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STUDIES ON THE INFLUENCE OF VARIOUS FACTORS ON LIPASE PRODUCTION USING *PSEUDOMONAS CEPACIA*

B.DHEVAHI[*1] AND R.GURUSAMY[2]

[1] Department of Science and Humanities, Kongunadu College of Engg & Tech, Tholurpatti, Thottiyam-Tk, Trichy-Dt- 621215, TamilNadu, India
[2] Department of Science and Humanities, Hindusthan Institute of Technology, Coimbatore- 641032, Tamil Nadu, India

ABSTRACT

Food Processing industry is always expecting economic and green technologies for the fat and oil modification. Microbial lipases are of immense importance and have gained special industrial attention in almost all industries . A new strain of lipolytic *Pseudomonas cepacia* was isolated from the oil industrial waste soil on a selective medium that contained olive oil as the only source of carbon and energy. The isolated strain was cultivated for lipase production in shake flasks at 30±1^{0}C and the effect of various fermentation conditions were studied. The maximum extracellular lipase activity of 15.15 U/ml and 15.30 U/ml were noticed for 30 g/l sucrose and 25 mg/l yeast extract respectively. The optimal cultural temperature was 40^{0}C and pH 6.0. Metal iron (Fe) also enhanced the lipase yield as supplementary.

KEYWORDS: ***Pseudomonas cepacia,*** **Sucrose, Yeast Extract, Iron, Temperature, Correlation**

B.DHEVAHI
Department of Science and Humanities, Kongunadu College of Engg & Tech, Tholurpatti, Thottiyam-Tk, Trichy-Dt- 621215, TamilNadu, India

INTRODUCTION

Lipases are hydrolytic enzymes that act in aqueous-organic interfaces, catalyzing the cleavage of ester bonds in triglycerides and producing glycerol and free fatty acids[1]. However, in environments with low water availability, lipases are able to catalyze esterification, interesterification and transesterification reactions, being thus very versatile biocatalysts[2,3,]. The growth and application of commercial enzymes are very significant and promising. Lipases occur widely in nature, but microbial lipases are commercially significant because of low production cost, greater stability and wider availability than plant and animal lipases. Lipases produced from microorganisms are formed extracellularly and may originate from bacteria or fungi. Especially microbial lipases have different enzymological properties and substrate specificities, many species of bacteria, yeast and molds are found to produce lipases[5]. Lipolytic enzymes are currently attracting an enormous attention because of their biotechnological potential[6]. They constitute the most important group of biocatalysts for biotechnological applications. Furthermore, novel biotechnological applications have been successfully established using lipases for the synthesis of biopolymers and biodiesel, the production of enantiopure pharmaceuticals, agrochemicals, and flavor compounds[7]. The chemo-regio and enantio-specific behavior of these enzymes caused tremendous interest among scientists and industrialists[8].

Lipases are produced by several microorganisms namely bacteria, fungi, archea, eucarya as well as by animals and plants. Commercially useful lipases are usually obtained from microorganisms that produce a wide variety of extracellular lipases[9]. Microbial lipases are of special interest because of their stability in organic solvents and their lack of requirement for cofactors, their broad substrate specification and their high enantioselectivity[10]. Lipases have also been used for addition to food to modify flavour by synthesis of esters of short chain fatty acids and alcohols, which are known as flavour and fragrance compounds [11]. Psychrotrophic Gram-negative bacteria, such as *Pseudomonas* species, pose a significant spoilage problem in refrigerated meat and dairy products due to secretion of hydrolytic enzymes, especially lipases and proteases and that study characterized the enzymes produced by strains of *P. fluorescens* isolated from pasteurized milk[12]. In this paper, we demonstrated the optimum conditions required for the lipase production by *Pseudomonas cepacia* in shake flask cultures. Because the composition of the medium affect the production of lipase dramatically, it is important to understand the influences of the various factors and determining the optimum cultivation conditions. Furthermore, the ability to increase the productivity of the lipase may be of great benefit because lower production costs could lead to new industrial applications.

MATERIALS AND METHODS

(i) Microorganism and Inoculum's

Pseudomonas cepacia was isolated from the oil contaminated soil by serial dilution and pour plate techniques[13]. The isolate was identified as *Pseudomonas cepacia* by biochemical tests which were carried out according to Bergey's Manual of Determinative Bacteriology[14]. The strain was grown in nutrient broth at 37 ºC for 24 hrs and it was screened for the ability to produce lipase with olive oil. Culture was maintained on nutrient agar media and transferred to a fresh agar plate every month, grown at 28 ^{0}C for approximately 3 weeks and stored at 4 ^{0}C. The nutrient agar medium was incorporated with yeast extract (1.5 g/l), Beef Extract (1.5g/l), NaCl (5.0g/l), Agar (15g/l), tryptone (5 g/l), KH_2PO_4 (1 g/l), $MgSO_4 \cdot 7H_2O$ (1 g/l), thiamine (1 g/l), glucose (5 g/l).

Fermentation by Shake flask culture

Fermentation was carried out in shake flasks using a complex medium as follows: Olive oil - 20 g /l (emulsified in 2% gum acacia), Egg yolk- 10 g/l, Ammonium chloride- 4.0 g/l, Magnesium sulphate heptahydrate -0.25 g/l, Dipotassium phosphate - 0.5 g/l, Calcium carbonate - 5.0g/l.

The fermentation media was then inoculated with 5ml of cells suspension and incubated at 35±1°C on a rotary shaker (120 rpm) for 48hrs. The initial pH of the culture medium was about 7. The effect of different concentrations of sucrose as carbon source, yeast extract as nitrogen source and iron on lipase production was studied individually. The effect of these data was obtained using 100 ml of basal medium at an initial pH 6, at 30 ^{0}C and with agitation at 150 rpm/min for 3 days. The same organism were subjected to different environmental conditions and the effects of the condition were studied. The effect of different pH values (3 to 7) and different initial temperature (25 to 50^{0}C) on the bacterial culture was studied using shake flask cultures. The pH was adjusted to the desired value by addition of either 1N NaOH or 1N HCl. The pH and temperature was measured using a digital pH meter and thermometer respectively. All experiments were carried out at least in duplicate to ensure reproducibility.

(ii) Lipase assay

Lipase activity in the broth was determined titrimetrically on the basis of olive oil hydrolysis[15]. One ml sample solution was added to the assay substrate containing 10 ml of 10% homogenized Olive oil in 10% gum acacia, 2 ml of 0.6% $CaCl_2$ solution and 5 ml of 0.2 mol/L citrate buffer, pH 7.0. The above mixture was incubated at 37°C for 1 hr in an orbital shaker.To stop the reaction, 20 ml of ethanol acetone mixture (1:1) was added to the reaction mixture. Liberated fatty acids were titrated with 0.1 mol/l NaOH and the extracellular lipase activity was expressed as units per ml of the broth. One 'lipase unit' (U) was defined as the amount of the enzyme that released one mole fatty acid per minute.

(iii) Statistical Analysis

All the data's were statistically analyzed by MINITAB 12.2 software (http:/www.minitab.com) and found the correlation between the cultural factors and lipase activity.

RESULTS AND DISCUSSION

In this study, fermentation was carried out in shake flasks using a complex medium amended with different concentration of sucrose (5, 10, 15, 20, 25, & 30 g/l) and yeast extract (5, 10, 15, 20, 25, & 30 mg/l) as a carbon source and nitrogen sources respectively. On the 4th day the culture was filtered and its supernatant was used for the analysis of enzymatic activity. The results were given in Fig. 1 and 2. The maximum lipase production was found to be 15.15 U/ml and 15.30 U/ml at 30 g/l sucrose and 25 mg/l yeast extract respectively. Various operating strategies have been evaluated to improve the yield of these enzymes[16, 17, 18]. In general, extracellular enzyme production depends greatly on the composition of the medium[19, 20]. Both the substrates sucrose and yeast extract showed a positive correlation (Correlation value for sucrose 0.718, and yeast extract 0.970) between their concentration and enzyme production and hence the bacterial growth depend on the concentration of sucrose and yeast extract. Lipase synthesis was found to increase in the presence of organic nitrogen sources[21]. Yeast extract was found to be very effective for the production of lipase in *Cryptococcs* sp S2[22].

Figure 1
Effect of sucrose on lipase production using Pseudomonas cepacia.

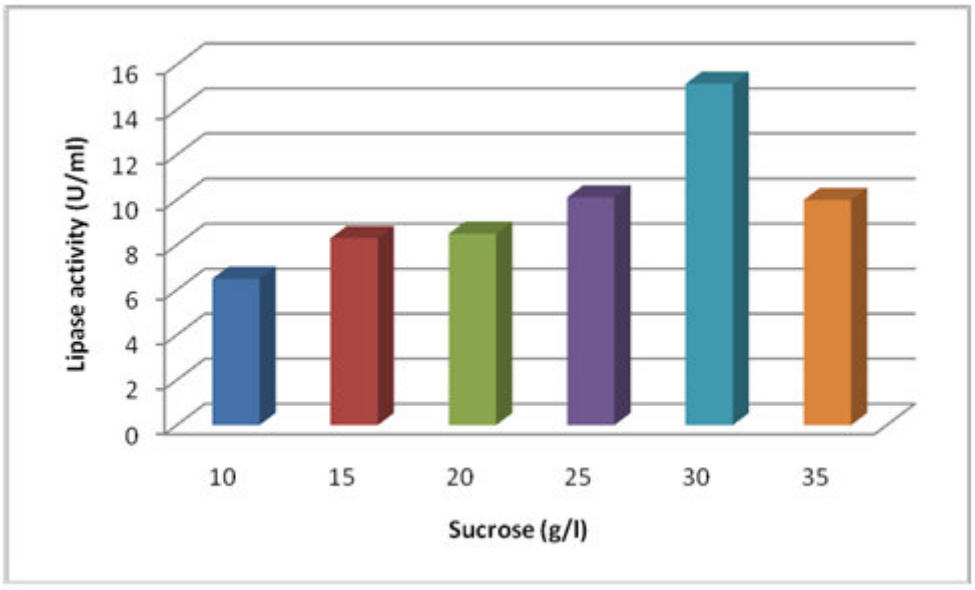
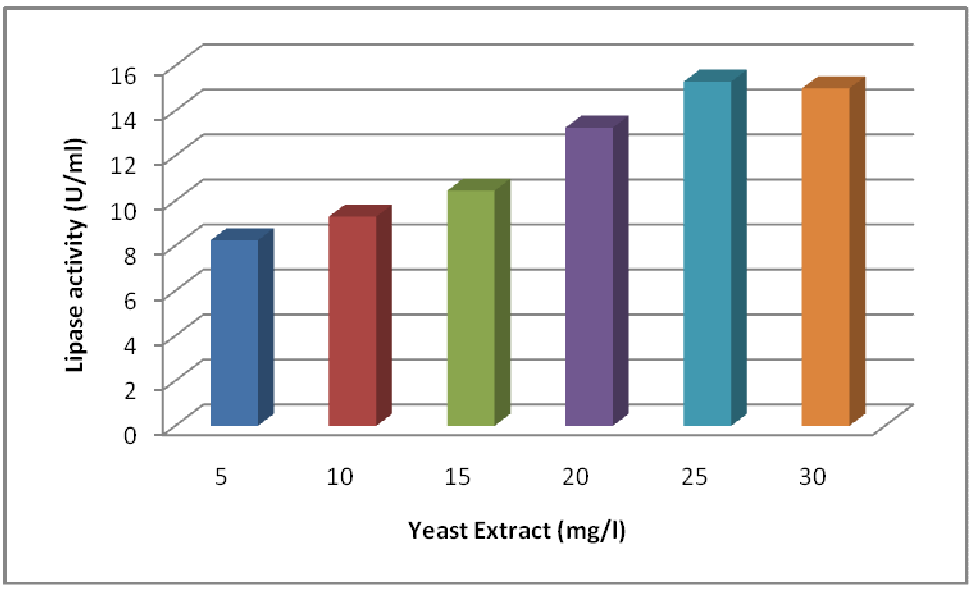
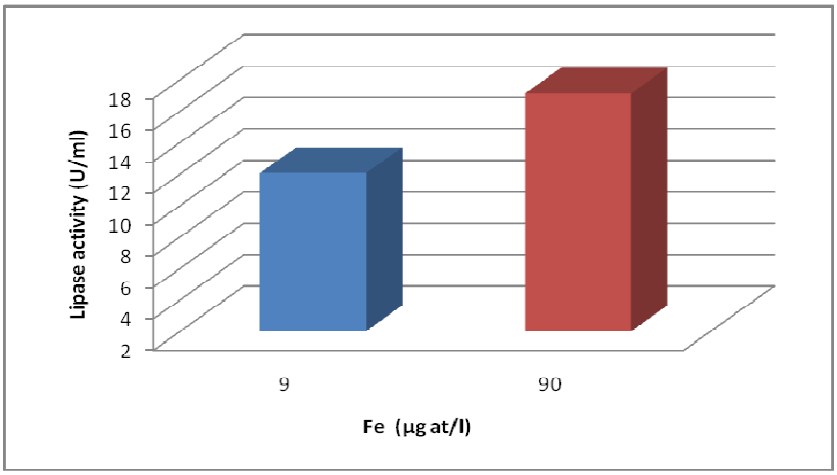


Figure 2
Effect of Yeast extract on lipase production using Pseudomonas cepacia.



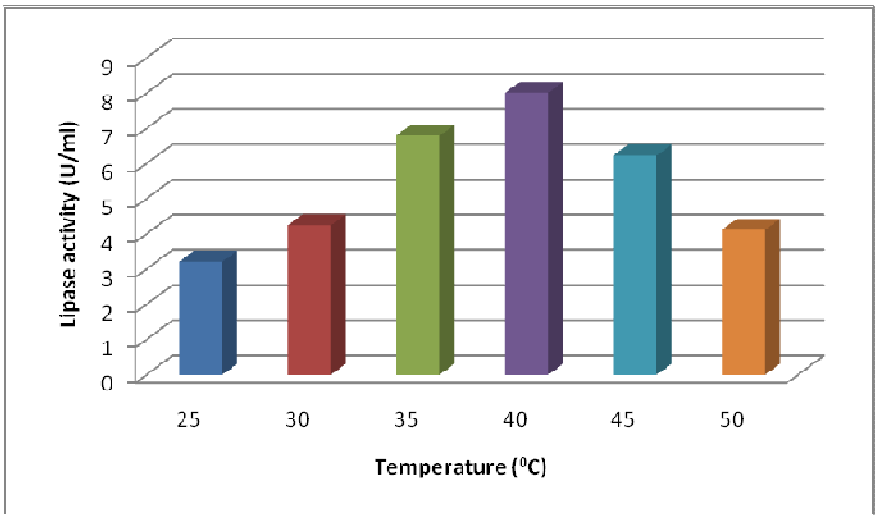
In this study we used different concentrations of Iron (Fe^{2+}) (9 and 90 µg/l) as additive in the basal medium to determine its stimulatory or inhibitory effect on lipase production. The results were given in Fig.3. Several authors have reported the stimulatory effects of metal ions on the lipase production of different organisms[23, 24]. In the present investigation the maximum lipase production was found at 90µg/l Fe. Both the concentration showed a positive correlation and hence Fe and lipase production are inter dependable.

Figure 3
Effect of Iron (Fe) on lipase production using Pseudomonas cepacia.



Influence of culture temperature on the lipase production by *Pseudomonas cepacia* was studied in the range of 25 to 50 ^{0}C, while keeping all other conditions as constant. The results were given in Fig. 4. The influence of higher temperature also showed significant when compared to the effect of lower temperature (25^{0}C) on lipase production. The optimal temperature determined for lipase production by *P. cepacia* was found to be 40^{0}C. The correlation of temperature and lipase production showed positive correlation (0.338). Even at high temperature (50^{0}C) the bacteria *P. cepacia* had shown the significant lipase activity and hence this new isolate would be thermopiles. A novel extracellular lipase with organic solvent tolerance was isolated from a local *Pseudomonas* species[25] This enzyme thermo stability was determined at various temperatures ranging from 20 to 90 °C. The maximum 100% lipase basal activity was achieved after being incubated at 37°C for 30 min and 96% activity was achieved when incubated at 45°C for 30 minutes. At 90°C the enzyme activity was 1%. The optimum value of temperature was determined as 55 °C[25].

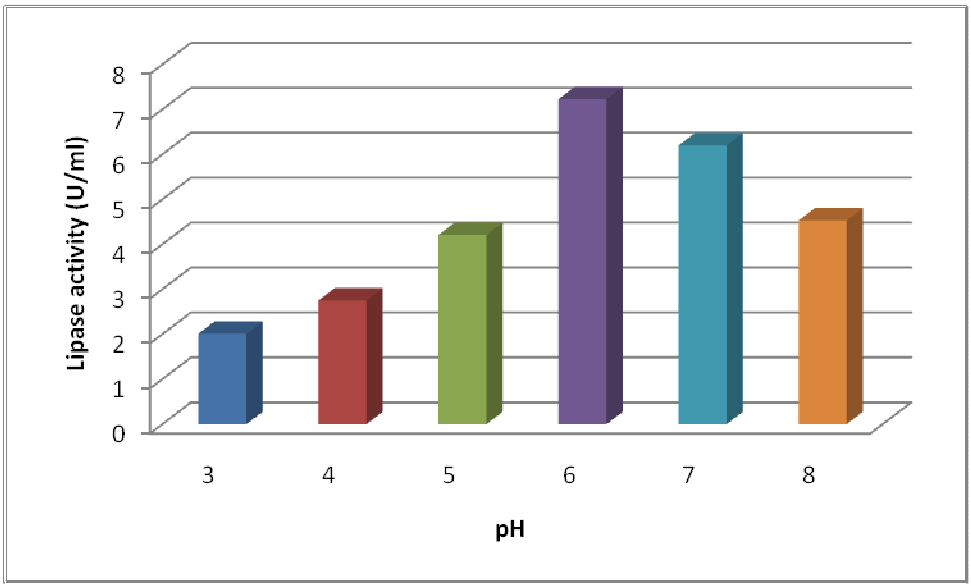
Figure 4
Effect of media temperature on lipase production using Pseudomonas cepacia.



The pH of the culture broth is one of the most critical environmental parameter which affects the microbial growth and their products. The pH profile for lipase from *P. cepacia* was

determined in basal medium adjusted to different pH values ranging from 3.5–7.0. The result shown in Fig. 5 indicated that the maximum enzymatic activity was observed when the initial pH of the medium was 6.0. Increasing culture medium pH from 6 to 8.0 had a significant inhibitory effect on the lipase activity. Correlation of pH and lipase activity was 0.700. The correlation is positive so lipase activity depends on the media pH. The bacteria *Bacillus sp LBN 4* produced maximum lipase at optimum temperature 50^0C and pH 7.0[26]. The *Bacillus licheniforms KDP* organism produced the maximum extracellular lipase at pH 7 and temperature 45°C[27] The optimal initial pH for lipase production by the fungi *Rhizopus* strain JK-1 in the basal medium was found to be 7.5[28].

Figure 5
Effect of media pH on lipase production using Pseudomonas cepacia.



CONCLUSION

For lipase production by microorganisms, an effective and inexpensive medium is very important. In this study we determined the environmental factors and nutritional requirements as main factors to develop an optimal medium for the production of the lipase by *P. cepacia*. From a series of experiments, we determined that the initial concentration of sucrose, yeast extract, Iron (Fe), pH and the temperature are the factors which mostly affect the production of lipase by *P. cepacia*. The optimum pH and incubation temperature for the lipase production were approximately 6.0 and 40^0C respectively. The maximum lipase activity was found to be 30g/l for sucrose, 25mg/l for yeast extract and 90µg/l for Fe. This preliminary study results prove that controlling the culture conditions and modifying the concentration of medium composition can dramatically enhance the production of lipase by *P.cepacia* because all the factors and the lipase production are interdependable. In the present study we optimized the influences of the various factors on the cultivation conditions for lipase production. In further studies purification and finding the Km value of the purified enzyme will be conducted.

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