The Effect of Oils and Fatty Acids on the Production of Filipin

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Filipin is a new antifungal antibiotic first reported by Ammann *et al.* (1955). It is produced by *Streptomyces filipinensis, n. sp.* Chemically it is a polyene and a member of a considerable group of antifungal antibiotics with polyene characteristics (Whitfield *et al.*, 1955). Because a compound of this type would be expected to have a long aliphatic chain in its structure, it was of interest to see what effect aliphatic substances such as oils and fatty acids would have on filipin production. After this work was begun, a paper was published by McCarthy *et al.* (1955) on the effect of oils and fatty acids on the production of fungichromin, an antibiotic somewhat similar to filipin.

MATERIALS AND METHODS

Fermentations were carried out in 500-ml flasks containing 100 ml of medium. Flasks were incubated at 28 C on a rotary shaker at 250 rpm with a 2-inch stroke. All media were adjusted to pH 7.0 before autoclaving.

Inoculation was with 1 per cent vegetative growth from a 3-day-old shake flask grown in 3 per cent cottonseed flour, 2 per cent glucose medium which had been heavily inoculated with spores from an agar slant. Flasks were sampled usually at 3, 4, and 5 days after inoculation. Samples of fermented broth were diluted with 95 per cent ethanol, extracting the antibiotic from the mycelium. After standing for 30 min the clear supernatant was decanted and assayed spectrophotometrically by the method described by Ammann *et al.* (1955). The results are obtained in μg per ml of filipin.

RESULTS

A study was made first of the production of filipin in media containing natural substances. As table 1 shows, a variety of oils and fats gave increased yields over the glucose control. Inasmuch as these substances are derived from a wide variety of sources, it would seem that the stimulation noted is not a specific effect but one characteristic of substances containing long aliphatic chains. The two waxes tried were without effect.

The next step was to determine the effect of individual fatty acids and their derivatives on yield. A summary of some of the results is presented in table 2. Compounds that gave high yields were palmitic acid and its esters, oleic acid, methyl oleate, triolein, 12-hydroxystearic acid, tetradecanol, hexadecanol, and methyl myristate. Stearic acid, methyl stearate, methyl laurate, and glycerol were better than the glucose control but were not as good as the natural substances. Myristic and lauric acids appeared to be toxic, while their esters were not. It is interesting that of the triglycerides tested, only triolein produced high yields, tripalmitin and the others being completely ineffective. This may be due to the high insolubility or poor dispersibility of these latter triglycerides. Triolein, on the other hand, is a liquid and consequently more readily dispersed. Various attempts to increase the dispersibility of tripalmitin were unsuccessful.

An obvious interpretation from table 2 is that it is the aliphatic portion of the natural fats and not the glycerol that causes the high yields. A number of other aliphatic substances that have been tried and found to be ineffective are octanoic acid, hexanoic acid, decanoic acid, butyric acid, calcium proprionate and acetic acid. Several of these are probably toxic. Yields of 1800 and 680 μg per ml were obtained with trioctanoin and trihexanoin, respectively. The fact that these substances are easily dispersed may explain their activity.

An experiment was set up to determine the optimum concentration of palmitic acid for maximum yield. From the results in table 3 it can be seen that there is little increase in yield at concentrations above 5 per cent palmitic acid and from an economic standpoint this could be considered the maximum concentration to use.

Various nitrogen sources were tested in a palmitic acid medium and it can be seen in table 4 that good yields were obtained with several widely different substances. Peptone, however, was completely unsatisfactory. McCarthy *et al.* (1955) found that fungichromin was produced quite well in a medium containing peptone and palmitic acid.

The time course of filipin production can be seen in table 5. An especially interesting point is the change in pH with time. On the low yielding glucose medium, the pH rises rapidly from 7.0 and exceeds 8.0 within 3 days. In media in which an aliphatic substance effective in filipin production is present, the pH remains around 7.0 for a relatively long time. In other media, especially those containing oleate, the pH usually drops to around 6.0, probably due to the hydrolysis of the oleate to

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TABLE 1. *Effect of natural oils, fats and waxes on filipin production*

Carbon Source*	Filipin Peak Yield
	µg/ml
Glucose	235
Soy oil	1170
Corn oil	1520
Coconut oil	1046
Sperm oil	2600
Palm oil	1204
Lard oil	2040
Lecithin (vegetable)	4206
Lecithin (animal)	2392
Mutton tallow	620
Japan wax	133
Spermaceti	80

* All media contain carbon source 2 g and soy flour 3 g per 100 ml, adjusted to pH 7.0.

TABLE 2. *Effect of aliphatic compounds on the production of filipin*

Carbon Source	G Per 100 Ml*	Filipin Peak Yield
		µg per ml
Glucose	2	235
Palmitic acid	2	2531
Ethyl palmitate	2	2470
Methyl palmitate	2	3130
Glyceryl monopalmitate	2	1562
Oleic acid	2	1749
Methyl oleate	2	4330
12-Hydroxystearic acid	2	2339
Tetradecanol	2	1970
Hexadecanol	2	2799
Stearic acid	2	405
Methyl stearate	2	560
Myristic acid	2	182
Methyl myristate	2	3560
Lauric acid	1	35
Methyl laurate	2	596
Tripalmitin	2	103
Tristearin	2	64
Triolein	2	2500
Trimyristin	2	76
Glycerol	2	447

* All media contain soy flour 3 g per 100 ml, adjusted to pH 7.0.

oleic acid which has considerable effect on the pH. Palmitic acid has little effect on the pH of the medium.

These pH changes indicate that there is a different sort of metabolic picture in the presence of an aliphatic substance than in a medium with carbohydrate.

DISCUSSION

Only two previous papers have dealt specifically with the effect of fatty acids and oils on antibiotic production in actinomycetes. The publication of McCarthy *et al.* (1955) has been discussed above. An earlier paper by Perlman and Wagman (1952) dealt with streptomycin and showed the effect of oils on its production. In streptomycin the replacement of glucose by oil was best made on a caloric basis and it is apparent that the oil did not have a specific effect but served only as an energy source. Oils and fatty acids contain more energy per unit weight than glucose. In the present case, and also in that of fungichromin, the

TABLE 3. *Effect of palmitic acid concentration on filipin production*

Carbon Source	G Per 100 Ml*	Filipin Peak Yield
		µg per ml
Palmitic acid	0.1	188
Palmitic acid	0.5	1120
Palmitic acid	1	2077
Palmitic acid	2	2531
Palmitic acid	3	3496
Palmitic acid	4	4320
Palmitic acid	5	5692
Palmitic acid	6	6200
Palmitic acid	10	6800

* All media contain soy flour 3 g per 100 ml, adjusted to pH 7.0.

TABLE 4. *Effect of nitrogen source on production of filipin*

Nitrogen Source*	Filipin Peak Yield
	µg per ml
Soy flour	2531
Cottonseed meal	2420
Cottonseed flour	2880
Brewer's yeast (Pabst)	3350
Corn steep liquor	2670
Peanut meal	1890
Yeast extract	1870
Wheat germ meal	1920
Liver extract	1680
Linseed meal	1600
Corn gluten meal	1320
Peptone	50

* All media contain 2 g palmitic acid and 3 g nitrogen source per 100 ml, adjusted to pH 7.0.

TABLE 5. *Time course of filipin production*

Incubation	Medium A*		Medium B*		Medium C*	
	pH	Filipin	pH	Filipin	pH	Filipin
days		*µg per ml*		*µg per ml*		*µg per ml*
2	7.8	255	6.8	1220	6.15	1136
3	8.15	255	6.8	1960	6.0	2952
4	8.3	256	7.1	2440	5.7	2520
5	8.6	232	7.25	2100	6.4	2800
6	8.6	206	7.7	2068	—	—
7	8.4	195	7.6	1968	—	—

* Medium A: soy flour 3 per cent, glucose 2 per cent. Medium B: soy flour 3 per cent, palmitic acid 2 per cent. Medium C: soy flour 3 per cent, methyl oleate 2 per cent. Initial pH of all media, 7.0.

increased yield in the presence of fatty acids and oils cannot be attributed to the added energy available, since the yields are greater than would be expected from the additional energy supplied.

It would appear from the present results that high filipin production cannot be attributed to any specific aliphatic substance, but rather that there are a limited number of compounds which are favorable. It is difficult to decide from the data what the exact requirements in a substrate are, since related materials such as stearic and 12-hydroxystearic acids show such wide differences. It may be necessary to separate toxicity from unavailability. The shorter-chain compounds that are ineffective may be toxic, while the longer-chain ineffective compounds may be unavailable due to inadequate dispersability.

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SUMMARY

This paper has dealt with the production of filipin, a polyene antifungal antibiotic, by *Streptomyces filipinensis, n. sp.* Antibiotic yields have been markedly increased by the presence of oils or specific fatty acids. The increase in yield cannot be due merely to the increased energy available since the yields are many times greater than would be expected on this basis.

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Thermal Destruction of *Micrococcus freudenreichii* and *Streptococcus thermophilus* with Particular Reference to Pasteurization without Holding

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Standards most commonly used to limit the minimum treatments in pasteurization of milk are 143 F for 30 min (vat method) and 161 F for 15 sec (HTST, or high-temperature short-time method). Interest has been shown in the development of methods that would employ temperatures high enough to permit elimination of holding periods (Dahlberg *et al.*, 1941; Barber, 1953; Tobias *et al.*, 1953; Litsky *et al.*, 1954; Herreid, 1954; and Tobias, 1955). In such processes the entire lethality would be accounted for in heating and cooling the product.

Although most results used for determining the thermal death time curves of vegetative cells have not included experimental data on higher temperatures and shorter times than those used in conventional HTST pasteurization, most investigators accept that thermal death time and thermal resistance curves obtained by plotting destruction times on a logarithmic scale against temperature on an arithmetic scale are straight lines (Schmidt, 1954). Extrapolation of a straight line beyond the temperature range for which experimental data are available has provided helpful predictions of the lethality of processes involving high temperatures for very short times (Bendixen *et al.*, 1937; Burton, 1951). Deviations from linearity have been reported at high temperatures in some studies (Holland and Dahlberg, 1940), though they might have been the result of experimental difficulties in applying and evaluating short treatments at high temperatures.

Some procedures used for studying the thermal resistance of bacteria involve such rapid heating and cooling that the lethal effects during the heating and the cooling periods may be ignored. Use of such methods is

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