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THE LIPOLYTIC POTENTIAL OF *FUSARIUM OXYSPORUM* F. SP. *VASINFECTUM*, A PHYTOPATHOGEN

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ABSTRACT

The capability of the plant pathogenic fungus *Fusarium oxysporum* f. sp. *vasinfectum* to elaborate extracellular lipases in the submerged cultures was assyed on Tween-20 agar. The pathogen produced 95 U/ mL of lipase after 7 d incubation at 25 DC under submerged fermentation. Glucose was found to be the best carbon source whereas sodium nitrate proved to be the best nitrogen source for lipase activity. The optimum lipase activity was achieved at. pH 7 and 25 DC. Tween-80 and olive oil were found to be the best inducers of the enzyme.

Key words: *Fusarium oxysporum* f. sp. *vasinfectum, phytopathogen, submerged fermentation, inducers.*

Plant pathogens produce an array of enzymes capable of degrading plant cell wall components (Baer and Gudmestad, 1995). Among the economically important plant pathogens, *F. oxysporum* is a ubiquitous phytopathogenic fungus attacking a wide range of plants. *F. oxysporum* f. sp. *vasinfectum* causes the vascular wilt of cotton and also occurs on species of *Cajanus, Coffea, Heavea, Hibiscus, Medicago, Ricinus, Solanum,* and *Vigna.* Generally, pathogenic fungi are believed to secrete various extracellular enzymes some of which have. been implicated in virulence (Wanjiru *et al.,* 2002). Among these, triacylglycerollipases (EC 3.1.1.3) constitute an extensive family, and catalyze both hydrolysis and the synthesis of the ester bonds. The biological function of lipases is the hydrolytic degradation of triacylglycerols into glycerol and free fatty acids. In nature, lipases are ubiquitous (Borgstrom and Brockman, 1984), having been found in animals, plants, fungi, and bacteria (Mukherjee and Hills,1994. Due to enantio specificity, lipases constitute an important group of biocatalysts (Jaeger and Reetz, 1998) and have many industrial applications (Schmid and Verger, 1998). Lipases have been implicated in pathogenesis of various plants. Commenil *et al.,* (1998) have indicated that a cutinolytic lipase from *Botris cinerea* was required for the penetration of the cuticle of the host plant and for the consequent infection process in tomato leaves. Voigt *et al.,* (2005) observed that a secreted lipase of *F. graminearum* is a virulence factor for infection of certain cereals. The objective of this study was to investigate the influence of the culture environment on extracellular lipase secretion and to optimize the same from this phytopathogen.

MATERIALS AND METHODS

Fungal strain

F. oxysporum f. sp. *vasinfectum* was obtained from the culture collection, CAS in Botany, University of Madras. The cultures were maintained at 25 °C on potato dextrose agar plates (plate 1).

Lipase activity on Tween-20 agar

The culture medium was prepared by dispensing peptone 10.0g, NaCI 5.0g, $CaCl_2$ O.l g and agar 20.0g in 1000ml water. 10ml Tween-20 was separately sterilized and added to the autoclaved medium, and the pH was adjusted to 6.0. The plates were inoculated at the center using a pinpoint inoculum of the test fungus. Lipolytic activity was indicated by the appearance of a visible precipitate, resulting from the deposition of crystals of the calcium salt formed by the fatty acids liberated by the enzyme, or as a clearing of such a precipitate around a colony due to complete degradation of the salt of the fatty acids. At regular intervals of 24h, each plate was examined and measurements were made to monitor the lipolytic activity (Sierra 1957).

Lipase production medium

The medium (Sierra ,1957) comprised of 10g peptone, 5g Nacl, O.lg $CaCl_2$.$2H_20$ in 1000ml distilled water. The pH was adjusted to 6 before autoclaving. 10ml Tween-20 was separately sterilized and added to the autoclaved medium. The flasks were inoculated with *F. oxysporum* f. sp. *vasinfectum* and incubated at 25 °C for a period of seven days. The experiments were carried out in triplicates.

Spectrophotometric assay for lipase activity

Lipase activity was assayed quantitatively by using para-nitro phenyl palmitate as the substrate (Winkler and Stuckmann, 1979). 10 ml isopropanol containing 30mg p-nitro phenyl palmitate (Sigma) was mixed with 90 ml 0.05 M sodium phosphate buffer (pH 8.0) containing 207 mg sodium deoxycholate and 100 mg gum arabic. A total volume of 2.4 ml freshly prepared substrate solution was prewarmed at 37 ^{0}C and mixed with 0.1 ml enzyme solution. After 15 min incubation at 37 ^{0}C , absorbance at 410 nm was measured against a blank. One enzyme unit was defined as 1umol of p-nitrophenol enzymatically released from the substrate in milliliters per minute.

Study of the environmental factors

Lipase liquid medium was amended with the various carbon and nitrogen sources as given in the results and with various inducers at 1 % and were incubated at different pH and temperature values. Lipase activity was measured spectrophotometrically for each of these factors and was expressed as lipase units.

RESULTS AND DISCUSSSION

Lipase production by filamentous fungi has been widely reported although most of the studies were carried out using rich media. This is undesirable for large-scale commercial production. There have been a number of techno-economic constraints for the production of industrial enzymes. In this investigation we have employed materials and methods that are cost-effective. A cheap lipase production medium has been used throughout to evaluate the effect of the environmental factors on lipase secretion by *F. oxysporum* f. sp. *vasinfectum* which is a well-known phytopathogen. *F. oxysporum* f. sp. *vasinfectum* has been explored for lipase production in view of the reported involvement of lipases in pathogenesis. *F. oxysporum* f. sp. *vasinfectum* showed extracellular lipolytic activity in Tween-20 agar indicated by the appearance of a visible precipitate, resulting from the deposition of crystals of the calcium salt formed by the fatty acid liberated by the enzyme, or as a clearing of such a precipitate around a colony due to complete degradation of the salt by the fatty acid (Plate 2).

Effect of pH and temperature

pH and temperature are the two important environmental factors which affect extracellular lipase production. *F. oxysporum* f. sp. *vasinfectum* was inoculated in the lipase production medium and incubated at different pH's viz. 5, 6, 7, 8, 9, 10 and 11. At pH 7 maximum lipase activity (207 U ml-1) was observed. *F. oxysporum* f. sp. *vasinfectum* was inoculated in lipase production medium and incubated at different temperatures viz. 4, 25, 30, 35 and 40°C. The optimum, temperature for extracellular lipase production was found to be 25°C yielding 160 U ml-I. A neutral pH 7 supported the best lipase production while the optimum temperature for the same has been 25 °C (Fig. 1 & 2).

Effect of carbon and nitrogen sources

Different carbon sources namely Glucose, Maltose, Galactose, Mannitol and Xylose were amended to the lipase production medium at 1 %. Glucose favoured highest lipase activity (98 U mI-I) followed by Xylose (82 U mI-I) and Mannitol, Maltose and Galactose (67 U mI-I, 58 U ml-I and 42 U ml-I respectively) (Fig. 3). Different nitrogen sources were amended in the lipase production medium at 1 %. Sodium nitrate favoured highest lipase production of 81 U ml-I followed by salts of ammonia NH_4Cl, (NH4)2S04 and $NaNO_3$ showing lipase activity of 64 UmI-I, 75 Uml-I and 53 U ml-I respectively (Fig. 4).

Effect of lipid substrates and vegetable oils

Different lipid substances namely Tween-20, Tween-80, Cholesterol, Stearic acid and Lecithin at 1 % were amended to the lipase production medium. Likewise, different vegetable oils namely olive oil, sunflower oil, castor oil, gingelly oil and coconut oil at 1% were amended to the lipase production medium. Tween-80 induced highest lipase activity (185 U ml-I). Olive oil showed highest lipase activity of 98 U mI-I. The substrate that induces enzyme production in any organism is of great relevance. In the case of lipases it is needless to say that the lipid substances provide good raw materials. The production of lipase was more significant in culture medium amended with lipids as the carbon source than without lipids (fig. 5 & 6). Thus, the brief study showed that lipases can be produced by employing certain cheap materials and inexpensive methods on a laboratory scale. This investigation might come in handy for those who might undertake scaling-up processes by exploiting *F. oxysporum* f. sp. *vasinfectum* for lipase production.

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Plate 1. Culture morphology of *F. oxysporum* f. sp. *vasinfectum*

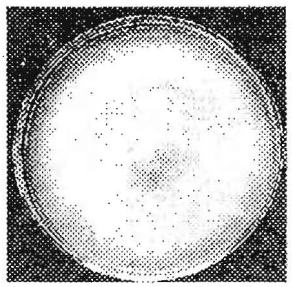

Plate 2. Lipolytic activity of *F. oxysporum* f. sp. *vasinfectum* (halo around the colony)

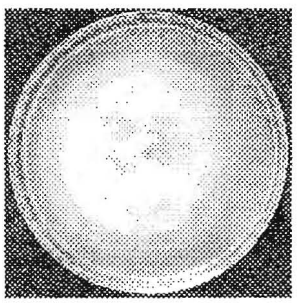

Fig. 1: Effect of pH on extracellular lipase production by *F. oxysporum* f. sp. *vasinfectum*

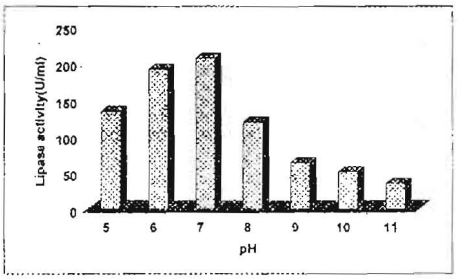

Fig. 2 : Effect of temperature on extracellular lipase production by *F. oxysporum* f. sp. *vasinfectum*

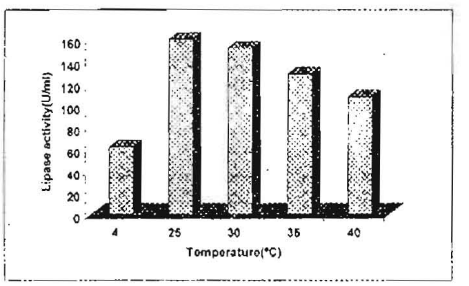

Fig. 3: Effect of carbon sources on extracellular lipase production by *F. oxysporum* f. sp. *vasinfectum*

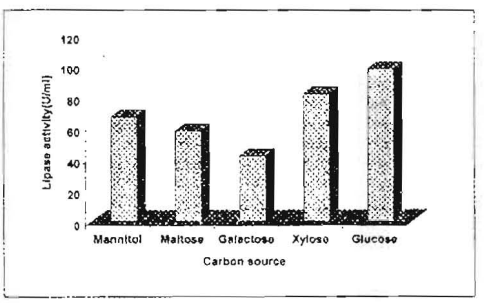

Fig. 4: Effect of nitrogen sources on extracellular lipase production by *F. oxysporum* f. sp. *vasinfectum*

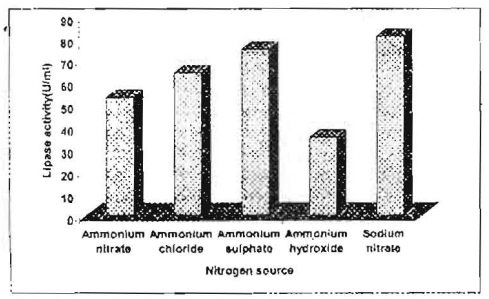

Fig. 5: Effect of lipid substrates on extracellular lipase production by *F. oxysporum* f. sp. *vasinfectum*

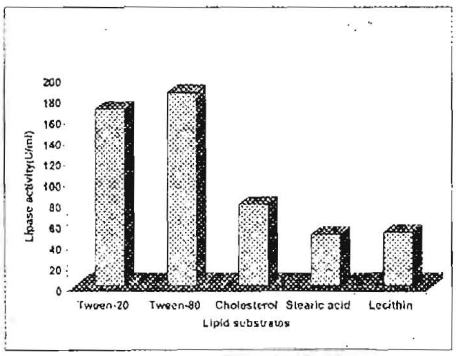

Fig. 6: Effect of vegetable oils on extracellular lipase production by *F. oxysporum* f. sp. *vasinfectum*