Fatty acid composition of the estuarine *Flexibacter* sp. strain Inp: effect of salinity, temperature and carbon source for growth

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(Received 2 October 1990; revised 25 January 1991; accepted 6 March 1991)

The total fatty acid content of the estuarine *Flexibacter* sp. strain Inp and the relative proportions of its constituent fatty acids were affected by growth temperature and salinity. Whilst both the proportion and concentration of the polyunsaturates were markedly stimulated by increases in salinity, the total amount of fatty acid per mg cell protein decreased. The highest concentration of fatty acid per mg cell protein did not coincide with the highest percentage of polyunsaturated fatty acids, which occurred when the bacterium was grown on glucose. The presence of an inverse relationship between C16:1w5 and C18:1w9 are regarded as evidence that two different pathways exist for the biosynthesis of unsaturated fatty acids in *Flexibacter* strain Inp.

Introduction

Changes in temperature, salt concentration and carbon source in the medium are known to produce variations in the membrane lipids of bacteria (Marr & Ingraham, 1962; Okuyama *et al.*, 1977; Kawagushi & Seyama, 1984; Russell & Kogut, 1985; Kates, 1986a; Rose, 1989). Variations in fatty acid composition due to changes in temperature and salinity differ between Gram-negative and Gram-positive bacteria. For example, lowering the growth temperature of the Gram-positive halotolerant *Planococcus* sp., results in increased amounts of branched and monounsaturated fatty acids (MUFAs), while the concentration of aC15:0 increases as the salt content is raised (Miller, 1985, 1986). In *Deleya halophila*, a moderately halophilic, Gram-negative bacterium, there is a decrease in MUFAs with a concomitant increase in cyclopropane fatty acids as the salt concentration in the medium is raised (Monteoliva-Sanchez *et al.*, 1988).

Reports on the fatty acid composition of members of the genus *Flexibacter* vary. Fautz *et al.* (1979) and Poen *et al.* (1984) reported branched as well as hydroxy fatty acids to be the main components. Nichols *et al.* (1986), working with several species, reported monounsaturated and branched fatty acids as the most important groups,

and Johns & Perry (1977) reported the presence of high amounts of long chain polyunsaturated fatty acids (PUFAs) in *F. polymorphus*.

PUFAs have been considered to be absent from bacteria (Shaw, 1974). However, recent work on bacteria from the marine environment has indicated that PUFAs may be more widespread than thought hitherto. Delong & Yayanos (1986) and Wirsen *et al.* (1987) examined a number of deep-sea bacteria and found significant amounts of PUFAs, as did Johns & Perry (1977) working with the marine species *F. polymorphus*. More recently, Yazawa *et al.* (1988) showed that 1.6% of isolates from fish intestines produced C20:5w3; in one strain, which was similar to the genus *Alteromonas*, this compound comprised 36% of the total fatty acids. The ecological role played by PUFA-producing bacteria in the sea remains obscure, but it is possible that they contribute to the PUFA intake of larval forms including commercially important crustacean species.

The bacterium *Flexibacter* sp. strain Inp, used in this study, is widely distributed in the Ecuadorian shrimp ponds from which it was isolated. These ponds are subjected to wide variations in temperature and salinity. *Flexibacter* strain Inp is orange pigmented and forms filamentous threads with spreading growth on plates; gliding is not very active. It can utilize nitrate and glutamate as nitrogen sources and peptone can be used as both carbon and nitrogen source. It can degrade gelatin and starch, but not agar or alginate. It is sensitive to lauryl sulphate, does not survive in fresh water, but grows on sea water media, tolerating up to 120 p.p.t. salinity. It

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Abbreviations: FAME, fatty acid methyl ester; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; p.p.t., parts per thousand (salinity).

is catalase- and oxidase-positive. Reichenbach (1989), in the most-recent edition of *Bergey's Manual of Systematic Bacteriology*, suggests that marine *Flexibacter*-like strains should be considered as members of the genus *Microscilla*.

Methods

Culture techniques. Cultures were maintained at 25 °C on ZoBell 2216-E solid medium (Oppenheimer & ZoBell, 1952). Before starting each experiment a subculture, taken from a 1- or 2-weeks-old ZoBell culture plate, was suspended in the medium to be tested; with the exception of the carbon source, this medium consisted of 2 g ammonium sulphate, 0.2 g dipotassium hydrogen phosphate, 0.5 g yeast extract, 0.5 g Tris/HCl and 1 ml of trace metal solution (Reichenbach & Dworkin, 1981) in 1 litre of sea water of salinity 30 p.p.t., adjusted to pH 7.6. Cycloheximide (0.1 g l⁻¹) was used to inhibit growth of eukaryotes. Since sea water contains a considerable number of different ions, salinities are quoted in the oceanographic sense, as parts per thousand (p.p.t.) (Riley & Chester, 1971). Salinities were measured with an inductive salinometer.

Temperature experiments. The temperatures tested were 18, 24 and 30 °C. Experiments were done in 250 ml flasks with 50 ml of culture medium and 5 ml of inoculum. Cultures were bubbled with 200:10 ml of an air/nitrogen mixture min⁻¹. The flasks were immersed within a water/bath controlled at the appropriate temperature.

Salinity relationships. Experiments were done using either starch or ZoBell 2216-E medium as carbon source, at 15, 30, 45 and 60 p.p.t., or 30, 45, 60 and 120 p.p.t., respectively. Cultures were bubbled with 100:20 ml of an air/nitrogen mixture min⁻¹, and incubated at 24 °C in a water-bath. Salinity was decreased by mixing the sea-water medium (salinity 30 p.p.t.) with distilled water, or increased by adding the mixture of marine salts described in Reichenbach and Dworkin (1981).

Inclusion of betaine (10 mM) in the medium was necessary to obtain growth of strain Inp at high salinities (45, 60 and 120 p.p.t.). Betaine and related compounds are known to promote growth of bacteria in media of high osmotic strength (Le Rudulier & Bouillard, 1983; Imhoff & Rodriguez-Valera, 1984; Le Rudulier *et al.*, 1984).

Carbon sources. A mixture of sterile air/nitrogen (100:20) was used to aerate the cultures. The salinity of the medium was 30 p.p.t. and the temperature was 24 °C. Except for the carbon source and the exclusion of yeast extract, the medium used was as described above. The carbon sources and their concentrations were as follows: starch, glucose, lactose, gluconate and Casamino acids (5.0 g l⁻¹); glycerol (3.9 ml l⁻¹); Tween 80 (12.6 ml l⁻¹). Except for Casamino acids and Tween 80, these values were chosen to normalize the carbon:nitrogen ratio of the medium to 5:1. ZoBell 2216-E medium diluted to half-strength (1/2-ZoBell) by the addition of sea water was also used.

Cultures were harvested during the late exponential phase, which ranged from 25 to 50 h depending on temperature, salinity and the carbon source used, by centrifugation at 10000 g for 15 min at 10 °C; the bacteria were then washed once in NaCl (0.5 M) and centrifuged again. The bacterial pellet was resuspended in 10 ml of potassium phosphate buffer (50 mM, pH 7.5) (White *et al.*, 1979) and stored at -20 °C.

Lipid extraction. Lipids were extracted by adding enough chloroform/methanol (1:2, v/v) to a thawed cell pellet to obtain a single phase chloroform/methanol/phosphate buffer mixture (1:2:0.8, by vol.). This extract was left at 4 °C overnight, then water and chloroform were

added to form two separate phases (Bligh & Dyer, 1959; White *et al.*, 1979). The total cell fatty acids, present in the lower layer, were extracted using a technique based on that of Moss *et al.* (1974) and Larsson & Odham (1984). The lipid extract was dried under a stream of nitrogen and then saponified with either 5% (w/v) NaOH or KOH in 50% (v/v) methanol at 100 °C for 45 min. The methanolysates were cooled, and adjusted to pH 2 with HCl (6 M). Chloroform (1 ml) was added and the mixture was shaken vigorously and allowed to separate into two phases. The upper phase was discarded and the lower fatty-acid-containing phase was dried under a stream of nitrogen. The fatty acids were esterified with 2 ml 10% (v/v) BCl₃ in methanol at 80 °C for 10 min. After cooling, the fatty acids methyl esters (FAMES) were extracted by adding 1 ml saturated NaCl and 2 ml hexane/chloroform (4:1, v/v). The extract was evaporated under a stream of nitrogen, redissolved in chloroform and spotted on to a TLC plate (precoated silica gel 60, Merck) to separate FAMES from other reaction products. The plates were developed for 90 min in a continuous elution tank using hexane/diethyl ether (90:10, v/v) as the solvent system. FAMES were located using iodine vapour and areas of silica gel containing FAMES were scraped from the plates and the FAMES dissolved in chloroform/methanol (2:1, v/v). The solvent was evaporated to dryness and the FAMES were redissolved in 200 µl hexane.

FAMES were analysed using a Carlo Erba gas chromatograph (model Vega 6180) with on-column injection into a 30 m × 0.25 mm i.d. DB-225 fused silica column (J & W Scientific) or an Econo-cap, Carbowax 30 m × 0.25 mm i.d. capillary column (Alltech). Hydrogen was the carrier gas (2.0 ml min⁻¹). The oven temperature was programmed to rise from 50 to 150 °C at a ramp rate of 49.9 centigrade degrees min⁻¹ and then 3 centigrade degrees min⁻¹ to 230 °C, and held for 10 min. The temperature was 250 °C. Uniform response was assumed for all components. Quantification was based on comparison of peaks areas with an internal standard (C23:0). FAMES were identified by comparison of their retention times with known standards. The identity of PUFAs was confirmed by GC/MS using a Finnigan gas chromatograph/mass spectrometer fitted with a 30 m × 0.25 mm i.d. OV1 capillary column, and with helium as the carrier gas. Samples were injected in splitless mode at 180 °C. The oven temperature was programmed to rise from 60 to 280 °C at a ramp rate of 6 centigrade degrees min⁻¹. The electron impact ionization voltage was 70 eV. Hydrogenation was also used to confirm the presence of double bonds (Kates, 1986b), whose position was determined by MS analysis of the Diels-Alder adducts, formed by reacting FAMES with 5,5-dimethoxy-1,2,3,4-tetrachlorocyclopentadiene (Aldrich) (Kidwell & Bieman, 1982; Nichols *et al.*, 1985).

Protein. Protein was determined by a modification of the method of Lowry, after extracting the cells in NaOH (1 M) (Hanson & Phillips, 1981). Bovine serum albumin was the standard used.

Chemicals. All reagents used were analytical grade (Sigma and BDH). Solvents were from Rathburn.

Statistics. Analyses were done using the Minitab statistical package on a Vax mainframe computer.

Results

Gas chromatography/mass spectrometry

The mass spectra of commercial standards of linoleic and linolenic acid were found to be identical to that of the compounds found in *Flexibacter* strain Inp, and identified as linoleic and linolenic acids from their retention times. The ion fragments of both the C16:1w5 and

Fig. 1

Fig. 1. Mass spectrum of the Diels-Alder adducts of C16:1w5.

Fig. 2

Fig. 2. Mass spectrum of the Diels-Alder adducts of C18:1w9.

Table 1. Fatty acid composition of strain Inp after growth at different temperatures

The composition is expressed as a weight percentage of the total fatty acids. The results presented are means of two experiments at each temperature; values in parentheses represent ± 1 SD.

Fatty acid	Growth temp. (°C)	Fatty acid composition (%)		
		18	24	30
14:0		0.53 (0.55)	1.15 (0.88)	1.69 (0.05)
15:0		3.09 (2.02)	5.00 (1.48)	8.68 (2.98)
15:1		0.29 (0.07)	0.16 (0.21)	0.93 (1.30)
16:0		0.49 (0.08)	0.26 (0.36)	0.25 (0.35)
16:1		11.96 (2.97)	14.92 (4.70)	18.40 (1.85)
16:1w9		3.03 (0.70)	1.58 (2.23)	0.03 (0.04)
16:1w7		—	0.41 (0.57)	0.70 (0.98)
16:1w5		53.80 (2.70)	48.10 (0.33)	49.01 (0.00)
Unknown		1.00 (1.42)	0.39 (0.55)	1.26 (1.74)
Unknown		0.49 (0.21)	0.06 (0.08)	0.10 (0.15)
17:0		0.40 (0.09)	0.06 (0.07)	1.06 (0.98)
18:0		2.74 (1.00)	2.50 (0.96)	4.50 (2.18)
18:1w9		9.40 (1.54)	10.40 (0.39)	5.82 (1.74)
18:1w7		3.26 (3.90)	0.98 (1.40)	1.15 (1.62)
18:2w6		8.72 (2.51)	11.74 (1.10)	4.69 (1.96)
18:3w3		0.45 (0.35)	1.07 (0.30)	0.14 (0.20)
Total fatty acid content [$\mu\text{g (mg protein)}^{-1}$]		56.6 (4.6)	70.8 (5.4)	158 (22)
Percentage unsaturation		78.2 (1.8)	74.3 (5.5)	61.5 (3.7)
Unsaturation index*		0.88 (0.04)	0.88 (0.07)	0.66 (0.03)

* Unsaturation index = [(% monounsaturates) + 2(% diunsaturates) + 3(% triunsaturates)]/100.

C18:1w9 Diels-Alder adducts confirmed unequivocally the identification of the two main monounsaturated fatty acids in strain Inp (Figs. 1 and 2).

Effect of temperature

As shown in Table 1, C16:1w5 was the major fatty acid found under all conditions, ranging from 48.1 to 67.7% of

the total fatty acid content. Palmitic acid (C16:0) also formed a significant fraction of the total. Oleic acid (C18:1w9), and linoleic acid (C18:2w6) were next in order of abundance. The PUFAs linoleic (C18:2w6) and linolenic acid (C18:3w3) ranged from 4.7 to 11.7% and 0.1 to 1.1% respectively. The degree of unsaturation, measured as percentage unsaturation and unsaturation index, tended to decrease with increasing temperature (Table 1).

Table 2. *Fatty acid composition of strain Inp after growth at different salinities on starch as carbon source*

The composition is expressed as a weight percentage of the total fatty acids. The results presented are means of three experiments at each salinity; values in parentheses represent ± 1 SD.

Fatty acid	Salinity (p.p.t.) . . . 15	Fatty acid composition (%)			
		30	45	60	
14:0	1.00 (0.26)	0.69 (0.16)	0.67 (0.19)	0.60 (0.20)	
i15:0	9.31 (1.70)	3.72 (0.50)	3.26 (0.57)	1.27 (0.41)	
a15:0	2.14 (1.50)	2.76 (0.50)	1.32 (0.53)	0.33 (0.36)	
15:0	0.39 (0.12)	0.19 (0.01)	0.29 (0.30)	0.22 (0.09)	
16:0	8.57 (2.15)	7.22 (2.01)	9.99 (2.23)	10.40 (3.63)	
16:1w9	1.36 (0.40)	2.26 (1.79)	2.14 (2.33)	2.60 (2.27)	
16:1w7	2.56 (2.23)	5.22 (2.96)	1.50 (1.01)	5.12 (3.70)	
16:1w5	65.30 (2.62)	69.00 (1.10)	44.07 (5.55)	19.01 (2.88)	
Unknown	0.32 (0.55)	—	0.21 (0.38)	0.23 (0.39)	
Unknown	0.20 (0.34)	0.23 (0.28)	0.05 (0.09)	—	
17:0	—	—	0.08 (0.14)	0.44 (0.38)	
18:0	0.39 (0.14)	0.26 (0.08)	1.84 (0.88)	3.52 (1.74)	
18:1w9	2.68 (0.29)	2.09 (1.07)	13.88 (4.13)	26.70 (3.06)	
18:1w7	0.55 (0.05)	0.73 (0.24)	6.38 (2.14)	6.30 (1.71)	
18:2w6	2.23 (0.58)	2.30 (0.93)	11.44 (0.88)	20.45 (3.90)	
18:3w3	0.31 (0.09)	0.20 (0.08)	0.57 (0.41)	1.90 (0.70)	
Total fatty acid content [$\mu\text{g (mg protein)}^{-1}$] . . .	119.6 (10.0)	81.28 (23.0)	38.5 (10.3)	31.6 (3.08)	

Table 3. *Fatty acid composition of strain Inp after growth at different salinities on 1/2-ZoBell medium*

The composition is expressed as a weight percentage of the total fatty acids. The results presented are means of three experiments at each salinity; values in parentheses represent ± 1 SD.

Fatty acid	Salinity (p.p.t.) . . . 30	Fatty acid composition (%)			
		45	60	120	
14:0	1.18 (0.06)	1.25 (0.04)	1.54 (0.22)	0.79 (0.08)	
i15:0	17.67 (0.96)	20.95 (1.58)	14.20 (1.40)	0.98 (0.34)	
a15:0	0.29 (0.07)	0.33 (0.10)	0.20 (0.11)	0.10 (0.10)	
15:0	0.67 (0.00)	0.58 (0.04)	0.55 (0.03)	0.11 (0.10)	
15:1*	1.93 (1.72)	1.38 (0.12)	0.72 (0.08)	0.14 (0.13)	
16:0	4.18 (0.40)	4.11 (1.58)	11.80 (0.61)	22.99 (1.54)	
16:1w9	0.87 (0.37)	1.31 (0.68)	1.58 (1.61)	0.19 (0.16)	
16:1w7	0.37 (0.21)	0.15 (0.25)	0.22 (0.22)	0.35 (0.31)	
16:1w5	55.49 (2.50)	49.90 (2.92)	53.79 (2.64)	4.75 (2.69)	
Unknown	0.44 (0.03)	0.24 (0.09)	0.14 (0.05)	—	
Unknown	0.81 (0.02)	1.57 (0.10)	1.56 (0.11)	—	
Unknown	0.94 (0.04)	0.86 (0.18)	0.27 (0.03)	0.08 (0.14)	
17:0	0.09 (0.09)	0.15 (0.08)	0.20 (0.02)	0.17 (0.15)	
18:0	0.78 (0.12)	1.08 (0.58)	1.50 (0.28)	5.68 (0.41)	
18:1w9c	5.88 (1.10)	6.04 (0.92)	4.57 (0.42)	27.14 (4.17)	
18:1w9t	0.81 (0.27)	0.95 (0.22)	0.70 (0.11)	4.16 (0.30)	
18:1w7	0.66 (0.33)	0.80 (0.11)	0.47 (0.05)	3.53 (0.64)	
18:2w6	5.06 (1.09)	4.91 (0.53)	3.63 (0.10)	22.35 (3.52)	
18:3w3	0.47 (0.09)	0.47 (0.10)	0.70 (0.11)	2.01 (0.50)	
Total fatty acid content [$\mu\text{g (mg protein)}^{-1}$] . . .	56.7 (17.9)	34.7 (2.50)	30.9 (9.66)	7.7 (0.02)	

* Double bond position not determined.

Table 4. Fatty acid composition of strain *Inp* after growth on different carbon sources

The composition is expressed as a weight percentage of the total fatty acids. The results presented are means of three experiments (for Casamino acids, Tween 80, glycerol, glucose and starch) or of two experiments (for lactose and gluconate); values in parentheses represent ± 1 SD.

Fatty acid	Carbon source . . .	Fatty acid composition (%)						
		Casamino acids	Tween 80	Glycerol	Glucose	Starch	Lactose	Gluconate
14:0		1.09 (0.04)	3.30 (0.49)	1.27 (0.09)	0.55 (0.02)	1.76 (0.23)	0.54 (0.00)	1.18 (0.06)
i15:0		12.47 (1.24)	3.17 (0.45)	10.81 (1.42)	3.34 (0.37)	8.5 (0.92)	3.34 (0.68)	8.24 (0.36)
a15:0		0.52 (0.16)	0.29 (0.01)	0.65 (0.15)	0.11 (0.1)	0.78 (0.33)	0.16 (0.05)	1.37 (0.60)
15:0		0.66 (0.03)	0.78 (0.08)	1.00 (0.26)	tr	0.60 (0.05)	0.19 (0.05)	2.40 (0.55)
15:1*		0.68 (0.01)	0.20 (0.20)	0.75 (0.32)	0.11 (0.19)	0.30 (0.04)	—	1.05 (0.10)
16:0		6.43 (0.10)	19.17 (2.70)	7.69 (0.78)	18.02 (2.26)	13.77 (2.47)	20.40 (0.23)	12.42 (1.07)
16:1w9		2.86 (1.49)	1.44 (0.18)	1.55 (0.60)	0.65 (0.27)	—	0.13 (0.18)	3.10 (2.25)
16:1w7		0.50 (0.20)	4.60 (0.37)	0.48 (0.10)	0.11 (0.19)	3.48 (1.92)	0.23 (0.18)	—
16:1w5		35.18 (1.80)	17.90 (3.94)	34.61 (0.72)	30.11 (2.5)	60.15 (2.85)	59.94 (2.56)	57.62 (2.32)
Unknown		1.79 (0.54)	1.02 (0.22)	0.65 (0.27)	0.51 (0.07)	0.53 (0.47)	—	0.35 (0.02)
Unknown		0.99 (0.18)	0.08 (0.13)	1.00 (0.71)	—	0.51 (0.11)	—	1.51 (0.12)
Unknown		0.74 (0.07)	0.50 (0.07)	0.80 (0.41)	—	0.30 (0.07)	—	0.47 (0.08)
i17:0		—	—	0.27 (0.00)	—	—	—	0.44 (0.10)
a17:0		0.14 (0.12)	—	—	—	—	—	—
17:0		0.17 (0.16)	0.44 (0.10)	0.03 (0.06)	0.07 (0.11)	0.08 (0.13)	—	—
18:0		1.82 (0.09)	4.65 (1.97)	1.88 (0.40)	3.23 (1.11)	0.78 (0.17)	1.08 (0.25)	0.83 (0.08)
18:1w9c		12.13 (1.06)	29.03 (3.96)	14.92 (2.80)	16.08 (1.16)	1.72 (1.18)	4.40 (1.77)	3.31 (0.12)
18:1w9t		1.93 (0.07)	—	1.98 (0.34)	2.25 (0.89)	—	0.72 (0.27)	0.54 (0.02)
18:1w7		1.52 (0.17)	4.02 (0.86)	1.97 (0.29)	2.60 (0.40)	0.17 (0.07)	0.61 (0.20)	0.51 (0.00)
18:2w6		10.53 (0.17)	1.42 (0.25)	12.07 (1.47)	17.77 (1.26)	1.14 (0.70)	2.77 (0.10)	3.11 (0.25)
18:3w3		0.97 (0.08)	—	1.14 (0.15)	2.25 (0.89)	0.22 (0.03)	0.22 (0.05)	0.24 (0.07)
Total fatty acid content [μg (mg protein) ⁻¹] . . .		40.0 (6.24)	43.6 (15.2)	64.8 (10.1)	43.9 (0.50)	78.2 (3.50)	64.5 (1.36)	52.8 (13.1)

* Double bond position not determined.

Effect of salinity

Increase in salinity was associated with a marked decrease in the total fatty acid content per mg cell protein (Tables 2 and 3). Both iC15:0 and C16:1w5 decreased markedly as salinity increased, while C18:1w9 and PUFAs increased. Linoleic and linolenic acid ranged from 2.2 to 22.4% and 0.2 to 2.0% respectively. Analysis of variance showed that both the proportions and the concentrations of the major fatty acids were significantly different over the range of salinities tested ($P > 0.1$). Increasing the salinity from 30 to 120 p.p.t. in 1/2-ZoBell medium resulted in a marked decrease in iC15:0 with a concomitant increase in C16:0. The percentage of C16:1w5, the major fatty acid in strain *Inp*, was constant at salinities from 30 to 60 p.p.t., but at 120 p.p.t. it decreased from about 60% to 4%, whereas both oleic acid and PUFAs increased markedly.

Effect of carbon source

The highest concentration of fatty acid per mg cell protein was found in cells grown on starch (Table 4) followed by cells grown on gluconate or glycerol.

Both the highest concentration per mg cell protein and the highest percentage of PUFAs were found in cells grown on glucose, glycerol or Casamino acids (Table 4). iC15:0, the main branched fatty acid present in strain *Inp*, was found in especially high concentrations in cells grown on Casamino acids. The concentration and proportion of C16:1w5 were inversely proportional to those of PUFAs. The fatty acid composition of strain *Inp* grown on Tween 80 was influenced by the presence of the dominant fatty acid in the growth medium.

Discussion

In general, the fatty acid composition of *Flexibacter* strain *Inp* resembles that found by Nichols *et al.* (1986) for several *Flexibacter* species. These authors found that iC15:0, C16:0 and MUFAs with carbon chain lengths of either 16 or 18 comprised the bulk of the fatty acids. The position of the double bond in the monounsaturates and the presence and relative importance of branched fatty acids varied between species. Amongst the species examined by these authors *F. flexilis*, which has C16:1w5 as its major fatty acid, is perhaps the most

closely related to strain Inp. Reports of C16:1w5 in micro-organisms are uncommon, although it has been found in *Cytophaga hutchinsonii* (Walker, 1969) and in *F. polymorphus* (Johns & Perry, 1977). The differences in the proportions of the major fatty acids between *F. flexilis* and strain Inp could be due to differences in the composition of the growth media used. In their experiments, Nichols *et al.* (1986) grew *F. flexilis* in a protein-rich medium. It is known that branched fatty acids can be synthesized from branched amino acid precursors (Boulton & Ratledge, 1985). In addition, *F. flexilis* is a fresh-water species. It is not uncommon to find differences in fatty acid composition between fresh-water and marine species of the same genus (Wood, 1974).

There is an apparent discrepancy in the fatty acid compositions of strain Inp as determined in experiments in which temperature or salinity were varied. This difference could be due to the different air/nitrogen bubbling rates used in the two types of experiment.

The drop in fatty acid yield per unit protein as salinity increased is similar to that found by Pugh *et al.* (1971), who suggested that a high salt concentration could inhibit fatty acid synthetase. It is interesting to note that whereas cells of strain Inp grown on 1/2-ZoBell or on starch medium contained similar concentrations of fatty acid per mg cell protein at 60 p.p.t. salinity, the proportion of PUFAs in cells grown on starch medium at 60 p.p.t. salinity was similar to that in cells grown on 1/2-ZoBell at 120 p.p.t. This suggests that the possible inhibition of fatty acid synthesis by increasing salt concentration in strain Inp was not directly coupled to the stimulation of PUFAs by increasing salinity.

The fatty acid composition of strain Inp when fatty acids present in Tween 80 were used as the carbon source reflected the major fatty acid present in the medium. Analysis of Tween 80 showed that 80% of its total fatty acid was C18:1w9. The absence of PUFAs from strain Inp when using Tween 80 as carbon source could be due to repression of the fatty acid synthesis enzymes by the free fatty acids present in the culture medium. This occurs in many species of bacteria (Weeks & Wakil, 1970; Nunn, 1986; Schweizer, 1989).

Of the major fatty acids found in strain Inp, C18:1w9, C18:2w6 and C18:3w3 are likely to be related by a common biosynthetic mechanism as is found in green algae and higher plants, namely the aerobic pathway for synthesis of unsaturated fatty acids (Harwood & Russell, 1984). If strain Inp has only the aerobic pathway, then two desaturases would be required, one specific for the $\Delta 9$ carbon and another for the $\Delta 11$ carbon. The presence of two desaturases has been reported in some bacteria (Fulco, 1970, 1983). If two such enzymes are present in strain Inp, the $\Delta 11$ and $\Delta 9$ desaturases are almost exclusively specific for fatty acids with carbon chain

lengths of 16 and 18, respectively. Additionally, the product of elongation of C16:1w5 would be either C18:1w5 or C18:1w7, depending at which end the two carbon atoms are inserted, but it is mostly likely to be C18:1w5. Hence the oleic acid could not originate from C16:1w5. Therefore, it is reasonable to hypothesize that strain Inp possesses both the anaerobic and aerobic pathways for synthesis of unsaturated fatty acids. Although Scheuerbrand & Bloch (1962) suggested that both pathways were mutually exclusive, more recently Wada *et al.* (1989) have demonstrated the presence of both pathways in a psychrotropic *Pseudomonas*.

The inverse relationship between the amounts in strain Inp of the two major fatty acids, C16:1w5 and C18:1w9, both of which were identified unequivocally by GC and GC/MS, can be explained in terms of a linked flux mechanism so that when one pathway is dominant the other is recessive, or vice versa.

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