LIPOLYTIC ACTIVITY OF SOME DERMATOPHYTES. II. ISOLATION AND CHARACTERISATION OF THE LIPASE OF *EPIDERMOPHYTON FLOCCOSUM*

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SUMMARY. The physicochemical properties, stability and localisation of lipase from the anthropophilic dermatophyte *Epidermophyton floccosum* have been investigated. The lipase was best secreted by older cultures at an optimum pH of 6.5–7 in the temperature range 40–45°C. Neither the skin-surface lipids nor the body temperature of man significantly affected the activity of the lipase.

INTRODUCTION

The distribution of dermatophyte lipids and their importance in the pathogenesis of dermatophytosis has been recently reviewed by Vincent (1978). The lipolytic activity of *Epidermophyton floccosum*, *Microsporum canis*, *Trichophyton rubrum* and *T. mentagrophytes* has also been screened as a function of different substrates (Hellgren and Vincent, 1980).

Lipolytic enzymes are important for the development of dermatomycosis because dermatophytes, in their first phase of growth, depend solely on their ability to split surface lipids to reach the stratum corneum. However, there is little information on the lipolytic systems and their physiological functions in dermatophytes. It is therefore important that these fungal lipases be more thoroughly characterised. In this study we investigated the physicochemical properties, stability and localisation of lipase isolated from an anthropophilic dermatophyte, *Epidermophyton floccosum*.

MATERIALS AND METHODS

Growth conditions. Freshly isolated strains of *E. floccosum* were cultured on the liquid medium of Blain, Patterson and Shaw (1978). For enzyme recovery the substrate was inoculated with 0.1 ml of standard mycelial suspension (Vincent, 1972) of the dermatophyte and incubated for 1 month at 30°C. Each batch was run in a series of replicates with at least seven separate cultures. The mycelial mass was removed by combined filtration and centrifugation of the substrate and the culture filtrates were directly used for measurements of lipolytic activity.

Lipase assay. Activity of the lipase was determined titrimetrically. The reaction mixture, composed of 5 ml of culture filtrate, 4.5 ml of lipase buffer (Borgström and Erlanson, 1973) and 0.5 ml of tributyrine, was incubated at 35°C for 3 h. Immediately after incubation, 20 ml of acetone:ethanol mixture (1:1) was added and the liberated fatty acids titrated with 0.05N NaOH. In the same manner, enzyme temperature and pH optima were established by adjustment of the assay mixture to different pH or by incubation at different temperatures. To confirm the velocity of some reactions, the hydrolysis products were extracted and analysed by thin-layer chromatography.

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RESULTS

The lipolytic activity of *E. floccosum* was observed during the whole growth period. The excretion rate increased continuously with age of the culture (fig. 1). This was confirmed by chromatographic identification of hydrolysis products. The optimum pH for *E. floccosum* lipase (fig. 2) lay in the range 6.5–7.0, and greater changes in pH did not abolish enzyme activity; e.g., at pH 5.5 or 9.0 up to ~50% of the activity could be recorded. The intensity of the substrate degradation increased with rising temperature up to 40–45°C and then rapidly fell. However, for routine measurements a temperature of 35°C is recommended (fig. 3).

The stability of *E. floccosum* lipase was also evaluated. We concluded that this lipase is a relatively slowly acting enzyme. Moreover, when the enzyme was incubated at 50–70°C its activity rapidly decreased. Incubation for 15–30 min at 60°C resulted in a drastic decline of the lipolytic activity. This thermosensitivity of the lipase indicates a protein character.

Finally, in our experimental conditions, we failed to establish whether or not there was also a passive liberation of endoenzymes, especially in the late stationary and autolytic growth phase.

FIG. 1.—Relationship between the growth of *E. floccosum* and lipase activity (expressed as titratable fatty acids liberated over a 3-h period). The experimental conditions are described in the text.

DISCUSSION

Even if *E. floccosum* generates lipase during its whole growth period, it is in the older cultures that lipase production predominates. Dermatophyte lipases are not labile, and this facilitates their action in the skin before the fungus reaches the keratin-rich layers of the epidermis. It is

FIG. 2.—Effect of pH on lipase activity in culture filtrate of *E. floccosum*.

FIG. 3.—Effect of temperature on lipase activity in culture filtrate from *E. floccosum*.

noteworthy that, despite the fact that the optimum pH of *E. floccosum* lipase was found close to pH 7, the acid mantle of the skin seems only partially to affect fungal enzymatic activity. Fatty-acid content in the skin surface lipids never reaches levels that could inhibit the growth of dermatophytes and the extent of lipolysis seems thus to be controlled by a feedback mechanism. This is probably due to the active alkalisation of the substrate by dermatophytes (Paveia, 1975) which in turn leads to the skin buffer capacity being overcome. Moreover, the keratinous structures swell in alkaline milieu, and this facilitates an easier penetration of the dermatophytes into the stratum corneum. Zucker (1964) reported a constitutional higher pH value associated with a decreased buffering capacity of the skin in patients with dermatomycosis compared with healthy controls; and this may suggest a predisposition to the disease in certain individuals.

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