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# Changes in fatty acids of *Pseudomonas nautica*, a marine denitrifying bacterium, in response to *n*-eicosane as carbon source and various culture conditions

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## Abstract

This study determined the effects of shifts in environmental conditions on the fatty acid composition of a sedimentary facultative anaerobic denitrifying marine bacteria (*Pseudomonas nautica* strain IP 617). The effects of carbon source (*n*-eicosane, sodium acetate and rich medium), temperature (13, 20 and 30°C), presence or lack of oxygen and growth phase (stationary and exponential) were investigated. As demonstrated by correspondence analysis, the effect of the various conditions tested, in descending order of importance, were carbon source > temperature > growth phase ≥ oxygen. Among the different growth substrates, *n*-eicosane (*n*C<sub>20</sub>) led to the most distinct FA profiles, characterised by high amounts of saturated and branched FA, the appearance of 20-carbon acids (20:1ω9 and 20:0) and a Δ<sup>10</sup> methyl branched series with mainly the 10Me16:0. With regard to temperature effects, *P. nautica* showed a mean acyl chain length thermoregulation process for the major monounsaturated fatty acids which led to increased values of the ratio ΣC<sub>18:1</sub>/ΣC<sub>16:1</sub> with increasing temperatures. The effect of growth phase and anaerobiosis were less marked. The analysis of bacterial fatty acids could enable the detection of hydrocarbonoclastic bacterial communities in marine sediments contaminated by hydrocarbons. © 1999 Published by Elsevier Science B.V. All rights reserved.

**Keywords:** Fatty acid; *Pseudomonas nautica*; Hydrocarbon; Culture condition

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## 1. Introduction

Lipids have been used for many years as biological

markers to determine sources of organic matter (terrestrial vs marine, natural vs anthropogenic, prokaryotic vs eucaryotic) and its transformation processes in various environments. In recent years, a special interest has been generated for the analysis of microbial fatty acids in sediments, which are specific for different groups of bacteria [1–9]. However,

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it is difficult, in marine sediments, to accurately relate the observed fatty acid profiles to the contributing bacteria, due to the need for further information on lipid chemistry of marine bacteria, and to the numerous eucaryotic contributions.

Changes in salt concentration, temperature or carbon source may produce variations in the membrane and wall lipid fatty acids of bacteria [9–14]. Such variations are certainly rare in unpolluted areas, but in the case of accidental or chronic anthropogenic inputs, like oil spills, community structures [15] can be altered.

The aim of this study was to determine the effect of shifts in environmental conditions on the lipid composition, and hence the biomarker reliability, of the sedimentary marine bacteria *Pseudomonas nautica* strain IP 617. The fatty acid composition of *P. nautica* was determined after culture under different important physicochemical and biological conditions, such as carbon source (*n*-eicosane, sodium acetate and rich medium), temperature, presence or lack of oxygen and growth phase. The goals were: (1) to assess if the 11 different FA profiles remained characteristic for the bacterium; (2) to understand the relative importance and the hierarchy of the different culture conditions; and (3) to identify possible characteristic biomarkers for *n*-alkane utilising cells.

## 2. Materials and methods

### 2.1. Bacterial strain and culture conditions

*P. nautica* strain IP 617 (Institut Pasteur culture collection 617/1.85), was used in this study. It is a representative marine denitrifying facultative anaerobic bacterium, able to grow either under aerobic conditions with hydrophobic substrates (hydrocarbons) or under anaerobic conditions with soluble substrates (acetate) and nitrate as electron acceptor [16]. *P. nautica* was precultured and cultured in 500 ml inverted T-shaped flasks, containing 175 ml of Mineral Salt Medium (MSM) for aerobic cultures or in 1.1-l serum flasks containing 1 l MSM for anaerobic cultures.

The MSM had the following composition (in g l<sup>-1</sup> distilled water): NaCl, 23 g; Tris(hydroxymethyl)-aminomethane, 5 g; KCl, 0.75 g; NH<sub>4</sub>Cl, 1 g for

aerobic and 3 g for anaerobic cultures; MgSO<sub>4</sub>·7H<sub>2</sub>O, 3.91 g; MgCl<sub>2</sub>, 5.08 g; KNO<sub>3</sub>, 1 g for anaerobic culture only; FeSO<sub>4</sub>, 0.002 g; K<sub>2</sub>HPO<sub>4</sub>, 0.074 g. The pH was adjusted to 7.8 with 1 M HCl. Depending on the experiment, the added carbon sources in g l<sup>-1</sup> of MSM were either 10 g of rich medium (5 g of yeast extract plus 5 g of bacto-peptone), 3 g of sodium acetate or 1 g of *n*-eicosane (*n*C<sub>20</sub>).

Aeration for aerobic cultures was maintained by agitation on a reciprocal shaker (96 rpm). For anaerobic cultures, oxygen was eliminated by N<sub>2</sub> flushing for 2 h. Growth was measured by monitoring optical density at 450 nm with a Shimadzu UV-Visible spectrophotometer. The cells were harvested by centrifugation (23 000 × *g*, 20 min, 10°C) at late exponential or during stationary growth phase and were washed twice with MSM (same centrifugation conditions).

The different physicochemical and biological conditions tested were temperature (13, 20 and 30°C), oxygen (presence vs lack of), carbon source (bacto-peptone+yeast extract, acetate and *n*-eicosane) and growth phase (stationary vs exponential).

The reference condition was *P. nautica* grown aerobically at 30°C, with rich medium as carbon source, and harvested during the exponential phase.

### 2.2. Fatty acid extraction

Bacterial cells were extracted as wet pellets using a modified Folch method [17,18]. Cell pellets (1 g) were sonicated for 30 min in 30 ml of chloroform–methanol 2:1 (v/v) (four times) at room temperature. Organic phases were filtered (Whatman GFC). One quarter of the total volume of 0.88% KCl in water (w/v) was added. After settling, the aqueous layer was drawn off and one quarter of the volume of the organic phase of methanol–KCl solution 1:1 (v/v) was added, and the washing procedure was repeated twice. The bottom layer, containing the purified lipids was dried over Na<sub>2</sub>SO<sub>4</sub>, and the solvent removed under a nitrogen stream. Hydrolysis of the total extract was conducted in a molar solution of KOH (in ethanol–water 95:5 v/v) by reflux for 1 h. The solution was cooled, and acidified with 2 M HCl after addition of 250 ml of water. Fatty acids and non-saponifiable materials were extracted with tert-butyl methyl ether (TBME) (3 × 50 ml). Solvent ex-

tracts were then washed with water and dried over  $\text{Na}_2\text{SO}_4$ .

Fatty acids and non-saponifiable compounds were separated by ion exchange column chromatography [19]. The column was packed with 30 g of Amberlyst A26 SBR, first activated with aqueous 2 M KOH (6 ml per gram of resin), washed with water until neutrality then by TBME. The lipid extract (TBME concentrated solution) was placed at the top of the column, non-saponifiable compounds were eluted by 90 ml of TBME, fatty acids (FA) were recovered with 90 ml of 2 M HCl/MeOH. The fatty acid solution was then directly refluxed for 3 h in order to obtain the fatty acid methyl esters (FAME) derivatives. The solution was cooled, and FAME were thoroughly extracted with  $3 \times 50$  ml of *n*-hexane after the addition of 80 ml NaCl saturated solution. The FAME solution was washed until neutrality, successively with  $\text{Na}_2\text{CO}_3$  5% (w/v) and with distilled water.

*N*-Acylpyrrolidide derivatives were used for the GC/MS confirmation of double bonds and methyl branching positions on the basis of their characteristic fragmentations, as previously described [20,21]. These derivatives were prepared by direct treatment of methyl esters (2 mg) with pyrrolidine-acetic acid 10:1 (v/v) (1 ml) under nitrogen purged reflux (2 h). The solutions were cooled, amides were taken up in  $\text{CH}_2\text{Cl}_2$  ( $3 \times 5$  ml) and washed repeatedly with 2 M HCl. Solvent extracts were then washed with water and dried over  $\text{Na}_2\text{SO}_4$ .

### 2.3. Fatty acid analyses

GC/FID analyses were carried out with a CP 9000 Chrompack chromatograph equipped with a J&W Scientific DB5-MS fused silica capillary column ( $60 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$ ). The injector was 'on column' ( $1 \text{ ml min}^{-1}$  of helium as carrier gas), the FID ( $\text{H}_2 = 30 \text{ ml min}^{-1}$ ;  $\text{N}_2 = 30 \text{ ml min}^{-1}$ ; air =  $250 \text{ ml min}^{-1}$ ) with a temperature of  $310^\circ\text{C}$ . The oven temperature was programmed from 60 to  $160^\circ\text{C}$  ( $20^\circ\text{C min}^{-1}$ ), then from 160 to  $290^\circ\text{C}$  ( $2^\circ\text{C min}^{-1}$ ) and finally isothermal (50 min). A comparison of retention times with known standards of FAME (Matreya, USA; bacterial acid methyl ester mixture, reference 1007) was made for tentative peak identification of compounds separated by GC. The quantitative analysis was carried out using docosanoic

acid added to the sample, before any treatment, as internal standard.

GC/MS analyses were carried out with a Hewlett Packard 5890 series II chromatograph with the same column as used for GC/FID analysis. The injector was 'splitless', operated in constant pressure mode ( $1 \text{ ml min}^{-1}$  of helium as carrier gas), with a 60-s venting time at  $300^\circ\text{C}$ . The oven temperature was programmed: (a) for FAME, from  $30^\circ\text{C}$  (for 1 min) to  $70^\circ\text{C}$  ( $50^\circ\text{C min}^{-1}$ ), then from 70 to  $120^\circ\text{C}$  ( $10^\circ\text{C min}^{-1}$ ), from 120 to  $290^\circ\text{C}$  ( $2^\circ\text{C min}^{-1}$ ) and finally isothermal (10 min); and (b) for *N*-acylpyrrolidides, from 30 (for 1 min) to  $100^\circ\text{C}$  ( $50^\circ\text{C min}^{-1}$ ), from 100 to  $200^\circ\text{C}$  ( $20^\circ\text{C min}^{-1}$ ), from 200 to  $290^\circ\text{C}$  ( $2^\circ\text{C min}^{-1}$ ) and finally isothermal (20 min).

The mass spectrometer was a Hewlett Packard 5989A MS Engine. Mass spectra were collected in full scan mode ( $m/z$  40–600) under the following conditions: interface temperature,  $295^\circ\text{C}$ ; source temperature,  $240^\circ\text{C}$ ; ionisation energy, 70 eV; electron multiplier, 1700 V;

GC/FTIR analyses were carried out with a Carlo Erba HRGC MEGA chromatograph equipped with a Chrompack CP SIL 8 fused silica capillary column ( $30 \text{ m} \times 0.32 \text{ mm} \times 0.25 \mu\text{m}$ ). The injector was 'on column' ( $2 \text{ ml min}^{-1}$  of helium as carrier gas). The oven was programmed from 70 to  $120^\circ\text{C}$  ( $10^\circ\text{C min}^{-1}$ ), from  $120^\circ\text{C}$  to  $210^\circ\text{C}$  ( $2^\circ\text{C min}^{-1}$ ), from 210 to  $280^\circ\text{C}$  ( $10^\circ\text{C min}^{-1}$ ), then isothermal (10 min). The light-pipe ( $160 \text{ mm} \times 1.2 \text{ mm}$ ) and the transfer lines were maintained at a temperature of  $285^\circ\text{C}$ . FTIR spectrometer was a 20 SXB Nicolet, 16 data scans were collected per spectra file with a resolution of  $8 \text{ cm}^{-1}$  using a medium-range MCT detector ( $4000\text{--}650 \text{ cm}^{-1}$ ) [22]. The sensitivity and the chromatographic resolution of the method did not allow detection of *trans* derivatives. So in the following discussion, we assume that all the unsaturated FA were *cis* isomers.

### 2.4. Fatty acid nomenclature

The shorthand nomenclature used is in the form of numbers separated by a colon [8]. The number before the colon indicates the carbon chain length and the one after corresponds to the number of double bonds. The double-bond position (from the methyl end of the molecule) is indicated by the number after

the 'ω'. The prefixes 'i' and 'a' mean iso and anteiso branchings, respectively. Methyl branching is indicated as the position of the additional methyl from the 'δ' (carboxylic) end of the molecule.

### 2.5. Statistical analysis

A factorial correspondence analysis (FCA) was performed on the two-way table (Table 1) using the 11 culture conditions (columns) and the percentages of the 36 measured fatty acids (rows). This exploratory multivariate statistical method, suitable for the analysis of percentage tables [23], was chosen because it provides graphical displays of differences and similarities between columns and between rows in an orthonormal axis system. The axes (factors) were ordered by decreasing contribution to the inter-row or inter-column distances, the same axes being used, with different scales, for culture conditions (columns) and fatty acids (rows). The importance of each culture condition relative to changes in fatty acids could be assessed [23]. In the same way, the importance of each fatty acid in the differences between culture conditions could also be assessed. The FCA used a generalised Euclidian metric ( $\chi^2$ -metric) for calculating dissimilarities [23,24].

## 3. Results and discussion

In order to test the effects of the different culture conditions, we examined 11 different cultures (Table 1). The FAME percentage distribution of *P. nautica* grown under reference conditions (rich medium, 30°C, exponential phase and aerobiosis) is shown in Table 1 (culture 3). The main FAME were even-numbered saturated (SAFA) and monounsaturated (MUFA), in the range of C<sub>12</sub>–C<sub>19</sub> which are characteristic of bacteria [9]. High ratios of both unsaturated and even-numbered FA were observed (respectively, 65.8 and 92.1%). Odd-numbered and branched FA were also present but in relatively low contributions (respectively, 7.4 and 0.6%).

A previous fatty acid analysis of the marine strain *Pseudomonas perfectomarinus* showed as major compounds 16:0 (22.4%), 16:1 (24.2%), 18:1 (48.6%) [25]. In contrast, *P. nautica* major compounds were 16:0 (25.7%), 16:1ω9 (20.6%), 16:1ω7 (3.9%) and

18:1ω9 (36.4%). The major MUFA were ω9: 16:1ω9 (20.6%), 18:1ω9 (36.4%). Traces of 15:1ω9 (0.1%) and 20:1ω9 (0.1%) were also present. However, ω7 MUFA were also present, but at lower concentrations: 16:1ω7 (3.9%), 17:1ω7 (3.8%, which was the major odd MUFA), and 18:1ω7 (0.6%). The predominance of the ω9 double bonds and the high values found for 16:1ω9 and 18:1ω9 differ from previous results with other *Pseudomonas*, e.g. *Pseudomonas atlantica* [26] and *Pseudomonas putida* [27], both being dominated by ω7 double bonds with high concentrations of 16:1ω7 and 18:1ω7.

Some bacterial characteristic branched and hydroxylated FA [1] [3,15,28,29] were detected as traces, mainly i-14:0, i-15:0, i-17:0 and 2-OH 12:0. Cyclopropyl FA, which are characteristic of some bacteria [1,30], including some anaerobic bacteria [31], were absent.

### 3.1. Effects of culture conditions

Table 1 shows the variations in individual FA and on FA groups due to different culture conditions. Table 2 shows the effect of culture conditions on the FA composition of *P. nautica* in comparison to growth under reference conditions (culture 3). Negative values indicate fatty acid group decreased compared to control, and the opposite for positive values.

### 3.2. Effects of aerobiosis/anaerobiosis

Lack of oxygen (culture 10) had little effect on the overall FA composition (small decrease  $\approx$ 1% for unsaturated and odd-numbered fatty acids, Table 2). Nevertheless, there was a general decrease of C<sub>16</sub> fatty acids, particularly 16:1ω9 (from 20.6 to 12.8%) which coincided with a general increase of C<sub>18</sub> fatty acids, in particular 18:1ω9 which increased from 36.4 to 43.4% (Table 1).

### 3.3. Effects of growth phase

Significant changes in the overall FA composition occurred as a result of different growth phases (culture 11), with an increase in SAFA (+11.5%) and a corresponding decrease in MUFA (−12.2%). These changes were in agreement with previous results with

Table 1

Composition (% total fatty acids) of *P. nautica* fatty acids under the different culture conditions with culture 3 as reference.

Culture:	3	1	2	4	5	6	7	8	9	10	11
Carbon source:	R.M.	R.M.	R.M.	Acet.	Acet.	Acet.	<i>n</i> C <sub>20</sub>	<i>n</i> C <sub>20</sub>	<i>n</i> C <sub>20</sub>	R.M.	R.M.
Temperature:	30°C	13°C	20°C	13°C	20°C	30°C	13°C	20°C	30°C	30°C	30°C
Oxygen:	+O <sub>2</sub>	+O <sub>2</sub>	+O <sub>2</sub>	+O <sub>2</sub>	+O <sub>2</sub>	+O <sub>2</sub>	+O <sub>2</sub>	+O <sub>2</sub>	+O <sub>2</sub>	−O <sub>2</sub>	+O <sub>2</sub>
Phase:	Exp.	Exp.	Exp.	Exp.	Exp.	Exp.	Exp.	Exp.	Exp.	Exp.	Stat.
Fatty acid											
10:0	–	–	0.2	–	–	–	–	–	–	–	–
2-OH 10:0	0.1	–	–	–	–	–	–	–	–	–	–
12:0	0.1	0.2	0.1	–	0.6	0.2	0.2	0.1	0.1	0.3	0.1
i-13:0	0.1	–	0.1	–	–	–	–	–	–	–	–
13:0	0.1	0.1	0.5	–	–	–	–	–	0.1	1.4	0.1
2-OH 12:0	–	–	–	–	–	–	–	–	–	0.3	–
3-OH 12:0	–	–	–	–	1.0	–	–	–	–	–	–
i-14:0	0.1	–	–	–	–	–	–	–	–	–	0.1
14:1 $\omega$ 9	–	0.1	0.1	–	0.4	–	–	0.2	–	–	–
14:0	2.9	1.7	2.3	1.3	2.3	2.7	1.0	2.0	1.5	2.7	3.5
i-15:0	0.2	0.1	0.2	–	–	–	–	–	–	0.3	0.3
a-15:0	–	–	0.1	–	–	–	–	–	–	–	–
15:1 $\omega$ 9	0.1	0.4	0.3	–	–	–	1.1	0.1	0.1	0.3	0.1
15:0	1.2	0.9	1.2	–	–	0.1	0.7	0.6	0.4	0.9	1.4
16:1 $\omega$ 9	20.6	29.7	23.7	15.3	14.7	11.1	18.6	7.4	5.9	12.8	17.4
16:1 $\omega$ 7	3.9	10.4	4.9	11.6	5.2	1.8	5.3	5.9	5.7	3.8	1.5
16:1 $\omega$ 5	0.2	0.4	0.3	0.4	0.5	0.2	–	–	0.1	0.3	0.2
16:0	25.7	17.3	23.8	24.6	22.8	30.5	30.5	37.7	44.3	24.9	36.3
10Me 16:0	0.1	–	–	–	–	–	0.7	4.9	2.4	–	0.5
i-17:0	0.1	0.1	0.1	–	–	–	–	–	–	0.2	0.1
17:1 $\omega$ 9	–	0.1	0.1	–	–	–	0.9	0.4	–	0.1	0.1
17:1 $\omega$ 7	3.8	3.7	3.4	–	–	0.1	–	–	0.2	2.2	1.7
17:0	1.6	0.9	1.4	–	0.1	–	0.6	0.4	0.3	1.2	1.8
2-OH 16:0	–	0.2	0.1	–	–	–	–	–	–	–	–
10-Me17:0	–	–	–	–	–	–	–	0.1	–	–	0.1
18:2 $\omega$ 6,9	–	0.1	0.3	–	–	0.1	–	0.2	0.1	0.5	–
18:1 $\omega$ 9	36.4	31.8	34.2	41.0	49.3	49.4	35.6	32.2	33.2	43.4	31.4
18:1 $\omega$ 7	0.6	1.1	0.8	2.5	–	0.6	–	0.3	0.2	1.5	1.1
18:0	1.5	0.8	1.6	3.4	3.0	3.2	2.6	3.5	2.2	2.8	1.5
10Me 18:0	–	–	–	–	–	–	0.1	1.0	0.4	–	0.1
19:1 $\omega$ 9	–	–	0.1	–	–	–	–	0.1	–	–	–
19:0	0.1	–	–	–	–	–	–	–	–	–	0.2
20:1 $\omega$ 9	0.1	–	0.2	–	–	0.2	1.9	1.6	1.6	–	–
20:0	0.1	0.1	0.1	–	–	0.1	0.3	1.3	1.1	–	0.1
Saturated	33.4	21.9	31.1	29.3	28.9	36.6	35.9	45.6	50.1	34.2	44.9
Unsaturated	65.8	77.7	68.4	70.7	70.1	63.3	63.3	48.4	47.0	64.8	53.6
Branched	0.6	0.2	0.4	0.0	0.0	0.0	0.8	6.0	2.7	0.5	1.1
Odd	7.4	6.1	7.2	0.0	0.1	0.1	4.0	7.4	3.9	6.3	6.2
Even	92.1	93.6	92.5	100.0	98.9	99.8	96.0	92.6	96.0	92.9	93.2

R.M., rich medium; *n*C<sub>20</sub>, *n*-eicosane; acet., sodium acetate; Exp., exponential phase; Stat., stationary phase; +O<sub>2</sub> and −O<sub>2</sub>, respectively, oxic and anoxic conditions; –, not detected. Saturated,  $\Sigma$  saturated (normal) fatty acids % (FA %); Unsaturated,  $\Sigma$  unsaturated FA %; Branched,  $\Sigma$  branched FA %; Odd,  $\Sigma$  odd-numbered carbon FA %; Even,  $\Sigma$  even-numbered carbon FA %.

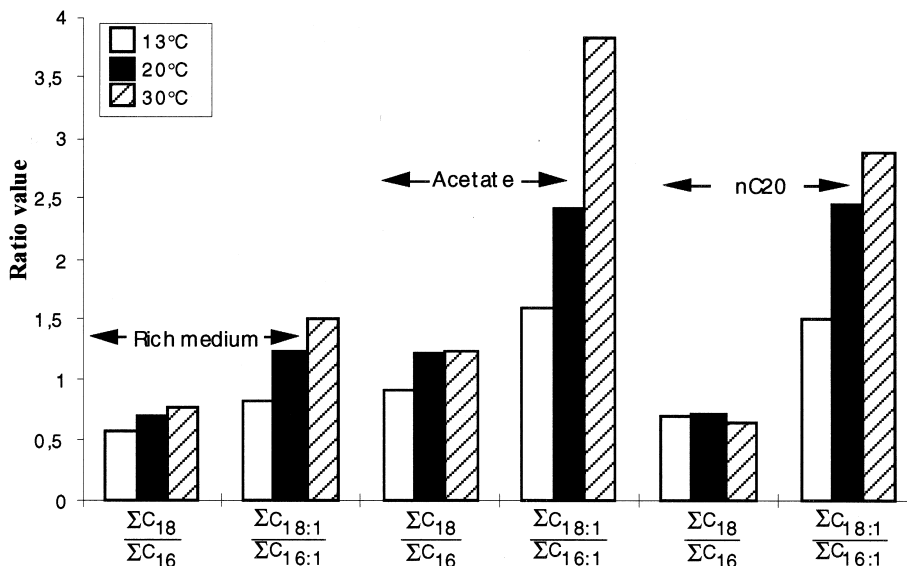


Fig. 1.  $\Sigma C_{18}/\Sigma C_{16}$  and  $\Sigma C_{18:1}/\Sigma C_{16:1}$  for *P. nautica* cultured at 13, 20 and 30°C with rich medium, acetate and *n*-eicosane as carbon source.

*Deleya halophila* [11] and *Leuconostoc oenos* [32]. The latter work showed a decrease of the unsaturated/saturated ratio when the cells had grown from early growth to their stationary phase (from 3.3 to 2.0 at pH 2.9 and from 1.9 to 1.4 at pH 3.3). A similar trend was observed for *P. nautica* with unsaturated/saturated ratio values of 2 in exponential phase and 1.2 in stationary phase.

### 3.4. Effects of growth temperature

The main effect of growth temperature on the FA composition of *P. nautica* was a significant increase ( $\approx +12\%$ ) in MUFA when the temperature decreased from 30 to 13°C (culture 1, Table 2). This trend was irrespective of the carbon source (Table 1), and coincided with a significant decrease in SAFA (Tables 1 and 2). These observations were in good agreement with previous research related to maintenance of membrane viscosity [10,11,25,33–35].

Additionally, the increased amounts of 16:1 MUFA coincided with the decreasing amounts of 18:1 MUFA (Table 1). This was previously reported for *Vibrio marinus* cultured at 25 and 15°C [25]. For *P. nautica* (cultured on rich medium), the observed changes for 16:1 and for 18:1 FA ranged from 25 to 40% and from 37 to 33%, respectively, with temper-

ature conditions varying from 30 to 13°C. This also might be explained by maintenance of membrane viscosity. This variation in FA chain length may be regarded as an alternate response to control membrane viscosity with change in temperature. Previous work [36] showed similar trends for the barotolerant strain RS 103 cultured at 40 and 20°C: the overall unsaturation increased, and while the 16:1 increased significantly (8–32%), the 18:1 remained comparatively stable (8–10%).

For *P. nautica* cultured on acetate and on rich medium, there was a systematic increase in MUFA when the temperature decreased and this corresponded to a decrease of the  $\Sigma C_{18}/\Sigma C_{16}$  ratio (which is a good measure of the mean acyl chain length [37] (Fig. 1). Of particular interest was the comparison of this ratio with  $C_{18:1}/C_{16:1}$  which showed the greatest changes (Fig. 1). This seemed to indicate, for *P. nautica*, a mean acyl chain length thermoregulation process focused on  $C_{16}$  unsaturated FA.

### 3.5. Effects of carbon source

Previous analysis [38] of the total lipids of *P. nautica* cultured on acetate and *n*-eicosane were 86 and 132 mg lipids per mg cell protein, respectively, was in agreement with other results [13] showing increasing

lipid content with *n*-alkane growth. Thus the substrate appeared to be a very important ‘controlling factor’ for the FA composition of *P. nautica*. This was confirmed, in Table 2, with the highest FA variations occurring with growth on *n*-eicosane. The highest variations for saturated (+16.7%), unsaturated (−18.8%) and branched (+2.1%) FA were observed (culture 9). For odd and even FA, culture with acetate produced the highest variations (respectively, −7.3 and 7.7%).

With the rich medium substrate as reference, growth on sodium acetate resulted in a drastic simplification of the FA pattern (culture 6, Table 1). No odd and branched FA were synthesized. Previous studies [39,40] showed significant levels of odd and branched FA in various bacteria (*Vibrio* sp., *Rhodothermus marinus*) cultured on complex media (soy broth, yeast extract and tryptone). These FA decreased and were replaced by even-numbered normal FA when the strains were cultured with ‘minimum media’ (1 g l<sup>−1</sup> of glucose as carbon source). A strong domination (≈100%) by even-numbered FA at the expense of the odd- and branched FA in this minimal medium indicated that acetyl-coenzyme A, derived from the central metabolic pathway, served almost exclusively as the chain initiator in fatty acid biosynthesis. These results were similar to those for *P. nautica* grown on acetate (Table 1, cultures 4–6).

Culture with *n*-eicosane (culture 9, Table 1) gave rise to a FA pattern very different from those with acetate or rich medium at 30°C and under aerobic conditions. The main changes were: (1) a maximum amount of SAFA (≈50%), mainly because of the significant increase in 16:0 (≈44%); (2) a maximum amount of BFA (≈2.7%), mainly due to the pres-

ence of the 10Me16:0 (≈2.4%); and (3) a marked increase in 20-carbon acids (20:1ω9 and 20:0) which accounted for 2.7% of the total FA. These trends were in good agreement with previous works [13,14,41–43] which showed the direct incorporation in cellular lipids of monoterminally oxidized products (parent acids) and β-oxidation products.

Additionally, we noted the presence of two Δ<sup>10</sup> methyl branched saturated FA (10Me16:0 ≫ 10Me18:0) in maximum amounts when *n*-alkane is the carbon source (culture 9, Table 1). These results were similar for *P. nautica* cultured with *n*C<sub>20</sub> at 13 and 20°C (Table 1, cultures 7 and 8). In addition, 10Me17:0 was present at 20°C (culture 8, Table 1).

10Me16:0 was reported as characteristic of *Desulfobacter* sp. [44–46] although this has been questioned by other workers [8,47]. Indeed, Δ<sup>10</sup> series were reported in several marine microorganisms as *Actinomycetes* sp. [43]. The appearance of 10Me16:0 in significant amounts for *P. nautica* cultured with *n*C<sub>20</sub> (4.9% at 20°C) was in agreement with previous observations of this compound in alkane-utilising bacteria [6,42].

An *n*-alkane-utilisation index ( $[\Sigma \text{ saturated FA} + \Sigma \text{ branched FA}] / [\Sigma \text{ unsaturated FA}]$ ) enabled discrimination between soluble carbon growth substrates and the *n*-alkane, values were 0.5 for rich medium, 0.6 for acetate and 1.1 for *n*C<sub>20</sub> (respectively, cultures 3, 6 and 9, Table 1).

### 3.6. Statistical analysis

It was difficult to distinguish general patterns from Tables 1 and 2. However, fatty acid profiles seemed to be closely controlled by temperature and/or growth medium. In order to understand the respec-

Table 2  
Effect of the culture conditions on the fatty acid composition of *P. nautica* (difference %) compared to reference conditions

FA (%)	Oxygen	Growth phase	Temperature	Carbon source	
	(10-3)	(11-3)	(1-3)	Acet. (6-3)	<i>n</i> C <sub>20</sub> (9-3)
Saturated	0.7	11.5	−11.5	3.2	16.7
Unsaturated	−1.0	−12.2	11.9	−2.5	−18.8
Branched	−0.1	0.5	−0.4	−0.6	2.1
Odd	−1.1	−1.2	−1.3	−7.3	−3.5
Even	0.8	1.1	1.4	7.7	3.9

*n*C<sub>20</sub>, *n*-eicosane; acet., sodium acetate; Saturated, Σ saturated (normal) fatty acids % (FA %); Unsaturated, Σ unsaturated FA %; Branched, Σ branched FA %; Odd, Σ odd-numbered carbon FA %; Even, Σ even-numbered carbon FA %. Refer to Table 1 for culture conditions.



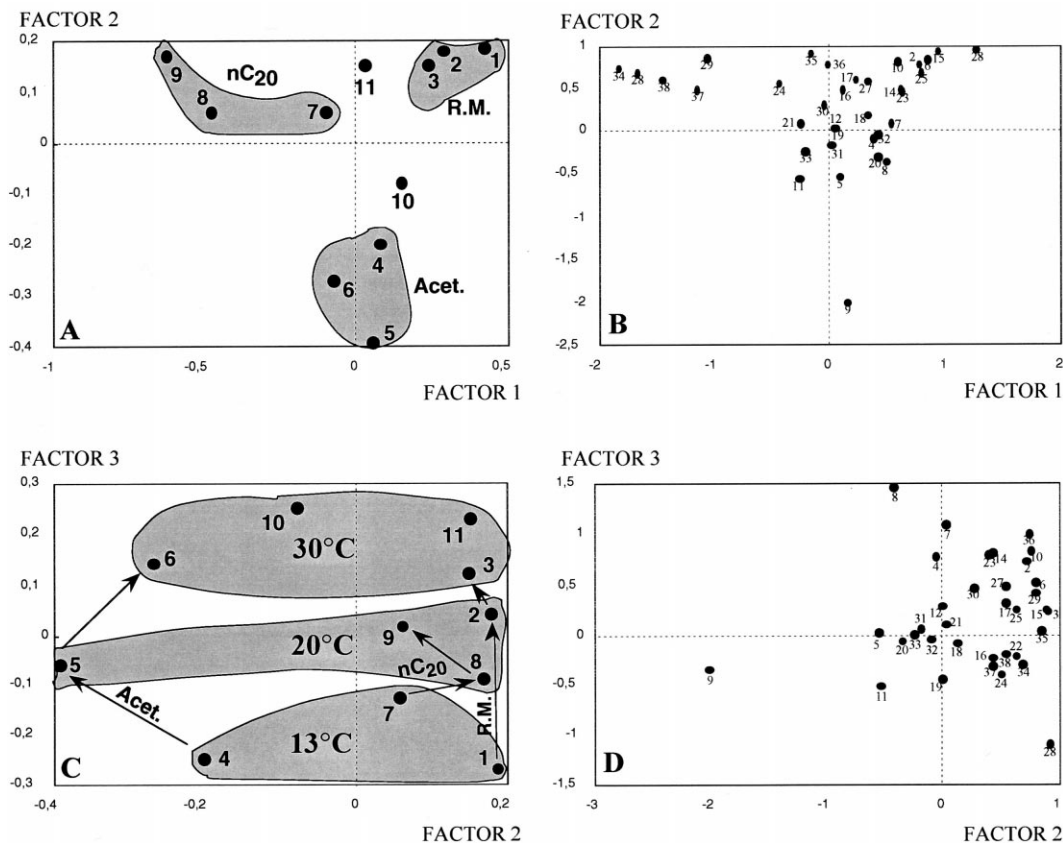


Fig. 2. Correspondence factorial analysis projection plots of culture conditions (A,C) and FAME (B,D) in planes F1, F2 and F2, F3 (F1, F2 and F3 are the first three factors accounting for 74.7% of the total variability). Attributions for cultures and FAME are given in Table 1.

tive importance and the hierarchy of the different culture conditions, we applied an FCA to the data matrix shown in Table 1. This enabled the identification of three factors accounting for 74.7% of the total variability (Fig. 2). The culture conditions (Fig. 2A,C) and the fatty acids profiles (Fig. 2B,D) were represented on separate plots in order to facilitate interpretation.

Factor 1 accounted for 42.7% of the differences in fatty acid profiles or in culture conditions. It allowed the differentiation of rich medium experiments (13, 20 and 30°C) and *n*-eicosane experiments (Fig. 2A). The rich media were well grouped (except cultures 10 and 11) and were located at the positive extremity of this factor. The corresponding loading plot for FAME (Fig. 2B) showed that 2-OH 16:0, *a*-15:0, 10:0, *i*-13:0, 17:1 $\omega$ 7, *i*-17:0, *i*-14:0 (in decreasing or-

der of absolute contributions) were characteristic of these culture conditions. This means that FA profiles for rich medium experiments were enriched with these compounds. The *n*-eicosane cultures, were more scattered than those for rich medium and were located at the opposite side of factor 1. These culture conditions were well described by the  $\Delta^{10}$  methyl branched series with mainly the 10Me16:0 and 10Me18:0 and the  $C_{20}$  FAs (20:1 $\omega$ 9 and 20:0). These results showed that factor 1 was clearly substrate-dependent.

Factor 2 accounted for 18.5% of the total variability and allowed discrimination between rich media and *n*-eicosane experiments and the acetate experiments. These experiments were located in the lower (negative) part of the factor 2 (Fig. 2A). These culture conditions were enriched in 3-OH 12:0, 12:0,

14:1 $\omega$ 9 and 18:0. Factor 2 was also growth-substrate-dependent.

Factor 3 accounted for 13.5% of the total variability and showed the effect of temperature (Fig. 2C). The 13°C experiments were present in the lower part of the third factorial axis, whereas the 30°C experiments were in the opposite end. For each medium, the cultures were ordered on the basis of the increasing temperatures (see arrows in Fig. 2C). Factor 3 was clearly temperature-dependent. However, the temperature effects were also dependent on the carbon source. For example, *n*C<sub>20</sub> cultures were less temperature-sensitive than acetate cultures.

The fatty acid profiles at 13°C were essentially characterised by high proportions of 16:1 $\omega$ 7 and 16:1 $\omega$ 9 and low proportions of 14:0. These proportions were inverted at 30°C. The cultures either anaerobic grown or stationary phase cells (cultures 10 and 11) were located in the 30°C zone, showing a predominance of the temperature effect. For anaerobic culture (culture 10, Table 1) 2-OH12:0 and 13:0 seemed to be the most characteristic FA. Finally, the stationary phase culture was similar to the reference culture (culture 3, Table 1).

#### 4. Summary

Factorial correspondence analysis demonstrated that it is the carbon source (up to 62% of the total variability) which appeared to dominate the changes of FA composition of *P. nautica*. Culture temperature, with 13.5% of the total variability had less effect, and growth phase and oxygen were the least significant controlling factors. With regard to the effect of temperature, *P. nautica* showed a thermoregulation process, related to maintenance of membrane viscosity, consisting in regulation of the mean acyl chain length with the C<sub>16</sub> unsaturated fatty acids. *P. nautica* grown on *n*-eicosane gave rise to distinct FA profiles, generally characterised by high amounts of saturated and branched FA and the presence of C<sub>20</sub> acids (20:1 $\omega$ 9 and 20:0) and a  $\Delta$ <sup>10</sup> methyl branched series (largely 10Me16:0).

The reported results revealed a striking effect of the carbon source. This point is of major importance when considering hydrocarbon contaminated environments. Indeed we may reasonably expect that

the bacterial fatty acid composition could reveal the presence of hydrocarbonoclastic bacterial communities in marine sediments exposed to hydrocarbons and thus could be used as a bioremediation index. The study of such developments is currently in progress.

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