HOLLOWAY, B. W. (1956). *J. gen. Microbiol.* **15**, 221-224

Self-fertility in *Pseudomonas aeruginosa*

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SUMMARY: Two interfertile strains of *Pseudomonas aeruginosa* were re-examined for evidence of self-fertility. One strain was self-fertile, the frequency of recombination being affected by the manner in which the parent organisms were grown before being combined. The other strain gave no evidence of self-fertility.

Genetic recombination has been shown to occur in *Pseudomonas aeruginosa* in a manner similar to that which occurs in *Escherichia coli* (Holloway, 1955). One conclusion reached in that work was that the strains used were interfertile in certain combinations but self-sterile. All crosses between mutant strains of the same parental strain were made with organisms grown on agar slopes. Some anomalous results were later obtained with certain inter-strain crosses in which recombination frequencies for bacteria grown in aerated broth were higher than for those grown on agar slopes; this was contrary to earlier experiments. The lack of recombination exhibited in the earlier experiments on intra-strain crosses may therefore have been due to the manner in which the organisms had been grown rather than to an inherent self-sterility. This possibility was strengthened by the findings of Loutit (1955), who obtained results consistent with the occurrence of genetic recombination between biochemical mutants derived from the one parental strain, and in these experiments the bacteria were grown in broth.

METHODS

Except where stated, the methods and media used were the same as those described previously (Holloway, 1955). Aeration of cultures was achieved by agitation on the shaking machine described by Kantorowicz (1951), at a frequency of 116 oscillations/min., and an amplitude of $1\frac{1}{4}$ in. Cultures were grown in 100 ml. of Difco nutrient broth + 0.5% Difco yeast extract in 8 oz. medical flat bottles (capacity 235 ml.). The strains used were various auxotrophic mutants of the parent strains 1 and L described earlier (Holloway, 1955), these parent strains being interfertile when certain auxotrophic mutants are crossed.

As some difficulty had been experienced in maintaining stock cultures in a completely unaltered state, the following procedure was adopted. At the beginning of this set of experiments all the strains to be used were each grown separately in aerated broth overnight, and about 1 ml. quantities of each culture were then sealed in thin-walled glass ampoules. The ampoules were quick-frozen in a solid CO₂ + ethanol mixture and stored at -15°. When a particular strain was required, an ampoule was thawed and used for the inoculum. With this method no variation in stock cultures has yet been detected.

RESULTS

Crosses were usually set up so that not only could prototrophic recombinants be detected but also recombination for either or both of the non-selective markers streptomycin (S) or chloramphenicol (C). The influence of the manner in which the bacteria were grown before crossing was investigated for a number of crosses. It was found that for most inter-L crosses the frequency of recombination when the bacteria were grown in aerated broth was appreciably higher than when they were grown on agar slopes. The results of a typical experiment are shown in Table 1. Growth in non-aerated broth gave lower recombination values than that for aerated broth, while growing the two strains together in aerated broth conferred no advantage. Although the frequency of recombination when the parents were grown on agar slopes was very low, nevertheless recombination did occur, as was shown by the formation of the non-parental type $Ad^+T^+S^rC^s$. This low frequency of recombination when the parents were grown on agar slopes led to the previous erroneous conclusion that strain L was self-sterile.

Table 1. *Variations in recombination frequency and segregation ratios for the markers S and C, for the cross $L(Ad^-)S^rC^r \times L(T_1^-)S^sC^s$ under different cultural conditions*

Cultural conditions	Recombination frequency*	S and C response among prototrophs			
		S^rC^r	S^sC^r	S^rC^s	S^sC^s
Grown together, aerated broth	5.7	13	0	55	4
Grown together, non-aerated broth	1.9	13	0	27	4
Grown separately, aerated broth	16.9	0	0	61	11
Grown separately, non-aerated broth	1.1	7	0	13	7
Grown separately, agar slopes	0.5	6	0	4	1

* Prototrophs/ 10^8 parent organisms. Corrected for back mutation to prototrophy of the parents from information derived from plate counts.

A number of crosses were carried out between various mutants of strain L to confirm the presence of genetic recombination in such crosses and to compare the results with the $1 \times L$ crosses previously examined. These crosses were all made with parents grown overnight in aerated broth, and the results are given in Table 2. There can be no doubt that genetic recombination had occurred, as is seen by the various non-parental types recovered. In the case of the cross $L(Ad^-) \times L(T_1^-)$, crosses were set up with the markers S and C entering the cross in all possible combinations. The results indicate that the progeny always tended to resemble the $L(Ad^-)$ parent with respect to the marker C and the $L(T_1^-)$ parent with respect to S. In much the same way as was found previously for the $1 \times L$ crosses, different combinations of selective markers resulted in different recombination frequencies and different ratios of sensitivity and

resistance of S and C. In most cases complete reliance cannot be placed on the observed ratios of S and C amongst the prototrophs tested, for with the low recombination frequencies observed, a certain proportion of the colonies picked off were undoubtedly back-mutations to prototrophy of the parent auxotrophic strains.

Table 2. *Recombination frequencies of L × L matings, and streptomycin and chloramphenicol resistance of the resulting prototrophs*

Cross	Recombination* frequency	S and C response among prototrophs			
		S ^r C ^r	S ^s C ^r	S ^r C ^s	S ^s C ^s
L(IV ₁ -S ^r C ^r) × L(LIL-S ^s C ^s)	3.9	38	1	0	27
L(IV ₁ -S ^r C ^r) × L(Thr-S ^s C ^s)	0	—	—	—	—
L(IV ₁ -S ^s C ^s) × L(T ₁ -S ^r C ^r)	2.4	39	204	3	156
L(IV ₁ -S ^r C ^s) × L(Ad-S ^s C ^r)	3.7	2	56	11	2
L(IV ₁ -S ^r C ^r) × L(P-S ^s C ^s)	0.2	—	—	—	—
L(LIL-S ^r C ^s) × L(P-S ^s C ^r)	0	2	2	58	0
L(LIL-S ^s C ^s) × L(Ad-S ^r C ^r)	3.8	15	6	5	45
L(LIL-S ^r C ^s) × L(Thr-S ^s C ^r)	0.1	0	1	71	0
L(Thr-S ^s C ^s) × L(T ₁ -S ^r C ^r)	0.2	—	—	—	—
L(Thr-S ^s C ^s) × L(Ad-S ^r C ^r)	1.4	3	8	6	11
L(Thr-S ^s C ^s) × L(P-S ^r C ^r)	0	—	—	—	—
L(T ₁ -S ^r C ^r) × L(Ad-S ^s C ^s)	8.7	11	86	2	7
L(T ₁ -S ^r C ^s) × L(Ad-S ^s C ^r)	25.4	0	1	14	93
L(T ₁ -S ^s C ^r) × L(Ad-S ^r C ^s)	34.3	151	22	7	0
L(T ₁ -S ^s C ^s) × L(Ad-S ^r C ^r)	19.7	2	0	154	24
L(T ₁ -S ^r C ^r) × L(P-S ^s C ^s)	0.7	5	27	1	1
L(Ad-S ^r C ^r) × L(P-S ^s C ^s)	3.8	0	9	21	42

* Prototrophs/10⁹ parent organisms. Corrected for back mutation to prototrophy of both parents.

With regard to strain 1, crosses were carried out which involved most of the possible matings of the auxotrophic mutants 1(T₁-), 1(LIL-), 1(IV₁-), 1(IV₂-), 1(M₁-), 1(Se-), 1(M₁-T₁-). In no case did the number of prototroph colonies exceed significantly the number of colonies due to back-mutation shown by controls of the parents plated on their own, and in no case was any segregation of the markers S and C demonstrated when the colonies were picked off and tested in the usual way. For strain 1, the previous conclusion of self-sterility is confirmed.

DISCUSSION

On the basis of the segregation of certain markers in *Pseudomonas aeruginosa* it has previously been found that strain 1 acts as an acceptor strain and strain L as the donor strain. In *Escherichia coli* the donor strain is F⁺ and self-fertile while the F⁻ acceptor strain is self-sterile. Although it has not been possible to demonstrate any F agent in *Pseudomonas aeruginosa*, the similarity in behaviour between the two mating systems is obvious. Furthermore, it is known that variations in cultural conditions can alter the F status of *E. coli* K12. The effect of cultural conditions on recombination frequency in *P. aeruginosa*

described above is additional evidence for suggesting that the basic mechanism underlying genetic recombination in these two organisms may be similar.

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REFERENCES

- HOLLOWAY, B. W. (1955). Genetic recombination in *Pseudomonas aeruginosa*. *J. gen. Microbiol.* **13**, 572.
KANTOROWICZ, O. (1951). Shaking apparatus for the aeration of bacterial cultures. *J. gen. Microbiol.* **5**, 276.
LOUTIT, J. S. (1955). Auxotrophic mutants of *Pseudomonas aeruginosa*. *Nature, Lond.* **176**, 74.

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