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Purification and Characterization of Lipase from the Anaerobic Lipolytic Bacterium *Selenomonas lipolytica*

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Abstract Two different extracellular lipases were produced by an anaerobic bacterium, *Selenomonas lipolytica*. A major lipase, lipase I, was isolated, which showed optimum activity at pH 6.0 and at 45°C. It showed a molecular weight of 240 kDa and was a tetramer of a subunit having molecular weight of 60 kDa, which is different from the known bacterial lipases.

Key words: Anaerobe, *Selenomonas lipolytica*, novel lipase

Lipases (glycerol ester hydrolases, E.C.3.1.1.3) have received much attention because of their applications in detergent and food industry, as well as in the production of optically active compounds, which serve as building blocks in the synthesis of pharmaceuticals [5]. Many aerobic bacteria and fungi have already been reported as lipase-producing microbes. Our present knowledge of microbial lipases in terms of the biochemical nature and the process of lipolysis is restricted to enzyme preparations from aerobic bacteria and fungi [1, 5, 13], whereas reports on lipases of obligately anaerobic bacteria are rare [2, 3, 15]. In the present paper, we describe the purification and characterization of lipase produced by a novel anaerobe, *Selenomonas lipolytica*[T] MCM B505, isolated from an oil mill waste [2]. This is the first report of a high molecular weight lipase from anaerobic bacteria.

The medium used for the enzyme production from *S. lipolytica* had the following composition (g/l): 10.0 glucose, 20.0 peptone, 1.0 NH_4Cl, 0.9 KH_2PO_4, 7.0 NaCl, 1.015 $MgCl_2 \cdot 6H_2O$, 0.3 $CaCl_2 \cdot 2H_2O$, 1 ml Tween-80 as lipase inducer, 1 ml of a trace element solution [14], 1 ml of a trace vitamin solution [16], 1 ml of a resazurin solution (0.1% w/v), and 0.5 cysteine-HCl, with pH adjusted to 7.0. The medium was prepared under anaerobic conditions [4, 11] with H_2:CO_2 (80:20, v/v) as the gas phase. The culture was grown in this medium at 35°C for 60 h. All the steps of the isolation procedure were carried out at 4°C. The cells were separated by centrifugation and the proteins were precipitated from the supernatant by addition of an equal volume of chilled acetone. The resultant precipitate was collected by centrifugation, dissolved in a minimum volume of 0.2 M Glycine-NaOH buffer (pH 8.0) containing 0.1 mM of PMSF as protease inhibitor, and dialyzed for 24 h with the same buffer. Addition of PMSF was essential, as the enzyme extract showed protease activity. The dialyzate was centrifuged and the supernatant loaded on a Sephadex G 200 (Sigma, St. Louis, U.S.A.) column equilibrated with the same buffer. Those fractions exhibiting lipase activity were pooled and loaded on a DEAE Sephacel (Sigma, St. Louis, U.S.A.) column, which was previously equilibrated with 0.2 M Glycine-NaOH buffer (pH 8.0) and washed with two column volumes of the same buffer. A linear gradient of KCl from 0.0 to 1.0 M was applied at a flow rate of 1 ml/min. Active fractions were pooled and used for further studies. The lipase activity was routinely measured at 35°C and at pH 8.0 using Triolein (Sigma, St. Louis, U.S.A.) as the substrate, and the liberated oleic acid was measured after esterification by gas chromatography (Chemito 8510, Toshniwal, India) [2, 8]. One unit of enzyme is defined as one micromole of oleic acid liberated by one ml of enzyme per minute under the assay conditions, and specific activity is presented as the units of enzyme per milligram of protein. The protein was determined using the method of Lowry *et al.* [10] using bovine serum albumin as the standard.

During the DEAE fractionation step of purification, two different pure lipases were obtained, namely, lipase I and lipase II (Table 1). When freshly prepared lipase I was electrophoresed on acrylamide gel (7%) and protein bands were stained with Coomassie Brilliant Blue R250 [12], only one band of protein was detected, which exhibited lipase activity (data not shown). The molecular weight of the

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Table 1. Purification of lipase from *Selenomonas lipolytica.*

Purification step	Total volume (ml)	Total activity (Units)	Total protein (mg)	Specific activity (Units/mg)	Yield (%)
Crude	1000	42.0	1050	0.04	-
Acetone precipitated	35	9.1	10.15	0.90	100
Sephadex G 200	25	3.75	0.3	12.50	57
DEAE Sephacel: Lipase I	5	0.365	0.01	36.50	28
Lipase II	5	0.125	0.005	25	9.6

native enzyme was estimated to be 240 kDa by gel filtration on a calibrated Sephacryl 200 column (Fig. 1). A subunit having molecular weight of 60 kDa (Fig. 2), was estimated by SDS-PAGE, as described by Laemmli [9]. These results suggest the lipase I to be a high molecular weight lipase, existing as a tetramer. No known bacterial lipases showed such a high molecular weight. Since lipase I with a molecular weight of 240 kDa has not yet been classified in any of the existing lipases [5], an additional new group of high molecular weight lipases is suggested. Lipase I exhibited a better specific activity (36.5 U/mg) and showed more affinity toward triacetin as compared to tributyrin and triolein. It was further investigated to determine its optimum activity at various pH values and temperatures. It showed optimum activity at pH 6.0 and at 45°C, while lipase II had optimum activity at pH 7.0 at 40°C.

The N-terminal amino acid sequence analysis of lipase I for the first ten residues was found as ANVDLSQYVI. This sequence was compared with all other N-terminal lipases sequenced at NCBI (http://www.ncbi.nlm.nih.gov/Entrez/). The sequence similarity was searched using BLAST. Interestingly, no homology was observed for this sequence with N-terminal end of any of the other reported lipases. Here, again, the enzyme could not be placed into any known family of lipases suggested by Jaeger and Arpigny on the basis of amino acid sequence [6].

At present, there has been only one other report on a lipase from an anaerobic bacterium - *Anaerovibrio lipolytica* [3]. The results presented in Table 2 indicate that the isolated lipase I from *S. lipolytica* is distinctly different from the lipase from *A. lipolytica*. This observation, coupled with the high molecular weight of lipase I from *S. lipolytica*, suggests the need for further studies on lipases from anaerobic lipolytic bacteria.

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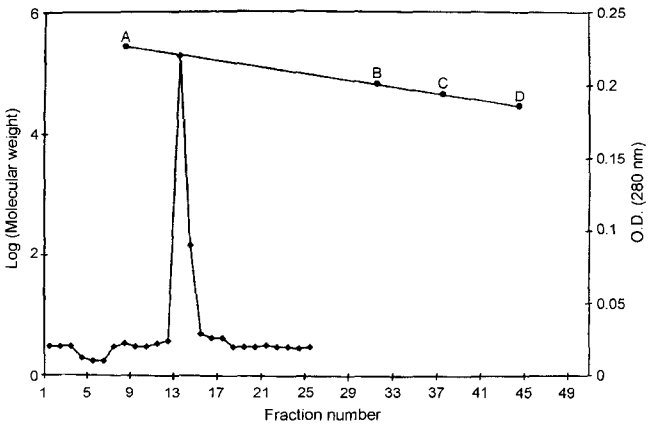

Fig. 1. Gel chromatographic estimation of molecular weight of lipase I.
-◆- Lipase I; -●- Eluted fraction of standard proteins. A, Urease (272 kDa); B, BSA (66 kDa); C, Chicken egg albumin (45 kDa); D, Carbonic anhydrase (29 kDa).

Fig. 2. SDS-PAGE of lipase I.
Lane A: lipase I; Lane B, Molecular weight standard markers, Bovine serum albumin (66 kDa); Ovalbumin (43 kDa); Carbonic anhydrase (29 kDa); Lysozyme (14 kDa). The gel was stained for protein with Coomassie Brilliant Blue R250.

Table 2. Comparison of lipase I with lipase from *A. lipolytica.*

Activity characteristics	*Selenomonas lipolytica*	*Anaerovibrio lipolytica* [3]
Optimum pH	6.0	7.4
Optimum temperature	45°C	20–22°C
Substrate specificity	Triacetin	Trilaurin
Molecular weight	240 kDa	not reported
Specific activity	36.5	38.40

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