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THE FLAGELLATION AND TAXONOMY OF SPECIES OF *ACETOBACTER*

by

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The taxonomy of the genus *Acetobacter* and its various species has been based mainly on cultural and physiological characteristics. All motile species of the genus have been described by VAUGHN (1942) as having polar monotrichous flagella. In BERGEY's Manual of Determinative Bacteriology 6th Ed. (1948) the motile species are also described as having polar monotrichous flagella. My study of 30 strains of the genus, representing many of the commonly recognized species, failed to show any with polar monotrichous flagella. Two distinct types of flagellation were found: A polar multitrichous type having from 3 to 8 flagella with unusually short wavelength, and a peritrichous type with rather few flagella of longer wavelength.

EXPERIMENTAL DATA.

All of the cultures studied were received from outside sources and were regarded by the donors as typical species of the genus *Acetobacter*. Each culture was studied physiologically and culturally to confirm its identity according to the classification in BERGEY's Manual, 6th Ed. A culture was regarded as a species of *Acetobacter* if it had the following characteristics: rod shaped; gram negative; generally pleomorphic; motile or nonmotile; strictly aerobic; ability to oxidize glucose and ethyl alcohol to acids; scant growth in peptone media without added carbohydrate, alcohol, etc.; growth in media at pH 5 to 6 better than at pH 7.

According to VAUGHN and BERGEY's Manual, 6th Ed., the oxidation of acetic acid to CO_2 and H_2O is of primary taxonomic importance for the separation of the species. Several basic media

were employed in this study using peptone and yeast extract in various concentrations and combinations. Liquid, semisolid and solid media were used. The acetate was added in concentration from 0.1% to 1.0%. Bromthymol blue and chlorphenol red were used as indicators. Overall best results were obtained in the following medium: Peptone 0.3%, yeast extract 0.2%, sodium acetate 0.2%, bromthymol blue 0.002%, pH 6.5. With some strains more rapid alkalinity was obtained with the medium in the form of agar slants. Incubation at 30°C. for two weeks was generally sufficient to bring out the final reaction. In the basic medium alone (i.e. without acetate), using Casitone as peptone, some strains of *Acetobacter* produce an acid reaction, some leave the medium neutral, and some produce a slightly alkaline reaction. Practically all species of gram negative bacteria which I have studied produce an alkaline reaction in this type of medium. If the acetate is oxidized to completion the medium becomes strongly alkaline. All of the strains tested, with one exception, gave comparable results with lactate substituted for acetate. The one exception was a strain labeled *A. mesoxydans vini* which produced an alkaline reaction in the lactate medium but not in the acetate medium.

The other differential tests employed in BERGEY's Manual need little comment. Ability to grow in a medium with ammonium salts as the sole source of nitrogen, such as Hoyer's solution, is used to differentiate *A. aceti* from *A. xylinum* and *A. rancens*. My experience did not confirm the usefulness of this test for the above purpose. All four of my strains of *A. xylinum*, having the characteristic leathery pellicle, grew well in Hoyer's solution. Serial transfer in the medium was not done and this might have given different results. Two of the cultures received as *A. suboxydans* produced a pinkish pellicle in glucose broth on prolonged incubation at 20°C. The pigment was limited to the bacterial growth and apparently water insoluble. I assume these to be strains of *A.roseum*. According to FRATEUR (1950) production of pink pigment is not a sound criterion for species differentiation. He suggests that it is better to label these strains as roseum varieties of some species. Following this suggestion my two cultures should probably be labeled *A. suboxydans* var. *roseum*. None of the cultures in my collection, including one labeled *A. oxydans* (ATCC 9433), grew as well or as rapidly at 21 as at 30°C., and a typical strain of this species is therefore not represented in the Collection. According to FRATEUR the original de-

scription of *A. oxydans* by HENNEBERG is insufficient for positive identification and he suggests that the organism is probably a variety of *A. suboxydans*.

Motility was determined by direct microscopic observation. The organisms were observed from a variety of media differing in composition, pH, temperature and length of incubation. The best motility was obtained in somewhat acid media, pH 5 to 6, and at incubation temperatures of 20 to 30°C. At 37°C. several strains were nonmotile which were motile at the lower temperatures. A typical strain of *A. aceti* (F-4) showed good motility at pH 6 but not at pH 7. In general the motility was better at 21° than at 30°C. Two types of motion were observed: A rapid, linear motion typical of polarly flagellated bacteria; and a wriggling motion typical of nonpolar or peritrichously flagellated bacteria. The flagella were stained by the method of LEIFSON (1951) using glucose broth cultures. Good stains were more difficult to obtain than with most bacteria, possibly because of the high acidity of the medium, the presence of capsular material, and the tendency of all the strains to clump when washed in the centrifuge. 5% formalin was added to each culture and the organisms washed three times with distilled water using the centrifuge method. The flagellation and physiological reactions of the various strains are recorded in table I.

In figures 1 to 8 are reproduced photomicrographs showing the typical flagellation of several species. The polar multitrichous flagellation of *A. melanogenum* and *A. suboxydans* is unique in my experience. Based on the measurement of 20 flagella on separate organisms from each of 6 strains, a total of 120 measurements, the average wavelength was found to be 1.4 μ. Similar measurements of the flagella of several typical polar multitrichous *Pseudomonas* strains gave an average wavelength of 2.2 μ. Unless other bacteria are discovered in the future with similar shape and grouping of the flagella, a flagella stain alone is sufficient for identification of these species of *Acetobacter*. The peritrichous flagellation of *A. aceti*, *A. rancens* and *A. orleanense* is quite orthodox. The number of flagella per organism tends to be few and the arrangement is very uneven. The flagellation resembles that of the colon bacteria, *Agrobacterium* species, *Achromobacter* species, etc. Clumping on centrifugation is very pronounced and there are few individual organisms on the stained slides. The individual strains showed a greater variation in

TABLE I.

Some Comparative Characteristics of Species of the Genus *Acetobacter*.

strain	received as	reaction in peptone	reaction in acetate	reaction in lactate	growth in Hoyer's medium	leathery pellicle	pigment	catalase	flagellation	sug-gested genus
B-746	*A. aceti*	sl.alk.	v.alk.	v.alk	—	—	—	—	peritrichous	
B-1036	*A. aceti*	,,	,,	,,	—	—	—	—	peritrichous	
F-4	*A. aceti*	,,	,,	,,	+	—	—	+	peritrichous	
F-6	*A. aceti* var. *muciparum*	,,	,,	,,	+	—	—	+	none	
B-55	*A. orleanense*	,,	,,	,,	?	—	—	+	peritrichous	
B-679	*A. acetum* var. *nairobiense*	,,	,,	,,	?	—	—	+	none	
B-145	*A. acetum-mocosum*	,,	,,	,,	—	—	—	+	none	
B-578	*A. aceti*	,,	,,	,,	—	—	—	+	none	
F-2	*A. rancens*	,,	,,	,,	—	—	—	+	none	
F-5	*A. rancens* var. *turbidans*	,,	,,	,,	—	—	—	+	peritrichous	
B-1025	*A. turbidans*	,,	,,	,,	—	—	—	+	none	
H-277	*A. rancens*	,,	,,	,,	—	—	—	+	none	
H-263	*A. oxidans*	,,	,,	,,	—	—	—	+	none	
F-12	*A.rancens* var. *saccharovorans*	neutral	sl.alk.	f.alk.	—	—	—	+	none	
F-3	*A. xylinum*	sl.alk.	f.alk.	f.alk.	+	+	—	+	none	
F-9	*A. xylinum* var. *maltovorans*	,,	,,	,,	+	+	—	—	none	
B-43	*A. xylinum*	,,	,,	,,	+	+	—	+	none	
B-581	*A. aceti*	,,	,,	,,	+	+	—	+	none	
F-10	*A. mesoxydans vini*	neutral	neutral	sl.alk.	—	—	—	—	none	
F-14	*A. mesoxydans* var. *saccharovorans*	,,	?	neutral	—	—	—	—	none	

Acetobacter

TABLE I (continued).

strain	received as	reaction in peptone acetate lactate			growth in Hoyer's medium	leathery pellicile	pigment	catalase	flagellation		sug-gested genus
F-1	*A. suboxydans* var. *biourgianum*	sl.acid	sl.acid	sl.acid	—	—	pink	+	polar	multitrichous	*Acetomonas*
F-16	*A. suboxydans*	,,	,,	,,	—	—	pink	—	,,	,,	
F-15	*A. suboxydans* var. *muciparum*	,,	,,	,,	—	—	—	+	,,	,,	
B-72	*A. suboxydans*	,,	,,	,,	—	—	—	+	,,	,,	
B-1225	*A. capsulatum*	,,	,,	,,	—	—	—	+	,,	,,	
B-1226	*A. viscosum*	,,	,,	,,	—	—	—	+	,,	,,	
F-8	*A. melanogenum* var. *maltovorans*	,,	,,	,,	—	—	brown	—	,,	,,	
F-13	*A. melanigenum* var. *maltosaccharovorans*	,,	,,	,,	—	—	brown	+	,,	,,	
B-58	*A. melanogenum*	,,	,,	,,	—	—	brown	+	,,	,,	
B-63	*A. melanogenum*	,,	,,	,,	—	—	brown	+	none		

Legend: sl. — slightly; f. — fairly; v. — very; alk. — alkaline; neutral — no change of reaction.

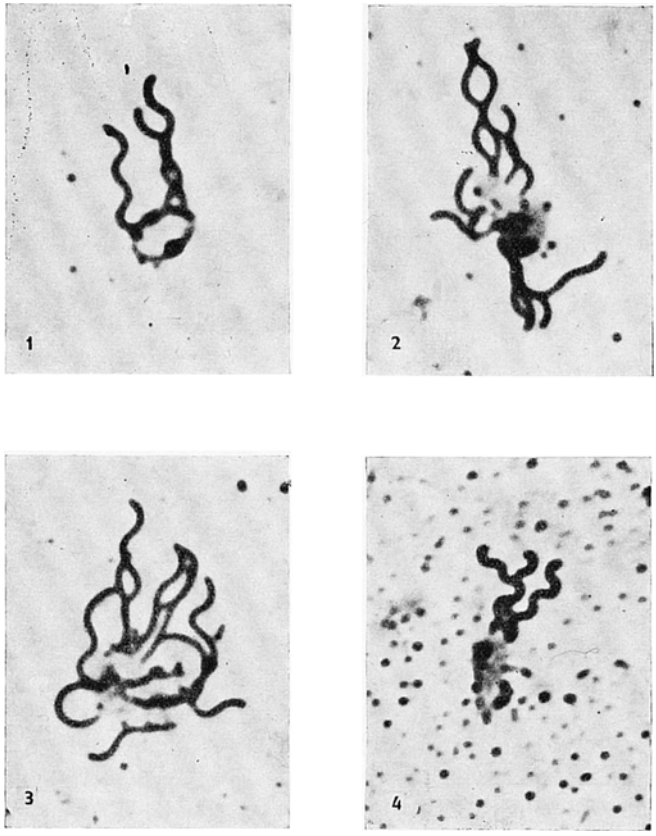
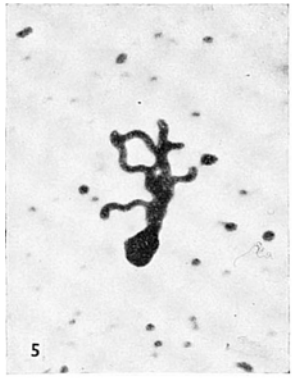


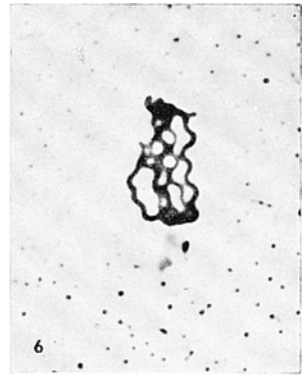
Fig. 1. *Acetobacter aceti* showing peritrichous flagella. Only the periphery of the soma took the flagella stain. The center was stained blue which shows light on the photograph. Leifson flagella stain. Photomicrograph × 3600.

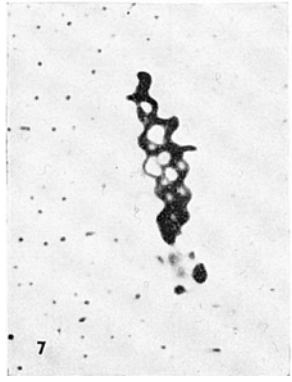
Fig. 2. *Acetobacter rancens* showing peritrichous flagella. Apparently a clump of two organisms. Leifson flagella stain. Photomicrograph × 3600.

Fig. 3. *Acetobacter aceti* showing peritrichous flagella. Several organisms in one clump. Leifson flagella stain. Photomicrograph × 3600.

Fig. 4. *Acetomonas suboxydans* var. *roseum* showing 3 polar flagella. Leifson flagella stain. Photomicrograph × 3600.







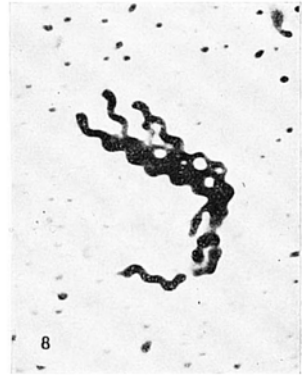


Fig. 5. *Acetomonas suboxydans* showing several polar flagella. The soma is atypical in staining solid with the flagella stain. Leifson flagella stain. Photomicrograph × 3600.

Fig. 6. *Acetomonas melanogena* showing several polar flagella. The soma which was stained blue appears rather faintly as a vertical rod below the clump of flagella. Leifson flagella stain. Photomicrograph × 3600.

Fig. 7. *Acetomonas melanogena* showing several polar flagella. The soma being stained blue appears rather faint on the photograph. Leifson flagella stain. Photomicrograph × 3600.

Fig. 8. *Acetomonas suboxydans* showing the characteristic polar multitrichous flagellation of the one organism and one flagellum on the other. Leifson flagella stain. Photomicrograph × 3600.

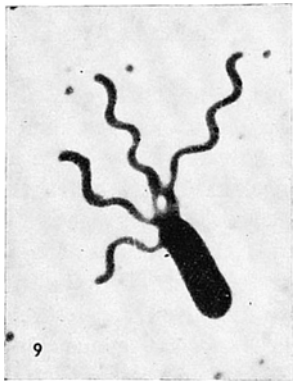


Fig. 9. *Pseudomonas* sp. showing polar multitrichous flagellation. Note the relatively much greater wavelength of these flagella compared to those of *Acetomonas*. Leifson flagella stain. Photomicrograph × 3600.

wavelength of the flagella than was the case with the polar types. Based on the measurement of 20 flagella on separate organisms from each of 4 strains, the average wavelength was found to be 2.9 μ.

DISCUSSION.

The data presented in this paper confirm the soundness of previous classifications of the genus, such as that of VAUGHN. All of the motile strains studied which were able to oxidize acetate to CO_2 and H_2O were found to be peritrichously flagellated. All of the motile strains studied which failed to oxidize acetate to CO_2 and H_2O were found to have polar multitrichous flagella. In spite of certain cultural and physiological similarities it would seem taxonomically sound to separate the present genus *Acetobacter* into two genera. It is suggested that the generic name *Acetobacter* be retained for the species which oxidize acetate and/or lactate to CO_2 and H_2O, and which are peritrichously flagellated or nonflagellated. The logical type species would seem to be *A. aceti*. For the species which do not oxidize acetate or lactate to CO_2 and H_2O, and which have polar multitrichous flagella, or no flagella, the genus *Acetomonas* gen.nov. is suggested. *Acetomonas suboxydans* seems to be the logical type species. The genus *Acetomonas* seems most appropriately placed in the family *Pseudomonidaceae*, but the redefined *Acetobacter* genus seems more logically fitted into another family.

Summary.

The *Acetobacter* genus as presently constituted include two distinctly different morphological types, one having peritrichous flagella with a wavelength averaging 2.9 μ and the other having polar multitrichous flagella with a wavelength averaging 1.4 μ. All of the peritrichously flagellated types actively oxidized acetic and lactic acids to CO_2 and H_2O, while none of the polar flagellated types showed this property. It is proposed that the present *Acetobacter* genus be divided into two genera: *Acetobacter* and *Acetomonas* gen.nov. The redefined *Acetobacter* genus should include only peritrichously flagellated species and nonflagellated species with similar physiology. *Acetomonas* gen.nov. should include only polar multitrichous species and nonflagellated species of similar physiology. Typical species of the redefined *Acetobacter* genus should oxidize acetic or lactic acid to CO_2 and H_2O while typical species of *Acetomonas* gen.nov. should not have this property.

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