Differentiation of *Acetomonas* and *Pseudomonas*

By J. L. SHIMWELL

Research Department, British Vinegars Ltd., Frome, Somerset

J. G. CARR

Research Station, Long Ashton, Bristol

AND MURIEL E. RHODES

Department of Microbiology, University of Reading

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SUMMARY

A comparative study of 50 strains of *Acetomonas* and 165 strains of *Pseudomonas* showed that the two genera could be easily and rapidly differentiated by the use of four criteria; these were: (a) production of acid on ethanol + CaCO₃ agar plates, (b) oxidation of calcium lactate to carbonate, (c) production and accumulation of dihydroxyacetone in glycerol media, (d) growth at (initial) pH 4.5. *Acetomonas* was positive with (a), (c) and (d), but negative with (b); *Pseudomonas* was positive only with (b). Liquefaction of gelatin and production of a greenish yellow fluorescent pigment, being variable with *Pseudomonas* and absent from *Acetomonas*, were useful only when positive.

INTRODUCTION

The belief in the universal polar flagellation of all motile acetic acid bacteria seems to have originated from the publication by Zeidler (1898) of a photomicrograph showing a single polar flagellum on several cells of *Termobacterium aceti*. Later, Vaughn (1942, 1943) published two papers with the stated purpose of drawing attention to the insufficiently recognized polar flagellation of all motile acetic acid bacteria, together with a photomicrograph of a cell with a single polar flagellum. These papers became widely known, and probably largely on this account Stanier (1947) was led to say 'In view of the extensive morphological and biochemical parallelism between acetic acid bacteria and organisms of the *P. fluorescens* type, it seems indefensible any longer to maintain a family Acetobacteriaceae; its members should be incorporated in the family Pseudomonadaceae'. At the time this seemed supported by his statement that 'some fluorescent pseudomonads' could apparently produce some acid from ethanol when CaCO₃ was present in the medium to neutralize free acid as soon as formed, and to prevent a decrease in pH value, for below pH 5.0 the bacteria rapidly died. It seems probable that the belief in the universal polar flagellation of the acetic acid bacteria became widely accepted for so long that it was thought unnecessary to stain the flagella of motile strains; they seem automatically to have been recorded as possessing 'a single polar flagellum'. Thus in the 6th edition of *Bergey's Manual* (1948) all motile *Acetobacter* strains were so described.

The work of Leifson (1954), confirmed by Shimwell (1958, 1959) and Asai & Shoda (1958), however, has changed the picture. It is now known that motile

acetate-oxidizing acetic acid bacteria have peritrichous flagella, and that it is only the motile ones which do not oxidize acetate (the 'suboxydans group' of Frateur, 1950) which have one or more (usually more) polar flagella. Although three years elapsed between Leifson's publication and the 7th edition of *Bergey's Manual* (1957) his findings were apparently not investigated, and the statement regarding the monotrichous polar flagellation of all motile strains of *Acetobacter* was repeated, on the grounds that Leifson's photomicrographs needed confirmation by unequivocal ones, or by electron micrographs, before his proposal to divide the acetic acid bacteria into *Acetobacter* (peritrichously flagellate, fully oxidizing) and *Acetomonas* (polarly flagellate, only partly oxidizing) could be accepted. This has since been done (Shimwell, 1958, 1959; Asai & Shoda, 1958; Shimwell & Carr, 1959), and there is no longer any doubt of the need to divide the heterogeneous acetic acid bacteria into their two component genera. Plate 1, fig. 1, shows the peritrichous flagella of a strain of *Acetobacter rancens*; Pl. 1, fig. 2, the polar ones of *Acetomonas oxydans*, the proposed type-species (Shimwell & Carr, 1959).

Although Stanier (1947) was thus wrong in his insistence that all acetic acid bacteria should be recognized as pseudomonads and transferred from Acetobacteriaceae to Pseudomonadaceae, there still remained, however, the question whether the acetic acid production by some strains of *Pseudomonas fluorescens* constituted sufficient grounds for regarding them as acetic acid bacteria taxonomically indistinguishable from *Acetomonas*; or, conversely, whether *Acetomonas* strains should be included in *Pseudomonas*, there to swell the number of already overcrowded and often ill-defined species. The examination of this point is the main object of this paper.

To those only familiar with either *Acetomonas* or *Pseudomonas* a written list of the properties common to both genera, derived from the literature and specially selected to emphasize resemblances, might suggest that it is justifiable to put the two genera together. To those who have handled and studied members of both genera there seems less possibility of mistaking one for the other. Frateur (1950) enumerated some of the major differences between *Pseudomonas* and his suboxydans group (= *Acetomonas*) such as the inability of the acetic acid bacteria to liquefy gelatin, to show appreciable growth on peptone agar, to produce yellowish green fluorescent water-soluble pigments (admittedly not found in all *Pseudomonas* species), to grow on potato, to metabolize protein and so on. In addition to these negative properties of *Acetomonas*, however, we consider it important to emphasize their outstanding positive properties which are not possessed by *P. fluorescens* and similar strains, and which sharply distinguish these two different types of bacteria.

METHODS

Strains used. These comprised the 50 *Acetomonas* strains previously studied by Shimwell & Carr (1959), and the 165 *Pseudomonas fluorescens* and similar strains studied by Rhodes (1959; excluding *Aeromonas* strains).

Biochemical methods. These were mainly those described by Frateur (1950; some as modified by Shimwell & Carr, 1959), and those of Rhodes (1959). It is convenient to give the methods used to obtain the results in Table 1.

(1) The production of acetic acid from ethanol was detected by the presence on agar plates of 1% (w/v) Difco yeast-extract + 2% (v/v) ethanol + 2% (w/v) CaCO_3 ,

of clear zones of dissolved CaCO_3 round the implanted (*c.* 1 cm.²) bacterial masses after incubation for 14 days at 26°, or sooner.

(2) *Growth at pH 4.5.* In the case of *Pseudomonas* strains this was studied in YE liquid medium adjusted to pH 4.5 with HCl (Rhodes, 1959) and in similarly adjusted 1 % (w/v) Difco yeast extract + 2 % (v/v) ethanol, or similar alcoholic media in the case of *Acetomonas* strains, since the latter grow poorly (if at all) on YE liquid medium alone.

(3) *Oxidation of calcium lactate through acetate to carbonate.* This was studied on 1 % (w/v) Difco yeast extract + 1 % (w/v) calcium lactate agar plates, the production of carbonate being shown by the appearance of nodular crystals of CaCO_3 under and/or around the implanted bacterial masses (*c.* 1 cm.²) as seen through the bottom of inverted plates at a magnification of $\times 40$.

Production and accumulation of dihydroxyacetone in media containing glycerol. Agar plates containing 1 % (w/v) Difco yeast extract + 2 % (v/v) glycerol were used. After various periods of incubation (1–14 days) at 26°, the plates were flooded, in the cold, with Fehling's solution, a quick (10 min.) production of an aureole of yellow to red copper oxide(s) round the implanted masses indicating the presence of dihydroxyacetone.

RESULTS

The results obtained with the primary differentiating criteria are recorded in Table 1. With the one exception in a total of 215 strains examined any one of the primary differentiating criteria served to eliminate either *Acetomonas* or *Pseudomonas*; any two of these criteria did so without exception (the one apparently anomalous strain will be referred to in the Discussion).

Table 1. *The results of using the four primary differentiating tests with 50 Acetomonas and 165 Pseudomonas strains*

Test	<i>Acetomonas</i> No. strains positive	<i>Pseudomonas</i> No. strains positive
(a) Production of acetic acid on ethanol + CaCO_3 agar	50 (in 2 days)	0 (in 14 days)
(b) Oxidation of lactate to carbonate	0	165
(c) Dihydroxyacetone from glycerol	*49 (in 18 hr.)	0 (in 14 days)
(d) Growth at pH 4.5	50	0

* See Discussion.

There were secondary differentiating criteria which concerned characteristics which were never possessed by the *Acetomonas* strains, but which were usually (but not always) possessed by the *Pseudomonas* strains studied. These were the liquefaction of gelatin and the production of fluorescent yellow-green water-soluble pigment. These secondary differentiating characteristics, when positive, almost certainly rule out *Acetomonas*, but when negative are inconclusive. Other similar secondary criteria (e.g. peptonization of milk, etc.) are excluded as superfluous.

DISCUSSION

It is submitted that *Acetomonas* can readily be distinguished from *Pseudomonas*; indeed, it is virtually impossible to confuse them. We submit also that the differences shown in Table 1 are adequate and suitable for decisive, easy and rapid laboratory

differentiation. Moreover, when these primary characters are considered in conjunction with the secondary characteristics, together with those listed by Frateur (1950) and by Rhodes (1959), it seems that there are relatively few resemblances between the genera *Acetomonas* and *Pseudomonas* apart from polar flagellation and an oxidative type of metabolism.

With regard to the one strain of *Acetomonas* which did not give dihydroxy-acetone from glycerol, it was reported (Shimwell & Carr, 1959) that this strain (*A. suboxydans*, NCIB 3734) also differed from the remaining 49 strains in not producing brown water-soluble pigment from any sugar used. As it was also non-motile, its differentiation from non-motile *Acetobacter* strains had to be based solely on its inability to oxidize acetic acid, lactate or acetate. It was suggested, however, (Shimwell, 1957) that in view of the facile mutability in *Acetobacter*, it was conceivable that even the ability to oxidize acetate might be capable of loss through mutation; and that if this were so, such a mutated *Acetobacter* strain might if non-motile be difficult to distinguish from a non-motile *Acetomonas* strain. We think that the anomalous strain in question may be an example of this possible situation. However, there is no question of the organism NCIB 3734 being a *Pseudomonas* strain, for it is a strong producer of acetic acid from ethanol, grows readily at pH 4.5, and does not oxidize acetic acid, acetate or lactate.

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REFERENCES

- ASAI, T. & SHODA, K. (1958). The taxonomy of *Acetobacter* and allied oxidative bacteria. *J. gen. appl. Microbiol.* **4**, 289.
- BERGEY'S *Manual of Determinative Bacteriology* (1948). 6th ed.; (1957) 7th ed. London: Baillière, Tindall and Cox.
- FRATEUR, J. (1950). Essai sur la systématique des acetobacters. *Cellule*, **53**, 287.
- LEIFSON, E. (1954). The flagellation and taxonomy of species of *Acetobacter*. *Leeuwenhoek ned. Tijdschr.* **20**, 102.
- RHODES, M. E. (1959). The characterization of *Pseudomonas fluorescens*. *J. gen. Microbiol.* **21**, 221.
- SHIMWELL, J. L. (1957). A pattern of evolution in the genus *Acetobacter*. *J. Inst. Brew.* **63**, 45.
- SHIMWELL, J. L. (1958). Flagellation and taxonomy of *Acetobacter* and *Acetomonas*. *Leeuwenhoek ned. Tijdschr.* **24**, 187.
- SHIMWELL, J. L. (1959). A re-assessment of the genus *Acetobacter*. *Leeuwenhoek ned. Tijdschr.* **25**, 49.
- SHIMWELL, J. L. & CARR, J. G. (1959). The genus *Acetomonas*. *Leeuwenhoek ned. Tijdschr.* **25**, 353.
- STANIER, R. Y. (1947). Acetic acid production from ethanol by fluorescent pseudomonads. *J. Bact.* **54**, 191.
- VAUGHN, R. H. (1942). The acetic acid bacteria. *Wallerstein Labs. Commun.* **5**, 5.
- VAUGHN, R. H. (1943). Motility in the genus *Acetobacter*. *J. Bact.* **46**, 394.
- ZEIDLER, A. (1898). Photographisches Bild des *Termobacterium aceti* mit Geissel. *Zbl. Bakt.* **4**, 669.

EXPLANATION OF PLATE

- Fig. 1. *Acetobacter rancens* strain. Electron micrograph showing peritrichous flagella. $\times 15,000$.
- Fig. 2. *Acetomonas oxydans*, cider strain. Electron micrograph showing multitrichous polar flagella. $\times 15,000$.

Fig. 1

Fig. 2