ADANSONIAN ANALYSIS AND DEOXYRIBONUCLEIC ACID BASE COMPOSITION OF SOME GRAM-NEGATIVE BACTERIA

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ABSTRACT

COLWELL, R. R. (Georgetown University, Washington, D.C.), AND M. MANDEL. Adansonian analysis and deoxyribonucleic acid base composition of some gram-negative bacteria. *J. Bacteriol.* 87:1412-1422. 1964.—The deoxyribonucleic acid (DNA) base compositions and S values for a minimum of 134 coded properties were determined for representative cultures of the genera *Pseudomonas*, *Xanthomonas*, *Aeromonas*, *Vibrio*, *Aerobacter*, *Escherichia*, *Alcaligenes*, and *Flavobacterium*. Those cultures having a high degree of similarity by the criterion of numerical taxonomy were found to have similar DNA base compositions. The relative affinities of clusters of cultures suggest taxonomic relations. Eleven species of *Xanthomonas* might be a single species, and *V. metschnikovii* was shown to be more closely related to enteric bacteria than to other vibrios which, in turn, were found to be like pseudomonads. *Aeromonas* was found to be intermediate in similarity to enterics and pseudomonads and divisible into at least two, but possibly three, species. *F. aquatile* was unlike any of the other organisms studied, and its DNA also differed greatly in composition from other representatives of the genus.

The Adansonian method has been applied to taxonomic studies of several bacterial groups, and the results have been published (reviewed by Sneath, 1962), showing a measure of success with this approach to the handling of taxonomic data. Recent work in this new field has been involved with coding procedures and computer processing of data output (Gilardi et al., 1960; Floodgate, 1962a, b; Beers and Lockhart, 1962; Liston, Wiebe, and Colwell, 1962, 1963). A numerical taxonomy based on the Adansonian principle that all characters have equal weight can be constructed from mutual similarity calculations. Ideally, every feature of a given organism is studied, but, by use of the asymptote

hypothesis (Sokal and Sneath, 1963), a statistically significant cross section of the phenotypic traits permits the ordering of isolates according to their calculated affinities.

With the development of high-speed computer techniques for data handling and analysis, the significance of the deoxyribonucleic acid (DNA) base composition and its usefulness in classification have been recognized (Lee, Wahl, and Barbu, 1956; Sueoka, Marmur, and Doty, 1959; Lanni, 1960; Sueoka, 1961; Mandel and Rownd, 1963; Marmur, Falkow, and Mandel, 1963). The characteristics which are tabulated and analyzed by the Adansonian method are presumably phenotypic expressions of the information stored in the DNA. Since the base composition of the DNA can be readily determined, it should be useful to combine the results obtained by this method with those of Adansonian analysis.

The purpose of this study was to investigate any correlation between Adansonian analysis and DNA base composition in a group of gram-negative bacteria of diverse taxa.

MATERIALS AND METHODS

The procedure described previously by Colwell and Liston (1961a) for computer analysis of the taxonomic data was modified slightly to facilitate the handling of the larger $i \times m$ data matrix obtained in the analysis. Each bacterial strain was tested for morphological, physiological, and biochemical characteristics and, for each strain, 134 coded properties were the minimal number for the computations. Additional tests carried out in the studies for the pseudomonads are being presented separately (Colwell, *Can. J. Microbiol. in press*). Similarity ("S") has been computed as $S = N_s / (N_s + N_D)$. The coding procedure has been described (Colwell and Liston, 1961a); the computer programs for similarity calculations and group formation used were written for the IBM

1620 40,000 K, and are described elsewhere (Colwell, 1963). Copies of the programs are available and may be obtained from the authors.

Isolation of DNA. Highly polymerized DNA in the native configuration was prepared by Marmur's (1961) method from bacteria harvested at the end of the exponential phase. The bacteria were grown at or near their temperature optima in double-strength nutrient broth (Difco) fortified with 0.2% yeast extract. One batch of *Flavobacterium aquatile* cells was grown in thiamine broth (Weeks and Beck, 1960) which provides luxuriant growth. Cultures were aerated by continual agitation on a rotary shaker. All DNA samples were dissolved in 0.15 M NaCl plus 0.015 M sodium citrate at pH 7.0 (SSC) and stored at 2 C over chloroform.

Determination of base compositions. The temperature profile of the helix-to-coil transition was found according to Marmur and Doty's (1959) method. Temperatures were observed on a thermometer graduated in 0.2 C intervals which was calibrated at the immersion level of the portion extending into the cuvette heating chamber against a National Bureau of Standards calibrated reference thermometer. T_m was determined as the midpoint of the increased absorbance measured at 260 m μ at the observed temperature with respect to the absorbance at 25 C. Corrections were applied for the absorbance at 320 m μ and for the expansion of water at the given temperature. All DNA samples were dialyzed against the same batch of SSC in four groups to eliminate the effects of any polyamine contamination (Mandel, 1962) and to insure equal ionic strength in all thermal denaturation determinations. Samples were heated at a concentration of 20 μ g/ml, and the base compositions were estimated from the relation of T_m to base composition established by Marmur and Doty (1962) and corrected for the true temperature and for departure from linearity at the extremes. The nomograph from which these calculations were made is presented in Fig. 1. Base composition is expressed as the molar ratio of guanine plus cytosine (per cent GC).

Base compositions of some of the samples were also determined by measurement of the buoyant density in CsCl (Meselson, Stahl, and Vinograd, 1957). Centrifugation in approximately 5.7 M CsCl (optical grade; Maywood Chemical Works,

Maywood, N.J.) (refractive index, 1.4010 ± 0.0005) established equilibrium in 20 to 22 hr in a Spinco model E analytical ultracentrifuge at 44,770 rev/min at 25 C. Ultraviolet-absorption photographs on Kodak professional film were traced with a Joyce-Loebl double-beam recording densitometer. Each cell contained 1 μ g of the DNA to be determined and 0.5 μ g of N^{15} -substituted *Pseudomonas aeruginosa* DNA of a density of 1.742 g/cc as a reference. The buoyant density and the base composition were calculated as described by Schildkraut, Marmur, and Doty (1962).

Bacterial strains. The bacterial strains included in the computer studies are listed in Table 1.

Media. Except for the *F. aquatile* strain which was cultured in thiamine broth and on thiamine agar (Weeks and Beck, 1960), all strains were maintained on a medium of the following composition (per liter): yeast extract (Difco), 3 g; proteose peptone (Difco), 10 g; and NaCl, 5 g. The pH was adjusted to 7.2 with 0.1 N NaOH. The various test media were prepared as described or according to standard methods (Society of American Bacteriologists, 1957).

FIG. 1. Temperature of thermal transition (T_m) of deoxyribonucleic acids as a function of guanine plus cytosine content. Modified from the relation shown by Marmur and Doty (1963).

TABLE 1. *Bacterial strains used in computer analysis*

Organism	Source	No. of strains	Reference, listed if additional information given for the strains
<i>Pseudomonas aeruginosa</i>	ATCC	34	Liston, Wiebe, and Colwell (1963)
<i>P. aeruginosa</i>	Marine specimens	4	Colwell and Liston (1961a)
<i>P. aeruginosa</i>	Clinical specimens	7	Hoff (1963)
<i>P. aeruginosa</i>	Food samples	1	J. C. Ayres, Iowa State University, Ames
<i>P. chlororaphis</i>	—	3	Haynes and Rhodes (1961)
<i>P. fluorescens</i>	ATCC	5	—
<i>P. solanacearum</i>	—	5	Colwell and Liston (1961b)
<i>Xanthomonas</i> sp.	—	16	Colwell and Liston (1961b)
<i>Aeromonas punctata</i>	ATCC	1	—
<i>A. punctata</i>	Marine specimens	2	—
<i>A. formicans</i>	—	5	Crawford (1954)
<i>Vibrio</i> sp.	ATCC	5	—
<i>Aerobacter aerogenes</i>	ATCC	1	—
<i>A. aerogenes</i>	Marine specimens	3	—
<i>Escherichia coli</i> I (K-12)	Clinical specimens	1	—
<i>E. coli</i> 4157	ATCC	1	—
<i>Alcaligenes faecalis</i>	ATCC	1	—
<i>Flavobacterium aquatile</i>	ATCC	1	—
Total		96	

RESULTS

Computer analyses. A matrix of 4,608 S values was obtained from the data coded for the 96 strains used. The strains were clustered into approximate groupings by the highest-link sorting method (Colwell and Liston, 1961a). The intragroup and the intergroup similarity values (ΔS , or triangular means) are given in Table 2. The success of the groupings is indicated by the intragroup S values. At 75% S, or greater, the "species level" groupings were obtained (Colwell and Liston, 1961a; Liston et al., 1963), with the exception of *Aeromonas punctata* and the "*Vibrio*" strains. The *A. punctata* strains, as a species cluster, were as similar to the *Aerobacter* strains as to one another within the group. The overlap between these genera has been observed, making identification and classification a serious problem (Clement and Gibbons, 1960). The "*Vibrio*" strains included in this analysis (*Vibrio* sp. 11171; *V. cuneatus*, *V. tyrogenus*, and *V. percolans*) were clearly separated from *V. metschnikovii* on the basis of the computer results and showed highest similarity to the *P. fluorescens* strains. As was noted in an earlier study, (Colwell and Liston, 1961a), *V. cuneatus* produced an abundant green fluorescent pigment. Strains

which could not be grouped with any of the others studied were *Alcaligenes faecalis* ATCC 8750 and *F. aquatile* ATCC 11947. The *F. aquatile* strain and the *Xanthomonas* grouping of *X. tamarindi*, *X. vesicatoria*, and *X. begoniae* were more similar to each other, on the basis of intergroup S value, than to any of the other strains or groups of strains (Table 2).

To estimate the ΔS spread which could be expected, the intragroup and intergroup ΔS was calculated for ten substrains of each of nine strains representing the genera *Escherichia*, *Aerobacter*, *Aeromonas*, and *Pseudomonas* (Table 3). The 4,050 S value output matrix was condensed, by programmed computer instruction, into the format of Table 3. The *Pseudomonas* strains demonstrated a high level of mutual similarity ($\Delta S = 75\%$), suggesting a species level relationship (Colwell and Liston, 1961a).

The results of the calculations performed on data obtained from the representatives of *Pseudomonas* were inspected carefully and are given in Fig. 2. Strains received as *P. fluorescens* and one ostensible *P. chlororaphis* were found to cluster within the *P. aeruginosa* group. In addition to the intrageneric relationships within *Pseudomonas*, the *Xanthomonas-Pseudomonas* relationships are

TABLE 2. Intragroup and intergroup *S* values (% similarity) for all strains used in this study

Organism	<i>Escherichia coli</i>	<i>A. aerogenes</i>	<i>A. punctata</i>	<i>A. formicans</i>	<i>A. faecalis</i>	<i>V. metschnikovii</i>	<i>P. aeruginosa</i>	<i>P. chlororaphis</i> B-560	<i>P. chlororaphis</i> B-977	<i>P. chlororaphis</i> 9446	<i>P. fluorescens</i> 11172 12633	" <i>Vibrio</i> "	<i>P. solanacearum</i>	<i>Xanthomonas</i>	<i>X. tamarindi, vesicatoria, begoniae</i>	<i>F. aquatile</i>
<i>Escherichia coli</i>	76.3															
<i>Aerobacter aerogenes</i> ...	59.9	77.1														
<i>Aeromonas punctata</i> ...	56.6	56.3	57.3													
<i>A. formicans</i>	39.6	42.7	49.5	66.0												
<i>Alcaligenes faecalis</i> *..	32.2	34.8	38.1	37.4	100.0											
<i>Vibrio metschnikovii</i> *..	38.9	37.3	41.2	55.0	29.3	100.0										
<i>Pseudomonas aeruginosa</i>	33.4	37.9	41.7	49.9	42.2	44.8	86.6									
<i>P. chlororaphis</i> B-560...	33.3	42.0	45.7	53.9	44.7	45.9	61.1	92.3								
<i>P. chlororaphis</i> B-977..	33.7	44.7	46.7	57.2	51.2	46.7	63.6	58.8	100.0							
<i>P. chlororaphis</i> 9446...	37.1	46.3	49.5	59.8	45.2	51.2	62.7	60.0	88.2	100.0						
<i>P. fluorescens</i> 11172 12633	32.7	38.7	44.3	46.9	57.0	41.4	55.7	55.8	63.5	63.1	85.7					
" <i>Vibrio</i> ".....	29.9	32.2	39.5	40.1	47.8	42.7	51.0	49.2	50.4	48.9	55.0	55.5				
<i>P. solanacearum</i>	30.6	31.8	39.8	46.2	43.1	42.6	50.7	42.8	50.2	51.2	59.0	51.8	74.2			
<i>Xanthomonas</i>	31.7	34.0	43.7	46.1	40.1	45.6	50.7	59.0	53.3	52.6	54.3	47.6	47.5	69.3		
<i>X. tamarindi, vesicatoria, begoniae</i>	27.3	29.1	36.2	32.6	40.8	40.2	38.9	45.7	37.3	37.1	46.6	43.7	44.7	57.8	75.1	
<i>Flavobacterium aquatile</i>	26.8	23.4	31.9	30.5	38.7	33.1	31.1	34.3	32.6	33.3	41.1	41.5	44.6	45.0	60.0	100.0

* Single strains.

TABLE 3. Intragroup and intergroup *S* values (% similarity) for bacterial strains of several genera

Organism	<i>E. coli</i> 4157	<i>A. aerogenes</i> 211	<i>A. punctata</i> 11163	<i>A. formicans</i> 13137	<i>P. fluorescens</i> 12633	<i>P. aeruginosa</i> 8707 8709	<i>P. fluorescens</i> 10796	<i>P. chlororaphis</i> B-560 [sic]
<i>Escherichia coli</i> 4157.....	92.0							
<i>Aerobacter aerogenes</i> 211.....	54.9	93.0						
<i>Aeromonas punctata</i> 11163.....	50.4	52.8	90.0					
<i>A. formicans</i> 13137.....	52.0	57.3	63.4	94.9				
<i>Pseudomonas fluorescens</i> 12633.....	32.1	37.6	44.3	56.5	91.4			
<i>P. aeruginosa</i> 8707 8709.....	36.3	45.2	52.1	71.5	75.0	94.7		
<i>P. fluorescens</i> 10796.....	35.6	40.8	49.9	65.2	71.2	76.3	94.9	
<i>P. chlororaphis</i> B-560 [sic].....	39.2	42.4	56.4	68.6	69.4	81.2	76.1	92.5

given in Fig. 3. The *Xanthomonas* strains, with the exception of the three strains, *X. tamarindi*, *X. vesicatoria*, and *X. begoniae*, sorted out by computer analysis at a generic level of similarity with *Pseudomonas*, based on the maximal *S* value relationships (given in Fig. 3). Parenthetically, it may be stated that 11 of the *Xanthomonas* species, *X. carotae*, *X. incanae*, *X. hederiae*, *X. malvacearum*, *X. campestris*, *X. phaseoli*, *X. pisi*, *X.*

pelargonii, *X. juglandis*, *X. physalidicola*, and *X. corylina*, could not be considered as exclusive species but perhaps as a single species, on the basis of the computer data.

DNA base composition analyses. Certain of the strains of *Pseudomonas* (Fig. 2) were studied to determine the base composition of the purified DNA extracted from batch cell cultures. Since particular interest was centered on the strains of

FIG. 2. Highest link sorting of *S* value data for *Pseudomonas aeruginosa*.

FIG. 3. Relationships between groupings obtained from Adansonian analyses, based on maximal inter-*S* values.

P. fluorescens, ATCC 12121 and ATCC 10796, and the *P. chlororaphis* which clustered with *P. aeruginosa*, a number of determinations were carried out on portions of the sample and on different preparations from the same source. From the results, a remarkable degree of identity was noted, with maximal error in estimation of T_m approximating ± 0.3 C (Table 4). In the *P. chlororaphis* cultures studied, the buoyant density of DNA in a CsCl density gradient was also run, serving as confirmation of the results obtained by thermal denaturation temperature measurements.

The results of analyses carried out with DNA from the "Vibrio" strains proved equally interesting. Expressed as moles per cent GC content, the DNA of the "Vibrio" group and the *Pseudo-*

monas appear to be similar, bearing out the computer results presented, in part, in Fig. 3. Finally, it can be noted from Table 4 that the *V. metschnikovii* ATCC 7708 and the *F. aquatile* ATCC 11947 strains do not have a high degree of similarity to any of the other groupings obtained. Data for two additional cultures of *Flavobacterium* are also given, pointing out the relative heterogeneity of this grouping. These data would suggest that the genus *Flavobacterium* may contain some very odd bedfellows, very likely strays more properly classified into other genera.

The cultures of *Xanthomonas* grouped together by computer analysis, which were also subjected to DNA base composition studies, are listed in Table 5, together with the results. The GC content of these strains was approximately 64 moles

TABLE 4. DNA base composition of *Pseudomonas*, *Vibrio*, and *Flavobacterium* strains

Organism	Strain	Thermal transition			Buoyant density	GC content
		No. of determinations	T _m	σ		
			C		g/cc	moles %
<i>P. aeruginosa</i>	ATCC 8707	8	97.5	0.3	1.727	64.0
<i>P. aeruginosa</i>	ATCC 8689	4	97.7	0.2	—	64.4
<i>P. (fluorescens)</i> [sic].....	ATCC 12121	3	97.5	—	—	64.0
<i>P. (fluorescens)</i> [sic].....	ATCC 10796	3	97.7	—	—	64.4
<i>P. chlororaphis</i>	NRRL B560 [sic]	4	97.7	0.2	1.728	64.2
<i>P. chlororaphis</i>	NRRL B977	1	96.1	—	1.723	61.0
<i>P. fluorescens</i>	ATCC 12633	6	96.0	0.3	1.721	61.0
<i>V. percolans</i>	ATCC 8461	3	97.0	—	—	65.0
<i>V. tyroginus</i>	ATCC 7085	2	96.3	—	—	61.7
<i>V. cuneatus</i>	ATCC 6972	2	96.1	—	—	61.2
<i>V. metschnikovii</i>	ATCC 7708	2	88.6	—	1.703	43.4
<i>F. vitrumen</i>	ATCC 10234	3	97.2	—	—	63.3
<i>F. suaveolens</i>	ATCC 958	3	97.8	—	—	64.5
<i>F. aquatile</i>	ATCC 11947	6	83.5	0.4	1.689	0.0

per cent, within the range encompassed by the pseudomonads studied.

Similar data for *Escherichia*, *Aerobacter*, *Aeromonas*, and *Alcaligenes* are given in Table 6. The situation which presents itself here is not quite so obvious as with the pseudomonads. A comparison of the biochemical and the computer data is presented in Fig. 4. The interrelationships constructed by the computer data appear to be strongly correlated with the base composition values. These data are in agreement with previously published results of other workers (Marmur and Doty, 1962).

DISCUSSION

Classification, as a philosophy, is one of the main elements in the use of language and is not an end in itself. Usually classifications are divided into "natural" versus "artificial," and the aura of respectability hovers over the one claiming the title of a "natural" classification, even though the distinction may be more apparent than real. The construction of a classification requires a rigorously defined terminology, as well as a statement of ultimate aims and purpose of the classification to be established. A natural classification is one based on likeness in a large number of attributes, an overall affinity, whereas an artificial classification can be constructed from likeness in only a small number of attributes, perhaps only one attribute. Furthermore, a natural classification is not necessarily phylogenetic and, in the case of

TABLE 5. DNA base composition of *Xanthomonas* species

Organism	Strain	Thermal transition		GC content
		No. of determinations	T _m	
			C	moles %
<i>X. hederae</i>	XH5	3	96.7	62.0
<i>X. pelargonii</i>	J. Koths	2	96.8	62.4
<i>X. pisi</i>	XP171	4	97.4	63.8
<i>X. phaseoli</i>	XP8	3	97.1	63.2
<i>X. phaseoli</i>	XP163	2	97.1	63.2

microorganisms, it may not be possible to construct a phylogenetic classification although some effort is being made in this direction (Ravin, 1963).

The magnitude of the problem of data handling has been a major obstacle to the establishment of a natural classification of microorganisms. With the technological advances in computer techniques and the initiation of the methodology of numerical taxonomy into biology, this handicap is easily overcome. In fact, the resurgence of interest in bacterial taxonomy occurring in recent years parallels the wider application of molecular genetics and of high-speed data handling techniques (Sneath, 1962; Luria, 1962; Ehrlich and Holm, 1962). It seems appropriate to combine these two approaches to the problems

TABLE 6. DNA base composition of "enteric" and aeromonad strains

Organism	Strain	Source	Thermal denaturation			Buoyant density	GC content
			No. of determinations	T _m	σ*		
				C		g/cc	moles %
<i>Escherichia coli</i>	T94A	H. P. Treffers	—	91.4†	—	1.710	50.0
	K12-IIIc	J. Marmur	—	91.4†	—	1.710	50.0
	B	S. E. Luria	—	91.5†	—	1.710	50.0
	44B	University of Massachusetts	—	91.5†	—	1.710	50.0
	9723d	ATCC	—	91.5†	—	1.710	50.0
	K12	University of Washington	9	91.5	0.2	1.710	50.0
	4157	ATCC	1	91.5	—	1.710	50.0
	Row (col ⁻)	H. Ozeki	3	91.6	—	1.710	50.5
	12317 (E2)	H. Ozeki	—	91.7†	—	1.710	50.7
	Row (17)/str-r	Derived from Row (col ⁻)	2	91.8	—	1.710	51.0
<i>Aerobacter aerogenes</i>	14308	ATCC	4	93.0	0.3	1.712	54.0
	13048	ATCC	—	93.2†	—	1.712	54.3
	MEG	University of Massachusetts	—	93.4†	—	1.712	54.4
	"Indiana"	J. Smith-Sonneborn	4	93.5	0.3	1.713	55.5
	211	ATCC	5	94.0	0.5	1.715	55.5
	"Balamuth"	University of California	2	94.5	—	1.715	56.5
<i>Aeromonas shigelloides</i>	14029	ATCC	—	92.3†	—	—	52.0
<i>A. hydrophila</i>	9071	ATCC	—	93.6†	—	—	55.7
<i>A. punctata</i>	11163	ATCC	4	94.4	0.5	1.716	56.5
<i>A. salmonicida</i>	14174	ATCC	3	94.5	—	—	56.7
<i>A. formicans</i>	(PG) 13137	ATCC	4	95.2	0.5	—	58.8
<i>A. liquefaciens</i> †	B-966	P. V. Liu	3	95.4	—	—	59.0
<i>A. liquefaciens</i>	Quilcene	J. Ross	3	95.4	—	—	59.0
<i>Alcaligenes faecalis</i>	8750	ATCC	3	93.6	—	1.715	54.8

* Standard error of the mean calculated only for data derived from four or more determinations.

† From Mandel and Rownd (1963).

‡ *Pseudomonas caviae* (Scherago).

of bacterial classification to obtain arrangements of individuals with a maximum of information storage in the taxa. A maximum of predictions and deductions should be possible from data of this kind. A comparable set of information from studies of the organization of the hereditary materials will permit confidence that these "taxospecies" may be equated with the "genospecies" (Ravin, 1963).

Lee et al. (1956) pointed out that certain

relationships could be detected between the results from taxonomic and biochemical studies of microorganisms; this was pursued further by a number of workers [reviewed by Lanni (1960) and by Sueoka (1961)]. The near identity in overall DNA base composition now can be considered a highly correlative feature of genetically interacting microbes. These organisms which are genetically most related, possessing homologous base sequences and forming molecular hybrids (Mar-

FIG. 4. Grouping of "enteric" and aeromonad strains on the basis of computer data and DNA base composition.

mur, Schildkraut and Doty, 1961), do, in fact, demonstrate a marked identity in overall base compositions in their DNA.

It is important to know, then, how results of numerical taxonomy can be interpreted in light of developments in molecular taxonomy. Two questions arise. Will the phenetic groupings obtained by numerical analysis be indicative of homology in terms of genetic compatibility and, at the least, be homogeneous groups of similar DNA base composition? Will it be possible to combine usefully the methods of computer analysis and DNA base composition analysis for description and ordering of microbial groups?

From the machine-processed data obtained in this study, significant clustering of strains was observed. Most of the clusters which formed were not unexpected in the terms of classical bacterial taxonomy (Breed, Murray, and Smith, 1957), but there were great variations in the degree of homogeneity as measured by group mean ΔS . The large sample of *P. aeruginosa* strains were very homogeneous (87% ΔS); the *Aeromonas punctata* and the "Vibrio" strains were each unlike the other similarly named strains. Some strains of *P. fluorescens*, 12121, 10796, and 11251, as well as one *P. chlororaphis* appeared to be patently misnamed, belonging more appropriately within the *P. aeruginosa* cluster. The S

values for these strains with *P. aeruginosa* were $>75\%$. The case history of the first strain of *P. chlororaphis* is of particular interest. Although this culture was labeled in our collection as having been received from W. C. Haynes as NRRL B-560, the machine sorting and DNA base composition indicated a high degree of similarity to *P. aeruginosa*. Indeed, the DNA of this culture formed a molecular hybrid with labeled *P. aeruginosa* NRRL B-23 DNA in the proportion indicative of extensive base sequence homology (Mandel and Marmur, unpublished data), and this culture is susceptible to certain bacteriophages active against *P. aeruginosa* (Holloway, personal communication). New subcultures of *P. chlororaphis* NRRL B-977 and B-560 (ATCC 9446) were examined for base composition; these latter show near identity to *P. fluorescens* rather than *P. aeruginosa*. Undoubtedly, some accident had befallen the label of the first culture. Nonetheless, this incident serves to illustrate the ability of the techniques involved to identify an unknown culture.

A definite ordering of the clusters was apparent from the sorted data output (Table 2), and this pattern appeared consistent even after experimental manipulation of certain of the representative strains (Table 3). The analysis of the sub-strains gave comparable results, as was shown in

a previous study (Liston, Wiebe, and Colwell, 1963). The procedure used for the measurement of the relationships gave consistent and reproducible results.

The *Pseudomonas* strains which were selected for base composition studies were those which sorted out centrally and distally within the *P. aeruginosa* cluster (Fig. 2) and cultures for which there was good reason to suspect a misclassification. From Table 4, it can be seen that there was a striking similarity of overall base compositions of those strains occurring within the *P. aeruginosa* species group. Furthermore, the predicted generic similarity of "*Vibrio*" and *Pseudomonas* was confirmed by the base composition studies.

The strains which could not be grouped by computer studies, *V. metschnikovii* and *F. aquatile*, were shown to have very low GC content as compared with high GC content of the DNA of most of the other strains studied.

The nonaffinity of *V. metschnikovii* to the other so-called vibrios was very clear. *V. metschnikovii* could be considered as more related to the strains belonging to the family Enterobacteriaceae than to any of the other clusters. Moreover, it appears to belong to the group of true vibrios reported by Davis and Park (1962), i.e., the *V. comma* and *V. eltor* strains. The strains *V. tyrogenus*, *V. cuneatus*, *V. percolans*, and *Vibrio* sp. ATCC 11171 [the *Vibrio* 01 of Happold and Key (1932)] should all be considered to be pseudomonads according to the computer studies and the DNA base composition analyses. Sebald and Véron (1963) also examined the DNA base compositions of many of these species of *Vibrio* and of other genera. In general, their conclusions are similar, but they propose that the *Vibrio* 01 (38.2% GC) be assigned to the genus *Moraxella*.

The *Xanthomonas* cluster was inspected as described above, and the results are given in Table 5. A slightly greater species group variation in GC content was noted as compared with the *P. aeruginosa* base compositions. However, the hypothesis that the 11 "species" of *Xanthomonas* are a single species appears to receive support from the data which are listed (Table 5).

The situation within the enteric and aeromonad groups is not clear-cut. The taxonomy of the latter grouping, particularly, is very much a matter of dispute (Ewing, Hugh, and Johnson, 1961; Liu, 1961; Snieszko, *personal communica-*

tion). The results (Table 3) suggest two species groupings of *Aeromonas*: *A. formicans* and *A. punctata*. However, the *A. punctata* strains did not share high affinities with each other, and the possibility that these strains represent a more diffuse grouping, not a single genus, cannot be discounted. Additional data should be accumulated for these strains and the relationships investigated more fully. From the data listed in Table 6, the view of Ewing et al. (1961) and Liu (1961) that the genus *Aeromonas* should comprise three species, *A. hydrophila* (syn. *A. liquefaciens*, *A. punctata*), *A. salmonicida*, and *A. shigelloides* will need serious consideration. It has been proposed (Eddy, 1960, 1962) that the species *A. salmonicida*, *A. liquefaciens*, and *A. formicans* be recognized but, according to nomenclature rulings, the correct names for the latter two strains should be *Aeromonas punctata* (Zimmerman, 1890, *sensu* Lehman and Neuman, 1896) Snieszko comb. nov. and *Aeromonas caviae* (Scherago) comb. nov., respectively. *A. shigelloides* probably merits separate consideration, and Sebald and Véron (1963) have proposed the new genus *Fergusonia* with *F. shigelloides* as the single species.

If the objective of any system of bacterial classification is ultimately to reflect the natural relationships of the individuals enclosed in the system (Kluyver and van Niel, 1936; van Niel, 1946), then we must admit to no more than a beginning. The advantage of this "conjoint" approach is the ability to indicate which cultures are of a sufficiently high degree of similarity to be considered as having close relationship. The possibilities of genetic relatedness (Marmur, Seaman, and Levine, 1963) and of base sequence homologies in the DNA (McCarthy and Bolton, 1963) can then be investigated in a rational manner, leading eventually to the construction of new classifications of the bacteria.

It seems premature to discard taxonomy established for the bacteria before the numerical taxonomist has been able to fill in the current, very inadequate descriptions of the named bacterial species. The improvement and revision of the current classification on the basis of genetic criteria (Luria, 1962) is a conservative view which should receive support from both the diagnostic bacteriologist and the scholastic biochemist. The species units which exist in nature, whether termed as such or called phenons

or biological clusters, serve a useful function of communication. However, it would be infinitely more useful to unite the nodal distribution of phenotypes, which are the taxa of the numerical taxonomist, with those nodal distributions observed about mean values of DNA base composition observed by the biochemist.

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