MICROBIAL LIPASES

Overview

- Microbial lipases (triacylglycerol acyl-hydrolases, EC 3.1.1.3) catalyze both the hydrolysis and synthesis of long-chain acylglycerols.
- Compared to plants and animals, microorganisms have been found to produce high yields of lipases.
- Based on three-dimensional structure of various lipases, all have been classified as serine hydrolases. This is because of the active site that composed of the catalytic triad Ser-Asp (Glu)-His is similar to serine proteases.
- Under normal conditions, lipases catalyze the hydrolysis of ester bonds of triglycerides to glycerol and free fatty acids.

Catalysis by lipases

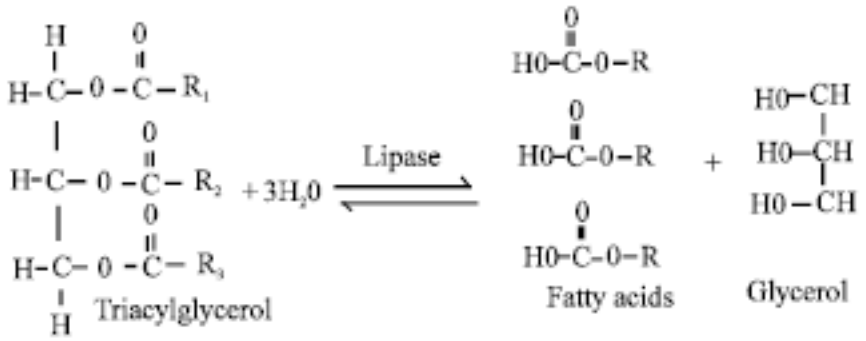


Fig. 1: The catalytic action of lipases: A triglyceride can be hydrolyzed to form glycerol and **fatty acid**s, or the reverse (synthesis) reaction can combine glycerol and **fatty acid**s to form the triglyceride

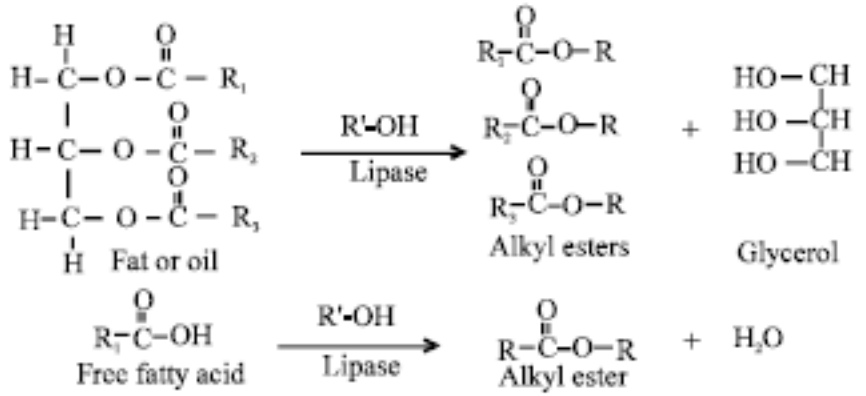


Fig. 2: Lipase mediated alcoholytic transesterification of fats or oils and free **fatty acid**s Freedman *et al.* (1986)

This reverse reaction form glycerides from fatty acids and glycerol through esterification reaction. Lipases are also used to catalyze the transesterification reaction using alcohol together with fats, oils and free fatty acids to produce alkyl esters (biodiesel).

What exactly is a lipase?

Until recently, two criteria have been used to classify a lipolytic enzyme as a “true” lipase (EC 3.1.1.3):

1. it should be activated by the presence of an interface, that is, its activity should sharply increase as soon as the triglyceride substrate forms an emulsion. This is commonly known as interfacial activation.
2. It should contain a “lid” which is a surface loop of hydrophobic oligo-peptide covering the active site of the enzyme and moving away on contact with the interface and immediately the substrate enter the binding pocket. (Exceptions exist).

Some microbial species reported to produce these enzymes include

1. *Bacillus sp.,*
2. *Pseudomonas sp.,*
3. *Burkholderia sp.,*
4. *Candida rugosa,*
5. *Candida antarctica,*
6. *Galactomyces geotricum,*
7. *Saccharomyces cerevisiae,*
8. *Trichosporon fermantans,*
9. *Cryptococcus albidus,*
10. *Aspergillus flavus.*

Detection methods for lipase activity: Using Tween 80

- A simple and reliable method for detecting lipase activity in microorganisms **uses the surfactant Tween 80** in a solid medium to identify a lipolytic activity.
- The formation of opaque zones around the colonies is an indication of lipase producing organisms.
- Modifications up on this assay have been carried out, like the use of various Tween surfactants in combination with Cu^{2+} salts.
- Around the colonies with lipolytic activity there appears a well visible halo which is due to crystals of the calcium salt of the fatty acid liberated by lipolysis.

Qualitative estimation of lipase activity using Tween 80

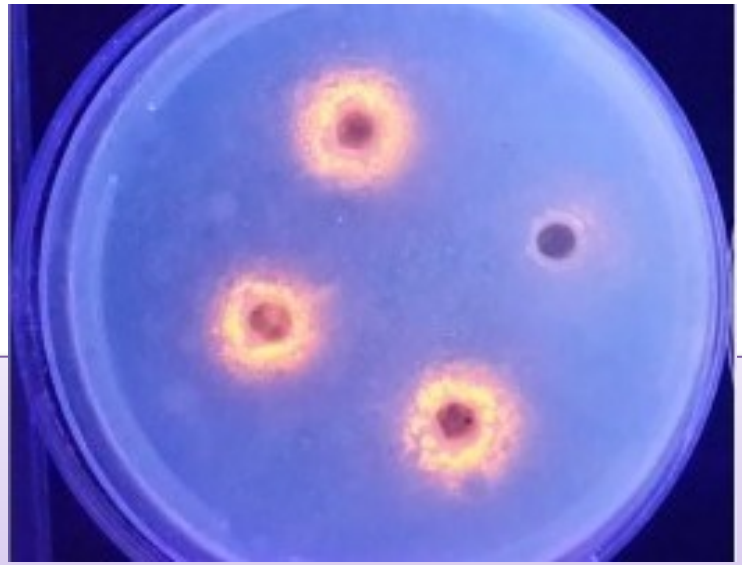
1. A precipitation test using Tween 80 agar plates is carried out to confirm lipolytic activity.
2. A white precipitation around the boundary of the colony was indicative of lipase activity

This method is based on the principle of calcium salt precipitation. The hydrolysis of tween releases fatty acids which bind with the calcium in the medium to form insoluble crystals around the point of inoculation. Tween 80 is used for the detection of lipases as it contains esters of oleic acid.

Plate screening assays for lipolytic activity: Phenol red method

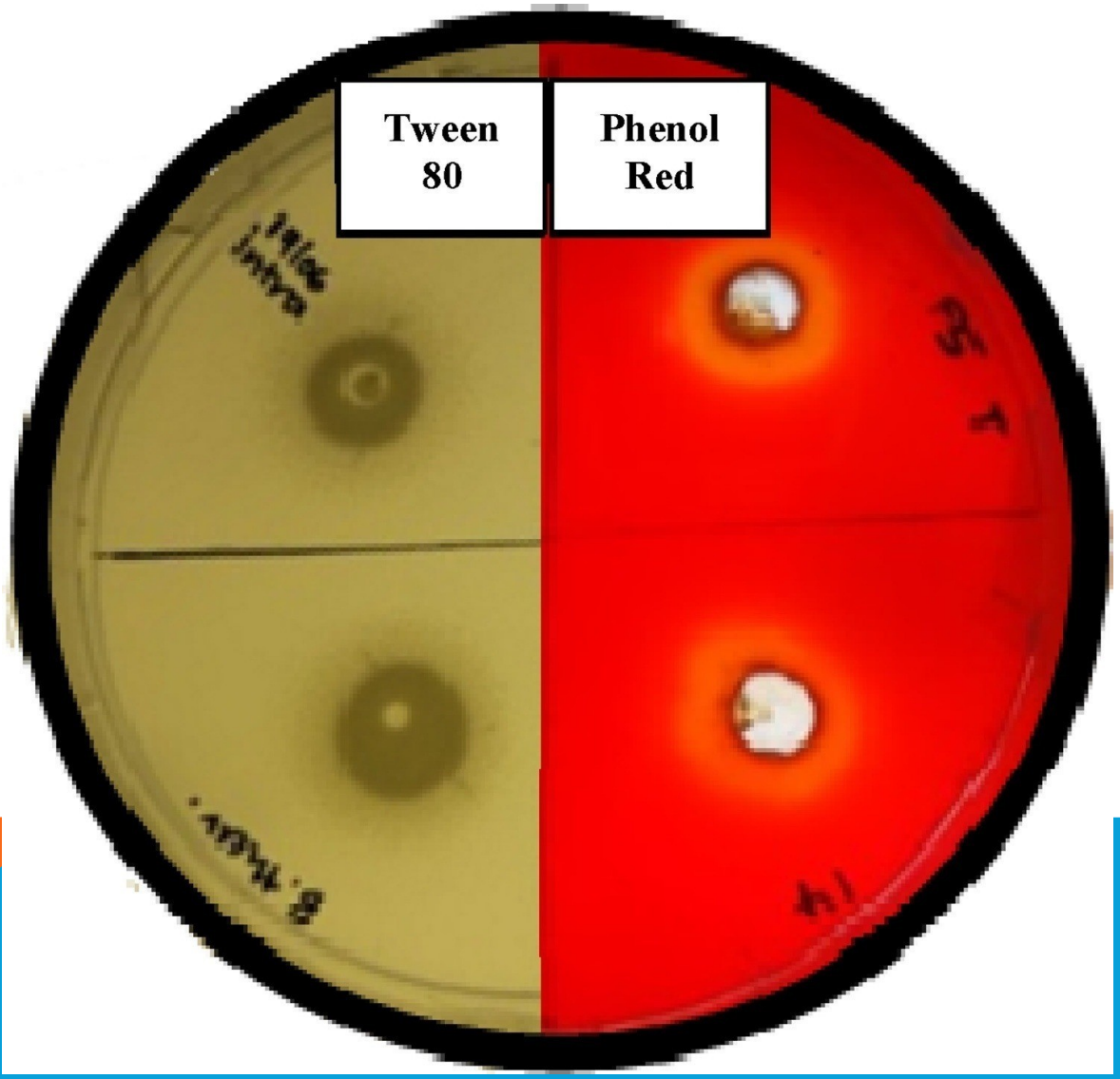
1. Phenol red olive oil agar plates are prepared.
2. Organisms are inoculated onto the phenol red agar plates supplemented with 1% substrate and incubated at 37 °C for 2-4 days.

The principle behind this assay is that a slight drop in pH from 7.3 (end point of the phenol red dye) to a more acidic pH will result in a change of colour from red to orange. The increase in acidity is due to the release of fatty acids following lipolysis.



1% olive oil plate screening assays for the detection of lipase activity of pure bacterial isolates

1% Tween 80 agar plates (left) and 1% tributyrin phenol red agar plates (right) for the detection of lipase activity, respectively, of pure bacterial isolates



Detection methods for lipase activity: Enzyme assays

- Screening systems making use of chromogenic substrates have also been described. The most widely used substrates are tributyrin and triolein which are emulsified mechanically in various growth media and poured into a plate.
- Lipolytic activity is determined spectrophotometrically by measuring the release of *p*-nitrophenol.
- *P*-nitrophenyl (*p*-NP) esters are used to determine lipase activity.
- The substrate mixture consist of 0.5 mM *p*-NP substrate in methanol, 50 mM tris-HCl buffer (pH 8) and 0.1% Triton X-100.
- The standard assay mixture contains 200 µl of substrate mixture and 20 µl of the crude supernatants which are incubated at 37 °C for 1 h.
- The enzyme activity is determined by measuring the release of *p*-NP at an absorbance of 405 nm.