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Fatty acid metabolism in *Serratia marcescens:* IV. The effect of temperature on fatty acid composition

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SUMMARY

An analysis of the fatty acids of *Serratia marcescens* grown at different temperatures shows that three medium-chain hydroxy acids, present in cells grown at 30°, are greatly decreased in quantity when the cells are grown at 37°. The content of unsaturated and cyclopropane acids remains unchanged. As the synthesis of unsaturated and cyclopropane acids in microorganisms requires hydroxy acids as intermediates, it is suggested that the temperature-sensitive reaction is a reaction leading to the trapping of the hydroxy acids in a bound form. An analysis of the cellular fatty acids of a nonpigmenting strain of *S. marcescens* has revealed the presence of 12 unidentified acids. These acids, which constitute 36% of the total fatty acids of the "free" lipid of cells grown at 30° are almost entirely absent in cells grown at 37°.

In the preceding paper (1), an analysis of the fatty acid content of *Serratia marcescens* was reported. This organism is notable in that, while it produces abundant red pigment when grown under favorable conditions, it is completely colorless when grown at 37°. However, the organism will pigment at 37° if grown in the presence of certain long-chain unsaturated fatty acids (2).

This communication describes the effect of temperature on the fatty acid composition of *S. marcescens*.

METHODS

Three strains of *S. marcescens* were employed. Strain 330 (NCTC 1377) and strain 120 formed abundant red pigment when grown at 30° on Bunting's medium (3) but were white when grown at 37°. Strain 121 was a naturally occurring mutant of 120 and formed no pigment under any conditions.

The techniques employed have been described previously (1). The cellular lipid from the contents of 24 Roux bottles was fractionated into "free" and "bound" fractions and the methyl esters of the fatty acids from these fractions were analyzed by gas-liquid chromatography (GLC).

RESULTS

The fatty acid composition of the "free" lipid of cells of strain 330 grown at 30° and 37° are shown in Table 1. It can be seen that the content of unsaturated and cyclopropane acids is very similar at the two temperatures, but that the content of the three hydroxy acids drops markedly at 37°. As the hydroxy acids are present almost entirely in the "free" lipid (1), the composition of the "bound" lipid does not vary significantly between 30° and 37°.

There is, therefore, a temperature-sensitive reaction that parallels the loss of pigmentation between 30° and 37°, and an examination of the fatty acid content of cells grown below 30° would be of interest.

The results of an analysis of the fatty acids from the "free" lipid of cells of 330 grown at 20° are shown in Table 1. Although the lowering of growth temperature does not visibly affect pigmentation, there is a drop in the amounts of hydroxy acids detected, but it is not as great as that found when the growth temperature is increased from 30° to 37°. However, the change in hydroxy acid content at 20° is overshadowed by a large increase in the amount of hexadecenoic acid at the expense of the cyclopropane acid, 9,10-methylenehexadecanoic acid. A similar effect is noted in the amount of octadecenoic acid and 11,12-methyleneoctadecanoic acid. This latter effect has not yet been investigated further.

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TABLE 1. THE EFFECT OF TEMPERATURE ON THE FATTY ACID COMPOSITION OF "FREE" LIPID OF *S. marcescens*, STRAIN 330

	Cells Grown at:		
	30°	37°	20°
Wt of dry cells (mg)	5070	2950	4780
Wt of "free" lipid (mg)	237	142	211
Wt of acid from "free" lipid (mg)	152	95	127
Fatty Acid	Percentage of Total Fatty Acids*		
Hydroxy acids			
3-Hydroxydecanoic	4.3	0.7	2.1
3-Hydroxy-5-dodecenoic	0.8	+	0.6
3-Hydroxydodecanoic	1.6	+	0.6
Unsaturated acids			
Hexadecenoic	1.9	2.0	28.2
Octadecenoic	6.7	5.5	21.6
Cyclopropane acids			
9,10-Methylenehexadecanoic	33.4	30.2	12.0
11,12-Methyleneoctadecanoic	4.7	4.0	+

* The remainder of the fatty acids (not shown) were *n*-saturated acids. The percentage of these acids does not change significantly with growth temperature (1). A + sign indicates that the component was detected and identified on the basis of retention times but that it did not occur to an extent greater than 0.2%.

Table 2 shows the variation with temperature of the content of hydroxy, unsaturated, and cyclopropane acids in the "free" lipid of strain 120. Once again, increase in growth temperature leads to a decrease in content of hydroxy acids, without significant change in unsaturated or cyclopropane acids. Strain 120 contains a higher content of unsaturated acids than strain 330; this increase is at the expense of the corresponding cyclopropane acids.

If there is some relationship between content of hydroxy acids and pigmentation, the analysis of the fatty acids of a nonpigmenting strain would be interesting. Such an analysis on the "free" lipid of strain 121 grown at 30° is shown in Table 3.

The results were quite unexpected. The "free" lipid contained a group of 12 unidentified acids, all eluted from the column ahead of methyl hexadecenoate. These unidentified acids comprised 36.5% of the total fatty acids. Investigations of their retention volumes on Apiezon L and PEGA columns under different conditions showed that none of the six major components of this mixture were *n*-saturated acids; only three of the group could be hydrogenated in the presence of platinum oxide in methanol.

In addition to this group of acids, the three hydroxy acids could also be detected. However, while 3-hydroxy-5-dodecenoic acid and 3-hydroxydodecanoic acid were present in only slightly larger amounts than in the parent red strain, 3-hydroxydecanoic acid constituted 21% of the total fatty acid of the "free" lipid. It was found that the group of unidentified acids, like the hydroxy acids, were present almost entirely in the "free" lipid.

The acids eluted ahead of methyl hexadecenoate from strain 121 grown at 30° constituted 64% of the total fatty acid of the "free" lipid compared with 13% for the same fraction of the parent red strain 120 grown under the same conditions.

Table 3 also shows an analysis of the fatty acids from the "free" lipid of strain 121 grown at 37°. In this case, not only the hydroxy acids but also the whole group of unidentified acids have almost entirely disappeared. The acids eluted ahead of methyl hexadecenoate constitute only 9.3% of the total fatty acid (5.4% of this fraction being methyl tetradecanoate), compared with 64% for cells grown at 30° (1.2% is methyl tetradecanoate).

The structure of the individual components in the group of unidentified acids is being investigated. Preliminary tests have shown that the group as a whole contains a factor or factors that can induce pigment formation in cells grown at 37°. However, a test for pyrrole on the group of acids proved negative.

TABLE 2. THE EFFECT OF TEMPERATURE ON THE FATTY ACID COMPOSITION OF "FREE" LIPID OF *S. marcescens*, STRAIN 120

	Cells Grown at	
	30°	37°
Wt of dry cells (mg)	3000	3530
Wt of "free" lipid (mg)	138	132
Wt of acid from "free" lipid (mg)	76	96
Fatty Acid	Percentage of Total Fatty Acids*	
Hydroxy acids		
3-Hydroxydecanoic	4.0	0.8
3-Hydroxy-5-dodecenoic	1.1	+
3-Hydroxydodecanoic	1.6	+
Unsaturated acids		
Hexadecenoic	8.8	8.3
Octadecenoic	12.0	12.1
Cyclopropane acids		
9,10-Methylenehexadecanoic	26.9	29.0
11,12-Methyleneoctadecanoic	2.4	1.6

* The remainder of the fatty acids (not shown) were *n*-saturated acids. The percentage of these acids does not change significantly with growth temperature. A + sign indicates that the component was detected and identified on the basis of retention times but that it did not occur to an extent greater than 0.2%.

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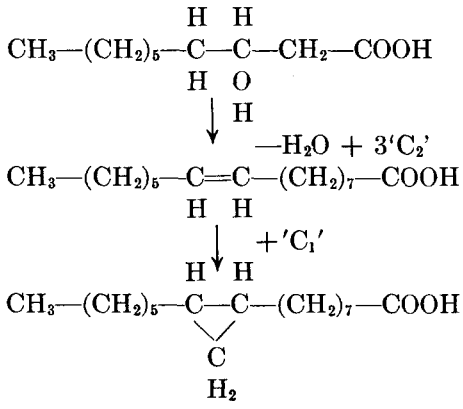
Tests showed that 3-hydroxydecanoic acid and DL-serine, alone or in combination, would not induce pigment formation at 37°.

DISCUSSION

The three strains of *S. marcescens* examined all showed a significant drop in their content of hydroxy acids with an increase in growth temperature from 30° to 37°. However, the concentration of unsaturated and cyclopropane acids remained constant.

It has now been fairly well established that, in bacteria, unsaturated fatty acids are formed from hydroxy acids (4, 5) while cyclopropane acids are formed by the addition of a 1-carbon unit across the double bond of a fatty acid, the 1-carbon unit probably being derived from an S-methyl carbon atom of methionine (6, 7, 8). The relationship between 3-hydroxydecanoic acid, 9,10-hexadecenoic acid, and 9,10-methylenehexadecanoic acid is shown below.



It is apparent from this scheme that if the production of hydroxy acids is limited, there should also be a deficiency of the unsaturated and cyclopropane acids. However, such a deficiency does not exist in cells grown at 37° and it must be assumed that the synthesis of hydroxy acids is not the temperature-dependent reaction. It is suggested, therefore, that a reaction leading to the trapping of hydroxy acids in a bound form is temperature-sensitive. In the case of 3-hydroxydecanoic acid, the synthesis of serratamic acid (*N*-3-hydroxydecanoyl-serine) (9) or serratamolide (a dilactone of serratamic acid) (10) may be such a reaction. Similar trapping reactions may apply in the case of 3-hydroxy-5-dodecenoic acid and 3-hydroxydodecanoic acid. It has been found that production of a glycolipid composed of 2 moles each of L-rhamnose and 3-hydroxydecanoic acid by *Pseudomonas aeruginosa* is also sensitive to growth temperature (11).

It is considered that in certain organisms the degree of saturation of fatty acids is higher after growth at an elevated temperature and that the lipids have higher melting points (12, 13). It is also well known that nutritional requirements and enzyme formation in microorganisms are affected by increases in growth temperature (14, 15).

TABLE 3. THE EFFECT OF TEMPERATURE ON THE FATTY ACID COMPOSITION OF "FREE" LIPID OF *S. marcescens*, STRAIN 121

	Cells Grown at:	
	30°	37°
Wt of dry cells (mg)	4870	3480
Wt of "free" lipid (mg)	501	198
Wt of acid from "free" lipid (mg)	400	124
Fatty Acid	Percentage of Total Fatty Acids*	
Hydroxy acids		
3-Hydroxydecanoic	21.0	1.2
3-Hydroxy-5-dodecenoic	2.7	+
3-Hydroxydodecanoic	1.8	+
Unidentified acids	36.5	1.5
Unsaturated acids		
Hexadecenoic	5.6	7.5
Octadecenoic	6.0	8.0
Cyclopropane acids		
9,10-Methylenehexadecanoic	8.3	27.9
11,12-Methyleneoctadecanoic	0.8	2.7

* The remainder of the fatty acids (not shown) were *n*-saturated acids. The percentage of these acids varied between 30° and 37° due to the fluctuation in the content of hydroxy acids and unidentified acids, but the total amount of *n*-saturated acids remained constant at the two temperatures. A + sign indicates that the component was detected and identified on the basis of retention times but that it did not occur to an extent greater than 0.2%.

As shown in Table 1, the relative content of unsaturated fatty acids was the same in cells grown at 30° and 37° but increased markedly in cells grown at 20°. The conversion of an unsaturated acid to its corresponding cyclopropane derivative results in the production of a fatty acid with a melting point intermediate between that of the unsaturated acid and the corresponding *n*-saturated acid, although the cyclopropane acid contains one more carbon atom. No function has yet been found for cyclopropane acids in microorganisms. It may be that they provide an acid with very suitable physical characteristics.

The influence of temperature on the metabolism of *S. marcescens* has always been apparent, on account of the sensitivity of pigment production to temperature. Evidence has accumulated that this lipid-soluble pigment exists as a pigment–protein–carbohydrate complex in the outer structures of the cell (16–19). Taylor and Williams (18) prepared an extract con-

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taining pigment, protein, and carbohydrate from cells of *S. marcescens*. The extracts were examined in an ultracentrifuge and it was found that the peak corresponding to pigment in cells grown at 27° was not present in cells grown at 37°.

An effect of temperature on the formation of bacterial flagella has been frequently reported (20). Preliminary examination of the cells of *S. marcescens* used in the present investigation indicates that flagella are present in cells grown at 30° but absent in those grown at 37°.

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