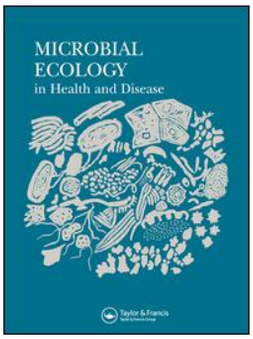

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ORIGINAL ARTICLE

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Phospholipid Analogue Profiles of Human *Porphyromonas gingivalis* Isolates from Different Geographical Locations

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Porphyromonas gingivalis displays interesting phospholipid analogue profiles, which can vary between strains from different animal species. The purpose of this study was to compare human strains, isolated in different parts of the world, with respect to their phospholipid profiles. In all, 24 isolates from three continents were cultured and their lipids were extracted with chloroform-methanol and characterized by fast atom bombardment mass spectrometry (FAB-MS) in negative ion mode. Both lower mass (carboxylate) and higher mass (phospholipid analogue) anions were measured in each mass spectrum. Calculation of Pearson correlation coefficients for each strain pair was then followed by average linkage cluster analysis. The major lower mass anion observed for all strains analysed in this study was of mass-to-charge (m/z) 241, which is consistent with previous FAB-MS studies on *P. gingivalis*. The major phospholipid analogue anion observed for isolates from Manchester, UK, was m/z 960 which is an unknown phospholipid. Other major peaks were of m/z 662 and 663, which may be identified respectively as PE (30:0) and PG (28:1) with contributions from the first isotope of PE (30:0). It was observed that isolates from the same geographical location displayed similar phospholipid profiles. It is concluded that phospholipid profiles of human *P. gingivalis* isolates are diverse and that there can be differences between *P. gingivalis* isolates obtained from different geographical locations. *Key words: Porphyromonas gingivalis*, phospholipids, FAB-MS, human, mass spectrometry.

INTRODUCTION

The periodontal pathogen, *Porphyromonas gingivalis,* has revealed some heterogeneity when examined by various analytical methods. The latter have included DNA-DNA homology (1), crossed immunoelectrophoresis (2), arbitrarily primed PCR (3), ribotyping (4) and phospholipid analogue profiling by fast atom bombardment mass spectrometry (FAB-MS) (5).

FAB-MS is just one strand of a rich tapestry of taxonomic methods and has proved especially valuable when applied to *Porphyromonas* spp. For example, phospholipid analogue distributions alone have been used to determine whether cat strains of *P. gingivalis* originated in Australia or in the USA (6). Computer analysis of phospholipid profiles of *P. gingivalis* has also permitted separation of cat strains from those of other animals and from humans (5). In the case of human isolates of *P. gingivalis* less is known of the relationship between geographical source and biotype or chemotype. However, human isolates of another anaerobic pathogen, *Clostridium difficile*, have shown geographical differences when English, French and Belgian strains have been examined by FAB-MS (7). The purpose of this study was to compare phospholipid molecular species distributions in human strains of *P. gingivalis* isolated in different parts of the world.

MATERIALS AND METHODS

Strains examined

The isolates examined included three fresh clinical isolates (S5, S6 and S7) obtained from periodontal pockets of patients with generalized aggressive periodontitis, attending the University Dental Hospital of Manchester, Unit of Periodontics. In addition, one culture collection strain (ATCC 33277^T), six Finnish, two Australian and 12 US

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isolates were also analysed. The origin and geographical distributions of all *P. gingivalis* isolates used in this study are shown in Table I (8). Strains were checked for purity using microscopic and colonial morphology. Their identities were confirmed using biochemical characterization employing RapID ANA II (Pro-Lab Diagnostics, Atlanta, GA, USA). Ethical approval was obtained from the various human ethic committees for collection of plaque samples.

Growth and harvesting

Isolates were inoculated onto triple plates of 5% (v/v) horse blood-fastidious anaerobe agar (Lab-M, Bury, UK). These were incubated in a compact M anaerobe work station (Don Whitley Scientific, Shipley, UK) in an anaerobic atmosphere of 80% N_2/10% CO_2/10% H_2 (v/v) at 37°C for 48 h. Cells were then harvested with a saline-moistened cotton wool swab to ensure efficient removal of bacteria from the plates. Harvested cells were suspended in 10 ml aliquots of 0.01 M phosphate-buffered saline, pH 7.4 (PBS; Sigma, Poole, Dorset, UK) by centrifugation at 3000 *g* for 20 min. Cells were then re-suspended in saline and the washing procedure was repeated.

Table I

Site of isolation and geographical origin of P. gingivalis strains studied

P. gingivalis strains	Species of origin	Geographical origin
S5	Human (oral)	Manchester, UK
S6	Human (oral)	Manchester, UK
S7	Human (oral)	Manchester, UK
ATCC 33277^T	Human (oral)	American Type Culture Collection
AHN 12631	Human (oral)	Helsinki, Finland
AHN 8448	Human (oral)	Helsinki, Finland
AHN 9188	Human (oral)	Helsinki, Finland
AHN 11190	Human (oral)	Helsinki, Finland
AHN 1885	Human (oral)	Helsinki, Finland
AHN 19950	Human (oral)	Helsinki, Finland
RMA 4165	Human (oral)	Los Angeles, USA
RMA 3725	Human (oral)	Los Angeles, USA
UQD 602	Human (oral)	Queensland, Australia
UQD 603	Human (oral)	Queensland, Australia
CC 666	Human (oral)	Los Angeles, USA
CC 870	Human (oral)	Los Angeles, USA
CC 647	Human (oral)	Los Angeles, USA
CC 1868	Human (oral)	Los Angeles, USA
CC 888	Human (oral)	Los Angeles, USA
CC 1103	Human (oral)	Los Angeles, USA
CC A7A1-128	Human (oral)	Los Angeles, USA
CC 1225	Human (oral)	Los Angeles, USA
CC 1109	Human (oral)	Los Angeles, USA
CC 852	Human (oral)	Los Angeles, USA

Key: S5 = *P. gingivalis*; S6 = *P. gingivalis*; S7 = *P. gingivalis* isolated from Manchester University Dental Hospital; ATCC 33277^T = *P. gingivalis* type strain; AHN = National Public Health Institute, Helsinki, Finland; RMA = RM Alden Research Laboratory, Santa Monica, UCLA; UQD = University of Queensland, Australia; CC = USC School of Dentistry, LA. ATCC 33277^T was isolated from human gingival sulcus and deposited with ATCC by J. Slots (8).

Lyophilization (freeze-drying)

The cell pellets were lyophilized under vacuum (10^{-2} Torr) until completely dry (approximately 5 h), using a Modulyo freeze drier (Edwards, Crawley, UK) and were stored in screw-capped bottles at ambient temperature until required.

Polar lipid extraction and analysis

Contamination precautions. All glassware was washed then rinsed thoroughly in chloroform-methanol (1:2, v/v) to remove all traces of lipids, grease and other contaminants. Care was taken to reduce contamination of the samples by using solvent-resistant gloves, avoiding the use of plastic ware and rubber bottle cap liners and transferring solvents by pipetting instead of pouring.

Extraction. Dried cells (10 mg) were transferred to a universal glass bottle. Lipids were extracted from cells using 2 ml of Analar methanol-chloroform (2:1, v/v). The extraction was vortex mixed and left to stand with occasional re-mixing for 4 h. The extracted cells were sedimented by centrifugation at 3000 *g* for 20 min and the supernatant fluid (extract) was transferred to a 5 ml screw-capped bijou bottle. The extraction procedure was repeated by adding a further 2 ml of solvent to the pellet. All extracts were dried using a vacuum desiccator over silica gel. Dried pooled extracts were re-dissolved in 1 ml of chloroform, then washed in 1 ml of sterile distilled water. After phase separation, the lower chloroform phase was transferred, using a glass pipette, to a 1.2 ml vial and re-dried in a vacuum desiccator. The dried lipid extract was then stored at −20°C until required for FAB-MS analysis.

FAB mass spectrometry. Lipid extracts were reconstituted in 10 µl of methanol and suspended in 10 µl of m-nitrobenzyl alcohol (m-NBA). After allowing the methanol to evaporate on a copper probe, samples were placed in the sample chamber of a Concept IS mass spectrometer (Kratos, Manchester, UK). The polar lipid molecules, which concentrated at the matrix surface, were ionized by fast atom bombardment with the inert gas, xenon. Anions generated at the surface of the m-NBA, by loss of a proton, were separated according to their mass-to-charge (m/z) ratio. Data from triplicate scans were obtained for ion intensities over a range of m/z values in the range 200–1000. Repeat scans were averaged and also printed out for data analysis.

Data analysis

Correct 'nominal' m/z values were calculated by truncating 'accurate' masses for anions, where m/z = (molecular weight − 1). The most intense lower mass (m/z 200–300) and higher mass (m/z 500–1000) peaks were analysed separately. In both cases, the most intense anions were

selected and the relative peak heights of major peaks were used to calculate normalized identities where the sum of peak height equals 100. Repeat analyses were averaged so that percentage relative intensity values could be listed in data tables for each m/z for all strains. The nominal m/z value was used for putative peak identifications. Calculations were performed with Microsoft Excel Version 8.0 and then exported to Statistical Package for Social Science, SPSS/PC+, to calculate Pearson coefficients of linear correlation for all isolate pairs of *P. gingivalis*. These coefficients were used to produce a dendrogram following single-linkage cluster analysis.

RESULTS

Carboxylate anions

A representative spectrum for *P. gingivalis* UQD 603, (University of Queensland isolate) is shown in Fig. 1. Normalized peak intensities were calculated for carboxylate anions ranging from m/z 211 to 293. The most intense lower mass anion observed for all strains analysed in this study was of m/z 241, consistent with the expected presence of $C_{15:0}$ mono-carboxylate anion (Table II). The second most intense carboxylate anion, m/z 242, was observed for all isolates from Manchester and Australia, the four US isolates CC852, CC A7A1-128, CC 1868, CC 666 and the *P. gingivalis* type strain ATCC 33277^T. Other major anions (Table II) observed were of m/z 211, 227, 279, 253, 281 and 291.

Phospholipid anions

Major phospholipid anions were recorded in the range m/z 619–961 for each human *P. gingivalis* strain. Phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) were the most prevalent polar lipid families present (Table III) in all the strains analysed. Unknown peaks were observed at m/z 722, 932, 933, 946, 947, 960 and 961. The major higher mass anion for all isolates from Manchester, UK was m/z 960. For even m/z values, anions may contain a single nitrogen atom, according to the 'nitrogen rule'. Although the unknown homologous series of m/z 932, 946 and 960 was observed in all isolates in this study, the intensity of the higher mass anions showed strain variation. The non-Manchester strains displayed m/z 662 as the major peak with the exception of AHN 1885 which revealed a major peak at m/z 663. The peak with m/z 662 is a phosphatidylethanolamine analogue, PE (30:0), having combined fatty acyl substituents at sn1 and sn2 positions on glycerol with 30 carbons and no sites of unsaturation. The peak at m/z 663 is consistent with the expected presence of PG (28:1) and first isotope peak of m/z 662, calculated from known abundance of C^{13}. Other major phospholipid anions were observed at m/z 619, 634, 635, 648, 676, 677, 691, 705, 720, 932, 946 and 960. The first nine of the latter peaks were identified tentatively as anions of the phospholipids PG

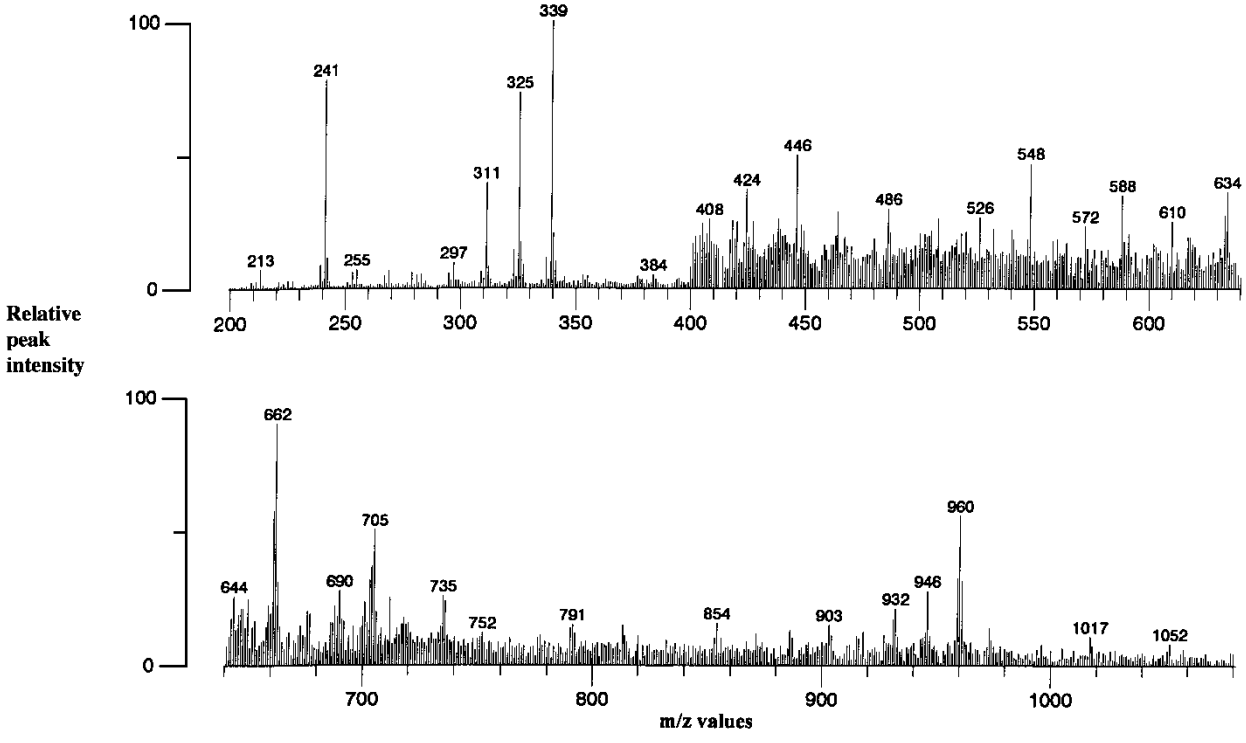

Fig. 1. Negative ion fast atom bombardment mass spectrum of *P. gingivalis* UQD 603 isolated at the University of Queensland, Australia. Mass-to-charge values are shown as nominal masses. From m/z 400 to 1080, intensities are shown with $\times 8$ magnification factor.

Table II

Carboxylate peaks for human P. gingivalis strains

Carboxylate anions		Mean normalized peak intensity for human *P. gingivalis* strains																							
m/z	ID	S5	S6	S7	ATCC 33277^T	AHN 12631	AHN 8448	AHN 9188	AHN 11190	AHN 1885	AHN 19950	UQD 602	UQD 603	RMA 4165	RMA 3725	CC 666	CC 870	CC 647	CC 1868	CC 888	CC 1103	CC A7A 1-128	CC 1225	CC 1109	CC 852
211	$C_{13:1}$	1.7	2.6	4.2	3.6	4.8	3.1	2	3.1	2.3	3.2	2.3	2.3	2.5	4.9	4.3	2.8	1.6	1.4	1.4	0.6	0.3	1.0	3.0	4.7
213	$C_{13:0}$	4.6	4.2	3.2	6.5	10	9.0	9.9	12.8	4.7	12.9	4.5	6.2	12.5	6.6	6.2	5.6	4.7	8.4	4.2	1.3	7.7	1.9	8.9	7.1
221	$C_{14:3}$	3	3	3	3.4	3.9	3.4	3.1	2.8	5	4	2.3	2.3	2.2	3.2	3.4	2.8	1.6	1.4	0.1	0.6	0.3	1.0	1.5	3.3
223	$C_{14:2}$	1.8	1.6	1.8	2.1	2.4	2.2	1.7	2.2	1.8	1.9	1.1	0.8	1.4	2.1	2.4	2.8	1.6	3.5	0.0	1.3	2.6	1.9	1.5	2.5
225	$C_{14:1}$	2.8	3.2	3.3	2.8	3.4	2.5	2.7	3.1	2.5	1.9	2.3	2.3	2.1	2.2	3.6	2.8	3.1	2.8	2.1	0.6	0.3	1.9	3.0	3.5
227	$C_{14:0}$	2.1	3	3.3	4	4.4	4.9	4.8	5.3	2.9	3.5	1.7	2.3	6.8	2.6	3.4	2.8	3.9	2.8	2.8	1.9	2.6	1.9	4.5	3.3
235	$C_{15:3}$	1.5	1.5	1.5	1.4	1.5	2.0	1.4	1.3	1.8	1.3	1.1	0.8	1	1.7	1.2	0.0	1.6	0.1	0.1	0.0	0.3	1.0	0.3	1.3
237	$C_{15:2}$	2.3	2.6	2.5	2	2.1	2.2	1.4	2.8	2.3	2.3	1.1	0.8	3.4	4.1	1.9	2.8	1.6	0.3	0.3	0.0	0.3	0.2	1.5	4.4
239	$C_{15:1}$	4.6	5.3	5.5	6.3	5.7	4.6	4.8	4.7	5.6	5.5	5.6	6.2	3.5	3.8	5.6	8.5	4.7	7.0	35.0	0.0	5.2	3.8	6.0	6.0
241	$C_{15:0}$	33.4	34	29.9	36.6	30.7	31.7	38.2	35.1	31.5	36.3	48.6	43.4	22.1	24.5	27.1	36.6	37.5	46.4	39.2	57.3	56.7	30.7	38.7	26.7
242	*$C_{15:0}$	15.5	10.2	12.1	6.8	6.3	7.7	7.5	6.6	6.5	7.5	7.9	7.8	5.6	5.2	20.1	5.6	5.5	8.4	7.0	12.7	7.7	5.7	7.4	9.2
251	$C_{16:2}$	1.5	1.3	1.4	1.7	1.4	1.6	1	1.6	1.4	1	1.1	1.6	1.8	2.2	1.4	2.8	1.6	0.0	0.1	0.0	0.0	0.2	1.5	4.7
253	$C_{16:1}$	2.2	2.4	2.2	2.9	3.2	2.0	2	2.2	2.4	2.6	3.4	3.1	4.7	4	3.1	2.8	4.7	2.8	2.1	0.6	1.3	4.8	3.7	3.0
255	$C_{16:0}$	4.1	6.5	9.6	5.5	4.4	5.9	4.8	5.6	7.1	6.8	4.5	5.4	1.1	1.6	3.6	4.2	6.3	7.0	4.2	19.1	7.7	23.0	6.0	4.0
263	$C_{17:3}$	1.5	1.5	1.7	1.5	1	1.2	0.7	0.6	2.6	1.3	1.1	0.8	6.6	6.3	0.7	1.4	1.6	0.1	0.1	0.0	0.3	0.0	1.5	0.9
265	$C_{17:2}$	2.5	1.5	1.9	1.3	1.2	1.6	1.4	0.9	1.4	1.3	1.1	1.6	3.5	3.7	0.9	1.4	1.6	0.1	0.3	0.0	0.3	1.9	1.5	3.5
277	$C_{18:3}$	7	6.9	4	3.9	3.2	3.0	1.7	1.9	3.4	1.3	1.1	1.6	13.8	14	2.4	2.8	3.1	1.4	0.1	0.6	1.3	1.0	1.5	1.9
279	$C_{18:2}$	2.8	2.9	2.5	1.6	4.6	4.0	6.1	2.8	5	1.9	3.4	3.9	1.7	2.5	3.6	2.8	4.7	1.4	0.1	0.6	1.3	3.8	1.5	5.5
281	$C_{18:1}$	1.6	2.5	3.1	2	3.2	4.6	2.7	2.5	6.9	2.3	3.4	3.9	1.1	1.7	3.4	2.8	1.6	2.8	0.3	1.3	0.3	11.5	4.5	2.5
291	$C_{19:3}$	1.6	1.3	1.5	2.8	1.2	1.4	0.7	0.9	1.7	0.6	1.1	1.6	2.1	2.6	0.7	2.8	4.7	0.1	0.1	0.6	2.6	1.0	1.5	0.8
293	$C_{19:2}$	1.9	2	1.8	1.3	1.2	1.6	1.4	0.9	1.4	0.6	1.1	1.6	0.5	0.5	1.0	2.8	3.1	1.4	0.1	0.6	1.3	1.9	0.7	0.9

Key: m/z values = mol. wt-1; S = isolates from University of Manchester Dental Hospital; ATCC 33277^T = *P. gingivalis* type strain; AHN = National Public Health Institute, Helsinki, Finland; RMA = RM Alden Research Laboratory, Santa Monica, UCLA; UQD = University of Queensland, Australia; CC = University of Southern California, School of Dentistry, LA.
*$C_{15:0}$ probably additional contributions in addition to any first isotope; see text for details.

Table III

Phospholipid peaks for human P. gingivalis strains

Phospholipid anions		Mean normalized peak intensity for human *P. gingivalis* strains																							
m/z	ID	S5	S6	S7	ATCC 33277^T	AHN 12631	AHN 8448	AHN 9188	AHN 11190	AHN 1885	AHN 19950	UQD 602	UQD 603	RMA 4165	RMA 3725	CC 666	CC 870	CC 647	CC 1868	CC 888	CC 1103	CC A7A 1-128	CC 1225	CC 1109	CC 852
619	PG (25:2)	2.5	2.4	2.5	3.1	5.1	5.0	3.8	4.4	5.2	6.0	3.1	4.8	2.8	3.3	3.5	4.0	3.8	3.7	3.2	2.3	3.4	3.3	3.5	2.9
620	PE (27:0)	2	2.1	2	2.8	4.6	3.1	2.9	4.1	3.4	3.3	3.4	3.6	5.5	2.8	1.7	2.8	3.4	3.3	2.3	2.6	3.0	2.2	3.2	2.0
634	PE (28:0)	3.3	2.9	2.7	5.4	10.3	8.2	7.0	10.7	5.9	8.4	6.2	8.8	10.5	7.6	7.8	7.1	5.9	6.3	6.4	4.7	6.1	4.4	6.4	4.9
635	PG (26:1)	3	2.7	2.5	2.6	5.0	4.4	3.2	4.6	3.9	4.3	2.5	2.7	4.6	4	2.6	3.1	5.2	5.9	5.0	3.0	5.1	3.3	2.5	2.0
648	PE (29:0)	3	3	3	3.5	4.8	6.2	5.1	7.7	4.7	6.9	3.7	4.5	6.6	4.5	4.3	4.7	4.0	5.0	4.6	3.9	5.0	3.7	6.4	4.9
649	PE (27:1)	1.5	1.7	1.3	2.1	3.9	4.5	2.9	4.2	3.1	6.0	2.5	3.0	3.2	2.6	2.6	3.5	3.8	3.9	3.9	3.4	4.8	3.3	4.3	2.0
662	PE (30:0)	9	8	7.5	11.8	13.8	10.4	9.8	14.9	8.4	12.3	15.5	14.5	14.5	13.9	9.6	13.6	9.8	12.6	13.0	7.8	11.5	9.9	8.9	13.7
663	PE (30:0)/ PG (28:1)	7	7.5	6	5.4	7.5	8.0	5.4	7.8	9.1	5.7	5.6	6.7	6.8	6.9	7.8	11.5	9.5	4.6	12.6	7.7	10.3	8.1	7.1	9.8
676	PE (31:0)	3	3.1	3.4	4.2	3.8	3.8	5.7	5.1	5.3	6.2	3.4	3.9	5	4.4	3.5	5.9	4.1	4.8	5.7	5.4	5.3	4.0	5.3	3.9
677	PG (29:1)	2.1	2.5	2.6	2.4	5.0	7.2	4.4	5.1	5.3	4.3	3.4	2.4	3.5	5	2.6	3.5	3.8	4.4	3.9	5.3	3.4	4.0	3.9	2.9
690	PE (32:0)	4.4	4	3.3	3.8	4.7	5.7	5.1	4.4	4.1	6.2	5.0	6.7	3.6	3.7	7.8	5.4	4.8	6.1	4.8	7.1	6.7	8.8	8.2	2.9
691	PG (30:1)	6.1	6	6.3	3.8	4.3	5.4	7.9	4.3	3.3	4.8	3.1	4.2	2.5	4.3	3.5	3.8	4.5	4.8	3.7	6.6	5.1	4.8	4.3	8.8
705	PG (31:1)	2.6	2.9	2.9	6.9	3.5	5.3	5.4	3.9	5.7	5.1	8.7	7.6	3.6	4.9	6.1	4.5	5.0	8.5	6.4	7.1	3.4	6.3	3.5	5.9
720	PS (31:0)	1.5	1.4	1.3	1.9	2.5	2.7	2.2	2.0	3.4	2.4	2.8	1.8	2.7	3.7	3.5	2.4	2.4	2.4	2.3	6.4	2.2	5.5	1.8	2.9
722	UNK	3.3	4	4.1	1.9	2.3	2.5	1.9	1.3	2.3	2.0	1.6	1.5	3.5	2.7	2.6	2.6	2.1	3.5	2.5	3.7	2.8	4.0	2.1	2.9
932	UNK	7	6	8	3.6	4.5	2.8	4.8	3.2	4.6	1.7	3.7	2.7	2.4	3.4	3.5	4.2	5.0	3.1	3.7	3.9	4.6	2.9	7.1	5.9
933	UNK	4	4	4	3.5	2.5	1.5	1.6	1.5	2.8	1.6	1.9	1.8	2.3	3.7	0.9	3.1	4.8	2.2	2.1	2.0	2.8	2.6	4.6	2.9
946	UNK	9	9	9	5.0	2.5	2.8	7.6	2.7	5.1	2.9	5.0	3.3	1.9	4.2	8.7	4.0	5.5	4.6	3.4	6.6	5.3	4.4	6.7	5.9
947	UNK	6	7	6.5	5.6	2.0	2.5	4.1	2.0	3.5	2.1	1.9	2.7	5.8	3.1	4.3	2.1	3.1	1.7	2.5	3.0	2.4	2.6	3.5	3.9
960	UNK	10	11	11	9.0	3.3	3.3	3.8	2.5	5.6	2.3	9.6	7.3	1.5	3.3	6.1	3.5	4.5	4.1	4.3	4.0	3.0	5.9	2.8	4.9
961	UNK	8.7	7.3	8.8	10.2	1.6	2.1	3.5	1.4	2.7	2.1	5.6	3.3	4	5.5	4.3	2.6	3.1	2.8	2.3	2.0	1.8	3.3	1.8	2.9

Key: UNK = unknown; PE = phosphatidylethanolamine; PS = phosphatidylserine; PG = phosphatidylglycerol; S = isolates from University of Manchester Dental Hospital; ATCC 33277^T = *P. gingivalis* type strain; AHN = National Public Health Institute, Helsinki, Finland; RMA = RM Alden Research Laboratory, Santa Monica, UCLA; UQD = University of Queensland, Australia; CC = University of Southern California, School of Dentistry, LA. The peak with m/z 663 can be attributed to a mixture of the anion of PG (28:1) and the first isotope of m/z 662, calculated from known abundance of C^{13}.

(25:2), PE (28:0), PG (26:1), PE (29:0), PE (31:0), PG (29:1), PG (30:1), PG (31:1) and PS (31:0). Peaks below m/z 200 were not recorded because the mass spectrometer was not optimized for very low m/z values. This was because a compromise was required to be able to measure m/z 960 accurately as well as a range of lower mass anions.

Clustering of isolates

The dendrogram was produced by single-linkage clustering of correlation coefficients (*r* values) on the basis of their phospholipid analogue profiles, and revealed the diversity of human *P. gingivalis* strains (Fig. 2). Four distinct clusters were observed. The 'tightest' cluster consisted of all three Manchester isolates (S5, S6 and S7). A second cluster consisted of Finnish isolates AHN 12631 AHN 11190, AHN 19950 and AHN 8448, plus the Santa Monica isolate RMA 4165. The third cluster was comprised of Los Angeles strains CC 870, CC 888, CC A74-128, CC 647 plus Finnish isolate AHN 1885. The fourth cluster consisted solely of the two Australian strains. No discrimination between US strains from different laboratories was observed.

DISCUSSION

Experimental growth conditions were strictly controlled because of the effects that age of culture, pH, temperature and nutrients have on cellular lipid composition (9).

All the human isolates analysed were of biotype 1 (2, 10) and are distinct from the catalase-positive animal isolates designated as biotype 2. This latter biotype has chemical variants that were also correlated with geographical source (6). This study is definitely not a re-classification and if it were a wide range of genetic and phenotypic characters would have been necessary. The study simply seeks to answer the question as to whether phospholipid molecular species distributions in *P. gingivalis* vary with differing geography of isolates.

The carboxylate anion data include peaks shown in previous studies using FAB-MS (11). The major carboxylate anion m/z 241 corresponding to pentadecanoate $C_{15:0}$ is derived from a compound of nominal molecular weight 242 and is consistent with earlier studies on *P. gingivalis* (5, 6, 11). Earlier gas-liquid chromatographic studies have shown that the $C_{15:0}$ carboxylate peak is due to *anteiso*-$C_{15:0}$ (12). One failing of FAB-MS is that it cannot distinguish between carboxylate anions of the same molecular weight because of the lack of ion fragmentation. The peak with m/z 242 is not simply the first isotope (M+1) of pentadecanoate because the proportion (M+1)/M is much higher than the 16.75% expected by theoretical calculation. It may include a lipid secondary ion or an ion derived from small lipophilic molecules. In the case of some isolates, there were intense peaks (Fig. 1) out of range, e.g. m/z 311, 325 and 339. These values can be attributed to the presence of C_{20}, C_{21}, C_{22} carboxylate anions.

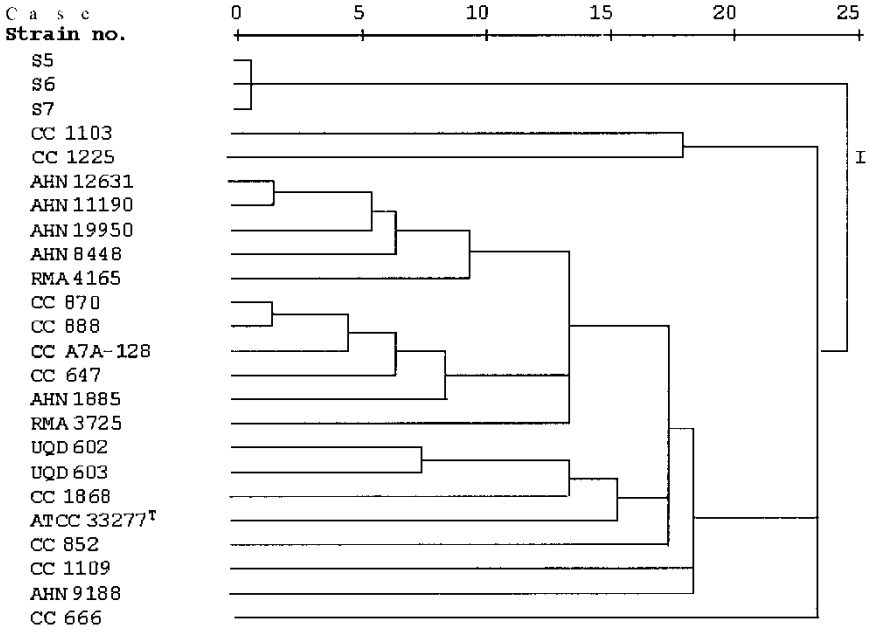

Fig. 2. Comparison of human *P. gingivalis* isolates on the basis of phospholipid analogue distributions. Dendrogram calculated by single-linkage cluster analysis of Pearson coefficients of linear correlation for strain pairs. Key: S = isolates from University of Manchester Dental Hospital; ATCC 33277 = *P. gingivalis* type strain; AHN = National Public Health Institute, Helsinki, Finland; RMA = RM Alden Research Laboratory, Santa Monica, UCLA; UQD = University of Queensland, Australia; CC = University of Southern California, School of Dentistry, LA.

The major families of phospholipids found in the present study, i.e. PE and PG, are entirely consistent with earlier studies (5, 6, 11). PE was also found to be the major type of phospholipid found in *Bacteroides* (13). In contrast, Gram-positive genera examined by FAB-MS mostly contain phosphatidylglycerol molecular species (14).

When phospholipid molecular species distributions were examined, each isolate of *P. gingivalis* analysed in the present study displayed a unique profile. The most intense higher mass peak, at m/z 662, was observed for all isolates in the present study except for the three Manchester isolates and Finnish isolate AHN 1885. This peak has been observed as the predominant phospholipid analogue anion for other groups including *Bacteroides* strains (14), *Capnocytophaga ochracea* (15) and *P. corporis* (16). Isolates from Manchester displayed a major peak at m/z 960. Although this peak was observed in all *P. gingivalis* polar lipid profiles from both this and earlier studies (6, 11), it was not the most intense higher mass peak for all isolates. This unknown phospholipid may correspond to a homologous series (m/z 932, 946 and 960) separated by single methylene groups in an acyl side chain. All studies on phospholipid profiles of *P. gingivalis* have revealed the presence of this homologous series (6). These anions appear to be phospholipids on the basis of their reaction with molybdenum blue reagent when separated by TLC from other lipids present. They must of course be surface active to be detected by FAB-MS, which depends upon congregation of solute at the surface of a matrix for ionization. They are not sphingolipids because of their even m/z value and their much greater TLC Rf value.

The species heterogeneity observed confirms previous findings obtained with FAB-MS (11) and other, genetic, techniques (17, 18). The latter have shown that *P. gingivalis* isolates obtained from different countries display considerable genetic diversity (3, 17). It has also been shown that there is heterogeneity among feline isolates of *P. gingivalis*, on the basis of their phospholipid profiles. Conversely, some strain similarities in phospholipid molecular species distributions were noted which appeared to correlate with geographical source of isolates as they had for biotype 2 strains (6). Understandably, phospholipid profiling failed to discriminate between isolates from within the same locality (isolates from University of Southern California and RMA Santa Monica, UCLA).

It is concluded that heterogeneity exists among human *P. gingivalis* isolates. Furthermore, human *P. gingivalis* isolates show quantitative differences in their phospholipid molecular species distributions that partially correlate with country of isolation. Because the phospholipid profiles were obtained under the same environmental conditions, differences between strains must be genetic. Future work will seek polymorphisms in genes involved in phospholipid synthesis.

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REFERENCES

1. Love DN. Characterization of the catalase of the genus *Porphyromonas* isolated from cats. J Appl Bacteriol 1994; 77: 421–5.
2. Parent R, Mouton C, Lamonde L, Bouchard D. Human and animal serotypes of *Bacteroides gingivalis* defined by crossed immunoelectrophoresis. Infect Immun 1986; 51: 909–18.
3. Menard C, Mouton C. Randomly amplified polymorphic DNA analysis confirms the biotyping scheme of *Porphyromonas gingivalis*. Res Microbiol 1993; 144: 445–55.
4. van Steenbergen TJM, Menard C, Tijhof CJ, Mouton C, de Graaff J. Comparison of three molecular typing methods in studies of transmission of *Porphyromonas gingivalis*. J Med Microbiol 1993; 39: 416–21.
5. Tavana AM, Korachi M, Boote V, Hull PS, Love DN, Drucker DB. Phospholipid analogues of *Porphyromonas gingivalis*. J Appl Microbiol 2000; 88: 791–9.
6. Korachi M, Love DN, Goldstein EJ, Citron DM, Blinkhorn AS, Boote V, Drucker DB. Comparative phospholipid analogue distributions of *Porphyromonas gingivalis* isolated from cats in Australia and the USA. Vet Microbiol 2001; 81: 153–63.
7. Korachi M, Rupnik M, Blinkhorn AS, Boote V, Drucker DB. Comparison of polar lipid profiles of *Clostridium difficile* isolates from different geographical locations. Anaerobe 2002; 8: 35–40.
8. Coykendall AL, Kaczmarek FS, Slots J. Genetic heterogeneity in *Bacteroides asaccharolyticus* (Holdeman and Moore 1970) Finegold and Barnes 1977 (Approved Lists, 1980) and proposal of *Bacteroides gingivalis* sp. nov. and *Bacteroides macacae* (Slots and Genco) comb. nov. Int J Syst Bacteriol 1980; 30: 559–64.
9. Tavana AM, Drucker DB, Boote V. Phospholipid molecular species distribution of *Porphyromonas asaccharolytica* ATCC 25260T: effects of temperature, culture age and pH. J Appl Microbiol 1998; 85: 1029–35.
10. Laliberte M, Mayrand D. Characterization of black-pigmented *Bacteroides* strains isolated from animals. J Appl Microbiol 1983; 55: 247–52.
11. Drucker DB, Tavana AM, Boote V, Love DN. Is *Porphyromonas gingivalis* heterogenous? Rev Med Microbiol 1997; 8: S11–2.
12. Shah HN, Gharbia SE. Biochemical and chemical analyses of black-pigmented Gram-negative anaerobes. FEMS Immunol Med Microbiol 1993; 6: 89–96.
13. Wardle HM, Drucker DB, Joseph LA. Phospholipid molecular species of *Bacteroides*. J Appl Bacteriol 1996; 80: 551–6.
14. Radcliffe CE, Drucker DB, Boote V, Fletcher-Williams G, Claydon MA. Phospholipid analogue profiles of *Peptostreptococcus*, *Micromonas*, and *Finegoldia* species analysed by fast atom bombardment mass spectrometry. Can J Microbiol 2001; 47: 96–101.
15. Drucker DB, Aluyi HS, Boote V, Wilson JM, Ling Y. Polar lipids of strains of *Prevotella*, *Bacteroides* and *Capnocytophaga* analysed by fast atom bombardment mass spectrometry. Microbios 1993; 75: 45–6.

16. Tavana AM, Drucker DB, Hull PS, Boote V. Phospholipid molecular species distribution of oral *P. corporis* clinical isolates. FEMS Immunol Med Microbiol 1998; 21: 57–64.
17. Loos BG, Dyer DW, Whittam T, Selander R. Genetic structure of populations of *Porphyromonas gingivalis* associated with periodontitis and other oral infections. Infect Immun 1993; 61: 204–12.
18. Loos BG, Mayrand D, Genco RJ, Dickinson DP. Genetic heterogeneity of *Porphyromonas* (*Bacteroides*) *gingivalis* by genomic DNA fingerprinting. J Dent Res 1990; 69: 1488–93.