BRIEF REPORT

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Estimatioı of Lipase Activity by the Diffusion Plate Method

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ABSTRACT. A diffusion plate method for the assay of the activity of lipases in large series of samples was worked out. When using this method the maximum deviation was found to be ±3.8 %.

When determining the activity of esterases and lipases in large series of samples potentiometric methods (Brockmann 1981), chromatographic methods (Plattner 1981) and turbidimetric assay (Junge *et al.* 1983) are used. All the methods yield accurate results but relatively complex and expensive devices are required.

It was the aim the present work to design a simple method for the assay of lipase activity on the basis of several literature data and to develop a procedure utilizing substrate degradation in an agar gel layer.

The use of traditional substrates (various oils, triolein, *etc.*) was not suitable as their emulsions are not stable in the solid agar medium. The use of various emulsifiers (in %): Slovasol EL 0.5–2, emulsifier C 0.5–2, sodium deoxycholate 5, Tween 20, 40, 60 and 80 1–2 (all compounds from Lachema, Czechoslovakia) was also unsuccessful, in spite of the fact that the emulsions were prepared in various ways and that various mutual ratios of emulsifiers and substrates were tested. Therefore, a substrate that is degraded by lipase (triacylglycerol lipase, EC 3.1.1.3), such as triolein, and that forms stable suspensions in both liquid and solid media was sought. Such a substrate should also, under suitable conditions, be degraded in the agar plate. In experiments, in which Tween 20, 40, 60 and 80 were used as substrates in a liquid medium (0.1 M phosphate buffer, pH 7.4) it was found that these compounds are degraded by lipase.

When testing the activity of lipase (porcine pancreatic lipase, Reachim, Soviet Union; commercial lyophilized preparation was dissolved in 40 % (*W*/*W*) glycerol in water, lipase activity of the standard was determined potentiometrically) a solid medium of the following composition was proposed: meat-peptone broth (0.3 % beef extract, 1 % peptone, 0.5 % NaCl in water) 1 L, agar 25 g, Tween 80 20 g. This medium (105 mL) was poured in Petri dishes 180 mm in diameter, solidified and 0.1 mL portions of the

TABLE I. Lipase activity of some microorganisms as assayed by the diffusion plate method

Microorganism	Specific lipase activity[a]
Candida lipolytica CS[b]	12.7
Candida lipolytica YU[b]	49.7
Streptomyces cinnamonensis[c]	23.0
Streptomyces albus[d]	0
Streptomyces spectabilis[e]	4.2

[a] Fermentation broth was assayed; oleic acid as product, nkat per mg dry mass.
[b] Munk *et al.* (1969).
[c] Královcová *et al.* (1984).
[d] Procházka *et al.* (1983); fermentation without oil.
[e] Lipavská, personal communication.

sample were pipetted into holes 8.5 mm in diameter. Zones were evaluated after a 20-h incubation at 28 °C. The optimum pH at which sharp zones were formed was 8.5. The reaction products were detected with the aid of different indicators (methylene blue, bromothymol blue, Nile blue, 0,5—2 % in an agar medium). For the assay of activity 1 % Nile blue was chosen as indicator. Light green zones on a dark blue background were evaluated (the diameter of minimum discernible zones was 10 mm, that of maximum zones observed was 17 mm). A linear relationship between the logarithm of lipase activity (1—40 nkat) and the zone diameter (10—13.5 mm) was found. The maximum deviation was ±3.8 %.

The method was used to assay the lipase activity in several microorganisms (Table I). In these assays it was found that the affinity of the studied microbial lipases for Tween 80 is probably higher by more than an order of magnitude than that of porcine lipase.

As compared with similar methods, in which Fabris and Gorini (1966), Alford and Steinle (1967), Mates (1973) and Goldberg and Pagast (1976) used triglycerides as substrates and detected reaction products primarily in the presence of calcium ions, the procedure described here yields sharper zones that can be, owing to colour discrimination, readily evaluated.

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