Wavelengths of Bacterial Flagella

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SUMMARY: In sunlight dark-ground microscopy the flagella of a packet of motile sarcina appear as a smooth tail. The tails often stiffen into helices of two different wavelengths, one being twice as long as the other. The short and the long wavelengths are identical in *Sarcina ureae* and *S. agilis*. This phenomenon of two wavelengths, one twice as long as the other, was also found, though less frequently, in certain other bacteria. Otherwise the wavelengths of the flagella of these bacteria appeared specific.

It has been claimed that bacterial flagella are not homologous (Pijper, 1949*a*, *b*; 1951; Pijper, Crocker, van der Walt & Savage, 1953) but fall into different natural groups. It is therefore significant that the novel flagellar feature described here apparently is the exclusive property of one of these groups.

MATERIALS AND METHODS

We are indebted to Dr J. P. van der Walt, now of the South African Council for Scientific and Industrial Research, for isolating *Sarcina ureae* from local garden soil, and to Prof. Annelyse Winkler of Göttingen and Dr E. G. Pringsheim of the Botany School, Cambridge, for a strain of *S. agilis*, and of *Caryophanon latum*, respectively. An H901 culture of *Salmonella typhi* we owe to Dr A. Felix. For flagellar wavelengths of other bacteria we used photomicrographs published by others.

The two *Sarcina* and *Caryophanon* spp. were examined as agar cultures, the flagella being slightly thickened by precipitated agar without having their wavelengths affected (Pijper, 1949*b*). Similarly, *Sal. typhi* was suspended in 0.5% (w/v) methylcellulose in saline.

The sunlight dark-ground microscope technique used was described by Pijper (1946, 1949*b*). Flagellar measurements on the sarcina were made from dark-ground photomicrographs produced by a $\times 60$ Zeiss objective, and a Kontax camera, resulting in a magnification of $\times 300$. The negatives, through a photographic enlarger, were projected at a magnification of $\times 3000$ and the flagellar shapes traced with pencil on paper. The tracings were measured with a pair of compasses and a millimeter scale. Pl. 1, figs. 2-7, and Pl. 2, figs. 8-12, illustrate some of the flagella measured. Assuming that measurements were accurate to 0.3 mm., actual wavelengths (distance from crest to crest) could be expressed with an accuracy of 0.1 μ .

RESULTS

Wavelengths of flagella of sarcina

Packets of *S. ureae* in sunlight dark-ground microscopy showed their flagella (word here used without prejudice) as a faint smooth tail (Pl. 1, fig. 1), very much like the fuzzy-looking tail previously described for *Sal. typhi* (Pijper, 1946, 1949b). These tails changed their shape in various ways, but the most common change was a sudden stiffening into a helix (Pl. 1, figs. 2, 3). This transition did not directly affect the motility of the packet. Its causation remains obscure. It might happen to one of several packets in a field, or to most packets in a field. Older cultures showed it more often. After several days of growth most packets had these helical flagella, or the helices had broken off and were floating by themselves. Helices whether loose or still attached tended to combine. Lengthwise 'linking' is shown in Pl. 1, figs. 4-6. The regularity of the coils facilitated transverse 'packing', bringing about the more solid structures of Pl. 1, figs. 5 and 7. Entanglement of 'linked' and 'packed' helices caused pseudo-agglutination (Pl. 1, fig. 5). 'Packing', whilst increasing the width of the helices to a slight degree, did not affect wavelengths.

Gradually the impression was gained that *S. ureae* produced helices of two different wavelengths, and that the one wavelength was double the other. This is clear in Pl. 1, fig. 6, where examples of the two wavelengths are shown in one field, and in Pl. 1, fig. 7, where flagella of two wavelengths are accidentally linked up.

An investigation of *S. agilis* showed similar conditions. Faint fuzzy tails stiffened into helices of short wavelength (Pl. 2, fig. 8) or long wavelength (Pl. 2, fig. 9). Lengthwise linking of short-wavelength helices is shown in Pl. 2, fig. 10, and accidental linking of short and long wavelength flagella in Pl. 2, fig. 11. In Pl. 2, fig. 12, examples of the two wavelengths are seen in the same field. The fundamental wavelengths in *S. ureae* and *S. agilis* appeared the same. *S. ureae* habitually produced more helices of long wavelength than of short wavelength, and *S. agilis* did the opposite.

A large number of measurements seemed needed to decide what the relations really were. This was done as described above, the results are given in Table 1.

Table 1 allows the conclusion that the tails of both *S. ureae* and *S. agilis* change into helices of two kinds, and that in both organisms the one kind of helix has a wavelength of 1.6μ , and the other a wavelength of 3.2μ , thus confirming the impression that one wavelength is double the other.

Observations on other bacteria

The occurrence of helical flagella of two wavelengths, one double the other, was looked for in other bacteria. Our collection of dark-ground photomicrographs of *Sal. typhi* showed several instances, of which Pl. 2, figs. 13 and 14, are examples. The two wavelengths are about 1.1 and 2.2μ . In *Caryophanon latum* straight fuzzy tails can also stiffen into helical structures; Pl. 2,

fig. 15, shows two helical flagella of different wavelength attached to one cell, and one wavelength is double the other.

Table 1. *Statistical analysis of measured wavelengths of flagella of S. ureae and S. agilis*

	<i>S. ureae</i>		<i>S. agilis</i>	
	Short	Long	Short	Long
Mean wavelength (μ .)	1.639	3.193	1.638	3.188
Standard error	0.0054	0.0048	0.0038	0.0068
No. of measurements	141	615	304	298
Confidence limits for mean at:				
5 %	1.639 $\pm$.011	3.193 $\pm$.009	1.638 $\pm$.007	3.188 $\pm$.013
1 %	1.639 $\pm$.014	3.193 $\pm$.012	1.638 $\pm$.010	3.188 $\pm$.018
Ratio between short and long wavelengths				
	Confidence limits (%)	Minimum	Maximum	
<i>S. ureae</i>	5	1.930	1.967	
	1	1.924	1.972	
<i>S. agilis</i>	5	1.930	1.963	
	1	1.923	1.969	

Transitions from smooth tails into helical flagella were described for *Proteus vulgaris* (Pijper, 1946, 1949*b*) and for *Bacillus cereus* (Pijper, 1949*b*). At that time no helices of different wavelengths were noticed or photographed. Pietschmann (1942) published a dark-ground photomicrograph of *B. cereus* in 1 % (w/v) tragacanth solution in water where in one field two different wavelengths were visible in stiffened helical flagella. The wavelengths were approximately 2.4 and 1.2 μ . Her only comment was that flagella of *B. cereus* which had stopped moving could show narrow or wide windings. She also showed similar photomicrographs of two bundles of flagella of *Pr. vulgaris*, one having a wavelength double the other, without comment. Neumann (1928) published, also without comment, two photomicrographs of bundles of helical flagella of *Pr. vulgaris* suspended in 5 % (w/v) gum arabic in water; the wavelength of one was *c.* 0.9 μ , and of the other *c.* 1.9 μ .

DISCUSSION

The hard horny excrescences called flagella in spirilla have nothing in common with the soft and fuzzy-looking structures called flagella in sarcina, *Sal. typhi*, *Pr. vulgaris*, *B. cereus*, *C. latum* and allied bacteria. In keeping with this, the flagella of *Spirillum* spp. do not occur with two different wavelengths; their very structure would seem to exclude this (Pijper, 1949*a, b*, 1951; Pijper *et al.* 1953). This constitutes a further argument why bacterial flagella cannot be regarded as homologous. Bacterial flagella are generally supposed to have a wavy, spiral (or better, helical) appearance. Flagellar wavelength has not been studied much, probably because the traditional dry preparations were regarded as misleading. Leifson (1951), however, although he only dealt with dried and stained preparations, suggested that perhaps wavelengths might be

more important than length. Leifson & Hugh (1954) since found $0.62\ \mu$ the average wavelength of twenty-five fixed and stained flagella of a pseudomonad. On this unusually short wavelength they wished to found a new species. Our findings suggest a need for measurements on wet preparations and on larger numbers. Reichert (1909) from dark-ground observations in colloid solutions concluded that in *Sal. typhi* the pitch of the screw-like flagella was $2.5\ \mu$, and of *Pr. vulgaris* $2\ \mu$. In two motile sarcinas he estimated the pitch in dead individuals to be sometimes 2 or $1.8\ \mu$., but usually $3\ \mu$. Weibull (1949) precipitated shaken-off flagella of *Pr. vulgaris* and *B. subtilis* with ammonium sulphate and described the wet flagella as having a period of $2\ \mu$. in *Pr. vulgaris* and $2.5\ \mu$. in *B. subtilis*. Later (1950) he measured the precipitated flagella of ten strains of *Proteus* spp. and confirmed his value of $2\ \mu$. From this he concluded that a definite spiral period is a characteristic feature of bacterial flagella. Neither author had noticed the occurrence, to which we here call attention, of the two wavelengths, one twice the other. Their figures therefore require correction. With this important reservation we confirm that flagellar wavelength is a characteristic feature. Its practical value for classification, however, is further limited by the necessity to perform many measurements on wet preparations, and by the fact that the range of available wavelengths is very limited. Leifson's (1951) observation of a strain of *Sal. wichita* which dissociated into two variants, one showing flagella twice the wavelength of the other, has no bearing on our phenomenon. The two kinds of wavelength were not seen by him in one and the same culture but only in two separate cultures, and moreover in fixed and stained preparations which easily give rise to artefacts. Also, our strain of *Sal. typhi* H 901 can hardly contain two variants. The central coiled filament of the flagellum of *B. brevis* indicated in the electron photographs of de Robertis & Franchi (1951) and the helical fine structure of the flagella of a motile diphtheroid appearing in the electron photographs of Starr & Williams (1952) appear to be of rather too different an order to cause the phenomenon we have described here. Our phenomenon may find its explanation in the molecular structure of this particular kind of flagellum.

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EXPLANATION OF PLATES

PLATE 1

- Fig. 1. Fast moving small packet of *S. ureae*, with tail; $\times 1000$.
- Fig. 2. Small packet of *S. ureae*, tail stiffened into helix of long wavelength; $\times 1500$.
- Fig. 3. Small packet of *S. ureae*, tail stiffened into helix of short wavelength; $\times 1500$.
- Fig. 4. Several helices of short wavelength of *S. ureae* linked up lengthwise; $\times 1500$.
- Fig. 5. Transverse 'packing' and lengthwise 'linking' of helices of long wavelength of *S. ureae*; $\times 1500$.
- Fig. 6. Helical flagella of both long and short wavelength of *S. ureae* in same field, several helices linked up lengthwise; $\times 1500$.
- Fig. 7. 'Packed' helix of short wavelength linked up accidentally with helix of long wavelength, both of *S. ureae*; $\times 1500$.

PLATE 2

- Fig. 8. Small packet of *S. agilis*, tail stiffened into helix of short wavelength; $\times 1500$.
- Fig. 9. Large packet of *S. agilis*, tail stiffened into helix of long wavelength; $\times 1500$.
- Fig. 10. Long stretch of helices of short wavelength of *S. agilis* linked up lengthwise; $\times 1500$.
- Fig. 11. Helices of long and short wavelength of *S. agilis*, accidentally linked up; $\times 1500$.
- Fig. 12. Helices of long and short wavelength of *S. agilis* in one field; $\times 1500$.
- Fig. 13. *Sal. typhi* with helix of short wavelength; $\times 1500$.
- Fig. 14. *Sal. typhi* with numerous helices of long wavelength; $\times 1500$.
- Fig. 15. *Caryophanon latum* with one helix of short wavelength and one helix of long wavelength; about $\times 600$.

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A. PIJPER & G. ABRAHAM—WAVELENGTHS OF BACTERIAL FLAGELLA. PLATE 1