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Characterization of lipid producing fungi isolated from soils

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Abstract: **Microbial lipids are in the form of triacylglycerols (TAGs). Glucose was used as carbon source for accumulation of triacylglycerols. In this study, isolated fungi were screened for their lipid producing capacities by Sudan Black B staining method. Biochemical and molecular characterizations were done for identifications of potential lipid producers. The fungus *Epicaleosporium ramularioids* and *Podosphaera xanthii* showed its lipid productivity 24.2736% and 45.67% respectively. The extracted lipids were analyzed by Gas chromatography with mass spectroscopy for fatty acid determination.**

Keywords: **Lipids, Oleaginous, Sudan Black B stain, GC-MS.**

1. INTRODUCTION

Microorganisms can accumulate lipids more than 20% of their dry weight is called oleaginous. The obtained lipids are considered as raw material for production of biodiesel (Ratledge and Wynn, 2002). Subsequently, the production of biofuels like bioethanol and biodiesel has expanded worldwide for more than 10 years. Biodiesel, which is gotten from vegetable and animal fats, is for the most part a blend of unsaturated fat methyl esters (FAMEs). Utilization of biodiesel has been expanding quickly as an alternative source of energy (Meng *et al*., 2009).

Nowadays, the exploration of oil producing microorganisms primarily center around the improvement of fermentative states of the current strain (Li *et al*., 2007) as well as screening functional oil producing microorganisms (Patnayak and sree, 2005). However, very few studies had been directly on isolating microorganisms from natural environment, which convert substrate to biodiesel. 20 strains isolated from oil rich soil and nut samples, which belong to 13 different species of fat yeast. This indicates there is abundant oil producing microorganisms in nature environment (Pan *et al*., 2009). Therefore, fat producing microorganisms are screening from natural environment, on one hand, it will expand the resources of oil microorganism sp., on the other hand, it also possibly obtains oil microorganism that have many industrial applications.

2. MATERIALS AND METHODS

Isolation of lipid producing fungi

The soil samples were obtained for the isolation of microorganisms. Upon collection of soil samples were allowed to refrigerator condition at 4^{o} C for further use. For isolation 0.1 ml of diluted soil suspension spread on yeast peptone dextrose agar medium (YPD) having the pH 5.5 and 6.5, the inoculated plates were kept for incubation at lab temperature for 3 days.

Screening of potential lipid producers

The oleaginous fungus was stained with qualitative analysis of Sudan Black B staining method and observes under a microscope for presence of intracellular lipids (Thakur, 1989).

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Biochemical characterization of isolated microorganisms

Some biochemical tests, such as Carbohydrate utilization test, Growth at different temperature (37°C and 4°C), Tolerance of 1 % acetic acid, Formation of extracellular amyloid compounds, Hydrolysis of urea, Growth in media of high osmatic condition, Gelatin liquefaction, and Production of lipase, were done for characterization of fungal isolates (Kurtzmann *et al*., 2011).

Extraction of lipids and identification of fungi

Lipid extracted by Bligh and Dyer method and selected potential strains were identified by 18S rRNA sequencing. The sequence alignments performed by MEGA blast with NCBI database (Bligh and Dyer, 1959; Edwards *et al*., 1989; Edgar, 2004).

Analysis of lipids by GC-MS

The selected fungi exhibited maximum lipid accumulation. Gas chromatography-Mass spectrometry **(GC:** Agilent 7890A **MS:** 5975C MSD) fitted with DB 5 MS column **(**Dimensions: 30m L x 0.25mm ID x 0.25um film thickness) was used to determination of fatty acid composition in lipids, and 2, 6 dihydroxyacetophenone used as a standard for quantification.

3. RESULTS

Isolation and screening of potential lipid producers

More than hundred isolates obtained from soil samples and they were screened by Sudan Black B staining for determine their capacity of intracellular lipids. Among all isolates only twelve were identified as oleaginous or potential lipid producers, out of twelve isolates only two fungi were selected for further studies as indicated in the name of MBY-32 and MBY-09.

Biochemical characterization of fungi as shown in Table.1

Tests	**MBY-32 at pH 5.5** (*Epicaleosporium ramularioids*)	**MBY-09 at pH 6.5** (*Podosphaera xanthii*)
Fermentation of carbohydrates	**+ve**	**+ve**
Growth at 37° C	**+ve**	**+ve**
Growth at 4° C	**-ve**	**-ve**
Tolerance of 1% acetic acid	**-ve**	**-ve**
Formation of extra cellular amyloid compounds	**+ve**	**+ve**
Hydrolysis of urea	**-ve**	**+ve**
Growth in media of high osmotic condition	**+ve**	**-ve**
Gelatin liquefaction	**+ve**	**+ve**
Lipase activity	**+ve**	**+ve**

Lipid extraction and identification of fungi

Extraction of lipids from fungus (MBY-32) showed its lipid productivity 24.2736%, and another fungus (MBY-09) showed its lipid productivity 45.67%. These two oleaginous fungi were characterized 18S rRNA typing and identified as *Epicaleosporium ramularioids* and *Podosphaera xanthii*.

Lipid components analysis by GC-MS

The various compounds present in lipids, extracted from *Epicaleosporium ramularioids* and *Podosphaera xanthii* have shown in following graph.

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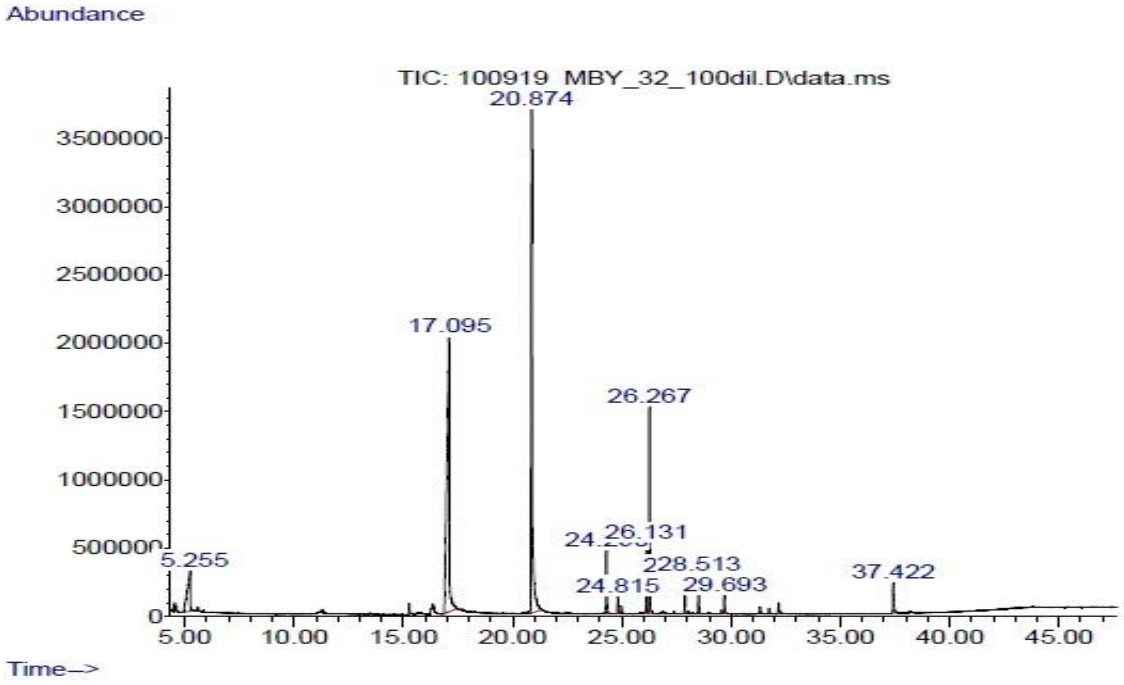
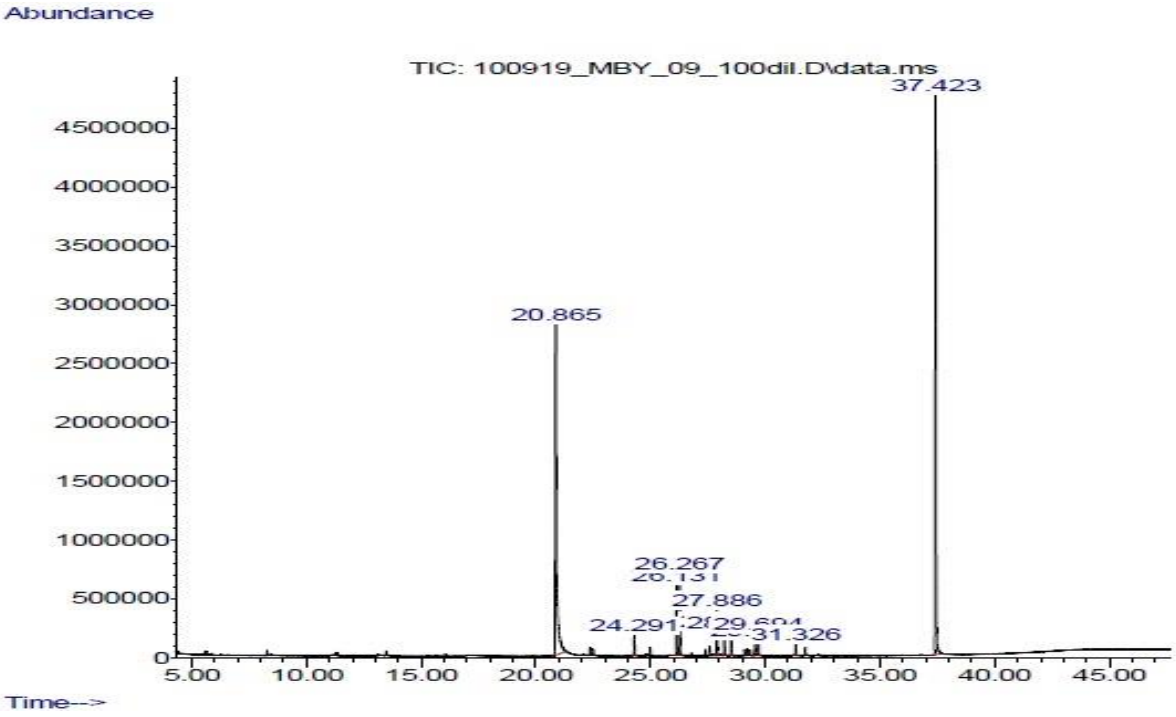


Fatty acids profile of *Epicaleosporium ramularioids* by GC-MS

The fatty acids profiles of lipids showed saturated fatty acids, such as Tridecanoic acid, 12-methyl-,methyl ester (2.527%), Pentadecanoic acid, methyl ester (2.899%), Methyl 13-methyltetradecanoate (8.425%), Pentadecanoic acid, 14-methyl-,methyl ester (1.515%), Hexadecanoic acid, methyl ester (1.545%), Methyl 15- methylhexadecanoate (0.762%). Other compounds such as isocrotonic acid (7.447%), Ethyl cyclopropanecarboxylate (39.685%), 2', 6'-Dihydroxyacetophenone (32.828%) and Oxazolidin-2-one, N-[(E)-butenoyl]-(0.788%), compounds were present in total amount of lipids.



Fatty acids profile of *Podosphaera xanthii* by GC-MS

Another above graph indicates the fatty acids profiles of lipids showed saturated fatty acids, such as Tridecanoic acid, 12-methyl-, methyl ester (1.331%), Pentadecanoic acid, methyl ester (4.593%), Tetradecanoic acid, 12-methyl-, methyl ester

(5.583%), Pentadecanoic acid, 14-methyl-, methyl ester (2.907%), Hexadecanoic acid, methyl ester (1.620%), Heptadecanoic acid, methyl ester (0.964%), Hexadecanoic acid, 14-methyl-, methyl ester (1.486%), and monounsaturated fatty acids, such as 9-Hexadecenoic acid, methyl ester, (Z)- (1.638%) and 9-Octadecenoic acid, methyl ester, (E)- (0.859%) and other compounds 2',6'Dihydroxyacetophenone (37.743%) and 1,2-Benzenedicarboxylic acid, diisooctyl ester (37.421%) were present in total amount of lipids.

4. DISCUSSION

Carbon source is playing an important role in accumulation of intracellular lipids in microorganisms. In this study, isolation of fungi from soils was collected from different gardens, and screening of lipid production by Sudan Black B staining method. Various biochemical parameters were considered to differentiate fungal isolates, these tests included several physiological aspects of fungi. The fungi *Epicaleosporium ramularioids* showed its lipid productivity is 24.2736%, which has isolated at pH 5.5 and another fungus *Podosphaera xanthii* showed its lipid productivity 45.67%, which has isolated at pH 6.5. The other oleaginous fungi *Galactomyces geotrichum* showed its dry biomass 2.6 g/L, Lipid yield 0.352 g/L and Lipid productivity 17% (Neema and Kumari, 2013). These extracted lipids were analyzed by GC-MS that showed numerous saturated and monounsaturated fatty acids, which were identified as qualitatively and quantitatively.

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