

Short Communication

Esterification of Fatty Acids by *Aspergillus flavus*¹

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Aspergillus flavus, grown on soybean oil fatty acids as the sole carbon source, produced triglycerides. While most of the triglycerides were intracellular, considerable amounts also were found extracellularly. The latter originated most likely from esterification of fatty acids by a cell-bound lipase. Although the fatty acids of these fungal triglycerides were the same as those of soybean oil fatty acids, polyunsaturated acid content was greater than expected from the added substrate.

In a previous communication (1), we reported that *Aspergillus flavus* (NRRL 1957), hydrolyzed soybean oil to fatty acids completely in three days of fermentation. However, continued fermentation resulted in a mixture of triglycerides and fatty acids in the fermentation medium. To confirm that these triglycerides are formed from fatty acid substrates, *A. flavus* was grown on soybean oil fatty acids as the sole carbon source. The results of the fermentation are discussed in this short communication.

EXPERIMENTAL

Fermentations were carried out at 28 C in Fernbach flasks as reported previously (1). The synthetic medium consisted of 0.2 g asparagine, 0.1 g K₂HPO₄, 0.05 g MgSO₄, 0.5 mg thiamine hydrochloride, 0.145 mg Fe(NO₃)₃·9H₂O, .088 mg ZnSO₄·7H₂O, .031 mg MnSO₄·4H₂O/100 ml water. Seven hundred fifty ml sterilized medium and 20 ml (16.8 g) distilled soybean fatty acids (16:0 = 10.4%; 18:0 = 3.8%; 18:1 = 22.0%; 18:2 = 55.6%; 18:3 = 8.2%) were fermented aerobically with a loopful of *Aspergillus flavus* for five days. The extracellular and intracellular lipids were extracted and analyzed as described previously (1).

RESULTS AND DISCUSSION

A. flavus grew well with soybean oil fatty acids as the sole carbon source. After five days of fermentation the yield of lipid-free dry cells was 4.14 g. The intracellular and extracellular lipid fractions obtained by extraction with CHCl₃-MeOH and hexane, respectively, were analyzed by gas chromatography, and the results are given in Table 1. At the end of fermentation, the extracellular lipids consisted of triglycerides (14%) and free fatty acids (FFA). Thin layer chromatography indicated only traces of di- and monoglycerides. Intracellular lipids were essentially triglycerides with 5% FFA. Previously (1), when soybean oil was the substrate, the presence of triglycerides following complete hydrolysis was explained on the basis of cell lysis. The large amount (14% of 7.9 g or 1.1 g) of extracellular triglyceride could not come from cell lysis based on the following calculation. The intracellular lipid content calculates to 33% of the total dry cell mass (2 g of lipid in 4.14 g of lipid-free cells). Therefore, 3.3 g of cells would have to be lysed to release 1.1 g triglycerides into the medium. Such a large amount of lysed cells (3.3 g/6.14 g or >50%) is not plausible.

These triglycerides are more likely formed by an extracellular cell-bound lipase. Such an enzyme system has been described for *Saccharomycopsis lipolytica* (2). Of the 16.8 g soybean fatty acids added, only 9.9 g was extracted. The remainder was utilized for cell growth and metabolism. Glycerol for triglyceride synthesis was presumably synthesized by *A. flavus*.

The fatty acid compositions of the intracellular and extracellular triglycerides were similar. Their linolenate and linoleate contents were higher than that of the substrate, soybean oil fatty acids. In contrast, the FFA fractions contained comparatively less linolenic acid and higher amounts of oleic acid than the substrate.

TABLE 1

Fatty Acid Compositions of Lipid Fractions from *A. flavus* Fermentation

Fraction	wt g	FFA %	Fatty Acid Composition											
			Free fatty acid fraction					Triglyceride fraction						
			16:0	18:0	18:1	18:2	18:3	16:0	18:0	18:1	18:2	18:3		
Soybean oil fatty acid	-	-	10.4	3.8	22.0	55.6	8.2							
Hexane extract	7.9	86	11.0	5.4	28.1	51.2	4.2	10.0	1.9	11.6	65.4	11.1		
CHCl ₃ -MeOH extract	2.0	5	12.5	5.9	23.6	53.3	4.7	8.5	1.8	14.2	66.2	9.4		
Total extract ^a	-	-	10.4	4.4	23.6	55.6	6.0							

^aCalculated fatty acid composition of combined extracts from fermentation broth and cells.

¹Part of a presentation at the American Oil Chemists' Society Annual Meeting in Phoenix, AZ, in May 1988.

FATTY ACID ESTERIFICATION

The calculated fatty acid composition of the total lipids was similar to the original soybean fatty acids except for decreased linolenic acid. This decrease in linolenic acid was not due to autoxidation because there was no change in the fatty acid composition in a control experiment in which *A. flavus* was omitted. It appears that *A. flavus* preferentially metabolized linolenic acid. This ability to consume linolenic acid might have potential industrial application.

REFERENCES

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2. Ota, Y., K. Gomi, S. Kato, T. Sugiura and Y. Mionda, *Agric. Biol. Chem.* 46:2885 (1982).

[Received September 18, 1987;
accepted May 2, 1988]