Hydrolysis of Corn Oil by Lipase from *Helminthosporium maydis* Race T

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ABSTRACT

Corn oil was used as the sole carbon source for growth of *Helminthosporium maydis* Race T (NRRL 5128) in shaken flasks at 28 C. Oil recovered by hexane extraction from culture medium and mycelia at 2-day intervals was analyzed by thin layer chromatography and gas chromatography. Triglyceride content of the oil was reduced, whereas free fatty acid, monoglyceride, and diglyceride contents increased as a result of *Helminthosporium maydis* growth. Free sterol and steryl ester contents were unaffected. Lipase production was demonstrated by *Helminthosporium maydis* cells grown on corn oil, but the enzyme was not detected in cells grown on sucrose.

INTRODUCTION

An epidemic of southern corn leaf blight caused by *Helminthosporium maydis* Nisikada et Miyake occurred throughout the U.S. in 1970. This epidemic was caused by Race T of the fungus and was especially destructive on corn planted late in the Midwest corn belt (1). This disease reduced crop yields, gave low test wt for dry milling, and caused excessive amounts of small, damaged kernels (2). Proximate analyses of blighted corn from the 1970 crop by Cavins et al. (3) showed an increase in fat acidity values from 29 to 100 as blight damage increased. Heavily damaged kernels contained ca. 5% less oil and had a slightly lower starch content than did good kernels. Ellis et al. (4) have shown that *H. maydis* Race T and O and *H. carbonum* Ullstrup Race I grow well on fatty acids having 12 or more carbon atoms. We have now determined that corn oil, as a sole carbon source, supports growth of *H. maydis* Race T and that hydrolysis of the oil occurs as a result of induced lipase production.

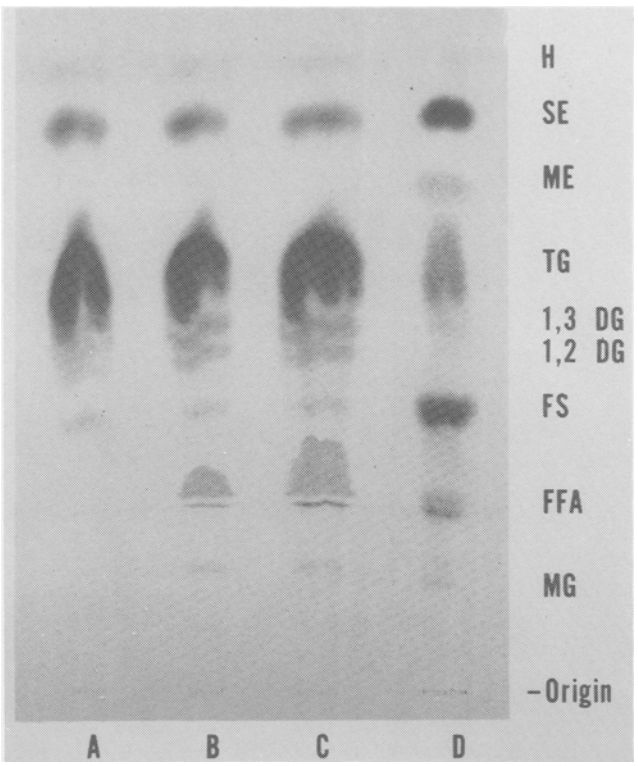

FIG. 1. Separation by thin layer chromatography of control oil (A), oil recovered from medium (B), and mycelia (C) after 8 days growth of *Helminthosporium maydis*, and standards (D). Identity of lipid classes (from top to bottom): hydrocarbons (H), steryl esters (SE), methyl esters (ME), triglycerides (TG), 1,3-diglycerides 1,3-DG), 1,2-diglycerides (1,2-DG), free sterols (FS), free fatty acids (FFA), and monoglycerides (MG).

EXPERIMENTAL PROCEDURES

Strain NRRL 5128 *H. maydis* Race T was grown on corn oil as the sole carbon source as described by Ellis et al. (4).

Corn oil recovered from medium and cells by hexane extraction (4) was analyzed on thin layer chromatographic (TLC) plates (20 x 20 cm) coated with Silica Gel G (0.5 mm) and activated 1 hr at 100 C. Oil samples were diluted 1:10, and 10 µl was applied as a 2-cm streak to the plates, which were then developed in the double development system of Freeman and West (5). The first solvent system of benzene:ether:ethanol:acetic acid (50:40:2:0.2, v/v) was allowed to migrate 14 cm. After air drying, plates were placed in the second solvent system of hexane:ether (94:6, v/v) and allowed to develop to the top. Zones, representing lipid classes, were visualized with iodine vapors or by charring with 50% sulphuric acid. Reference standards (Supelco, Inc., Bellefonte, PA) were chromatographed alongside the oil samples. Free fatty acids (FFA) in corn oil recovered from cultures of *H. maydis* were isolated by column chromatography (6) and analyzed by gas chromatography (GC). Methyl esters were prepared with borontrifluoride (14% wt/v) in methanol. GC analyses were done on a Bendix 2500 chromatograph equipped with flame ionization detectors and glass columns (6 ft x 2 mm) packed with 5% ethylene-glycoladipate (Supelco, Inc., Bellefonte, PA). Oven temperature was 180 C. Fatty acid methyl esters were identified by comparing their retention times to those of authentic standards.

A double-layered plating procedure (7) was used to detect lipase production by cells of *H. maydis* grown on corn oil or sucrose. The base layer consisted of corn oil (5 ml), 1:1500 Victoria Blue B (Allied Chemical, Morristown, NJ) (40 ml), and agar (Difco Laboratories, Detroit, MI) (6 g) in water (365 ml). This layer (6 ml per petri dish) was overlayed with a nutrient layer consisting of yeast extract (1.2 g), tryptone (2.8 g), sodium chloride (2.0 g), agar (80 g), and water (400 ml). Plates were inoculated in triplicate with cells grown on either corn oil or sucrose. Plates were incubated at 28 C and observed daily for 14 days.

RESULTS AND DISCUSSION

Dark blue coloration was observed under all cell colonies; however, only a subsequent clearing of the oil emulsion around the colony was regarded as positive evidence of lipase production. Cells grown on corn oil gave positive results after 4-6 days, whereas sucrose-grown cells did not cause a clearing of the emulsion within 14 days.

TLC separation of control oil (A) and oil recovered from medium (B) and mycelia (C) after 8 days growth of *H. maydis* is shown in Figure 1. Reference standards (D) are

from top to bottom: hydrocarbons (n-eicosane), steryl esters (cholesterol oleate), methyl esters (methyl oleate), triglycerides (triolein), 1,3-diglycerides (1,3-dipalmitin), 1,2-diglycerides (1,2-dipalmitin), free sterols (cholesterol), FFA (oleic), and monoglycerides (monopalmitin). Only trace quantities of hydrocarbons were detected, and no variation in concentration as a result of *H. maydis* growth could be observed on TLC plates. Zones representing steryl esters and free sterols in corn oil were unchanged. Diglycerides, FFA, and monoglycerides showed definite increases in concentrations. The most marked change in oil composition as a result of *H. maydis* growth is the increase in FFA. Expressed oil from 8-day cells (C) had a higher concentration of FFA than did free oil recovered from the medium (B). This increase in FFA probably results from the lipase produced by the cells hydrolyzing more corn oil than they used for growth. Analysis of FFA methyl esters by GC showed no significant difference in relative amounts of acids in control and experimental oil, indicating that *H. maydis* cells did not selectively utilize a particular fatty acid.

We have demonstrated that one strain of *H. maydis* Race T can readily use corn oil as its sole carbon source for growth, in vitro. The primary effect of *H. maydis* growth increases FFA content due to lipolytic activity of the growing fungi.

REFERENCES

1. Jellum, M.D., Cereal Chem. 48:663 (1971).
2. Brekke, O.L., A.J. Peplinski, E.L. Griffin, Jr., and J.J. Ellis, Ibid. 49:466 (1972).
3. Cavins, J.F., O.L. Brekke, E.L. Griffin, Jr., and G.E. Inglett, J. Agric. Food Chem. 20:1146 (1972).
4. Ellis, J.J., G.A. Bennett, and C.W. Hesseltine, Mycologia 65:539 (1973).
5. Freeman, C.P., and D. West, J. Lipid Res. 7:324 (1966).
6. McCarthy, R.D., and A.H. Duthie, Ibid. 3:117 (1962).
7. Alford, J.A., and E.E. Steinle, J. Appl. Bacteriol. 30(30):488 (1967).

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