GENETIC MAPPING OF PYOCIN R1 FACTOR IN *PSEUDOMONAS AERUGINOSA*

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The genetic factor responsible for the production of pyocin R1 was found to be transferable from the original strain P15 to PAO sublines by F116 transduction. Similar to pyocin R2 and R3 factors, R1 factor was cotransduced with *trp-1* marker. The behavior of the R1 factor was studied in PAO comparing with other pyocin factors. The results suggest that all these pyocin factors occupy a similar position close to *trp-1* in the chromosome of PAO strains.

A group of pyocins which resembles a certain bacteriophage tail was found by us and referred to as R-type pyocins (1). The genetic character of pyocinogenic factors was also studied, and pyocin R2 as well as R3 factor was found to be incorporated into the bacterial chromosome at a certain position (2, 3). This paper will show that the genetic determinant for pyocin R1 production is also of chromosomal nature. Pyocin R1 (hitherto referred to as pyocin R) was the first pyocin to be purified and characterized (4). It has a complex structure composed of a core, sheath, base plate, and fibers (5). The particle weight was estimated to be about 10^7 (4), and subunit analyses revealed that one particle consisted of more than 400 molecules of about 20 different protein species (6, 7). The immunological cross reaction was found among pyocin R1 and R2 or R3 (1).

For the genetic analysis of pyocin R1 factor, the transfer of this factor from the original strain P15 to PAO strains was attempted, since PAO strains are genetically the best analysed *Pseudomonas* (8). We found that pyocin R1 factor can be transferred from P15 to PAO by F116-mediated transduction, although at a very low frequency. The behavior of R1 factor thus transferred was studied in PAO strains comparing to that of R2 or R3 factor.

MATERIALS AND METHODS

Strains. *Pseudomonas aeruginosa* strains used in this study are listed in Table 1. Strains M182 and M197 were obtained from M12 by repeated treatment with N-methyl-N'-nitro-N-nitrosoguanidine. Thus the pyocin-less character of strain M197 is independent of those of M125 or M127, which are derived from M47 (2). Although M197 or M47 shows no pyocin R2 activity, Mitomycin-C induced lysate of either culture displays a partial activity in neutralizing anti-pyocin R2 serum. Indicators used are also shown in Table 1. It was very easy and clear to distinguish pyocin R1 from R2 or R3 by this set of indicators. The phages F116 (M15) or F116 (M16) were prepared from the confluent lysis plates employing P15 or M16 (3). In other cases, cultures of F116 lysogens (M145, M146, or M261) were induced with Mitomycin-C and high titer phage lysates thus obtained were used. These preparations are referred to as F116 (M145), F116 (M146), or F116 (M261), respectively. Transduction experiments were carried out as previously described (3). Media used are the same as those of the previous work (1).

Table 1. Strains used.

Strain	Character	Origin (reference)
M16	PF2 ⁺ , <i>leu</i> -1, <i>str</i> ^r , pyocin R2 ⁺	PAO (1)
M2004	FP2 ⁺ , prototroph, <i>fpa</i> , pyocin R3 ⁺	PAT (9)
P15	prototroph, pyocin R1 ⁺	(4)
M125	FP ⁺ , <i>trp</i> -1, <i>pro</i> -3, <i>str</i> , pyocin ⁻	PAO (3)
M127	FP ⁺ , <i>trp</i> -1, <i>lys</i> -2, <i>str</i> , pyocin ⁻	PAO (3)
M145	FP ⁺ , <i>lys</i> -2, <i>str</i> , pyocin R3 ⁺ , F116 ¹	PAO (9)
M146	FP ⁺ , <i>lys</i> -2, <i>str</i> , pyocin R2 ⁺ , F116 ¹	PAO (9)
M182	FP ⁻ , <i>trp</i> -1, <i>his</i> -8, <i>ilv</i> -9, pyocin R2 ⁺	PAO, this paper
M197	FP ⁻ , <i>trp</i> -1, <i>his</i> -8, <i>ilv</i> -9, <i>met</i> -18, pyocin ⁻	PAO, this paper
M261	FP ⁺ , <i>lys</i> -2, <i>str</i> , pyocin R1 ⁺ , F116 ¹	This paper
Indicators		
P14	prototroph, pyocin R1 ^s , R2 ^s , R3 ^s	(10)
P14 b	prototroph, pyocin R1 ^s , R2 ^s , R3 ^r	(10)
P14 d	prototroph, pyocin R1 ^s , R2 ^r , R3 ^r	(10)
P14 f	prototroph, pyocin R1 ^r , R2 ^s , R3 ^s	(10)

FP: *Pseudomonas* fertility factor, *fpa*: *p*-fluorophenylalanine resistance. *str*: streptomycin resistance.

RESULTS

Plating efficiency of F116 preparations on various Pseudomonas strains

When F116 prepared on PAO was tested on PAO and P15, the number of plaques formed was not the same on these two indicators. Actual values fluctuated from one experiment to another, but the efficiency of plating of

F116 (PAO) on P15 was usually below 10%. On the other hand, F116 propagated on any of these strains gave the same number of plaques on PAO (Table 2). Therefore, an F116 plaque was isolated, purified, and propagated on P15 and used for transduction experiments.

Table 2. Plating efficiency of F116*.

	Indicator PAO	PAT	P15
Phage			
F116 (PAO)	1	0.1-0.2	0.03-0.1
F116 (PAT)	1	1	0.03-0.1
F116 (P15)	1	0.2	1

* Plated at 30° overnight on nutrient agar.

Transduction of R1 factor from P15 to PAO

As reported in the preceding paper, the phage F116 was capable of transducing pyocin R3 factor from PAT to PAO strains (9). The same kind of experiment was attempted to see if any marker could be transferred from P15 to PAO. Parallel experiments were performed with F116 (M16). Two pyocin-less PAO strains were employed as recipients, and the results are shown in Table 3. Although there was no difference in the efficiency of plating on these recipients between

Table 3. Transduction of R1 factor to PAO.

Recipient	Phage	m.o.i. ^a	Frequency of transduction	Pyocinogenic transductant
M125	F116 (P15)	5.7	<i>trp-1</i> ⁺ : 5×10^{-8} <i>pro-3</i> ⁺ : $< 2 \times 10^{-8}$	0/3 —
	F116 (M16)	5.7	<i>trp-1</i> ⁺ : 1.5×10^{-6} <i>pro-3</i> ⁺ : 1×10^{-6}	9/30 0/30
M127	F116 (P15)	4.9	<i>trp-1</i> ⁺ : 2.5×10^{-8} <i>lys-2</i> ⁺ : 4×10^{-7}	5/7 0/30
	F116 (M16)	4.9	<i>trp-1</i> ⁺ : 1.3×10^{-6} <i>lys-2</i> ⁺ : 1.3×10^{-6}	13/30 0/30

^a m.o.i. = multiplicity of infection.

F116 (P15) and F116 (M16), a considerable disparity was found in the efficiency of transduction. As the transduction among P15 sublines occurred at the similar level of frequency with the F116(P15) preparation (10^{-6} to 10^{-7} , experiments not shown), the low level of transduction in these cases could be reflective of the difference in DNA specificity or homology. The pyocinogenic character was examined with these transductants. In agreement with the previous observation, a high level of cotransduction of pyocin factor was found in *trp-1*⁺

recombinants with F116(M16). In the case of F116(P15), the number of transductants obtained was very small, but pyocin-positive colonies were found among *trp-1*⁺ transductants at a high level of linkage. In these experiments, pyocins produced by F116 (P15) transductants were all of R1 nature and those by F116 (M16) were of R2.

Transduction of pyocinogenic factors among PAO strains

While the efficiency of interstrain transduction was very low, the R1 factor once transferred into PAO was transducible at much higher rates between PAO strains. Therefore the behavior of R1 factor transferred into PAO was studied in comparison with other pyocin factors. An R1⁺ colony (M261) was selected among the *trp-1*⁺ transductants obtained from the experiment shown in Table 3. The culture of M261 was treated with Mitomycin-C to induce F116 production. The high titer phage lysate thus obtained was used for the next experiment. The same kind of phage lysates were prepared with M145 and M146 which are derived from M127 by F116 (M2004) or F116 (M16) transduction. The characters of these 3 strains are essentially the same except for pyocin factors; M145 carries R3, M146 R2, and M261 R1 (See Table 1). Transduction experiments were performed with these 3 phage preparations employing M127 as a recipient and the results are summarized in Table 4. Since both the recipient and donors have

Table 4. Transduction of pyocinogenic factors.

Recipient	Phage	m.o.i.	Transduction frequency	Pyocin ⁺ /transductant	Pyocin type
M127	F116 (M146)	7.2	<i>trp-1</i> ⁺ : 2 × 10 ⁻⁷	16/60	R2
M127	F116 (M145)	9.4	<i>trp-1</i> ⁺ : 4 × 10 ⁻⁷	24/120	R3
M127	F116 (M261)	14	<i>trp-1</i> ⁺ : 6 × 10 ⁻⁷	49/90	R1
M197	F116 (M261)	5.2	<i>his-8</i> ⁺ : 1 × 10 ⁻⁷	0/30	—
			<i>ilv-9</i> ⁺ : 7 × 10 ⁻⁷	0/60	—
			<i>met-18</i> ⁺ : 10 ⁻⁸	0/3	—
			<i>trp-1</i> ⁺ : 7 × 10 ⁻⁷	12/60	R1
			(str ^r 18/60)		

the same *lys-2* marker, no *lys*⁺ recombinant was obtained. Transductants of *trp-1*⁺ were obtained at essentially the same level of frequency with these phages. (The frequency was calculated per recipient bacterium.) The pyocinogenic character of transductants was analysed and is listed in Table 4. In this series of experiment, the nature of pyocins transduced was the same as that of the donors. The linkage values of pyocin factors to *trp-1* were all high in these cases. In particular that of pyocin R1 was very high. More than half of the *trp-1*⁺ transductants were pyocin R1 positive.

A result of another kind of transduction experiment is also given in Table 4. M197, a strain showing a multiple amino acid requirement, was used as a recipient,

and the transduction of each marker as well as its linkage to pyocin factor was studied with F116 (M261). As expected from the previous observation, no linkage of pyocin R1 to *his-8*, *ilv-9*, or *met-18*, except *trp-1*, was found. About 20% of *trp-1*⁺ were pyocin R1⁺ in this case. The streptomycin-resistant marker was also transferred linked to *trp-1*. There was one transductant which acquired pyocin R1, *trp-1*, and *str*^r all together.

Transduction of pyocin R1 factor into a pyocin R2⁺ strain

Experimental results described above indicate that pyocin R1 factor may probably be incorporated into the bacterial chromosome close to *trp-1*, like R2 or R3 factor. Therefore, an experiment was designed to see the relation of two factors, R1 and R2, upon transduction. We can distinguish four possible combinations of these two factors, R1⁻·R2⁻, R1⁺·R2⁻, R1⁻·R2⁺, and R1⁺·R2⁺, by our set of indicators. R1 kills P14d but not P14f and R2 kills P14f but not P14d, while R1⁺·R2⁺ should kill both P14d and P14f as long as the doubly pyocinogenic cell produces both pyocins. The experiment was carried out with M182, an R2⁺, *his*, *ilv*, *trp* strain, as a recipient. As indicated in Table 5, transductants were obtained with any of the 3 markers. The pyocin character of all the *his*⁺ and *ilv*⁺ transductants was of pyocin R2, the recipient type. On the other hand, one-half of *trp-1*⁺ transductants showed the activity of pyocin R1. No pyocin-less nor doubly pyocinogenic colonies were obtained. A considerable number of *str*^r were also obtained among *trp-1*⁺ transductants, but they were all of R2 type. These results suggest that both R1 and R2 factors are located at a similar or overlapping position on one side close to *trp-1* and the *str* probably on the other side across *trp-1*.

Table 5. Transduction of pyocin R1 factor into pyocin R2⁺ strain.

Recipient	Phage	m.o.i.	Frequency of transduction
M182	F116 (M261)	3.5	<i>his-8</i> ⁺ : 2.5×10^{-7} <i>ilv-9</i> ⁺ : 1×10^{-6} <i>trp-1</i> ⁺ : 4×10^{-7}
Distribution of unselected markers:			
	Pyocin R1 ⁺	Pyocin R2 ⁺	<i>str</i> ^r
<i>his</i> ⁺	0/60	60/60	0/60
<i>ilv</i> ⁺	0/60	60/60	0/60
<i>trp</i> ⁺	30/60	30/60	14/60

DISCUSSION

Pyocin R1 has been most extensively studied among R-type pyocins from the standpoints of chemical character (4), morphology (5), action mechanism (11, 12), subunit structure (6, 7), biosynthesis, and morphogenesis (7, 13). However,

its genetic study has not been made. A fertility factor (FP2) was found by HOLLOWAY in certain strains of *Pseudomonas aeruginosa* and genetic analyses of this bacterium have been carried out by his group and others (8). As we found that their strains (PAO and PAT) also produced the same kind of pyocins (R2 and R3) (1), we analysed the genetic character of these pyocinogenic factors and found that both were incorporated into the bacterial chromosome (2, 3, 9). For comparative study of R-type pyocins, we attempted to transfer pyocin R1, the best analysed pyocin, into PAO strains. As described in this paper, pyocin R1 was transferred by F116-mediated transduction linked to *trp-1* marker. This result suggests that pyocin R1 factor is also incorporated into the chromosome of P15 strain close to *trp-1*. The frequency of this transduction was very low, but the linkage of pyocin R1 to *trp-1* was very high. The locus of transferred pyocin R1 in PAO was also confirmed by transduction with F116. Parallel experiments carried out with the corresponding R2 or R3 donors showed that all three pyocins were linked to *trp-1*. The extent of this linkage to *trp-1* was apparently highest with R1 over R2 or R3 (Table 4). This fact might signify that pyocin R1 factor occupies a position much closer to *trp-1* than R2 or R3 factor. Therefore, an experiment was carried out to transfer R1 factor into an R2-positive recipient under the supposition that if two loci were apart and different, doubly pyocinogenic or pyocin-less transductants should emerge. No such transductants were obtained; instead a considerable number of transductants were observed in which the place of pyocin R2 was taken by R1 (Table 5). In the work described in the preceding paper (9), we could not distinguish $R2^+ \cdot R3^+$ colonies from $R2^+ \cdot R3^-$ ones, but in the present case, it was very simple and easy to detect the pyocin character of $R1^+ \cdot R2^+$, $R1^- \cdot R2^+$, and $R1^+ \cdot R2^-$ by the difference of their action spectra. The above result indicates that pyocin R1 and R2 factors are not separate markers but sit at a similar or, most probably, a partially overlapping position in the chromosome of PAO strains. As these pyocins consist of about 20 kinds of subunit and as they show a cross immunological reaction, some of the subunit proteins might be common between these pyocins. In the preceding work, a few $R2^+$ recombinants were obtained from the cross of $R3^+ \times R2$ defectives (9). In the present series of experiment, however, no $R2^+$ was so far obtained when R1 was introduced into R2-defective recipients. We do not know at this time whether this is because of the difference in the composition or arrangement of subunit genes or difference in the finer structure of genes, or something else. For more precise comparison of R-type pyocins from genetical as well as biochemical aspects, we should await further investigation.

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