African Journal of Biotechnology Vol. 6 (18), pp. 2174-2176, 19 September 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Short Communication

Determination of the growth rate and volume of lipid produced by *Lipomyces* species isolated from shear butter leaf (*Vitellaria paradoxa*)

Oladipo I. C.[1], Adebiyi A. O.[2], Ayandele A. A.[2], Adebiyi A. P.[3] and Adelowo O. O.[2]

[1]Science Laboratory Technology Department, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Nigeria,
[2]Pure and Applied Biology Department, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Nigeria.
[3]Food Science and Engineering Department Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Nigeria.

Accepted 26 July, 2007

A *Lipomyces* strains was isolated from shear butter leaf (*Vitellaria paradoxa)* by placing the leaf sample in 10 ml of sterile distilled water containing 0.002 g of potassium dihydrogen phosphate and incubated for 3 days at 28°C. A drop of this was subsequently streaked nitrogen free medium. For determination of growth rate and volume of lipid produced, 24 h culture of the *Lipomyces* species isolated was washed into each of the following medium: yeast extract both (YE), nitrogen free broth (NF), maize broth free of salts (MF), maize broth with salts (MB), sorghum broth with salts (SB) and sorghum broth free of salts (SF). These were incubated for 7 days at 28°C on a shaker, and the lipid produced was extracted by using diethyl ether. The *Lipomyces* species was found to be able to grow and produce lipid more efficiently in yeast extract broth than in other medium used. The organism produced 25 ml of lipid per 8 g of glucose in yeast extract broth.

Key words: *Lipomyces* species*, Vitellaria paradoxa,* yeast extract broth, lipid.

INTRODUCTION

Lipids are heterogeneous class of naturally occurring organic compounds grouped together not by the presence of distinguishing functional group or structural feature but rather on the basis of common solubility properties. Lipids are all insoluble in water and soluble in one or more organic solvents (Ralph and Joan, 1982). It has been well established that lipids are produced by various micro-organisms, and such lipids producing organisms are termed oleaginous organism (Bail et al., 1984). Notably among such micro-organism are certain yeasts, filamenttous fungi as well as tubercle bacilli and a species of *Azotobacter.* The tubercle bacilli and other acid-fast bacteria contain 20 to 40% lipid on dry weight basis. Even, some of the common non-acid-fast bacteria may contain considerable amounts of lipids. Yeasts have received the most attention (Rose and Hunter, 1971) and have shown the most promise through the years (Tulloch and Spencer, 1964) as source of single cell oil through the result of Lindner and associates, who developed a process for the production of fat from carbohydrates by *Endomyces vernalis* in Germany during World War I (Fink and Haehn, 1973; Prescott and Dunn, 1940). Fungi have generally been preferred over bacteria and algae as sources of oil because of the higher yield obtainable with some species, the quality of the oil produced, the lack of toxic contaminant and the relative ease of growing the organism (Bail et al., 1984). Generally, yeast has been given the nod over molds because their single cell mode of growth has been easier to handle than the mycelial growth of molds and they tend to convert substrate to lipid more efficiently (Bail et al., 1984).

Considerably attention has been showered on intercellular lipid of yeast since the initial work of Nageli and Loew (1878) on fat production by beer yeast. The study of extracellular lipids of yeast on the other hand, is of

*Corresponding author. E-mail: xtiecoker@yahoo.com. Tel. 2348032183477.

rather recent origin. The first report of extracellular lipid production by yeast came from DiMenna (1958), Deinema (1961) and Deinema and Llandheer (1960) who showed that young cells of new yeast from New Zealand pasture grass secrete lipids into the culture medium. The formation of extracellular has been observed in over 200 strains of yeasts and most of these have been isolated from a neglected habitat, the phyllosphere, the importance of which was first demonstrated by Last (1955) during a study of fungus incidence on cereal leaves in England.

In view of the importance of single cell oil, this work is aimed at isolation and identification of a lipid producing organism from leaves sample and determination of growth rate and volume of lipid produced by the isolate in different growth medium (Table 1)

Table 1. Growth rate and volume of lipid produced by *Lipomyces* species in different medium.

Medium	Growth rate	Volume of lipid produced
Yeast Extract Broth	++++	25 ml
Nitrogen Free Broth	A	0.9 ml
Maize Broth + Salts	+++	0.2 ml
Maize Broth Free of Salts	+++	Nil
Sorghum Broth + Salts	+++	Minute
Sorghum Broth Free of Salts	+++	Nil

++++ = Very good, +++ = Good, A = aggregate.

MATERIALS ANDMETHOD

Isolation

Leaves sample collected were placed in 10 ml of sterile water containing 0.002 g of potassium dihydrogen phosphate inside sterile Petri-dishes. The Petri-dishes containing the leaves sample were then incubated for 3 days at 28°C. After the 3 days, a drop of the solution was aseptically streaked on a nitrogen free medium. The streaked plates were then incubated for 48 h at 28°C. Pure colonies were subcultured aseptically from the nitrogen free agar plates to the yeast extract agar plates. The plates were then incubated at 20°C for three days (Stodola et al., 1967).

A drop of sterile water was placed on a clean slide and a small loopful of the isolate was emulsified in the water. This was mixed thoroughly and a thin smear was made; the smear heat fixed. It was then stained with crystal violet for 2 min followed by rinsing with gram's iodine solution. The iodine was allowed to react for 1 min before washing with 95% alcohol until no more violet runs from the slide. It was then rinsed under tap water and counter stain with safranin for 2 min. Finally, it was washed with water and blotted dry before examination under microscope using oil immersion objective (Kings and Cheethan, 1984; Olutiola et al., 1991).

Determination of the rate of growth and volume of lipid produced

Heavy inoculums of the isolate was grown on different plates containing yeast extract agar and incubated for 24 h at 28°C. Then, the 24 h old culture grown on yeast extract agar were washed separately into conical flask containing yeast extract broth (YE) and nitrogen free broth (NF), aseptically. The cultures were then incubated for thirteen days at 28°C on a shaker. 24 h culture of the isolate was also aseptically washed into maize broth with salts (MB), maize broth free of salts (MF), sorghum broth with salts (SB), sorghum broth free of salts (SF) and were incubated for seven days at 28°C also on a shaker Ejiofor and Okafor (1989).

Extraction of the lipid produced

After seven and thirteen days incubation, the cells of the organism were separated from the lipids produced by adding 20 ml of diethyl ether to each of the broth in which the isolate was cultured. The lipid was obtained by shaking the solution in a separating funnel to get the lipid out. The broth solution was at the bottom, the cells at the middle while the lipid floated on the uppermost surface. The tap of the separating funnel was loosed to release the broth solution containing cells while the floating lipid was poured into a container and kept in a cool place to allow diethyl ether to evaporate (Woodbine, 1959).

Identification of the organism

The identification was carried out by culturing the isolate on malt extract agar for 24 h at 25°C with the cap of the McCartney bottle slightly loosed to enhance sporulation (Campbell and Duffus, 1988). Spore staining was carried out for the isolate culture grown on sodium acetate agar. A drop of methylene blue was placed on a clean slide and a small loopful of the organism from sodium acetate agar was emulsified in the methylene blue. This was thoroughly mixed and a thin smear was made. It was then covered with a cover slip and examined under the microscope using oil immersion objective (Lodder, 1952; Pandey and Trivedi, 1969).

RESULTS AND DISCUSSION

Growth was recorded on the plates cultured with solution from the leaves sample placed in sterile water containing potassium dihydrogen phosphate. The colonies on the nitrogen free agar after being transferred to the yeast extract agar was more pronounced and globulus. Gram staining was performed for single colony picked from the plate. Microscopic examination showed that the isolate has notable characteristics spherical structure of yeast cells. When the isolate was cultured in yeast extract broth, growth was found to be very good and 25 ml of lipid was produced, while in nitrogen free broth the isolate produced aggregate and 0.9 ml of lipid. The isolate was further grown in maize broth to which potassium dihydrogen phosphate and magnesium sulphate salts were added. It was discovered that the growth was good and it produced 0.2 ml of lipid. Also, in maize broth free of salts, the growth of isolate was found to be good, but lipid was not produced in this broth. This might be due to the fact that the broth was able to support the growth of the isolate but contained no nutrient to facilitate lipid production. Furthermore, the isolate was grown in sorghum broth to which salts were added, growth was good and

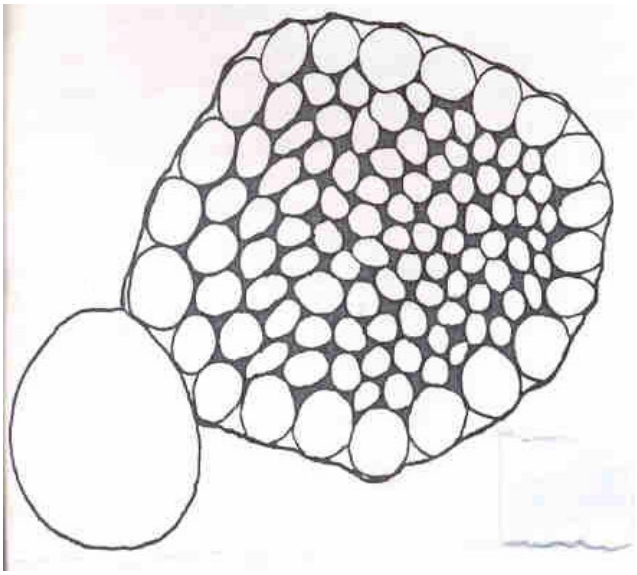

Figure 1. Microscopic appearance of the organism *Lipomyces* species.

minute amount of lipid was produced. While in sorghum broth free of salts, growth was good but lipid was not produced. This might also be due to the fact that the broth did not contain nutrient that will facilitate lipid production by the organism. Spore staining of isolate cultured in sodium acetate agar was carried out. An ascus separated from the vegetative cell was seen on the stained slide under the microscope.

In the course of this work, it was discovered that the isolate demonstrated quite a good considerable amount of growth in both the yeast extract broth and the fabricated media. Furthermore, the composition of the medium was also found to have profound effect on lipid production (Starkey, 1946). It was only in yeast extract broth that the isolate produced high amount of lipid. This shows that the medium contain nutrient that facilitated lipid production by the organism. The yield in nitrogen free broth was low compared to that from yeast extract broth. This indicates that the nitrogen free broth contained low source of nutrient that facilitate production of lipid by the organism.

The spore staining indicated that the organism was found to be a strain of *Lipomyces* (Figure 1) scovered that the isolate thrives well in yeast extract broth than in nitrogen free broth or other fabricated media. This may be due to the fact that organism was able to utilize the nutrients present in the yeast extract medium effectively. The isolate was found to be highly oleaginous because it was able to produce 25 ml of lipid per 8 g of glucose substrate in yeast extract broth. According to Bail et al. (1984), 22 - 25 g of lipid is obtainable from oleaginous organism per 100 g of glucose substrate. That shows, for the isolate about 312.5 ml of lipid will be obtainable per 100 g of glucose substrate.

Bail et al. (1984) reported that lipid produced by an oleaginous organism is edible, can be used as fuel oil additive and for other industrial purposes. With further investigations, the oil produced by the *Lipomyces* strain can be used extensively in industries and a cheap medium can be designed to support high lipid production on commercial scale.

REFRENCES

Bail N, Hammond EG, Glatz BA (1984). JAOCS 61, 1743-1746. Davies RJ (1988). In: Single cell oil, Moreton RS, ed. pp 99-143, London.

Campbell I, Duffus JH (1988). Culture, Storage, Isolation and identification of yeasts. Yeast, a practical approach. pp. 272-278.

Deinema MH (1961). Intracellular and extracellular Lipid Production by yeasts. 61: 1-54

Deinema MH, Llandheer CA (1960). Extracellular Lipid Production by a strain of *Rhodotorula graminis*. Biochim. Biophys. Acta. 37: 178-179.

Ejiofor AO, Okafor N (1989). Production of Mosquito Larvicidal *Bacillus Thuringiensis* serotype H-14 on raw media from Nigeria. J. Appl. Bacteriol. 67: 5-9.

King RD, Cheethan PSJ (1984). Biotechnology applied to fats and oils. Food Biotechnol. 1: 191-199.

Last FT (1955). Seasonal incidence of *Sporobolomyces* on Cereal leaves. Bri. Mycol. Soc. Trans. 38: 221-239

Lodder J (1952). *Lipomyces* lodder et kreger-van Rij. The yeasts. pp. 378-402.

Olutiola PO, Famurewa O, Sonntag HG (1991). An introduction to general micro-biology ,a practical approach. pp. 48-55

Pandey SN, Trivedi PS (1969). The yeasts. Textbook of Botany. 1: 430-439

Rose AH, Hunter K (1971). Yeast. lipids. and. membranes, p. 211-270. In Rose AH, Harrison JS (ed.), The yeasts, vol. 2. Academic, Press.

Starkey RL (1946). Lipid production by a soil yeast. J. Bacteriol. 51: 33-50.

Stodola FH, Deinema MH, Spencer JFT (1967). Extracellular lipids of yeast. Bacteriol. Rev. 31(3): 194-213.

Tulloch AP, Spencer JFT (1964). Extracellular glycolipids of *Rhodotorula* species. Isolation and synthesis of 3-D Hydroxyl palmitic and 3-D-hydroxy stearic Acids. Can. J. Chem. 42: 830-835.

Woodbine M (1959). Microbial fat. Micro-organisms as potential Fat producers. Prog. Ind. Microbiol. 1: 181-245.