Characterization of *Yersinia* Species by Analysis of Whole-Cell Fatty Acids

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The cellular fatty acid composition of *Yersinia enterocolitica* (50 strains, including all recognized biovars), *Y. pseudotuberculosis* (7 strains), and *Y. pestis* (1 strain) was determined by gas-liquid chromatography. All species showed a generally similar pattern characterized by relatively large amounts of 3-OH-14:0, 16:1, 16:0, 17:0-cyclo, and 18:1. The heterogeneity of *Y. enterocolitica* as revealed by biochemical reactions and by deoxyribonucleic acid-deoxyribonucleic acid hybridization was not found to be reflected in the fatty acid composition. Only small interspecies differences were detected. *Y. enterocolitica* differed slightly from *Y. pseudotuberculosis* by its higher concentrations of 12:0, 16:0, and 17:0 and its lower content of 14:0. *Y. pestis* was characterized by its high content of 18:1, low content of 15:0, and absence of 13:0. A reference group of five *Salmonella typhi* strains showed a fatty acid pattern grossly comparable to that of yersiniae, but the following differences were noted: the presence in *S. typhi* of an additional compound, 2-OH-14:0, relatively high concentrations of 14:0 and 16:0, and low amounts of 15:0, 16:0, and 17:0. Thus, the genus *Yersinia* appears relatively homogeneous with respect to fatty acid composition, and the present results do not support per se a splitting of *Y. enterocolitica* into additional species. Furthermore, the high degree of similarity of the fatty acid patterns of yersiniae and *S. typhi* agrees well with the present placement of *Yersinia* in the family *Enterobacteriaceae*.

The genus *Yersinia* presently is placed in the family *Enterobacteriaceae* and consists of three species: *Yersinia enterocolitica*, *Y. pseudotuberculosis*, and *Y. pestis* (6). Due to the clinical significance of these three species, their taxonomy has been extensively studied (2, 3, 13-15, 19, 21, 24), but some points are still unclear.

Deoxyribonucleic acid (DNA)-DNA hybridization experiments (3, 19) and numerical analysis of biochemical tests (13) define *Y. pseudotuberculosis* and apparently also *Y. pestis* as homogeneous groups compatible with the concept of single species. *Y. enterocolitica* is more heterogeneous, and several subgroups have been proposed mainly on the basis of biochemical characteristics (3, 14, 21, 24). A splitting of this group into two or more species has also been suggested (3, 14).

Regarding interspecies relatedness, the literature is conflicting. On the basis of DNA-DNA hybridization, the taxonomic position of *Y. enterocolitica* seems to be relatively distant from both *Y. pseudotuberculosis* and *Y. pestis* (3, 19). Actually, the genetic affinities between *Y. enterocolitica* and *Y. pseudotuberculosis* strains were found to be so weak that Moore and Brubaker (19) questioned the inclusion of *Y. enterocolitica* in the genus *Yersinia*. The DNA

homology between *Y. pseudotuberculosis* and *Y. pestis* is apparently at a higher level (3, 19). On the other hand, comparative analyses of protein antigens indicated a strong relationship between *Y. enterocolitica* and *Y. pestis* (2), and all three species contain "enterobacterial common antigen" (17, 18).

Fatty acid patterns of bacterial whole cells or extractable lipids have proved to be a useful aid in taxonomic evaluations (1, 8, 12, 16, 22). In this study we wanted to analyze representative strains of *Yersinia* for fatty acid composition. The main objectives were to find out to what extent fatty acid patterns could be used to differentiate *Y. enterocolitica*, *Y. pseudotuberculosis*, and *Y. pestis* and to confirm the reported heterogeneity of *Y. enterocolitica*. Five strains of *Salmonella typhi*, which served mainly as representatives of an unequivocal *Enterobacteriaceae* species, were also examined.

MATERIALS AND METHODS

Bacterial strains. Fifty-eight strains of *Yersinia* and five of *Salmonella typhi* were analyzed (Table 1). H. H. Mollaret, Institut Pasteur, Paris, kindly provided 31 *Y. enterocolitica* strains, whereas the 7 *Y. pseudotuberculosis* strains were a gift from S. Winblad, Malmö, Sweden. One *S. typhi* strain (NCTC 57) and

TABLE 1. *Bacterial strains examined in this study*

Strain	Source	Obtained from:	Serogroup	Biovar ^a				Indole
				W	N	K/T	B	
<i>Y. enterocolitica</i>								
I.P. 6	Chinchilla	Institut Pasteur	1	3	3	1		-
I.P. 8	Hare	Institut Pasteur	2	5	5	4	S-	-
I.P. 85	Pig	Institut Pasteur	3	4	4	1		-
I.P. 96	Chinchilla	Institut Pasteur	4-32	4	4	1		-
I.P. 1476	Water	Institut Pasteur	4-33	1	1	3		+
I.P. 123	Cow	Institut Pasteur	5A	1	1	3		+
I.P. 47	Monkey	Institut Pasteur	5B	4	4	1		-
I.P. 885	Dog	Institut Pasteur	5-27	2	2	2		+
I.P. 102	Human	Institut Pasteur	6-30	1	1	3		+
I.P. 1477	Water	Institut Pasteur	6-31	1	1	3		+
I.P. 106	Guinea pig	Institut Pasteur	7-8	2	1	3		+
I.P. 107	Human	Institut Pasteur	8	2	2	2		+
I.P. 842	Human	Institut Pasteur	8-19	1	1	3		+
I.P. 201	Human	Institut Pasteur	9	2	2	2		+
I.P. 551	Human	Institut Pasteur	10	1	1	3		+
I.P. 105	Human	Institut Pasteur	11-23	2	1	3		+
I.P. 841	Human	Institut Pasteur	11-24	1	1	3		+
I.P. 490	Hare	Institut Pasteur	12-25	5	4 (S-) ^b	4	S-	-
I.P. 103	Sheep	Institut Pasteur	12-26	2	2 (S-)	4	S-	+
I.P. 553	Human	Institut Pasteur	13-7	1	1	3		+
I.P. 480	Human	Institut Pasteur	14	1	1	3		+
I.P. 614	Human	Institut Pasteur	15	4	4	1		-
I.P. 1475	Water	Institut Pasteur	16	3	3	1	S-	-
I.P. 867	Human	Institut Pasteur	16-29	1	2	4	Rh+	+
I.P. 955	Water	Institut Pasteur	17	1	1	4	M+R+Rh+	+
I.P. 846	Human	Institut Pasteur	18	1	1	3		+
I.P. 845	Human	Institut Pasteur	20	2 (L-)	2 (L-)	2		+
I.P. 1110	Human	Institut Pasteur	21	1	2	2		+
I.P. 1367	Human	Institut Pasteur	22	1	2	2		+
I.P. 1474	Water	Institut Pasteur	28	2	2 (S-)	4	S-	+
I.P. 1501	Institut Pasteur		34	1	1	3		+
SIFF 201/75	Water	Our collection	5	2 (L-)	1 (L-)	3		+
SIFF 202/75	Water	Our collection		2 (ODC-)	1 (ODC-)	4	M+R+Rh+	+
SIFF 203/75	Water	Our collection		4 (ODC-)	4 (ODC-)	4	M+R+Rh+	-
SIFF 204/75	Water	Our collection		2 (L-)	1 (L-)	4	M+R+Rh+	+
SIFF 205/75	Water	Our collection		2	1	4	M+R+Rh+	+
SIFF 206/75	Water	Our collection		2	1	4	Rh+	+
SIFF 207/75	Water	Our collection	4-33	2 (L-)	1 (L-)	4	Rh+	+

SIFF 208/75	Water	Our collection	4-33	2	1	4	Rh+	+
SIFF 209/75	Water	Our collection	6-31	1	1	3		+
SIFF 210/75	Water	Our collection	6-31	1	1	3		+
SIFF 211/75	Water	Our collection	6-31	1	1	3		+
SIFF 212/75	Water	Our collection		1	1	3		+
SIFF 213/75	Water	Our collection		2	1	4	Rh+	+
SIFF 214/75	Water	Our collection		2 (L-)	1 (L-)	4	R+Rh+	+
SIFF 215/75	Water	Our collection		2	1	3		+
SIFF 216/75	Water	Our collection		3	3	3 (I-)		+
SIFF 217/75	Water	Our collection		2 (ODC-)	1 (ODC-)	3 (ODC-)		-
SIFF 218/75	Water	Our collection		2 (L-)	1 (L-)	3		+
SIFF 301/75	Rodent	Our collection		1	1	3		+
<i>Y. pseudotuberculosis</i>								
SIFF 402/75		S. Winblad	IA					
SIFF 403/75		S. Winblad	IB					
SIFF 407/75		S. Winblad	IIA					
SIFF 401/75		S. Winblad	IIB					
SIFF 405/75		S. Winblad	III					
SIFF 404/75		S. Winblad	IV					
SIFF 406/75		S. Winblad	V					
<i>Y. pestis</i>								
NCTC 5923		National Collection of Type Cultures, London						
<i>S. typhi</i>								
NCTC 57		National Collection of Type Cultures, London						
SIFF 5924/76		Our collection						
SIFF 7306/76		Our collection						
SIFF 8689/76		Our collection						
SIFF 752/75		Our collection						

^a All strains were tested by Lassen and Kapperud (unpublished data) by the methods of Wauters (24), and the results are presented according to the biovar (= biotype) systems of: W, Wauters (24); N, Nilehn (21); K/T, Knapp and Thal (14); and B, Brenner et al. (3) (atypical strains). Abbreviations: S, sucrose; L, lactose; ODC, ornithine decarboxylase; I, indole; M, melibiose; R, raffinose, and Rh, rhamnose.

^bDeviating test results (Lassen and Kapperud, unpublished data) in brackets.

the single strain of *Y. pestis* were obtained from the National Collection of Type Cultures, London. The remaining strains were from our own collection, and their biochemical properties are indicated in Table 1 (15; Lassen and Kapperud, unpublished data).

Medium. All strains were maintained and cultivated on blood agar base (Oxoid) supplemented with 5% citrated horse serum, pH 7.2. A single batch of medium was used for cultivation. Incubations were performed for 24 h at 37°C. The bacteria were carefully removed from the agar surface by the aid of a phosphate buffer (0.5 M, pH 7.2) and were then centrifuged and washed twice by distilled water before freeze drying. The medium composition and growth conditions were carefully standardized.

Chemicals. Solvents of analytical grade were distilled before use. The standards of fatty acid methyl esters were obtained from Applied Science Laboratories Inc., State College, Pa.

Chemical procedures and gas chromatography. Dried bacterial cells (1 to 10 mg) were methanolized by 2 N HCl in methanol (10, 11). Since the strains of the present study contain cyclopropane fatty acids, which largely are destroyed by acidic methanolysis, a saponification-reesterification procedure was also used (12).

Fatty acid methyl esters were analyzed on a Hewlett-Packard 5750 gas chromatograph, as described previously (10, 11). Two columns, packed with either 10% SE-30 or EGA on Gas Chrom Q (100 to 120 mesh), were used. In the later stage of the study, the EGA column was replaced by a 10% CP-10 on Gas Chrom Q column (Applied Science Laboratories Inc.).

Gas chromatographic peaks were primarily characterized by comparing retention times of unknown peaks to those of fatty acid methyl ester standards. The identities of the cyclopropane fatty acids were confirmed by the reduction procedure of Brian and Gardner (4). For identification, hydroxy fatty acid methyl esters were separated from the other esters by chromatography on silica (20) and were gas chromatographed separately. Generally, the fatty acid identities were confirmed by mass spectrometry, in which a Hewlett-Packard 5992A gas chromatograph-mass spectrometer-computer system equipped with a glass column (200 by 0.2 cm) packed with 1% SE-30 on Gas Chrom Q was used. The quadrupole mass spectrometer was automatically calibrated (AUTOTUNE program) with a perfluorotetrabutylamine (PFTBA) reference compound to a fixed set of mass spectral conditions; the intensity ratio of the peaks at m/e 69, 219, and 502 was adjusted to 100:35:2. The ionization conditions used were 70 eV at 170°C.

Numerical methods. The percent fatty acid composition of each bacterial strain was calculated on the basis of the integrator (Hewlett-Packard 3370B) printouts of two chromatograms. The primary data (percent peak area) were obtained after acidic methanolysis and chromatography on the SE-30 column (see above). These values were then adjusted by the corresponding peak area data obtained by the second chromatographic run, e.g., that of the alkaline methanolysates on the EGA or CP-10 column.

The fatty acid compositions of the 63 strains are not presented. Instead, these values were reduced to mean

values, ranges, and standard errors for each species and certain biovars of *Y. enterocolitica* (Table 2). The evaluation of the taxonomic distances between pairs of groups and species (Table 3) was based on the differences between pairs of corresponding fatty acid mean values and their associated standard errors. Two significance levels of differences were defined for two ($P \sim 0.07$) and three times ($P \sim 0.02$) the levels of standard error, respectively.

A numerical expression of fatty acid-based relatedness was achieved by use of the following similarity index:

$$\frac{1}{n} \sum_{i=1}^n \sqrt{\frac{(\bar{x}_{1i} - \bar{x}_{2i})^2}{(\sigma_{1i} - \sigma_{2i})^2}}$$

$\bar{x}_{1i}$ and $\bar{x}_{2i}$ express the mean fatty acid concentrations of two bacterial groups to be compared, respectively; σ_{1i} and σ_{2i} denote the associated standard deviations; and n is the number of fatty acids, i.e., 16.

RESULTS

As shown in Table 2, all *Yersinia* strains exhibit a similar fatty acid pattern characterized by high levels of the acids 3-OH-14:0, 16:1, 16:0, 17:0-cyclo, and 18:1 (see Table 2 for nomenclature and abbreviations). Also found in almost all strains but in lower and more variable concentrations were 12:0, 3-OH-13:0, 14:0, 15:0, 17:0, and 19:0-cyclo fatty acids.

All strains contained hydroxy- and cyclopropane fatty acids in addition to the straight-chain saturated and unsaturated acids. These two classes of fatty acids have been shown to be interesting chemotaxonomic markers of bacteria (16, 22). However, cyclopropane fatty acids are degraded by methanolic hydrochloride and require alkaline conditions for methyl ester formation (4, 23). Optimal yield of hydroxy fatty acids, on the other hand, is apparently dependent on an acidic catalyzed methanolysis (12). Accordingly, all strains were methanolized under both acidic and alkaline conditions.

The biosynthesis of cyclopropane fatty acids tends to be especially dependent on growth conditions and the stage of the life cycle of the cells (7); this is probably the reason for the relatively high variation of 17:0-cyclo fatty acid, as well as its precursor 16:1 (7), observed for all species (Table 2). The quantitative value of these two acids is therefore questionable in taxonomic evaluations. On the other hand, their sum showed a more normal variation, with a standard error below 3% (Table 2).

The presence of hydroxy fatty acids appeared to be of particular taxonomic interest (see below). Their occurrence was, therefore, determined by separate analysis, which included preparative thin-layer chromatography (20) and subsequent gas chromatography-mass spec-

TABLE 2. Composition of fatty acids from *Yersinia* species and *S. typhi*

Fatty acid ^a	<i>Y. enterocolitica</i>		<i>Y. pseudo-tuberculosis</i> (7 strains)	<i>Y. pestis</i> (1 strain)	<i>S. typhi</i> (5 strains)
	Biovars 1 and 2 (39 strains)	Biovars 3, 4, and 5 (11 strains)			
12:0	2.3 ^b	2.2	1.2	1.0	0.9
	1.5-3.3 ^c	1.3-3.3	0.6-2.6		0.1-1.6
	0.08 ^d	0.23	0.27		0.25
13:0	0.5	0.4	0.3	— ^e	0.2
	0.1-1.0	0.1-0.8	0.1-0.5		0.1-0.4
	0.04	0.08	0.06		0.09
3-OH-13:0	1.1	1.4	1.7	0.5	0.1
	0.2-2.1	0.3-2.6	1.0-2.0		0-0.2
	0.06	0.19	0.14		0.04
14:0	0.7	1.1	0.4	0.4	4.5
	0.4-1.7	0.5-1.8	0.2-0.4		3.9-5.0
	0.04	0.15	0.03		0.20
2-OH-14:0	—	—	—	—	0.4 0.2-0.5 0.17
3-OH-14:0	9.7	10.5	9.5	10.1	11.4
	8.0-12.2	9.2-11.9	9.2-10.3		10.9-12.6
	0.13	0.33	0.22		0.32
15:0	5.7	6.2	7.0	1.7	1.4
	1.4-9.3	1.4-11.9	5.0-8.0		0.7-2.7
	0.32	1.02	0.44		0.34
16:1	22.2	25.6	23.4	25.5	8.5
	14.3-29.1	20.8-28.2	10.6-28.3		3.8-17.8
	0.59	0.95	2.36		2.90
16:0	20.5	20.6	16.5	21.9	34.9
	15.6-30.6	13.5-29.2	14.7-19.8		31.9-36.8
	0.48	1.58	0.64		1.05
17:0-cyclo	16.2	11.8	17.4	13.9	18.5
	9.1-22.7	4.9-16.2	12.2-27.4		8.7-24.0
	0.58	1.06	2.03		3.41
17:0	3.8	4.2	6.9	2.1	0.8
	0.7-5.4	0.7-7.0	4.4-8.1		0.2-1.7
	0.17	0.63	0.51		0.25
18:2	5.3	4.0	4.6	2.1	5.5
	2.8-7.4	2.6-5.6	3.5-9.2		1.9-7.8
	0.19	0.28	0.85		1.43
18:1	11.3	11.4	9.8	20.6	12.8
	8.8-13.6	9.6-12.5	5.3-12.4		8.0-19.4
	0.20	0.34	0.90		2.51
18:0	0.6	0.5	0.4	1.4	0.9
	0.1-1.3	0.1-1.0	0.1-0.7		0.1-1.7
	0.05	0.1	0.11		0.31
19:0-cyclo	0.2	0.1	0.6	0.3	0.2
	0-0.6	0-0.3	0-2.9		0.1-0.2

TABLE 2—continued

Fatty acid ^a	<i>Y. enterocolitica</i>		<i>Y. pseudotuberculosis</i> (7 strains)	<i>Y. pestis</i> (1 strain)	<i>S. typhi</i> (5 strains)
	Biovars 1 and 2 (39 strains)	Biovars 3, 4, and 5 (11 strains)			
	0.05	0.04	0.42		0.04
16:1 +	38.4	37.4	40.6		27.0
17:0-cyclo	33.5–40.8	35.4–39.4	38.0–42.6	39.4	24.7–28.3
	0.22	0.43	0.72		0.66

^a The figure in front of the colon indicates the number of carbon atoms in the chain, and the figure after the colon indicates the number of double bonds; 2-OH and 3-OH indicate hydroxyl groups and their positions. The term "cyclo" refers to a cyclopropane ring in the chain.

^b Mean value of the relative amount (percentage of total) of fatty acids.

^c Range among strains.

^d Standard error.

^e —, Below the limit of detection

trometry. These analyses were performed for two strains of each species (one of *Y. pestis*). In general, the results confirmed the identification obtained by gas-liquid chromatography (GLC) retention times on unfractionated whole-cell methanolsates.

All biovars included in the study were members of *Y. enterocolitica* (Table 1). None of the biochemical distinctions of the biovars was clearly reflected in the fatty acid patterns of the strains. Also, when indole-positive strains (biovars 1 and 2, according to Wauters) were compared with indole-negative strains (biovars 3, 4, and 5), almost negligible differences were noted (Table 3). Furthermore, no clear correlation between source of origin (Table 1) and fatty acid composition could be seen.

The differences between *Y. enterocolitica* and *Y. pseudotuberculosis* are also small (Tables 2 and 3). *Y. enterocolitica* contained slightly higher concentrations of 12:0, 14:0, and 16:0 and a lower concentration of 17:0. Only one strain of *Y. pestis* was analyzed. This strain differed from the other yersiniae by its lack of 13:0, its low concentration of 15:0, and its high concentration of 18:1.

The taxonomically distant group of *S. typhi* strains showed a fatty acid composition of pattern grossly similar to that of yersiniae (Table 2), but there were several marked differences in concentration. Thus, the *S. typhi* strains contained the additional compound 2-OH-14:0 and relatively large amounts of 14:0 and low amounts of 16:1 and 17:0 fatty acids.

DISCUSSION

Only a few strains of *Yersinia* have previously been examined for cellular fatty acids. Tornabene et al. (9, 23) found 16:1, 16:0, and 17:0-cyclo to be the principal acids of extractable lipids and

3-OH-14:0 to be the principal acid of the somatic lipopolysaccharides isolated from *Y. pseudotuberculosis* and *Y. pestis*; these four acids have also been found to be characteristic of other members of *Enterobacteriaceae* (16, 22). As shown in Table 2, these and some additional acids are characteristic of *Y. enterocolitica*. This species exhibits a fatty acid composition very similar to that of *Y. pseudotuberculosis* and of most enterobacterial groups (unpublished results).

The degree of intraspecies homogeneity within *Y. enterocolitica* is presently unclear. In terms of biochemical profiles, the species contains several distinct groups (biovars). DNA-DNA hybridization experiments have also revealed distinct groups. Brenner et al. (3) found biovars (= biotypes) 1, 2, and 3 of Knapp and Thal (15; Table 1) to belong to the same DNA relatedness group, whereas biovar 4 (atypical strains) formed two or three separate relatedness groups different from that of typical *Y. enterocolitica* strains. Moore and Brubaker (19), on the other hand, found high relatedness values among strains of *Y. enterocolitica* by use of the same technique, but on a more restricted number of strains. They stated that *Y. enterocolitica* constitutes a valid species, at least with respect to biovars 1 through 4 of Wauters (24; Table 1).

This biochemical or DNA-DNA hybridization-related heterogeneity within *Y. enterocolitica* was not reflected in the fatty acid composition. For example, only minute differences, if any, in fatty acid composition could be observed between the individual biovars and the indole-positive and -negative strains as well (Table 3). Thus, fatty acid composition is unlikely to be of value in resolving intraspecies heterogeneity.

The three *Yersinia* species studied exhibited fatty acid patterns which were only slightly dif-

TABLE 3. Significant fatty acid differences between species/biovars^a

Pairs of biovars or species compared ^b	12:0 ^c	13:0	3-OH-13:0	14:0	2-OH-14:0	3-OH-14:0	15:0	16:1	16:0	17:0-cyclo	18:2	18:1	18:0	19:0-cyclo	16:1 + 17:0-cyclo	Similarity index ^d
<i>Y. enterocolitica</i> biovar 1 versus <i>Y. enterocolitica</i> biovar 2			*				*									0.07
<i>Y. enterocolitica</i> biovar 1+2 versus <i>Y. enterocolitica</i> biovar 3+4+5				*				*		*						0.08
<i>Y. enterocolitica</i> versus <i>Y. pseudotuberculosis</i>	**	*	*	**				**	**	**				*		0.15
<i>Y. enterocolitica</i> versus <i>Y. pestis</i>	**	**										**	**	**		
<i>Y. pseudotuberculosis</i> versus <i>Y. pestis</i>		**					**		**	**	**	**	**			
<i>S. typhi</i> versus <i>Y. enterocolitica</i>	**	*	**	**	**	**	**	**	**	**	**	**	**		**	0.47
<i>S. typhi</i> versus <i>Y. pseudotuberculosis</i>		**	**	**	**	**	**	*	**	**	**				**	0.74
<i>S. typhi</i> versus <i>Y. pestis</i>		**		**	**	**		**	**	**	**	**	**		**	

^a * A difference two times its standard error; ** a difference three times its standard error.

^b The biovars (= biotypes) indicated are those of Wauters (24). Biovars 1, 2, 3, 4, and 5 include 19, 20, 3, 6, and 1 strains, respectively. Only one strain of *Y. pestis* was analyzed. Significant differences between this species and the others were defined as those of fatty acid concentrations which fell outside the range of the corresponding fatty acid concentrations of the compared species.

^c See Table 2 for fatty acid nomenclature and abbreviations.

^d The formulae used for determining the similarity indexes are given in the text.

ferent from each other. The smallest deviations were observed between *Y. enterocolitica* and *Y. pseudotuberculosis*, whereas the single strain of *Y. pestis* was the most different from the other yersiniae. Since only one member of *Y. pestis* was analyzed, however, the taxonomic significance of this difference is questionable. Thus, the genus *Yersinia* appears to be homogeneous, and the fatty acid data on the yersiniae do not reflect the apparent taxonomic distance between *Y. enterocolitica* and *Y. pseudotuberculosis* as observed by DNA-DNA hybridization experiments (3, 19).

A reference group of five strains of *Salmonella typhi* was included in the study to determine the taxonomic position of *Yersinia* relative to another group of *Enterobacteriaceae*. Although the overall fatty acid pattern of *S. typhi* was found to be grossly comparable to that of *Yersinia*, several distinct differences were detected (Table 3). Of these, the presence of 2-OH-14:0 in *S. typhi* is interesting. This hydroxy fatty acid occurs together with the considerably more abundant isomer 3-OH-14:0 as a constituent of the endotoxin lipopolysaccharide and appears to be a stable taxonomic marker of the genera *Salmonella*, *Klebsiella*, and *Serratia* but appears to be lacking in *Escherichia*, *Enterobacter*, *Shigella*, and *Proteus* (5; unpublished data). These observations could indicate a closer relationship of *Yersinia* to the latter group of genera than to the former.

Compared with *Pasteurella multocida*, the fatty acid composition of *Yersinia* is markedly different. *P. multocida* lacks cyclopropane fatty acids and exhibits quite different concentrations of saturated and unsaturated straight-chain acids (unpublished results). Thus, the transfer of *Y. pseudotuberculosis* and *Y. pestis* from the genus *Pasteurella* to a genus of the family *Enterobacteriaceae* (6) appears justified on the basis of fatty acid composition.

REPRINT REQUESTS

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LITERATURE CITED

- Abel, K., H. DeSmertzing, and J. I. Peterson. 1963. Classification of microorganisms by analysis of chemical composition. I. Feasibility of utilizing gas chromatography. *J. Bacteriol.* **85**:1039-1044.
- Barber, C., and E. Eylan. 1976. Immunochemical relations of *Yersinia enterocolitica* with *Yersinia pestis* and their connection with other *Enterobacteriaceae*. *Microb. Lett.* **3**:25-29.
- Brenner, D. J., A. G. Steigerwalt, D. P. Falcão, R. E. Weaver, and G. R. Fanning. 1976. Characterization of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* by deoxyribonucleic acid hybridization and by chemical reactions. *Int. J. Syst. Bacteriol.* **26**:180-194.
- Brian, B. L., and E. W. Gardner. 1968. A simple procedure for detecting the presence of cyclopropane fatty acids in bacterial lipids. *Appl. Microbiol.* **16**:549-552.
- Bryn, K., and E. Rietschel. 1978. L-2-Hydroxytetradecanoic acid as a constituent of *Salmonella* lipopolysaccharides (Lipid A). *Eur. J. Biochem.* **86**:311-315.
- Buchanan, R. E., and N. E. Gibbons (ed.). 1974. *Bergey's manual of determinative bacteriology*, 8th ed. The Williams and Wilkins Co., Baltimore.
- Cronan, J. E., Jr. 1968. Phospholipid alterations during growth of *Escherichia coli*. *J. Bacteriol.* **95**:2054-2061.
- Drucker, D. B. 1977. Gas-liquid chromatographic chemotaxonomy, p. 51-125. In J. R. Norris and D. W. Ribbons (ed.), *Methods in microbiology*, vol. 9. Academic Press Inc., London.
- Hartley, J. L., G. A. Adams, and T. G. Tornabene. 1974. Chemical and physical properties of lipopolysaccharide of *Yersinia pestis*. *J. Bacteriol.* **118**:848-854.
- Jantzen, E., K. Bryn, T. Bergan, and K. Bøvre. 1974. Gas chromatography of bacterial whole cell methanolysates. V. Fatty acid composition of neisseriae and moraxellae. *Acta Pathol. Microbiol. Scand. Sect. B* **82**:767-779.
- Jantzen, E., K. Bryn, and K. Bøvre. 1974. Gas chromatography of bacterial whole cell methanolysates. IV. A procedure for fractionation and identification of fatty acids and monosaccharides of cellular structures. *Acta Pathol. Microbiol. Scand. Sect. B* **82**:753-766.
- Jantzen, E., K. Bryn, N. Hagen, T. Bergan, and K. Bøvre. 1978. Fatty acids and monosaccharides of *Neisseriaceae* in relation to established taxonomy. *NIPH Ann. (Oslo)* **1**:59-71.
- Johnson, R., R. R. Colwell, R. Sakazaki, and K. Tamura. 1975. Numerical taxonomy study of the *Enterobacteriaceae*. *Int. J. Syst. Bacteriol.* **25**:12-37.
- Knapp, W., and E. Thal. 1973. Differentiation of *Yersinia enterocolitica* by biochemical reactions. *Contrib. Microbiol. Immunol.* **2**:10-16.
- Lassen, J. 1972. *Yersinia enterocolitica* in drinking water. *Scand. J. Infect. Dis.* **4**:125-127.
- Lechevalier, M. P. 1977. Lipids in bacterial taxonomy—a taxonomists view. *CRC Crit. Rev. Microbiol.* **5**:109-210.
- Le Minor, C., A.-M. Chalon, and M. Véron. 1972. Recherches sur la présence de l'antigène commun des "Enterobacteriaceae" (antigène Kunin) chez les "Yersinia," "Levinea," "Aeromonas" et "Vibrio." *Ann. Inst. Pasteur (Paris)* **123**:761-774.
- Maeland, J. A., and A. Digranes. 1975. Common enterobacterial antigen in *Yersinia enterocolitica*. *Acta Pathol. Microbiol. Scand. Sect. B* **83**:382-386.
- Moore, R. L., and R. R. Brubaker. 1975. Hybridization of deoxyribonucleotide sequences of *Yersinia enterocolitica* and other selected members of *Enterobacteriaceae*. *Int. J. Syst. Bacteriol.* **25**:336-339.
- Morris, L. J., H. Hayes, and R. T. Holman. 1961. Naturally-occurring epoxy acids. III. Methods for their isolation. *J. Am. Oil Chem. Soc.* **38**:316-321.
- Nilehn, B. 1969. Studies on *Yersinia enterocolitica*. *Acta Pathol. Microbiol. Scand. Suppl.* **206**:1-48.
- Shaw, N. 1974. Lipid composition as a guide to the classification of bacteria. *Adv. Appl. Microbiol.* **17**:63-108.
- Tornabene, T. G. 1973. Lipid composition of selected strains of *Yersinia pestis* and *Yersinia pseudotuberculosis*. *Biochim. Biophys. Acta* **306**:173-185.
- Wauters, G. 1973. Correlation between ecology, biochemical behavior and antigenic properties of *Yersinia enterocolitica*. *Contrib. Microbiol. Immunol.* **2**:38-41.