DEOXYRIBONUCLEIC ACID HYBRIDS OF ACETIC ACID BACTERIA

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ABSTRACT

De Ley, J. (State University, Ghent, Belgium), and S. Friedman. Deoxyribonucleic acid hybrids of acetic acid bacteria. J. Bacteriol. 88:937-945. 1964.—Deuterated $^{14}$-labeled deoxyribonucleic acid (DNA) from Acetobacter acetii (mesoxydans 4) forms hybrids with ordinary DNA from other species of this genus (A. zylinum, A. pasteurianus, A. estunensis, and possibly A. zylinoides) when the guanine plus cytosine base composition does not vary by more than 1 to 2%. Beyond this limit (A. acetii Ch31 and A. muciparus 5) no hybrids are formed. The hybrids are apparently derived from an asymmetrical part of the compositional distribution. The results lend strength to the concept of a genetic species rather than to a division of a genus into sharply separated species, based on small phenotypic differences. Taxonomic implications are discussed.

An improved bacterial classification appears to be on the verge of emerging. The present system is based nearly solely on morphology, physiology, biochemistry, and serology. Other available information indicates that genetic exchange, molecular biology (Marmur, Falkow, and Mandel, 1963), adansonian analysis (Sneath, 1962), and comparative biochemistry (De Ley, 1962; Ann. Rev. Microbiol., in press) might be the main sources upon which improvements will depend.

In the present paper, we shall only be concerned with changes to be expected at the subgeneric level.

The concept of the bacterial species in its present state and practice is not satisfying, because it is a taxonomic unit which is only vaguely defined and which allows too much personal bias. Whenever a new strain is even slightly different from previous described ones, a new Latin species name can be given in the Linnean binomial nomenclature, resulting in the presently existing enormous profusion of species names. From the determinative keys, it is easily seen that species differentiation is often based on one or, at most, a few properties, which makes it doubtful that gross genetic differences would be involved. A Latin species name in bacteriology has often only the value of a strain number or a label.

A more logical and natural unit of classification emerges from bacterial genetics and molecular biology as a "genospecies" (Ravin, 1963), i.e., a group of strains which are potentially able to contribute to or to share in a common gene pool. Phenotypically, this results in a cluster of satellite strains around a central core. Such a situation was foreshadowed in the biotype concept of Winogradsky (1952). Each cluster would often encompass many of the present taxonomic species, and sometimes even genera (e.g., the group Escherichia-Shigella-Salmonella). Several examples are reviewed by Marmur et al. (1963). Comparative physiology and biochemistry of many strains within a genus likewise point in this direction. Two examples may suffice. Smith, Gordon, and Clark (1962) retained only 19 biotypes out of 175 species names of Bacillus. De Ley (1961) proposed to retain only two biotypes of acetic acid bacteria, namely Gluconobacter oxydans and Acetobacter acetii, and to degrade the taxonomic species names to the level of a label. The results of the adansonian analysis of many other groups likewise strengthen the new concept, by showing that a pleistos consists of strains with many intertwined properties.

In the present paper, we report on the formation of deoxyribonucleic acid (DNA)-hybrids between strains of Acetobacter. Previous results on the physiological and biochemical properties (De Ley, 1961) and on the DNA base composition (De Ley and Schell, 1963) of the acetic acid bacteria allowed the prediction that hybrids would be formed between DNA from strains with similar guanine and cytosine (G + C) content, thus proving their genetic relationship. This prediction was fulfilled. Unfortunately, the method of DNA-hybridization has several
limitations which prevent extensive testing, such as the following. (i) Only very few strains grow with sufficient yield on N\textsuperscript{15} and deuterium oxide. (ii) Strains which are morphologically, physiologically, and biochemically almost identical, but which differ by more than 1 to 2% G + C do not form DNA-hybrids. (iii) DNA-hybridization probably depends on almost perfect identity in DNA base sequence. The results corroborate the concept of a biotype or “genosppecies.”

**Materials and Methods**

**Organisms used.** The strains of Acetobacter and *Glucconobacter* were the same as used in a previous paper by one of us (DeLey, 1961). The same nomenclature shall be adhered to. *Aeromonas hydrophila* strain AB 883 was obtained through the courtesy of H. Lautrop, Statens Serum Institutet, Copenhagen, Denmark, and *Agrobacterium tumefaciens* strain 4720 was obtained from M. Bernaerts, Central Laboratory, Ministry of Economic Affairs, Brussels, Belgium.

**Cultivation techniques.** The acetic acid bacteria were grown at 30°C for 2 to 3 days in Roux flasks on a medium containing (w/v) 5% glucose, 3% CaCO\textsubscript{3}, 1% yeast extract (Nederlandsche Gist- und Spiritusfabriek, Brugge, Belgium), and 2.5% agar (Difco). Both *Aeromonas* and *Agrobacterium* were grown at 25°C for about 3 days in Roux flasks containing, for the former organism, 0.5% peptone (Difco), 0.25% yeast extract, 0.5% meat extract (Compagnie Liebig, Antwerp, Belgium), 0.01% MgSO\textsubscript{4}.7H\textsubscript{2}O, 0.01 M phosphate buffer, and 2.5% agar with a final pH of 7.0; and, for the latter bacteria, 1% glucose, 1% yeast extract, and 2.5% agar.

**Cultivation of deuterated N\textsuperscript{15}-labeled Acetobacter aceti (mesozydans) strain 4.** Because acetic acid bacteria prefer a very complex medium, a synthetic medium was devised to allow the uptake of (N\textsuperscript{15}H\textsubscript{4})\textsubscript{2}SO\textsubscript{4} as sole nitrogen source. Carbon sources which would not alter the pH drastically during growth were selected. The selection of a deuterated N\textsuperscript{15}-labeled strain was made in the following three steps.

**Growth on a synthetic medium with (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} as sole nitrogen source.** All the strains (see De Ley and Schell, 1963) were inoculated in a basic medium containing (w/v) 0.545% KH\textsubscript{2}PO\textsubscript{4}; 0.955% Na\textsubscript{2}HPO\textsubscript{4}.12H\textsubscript{2}O; 0.025% MgSO\textsubscript{4}.7H\textsubscript{2}O; 0.0005% FeCl\textsubscript{3}; 0.1% NaCl; 0.1% (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}; a carbon source (either 1% glucose, 1% mannitol + 0.1% glucose, 1% glycerol + 0.1% glucose, or 1% fructose + 0.1% glucose); trace elements according to Atkinson (1956); 10 mg/ml each of d-L-alanine, L-asparagine, L-glutamic acid, L-aspartic acid, L-valine, L-isoleucine, and L-histidine; 3 mg/ml of biotin; 10 mg/ml of calcium pantothenate; 2 mg/ml of p-aminobenzoic acid; 10 mg/ml of thiamine; 20 mg/ml of riboflavin; 20 mg/ml of inositol; 4 mg/ml of nicotinamide; 4 mg/ml of pyridoxine; final pH, 6.5. From the original group of 19 Acetobacter strains, only 5 showed sufficient growth; from the 9 Glucconobacter strains, only 3 were promising.

**Growth on the synthetic medium with (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4} and heavy water.** The complete medium was prepared and lyophilized overnight. It was then redissolved in either 20, 40, 60, 80, or 100% heavy water, and, after the addition of agar (2.5%, w/v), the medium was sterilized for 20 min in a pressure cooker at 120 C. The bacteria were adapted to the heavy water by serial transfer onto slants of increasing concentrations of deuterium oxide. On the higher concentrations of deuterium oxide, 1 week was required for full growth as compared with 2 to 3 days on ordinary water. Only *A. aceti* (mesozydans) 4 and *G. oxydans* (suboxydans SU) gave sufficient growth for further experiments.

**Mass cultures of deuterated N\textsuperscript{15}-labeled A. aceti (mesozydans 4) and G. oxydans (suboxydans SU).** Both strains were grown in Roux flasks on the synthetic medium described above with 1% glucose for the former strain and 1% mannitol + 0.1% glucose for the latter. The salt-amino acid-vitamin mixture was lyophilized and then dissolved in heavy water. Agar and the anhydrous carbon source were added, and the entire mixture was sterilized for 20 min at 120°C in a pressure cooker. The Roux flasks were inoculated from a slant with fully adapted organisms, and growth was spread evenly after a few days. The flasks were incubated at 30°C for 1 week before sufficient organisms were available for harvesting.

**Preparation of DNA.** DNA was prepared according to Marmur (1961). The yield of DNA recovered was higher when the cells were lyophilized overnight before extraction.

**Heating and annealing of deuterated N\textsuperscript{15} and ordinary DNA.** The method of Schildkraut, Marmur, and Doty (1961) was modified as follows. The solutions (usually 1 ml) of deuterated N\textsuperscript{15},DNA and of ordinary DNA in 1.9 × SSC buffer (0.285 M NaCl and 0.0285 M sodium citrate) at pH 7.0 were placed in a 5-ml glass-stoppered
tube and completely immersed in a water-glycerol bath at 105 °C. After 10 min, the tube was immediately transferred to a thermostated water-bath at 75 °C, and was held there for 2 hr [temperature for optimal reformation rate according to Marmur and Doty (1961)]. After an additional hour at 70 °C, the temperature was lowered in steps of 5 °C every 15 min to 25 °C. Before CsCl centrifugation, the hybrid preparations were treated with the phosphodiesterase according to Schildkraut et al. (1961). In our case, however, we had to incubate for 8 hr with 10 units of enzyme (107 μg of protein) in 0.85 ml of DNA solution. E. coli phosphodiesterase was prepared according to Lehman (1963). We are indebted to I. R. Lehman, Stanford University, Palo Alto, Calif., for supplying the strain. The activity of our enzyme preparation on single-stranded DNA was confirmed by incubating the enzyme with a mixture of single- and double-stranded DNA from A. acetii(mesozydans 4) for several periods of time up to 8 hr and by determining the amount of single-stranded DNA left after CsCl density-gradient centrifugation. After heating and annealing DNA solutions according to the above procedure, only a small percentage of single-stranded DNA remained, which was completely eliminated by the phosphodiesterase activity.

CsCl density-gradient centrifugation. To 0.85 ml of the phosphodiesterase-treated DNA solution was added ca. 1 μg of reference DNA (Cytophaga sp.n. 292) in 0.01 ml. Solid CsCl (no. 2041; E. Merck, Darmstadt, Germany) was added to obtain a refractive index of 1.4022, measured with an Abbe 60 refractometer (Bellingham & Stanley, London, England) at 25 °C. The above brand of CsCl was selected because it was found to be almost free from ultraviolet-absorbing substances and contained less then 0.05% Rb. It needed no further purification. Centrifugation was carried out according to Schildkraut et al. (1961) in a model E Spinco analytical ultracentrifuge at 44,770 rev/min for 21 to 23 hr with the temperature regulator at 25 °C, with rotor An-D, cells with Kel-F centerpieces, and green-anodized 1-degree negative wedge window. Photographs were taken with ultraviolet light on Kodak sheet film CF8 and developed with Kodak Microdol. A cardboard mask below the Cl2 and Br2 filters is required to eliminate stray light and to obtain an even background. Tracings of the picture (enlarged 5 ×) were made with a Joyce-Loebl double-beam recording microdensitometer MKIIIB with an effective slit width of 0.5 mm. The densities of the DNA bands were calculated graphically by reference to the Cytophaga DNA.

Reference DNA. Strain 292 of Cytophaga sp. (National Collection of Marine Bacteria, Torry Research Station, Aberdeen, Scotland) was selected because of its low percentage of G + C (33.6 corresponding to a buoyant density of 1.6929), its high DNA content, and the narrow compositional distribution of the DNA (De Ley and Van Muylem, 1963). The organisms were grown as described in the latter paper, and the DNA was purified according to the method of Marmur (1961). From 23 ultracentrifugal runs, the density was determined to be 1.6931, in excellent agreement with the above calculated value.

Results

Hybrid formation between ordinary and deuterated N15-labeled DNA from A. acetii(mesozydans 4). Deuterated N14-DNA from this strain was found by CsCl density-gradient centrifugation to have a buoyant density of 1.7467 ± 0.0003 (average of 20 estimations). Ordinary DNA from this strain was previously found (De Ley and Schell, 1963) to have a DNA base composition of 60.6% G + C, from which a buoyant density of 1.7194 can be calculated. This value was confirmed in our CsCl density-gradient runs. Density-gradient centrifugation of a mixture of native ordinary and deuterated N15-DNA resulted in two completely separated peaks (Fig. 1a). Upon heating and annealing both kinds of DNA together, a major hybrid peak appeared, with renatured ordinary and deuterated N16-DNA visible as two shoulders (Fig. 1b). Due to the presence of single strands, the density of the intermediate peak was slightly higher than expected for the hybrid. Phosphodiesterase removed the single strands and reduced the density of the major peak to the value of 1.733, expected for the hybrid (Fig. 1c). Identical results were seen when hybridization was carried out with either 5, 10, or 15 μg/ml of each DNA.

These experiments served a double purpose. (i) By hybrid formation they proved that the deuterated N14-DNA was indeed derived from A. acetii(mesozydans 4) and not from a contaminant. (ii) They showed the optimal type of hybridization to be expected for further hybridization experiments with other strains. In the experiments of Schildkraut et al. (1961) and of
whereas in our case *A. aceti* (mesozydans 4) DNA appeared to be only partially labeled; the density difference was only 0.027, resulting in more confluent bands.

*Hybrid formation between DNA of the strain* *A. aceti* (mesozydans 4) and *A. aceti* (estunensia E), (xylinum 8747), (xylinoides 4940), and (pasteurianus 11). Heating and annealing together of the DNA from the strains mesozydans and estunensia E presented the most clear-cut case for hybridization (Fig. 2). Three distinct bands (Fig. 2b) were seen, the middle one being either a hybrid, an aggregate, or some single-stranded DNA. The latter two possibilities were ruled out after phosphodiesterase treatment, which flattened the bands but left the hybrid quite visible (Fig. 2c). Attempts to improve the picture by centrifugation with higher concentrations of DNA in the gradient resulted in a distinct shoulder (Fig. 2d).

Two slightly different cases were encountered with strains *pasteurianus* 11 and *xylinum* 8747. The amount of hybrids formed was rather small and, for reasons discussed below, their densities were not the average of the ordinary and deuterated N15-DNA. Therefore, the presence of these hybrids was partially obscured by the bulk of the unreacted renatured parental DNA. With strain *pasteurianus* 11, a distinct shoulder was obtained (Fig. 3b), which, although diminished, persisted after enzyme treatment (Fig. 3c).

The use of unequal amounts of ordinary and deuterated N15-DNA for hybridization was expected to shift the density of the heavy peak to the light side, which was found to be the case (Fig. 3d). Heating and annealing both types of DNA separately, followed by mixing and phosphodiesterase treatment, failed to produce any shoulder and was additional evidence that the shoulder was a hybrid and not single-stranded DNA. After the heating and annealing together of the DNA from strains mesozydans 4 and *xylinum* 8747 (Fig. 4) at low concentrations (10 μg/ml of each DNA), only two bands were seen (Fig. 4b and c). The density of the xylinum-DNA band, after phosphodiesterase treatment, was definitely too heavy, which suggested the presence of a small amount of hybrid, obscured by a much larger amount of renatured xylinum-DNA. By the use of unequal amounts of DNA for hybridization, the presence of the hybrid was shown in a double band, with the parental xylinum-DNA at the correct density (Fig. 4d).
It is possible that a small amount of *mesoxydans* 4-xylinoides 4940 hybrid was formed. It was, however, barely resolvable (Fig. 5a).

The amount of hybrid formed in each case was roughly estimated by calculating the difference of the total area under the curve minus the area from the renatured parental DNA. This per cent difference was then expressed as the per cent maximal possible hybridization, considering that for total hybridization the ratio of renatured parental ordinary DNA to hybrid to renatured parental deuterated N\(^{15}\)-DNA is 1:2:1. These results are given in Table 1.

**Attempted hybridization between mesoxydans deuterated N\(^{15}\)-DNA and DNA from other strains.** No hybrids were detected between *A. aceti* (mesoxydans 4) on the one hand and *A. aceti* Ch31, *A. aceti* var. muciparum 5, *Aeromonas hydrophila* AB883, *Agrobacterium tumefaciens* 4720, and *G. oxydans* (melanogenus 116) on the other hand.

**Attempted hybridization within the genus Gluconobacter.** From our collection of nine strains, only strain *suboxydans* SU grew reasonably well on the deuterium oxide-N\(^{15}\) medium. Deuterated N\(^{15}\)-DNA was prepared from this strain by the methods described above. Because the percentage of G + C of this strain is rather far removed from that of the other strains of this genus (De Ley and Schell, 1963), it was not so surprising that no hybrids were obtained between this DNA and ordinary DNA from the following Gluconobacter strains: *melanogenus* 116, *capsulatus* 4943, *gluconicum* 4739, *melanogenus* 49, and *melanogenus* 8086. Other methods will have to be used in the future to determine the relatedness of these strains.

**DISCUSSION**

**Molecular biological considerations.** The results of the hybridization experiments are summarized in Table 1. Hybrids are only formed between strains of the same genus and with DNA of almost the same base composition. When the G + C content of the strains varies by more than 1 to 2%, no hybridization occurs in spite of great morphological, physiological, and biochemical similarities. This confirms the findings of Schildkraut et al. (1961). This also accounts for the failure to produce hybrids between *G. oxydans* (suboxydans SU) and other members of this genus. The DNA base composition of this strain is the lowest of this genus (see Fig. 5; De Ley and Schell, 1963). Its G + C content varies by 3.4%
FIG. 3. Hybrid formation between deuterated N¹⁵-DNA from Acetobacter aceti (mesoxydans 4) and ordinary DNA from A. aceti (pasteurianus 11); 1 μg of Cytophaga DNA was added as reference. See text for methods. (a) Both types of DNA heated and annealed separately at 15 μg/ml; treated with phosphodiesterase; 6.8 μg in the gradient, prior to enzyme hydrolysis. (b) Both types of DNA heated and annealed together at 15 μg/ml each; not treated with phosphodiesterase; 7.5 μg in the gradient. (c) Same as (b) but treated with phosphodiesterase. (d) Mesoxydans deuterated N¹⁵-DNA (45 μg/ml) and pasteurianus ordinary DNA 15 μg/ml, heated and annealed together; treated with phosphodiesterase; 10 μg in the gradient, prior to enzyme hydrolysis.

FIG. 4. Hybrid formation between deuterated N¹⁵-DNA from Acetobacter aceti (mesoxydans 4) and ordinary DNA from A. aceti (xylinum 8747); 1 μg of Cytophaga DNA was added as reference. See text for methods. (a) Both types of DNA were heated and annealed separately at 15 μg/ml, mixed, and treated with phosphodiesterase; 6.8 μg in the gradient, prior to enzyme hydrolysis. (b) Both types of DNA were heated and annealed together at 15 μg/ml each; not treated with phosphodiesterase; 5.7 μg in the gradient. (c) Same as (b) but treated with phosphodiesterase. (d) Mesoxydans deuterated N¹⁵-DNA (16 μg/ml) and ordinary xylinum DNA (45 μg/ml) were heated and annealed together; treated with phosphodiesterase; 8.5 μg in the gradient prior to enzyme hydrolysis.
The DNA hybrids of Acetobacter strains do not have densities midway between the densities of parental DNA. These hybrids are either heavier (mesoxydans-pasteurianus) or lighter (mesoxydans-xylinum and mesoxydans-estunensis) than the theoretically expected values. The hybrid between ordinary and deuterated N$^{18}$-DNA of the same strain (mesoxydans) has exactly the expected intermediate density of 1.733. In the latter case, nearly the theoretical amount of ordinary DNA molecules will find their heavy counterparts during annealing. For the other hybrids, this does not appear to be the case. The position of the hybrid band allows an estimate of the type of molecules reacting. The mesoxydans-pasteurianus hybrids probably arose from G + C-rich DNA from both strains. The mesoxydans-xylinum hybrids might have been formed from the DNA molecules low in G + C from both strains. The mesoxydans-estunensis hybrids may have arisen from G + C-rich DNA from mesoxydans, with an approximately average contribution from estunensis. These considerations offer the most obvious explanation, although other situations (e.g., loops in the hybrid molecules) might also account for it.

**Taxonomic implications.** The above hybridization experiments not only are of importance for the acetic acid bacteria, but also, in conjunction with the results of Schildkraut et al. (1961), Marmur et al. (1963), and McCarthy and Bolton (1963), bear on the species concept of bacteria in general.

Comparative biochemistry and physiology (De Ley, 1961), DNA base composition (De Ley and Schell, 1963), and infrared absorption spectra (Scopes, 1962) of Acetobacter and Gluconobacter show that these genera cannot be regarded as a collection of sharply delineated species, but that they have to be considered as a main theme with many minor variations. This implies that the strains of each genus have many genes in common, and that, for closely related strains, long strands from that of its nearest neighbor, G. oxydans (melanogenus 119), and varies even more from that of the other strains. Unfortunately, it was technically impossible to prepare deuterated N$^{18}$-DNA from other Gluconobacter strains because they grow far too poorly in N$^{18}$-deuterium oxide media. This is one of the shortcomings of the hybridization method.
of DNA would be identical. The more the strains differ in their G + C content, the less similarity in DNA there is and the less hybrids can be expected. This is precisely the result of the present experiments (Table 1). It has to be stressed that hybridization occurred between long DNA molecules, with a molecular weight of about $5 \times 10^{9}$, equalling some 17,000 bases per strand, which are thus completely identical. Furthermore, the fact that only 10 to 25% of the DNA molecules hybridized does not mean that all the others are completely different. Hybridization presumably depends on a perfect or nearly perfect complementary base sequence. It may suffice that here and there in these very long chains a gene or maybe even some bases are different, thus preventing hybridization. One can therefore conclude that in the above cases at least 10 to 25% of the genes are identical. Although it was technically unfeasible to prepare sufficient quantities of deuterated N14-DNA from other strains, it seems probable that other crosses with other combinations of closely related strains are possible. Schildkraut et al. (1961) and Marmur et al. (1963) prepared DNA-hybrids between B. subtilis and either B. subtilis var. acclinis or B. natto. From their results it can be calculated that about 65% of the B. subtilis-B. acclinis DNA, and about 80% of the B. subtilis-B. natto DNA, hybridized. These three strains are morphologically, physiologically, and biochemically almost identical; B. natto is a Japanese strain of B. subtilis and the B. acclinis variety is a pigmented strain which easily loses its pigment and becomes indistinguishable from other B. subtilis strains. These three strains are thus only varieties of the same basic type. This situation is thus similar to the behaviour of the DNA from our strains mesoxydans, xylinoides, estunensis, and possibly zylinoides. The results are thus not in favor of definite, sharply separated species in Acetobacter. They are more compatible with the concept of a biotype or "genospecies."

A lack of DNA-hybridization does not necessarily mean that strains are completely unrelated. This is illustrated by Escherichia-Salmonella (Marmur et al., 1963). From the absence of DNA-hybridization between Acetobacter and Aeromonas, Agrobacterium, or Gluconobacter, it cannot be concluded that Acetobacter would be unrelated to these genera.

### ACKNOWLEDGMENTS

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### Table 1. Synopsis on hybrid formation between deuterated N14-DNA from Acetobacter aceti (mesoxydans) and ordinary DNA from other organisms

<table>
<thead>
<tr>
<th>Strain</th>
<th>Per cent hybrid formed</th>
<th>Mean per cent G + C</th>
<th>σ</th>
<th>Compositional distribution of DNA molecules expressed as per cent G + C ± 2σ</th>
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<tbody>
<tr>
<td>Acetobacter aceti (zylinoides) 4940</td>
<td>ca. 10</td>
<td>62.4</td>
<td>1.5</td>
<td>59.5-65.4</td>
</tr>
<tr>
<td>A. aceti (estunensis) E</td>
<td>ca. 25</td>
<td>62.2</td>
<td>1.1</td>
<td>60.0-64.4</td>
</tr>
<tr>
<td>A. aceti (zylinum) 8747</td>
<td>ca. 20</td>
<td>60.7</td>
<td>2.25</td>
<td>56.2-65.2</td>
</tr>
<tr>
<td>A. aceti (mesoxydans) 4</td>
<td>Complete</td>
<td>60.6</td>
<td>1.6</td>
<td>57.4-63.8</td>
</tr>
<tr>
<td>A. aceti (pasteurianus) 11</td>
<td>ca. 15</td>
<td>59.8</td>
<td>1.75</td>
<td>56.3-63.3</td>
</tr>
<tr>
<td>A. aceti Ch 31</td>
<td>0</td>
<td>59.6</td>
<td>1.6</td>
<td>56.4-62.8</td>
</tr>
<tr>
<td>A. aceti (var. muciparus) 5</td>
<td>0</td>
<td>59.5</td>
<td>1.5</td>
<td>56.5-62.5</td>
</tr>
<tr>
<td>Gluconobacter oxydans (melanogenus) 116</td>
<td>0</td>
<td>60.6</td>
<td>0.87</td>
<td>58.9-62.3</td>
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<tr>
<td>Aeromonas hydrophila AB 883</td>
<td>0</td>
<td>59.5</td>
<td>1.25</td>
<td>57.0-62.0</td>
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<tr>
<td>Agrobacterium tumefaciens 4720</td>
<td>0</td>
<td>61.0</td>
<td>0.12</td>
<td>60.8-61.2</td>
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</table>

* Per cent hybrid formed is expressed relative to the theoretical amount possible.

† The per cent G + C and σ values (variance of the compositional distribution) of Acetobacter and Gluconobacter were taken from the paper of De Ley and Schell (1963); the other values were taken from unpublished results.
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LITERATURE CITED


