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SHORT PAPERS

Phage-enhanced suppression of the growth of revertants by auxotrophs in *Salmonella typhimurium*

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1. INTRODUCTION

Ryan & Schneider (1949) have shown that histidine-independent revertants of histidine-requiring *Escherichia coli* are inhibited in the presence of large numbers of auxotrophic cells. Similar results have since been reported for a wide range of mutations in other species (references in Grigg, 1958). This account presents evidence for inhibition of proline-independent revertants of proline-requiring strains of *Salmonella typhimurium*, the degree of which is increased in the presence of homologous transducing phage.

2. MATERIALS AND METHODS

The nomenclature, materials and methods in these experiments were essentially those described by Smith-Keary (1960); MM = minimal medium and EMM = minimal medium enriched with Difco nutrient broth (0·01 %). The glucose concentration was 0·2 % unless otherwise stated. HLMT = supplements of histidine, leucine, methionine and tryptophan, to final concentrations of 0·002 % of the L-amino acid; these were required, in addition to proline, by the strain carrying *pro B401*. The origin of *proB401* is described by Smith-Keary & Dawson (1964); all other mutants were obtained from the collection of the Carnegie Department of Genetics, Cold Spring Harbor, Long Island, New York. *proB25* (Miyake & Demerec, 1960) and *trpA52* (Riyasaty & Dawson, 1967) are multisite mutants.

Homologous transducing phage P22 was added to bacterial suspensions at a multiplicity of infection of 5·0. Phage-free suspensions were diluted with T2 buffer, before plating on medium selective for revertants, so as to contain a similar number of bacteria to those surviving infection in suspensions treated with phage. Colonies were scored after 6 days incubation.

3. RESULTS AND DISCUSSION

During crosses between ten *S. typhimurium proB* mutants, it was observed that only one strain (*proB11*) produced a similar number of proline-independent colonies when plated on selective medium with homologous transducing phage P22 and without phage. One strain (*proB33*) produced conspicuously more colonies, and eight strains produced fewer colonies, when phage was present (Table 1). Since phage had the greatest effect on the multisite mutant *proB25* (Table 2), reconstruction experiments were carried out using this strain. Mixtures were prepared containing *proB25* (4×10^9 cells/ml.) added to dilute suspensions of purified slow-growing revertant cells such that, if all the latter grew on selective medium, a known number of colonies (25 or 50 per plate) would appear in addition to any revertants arising *de novo* from the auxotroph. These mixtures, and also

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Table 1. *Suppression of revertants; colonies on four EMM-HLMT plates in presence and absence of homologous transducing phage;* c. 10^8 *surviving bacteria per plate*

Strain	Phage present	Phage absent
proB3	82	156
proB7	91	141
proB9	45	449
proB11	513	509
proB25	2	133
proB31	86	223
proB33	431	128
proB34	146	266
proB45	47	119
proB401	35	51

Table 2. *Revertants of* proB25 *on four plates of enriched and unenriched selective media in presence and absence of homologous transducing phage;* c. 10^8 *surviving bacteria per plate; two repeat experiments*

Medium	Phage present	Phage absent
EMM-HLMT	5	131
	2	89
MM-HLMT	3	92
	3	77

Table 3. *Suppression of growth of added cells on four plates in presence and absence of homologous transducing phage;* c. 10^8 *surviving bacteria per plate*

	proB25 background on EMM-HLMT		*proB25* background on EMM		*trpA52* background on EMM	
Phage present	+	−	+	−	+	−
No cells added	1	92	9	12	0	0
Cells added*						
proB25-1: 100	3†	35	0	19	50	95
proB25-1: 200	0	55	0	40	119	180
proB25-2: 100	2	14	0	13	71	108
proB25-2: 200	1	71	0	11	97	237
WT: 100	.	.	123	126	124	121
WT: 200	.	.	254	223	217	230

* Stock added, and number of colonies expected on four plates, in addition to those arising from the background *de novo*, if all added cells form colonies. *proB25-1* and *proB25-2* are two slow-growing revertants of *proB25*; *WT* = wild type.

† Number of colonies observed in excess of those scored on control platings (row 1) of suspensions without added cells.

control mixtures with added wild-type cells and without any added cells, were plated with and without homologous transducing phage on selective medium. Further mixtures were prepared in which the non-reverting multisite mutant *trpA52* was substituted for *proB25*. The results (Table 3) show that:

(*a*) 65–95 % of added revertant cells were suppressed in the presence of *proB25* when phage was absent.

(*b*) Almost all added revertant cells were suppressed in the presence of *proB25* when phage was present.

(*c*) Added revertant cells were unsuppressed in the presence of *trpA52* when phage was absent, but about 50 % were suppressed when phage was present.

(*d*) Wild-type cells were unsuppressed.

It was also found that the survival of revertants, when plated alone with phage, was similar to that of auxotrophic cells.

It is concluded that added revertant cells were inhibited when plated with large numbers of auxotrophs, and that the degree of inhibition was increased in the presence of phage. A greater degree of inhibition was noted in the presence of *proB25* than in the presence of *trpA52*. The proportion of revertant cells suppressed did not depend on the number added; thus there is no evidence for mutual suppression of revertant colonies under the conditions of these experiments.

Grigg (1958, 1965) found that suppression of revertants of *Escherichia coli* and *Neurospora crassa* resulted from removal of carbohydrate from the selective medium by the auxotrophic population, rendering it unavailable for the growth of revertants. Table 4 shows that an increase in the glucose concentration of selective medium from 0·2 to 0·5 % allowed an approximately twofold increase in the number of revertants in the present study, both in the presence and in the absence of phage. No further increase could be

Table 4. *Relief of suppression in presence of* proB25*; colonies on four plates with raised glucose concentration in presence and absence of homologous transducing phage*

(*a*) Alteration in glucose concentration of MM; *c.* 10^8 surviving bacteria per plate. Two repeat experiments

Glucose concentration (%)	Phage present	Phage absent
0·2	16	49
	9	14
0·5	20	84
	23	80

(*b*) Suppression of growth of added cells on EMM containing 0·5 % glucose; *c.* $2{\cdot}5 \times 10^7$ surviving bacteria per plate. Compare Table 3

	Phage present	Phage absent
No cells added	16	61
Cells added*		
proB25-1: 200	114†	151
proB25-2: 200	90	198

*, † As in Table 3.

obtained, however, either by raising the glucose concentration higher than 0·5 % or by growth of cells in the presence of an energy source (arabinose) to which the auxotroph was unadapted. While it is possible that suppression was caused by production of some inhibitory substance by auxotrophs in low concentrations of glucose, these data, and also those of Grigg, are most easily explained as the result of competition of revertants and auxotrophs for glucose.

The enrichment of selective medium by nutrient broth increased the number of revertant colonies of *proB25* (Table 2); similar results were noted in ultraviolet induction

experiments by Witkin (1956), who found that the frequency of induced prototrophs in an auxotrophic population was determined by the rate of protein synthesis during the first hour of postirradiation incubation. Although this effect cannot contribute to the suppression of cells added in reconstruction experiments (Tables 3, 4), it is possible that interference with bacterial metabolism results in suppression of some newly revertant cells when phage is present. Experiments designed to elucidate this effect are in progress.

SUMMARY

Salmonella typhimurium proB25 produced less colonies on selective medium in the presence of homologous transducing phage than in its absence. This is the result of suppression of revertants in the presence of a large population of auxotrophic cells, the degree of which is increased when phage is present.

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