Phenotypic variation of lipid composition in *Burkholderia cepacia*: a response to increased growth temperature is a greater content of 2-hydroxy acids in phosphatidylethanolamine and ornithine amide lipid

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***Burkholderia cepacia* produces an unusual range of polar lipids, which includes two forms each of phosphatidylethanolamine (PE) and ornithine amide lipid (OL), differing in the presence or absence of 2-hydroxy fatty acids. By using chemostat cultures in chemically defined media, variations in the lipid content and the proportions of individual lipids have been studied as a function of (a) growth temperature, (b) growth rate and (c) growth-limiting nutrient (carbon, magnesium, phosphorus or oxygen). Total cellular lipid in carbon-limited cultures was lowest at high growth temperatures and low growth rates. Increases in growth temperature over the range 25–40 °C led to increases in the proportions of molecular species of PE and OL containing 2-hydroxy acids, without changing the PE:OL ratio. Growth temperature did not alter the balance between neutral and acidic lipids, but the contribution of phosphatidylglycerol to the latter increased with rising growth temperature and growth rate. Pigmentation of cells and the presence of flagella were also temperature-dependent. Change in growth rate also affected the PE:OL ratio and the extent to which monoenoic acids were replaced by their cyclopropane derivatives. Whereas similar lipid profiles were found for carbon-, magnesium- and oxygen-limited cultures, ornithine amides were the only polar lipids detected in phosphorus-limited cells.**

Keywords: *Burkholderia cepacia*, lipids, phenotypic variation, 2-hydroxy acids

INTRODUCTION

Burkholderia cepacia is now recognized as a significant opportunistic pathogen, particularly in relation to cystic fibrosis (Govan & Deretic, 1996; Govan *et al.*, 1996). In consequence, considerable effort has been expended on the study of potential virulence factors (Nelson *et al.*, 1994; Wilkinson & Pitt, 1995b) and on methods of strain characterization for use in epidemiological investigations (Wilkinson & Pitt, 1995a). One of the most striking features of the surface chemistry of the organism is the range of polar lipids, which includes two forms each of phosphatidylethanolamine (PE) and

ornithine amide lipid (OL), differing in the presence or absence of an ester-linked 2-hydroxy fatty acid (Kawai *et al.*, 1988b; Cox & Wilkinson, 1989), as well as phosphatidylglycerol (PG) and/or bis(phosphatidyl) glycerol (DPG) and an uncharacterized glycolipid (Yabuuchi *et al.*, 1992). OL consists of various molecular species based on the general α -N-acyloxyacylornithine structure (1) applicable to such lipids in diverse bacteria (Wilkinson, 1988).

Abbreviations: DPG, bis(phosphatidyl)glycerol; OL, ornithine amide lipid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PHB, poly(3-hydroxybutyrate).

This unusual lipid profile for *B. cepacia* is characteristic of other *Burkholderia* spp. (Galbraith & Wilkinson, 1991; Yabuuchi *et al.*, 1992; Phung *et al.*, 1995a), whereas certain species recently excised from *Burkholderia* and *Alcaligenes* to form the new genus *Ralstonia* lack both forms of OL (Galbraith & Wilkinson, 1991; Yabuuchi *et al.*, 1995). Phenotypic variation of lipid composition in bacteria, including the stimulation of OL production by growth under phosphorus-limiting conditions for at least some Gram-negative species, is a well-known phenomenon (Wilkinson, 1988). Also, by virtue of its amphiphilicity and endotoxin-like structure, OL possesses various biological activities, including haemagglutination, macrophage activation, hypothermic response and adjuvant activity (Kawai & Yano, 1983; Kawai & Akagawa, 1989; Kawai *et al.*, 1996; Kato & Goto, 1997). Thus to confirm the chemotaxonomic value and evaluate any pathogenic potential of these lipids, it was desirable to verify that the profile was a stable characteristic of *Burkholderia* spp. and to identify any effects of growth parameters on the lipid profile. The well-studied strain NCTC 10661 of *B. cepacia* was selected for this purpose.

METHODS

Organism and growth conditions. *B. cepacia* strain NCTC 10661 was grown in continuous culture using a chemostat (1.5 l) and a chemically defined medium (CDM) with succinate as the source of carbon. In studies of the effects of growth temperature, carbon was the growth-limiting nutrient in a CDM of the following composition (g l⁻¹): MgSO₄·7H₂O, 0.40; K₂SO₄, 0.40; H₃PO₄ (88%, w/w), 1.75; FeSO₄·7H₂O, 0.025; MnSO₄·4H₂O, 0.0041; ZnSO₄·7H₂O, 0.0044; CuSO₄·5H₂O, 0.00079; CaCl₂·2H₂O, 0.073; (NH₄)₂SO₄, 3.00; disodium succinate, 5.00. Cultures were maintained at pH 7.0±0.1 and controlled temperature (±0.5 °C) in the range 25–40 °C, with aeration at 0.8 l min⁻¹, stirring at 750 r.p.m. and a dilution rate of 0.15 h⁻¹. For studies of the effect of growth rate (range 0.05–0.40 h⁻¹), cultures were grown at 34 °C and the succinate concentration of CDM was increased to 10.0 g l⁻¹. This enhanced concentration was also used in cultures limited by the availability of phosphorus (88%, w/w, H₃PO₄; 0.08 g l⁻¹), magnesium (MgSO₄·7H₂O, 0.10 g l⁻¹) or oxygen (aeration, 0.05 l min⁻¹; additional nitrogen, 0.75 l min⁻¹): in each case, the dilution rate was 0.15 h⁻¹ and the temperature 34 °C. Cultures were monitored continuously for pH, dissolved oxygen tension (Ingold polarographic electrode), CO₂ output (IR gas analyser) and optical density (OD₅₄₀; Pharmacia LKB Novaspec II spectrophotometer) to verify the establishment of steady-state growth. Residual succinate was assayed by using succinyl-CoA synthetase (EC 6.2.1.5; Boehringer), ammonium by the method of Chaney & Marbach (1962) and magnesium by plasma emission spectroscopy (Plasma 40ICP; Perkin-Elmer). Cells were collected from steady-state cultures by centrifugation (35 000g, 30 min, 4 °C), washed twice (water), and freeze-dried.

Extraction of lipids. Lipids were extracted by stirring dry cells (1.0–1.4 g) with chloroform/methanol (2:1, v/v; 100 ml) for 2 h at room temperature. The suspension was filtered (no. 4 sinter), the insoluble residue was washed with further solvent, and the combined filtrates were rotary-dried. The lipid residue

was redissolved in chloroform/methanol and stored at –20 °C.

Identification of lipids. Phospholipids were identified by (a) TLC, (b) ³¹P NMR spectroscopy (London & Feigenson, 1979) and (c) high-voltage paper electrophoresis at pH 5.3 of the water-soluble products obtained on mild alkaline methanolysis (Wilkinson & Bell, 1971). Lipids containing ornithine were identified by TLC comparisons and by analysis (*vide infra*) of the total lipids for ornithine after hydrolysis (6.1 M HCl, 105 °C, 4 h). The method of Bartlett (1959) was used for the assay of phosphorus, and that of Braunege *et al.* (1978) for the assay of poly(3-hydroxybutyrate) (PHB).

Identification of fatty acids. Ester-bound fatty acids were released as methyl esters by mild alkaline methanolysis (Wilkinson & Bell, 1971). Total fatty acids were obtained by acid hydrolysis (6.1 M HCl, 105 °C, 4 h), followed by extraction into light petroleum (b.p. 60–80 °C) and esterification with ethereal diazomethane or methanolic HCl (Bryn & Jantzen, 1982). The methyl esters were identified by GLC and MS.

Chromatographic methods. TLC separations were carried out on silica gel 60F₂₅₄ (Merck) with the following solvent systems: A, chloroform/methanol/water (65:25:4, by vol.); B, chloroform/methanol/acetic acid (65:25:10, by vol.). Amino lipids were detected with ninhydrin and phospholipids with the reagent of Dittmer & Lester (1964). For improved chromatography, PHB was removed from extracts by precipitation with diethyl ether, and non-lipid contaminants by a Folch wash (Duthie & Patton, 1965). Photodensitometric evaluation of chromatograms was carried out with a Chromoscan 3 (Joyce-Loebl) with a scan length of 10 cm, an aperture of 0.3 mm, a green filter (IFL 2134) for phospholipids and a red filter (IFL 2164) for amino lipids. Replicate scans on the same and duplicate plates were used to obtain mean values.

GLC separations of fatty acid methyl esters were performed at 180 °C with a fused-silica capillary column (25 m × 0.2 mm) of BP1 (SGE) in a Mega 5160 chromatograph (Carlo Erba), and hydrogen as the carrier gas. For combined GLC-MS, the chromatograph was coupled to a Finnigan 1020B mass spectrometer. A column (25 m × 0.25 mm) of BP20 (SGE) at 145 °C with nitrogen as the carrier gas and methyl benzoate as the internal standard was used for GLC analyses of methyl 3-hydroxybutyrate. High-performance anion-exchange chromatography with a PAC PA1 column (250 mm × 4 mm) in a Dionex DM-300 instrument, and pulsed amperometric detection, was used to identify and estimate ornithine. Arginine was used as an internal standard and 23 mM NaOH containing 7 mM sodium tetraborate as the eluent, giving the following approximate retention times: arginine, 3 min; ornithine, 6 min.

Electron microscopy. Washed cells and media from cultures grown at different temperatures were air-dried on 300-mesh copper grids, negatively stained with 0.5% (w/v) phosphotungstic acid and examined by transmission electron microscopy in a JEOL 100C instrument operated at 80 kV.

RESULTS

In preliminary studies (data not shown), glucose, gluconate and succinate were compared as alternative carbon sources using aerated batch cultures of *B. cepacia* in a phosphate-buffered CDM. Using equal concentrations (10 g l⁻¹) of each substrate, the maximum specific growth rates (0.36–0.48 h⁻¹), durations of active

Table 1. Variation in culture density and cellular lipid content with growth temperature for carbon-limited cultures

Cultures were maintained at pH 7.0 and dilution rate 0.15 h^{-1} . Data are presented for duplicate cultures.

Temp. (°C)	Culture density (OD ₅₄₀)	Lipid content (%, w/w)	Lipid phosphorus (%, w/w)
25	0.26; 0.26	11.1; 12.3	2.1; 2.2
30	0.28; 0.27	13.6; 13.1	1.8; 1.7
34	0.30; 0.30	9.7; 9.8	2.0; 1.9
37	0.27; 0.28	5.2; 5.0	1.9; 1.9
40	0.23; 0.25	5.3; 5.9	2.4; 2.3

growth (~20 h) and final culture densities (OD₅₄₀ ~1.2) were similar, though the succinate-based culture showed the largest change in pH (7.0 to 8.8). With glucose as carbon source, the shortest lag phase was observed for growth at 34 °C, which was therefore chosen as the standard temperature for chemostat cultures. Initial studies using nitrogen-limited chemostat cultures with glucose as carbon source showed that it was not possible to attain a steady state, as revealed by variations in culture density, CO₂ output and residual glucose concentration. Alkali consumption to maintain pH 7.0 was high, and the formation of gluconate and 2-ketogluconate (Lessie & Phibbs, 1984) was confirmed. Further chemostat work was therefore based on succinate-containing CDM, and carbon limitation was routinely used in an attempt to avoid or minimize problems associated with an excess of carbon and energy source (the accumulation of reserve material such as PHB; Dawes, 1992).

Effects of growth temperature on carbon-limited cultures

Variation of the growth temperature produced visible changes in the organisms. At the highest temperatures (37 and 40 °C), the cells were a pale cream, but those grown at 34 °C or below were a dark olive green. Lipid extracts from the latter cells contained a brown pigment which migrated at the solvent front on TLC, but which was not studied further. Cells grown at 25 °C were short rods (typically about $1.3 \times 0.25\text{ }\mu\text{m}$) with several polar flagella. As the growth temperature was increased, the cells became somewhat larger and pleomorphic, with fewer flagella: at 40 °C, flagella were absent from the cells and the medium, indicating that their production was switched off. At all growth temperatures, cells contained granules indicative of the storage of PHB. Analysis of lipid extracts confirmed the presence of this polymer (range 9–13 %).

The lipid content of cultures varied markedly, peaking at 30 °C and tailing off at the higher growth temperatures (Table 1). However, the phosphorus content of the lipids was relatively constant and indi-

Table 2. Distribution of lipid phosphorus and its variation with growth temperature for carbon-limited cultures

Data are expressed as percentages of the total phosphorus as determined by TLC and by ³¹P NMR spectroscopy (values in parentheses). TLC data are means from duplicate scans of duplicate plates. PE1 incorporates only non-hydroxy acids; in PE2, these are partly replaced by 2-hydroxy acids.

Temp. (°C)	PE1 + PE2 (%)	DPG + PG (%)
25	68 (69)	32 (31)
30	73 (60)	27 (40)
34	72 (65)	28 (35)
37	73 (60)	27 (40)
40	74 (58)	26 (42)

cated that phospholipids constituted about 50% of the total in all extracts. The phospholipids present were DPG, PG, and the two forms of PE: PE1 containing only non-hydroxy acids and PE2 containing 2-hydroxy acids as well. No attempt was made to verify the presence of a minor glycolipid (Yabuuchi *et al.*, 1992), but efforts were made to quantify the relative amounts of the phospholipids by ³¹P NMR spectroscopy and by TLC (solvent system B) followed by densitometry. In contrast with previous studies (Cox & Wilkinson, 1989; Galbraith & Wilkinson, 1991), broad signals on a noisy background were obtained by the NMR method, so that separate PE peaks were not observed and there was partial overlap of those for DPG and PG. Chromatographic resolution on TLC was also incomplete, so the data obtained by both methods (Table 2) are only given for lipid pairs. Although quantitative agreement is only modest because of the intrinsic limitations of the methods, it appears that the balance between neutral (PE) and acidic (DPG and PG) lipids was conserved, with the former predominating at all growth temperatures. Visual evaluation of chromatograms and NMR spectra showed that PG was the more abundant acidic phospholipid under all conditions, but the distribution of phosphorus between PG and DPG (TLC) varied from about 1.5:1 (25 °C) to 2.5:1 (40 °C).

The relative proportions of PE1 and PE2, as well as those of the two ornithine-containing lipids (OL2 containing 2-hydroxy acids), were determined by TLC (solvent system A) and densitometry (Table 3). The ratio of total PE:total OL remained almost unchanged (~2:1), but the proportions of hydroxylated lipids increased with rising growth temperature, so that the relative amounts of OL1 and OL2 were reversed and the ratio PE1:PE2 fell from 8:1 (25 °C) to 2:1 (40 °C). Analyses of the total lipids by high-performance anion-exchange chromatography showed no major difference or trend in ornithine content (range 5.8–7.8 %).

The major effect of increased growth temperature on the lipid profile of *B. cepacia* NCTC 10661 is greater

Table 3. Distribution of amino lipids and its variation with growth temperature for carbon-limited cultures

Data are expressed as percentages of the total colour produced with ninhydrin. Values are means of duplicate scans of duplicate plates, with standard deviations in parentheses. PE1 and PE2 are differentiated by the presence in the latter of 2-hydroxy acids. In OL2, non-hydroxy acids are replaced by 2-hydroxy acids.

Temp. (°C)	PE1 (%)	PE2 (%)	OL1 (%)	OL2 (%)
25	63.1 (0.6)	7.7 (0.2)	19.8 (0.3)	9.4 (1.1)
30	53.4 (1.4)	10.0 (1.5)	20.2 (0.5)	16.4 (1.2)
34	55.4 (0.8)	13.4 (0.8)	16.5 (0.6)	14.7 (1.0)
37	47.5 (0.4)	18.0 (1.8)	12.8 (0.9)	21.7 (0.6)
40	45.5 (0.9)	22.0 (2.3)	8.8 (1.9)	23.7 (0.4)

production or incorporation of 2-hydroxy acids into zwitterionic lipids (PE and OL). The incorporation is specific to these lipids and, in the case of PE, to the *sn*-2 position (Yabuuchi *et al.*, 1992). Changes in overall composition of the ester-linked fatty acids (Table 4) should reflect this replacement and any other adaptive responses (e.g. effects on mean chain length, degree of unsaturation or extent of cyclopropanation). Several features were apparent: (a) cyclopropanation was least at 25 °C, and variations with temperature were more pronounced for 19:cyc than for 17:cyc; (b) the sum of 16:1 and 17:cyc was almost constant, whereas the sum of 18:1 and 19:cyc was greatest at low growth temperatures; (c) overall, the proportion of 16:0 showed a modest increase with rising growth temperature. Repeated attempts to monitor temperature-dependent changes in the ester-linked 2-hydroxy acids (mainly 2-OH-16:0, 2-OH-16:1, 2-OH-18:1 and 2-OH-19:cyc)

were unsuccessful, because of the relatively small, tailing peaks on GLC as previously found for total lipid mixtures (Cox & Wilkinson, 1989). However, analyses of acid hydrolysates for 3-hydroxyhexadecanoic acid (3-OH-16:0), the dominant amide-linked acid in OL1 and OL2, showed relatively little variation with temperature (range 3.6–4.7%), supporting other evidence for an almost constant proportion of total OL.

Effects of growth rate on lipid composition of carbon-limited cultures

For organisms grown at 34 °C with carbon as the limiting nutrient, growth rate affected both lipid content and distribution. The lipid content was highest (9.8%) at the dilution rate of 0.15 h⁻¹ and least (3.2%) at the rate of 0.05 h⁻¹. As determined by TLC, the proportion of total PE did not change significantly with growth rate (range 70–78%), but the ratio of DPG:PG was highest (2.3:1) in slow-growing cells (dilution rate 0.05 h⁻¹) and lowest (0.6:1) in fast-growing cells (dilution rate 0.40 h⁻¹). The PE:OL ratio also varied with growth rate, rising from 1.6:1 (dilution rate 0.05 h⁻¹) to 3:1 (dilution rate 0.40 h⁻¹). The proportions of OL1 and OL2 were similar at all growth rates and the ratio PE1:PE2 was about 4:1 except at the highest growth rate (9:1 at the dilution rate of 0.40 h⁻¹). Variations in the composition of ester-linked, non-hydroxy acids are shown in Table 5. The most striking trend is the progressive decline in cyclopropanation of the monoenoic acids as the growth rate was increased.

Effects on lipid composition of the growth-limiting nutrient

When cells were grown under standard conditions (34 °C, dilution rate 0.15 h⁻¹) but with either magnesium or oxygen as the limiting nutrient, the polar lipid and

Table 4. Ester-linked, non-hydroxy fatty acids in the total lipids and their variation with growth temperature for carbon-limited cultures

Data are expressed as percentages of the total peak area on GLC of the methyl esters obtained by mild alkaline methanolysis. Values are means from duplicate chromatograms and duplicate experiments, with standard deviations in parentheses.

Fatty acid*	Growth temperature (°C)				
	25	30	34	37	40
14:0	0.6 (0.0)	1.0 (0.1)	Trace	Trace	Trace
16:0	16.4 (0.6)	15.7 (0.5)	19.1 (0.9)	22.7 (0.4)	21.1 (0.5)
16:1	19.6 (0.9)	13.2 (1.5)	15.8 (1.0)	14.1 (0.2)	14.3 (0.7)
17:cyc	10.2 (0.6)	18.6 (2.3)	18.3 (0.8)	20.6 (1.2)	19.5 (0.3)
18:0	3.6 (0.4)	2.6 (0.4)	2.4 (0.4)	2.1 (0.7)	3.8 (0.5)
18:1	46.6 (0.6)	30.6 (0.5)	33.2 (2.0)	33.5 (0.1)	32.0 (0.1)
19:cyc	3.0 (0.1)	18.3 (1.2)	11.2 (3.3)	7.0 (0.6)	9.3 (0.1)

* Shorthand designation: the number before the colon indicates the total number of carbon atoms, the number after the colon indicates the number of double bonds, and cyc indicates the presence of a cyclopropane ring.

Table 5. Variations with growth rate of ester-linked, non-hydroxy fatty acids in the lipids of carbon-limited cultures

Data are expressed as percentages of the total peak area on GLC of the methyl esters obtained by mild alkaline methanolysis. Values are the means from duplicate chromatograms, with standard deviations in parentheses. See Table 4 for the fatty acid shorthand.

Fatty acid	Dilution rate (h ⁻¹)			
	0.05	0.15	0.20	0.40
14:0	0	Trace	Trace	0
16:0	33.8 (0.8)	20.8 (1.7)	20.9 (0.6)	30.3 (0.5)
16:1	8.5 (0.9)	13.9 (0.4)	18.6 (1.3)	27.0 (0.2)
17:cyc	23.3 (0.6)	19.8 (0.8)	16.6 (0.8)	4.5 (0.3)
18:0	Trace	2.4 (1.1)	1.8 (1.6)	Trace
18:1	23.5 (0.3)	33.6 (0.7)	37.6 (0.3)	38.2 (1.4)
19:cyc	10.9 (0.7)	9.5 (0.6)	4.4 (0.4)	0

fatty acid profiles were very similar to those described above for carbon limitation (data not shown). However, when phosphorus was the growth-limiting nutrient, OL1 and OL2 were the only polar lipids detected and 3-OH-16:0 was the major fatty acid in an acid hydrolysate (34% of the total).

DISCUSSION

Profiles of polar lipids

Although phospholipid profiles constituted from PE, PG and DPG are commonplace among Gram-negative bacteria, and OL is also rather often encountered (Wilkinson, 1988), the occurrence of two forms each of PE and OL appears to be unique to *Burkholderia* spp. (Galbraith & Wilkinson, 1991; Yabuuchi *et al.*, 1992). One anomaly (Yabuuchi *et al.*, 1995) is posed by *Burkholderia andropogonis*, which contains both forms of PE but neither form of OL, thus resembling *Ralstonia* spp. in this respect (Galbraith & Wilkinson, 1991; Yabuuchi *et al.*, 1992, 1995). Other bacteria known or suspected to produce two forms of PE analogous to PE1 and PE2 include some actinomycetes (Kawanami *et al.*, 1969; Yano *et al.*, 1970; Pramanik *et al.*, 1990; Hoischen *et al.*, 1997) and myxobacteria (Yamanaka *et al.*, 1988). Non-hydroxy and hydroxy forms of OL comparable with OL1 and OL2 are reported to occur in *Flavobacterium* spp. (Kawai *et al.*, 1988a; Asselineau *et al.*, 1988) and at least some *Cytophaga* strains (Pitta *et al.*, 1989; Kawazoe *et al.*, 1992). Lipids of the general structure 1 with only 2-hydroxy fatty acids in ester linkage (like OL2) are present in *Thiobacillus thiooxidans* (Knoche & Shively, 1972) and *Gluconobacter cerinus* (Tahara *et al.*, 1976).

Effect of phosphorus-limited growth

The present study has shown that for *B. cepacia*, at least, the unusual profile of polar lipids is a rather stable

phenotype. Only under phosphorus-limited growth is the profile drastically changed, with total replacement of PE by OL. Similar effects of phosphorus deprivation have been described for *Pseudomonas fluorescens* (Minnikin & Abdolrahimzadeh, 1974; Dorrer & Teuber, 1977) and *Rhodobacter sphaeroides* (Benning *et al.*, 1995), and probably apply also to *Shewanella putrefaciens* (*Pseudomonas rubescens*; Wilkinson, 1972) and many other OL-producing species. However, a low-phosphate medium had no significant effect on *Paracoccus denitrificans* (Wilkinson *et al.*, 1982) whereas the proportion of OL was enhanced by a deficiency of divalent cations (Wee & Wilkinson, 1988). Whether the simultaneous loss of PG and DPG in phosphorus-limited cultures of *B. cepacia* was compensated for by the production of an acidic glycolipid [as described for *Brevundimonas* (*Pseudomonas*) *diminuta* (Minnikin *et al.*, 1974) and *R. sphaeroides* (Benning *et al.*, 1995)] was not established by this study, although the detection of an uncharacterized glycolipid in *B. cepacia* (Yabuuchi *et al.*, 1992) raises that possibility. A glycolipid in *Burkholderia pseudomallei* with similar low mobility on TLC contains both 2-hydroxy and non-hydroxy acids (Phung *et al.*, 1995a, b).

Effects of growth temperature

Many studies of the effects of growth temperature on bacterial lipid composition have been carried out (Ratledge & Wilkinson, 1988; Suutari & Laakso, 1994), but in most cases the inferences are, to some degree, compromised by failure to disentangle possible effects of growth temperature from those of growth rate, in particular. Thus opportunities for direct comparison of the present results with those of previous studies are limited. The marked dependence on growth temperature of cellular lipid content for carbon-limited cultures of *B. cepacia* (Table 1) was not observed for similar cultures of *P. fluorescens* (Gill, 1975) and other psychrotrophic

pseudomonads (Bhakoo & Herbert, 1980), nor for batch cultures of psychrotrophic pseudomonads (Cullen *et al.*, 1971; Wada *et al.*, 1987) and *Yersinia enterocolitica* (Nagamachi *et al.*, 1991), for example. However, trends towards higher lipid contents at lower growth temperatures have been recorded in some studies of *Y. enterocolitica* (Abbas & Card, 1980) and psychrophilic, marine *Vibrio* strains (Bhakoo & Herbert, 1979). In the case of *B. cepacia*, the variations in cellular lipid are apparently not attributable to variable production of PHB, nor to major changes in the relative proportions of polar and apolar lipids, but the lower lipid content of cells grown at higher temperatures may partly be explained by their greater size.

As shown (Tables 2 and 3), growth temperature has no significant effect on the ratios of total PE:total OL:PG+DPG in *B. cepacia*. Similar stability of the polar lipid composition has been described for other Gram-negative bacteria, including carbon-limited chemostat cultures of psychrotrophic pseudomonads (Bhakoo & Herbert, 1980), and also batch-grown *Escherichia coli* (Haest *et al.*, 1969), *P. fluorescens* (Cullen *et al.*, 1971), another psychrotrophic pseudomonad (Wada *et al.*, 1987) and *Y. enterocolitica* (Abbas & Card, 1980; Tsuchiya *et al.*, 1987; Nagamachi *et al.*, 1991). A rise in PG:DPG ratio with increasing growth temperature, as found for carbon-limited cultures of *B. cepacia*, was also reported for nitrogen-limited chemostat cultures of *P. fluorescens*, but the proportions of both acidic phospholipids increased (relative to PE) with temperature when the latter organism was grown under carbon-limiting conditions (Gill, 1975). More complex fluctuations in phospholipid profiles have been described for various halophilic and psychrophilic organisms (Bhakoo & Herbert, 1979; Ohno *et al.*, 1979; Adams & Russell, 1992).

The aspect of bacterial lipids most extensively studied in relation to thermal adaptation is fatty acid composition (Suutari & Laakso, 1994). The cellular response to reduced growth temperature, which may be an increase in the proportion of unsaturated fatty acids, a decrease of mean chain length, an increase in the extent or change in location of branching, or a decrease in cyclopropanation of monoenoic acids (Russell, 1984), is normally interpreted as a mechanism for the preservation of membrane fluidity (homoviscous adaptation; Sinensky, 1974), although other explanations may be invoked (Hazel, 1995). The present results for non-hydroxy acids in *B. cepacia* (Table 4) are broadly consistent with those for other, typical Gram-negative bacteria, but the most striking trend is the increased production and specific incorporation of 2-hydroxy acids into PE and OL with rising growth temperature. Relatively little is known about the formation and lipid incorporation of 2-hydroxy acids in bacteria, although characteristics of the α -hydroxylase in *Sphingomonas paucimobilis*, an organism producing glycosphingolipids rather than lipopolysaccharide (Kawasaki *et al.*, 1994), have been determined (Matsunaga *et al.*, 1994, 1996). The effect of a 2-hydroxy group on the gel-to-

liquid crystalline phase transition temperature may depend on the parent lipid and its environment, although 2-hydroxylation of a glycosphingolipid in a bilayer of phosphatidylcholine appeared to disrupt packing of the acyl chains (Singh *et al.*, 1992). The consequences for membrane fluidity and thermal transition of the increased hydroxylation of PE and OL in *B. cepacia* at higher growth temperatures have yet to be determined.

Effects of growth rate

Change of growth rate at constant temperature (34 °C) with carbon as the limiting nutrient affected both the polar lipid and the fatty acid composition of *B. cepacia*, as well as the total lipid content. In contrast, little or no change in lipid content of carbon-limited cultures was found for *E. coli* (Damoglou & Dawes, 1968; Calcott & Petty, 1980) or *P. aeruginosa* (Gilbert & Brown, 1978). Also, the DPG:PG ratio rose markedly with increasing growth rate for *E. coli* (Calcott & Petty, 1980), and slightly for *P. aeruginosa* (Gilbert & Brown, 1978), but fell for *B. cepacia*. The accumulation of DPG by Gram-negative bacteria is often observed as the growth rate declines and cells enter the stationary phase in batch culture (Wilkinson, 1988). In earlier studies using batch cultures of *B. cepacia* NCTC 10661 (equivalent to ATCC 17759 and the O7 reference strain), the PG content was reported as little or none (Cox & Wilkinson, 1989), minor (DPG:PG ratio about 6:1; Kawai *et al.*, 1988b) or the sole acidic phospholipid (Anwar *et al.*, 1983a). The last result was also reported for the type strain (Yabuuchi *et al.*, 1992). Low growth rates in chemostat cultures and ageing of batch cultures also often promote the conversion of monoenoic acids into their cyclopropane derivatives (Gill & Suisted, 1978; Leach *et al.*, 1997), as found for *B. cepacia* (Table 5), although the contrary effect was noted in one study of *E. coli* (Arneborg *et al.*, 1993). Growth rate and other cultural parameters have been shown to influence potential virulence factors of *B. cepacia* and sensitivity of the organism to antibacterial action (Anwar *et al.*, 1983b; Cozens & Brown, 1983; McKenney *et al.*, 1994; McKenney & Allison, 1995, 1997), and the present study shows that variation of the lipid profile also needs to be considered in these contexts.

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