Psychrophilic Yeast Isolates for Cold-Active Lipase Production

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Abstract - Lipases are glycerol ester hydrolases which hydrolyze triglycerides to glycerol and free fatty acids. Different cold active lipase producers which were bacterial and yeast isolates screened from cold stored spoilt coconuts, milk samples, nuts and vegetables. Cold-adapted and psychrophilic microorganisms are a good source of cold-active enzymes that have potential applications. Different lipolytic isolates both bacteria and yeasts were obtained, and amomg these the yeasts were selected for further study as they are Generally Regarded as Safe (GRAS). Among the six lipolytic yeasts two isolates identified as Geotrichum and Rhodotorula sps was found to be good cold active lipase producers and hydrolyzed palm olein on a selective agar incorporated with Nile Blue sulphate and palm olein. Lipolytic activity of the isolates was indicated by accumulation of blue droplets beneath the colony. Lipases isolated from different sources have a wide range of properties depending on their sources with respect to thermo stability, positional specificity, pH optimum and fatty acid specificity etc. Palm olein was used as an inducer for inducing lipase production. The selected yeast isolates showed efficient enzyme production at 25^0C and pH 7.2. The enzyme produced was active at both 15^0C and 20^0C, thus making it a potential enzyme for application in dry cleaning industry. Cold active lipases also have potential application in industries like food, cosmetic and pharmaceutical. They also have significant environmental applications in psychrophilic environment and so the present isolate a cold-active lipase producer is of both industrial and environmental significance.

Keywords: Cold-active, Geotrichum sps, Lipase, Palm-olein, Rhodotorula sps.

I. INTRODUCTION

Lipases are triaclyglycerol acylhydrolases (EC 3.1.1.3) which catalyze the hydrolysis of triglycerides to free fatty acids and glycerol [1]. They constitute the most important group of biocatalysts for biotechnological applications. They also express other activities such as cholesterol esterase, phospholipase, isophospholipase, cutinase, amidase, cholesterol esterase etc.[2]. Lipases also have the ability for biotransformation and are hence popular in industries like food, detergent, cosmetic, leather, textile and pharmaceutical industries [3]. They are also employed for production of biodiesel, degradation of oil spills, effluent treatment etc. They thus have an environmental application [4]. Microbial lipases have special attention industrially due to their stability towards extremes of temperature, pH and also because they have broad substrate specificity [5], [6]. Lipases are ubiquitous in nature and are active at different temperatures. The cold active lipases have good activity in the temperature range of 0-30^0C [7]. Many microbes like bacteria, yeast and fungi are known to be potential producers of cold active lipases [8]. Cold active lipases from yeast are preferred as they are GRAS organisms, have shorter fermentation cycle and also carry out reactions at low temperatures which directly reduces the energy needs. In the present study cold-active lipase producing yeasts identified morphologically and biochemically as *Geotrichum sps* [9] and *Rhodotorula sps* were isolated and tested for their ability to degrade different oils. The selected yeast isolates produced cold active lipase at 25^0C and the enzyme was active at 15^0C and 20^0C. Thus these good cold active lipase producers have potential industrial and environmental significance.

II. DESIGN OF THE STUDY

The design of the study was collection of source samples for isolation of lipolytic yeasts by enrichment culture technique. Potential psycrophilic cold-active lipase producing yeasts were selected based on enzyme production at 30^0 C. The selected isolates were tested for production using diverse lipid rich sources like oils and fats. The enzyme activity was tested at 15^0 and 20^0 C so that these enzymes could be used commercial for food application or oil degradation at low temperatures.

III. PREVIOUS WORK

A wide range of cold-adapted lipases have been reported from psychotropic and psychrophilic microorganisms which are found inhabiting extreme cold regions like Antarctica, deep sea environments and refrigerated food samples. [10], [11], [12]. Mostly the important lipase-producing organisms belonging to bacterial genera are *Bacillus, Pseudomonas*

and Burkholderia [13] and fungal genera include [14] *Aspergillus, Penicillium, Rhizopus, Candida.* Some of the lipase producing yeasts belong to seven different genera which include *Zygosaccharomyces, Saccharomyces, Kluyveromyces, Pichia, Lachancea, Candida,* and *Torulaspora* [15]. A mesophilic yeast of the subtropical region identified as *Geotrichum sp.,* was reported to produce two kinds of cold active lipases which are found to be stable at room temperature,[7] a bacterial isolate namely *Pseudomonas taiwanensis* produced cold active lipase active at 15^0C [4]. An efficient isolate identified as *Pseudomonas gessardii* isolated from oil spilled soil from vegetable oil processing factories is reported to produce extracellular lipase at mesophilic temperature [3]. An isolate named as DVL2 which was isolated from common city garbage produced both extra and intra cellular lipases that were active at mesophilic temperature[16].

IV. METHODOLOGY

COLLECTION OF SOURCE SAMPLES: Diverse sources like cold stored spoilt coconuts, milk samples, nuts and vegetables were screened for lipase producers using enrichment culture technique.

PRIMARY SCREENING: Modified Nutrient Agar, and Modified YEPD agar were used for screening of the primary isolates in which palm olein oil and olive oil were used as lipid sources. Plates were incubated at 30^0 C and observed for growth.

SECONDARY SCREENING: The Lipolytic activity of primary isolates was indicated by the accumulation of insoluble blue droplets beneath the colonies on addition of Palm olein and 0.1% aqueous Nile blue sulphate[17] indicating the release of free fatty acids. Positive lipolytic isolates from the oil agar medium were further cultured and assayed for lipase production in broth at 15^0 and 20^0C . The isolates were identified based on cultural, microscopic and biochemical studies.

LIPASE PRODUCTION: Enzyme was produced by submerged fermentation in 250 ml Erlenmeyer flasks containing 50 ml of Lipase induction broth containing, peptone-0.1%, glucose-0.2%, yeast-extract-2 %, dipotassium hydrogen phosphate-0.1%, 0.5 % ammonium sulphate -0.1% along with 2% w/v palm olein and a pH of 7.2 [18].The flasks were inoculated with actively growing yeast cultures and incubated at 30^0C. The broth samples were collected after every 24 hrs and assayed for lipase activity both at 15^0 and 20^0C. Yeast isolates with good lipase activity were selected and studied for degradation of different commercial oils at 15^0 and 20^0C.

LIPASE ASSAY: One ml of culture broth was cold centrifuged at 4^0C, 5000 rpm for 10 minutes. Supernatant was taken as the enzyme source. The enzyme assays were carried out titrimertrically using olive oil as a substrate at both 15^0 & 20^0C against NaOH using thymolopthalein indicator.

V. RESULTS

Lipolytic organisms were screened from diverse sources. Diverse bacterial and yeast isolates were obtained (Fig 1). Lipolytic isolates that hydrolyzed palm olein produced blue coloured droplets that accumulated beneath the colony indicating the release of free fatty acids on the selective agar medium (Fig 2 A). The lipolytic activity of the enzyme in broth was studied by agar well assay and this was indicated by presence of blue droplets in hydrolysis zone around the agar well (Fig 2 B). Free fatty acids were released in the medium and this was indicated by the decrease in pH of the medium from pH 7.2 to 5. Formation of insoluble blue droplets were also observed even in the broth medium both at 200 & 250C when tested for lipolysis with incorporation of 0.1% aqueous Nile blue sulphate using different oils like olive, ghee, vanaspathi, sunflower, coconut and palm olein and also indicating a decrease in pH (Fig 3).

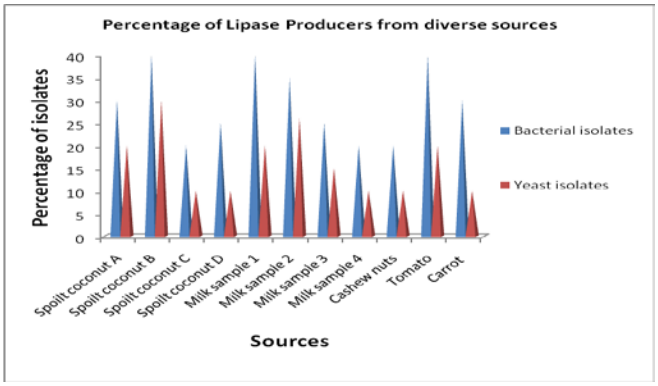
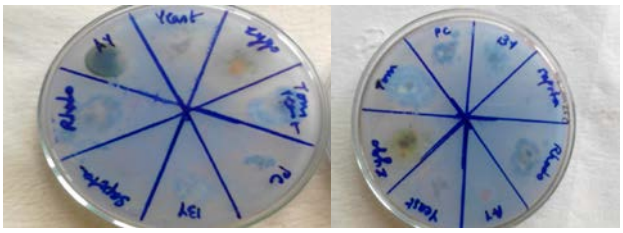


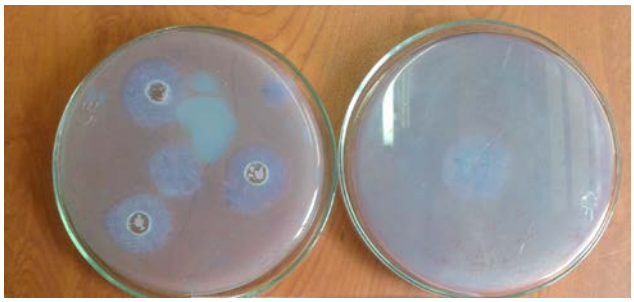
Fig 1: Different lipolytic bacteria and yeasts from diverse sources.

Six lipolytic yeasts were obtained of which two of the yeast isolates identified culturally, morphologically and biochemically as Rhodotorula sp. and Geotrichum sp were found to be a good cold-active lipase producers.

Cold-active enzyme yield was more when grown at 250C and assayed at 150 & 200C (Figure 4 & 5). Thus making the isolates potential cold-active lipase enzyme producers. The peak production time in fermentation cycle for the two isolates was noted and the isolates were tested for lipase production at 150 & 200C using different oils (Figure 6 & 7).



A



B

Fig 2: A: Lipolysis indicated by accumulation of blue droplets beneath the colony.

B: Accumulation of blue droplets outside the agar well indicating lipolysis by cold active lipase in broth.

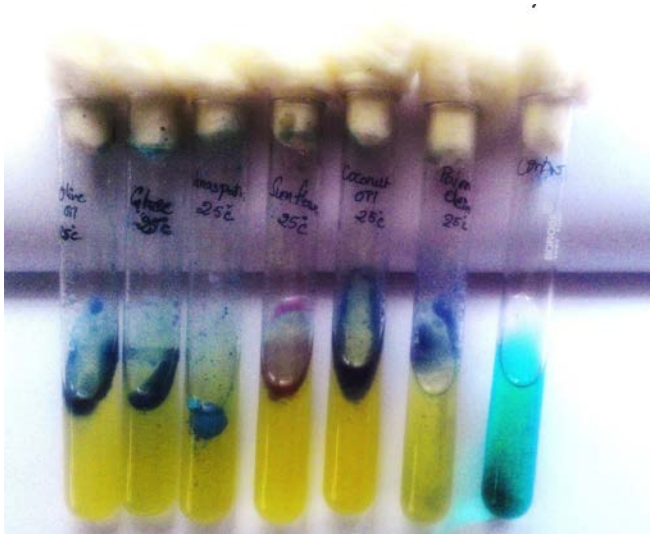


Fig 3: Formation of insoluble blue droplets in broth indicating the lipolysis of different oils.

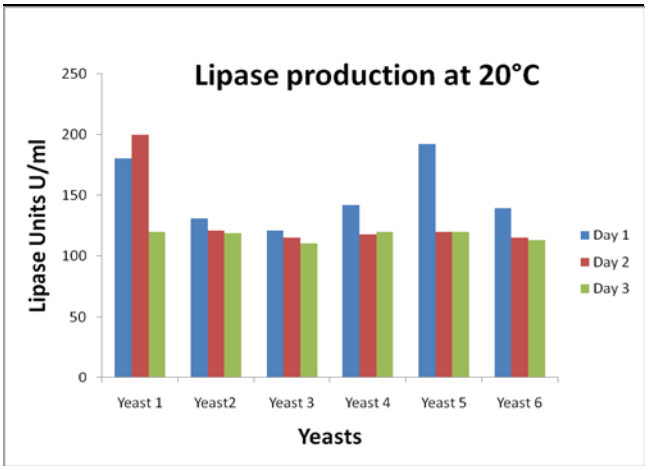


Fig 4: Lipase production of the primary yeast isolates at 20^0C

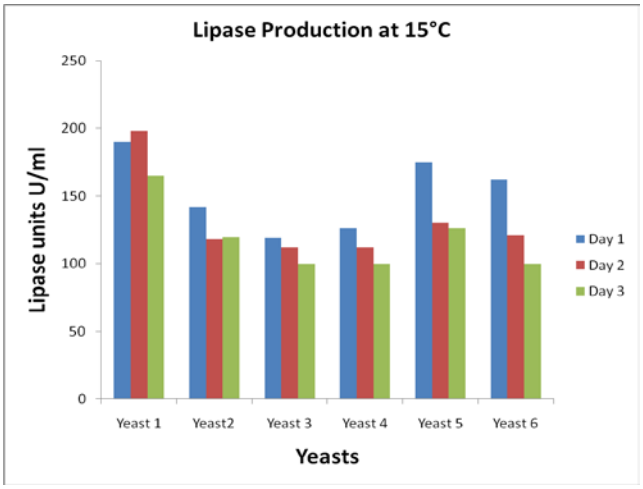


Fig 5: Lipase production of the primary yeast isolates at 15^0C

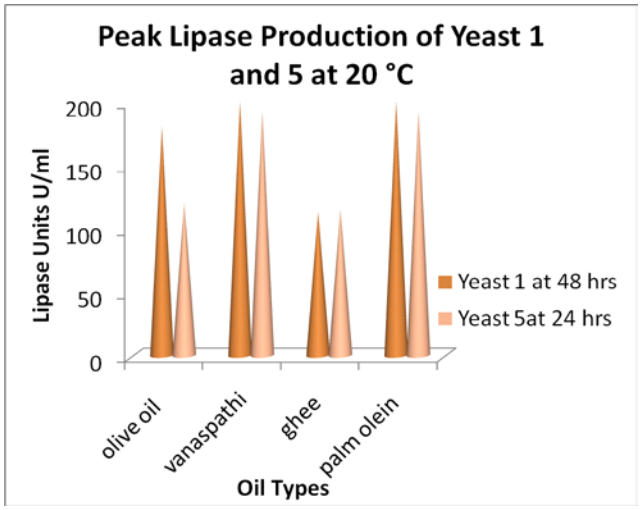


Fig 6: Peak lipase enzyme production at 20^0C by the selected yeast isolates using different oils

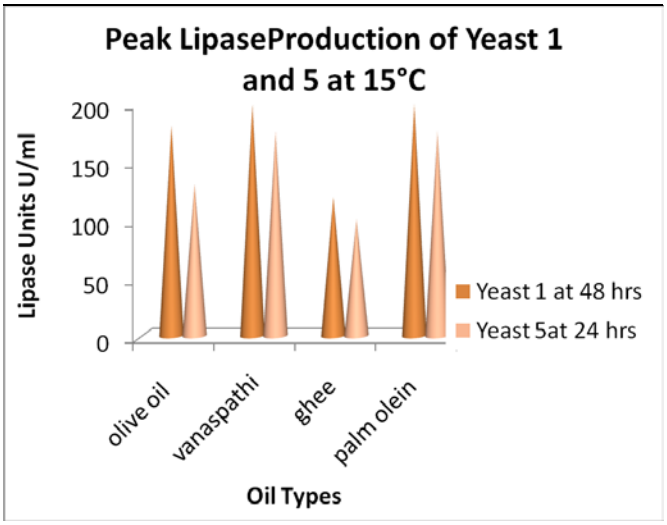


Fig 7: Peak lipase production of selected yeast isolates using different oils at 15^0C.

VI. CONCLUSION

Different types of yeast isolates were obtained from souces like cold stored spoilt coconut and milk samples... Two efficient lipolytic isolates with highest cold-active lipase production namely yeast 1 and yeast 5 identified as Geotrichum sp and Rhodotorula sp respectively were selected. Lipase production by the selected strains was tested in a wide range of commercial oils like sunflower oil, olive oil, coconut oil, vanaspathi and ghee at 300C and cold-active enzyme activity was tested at 150 and 200C.

The highest cold-active lipase activity for Geotrichum was observed at 48 hrs and at 24 hrs for Rhodotorula at both 200C & 150C indicating them to be potential sources for cold active enzyme application at a commercial scale.

VII. FUTURE SCOPES

Thus these isolates could be exploited for cold-active lipase production which has major role in food cosmetic, detergent and pharma industry along with environmental applications.

VIII. ACKNOWLEDGMENT

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