DETECTION OF MICROBIAL LIPASE BY COPPER SOAP FORMATION

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Received for publication August 1, 1932

Copper soap formation as a test for the hydrolysis of fat (Carnot and Mauban, 1918) may be used for the detection of microbial lipase.

METHOD

To standard nutrient agar in the tube add 0.5 cc. per tube of melted butter of good quality and sterilize in the usual manner. The melted fat agar is cooled to 45°C., the fat well mixed in and the medium poured at once into a cold petri dish. On solidification the medium is inoculated by the streak method with the organism under test, and incubated at suitable temperature for forty-eight hours. The plate is then flooded with a saturated aqueous solution of $CuSO_4$ for ten minutes and rinsed gently with water.

When the fat has been attacked conspicuous bluish-green streaks of insoluble copper soap appear on the plate. The intensity of the color is a rough gauge of lipolytic activity.

In the case of palmitin the reactions involved may be represented thus:

$$\underset{\text{Palmitin}}{2C_3H_5(OOCC_{15}H_{31})_3} \quad \underset{\text{(Lipase)}}{+} \quad \underset{\text{Water}}{+ 6H_2O} = \underset{\text{Glycerine}}{2C_3H_5(OH)_3} + \underset{\text{Palmitic acid}}{6C_{15}H_{31}COOH} \text{ and}$$

$$6C_{15}H_{31}COOH + 3CuSO_4 = 3H_2SO_4 + \underset{\text{Copper palmitate (blue-green)}}{3Cu(OOCC_{15}H_{31})_2}$$

Among organisms giving positive tests are: *Staphylococcus aureus, Staph. albus, Sarcina lutea, Pseudomonas fluorescens,*

Penicillium expansum and other molds. Among the negative organisms are *Escherichia coli, Bacillus subtilis, Bacillus mycoides,* and yeasts generally.

Cottonseed oil or soy bean oil may be used instead of butter.

The method is well adapted to the classroom as well as to the research laboratory.

REFERENCE

CARNOT, P., AND MAUBAN, H. 1918 Compt. rend. Soc. biol., **81**, 98.