Ancestral Remnants in the Deoxyribonucleic Acid
from Pseudomonas

J. De Ley* and In Won Park

*Laboratory of Microbiology and Microbial Genetics,
State University of Gent, Gent, Belgium, and Department of Chemistry,
College of Liberal Arts and Sciences, Seoul National University, Seoul, Korea
(Received August 12, 1969)

Deoxyribonucleic Acid 속의
Pseudomonas 로 부터의 Ancestral Remnants

J. De Ley* 박 인 원

Laboratory of Microbiology and Microbial Genetics, State University of Gent, Gent, Belgium.

(1969. 8. 12 접수)

ABSTRACT

Cross-hybridizations between DNA of two pseudomonads and a xanthomonad suggested that the three DNA types had a considerable section in common. The existence of this common part was proved by hybridization of pre-selected DNA, i.e. DNA resulting from a previous hybridization between any one set of two DNA types, with the third type. It was thus shown that about 50% of the DNA of the three organisms was similar. This common part was isolated in pure state and its % (G+C) was found to be identical to the overall base composition of the native DNA. The evolutionary drift in % (G+C) could thus not be detected. The total molecular weight of the chromosomal DNA/bacterial nucleoid was determined to be $2.4 \times 10^9$ daltons. It can therefore be estimated that the common putida-fluorescens-pelargonii DNA part consists of some $2 \times 10^3$ cistrons. P. putida and P. fluorescens share an additional $1,300$ cistrons, and all xanthomonads share at least an additional $1,000$ cistrons.

요 약

Pseudomonad DNA 의 공통부분

pseudomonad DNA 와 Xanthomonad DNA 의 cross-hybridization 의 결과는 pseudomonas putida, pseudomonas fluorescens 의 pseudomonas compestris var. pelargonii 로의 DNA 는 그 구조에 상당한 방위의 공통된 부분을 가진 것으로 나타났다. 이러한 공통부분의 존재는 두 종류의 DNA 사이의 hybridization 으로 미리 선택됨 부분을 세 번째의 DNA 와 hybrid 로 형성시킴으로써 증명되었다. 이러한 실험결과에 의하여 위의 세 pseudomonad DNA 는 약 50%의 공통부분을 서로 가지고 있다는 것을 알 수 있었다.
INTRODUCTION

Deoxyribonucleic acid from pseudomonads and xanthomonads appears to have many nucleotide sequences in common (1). About 60–80% of the DNA from P. campestris var. pelargonii is similar to the DNA from several pseudomonads. Conversely some 40–70% of the pseudomonad fluorescens DNA is similar to the DNA of several xanthomonads (1). From the above reciprocal DNA-hybridizations between P. fluorescens, P. putida and P. campestris var. pelargonii it can be calculated that the DNA of these organisms might contain a number of similar nucleotide sequences, involving up to 54% of each chromosome. If the existence of such a common part could be demonstrated it would be a new argument in favour of a common ancestral origin for pseudomonads and xanthomonads, possibly from some primitive pseudomonads. The common DNA part would thus represent the remnant of this ancestral chromosome which has remained nearly unchanged during the evolutionary divergencies leading to these two organisms existing at present.

This work is concerned with the experimental confirmation of the existence of this common DNA part and of some of its properties, such as, base composition and number of nucleotide pairs.

MATERIALS AND METHODS

Determination of the molecular weights of DNA preparations. The molecular weight of the intact chromosomal DNA of our organisms are 2.4–3×10^9 daltons (see Table 2). Unavoidable fragmentation of the DNA molecules occurs during the preparation. For the hybridization experiments it is important to have DNA molecules with an average molecular weight of, at least, 2×10^8 daltons. Therefore the molecular weight of many pure DNA samples was determined by ultracentrifugation.

A solution of 70 μg DNA/ml, 1×SSC buffer, pH 7.0, was centrifuged with an An-D rotor in an analytical ultracentrifuge, Spinco, model E at 37,020 rpm for 45 min., at 25°C. Every 4 min. photograph was taken in UV light. The photographic plates were developed and converted into a transmittance graph by means of a Joyce-Loebl Densitometer.

Hybridization with pre-selected C^-DNA fragments and estimation of the common part between the P. putida, P. fluorescens and P. campestris var. pelargonii. The principle of the method of this experiment is the same as described previously (1). One difference in the present procedure was that pre-selected C^-DNA fragments were denatured again and used for further hybridization with the DNA of the other strains. One series of experiments will be given as an example. The complete scheme of these experiments is shown diagrammatically in Fig. 1. In a closed glass bottle containing 5 ml of 2×SSC buffer, 140 μg of sheared denatured C^-DNA (sp. act., 580 cpm/μg DNA) from the xanthomonad sp 121 was incubated for 16 hours at 60°C with 1,400 μg of unlabeled, unheated, denatured DNA from P. fluorescens, trapped in 3.599 g of 3% agar. The mixture was then transferred into a water-jacketed chromatographic column, thermostatted at 60°C. It was washed with 160 ml of 2×SSC buffer until negligible radioactivity due to unhybridized DNA fragments, was eluted. The total radioactivity of these washings was 46,870 cpm. The hybridized C^-DNA was eluted from the agar column with 13 ml of 0.01×SSC buffer at 75°C and collected in 10 ml fractions. The total radioactivity of these 13 fractions was 34,990 cpm and therefore 42.7% of the added C^-DNA had been hybridized. The first four 10 ml fractions thus collected, containing most of the eluted C^-DNA, were pooled, lyophilized and
dissolved in 5 ml of distilled water. To remove the small amount of agar particles, leached and carried with the eluant, the dissolved lyophilisate was centrifuged for 10 min. at 30,000 × g. This procedure was repeated until all agar particles were removed. The agar particles thus washed were found to be free of detectable radioactivity. The agar-free lyophilisate was dissolved in enough distilled water and 10 × SSC buffer to yield a final volume of 0.014 ml with a 0.25 × SSC concentration. The dissolved DNA was denatured at 107-110°C for 5 min., quick-cooled and adjusted with 10 × SSC buffer to a final concentration of 2 × SSC and a final volume of 2.48 ml. Total recovered, hybridized C*DNA fragments were 55 μg. Three portions of this solution, each containing 15 μg of C*DNA fragments were further hybridized separately with 150 μg of ordinary DNA from each of the three strains, which had been trapped in some 500 μg of agar. Determination of degree of hybridization was carried out as above in the DNA-agar column. The results of the above example are illustrated in Fig. 2.

Determination of the molecular weight of chromosomal DNA/nucleoid of P. putida, P. fluorescens and P. campestris var. pelargonii. Estimation of the molecular weight was carried out with cells in the early, middle and late log phase, as well as in the early and late stationary phase of growth. The organisms grown in liquid media of appropriate composition at the optimum temperature as described in our previous work(19). Growth of the cultures was followed by measuring the turbidity changes with a Klett Colorimeter. Thirty minutes before the harvest, 50 μg of chloramphenicol/ml of medium was added. After harvesting the cells were washed several times with 1 × SSC buffer and a dense suspension of about 1011 cells/ml was made. Aliquots of this suspension were used for:

1) Measuring the density of cell suspension,
2) Counting the average number of nucleoids per cell and
3) Chemical determination of the total DNA content of one cell.

The density of the original dense suspension was determined by counting the number of cells in a certain volume of this suspension. The original suspension was diluted to a suitable density and the cells were counted with a petri-Hauser counting chamber.

2 × 108 bacterial cells were centrifuged onto 1.5% agar, the thin surface layer of which was cut out of the tube and placed on glass beads over a solution of 2% OsO4 for 3 min. to fix the bacteria. The method of Smith(27) was modified for staining the nucleoids. The fixed bacteria on the agar were smeared on a cover-silde and hydrolyzed in 1N HCl for 20 min. at 60°C, rinsed several times with distilled water and stained with Giemsa dye for various lengths of time, 5-15 min., at 37°C. Giemsa stock solution diluted 50 times was suitable for P. fluorescens, 100 times diluted solution for the other strains. The average number of nucleoids was determined by examining 200 to 500 randomly selected cells. A Leitz phase contrast microscope, model Ortho Lux, was used for counting the cells (magnification: 12 × 40) and the nucleoids (magnification: 12 × 90).

2 to 7 × 104 cells were digested in 0.5 N NaOH for 20 to 24 hours at 37°C. After adjusting the digest to 0.6 N HClO4 and cooling for 30 min in an ice bath, 2 ml (about 2 volumes of 0.6 N HClO4 solution) of cold acetic acid were added. After 2 hours the precipitate was centrifuged and washed twice with 1 ml of cold 0.6 N HClO4. The DNA was hydrolyzed with 1.5 ml of 0.6N HClO4 for 20 min at 100°C. The protein residue was removed from the hydrolysate and an aliquot of acid soluble supernatant was treated with diphenylamine for the estimation of DNA(19). The optical density was read at 600 μm and compared against a standard curve prepared by running the same test with pure DNA from P. fluorescens 458.

On the likely assumption that all the detected DNA was of chromosomal origin, the molecular weight of the chromosomal DNA calculated from the following formula:

\[
\text{Mol. wt. of chromosomal DNA (daltons)} = \text{Avogadro's number} \times \text{DNA content (g) per nucleoid}
\]

The determination of the molecular weight of chromosomal DNA from Escherichia coli B was performed for the purpose of comparing our result with the value reported by other authors and ascertaining at the same time our techniques.
Three sets of experiments are depicted, each one with a different type of C\(^{14}\) DNA, represented at the top of each pyramid. Arrows show the subsequent hybridization steps. Each rectangle represents a hybridization with ordinary, high molecular, denatured DNA of the organism whose name is next to it; the number at the upper right hand corner is the absolute percent hybridization with the C\(^{14}\) DNA added.

The numbers at the bottom right hand corner are the % homology, the % hybridization of the C\(^{14}\) DNA with ordinary DNA of the same organism being taken as 100. These values are also graphically represented as the shaded areas; since the area of each rectangle is proportional to the length of the corresponding chromosome, the shaded areas represent the fraction which is similar to the labeled strain used.

**RESULTS AND DISCUSSION**

The molecular weight of Pseudomonas-DNA. A representative set of results is listed in Table 1. The average molecular weight of DNA/nucleoid, calculated from these and other data, is given in Table 2. The results from the latter table can be only regarded as estimates, since determinations of this type are subject to such uncertainties as: continuous DNA synthesis in one nucleoid, the errors associated with

<table>
<thead>
<tr>
<th>Time in hr.</th>
<th>Logarithmic phase Early</th>
<th>Logarithmic phase Late</th>
<th>Stationary phase Early</th>
<th>Stationary phase Late</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas fluorescens</td>
<td>3.5</td>
<td>6</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Turbidity in Klett units</td>
<td>55</td>
<td>165</td>
<td>370</td>
<td>405</td>
</tr>
<tr>
<td>(\mu g) DNA per cell (\times 10^9)</td>
<td>7.69</td>
<td>6.07</td>
<td>10.46</td>
<td>4.64</td>
</tr>
<tr>
<td>Average number of nucleoids per cell</td>
<td>2.2</td>
<td>2.1</td>
<td>2.0</td>
<td>1.4</td>
</tr>
<tr>
<td>Molecular weight in daltons (\times 10^{-9})</td>
<td>2.10</td>
<td>1.74</td>
<td>3.15</td>
<td>1.91</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time in hr.</th>
<th>Logarithmic phase Early</th>
<th>Logarithmic phase Late</th>
<th>Stationary phase Early</th>
<th>Stationary phase Late</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas putida</td>
<td>3.5</td>
<td>5</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>Turbidity in Klett units</td>
<td>95</td>
<td>270</td>
<td>465</td>
<td>425</td>
</tr>
<tr>
<td>(\mu g) DNA per cell (\times 10^9)</td>
<td>9.0</td>
<td>8.56</td>
<td>6.57</td>
<td>6.77</td>
</tr>
<tr>
<td>Average number of nucleoids per cell</td>
<td>2.0</td>
<td>1.9</td>
<td>1.6</td>
<td>1.2</td>
</tr>
<tr>
<td>Molecular weight in daltons (\times 10^{-9})</td>
<td>2.71</td>
<td>2.71</td>
<td>2.48</td>
<td>3.40</td>
</tr>
</tbody>
</table>
Table 2. The average molecular weight of DNA, average number of nucleotide pairs and calculated number of cistrons in one nucleoid of two pseudomonads and one xanthomonad.

618 was taken as average molecular weight of one nucleotide pair, linked in DNA. The number of nucleotide pairs in one cistron was assumed as 1,000. Escherichia coli was included as a control.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Number of determinations</th>
<th>Molecular weight of DNA/ nucleoid in daltons x 10^6</th>
<th>Number of nucleotide pairs per nucleoid x 10^5</th>
<th>Assumed number of cistrons per nucleoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. fluorescens 488</td>
<td>7</td>
<td>2.5 ± 0.7</td>
<td>4.0</td>
<td>4,000</td>
</tr>
<tr>
<td>P. putida 520</td>
<td>6</td>
<td>2.7 ± 0.3</td>
<td>4.4</td>
<td>4,400</td>
</tr>
<tr>
<td>P. campestris var. pelargonii XP 121</td>
<td>5</td>
<td>2.1 ± 0.3</td>
<td>3.4</td>
<td>3,400</td>
</tr>
<tr>
<td>E. coli B</td>
<td>5</td>
<td>3.1 ± 0.2</td>
<td>4.9</td>
<td>4,900</td>
</tr>
</tbody>
</table>

counting and frequent difficulty in determining the exact number of mycoloids/cell. Therefore the differences between the molecular weight of the pseudomonad and xanthomonad-DNA appear not to be significant. Since the average molecular weight of the DNA (2.4 ± 0.4 x 10^6 daltons), it contains some 3.9 x 10^6 nucleotide pairs and could thus accommodate some 3900 cistrons (assuming some 1000 nucleotide pairs per cistron).

The value for the molecular weight of E. coli DNA (Table 16) is in agreement with the value of 2.8 x 10^6 daltons reported by Cairns(6). The Pseudomonas chromosome is thus only 80% as long as that from Escherichia coli.

Only one symmetrical unimodal band was observed in the density gradient distributions of the native DNA from our three organisms. Thus the existence of “episome-like” or satellite DNA, largely different from the bulk of the pseudomonad DNA, may be excluded. If future work should show that the pseudomonads would have a circular chromosome, it seems likely that the molecular weight of its DNA would be 2.4 x 10^6 daltons.

Reciprocal hybridizations between DNA from the three pseudomonads. The results, shown in Table 3 and in excellent agreement with the results from previous experiments in which the same strains were used(1). Reciprocal crosses between the DNA from P. putida and P. fluorescens show 83 and 82% DNA homology respectively; between P. fluorescens and the xanthomonad the DNA homology is 53 and 57% respectively and between P. putida and the xanthomonad the homology is respectively 51 and 54%. Since within error the percent hybridization for each reciprocal cross is about the same, the size of the three genomes must also be approximately equal, thus confirming...
TABLE 3. Reciprocal Hybridization Between DNA from two Pseudomonads and One Xanthomonad.

The results are expressed as the percentage of DNA homology 100% being the hybridization of C14-DNA with ordinary DNA from the same strain. An estimate of the number of cistrons involved. Every result is an average of 2-5 experiments. For methods, see text.

<table>
<thead>
<tr>
<th>Material</th>
<th>C14-DNA from P. putida</th>
<th>C14-DNA from P. fluorescens</th>
<th>C14-DNA from P. campestris var. pelargonii</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% hybridization with C14-DNA</td>
<td>Estimated number of cistrons shared</td>
<td>% hybridization with C14-DNA</td>
</tr>
<tr>
<td>P. putida</td>
<td>100</td>
<td>3900 total</td>
<td>82 ± 13</td>
</tr>
<tr>
<td>P. fluorescens</td>
<td>83 ± 13</td>
<td>3200 total</td>
<td>100</td>
</tr>
<tr>
<td>P. campestris var. pelargonii</td>
<td>51 ± 1</td>
<td>200 total</td>
<td>53 ± 9</td>
</tr>
</tbody>
</table>

TABLE 4. DNA Base Composition in the Common Parts of the Chromosomes of Two Pseudomonads and a Xanthomonad.

For methods, see reference (19). The agreement in % (G+C), determined by either paper chromatography or thermal denaturation, is excellent for both pseudomonads. The difference for the xanthomonad-DNA is not surprising because the formula to calculated % (G+C) from thermal transition is only an average.

<table>
<thead>
<tr>
<th>Material</th>
<th>Number of estimations</th>
<th>% A</th>
<th>% T</th>
<th>% G</th>
<th>% C</th>
<th>A/T</th>
<th>G/C</th>
<th>% (G+C) from thermal transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common part pelargonii</td>
<td>fluoroscens-putida</td>
<td>7</td>
<td>19.9</td>
<td>18.0</td>
<td>28.0</td>
<td>34.1</td>
<td>1.11</td>
<td>0.82</td>
</tr>
<tr>
<td>Native DNA from P. campestris var. pelargonii</td>
<td>9</td>
<td>19.5</td>
<td>18.2</td>
<td>30.2</td>
<td>32.1</td>
<td>1.07</td>
<td>0.94</td>
<td>62.3±0.9</td>
</tr>
<tr>
<td>Common part putida</td>
<td>fluoroscens-pelargonii</td>
<td>13</td>
<td>18.8</td>
<td>17.2</td>
<td>30.1</td>
<td>33.9</td>
<td>1.09</td>
<td>0.89</td>
</tr>
<tr>
<td>Native DNA from P. putida</td>
<td></td>
<td>6</td>
<td>18.8</td>
<td>17.3</td>
<td>31.8</td>
<td>32.1</td>
<td>1.09</td>
<td>0.99</td>
</tr>
<tr>
<td>Common part fluorescens</td>
<td>pelargonii-putida</td>
<td>1</td>
<td>19.1</td>
<td>19.6</td>
<td>29.0</td>
<td>32.3</td>
<td>0.97</td>
<td>0.90</td>
</tr>
<tr>
<td>Native DNA from P. fluorescens</td>
<td>7</td>
<td>20.0</td>
<td>18.6</td>
<td>30.2</td>
<td>31.2</td>
<td>1.08</td>
<td>0.97</td>
<td>61.4±0.6</td>
</tr>
</tbody>
</table>

The above conclusion from direct molecular weight measurements. Although it is conceivable that differences in molecular weight up to some 20% could exist between the three genomes without being detectable by the above methods, a difference of this order would not be expected to alter significantly the conclusions drawn on the existence and the relative size of the common parts.

The similar size of the xanthomonad and the pseudomonad genome is what would be expected if the xanthomonads constitute a genetic species in the genus Pseudomonas, as proposed (19).

The results from Table 3 also indicate that a certain part of each of the three genomes might be similar or identical. This we call the "common part", or the "putida-fluorescens-pelargonii part". In P. putida-DNA this part might amount to at least 83—49=34% and at most 51% of its length. The common part in the fluorescens-chromosome might range from 35 to 53% and in the pelargonii-chromosome from 21 to 54%.

The confirmation of its existence and more precise estimations on the total number of nucleotide sequences involved, were obtained by experiments described in the next section.

DNA common parts in the fluorescens, putida and pelargonii-chromosome. C14-DNA from each of the three organisms was hybridized with each of the other two DNA types; the homologous fragments were released and hybridized again with each of the three DNA types (see Materials and Methods). In each case the nucleotide sequences common to one pair of organisms, also hybridized with the third organism. It may be concluded therefore that the DAN from the three organisms have nucleotide sequences in
Deoxyribonucleic Acid \\textit{Pseudomonas} \\& \textit{putida} Ancestral Remnants

common. However, the common part from each of the three organisms while similar, is not identical, as can be seen from the following consideration. If a perfectly identical common part would exist, amounting to \( \times \% \) of each of these three DNA types, then DNA fragments from organism A hybridized onto B and subsequently onto C, and the alternative A to C to B, should yield the same value of \( \times \%. \) Furthermore, DNA fragments from organism A, after duplicating with B, being released, and again hybridized with DNA from both A and B, ought likewise to give the same degree of hybridization. That this is not the case is illustrated e.g. by the three sets of second hybridizations at the right side of Fig. 1. Therefore, the "common parts" of the three organisms are similar, but not identical. Bolton et al.\(^1\) have pointed out that \( 5\% \) differences in the nucleotide sequences are permissible to allow a stable hybridization at 60°C.

The results show that \( P. \) putida and \( P. \) fluorescens are more closely related to each other than to \( P. \) campestris var. pelargonii and that the latter is more related to \( P. \) fluorescens than to \( P. \) putida.

From the results one can make a reasonable estimate of the structure of each DNA type and of the relative size of the common parts. The putida- and fluorescens-chromosomes share some \( 83\% \) of their base sequences. In the fluorescens chromosome some \( 62\% \) of this part (or \( 46\% \) of the total chromosome) is similar to the pelargonii-chromosome. In the putida chromosome, the part common to pelargonii and fluorescens might be somewhat larger, amounting to some \( 62\% \pm 6\% \). Comparison between the first and the second hybridization shows that these two chromosomes probably do not contain detectable separate pelargonii-putida or pelargonii-fluorescens parts, but that the part similar with the \textit{xanthomonad} DNA is most likely contained within the fluorescens-putida common part.

The results on the composition of the \textit{xanthomonad} chromosome show similarly that it contains some \( 44 \pm 5\% \) which is similar to both the fluorescens and the putida chromosomes. It might be but it is not certain that this chromosome contains some \( 10\% \) of its length as a pelargonii-fluorescens part. Most likely, however, this part is more similar to fluorescens than to putida.

When the \( 5\% \) error on experiments of this type is taken into account, the picture may be simplified as shown in Fig. 8 D. The common part of about \( 50\% \) is made up of some 2 million nucleotide pairs, which could accommodate some 2000 cistrons, common to the three organisms. The cistrons, which are present in this common part must be those which determine the phenotypic properties common to all three organisms. Several of these features are known: they are the cell shape, aerobic character, polar implantation of the flagella. The existence of many enzymes, common to all three organisms has been established in this laboratory (Kersters, unpublished data): the cytoplasmic, soluble glucose-6-phosphate and glutamic-6-phosphate dehydrogenases, the Enter-Doudoroff enzymes, catalase, the particulate succinate and malate dehydrogenases, many enzymes involved in the oxidation of hexoses, pentoses, lactate, formate, succinate, L-alanine and D-aspartic acid. The cistrons for all these features may thus be assumed to be located on the common part.

The fluorescens-putida common part amounting to some \( 33\% \) of the DNA, could accommodate some 1300 cistrons for phenotypic features typically shared by both organisms, such as the production of a greenish pigment, the enzymes for the oxidation of glutamate, the enzymes for the use of nitrite, ammonia and urea as nitrogen sources and numerous particulate enzymes (Kersters, unpublished) such as the dehydrogenases for D-ribose, D-xylene, D-arabinose, D-glucose, D-galactose and D-mannose. The remaining \( 17\% \), or roughly 650 cistrons, would be responsible for the species differentiation between \( P. \) putida and \( P. \) fluorescens. From Lysenko's\(^2\) results on these same organisms in conjunction with our data, it can be assumed that the cistrons for the hydrolysis of gelatin and casein would be located on the latter piece of the fluorescens-DNA; the cistrons for the consumption of xylene, glycerol, etc. would be specifically located on the putida-DNA. Each of the species \( P. \) fluorescens and \( P. \) putida consists of numerous individual strains, all slightly different from each other, It may be
recalled that the strains 520 and 488 were selected for the hybridization work, because they are typical for these two species. Therefore it may be assumed that the number of 650 species-specific cistrons will be fairly representative for all strains of both species. If DNA from many strains from one species would be cross-hybridized, it can be expected that most differences would be among the 650 cistrons, although some differences would also be expected in the common parts. The latter conclusion emerges by comparison with the extensive phenotypic analysis of 354 strains of *P. aeruginosa*.

By combining the present results with our previous work, it is tempting to conclude that the 50% piece of the pelargonium-DNA, which is different from the pseudomonad DNA, might itself consist of two sections: 1) a general xanthomonad part, totalling some 25% of the DNA, shared by most if not all strains of xanthomonads, and 2) a typical part for the variety pelargonii. This general xanthomonad-DNA part might accommodate about 1000 cistrons. As above, one can attempt a correlation with known phenotypic features shared by all xanthomonads. The general xanthomonad DNA part might thus include the cistrons for the production of the carotenoid pigment, phytochromenicity, polysaccharide production, possible beta-glucosidase, etc. The dissimilar part, consisting of 25% or less of the chromosome, accounts for the differences between the xanthomonad strains.

Finally, the above attempt correlation between common and dissimilar parts on the one hand and phenotypic properties on the other hand applies strictly to the model system of the three organisms used. When these experiments will be extended to more organisms in the genus *Pseudomonas*, the quantitative aspect of the common part will approach a more precise definition.

From the pioneering experiments of McCarthy and Bolton and Bolton et al., one can make a reasonable estimate of the relationships between genera in the Enterobacteriaceae, although more hybridizations will be needed for further confirmation. It would appear then that the central group of *Escherichia coli*, *Salmonella* and *Shigella* share some 75% of their chromosome; *Aerobacter* shares some 50% with the above three genera and *Klebsiella* about 25%. *Proteus vulgaris*, *Proteus morgani*, *Providencia* and *Serratia* appear to have only some 10% of their nucleotide sequences in common with the central group. For *Serratia* this agrees very well with our finding that one strain of this genus exhibited 31-36% DNA homology with the pseudomonads indicating that *Serratia* might be more related to the pseudomonads than to the Enterobacteriaceae and that the concept of bacterial families is unsettled.

Base composition of the pseudomonad-xanthomonad common DNA part. Bacterial chromosomes obviously underwent a drift in % GC during their evolutionary changes. Theoretical aspects of this problem have been studied by Freeze. Whether the direction of this drift is towards higher or lower % GC is not known. Since the common pseudomonad-xanthomonad part very likely represents the mutationally stable part of the ancestral pseudomonad chromosome, it was hoped that the % GC of this fraction would be different from the mean GC content of the total DNA and might thus reveal the direction of the drift. Therefore the common part was isolated from each of the three DNA types on a rather large scale and its % GC was determined by paper chromatography (see Materials and Methods). For comparison and as a control the % GC of each of the native DNA types was also determined in the same way. The results are listed in Table 4. The ratio C/G from the native DNA types is close to 1, as it should be, however, for the common parts it is about 1.15. The reason for this rather high C content in the common part is not clear, but might be linked to the fact that these determinations were effected with single stranded samples and that for steric reasons unequal matching with DNA from the recipient strain may have occurred. Nevertheless, the % GC in the common part is exactly the same as in the complete DNA. The % GC of the three common parts are very similar but not the same as one might expect. Yet this is not surprising, since the paper chromatographic method has a mean deviation of 1% and because the three common parts are not perfectly identical, as pointed...
out above. The % GC of the dissimilar DNA stretches in all three organisms is thus also similar to the entire DNA molecule. Their rates of transition GC→AT and AT→GC were thus approximately the same and the evolutionary GC drift can not be detected by using closely related organisms.

Evolutionary implications. The interpretation of the data, exemplified in Fig. 2 D, is that the three organisms derived from a pool of common ancestors and that about half of each chromosome is still very closely related to the original ancestral part. It is unlikely that either one of the three organisms is the ancestor of the other two. From the above attempted correlation between the common part and the phenotypic properties some properties of the ancestors can tentatively be reconstructed: it may have been a polarly flagellate, aerobic rod-like organism, containing catalase, the Enter-Douderoff and shunt pathways and it was able to oxidize a number of carbohydrate substrates. Its % GC was probably in the vicinity of 62%. In other phenotypic properties the ancestral organism may have differed considerably from the present-day living pseudomonads. It seems that, from the three strains under consideration, the xanthomonad diverged first or faster and that the split between P. fluorescens and P. putida was a more recent event.

The hybridizations between many other pseudomonads and xanthomonads and the same three organisms allow some further tentative suggestions. We shall only consider the pseudomonads proper and exclude organisms of doubtful generic status, such as P. iedinum, P. diminuta and P. atlantica. It would seem that P. aureofaciens, P. maltophilia, P. aeruginosa and the xanthomonads diverged first or faster from the common pool of ancestors, which explains why they have the smallest similarity with the three above organisms. The other species such as P. fragi, P. stutzeri, etc. would have originated later. The further splitting of each species into separate varieties, races and strains occurred apparently still later. The subdivision of the xanthomonads with their high degree of DNA homology is an example of this.

REFERENCES

2) Smith, A.C., J. Bacterial., 59, 575 (1950).
7) Jensen, O. *Pseudomonas aeruginosa and other green fluorescent pseudomonads*. Munksgaard, Copenhagen (1965).