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SHORT PAPERS

A method for isolating paralysed (*mot*$^-$) mutants from non-flagellated cells of Salmonella

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1. INTRODUCTION

In Salmonella, paralysis and absence of flagella are caused by mutations in *mot* (motility) and *fla* (flagellation) genes, respectively (Enomoto, 1966; Joys & Stocker, 1965; Iino & Enomoto, 1966). *ah1*$^-$ mutations which cause inactivation of *H1* (the phase-1 flagellar antigen gene) and map at sites closely linked to it also result in absence of flagella in phase-1 (flagella in phase-2 are normally produced) (Iino, 1961). *mot*$^-$ and *fla*$^-$ mutants, produced in a population of motile bacteria by spontaneous mutations or by transduction of the mutant gene, can be easily accumulated by selection for resistance to phage *x* which attacks only motile bacteria (Meynell, 1961) and isolated on NGA (nutrient gelatin agar) medium as SD- and LP-type colonies; the former is a small and dense colony characteristic of the *mot*$^-$ mutant and the latter is a large and pale colony characteristic of the *fla*$^-$ mutant (Enomoto & Iino, 1963). *mot*$^-$*fla*$^-$ double mutants can be obtained by isolating LP-type sectors which are produced on NGA medium at the margin of inoculation site of the parental *mot*$^-$ mutant after prolonged incubation for 48 h at 37 °C (Enomoto, 1967). However, there has been no method for isolating *mot*$^-$ double mutants. In this report, an efficient method for concentrating a small number of *mot*$^-$ cells previously mixed with a large number of *fla*$^-$ cells is presented and the application of this method to the isolation of the *motA*$^-$ *motC*$^-$ double mutant which was produced by transduction of the *motC*$^-$ gene to the *motA*$^-$*ah1*$^-$ mutant is shown.

2. MATERIALS AND METHODS

The bacterial strains used were the wild-type strain TM2 (*H1i:H21, 2*) of *Salmonella typhimurium* and three kinds of non-motile mutants derived from it; *mot*$^-$ (flagella-paralysed), *fla*$^-$ (non-flagellated), and *ah1*$^-$*mot*$^-$ mutants. The mutant *motC244* has a mutational site cotransducible with *H*1 at a frequency of 52 % (Enomoto & Yamaguchi, 1969). *flaD42* is a stable non-flagellated mutant (Iino & Enomoto, 1966). The double mutant *ah1-14motA257* which was obtained in the course of isolation of *motA257fla* double mutants is non-flagellated in phase-1 and shows paralysed flagella in phase-2 due to the O–H variation (Iino, 1961). Phage P22 was used for all transductions except when phage-sensitive transductants were required, when its mutant P22L4 (Smith & Levine, 1967) was used.

The composition of media, preparation of phage stocks and transduction procedures were described by Enomoto (1966). Anti-*i* serum with a titre of about 20000, which was prepared against SJ847 (*S. abortus-equi* SJ241 with *H1i* from *S. typhimurium*) and absorbed completely with the O-antigen of SJ241, was used at a final concentration of 0·1 % (v/v).

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Preparation of dead cells. A 250 ml shaken broth culture of TM2 cells expressing the phase-1 flagellar antigen *i* was centrifuged after incubation for 5 h at 37 °C. The cells were suspended in buffered saline (0·85 % NaCl in M/150 phosphate buffer, pH 7·0; Levine, 1957) containing 1 % (v/v) formalin, left at room temperature for 36 h, washed twice with buffered saline after sterility test, and resuspended in 10 ml buffered saline (about 2×10^{10} cells/ml).

Concentration of mot⁻ *cells.* A tenfold dilution (about 10^8 cells/ml) of 0·05 ml of an overnight broth culture of *motC244* expressing the flagellar antigen *i* was mixed with 5·0 ml of an overnight broth culture of *flaD42* (about 10^9 cells/ml) and 0·3 ml of the mixture was stored at 4 °C as an original sample. To the remaining mixture (about 4·7 ml), 0·25 ml suspension of TM2 dead cells was added, shaken well, then 0·05 ml tenfold dilution of anti-*i* serum was added, and the mixture was shaken gently at 37 °C for 90 min to agglutinate a small number of living *motC244* cells with a large number of dead cells. The mixture was centrifuged at about 30 ***g*** for 15 min to deposit only the agglutinated cells, which were washed once by the same centrifugal force; unagglutinated cells in supernatant were sucked out by a capillary tube with the tap aspirator. The deposit was suspended in 2 ml buffered saline, shaken vigorously (about 10^3 strokes/min, 25 mm amplitude) for 30 min for deflagellation, centrifuged at about 2000 ***g*** for 10 min, resuspended in 5 ml broth and incubated shaken for 5 h at 37 °C. A 0·3 ml sample was taken and stored at 4 °C. This was the first treatment and the procedure was repeated for further concentration. The samples from each treatment for concentration were plated on NGA medium after appropriate dilution, incubated overnight at 37 °C and the number of LP- and SD-type colonies were counted. Deflagellation by shaking has the advantage that the cells freed from the aggregate are prevented from reagglutination and the dead cells can be discarded at the next repeat of concentration as the unagglutinated cells; the living *motC244* cells which regenerate flagella in the following incubation are agglutinated with the flagellated dead cells newly added in the next repeat.

Isolation of the motA⁻motC⁻ *double mutant.* A 4·0 ml overnight broth culture of the *ah1-14motA257* mutant (about 10^9 cells/ml) which is in non-flagellate state of phase-1 was mixed with a 1 ml phage P22L4 lysate of the *motC244* mutant ($5{\cdot}0 \times 10^{10}$ p.f.u./ml) expressing the phase-1 antigen *i*. The mixture was incubated for 2 h at 37 °C and treated with the same procedure for concentration as described above. Three kinds of clones expressing the phase-1 flagellar antigen *i* are expected to appear in the mixture; the revertant from *ah1*⁻ to *ah1*⁺, the transductant receiving the wild-type allele of *ah1*, and the contrasductant receiving the mutant gene *motC244* as well as the *ah1* allele. The last one is required. Accordingly, the SD-type colonies expressing the flagellar antigen *i* were examined by transduction tests from *motA257*, *motC244* and TM2 as to the production of swarms (complete *mot*⁺ transductants) and trails which are produced by non-motile cells made temporarily motile by abortive transduction of the complementary *mot* gene (Lederberg, 1956; Stocker, 1956). The clones which produce neither swarms nor trails in the transduction from *motA257* and *motC244* were regarded as the double mutant.

3. RESULTS

A series of concentration experiments on *motC244* cells mixed with *flaD42* cells at a ratio of about 1:1000 produced the good results shown in Table 1. By three concentration steps the ratio of SD-type colonies to the total increased to 30 %, about 462 times as high as the original ratio. Approximately tenfold concentrations were achieved in each of the first two steps.

Concentration for obtaining the double mutant *motA257motC244* was performed five times over. The results are shown in Table 2. The ratio of SD-type colonies was raised to 61 % by the five concentration steps. Concentration was about 10 times per treatment

except the fifth. Forty-six SD-type colonies from the fourth treatment and 113 from the fifth were examined for their flagellar antigens by slide agglutination tests; all of them showed the phase-1 flagellar antigen *i*. Therefore the number of cells expressing the antigen *i* in the original transduction mixture was inferred to be about three per 10^6 non-flagellated cells. On thirty clones of the 113 SD-type colonies, the transduction was carried out from *motA257*, *motC244* and TM2. Fifteen clones produced many swarms and trails in the transduction from *motC244* and TM2, though they did not produce any swarms and trails in the transduction from *motA257*; these were regarded as revertants or transductants to *ah1*$^+$. The remaining fifteen produced neither swarms nor trails in the transductions from *motA257* and *motC244*; a few trails were produced by treatment of the TM2 lysate. These were regarded as the double mutants, forming 50% of the SD-type colonies.

This method may be applied to the isolation of a paralysed mutant having the *H* gene introduced by transduction from various *Salmonella* serotypes.

Table 1. *Concentration of* mot$^-$ *cells mixed with a large number of* fla$^-$ *cells*

No. of concentration steps	No. of colonies			
			SD type	
	Total	LP type	No.	%
0	3073	3071	2	0·07
1	3037	3018	19	0·63
2	3318	3075	243	7·32
3	589	412	177	30·1

Table 2. *Concentration of cells expressing the flagellar antigen* i *in the transduction mixture*

No. of concentration steps	No. of colonies			
			SD type	
	Total	LP type	No.	%
3	1591	1587	4	0·3
4	1451	1401	50	3·4
5	1207	469	738	61·1

SUMMARY

A method for concentrating flagella-paralysed (*mot*$^-$) cells existing in a large number of non-flagellated cells of Salmonella was invented. The *mot*$^-$ cells expressing the phase-1 flagellar antigen *i* were agglutinated by anti-*i* serum with carriers, the dead cells of TM2 expressing the phase-1 antigen *i*; only the agglutinated cells were harvested by a low speed of centrifugation and incubated in broth. About tenfold concentration was brought about by one treatment, which was repeated for further concentration. By this method, the *motA257motC244* double mutant which was produced by transduction at a frequency of about 3×10^{-6} per non-flagellated recipient cell was isolated from the broth culture concentrated five times, in which about 30% of the cells were the double mutant.

REFERENCES

ENOMOTO, M. (1966). Genetic studies of paralysed mutants in Salmonella. I. *Genetics* **54**, 715–726.

ENOMOTO, M. (1967). Composition of chromosome fragments participating in phage P22-mediated transduction of *Salmonella typhimurium*. *Virology* **33**, 474–482.

ENOMOTO, M. & IINO, T. (1963). Colonial dimorphism in non-motile *Salmonella*. *J. Bact.* **86**, 473–477.

ENOMOTO, M. & YAMAGUCHI, S. (1969). Different frequencies of cotransduction of *motC* and *H1* in Salmonella. *Genet. Res.* (in the press).

IINO, T. (1961). Genetic analysis of O–H variation in Salmonella. *Jap. J. Genetics*, **36**, 268–275.

IINO, T. & ENOMOTO, M. (1966). Genetical studies of non-flagellate mutants of Salmonella. *J. gen. Microbiol.* **43**, 315–327.

JOYS, T. M. & STOCKER, B. A. D. (1965). Complementation of non-flagellate Salmonella mutants. *J. gen. Microbiol.* **41**, 47–55.

LEDERBERG, J. (1965). Linear inheritance in transductional clones. *Genetics* **41**, 845–871.

LEVINE, M. (1957). Mutations in the temperate phage P22 and lysogeny in Salmonella. *Virology* **3**, 22–41.

MEYNELL, E. W. (1961). A phage, Φx, which attacks motile bacteria. *J. gen. Microbiol.* **25**, 253–290.

SMITH, O. H. & LEVINE, M. (1967). A phage P22 gene controlling integration of prophage. *Virology* **31**, 207–216.

STOCKER, B. A. D. (1956). Abortive transduction of motility in Salmonella; a non-replicated gene transmitted through many generations to a single descendant. *J. gen. Microbiol.* **15**, 575–598.