0.006	0.010	0.007	0.007	0.017	0.006	0.017
C 14:0	0.012	0.013	0.028	0.129	0.139	0.062	0.045
C 15:0	n.d	0.005	0.012	0.036	0.020	0.014	0.024
C 16:0	0.190	0.433	0.466	0.885	0.497	0.948	0.486
C 17:0	0.006	0.017	0.009	0.023	0.012	0.019	0.019
C 18:0	0.068	0.112	0.182	0.155	0.160	0.241	0.145
C 20:0	0.003	0.005	0.013	0.009	0.008	0.024	0.007
C 21:0	n.d	0.005	n.d	n.d	n.d	n.d	0.002
C 22:0	0.004	0.008	0.022	0.020	0.017	0.020	0.008
C 23:0	0.002	0.006	0.007	0.006	0.003	0.005	0.004
C 24:0	0.018	0.024	0.054	0.088	0.079	0.051	0.026
Total SAFA	**0.320**	**0.644**	**0.879**	**1.405**	**1.045**	**1.415**	**0.822**
C 14:1	n.d	n.d	n.d	0.006	0.009	0.008	0.005
C 15:1	n.d	n.d	n.d	n.d	n.d	0.013	n.d
C 16:1	0.008	0.010	0.078	0.516	0.412	0.274	0.109
C 17:1	0.009	0.042	0.024	0.031	0.047	0.048	0.037
C 18:1ω9C	0.420	0.833	1.843	1.180	1.121	1.500	1.559
C 20:1	0.004	0.006	0.020	0.012	0.015	0.021	0.006
C 22:1	n.d	n.d	0.003	0.003	n.d	0.005	0.003
Total MUFA	**0.441**	**0.891**	**1.968**	**1.747**	**1.604**	**1.869**	**1.718**
C 18:2ω6	0.292	0.479	0.801	1.330	0.269	1.606	0.583
C 18:3ω3	0.039	0.024	0.025	0.221	0.013	0.116	0.054
C 18:3ω6	0.003	0.008	n.d	0.234	0.243	0.239	0.032
C 20:2	n.d	0.006	0.005	0.004	n.d	0.008	0.005
Total PUFA	**0.334**	**0.517**	**0.831**	**1.790**	**0.525**	**1.969**	**0.674**
Total FA	1.094	2.052	3.677	4.941	3.173	5.253	3.214
Omega 3	0.039	0.024	0.025	0.221	0.013	0.116	0.054
Omega 6	0.295	0.487	0.801	1.564	0.511	1.845	0.616
Omega 9	0.420	0.833	1.846	1.183	1.121	1.504	1.561
Oleat	0.420	0.833	1.843	1.180	1.121	1.500	1.559
Linoleic acid	0.292	0.479	0.801	1.330	0.269	1.606	0.583
Linolenic acid	0.042	0.032	0.025	0.455	0.256	0.356	0.086

SAFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids
n.d, not detected

TABLE 7. The amount of beta-glucan (glucan-S1) content during the five-day cultivation period in 100 ml PDB medium.

Microfungi	Cultivation period (days)	Dry weight (gram)	Beta-glucan (glucan-S1) (gram/dry weight)	% Beta-glucan (glucan-S1)
T. harzianum	5	0.733±0.1	0.207±0.1	28.18±0.2
M. theicola	5	0.650±0.2	0.173±0.1	26.67±0.2
T. lentiforme	5	0.590±0.1	0.277±0.0	46.89±0.0
T. hamatum	5	0.407±0.1	0.270±0.0	66.39±0.1
M. circinelloides	5	0.420±0.1	0.280±0.0	66.67±0.2
L. theobromae	5	0.467±0.1	0.260±0.0	55.71±0.2
F. oxysporum	5	0.413±0.0	0.170±0.1	41.13±0.2

PDB, potato dextrose broth

TABLE 8. Percentage of EAAs in total protein content for each microfungus after a five-day cultivation period.

EAA	*T. harzianum*	*M. theicola*	*T. lentiforme*	*T. hamatum*	*M. circinelloides*	*L. theobromae*	*F. oxysporum*
Methionine	0.54%	0.62%	0.61%	0.46%	0.56%	0.79%	0.61%
Arginine	2.22%	2.42%	2.29%	1.59%	1.82%	2.12%	2.41%
Lysine	2.60%	2.66%	4.17%	2.30%	3.15%	3.37%	3.60%
Histidine	1.13%	1.13%	1.36%	0.82%	1.00%	1.11%	1.22%
Isoleucine	1.56%	2.04%	2.24%	1.49%	1.68%	2.01%	2.26%
Leucine	2.59%	3.25%	3.36%	2.50%	2.57%	3.24%	3.51%
Threonine	2.04%	2.55%	2.97%	1.77%	1.97%	2.27%	3.15%
Phenylalanine	1.59%	2.18%	2.05%	1.44%	1.62%	2.01%	2.21%
Valine	1.90%	2.56%	2.95%	1.98%	2.18%	2.65%	2.93%
Tryptophan	1.00%	0.96%	1.15%	0.80%	0.76%	1.21%	1.10%
Total	**17.16%**	**20.37%**	**23.16%**	**15.16%**	**17.31%**	**20.77%**	**23.00%**

TABEL 9. The proportion of the total crude protein, crude lipid, ash, EAA, EFA, and beta-glucan (glucan-S1) from seven microfungi after a five-day culture period.

Microfungi	Crude protein (%)	Crude lipid (%)	Ash (%)	EAA (%)	EFA (%)	Beta-glucan (gram/dry weight)	Total (%)
T. harzianum	35.94	4.33	6.04	6.17	1.51	0.207	54.20
M. theicola	37.62	4.12	5.28	7.66	2.69	0.173	57.54
T. lentiforme	34.82	2.22	4.66	8.06	5.34	0.277	55.38
T. hamatum	37.54	6.54	5.45	5.69	5.93	0.285	61.44
M. circinelloides	45.58	4.32	5.40	7.89	3.29	0.280	66.76
L. theobromae	31.56	4.14	8.48	6.55	6.93	0.260	57.92
F. oxysporum	41.34	5.20	5.78	9.51	4.46	0.170	66.46

TABLE 10. Comparison of essential amino acids from seven microfungi to fish and shrimp feed requirements (percentage of dietary protein).

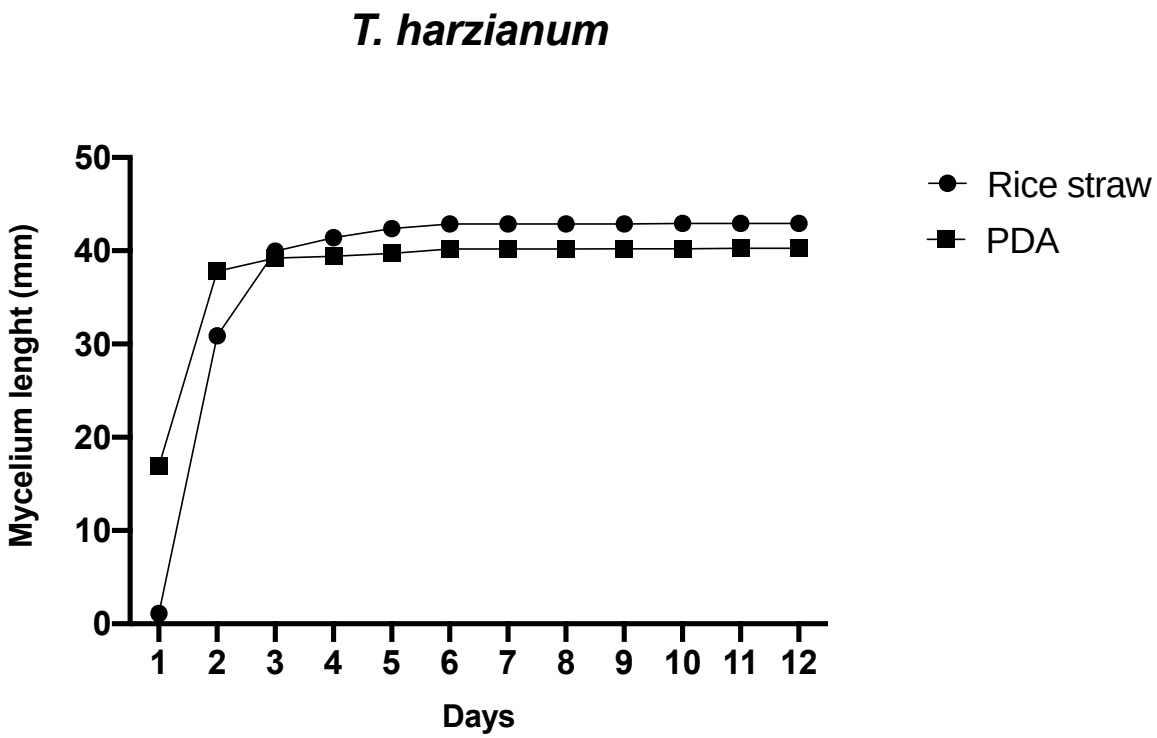
Amino acids	Microfungi (%)							Fish (%) (Molina-Poveda, 2016; NRC, 1993)								Shrimps (%) (Akiyama et al., 1992; Molina-Poveda, 2016)			
	THZ	**MTC**	**TLF**	**THM**	**MCC**	**LTB**	**FOX**	**Salmon**	**Sea Bass**	**Sea Bream**	**Rainbow trout**	**Common Carp**	**Catfish**	**Tilapia**	**Eel**	**Shrimps**	***P. monodon***	***L. vannamei***	***M. japonicus***
Methionine	0.54	0.62	0.61	0.46	0.56	0.79	0.61	3-4	1.8–2.2	1.4	0.7–1.9	3.1	2.3	1.6–2.8	3.2	2.4	2.4–2.9		1.4
Arginine	2.22	2.42	2.29	1.59	1.82	2.12	2.41	5.8-6	3.8–3.9	1.7	3.5–4.2	4.3	4.3	4–4.2	4.5	5.8	5.3–5.5	5.6	3.2–5.3
Lysine	2.60	2.66	4.17	2.30	3.15	3.37	3.60	2-4.8	4.4–4.5	1.7	3.0–8.4	5.7	5.1	4.1–5.7	5.3	5.3	5.2	3.9–4.7	3.8
Histidine	1.13	1.13	1.36	0.82	1.00	1.11	1.22	1.6-1.8			1.0–1.2	2.1	1.5	1.72	2.1	2.1	2.2		1.2
Isoleucine	1.56	2.04	2.24	1.49	1.68	2.00	2.26	2.2-2.4			1.5–2.8	2.5	2.6	3.11	4.0	3.5	2.7	3.9	2.6
Leucine	2.59	3.25	3.36	2.50	2.57	3.24	3.51	3.8-3.9			2.3–9.2	3.3	3.5	3.39–3.4	5.3	5.4	4.3	5.8	3.8
Threonine	2.04	2.55	2.97	1.77	1.97	2.27	3.15	2.2-3	2.3–2.6		2.60	3.9	2.0	3.75–4.7	4.0	3.6	3.5	3.5–3.8	2.6
Phenylalanine	1.59	2.18	2.05	1.44	1.62	2.01	2.21	5.1-6.3			2.0	6.5	5.0	3.75–3.8	5.8	4.0	3.7		3.0
Valine	1.90	2.56	2.95	1.98	2.18	2.65	2.93	1.3-3			1.7–3.4	3.6	3.0	2.8	4.0	4.0	3.4		2.8
Tryptophan	1.00	0.96	1.15	0.80	0.76	1.21	1.10	0.5-0.7		0.6	0.3–0.9	0.8	0.5	1.0	1.1	0.8	0.5		0.8

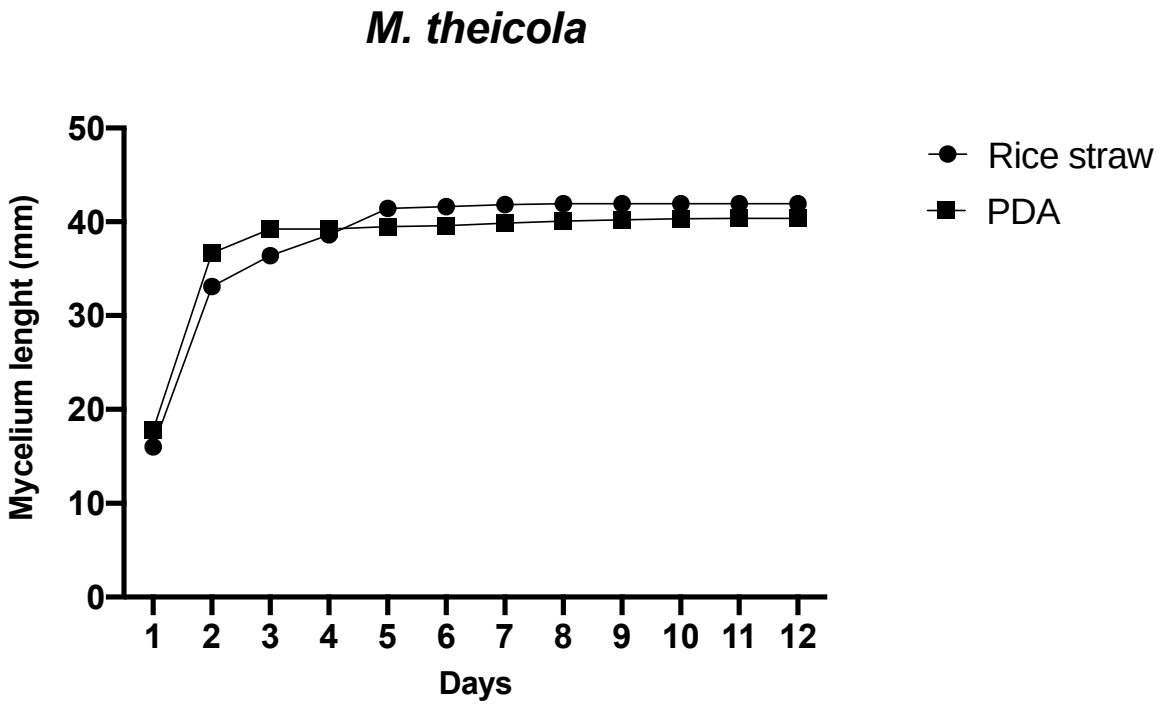
TABLE 11. Comparison of essential fatty acids from seven microfungi to fish and shrimp feed requirements.

EFA	Microfungi (%)							Fish (%) (Akiyama et al., 1992; Molina-Poveda, 2016; NRC, 1993; 2011)							Shrimps (%) (Akiyama et al., 1992; Molina-Poveda, 2016; NRC, 1993; 2011)					
	THZ	**MTC**	**TLF**	**THM**	**MCC**	**LTB**	**FOX**	**Salmon**	**Sea Bass**	**Sea Bream**	**Rainbow trout**	**Common Carp**	**Catfish**	**Tilapia**	**Eel**	***M. rosenbergii***	***P. monodon***	***L. vannamei***	***M. japonicus***	***F. aztecus***	***F. chinensis***
Linoleic acid	0.29	0.48	0.80	1.33	0.27	1.61	0.58	1–2.5				0.5–1		0.5–1	0.5		1–1.5				
Linolenic acid	0.04	0.03	0.02	0.46	0.26	0.36	0.09	1.0			0.8–1	1.0	1–2		0.5		1–1.5			0.7–1	
ARA	n.d	n.d	n.d	n.d	n.d	n.d	n.d									0.08	0.9	0.5	1.1		
EPA	n.d	n.d	n.d	n.d	n.d	n.d	n.d		0.5	0.5–1.0			0.5–0.75	1.0			0.9–1.2	0.5	1.1		
DHA	n.d	n.d	n.d	n.d	n.d	n.d	n.d		0.5	0.5			0.5–0.75			0.075	0.9–1.2	0.5	1.1		1.0

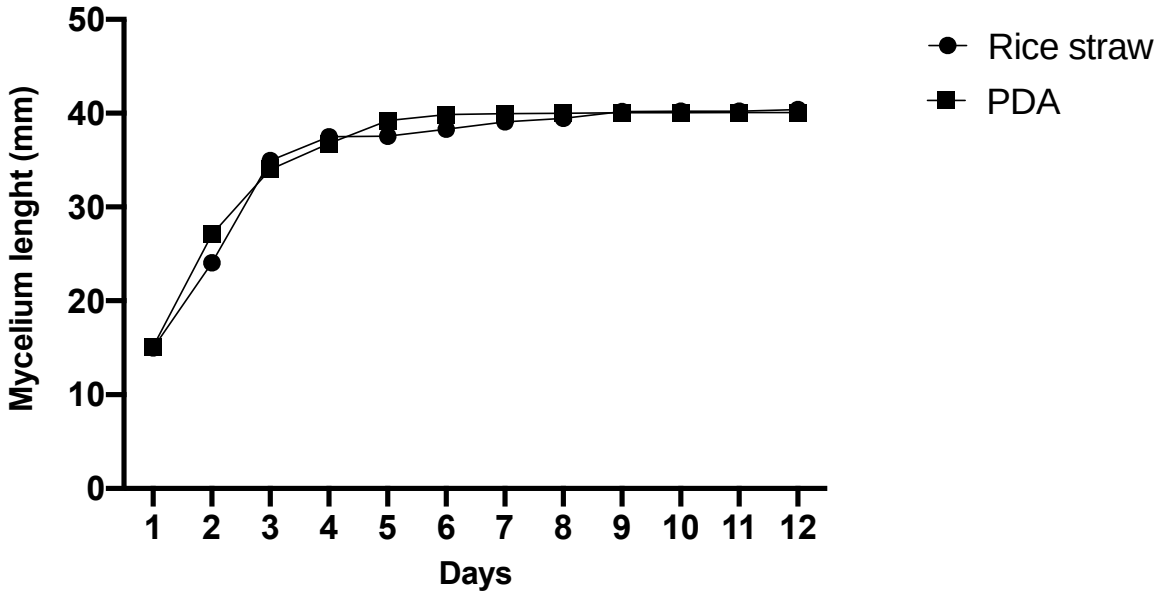
THZ, *T. harzianum*; MTC, *M. theicola*; TLF, *T. lentiforme*; THM, *T. hamatum*; MCC, *M. circinelloides*; LTB, *L. theobromae*; FOX, *F. oxysporum*
EFA, essential fatty acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid
n.d, not detected

Figures:

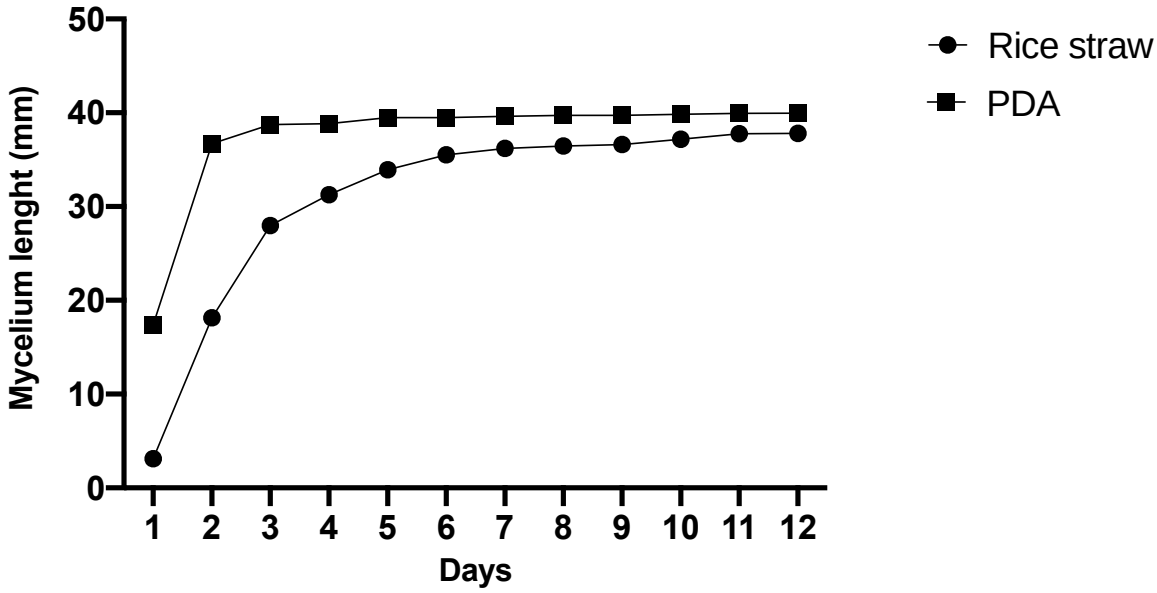

T. harzianum
Mycelium lenght (mm)
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12
Days
Rice straw
PDA

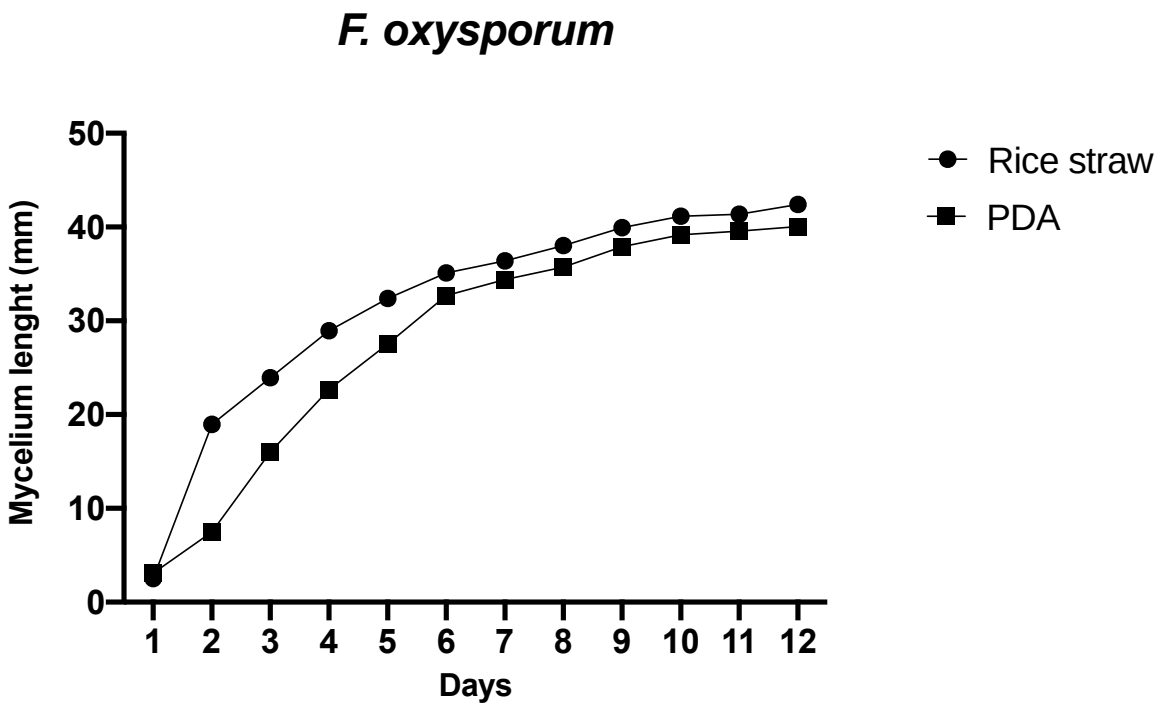

M. theicola
Mycelium lenght (mm)
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12
Days
Rice straw
PDA

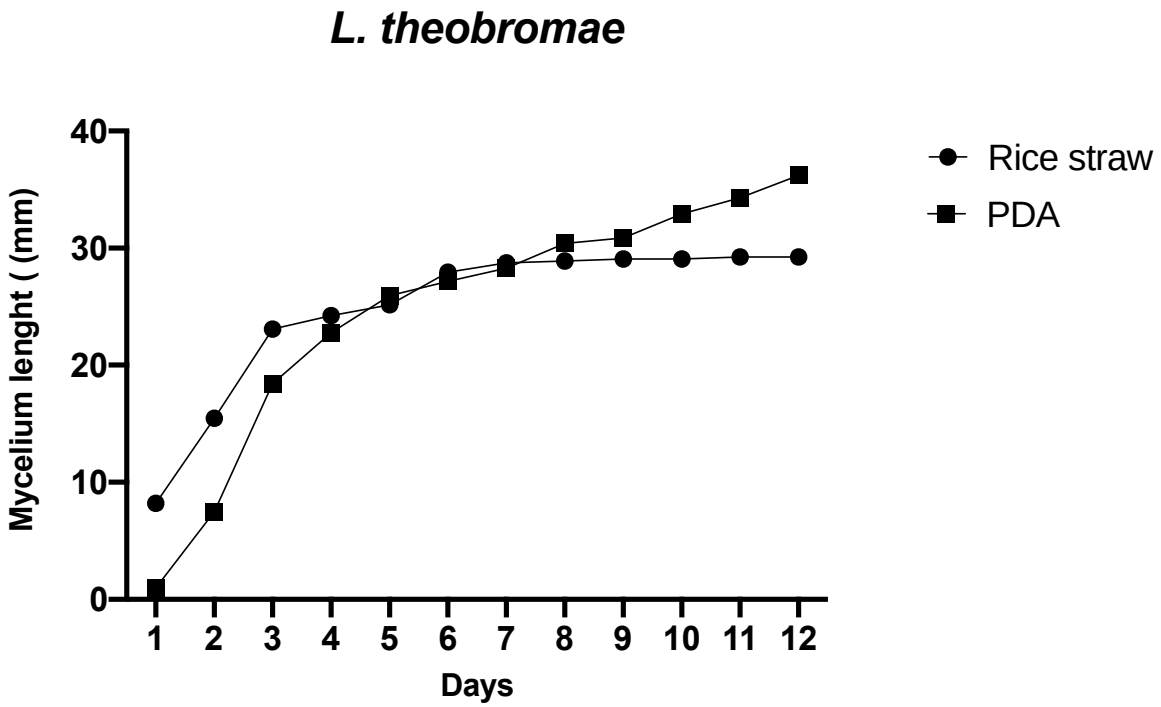
M. circinelloides



T. lentiforme




F. oxysporum
Mycelium lenght (mm)
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12
Days
Rice straw
PDA


L. theobromae
Mycelium lenght ((mm)
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12
Days
Rice straw
PDA

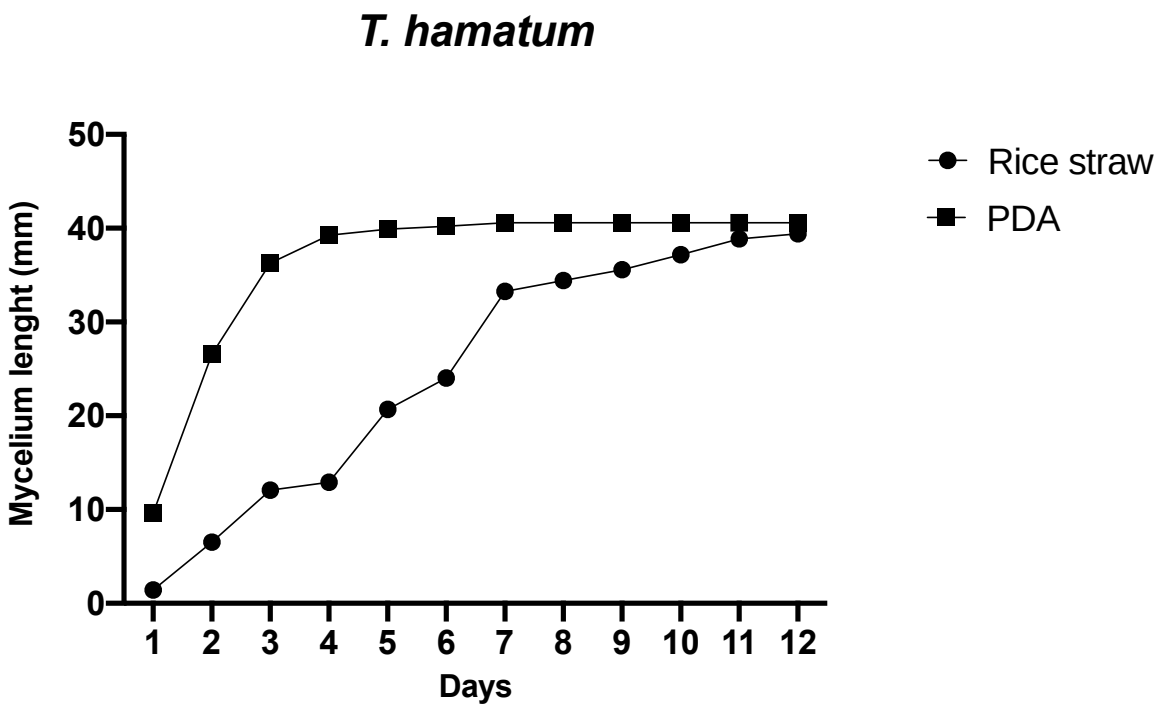
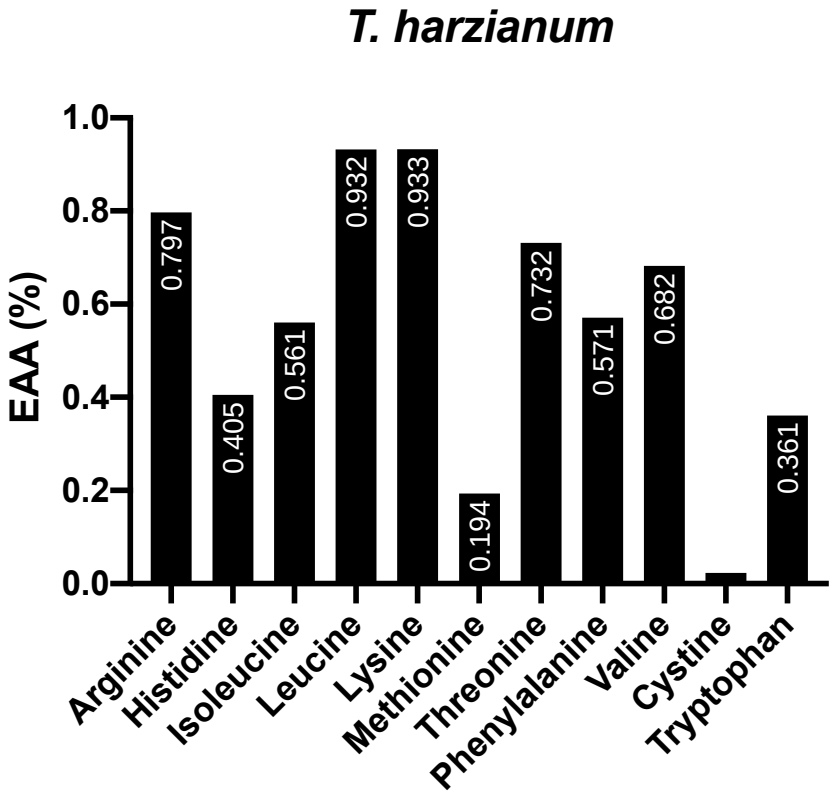
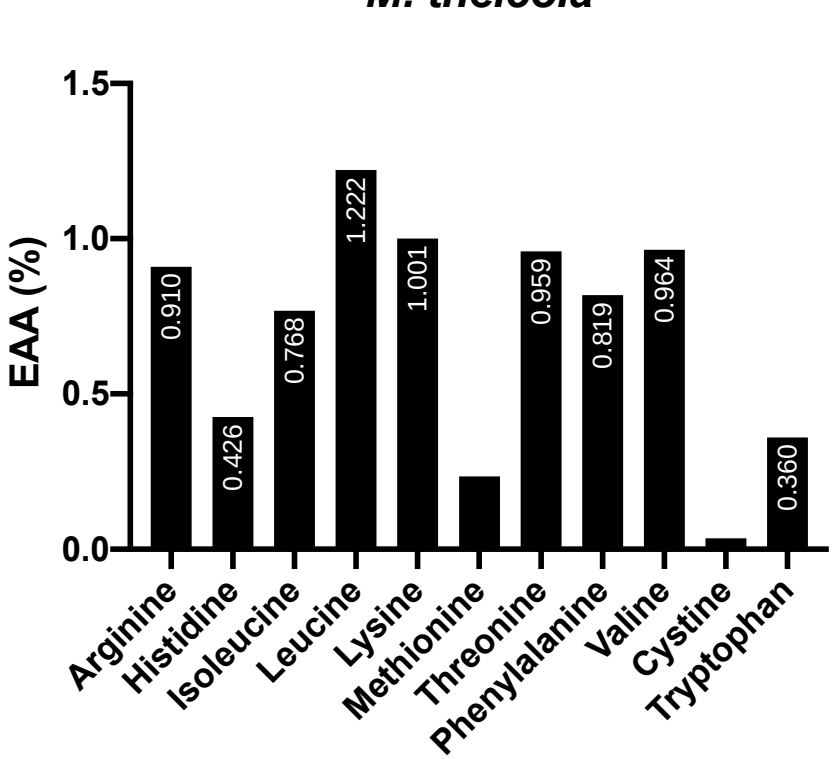
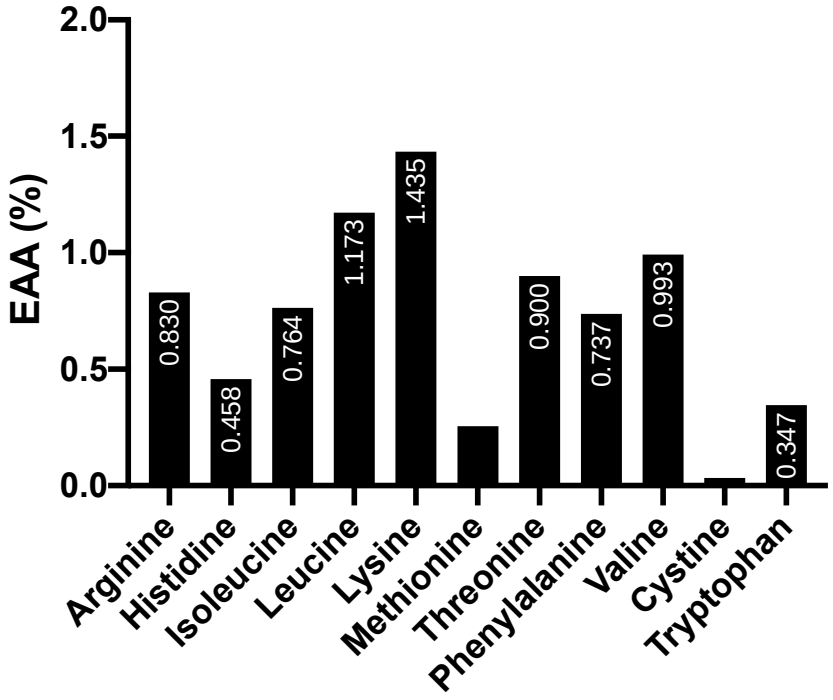


FIGURE 1. Radial growth (mycelium length) of seven microfungi grown in rice straw media and PDA during 12 days cultivation period. The optimum growth of microfungi occurred on the third to fifth days.

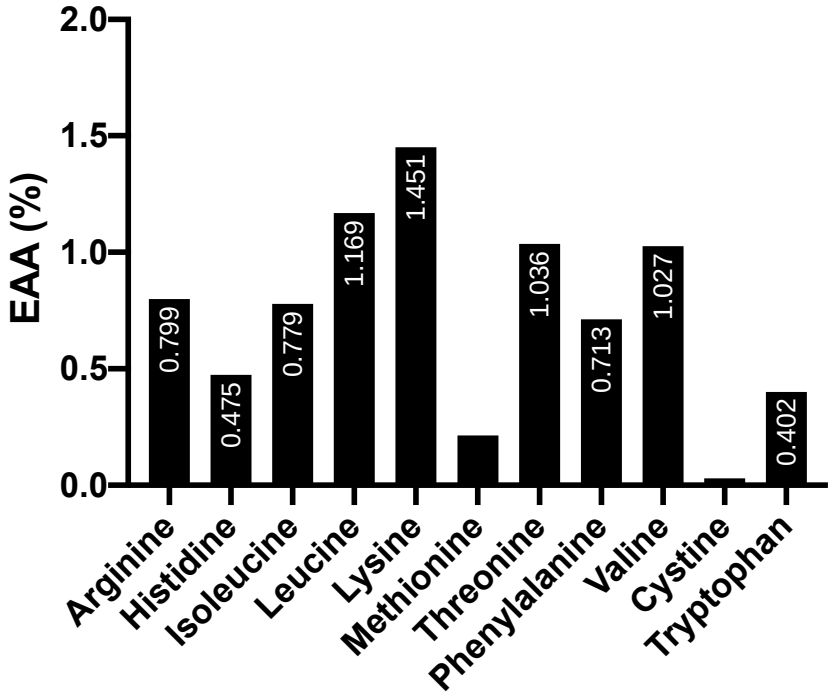

T. harzianum
EAA (%)
1.0
0.8
0.6
0.4
0.2
0.0
0.797
0.405
0.561
0.932
0.933
0.194
0.732
0.571
0.682
0.361
Arginine
Histidine
Isoleucine
Leucine
Lysine
Methionine
Threonine
Phenylalanine
Valine
Cystine
Tryptophan

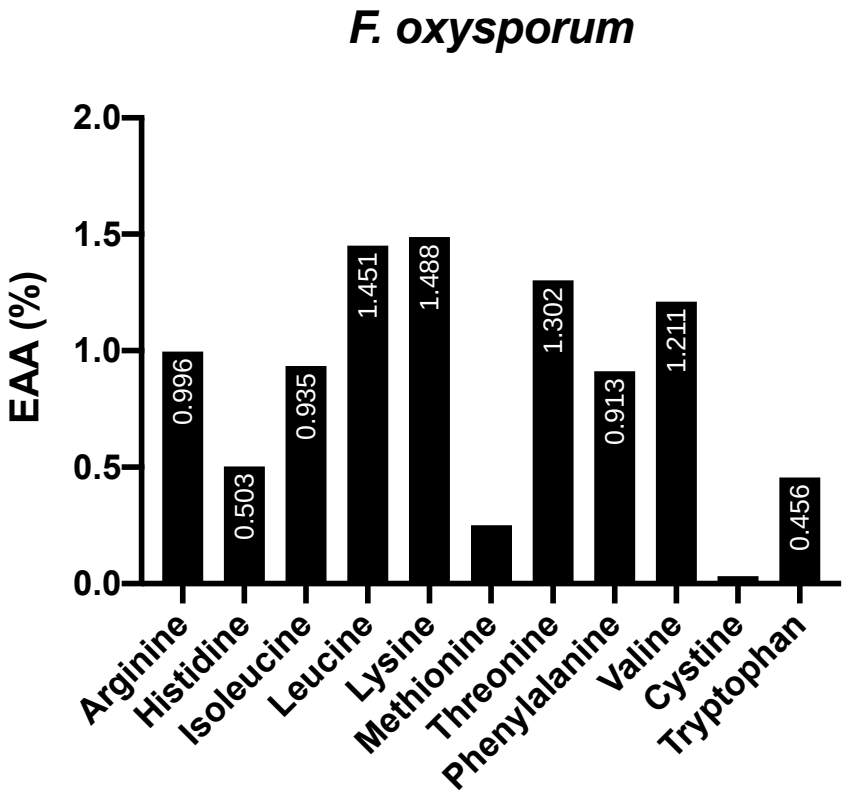

M. theicola
EAA (%)
1.5
1.0
0.5
0.0
0.910
0.426
0.768
1.222
1.001
0.959
0.819
0.964
0.360
Arginine
Histidine
Isoleucine
Leucine
Lysine
Methionine
Threonine
Phenylalanine
Valine
Cystine
Tryptophan

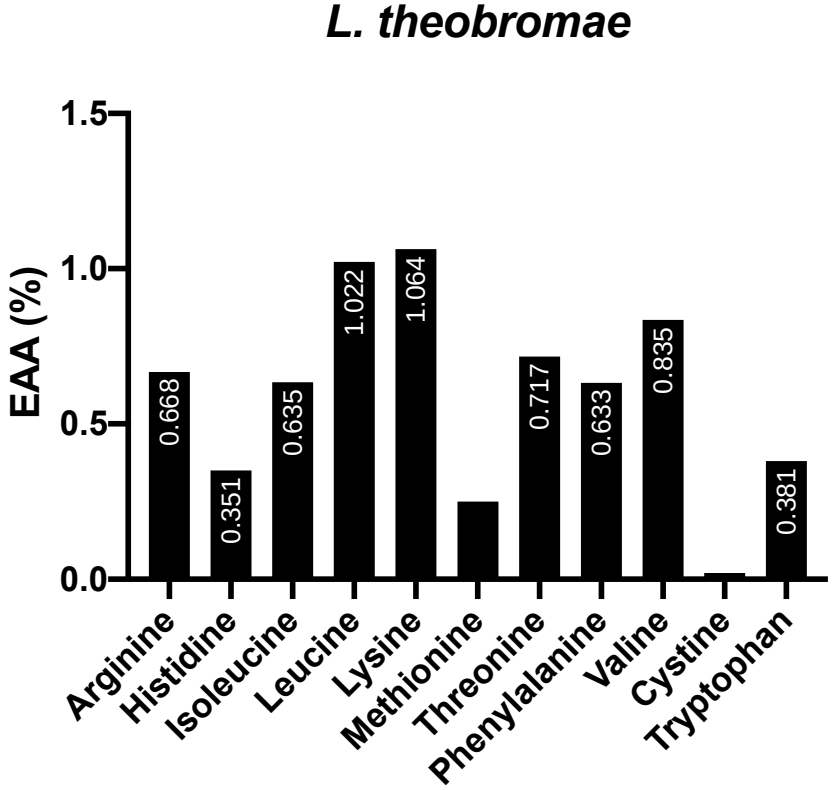
M. circinelloides



T. lentiforme




F. oxysporum
EAA (%)
2.0
1.5
1.0
0.5
0.0
0.996
0.503
0.935
1.451
1.488
1.302
0.913
1.211
0.456
Arginine
Histidine
Isoleucine
Leucine
Lysine
Methionine
Threonine
Phenylalanine
Valine
Cystine
Tryptophan


L. theobromae
EAA (%)
1.5
1.0
0.5
0.0
0.668
0.351
0.635
1.022
1.064
0.717
0.633
0.835
0.381
Arginine
Histidine
Isoleucine
Leucine
Lysine
Methionine
Threonine
Phenylalanine
Valine
Cystine
Tryptophan

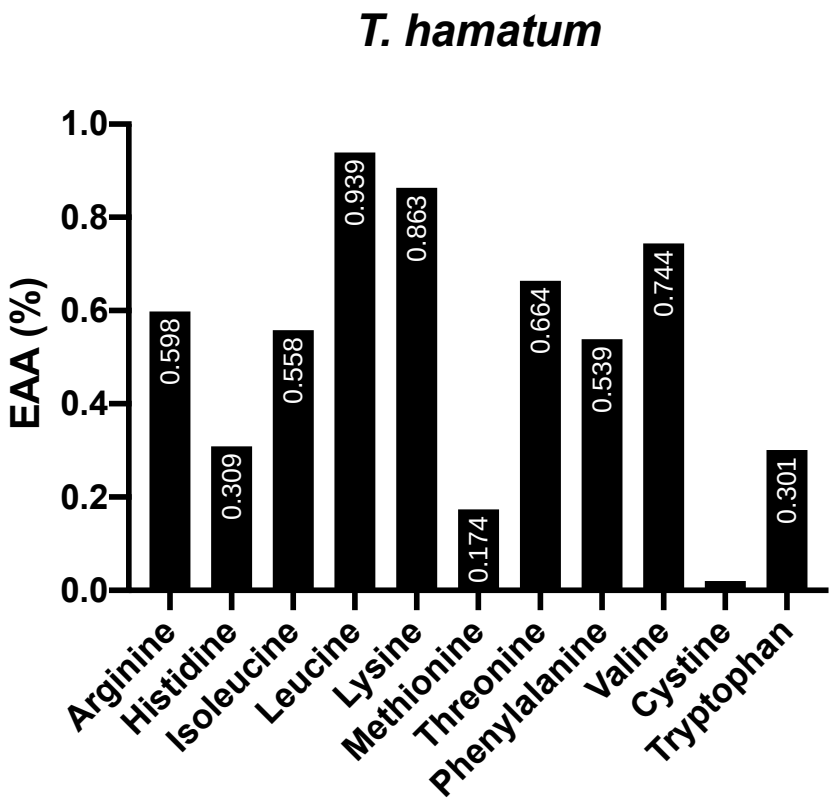
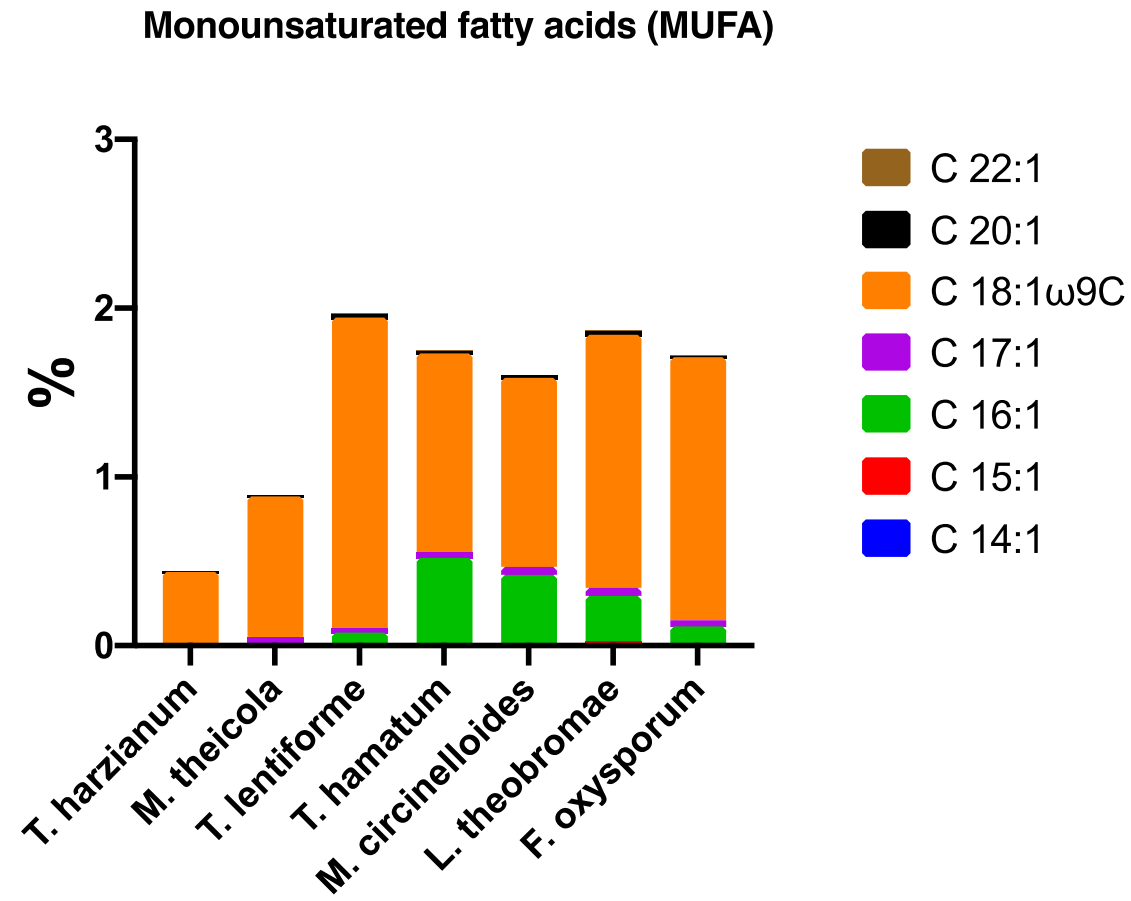
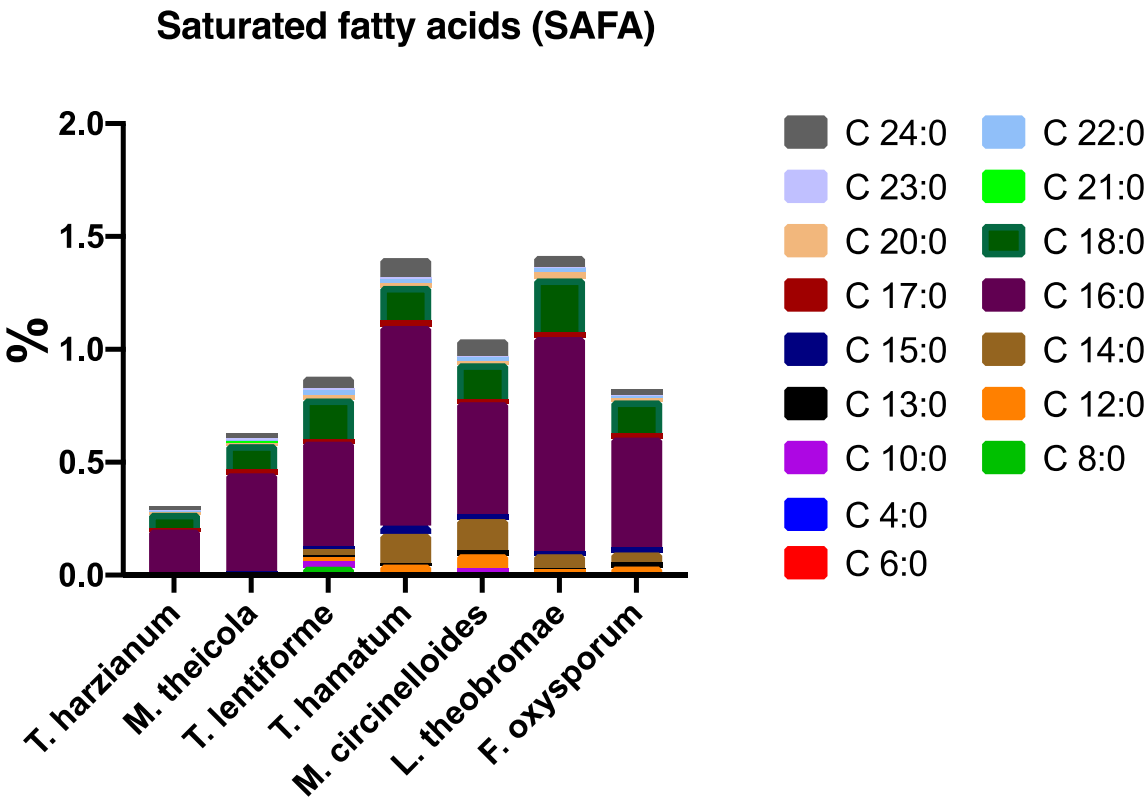


FIGURE 2. Essential Amino Acids (EAA) composition of seven microfungi after a five-day cultivation period.


Monounsaturated fatty acids (MUFA)
%
3
2
1
0
T. harzianum
M. theicola
T. lentiformе
T. hamatum
M. circinelloides
L. theobromae
F. oxysporum
C 22:1
C 20:1
C 18:1ω9C
C 17:1
C 16:1
C 15:1
C 14:1


Saturated fatty acids (SAFA)
%
2.0
1.5
1.0
0.5
0.0
T. harzianum
M. theicola
T. lentiformе
T. hamatum
M. circinelloides
L. theobromae
F. oxysporum
C 24:0
C 22:0
C 23:0
C 21:0
C 20:0
C 18:0
C 17:0
C 16:0
C 15:0
C 14:0
C 13:0
C 12:0
C 10:0
C 8:0
C 4:0
C 6:0

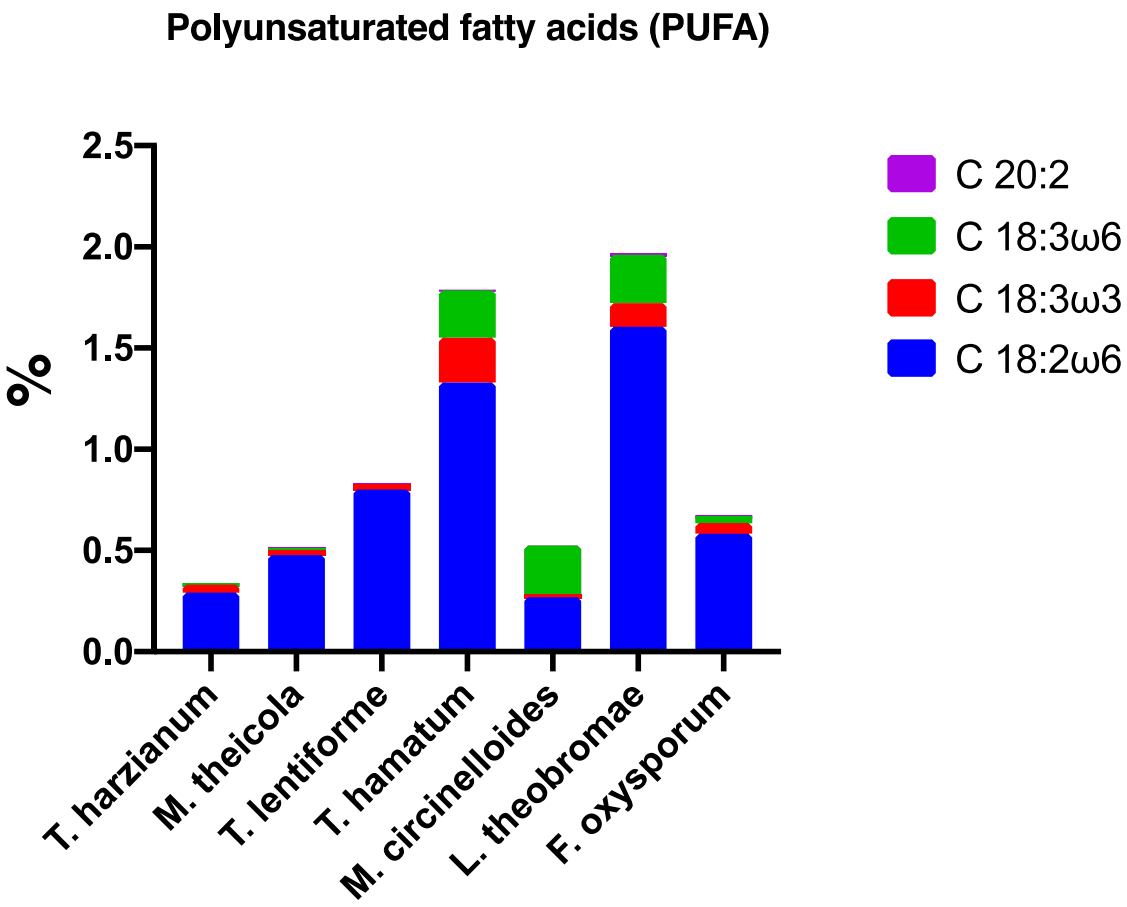


FIGURE 3. The composition of fatty acids (SAFA, MUFA, PUFA) from seven microfungi after a five-day cultivation period.

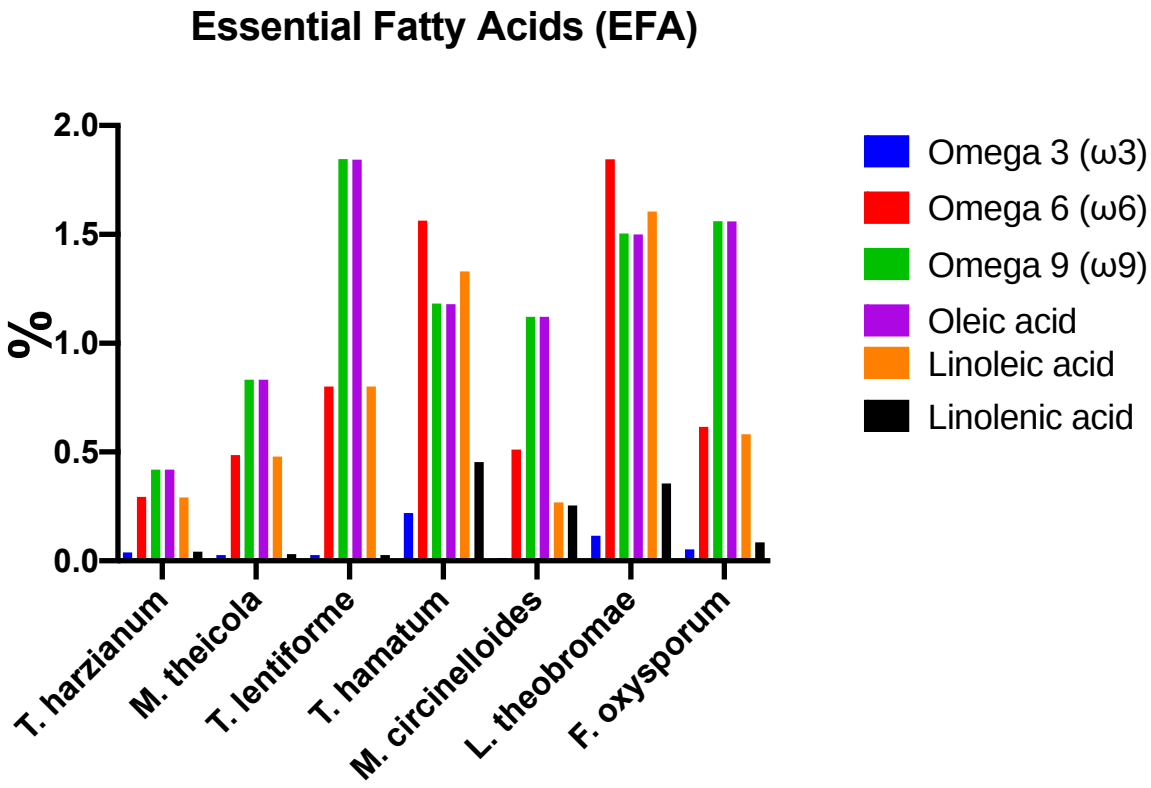


FIGURE 4. The proportion of essential fatty acids (EFA) from seven microfungi after a five-day cultivation period.