Fatty Acid Composition of *Caulobacter crescentus*

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SUMMARY

The most abundant fatty acids of *Caulobacter crescentus*, as determined by gas-liquid chromatography, were 18:1, 16:1, 16:0, 12:0, 15:0, 17:0 and cyclopropane acid 17 Δ . The composition of the two morphological forms of this bacterium differed, in that the stalked bacteria and isolated stalk fragments contained more 16:0 and 17 Δ , and less 16:1 than the swimmers.

INTRODUCTION

Caulobacter crescentus has a dimorphic life cycle in which motile swimmers differentiate into stalked non-motile cells. The stalked bacteria develop into predivisive forms which subsequently undergo asymmetric binary fission, each giving rise to a daughter swimmer and a non-motile daughter with the stalk of the parent (Poindexter, 1964). No information concerning the fatty acid composition of these differentiating Gram-negative bacteria has been available. In this report, the fatty acid methyl ester contents of the wild-type *C. crescentus* CB15 (ATCC19089) and a morphological mutant, SK1 418, derived from CB15 and characterized by long stalk formation, have been examined. The long-stalked mutant is amenable to separation of morphological forms and isolation of substantial amounts of stalk material, so that the fatty acid composition of the swimmers, stalked bacteria and isolated stalk fragments could be determined and compared.

METHODS

Cultures were grown in complex broth [0.2 % (w/v) peptone, 0.1 % (w/v) yeast extract, and 1 % (v/v) Hutner's vitamin-free mineral base] (Cohen-Bazire, Sistrom & Stanier, 1957), at 25 °C for 38 to 41 h. The cultures were shaken at 150 rev./min. The bacteria were harvested in a refrigerated Sorvall RC2-B centrifuge (15000 *g*) and washed twice with distilled water. Swimmers and stalked bacteria were separated from each other by differential centrifugation at 6900 *g* to obtain stalked bacteria and 4000 *g* for swimmers (Stove & Stanier, 1962). Repeated centrifugations and distilled water washings were required for adequate segregation of the two morphological types. To evaluate segregation results, Gray's flagella stain (Gray, 1926) was used. Approximately 90 % homogeneity in the two different populations was obtained. Stalk fragments were removed from strain SK1 418 by shear treatment with a Waring blender, and isolated free of bacterial cells and gross bacterial debris by repeated cycles of differential centrifugation at 20000 and 4500 *g* for 10 min.

Lyophilized bacteria and stalk samples (200 or 400 mg) were saponified with 10 ml 2.7 N-KOH in 50 % (v/v) aqueous methanol for 3 to 4 h at 64 °C. After removal of cell debris by centrifugation, non-saponified lipid was extracted with 0.2 vol. petroleum ether

(b.p. 30 to 60 °C). The aqueous phase was acidified to pH 2, extracted five times with petroleum ether, and the extract was dried over anhydrous Na_2SO_4 and NaHCO_3 (4:1, w/w) or with Na_2SO_4 only. Evaporations were performed at 38 °C under nitrogen. Three methylation procedures were compared: (1) 14 % BF_3 -methanol reagent (Applied Science Laboratories, Inc., State College, Pennsylvania, U.S.A.) for 2 min at 100 °C (Metcalf & Schmitz, 1961); (2) 2 N- H_2SO_4 in anhydrous methanol for 5 min at 100 °C (Makula & Finnerty, 1972; (3) 3 ml of 5 % (v/v) HClO_4 in dry methanol for 15 min at 55 °C (Theodore & Panos, 1973). After extraction and evaporation, the fatty acid methyl esters were separated by gas-liquid chromatography (Perkin-Elmer Model 990) with N_2 as the carrier gas, using a 6 ft $\times$ 0.25 in column of 10 % diethylene glycol succinate (DEGS) on Chromosorb W AW at 140 °C, and a 12 ft $\times$ 0.25 in column of 1 % Apiezon L (ApL) on Chromosorb W AW at 180 °C. Qualitative identifications were established by comparison of retention times with those of standard fatty acid methyl esters obtained from Applied Science Laboratories, Inc. Peak areas were determined by planimetry and triangulation. The relative percentage of each peak was calculated from the ratio of its area to the total peak area.

RESULTS

Caulobacter crescentus CB15 and its long stalked mutant sk1 418 are qualitatively similar in their fatty acid composition (Table 1). Some minor quantitative differences exist between the parent CB15 and mutant sk1 418, particularly with respect to dodecanoic acid (12:0), hexadecanoic acid (16:0) and *cis*-9,10-methylene hexadecanoic acid (17 Δ) (see Table 1 for symbols). Among the fatty acid methyl esters identified [methyl esters of 11:0 through *cis*-9,10-methylene octadecanoic acid (19 Δ)] the most abundant fatty acid is octadecenoic acid (18:1), comprising about one-third of the total fatty acid composition. Octadecenoic (18:1), hexadecenoic (16:1) and hexadecanoic (16:0) acids account for more than 60 % of the total in *C. crescentus* under the growth conditions used.

Comparison was made of the fatty acid methyl esters prepared by three different methylation procedures from similar samples obtained from an unsegregated culture and from each segregated morphological type of strain sk1 418. No substantial differences were observed as a consequence of the methylation method, although a few peaks, present in trace amounts in samples treated with BF_3 or with H_2SO_4 , were usually not present in the preparations methylated with perchloric acid. Tridecanoic acid (13:0) was the most prominent minor fatty acid methyl ester peak identified as characteristic of the BF_3 catalyst-treated preparations. The data reported in Table 1 represent results from samples methylated with BF_3 -methanol.

Comparison of fatty acid compositions of the populations of segregated morphological types of *C. crescentus* sk1 418 revealed no notable qualitative differences. However, the swarmer population possessed strikingly more 16:1 and less 16:0, 17 Δ , and octadecanoic acid (18:0) than did the stalked bacteria. The isolated stalk fragments have a fatty acid composition similar to that of the intact stalked cell population, and accordingly differ from the swarmers particularly with respect to the fatty acids 16:1, 16:0, 17 Δ and 18:0.

DISCUSSION

The fatty acid composition of no *Caulobacter* species has previously been reported. Among bacteria which bear some similarity to caulobacters in physiological properties and certain morphological features are members of the *Pseudomonas* genus. Both genera are comprised of aerobic Gram-negative rods or vibrios with polar flagella, and the guanine+

Table 1. Fatty acid composition of *Caulobacter crescentus* swarmer and stalked bacteria and isolated stalks

Fatty acid (%)	Bacterial population
19Δ	T T T T —
n-18:2	T T T T —
2-OH 18:0	— T T — T
UN	— T T T T
n-18:0	6 4 2 2 5
n-18:1	34 33 34 34 36
n-17:0	4 3 4 4 T
17Δ	4 7 3 2 5
a-17:0	5 7 11 8 10
UN	— T T T T
n-16:0	14 10 13 10 13
n-16:1	16 15 15 26 13
i-16:0	T T T T T
UN	T T T T T
n-15:0	7 7 4 5 8
a-15:0	T T T T T
UN	T T T T —
n-14:0	2 2 1 3 1
i-14:0	— T T T T
13:0	T T T T T
12:0	7 12 11 10 9
11:0	T T T T —

Number to the left of the colon refers to the number of carbon atoms; number to the right refers to the number of double bonds. UN, unidentified; T, trace (less than 1 %); Δ, cyclopropane fatty acid; 2-OH, a hydroxy acid; n, a straight chain; i, branched chain, *iso*; a, branched chain, *ante-iso*.

cytosine ratios of species of these genera are quite similar: 62 to 67 mol % for *Caulobacter* species (Poindexter, 1964) and 60 to 67 mol % for *Pseudomonas* species (Mandel, 1966; Skerman, 1967). Although nucleic acid homologies within the genus *Pseudomonas* have been extensively examined (Palleroni & Doudoroff, 1972; Palleroni, Kunisawa, Contopoulou & Doudoroff, 1973), no study has been made of the genetic homology between *Caulobacter* and *Pseudomonas* species. However, several reports of the lipid compositions of various *Pseudomonas* species can be cited for comparison with the fatty acid content of *C. crescentus*.

The three acids (18:1, 16:1 and 16:0) which together make up more than half the fatty acid composition of *C. crescentus* are also present as the major components of *Pseudomonas aeruginosa*, both in readily extractable lipids (Bobo & Eagon, 1968) and in whole cell alkaline hydrolysates (Moss, Samuels & Weaver, 1972). These same three fatty acids are the major constituents of several other *Pseudomonas* species, including *P. putida*, *P. acidovorans*, and *P. stutzeri* (Moss *et al.* 1972). Hydroxy acids in *C. crescentus* were difficult to detect and identify, and appeared to be present in no more than trace amounts. The occurrence of certain hydroxy acids (2- or 3-hydroxytetradecanoic and 2- or 3-hydroxyhexadecanoic acids) in *Pseudomonas* species varies with the species, and in some, hydroxy acids appear as major constituents, mainly in lipopolysaccharide (Hancock, Humphreys & Meadow, 1970; Moss *et al.* 1972; Samuels, Moss & Weaver, 1973). A preliminary report has indicated that the water-soluble lipopolysaccharide of *C. crescentus* contains no 3-hydroxytetradecanoic acid (Shapiro, Agabian-Keshishian & Bendis, 1971). Other *Caulobacter* species should be examined to determine whether a lack of hydroxy fatty acids is characteristic of this genus.

Cyclopropane fatty acids accumulate toward the end of growth, i.e. with ageing of the bacterial culture (Marr & Ingraham, 1962; Law, Zalkin & Kaneshiro, 1963). Here we may have observed an ageing phenomenon associated with procaryotic cell differentiation – ageing of the bacterium as a consequence of its stalk formation. The stalk portion is passed on to the stalked daughter from a parent stalked bacterium; the other daughter cell, a swarmer, occurs at the opposite pole of the divided cell and does not receive any of the stalk material of the parent. The stalked bacterium does not represent an 'old' or stationary phase cell because it will continue to undergo division, via asymmetrical fission, and synthesize new cellular material. Some new stalk material may be synthesized with each of its generations, but a portion of the stalk is aged and originates from the first generation of the stalked bacterium's existence (Schmidt & Stanier, 1966). It is not known if the higher cyclopropane (17 Δ) acid content (and corresponding decrease in 16:1) is uniquely associated with stalk material only, or if the entire stalked bacterium's lipid content reflects the quantitative differences from the swarmer. This situation is due to the fact that, while it is possible to isolate stalk fragments free of bacteria and bacterial debris, the reverse is not technically feasible. The method of removing stalk fragments from the stalked caulobacters is not completely efficient, and some stalk fragments remain trapped in the much more substantial bacterial cell mass during the centrifugal purification procedures used to obtain cell-free stalk fragments. Thus we have not been able to examine the fatty acid composition of a purified 'destalked' *C. crescentus* population devoid of stalk material.

The cytoplasmic membrane and outer wall envelope of Gram-negative bacteria are rich in lipid, and these structures are present in the caulobacter stalks. The quantitative differences in several fatty acids between swarmers, stalked bacteria and stalk fragments suggest that there may be differentiation affecting the lipids of these wall and membrane structures in *Caulobacter crescentus*.

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