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Fatty Acids of *Myxococcus xanthus*

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Fatty acids were extracted from saponified vegetative cells and myxospores of *Myxococcus xanthus* and examined as the methyl esters by gas-liquid chromatography. The acids consisted mainly of C_{14} to C_{17} species. Branched acids predominated, and *iso*-pentadecanoic acid constituted half or more of the mixture. The other leading component (11–28%) was found to be 11-*n*-hexadecenoic acid. Among the unsaturated acids were two diunsaturated ones, an *n*-hexadecadienoic acid and an *iso*-heptadecadienoic acid. No significant differences between the fatty acid compositions of the vegetative cells and myxospores could be detected. The fatty acid composition of *M. xanthus* was found to be markedly similar to that of *Stigmatella aurantiaca*. It is suggested that a fatty acid pattern consisting of a large proportion of *iso*-branched C_{15} and C_{17} acids and a substantial amount of an *n*-16:1 acid is characteristic of myxobacteria.

Myxococcus xanthus is a gram-negative rod of the order *Myxobacterales*. It is an obligate aerobe with gliding motility and is found normally in soil. The organism forms fruiting bodies as part of a normal developmental cycle. Within these fruiting bodies, the vegetative rods are transformed into metabolically quiescent, resting cells called myxospores (microcysts). Formation of these myxospores can be induced in liquid culture by the addition of certain alcohols, notably 0.5 M glycerol (11). The naturally formed, as well as the chemically induced, myxospores have increased resistance to desiccation, ultraviolet light irradiation, sonic vibration, and heat (29).

As part of a study of this morphogenetic process, we have examined the fatty acid composition of both vegetative cells and myxospores in *M. xanthus*. In addition, the data have been compared with those for *Bacillus subtilis*, *Cytophaga hutchinsonii*, and one other myxobacterium, *Stigmatella aurantiaca*, which was reexamined in order to characterize the fatty acids, particularly the branched acids, more closely.

(A preliminary report of these findings was presented at the 71st Annual Meeting of the American Society for Microbiology, Minneapolis, Minn., 2–7 May 1971.)

MATERIALS AND METHODS

Culture conditions: vegetative cells. *M. xanthus* strain FB was grown in 1% Casitone containing 0.01 M phosphate buffer, pH 7.6, and 0.008 M $MgSO_4$ at 32 C with shaking. When the cells were harvested, the autolytic enzymes were inactivated by adding Formalin to a final concentration of 1%. The mixture was kept at room temperature for 30 min, cooled to 4 C, and centrifuged at 8,300 × *g*. The cells were suspended and washed two times with distilled water before treatment to extract fatty acids.

The defined medium for growth of *M. xanthus* consisted of amino acids, $MgSO_4$, and phosphate buffer (16).

Myxospores. Vegetative cells were grown in 1% Casitone and 0.008 M $MgSO_4$ without buffer to mid-log phase. An autoclaved glycerol-water mixture (1:1, vol/vol) was added to make the final glycerol concentration 0.5 M. Six hours after induction, the myxospores were harvested (without Formalin treatment), washed two times with distilled water, and stored at −60 C if not used immediately.

S. aurantiaca was strain Cro Cl I described by Reichenbach and Dworkin (26). It was grown in 1% Casitone containing 0.0125 M $MgSO_4$.

Extraction of fatty acids. The washed cells were suspended in 1 N sodium hydroxide (about 100 ml per 35 g of wet cells) in a screw-cap Erlenmeyer flask, and the mixture was flushed with nitrogen and treated either at 90 C for 2 h or at 50 C for 6 h. The mixture was centrifuged at 12,000 × *g* to remove debris, principally cell walls, and then acidified with HCl. The acids were extracted successively with ether and chloroform.

The myxospores have a highly resistant capsule, and we found it advantageous to grind them with levigated alumina (3 g of alumina per 1 g of dry myxospores) before hydrolysis at 90 C for 3 h. The cooled mixture was centrifuged at 3,000 × *g* to remove a small amount of debris, and then extracted with ether. The aqueous solution was then acidified with

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concentrated HCl, and the fatty acids were extracted successively with ether and chloroform. A large amount of material precipitated at this point, and low-speed centrifugation was necessary to separate the solvent layers. The combined organic solvent fraction was dried over anhydrous $MgSO_4$ and evaporated to dryness under a stream of nitrogen.

In one experiment the lipids were extracted from the wet cells by the method of Ames (3).

Methyl esters were prepared by heating the acids with boron trifluoride-methanol reagent (23). In addition, a portion of one preparation was esterified by treating the acid mixture with diazomethane in ether (24).

Hydrogenation of methyl esters. Methyl ester hydrogenation was carried out in methanol solution by using platinum oxide catalyst. The mixture was subjected to hydrogen pressure (2.8 kg/cm^2) for 2 h at room temperature with shaking. The catalyst was removed by filtration, the solvent was evaporated under a stream of nitrogen, and the methyl esters were dissolved in a small amount of hexane for chromatography.

Bromination. Bromination consisted of adding a few drops of bromine to a hexane solution of methyl esters until a brown color persisted, and holding the mixture in a hood out of the light for several hours. The solution was then evaporated with a stream of nitrogen and redissolved in hexane for chromatography.

Gas chromatography. A Beckman GC4 gas chromatograph with a flame ionization detector was used. Matched, packed columns were purchased from Beckman Instruments Co., Fullerton, Calif. Helium was used as the carrier gas at a flow rate of 45 ml/min. All materials were injected in acid-washed, distilled hexane. A diethylene glycol succinate (DEGS) column (244 by 0.32 cm) at 130 to 150 C and a Dexsil 300 column (366 by 0.32 cm) at 190 to 240 C were used for the analyses. The Dexsil column afforded better resolution of the many C_{16} and C_{17} species present (eight distinguishable peaks for C_{16} and C_{17} species versus five with the DEGS column) and therefore was used almost exclusively after preliminary studies. Fatty acids from C_{10} to C_{30} could have been detected under these conditions.

Standard mixtures of straight-chain saturated and unsaturated fatty acid methyl esters were obtained from Hormel Institute (Austin, Minn.). The fatty acids extracted from *B. subtilis* and *Escherichia coli* have been characterized (7, 17–19) and were also used as standards.

Identification of most fatty acids is based upon comparison of the relative retention times of the methyl esters with those of standards and upon plots of log retention time versus chain length (1). Bromination eliminated unsaturated esters and cyclopropane-containing fatty acid methyl esters from the mixture, as did catalytic hydrogenation; the latter converted the unsaturates to saturates, thereby aiding in their identification.

The relative amounts of fatty acid esters were calculated from their relative peak areas.

Argentation chromatography. Scrupulously clean glass plates were spread to a depth of 0.25 μm with silica gel that had been slurried with 5% aqueous silver nitrate solution. The plates were heated at 110 C for 1.5 h before use and were developed at room temperature in toluene. Iodine was used for localization of the esters. The spots were scraped off, and the esters were extracted with diethyl ether.

Ozonolysis. The monoenoic and dienoic ester fractions, separated by argentation chromatography, were injected separately into a EGS column (244 by 0.32 cm) at 163 C, and separate fractions were collected (two from the diene mixture and eight from the monoene mixture). The individual fractions were rechromatographed. Half of each fraction was hydrogenated, while the other half was ozonized in pentane at −65 C by the procedure of Sand et al. (27) and then reduced with triphenylphosphine oxide. Both treated fractions were rechromatographed on the EGS column.

RESULTS

Fatty acids were extracted by shaking the cells with 1 N sodium hydroxide at 50 or 90 C for 1 to 4 h. The yield of fatty acids was highest at 90 C and amounted to 4% of the dried cell weight; a lipid extract amounted to 9% of dried cell weight. The composition of the fatty acid mixture did not vary significantly with the temperature of hydrolysis. Chromatograms of a typical ester mixture, as determined both on Dexsil 300 and on DEGS columns, are shown in Fig. 1. Over 90% of the fatty acids had carbon skeletons from 12 to 18 carbon atoms in length; the fatty acid patterns are presented in Table 1. Cells in two stages of growth, as well as those grown in defined medium, were studied.

On hydrogenation, three C_{16} and three C_{17} unsaturated peaks disappeared from the chromatogram, and there was a concomitant increase in the areas of the *n*-16:0 and *i*-17:0 peaks (Table 1, ester preparation 1). An example of the change in the chromatogram on bromination is shown in Fig. 2. The same six peaks disappeared as did after hydrogenation. These results suggest that cyclopropane acids were not present.

We found that the esterification with boron trifluoride-methanol reagent (23) could produce a number of peaks similar to those of the methyl esters. Many of these, however, had retention times greater than those of the methyl esters obtained from the organisms studied. These artifacts were identified as such by comparison of the chromatogram with those from a reagent blank and from a fatty acid ester preparation that had been esterified with diazomethane. The origin(s) of the extra peaks was not investigated.

The unsaturated esters were further charac-

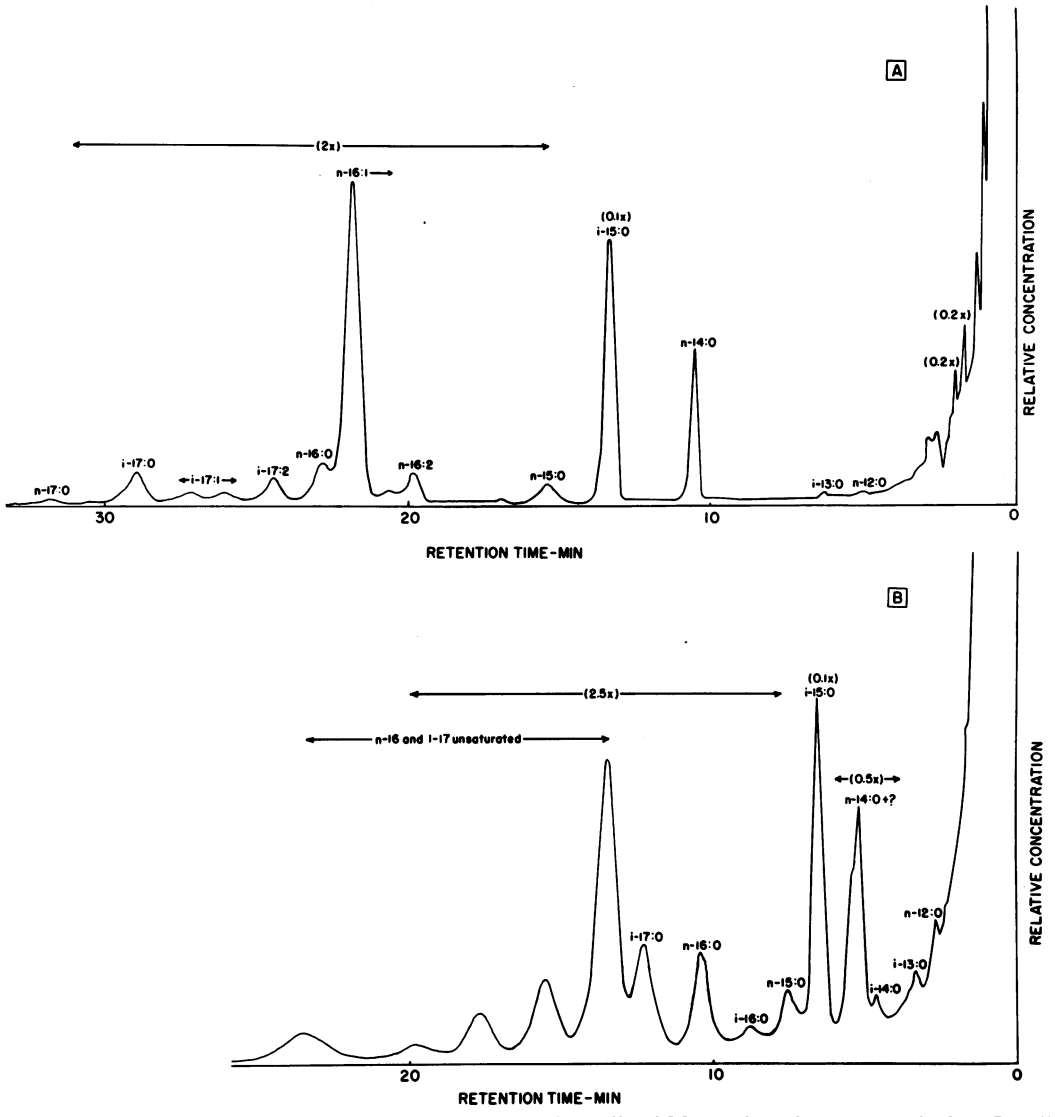

FIG. 1. *(a) Methyl ester mixture from log-phase vegetative cells of M. xanthus chromatographed on Dexsil 300 at 190 C. (b) Methyl ester mixture from log-phase vegetative cells of M. xanthus chromatographed on DEGS at 154 C. The figures 2×, 0.1×, etc., refer to the extent of peak amplification.*

terized by thin-layer chromatography on silver nitrate-impregnated silica gel. Two bands of unsaturated material were located with iodine. Their R_f values were 0.4 and 0.2. After comparison with standards, these were taken to be monounsaturated and diunsaturated esters, respectively. On gas-liquid chromatography, the diunsaturated fraction was found to consist almost entirely of two components (Fig. 3b). When a portion of the fraction was hydrogenated and rechromatographed, *n*-16:0 and *i*-17:0 esters were found to have been formed. When the separate esters were collected and ozonized and the products were rechromatographed, the *i*-17:2 ester yielded a C_{10} aldester (chain length determined graphically with the aid of standards). The ozonolysis product from the *n*-16:2 ester could not be determined because of technical difficulties.

The monounsaturated fraction from chromatographic examination (Fig. 3a) was found to be complex. The components of both monounsaturated and diunsaturated ester fractions, along with probable carbon skeleton (based

TABLE 1. *Fatty acid as methyl esters*[a]

Nature of cells	Methyl esters (area percent)																			
	n-12:0	*i*-13:0	*n*-13:0	*i*-14:0	*n*-14:0	*i*-15:0	*n*-15:0[b]	*n*-16:2 + *i*-16:0[c]	*n*-16:1[d]	*n*-16:1[e]	*n*-16:0	*i*-17:2	*i*-17:1	*i*-17:1	*i*-17:0	*n*-17:0	*n*-18:1	*n*-18:0	Unidentified	Total unsaturated	Total branched chain
Myxospores																					
Whole, dried	1.0	0.6			9.0	49.0	0.4	3.0	0.3	28.0	3.0	0.7	0.2	0.3	0.5		0.2		4.0	33.0	51.0
Frozen, ground pellet	1.0	0.7	0.1		5.0	63.0	1.0	3.0	0.6	13.0	4.0	2.0	0.4	1.0	2.0	0.1	0.2		3.0	20.0	69.0
Supernatant	0.3	0.3	TR		5.0	67.0	2.0	3.0	0.4	16.0	2.0	1.0	0.3	1.0	2.0			0.2		21.0	73.0
Vegetative cells																					
Grown in defined medium	0.3	0.1	0.1	0.2	2.0	62.0	2.0	3.0	2.0	17.0	4.0	0.2	0.8	0.8	2.0				3.0	24.0	70.0
Grown in Casitone post-log phase	0.1	0.2			1.0	67.0	2.0	1.0	0.3	24.0	0.5	0.7	0.3	0.8	0.7				2.0	27.0	70.0
											3.0				6.0				0.5	25.0[j]	
Log-phase, lipid extract[f]	0.3	0.5		0.2	8.0[b]	54.0	0.8	0.5													
NaOH-treated whole cells																					
1	3.0	0.3			1.0	49.0	4.0	3.0	1.0	11.0	2.0[h]	6.0	2.0	4.0	5.0[i]				9.0	28.0	66.0
2	0.1	0.2			6.0	60.0	2.0	2.0	0.9	18.0	3.0	2.0	0.6	0.7	3.0	TR			1.0	23.0	66.0
3	0.3	0.9			3.0[b]	57.0	4.0	1.0			4.0				4.0					26.0[g]	

[a] The cells were grown and the fatty acids were isolated and esterified as described. Gas-liquid chromatography was on the Dexsil 300 column unless otherwise noted, and results are expressed as percentage of total area of gas-liquid chromatography peaks for the esters. TR, trace. The acids are listed in order of increasing retention time on the Dexsil column.

[b] The peak had a small amount of a poorly resolved, unsaturated component.

[c] Bromination experiments indicated that only trace amounts of *i*-16:0 were present.

[d] Δ-9, 10.

[e] Δ-11, 12.

[f] These data are from chromatography on a DEGS column. The lipid extract was hydrolyzed for 2 h at 50 C in 0.2 M NaOH in ethanol. The mixture was then poured into water and extracted with ether. The fatty acids were extracted from the acidified aqueous layer and esterified.

[g] The DEGS column did not separate all of the *n*-16 and *i*-17 unsaturated esters.

[h] After catalytic hydrogenation, the percentage was 21.

[i] After catalytic hydrogenation, the percentage was 18.

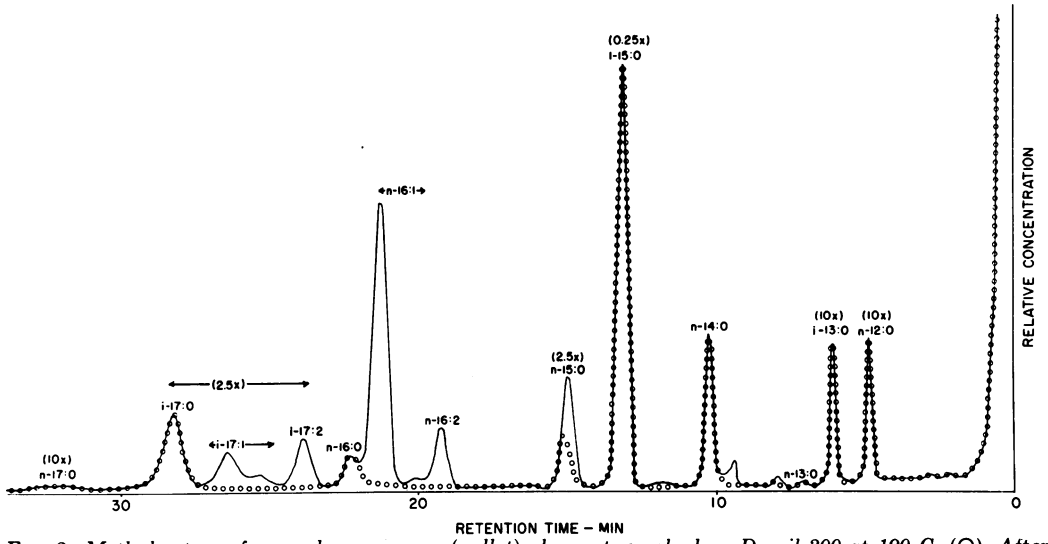

FIG. 2. *Methyl esters of ground myxospores (pellet) chromatographed on Dexsil 300 at 190 C. (O), After bromination.*

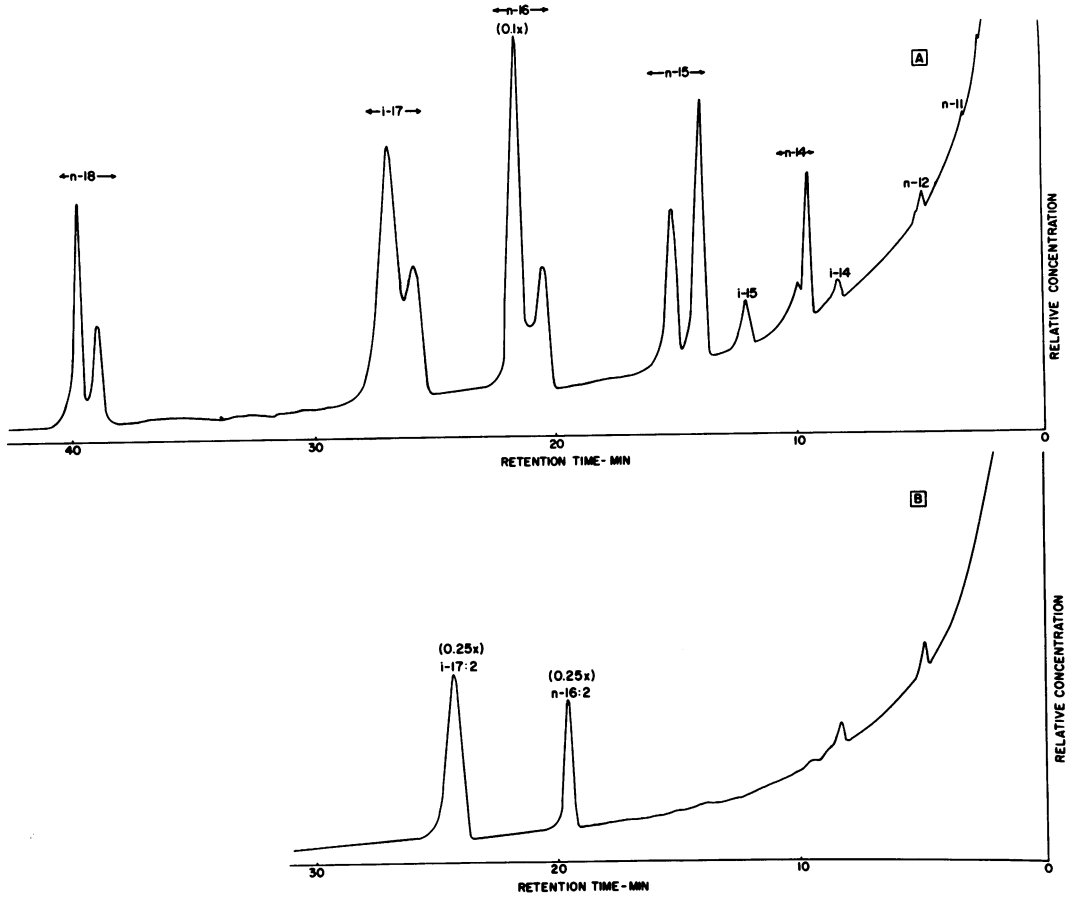

FIG. 3. *Separation of unsaturated esters by argentation thin-layer chromatography and chromatography of the fractions obtained on Dexsil 300 at 190 C. (a) Monounsaturate fraction; (b) diunsaturate fraction.*

upon retention times), are listed in Table 2. The nonpolar Dexsil column separated the components of the ester mixture without overlap as to chain length (e.g., *n*-16:0 ester was eluted well in advance of *i*-17:2, the next component to be eluted), thus enabling us to assign chain lengths to the various components of the monoene mixture. These assignments were supported by the formation of the anticipated hydrogenation products.

The smaller of the *n*-16:1 peaks was found to have retention time identical to that of palmitoleic acid (9-hexadecenoic acid). The larger *n*-16:1 peak had a higher retention time, and thus it should have had its position of unsaturation further from the carboxyl terminus (1). On ozonolysis, it yielded the C_{11} aldester, indicating that the double bond was in the 11, 12-position (11-hexadecenoic acid). The *n*-18:1 acids were found by comparison with authentic materials to be oleic (Δ-9, 10) and vaccenic (Δ-11, 12) acids. Only the hexadecenoic ester was available in sufficient quantity to make an unequivocal identification of the ozonolysis products possible. There was no discernible pattern in the equivalent chain lengths.

An ester preparation from *S. aurantiaca* was examined as well in order to characterize more closely the "branched" acids reported by Schröder and Reichenbach (28). These were found to be *iso*-acids on the basis of retention times. Our results, which are in reasonable agreement with those of Schröder and Reichenbach, are listed in Table 3. The *n*-16:1 ester had a retention time corresponding to that of 11-hexadecenoic methyl ester, and the *i*-17:1 ester had a retention time corresponding to that of the second *i*-17:1 peak observed from *M. xanthus* and, therefore, the isomer with the double bond farther from the carboxyl end. There was no evidence for the formation of other *n*-16:1 and *i*-17:1 isomers or, apparently, for diunsaturated esters.

TABLE 2. *Unsaturated fatty acids of M. xanthus examined after separation by argentation chromatography*

Fatty acid	Equivalent chain length[a]	Total diunsaturates (%)	Total monounsaturates (%)
n-16:2	15.59	40	
i-17:2	16.15[b]	60	
n-11:1	10.90		0.1
n-12:1	11.88		0.2
i-14:1	13.77		1.0
n-14:1	13.68, 13.79		0.5, 0.5
i-15:1	14.32		0.5
n-15:1	14.70, 14.93		3, 2
n-16:1	15.69[c], 15.84		2, 79
i-17:1	16.72, 16.83		2, 6
n-18:1	7.65,[c] 17.85[d]		1, 2

[a] See reference 1.
[b] Proximal double bond at 10, 11.
[c] Δ-9, 10.
[d] Δ-11, 12.

TABLE 3. *The fatty acids of S. aurantiaca vegetative cells*

Acid	Present work[a]	Schröder and Reichenbach[a,b]
n-12:0	0.7	TR
i-13:0	0.3	TR
i-14:0	0.5	TR
n-14:0	1	2
i-15:0	39[c]	36
n-15:0	5	8
i-16:0	8	8
n-16:1	21[d]	21
n-16:0	13	7
i-17:1	0.5	2
i-17:0	10	2
n-17:0		2

[a] Data are reported as percent of total area of gas-liquid chromatography peaks for the esters, as determined on Dexsil 300. TR, trace.
[b] Data of reference 28; average of results of two experiments.
[c] A poorly resolved peak was located immediately following the *i*-15:0 peak. Schröder and Reichenbach (28) report 15:1 (3%), which could be the same component.
[d] Δ-11, 12.

DISCUSSION

The complexity of the fatty acid mixture found in *M. xanthus* is not usual among gram-negative organisms, which in many instances have only a few acids, usually straight-chain acids with even numbers of carbon atoms (4). Such complexity may reflect to some extent increased sensitivity of analytical procedures. The Dexsil column with its stated 500 C upper limit of temperature stability (12) extends greatly the temperature range available for gas-liquid chromatography without deterioration of the column. Because of its thermal stability, the rather slow elution times of the 12-ft (3.66 m) Dexsil column were not a disadvantage, and some good separations of closely related materials could be made (e.g., the separation of the *n*-16 and *i*-17 unsaturates). It would be advantageous if the older bacterial fatty acid studies (before 1960, for example) were repeated with modern techniques.

Since the 11-hexadecenoic acid made up nearly 80% of the monounsaturated fraction

and, at the same time, represented about 15% of the total fatty acids (Table 1), it is clear that many of the esters listed in Table 2 were present in very small quantities indeed. These, therefore, might not have been detected in the gas-liquid chromatography of the unseparated ester preparation. Regrettably, insufficient material was available for a further study of the unsaturated acids.

The predominance of branched acids is not common among gram-negative bacteria (4). They have been observed in *Ruminococcus flavifaciens* (2), in flexibacteria (5), in *S. aurantiaca* (28), and *Cytophaga hutchinsonii* (Walker, R. W., G. L. Howard, and W. Litsky. Bacteriol. Proc., p. 124, 1968) among others. It is unlikely that the high proportion of branched fatty acids is simply a medium effect in *M. xanthus*. The fatty acid composition observed in the organism grown on defined medium (amino acids and salts) is not greatly different from that of organisms grown on Casitone. Indeed, the high leucine level in the defined medium could be a reflection of the need for precursors of *i*-15 and *i*-17 species. Alternatively, the high leucine level could reflect a need to overcome permeability difficulties or antagonistic effects among some amino acids (10).

The substantial proportion of unsaturated fatty acids (26%) in *M. xanthus* is usual among gram-negative organisms. Diunsaturated acids have not often been observed in bacteria. Fatty acids of cell walls of *Brucella abortus* contained 19.7% *n*-18:2 (6); this acid has also been reported in *Aerobacter aerogenes*, *Serratia marcescens*, and *Pseudomonas* sp. (8). Acids 14:2, 16:2, and 18:2 have been found in several strains of *Leptospira* (22). In the first and last cases, the dienoic acid could have come from the culture medium; culture conditions for the others were not specified. The only bacterium now known to synthesize a dienoic acid is *Bacillus licheniformis* (13), which forms 5, 10-hexadecadienoic acid. Since *M. xanthus* produces dienoic acids even when grown in defined medium, it is reasonable to conclude that it synthesizes them as well.

The large number of monounsaturated fatty acids detected is also unusual, but again it may reflect to some degree more refined analytical techniques. The occurrence of 11-hexadecenoic acid has been noted only occasionally in bacteria and in substantial quantity only in *C. hutchinsonii* (30). The present investigation shows it to be a major component also in *S. aurantiaca* and *M. xanthus*.

The differences in the fatty acid composition of *M. xanthus* vegetative cells and myxospores cannot be regarded as significant in view of the wide scatter in the experimental results. Neither can any differences by discerned between log-phase and post-log-phase cells. No explanation can be offered for the variance of results from dried and frozen myxospores. Data from both the soluble and insoluble components of ground, frozen myxospores are included to demonstrate that no acids were preferentially solubilized. It would appear that myxospore formation is not associated with the synthesis of detectable quantities of different fatty acids; Schröder and Reichenbach came to the same conclusion with regard to *S. aurantiaca* (28). Thus, the study of the fatty acid composition of *M. xanthus* vegetative cells and myxospores has not provided any information with regard to the mechanism of the transformation.

In Table 4 the fatty acid spectra of the two myxobacteria that we have investigated are contrasted with that of *C. hutchinsonii*, another gliding bacterium, and that of *B. subtilis*, another spore-former. There are marked similarities between the two myxobacteria. *Iso*-branched fatty acids predominate in each case, and in each a large quantity of 11-hexadecenoic acid has been found. The *Cytophaga*, a gliding bacterium that is not closely related to the myxobacteria, still contains a substantial amount of 11-hexadecenoic acid as well as a high proportion of branched chains. In this case, however, *ante-iso* acids represent over half of the branched acids. There could be a medium effect involved inasmuch as *C. hutchinsonii* was grown on glucose-salts, which does not support the growth of *M. xanthus* (10).

Another genus of bacteria known to contain high proportions of branched acids is *Bacillus* (21), which, like *M. xanthus*, is rod-shaped and goes through a resting phase (spores). The function of branched acids in bacteria is not well documented. Bacilli do not usually have large amounts, if any, of unsaturated acids (20), and, although it might be argued that branched chains confer liquidity at lower temperatures in *Bacillus* membranes, this would not seem to be necessary in *M. xanthus* because it also has substantial amounts of unsaturated fatty acids. It has been suggested that branched acids might give a cell envelope greater flexibility (8), but such a possibility would depend very much upon their alignment and function in the cell envelope. It may be that the presence of a large proportion of *iso*-branched C_{15} and C_{17} acids and a substantial quantity of a 16:1 acid is characteristic of myxobacteria. G. Rosenfelder (personal communication) has also shown that *i*-15:0 fatty acid is the major fatty acid in the

TABLE 4. *A comparison of the percent distribution of the major fatty acids of two myxobacteria, Cytophaga hutchinsonii and Bacillus subtilis*

Fatty acid as methyl ester	Distribution (%)			
	S. aurantiaca[a]	*C. hutchinsonii*[b]	*M. xanthus*[a]	*B. subtilis*[c]
i-14:0	0.5			3
n-14:0	1	0.7	4	1
a-15:0		1		42
i-15:0	39	16	55	9
n-15:0	5	2	3	
i-16:0	8		tr	10
n-16:0	13	18	3	10
n-16:1	21[d]	27[e]	16[d]	
a-17:0		28		17
i-17:0	10		4	8
i-17:1	0.5		3	
Unsaturated fatty acids	22	27	24	0
Branched chains	58	45	66	89

[a] Grown on Casitone; present work.
[b] Grown on glucose-salts (R. W. Walker et al., Bacteriol. Proc., p 124, 1968).
[c] Grown on dextrose, peptone, and yeast extract (17).
[d] Over 97% is the Δ-11, 12 isomer.
[e] Over 90% is the Δ-11, 12 isomer.

lipopolysaccharide of a number of myxobacteria. Furthermore, he has shown the presence of 3-OH *i*-15:0 and 3-OH *i*-17:0 fatty acids in these organisms. We did not attempt to identify any of the 3-OH fatty acids.

Namba et al. (25) found in *B. subtilis* α-ketoisovalerate dehydrogenase, an enzyme which converted various branched α-keto acids, most effectively α-ketoisovaleric acids, to the corresponding decarboxylated coenzyme A (CoA) derivatives. A specific enzyme then condensed the branched CoA with several units of malonyl CoA to form the fatty acids that were observed. It would be of interest to know whether myxobacteria possess a similar enzyme system.

Artifacts arising from the use of boron trifluoride-methanol reagent for esterification have been noted before (9, 14). The method was selected because of its simplicity and mild conditions, which would not be expected to alter the fatty acids. It is recommended that it be used (if at all) only with a suitable "blank." The diazomethane procedure, although it is more cumbersome and potentially hazardous, is a "clean" reaction that proceeds under very mild conditions and, under the condition we used, without side reactions. It would be the preferable method, at least where only very small amounts of acids are available.

Culture filtrates of myxobacteria can lyse living gram-positive bacteria; on the other hand, lysis of gram-negative bacteria requires either direct contact with the myxobacterial cell or some prior treatment which disrupts the cell envelope (e.g., butanol treatment of autoclaving; see reference 15 for a discussion of this effect.) Haskä et al. (15) have found that, in addition to the above treatments, a mixture of *iso*-branched and unbranched fatty acids with 11 to 15 carbon atoms could sensitize other resistant bacteria to the action of lysozyme or bacteriolytic enzymes from *M. virescens*. It was suggested that the fatty acids had altered permeability in the bacterial cell envelope, rendering it more susceptible or accessible to the activity of the wall-lytic enzymes. Haskä et al. (15) also claim to have demonstrated the presence of fatty acids in the culture filtrate of *M. xanthus*. It is thus possible that some of the unusual fatty acids present at high concentrations in *M. xanthus* play a role in the lysis of gram-negative bacteria.

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