

NOTES

Phenocopies of a Heteromorphous Flagellar Mutant in *Salmonella*

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So-called "curly" bacterial flagella are characterized by having half the wavelength of normal flagella. It has been shown that, in a medium containing *p*-fluorophenylalanine (FPA) in place of phenylalanine (PA), a normally flagellated strain of *Salmonella* regenerates curly flagella after the normal flagella have been removed by shaking (D. Kerridge, *Biochim. Biophys. Acta* **21**:593, 1959). Among other *Salmonella* mutants with aberrant flagellar forms, a "heteromorphous" mutant has been described which regularly produces some cells (about 2%) with a flagellum made up of both normal and curly portions within a clone of normally flagellated cells (T. Iino and M. Mitani, *J. Gen. Microbiol.* **44**:27, 1966). We report now the induction of phenocopies of this heteromorphous mutant in a medium containing both PA and FPA.

Mutant strain SL448 of *S. typhimurium* cannot grow in media lacking PA, whereas, in media containing 10 μ g of PA per ml, it grows as well as the wild strain and the cells produce normal flagella. SL448 was cultivated in 10 ml of Penassay Broth (Difco) at 37 C with aeration for about 3 hr (until the cell concentration reached 10^9 per milliliter). The cells were then centrifuged and washed twice with minimal medium (MM; 0.7% K_2HPO_4 , 0.2% KH_2PO_4 , 0.05% sodium citrate \cdot $5H_2O$, 0.01% $MgSO_4 \cdot 7H_2O$, and 0.2% glucose in distilled water) and resuspended in 20 ml of MM. This suspension was shaken (ca. 750 strokes/min, 25-mm amplitude) for 45 min to deflagellate the cells. They were then centrifuged at $1,200 \times g$ for 15 min, and the pellet (composed mainly of deflagellated cells) was suspended in 10 ml of regeneration medium (RM) and incubated at 37 C. RM consisted of MM supplemented with the complete amino acid mixture (0.1 mg of each L-amino acid/ml). When PA was replaced by FPA, the composition of the medium is represented by RM ($x:y$), where $x:y$ indicates the

weight ratio of PA to FPA. After 2.5 hr of incubation, the morphology of the regenerated flagella was examined by electron microscopy as described by M. Mitani and T. Iino (*J. Bacteriol.* **90**:1096, 1965).

Before the flagella were sheared off, 27% of the cells were flagellated. Average numbers of flagella per flagellated cell (*f/c*) and waves per flagellum (*w/f*) were 2.7 and 2.1, respectively. After deflagellation, the fraction of the flagellated cells decreased to 6%, and *f/c* and *w/f* to 1.4 and 0.9, respectively. The deflagellated cells were distributed to RM (1:0), (9:1), (7:3), (5:5), (3:7), (2:8), (1:9), and (0:1) at the final concentration of 2.2×10^7 per milliliter. After 2.5 hr in RM (1:0), the cell concentration had increased 100-fold, and 26% of the cells were found to be flagellated. Their *f/c* was 2.8 and *w/f* was 1.4. That is, in RM (1:0), after 2.5 hr, the cells had recovered their normal number of flagella, but these newly synthesized flagella were shorter than the original ones. In RM containing both PA and FPA, both cell multiplication and flagellation were depressed proportionally to the concentration of FPA. In RM (1:9) and RM (0:1), cells had multiplied 10-fold and 8-fold, respectively, but, among 100 cells examined, no flagellated cells were detected. This may indicate that, in these media, *de novo* flagellar formation did not occur and the number of flagella per cell was diluted out by cell multiplication to less than 1%. Cells presenting simultaneously both normal and curly flagella will be referred to as heteromorphous cells. On the other hand, the term heteromorphous flagella will describe flagella with both normal and curly waves in one fiber. The shape of flagella was normal in RM (1:0), whereas only curly flagella were present in RM (2:8). In RM (9:1) through RM (3:7), normal, curly, and heteromorphous flagella appeared; none of the flagella had intermediate shapes (Fig. 1, 2; Table 1). Cells carrying both normal and curly waves on a single fiber were occasionally ob-

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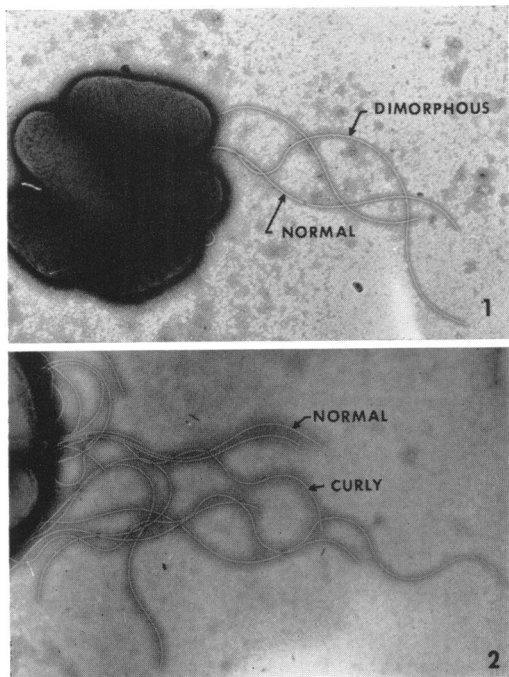


FIG. 1. Appearance of flagella in medium containing an FP-FPA ratio of 9:1.

FIG. 2. Appearance of flagella in medium containing an FP-FPA ratio of 8:2.

served. Length of one turn of the helix and the wavelength-amplitude ratio of the normal flagella were $2.96 \pm 0.19 \mu$ and $6.8 \pm 0.1 \mu$, respectively (at the 95% confidence level), and those of the curly were $1.52 \pm 0.06 \mu$ and $4.5 \pm 0.2 \mu$. These values were not significantly different among flagella in RM (1:0) through (2:8).

Efficiency of flagellation was higher with increasing PA-FPA ratios. In RM (3:7), curly flagella were produced more abundantly than the normal; in RM (5:5), (7:3), and (9:1), numbers of normal flagella predominated. Proportions of heteromorphous cells and heteromorphous flagella were largest in RM (7:3), in which the ratio of normal to curly was close to 1.

A characteristic feature of the heteromorphous flagella was their being curly at the region proximal to the cell and normal near the tip (Table 2). They presented on the average less than one normal wave on each flagellum, whereas curly waves ranged from one to six. A few hetero-

TABLE 1. Distribution of normal and curly flagella regenerated in media containing varying ratios of phenylalanine and p-fluorophenylalanine

Determination	PA-FPA ratio			
	9:1	7:3	5:5	3:7
Total no. of cells examined.....	134	153	159	111
No. of flagellated cells.....	48	45	44	16
No. of cells				
Normal.....	23	18	28	2
Curly.....	8	7	8	11
Heteromorphous.....	17	20	8	3
No. of flagella				
Normal.....	60	45	62	6
Curly.....	28	34	21	20
Heteromorphous.....	8	17	2	0
Avg no. of flagella per flagellated cell.....	2.0	2.1	1.9	1.6
Avg no. of waves per flagellum ^a .	1.3	1.3	0.9	1.0

^a Number of normal waves; one curly wave was counted as 0.5.

TABLE 2. Distribution of curly and normal waves in heteromorphous flagella

Arrangement of waves ^a	Cells observed in media containing FA and FPA in proportions:				Total
	9:1	8:2	7:3	5:5	
Cell-n-c	0	3	0	1	4
Cell-c-n	18	39	17	3	77
Cell-n-c-n	0	1	0	0	1
Total	18	43	17	4	82

^a Cell-n-c means that flagellar waves were normal at the region proximal to the cell and curly near the tip.

morphous flagella were curly at the distal region, and 1 among 82 heteromorphous flagella was found to be normal at both distal and proximal regions and curly in between.

In summary, the regenerated flagella are either normal or curly and never manifest intermediate shapes, even over a range of ratios of PA to FPA.