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Preliminary Report

Specialized Transduction of the *Tryp* Gene by the Temperate Phage ϕ80

The relationship between the prophage and its host bacterial chromosome is a major problem in lysogeny. This problem has been analysed extensively in strain K12 of *Escherichia coli*, a strain widely used for genetic recombination studies. This strain is naturally lysogenized by temperate phage λ and the distinctive features of this K12-λ system, especially of the *Gal*-transducing particles, have already been clarified by Morse, Lederberg & Lederberg (1956a, b)[1,2], Arber (1957)[3], Champbell (1957)[4] and others.

In the present series of papers, it has been reported that the prophage ϕ80 locus is closely linked on the K12 chromosome to the *tryp* marker, which was extensively investigated by Yonofsky and Bonner's group. The transduction by this phage of the *tryp*+ gene to *tryp*− mutants of *E. coli* K12 is described in this preliminary report.

Using various *tryp* mutants, tests were made to see whether the *tryp* gene was transduced by ϕ80. With induced ϕ80 grown on the *tryp*+ strain, the *tryp*+ character was successfully transduced into *tryp*− strains (Fig. 1) (Table 1). With *indole*−, which is mutants of the A protein of tryptophan synthetase (Yanofsky, 1959)[5], transductions were also found (Table 1). Attempts to transduce genes other than those on the *tryp* loci were unsuccessful and the unique quality of the ϕ80 transduction system was established.

Table 1. Transduction-experiment at Various Loci

Marker	Strain	Plate used for the detection of transduction	Transduction
Tryp. T41	T41 (ϕ80)$^+$S^r	M.M.**	+
A11	A11 (ϕ80)$^+$S^r	M.M.	+
A23	A23 (ϕ80)$^+$S^r	M.M.	+
A-mutant	3623(ϕ80)$^+$S^r	M.M.	+
B-mutant	4627(ϕ80)$^+$S^r	M.M.	+
Anth. T16	T16 (ϕ80)$^+$S^r	M.M.	+*
3977	3977(ϕ80)$^+$S^r	M.M.	+*
Cys. -B	C-4 (ϕ80)$^+$S^r	M.M.	−
	C$^-$P$^-$(ϕ80)$^+$S^r	M.M.	−
Proline	C$^-$P$^-$(ϕ80)$^+$S^r	M.M.+Cys.	−
Threonine	Y70 (ϕ80)$^+$S^r	M.M.+L+B_1	−
Leucine	Y70 (ϕ80)$^+$S^r	M.M.+T+B_1	−
B_1	Y70 (ϕ80)$^+$S^r	M.M.+L+T	−
	Hayes Hfr (ϕ80)$^+$S^r	M.M.	−
Methionine	3637(ϕ80)$^+$S^r	M.M.	−
	A$^-$M$^-$	M.M.+Arg.	−
Purine	4801	M.M.	−
Thymine	Thy$^-$	M.M.	−

Gal. Gal_6	4627 $(\phi 80)^+S^r$	EMBGal	—
Gal_4	3104	EMBGal	—
Gal_5	Y70 $(\phi 80)^+S^r$	EMBGal	—
Lac.	4627 $(\phi 80)^+S^r$	EMBLac	—
	1027	EMBLac	—
	Y70 $(\phi 80)^+S^r$	EMBLac	—
Mal.	4627 $(\phi 80)^+S^r$	EMBMal	—
Xyl.	4627 $(\phi 80)^+S^r$	EMBXyl	—
Arab.	4627 $(\phi 80)^+S^r$	EMBArab	—

* very rare ** minimal medium

Table 2. Transduction Rate of Tryp$^-$ Cultures by Lysates of Tryp$^+$

Recipient Tryp$^-$	Donor		Number of Tryp$^+$ transductants		Tryp$^+$ transductants per 10^8 plaque forming centers
	Strain	Plaque forming titer in ml	Control (no lysate)	1.0ml lysate	
T41$(\phi 80)^+$	Tryp$^-$	4.0×10^{10}	12	685	1.7
4627$(\phi 80)^+$	〃	〃	0	361	0.9
3623$(\phi 80)^+$	〃	〃	4	240	0.6

Fig. 1 Transduction of tryp$^+$ marker
upper plate: tryp$^+$ colonies on minimal medium transduced by LFT-ϕ80.
lower left: control of transducing phage alone
lower right: control of recipient tryp$^-$ (T41) bacteria alone

From the data shown in Table 2 the ratio of transducing particles to plaque

forming centers in a lysate may be calculated. In (ϕ80)$^+$ recipient, *tryp*$^+$ transductants occur at a frequency of about one per 10^8. This frequency approaches or is of the same order as that of the λ-*gal* transduction system. This one per 10^7 — 10^8 phage will be referred to as a LFT-ϕ80 (low frequency transducer) corresponding to the LFT-λ. The failure to observe *gal*-marker transduction with lytic lambda has been reported by Morse *et al.* (1956)[1]. This is also true with the ϕ80-*tryp* transduction system.

The *tryp*-positive colonies arising in transduction experiments were routinely subjected to single colony isolation for the establishment of heterogenotic clones. Many transductants F$^-$ *tryp*$^+$, *gal*$^-$ were grown side by side on the master plate replicated on a galactose minimal agar seeded with a F$^-$*tryp*$^-$ *gal*$^+$ culture and were induced by UV-light. The donor and recipients should not both be able to grow on this plate. After 48 hours incubation, as shown in Fig. 2, mass colonies of transductants were formed corresponding to heterogenotic clones which gave HFT (high frequency transducing) lysates. The HFT-producing heterogenote of the transductional clones appeared with as high frequency as 76 out of 349 clones (21.5%), as shown in Table 3. This value is slightly lower than that of the λ-*gal* heterogenote (30%).

The number of transducing particles in a HFF-ϕ80 lysate has been estimated by plating suitably diluents of the lysate with *tryp*$^-$(ϕ80)$^+$ recipient on minimal

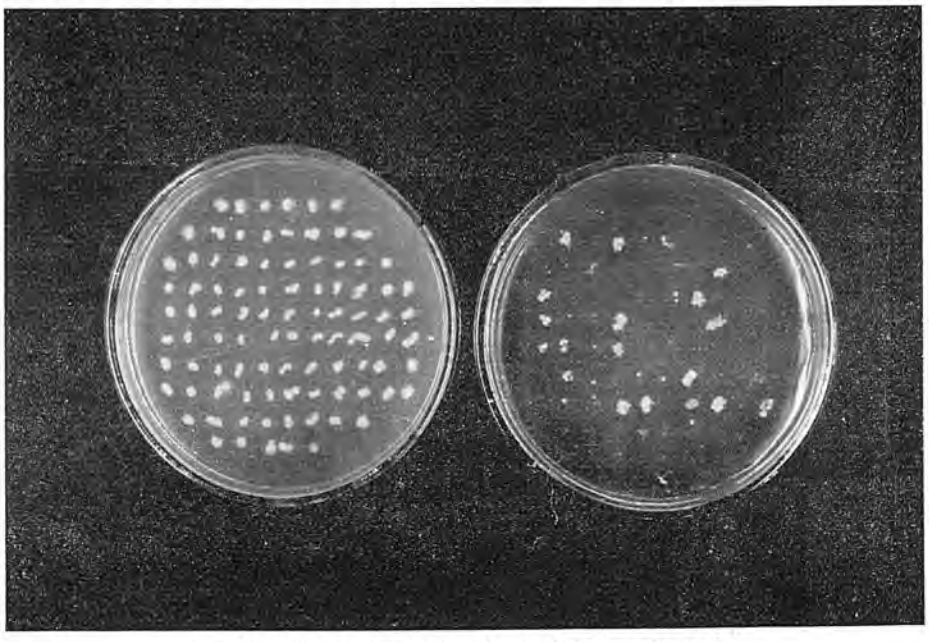

Fig. 2 Replica plating looking for the Heterogenotic strains.
left : master plate of transductants
right : replica plate

Table 3. Frequency of Heterogenote (HFT-producer) for Tryp Marker among the Tronsductional Clones

Recipient cells	Heterogenotes/total examined	% heterogenote
4627 (ϕ80)$^+$ Tryp$^-$, Gal$^-$	39/172	22.7
3623 (ϕ80)$^+$ Tryp$^-$, Gal$^-$	36/177	20.3

Table 4. The Plaque Forming Titer and the Transducing Titer of the High Frequency of Transduction (HFT)-Lysates

Heterogenote No; endogen/exogen	transducing titer per ml	plaque forming titer per ml	transductions per plaque forming unit
4-1 4627/Tryp$^+$	1.46×10^9	2.80×10^{10}	1/19.1
4-2	1.13×10^9	7.80×10^{10}	1/69.0
4-3	0.91×10^9	5.64×10^{10}	1/62.7
5-1	1.94×10^9	4.85×10^{10}	1/25.0
5-2	2.03×10^9	3.90×10^{10}	1/19.5
5-3	3.09×10^9	8.15×10^{10}	1/26.3
5-4	2.26×10^9	5.63×10^{10}	1/25.0
6-1 3623/Tryp$^+$	0.77×10^9	9.84×10^{10}	1/127
6-3	1.22×10^9	7.53×10^{10}	1/61.5
6-4	1.40×10^9	8.00×10^{10}	1/57.1

agar. The results of Table 4 show that HFT lysates contained more than 10^9 transducing particles. There were quantitative variations between lysates.

The HFT-lysate of λ-phage contains two types of particles; ordinary λ and a particle referred to as *λ-gal* or λdg (Arber *et al.*, 1957[3]; Campbell, 1957[4]; Weigle *et al.*, 1959[6]). The nature of the transducing particles in this lysate is now under investigation.

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AIZO MATSUSHIRO

Department of Parasitology,
The Research Institute for Microbial Diseases,
Osaka University, Osaka

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