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Comparison of *Yersinia pestis* to other closely related *Yersinia* species using fatty acid profiles

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ABSTRACT

Capillary gas chromatography with flame ionisation detection (GC–FID) was used to determine the cellular fatty acid (CFA) profiles of six *Yersinia pestis* strains. The profiles were then compared with the CFA profiles of other closely related *Yersinia* species including: *Y. pseudotuberculosis*, *Y. enterocolitica, Y. intermedii, Y. kristensenii* and *Y. frederiksenii*. For GC–FID analysis, whole cell fatty acid methyl esters (FAMEs) from cells cultured on brain–heart infusion (BHI) agar at 35 °C for 24 h were obtained by saponification, methylation and extraction into hexane/methyl *tert*-butyl ether. A data set for each *Yersinia* species was prepared using fatty acid profiles from five replicates prepared on different days. Major fatty acids of the 26 *Yersinia* strains evaluated in this study were straight-chain 12:0, 14:0, 15:0, 16:0 and unsaturated summed 16:1 ω7c/16:1 ω6c, 18:1 ω7c, and summed 14:0 3OH/16:1 iso, and 17:0 ω cyclo 7–8. The CFA profiles for *Y. pestis* and *Y. pseudotuberculosis* are similar, but there are several fatty acids, 16:1 ω5c, 16:0, 17:1 ω7c, 17:0 ω cyclo 7–8, 19:0 and summed 18:2 ω6c, 9c/18:0 ante, that differ significantly between these two species. Analysis of FAMEs from *Yersinia* strains grown on BHI agar by a rapid GC–FID method can provide a sensitive procedure for the identification of these organisms, and this analytical method provides a procedure for the differentiation of *Y. pestis* strains from closely related *Yersinia* species.

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1. Introduction

Yersinia is a Gram-negative, rod-shaped bacteria of the family *Enterobacteriaceae*. The genus *Yersinia* includes 11 species, three of which are potentially pathogenic to humans: *Yersinia enterocolitica*, *Y. pseudotuberculosis* and *Y. pestis* (Perry & Fetherston, 1997). In the US, most human illnesses are caused by *Y. enterocolitica*, however, many *Y. enterocolitica* isolates have been found not to be pathogenic (US Food and Drug Administration (FDA), 1991). Infection may occur by consumption of contaminated food products such as meat, vegetables, and milk-derived products (FDA, 1991, 2001, Chap. 8). Symptoms of infection with *Y. enterocolitica* include fever, abdominal pain and diarrhoea. *Y. pseudotuberculosis* is the least common species of *Yersinia* causing disease in humans.

The taxonomy of *Yersinia* was revolutionized in 1976 by Brenner, Steigerwalt, Falxo, Weaver, and Fanning (1976) who used DNA–DNA hybridization and biochemical tests for the classification of *Y. enterocolitica* and *Y. pseudotuberculosis* (Brenner et al., 1976). As a result of these studies, three new *Yersinia* species were identified, *Y. frederiksenii*, *Y. intermedii* and *Y. kristensenii* (Bercovier et al., 1980; Brenner et al., 1980; Ursing et al., 1980). Loftus, Harewood, Cockerill, and Murray (2002) have described the clinical features of patients infected with these *Yersinia* species and found the most common symptom to be diarrhoea. Between 1985 and 1999, 194 patients had *Yersinia* species isolated from stool specimens (Loftus et al., 2002). They concluded that *Y. frederiksenii*, *Y. intermedii* and *Y. kristensenii* may represent up to 20% of all *Yersinia* isolates (Loftus et al., 2002).

Y. pestis was discovered in 1894 by Alexandre Yersin during an epidemic of plague in Hong Kong (Bendiner, 1989; Yersin, 1894). *Y. pestis* has undergone several nomenclature changes since being named *Pasteurella pestis* after Yersin's mentor (Bendiner, 1989). Humans may develop plague through the bite of infected fleas, direct contact with contaminated tissue, or inhalation of the bacterium. Human plague is usually present as bubonic, septicaemic, and pneumonic forms (Perry & Fetherston, 1997). Pneumonic plague is the most deadly form of the disease and the most rapidly transmitted. It would most likely be the form found if *Y. pestis* were used in a bioterrorism event.

The first study to analyse whole cell fatty acid concentrations for three *Yersinia* species was reported by Jantzen and Lassen (1980) of Norway. In the current study, a rapid GC–FID method was used to determine whether it could identify *Yersinia* strains and whether *Y. pestis* could be differentiated from closely related *Yersinia* species.

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2. Materials and methods

2.1. Bacterial agents and growth conditions

Twenty-six *Yersinia* strains from an FDA collection were analysed, including *Y. enterocolitica* (5 strains), *Y. pseudotuberculosis* (6 strains), *Y. frederiksenii* (2 strains), *Y. intermedii* (4 strains), *Y. kristensenii* (3 strains) and *Y. pestis* (6 vaccine strains) (Table 1). All bacteria were grown on brain–heart infusion (BHI) agar (Difco, Detroit, MI, USA) and were initiated from frozen stocks. The growth medium was prepared with 52 g BHI agar/l distilled water, pH 7.0. Twenty millilitres of medium was added to each 100 mm petri plate. All bacteria were incubated at 35 ± 1 °C for 24 h.

2.2. Chemical procedures and gas chromatography analysis

For gas chromatography with flame ionisation detection (GC–FID) analysis, bacterial cells were harvested from the culture plates. Whole cell fatty acid methyl esters (FAMEs) were prepared by saponification, methylation and extraction into hexane/methyl *tert*-butyl ether. Using a sterile disposable wooden stick, ~25 mg of bacterial cells were harvested from the culture plates and placed in sterile 13 × 100 mm tubes. One millilitre of 3.75 mol/l NaOH (1:1, methanol/distilled water) was added to each tube containing the bacteria to saponify the fatty acids. The tubes were heated in a boiling water bath for 5 min, vortexed, heated for an additional 30 min in a boiling water bath, and then cooled in tap water. Two millilitres of 3.25 mol/l HCl (1:1.18, methanol/6 mol/l HCl) was added for methylation of the fatty acids, and the tubes were heated for 10 min at 80 °C. The tubes were cooled and the FAMEs were extracted by addition of 1.25 ml of 1:1 hexane/methyl *tert*-butyl ether with gentle tumbling for 10 min. The lower phase was removed, and 3.0 ml of 0.3 mol/l NaOH was added to the organic phase as a base wash and tumbled for an additional 5 min. The organic phase was then removed for GC analysis. The FAMEs were analysed by GC using the rapid Microbial Identification System (MIS, MIDI Inc., Newark, DE, USA) software (RCLN50 and RBTR20) to identify the relative amounts of fatty acids in the bacteria. Results were expressed as a percentage of the total fatty acids. The GC used was an Agilent 6890 with a flame ionisation detector and an Agilent auto sampler and injector (Agilent 7683) (Agilent Technologies, Palto Alto, CA, USA). A 25 m (length) × 0.2 mm ID × 0.33 μm film thickness, cross-linked 5% phenylmethyl silicone fused silica capillary column (Agilent 19091B-102) was used to separate the fatty acids. Operating conditions were as follows: initial temperature was 170 °C and was increased at a rate of 28 °C /min to 288 °C and then increased to 310 °C at 60 °C/min and held for 1.25 min. Hydrogen was used as the carrier gas at a constant flow rate of 1.3 ml/min. A calibration analysis was used for the first two injections of every sequence and was automatically reanalysed after every 11th sample injection using calibration standard no. 1300-AA (Microbial ID, Inc., Newark, DE, USA). The similarity index (SI) is a numerical value that expresses how closely the fatty acid composition of an unknown compares with the mean fatty acid composition of the strains used to create the library entry. As each fatty acid varies from the mean percentage, the SI will decrease in proportion to the cumulative variance between the composition of the unknown and the library entry (Sasser, 1997). Samples with a similarity of 0.500 or higher with a separation of 0.100 between the first and second choice are considered good library comparisons.

Table 1
Bacterial agents analysed by gas chromatography.

Microorganism	Strain designation
Yersinia enterocolitica	Y2
	Y38
	Y105
	Y106
	Y120
Y. pseudotuberculosis	Y188
	Y199
	Y270
	Y281
	Y283
	2390
Y. frederiksenii	Y225
	Y226
Y. intermedii	Y227
	Y228
	Y229
	2393
Y. kristensenii	Y231
	Y232
	Y233
Y. pestis	2388
	2389
	D27
	D27X
	D28
	D28X

2.3. Statistical analysis

Differences in fatty acids amongst bacterial strains were assessed by analysis of variance (ANOVA) (Snedecor & Cochran, 1980). Values are expressed as means with their standard deviations. The Duncan multiple comparison method was used to differentiate amongst means for variables that were significantly different (Snedecor & Cochran, 1980).

3. Results and discussion

GC–FID analysis of chemical components of bacterial cells has provided useful information for rapid detection and identification of bacteria in clinical and diagnostic bacteriology laboratories and currently has increased significance for both food safety and security (Daneshvar, Douglas, & Weyant, 2001; Whittaker, Day, Curtis, & Fry, 2007; Whittaker et al., 2005). In this study, the cellular fatty acid (CFA) profiles of *Y. pestis* strains were compared to the CFA profiles of five closely related *Yersinia* species. Fig. 1 is an example of a chromatogram for a strain of *Y. pestis* (strain # 2389).

A database for the six *Yersinia* species was prepared using fatty acid profiles from the strains listed in Table 1. The CFA profiles for the *Yersinia* species are shown in Table 2. The *Yersinia* species analysed have a similar fatty acid pattern. The major fatty acids for distinguishing the strains evaluated in this study were straight-chain 12:0, 14:0, 15:0, 16:0, unsaturated summed 16:1 ω7c/16:1 ω6c, 18:1 ω7c, summed 14:0 3OH/16:1 iso, and 17:0 ω cyclo 7–8, indicating Gram-negative bacteria. The highest concentrations were found for 16:0 fatty acid, followed by 17:0 ω cyclo 7–8 for all of the *Yersinia* species analysed. Similar results showing that *Yersinia* species have analogous fatty acid patterns have been reported (Leclercq, Guiyoule, El Lioui, Carniel, & Decallonne, 2000; Leclercq, Wauters, Decallonne, El Lioui, & Vivegnis, 1996). Results from previous studies (Jantzen & Lassen, 1980; Leclercq et al., 1996; Leclercq et al., 2000) also showed 12:0, 14:0, 14:0 3OH, 16:0, 16:1, 17:0 cyclo and 18:1 as major fatty acids with 16:0 and 17:0 cyclo having the highest concentrations. These studies (Jantzen & Lassen, 1980; Leclercq et al., 1996; Leclercq et al., 2000) used different growth conditions including time and temperature of incubation, and type of media, which would result in differences in concentrations of fatty acids. Leclercq et al. (1996) evaluated the pathogenic-

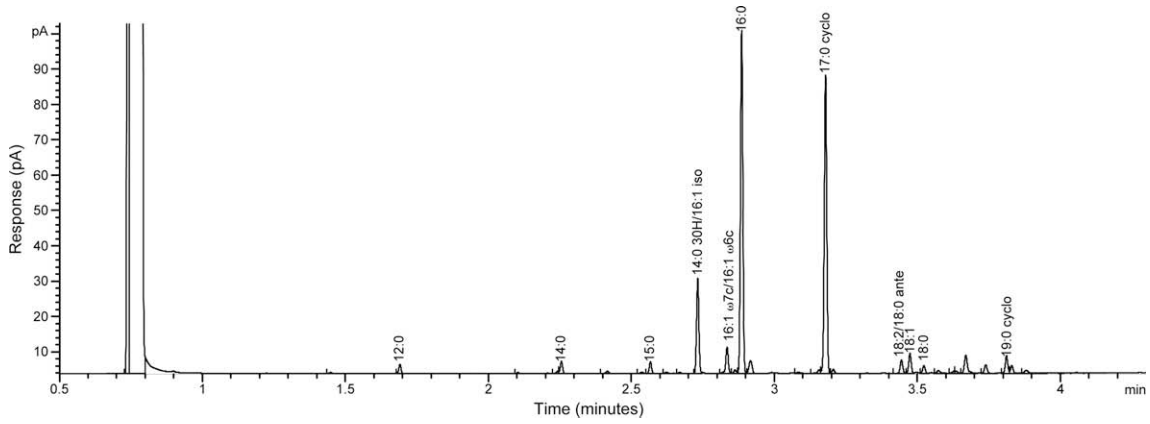

Fig. 1. Chromatogram for a strain of *Yersinia pestis* (strain # 2389).

Table 2
Comparison of cellular fatty acid profiles for various strains of *Yersinia*.

Cellular Fatty Acid	*Y. pestis* (%)	*Y. pseudotuberculosis* (%)	*Y. enterocolitica* (%)	*Y. intermedii* (%)	*Y. kristensenii* (%)	*Y. frederiksenii* (%)	*P*
12:0	1.05 ± 0.16[*,a]	1.02 ± 0.28[a]	3.80 ± 0.32[b]	6.65 ± 0.60[c]	6.50 ± 0.39[c]	6.64 ± 0.31[c]	<0.001
13:0	0.00 ± 0.00[a]	0.03 ± 0.05[a]	0.18 ± 0.14[b]	0.51 ± 0.11[c]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	<0.001
12:0 3OH	0.08 ± 0.07[b]	0.07 ± 0.07[b]	0.00 ± 0.00[a]	0.05 ± 0.04[b]	0.02 ± 0.03[a]	0.47 ± 0.07[c]	<0.001
Unknown13.951	0.26 ± 0.06[a]	0.29 ± 0.12[a]	0.56 ± 0.06[b]	0.73 ± 0.08[d]	0.70 ± 0.05[c,d]	0.64 ± 0.03[c]	<0.001
14:0	1.48 ± 0.14[a]	1.53 ± 0.38[a]	4.82 ± 0.51[d]	1.84 ± 0.13[b]	2.75 ± 0.13[c]	2.60 ± 0.26[c]	<0.001
Unknown 14.502	0.31 ± 0.13[a]	0.35 ± 0.19[a]	0.86 ± 0.11[c]	1.07 ± 0.11[d]	0.79 ± 0.04[c]	0.66 ± 0.23[b]	<0.001
15:0	1.43 ± 0.71[b]	1.29 ± 1.25[b]	2.53 ± 1.47[c]	3.93 ± 0.98[d]	0.49 ± 0.09[a]	0.13 ± 0.09[a]	<0.001
14:0 2OH	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.12 ± 0.20[b]	0.00 ± 0.00[a]	0.17 ± 0.01[b]	0.13 ± 0.05[b]	<0.001
16:1 ω5c	0.48 ± 0.05[d]	0.31 ± 0.06[b]	0.35 ± 0.03[c]	0.53 ± 0.04e	0.26 ± 0.01[a]	0.26 ± 0.04[a]	<0.001
16:0	36.29 ± 1.17[c]	38.87 ± 2.88[d]	34.83 ± 1.89[b]	30.49 ± 0.63[a]	36.60 ± 0.94[c]	39.26 ± 1.02[d]	<0.001
17:0 Iso	0.06 ± 0.10	0.04 ± 0.08	0.08 ± 0.10	0.06 ± 0.07	0.07 ± 0.07	0.09 ± 0.11	0.672
17:1 ω7c	0.41 ± 0.15[b]	0.30 ± 0.17[a]	0.35 ± 0.03[b]	0.32 ± 0.14[b]	0.22 ± 0.12[a]	0.22 ± 0.15[a]	<0.001
17:0 ω Cyclo 7-8	32.16 ± 1.86[d]	28.50 ± 4.36[c]	29.13 ± 1.29[c,d]	20.09 ± 1.99[a]	23.36 ± 1.23[b]	28.58 ± 0.50[c]	<0.001
17:0	0.54 ± 0.19[c]	0.72 ± 0.71[c]	1.13 ± 0.50[d]	1.52 ± 0.39e	0.17 ± 0.04[b]	0.06 ± 0.07[a]	<0.001
18:1 ω7c	3.21 ± 0.92[c,d]	3.66 ± 1.32[d]	2.14 ± 1.02[a]	5.62 ± 0.32e	2.85 ± 0.18[b,c]	2.47 ± 0.44[a,b]	<0.001
18:0	0.74 ± 0.12[c]	0.76 ± 0.17[c,d]	0.60 ± 0.16[b]	0.85 ± 0.17[d]	0.46 ± 0.13[a]	1.25 ± 0.32e	<0.001
19:0 Cyclo ω8c	1.38 ± 0.62[c]	1.25 ± 0.78[c]	1.30 ± 0.57[c]	0.68 ± 0.25[b]	0.28 ± 0.05[a]	1.59 ± 0.08[c]	<0.001
19:0	0.89 ± 0.17[d]	0.77 ± 0.15[c]	0.70 ± 0.10[c]	0.49 ± 0.15[a]	0.48 ± 0.05[a]	0.62 ± 0.04[b]	<0.001
18:1 2OH	0.16 ± 0.22[b]	0.21 ± 0.21[b]	0.18 ± 0.20[b]	0.01 ± 0.04[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	<0.001
Summed 14:0 3OH/16:1 iso	11.30 ± 0.98[c]	10.97 ± 1.34[c]	10.69 ± 0.69[c]	9.16 ± 0.74[a]	9.89 ± 0.51[b]	9.69 ± 0.57[a,b]	<0.001
Summed 16:1 ω7c/16:1 ω6c	6.14 ± 2.47[b]	7.60 ± 4.25[b]	4.36 ± 1.88[a]	14.18 ± 2.35[d]	12.34 ± 1.03[c]	3.44 ± 0.54[a]	<0.001
Summed 18:2 ω6, 9c/18:0 ante	1.55 ± 0.25[c]	1.37 ± 0.18[d]	1.23 ± 0.17[c]	0.85 ± 0.23[a]	0.86 ± 0.12[a]	1.10 ± 0.10[b]	<0.001
Similarity Index	0.684 ± 0.075	0.655 ± 0.068	0.674 ± 0.115	0.890 ± 0.073	0.796 ± 0.051	0.777 ± 0.023	
n	30	30	25	20	15	10	

[*] Mean ± standard deviation. Means for a variable not sharing a common superscript letter are significantly different ($P < 0.05$) as determined by the Duncan multiple comparison method, which was applied only if significant differences were determined to exist by ANOVA.

ity of *Yersinia* strains by determining the ratio of fatty acids 12:0 to 14:0 and determined that potentially pathogenic strains had a significantly lower ratio. The 12:0/14:0 ratio's found in the present study for *Y. enterocolitica*, *Y. pseudotuberculosis*, and *Y. pestis* are similar to the ratio reported by Leclercq et al. (2000). Nagamachi et al. (1991) showed that *Y. enterocolitica* is capable of growing at temperatures ranging from approximately 4 to 45 °C and they examined how this organism alters its membrane fatty acids in response to a change in growth temperature. With a rise in temperature, saturated and cyclopropane fatty acids increased and unsaturated decreased. The fatty acid concentrations were reversed when the culture temperature was lowered. They concluded that *Y. enterocolitica* maintains its membrane rigidity and fluidity in response to growth temperature by changing the membrane fatty acid composition (Nagamachi et al., 1991). This study is important for emphasising the importance of standardising the growth conditions when determining fatty acid profiles for various bacterial strains.

Table 2 compares the CFA profiles for *Y. pestis* to the other *Yersinia* species. Based on these CFA profiles, there is clear separation of the various *Yersinia* species. The CFA profiles for *Y. pestis* and *Y. pseudotuberculosis* are similar, but there are several fatty acids, 16:1 ω5c, 16:0, 17:1 ω7c, 17:0 ω cyclo 7–8, 19:0 and summed 18:2 ω6c, 9c/18:0 ante, that differ significantly in percentage between these two species (Table 2). *Y. pestis* is genetically very similar to *Y. pseudotuberculosis* (FDA, 1991). Results from other studies also confirm a close relationship between *Y. pestis* and *Y. pseudotuberculosis* (Frolov, Ruban, & Vasyurenko, 1989; Tornabene, 1973). The mean SI value for the *Yersinia* strains ranged from 0.655 for *Y. pseudotuberculosis* to 0.890 for *Y. intermedii* (Table 2).

In summary, fatty acids were extracted from whole bacterial cells of various *Yersinia* species, and derivatized into methyl esters. The FAMEs were identified and quantified using GC–FID, which is a rapid and sensitive procedure. The cellular fatty acids were extracted in approximately 1 h and run on the GC–FID for 5 min. The data in this study show that *Yersinia* species can be identified after 24 h of growth on BHI agar. This study demonstrates the importance of being able to construct a unique library for identification of *Yersinia* species, based on type of media and specific growing conditions. The percentages and unique fatty acids

isolated from *Yersinia* strains can provide a sensitive method for identification. This analytical method provides a procedure for the differentiation of *Y. pestis* strains from closely related *Yersinia* species.

4. Safety

Standard microbiological safety precautions were observed whilst working with the bacterial strains. Whilst performing experimental procedures, safety glasses, laboratory coats and disposable gloves were utilised. The bacteria were completely inactivated using sodium hydroxide that was heated in a boiling water bath before the fatty acid extraction procedure.

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References

Bendiner, E. (1989). Alexandre Yersin: pursuer of plague. *Hospital Practice, 24*, 121–128. 131–132, 135–138.

Bercovier, H., Ursing, J., Brenner, D. J., Steigerwalt, A. G., Fanning, G. R., Carter, G. R., et al. (1980). *Yersinia kristensenii*: A new species of *Enterobacteriacae* composed of sucrose-negative strains (formerly called atypical *Yersinia enterocolitica* or *Yersinia enterocolitica*-like). *Current Microbiology, 4*, 219–224.

Brenner, D. J., Bercovier, H., Ursing, J., Alonso, J. M., Steigerwalt, A. G., Fanning, G. R., et al. (1980). *Yersinia intermedia*: A new species of *Enterobacteriacae* composed of rhamnose-positive, melibiose positive raffinose-positive strains (formerly called atypical *Yersinia enterocolitica* or *Yersinia enterocolitica*-like). *Current Microbiology, 4*, 207–212.

Brenner, D. J., Steigerwalt, A. G., Falxo, D. P., Weaver, R. E., & Fanning, G. R. (1976). Characterization of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* by deoxyribonucleic acid hybridization and by biochemical reactions. *International Journal of Systematic Bacteriology, 26*, 180–194.

Daneshvar, M. I., Douglas, M. P., & Weyant, R. S. (2001). Cellular fatty acid composition of *Lautropia mirabilis*. *Journal of Clinical Microbiology, 39*, 4160–4162.

Frolov, A. F., Ruban, N. M., & Vasyurenko, Z. P. (1989). Fatty acid composition of lipopolysaccharides of the strains of different species of *Yersinia*. *Journal of Hygiene, Epidemiology, Microbiology and Immunology, 33*, 55–61.

Jantzen, E., & Lassen, J. (1980). Characterization of *Yersinia* species by analysis of whole-cell fatty acids. *International Journal of Systematic Bacteriology, 30*, 421–428.

Leclercq, A., Guiyoule, A., El Lioui, M., Carniel, E., & Decallonne, J. (2000). High homogeneity of the *Yersinia pestis* fatty acid composition. *Journal of Clinical Microbiology, 38*, 1545–1551.

Leclercq, A., Wauters, G., Decallonne, J., El Lioui, M., & Vivegnis, J. (1996). Usefulness of cellular fatty acid patterns for identification and pathogenicity of *Yersinia* species. *Medical Microbiology Letters, 5*, 182–194.

Loftus, C. G., Harewood, G. C., Cockerill, F. R., & Murray, J. A. (2002). Clinical features of patients with novel *Yersinia* species. *Digestive Diseases and Science, 47*, 2805–2810.

Nagamachi, E., Shibuya, S., Hirai, Y., Matsushita, O., Tomochika, K., & Kanemasa, Y. (1991). Adaptational changes of fatty acid composition and the physical state of membrane lipids following the change of growth temperature in *Yersinia enterocolitica*. *Microbiology and Immunology, 35*, 1085–1093.

Perry, R. D., & Fetherston, J. D. (1997). *Yersinia pestis* – Etiologic agent of plague. *Clinical Microbiology Reviews, 10*, 35–66.

Sasser, M. (1997). Identification of bacteria by gas chromatography of cellular fatty acids. *MIDI Technical Note No. 101*, MIDI, Newark, DE.

Snedecor, G.W., & Cochran, W.G. (1980). *Statistical Methods*, 7th ed. Ames, IA: The Iowa State University Press. (pp. 175–193, 215–237).

Tornabene, T. G. (1973). Lipid composition of selected strains of *Yersinia Pestis* and *Yersinia Pseudotuberculosis*. *Biochimica et Biophysica Acta, 306*, 173–185.

Ursing, J., Brenner, D. J., Bercovier, H., Fanning, G. R., Steigerwalt, A. G., Brault, J., et al. (1980). *Yersinia frederiksenii*: A new species of *Enterobacteriacae* composed of rhamnose-positive strains (formerly called atypical *Yersinia enterocolitica* or *Yersinia enterocolitica*-like). *Current Microbiology, 4*, 213–217.

US Food and Drug Administration. (1991). Foodborne pathogenic microorganisms and natural toxins handbook. *Yersinia enterocolitica*, Available from:<http://vm.cfsan.fda.gov/mow/chap5.html>.

US Food and Drug Administration. (2001). Bacteriological analytical manual. *Yersinia enterocolitica*, Available from: <http://vm.cfsan.fda.gov/ebam/bam-8.html>.

Whittaker, P., Day, J. B., Curtis, S. K., & Fry, F. S. (2007). Evaluating the use of fatty acid profiles to identify *Francisella tularensis*. *Journal of AOAC International, 90*, 465–469.

Whittaker, P., Fry, F. S., Curtis, S. K., Al-Khaldi, S. F., Mossoba, M. M., Yurawecz, M. P., et al. (2005). Use of fatty acid profiles to identify food-borne bacterial pathogens and aerobic endospore-forming bacilli. *Journal of Agricultural and Food Chemistry, 53*, 3735–3742.

Yersin, A. (1894). La peste bubonique a Hong-Kong. *Annuals Institute Pasteur, 8*, 662–667.