Journal of General Microbiology (1981), **125**, 213–216. *Printed in Great Britain*

SHORT COMMUNICATION

A Simple Method for the Isolation of Flagellar Shape Mutants in *Salmonella*

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(*Received 20 March 1981*)

During prolonged static cultivation of liquid cultures of *Salmonella* strains with normal flagella, flagellar shape mutants occurred spontaneously and their proportion in the cultures increased so that they were easily isolated by spreading dilutions of the cultures on semisolid medium. By this method various types of flagellar shape mutants, including curly, straight and polymorphous mutants, were obtained.

INTRODUCTION

In general, bacterial flagella show a helical form with a wavelength and wave height characteristic of each strain (Leifson, 1960). In various strains, mutants carrying flagella with genetically altered shape have been isolated. Representative types of flagellar shape mutants are 'curly' in *Salmonella* (Leifson, 1960; Iino, 1962), 'straight' in *Bacillus subtilis* (Martinez *et al.*, 1968), *Salmonella typhimurium* (Iino & Mitani, 1967) and *Escherichia coli* (Kondoh & Yanagida, 1975), and 'polymorphous' in *S. typhimurium* (Iino *et al.*, 1974). Curly flagella have a wavelength approximately half the normal. Flagella of polymorphous mutants are either straight or possess one of the four distinct wave forms C, N, S or M, whose wavelength and wave height decrease in that order (Iino *et al.*, 1974). Genetic analysis of flagellar shape mutations has been carried out most extensively with *Salmonella* mutants. Transduction analysis in *Salmonella* indicated that the mutations were in *H1* or *H2*, the structural genes for flagellin, which is the sole component of the filament of bacterial flagella (Iino, 1962; Iino & Mitani, 1967; Iino *et al.*, 1974; Horiguchi *et al.*, 1975). However, only a few flagellar shape mutants have so far been isolated, and their genetic backgrounds vary. For more detailed investigation of the genetic determination of flagellar shape, the isolation of more mutants with the same genetic background would be desirable. Recently, we found that during static cultivation of wild-type bacteria, various types of flagellar shape mutants occurred spontaneously and increased to a high proportion in the cultures. In this communication, we report the isolation of flagellar shape mutants from three *Salmonella* strains carrying different alleles of the flagellin genes *H1* or *H2*.

METHODS

Organisms. The *Salmonella* strains used were SJW1103, SJW797 and SJW806. Strains SJW1103 *H1-i* and SJW797 *H1-gt* are phase-1 stable strains with flagella of antigen type 'i' and 'gt', respectively. Strain SJW806

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0022-1287/81/0000-9849 $02.00

H2-enx is a phase-2 stable strain with flagella of antigen type 'enx'. All of them are transductional derivatives of *Salmonella typhimurium* TM2. Their flagella are normal, i.e. their average wavelength is between 2·1 and 2·3 μm and their average wave height is between 0·40 and 0·45 μm.

Media and cultivation. Nutrient broth was composed of 1% (w/v) polypeptone and 1% (w/v) meat extract, adjusted to pH 7·0 with NaOH. Nutrient agar was prepared by the addition of 1·5% (w/v) agar to the nutrient broth, and semisolid medium by the addition of 0·3% (w/v) agar and 8% (w/v) gelatin. All cultures were incubated at 37 °C.

Identification of flagellar shape mutants. These were identified by their colony morphology on semisolid medium. On this medium, wild-type bacteria form swarms, while most of the flagellar mutants including non-flagellate (*fla*), paralysed (*mot*) and flagellar shape mutants form compact colonies, the detailed appearance of which varies with the flagellar characters (Iino, 1962; Enomoto & Iino, 1963; Iino & Mitani, 1967). *Fla* and *mot* mutants form LP (large and pale) and SD (small and dense yellow) colonies, respectively, whereas flagellar shape mutants form dense yellow and flat colonies. These colony forms can be distinguished from each other by the use of a binocular microscope at ×25 or ×40 magnification.

In liquid medium, curly and polymorphous mutants show rotational movement and have a tendency to form characteristic aggregations (Iino *et al.*, 1974), while straight mutants are non-motile. Thus, flagellar shape mutants judged from colony morphology were examined for behaviour in nutrient broth. Precise examination of their flagellar shape was carried out by electron microscopy.

Electron microscopy. Bacteria fixed with 5·0% (w/v) formalin were negatively stained with 2% (w/v) phosphotungstic acid solution (pH 6·8) and examined in a JEM-T7 electron microscope.

RESULTS AND DISCUSSION

Isolation of flagellar shape mutants

Individual colonies of the parental strains—SJW1103, SJW797 and SJW806—on nutrient agar plates were inoculated in 1·0 ml nutrient broth in 12 × 90 mm test tubes, and the cultures were kept at 37 °C without shaking. The titre of viable bacteria reached a maximum (about 2×10^9 ml^{-1}) after 24 h incubation, and decreased gradually thereafter. After 4 d incubation, when the titre was about 1×10^8 ml^{-1}, the cultures were diluted to about 2×10^3 bacteria ml^{-1} with saline and portions of the dilutions were spread on semisolid medium plates so as to give isolated colonies. After overnight incubation, compact colonies appearing among confluent swarms of wild-type bacteria were examined for their morphology. From almost every culture, dense yellow and flat colonies characteristic of flagellar shape mutants were detected. Bacteria from these colonies were then examined for behaviour in nutrient broth. Most of the clones showed the rotational movement and aggregation characteristic of curly and polymorphous mutants, the remainder being non-motile. Electron microscopy showed that all the non-motile clones were straight mutants, while those showing rotation and aggregation comprised not only curly and polymorphous mutants but also various other types of flagellar shape mutants, as described below.

In order to count bacteria of every flagellar character, including the wild-type, dilutions of cultures were first spread on nutrient agar plates to obtain isolated colonies, and the colonies that appeared were then examined for their flagellar characters (Table 1). A large number of flagellar shape mutants, especially 'curly' mutants, as well as paralysed ones (*mot*) were detected. In control experiments, in which bacteria were subcultured to new broth every 12 h and incubated with shaking, the only mutants that appeared were a few *fla* mutants in some of the cultures. Why the proportion of flagellar shape and *mot* mutants increased during prolonged static cultivation is unknown.

Characteristics of the mutants obtained

By the above procedure, mutants showing rotation and aggregation in broth were obtained from more than 90% of the tube cultures of all three parental strains. From about 40% of the cultures of strains SJW1103 and SJW797, straight mutants were also obtained. From strain SJW806, however, no straight mutant was obtained, even though more than 200 tube cultures were examined. This suggests that, in contrast to the flagellins of the other two strains, the flagellin of SJW806 has a structure that does not readily assemble into straight flagella.

Table 1. *Occurrence of flagellar mutants in static cultures of Salmonella strains with normal flagella*

Appropriate dilutions of 1 d and 4 d cultures were spread on nutrient agar to obtain isolated colonies. Individual colonies appearing on the plates were examined for flagellar characters (see Methods). Among the flagellar shape mutants, those showing rotation and aggregation in nutrient broth were grouped as 'curly'.

Parental strain	Incubation	Tube no.	Incubation time (d)	Total no. of colonies examined	No. of colonies with each flagellar character*				
					Normal	'Curly'	Straight	*fla*	*mot*
SJW1103 *H1-i*	Static	1	1	405	405	0	0	0	0
			4	464	142	227	1	1	93
		2	1	406	406	0	0	0	0
			4	463	210	56	0	0	197
		3	1	406	406	0	0	0	0
			4	418	123	102	0	0	193
SJW797 *H1-gt*	Static	1	1	310	310	0	0	0	0
			4	444	411	13	0	16	4
		2	1	313	312	0	0	0	1
			4	457	400	13	1	1	42
		3	1	290	290	0	0	0	0
			4	464	446	7	2	5	4
SJW806 *H2-enx*	Static	1	1	453	453	0	0	0	0
			4	506	452	48	0	4	2
		2	1	343	343	0	0	0	0
			4	411	398	8	0	0	5
		3	1	341	341	0	0	0	0
			4	494	345	120	0	1	28
SJW1103 *H1-i* (control)	Shaken, subcultured every 12 h	1	1	358	358	0	0	0	0
			4	358	358	0	0	0	0
		2	1	358	358	0	0	0	0
			4	358	355	0	0	3	0
		3	1	358	358	0	0	0	0
			4	357	356	0	0	1	0

* *fla*, non-flagellate mutants; *mot*, paralysed (flagellate but non-motile) mutants.

By electron microscopy, it was shown that the group of 'curly' mutants, showing rotation and aggregation in liquid medium, contained various types. Some had typical curly flagella (wavelength 1·1 ± 0·1 μm; wave height 0·4 ± 0·05 μm), some had flagella similar to one of the three forms (N, S and M) of the polymorphous mutant SJ4020 (Iino *et al.*, 1974), and some had polymorphous flagella of two or more different forms.

On the basis of a model assuming that flagellin can be transformed into two conformations (L- and R-states), it has been predicted that there should be two types of straight flagella, namely L- and R-types, that are made up exclusively of flagellins in either the L-state or the R-state (Calladine, 1978; Kamiya *et al.*, 1979). Ten straight flagellar mutants from SJW1103 were examined for their flagellar types by electron microscopy and optical diffraction; flagella of one mutant were R-type and those of the other nine were L-type (Kamiya *et al.*, 1980).

The mutation sites of all the mutants obtained here were shown by P22 phage-mediated transduction to be in one or other of the flagellin genes, *H1* and *H2*. Detailed mapping of the mutation sites within *H1* or *H2* is in progress.

We thank Mr K. Somiya for technical assistance.

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