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Lipase production by lactic acid bacteria in submerged and solid state fermentation

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ABSTRACT

Lactobacillus acidophilus NCIM 2909 has been found to produce acidic lipase during submerged fermentation. Production was optimized by varying several process parameters and solid substrate fermentation (SSF) was carried out using different oil cakes. Highest activity of lipase after 48h was attained with GOC: Mineral medium (35.7 kU/l) under conditions of 70% initial moisture content, 2.5ml inoculum size and 35°C incubation temperature.

KEYWORDS

Lactobacillus acidophilus;
Lipase;
Oil cakes;
SSF;
Submerged fermentation.

INTRODUCTION

Lipolytic activity is an important and ubiquitous enzymatic activity. Bacterial lipases, in general, are extra cellular enzymes, which are commercially and industrially important[1]. Most studies on lipolytic enzymes production by bacteria, fungi and yeasts have been performed in submerged cultures; however, there are only a few reports on lipase synthesis in solid state cultures. In recent years increasing attention has been paid to the conversion of processing industry wastes in lipase by solid state culture[2]. But several physicochemical and biological factors such as pH of the medium, temperature, and period of incubation, age, size, and type of inoculums, nature of substrate and type of micro-organism will determine the growth of organism and enzyme production. Lipolytic activities of several probiotic strains were observed in many works[3,4] but utilization of oil cakes by employing lactic acid bacteria was not reported so far. Moreover very little information is available in regard to the factors affecting the maximum enzyme for its application in industrial scale and the present work deals with it.

EXPERIMENTAL

Submerged fermentation

Flasks containing mineral solution (0.7% Na_2HPO_4, 0.3% KH_2PO_4 0.1%NH4Cl, 0.5% NaCl) supplemented with 0.2%(w/v) glucose, peptone substrates was inoculated with actively growing 1% inoculum (1×10^9 cfu/ml) of *L.acidophilus* culture and incubated at 35°C for 18h. An optimized medium was prepared by varying carbon, nitrogen substrates along with lipid inducers. After 18h incubation time, aliquots of samples collected were centrifuged at 10,000rpm for 10 minutes and the supernatant obtained, used as enzyme source for estimation of lipase activity.

Solid substrate fermentation

Different oil cakes used in this study [Coconut Oil Cake (COC), Groundnut Oil Cake (GnOC), Gingelly Oil Cake (GOC)] were dried in oven at 80°C (24hr) for determining dry weight and finely grounded in a blender to obtain an even particle size. 10g of different solid substrates taken in 250ml Erlenmeyer flasks were moistened with 4ml of either water or mineral medium and distilled water was added to adjust the required initial moisture level. SSF was also carried out to study the effect of various physico-chemical parameters, *viz.* initial moisture content of the substrate (60%, 65%, 70%, 75%), incubation time (12, 24, 36, 48 h), incubation temperature (30, 35, 40, 45°C), inoculum size (1.0, 1.5, 2.0, 2.5, 3.0ml) for optimized production of lipase. Unless otherwise mentioned, SSF was carried out with 2 ml of *L.acidophilus* culture (1×10^9 cfu/ml) on all oil cakes with the 60% initial moisture content and incubated at 35×C temperature for 48h under static conditions. At the end of SSF, sterilized distilled water with 0.1% Tween 80, added to the flasks and kept in shaking incubator (250 rpm) for one hour. Contents of the flasks were centrifuged and supernatant collected for protease assay. Duplicates were maintained for all the experiments and enzyme assaying was done in triplicate.

Enzyme assay

A titrimetric method was followed for lipase activity estimation[5]. One unit of lipase activity is defined as the amount of enzyme required to liberate one μmol equivalent fatty acid per minute and enzyme activity was expressed in kU/l for all the assays.

Enzyme characterization

The effect of pH and temperature on lipase enzyme activity and stability was studied by using different pH buffers [(Glycine-HCl Buffer (pH 2.0-4.0), Sodium phosphate buffer (pH 5.0 - 8.0), Glycine sodium hydroxide buffer (pH 9.0 -10.0)] and temperature conditions (25-60°C).

RESULTS AND DISCUSSION

One of the major draw backs in the industrial applications of enzymes from lactic acid bacteria is the low productivity, therefore it is essential to investigate the routes for maximizing enzyme production both by submerged and SSF. As the medium requirement varies between organisms, here optimization of medium was carried out for attaining maximum lipase activity under submerged conditions (TABLE 1). Glucose, peptone and olive oil gave maximum production of lipase (8.2 kU/l ,9.1 kU/l,8.6 kU/l) followed by lactose, yeast extract and palm oil (7.6 kU/l , 7.1 kU/l ,6.3 kU/l). Our results are in accordance with some other reports where, for production of extra cellular lipases, glucose as a carbon source[4,6] and peptone as nitrogen source[7] can be considered as best for lipase production. But here the presence of lipid inducer gave highest activity compared to sole carbon source utilization.

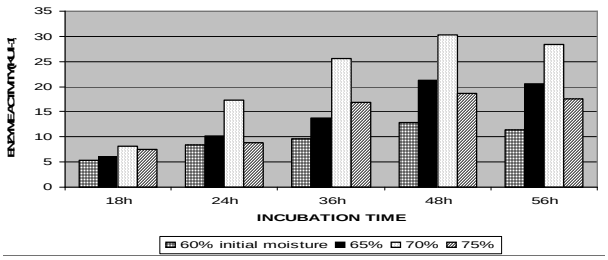

Figure 1 : Effect of incubation time and initial moisture content on lipase activity (kU/l) using GOC: mineral medium in SSF

In SSF experiments, instead of adding water alone as moistening medium, adequate amounts of mineral solution improved the lipase yield extensively. A combination of GOC: Mineral medium (70% initial moisture) followed by COC: Mineral medium (65% initial moisture) after 48h represents highest activities (TABLE 2). Lipase production in GOC: Mineral medium (70% initial moisture) was started after 24h and increased sharply to a maximum value of 30.3 kU/l (considered as 100% activity) after 48h (Figure 1). This enzyme activity was almost 3- fold higher than submerged fermentation where only 9.1 kU/l is the maximum enzyme activity. A very lower relative activity (40.3%) was observed for COC at 65%initial moisture where water is moistening medium. This value was increased to 72.9% if mineral solution was added. For GnOC: mineral medium also (65% initial moisture) a 70.3% relative activity was observed. Present study indicated that lower moisture level would improve the enzyme activity and is dependent on combination of substrates along with different salts and nutrients. Where as higher moisture

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TABLE 1 : Production of lipase by *L.acidophilus* using different carbon, nitrogen and lipid sources in submerged fermentation

Carbon source	Enzyme activity (kU/l)*	Nitrogen source	Enzyme activity (kU/l)*	Lipid inducer	Enzyme activity (kU/l)*
Chitin	3.73+0.68	Ammonium phosphate	2.65+0.51	Coconut oil	4.42+0.36
Glucose	8.26+0.65	Ammonium sulphate	2.81+0.43	Ground nut oil	3.85+0.41
Gum arabic	2.93+0.75	Casein	4.75+0.32	Gingelly oil	4.83+0.52
Lactose	7.62+0.86	Peptone	9.12+0.34	Olive oil	8.66+0.32
Starch	4.35+0.47	Yeast extract	7.16+0.15	Palm oil	6.35+0.28
Sucrose	5.26+0.37	Urea	1.87+0.25	Tween 80	2.33+0.16

***All the values are expressed in Mean + SD**

TABLE 2 : Effect of initial moisture content (%) in Substrate: water and Substrate: mineral medium combinations on lipase activity (kU/l)

Initial moisture content (%)	60%	65%	70%	75%
Substrate: water				
COC	10.6+3.1	12.2+2.9	13.4+3.4	16.8+3.1
GnOC	9.8+4.3	15.5+1.6	12.1+2.6	11.3+2.1
GOC	11.4+3.7	18.6+1.3	15.4+2.7	14.5+2.1
Substrate: mineral medium				
COC	18.6+1.7	22.1+1.5	17.8+1.6	16.7+2.5
GnOC	16.2+4.3	21.3+4.9	17.9+2.4	17.9+4.3
GOC	14.8+2.6	19.3+3.2	30.3+3.5	14.6+2.6

***All the values are expressed in Mean + SD**

level decreases porosity, promotes development of stickiness, increases the chances of contamination[8,9].

SSF was carried out with 2ml *L.acidophilus* culture on GOC: Mineral medium with the initial moisture content adjusted to 70% at 35°C for studying the effect of incubation time, temperature, inoculum size on lipase activity. After 24 h of incubation, 17.7 kU/l of the enzyme was produced, which exponentially increased to 30.3 kU/l after 48 h. Incubation beyond 48 h was undesirable as this resulted in decreased enzyme yields (Figure 1).

The reason for this might have been due to the denaturation of the enzyme caused by the interaction with other components in the medium[9]. Temperature is one of the important factors, which strongly affect the SSF process. It has been reported that during microbial cultivation in SSF, the temperature of the fermenting bed increased, which exerted harmful effects on the microbial activity[10,12]. In the present study, 35°C proved to be the best temperature where GOC: Mineral medium (70% initial moisture) used for the enzyme synthesis. Incubation at higher temperature affected the enzyme synthesis where only 64% relative activity was observed at 45°C than at 35°C (results not shown). During SSF, a large amount of heat is generated which is directly proportional to the metabolic activities of microorganism[10,12]. Lower inoculum size required longer time for the cells to multiply to sufficient number to utilize the substrate and produce the desired product. A balance between the proliferating biomass and available nutrient would yield an optimum condition at which the enzyme synthesis will be high. This was evident as the strain showed increased enzyme production with the increase in inoculum size from the lowest value of 1.0ml and showed maximum enzyme activity (35.7 kU/l) at 2.5ml inoculum (1×10^9 cfu/ml). However, further increase in the inoculum size resulted in decreased enzyme synthesis, indicating that limitation of nutrients occurred due to the increased microbial activity (results not shown).

Lipase produced by *Lactobacillus acidophilus* was optimally active at pH 5.0. It was stable in the range of pH 4.0-7.0 for 30 min. The optimum pH for lactobacillus may vary among species and it ranges from pH 5.0-7.0[3,4]. The optimum temperature for lipase activity is 40°C and it retained 100% activity for 30 min[11] Werasit et al.[12] showed a maximum lipase activity at 40°C for *Staphylococcus warmer.* Temperature maintenance during the incubation time will definitely affect the lipase activity and type of enzyme source also influences it[3,13]. The higher stability was observed at lower temperatures than higher temperatures and it ranges form 30-45°C.

CONCLUSION

Acidophilic nature of this enzyme could have potential application in food industries. In order to meet

the requirements of food and other industries process improvement of lipase production should me made. Here we have reported a novel application of oil cakes, cultured with *L.acidophilus* which is a GRAS (Generally Recognized As Safe) organism for maximum lipase production. *L.acidophilus* utilization for lipase production showed promising results. GOC proved to be the best source for lipase production compared to other oil cakes. As bioprocess usage of oil cakes is beneficial due to relatively cheaper availability of the oil cakes throughout the year, making it even more favorable when economics is considered.

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