AN ANALYSIS OF HISTIDINE REQUIRING MUTANTS IN *PSEUDOMONAS AERUGINOSA*

B. J. MEE AND B. T. O. LEE

Department of Genetics, University of Melbourne, Parkville N.2, Victoria, Australia

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AN analysis of auxotrophic mutants of *Salmonella typhimurium* (DEMEREC 1964) showed that the majority of the genes, which belong to phenotypically related groups, are arranged in clusters of two or more on the bacterial chromosome. It has been shown that the genes controlling the enzymes for histidine biosynthesis in Salmonella (AMES and HARTMAN 1962, 1963) and *Staphylococcus aureus* (KLOOS and PATTEE 1965) are clustered and do not function autonomously but constitute an operon (JACOB and MONOD 1961). In contrast to this, most of the genes controlling histidine biosynthesis in *Neurospora crassa* (AHMED, CASE and GILES 1964; WEBBER and CASE 1960) and *Saccharomyces cerevisiae* (FINK 1964) are widely distributed throughout the genome. However, three of the genes, which specify the enzymes for the second, third and last step of the biosynthetic pathway, are clustered and appear to constitute an operon (WEBBER 1965; FINK 1966). In a survey of 176 auxotrophic mutants of *Pseudomonas aeruginosa* strain 2 (FARGIE and HOLLOWAY 1965), it was reported that genes controlling related steps in a biosynthetic pathway are generally unlinked. Of the 21 histidine mutants examined, three unlinked groups of genes were delineated.

This paper describes the transduction analysis of histidine requiring mutants of *Pseudomonas aeruginosa* strain 1 in an attempt to determine the relationship of the genes controlling the biosynthesis of histidine in this microorganism to that found in the previous investigations. One hundred and seven histidine requiring mutants were obtained and these have been classified into five groups which are probably not linked.

MATERIALS AND METHODS

Media: Nutrient Broth (NB): Oxoid Nutrient Broth Number 2 2.5%, Difco Yeast Extract 0.3%. Nutrient Agar (NA): NB solidified with 1.5% Agar (Davis). Minimal Medium (MM): that of VOGEL and BONNER (1956) solidified where necessary with 1.5% Difco Bacto Agar or Ionagar Number 2 (Oxoid). Layer Agar (LA) for phage assays: Difco Nutrient Broth plus 0.5% Difco Yeast Extract and 1% Difco Bacto Agar. TNM buffer: Tris 0.01M, NaCl 0.15M, $MgSO_4$ 0.01M, pH 7.4.

Bacterial Strains: A list of the mutants employed in this work and the wild-type strain is given in Table 1.

Bacteriophage Strain: F116 (HOLLOWAY, MONK, HODGINS and FARGIE 1962).

General cultural procedures: The procedures were the same as used previously (HOLLOWAY 1955; HOLLOWAY and FARGIE 1960). The transducing phage, F116, was propagated by the layer agar technique. Phage assays and other techniques followed those of ADAMS (1959). The phage propagated on different donor strains was sterilized by filtration with a Millipore filter.

TABLE 1

Bacterial strains used in this study

Strain No.*	Description
1 *chl-r* FP$^-$	prototrophic chloramphenicol resistant (HOLLOWAY 1955)
1 *his-67*	histidine requiring
1 *his-68*	histidine requiring
1 *his-69*	histidine requiring
1 *his-70*	histidine requiring
1 *his-5031*	histidine requiring
1 *his-5034*	histidine requiring
1 *his-5035*	histidine requiring
1 *his-5037*	histidine requiring
1 *his-5039*	histidine requiring
1 *his-5044*	histidine requiring
1 *his-5060*	histidine requiring
1 *his-5065*	histidine requiring
1 *his-5067*	histidine requiring
1 *ade-66 his-3*	adenine requiring histidine requiring double mutant
1 *ade-66 his-23*	adenine requiring histidine requiring double mutant
1 *ade-66 his-26*	adenine requiring histidine requiring double mutant
1 *ade-66 his-28*	adenine requiring histidine requiring double mutant
1 *ade-66 his-30*	adenine requiring histidine requiring double mutant
1 *ade-66 his-35*	adenine requiring histidine requiring double mutant
1 *ade-66 his-42*	adenine requiring histidine requiring double mutant
1 *ade-66 his-47*	adenine requiring histidine requiring double mutant
1 *ade-66 his-49*	adenine requiring histidine requiring double mutant
1 *met-28 his-1*	methionine reguiring histidine requiring double mutant
1 *ser-3 his-3*	serine requiring histidine requiring double mutant
1 *try-4 his-4*	tryptophan requiring histidine requiring double mutant
1 *ade-36 leu-8* FP+	adenine requiring leucine requiring double mutant (HOLLOWAY and FARGIE 1960)
1 *met-5242*	methionine requiring
R101	histidine requiring recombinant
R102	histidine requiring recombinant
R103	histidine requiring recombinant
R104	histidine requiring recombinant
R105	histidine requiring recombinant
R106	histidine requiring recombinant

* This nomenclature follows DEMEREC *et al.* 1966.

Isolation of mutants: Histidine requiring mutants of *Pseudomonas aeruginosa* strain 1, were isolated after treatment with N-methyl N-nitro nitrosoguanidine (MNNG) at a concentration of 20 μg/ml and over a pH range from 5.0 to 6.2. An overnight NB culture was washed and resuspended in a citrate buffer at the appropriate pH; the mutagen was added to give a final concentration of 20 μg/ml, and incubated 2 hr at 37°C. Following this the cells were centrifuged, washed and transferred to NB and grown 3 hr at 37°C. These cultures were then diluted and aliquots of 0.1 ml were plated on MM supplemented with 1.2% NB. Small colonies and those with a diffuse morphology were picked off. These were streaked for single colonies, screened for auxotrophy and then for histidine requirement. In order to minimize the isolation of sibling mutants, the treated cultures were divided prior to expression into several aliquots and only one histidine requiring mutant retained from each aliquot.

Transduction experiments: Preparations of the transducing phage were assayed on the wild-type strain 1C and in all transduction experiments, the phage was used at a titre of 5×10^9 plague forming units/ml. The recipient strains were grown overnight in 40 ml NB shaken in a 150 ml McCartney bottle. Prior to transduction, the fresh overnight cultures were diluted 1:1 with TNM to give an approximate final concentration of 1×10^9 cells/ml. Equal volumes of phage and cells were mixed (multiplicity of infection = 5), incubated at 37°C for 20 minutes, and aliquots were plated onto appropriate selective media. Controls were included to measure the reversion frequency of the recipient cells and to confirm the absence of viable bacteria in the phage preparation. The plates were counted after 2 days incubation at 37°C. The qualitative transduction analysis was performed as follows. Aliquots of 0.1 ml of 1×10^9 cells per ml were spread evenly over the surface of a MM plate. This was allowed to dry and, by means of a loop, a volume of 0.02 ml of phage at 5×10^9 plaque forming units was spread onto areas one inch square. Recombinants appeared in the areas where transduction had occurred, and were counted after 2 days incubation at 37°. By using this method, ten phage preparations, including one which was the wild-type donor, could be tested on the same recipient.

Donor phenotype selection and cotransduction experiments: These were carried out quantitatively as described above. In order to examine the segregation of nonselected markers among the transductants formed, the recombinants were streaked for purity on the same medium on which the cross was performed and then were spot-tested on different supplemented and MM plates.

Mating experiments: The parents were grown separately, overnight at 37°C in a shaken NB. The mating procedure consists of mixing washed saline suspensions of the FP^- and FP^+ parents standardized to 2×10^9 cells/ml, and immediately plating aliquots onto MM with the appropriate amino acid supplement. The plates are counted after 48 hr incubation at 37°C.

RESULTS

Transduction: Preliminary classification of the histidine requiring mutants was carried out using growth response on histidinol and a qualitative transduction analysis. The growth responses though reliable were not entirely consistent with later evidence and require more detailed biochemical work which is at present being undertaken. Qualitative transduction analysis was used to classify over 100 independently isolated histidine auxotrophs all of which could be allotted to one of five groups. A total of 23 of these auxotrophs were chosen for a quantitative transduction analysis. Mutants which had low reversion rates and normal phage absorption were chosen so as to include representatives of each of the five groups. Transducing phage was grown on the selected histidine mutants and the wild-type strain from which these mutants were derived. Transduction was carried out with all the possible combinations of the mutants, using each one both as donor and as recipient. In addition, each mutant was treated with phage from the wild-type strain. A sample of the results of one such experiment is given in Table 2.

In these experiments, several phage preparations were made from the same bacterial strain. Therefore most of the transductions were performed at least twice with different phage preparations. There was a large variation in the efficiency with which prototrophs were produced by the different mutants when so infected. However, the relative efficiency with which a certain mutant recipient produced prototrophs when infected by a variety of donor strains including the wild type was fairly constant. This led to the expression of prototrophic yields as the percentage (CLOWES 1958):

TABLE 2

A sample of the numbers of transductants obtained in crosses between histidine-requiring mutants

Donor strains	Recipient strains								
	1.5034	1.5039	1.5060	1.67	1.5044	1.5065	1.70	1.4.4	1.5035
1C	55	42	23	67	92	40	87	108	126
1.66.23	6	0	60	147	104	23	144	75	261
R105	4	0	43	22	107	63	125	70	276
1.28.1	5	0	82	23	92	115	89	96	191
1.66.28	124	23	1	0	8	75	82	71	137
1.69	138	69	0	4	7	91	138	107	234
R102	83	116	3	2	5	20	112	105	180
1.66.42	69	41	3	0	9	96	62	94	207
1.68	116	104	0	0	3	50	173	112	198
1.67	73	59	1	0	6	40	162	62	14
R103	101	107	0	1	0	42	75	54	68
R101	152	57	60	54	166	0	105	146	112
1.70	155	42	34	133	113	40	0	0	78
R104	103	44	33	16	65	54	2	2	98
1.3.3	52	52	48	26	80	75	0	0	158
1.4.4	42	97	50	43	64	27	2	0	162
1.5035	82	54	85	129	97	69	175	54	0
1.5037	138	35	33	98	160	77	39	80	1

Each entry represents the number of transductants obtained per 5×10^8 bacteriophage on a single plate. Data of this type was used to obtain the normalized percentages shown in Table 3 only if a given recipient produced more than 20 histidine-independent transductants per 5×10^8 wild-type donor bacteriophage. The donor and recipient strains have been grouped according to their proposed linkage arrangement.

$$\frac{\textit{(Number of prototrophic colonies obtained with a phage grown on mutant)}}{\textit{(Number of prototrophic colonies obtained with a phage grown on wild type)}} \times 100$$

In Table 3, the figures represent this value for each bacterial mutant.

A value of approximately 100% would indicate that the two histidine markers are not linked, while low values indicate relative proximity of the locus of the donor and that of the recipient. From Table 3 it can be seen that the 23 selected mutants fall into five transduction groups. Two members of Group II, 1.5044 and 1.66.35 gave significant proto'roph formation when used as donors and recipients in transductions involving other mutants of Group II. However, they gave low values when tested against each other. On examination of accumulated intermediates by chromatography, most members of Group II did not accumulate Pauly positive intermediates while 1.66.35 accumulated imidazole glycerol phosphate (unpublished). The examination of accumulated intermediates in Pseudomonas is complicated by the fact that concentrations are low, possibly because Pseudomonas is capable of inducing enzymes for the metabolism of such compounds.

Many sources of error are inherent in the actual numbers of prototrophs formed in a transduction experiment (Table 2) for example, phage titre, phage transducing ability, multiplicity of infection, plating conditions, physiological state of the plating organisms, mode of incubation, etc. However, the uncertainty of plaque forming units as an assay of the transducing ability of a phage preparation appeared to be the most serious and uncontrollable variable. In order to overcome this problem, the following procedure was adopted.

Doubly marked mutants were selected from each transduction group, and these were mated with the strain 1.ade-36.leu-8.FP$^+$. Recombinants were selected which required only histidine. The mutants chosen and the recombinants obtained are shown in Table 4. These recombinants were used as donors in experiments designed to provide an internal control on the transducing ability of a given phage preparation. Recipients having another amino acid requirement in addition to their histidine marker, were transduced by phage prepared on these recombinants and transductants selected on both MM plus histidine and MM plus the other amino acid. The data obtained from these experiments is shown in Table 5. When selection is made for the other amino acid requirement the ratio of prototroph formation of wild-type donor phage to mutant donor phage can be used as the correction factor for each phage preparation. Using this correction factor, the percentage value, [*(Number of prototrophs obtained with phage grown on mutant)/(Number of prototrophs obtained with phage grown on wild type)*] $\times$ 100 can be amended to account for the different transducing ability of each given phage preparation. All the results obtained from these experiments using doubly auxotrophic recipients have been incorporated in Table 3 as the corrected percentage. It can be seen from Table 5 that the histidine marker is incorporated less frequently than the adenine, methionine or serine markers. Differences in incorporation between various markers has been observed by other workers both in Pseudomonas (FARGIE and HOLLOWAY 1965) and in Salmonella (HARTMAN 1956). In Salmonella histidine markers have been shown to be more frequently incorporated than many others.

Relationship to Pseudomonas aeruginosa *strain 2:* Three groups of histidine requiring mutants called *his 1*, *his 2* and *his 3* were described by FARGIE and HOLLOWAY (1965). These groups were defined as a result of an analysis by transduction and cross-feeding, using 21 histidineless mutants in strain 2 of *Pseudomonas aeruginosa*. Since we obtained five groups of histidineless mutants in strain 1 after analysis by transduction, it was decided to correlate this analysis with that of FARGIE and HOLLOWAY. Using strain 2 as donor, selected mutants were crossed by conjugation and transduction with mutants from each of the strain 1 groups. A qualitative measure of the prototroph formation in these experiments is shown in Table 6.

There are a number of factors which can influence the reliability of these correlations. For example, cross-feeding and intermediate feeding in *Pseudomonas aeruginosa* does not produce the well defined classification characteristic of most other species of microorganisms. Secondly, there is a reduction in the numbers of prototrophs formed in a transduction between the two strains. This

TABLE 3

Transduction analysis for 23 histidine-requiring mutants of Pseudomonas aeruginosa *strain 1*

RECIPIENT STRAINS

STRAINS	1.66.23	1.66.49	1.28.1	1.5034	1.5039	1.5060	1.66.30	1.66.28	1.69	1.66.26	1.66.42	1.68	1.67	1.66.35	1.5044	1.66.3	1.5065	1.70	1.66.47	1.3.3	1.4.4	1.5035	1.5037
1.66.23	3 0	5 2 0	0 0	10	0 0	100	100	100	100 100	43 59 100	100	15 100 100	40 100 100	87	100	100	72 100	100 100	83 60 100	100 67	80 74 81	100 100	100
1.66.49	5	3 0	0	8	0 0	100	100	85 100	100 100	82 100 100	100	100 35	73	100	100	100	100	46 100 79	100	100 100	54 89	60 100	100
1.28.1	1	0 0	0	3	0 0	100 100	83 100 94	100 100 100	100 100 80	100 65 100	80 68 100	100 63	100	96 100	74 64	100 81 100	94 56	100 100 24	46 100	92	75 100	88 66 56	75 81
1.5034	0 0	4 0	2	0	4 0	75 77 100	79 100	88 100	100	100	99	59 70 100	100 55	100	57 100	50	70 49	100 41	83 100	100	100 100	47 86 71	47 80
1.5039	0	0 0	9	0	0 0	72 100	100	100	45	80 100	100	38 100	80 100	100	47	100 100	81 100	100	100	100	68	43 100 100	42 100
1.5060	58 100	62 67 100	100	43	37 79 100	0 0	1	0 1	0	1	0	0 0	0 2	5	0	64 100	100 100	100	88 83 100	100	100	60 100 100	60 100
1.66.30	60 53 100	100 100	89 90	63	54 100 100	0 0	0	0 0	0	0	8 1	7	1	3	3	100 100	85 100	100 100	75 100	45 100	100 100	74 57 100	77 100
1.66.28	54 100 25	100 59 100	61 100	100	100	0	0	0 0	0	2	4 3	4	0	0 0	8 2	100	100	48 100 72	70 45	57 100 100	67 100	88	100
1.69	100	100	100	100	100	0	1 0	5 6 5	0	1 2	0	3	3 6	7	7	28 100	100	100 100	79 80	100 100	100	100 98	100
1.66.26	100	100 100	100 100	100	100	0	0	1 0	11	2 1	1 2	0	0	3 4	7 5	100	63	100 100 100	100	100 100 100	100	100	100 100
1.66.42	63 100	74 100	30 100	100	100	0	4	0 2	0 5 0	1 1	4 0	0	0	1 2	12 5	100 56 100	100	90 69 100	88 100	93 84	100 100	100 100	100 100

DONOR

1.60	97 87 100	100	100 100	100	100	3 0	0 0	0 0	6	0 4	1	0	0 0	3	3	92 100	100	100	100 100	100	100	100	100
1.67	70	52 63	42 100 100	100	100	0	0	0 0	5 3	0	0 1	0	0	1 8	8	30	100	100	92	100	84	64	100
1.66.35	58 100 100	83 100 82	58 100	100	74	0	3 3	2 0 5	14 7	1 2 2	4 1 3	4	5 1	0 0 1	0 0	46	100	100 100 60	100	100 100	72 98 60	28 52 49	100 2
1.5044	100	95 100	100	100	100 100	7 17	4	3	9	2	2	7	0	0	0	100	100	100	100	100	89	72	78
1.66.3	100 84	100 100	100 100	100	100	71	100 86 100	100 100 100	100 63	100 77 100	100	100 69	100 54	52 100	100	0 0 0	0 0	100 100 64	68	100 100 100	100 93	80	100 100
1.5065	100	100	100	100	73 100	89 100	95 85	93 74	100	83 53	100	100	59	100	90 100	4 0	1 0	100 53	100	96	100	83 100	58 100
1.70	100 100	100 100	100 100	100	77 100	100	100	100 100	92 100	100 100	100	100	100	100	100	100	100 100	0 0 0	0	0 3 0	0	100 100	100 100
1.66.47	100 100 52	100 91 100	100 100	100	100	100	100 100	100 100	100 42	100 100	100 100	100	100 100	80 85 100	90 100	100 100 100	100	0 2 6	0	1	3 0	64 80 70	100 73
1.3.3	100 100	57 100 100	100	100	100	100	100 100 82	100 100	100	72 100	59	100 100	100	100	70	60 100	100	1 0 0	2	2 0	0	100	100
1.4.4	100 100	68 100 100	100	100	100	100	100	100 100	100	100 100	100	90 100	100 100	100	56 82	85 100	58 100	0 0	0 0	2	0	57 100 100	100
1.5035	97 100	38 100	96	100	100 100	100	88 100	75 100	100	62 100	100	100 100	100 100	100	100 100	65 100	100 100	100 100	100	100	100 100	0 0 0	0 1
1.5037	100 30	100 100	100	100	31 100	100	100	100	100	100 86	100	100	100 64	100	100 66	100 100	100 43	43 38	100	100	100	0 1	1 0

The normalized frequencies of prototroph formation (see text) are presented as percentages for crosses between histidine-requiring mutants. Each entry represents the result of a single experiment and is derived from data similar to that presented in Tables 2 and 5. The *number of entries* for a cross represents the number of separate experiments performed, each on a single plate. The transduction frequencies for the crosses within each of the five boxes are significantly reduced and this is taken as evidence of linkage between the mutational sites involved.

TABLE 4

Original mutants and their recombinants following conjugation with 1 ade-36 leu-8 *FP+ after selection on histidine plates*

Original double mutant	Requirement	Recombinant
1.66.3	Ade His	R101
1.66.26	Ade His	R102
1.66.35	Ade His	R103
1.66.47	Ade His	R104
1.66.49	Ade His	R105
1.28.1	Met His	R106

reduction, about 100-fold, is due to restriction (ROLFE and HOLLOWAY 1966).

Because of these uncertainties, the histidineless mutants of strain 1 have been allotted group numbers in Roman numerals I, II, III, IV, V. However, the groups probably correspond in both strains.

Cotransduction and donor phenotype selection: In a qualitative survey, a large number of auxotrophs were transduced with phage preparations of two mutants from each linkage group as well as phage prepared on the wild-type strain. Any auxotroph which showed a decrease in the number of transductants formed with both members of a given linkage group (compared to wild type) was then tested quantitatively for cotransduction with that locus.

By this means, a methionine requiring mutant 1.5242, was found to be cotransducible with the histidine markers of group I. In transduction with the histidine mutants of group I so far examined, the methionine marker is cotransduced with a frequency of 75 to 88%. However, this marker shows no cotransduction with any other group of histidine requiring mutants.

Preliminary evidence indicated that the mutants of group I control more than one enzyme. This is based on the fact that some members of the group will grow on histidinol while others accumulate histidinol. Conclusive proof that group I is genetically heterogeneous depends on enzyme determinations which are at present being performed.

Donor phenotype selection (CLOWES 1958) between two histidineless mutants can be carried out when the donor cells can utilize an intermediate, histidinol, which does not support the growth of the recipient cells. Recombinants from the simultaneous transduction of two such markers will have the phenotype of the donor and grow only on MM supplemented with histidinol. Cotransduction of two such markers is expected only when they are closely linked (Figure 1).

Recipients which did not grow on L-histidinol at 30 μg/ml were transduced with a phage prepared on strains which could grow on this compound, and the recombinants formed on MM plus histidinol were examined for the nutritional characteristic of the donor. If the transductants formed are all prototrophic, it can be assumed that the two markers are unlinked. If, on the other hand, a percentage of these transductants will grow on MM plus histidinol but not on MM alone, linkage between the two genes can be assumed. Table 7 shows the percent-

TABLE 5

Number of prototrophs formed following transduction using all of the recombinants as donors and selecting for the corresponding donor allele of each of the requirements of the recipients

	Recipient strains, and contents of plates on which recombinants were selected																					
	1.66.23		1.66.49		1.28.1		1.66.30		1.66.28		1.66.26		1.66.42		1.66.35		1.66.3		1.66.47		1.3.3	
Donor strains	His	Ade	His	Ade	His	Met	His	Ade	His	Ade	His	Ade	His	Ade	His	Ade	His	Ade	His	Ade	His	Ser
1C	115	45	118	59	210	110	135	117	184	124	176	44	168	145	141	187	93	23	84	136	252	75
R105	30	1	64	0	217	1	103	122	78	81	202	88	80	115	50	225	9	5	53	91	217	109
R106	146	0	144	0	300	0	125	133	241	330	203	79	142	146	157	293	275	33	41	62	295	85
R102	205	75	120	66	276	108	205	2	220	1	311	3	114	1	181	6	112	39	70	108	204	109
1.68	98	26	181	82	300	187	108	1	275	0	234	3	159	2	280	7	81	18	83	106	314	111
R103	77	34	111	61	300	105	126	4	261	2	275	2	133	2	201	0	85	7	60	112	96	46
R101	119	38	122	68	300	119	73	75	125	70	141	54	91	61	68	82	24	0	67	107	301	80
1.70	130	63	54	40	300	103	116	108	168	107	67	71	13	49	70	139	15	3	39	0	198	4
R104	55	13	143	62	348	153	197	150	77	56	175	64	112	107	41	95	22	5	78	0	281	1

TABLE 6

Prototroph formation following both conjugation and transduction using strain 2 as donor

	Strain 1									
	I		II		III		IV		V	
Strain 2	C.	T.*	C.	T.	C.	T.	C.	T.	C.	T.
1	—	—	+	+	+	+	+	+	+	+
2	+	+	—	—	+	+	+	+	+	+
3	+	+	+	+	—	—	+	+	+	+

* C.—conjugation; T.—transduction.

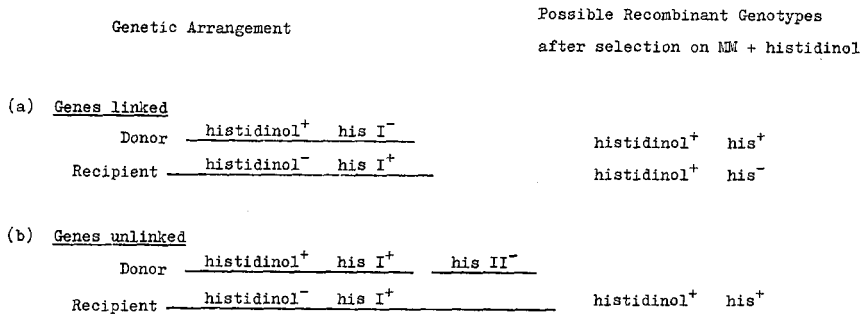

FIGURE 1.—Donor phenotype selection for two histidineless mutants differing in their ability to grow on histidinol.

age cotransduction between three members of Group I which will not grow on MM plus histidinol with members of every proposed group of histidineless mutants. The donor strain selected from group I, 1.5034, does grow on histidinol.

DISCUSSION

Five groups of genes controlling the biosynthesis of histidine in *Pseudomonas aeruginosa* strain 1 have been identified by a quantitative transduction analysis of 23 mutants. In general, the transducing phage, F116, prepared on the wild-type strain 1C was assumed to give the maximum prototroph formation. If this phage, prepared on any mutant, gave a transduction frequency similar to wild type with any particular recipient, it was assumed that the two mutant loci were not linked. If, on the other hand, there was a marked reduction of prototroph formation, cotransduction and linkage of the markers concerned was inferred. It can be seen from Table 3 that a marked reduction in prototroph formation occurred between members of the proposed groups, but prototroph formation in transduction between groups was invariably similar to that obtained with phage prepared on the wild-type strain. It can be concluded that the five groups of genes controlling histidine biosynthesis in *Pseudomonas aeruginosa* are not linked.

One of these groups, Group I was found to be cotransducible with a methionine

TABLE 7

Percentage cotransduction of markers selected on MM plus histidinol in donor phenotype selection experiments

Donor strains	Group No.	Recipient strains		
		1.5067	1.5031	1.66.49
1.5034	I	63	85	95
1.66.30	II	0	0	0
1.66.42	II	0	0	0
1.5065	III	0	0	0
1.4.4	IV	0	0	0
1.5035	V	0	0	0

marker at a high frequency. The other four groups showed no cotransduction with this marker. This is confirmatory evidence that Group I is not linked to the other four groups. However, definite evidence concerning the linkage arrangement of the four remaining groups depends upon locating markers which can be contransduced with each of the proposed groups. This lack of linkage can also be demonstrated by donor phenotype selection, which can be carried out using only those members of Group I that cannot grow on histidinol.

It has been shown (FARGIE and HOLLOWAY 1965; HOLLOWAY, HODGINS and FARGIE 1963) that genes controlling related biosynthetic steps in *Pseudomonas aeruginosa* are not closely linked. In their investigation they examined three groups of genes controlling histidine biosynthesis which were found to conform to this generalization. Three of the five groups described in this analysis have been correlated with the linkage groups of strain 2. The other two groups differ from these, and also appear to be unlinked.

AMES and HARTMAN (1962) have shown that there are ten enzymes concerned in histidine biosynthesis in the organism *Salmonella typhimurium.* At present, it is assumed that Pseudomonas synthesises histidine by the same series of biosynthetic steps. This asumption has been proved correct in a variety of microorganisms, including *Staphylococcus aureus* (KLOOS and PATTEE 1965), *Neurospora crassa* (WEBBER and CASE 1960), and *Saccharomyces cerevisiae* (FINK 1964). In both Salmonella and Staphylococcus, the genes controlling these enzymes are in the form of an operon and all of them map within one region of the chromosome. In Neurospora and Saccharomyces most of the genes for histidine biosynthesis are not linked and can be divided into seven linkage groups. In each of these organisms one of the linkage groups, the *hi-4* locus in yeast and the *hist-3* locus in Neurospora, is complex and forms an operon-type structure, each one consisting of the same three genes.

In Pseudomonas, preliminary evidence suggests that two of the groups of histidine loci are complex. Group II appeared to consist of at least two genes or cistrons. This was based on chromatography of accumulated intermediates and an increased recombination frequency for mutants 1.5044 and 1.66.35 with other members of Group II (Table 3). Group I also seemed to consist of at least two genes, based on chromatography of accumulated intermediates and growth responses on histidinol. Donor phenotype selection gave a value for the cotransduction frequency of the two different genetic loci present in Group I, thereby confirming the presence of at least two genes or cistrons. Thus, it appears that the genes for at least seven of the ten enzymatic steps in histidine biosynthesis for *Pseudomonas aeruginosa* can be distinguished. Hence, it is possible that other linkage groups will be isolated and classified, or that the groups reported here will be differentiated into genes controlling functionally distinct steps on this pathway. At present, it is not possible to investigate any biosynthetic pathways in Pseudomonas by complementation analysis although this would help to clarify the intragenic arrangement.

This study is in agreement with a similar investigation of four gene loci controlling the biosynthesis of isoleucine-valine in *Pseudomonas aeruginosa* (PEARCE

and LOUTIT 1965) where evidence was presented that linkage existed between the functionally related genes.

The conclusion which can be drawn from these results is that clustering of functionally related genes in Pseudomonas does occur, but not to the extent found for *Salmonella typhimurium*. The linkage relationships of the genes for histidine biosynthesis, rather than being clustered into an operon, are similar to that reported for the biosynthesis of arginine and the aromatic amino acids in the Enteric bacteria. As yet, little work has been carried out on regulation of biosynthetic enzymes in Pseudomonas. However, it has been reported (CRAWFORD and GUNSALUS 1966) that the enzyme, tryptophan synthetase, in *Pseudomonas putida* is induced by indoleglycerol phosphate, which has no effect on the synthesis of the enzymes earlier in the pathway. These enzymes appeared to be repressible by tryptophan in the usual manner. It has not been possible to carry out any form of genetic analysis in *Pseudomonas putida*; however, in *Pseudomonas aeruginosa* three groups of tryptophan requiring mutants are not linked (FARGIE and HOLLOWAY 1965). This situation is different from that found for tryptophan biosynthesis in *Salmonella typhimurium* (BLUME and BALBINDER 1966), *E. coli* (YANOFSKY 1960) and *B. subtilis* (ANAGNOSTOPOULOS and CRAWFORD 1961). As it has been shown in this paper that the genes for histidine biosynthesis in *Pseudomonas aeruginosa* are not all linked, it will be of interest to examine the regulatory mechanism in this organism.

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SUMMARY

The genetics of histidine biosynthesis in *Pseudomonas aeruginosa* was investigated in order to critically examine reports that functionally related genes in this organism are not clustered, and to compare this system with those previously reported. In contrast to the system established in the other bacteria Salmonella and Staphylococcus, histidine biosynthesis in Pseudomonas appears to be controlled by five groups of genes which are not linked and, consequently, it bears some similarity to the situation found in Neurospora and Saccharomyces. The distribution of these loci was determined by transduction analysis. Linkage of one of these groups to an unrelated biochemical marker has been shown by cotransduction experiments. This group has been studied by donor phenotype selection and it seems to control more than one biosynthetic step. A second group appears to be genetically heterogeneous, since two members of the group give significant values in transduction experiments with other members of that group. All of the 107 histidine requiring mutants so far examined can be placed in one of these five transduction groups.

LITERATURE CITED

ADAMS, M. H., 1959 *Bacteriophages*. Interscience Publishers, New York.

AHMED, A., M. E. CASE, and N. H. GILES, JR., 1964 The nature of complementation among mutants in the histidine-3 region of *Neurospora crassa*. Brookhaven Symp. Biol. **17**: 53–65.

AMES, B. N., and P. E. HARTMAN, 1962 Genes, enzymes and control mechanisms in histidine biosynthesis. pp. 322–345. Symp. Fundamental Cancer Res., 15th Houston, 1961. —— 1963 The histidine operon. Cold Spring Harbor Symp. Quant. Biol. **28**: 349–356.

ANAGNOSTOPOULOS, C., and I. P. CRAWFORD, 1961 Transformation studies on the linkage of markers in the tryptophan pathway in *Bacillus subtilis*. Proc. Natl. Acad. Sci. U.S. **47**: 378–390.

BLUME, A. J., and E. BALBINDER, 1966 The tryptophan operon of *Salmonella typhimurium*. Fine structure analysis by deletion mapping and abortive transduction. Genetics **53**: 577–592.

CLOWES, R. C., 1958 Investigation of the genetics of cysteineless mutants of *Salmonella typhimurium* by transduction. J. Gen. Microbiol. **18**: 154–172.

CRAWFORD, I. P., and I. C. GUNSALUS, 1966 Inducibility of tryptophan synthetase in *Pseudomonas putida*. Proc. Natl. Acad. Sci. U.S. **56**: 717–724.

DEMEREC, M., 1964 Clustering of functionally related genes in *Salmonella typhimurium*. Proc. Natl. Acad. Sci. U.S. **51**: 1057–1059.

DEMEREC, M., E. A. ADELBERG, A. J. CLARK, and P. E. HARTMAN, 1966 A proposal for a uniform nomenclature in bacterial genetics. Genetics **54**: 61–76.

FARGIE, B., and B. W. HOLLOWAY, 1965 Absence of clustering of functionally related genes in *Pseudomonas aeruginosa*. Genet. Res. **6**: 284–299.

FINK, G. R., 1964 Gene-enzyme relations in histidine biosynthesis in yeast. Science **146**: 525–527. —— 1966 A cluster of genes controlling three enzymes in histidine biosynthesis in *Saccharomyces cerevisiae*. Genetics **53**: 445–459.

HARTMAN, P. E., 1956 Linked loci in the control of consecutive steps in the primary pathway of histidine synthesis in *Salmonella typhimurium*. Carnegie Inst. Washington Publ. **612**: 35–61.

HOLLOWAY, B. W., 1955 Genetic recombination in *Pseudomonas aeruginosa*. J. Gen. Microbiol. **15**: 221–224.

HOLLOWAY, B. W., and B. FARGIE, 1960 Fertility factors and genetic linkage in *Pseudomonas aeruginosa*. J. Bacteriol. **80**: 362–368.

HOLLOWAY, B. W., L. M. HODGINS, and B. FARGIE, 1963 Unlinked loci affecting related biosynthetic steps in *Pseudomonas aeruginosa*. Nature **199**: 926–927.

HOLLOWAY, B. W., M. MONK, L. M. HODGINS, and B. FARGIE, 1962 Effects of radiation on transduction in *Pseudomonas aeruginosa*. Virology **18**: 89–94.

JACOB, F., and J. MONOD, 1961 On the regulation of gene activity. Cold Spring Harbor Symp. Quant. Biol. **26**: 193–211.

KLOOS, W. E., and P. A. PATTEE, 1965 Transduction analysis of the histidine region in *Staphylococcus aureus*. J. Gen. Microbiol. **39**: 195–207.

PEARCE, L. E., and J. S. LOUTIT, 1965 Biochemical and genetic grouping of isoleucine-valine mutants of *Pseudomonas aeruginosa*. J. Bacteriol. **89**: 58–63.

ROLFE, B., and B. W. HOLLOWAY, 1966 Alterations in host specificity of bacterial deoxyribonucleic acid after an increase in growth temperature of *Pseudomonas aeruginosa*. J. Bacteriol. **92**: 43–48.

VOGEL, H. J., and D. M. BONNER, 1956 Acetylorthinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**: 97–106.

WEBBER, B. B., 1965 Genetical and biochemical studies of histidine-requiring mutants of *Neurospora crassa*. III. Correspondence between biochemical characteristics and complementation map position of *his-3 mutants*. Genetics **51**: 263–273.

WEBBER, B. B., and M. E. CASE, 1960 Genetical and biochemical studies of histidine-requiring mutants of *Neurospora crassa*. I. Classification of mutants and characterization of mutant groups. Genetics **45**: 1605–1615.

YANOFSKY, C., 1960 The tryptophan synthetase system. Bacteriol. Rev. **24**: 221–245.