International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.6, pp 393-399, 2017

Optimization of *staphylococcus saprophyticus* Lipase isolated from windrow Compost

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Abstract : Lipases are versatile biocatalysts that are used extensively in detergent and pharmaceutical formulations. Their superior value arises from specificity and efficacy as compared to chemical catalysts. In this study we have isolated a bacterial strain *Staphylococcus saprophyticus* (WCS1C2) that showed high lipase production of 140 units / ml from windrow compost bed. The culture parameters were optimized with altered conditions like temperature, pH, incubation time, substrate specificity and metalions. The lipidic substrates tested were coconut oil, olive oiland trybutyrin. The optimized conditions where maximum lipaseof 644 units/ml producedwere found to be with pH 6.0, incubation temperature at 27^0C and incubation period of 120 hrs.The carbon, nitrogen and metal ion source to be optimal for the production of lipase was found to be with olive oil; peptone + yeast extract combination and sodium chloride 1% respectively. Under optimized conditions, lipase production by *Staphylococcus saprophyticus* increased by 5 fold compared to unoptimized conditions.
Keywords : Lipase, Optimization, Enzyme activity, *Staphylococcus saprophyticus.*

Introduction

Lipases are serine hydrolases which has uncommon potential of acting at the lipid–water interface. Due to unique properties of lipases, the enzyme has been proved to be useful for wide range of biotechnological applications[1,2]. Lipases have been isolated and purified from fungi, yeast, bacteria, plant and animal sources[3]. Bacterial lipases are more economical and stable[4]. Currently bacterial lipases are of great demand because of potential industrial applications[5].Different genera of bacteria including *Streptomyces* spp. are known to produce lipase but among them *Achromobacter* spp, *Alcaligenes* spp, *Arthrobacter* spp, *Pseudomonas* spp and *Chromobacterium* spp have been well exploited for lipase production[6]. *Staphylococci* is the another genera shown the potential of lipase production. Staphylococcal lipases are classified as true lipases[2, 7, 8].

In most instances lipase production ability of Staphylococci has been related to their pathogenecity. Thus with few exceptions, there are almost no reports of attempts to purify and characterize lipases synthesized by the coagulase negativestaphylococci which are believed to be nonpathogenic. The *Staphylococci* whose lipases are been studied till date are either isolated from medical samples (pathogenic) or does belong to commensal microfloara of skin[9, 10]. In the present study lipase producing *Staphylococcus saprophyticus* was isolated from windrow compost bed and fermentation media was optimizedfor the lipase production using different substrates, pH, temperature incubation time and different metal salts.

Materials and Methods

Microorganism

The bacterial strain used in this study was isolated from windrow compost bedprepared in the Solid Waste Managementunit at Mercy College campus, Palakkad.The lipase production by this strain was observed by using Tributyrin Agar Medium. The strain was characterized as *staphylococcus saprophyticus* by 16s rDNA sequencing.

Lipase production in Fermentation Media.

The bacterium was initially cultured using medium containing (w/v): peptone (2%), yeast extract (0.5%), sodium chloride (0.5%), sodium carbonate (0.025%), and olive oil (1%),at p^H 7.2 and 37°C for 48hrs.Then 1% of enriched 24hrs culture was inoculated into a 100ml medium for subculturing and the flasks were incubated at 37°C in a rotating shaker at 150 rpm for 5days. The clarified supernatant after centrifugation at 10000 rpm for 30 minutes in a refrigerator centrifugewas used as a source of extracellular lipase enzyme.

Lipase assay

Lipase activity was determined spectrophotometrically using p-NPP (para nitro phenyl palmitate) as a substrate. Reaction mixture was incubated at 37°C for 10min. After incubation 1mL of ethanol was added in order to terminate the reaction. A control (0.05mM phosphate buffer) was run simultaneously which contained the same contents but the reaction was terminated prior to addition of the enzyme. Absorbance of the resulting yellow colored product was measured at 410 nm in spectrophotometer. The amount of enzyme that liberated 1µmol of p - nitrophenol from pNPP per minute under the assay condition was defined as one unit of lipase activity as showed by the following formula (U/ml) = µmol/ml.

Min

Optimization of various Physico - Chemical parameters

Optimization of various parameters is one of the most important approaches used for achieving the over production of enzymes in large quantities to meet industrial demands[11]. A variety of factors such as pH, temperature, duration of incubation, carbon sources and nitrogen sources, oil sources acting as inducers, surfactants and agitation are known to affect the production of lipases. Apart from individual factors, interactions of determinative factors will also have asignificant influence on the production of the enzyme [12].The extracellular lipase produced by *staphylococcus saprophyticus* WCS1C2 was first examined by cultivation of bacteria in selected media. This prompted optimize various parameters to improve production of bacterial lipase.

Optimization of Fermentation Media:

The fermentation media prepared was optimized with following factors such as Incubation period, pH,Temperature, Lipidic Substrates and metal salts.

Effect of incubation time on Lipase production

To determine the optimum incubation period for lipase production, the isolate was cultured in the production medium and incubated at 37ºC for different time durations (12, 24, 48, 72, 96,120,144 hours) and then assayed for lipase. The optimum incubation period achieved by this step was fixed for subsequent experiments

Effect of pH on Lipase production

To study the effect of pH of the medium on lipase production were performed with medium of different pH. The pH was adjusted to 5.0,6.0,7.0,8.0,9.0 and 10.0 using 1N NaOH or 1N H Cl. Samples were drawn at 2, 3, 4 and 5 days interval. Enzyme activity (U/ml) and protein concentration (mg/ml) of the samples were determined. The optimum pH achieved by this step was fixed for subsequent experiments.

Effect of temperature on Lipase production

Investigations on the effect of cultivation temperatures on lipase production have been carried out by incubating the medium at different temperatures. The lipase production was carried out at 27°C, 37°C, 47°C, 57°C and 67°C keeping all other conditions at their standard levels and then assayed for lipase. The optimum temperatures achieved by this step was fixed for subsequent experiments

Effect of substrates on Lipase production

Lipase production was accelerated by incorporation of suitable lipidic substrate. Different substrates such as Coconut oil, Tributyrin and Olive oil at 1% concentration were supplemented separately in the basal medium. After incubation in an optimal condition, the lipase was quantified.

Effect of metal salts on Lipase production

In order to study the effect of metal salts of the medium on lipase production, experiments were performed with different metal salts such as NaCl, KCl, $MgCl_2$, NH_4Cl and $FeCl_3$(1%) and assayed for lipase and optimal condition for lipase production was achieved.

Optimized Condition

The final production of lipase enzyme was done by culturing the organism in the desired medium with optimized conditionspH 6, 27°C, olive oil as substrate with NaCl 1%. After culturing, the culture supernatant was collected and the lipase activity was tested and confirmed the highest production of lipase.

Results and Discussion

The lipolytic activity of *S.saprocphyticus* was observed by the formation of halo around the colonies upon hydrolysis of Tributyrin by the enzyme lipase (Figure 1)

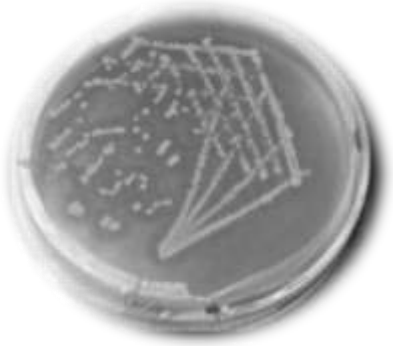

Figure 1: Lipase positive colonies on Tributyrin Agar

It was noted that the highest enzyme production was recorded on 5^{th} daywith 140 U/ml in basal medium and declined to 99U/ml after 5 days (Figure 2). In bacillus sp. Lipase production was found to be maximum after 7 days of incubation and then decreased gradually from 8^{th}day[13]. Among the tested substrates olive oil was emerged as the best substrate for maximum lipase production (140 U/ml). Among the different carbon sources used, olive oil was found to be the most suitable carbon source[14, 15, 16, 17].

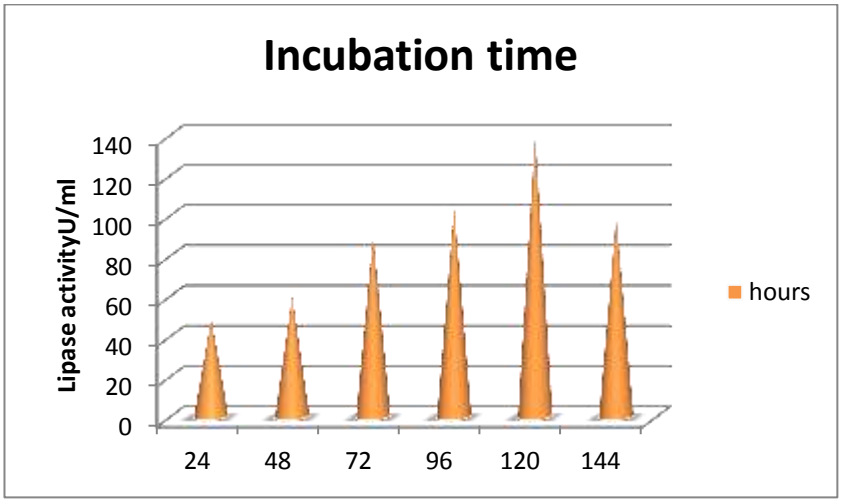

Figure 2: Effect of incubation period on lipase production by *Staphylococcus saprophyticus*

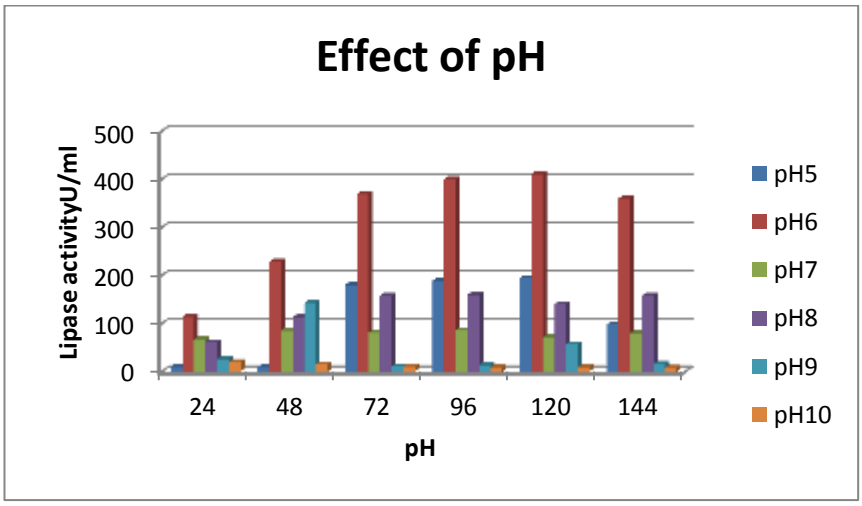

Figure 3: Effect of different pH levels on lipase production by *Staphylococcus saprophyticus* at different time interval

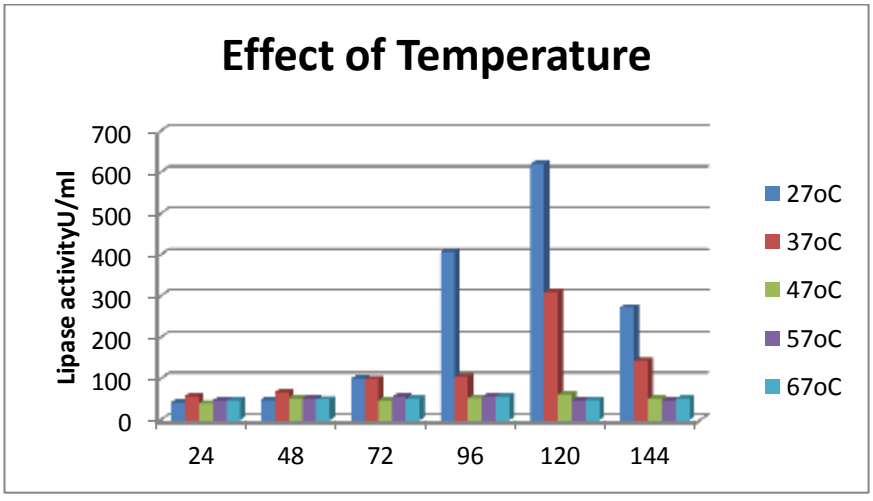

Figure 4: Effect of different incubation temperatures on lipase production by *Staphylococcus saprophyticus* at different time intervals.

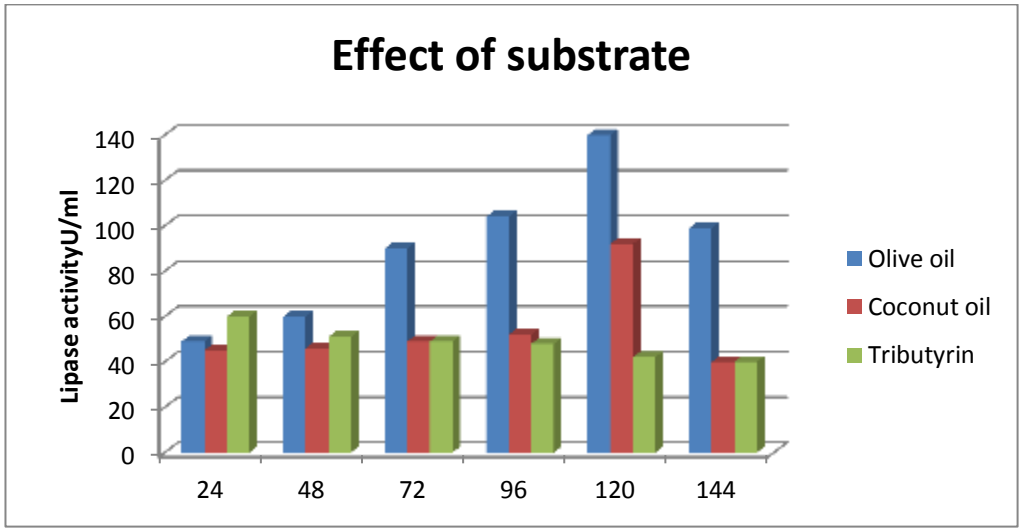

Figure 5: Effect of different lipid substrates on lipase production by *Staphylococcus saprophyticus* after 144hrs incubation

pH and temperature are the two important environmental factors which influences the lipase production. The pH of the production medium plays a critical role for the optimal physiological performances of the bacterial cell and the transport of various nutrient components across the cell membrane aiming at maximizing the enzyme yields. The effect of different medium pH at various incubation periods on lipase production resulted that pH 6 was the optimum for maximum lipase production (410U/ml) (Figure 3). The optimum temperature for lipase production was found to be 27°C showing lipase units of 620 U /ml (Figure 4).In *Candida rugosa* optimal condition for maximum lipase production noticed was pH 6.5at 30^{o}C. Among the carbon source used for identifying the most suitable substrate for lipase production olive gave maximum lipase production (140U/ml) while tributyrin gave minimum lipase production (42 U/ ml)(Figure 5).

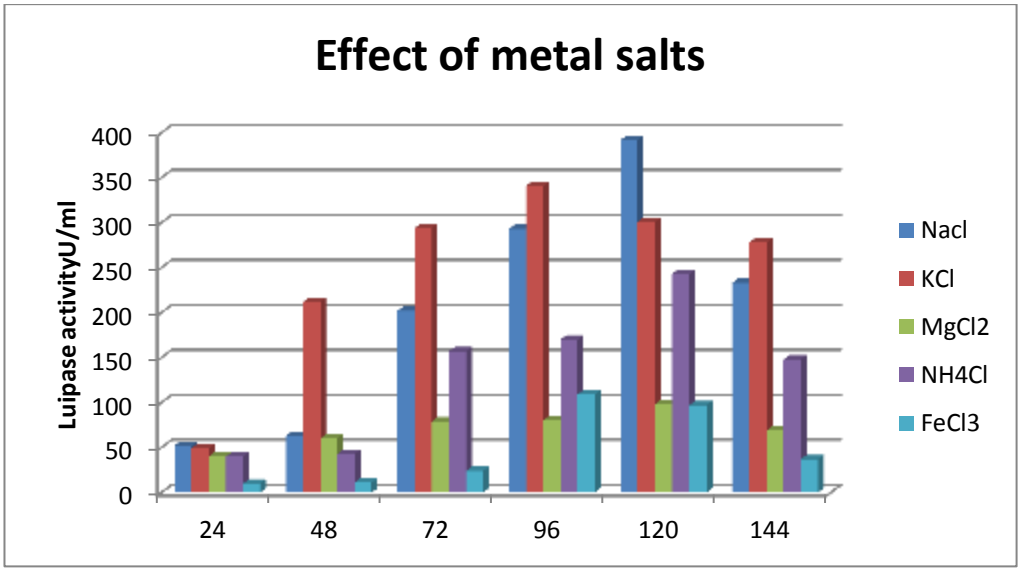

Figure 6:Effect of different metal ions on lipase production by *Staphylococcus saprophyticus*

The optimization with metal salts was found that the enzyme produced was maximum in the presence of sodium chloride (Figure 6). Lipase production was enhanced by using optimized conditions and substances. Minimal medium with 1% olive oil was selected as lipase production media. This medium contained only minerals and inorganic nitrogen source. The strain was enforced to produce lipase by using only olive oil as soulcarbon source. Olive oil was found to be the best among all the carbon sources used in case of *candiarugosa* lipase production[18].

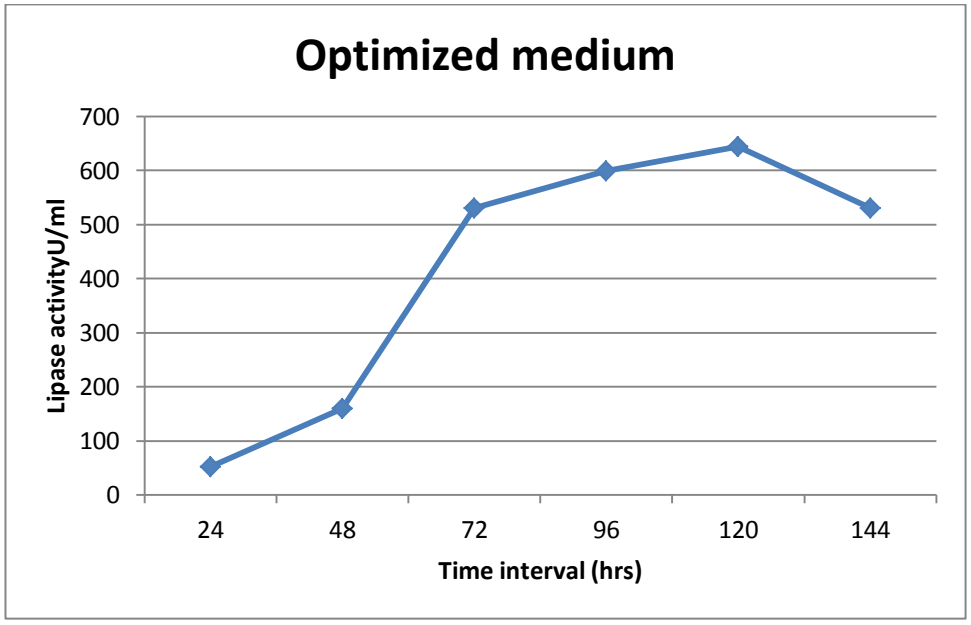

Figure 7: Lipase production by *Staphylococcus saprophyticus* in optimized medium

To find the optimum time for lipase production, at 27°C for 1-6 days and supernatant for each day was evaluated in the means of lipase activity using spectrophotometric lipase assay. The results showed that higher lipase activity (644U/ml) exhibited at incubation times of 5 days (Figure 7).

Conclusion

The bacterium that was positive towards lipase production was identified to be *staphylococcus saprophyticus*. Production media was prepared by incorporating olive oil in the basal media. The production media was incubated for 5 days and the extracellular enzyme thus produced was extracted and purified. The pure enzyme was thus assayed quantitatively and the efficiency was found to be 140 U/ml. The study was further focused to study the optimal conditions for the maximum enzyme production. The temperature and pH for maximum efficiency of lipase production was optimized to be 27^0C and pH 6 respectively.

The enzyme activity determined before optimization was 140U/ml and the lipase activity from the optimized medium kept at pH 6, temperature 27^0C with olive oil as the carbon source supplemented with NaCl was644 U/ml, indicating 5 fold increase in lipase production by *S.saprophyticus*. The extracellular lipase enzyme can be further purified and used in different industrial applications.

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