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# Lipase Production by Fungal Isolates Grown in Palm Oil Mill Effluent

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Author PNIN participated in all operations of this manuscript. Author OKA designed the study and wrote the protocol performed. Author PNIN wrote the first draft of the manuscript and managed the analyses of the study and literature searches. Author OKA revised the manuscript and author PNIN has the final responsible for all information presented. Both authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** The present work is aimed at determining the optimum conditions of pH, temperature and nitrogen concentration for lipase production by *Candida rugosa* and *Geotrichum candidum* in POME.

**Place and Duration of Study:** This work was carried out at the Department of Microbiology, Michael Okpara University, Umudike-Abia state, Nigeria from July to December, 2013. The organisms used in the study were isolated in a previous work by the authors.

**Methodology:** Five milliliter of sterile water was added to respective agar slant containing

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the two different fungal isolates while developed growth was scrapped with sterile needle and subsequently transferred into nutrient broth contained in 250 mL Erlenmeyer flasks. These flasks were further incubated for 48 h at 28ºC on rotary shaker at 180rmp. At this stage 0.1 mL of inoculum was transferred to the production medium (POME) and incubated for 144 h.
**Results:** At initial pH6.0, *C. rugosa* produce maximum lipase activity of 26.37 $UmL^{-1}$ while *G. candidum* showed highest activity of 29.4 $UmL^{-1}$ at pH 7. *C. rugosa* alo showed maximum lipase activity of 27.8 $UmL^{-1}$ at 30ºC while *G. candidum* produced highest lipase activity (24.9 $UmL^{-1}$) at 35ºC. The use of soybean meal in the optimization of production lipase revealed that at concentration of 3.5%w/v, the respective fungi isolates were best supported for lipase yield with maximum activity of 25.97 $UmL^{-1}$ by *C. rugosa* and 28.32 $UmL^{-1}$ by *G. candidum.*
**Conclusion:** This work evaluated the effect of three culture conditions (pH, temperature and nitrogen conc.) on lipase production by *G. candidum* and *C. rugosa* cultivated in POME. Results reveal that the factors were critical to growth and lipase production by the organisms and may be useful indices in the production of lipase even from other oil processing effluents.

*Keywords: Lipase activities; optimization; palm oil mill effluent.*

## 1. INTRODUCTION

Oil wastes from both crude and vegetable origin such as palm oil mill industries are notable pollutants that cause serious hazards when they are channeled in an untreated form into aquatic environments. These result in malodor, color change of the receiving water body. Oil remnant in the effluent may burn on the surface of receiving waters creating potential safety hazards also making filtration treatment difficult and consume dissolved oxygen necessary to forms of life in such water bodies. This in turn affects the fishes and causes eutrophication [1]. These negative effects of POME on aquatic habitats and environment may in turn result in complete damage of the ecology of seaside areas. Conventional (physical) treatment of POME is considered inadequate. Therefore, biological treatment is the most effective method for removing fat, oil and grease by degrading them into miscible molecules [2].

Lipases (triacylglycerol acylhydrolases, E.C. 3.1.1.3) are widely produced in nature by plants, animals and several microorganisms, but only microbial lipases are commercially significant [3]. Commercially valuable lipases are mostly obtained from microorganisms that produce a wide variety of extracellular lipases [4].

Lipases catalyze the hydrolysis of triacylglycerols to glycerol and free fatty acids unlike esterases, lipases are activated only when adsorbed to an oil–water interface [3,5]. They have emerged as one of the leading biocatalysts with high potentials in the enhancement of economy within untapped lipid technology in bio-industries [6]. Microbial lipases have been given special industrial consideration due to their stability, selectivity, and broad substrate specificity [7]. Various applications of lipases include organic syntheses, hydrolysis of fats and oils, modification of fats, flavor enhancement in food processing, resolution of racemic mixtures, and chemical analyses [3].

Its application in various fields of biotechnology has drawn the attention of researchers to use of these lipases in solving problems associated with oil pollution whereby they catalyze the hydrolysis of fats and oil [8]. Many microbial strains have been used for enzyme

production. The use of fungal lipases to improve processes and application is becoming very acceptable and so many of them have been characterized [9-11]. In the area of waste water treatment, especially the bioremediation of palm oil mill effluent (POME), the yeast *Yarrowia lipolytica* have been successfully utilized [12]. Universal yeast medium has been used to study lipase production by *C. rugosa* [13]. The versatility of lipase from *C. rugosa* has made it one of the most industrially used enzymes [14]. In fact, its activity in the synthesis and hydrolysis of fats, flavour enhancement, production of fatty acids, detergents and bulk chemicals have been widely studied [14-16].

Similarly, recent studies on the use of soy oil and other agro-industrial wastes as substrates for the extracellular production of lipase by *Geotrichum* sp have been reported [17,18]. In the present study, the conditions for the production of lipases by *C. rugosa and G.candidum* was investigated.

## 2. MATERIALS AND METHODS

### 2.1 Microorganisms

The selected fungal isolates *C. rugosa* and *G. candidum* used in this work were isolated from POME sample collected from Starline palm oil mill industries Umukalika Obingwa Abia state Nigeria and had been identified in previous study by Ibegbulam- Njoku and Achi [19].

### 2.2 Filtration

The raw POME used as medium was passed though a hand sieve of 0.5mm pore size to eliminate coarse solids such as broken shells, kernels and plant fibers before passing the filtrate through a Whatmann No 41 filter paper pore size of 20-25µm .

#### 2.2.1 Cultivation

The method used was as described by Pechsuth et al. [20] with slight modification. The respective fungi isolates were sub cultured on PDA Slant and incubated for 96 h at 28$^{o}$C. To agar slants containing each of the fungal isolates, 5mL of sterile water was added while developed growth was scrapped with sterile needle and subsequently transferred into nutrient broth contained in 250 mL Erlenmeyer flasks. These flasks were further incubated for 48 h at 28ºC on rotary shaker at 180 rpm. At this stage 0.1mL of this inoculums, counted as $10^{6}$cells/mL using haemocytometer was transferred into POME for further studies.

### 2.3 Lipase Assay (Titrimetric Method)

The lipase activity in crude extract of respective selected fungal isolates was measured using titrimetric method as described by Nwuche and Ogbonna [21] with slight modification. The assay for extracellular lipase was carried out using Palm oil as a substrate, gum Arabic (emulsifier) and (vehicle) water (4:1:2) to produce a thick creamy white emulsion. The partially purified lipase ((10 µL)) supernatant of the cell culture (20µL) was added to 5mL of emulsion and 2ml of 100 mM phosphate buffer pH 7.0 ($K_2HPO_4$) before incubating for 30 mins at 37ºC with shaking (120rpm). The reaction was stopped by addition of 1.0 ml of 1:1 acetone/ ethanol solution. The amount of fatty acid liberated was estimated by titrating with 0.05M NaOH using phenolphthalein. Blank assays were conducted adding the enzyme just before titration.

One unit (u) of lipase activity was defined as the amount of enzyme required to hydrolyze 1µmol of fatty acids from triglycerides indicator under assay condition [22].

$$\text{Lipase activity } (UmL^{-1}) = \frac{\text{Vol. of NaOH consumed (mL)} \times \text{Molarity of NaOH (M)}}{\text{Vol. of Lipase (mL)} \times \text{Reaction Time (min)}}$$

One unit (u) of lipase was defined as the amount of enzyme required to liberate 1µmol of fatty acids under the assay condition specified'' The fatty acids were liberated from the palm oil, not hydrolyzed.

#### 2.3.1 Effect of initial pH on lipase production

The method used was described by Dheeman et al. [23] with little modification. The effect of pH on lipolytic activity was determined using the following buffers (all at 50 mM): succinate-NaOH (pH 4.0 and 5.0), sodium phosphate (pH 6.0 and 7.0), Tris–HCl (pH 8.0 and 9.0) to improve initial pH of 100ml POME sample contained in 250mL Erlenmeyer flasks and inoculated with 0.1ml ($10^6$cells/mL) of respective selected culture. The flasks were incubated at 28ºC for 144 h on a rotary shaker with agitation speed of 180rpm. Culture (20 mL) was centrifuged at 12,000 rpm for 15 min. The supernatant was analyzed for extracellular lipase activity [24].

#### 2.3.2 Effect of incubation temperature on lipase production

Selection of optimum temperature for the production of lipase used was described by Rekha et al. [25] with slight modification in incubation temperature. Each POME sample (100mL) contained into 250 mL Erlenmeyer flasks was inoculated with 0.1mL ($10^6$cells/mL) of the respective selected fungal culture and incubated at different temperatures 25, 30, 35, 40, 45 and 50ºC accordingly for 144 h. The crude enzyme used for extracellular lipase activity was from culture broth (POME) after centrifugation. The enzyme assay was performed as discussed above.

#### 2.3.3 Effect of soybean on lipase production

The method used was as described by Reda et al. [26] with little modification in palm oil mill effluent. Different concentrations of soybean (1.5, 2.5, 3.5 and 4.5% w/v) were used to supplement 100mL of POME sample into 250 mL Erlenmeyer flasks, inoculated with selected fungal isolate ($10^6$ cells/mL), incubated and samples drawn for determination of lipase activity as described above.

## 3. RESULTS AND DISCUSSION

### 3.1 Effect of Initial pH on Lipase Production

The effect of initial pH on lipase production of *C. rugosa* and *G. candidum* in POME is shown in Fig 1. The effect of initial pH of culture medium was determined at pH 4-9.

The results showed that *G. candidum* was able to grow in a wide range of pH 4.0 to 7.0. Initial pH 7 promoted the highest lipase activity of 29.4 $UmL^{-1}$ in *G. candidum*. Previous reports of Vakhlu and Kour [14] showed *Geotrichum* produced active lipase on olive oil at pH 5.6 and 7.0. Also, Cho et al. [27] reported maximum lipase activity by *G. candidum* at initial pH 6 of the basal GKM medium. Although *C. rugosa* produced lipase in POME at pH 4.0, 5.0

and 7.0, the maximum lipase activity of 26.37 $UmL^{-1}$ was observed at pH 6.0. Prabhakar, et al. [28] reported that pH dependency of the culture had important role for growth of both fungi (*Rhizopus* sp: TP.St 02 and TP.St05) and on enzyme production. Maximum lipase production in both strains was observed at pH 6.0 for TP.St. 02, and 6.5 TP St.05 while minimum lipase was shown at pH 9. This is in line with studies of Rekha et al. [24] who reported optimal lipase activity of *C. rugosa* (51.25 $UmL^{-1}$) at pH 6. Falony et al. [29] also reported optimal lipase activity using *Aspergillus niger* at pH 6. However, lipase activity decreased with increase in the initial pH of medium, with least activities of 9.6 U $mL^{-1}$ for *G. candidum* and 9.0 $UmL^{-1}$ for *C. rugosa* at pH of 9.0.

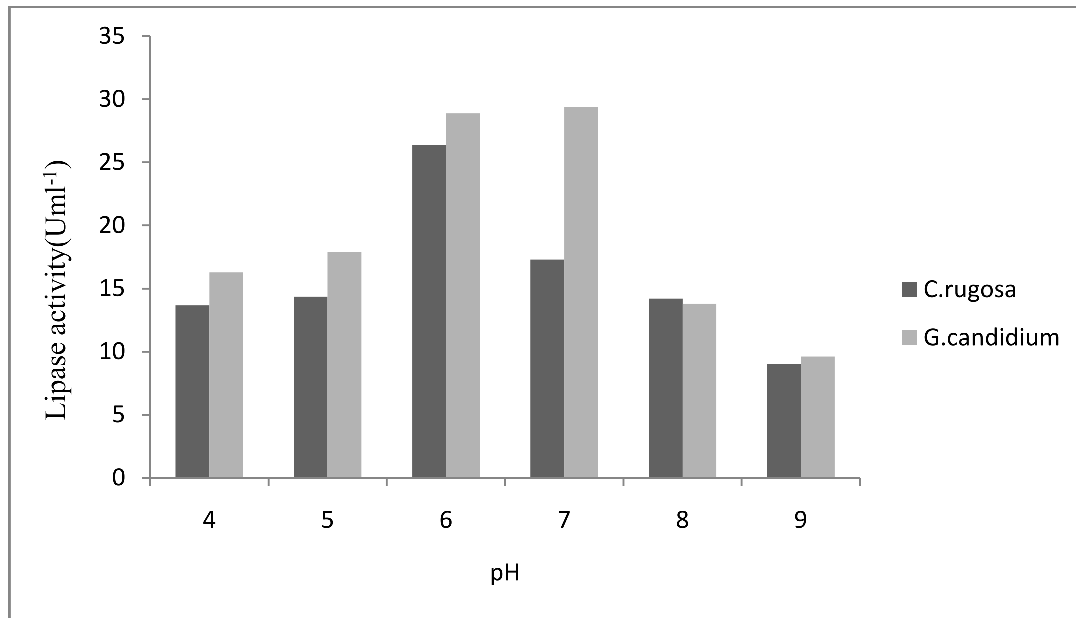


**Fig. 1. Effect of initial pH on lipase production by *C. rugosa* and *G. candidum* in POME**

### 3.2 Effect of Incubation Temperature on Lipase Production

Lipase activity at various temperatures (25ºC to 50ºC) is shown in Fig 2. Temperature of 35ºC supported the highest lipase activity during the growth of *G. candidum* with maximum activity of 24.9 $UmL^{-1}$ while *C. rugosa* at 30ºC showed highest lipase activity of 27.8 $UmL^{-1}$ in POME. However, higher temperature of 40 and 45ºC did not support lipase production in both fungal isolates. Cho et al. [27] reported optimal lipase production of 150 $UmL^{-1}$ by *G. candidum* at 30ºC. Rekha et al. [25] reported that optimum lipase production of 50.25 $UmL^{-1}$ by *C. rugosa* NCIM 3467 was obtained at incubation temperature of 32ºC. However, Savitha et al. [11] reported optimal temperature for fungal lipase activity on para- Nitrophenyl palmitate at 40ºC showing three- fold increase in activity compared to 37°C.

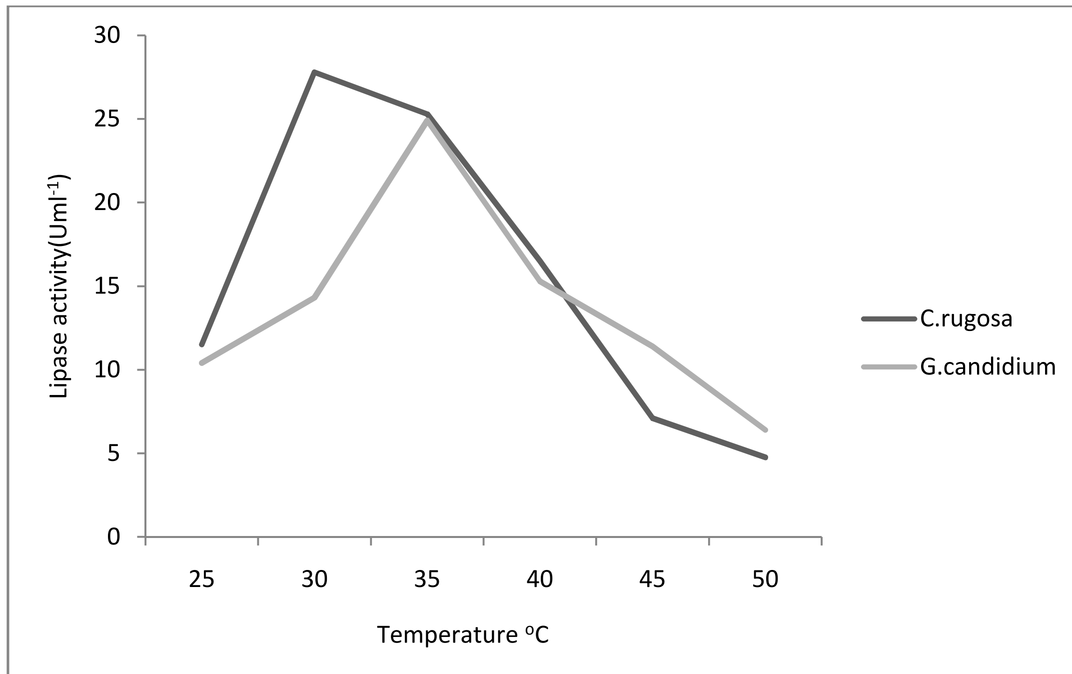


**Fig. 2. Effect of temperature on lipase production by *C. rugosa* and *G. candidum* in POME**

### 3.3 Effect of Soybean on Lipase Production

The effect of various concentrations of soybean meal (1.5, 2.5, 3.5 and 4.5%w/v) on fungal lipase production was shown in Figs. 3 and 4. The result revealed that soybean meal at 3.5%w/v best supported the lipase production using *C. rugosa* (Fig. 3) and *G. candidum* (Fig. 4) with maximum activities of 25.97 $UmL^{-1}$ and 28.32 $UmL^{-1}$ respectively. Many oil-seed cakes such as soybean meal are rich in protein, good food supplements for feed applications and are used in biotechnological processes as reported in previous studies [27,30]. This result of this work is in line with Iftikhar and Hussian [31], who reported that 1% soybean meal was used to obtain 69.65 $Ug^{-1}$ of lipase in mutant strain of *Rhizopus oligosporous* $T^{uv}$-31. Loo et al. [32] reported highest lipase activity (22.6 $UmL^{-1}$) from *G. candidum* using peptone and yeast extract after 54 h of fermentation.

Similarly, Kempka et al. [33] reported the use of soybean meal, sugar cane molasses, corn steep liquor, yeast hydrolyzed, yeast extract, sodium chloride, soybean oil, castor oil, corn oil, olive oil, and peptone in the production of lipase from *P. verrucosum* by solid substrate fermentation. The study proved soybean meal the best substrate in lipase production. Prabhakar et al. [28] equally examined the optimization of lipase in two strains of *Rhizopus* sp using different concentrations (0.1 to 1%) of peptone. It was observed that maximum activity of lipase occurred at 0.3%. However, Barbosa et al. [30] reported that the addition of soybean and castor meals in minimal salt medium strongly decreased lipase production *by Botryosphaeria ribis* EC-01. However, previous report by [34] contradicts this result by showing that the lipase production could be improved by inorganic nitrogen sources while cell growth was influenced by organic source.

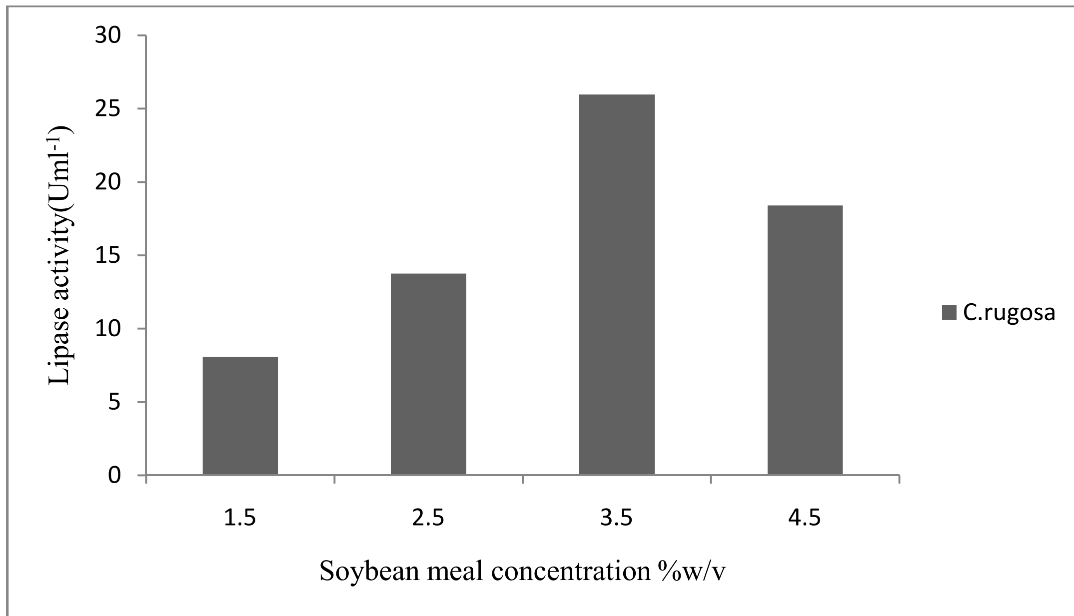


**Fig. 3. Effect of soybean on lipase production by *C. rugosa* in POME**

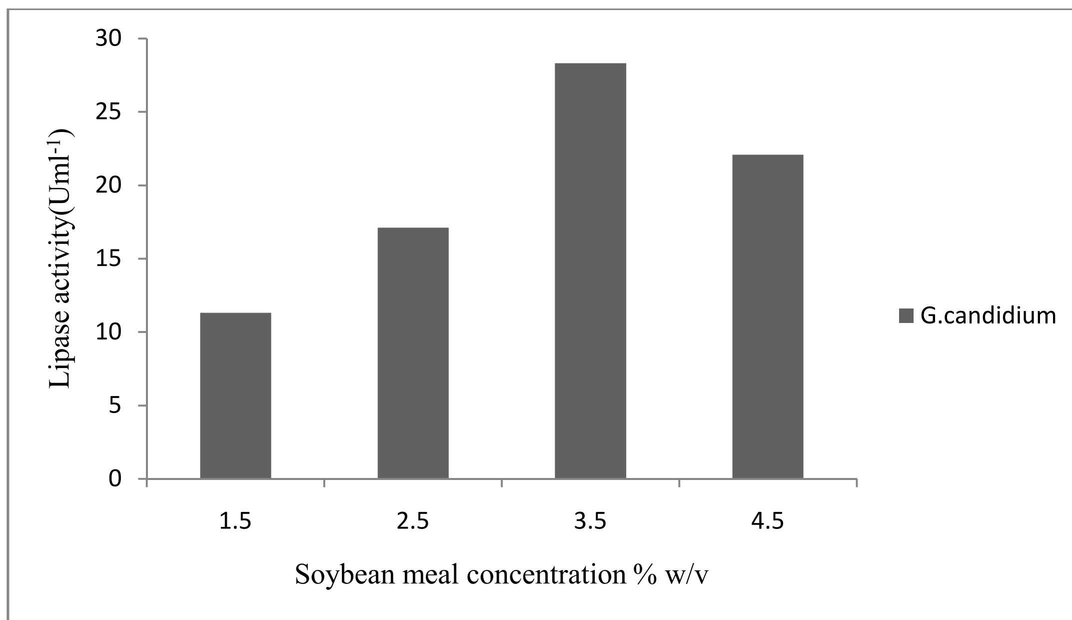


**Fig. 4. Effect of soybean on lipase production by *G. candidum* in POME**

## 4. CONCLUSION

This work evaluated three culture conditions (pH, Temperature and concentration of nitrogen source) for the production of lipase by *G candidum* and *C. rugosa*. The ability of *G. candidum* and *C. rugosa* to produce lipase in POME was demonstrated. However, the

investigation of lipase production in POME revealed that most culture conditions are better regulated for optimal lipase activities. Similarly, *G. candidum* and *C. rugosa* can be used in production of lipase using other industrial oil related effluents.

Use of POME as raw material for lipase production may reduce some of environmental problems associated with the disposal of POME in the environment thereby making POME a veritable tool to create wealth.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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