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European Journal of Zoological Research, 2012, 1 (1):23-25
(http://scholarsresearchlibrary.com/archive.html)

ISSN: 2278–7356

Optimization of lipase production by *vibrio* Sp. -A fish gut isolate

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ABSTRACT

Optimization of lipase production by fish gut isolate Vibrio sp. was investigated. Effect of different lipidic substrates on lipase production revealed that coconut oil sediment supported for maximizing the lipase production than other substrates during 48 h of fermentation. Effect of temperature and pH on lipase production showed that pH 7 and 37ºC was optimum for enhancing lipase production and this was also resulted at 48 h. Among the metal ions tested, magnesium sulphate was observed as a suitable metal ion for higher lipase production.

INTRODUCTION

Lipases (triacylglycerol acylhydrolase; E.C.3.1.1.3) have become one of the prominent industrial enzymes which catalyze both hydrolysis of triglycerideand synthesis of esters from glycerol and long chain fatty acids. A 'true' lipase is named as a carboxyl esterase, which governs the hydrolysis and synthesis of long-chain acylglycerols with triacylglycerol from the standard substrate (1).

Lipases posses the unique feature of acting at an interface between the aqueous and non-aqueous (i.e. organic) phase; this feature distinguishes them from esterases. Because of their wide-ranging significance, research on lipases is paying attention particularly on structural characterization, elucidation of mechanism of action, kinetics, sequencing and cloning of lipase genes, and general characterization of performance (2).

Lipases are of widespread occurrence throughout the living organisms, and they are mainly found abundantly in microbial flora comprising bacteria, fungi and yeast. *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Chromobacterium* and *Pseudomonas* are the important microbial genera used for commercial lipase production. The pancreas of mammals such as pigs and humans, and the higher plants such as castor bean (*Ricinus communis*) and rapeseed (*Brassica napus*) are other sources of lipases (3).

Lipolytic enzymes are currently attracting more attention because of their biotechnological potential. They mainly used in detergent, food, flavour industry, biocatalytic resolution of pharmaceuticals, esters and amino acid derivatives, making of fine chemicals, agrochemicals, use as biosensor, bioremediation and cosmetics and perfumery. The use of lipases, make it as a billion dollar business, consequently a strong need exists to identify the novel lipase genes and to optimize existing lipases with respect to desired properties of commercial. Hence media optimization is an essential one, and the enzyme production is generally induced by altering the media composition. Bacterial lipases are mostly extracellular and are greatly influenced by nutritional and physico-chemical factors,

such as temperature, pH, nitrogen and carbon sources, presence of lipids, inorganic salts, agitation and dissolved oxygen concentration etc. In the present study, a new lipolytic isolate sourced from fish intestine was optimized with different lipidic substrates, pH, and temperature and metal ions.

MATERIALS AND METHODS

Microorganism

The microorganism used in this experiment was isolated from the gut of the Tripod fish collected from the local market. This bacterial strain produced zone when streaking on tributyrin agar medium and confirmed as lipolytic activity. Further by using various biochemical testes this strain was identified as *Vibrio* sp.

Lipase production

The bacterium was initially enriched by using the medium containing (w/v): beef extract (0.15%), peptone (0.5%), sodium chloride (1.0%) and glucose (0.5%), pH 7, at 32°C for 24 h. Then, 5% of enriched seed culture was inoculated into a 50 ml medium (w/v) containing potassium peptone 0.5%, dihydrogen orthophosphate 0.1%; sodium chloride 1% and magnesium sulphate 0.01%. It was then incubated at 32°C at 150 rpm. After incubation it was centrifuged at 10000 rpm and the supernatant was used as lipase enzyme source.

Lipase assay

Lipase activity was assayed through spectrophotometric method by using p-nitrophenol palmitate as substrate. The reaction mixture containing 100 µl of 50 mM Tris buffer (pH-7.0), 50 µl of substrate solution (1mM p-NPP containing 1% Triton X-100), 350 µl of H_2O and the reaction was started by adding 100 µl of enzyme solution. After incubation of 10 min, the reaction was stopped by adding 1 ml of 2% sodium dodecyl sulphate (SDS) solution. The absorbance was recorded at 420 nm using UV–vis–spectorophotometer. One unit of lipase activity was defined as the amount of enzyme releasing 1 µmol of p-nitrophenol per minute (4).

Optimization of lipase production

Effect of different lipidic substrates on lipase production

The influence of different lipidic substrates on lipase production was determined by using coconut oil cake, groundnut oil cake, sesame oil cake, fish bone, coconut oil sediment. Their influence on lipase production was determined at different level concentrations (0.5, 1.0, 1.5 and 2.0%) and also at different time intervals (24, 48 and 72h).

Effect of different initial pH on lipase production

The effect of medium pH on lipase production was determined at different pH varied from 5, 7 and 9 during different time intervals of 24, 48 and 72 h.

Effect of different incubation temperature on lipase production

Effect of medium temperature on lipase production was determined by incubating the production media at different temperatures such as 27^{o}C, 37^{o}C and 47^{o}C for the time intervals of 24, 48 and 72h.

Effect of metal ions on lipase production.

The effect of different metal ions on lipase production was determined by using five different metals such as manganese sulphate, ferrous sulphate, copper sulphate, magnesium sulphate, calcium chloride at the level of 0.1% concentration.

RESULTS AND DISCUSSION

Lipase production by *Vibrio* sp. at different concentrations of lipidic substrates during various time intervals is shown in Fig. 1, 2 and 3. The results inferred that 1.5% was the optimum concentration for enhancing lipase production in all the tested substrates. Moreover among the selected substrates coconut oil sediment and fish bone produced maximum lipase then other substrates supplied. Almost in all the substrates, 48h was found to be the optimum time for maximum lipase production and at this period the lipase production was high when compared to 24 h and 72 h. It was reported that, coconut oil is the best and inexpensive substrate for lipase production. Lipids like coconut oil are found to be an inducer of lipase production and this was observed in lipase production by *Mucor*

griseocyanus (5). Supakdamrongkul et al (6) also reported that, lipase production by *Nomuraea rileyi* was high, when coconut oil is used as the substrate.

The effect of different initial medium pH at various incubation periods on lipase production resulted that neutral pH (pH 7) was the optimum for enhancing lipase production. This pH supported well for lipase production in all the tested incubation time intervals (24, 48 and 72 h) (Fig. 4). This results inferred that this strain prefers natural pH (7.0) for better growth and enzyme production. Dharmsthiti and Kuhasuntisuk (7) reported the effect of pH on lipase activity by *Pseudomonas aeruginosa* and they observed that pH 7-8 was optimum for increasing the lipase activity. Similarly Esakkiraj et al., (8) reported that lipase production by *Staphylococcus epidermidis* CMST-Pi 2 isolated from the gut of shrimp was maximum in pH 7.

The effect of different incubation temperatures on lipase production at various time interval revealed that 37ºC was the optimum temperature for maximum lipase production by *Vibrio* sp. The same temperature was noticed as the optimum for the various incubation period. Also it inferred that, the lipase production by *Vibrio* sp. at 47^{o}C was more when compared the production recorded at 27^{o}C and at the same time, it was low when compared the production realized at 37ºC (Fig. 5). This mesophilic temperature was observed as optimum for maximum lipase production in many species. Optimization of temperature is an important step because they play important role in cell growth and enzyme production. This was proved in solid-state lipase production by *Rhizopus oligosporous*, where it was maximum at 30^{o}C (9). Bapiraju *et al*., (10) observed optimum lipase production by *Rhizopus* sp. BTNT-2 at 28^{o}C. The same temperature was also observed as a optimum for lipase production in *Serratia rubidaea* (11).

Metal ions are the important minor nutrients that decide the successive lipase production by microbial strains. In the present study, five metal ions were screened and the results showed that magnesium sulphate was found to be more specific for higher lipase production by *Vibrio* sp. (Fig. 6). The other best metal ion observed for maximum lipase production was manganese sulphate. Zhen-qian and Chun-yun (12) reported that lipase production by *Enterobacter agglomerans* increased very much by Mg^{2+}. In consistence with the present study, Joseph et al. (13) reported that Mg^{2+} was the most suitable metal ion for lipase production by *Staphylococcus epidermidis*. Similarly Shariff *et al.* (14) reported that lipase production by *Bacillus* sp. strain L2 was increased upon the addition of Mg^{2+} and Ca^{2+} ions.

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