

# Production of a Polyunsaturated Isoprenoid Wax Ester during Aerobic Metabolism of Squalene by *Marinobacter squalenivorans* sp. nov.

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**This paper describes the production of 5,9,13-trimethyltetradeca-4E,8E,12-trienyl-5,9,13-trimethyltetradeca-4E,8E,12-trienoate during the aerobic degradation of squalene by a *Marinobacter* strain, 2Asq64, isolated from the marine environment. A pathway involving initial cleavage of the C<sub>10</sub>-C<sub>11</sub> or C<sub>14</sub>-C<sub>15</sub> double bonds of the squalene molecule is proposed to explain the formation of this polyunsaturated isoprenoid wax ester. The isoprenoid wax ester content reached 1.1% of the degraded squalene at the mid-exponential growth phase and then decreased during the stationary phase. The wax ester content increased by approximately threefold in N-limited cultures, in which the ammonium concentration corresponds to conditions often found in marine sediments. This suggests that the bacterial formation of isoprenoid wax esters might be favored in such environments. The bacterial strain is then characterized as a member of a new species, for which we propose the name *Marinobacter squalenivorans* sp. nov.**

The microbial communities of marine environments are often exposed to fluctuating conditions such as the availability of nutrients. Microorganisms have developed a variety of strategies that allow them to survive in these variable environments; the accumulation of storage lipids is only one of them (4). Storage compounds occur normally as intracellular inclusions in bacteria (42). While the accumulation of triacylglycerols occurs preferentially in eukaryotic organisms, triacylglycerols are not frequently found as storage compounds in bacteria (4). Bacteria usually accumulate specialized lipids such as poly(3-hydroxybutyric acid) or other polyhydroxyalkanoic acids (31, 44, 45). Only some bacteria of the genera *Mycobacterium* (6), *Streptomyces* (29), and *Rhodococcus* (3, 5, 50) accumulate triacylglycerols. Wax esters, which are trace constituents of numerous bacteria (e.g., *Escherichia coli* [28], *Serratia marcescens*, *Bacillus cereus* [22], *Nocardia* sp. [21], and *Thiobacillus thioparus* [15]), are generally considered as widespread energy storage components in the genus *Acinetobacter* (18). In fact, it was previously demonstrated that different strains of *A. calcoaceticus* increased their wax ester contents in N-limited cultures and that the accumulated wax esters were degraded to water-soluble molecules and CO<sub>2</sub> during C starvation (18). These results showed that these compounds may serve as an ATP-generating substrate during starvation.

We previously observed the production of several isoprenoid wax esters during the aerobic degradation of 6,10,14-trimethylpentadecan-2-one and phytol by four bacteria (*Acinetobacter* sp. strain PHY9, *Pseudomonas nautica* [IP85/617], *Marinobacter* sp. strain CAB [DSMZ11874], and *Marinobacter hydrocarbonoclasticus* [ATCC49840]) isolated from the marine en-

vironment (33). The amount of these esters increased considerably in N-limited cultures, in which the ammonium concentration corresponds to conditions often found in marine sediments. Similar isoprenoid wax esters have also been detected during the degradation of phytol by bacterial communities isolated from marine sediments under aerobic and denitrifying conditions (34, 35). These different results suggest that the bacterial formation of isoprenoid wax esters might be favored in marine sedimentary environments.

The aerobic degradation of squalene (2,6,10,15,19,23-hexamethyltetracos-2,6E,10E,14E,18E,22-hexaene), which is generally considered to be one of the most abundant biogenic hydrocarbons in the marine environment (1, 9, 24, 37), has been reported in detail for several microorganisms (8, 40, 41, 46, 47, 52, 53). In this paper, we report the formation of relatively large amounts of a polyunsaturated isoprenoid wax ester during aerobic metabolism of this triterpene by a new bacterial strain isolated from marine sediments (12). The bacterial strain (i.e., strain 2Asq64) is also characterized as representative of a new species of the genus *Marinobacter* on the bases of its genetic and physiological differences from the closest existing species of the genus. Thus, we propose the name *Marinobacter squalenivorans* sp. nov. for this strain.

## MATERIALS AND METHODS

**Procedure for isolation of the strain.** Strain 2Asq64 has been isolated from sediment collected at the Carteau Cove (Gulf of Fos, Mediterranean Sea, France). Its isolation was performed with squalene as the sole carbon source and was carried out under anaerobic denitrifying conditions (12).

**Growth conditions.** The basic growth medium consisted of artificial seawater (ASW) (7) supplemented with iron sulfate (0.1 mM), potassium phosphate (0.33 mM), and squalene (2.4 mM) as the organic substrate (i.e., Sq medium). For experiments in which growth was N limited, ammonium chloride was added to the Sq medium to a concentration of 0.1 mM rather than 60 mM. Cultures were incubated at 20°C in 250-ml Erlenmeyer flasks containing 50 ml of Sq medium and were agitated with a reciprocal shaker. For each experiment, two flasks with identical growth media were inoculated—the first for monitoring growth and the

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second for determination of substrate degradation and identification of metabolites. Sterile control experiments were carried out in parallel. Growth was monitored by measuring the optical density at 610 nm with a Shimadzu UV 240 spectrophotometer.

**PCR amplification of the 16S rRNA gene.** Cells were lysed and DNA was extracted as described by Zhou et al. (54). Briefly, the suspensions were repeatedly frozen and thawed and were subsequently incubated in the presence of sodium dodecyl sulfate and proteinase K. DNA was extracted by applying CTAB (hexadecyl-methylammonium bromide), phenol, chloroform, and isoamyl alcohol and was precipitated by the addition of isopropanol. The 16S rRNA gene of the strain was amplified by PCR with universal primers for eubacteria—EU3 and EU5—as previously described (32). The nucleotide sequence of the PCR product was determined by MWG Biotech Company (Ebersberg, Germany).

**Phylogenetic analysis and alignment.** The 16S rRNA gene sequences were aligned with the same region of the closest relatives. Sequences were obtained with the Blast program in BLAST version 2.1 (<http://www.ncbi.nlm.nih.gov/BLAST>) (2). The sequences were aligned by using the method of CLUSTAL W (48). A phylogenetic tree was constructed by using the neighbor-joining method (36). A bootstrap analysis with 1,000 replicates was carried out to check the robustness of the tree. Finally, the tree was plotted by using the TREEVIEW program (30).

**Characterization of the isolated bacteria.** Routine tests (Gram and spore staining and oxidase, catalase, and urease tests) were done as described by Smibert and Krieg (43). Isolates were tested under anaerobic conditions for nitrate utilization, nitrite accumulation, and nitrous oxide production in the presence of acetylene as previously described (10). Biolog GN microplates (Biolog TNC, Hayward, Calif.) were used to investigate the nutritional screening of the strains. Arginine dihydrolase, lysine decarboxylase, and ornithine decarboxylase activities were determined by the techniques of Stolp and Galdgari (46). The requirement for sodium chloride was tested aerobically in ASW medium without sodium and supplemented with lactic acid and ammonium acetate as the carbon sources. The pH was adjusted to 7.5 by using KOH.

**Bacterial morphology and numeration.** Detailed cell shapes and flagella were examined by transmission electron microscopy. The cells were negatively stained with phosphotungstic acid (1%) and observed with a Zeiss EM 912 transmission electron microscope at 100 kV.

For bacterial numeration, cultures were fixed with formaldehyde to a final concentration of 2% and refrigerated until needed. The samples were then diluted with 0.2- $\mu\text{m}$ -pore-size-filtered (No. 7182002; Whatman) ASW and gently sonicated in a Branson 2200 ultrasonic bath for 5 min. The samples were then vortexed for 15 s and incubated with DAPI (4',6-diamidino-2'-phenylindole dihydrochloride) (Boehringer Mannheim) to a final concentration of 2  $\mu\text{g ml}^{-1}$  in darkness for 15 min before filtration on prestained (Irgalen black) 0.2- $\mu\text{m}$ -pore-size membrane filters (GTBP; Millipore). Bacteria were counted immediately with an epifluorescence microscope equipped with a mercury lamp on randomly selected fields until 20 fields—or about 500 bacteria—were counted.

**Treatment of bacterial cultures.** At the end of the growth period, the contents of the flasks were acidified with hydrochloric acid (pH 1) and continuously extracted with chloroform over 24 h. The different extracts thus obtained were dried with anhydrous  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated by means of rotary evaporation.

**Hydrogenation.** Some extracts were hydrogenated (under an atmosphere of  $\text{H}_2$ ) for 12 h under magnetic stirring in methanol with Pd on  $\text{CaCO}_3$  (Aldrich; 10 to 20 mg per mg of extract) used as a catalyst. After hydrogenation, the catalyst was removed by filtration and the filtrate was concentrated by rotary evaporation.

**Derivatization.** Following evaporation of solvents, the residues obtained after treatment of the bacterial cultures were taken up in 400  $\mu\text{l}$  of a mixture of pyridine and BSTFA [Supelco; Bis(trimethylsilyl)trifluoroacetamide] (3:1, vol/vol) and allowed to react at 50°C for 1 h. After evaporation to dryness, the residue was taken up in an adequate amount of ethyl acetate and analyzed by gas chromatography and mass spectrometry.

**Quantification of residual substrate and identification of metabolites.** Structural assignments were based on interpretation of mass spectral patterns and confirmed by the comparison of retention times and mass spectra with those of authentic standard compounds. Quantitative determinations were based on calibration with external standards. Gas chromatography-electron impact mass spectrometry analyses were carried out with a Hewlett-Packard 5890 series II plus gas chromatograph connected to an HP 5972 mass spectrometer. The following operative conditions were employed. A 30-m  $\times$  0.25-mm (inside diameter) capillary column was coated with HP5 (Hewlett-Packard; film thickness, 0.25  $\mu\text{m}$ ); oven temperature was programmed from 60 to 130°C at 30°C  $\text{min}^{-1}$  and then from 130 to 300°C at 4°C  $\text{min}^{-1}$ ; carrier gas (He) pressure was maintained at 1.04 bar until the end of the temperature program and was then

programmed from 1.04 to 1.5 bar at 0.04 bar  $\text{min}^{-1}$ ; the injector (on column with a retention gap) temperature was 50°C; the electron energy was 70 eV; the source temperature was 170°C; and the cycle time was 1.5 s.

**Chemicals.** Squalene (Aldrich) was purified by column chromatography on silica gel (Kieselgel 60 plus 0.5%  $\text{H}_2\text{O}$ ) with hexane as the eluent. Geranylacetone was purchased from Aldrich. 5,9,13-Trimethyltetradecanoic acid was produced from 6,10,14-trimethylpentadecan-2-one by a previously described procedure (26). The synthesis of 5,9,13-trimethyltetradeca-4*E*,8*E*,12-trienoic acid required: (i) condensation of (*E*),(*E*)-farnesyl bromide with diethyl malonate and (ii) subsequent alkaline hydrolysis and decarboxylation of the foregoing diethyl farnesylmalonate (23). Reduction of these acids with  $\text{LiAlH}_4$  in dry diethyl ether afforded the production of the corresponding alcohols. 5,9,13-Trimethyltetradeca-4*E*,8*E*,12-trienyl-5,9,13-trimethyltetradeca-4*E*,8*E*,12-trienoate and 5,9,13-trimethyltetradecyl-5,9,13-trimethyltetradecanoate were prepared from alcohols and acids by the procedure previously described by Gellerman et al. (20).

**Nucleotide sequence accession number.** The sequence obtained in this study is available from the EMBL database under number AJ439500.

## RESULTS AND DISCUSSION

**Phylogenetic and physiological characterization of *Marinobacter* strain 2Asq64.** Analysis of the 16S rRNA sequence results in compelling information about the phylogenetic position of our strain. The rRNA gene sequence of the isolate was compared to sequences available from the database and showed nonambiguous affiliation of this bacterium within the gamma *Proteobacteria*. The closest relatives for which the sequences are currently known are various *Marinobacter* strains. The percentage of similarity between the sequence of our isolate and those of the strains belonging to the *Marinobacter* genus ranged between 96.5 and 93.8% (Fig. 1). The closest phylogenetically affiliated strain is *Marinobacter* sp. strain NK1, which showed 96.5% similarity to our isolate. Thus, according to the phylogenetic tree obtained and presented in Fig. 1, our strain 2Asq64 is fully included in the cluster composed of *Marinobacter* strains but is distant enough from the other species of the genus (95 and 96% of similarity) to be considered as a representative of a new species.

Like the other representatives of the genus, the cells of *Marinobacter* strain 2Asq64 were gram negative, non-spore forming, and motile. Visualized under electron microscopy with negative staining, they appeared as ovoid cells with flagella positioned at the poles (Fig. 2A). They presented an absolute NaCl ion requirement for growth. *Marinobacter* strain 2Asq64 was a facultative aerobic organism with a nonfermentative metabolism. Anaerobically, it was able to denitrify with  $\text{N}_2$  production. The strain exhibited oxidase and catalase activities. The arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, and urease tests were negative. *Marinobacter* strain 2Asq64 did not need growth factors. It degraded benzo[*b*] thiophene and various aliphatic hydrocarbons (tetradecane, 1-tetradecene, and perhydrofluorene) under aerobic conditions. As previously observed in other strains, the restricted nutritional profile was one of the main phenotypic characteristics of the genus *Marinobacter* (19, 32). In contrast to the type strain *M. hydrocarbonoclasticus* (ATTC 49840), *Marinobacter* strain 2Asq64 was able to use only pyruvate, succinate, acetate, citrate, lactate, formate, and some amino acids (L-Ala, L-Leu, L-Pro, and L-Phe) as the sole source of carbon and energy (12).

**Growth and metabolism of squalene by *Marinobacter* strain 2Asq64.** *Marinobacter* strain 2Asq64 was able to grow with squalene as the sole source of carbon and energy under aerobic conditions, with a doubling time of 29.5 h (Fig. 3). About 95%

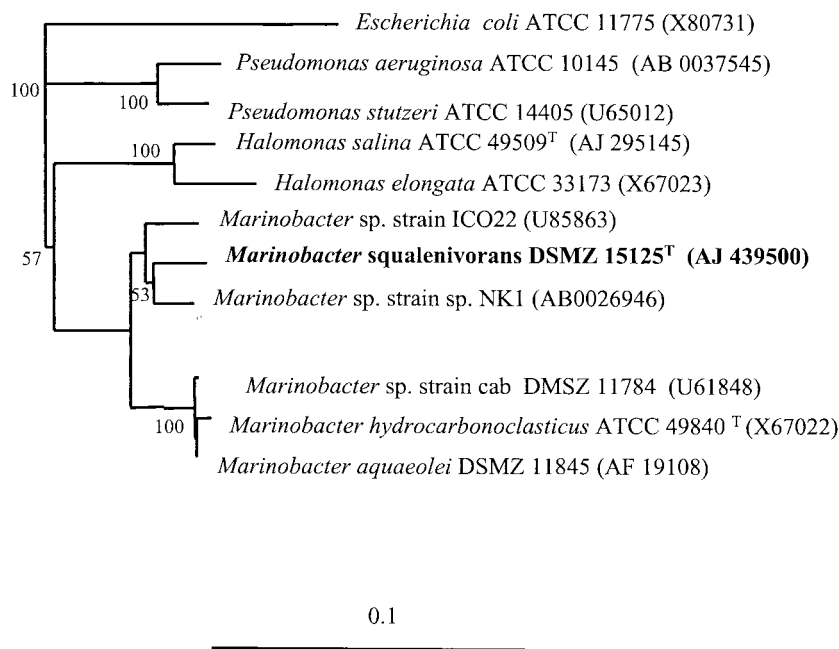


FIG. 1. Phylogenetic affiliation of *Marinobacter* strain 2Asq64 on the basis of comparison of the 16S rRNA gene sequence with those of the closest species. Comparisons were made using 1,408 unambiguous positions. The scale bar indicates one nucleotide difference per 1,000 nucleotides.

substrate degradation had occurred in bacterial assays after 10 days of incubation at 20°C, while 96% of residual squalene was recovered in sterile controls. It was essential to carry out sterile controls in parallel to aerobic cultures because of the presence of small amounts of peroxides in commercial squalene and in the solvents used for its purification. These compounds may induce processes that are, in fact, auto-oxidative and that might have led to the production of nonnegligible amounts of abiotic oxidation products of this unsaturated isoprenoid compound (27). After 10 days of growth, comparison of sterile controls and bacterial assays provided evidence of the bacterial production of small amounts (<5 µg liter<sup>-1</sup>) of geranylacetone (compound 1), 5,9,13-trimethyltetradeca-4*E*,8*E*,12-trien-1-ol (compound 2), and 5,9,13-trimethyltetradeca-4*E*,8*E*,12-trienoic acid (compound 3) as well as of a significant quantity (0.15 mg liter<sup>-1</sup>) of 5,9,13-trimethyltetradeca-4*E*,8*E*,12-trienyl-5,9,13-trimethyltetradeca-4*E*,8*E*,12-trienoate (compound 4) (Fig. 4A). These compounds were formally identified by comparison of their chromatographic retention times and electron impact mass spectra (before and after hydrogenation) with those of reference compounds. Owing to the involvement of strongly favored allylic cleavages, polyunsaturated isoprenoid compounds present poorly informative electron impact mass spectra dominated by a fragment ion at *m/z* 69 (Fig. 5A and 6A and C). Hydrogenation of the double bonds allowed us to obtain interesting molecular structure information (Fig. 5B and 6B and D). In the case of 5,9,13-trimethyltetradecyl-5,9,13-trimethyltetradecanoate (compound 5) (obtained after hydrogenation of compound 4 [Fig. 4B and 5B]), the fragment ion [RC(OH)=OH]<sup>+</sup> at *m/z* 271 formed after the rearrangement of two hydrogen atoms (25) allows an easy characterization of the acid moiety of this ester, whereas the alcohol moiety gives

a [C<sub>17</sub>H<sub>34</sub>]<sup>o+</sup> fragment ion at *m/z* 238 (Fig. 5B). The structure of this ester was also confirmed after alkaline hydrolysis, which results in the formation of 5,9,13-trimethyltetradecan-1-ol (compound 6) and 5,9,13-trimethyltetradecanoic acid (compound 7) (Fig. 4C).

The presence of these different metabolites suggests that, under aerobic conditions, the degradation of squalene by *Marinobacter* strain 2Asq64 involves cleavage of the C<sub>10</sub>-C<sub>11</sub> or C<sub>14</sub>-C<sub>15</sub> double bonds (Fig. 7). This cleavage produces geranylacetone (compound 1) and 5,9,13-trimethyltetradeca-4*E*,8*E*,12-trienal (compound 8). A similar oxygenase-catalyzed splitting of internal double bonds has been previously observed in the case of the aerobic degradation of squalene by *Arthrobacter* sp. (52). *Arthrobacter* sp. accumulated relatively large amounts of geranylacetone (until 56% of yield), whereas only traces of this ketone are present in cultures of *Marinobacter* strain 2Asq64. The metabolism of geranylacetone by *Marinobacter* strain 2Asq64 probably involves the oxidation of the keto-terminal methyl group and the subsequent decarboxylation of the resulting keto acid to the 5,9-dimethyldeca-4*E*,8-dienoic acid (compound 9). The acid 9 thus formed may be subsequently totally metabolized via alternating β oxidation and β decarboxymethylation reaction sequences (33, 34). Oxidation of the 5,9,13-trimethyltetradeca-4*E*,8*E*,12-trienal (compound 8) (resulting from the cleavage of the C<sub>10</sub>-C<sub>11</sub> or C<sub>14</sub>-C<sub>15</sub> double bonds) produces the corresponding acid 3, which may then be metabolized via alternating β oxidation and β decarboxymethylation reaction sequences. The production of significant amounts of the ester 4 implies the presence of a very active esterase system, since the experimental conditions exclude a chemical esterification. Indeed, this ester was lacking in the sterile controls, and it was not formed during the workup



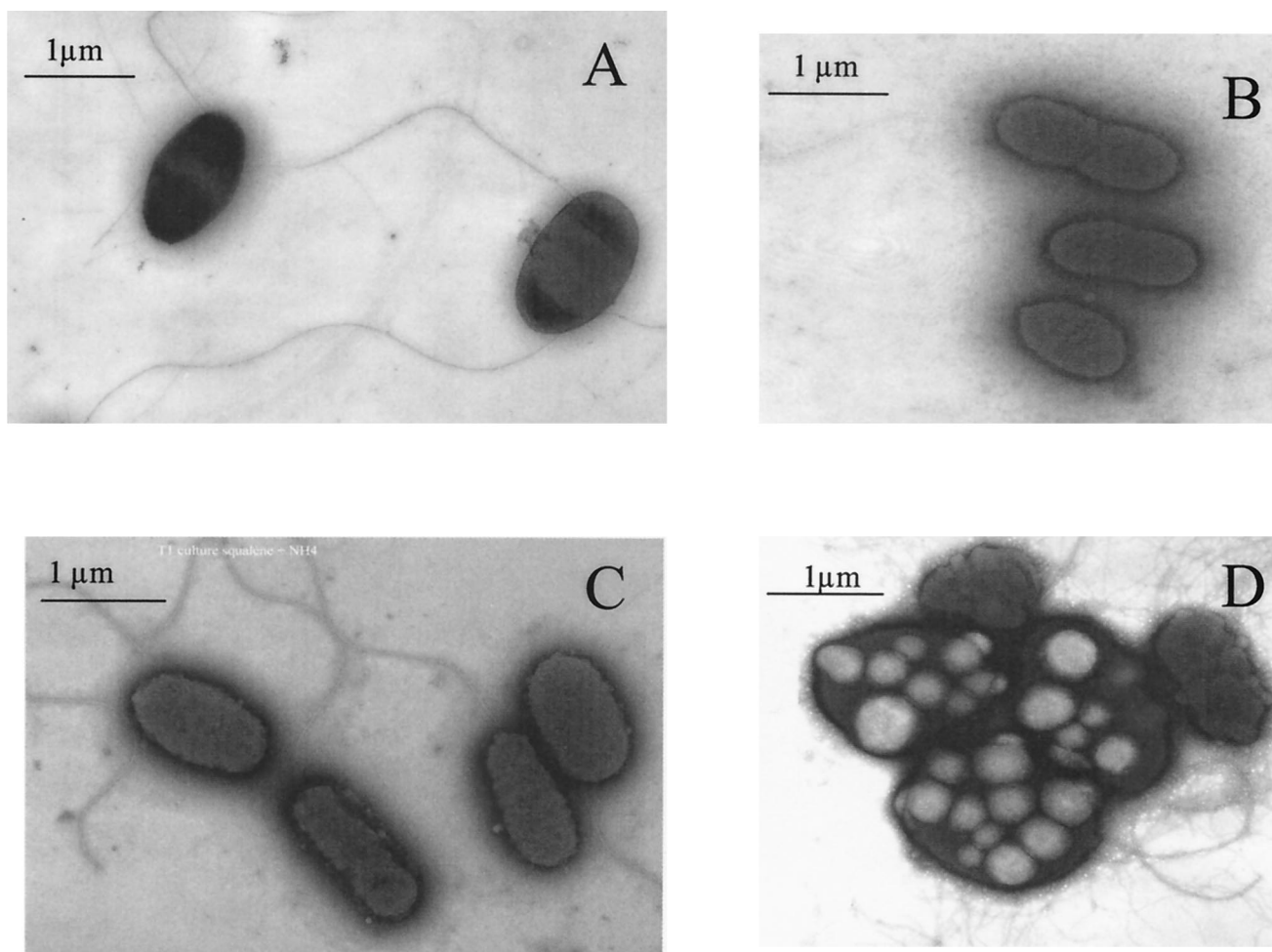


FIG. 2. Electron microscopy of *Marinobacter* strain 2Asq64. (A) Strain negatively stained with uranyl acetic acid or (B, C, and D) with phosphotungstic acid; (A and B) strain grown on medium containing yeast extract and bactopeptone as carbon sources ( $5 \text{ g liter}^{-1}$ ); (C) strain grown on squalene as carbon source; (D) strain grown in N-limited conditions in the presence of squalene.

procedure. The formation of this compound may be attributed to the reduction of a part of the 5,9,13-trimethyltetradeca-4*E*,8*E*,12-trienal (compound 8) to the corresponding alcohol 2 by a dehydrogenase and to the subsequent condensation of this

alcohol with the acid 3 (Fig. 7). Esterification activity seems to be confined to 5,9,13-trimethyltetradeca-4*E*,8*E*,12-trien-1-ol (compound 2) and 5,9,13-trimethyltetradeca-4*E*,8*E*,12-trienoic acid (compound 3), since we failed to detect significant amounts

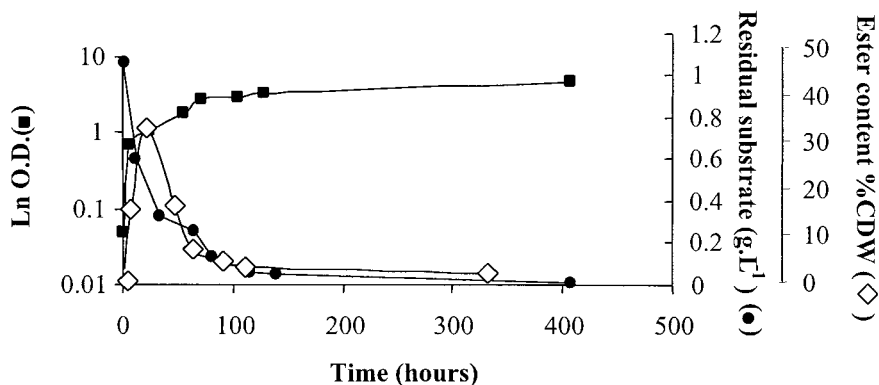


FIG. 3. Production and consumption of the ester 4 during the growth of *Marinobacter* strain 2Asq64 in the presence of squalene as the carbon source under aerobiosis.

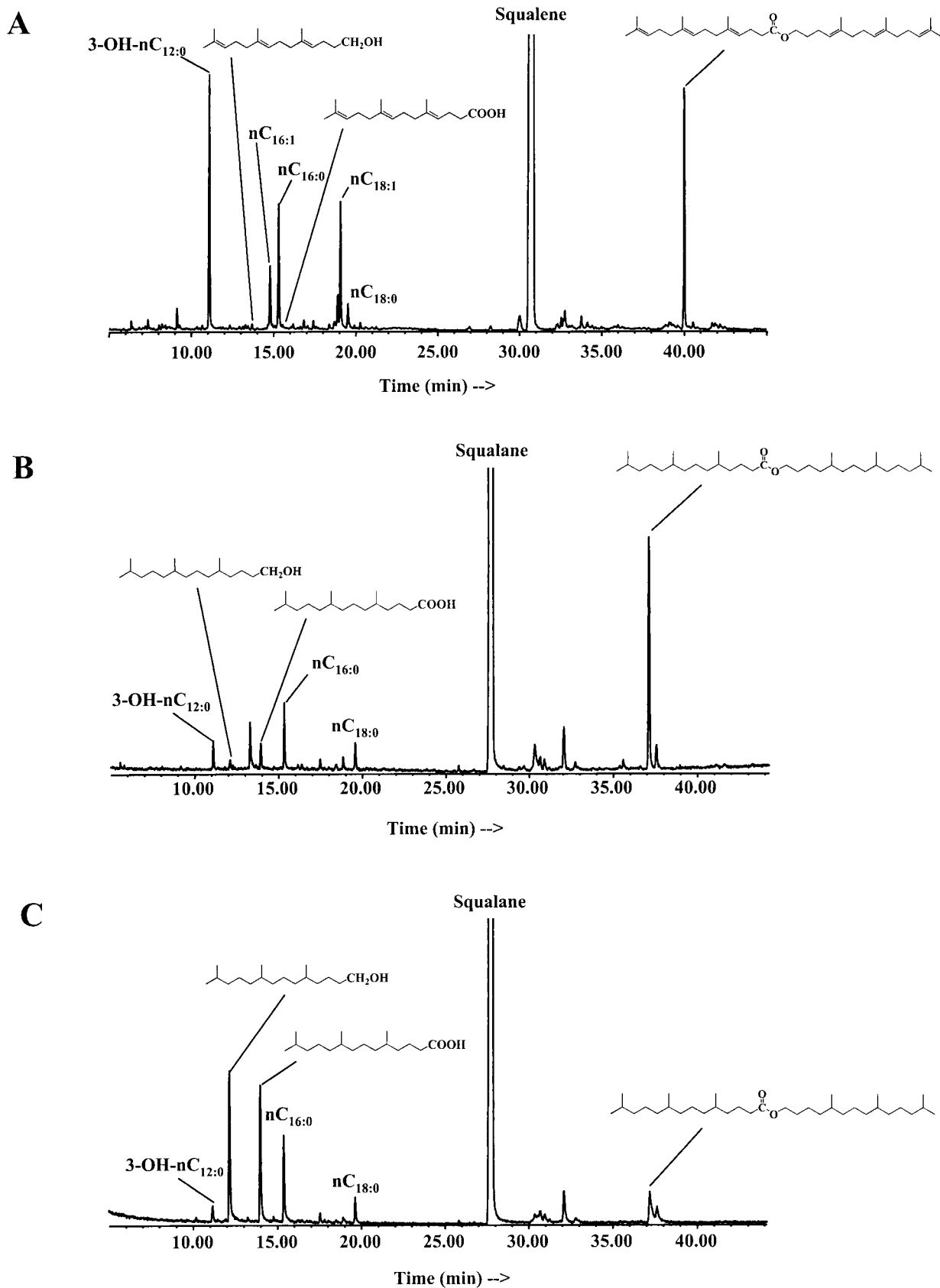


FIG. 4. Total ion chromatograms of nonhydrogenated (A), hydrogenated (B), and hydrogenated-saponified (C) extracts obtained after growth of *Marinobacter* strain 2Asq64 on squalene.

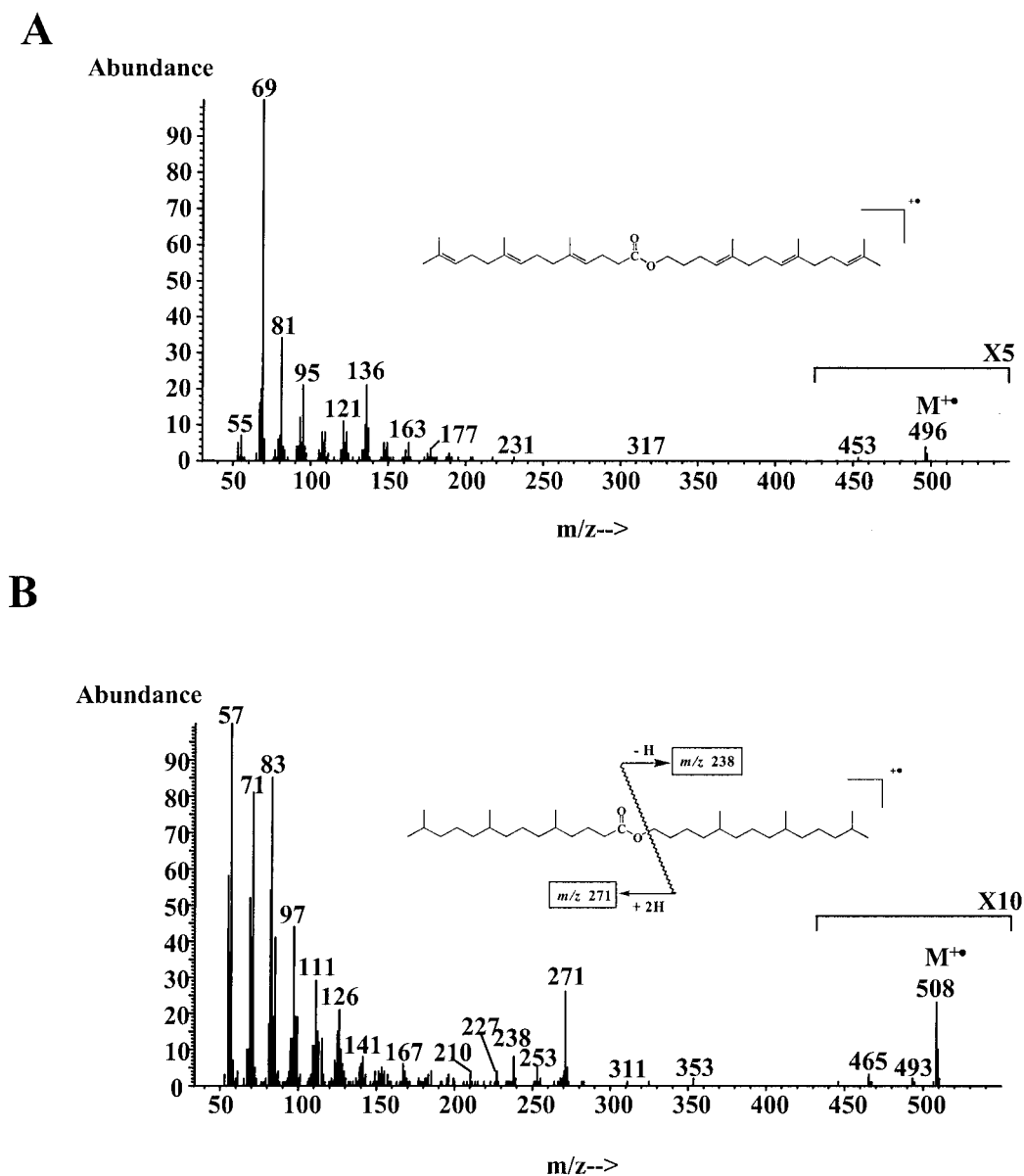


FIG. 5. Electron impact mass spectra of 5,9,13-trimethyltetradeca-4*E*,8*E*,12-trienyl-5,9,13-trimethyltetradeca-4*E*,8*E*,12-trienoate (compound 4) (A) and 5,9,13-trimethyltetradecyl-5,9,13-trimethyltetradecanoate (compound 5) (B).

of esters with an acyl moiety arising from the metabolism of the acid 3 (via the  $\beta$  oxidation or  $\beta$  decarboxymethylation reaction sequences).

Previous work (18) has demonstrated that the amount of wax esters produced increases considerably in N-limited cultures under conditions of low growth rate where carbon and energy are in excess. In non-N-limited cultures of *Marinobacter* strain 2Asq64, the isoprenoid wax ester 4 content reaches 10 mg liter<sup>-1</sup> (1.1% of the degraded squalene) at the mid-exponential growth phase and then decreases during the stationary phase (Fig. 3). The wax ester content increased by approximately threefold in N-limited cultures compared with that in nonlimited ones, while the growth yield decreased by approximately 10-fold ( $2.4 \times 10^9$  cells mg<sup>-1</sup> of removed squalene in the non-N-limited culture and  $2.7 \times 10^8$  cells mg<sup>-1</sup> of removed

squalene in the N-limited culture). It is interesting that the ammonium concentration used in the N-limited cultures (0.1 mM) corresponds to conditions that are often found in marine sediments (11). This result suggests, therefore, that the formation of isoprenoid wax esters might be favored in such environments when squalene is available.

The examination of the ultrastructure of negatively stained cells by electron microscopy showed intracytoplasmic electron transparent inclusions (Fig. 2). These inclusions were mainly detected in the N-limited squalene culture. On the bases of this observation and the previous detection of intracytoplasmic inclusions in cells of *Acinetobacter* species (39), which often produce wax esters as energy storage components (18), it is assumed that the wax ester 4 is stored in the cytoplasm of *Marinobacter* strain 2Asq64 cells.

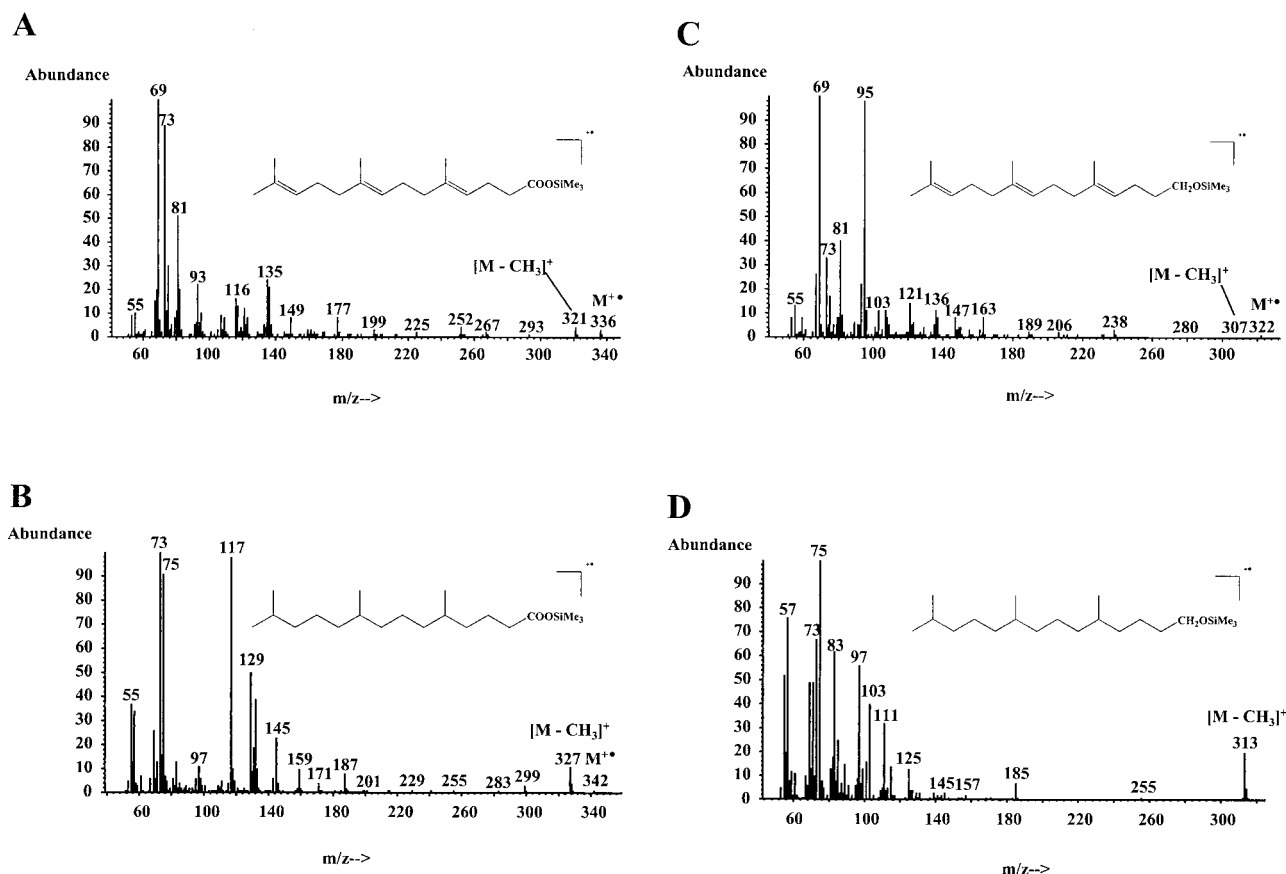


FIG. 6. Electron impact mass spectra of silylated 5,9,13-trimethyltetradec-4E,8E,12-trienoic acid (compound 3) (A), 5,9,13-trimethyltetradecanoic acid (compound 7) (B), 5,9,13-trimethyltetradec-4E,8E,12-trien-1-ol (compound 2) (C), and 5,9,13-trimethyltetradecan-1-ol (compound 6) (D).

Isoprenoid wax esters have been detected in few marine and lacustrine sediments varying in age from new to 50,000 years old (13, 16). Proposed sources have included mosses (17), bryophytes (14), dinoflagellates (51), or zooplankton (in which the presence of wax esters was attributed to the dietary intake of phytol) (38), but in most cases, no evidence was presented to substantiate this. The results obtained in the present study confirm some of our previous observations (33–35) and show that the bacterial degradation of isoprenoid compounds (notably of phytol and squalene, which are widely distributed in the marine environment [49]) might constitute another potential source of isoprenoid wax esters in marine sediments. Our results are in good agreement with the hypothesis of Volkman and Maxwell (49), who said in their review of acyclic isoprenoid compounds that esterification may be a significant process in microbially active sediments. Though in some environments isoprenoid wax esters can be rapidly hydrolyzed during early diagenesis (17), the detection of such compounds in lacustrine sediments that are 50,000 years old (16) suggests that, under some conditions, esterification can enhance the preservation of labile isoprenoid compounds.

Wax esters, which constitute useful metabolite traps, appeared to be particularly well suited to the study of the bacterial metabolism of isoprenoid compounds (33–35). It is interesting that bacteria producing large amounts of wax esters

belong to the genera *Marinobacter* (reference 33 and this work), *Pseudomonas* (33), and *Acinetobacter* (18, 33). Though several tests are needed to obtain definitive statements, the production of large amounts of wax esters seems to be a characteristic of bacteria belonging to the gamma subdivision of the *Proteobacteria*. According to its phylogenetic position in the *Marinobacter* cluster and its physiological characteristics—particularly squalene degradation—we propose that *Marinobacter* strain 2Asq64 should be a representative of a new species of the genus *Marinobacter* with the name *M. squalenivorans* sp. nov., which refers to its capacity to use squalene as its sole energy and carbon source.

**Description of *M. squalenivorans* sp. nov.** The Latin noun *squalenum* refers to squalene, a specific hydrocarbon molecule, and the Latin term *vorans* means devouring; therefore, *squalenivorans* means “squalene devouring.” The organisms consist of ovoid straight rod-shaped cells that typically have a width of 0.7  $\mu\text{m}$  and a length of 1.2  $\mu\text{m}$ , with motility accomplished by the use of a polar flagellum. Cells of this species are gram negative, nonsporulating, and chemoorganotrophic. They exhibit a respiratory type of metabolism, aerobic and anaerobic with oxygen or nitrate, respectively, as terminal electron acceptors. The cells are capable of denitrification and are not able to ferment. Electron donors and carbon sources for aerobic and anaerobic oxidative chemoorganotrophy include

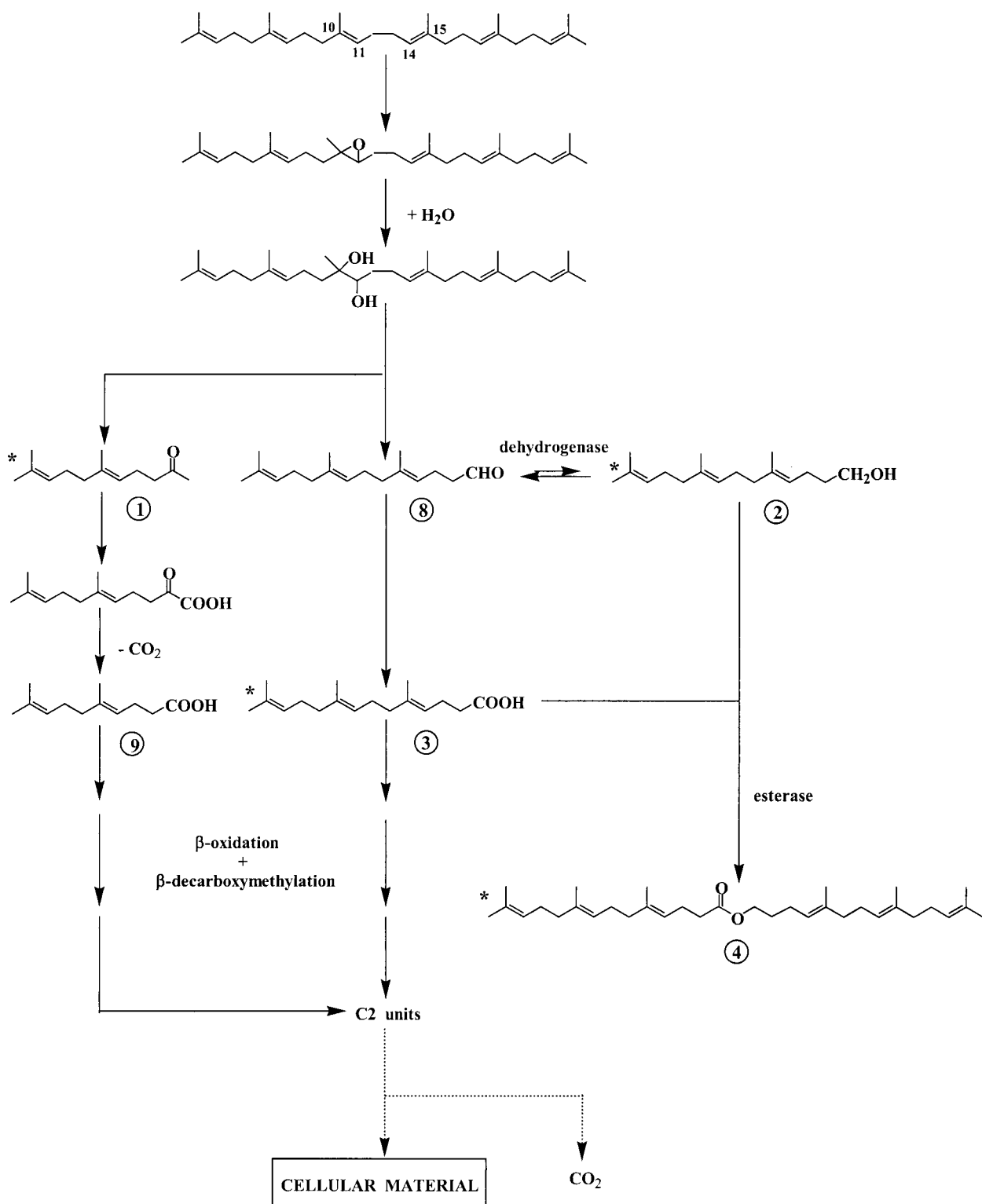


FIG. 7. Proposed pathways for the metabolism of squalene by *Marinobacter* strain 2Asq64 (\*, metabolites detected).

pyruvate, succinate, acetate, citrate, lactate, formate, L-alanine, L-leucine, L-proline, and L-phenylalanine. Indole is not produced. Tests for urease, arginine dihydrolase, lysine decarboxylase, and ornithine decarboxylase are all negative. Tests for

oxidase and catalase reactions are positive. The organism is able to aerobically use the following hydrocarbons as energy and carbon sources: benzothiophene, tetradecane, 1-tetradecene, perhydrofluorene, and squalene. The cells are slightly



halophilic, and the optimum temperature for growth is 32°C. The mol% G+C of DNA is 54.3 (as measured by high-pressure liquid chromatography). The habitat of the organisms is coastal marine anoxic sediment. The type strain for this species is strain 2A sq64, isolated from a coastal sediment contaminated by crude oil near Marseille, France, and has been deposited with the Deutsche Sammlung von Mikroorganismen under the number DSMZ 15125 and with the American Type Culture Collection under the accession number ATCC BAA-792. The 16S rRNA gene sequence of the strain has been deposited in the EMBL database under the accession number AJ439500.

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