ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

# SELECTION OF SUITABLE BASAL MEDIUM FORMULATION FOR EXTRACELLULAR LIPASE PRODUCTION AND GROWTH OF FIVE LIPOLYTIC FUNGI

**Toshi Wadia*, Sudhir Kumar Jain**

*School of Studies in Microbiology, Vikram University, Ujjain, Madhya Pradesh, India*

**Corresponding author: tshwadia@gmail.com*

## ABSTRACT

The main purpose of this study was to select the basal medium, suitable for the growth of fungal isolates, which favors the mycelium growth and extracellular lipase production. For this, five fungi were isolated from the oil contaminated sites of Ujjain city and identified as *Curvularia lunata* (Wakker) Boedijn, *Aspergillus flavus* Link, *Trichoderma harzianum* Rifai, *Aspergillus parasiticus* Speare and *Aspergillus niger* gr. Four media namely Medium–1, Medium–2, Medium–3 and Medium–4 with different composition were tested for mycelium growth and lipase production by these five fungi. Results showed that Medium–1 was found as the most suitable for the growth of lipase producing fungi and also for extracellular lipase production over other three media, while Medium-4 was found the least suitable medium for the growth and lipase production by all the five fungi. In Medium-1, *Trichoderma harzianum* Rifai, *Aspergillus niger* gr., *Curvularia lunata* (Wakker) Boedijn and *Aspergillus flavus* Link showed maximum 20 mm clearance zone of lipase activity with dry weight of biomass 3.00 g, 2.89 g, 1.96 g and 2.80 g respectively while *Aspergillus parasiticus* Speare showed 15 mm clearance zone of lipase activity with dry weight of biomass 1.98 g. The study can conclude that best suitable medium for lipase production should contain olive oil as lipid substrate, peptone as protein source and $Mg^{2+}$ and $K^{+}$ ions source. These not only support the mycelial growth but also the production of lipase, extracellularly in the liquid medium. In general this study support that selection of best suited basal medium for the growth of fungi should be done before the large scale production of lipase.

**Keywords:** Basal Medium, Biomass, Extracellular Lipase, Fungi

## 1. INTRODUCTION

Culture medium is any type of substance used for the cultivation of microorganisms and provides good nutrient availability so that they can flourishly grow and multiply. Basal medium can be defined as an unsupplemented medium which promotes the growth of many types of microorganisms, which do not require any special nutrient supplements [1]. An ideal and optimal nutrient medium must contain simple composition as well as should enhance optimum growth, so that fungi can show increase biomass and growth [2-4]. The selection of basal media was an important step for the cultivation of fungi and should be done prior to the study of optimization of culture conditions including large scale production of any material. Basal medium consists of mineral salts and sole carbon and nitrogen source and it is used to be supplemented by other additional components for the enhancement of yield of the desired product by the fungi. Researchers formulated basal medium as an alternative medium for the growth of various fungi [5]. In another research, the growth of fungi and their potential to produce extracellular lipase was compared using different culture media [6]. Different media that support the growth of lipase producing fungi were reported by researchers and the selection of basal medium was done on the basis of adequate mycelial mass formation and maximum extracellular lipase production occurred in it. These two parameters were used to select the suitable basal media by calculating dry weight of biomass of lipase producing fungi in grams(g)/100 milliliters(ml) and extracellular lipase production by determining lipase activity in millimeter (mm) by agar well diffusion method [7, 8]. In the present study, four media were tested by cultivating all five selected lipase producing fungi that is *Curvularia lunata* (Wakker) Boedijn, *Aspergillus flavus* Link, *Trichoderma harzianum* Rifai, *Aspergillus parasiticus* Speare, and *Aspergillus niger* gr. These fungi were isolated from the oil contaminated sites of the Ujjain city and identified, based on colony morphology and other cultural characteristics. The basal medium components

like nitrogen source, lipidic carbon source and minerals, were differ with respect to concentration and presence or absence in the media. The selection of suitable basal medium was made on the basis of yield of dry weight and lipase activity.

## 2. MATERIAL AND METHODS

### 2.1. Dry weight (biomass) determination

For the optimum growth of lipase producing fungi the selection of appropriate medium which act as the basal medium for further optimization of different physico-chemical parameters is essential. For this four media were used:

***Medium-1***: Composition for 100ml preparation - Peptone: 3.0g; $MgSO_4.7H_2O$: 0.05g; KCl: 0.05g; $K_2H_2PO_4$: 0.2g and olive oil: 1.0 ml (emulsified with glucose: 0.5g), pH 6.0 [9].

***Medium-2:*** Composition for 100ml preparation - Soluble starch: 2.0g; corn steep liquor: 5.0ml; $K_2HPO_4$: 0.2g; $MgSO_4.7H_2O$: 0.1g; $CaCO_3$: 0.5g; soybean oil: 0.5ml (emulsified with gum Arabic: 0.005g); pH 7.0 [10].

***Medium-3***: Composition for 100ml preparation - $KNO_3$: 0.25g; $KH_2PO_4$: 0.1g; $MgSO_4.7H_2O$: 0.05g; NaCl: 0.5g; and olive oil: 1.5 ml (emulsified with Tween 20: 0.5ml); pH 8.0 [7].

***Medium-4:*** Composition for 100 ml preparation - Glucose: 2.0g; $(NH_4)_2\ SO_4$: 3.0g; $KH_2PO_4$: 2.0g; KCl: 0.5g; $MgSO_4$: 0.5g; pH 4.0 [11].

All four above mentioned liquid media were separately taken in 100 ml quantity into 500 ml conical flask and sterilized in autoclave at 15 $lb/in^2$ for 15 minutes. Culture suspension of each fungus was prepared from active culture by washing 4-5 days old slants using distilled water with 0.1 % (v/v) Tween 80. After this 0.5 ml of spore suspension was inoculated aseptically into the labeled flasks with micropipette and incubated at 28°C for one week. This experiment was done in triplicate. After incubation the mycelium mat formed was separated by filtration through an already weighed Whatman no. 1 filter paper. The biomass was washed many times with saline and then dried at 70°C, for 24 hours in oven. After proper drying, dry weight of biomass was taken by weighing on digital single pan balance.

The actual biomass was determined by following formula:

Biomass (g/ml) = {Wt. of filter paper with biomass – Wt. of empty filter paper (g)}/Volume of sample (ml)

The dry weight of biomass for 100 ml of medium:

Dry wt. of biomass (g) = (Wt. of filter paper with dried biomass–Wt. of empty filter paper)

The obtained filtrate of each culture broth was centrifuged at 6000 rpm for 40 minutes at 4°C in cooling centrifuge (REMI, India). Supernatant was collected and filtered again through a sterile filter paper and this filtrate was used as crude extracellular lipase. This crude lipase extract used to determine the lipase activity.

### 2.2. Lipase activity determination by agar well diffusion assay method [12]

Estimation of lipase enzyme was done according to the lipase activity in the supernatant. For qualitative estimation of crude lipase this T.C.Z. (Tributyrin Clearing Zone) technique was employed. The clear zone obtained due to diffusion of lipase into the medium, was measured in mm which represents lipase activity and considered equivalent to milligram (mg) lipase per ml of sample. In this assay for 100ml preparation of medium- 200 µL Tributyrin was emulsified with 0.02g gum Arabic, and 2% agar powder was added for solidification. Using Tris buffer, the pH was adjusted at 8.8. After autoclaving the medium was poured in sterile Petri–plates after cool down and allowed to solidify. Wells were made in the centre of each plate using sterile cork-borer having size of 8mm diameter and filled with 0.1 ml of crude lipase enzyme extract obtained as a result of above method. Immediately incubate the plates at 28°C for 12–24 hours. After 24 hours, a clear circular zone was measured (mm) as lipase activity.

## 3. RESULTS AND DISCUSSION

According to the obtained values as the results of dry weight and lipase activity determination, mentioned in the Table 1, the most suitable medium for the growth of lipase producing fungi and also for extracellular lipase production was Medium-1. Therefore, it could be further selected as the basal medium for fermentation study. In Medium-1 *Trichoderma harzianum* Rifai, *Aspergillus niger* gr., *Curvularia lunata* (Wakker) Boedijn and *Aspergillus flavus* Link showed maximum 20 mm zone of lipase activity (as shown in Fig. 1) with dry weight of biomass 3.00g, 2.89g, 1.96g and 2.80g respectively while *Aspergillus parasiticus* Speare showed 15 mm zone of lipase activity (Fig. 1) with dry weight of biomass 1.98 g. Medium-4 was found the least suitable medium for the growth and lipase production

by all the five fungi. As data given in the Table 1, *Curvularia lunata* (Wakker) Boedijn showed no lipase activity when cultivated in this medium. For the selection of basal medium for the lipase production from all selected fungi isolates by submerged fermentation, four different media with varying concentration of different minerals, lipidic-carbon and nitrogen components were taken under study. Basal medium that showed maximum of both the parameters *i.e.*, biomass and extracellular lipase production could be selected as the most suitable medium and considered for the utilization in further fermentation related study. Since lipase considered as an inducible enzyme, medium employed in its production should contain some lipidic carbon source that induces fungi for the lipase production and perform lypolysis to utilize the lipid present in the surrounding medium and grow actively. Adham [13] studied different media for lipase production by strains of *Aspergillus niger*.

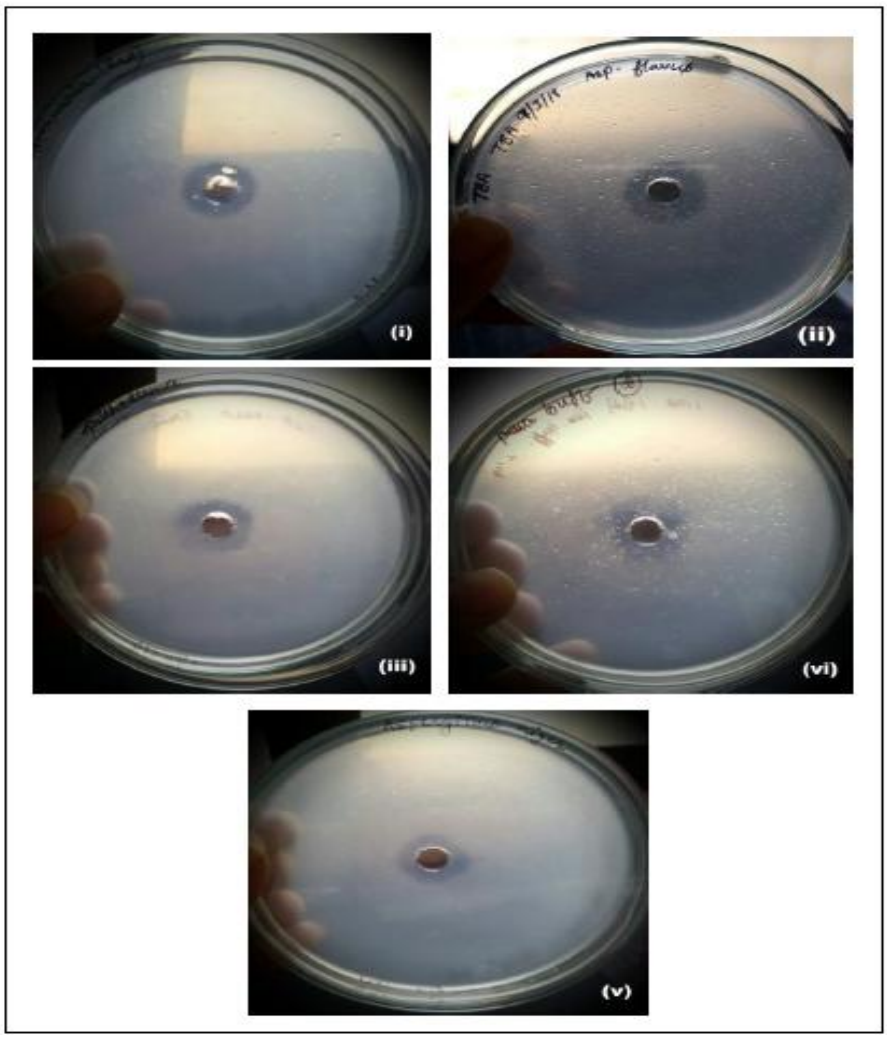


**Fig. 1: Agar Well Diffusion Lipase Assay on Tributyrin and Agar (2%) plate showing diffusion of crude lipase extract obtained from Medium-1 by (i) *Curvularia lunata* (Wakker) Boedijn; (ii) *Aspergillus flavus* Link; (iii) *Trichoderma harzianum* Rifai; (iv) *Aspergillus parasiticus* Speare; (v) *Aspergillus niger* gr.**

Medium-1 contained one ml olive oil and 3.0 g peptone per 100 ml, as the lipidic carbon and nitrogen source respectively and support the maximum growth and production of extracellular lipase in all five selected fungi, specifically *Trichoderma harzianum* Rifai with highest lipase activity 20mm and also highest dry weight of biomass 3.00g. Similarly, Medium-3 also contained 1.5ml olive oil per 100ml with different mineral salts and it also supported the moderate growth and extracellular lipase production. *Aspergillus flavus* Link showed highest lipase activity 19.5mm with dry weight of biomass 0.45g in it among other fungi. This is in the agreement with Maia [14] who reported that when medium supplemented with 1% (v/v) olive oil, highest

lipase activity observed and concentration of peptone below 3% (w/v) resulted in a reduction of lipase production. Medium-2 contained 0.5ml soybean oil and 2.0g soluble starch per 100 ml, as lipidic carbon and another carbon source respectively, also 5.0ml corn steep liquor as nitrogen source with mineral salts in different concentration. Results presented in the Table 1 show that this medium supported the mycelial growth of all five selected lipase producing fungi, dry weight of biomass for *Curvularia lunata* (Wakker) Boedijn, *Aspergillus flavus* Link, *Trichoderma harzianum* Rifai, *Aspergillus parasiticus* Speare and *Apergillus niger* gr. are 1.61g, 1.85g, 2.50g, 2.78g and 2.97g respectively but the lipase activity reported by them are comparatively low with respect to biomass production. The presence of soluble starch as main carbon source and corn steep liquor as nitrogen source supported the mycelial growth of fungi but 0.5ml soybean oil in the medium induced moderate lipase activity by all the fungal isolates in comparison with Medium-1. *Aspergillus niger* gr. showed 15.8 mm as highest lipase activity in the Medium–2. As work done in the past, this medium was utilized to determine the lipase activity and stability of extracellular lipase by *Humicola lanuginosa* at different temperature and pH [14]. According to Bornscheuer [15] for lipase production, olive oil has been referred as one of the best inductor and substrate for lipase production, among natural oils. Medium-4 contained 2.0g glucose as sole carbon source and 0.3g $(NH_4)_2SO_4$ and 0.56g $MgSO_4$ as nitrogen and $Mg^{2+}$ source respectively, but lacked any lipidic carbon source hence showed least biomass production and lipase production among all.

**Table 1: Production of biomass and extracellular lipase by selected fungal isolates in different medium**

| Medium | Fungi | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Curvularia lunata* (Wakker) Boedijn | | *Aspergillus flavus* Link | | *Trichoderma harzianum* Rifai | | *Aspergillus parasiticus* Speare | | *Aspergillus niger* gr. | |
| | Dry Weight (g) | Lipase Activity (mm) | Dry Weight (g) | Lipase Activity (mm) | Dry Weight (g) | Lipase Activity (mm) | Dry Weight (g) | Lipase Activity (mm) | Dry Weight (g) | Lipase Activity (mm) |
| **Medium–1** | 1.96±1.4 | 20±1.5 | 2.80±2.5 | 20±1.2 | 3.0±0.85 | 20±0.9 | 1.98±1.2 | 15±1.7 | 2.89±2.1 | 20±1.9 |
| **Medium–2** | 1.61±0.47 | 5.8±0.8 | 1.85±1.5 | 10.5±2.2 | 2.50±1.2 | 15±1.2 | 2.78±0.85 | 8.6±1.5 | 2.97±1.6 | 15.8±1.0 |
| **Medium–3** | 0.21±0.81 | 15.8±0.9 | 0.45±0.3 | 19.5±1.7 | 0.48±0.2 | 12±1.0 | 0.36±0.2 | 18±2.1 | 0.39±0.2 | 18±1.5 |
| **Medium–4** | 0.56±0.47 | 0.0 | 0.80±0.3 | 5.5±1.5 | 0.88±0.5 | 6.5±1.5 | 0.78±0.5 | 5.4±0.8 | 0.89±0.2 | 7.8±0.5 |

*Experiment conducted in triplicates each value represents mean±SD; g = grams; mm = millimeter*
*Biomass was determined in terms of dry weight in g and lipase production was determined in terms of lipase activity in mm*

## 4. CONCLUSION

The study can conclude that the best suitable basal medium for lipase production should contain olive oil as lipid substrate, mineral salts containing $Mg^{2+}$ and $K^{+}$ ions and peptone as protein source. This, not only support the mycelial growth but also the extracellular production of lipase from fungi, into the liquid medium. In general this study supports that the selection of best suited basal medium for the growth of fungi should be done before the large scale production of lipase.

## 5. REFERENCES


1. www.biologyonline.com›dictionary›basal-medium.
2. Pelczar MJ, Chan ECS, and Krieg NR. Microbiology. 5th edition: Tata McGraw-Hill, New Delhi, India: 1993.
3. Melentiadis J, Jacques FG, Meis M, Mouton JW, Verweij PE. *Journal of Clinical Microbiology,* 2001; **39 Suppl 2:**478-484.
4. Gabriel-Ajobiewe Ruth AO, Akinyele BJ, Mirrila EB. *Journal of Microbiology, Biotechnology and Food Sciences,* 2012; **1 Suppl 4**:1136-1151.
5. Adesemoye AO, Adedire CO. *World Journal of Microbiology and Biotechnology*, 2005; **21 Suppl 3**:329-336.
6. Ferreira Ana Beatriz Monteiro, Ivan Herman Fischer, Luís Garrigós Leite, Carlos Roberto Padovani, et al. *Arq. Inst. Biol.,* 2019; **86:**1-9.
7. Lanka S, Trinkle T. *International Journal of Pharmacognosy and Phytochemical Research,* 2017; **9 Suppl 7**:928-932.
8. Kakde RB, Chavan AM. *International Journal of Biology*, 2011; **3 Suppl 1:**94-100.

9. Akhtar MW, Mirza AQ, Chughtai MID. *Applied and Environmental Microbiology*, 1980; **40 Suppl 2:**257-263.
10. Arima K, Liu WH, Beppu T. *Agricultural and Biological Chemistry*, 1972; **36 Suppl 5:**893-895.
11. Petrovic SE, Skrinjar M, Becarevic IF, Banka L. *Biotechnology Letters*, 1990; **12 Suppl 4:**299-304.
12. El-Sheikh HH. Ph.D. thesis, Department of Botany, 1982; *Al-Azhar University, Cairo,* Egypt.
13. Adham NZ, Ahmed EM. *Indian Journal of Microbiology*, 2009; **49 Suppl 1:**77-83.
14. Maia MDMD, de Morais MMC, de Morais MA Jr., Melo EHM, et al. *Rev. Microbiol*, 1999; **30 Suppl 4:**304-309.
15. Bornscheuer UT. *Microbiology Review*, 2002; **26 Suppl 1:**73-81.