The IR spectra were taken on a UR-10 instrument and the UV spectra on a Hitachi instrument.

SUMMARY

It has been established that a complex lipase preparation from *Oospora lactis* possesses a high activity, causes no position and geometric isomerization of the fatty acids isolated with its aid, possesses no specificity of action, and splits off the fatty acids from all three positions of the triglyceride molecule.

The high activity of the complex lipase preparation permit its recommendation for the hydrolysis of fat in order to isolate the total fatty acids in the native state, which is important in the study of their chemical structure and also in the splitting of fats in industry.

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METABOLITES OF THE PATHOGENIC FUNGUS *Verticillium dahliae*.

IV. INFLUENCE OF THE SOURCE OF CARBON ON THE LIPID COMPOSITION OF THE FUNGUS *Verticillium dahliae*

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The dependence of the metabolism of the fungus *Verticillium dahliae* Kleb. on the external conditions is one of the reasons for the limited nature of information on its lipid metabolism.

There is information in the literature on the influence of the source of carbon-containing nutrient on the growth and metabolism of *V. dahliae* [1-3], but nothing is known about how the source of carbon affects its lipid composition. Continuing investigations of the lipid metabolism of *V. dahliae* [4-6], we have studied the influence of the source of carbon-containing nutriment and fermentation on its lipid composition.

We used as nutrient media: 1) starch (biomass — dark gray powder); the yield of lipid fraction amounted to 5.9%; 2) sucrose (biomass — gray powder); 13.6%; 3) glucose (biomass —

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black powder); 12.6; 4) potato pulp (biomass — light gray powder); 2.5; and 5) hulled millet grain (biomass — dark gray powder); 2.9%. As the control we took the lipid fraction isolated with a yield of 23% from the biomass of *V. dahliae* grown under stationary conditions on sucrose [6]. The amount of lipid fraction in the growth of the fungus in a fermenter was a maximum where the nutrient medium was sucrose and glucose and was considerably less than on growth under stationary conditions. All the oils isolated were yellow, liquid or semiliquid, with a characteristic unpleasant smell.

The qualitative and quantitative compositions of the neutral lipids of samples of *V. dahliae* grown in the various nutrient media (1-5, see above) determined by TLC [6] were as follows (%):

Fraction	Class of Lipids	R_f	*1*	*2*	*3*	*4*	*5*
5	Carbohydrates (CH)	0.96	11	19	11	20	12
	Sterol esters (SE)						
4	Mixture of phthalate (Phth), triglycerides (TG), and an unidentified substance X_3	0.66	38	56	54	57	50
3	Free fatty acids (FFA)	0.41	29	15	20	7	15
2	Unidentified substance X_2	0.22	15	3	6	8	13
1	X_1	0.00	7	7	9	8	10

The qualitative composition of the lipid fractions of the fungus changed only slightly with a change in the nutrient medium but the differences in the quantitative composition were more considerable. On the basis of the PMR, IR, and mass spectra of the lipid fractions isolated by preparative TLC it was established that they contained TG in trace amounts and methyl esters (ME) of fatty acids were practically absent. The formation of ME of fatty acids in trace amounts was observed only for sample 4, while in the lipid composition of the fungus grown under stationary conditions, the TG and fatty-acid ME fractions are predominating [6].

The absence of the TG and fatty-acid ME fractions from the neutral lipids of the fungus grown in the fermenter was confirmed by the coincidence (GLC results) of the fatty-acid compositions of the initial previously unhydrolyzed but diazomethane-methylated lipid fractions with the fatty-acid composition obtained after mild alkaline hydrolysis of the same fractions followed by methylation.

In the development of the fungus, TG are first synthesized from the carbohydrate nutrient medium, and these are then used as an endogenous energy-reserve material [8]. The results that we obtained indicate that, apparently, in the fermenter the fungus passes through its development cycle more rapidly and the TG can be split into the total fatty acids.

The composition of the total fatty acids (GLC) obtained after alkaline hydrolysis of the initial oils was as follows (%):

Acid	*1*	*2*	*3*	*4*	*5*
$C_{14:0}$ antiiso-	—	—	—	—	0.1
$C_{15:0}$	—	—	—	—	0.3
$C_{15:0}$	—	—	—	—	0.9
iso-$C_{16:0}$	—	—	—	—	0.3
$C_{16:0}$	17.5	27.2	18.8	24.4	10.5
$C_{16:1}$	0.6	1.0	0.7	1.3	0.8
$C_{18:0}$	2.6	4.0	3.3	3.6	2.8
$C_{18:1}$	43.4	48.0	47.1	38.7	35.1
$C_{18:2}$	31.6	19.8	26.3	27.0	44.8
$C_{18:3}$	4.3	—	3.8	5.0	4.4
$\frac{\text{Unsat.}}{\text{Sat.}}$	4.0	2.2	3.5	2.7	5.8

The qualitative acid compositions of all the samples investigated were basically similar. When sucrose was used as the nutrient medium, no formation of linolenic acid was observed either in the fermenter or under stationary conditions [6]. When the fungus was grown on millet (sample 5) the formation of pentadecanoic and saturated iso acids was ob-

served. In the quantitative respect, the saturated acid predominated, although the $\Sigma_{unsat}/\Sigma_{sat}$ ratio changed according to the medium in all the samples.

In all the samples, fraction 4 (R_f 0.66) predominated among the neutral lipids. Having isolated this fraction from the lipids of sample 2, we rechromatographed it under the same conditions of TLC and isolated a phthalate, which was identified as di-2-ethylhexyl phthalate [5]; TG, identified from their spectra [4]; and an unidentified substance giving a positive Beilstein test.

In view of the fact that the presence of halogen-containing components in the lipid fraction of *V. dahliae* grown under stationary conditions using sucrose as the source of carbon has been detected qualitatively previously [7], we made an attempt to determine quantitatively the amount of halogen in the lipid fraction of the mycelium of *V. dahliae* grown on Czapek—Dox medium with sucrose in a fermenter, and under stationary conditions.

Samples of the initial oils were subjected to severe alkaline hydrolysis to convert the chlorine into the water-soluble state. The halogen was determined by the method of precipitating titration with biamperometric indication of the final current. The high value of the blank experiment (approximately 50% of the amount of chlorine determined) due to the presence of inorganic chlorine in the reagents lowered the accuracy of the result but did not affect the correctness of the determinations.

No chlorine was found in samples 1, 3, 4, and 5.

Below we give information on the amounts of halogen in the neutral lipids of a sample of *V. dahliae* grown on sucrose (the corrections for the blank experiment have been deducted in the results):

Sample	Weight of oil, g	Cl^- found, μg and confidence intervals (α = 0.95)	Cl^- content, %
Grown under stationary conditions	2.1510	575.3 570.6 600.3	
	Mean:	575.4 ± 181.2	0.027 ± 0.008
Grown in a fermenter	1.9923	325.0 305.0 328.5	
	Mean:	316.2 ± 99.6	0.016 ± 0.005

We shall report the structure of the chlorine-containing compound later.

Thus, the nutrient medium has a strong influence on the qualitative and quantitative composition of the neutral lipids of *V. dahliae* which is undoubtedly reflected in the metabolism of the fungus as a whole, and also in its pathogenic properties.

EXPERIMENTAL

All the spectra were taken under the conditions described previously [4]. The *Verticillium dahliae* Kleb., initially isolated from a diseased cotton plant, was grown in fermenters on Czapek—Dox medium using various sources of carbon an nutrient material.

The lipid fraction was extracted and chromatographed under the conditions described previously [4-6].

Severe Alkaline Hydrolysis. A weighed sample of the initial oil (of the order of 2 g) was treated with a mixture of 2 g of KOH (accurately weighed, chda [pure for analysis]), 1 ml of double-distilled water, and 5 ml of methanol. The mixture was boiled on the water bath for 3 h. Then the condenser was rinsed with double-distilled water and the volume of the hydrolyzate was made up to 50 ml. The fatty part was extracted from the hydrolyzate with diethyl ether (40, 20, and 20 ml), and then the aqueous layer was made weakly acid to Methyl Orange with 1.5 N HNO_3 (chda) and extraction with diethyl ether was continued (50, 25, and 25 ml). The aqueous layer containing Cl^- ions was carefully evaporated in the water bath with the avoidance of the oxidation of the Cl^- by the nitric acid. The solution was transferred quantitatively to a 50-ml measuring flask and made up to the mark with double-distilled water.

Biamperometric titration was performed in the apparatus described previously [9], with two silver electrodes rotating about a common axis, with respect to the silver current.

The titration curves were obtained in clear form by the titration of the Cl^- in 50% acetone in the presence of a solution of gelatin and with the ice-water cooling of the solution undergoing titration [10]. A standard solution of $AgNO_3$ (0.05 N) was metered in by a plunger microburet, which enabled the reagent to be metered with an accuracy of 0.0005 ml [3]. As the support we used a saturated solution of KNO_3 (especially pure), and titration was performed at a voltage on the electrodes of 0.3 V. The equivalence point was found by the usual graphical method.

SUMMARY

The compositions of the neutral lipids and the fatty-acid compositions of *Verticillium dahliae* Kleb. grown in a fermenter with various sources of carbon-containing nutriment have been studied. It has been shown that the capacity of the fungus for synthesizing lipids and the quantitative and qualitative compositions of the neutral lipids and the fatty acids change according to the source of carbon-containing nutriment. Under the conditions of growth of the fungus in a fermenter, in some cases the formation of linolenic acid and of saturated iso acids is observed.

It has been established that when *V. dahliae* is grown on sucrose, the neutral lipids of the fungus include a chlorine-containing compound. The chlorine in the initial oil has been determined quantitatively.

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