Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Screening And Identification Of Thermophilic Fungi For The Production Of Extracellular Lipase From Vegetable Waste Compost.

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ABSTRACT

The lipase producing fungi were screened from the compost by both qualitative and quantitative by titrimetric method with the objective to findout the thermophilic fungus (*Malbranchea cinnamomea*) capable of producing an enzyme lipase. The thermophilic fungi were isolated from vegetable waste compost on Potato Dextrose Agar media following the serial dilution technique. The thermophilic fungal isolate was identified as *Malbranchea cinnamomea* by MTCC, Chandigarh. The higher lipolytic activity of 7.66 ±0.36^{bc} U/ml was recorded on 6th day of incubation period. The present study was under taken to investigate the production of an extracellular lipase activity from the thermophilic fungi *Malbranchea cinnamomea* at different incubation time and the maximum lipase activity was observed on 6th day of incubation period at pH 6.8.

KEY WORDS: Lipase, *Malbranchea cinnamomea*, Olive oil, Thermophilic fungi, Tween 80.

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INTRODUCTION

Thermophilic fungi are those micro-organisms that survive at high temperature. Among various eukaryotic organisms, only some species of fungi have the ability to thrive at temperature between 45 to 55ºC. These fungi were known as thermophilic and thermotolerant forms and were distinguished on the basis of their minimum and maximum temperature of growth.

Thermopilic fungi are generally found in soil, composts, stored grains, bird's nest, wood chips and municipal waste compost. Thermophiles provide a major advantage for industrial and biotechnological processes because of their efficient growth at higher temperature. Enzymes from thermophiles are generally more stable than mesophiles as it can catalyze all the reactions at high temperature.

In compost, thermophilic fungi have the ability to produce a variety of cell wall degrading enzymes[1]. The filamentous fungi are considered the best producers of extracellular lipases for large scale production[2]. The production of lipases is influenced by many factors such as pH, temperature, carbon, Nitrogen[3].

Interest in thermophilic fungi with thermostable enzymes mainly is due to the fact that most of the existing industrial enzyme processes run at high temperatures using enzyme from mesophilic sources[4,5]. There are many advantages in using thermostable enzymes in industrial processes as compared to thermolabile enzymes[6,7].

Lipases are ubiquitous hydrolytic enzymes which catalyze the hydrolysis of triglycerides to glycerol and free fatty acids. In addition, lipases also catalyze the hydrolysis, trans esterification and synthesis of other esters. Due to the capacity of lipases to catalyze precise chemical transformation, they are widely used in detergent, cosmetic, food, organic synthesis and pharmaceutical industries[8,9].

The interest in microbial lipase production has increased in the last decades, because of its large potental in manufacturing applications as additives for nutrition (flavour modification), fine chemicals (synthesis of esters), waste water treatment (decomposition and removal of oil substances), cosmetics, pharma, leather and medicine[10-12].

Microbial lipases have assumed a great deal of importance as industrial enzymes in view of their potential for use in various biotechnological processes. Fungi are the important producers since they produce enzymes extracellularly[13].

MATERIALS AND METHODS

Isolation:

Vegetable wastes (Cabbage, tomato and mixed vegetable) are collected from different markets in and around Bangalore and were brought to the Department of Botany Bangalore

University Bangalore and composted in heaps and pits. The samples were collected during thermophilic state of composting (50-55 ºC) in polythene bags and used for further process. The dilution plate technique[14] was employed to isolate fungi from vegetable waste composts. The thermophilic fungi were isolated on Potato Dextrose Agar media (PDA).

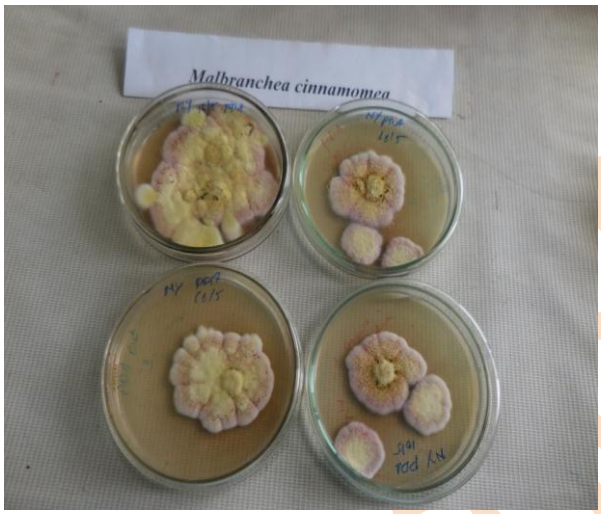

Fig 1:- Plates showing pure colonies of *Malbranchea cinnamomea* (5 days old).

Identification:

The culture were identified from Microbial Type Culture Collection Centre (MTCC, Chandigarh) as *Malbranchea cinnamomea* and deposited with reference number **MTCC 12145**.

Culture characteristics:

The genus *Malbranchea*, has a sclerotial conidioma, conidiophores reduced, hyaline, conidiogenous cells, thallic, arthic, conidia hyaline, in coiled chains, 1-celled.

Malbranchea cinnamomea is thermophilic, colonies appeared robust, almost filling the petridish, dense, thick, smooth, velvety, with coarse, creamy yellow tufts of hyphae. The colour is sulphur yellow with yellow to pink margin. Large deposits of dark brown exudates present, the medium turns dark brown to black. The arthroconidia are borne as curved or loosely coiled lateral branches arising from the broader vegetative hyphae, the conidia are cylindrical, often curved, thick walled, hyaline at first, later yellow 2.5-3.5 µm diameter.

Screening of fungi for the production of extracellular lipase:

The medium of Ullman and Blasins[15] was used which has the following composition (g/L).

Peptone-10g

$MgSO_4.7H_2O$-0.2g

$Cacl_2.2H_2O$-0.2g

Agar-20g

Distilled water-1000 ml

The medium was sterilized by autoclaving at 121 ºC for 20 minutes. Tween 80 (10ml) was autoclaved separately and added to the sterile and cooled basal medium. The medium was dispensed aseptically in sterile petridishes followed by inoculation by fungal isolates and petriplates were incubated at 50 ºC for 7 days, the lipolytic ability was observed as a visible precipitate due to the formation of crystals of calcium salt of the oleic acid liberated by the enzyme. The depth of precipitate (mm) was measured.

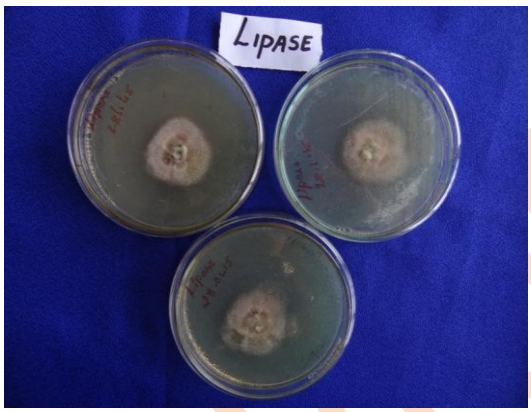

Fig 2: Deposition of calcium crystals indicating the presence of lipase activity from *Malbranchea cinnamomea*.

Lipase assay by titrimetry method:

Lipase activity in the broth or mycelia was determined titrimetrically on the basis of olive oil hydrolysis Macedo *et al*. [16]. One ml sample was added to the assay substrate containing 10 ml of 10% homogenised olive oil in, 2 ml of 0.6% $CaCl_2$ solution and 5 ml of 0.2 mol/L citrate buffer pH 7.0. The enzyme substrate mixture was incubated on orbital shaker with a shaking speed of 100 rpm at 37 ºC for an hour the reaction was stopped by adding 20 ml acetone mixture (1:1) to the reaction mixture. The fattyacids thus liberated was titrated against 0.1 mol//L NaOH. Intracellular lipase activity was expressed as units per gram mycelium and extracellular lipase activity as units per ml of the broth. One 'lipase unit' (U) was defined as the amount of the enzyme that released one milli mole fattyacid per minute.

DATA ANALYSIS

The data obtained was subjected to statistical analysis by one way/two way ANOVA, significant 'F' ratios between groups means were further subjected to Duncan's Multiple Range Test (DMRT) using statistical software SPSS version 20. Probability values <0.05 were considered as significant.

RESULT AND DISCUSSION

The lipase production ranged from 1.3 ± 0.07^{ab} to 7.66 ± 0.36^{bc}. The maximum lipase activity was found to be 7.66 ± 0.36^{bc}on 6th day of incubation at temperature of 55 ºC.

Table 1: Quantitative estimation of lipase activity from *Malbranchea cinnamomea*

Incubation time (days)	Lipase activity (U/ml)
1	1.3 ± 0.07^{ab}
2	3.4 ± 0.07^{ab}
3	4.73 ± 0.12^{bc}
4	6.0 ± 0.05^{bc}
5	7.03 ± 0.23^{bc}
6	7.66 ± 0.36^{bc}
7	6.1 ± 0.35^{bc}
8	5.03 ± 0.08^{bc}
9	4.6 ± 0.14

There was a gradual decrease in lipase activity from 8th day of the incubation period. The influence of temperature on lipase production was recorded. The enzyme activity was estimated at 24 h intervals in stationary culture filtrates.

Previous studies by Anuradhabalan *et al.* [17] reported the thermostable lipase from *Geobacillus thermodenitrificans*. The optimum temperature for thermostable lipase was 65 ºC. Thermostable lipase activity was highest at pH 7.0 and stable for 16 hours at this pH and 65 ºC temperature. According to Praveen kumar *et al.* [18], the *Trichoderma* sp. showed maximum extracellular protein and lipase activity of about 7.83 U/ml at 4th day of the incubation period.

CONCLUSION

The present study revealed that the potent lipase producing thermophilic fungi was isolated, identified as *Malbranchea cinnamomea* and screened from vegetable waste compost. The maximum lipase activity was shown at 6th day of incubation period. In conclusion the organism thus isolated from compost sample has an ability to produce large quantities of extracellular lipase at 55 ºC temperature and 6.8 pH.

ACKNOWLEDGEMENTS

The authors are grateful to DST for providing financial support under PURSE Programme, and also laboratory facilities provided by Department of Botany, Bangalore University, Bengaluru, India.

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