Variations of the Moles Percent Guanine plus Cytosine Within a Group of *Enterobacteriaceae* Belonging or Related to the Genus *Enterobacter*

D. IZARD, C. FERRAGUT, F. GAVINI, AND H. LECLERC

*Institut Pasteur de Lille, Unité INSERM 146, Domaine du C.E.R.T.I.A., B.P. 15,
59650 Villeneuve d'Ascq, France*

This study is a measurement of the moles percent guanine plus cytosine of 34 strains of bacteria belonging or related to the genus *Enterobacter*. All of the strains included in the sampling have been previously studied by numerical taxonomy, which has shown four new classes: H₁, H₂, H₃, and d. The average guanine-plus-cytosine contents for the defined species and of the new classes are: *Enterobacter cloacae* (10 strains), 54.5 mol% (standard deviation, 1.32); *Hafnia alvei* (5 strains), 48.1 mol% (standard deviation, 1.0); *Enterobacter agglomerans* (syn. *Erwinia herbicola*) (1 strain), 52.4 mol% (standard deviation, 0.41); *Serratia liquefaciens* (1 strain), 50.9 mol% (standard deviation, 1.22); *Enterobacter aerogenes* (1 strain), 53.5 mol% (standard deviation, 0.29); class H₁ (5 strains), 52.2 mol% (standard deviation, 1.3); class H₂ (5 strains), 53.4 mol% (standard deviation, 1.9); class H₃ (5 strains), 54.2 mol% (standard deviation, 0.1); class d (1 strain), 52.6 mol% (standard deviation, 1.45). The importance of the guanine-plus-cytosine contents for discriminating defined species and new classes is discussed.

The purpose of this study is to define the guanine-plus-cytosine contents (G+C) for the *Enterobacteriaceae* belonging or related to the genus *Enterobacter*. It is a continuation of the numerical taxonomy work of Gavini et al. (9) on 175 strains, which regrouped defined species such as *Enterobacter cloacae* (class G), *Hafnia alvei* (class I), *Enterobacter agglomerans*, syn. *Erwinia herbicola* (class a), *Serratia liquefaciens* (class b), *Enterobacter aerogenes* (class c), and new classes (H₁, H₂, H₃, and d).

MATERIALS AND METHODS

The 34 strains used are listed in Table 1; all were included in the study of Gavini et al. (9). The preparation of the deoxyribonucleic acid and the determination of the melting temperature (T_m) were described by Ferragut and Leclerc (8). The absorbance measurements at 260 nm have been corrected for the concentration dilution caused by solvent expansion at the higher temperature (15). The mol% G+C has been measured by the average T_m as described by De Ley (6).

RESULTS

The results of the G+C determinations are shown in Tables 2 (defined species) and 3 (undefined species). Table 4 compares our results with those cited in the literature. The precision of the determinations is $\pm 1.1\%$ at a 95% level of confidence. The standard deviation shows the homogeneity of the taxa.

Defined species. (i) *E. cloacae*. The average G+C content for *E. cloacae* (54.5 mol%) was measured from 10 strains and was the highest of all the analyzed species and classes. The range of results (standard deviation, 1.32) showed a certain heterogeneity previously revealed by numerical analysis. Very few determinations of *E. cloacae* G+C content are cited in literature. Ouellette et al. (19) determined the G+C content of *E. cloacae* CDC 1237-63 to be 56.9 mol% by the T_m method. This value is close to the upper limit of our results (52.6 to 56.4 mol%). The G+C content of *E. cloacae* ATCC 13047 was found by Starr and Mandel (22) to be 54.6 mol% by the buoyant density method and 55.4 mol% by the T_m method.

(ii) *H. alvei*. The average G+C content of this species was 48.1 mol% (five determinations), which is different from those of the other groups (*E. cloacae*, H₁, H₂, and H₃). The standard deviation (1.0) showed a good homogeneity in the results. The study of this group agrees with the conclusions of the numerical analysis. Our results confirmed the maintenance of the level of genus for the bacteria belonging to the *Hafnia* group (2, 12, 22, 23). Our measurements agree with those of Starr and Mandel (22), who cite 48 mol% (T_m) and 48.7 mol% (buoyant density) for *H. alvei* ATCC 23280. They are, on the other hand, very different from the range of 52 to 57 mol% given by Sakazaki (20) in the 8th edition of *Bergey's Manual*.

(iii) *E. agglomerans* syn. *E. herbicola*. The average G+C content of strain 18 was 52.4 mol%, which is close to the lower part of the range of 52.6 to 57.7 mol% derived from the 30 strains cited by Lelliot (14) in the 8th edition of *Bergey's Manual*.

(iv) *S. liquefaciens*. The strain analyzed was *S. liquefaciens* ATCC 14460. The G+C content was 50.9 mol%, which is close to the Mandel and Rownd (16) finding of 51.9 mol% (T_m) for the same strain. On the other hand, our determina-

tion disagrees with the values of 53.9 mol% by the T_m method and 52.6 mol% by the buoyant density method cited by Starr and Mandel (22) in a paper published more recently. The G+C content of this strain was also determined by the chemical method: Marmur et al. (18) found 50 to 52 mol%.

(v) *E. aerogenes*. The G+C content of the strain analyzed, *E. aerogenes* ATCC 13048, was 53.5 mol%. This value is close to those cited in literature by several authors who mention, for

TABLE 1. List of strains of bacteria used^a

Species (class)	Strain no.
<i>Enterobacter cloacae</i> (class G)	13, 35, 42, 45, 53, 87, 97, 127, 132, 137
<i>Hafnia alvei</i> (class I)	<i>H. alvei</i> ATCC 13337, 71, 94, 114, 120
<i>Enterobacter agglomerans</i> (class a)	18
<i>Serratia liquefaciens</i> (class b)	<i>S. liquefaciens</i> ATCC 14460
<i>Enterobacter aerogenes</i> (class c)	<i>E. aerogenes</i> ATCC 13048
Class H ₁ (undefined strains)	66, 86, 99, 134, 146
Class H ₂ (undefined strains)	80, 91, 133, 148, 171
Class H ₃ (undefined strains)	111, 123, 130, 145, 175
Class d (undefined strains)	160

^a All are included in the numerical analysis of Gavini et al. (9).

TABLE 2. G + C content of defined species

Species and strain no.	No. of determinations	Mean G + C content for the determinations ^a (mol %)
<i>Enterobacter cloacae</i>		
97	6	52.6
42	5	52.7
87	4	53.6
127	5	53.7
13	3	54.5
35	7	54.9
137	3	55.4
45	4	55.7
132	3	55.7
53	3	56.4
<i>Hafnia alvei</i>		
120	5	47.2
94	3	47.4
ATCC 13337	5	47.7
71	3	48.8
114	5	49.5
<i>Enterobacter agglomerans</i>	3	52.4
18		
<i>Serratia liquefaciens</i>	5	50.9
ATCC 14460		
<i>E. aerogenes</i> ATCC 13048	3	53.5

^a The average G + C content (moles percent) $\pm$ standard deviation values for the taxons *Enterobacter cloacae* and *Hafnia alvei* were 54.5 ± 1.32 and 48.1 ± 1.0 , respectively.

TABLE 3. G + C content of undefined classes of *Enterobacteriaceae*

Class and strain no.	No. of determinations	Mean G + C content for the determinations ^a (mol%)
Class H ₁		
86	3	50.3
146	3	51.4
66	5	52.6
99	4	53.0
134	3	53.6
Class H ₂		
133	4	51.3
148	4	52.0
80	4	53.0
91	3	54.7
171	3	55.9
Class H ₃		
175	4	53.2
111	4	53.8
123	3	53.9
145	3	54.3
130	3	55.8
Class d		
160	5	52.6

^a The average G + C content (moles percent) $\pm$ standard deviation values for the classes H₁, H₂, and H₃ were 52.2 ± 1.3 , 53.4 ± 1.9 , and 54.2 ± 0.1 , respectively.

TABLE 4. Comparison of our results with those in the literature

Species	G + C content: Izard et al. (mol%)	Strain	Data from other sources			Refer- ence
			mol% G + C by:			
			T_m method	Buoyant density method	Chemical method	
<i>Enterobacter cloacae</i>	52.6–56.4, 10 strains	ATCC 13047	55.4	54.6		22
		CDC 1237–63	56.9			19
<i>Hafnia alvei</i>	47.2–49.5, 5 strains	ATCC 23280	48.7	48.0		22
				52–57		20
<i>Enterobacter agglomerans</i>	52.4, 1 strain	30 strains		52.6–57.7		14
<i>Serratia lique- faciens</i>	50.9, ATCC 14460	1 strain	53.8			11
		ATCC 14460	53.9	52.6		22
		ATCC 14460	51.9			16
		ATCC 14460			50–52	18
<i>Enterobacter aerogenes</i>	53.5, ATCC 13048	ATCC 13048	54.9	53.1		22
		ATCC 13048		53.1		1
		ATCC 13048		54.3		4
		5 strains	54.0–56.5	54.0–56.5		4
		5 strains			55.2–58.9	3
		1088 (Harvard)	57.5			17
		1088 (Harvard)		57.0		21
		2 strains			57.7–59.3	19
		3 strains	57.8			7
9 strains	56.4–60.8			19		

the same strain, 54.9 mol% by the T_m method (22) and 53.1 to 54.3 mol% by the buoyant density method (1, 4, 22). Many authors have measured the base composition of *E. aerogenes* which is near that of *E. cloacae*: Ouelette et al. (19) (nine strains), 56.4 to 60.8 mol%; Colwell and Mandel (4) (five strains), 54.0 to 56.5 mol%; Falkow et al. (7) (three strains), 57.8 mol%; Belozerskii and Spirin (3) (five strains), 55.2 to 58.9 mol%; Marmur and Doty (17) and Schildkraut et al. (21) (the same strain [Harvard 1088]), 57.5 and 57.0 mol%, respectively. Like the G+C content of *E. cloacae*, that of *E. aerogenes* varies within a wide interval (53.1 to 60.8 mol%).

Undefined classes. The average G+C content of class H₁ was 52.2 mol% (five determinations); the standard deviation was 1.3. The average G+C content of class H₂ (53.4 mol%) was close to that of *E. cloacae*; this tends to confirm the conclusions of the numerical analysis. As for the species *E. cloacae*, the standard deviation was relatively high (1.9). The average G+C content of class H₃ (54.2 mol%) was approximately equal to that of *E. cloacae*. H₃ is very homogeneous (standard deviation, 0.1) and phenetically

close to the species *E. cloacae*. Class d consisted of three strains; the G+C content of strain 160 was 52.6 mol%. According to a previous numerical taxonomic study, these strains should belong to the group "*Citrobacter* lysine⁺" (Leclerc and Buttiaux [13]), syn. "*Citrobacter* like" (Crosa et al. [5]), class C (Gavini et al. [10]).

DISCUSSION

The results obtained agree with the conclusions of numerical analysis (*E. cloacae*, *H. alvei*, H₁, H₂, and H₃), but the discriminatory value of G+C content in the taxonomy of coliforms is low. It seems absolutely necessary to reproduce the measurements on a great number of strains of each species or class. The heterogeneity of G+C content within a species or a class seems to give an account of the different base compositions mentioned for a same species by the different authors. In fact, one must remember the continuum made by *E. cloacae* and classes H₁, H₂, and H₃ (54.5, 52.2, 53.4, and 54.2 mol%, respectively) and the well individualized position of the bacteria belonging to the group *Hafnia* (48.1 mol%).

ACKNOWLEDGMENTS

We thank Professor J. De Ley for his advice and for permission for one of us to work for some time in his laboratory, and we thank A. Bernigaud for his technical assistance.

REPRINT REQUESTS

Address reprint requests to: Daniel Izard, Institut Pasteur de Lille, Unité INSERM 146, Domaine du C.E.R.T.I.A., B.P. 15, 59650 Villeneuve d'Ascq, France.

LITERATURE CITED

1. Baptist, J. N., C. R. Shaw, and M. Mandel. 1969. Zone electrophoresis of enzymes in bacterial taxonomy. *J. Bacteriol.* **99**:180-188.
2. Bascomb, S., S. P. Lapage, M. A. Curtis, and W. R. Willcox. 1974. Identification of bacteria by computer: identification of reference strains. *J. Gen. Microbiol.* **83**:271-282.
3. Belozerskii, A. N., and A. B. Spirin. 1960. p. 147. In E. Chargaff and J. N. Davison (ed.), *The nucleic acids: chemistry and biology*, vol. 3. Academic Press Inc., New York.
4. Colwell, R. R., and M. Mandel. 1964. Adansonian analysis and deoxyribonucleic acid base composition of some gram-negative bacteria. *J. Bacteriol.* **87**:1412-1422.
5. Cross, J. H., A. G. Steigerwalt, G. R. Fanning, and D. J. Brenner. 1974. Polynucleotide sequence divergence in the genus *Citrobacter*. *J. Gen. Microbiol.* **83**:271-282.
6. De Ley, J. 1970. Reexamination of the association between melting point, buoyant density, and chemical base composition of deoxyribonucleic acid. *J. Bacteriol.* **101**:738-754.
7. Falkow, S., I. R. Ryman, and O. Washington. 1962. Deoxyribonucleic acid base composition of *Proteus* and Providence organisms. *J. Bacteriol.* **83**:1318-1321.
8. Ferragut, C., and H. Leclerc. 1976. Etude comparative des méthodes de détermination du T_m de l'ADN bactérien. *Ann. Microbiol. (Inst. Pasteur)* **127A**:223-235.
9. Gavini, F., C. Ferragut, B. Lefebvre, and H. Leclerc. 1976. Etude taxonomique d'entérobactéries appartenant ou apparentées au genre *Enterobacter*. *Ann. Microbiol. (Inst. Pasteur)* **127B**:317-335.
10. Gavini, F., B. Lefebvre, and H. Leclerc. 1976. Positions taxonomiques d'entérobactéries H_2S^- par rapport au genre *Citrobacter*. *Ann. Microbiol. (Inst. Pasteur)* **127A**:275-295.
11. Grimont, P. A. D., F. Grimont, H. L. C. Dulong de Rosnay, and P. H. A. Sneath. 1977. Taxonomy of the genus *Serratia*. *J. Gen. Microbiol.* **98**:39-66.
12. Johnson, R., R. R. Colwell, R. Sakazaki, and K. Tamura. 1975. Numerical taxonomy study of the *Enterobacteriaceae*. *Int. J. Syst. Bacteriol.* **25**:12-37.
13. Leclerc, H., and R. Buttiaux. 1965. Les *Citrobacter*. *Ann. Inst. Pasteur (Lille)* **16**:67-74.
14. Lefflot, R. A. 1974. Genus XII: *Erwinia*, p. 332-339. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams and Wilkins Co., Baltimore.
15. Mandel, M., and J. Marmur. 1968. Use of ultraviolet absorbance temperature profile for determining the guanine plus cytosine content of DNA. *Methods Enzymol.* **12B**:195-206.
16. Mandel, M., and R. Rownd. 1964. Deoxyribonucleic acid base composition in the *Enterobacteriaceae*: an evolutionary sequence, p. 585-597. In C. A. Leone (ed.), *Taxonomic biochemistry and serology*. Ronald Press, New York.
17. Marmur, J., and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J. Mol. Biol.* **5**:109-118.
18. Marmur, J., S. Falkow, and M. Mandel. 1963. New approaches to bacterial taxonomy. *Annu. Rev. Microbiol.* **17**:329-372.
19. Ouelette, C. A., R. H. Burris, and P. W. Wilson. 1969. Deoxyribonucleic acid base composition of species of *Klebsiella*, *Azotobacter* and *Bacillus*. *Antonie van Leeuwenhoek J. Microbiol. Serol.* **35**:275-286.
20. Sakazaki, R. 1974. Genus VIII: *Hafnia*, p. 325-326. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams and Wilkins Co., Baltimore.
21. Schildkraut, C. L., J. Marmur, and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its buoyant density in $CaCl_2$. *J. Mol. Biol.* **4**:430-443.
22. Starr, M. P., and M. Mandel. 1969. DNA base composition and taxonomy of phytopathogenic and other enterobacteria. *J. Gen. Microbiol.* **56**:113-123.
23. Veron, M., and L. Le Minor. 1975. Nutrition et taxonomie des *Enterobacteriaceae* et bactéries voisines. III. Caractères nutritionnels et différenciation des groupes taxonomiques. *Ann. Microbiol. (Inst. Pasteur)* **126B**:125-147.