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Fatty Acid and Aliphatic Hydrocarbon Composition of *Sarcina lutea* Grown in Three Different Media

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Sarcina lutea was grown in Trypticase Soy Broth, Nutrient Broth, and a chemically defined medium. Gas chromatographic analysis of lipid components demonstrated that the composition of the medium had an effect on the relative per cent composition of the aliphatic hydrocarbons and fatty acids present in the cells. The branched olefinic hydrocarbons from the organisms grown in Trypticase Soy Broth showed no predominance or only a slight predominance of odd-numbered carbon chains, whereas the hydrocarbons from cells grown in the other two media showed an obvious predominance of odd-numbered carbon chains. The monocarboxylic fatty acid content and distribution showed only minor differences, with all normal saturated fatty acids present in relatively small quantities for cells grown in Nutrient Broth and in a chemically defined medium.

Studies on the identification of the fatty acids and aliphatic hydrocarbons of *Sarcina lutea* have shown that the fatty acids are distributed in two families of pairs, or dyads, of saturated monocarboxylic acids, with and without methyl branching (12). The hydrocarbons are distributed in two families of tetrads (group of four isomers) of acyclic olefins, all showing methyl branching. The only difference between the tetrads pertaining to different families was found in the relative gas chromatographic retention times of the last two components of each group.

It is well established that culture conditions affect the nature and distribution of the components of the extractable lipid material (2, 4-8, 14). To determine the extent of the changes that could occur as a result of changes in the substrate composition and age of cultures, we have made a comparative study of the content, nature, and distribution of the fatty acids and aliphatic hydrocarbons of *Sarcina lutea*, at different growth phases and in different media (Trypticase Soy Broth, Nutrient Broth, and a chemically defined medium.

MATERIALS AND METHODS

Culture conditions. S. lutea (ATCC 533) was grown in three different media. Trypticase Soy Broth (soy peptone and pancreatic digest of casein) and Nutrient Broth (gelysate and beef extract) were obtained from BBL and were prepared as directed by the manufacturer. The chemically defined medium was a mineral medium containing 10 salts (1), with 5% of the 18-amino acid mixture described by Meister for α-casein (9) and 0.1% pyruvic acid.

A 3-liter amount of each sterile medium was inoculated with 3 ml of a 15-hr-old culture. The flasks were placed in water baths at 25 C and continuously aerated with filtered air (6 to 8 liters/hr). It was determined by turbidity measurements that the early stationary phase of growth was reached in all three media after about 48 hr of incubation. The cells were collected at 24, 48, and 96 hr by centrifugation and were washed three times with 0.15 M saline. The cells were frozen and then dried over P_2O_5 under a vacuum.

Material. The bacterial hydrocarbons and fatty acids were isolated from a benzene-methanol extract of 1.5 g of dried cells by use of a silica gel column with *n*-heptane, benzene, and methanol solvents. The aliphatic hydrocarbons found in the *n*-heptane eluate were further purified on an alumina column by elution with *n*-heptane. Other details of this technique have been presented elsewhere (11). The methyl ester fraction of the total fatty acids liberated from methanol eluate by alkaline hydrolysis (10) was prepared for gas chromatographic analysis according to a method described by Huston and Albro (3).

Gas chromatography and mass spectrometry. The aliphatic hydrocarbons and fatty acid methyl esters were analyzed on an F & M 810 gas chromatograph and an LKB 9000 gas chromatograph-mass spectrometer. The method of preparation of material for analysis and the application of the gas chromatograph and mass spectrometer combination to the identification of the lipid components have been described previously (11). All gas chromatograms shown were obtained on an F & M gas chromatograph with a stainless-steel column (155 meters by 0.076 cm, inner diameter) coated with Igepal CO 990 (General Aniline and Film Corp., New York, N. Y.) at a nitrogen pressure of 933 g/cm^2.

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RESULTS

Effects of growth. When analyzed by gas chromatography, the extracts taken from 24-, 48-, and 96-hr-old cells grown in each of the media did not show any significant pattern differences that could be attributed to age; therefore, only the analyses of extracts from cells grown for 48 hr (approximately the early stationary phase of growth in all three media) are presented.

The dried cells of *S. lutea* harvested at 48 hr were found to contain, on the average, 1.40% extractable lipid material. By measuring peak areas, it was calculated that the aliphatic hydrocarbons analyzed constituted 0.25% of the cell mass of the cells grown in the Trypticase Soy medium and approximately 0.18% of the cells grown in Nutrient Broth and chemically defined medium.

Fatty acids. The fatty acid patterns of the organism grown on the three different media are shown in Fig. 1A, B, and C (Trypticase Soy Broth, Nutrient Broth, and defined medium, respectively) and in Table 1. The upper portion of the major components is inset in each figure. Table 1 contains a summary of the identities and relative per cent composition. The Trypticase Soy medium appears to support the synthesis of normal fatty acids better than Nutrient Broth and chemically defined medium, as shown by the reduction of the *n*-C14 (peak 6) and *n*-C16 (peak 10) fatty acids (Figs. 1B and C). The components labeled a, b, c, and d in the fatty acid patterns obtained from cells grown in Nutrient Broth (Fig. 1B) and chemically defined medium (Fig. 1C) gave complex mass spectral patterns that have not been fully characterized at this time. There do not appear to be any other significant differences in regard to the presence of specific fatty acids synthesized by *S. lutea* in the three different media; however, there are some differences in the quantity of the particular components (Table 1).

Hydrocarbons. In Fig. 2A, B, and C (Trypticase Soy Broth, Nutrient Broth, and defined medium, respectively) and in Table 2, the qualitative compositions of the hydrocarbon components are closely comparable; however, there are significant differences in the relative percentages of the components (Table 2). The proportions of hydrocarbon components were more uniform on Trypticase Soy Broth, indicating that this is a more complete medium for *S. lutea*. In comparison to the hydrocarbons obtained from cells grown in the other two media, the cells grown in Trypticase Soy Broth showed the highest proportion of iso olefins and the lowest proportion of anteiso olefins.

Attempts were made to grow *S. lutea* in an additional chemically defined medium containing fewer amino acids. A slower growth rate was obtained with a medium containing 14 amino acids, and practically no growth was observed in defined media containing fewer than 14 amino acids. There is preliminary evidence that leucine and isoleucine are very important for cell growth, since changes in the quantity of these two amino acids in the chemically defined medium resulted in significant changes in the growth rate over a 48-hr period.

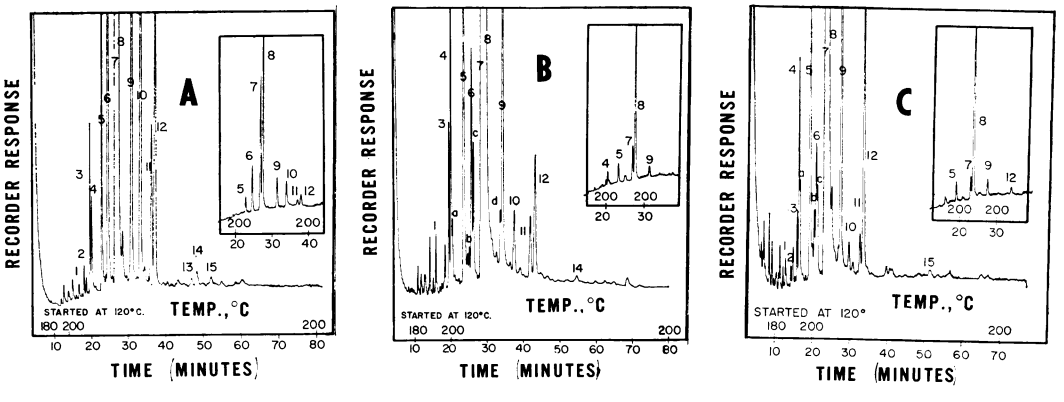

FIG. 1. *Gas chromatographic separation of the fatty acid methyl esters of Sarcina lutea on a stainless-steel column (155 meters by 0.076 cm, inner diameter) coated with Igepal. Nitrogen pressure, 933 g/cm²; no split. F & M 810 apparatus equipped with a flame ionization detector. Range, 10²; attenuation, 1. Temperature programmed at approximately 6 degrees per min from 120 to 200 C and held isothermally. From 1.5 g of extracted cells, 1/100 of the sample was injected for each of the preparations. (A) From cells grown in Trypticase Soy Broth. (B) From cells grown in Nutrient Broth. (C) From cells grown in a chemically defined medium.*

TABLE 1. *Relative per cent composition of the fatty acids of Sarcina lutea in three different media*[a]

	Compound	Trypticase	Nutrient Broth	Defined medium
1.	i 12:0	0.09	0.41	0.59
2.	12:0	0.14	—	0.55
3.	i 13:0	1.13	1.06	0.75
4.	ai 13:0	0.69	4.63	2.27
5.	i 14:0	3.45	6.49	4.6
6.	14:0	6.45	1.45	1.35
7.	i 15:0	25.74	10.88	5.03
8.	ai 15:0	44.35	63.86	69.58
9.	i 16:0	5.97	4.00	4.26
10.	16:0	5.4	0.63	0.63
11.	i 17:0	1.3	0.57	0.64
12.	ai 17:0	2.84	1.28	3.27
13.	18:0	0.14	—	—
14.	18:1	0.22	—	—
15.	18:2	0.14	—	—
	a	—	0.44	1.07
	b	—	0.87	0.71
	c	—	0.94	1.01
	d	—	0.42	—
	Others	1.95	2.07	3.69

[a] Symbols: i = iso; ai = anteiso. The per cent composition of the fatty acids was calculated on the basis of their gas chromatographic areas, which were obtained by multiplying the peak heights by the widths at half peak heights.

DISCUSSION

It has been shown by gas chromatographic analysis that the growth of *S. lutea* on different media can affect the relative per cent composition of the fatty acids and hydrocarbons contained in the cells without changing the nature of the compounds. The fatty acids from cells grown in different media showed (Fig. 1 and Table 1) a reduction of all normal fatty acids in Nutrient Broth and chemically defined medium, and, to a lesser degree, an increase in the proportions of the major anteiso fatty acids and a decrease of the major iso fatty acids.

Although the hydrocarbons show a significant difference in the relative per cent composition, there is still an indication that the hydrocarbons are generated by a unique pathway which involves a discreet balance of precursors (Table 2). The major differences observed in the distribution of the hydrocarbons are the following: (i) the iso olefins are in highest proportion for cells grown in Trypticase Soy Broth (with the exception of iso-Δ-C24, compound 9, Table 2); (ii) the first anteiso olefin of each tetrad is in the highest proportion for cells grown in nutrient broth (exceptions, anteiso-Δ-C27, compound 22, and anteiso-

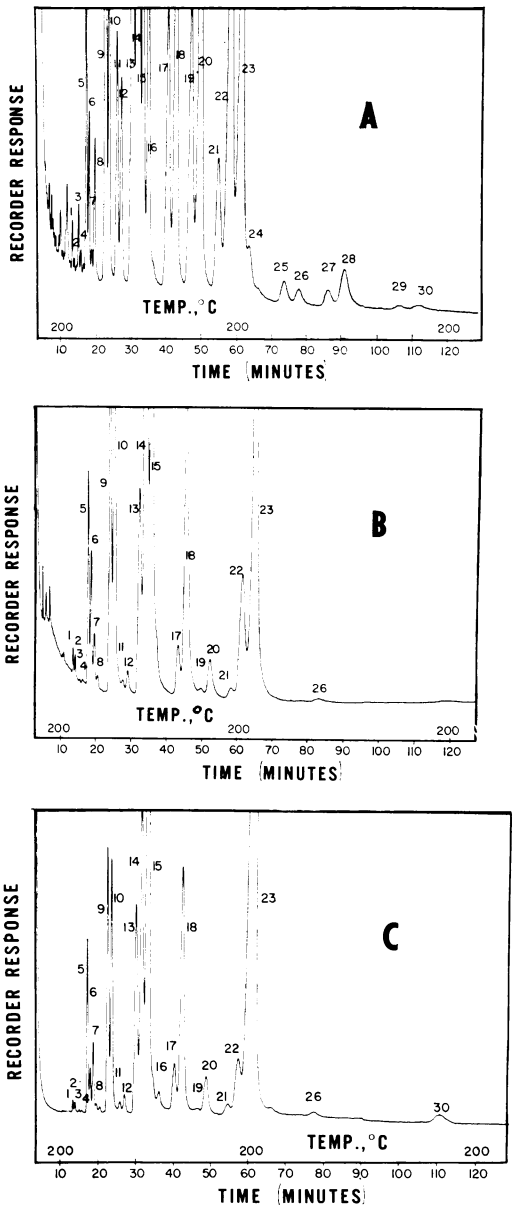

FIG. 2. *Gas chromatographic separation of the hydrocarbons of Sarcina lutea on a stainless-steel column (155 meters by 0.076 cm, inner diameter) coated with Igepal. Nitrogen pressure, 933 g/cm²; no split. F & M 810 apparatus equipped with a flame ionization detector. Range, 10²; attenuation 1. Isothermal at 200 C. From 1.5 g of extracted cells, 1/50 of the sample was injected for each preparation. (A) From cells grown in Trypticase Soy Broth. (B) From cells grown in Nutrient Broth. (C) From cells grown in a chemically defined medium.*

TABLE 2. *Relative per cent composition of the hydrocarbons of Sarcina lutea*[a]

	Compound	Trypticase	Nutrient Broth	Defined medium
1.	iso-Δ-C22	0.25	0.22	0.14
2.	anteiso-Δ-C22	0.01	0.18	0.11
3.	iso-Δ-C22	0.36	0.03	0.03
4.	anteiso-Δ-C22	0.11	0.03	0.02
5.	iso-Δ-C23	2.53	1.96	1.78
6.	anteiso-Δ-C23	0.84	1.25	0.56
7.	anteiso-Δ-C23	0.35	0.50	0.87
8.	iso-Δ-C23	0.71	0.19	0.18
9.	iso-Δ-C24	6.33	7.24	4.05
10.	anteiso-Δ-C24	3.09	7.26	3.89
11.	iso-Δ-C24	2.46	0.23	0.30
12.	anteiso-Δ-C24	1.57	0.41	0.45
13.	iso-Δ-C25	9.23	5.69	4.26
14.	anteiso-Δ-C25	10.49	21.81	8.15
15.	anteiso-Δ-C25	4.32	15.60	18.39
16.	iso-Δ-C25	4.61	?	0.91
17.	iso-Δ-C26	4.24	1.63	1.54
18.	anteiso-Δ-C26	6.65	9.55	7.52
19.	iso-Δ-C26	4.85	0.54	0.24
20.	anteiso-Δ-C26	9.05	1.42	1.57
21.	iso-Δ-C27	2.64	0.52	0.63
22.	anteiso-Δ-C27	10.05	5.81	3.44
23.	anteiso-Δ-C27	9.76	16.41	39.41
24.	iso-Δ-C27	1.24	—	—
25.	iso-Δ-C28	0.53	—	—
26.	anteiso-Δ-C28	0.32	0.14	0.18
27.	iso-Δ-C28	0.42	—	—
28.	anteiso-Δ-C28	1.24	—	—
29.	iso-Δ-C29	0.13	—	—
30.	anteiso-Δ-C29	0.21	—	0.54
	Others	1.51	1.38	0.84

[a] Symbols: Δ = double bond. The per cent composition of the hydrocarbons was calculated on the basis of their gas chromatographic area, which was obtained by multiplying the peak heights by the widths at half peak heights.

Δ-C28, compound 26); (iii) the relative proportion of the second anteiso olefin of each tetrad is different in the even- and odd-carbon chains. In the even-numbered chains, the proportion is higher in cells grown in Trypticase medium. In the odd-numbered chains, the proportion is higher in the cells grown in the chemically defined medium.

The distribution of hydrocarbons in the cells grown on Trypticase Soy Broth shows no predominance of compounds with an odd number of carbon atoms, with the exception of the two anteiso-Δ-C27 compounds (compounds 22 and 23). However, an obvious predominance of odd-carbon chains is observed in the hydrocarbons obtained from cells grown on Nutrient Broth and chemically defined medium.

The fact that *S. lutea* can grow on a chemically well-defined medium provides a sound basis for studying the specific nutrient requirements of this organism. It is hoped that a detailed study of modifications in this medium will permit the elucidation of the essential nutrients. It should then be possible to pinpoint the precursors of the fatty acids and hydrocarbons. This work could be facilitated by the use of radioactively labeled percursors, as described in an accompanying report (13).

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