Polar and Nonpolar Lipids and Their Fatty Acid Composition of a Few *Fusarium* Species

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A few species of *Fusarium* have been evaluated for their potential to produce lipids. The isolates under investigation exhibited wide variation with respect to the mycelial weight, total lipid content and percentage composition of polar and nonpolar lipids in which triglycerides were the major components (81–90%). Palmitic, stearic, oleic and linoleic acids were the major fatty acids in both the fractions. The polar lipids contained higher levels of linoleic acid, whereas nonpolar lipids contained oleic acid as the predominant acid. Nonpolar lipids were more saturated than polar lipids.

KEY WORDS: *Fusarium pallidoroseum*, lipids of *Fusarium*, nonpolar and polar lipids, unsaturation index of lipids.

The basic physiology of fat accumulation in oleagenic organisms has been the subject of several investigations for many years (1–6). Many species of *Fusarium* have been indicated as potential fat producers (7–9). They have proved to be particularly efficient in biotransformation of carbohydrates into fat, even at low sugar concentrations, an important criterion in the bioconversion of wastes into fat. However, only a few species among the large population of *Fusarium* have been examined for fat production.

The present investigation was undertaken to study the relative proportion of polar and nonpolar lipids of *Fusarium* isolates and the distribution of fatty acids between these fractions.

MATERIALS AND METHODS

Organisms. Fusarium isolates were collected from different sources. *Fusarium lini* ITCC 788, *F. lycopersici* ITCC 1322 and *F. oxysporum* ITCC 1635 were supplied by the Indian Agricultural Research Institute, New Delhi, India; *F. equeseti* and *F. moniliforme* were kindly given by the Applied Botany Department, University of Mysore, Mysore, India. One isolate of *F. oxysporum* and two strains of *F. pallidoroseum* were isolated from cereals at our laboratory. The cultures were maintained on potato dextrose agar slants at ±5°C by monthly subculture.

Inoculum. For inocula, each culture was grown at 25–27°C in 100 mL potato sucrose broth (potato extract from 200 g diced potato, 20 g sucrose per liter of the broth) in 250-mL conical flask on a shaker at 100 rpm for 3 d. The mycelial mass was filtered off aseptically through a muslin cloth. An aliquot of the filtrate was suitably diluted with sterile distilled water to get a spore density of 10^5 to 10^6/mL.

Culture conditions. The composition of the culture medium was as follows: (g/L); Sucrose, 100; $MgSO_4 \cdot 7H_2O$, 1.0; NH_4NO_3, 2.0; KH_2PO_4, 1.0; KCl, 0.5; yeast extract, 1.0; succinic acid, 1.0; and trace elements, 1.0 mL; pH 5.25. The composition of the trace element solution was: (g/L); $FeSO_4$, 5; $ZnSO_4 \cdot 7H_2O$, 1.0; $CuSO_4 \cdot 5H_2O$, 0.5; NH_4O, 0.5; $NH_4MoO_4 \cdot 2H_2O$, 0.1. The medium was distributed in 100-mL proportions into Roux bottles (10) and sterilized at 121°C for 15 min. One mL of spore suspension of the test isolate was added to each of ten flasks. The flasks were kept horizontal to provide maximum surface area in an incubator at 27 ± 1°C for 10 d. The mycelial mats were harvested by filtration, washed with water, dried at 50°C to constant weight and analyzed.

Total lipid content. For extraction of total lipids, 2 g dried mycelia was hydrolyzed with 1N HCl by steaming for 15 min. The digest was then mixed with Hyflo-Supercel (cp. 0.5 g) and filtered through Whatman No. 1 filter paper (Maidstone, England) in a Buchner funnel. The residue was washed with water until it was free from acid. The filtrates were pooled together in a separating flask and mixed with 2.5 vol of a mixtue of $CHCl_3$/MeOH (1:1, vol/vol) to recover the lipids lost during washing. The lower $CHCl_3$ layer was transferred to a 500-mL flask.

The residue along with the filter paper was further treated as explained earlier (9). Finally, all the extracts, including the chloroform extract of the acid filtrate, were combined and evaporated on a flash evaporator with a water bath temperature of 50°C under nitrogen atmosphere. The residue was redissolved in $CHCl_3$/MeOH (2:1, vol/vol) and passed through a sodium sulfate layer in a funnel to remove undissolved impurities and moisture, if any. The sodium sulfate layer was washed with the above solvent mixture several times to recover all the lipids. The filtrates were collected in a round-bottom flask and evaporated as above. The tared weight of the residue was taken, and the percentage of lipids was calculated.

Polar and nonpolar lipids. The lipid was fractionated into polar and nonpolar classes by countercurrent distribution followed by rubber dialysis. The method is briefly described below. The lipid to be fractionated was dissolved in 45 mL of upper layer of petroleum ether (40–60°C): ethyl alcohol (87%) mixture and treated as per the method of Galanos and Kapoulas (11). The polar fraction derived from this method after Folch washing, evaporation and drying was again dissolved in 20 mL petroleum ether (40–60°C) and further purified by rubber dialysis as described by Rattanchand and Srinivasan (10). The dialyzed fraction was mixed with the nonpolar fraction of the countercurrent distribution and evaporated to dryness. The weight of this fraction was used to calculate the percentage of nonpolar lipids in the original sample. The nondialyzable fraction was also evaporated to dryness and weighed, and the percentage polar lipids of the original sample was calculated.

Triglyceride content. The triglyceride content of the nonpolar lipids was determined by thin-layer chromatography (12).

Fatty acid composition. The polar and nonpolar lipids were saponified and methylated separately, and the fatty acid distribution in each fraction was determined by gas-liquid chromatography as described earlier (9).

RESULTS AND DISCUSSION

The weight of the mycelia and the percentage lipid content of different *Fusarium* isolates are given in Table 1.

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TABLE 1

Mycelial Weight and Lipid Content of *Fusarium* Isolates

Isolates	Mycelial weight (g/L)	Lipid (%)
F. equeseti	8.9	32.7
F. lini ITCC 788	9.4	35.8
F. lycopersici ITCC 1322	26.0	38.5
F. moniliforme	14.2	25.8
F. oxysporum	11.5	28.4
F. oxysporum ITCC 1635	13.3	28.1
F. pallidoroseum-1	37.0	33.1
F. pallidoroeum-2	25.0	49.7

TABLE 3

Distribution of Fatty Acids in Polar Lipids of *Fusarium* Isolates[a]

Isolates	14:0	16:0	18:0	18:1	18:2	18:3
F. equeseti	1.4	19.1	10.2	26.6	39.6	3.2
F. lini ITCC 788	—	16.6	6.2	24.9	52.2	—
F. lycopersici ITCC 1322	—	17.8	6.9	22.3	50.5	2.3
F. moniliforme	—	21.6	4.1	20.6	43.6	9.5
F. oxysporum	—	24.8	8.4	19.4	47.5	—
F. oxysporum ITCC 1635	—	26.0	11.2	30.3	32.5	—
F. pallidoroseum-1	1.0	19.7	8.5	18.3	46.9	6.0
F. pallidoroseum-2	—	24.6	4.6	23.6	46.0	—

[a]Values are mean determinations of three trials.

The isolates showed great variation with respect to the amount of mycelium and the lipid content. They contained from 25.8 to 49.7% lipids on a dry-weight basis. Among the isolates examined, *F. moniliforme* contained a relatively low percentage of lipid, and *F. pallidorosseum*-2 was the highest. *Fusarium lycopersici* and two strains of *F. pallidoroseum* produced more than 25 g of mycelial mass per liter of the medium. The results showed that *F. pallidoroseum*-2 had comparatively greater potential for efficient conversion of the substrate to biomass and lipids.

The percentage composition of polar and nonpolar fractions of lipids are given in Table 2. The polar lipids varied from 2.3 to 8.3% in different isolates. However, there was a significant difference in the levels of polar lipids of these isolates. *Fusarium moniliforme* had the highest level of polar lipids, whereas *F. pallidoroseum*-2 had the lowest level. The nonpolar fraction accounted for more than 90% of the total lipids in all isolates. *Fusarium lini, F. lycopersici* and two isolates of *F. pallidoroseum* had high proportions of nonpolar lipids. Triglycerides were the major components of the nonpolar fraction, and their concentration varied from 81 to 90% in different isolates.

The fatty acid profile of the polar and nonpolar lipids are presented in Tables 3 and 4. Qualitatively, the isolates examined had similar fatty acid compositions. Palmitic, stearic, oleic and linoleic acids were the major fatty acids in both fractions. Myristic and palmitoleic acids were present in trace amounts. However, the distribution of fatty acids between the two fractions were not the same. The polar lipids contained higher levels of 18:2 acid, whereas nonpolar lipids contained 18:1 as the predominant acid. Only *F. equeseti, F. moniliforme* and *F. pallidoroseum*-1 produced considerable amounts of 18:3, and the major portion of it was found in the polar fraction of the lipids of these isolates.

Total saturated and unsaturated acids are given in Table 5. In the majority of the isolates, nonpolar lipids were more saturated than the polar fractions. As the unsaturation index (USI) of fatty acids gives a truer picture of the degree of unsaturation, it was calculated by standard methods (13,14). The ratio of USI of polar lipids to that of nonpolar lipids is a measure of the relative prevalence of unsaturated acids in the two fractions. In the majority of isolates in the present investigation, this value was more than one (Fig. 1), indicating greater unsaturation of the polar lipids than the nonpolar. Therefore, the isolates appeared to have a greater tendency to incorporate unsaturated fatty acids into the polar lipids. However, in *F. oxysporum*, unsaturated acids were almost equally distributed between the polar and nonpolar fractions.

In summary, *Fusarium* species exhibit wide variation with respect to mycelial weight, total lipid content and percent composition of their polar and nonpolar fractions under any given conditions. Nonpolar lipids account for more than 90% of the total lipids. Triglycerides are the major components of nonpolar lipids. Palmitic, linoleic and oleic acids constitute 80 to 85% of the total fatty acids of both fractions in each species. Nonpolar lipids are more saturated than polar lipids. Most of the species show a greater tendency to incorporate unsaturated fatty acids into polar lipids.

TABLE 2

Polar and Nonpolar Fractions and Triglyceride Content of Lipids of *Fusarium* Isolates[a]

Isolates	Polar lipids (% total lipids)	Nonpolar lipids	
		Total (% total lipids)	Triglycerides (% nonpolar lipids)
F. equeseti	6.0	93.6	83.3
F. lini ITCC 788	3.5	96.5	90.0
F. lycopersici ITCC 1322	4.8	95.2	86.0
F. moniliforme	8.3	91.7	85.5
F. oxysporum	6.8	93.2	85.5
F. oxysporum ITCC 1635	7.8	92.2	86.6
F. pallidooseum-1	3.8	96.2	85.0
F. pallidoroseum-2	2.3	97.7	89.6

[a]Values are mean determinations of three trials.

TABLE 4

Distribution of Fatty Acids in Nonpolar Lipids of *Fusarium* Isolates[a]

Isolates	16.0	18.0	18.1	18.2	18.3
F. equeseti	20.4	14.9	35.4	26.6	2.6
F. lini ITCC 788	20.0	16.5	40.6	20.3	1.8
F. lycopersici ITCC 1322	26.2	9.0	40.8	20.8	2.2
F. moniliforme	19.2	8.0	39.6	31.6	2.6
F. oxysporum	21.0	9.3	41.7	28.0	—
F. oxysporum ITCC 1635	21.8	9.3	40.7	28.2	—
F. pallidoroseum-1	25.8	15.8	33.2	23.8	2.6
F. pallidoroeum-2	18.2	16.3	43.1	18.9	2.9

[a]Values are mean determinations of three trials.

TABLE 5

Total Saturated and Unsaturated Acids in Polar and Nonpolar Lipids of *Fusarium* Isolates

	Saturated		Unsaturated	
Isolates	Polar	Nonpolar	Polar	Nonpolar
F. equeseti	30.7	35.3	69.4	64.6
F. lini ITCC 788	22.8	36.5	77.1	62.7
F. lycopersici ITCC 1322	24.7	35.2	75.1	63.8
F. moniliforme	25.7	27.2	73.7	73.8
F. oxysporum	33.2	30.3	66.9	69.7
F. oxysporum ITCC 1635	37.2	31.1	62.8	68.9
F. pallidoroseum-1	29.2	41.6	71.2	59.6
F. pallidoroseum-2	29.2	34.5	69.6	64.9

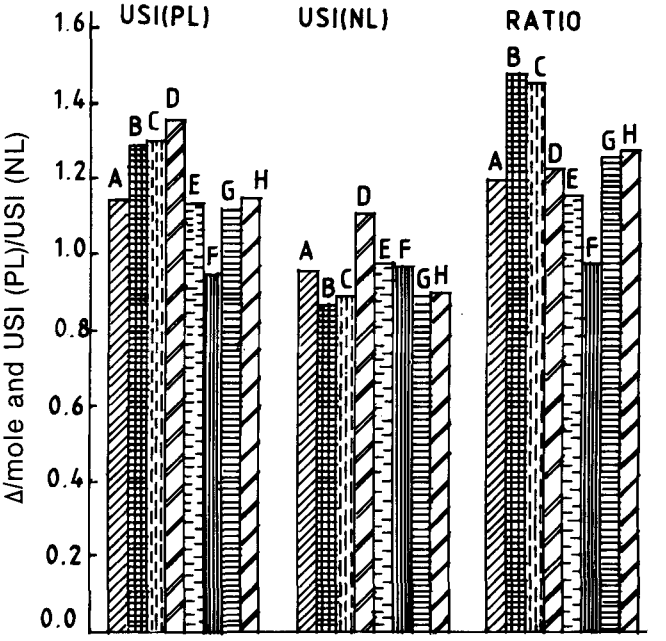

FIG. 1. Unsaturation indices (USI) of polar and nonpolar lipids and their ratios. PL, polar lipids; NL, nonpolar lipids; A—*F. equeseti*; B—*F. lini* ITCC 788; C—*F. lycopersici* ITCC 1322; D—*F. moniliforme*; E.—*F. oxysporum*; F—*F. oxysporum* ITCC 1635; G—*F. pallidoroseum* 1; H—*F. pallidoroseum* 2.

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