DNA Homology and Taxonomy of *Pseudomonas* and *Xanthomonas*

By J. DE LEY, I. W. PARK, R. TIJTGAT,
AND J. VAN ERMENGEM

*Laboratory for Microbiology, Faculty of Sciences,
State University, Gent, Belgium*

(Received 5 July 1965)

SUMMARY

The relatedness between several nomen species of the *Pseudomonas*–*Xanthomonas* group and some other organisms was numerically fixed through DNA homology and DNA base composition. For *Pseudomonas* the numerically analysed strains proposed by Lysenko as neotypes were used. The mean % (G + C) was in the range 60–67·5. ¹⁴C-DNA from either *Pseudomonas fluorescens* or *P. putida* was hybridized with DNA from 17 different species centres and the DNA homology was in the range 50–100 %. Genetic species differentiation in the genus *Pseudomonas* seems justified. In three border cases (*P. iodinum*, *P. diminuta* and *P. atlantica*) DNA homology was only 28–50 %, so the inclusion of these organisms in the genus *Pseudomonas* is uncertain. The species centres *P. pavonacea* and *P. rubescens* are omitted from the genus *Pseudomonas* because of their very low DNA homology and aberrant DNA base composition. Twenty-eight nomen species of *Xanthomonas* all form a narrow group in the range 63·5–69 % (G + C). With two exceptions DNA homology with a median strain *Xanthomonas pelargonii* was always over 75 % and frequently nearly complete. ¹⁴C-DNA from *P. fluorescens* hybridized with *Xanthomonas*-DNA to the same extent as with the pseudomonads proper. The *Xanthomonas* cluster overlapped perfectly with part of the *Pseudomonas* group. It is proposed therefore to gather all xanthomonads in a single genetic species *P. campestris*. This is such a dense cluster that the preservation of separate species names for the border cases seems undesirable. About one half to two-thirds of *Pseudomonas*- and *Xanthomonas*-DNA is identical. The genera *Rhizobium*, *Azotobacter* and *Azomonas* appeared to be rather closely related to *Pseudomonas* since they shared some 40–50 % of their DNA. The genus *Serratia* appeared to be more closely related to *Pseudomonas* than to *Escherichia*. The genera *Gluconobacter*, *Acetobacter*, *Serratia* and *Escherichia* shared some 20–30 % DNA with *Pseudomonas*, but *Bacillus*-DNA was almost entirely different. From a comparison between DNA homology and taximetric similarity, it appeared that most pseudomonads would not contain unused genes. The advantages of a classification based on % (G + C) and DNA homology are obvious.

INTRODUCTION

Assuming a molecular weight of 6×10^9 for bacterial chromosomal DNA and about 10^3 base pairs per cistron, it can be estimated that a bacterial genome contains some 10^4 cistrons. The present orthodox classification, as exemplified in

Bergey's Manual (1957), is built on phenotypic data, quite often on very few, thus encompassing only a minute fraction of the total genome. Numerical analysis is a much better approach to determine relatedness: because up to 200 characters may be used, and assuming that an average of 5–10 cistrons is involved in each character, it can be estimated that some 10–20 % of the genome is detected. Many properties still remain hidden and classification could be improved considerably by determining the degree of identity of chromosomal DNA. This is possible through the DNA-hybridization method of McCarthy & Bolton (1968). The present paper is a molecular biological approach to a more rational classification based on two DNA parameters: (i) base composition, expressed as a mean molar (guanine + cytosine) content (% (G + C)), and (ii) homology. It is likewise an attempt for a better understanding of the ill-defined concept of species and genus in bacteriology. As a model system we sought to clarify the relatedness between several nomen species within *Pseudomonas* and *Xanthomonas*, as well as the relatedness between the two groups. We selected the collection of *Pseudomonas* strains from Lysenko (1961) because their taximetric similarity is well known. By comparing DNA homology and taximetric similarity of the same strains, one might also detect whether a fraction of the genome remains unused. For *Xanthomonas* some 60-odd nomen species are listed in *Bergey's Manual* (1957). With nine of them 80–100 % of their DNA is identical and they can be gathered in one genetic species, *X. campestris* (Friedman & De Ley, 1965; De Ley & Friedman, 1965). These investigations were extended in the present paper with a total of 28 different nomen species and their DNA homology with a representative *Pseudomonas* was determined. The degree of DNA homology was determined between the pseudomonads and representatives of a few other genera in the same % (G + C) range (*Gluconobacter*, *Acetobacter*, *Serratia*, *Rhizobium*, *Azotobacter* and *Azomonas*) and in a different % (G + C) range (*Escherichia* and *Bacillus*).

METHODS

Organisms used. All organisms used are shown in Table 1. The strains of *Pseudomonas* (except strain PR 107) were received from Dr O. Lysenko, Laboratory of Insect Pathology, Prague; the strain numbers are from the Culture Collection of Entomogenous Bacteria (CCEB), Prague. Strain 293 was originally thought to be *P. synxantha* (Lysenko, 1961) but was later identified as *Serratia marcescens* (*Catalogue of Cultures*, 1964). Strain 607 was only tentatively labelled as *P. synxantha* (O. Lysenko, personal communication). All xanthomonads were received from Professor M. P. Starr from the International Collection of Phytopathogenic Bacteria, Department of Bacteriology, University of California, Davis, California. The strain PR 107 was received as *Pseudomonas* (*Xanthomonas*?) *rimaefaciens*. The results given in the present paper show it to belong in *Pseudomonas*. The *Rhizobium* strains were received from Professor C. Bonnier, Institut Agronomique de l'État, Gembloux, Belgium, and the *Azotobacter* strain was obtained from the National Collection of Industrial Bacteria (NCIB), Aberdeen, Scotland. In a future paper we shall bring evidence to show why the generic name *Azomonas* (Baillie, Hodgkiss & Norris, 1962), which is used in Table 1, is a better choice for strain 9218. The strains of *Gluconobacter*, *Acetobacter*, *Escherichia* and *Bacillus* were from our departmental collection.

Cultivation techniques. All organisms were grown on solid media in Roux flasks. The *Pseudomonas* and *Serratia* strains were grown for about 2 days at 30° on a medium containing (% w/v): Proteose peptone 1, yeast extract (Nederl. Gist-en Spiritusfabriek, Brugge, Belgium) 0.1, NaCl 0.5, glucose 1 and agar 2.5. The xanthomonads were grown at 25° for 3–4 days on a medium, selected for good growth and low polysaccharide production, and containing (% w/v): beef extract (Oxoid) 1, Difco-peptone 1, NaCl 0.5, agar 2.5. The acetic acid bacteria were grown for 3 days at 30° on (% w/v): glucose 5, CaCO₃ 3, yeast extract 1, agar 2.5. *Rhizobium* was grown for 3 days at 25° on (% w/v): mannitol 1, NaCl 0.02, K₂HPO₄ 0.05, MgSO₄·7H₂O 0.02, CaSO₄·2H₂O 0.01, CaCO₃ 0.01, yeast extract 0.1, agar 2.5. *Azotobacter* and *Azomonas* were grown for 2 days at 30° on (% w/v): glucose 1, K₂HPO₄ 0.1, MgSO₄·7H₂O 0.02, CaCO₃ 0.1, NaCl 0.02, Na molybdate 0.0005, agar 2.5. *Escherichia coli* and *Bacillus subtilis* were grown for 1–2 days at 30° on (% w/v): peptone 0.5, yeast extract 0.25 and agar 2.5.

Cultivation of ¹⁴C-labelled strains. *Xanthomonas pelargonii* P 121 was selected because its % (G + C) is in the middle of the group. For the same reason it had been used in previous hybridization experiments (Friedman & De Ley, 1965; De Ley & Friedman, 1965). *Pseudomonas putida* 520 and *P. fluorescens* 488 were selected because their % (G + C) (Table 1) and taximetric similarity (Lysenko, 1961) put them centrally in the *Pseudomonas* group. The organisms were grown at 30° on a reciprocal shaker in liquid medium in 1 l. Erlenmeyer flasks containing 200 ml. each of the medium. The medium contained (% w/v) proteose peptone (Oxoid) 1, yeast extract 0.1, NaCl 0.5, glucose 1, at pH 7.2. At the beginning of the log phase a sterile solution with 100 µc. uracil-2-¹⁴C/200 ml. of medium was added and the organisms were allowed to grow until the end of the log phase. They were harvested and washed several times in 0.01 M-phosphate buffer (pH 6).

SSC buffer contained 0.15 M-NaCl and 0.15 M-trisodium citrate at pH 7.0. It was also made up at quarter strength (0.25 × SSC) and double strength (2 × SSC) etc.

Preparation of DNA was done according to Marmur (1961). Its molecular weight was in the range 5–10 × 10⁶. High molecular weight denatured DNA agar was prepared according to Bolton & McCarthy (1962), with the exception that it was heated at 107–110° in 0.25 × SSC buffer instead of at 100°, because the ‘melting point’ of *Pseudomonas*- and *Xanthomonas*-DNA is rather high. Depending on the strain 1 g. of wet agar contained about 500 µg. DNA. ¹⁴C-DNA was sheared by passing it through the French pressure cell at 12,000 p.s.i. Its molecular weight was about 3 × 10⁵. It was denatured at 107–110° in 0.1–0.2 × SSC buffer for 5 min. followed by quick cooling in ice. Its final concentration was 50 µg./ml. 2 × SSC buffer. The specific activity was 680 cpm./min./µg. ¹⁴C-DNA from *X. pelargonii*, 336 cpm./min./µg. ¹⁴C-DNA from *P. fluorescens* 488 and 70 cpm./min./µg. ¹⁴C-DNA from *P. putida* 520.

Hybridization experiments; phosphodiesterase treatment. The method of McCarthy & Bolton (1963) was used. An amount of agar containing 350 µg. of high-molecular denatured DNA from different strains and 35 µg. of sheared denatured ¹⁴C-DNA from each of the three labelled strains was used. Disintegrations were counted as previously described (De Ley & Friedman, 1965). The correction factor for self-absorption in 2 × SSC buffer in the conditions used was 14.4 %; for 0.01 × SSC it was negligible.

To determine the percentage of unhybridized ¹⁴C-DNA which adhered as loose single-stranded ends to the DNA-agar after washing with 2 × SSC buffer, a treat-

Table 1. *Some physical properties ('melting point' T_m ; average (guanine + cytosine) content % (G + C)) of purified DNA and degree of DNA homology of several pseudomonads, xanthomonads and a few other groups.*

The pseudomonads are the neotype species centres as proposed by Lysenko (1961). The values with * are taken from De Ley & Friedman (1965), the values for T_m and % (G + C) for the acetic acid bacteria are from De Ley & Schell (1963), *Rhizobium* from De Ley & Russell (1965) and *Escherichia coli* from De Ley (1965). The 'melting point' T_m was determined with the automatic recording thermal spectrophotometer (De Ley & Van Muylen, 1963). The % (G + C) was calculated from T_m according to Marmur & Doty (1962). The method of McCarthy & Bolton (1963) was followed for the hybridization experiments.

Strain number	Organism	T_m	% (G + C)	Hybridization with ^{14}C -DNA from <i>P. fluorescens</i> 488		Hybridization with ^{14}C -DNA from <i>P. putida</i> 520		Hybridization with ^{14}C -DNA from <i>X. pelargonii</i> P121	
				% ^{14}C -DNA bound	% ^{14}C -DNA bound relative to <i>P. fluorescens</i>	% ^{14}C -DNA bound	% ^{14}C -DNA bound relative to <i>P. putida</i>	% ^{14}C -DNA bound	% ^{14}C -DNA bound relative to <i>X. pelargonii</i>
	<i>Pseudomonas</i>								
338	<i>geniculata</i>	97.0	67.5	35.8	64	28.5	51	—	—
513	<i>diminuta</i>	96.9	67.3	21.5	38	21.2	38	—	69*
481	<i>aeruginosa</i>	96.7	66.8	35.4	63	34.0	61	—	—
506	<i>atlantica</i>	96.6	66.5	15.7	28	16.2	29	—	75*
PR107	<i>rimae faciens</i>	96.6	66.5	35.8	64	—	—	33.5	55
380	<i>ovalis</i>	95.9	64.8	65.0	116	37.7	67.5	—	63*
522	<i>stutzeri</i>	95.8	64.5	42.4	75.5	33.5	60	—	—
520	<i>putida</i>	95.4	63.5	39.6	70.5	55.8	100	32.8	54
559	<i>chlororaphis</i>	95.3	63.3	36.5	65	34.6	62	—	—
518	<i>aureofaciens</i>	95.1	62.8	30.8	55	27.8	50	—	—
488	<i>fluorescens</i>	94.95	62.4	56.1	100	46.3	83	40.8	67
525	<i>denitrificans</i>	94.9	62.3	35.4	63	43	77	—	81*
607	<i>synzantha</i>	94.35	61.0	52.3	93.5	35.7	64	—	—
387	<i>fragi</i>	94.2	60.6	41.0	73	35.7	64	—	62*
512	<i>iodinum</i>	94.05	60.2	25.3	45	27.3	49	—	—
381	<i>taetrolens</i>	93.85	59.8	43.8	78	32.6	58.5	—	—
519	<i>rubescens</i>	88.25	46.1	1.7	3	3.3	6	—	—
538	<i>pavonacea</i>	88.05	45.6	3.9	7	5.5	10	—	—
	<i>Xanthomonas</i>								
C144	<i>celebensis</i>	97.7	69.2	18.5	33	—	—	32.8	54
H110	<i>hyacinthi</i>	97.4	68.5	33.1	59	—	—	45.6	75
C129	<i>campestris</i>	97.3	68.2	—	—	—	—	—	91*

Table 1 (continued)

Strain number	Organism	T_m	% (G + C)	Hybridization with ^{14}C -DNA from <i>P. fluorescens</i> 488		Hybridization with ^{14}C -DNA from <i>P. putida</i> 520		Hybridization with ^{14}C -DNA from <i>X. pelargonii</i> P 121	
				% ^{14}C -DNA bound	% ^{14}C -DNA bound relative to <i>P. fluorescens</i>	% ^{14}C -DNA bound	% ^{14}C -DNA bound relative to <i>P. putida</i>	% ^{14}C -DNA bound	% ^{14}C -DNA bound relative to <i>X. pelargonii</i>
T7	<i>translucens</i>	97.2	68.0	34.8	62	—	—	47.2	77.5
M16	<i>maculafoliogardeniae</i>	97.1	67.7	39.8	71	—	—	50.6	83
T20	<i>tamarindi</i>	97.1	67.7	—	—	—	—	—	104*
A121	<i>alfalfae</i>	96.9	67.3	33.7	60	—	—	54.1	89
H1	<i>hederae</i>	96.9	67.3	—	—	—	—	—	87*
B3	<i>begoniae</i>	96.85	67.1	—	—	—	—	—	92*
C104	<i>carotae</i>	96.8	67.0	—	—	—	—	—	114*
P10	<i>pruni</i>	96.7	66.8	28.6	51	—	—	48.7	80
L1	<i>lespedezae</i>	96.65	66.6	33.7	60	—	—	56	92
C5	<i>corylina</i>	96.65	66.6	41.0	73	—	—	52.3	86
P121	<i>pelargonii</i>	96.6	66.5	31.4	56	27.6	49.5	60.9	100
V136	<i>vesicatoria</i>	96.55	66.4	25.0	44.5	—	—	52.3	86
J107	<i>juglandis</i>	96.5	66.3	—	—	—	—	—	93*
I3	<i>incanae</i>	96.5	66.3	27.5	49	—	—	48.7	80
P137	<i>poinsettiaeicola</i>	96.4	66.0	29.7	53	—	—	50.9	83.5
P162	<i>phaseoli</i>	96.4	66.0	29.7	53	—	—	45.6	75
C110	<i>cassavae</i>	96.35	65.9	37.6	67	—	—	55.3	91
V118	<i>vignicola</i>	96.3	65.7	32.5	58	—	—	58.3	96
P5	<i>papavericola</i>	96.3	65.7	25.8	46	—	—	48.7	80
B109	<i>beticola</i>	96.3	65.7	26.4	47	—	—	46.9	77
M2	<i>malvacearum</i>	96.25	65.6	25.8	46	—	—	37.2	61
L4	<i>lactucae</i>	96.0	65.1	27.0	48	—	—	56.7	93
V24	<i>vasculorum</i>	95.9	64.8	25.3	45	—	—	46.3	76
T11	<i>taraxaci</i>	95.7	64.3	27.0	48	—	—	54.4	89.5
G1	<i>geranii</i>	95.4	63.5	25.8	46	—	—	53.2	87.5
8131	<i>Gluconobacter oxydans (viscosus)</i>	95.35	63.4	11.2	20	10.1	18	—	23*
20	<i>Acetobacter aceti (liquefaciens)</i>	95.6	64.0	19.1	34	15.6	28	—	37*
293	<i>Serratia marcescens</i>	93.9	59.9	20.2	36	17.3	31	—	—
I5	<i>Rhizobium meliloti</i>	95.0	62.5	21.6	38.5	—	—	—	—
6.2	<i>Rhizobium leguminosarum</i>	93.6	59.1	31.4	56	—	—	—	—
9125	<i>Azotobacter chroococcum</i>	96.4	66.0	—	—	27.9	50	—	—
9128	<i>Azomonas macrocytogenes</i>	93.3	58.4	—	—	23.5	42	—	—
B	<i>Escherichia coli</i>	90.9	52.5	16.8	30	9.5	17	—	19*
BQ2	<i>Bacillus subtilis</i>	88.9	47.7	3.4	6	6.1	11	—	—
Agar		—	—	0.5	1	—	—	1.2	2

ment with dogfish phosphodiesterase was carried out as follows. Hybridization of DNA and washing of the agar column with ten 10 ml. fractions of $2 \times \text{SSC}$ buffer at 60° was carried out as usual. All buffer was then eliminated with slight air pressure until the agar was still just moist. The temperature of the column was brought down to 37° . The agar was washed with 5 ml. of PD buffer (containing 1.5×10^{-3} M- MnCl_2 0.01 M-tris, 0.14 M- NaCl ; pH 8.0) and the eluate was collected. 1/50 diluted enzyme in 1 ml. buffer was added to the DNA agar and incubated at 37° for 1 hr, upon which the column was again washed at 60° with ten 10 ml. fractions $2 \times \text{SSC}$ buffer and samples taken for counting. The last step consisted in increasing the column temperature to 75° and washing with 10–15 10-ml. fractions of $0.01 \times \text{SSC}$ buffer.

Determination of the average base composition and the compositional distribution of the DNA molecules. Both values were calculated from the thermal denaturation curve of pure DNA. The latter was determined with the automatic recording thermal spectrophotometer as previously described (De Ley & Van Muylem, 1963). The mean base composition, expressed as % (G+C), was calculated from the 'melting point' T_m with Marmur & Doty's (1962) formula $\% (\text{G+C}) = (T_m - 69.3)/0.41$. A previous paper chromatographic check on this formula (De Ley & Schell, 1963) showed it to be valid in the range used. The standard deviation σ of the compositional distribution around the average % (G+C) was calculated according to Doty, Marmur & Sueoka (1959).

RESULTS

The main results are summarized in Table 1.

DNA base composition. The pseudomonads are divided into two groups. The largest has a T_m in the range of 93.8° to 97.0° or a % (G+C) from 59.8 to 67.5. The other group comprises only *P. rubescens* 519 and *P. pavonacea* 533 with a quite different % (G+C) of about 46%. The latter strains are thus genetically different from the main group. The % (G+C) range of the main group agrees very well with values of a few strains reported by Colwell & Mandel (1964), De Ley & Friedman (1965), Marmur, Falkow & Mandel (1963).

The 28 strains of *Xanthomonas* are all in the narrow range of T_m $96.6^\circ \pm 1.2^\circ$ or a % (G+C) from 63.5 to 69.2. These values confirm the previous conclusion from this laboratory, that these organisms form a narrow group. It may be noted that the % (G+C) values of *Xanthomonas* overlap with the higher range of *Pseudomonas* and that no distinction can be made between these groups on the basis of base composition alone. The T_m of *Serratia marcescens* 293 falls completely within the range of other serratias (Colwell & Mandel, 1965), although it might belong to a slightly aberrant subgroup. It is noticeable that the % (G+C) from *Serratia* overlaps with the lower range of *Pseudomonas*.

Compositional distribution of DNA molecules. Most of the pseudomonads and all xanthomonads have a σ value between 0 and 1 with an average of 0.3, a value which confirms our previous findings (De Ley & Van Muylem, 1963). The unusually high value of 2.5 for *Pseudomonas iodinum* is another argument that this strain possibly does not belong in *Pseudomonas*. The high σ value of 3.0 for *Serratia marcescens* 293 conforms to the values of 1.3–3 for other Enterobacteriaceae (De Ley & Van Muylem, 1963; De Ley, 1965). A word of caution is required on the calculation of

Table 2. *Determination of the fraction of single-stranded ¹⁴C-DNA, attached to DNA agar, which remains unhybridized.*

Species	Total c.p.m.* in wash	Total c.p.m. released by phospho- diesterase†	Total c.p.m. retained on DNA-agar after phospho- diesterase	% labelled DNA released by phospho- diesterase	With phosphodiesterase		Without phosphodiesterase	
					% labelled DNA bound	% labelled DNA bound relative to <i>P. fluorescens</i>	% labelled DNA bound	% labelled DNA bound relative to <i>P. fluorescens</i>
<i>P. fluorescens</i>	4736	744	4975	7.1	47.6	100‡	54.7	100‡
<i>P. aeruginosa</i>	7150	458	3315	4.2	30.3	63.7	34.5	61.4
<i>P. taetrolens</i>	6212	755	3930	6.9	36.1	75.8	43.0	76.5
<i>P. chlororaphis</i>	7184	355	3090	3.3	29.1	61.1	32.4	57.7
<i>P. stutzeri</i>	5800	194	3825	2.0	39.0	81.9	41.0	73.0

* C.p.m. = counts per minute.

† The phosphodiesterase treatment is described in the text.

‡ All values relative to *P. fluorescens* 488, which is taken as 100 %.

The method of McCarthy & Bolton (1963) was followed for the hybridization experiments.

the σ value according to Doty *et al.* (1959). When the compositional distribution is calculated from this σ value with, for example, the strains H110, P121 and G1, it appears that these strains would have very few DNA molecules in common, but the hybridization experiments show that most of the DNA molecules are identical. This means that the overlapping of the compositional distribution curves must be considerable, and thus that the σ values are too small because the correction factor of $3^\circ 0$, introduced by Doty *et al.* (1959), is too great. The best fit with our experimental data is obtained when no correction at all is applied; this means also that all σ values, calculated from thermal denaturation curves, including those mentioned above, would be some 7.5 % (G + C) too small.

Table 3. *Comparison between DNA homology and taximetric similarity of several strains of Pseudomonas.*

The former values are from Table 1 and the latter, for the same strains, are from Lysenko (1961). Values indicated by * are put as 100 %.

Species	Relative to <i>P. putida</i> 520		Relative to <i>P. fluorescens</i> 488	
	% DNA homology	% taximetric similarity	% DNA homology	% taximetric similarity
<i>P. geniculata</i>	51	44	64	62
<i>P. diminuta</i>	38	61	38	62
<i>P. aeruginosa</i>	61	73	63	72
<i>P. atlantica</i>	29	50	28	56
<i>P. ovalis</i>	67.5	81	116	77
<i>P. stutzeri</i>	60	52	77.5	68
<i>P. putida</i>	100*	100*	70.5	78
<i>P. chlororaphis</i>	62	80	65	86
<i>P. aureofaciens</i>	50	75	55	79
<i>P. fluorescens</i>	83	78	100*	100*
<i>P. denitrificans</i>	77	65	63	59
<i>P. fragi</i>	64	66	73	69
<i>P. iodinum</i>	49	65	45	59
<i>P. taetrolens</i>	58.5	58	78	68
<i>P. rubescens</i>	6	46	3	64
<i>P. pavonacea</i>	10	50	7	48

DNA-hybridization experiments. Hybridization with either *Pseudomonas fluorescens* 488 or *P. putida* 520 shows that all pseudomonads fall into two distinct groups. The low % (G + C) strains *P. rubescens* and *P. pavonacea* hybridize for only 3–10 %. The main group, made up of all the other strains, contains a few doubtful cases: *P. diminuta*, *P. atlantica* and *P. iodinum*, which hybridize to the extent of 28–50 %; all other strains show a high degree of relatedness. DNA of *P. fluorescens* 488, *P. ovalis* 380 and *P. 'synxantha'* 607 appears to be almost, if not completely, identical. The degree of DNA homology of many strains is about the same with both *P. fluorescens* and *P. putida*. All strains of *Xanthomonas*, except *X. celebensis* c144, have a DNA homology of 45–73 % with *P. fluorescens*, with an average of 54 %. This agrees closely with our previous conclusion based on a smaller collection of strains that about two-thirds of *Xanthomonas*-DNA is equal to *Pseudomonas*-DNA (De Ley & Friedman, 1965).

From the hybridization of DNA from all xanthomonads with *X. pelargonii* P121,

it follows that all strains, except *X. celebensis* c144 and *X. malvacearum* m2, hybridize for 75 % or more, with an average for the group of 87 %. Nine strains out of 27 hybridize for 90–100 % with *X. pelargonii*; eleven strains hybridize for 80–90 % and the remaining 5 for 76–79 %.

Reproducibility of the method. The values for DNA homology were in most cases reproducible to within 1–6 %, although in four cases errors of 9–13 % were observed. The number of erroneous results depends on the age of the DNA preparations. High-molecular, double-stranded DNA can be preserved in good condition in concentrated solution (about 1 or more mg. DNA/ml. SSC buffer) for several months at 4° with a drop of chloroform added. For reproducible results it is advisable to use freshly prepared DNA agar and sheared denatured ¹⁴C-DNA solution that is not over 2 weeks old.

The effect of phosphodiesterase. One set of experiments, representative for several others, is summarized in Table 2. It was invariably found that with every strain used, about 5 % of the total recovery is not hybridized; this corresponds to about one-tenth of the length of a ¹⁴C-DNA fragment. Since this value is about the same for both the reference strain and the others under investigation, it does not affect the final conclusion on DNA homology.

DISCUSSION

An approach to a rational classification, based on two independent parameters from the genome (mean base composition and DNA homology) is represented in Figs. 1–3.

Xanthomonas

With respect to the central reference strain *X. pelargonii* p121, all strains (except m2 and c144, see below) form one tight cluster (Fig. 1). These results throw a new light on the biological relatedness between organisms and on the species concept. They show that artificial pigeon-holing in the usual orthodox classifications of this genus do not reflect reality but they corroborate the concept of a genetic species. The genus *Xanthomonas* appears not to contain about 60 sharply separated species, but to be a tight cluster of strains, each one having 75–100 % of its DNA identical with that of the central reference strain, and probably also amongst themselves. It seems to us that at least the ten strains which have over 90 % DNA similarity (campestris, tamarindi, begoniae, carotae, lespedezae, pelargonii, juglandis, cassavae, vignicola and lactucae) can no longer be regarded as separate species and should be gathered into one. In view of the tightness of the group, it seems better to regard the entire group as one biological unit, one geno-species, for which the specific epithet 'campestris' is proposed (the generic name will be discussed below). This supports a previous suggestion (Friedman & De Ley, 1965; De Ley & Friedman, 1965), which was made after a similar study of a smaller group. The most widely divergent strains, e.g. pelargonii and phaseoli, differ by 25 % of their genome or about 2500 cistrons. Whether these organisms deserve separate species recognition is more a matter of opinion than of fact. For nomenclatural purposes it should be recalled that other organisms which differ by some 25 % of their genome, such as rhesus monkey and man (Hoyer, McCarthy & Bolton, 1964), may differ by as many as 8×10^6 cistrons ('total' mol. weight of mammalian DNA 2×10^{12} and assuming

Fig. 1. Relatedness within the *Xanthomonas* group and between the xanthomonads and the pseudomonads, on the basis of DNA base composition and DNA homology (hybridization relative to *X. pelargonii* P 121). The organisms, whose position on the map is indicated by $\times$ followed by a strain number, are named xanthomonads. The line ---- shows the approximate border of the proposed genetic group *Pseudomonas campestris*. It is seen that strain M 2 and C 144 are aberrant from the main group. The position of the named pseudomonads is indicated by $\bullet$ and the abbreviation of the species name. The named *Pseudomonas* group is delineated by — — —. Relatedness with a strain of *Acetobacter* (Ac) and *Gluconobacter* (Gl) is given for comparison. All data from Table 1.

10^3 base pairs per cistron) and that a few thousand genes are now thought to account for the differences within the species man.

The strains *malvacearum* M 2 and *celebensis* C 144 seem to fall outside the *Xanthomonas* group. The former strain, however, still groups with the other xanthomonads, with respect to *P. fluorescens* 488. Strain C 144 is the most remote one. We believe it to be still a xanthomonad because of its alleged phytopathogenicity and because we detected the typical xanthomonas-carotenoid (absorption maxima at 464 and

437 m μ and a shoulder at 418 m μ) after extraction according to Starr & Stephens (1964). Both strains might belong to separate species-centres, although it seems more likely that they are aberrant variations on the main theme. Other strains aberrant in % (G+C) are known, in *Chromobacterium* and in *Agrobacterium* (De Ley & Van Muylem, 1963; De Ley, 1964).

Pseudomonas

The strains 'rubescens' 519 and 'pavonacea' 533 fall completely outside the main group, since their DNA is almost completely different. They differ more from *Pseudomonas* than does, for example, *Escherichia*. We propose to remove them from the genus *Pseudomonas*. Their % (G+C) and physiological properties suggest a

Fig. 3. Relatedness within the pseudomonas group and between the pseudomonads and a few other genera on the basis of DNA base composition and DNA homology (hybridization relative to *P. putida* 520). Symbols and abbreviations as in Figs. 1 and 2.

possible relationship with *Vibrio* which might be worth investigating. All the other strains form one broad group in which the strains are clearly separated. Since each strain is proposed as a species centre on the basis of numerical analysis (Lysenko, 1961), the present results indicate that—in contrast to *Xanthomonas* species differentiation in *Pseudomonas* may be justified. Since DNA-homology of the three doubtful cases (*P. iodinum*, *P. atlantica* and *P. diminuta*) is small, their inclusion in *Pseudomonas* is open to doubt. In fact they are about as far removed from *P. fluorescens* as, for example, *Serratia*, *Acetobacter*, *Rhizobium* and *Azotobacter*. The strain 'rimaefaciens' PR 107, previously of uncertain generic status, appears to belong to *Pseudomonas*.

Previously we found that about 60–80% of *Xanthomonas*-DNA is identical to *Pseudomonas*-DNA (De Ley & Friedman, 1965). The reverse is also true since 45–73% of *Pseudomonas fluorescens*-DNA is identical with *Xanthomonas*-DNA.

Figure 2 confirms Fig. 1: in relation to *P. fluorescens* all xanthomonads form one tight cluster. They share about as much DNA with *P. fluorescens* as do, for example, *P. geniculata* or *P. aeruginosa*. Therefore, there seems to be no reason to keep the xanthomonads in a separate genus and we propose to gather them in a single genetic species *Pseudomonas campestris*. It may be recalled that the creation of the genus-name *Xanthomonas* (Dowson, 1939) was not inspired by genetic data, but by the desire to group together the yellow-pigmented, necrotic phytopathogenic pseudomonads.

Phenotypically non-expressed DNA in Pseudomonas. It is not known whether all or only part of bacterial DNA is phenotypically expressed. It is conceivable that a certain fraction would have no genetic function as for example, nonsense DNA, and unused genes. Comparison of taximetric similarity of characters and DNA homology of the same strains might provide an answer. If homologous, phenotypically non-expressed DNA is present, DNA homology will be higher than the similarity value. Such a comparison is made in Table 3. In 21 out of 30 cases DNA homology is not higher than the taximetric similarity. In many of the remaining 9 cases the difference lies within the limit of sensitivity of the methods. Therefore it seems that in most cases all DNA is used for phenotypic expression and that unused genes either do not exist or are rather scarce.

Imperfection of numerical analysis. In spite of its considerable advance over usual classification, taximetric analysis still deals with only a small part of the genome. Therefore occasional imperfections in its conclusions can be expected. It was previously (De Ley & Van Muylem, 1963) pointed out that the yellow-pigmented marine Cytophaga-like bacteria fall into widely divergent % (G + C) groups. The present paper provides another example with the strains rubescens and pavonacea. A classification based on % (G + C) and DNA homology appears to us to be more logical.

Relatedness of some other genera to Pseudomonas (Figs. 2 and 3). Genera such as *Gluconobacter*, *Acetobacter*, *Serratia* and *Escherichia* have some 20–30 % DNA similarity with *Pseudomonas*. They would have some 2000 cistrons in common with it. *Rhizobium*, *Azotobacter* and *Azomonas* appear to be more closely related to *Pseudomonas* than expected, since 40–56 % of their DNA seems to be *Pseudomonas*-like. Similarly, *Serratia* might be more closely related to *Pseudomonas* than expected. The chromosome of *Bacillus* is almost entirely different.

One of us (J.D.L.) is indebted to the Centre National de Biochimie et de Biologie Moléculaire for research and personnel grants. Another (I.W.P.) is indebted to the Patrimonium of the University of Gent for a study grant. We are indebted to the institutes and individuals who kindly sent us the strains, and to Dr M. Mandel, Texas, Medical Centre Houston, Texas, for a gift of phosphodiesterase.

REFERENCES

- BAILLIE, A., HODGKISS, W. & NORRIS, J. R. (1962). Flagellation of *Azotobacter* spp. as demonstrated by electron microscopy. *J. appl. Bact.* **25**, 116.
Bergey's Manual of Determinative Bacteriology. (1957). Ed. by R. S. Breed, E. G. D. Murray and N. Smith. Baltimore: Williams and Wilkins Co.
 BOLTON, E. T. & MCCARTHY, B. J. (1962). A general method for the isolation of RNA complementary to DNA. *Proc. natn. Acad. Sci., U.S.A.* **48**, 1390.

- Catalogue of Cultures, Czechoslovak Collections of Micro-organisms*, Brno, 1964.
- COLWELL, R. R. & MANDEL, M. (1964). Adansonian analysis and deoxyribonucleic acid base composition of some Gram-negative bacteria. *J. Bact.* **87**, 1412.
- COLWELL, R. R. & MANDEL, M. (1965). Adansonian analysis and deoxyribonucleic acid base composition of *Serratia marcescens*. *J. Bact.* **89**, 454.
- DE LEY, J. (1964). Effect of mutation on DNA-composition of some bacteria. *Antonie van Leeuwenhoek*, **30**, 281.
- DE LEY, J. (1965). DNA base composition of *Klebsiella rubiacearum*. *Antonie van Leeuwenhoek*, **31**, 203.
- DE LEY, J. & FRIEDMAN, S. (1965). Similarity of *Xanthomonas* and *Pseudomonas* deoxyribonucleic acid. *J. Bact.* **89**, 1306.
- DE LEY, J. & RASSEL, A. (1965). DNA base composition, flagellation and taxonomy of the genus *Rhizobium*. *J. gen. Microbiol.* **41**, 85.
- DE LEY, J. & SCHELL, J. (1963). Deoxyribonucleic acid base composition of acetic acid bacteria. *J. gen. Microbiol.* **33**, 243.
- DE LEY, J. & VAN MUYLEM, J. (1963). Some applications of deoxyribonucleic acid base composition in bacterial taxonomy. *Antonie van Leeuwenhoek*, **29**, 344.
- DOTY, P., MARMUR, J. & SUEOKA, N. (1959). The heterogeneity in properties and functioning of deoxyribonucleic acids. *Brookhaven Symp. Biol.* **12**, 1.
- DOWSON, W. J. (1939). On the systematic position and generic names of the Gram negative bacterial plant pathogens. *Zentbl. Bakt. (2 Abt. Orig.)*, **100**, 177.
- FRIEDMAN, S. & DE LEY, J. (1965). 'Genetic species' concept in *Xanthomonas*. *J. Bact.* **89**, 95.
- HOYER, B. H., MCCARTHY, B. J. & BOLTON, E. T. (1964). A molecular approach in the systematics of higher organisms. *Science*, **144**, 959.
- LYSENKO, O. (1961). *Pseudomonas*—an attempt at a general classification. *J. gen. Microbiol.* **25**, 379.
- MCCARTHY, B. J. & BOLTON, E. T. (1963). An approach to the measurement of genetic relatedness among organisms. *Proc. nat. Acad. Sci., U.S.A.* **50**, 156.
- MARMUR, J. (1961). A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J. mol. Biol.* **3**, 208.
- MARMUR, J. & DOTY, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J. mol. Biol.* **5**, 109.
- MARMUR, J., FALKOW, S. & MANDEL, M. (1963). New approaches to bacterial taxonomy. *Annu. Rev. Microbiol.* **17**, 329.
- STARR, M. P. & STEPHENS, W. L. (1964). Pigmentation and taxonomy of the genus *Xanthomonas*. *J. Bact.* **87**, 293.