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Aspergillus terreus Mekky221, as a potential candidate fungus for biodiesel production using sugar cane bagasse and rice straw as inexpensive carbon sources

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ABSTRACT

Fungi are considered one of the most important sources relied upon in the production of biodiesel, especially if the materials to be used are agricultural waste and are considered an inexpensive source of sugars. Aspergillus terreus Mekky221 was the most abundant of the Seven oleaginous fungal isolates that were isolated from Agriculture waste specimens were taken from cultivated land, and subsequently examined for biodiesel production in the current study. By using morphological (macroscopic and microscopic) analysis and molecular confirmation using 18S rRNA sequencing, the isolate's species identity was established and recorded on Gene Bank under accession number PQ182607. In order to enhance lipid accumulation, we also improved the culture conditions, Aspergillus terreus Mekky221 showed the highest lipid production (Dray biomass 4.89 ± 0.19 g/L giving lipid content 2.52 ± 0.06 g/L Equivalently 51.57 ± 3.03 %) in the existence of 100g/L sucrose and 10 g/L yeast extract at pH, 5 after five days of incubation at 30°C under static conditions. solid state fermentation medium was made: A 5-liter flask was filled with 40g of dry bagasse and rice straw as inexpensive carbon sources, mixture evenly moistened at a 60% level using sterile distilled water and peptone (5 g/1), autoclaved after cooling, spores added with known concentration, pH 5, temp. 30 and incubated for 5 days Aspergillus terreus Mekky221 showed lipid production (dray biomass 3.99 ± 0.17 g/L giving lipid content 2 .11 \pm 0.08 g/L Equivalently 52.8 \pm 0.13 %). Finally, the transesterification process is dependent on the one-step conversion of the fungal dry biomass's lipid to FAME and by using a gas chromatographic analysis of fatty acids. Aspergillus terreus Mekky221 is therefore thought to be a promising oleaginous filamentous fungus that can be used to biodiesel production from agricultural waste.

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Introduction

Diesel in the form of biodiesels is produced by extracting oil from renewable feedstock, including vegetable and animal oils as well as oils produced by microorganisms (fungi, bacteria, cyanobacteria, yeasts, and microalgae).

Since biodiesel emits few carbon emissions and contains no toxins, it may be the most practical green fuel



option for alternative energy sources (Rathore et al. 2022). Globally, biodiesel is becoming more and more popular because of its affordability, environmental friendliness, and biodegradability; nevertheless, it is low in sulfur and aromatic hydrocarbons (Neupane, 2022, Moharam et al. 2023, Elhalik et al. 2024).

Because of their large-scale rapid growth, short life cycle, independence from light energy, ability to produce large amounts of lipids using a variety of carbon sources, and abundance of fatty acids (both saturated and unsaturated) for the production of high-quality biodiesel, oleaginous filamentous fungi are incredibly ideal sources for the sustainable biodiesel industry (Al-Zaban & Abd El-Aziz, 2023 and Mohammed et al. 2024). Finding fungi species that can produce a significant quantity of lipids will be extremely helpful for improving process economics. Several oleaginous filamentous fungi, including *A. niger* and *Aspergillus terreus*, *Fusarium oxysporum*, and *Mucor circinelloides*, can be used as very inexpensive biological templates for the production of biodiesel (Al-Zaban & Abd El-Aziz 2023 and Mekky et al. 2024).

Lipid synthesis is complicated and cannot be mediated by a single gene or mechanism. Based on conserved areas of fatty acid pathways in oleaginous fungi, sequence information illuminates the molecular mechanisms controlling fatty acid synthesis (Zhang et al. 2022). The most popular reaction to produce biodiesel from fungal oil is called the transesterification process of triglycerides (TAG) with alcohol (methanol) in the presence of a catalyst mixed with oil to convert them into fatty acid esters and glycerol (Salaheldeen et al. 2021).

Because of its advantages for the environment, including biodegradability, lower sulfur and aromatic hydrocarbon content, which lowers emissions during fuel combustion, and lower emissions of CO, CO2, and particulate matter, biodiesel fuels are garnering more and more attention globally (Chauhan & Shukla 2011). Furthermore, it has a direct impact on human health because, in comparison to fossil diesel, it reduces cancer risk by 95% (Huang et al. 2010). Microorganisms exhibit tremendous biological variety, and some metabolic patterns may facilitate the buildup of lipids. (Linton et al. 2019 and Mekky et al. 2021). Under the right cultivation conditions, the oil content of certain bacteria can reach up to 70% of the total cellular dry weight (Ughy et al. 2023). The majority of the lipids found in microorganisms like bacteria, fungus, and algae are triacylglycerols (TAG), which are metabolites that are employed for energy storage. The majority of these lipids resemble traditional vegetable oils since they are made up of long chain fatty acids (Chandra et al. 2020). The production of microbial oil has several advantages over vegetable and animal fats,

Materials and Methods

Chemicals & Solvents

The majority of the chemicals and solvents used were bought from Sigma-Aldrich, Germany.

Isolation and purification Aspergillus terreus spp.

Agriculture waste samples were taken from cultivated land at Ezpet Mekky Belbies center Al-Sharqia Government, Egypt. Five grams of each sample were washed many times sterilized distilled water, then plated onto Oxoid (MEA) Malt Extract Agar. Plates were incubated under controlled conditions for three days at 30 °C. Single fungal colonies were isolated then transferred to new MEA plates repeatedly until obtain pure cultures (Fanele & Ndlovu 2023).

Lipid accumulation and cultivation conditions

The composition of the most potent production medium (g/l): Glucose 100, yeast extract 10, adjusted to pH 5.4. A 10% (by volume) mycelial suspension of the isolated pure cultures of *Aspergillus terreus* inoculated into a 500 ml flask containing 250 ml of broth and incubated at 30° C for 7 days (Ahmed et al. 2006).

Dry weight determination

After harvesting the cells by filtration and then washed 3 times with deionized water to remove the media remains then drying them at 55-60 °C for an overnight period or until their weight remained constant, the amount of biomass produced was calculated (Vicente et al. 2009).

Lipid extraction

To extract the lipid, 40 mL of a 2:1 chloroform-methanol combination was added to 1 g of dry biomass that had been ground and filtered through Whatman paper no. 1. Lipids were identified when the solvent containing them was separated and subsequently evaporated (Mishra et al. 2014).

Lipid quantification

The extracted lipid was quantified using sulfophospho vanillin method (SPV) (Suleiman et al. 2018). Vanillin (6%) and phosphoric acid (85%) were combined to create phosphovanillin reagent. The specimen was created by diluting 20 μ L of samples with 180 μ L of sulfuric acid, incubating at 100 °C for 10 minutes, and then allowing it to cool at room temperature. Next, phosphovanillin reagent was added and the sample was left for a while to develop color before being measured at 530 nm (Hashem et al. 2022).

Biodiesel production

The transestrification process is dependent on the onestep conversion of the fungal dry biomass's lipid to FAME using the technique of (Vicente et al. 2009). In this work, fungal lipids were extracted, and magnetic stirring was used to conduct reactions in glass-closed jars. The FAME layer was accumulated while the reaction was heated in a thermostatic bath. The top organic layers of the crude glycerol were mixed with the first layer of biodiesel after it had been rinsed five times with n-hexane and water. The reaction yield was determined by measuring the residual that included biodiesel (FAMEs).

The following formulas were used to determine the result (biodiesel) as a percentage of initial lipid and dry biomass: Biodiesel (% of dry biomass) = Weight of obtained FAME/ Weight of dry biomass X100.

Identification of most biodiesel producer isolate

In order to confirm the morphological characteristics of the *Aspergillus terreus* isolate, based on the culture characteristics (color, texture appearance, and diameter of the colonies) as well as microscopic investigations using both the light microscope (Mekky et al. 2021). Finally, the 18S-rRNA (partial sequence dependent on a specific pair of primers) has been used in the molecular technique to corroborate the fungal diagnosis. The PCR results were also sequenced using the Applied Biosystems Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit. Using the NCBI BLAST search software in the National Center for Biotechnology Information (NCBI), the resultant sequence was aligned with similar sequences with MEGAx, evolutionary analyses were carried out (Mega 2018).

Maximization biodiesel production

The impact of environmental and nutritional factors on fungal growth and biodiesel production was studied under static settings in an effort to improve *Aspergillus terreus* biodiesel production.

Effect of carbon sources on production of biodiesel We measured the impact of various carbon sources on lipid synthesis by adding 50g/L of Eight carbon sources (fructose, lactose, glucose, sucrose and maltose) to the basal medium and were analyzed in an equimolar amount (Youssef et al. 2021).

Effect of nitrogen sources on the production of biodiesel

In terms of the nitrogen supply the six nitrogen sources: yeast extract, ammonium nitrate, sodium nitrate, peptone, ammonium acetate, and casein were used at (0.5g/L) (Youssef et al. 2021).

Effect of the incubation periods on production of biodiesel

Incubation periods were adjusted between 2 and 8 days under static conditions (Youssef et al. 2021).

Effect of pH on production of biodiesel

The original pH of the medium was adjusted using 1 N HCl or 1 N NaOH. Several incubation times (between one and seven days) were investigated for their impact on lipid synthesis (Youssef et al. 2021).

Effect of temperature on the production of biodiesel

In this experiment, which was conducted under static conditions, the impact of various incubation temperatures $(20^{\circ}C, 25^{\circ}C, 30^{\circ}C, 35^{\circ}C, and 40^{\circ}C)$ on lipid synthesis was assessed (Youssef et al. 2021).

Solid- State Fermentation (SSF)

In this work, we employed sugarcane bagasse and rice straw as inexpensive carbon sources to promote the growth of oleaginous fungi. We gathered the sun-dried sugarcane bagasse and rice straw from the local market in our region, which is located in the Ezpet Mekky Belbies center of the Al-Sharqia Government, Egypt.

Bagasse pretreatment

The bagasse and Rice Straw were autoclaved after treating by distilled water and at 121° C for 15 minutes (Aguiar et al. 2010).

Media preparation

This is how the medium of solid state fermentation was made: A 5-liter flask was filled with 40g of dry bagasse and rice straw, mixture was evenly moistened at a 60% level using sterile distilled water and peptone (5 g/1) and it was autoclaved for 15 minutes at 121°C. and let to cool (Yafetto 2022).

Inoculum preparation

In this study, the inoculum for solid-state fermentation was made by fungus was growing on the MEA slants for 7 days at the optimal temperature. A sterile Tween-80 was added after that, 2-3 ml of to the fully sporulated fungal slant, using sterile needle with gently scraping spores removed from the sporangiophores. Prior to inoculation, spore count was carried out using a hemocytometer (Yafetto 2022).

Gas chromatography examination

Using an Agilent Technologies 6890N (Net Work GC system) in the USA, gas chromatographic analysis of fatty acids methyl esters (FAMEs) for the high producer isolate *A. terreus* Mekky221 was carried out in Central Laboratories of the National Research Centre (NRC).

Statistical analysis

Mini-Tab software (version 19) was used for statistical analyses, and all experiments were run in triplicate. Standard deviation, or mean \pm SD, is used to express values. Unless otherwise indicated, a significance level of p < 0.05 was taken into account.

Results

Isolation and screening of fungal isolates

Using dilution agar plating, a total of seven *A. terrus* isolates were isolated from the agricultural waste samples. Using lipid extraction, lipid measurement, and dry weight determination, the seven *A. terrus* isolates as depicted in figure 1.

Polyphasic identification of the most potent taxon

Molecular and classical characterization and identification of the higher producible for biodiesel *A. terrus* spp. isolates, were performed. One of the most used methods for identifying fungus is routine identification. Figure (2) shows microscopic and macroscopic characteristics of *A. terrus* Mekky221 isolate, when it was demonstrated that the 3-day-old culture moderate growth.

The top hit displayed 98% exact identity with at the molecular level of *A. terrus* strains. Fungal isolate *A. terrus* Mekky221 was identified as *A. terrus* and recorded in Gene-Bank under accession number PQ1826087 (Fig. 3).

Effect of temperature on production of biodiesel Figure 4 illustrated that *A. terrus* Mekky221 was incubated at various temperature ranges from 5 °C to 50 °C. The results proved that the better temperature to produce lipids by *A. terrus* Mekky221 was 2.52 ± 0.06 g/L at 30°C.



Fig. 1. Different fungal and bacterial isolated from Agriculture waste samples, (Figure 1.1, 1.2) *A. terreus* spp. after purification Plates were incubated at 30 °C for 3 days, (Figure 1.3) culturing isolate on broth media incubated at 30°C for 5 days and (Figure 1.4) Biomass of-*A. terreus* spp.



Fig. 2. Morphological and microscopic characteristic of Mekky221 fungal isolate *Aspergillus terreus* Mekky221 (A) colony of Mekky221 isolate *Aspergillus* sp. on CYA, (B) reverse colony of Mekky221 fungal isolate *Aspergillus* sp., (C and D), bright field microscope (X= 20×40).



Fig. 3. Phylogenetic tree of gene sequences of the *A*. *terrus* Mekky221 isolate with the sequences retrieved from NCB Gene Bank site, with accession number PQ182607.

However, a high temperature of 50°C showed the lowest production of lipids was 0.47 ± 0.02 g/L. Also, dry biomass and lipid content at 30°C were 4.89 ± 0.19 g/L and 51.57 ± 3.03 % respectively, but at 50°C were $2.29 \pm$ 0.04 g/L and 20.52 ± 0.6 % respectively. In all cases, as temperatures approached the maximum at 50°C were rapidly reduced the production of lipids by *A. terreus* Mekky 221. Thus, from the present study, it was found that there is an increase in the production of lipids at 30 °C and a further decrease in the production of lipids by *A. terreus* Mekky 221 was observed in higher temperatures.



Fig. 4. Effect of different temperature on lipid production by *A. terrus* Mekky 221. (Error bars are means \pm standard deviations (*n*=3).

Effect of pH on production of biodiesel

Also, pH factor is considered as an important factor for lipid production, therefore; seven levels of pH (2 and 8) were used to detect center point ofpH value which will be used in main optimization. The high and low pH ranges affected the production of lipid biomass by *A. terreus* Mekky221 at pH 5 high lipid produced was 1.96 ± 0.02 g/L and low at 8 was 0.47 ± 0.02 g/L. Also, dry biomass and lipid content at pH 5 were 4.60 ± 0.02 g/L and $42.50 \pm 1.31\%$ respectively, but at pH 8 were 2.21 ± 0.03 g/L and $21.09 \pm 0.94\%$ respectively. Therefore, the center point of pH 5 is better than others as shown in figure 5.



Fig. 5. Effect of different pH on lipid production by A. *terrus* Mekky 221. Error bars mean \pm standard deviations (n=3).

Effect of the incubation periods on production of biodiesel

Incubation period also is important to accumulate lipid inside cell wall of fungi. Different incubation periods were selected for lipid production from A. terrus Mekky221 2, 3, 4, 5, 6, 7, and 8 days were used to detect center point of the incubation period to use it in the main optimization. According to ANOVA one way in Minitab19 software, Tukey method can sort results from high to low production and give letters from A to Z according to production. The optimum incubation period of A. terreus Mekky 221 isolated for the production of lipids was found at 5 days with the higher maximum incubation periods and incubation conditions for production of lipids by A. terreus Mekky 221 under static condition was 1.16 ± 0.05 g/L. However, a low incubation period of 1 day showed the lowest production of lipids was 0.38 ± 0.03 g/L. Also, dry biomass and lipid content at 5 days were 3.05 ± 0.04 g/L and 37.94 ± 1.96 % respectively, but at 1 day it was 1.85 ± 0.25 g/L and 20.85 ± 1.52 % respectively (Figure 6).

Effect of carbon sources on production of biodiesel

Different carbon sources such as starch, lactose, maltose, glucose, sucrose, bagasse and cellulose. According to ANOVA one way in minitab19, all sources were ordered gradually from high to low lipid production, glucose is the highest for lipid production and carried symbol (a)

was 1.54 ± 0.08 g/L. However, a low carbon source of cellulose showed the lowest production of lipids was 0.61 ± 0.03 g/L. Also, dry biomass and lipid content were 3.04 ± 0.04 g/L and 50.81 ± 2.29 % respectively but cellulose was 2.04 ± 0.03 g/L and 29.91 ± 1.46 % respectively (Figure 7).



Fig. 6. Effect of different incubation periods on lipid production by *A. terreus* Mekky 221. Error bars mean \pm standard deviations (*n*=3).



Fig. 7. Effect of different carbon sources on lipid production by Aspergillus Mekky 221. Error bars are means \pm standard deviations (n=3).

Effect of nitrogen sources on the production of biodiesel Different nitrogen sources such as ammonium nitrate, ammonium sulphate, urea, tryptophane, yeast extract, peptone, and asparagine. Yeast extract gives the maximum values 3.4 ± 0.05 g/L. However, the minimum nitrogen source of asparagine showed the lowest production of lipids was 0.57 ± 0.02 g/L. Also, dry biomass and lipid content for yeast extract was $5.37 \pm$

0.07a g/L and 63.34 ± 0.62 % respectively, but asparagine was 2.35 ± 0.14 g/L and 24.21 ± 1.65 %, respectively (Figure 8). The current results suggested that optimum biodiesel production by *A. terreus* Mekky 221 was observed with glucose as a carbon source and yeast extract as a nitrogen source.



Fig. 8. Effect of different nitrogen sources on lipid production by *A. terreus* Mekky 221. Error bars are means \pm standard deviations (n=3).

Solid state fermentation medium from dry bagasse and rice straw

Solid state fermentation medium was made: A 5-liter flask was filled with mixture made with 40g of dry bagasse and rice straw as inexpensive carbon sources, mixture evenly moistened at a 60% level using sterile distil water and peptone (5 g/1), autoclaved after cooling, added with known concentration, pH 5, temp. 30 and incubated for five days *A. terreus* Mekky221 showed lipid production (dry biomass 3.99 ± 0.17 g/L giving lipid content $2 \cdot .11 \pm 0.08$ ag/L Equivalently 52.8 ± 0.13 %) as represented in figure 9.

In this study GC mass reveal, the existence of 20 molecules where six major molecules of methyl esters of fatty acids including oleic Acid (26.12%), myristic acid (22.99%), arachidic acid (10.84%), octadecanoic acid (8.81%), and α -linolenic acid (7.52%), stearic acid (3.23%) consecutively. Two molecules were present in moderate levels including: 9,12-Octadecadienoic acid (Z, Z)-(2.91%) and pentadecanoic acid 2.51%. The rest of molecules were present in minor percentages (Table 1).



Fig. 9. The process of biodegradation of agricultural waste using *A. terreus* Mekky221 fungal isolate. (A) sugar cane bagasse residue after cutting. (B) Rice straw after cutting. (C) Adding the mixture into a 5-liter flask and fungal growth on agricultural waste for lipid and biodiesel production. (D) Fungal biomass. (E) GC-MS chart for biodiesel produced by fungal isolate.

Table 1 Chemical analysis using the GC Mass device for fats used to manufacture biodiesel and produced by A. terreus

 Mekky221.

RT	Name	Peak	Molecular	Molecular Weight
10.11	1 Havadaganal 2 mathyl	1.12		256
10.11	I-Hexadecaliol, 2-methyl	1.12	C17H360	230
13.08	Dodecane	0.95	$C_{12}H_{26}$	1/0
23.7	Docosane	1.25	$C_{22}H_{46}$	310
28.7	Tetradecane	1.11	$C_{14}H_{30}$	198
32.7	Tetradecane, 2,6,10-trimethyl	1.41	C17H36	240
36.7	Hexadecane	0.98	$C_{16}H_{34}$	226
40.8	Tricosane	1.39	$C_{23}H_{48}$	324
46.8	Pentadecanoic acid	2.5	$C_{15}H_{30}O_2$	242
48.1	Docosane, 11-decyl	1.16	C32H66	450
49.2	Palmitoleic acid	1.92	$C_{16}H_{30}O_2$	254
49.3	Dotriacontane	0.93	C32H66	450
50.2	Myristic acid methyl ester	22.99	$C_{15}H_{30}O_2$	242
54.8	Isochiapin b	0.96	$C_{19}H_{22}O_{6}$	346
55.4	9,12-Octadecadienoic acid (Z,Z)-	2.91	$C_{18}H_{32}O_2$	280
55.9	Oleic Acid methyl ester	26.12	$C_{19}H_{36}O_2$	296
56.7	Octadecanoic acid methyl ester	8.81	$C_{19}H_{34}O_2$	294
63.6	Stearic acid methyl ester	3.23	$C_{19}H_{38}O_2$	298
67.1	Arachidic acid methyl ester	10.84	$C_{21}H_{42}O_2$	326
74.5	Rhodopin	1.90	$C_{40}H_{58}O$	554
88.2	α -linolenic acid methyl ester	7.52	$C_{19}H_{32}O_2$	292

Discussion

In the current study *A. terreus* isolates were isolated from the agriculture waste samples and the collected agricultural waste samples were a significant source of lipid producing fungi. And this is consistent with many studies that show that, the most significant species for biotechnology applications are fungi (Corbu et al. 2023).

Numerous studies on eukaryotic Species that thrive in a variety of settings make up the fungal kingdom. Additionally, fungi play vital functions in microbiota as parasites, endophytes, symbionts, and saprotrophs (Weston 2019).

The maximum biodiesel yield was achieved from the isolates Mekky221, whose fungal cells were cultured on baseline medium containing glucose and yeast extract as carbon and nitrogen sources, in order to get fungal biomass and biodiesel yield. Such results have been recorded by (Ibrahim et al. 2023).

In the current study, lipid and biodiesel were produced by *Aspergillus terreus* Mekky221 fungi using agricultural wastes such as sugarcane bagasse and rice straw, which are an inexpensive source of carbohydrates. The goal of creating more environmentally friendly manufacturing could be aided by fungi, which are a great option for producing a variety of goods like myco-leather and textiles, biofuels, construction materials, waste water treatment, and sustainable meat alternatives (Poorniammal et al. 2021 and Alshawwa et al. 2022).

According to research, fungi strains including *Rhodosporidium* sp., *Rhodotorula* sp., *Aspergillus* sp. and *Lipomyces* sp. can store intracellularlipids from 30 to 70% of their biomass dry weight. Microorganisms that can accumulate oils in lipid form more than 20% of their biomass are classified as oleaginous species. The carbon substrate is still being taken up by the cells in oleaginous bacteria and is being directed into the lipid biosynthesis pathway, which increases the formation of triacylglycerol (TAG) and its storage in lipid bodies (Athenaki et al. 2018 and Saied et al. 2023).

There is a strong correlation between the fatty acid composition of lipids which should have more SFA and MUFA and less PUFA and the quality of biodiesel (Hui et al. 2010). An essential stage in the creation of biodiesel is the separation of lipids, and this process is entirely reliant on the internal structure of the microbe used for obtaining the lipids. Prior to the transesterification reaction, cells must be disrupted in order to obtain lipids from biomass. This procedure, along with the necessary draining and water removal step prior to processing, requires a significant amount of energy and is a major barrier to the economic feasibility of producing biodiesel (McMillan et al. 2013). Fatty acid methyl esters (FAMEs) are exclusively formed by lipids that have fatty acid ester

bonds and free fatty acids; most other lipid types are synthesized by microorganisms and are unsuitable for the manufacture of biodiesel (Patel et al. 2018).

In the current study, the experiment showed that saturated fatty acids were more productive than unsaturated fatty acids at normal temperatures, but with reducing temperatures, the opposite was true, and this is consistent with many studies. The most powerful lipid generator, *A. terreus*, and the fatty acid profile suggested that SFA were more common than USFA. Based on its fatty acid profile, *A. terreus* was found to be a viable new commercial biodiesel feedstock (Youssef et al. 2021).

Oleic acid, myristic acid, arachidic acid, octadecanoic acid, α -linolenic acid, stearic acid, methyl esters are the main fatty acid esters compositions found in *A. terreus* and can be used as affordable biodiesel in accordance with published report (Al-Zaban & Abd El-Aziz 2024).

Conclusion

The current study temperature, pH, carbon and nitrogen supply, have a major impact on biodiesel production. By using morphological and molecular confirmation, the isolate's species identity was established and recorded on Gene Bank under accession number PO182607. Aspergillus terreus Mekky221 showed the highest lipid production (dray biomass 4.89 ± 0.19 g/L giving lipid content 2.52 \pm 0.06 g/L Equivalently 51.57 \pm 3.03 %) in the presence of 100g/L sucrose and 10 g/L yeast extract at pH, 5 after five days of incubation at 30°C under static conditions. solid state fermentation medium was made. A 5-liter flask was filled with 40g of dry bagasse and rice straw, mixture evenly moistened at a 60% level using sterile distilled water and peptone (5 g/1), autoclaved after cooling, spores added, pH 5, temp. 30 and incubated for 5 days Aspergillus terreus Mekky221 showed lipid production (dray biomass 3.99 ± 0.17 g/L giving lipid content 2 .11 \pm 0.08 g/L Equivalently 52.8 \pm 0.13 %). Aspergillus terreus Mekky221 is therefore thought to be a promising oleaginous filamentous fungus that can be used to biodiesel production from agricultural waste.

Conflict of interest

The authors declare that they have no conflict of interest.

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