5. B. I. Davies, Ann. N.Y. Acad. Sci., 121, 404 (1964).
6. K. L. Smith, Methods in Hormone Research, Vol. 2, Academic Press, New York (1962), p. 439.

ISOLATION AND PURIFICATION OF A LIPASE FROM THE FUNGUS *Oospora lactis*

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UDC 547.153.582

Under certain conditions, the fungus *Oospora lactis* produces an active exolipase (E.C. 3.1,1.3 — triglyceride hydrolase) [1]. In the present paper we discuss the isolation and purification of the extracellular lipase of the fungus *Oospora lactis* and some of its properties.

The mycelium of the fungus was separated by filtration through a paper filter. The enzyme was precipitated from the filtrate of the culture liquid with six volumes of isopropanol [1]. The enzyme was extracted from the "isopropyl powder" with 0.1 M phosphate buffer, pH 7.4. The part that did not dissolve was separated by centrifuging or by filtration through the paper filter. The clear solution of the enzyme was deposited on a column of Sephadex G-75 equilibrated with 0.1 M phosphate buffer. The dimensions of the column were 120 × 3 cm and the rate of elution 20 ml/h. Fractions with a volume of 5 ml were collected.

It can be seen from the elution graph (Fig. 1) that the protein issued in two peaks, and the lipase activity appeared between the two peaks (fractions 12-17). The active fractions were combined and were concentrated by freeze-drying or, in some cases, with the aid of dry, washed, Molselekt G-25 (Reanal) and redeposition on a column of Sephadex G-75 (2 × 100 cm). It was eluted with the initial buffer at the rate of 15 ml/h.

Activity was shown in the fractions of the second peak (Fig. 2). The specific acitivity of the enzyme had increase 100-fold in comparison with the isopropyl powder. A further attempt to purify the enzyme with the aid of DEAE-Sephadex A-50 gave no effect, the enzyme being eluted in one symmetrical peak. Disk electrophoresis in polyacrylamide gel [2] showed the presence of one band, which also gave the specific reaction for lipolytic enzymes [3].

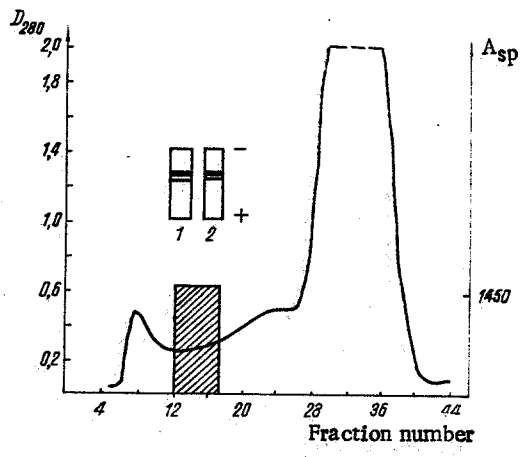

Fig. 1

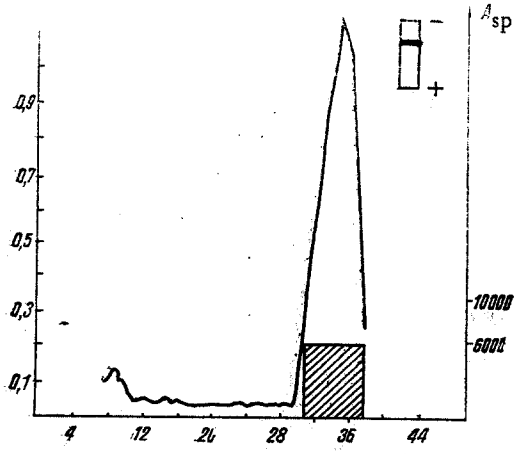

Fig. 2

Fig. 1. Separation of the total fraction on Sephadex G-75 (*Oospora lactis*): 1) electrophoretogram of the active fraction in polyacrylamide gel; 2) enzymogram (according to Abe) [3].

Fig. 2. Repeated gel filtration on Sephadex G-75 (*Oospora lactis*). For explanations, see Fig. 1.

Institute of Microbiology, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 544-545, July-August, 1978. Original article submitted March 3, 1978.

0009-3130/78/1404-0473$07.50 © 1979 Plenum Publishing Corporation

The results of the purification are given below:

Purification step	Fraction volume, ml	Amount of protein mg/ml	Total amount of protein	Activity 1 mg	Activity total	Degree of purification
Isopropyl powder	5	150	750	66	49500	1
Gel filtration on Sephadex G-75	3.5	11	3,85	1450	5600	22
Repeated gel filtration	8	0,1	0,8	6000	4800	90,9

Some properties of the purified enzyme have been studied: optimum pH 7.5, optimum temperature 32-37°C.

LITERATURE CITED

1. S. S. Shchelokova, M. Ya. Tabak, and M. Z. Zakirov, Uzb. Biol. Zh., No. 3 (1978).
2. K. Davranov, M. Rizaeva, and M. Z. Zakirov, Khim. Prirodn. Soedin., 636 (1976).
3. H. Maurer, Disk-Elektrophorese, Theorie und Praxis, Walter de Gruyter, Berlin (1968).