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Genetic Analysis of Lysine Auxotrophs of *Staphylococcus aureus*[1]

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The genetics of lysine biosynthesis in *Staphylococcus aureus* was examined by a transductional analysis of lysine auxotrophs. These mutants had previously been grouped according to their biochemical characteristics. The mutant sites appeared to be closely linked. Complementation was observed between different groups but not between mutant strains belonging to the same group. A strain was detected which seemed to have a mutant control region. Evidence is presented to support the hypothesis that the lysine biosynthetic region functions as an operon.

Genetic analysis of amino acid biosynthetic pathways in *Staphylococcus aureus* has been limited. The systems investigated include those of tryptophan (7), histidine (5), methionine (R. D. Humbert and J. N. Baldwin, Bacteriol. Proc., p. 31, 1963), and isoleucine-valine (8) biosynthesis. The studies associated the mutant subgroups with specific biochemical lesions and were able to show that the loci in each system appeared as a gene cluster. The regulation of these synthetic systems was not examined.

The biochemistry, regulation, and genetics of lysine biosynthesis have been extensively studied in *Escherichia coli*. A recent review (10) cites the work of Bukhari and Taylor in which the genes for diaminopimelic acid (DAP) and lysine synthesis have been mapped. With an overall map length of 90 min, the locus coding for dihydrodipicolinic acid (DHP) reductase and the two sites tentatively responsible for some step in the transformation of tetrahydrodipicolinic acid to succinyl-DAP are located in separable sites along the 0- to 3-min region. The genes governing synthesis of DHP synthetase and succinyl-DAP deacylase were located at 47 min, and the site for DAP decarboxylase, at 55 min. It can be readily seen that these loci do not occur in a single gene cluster. These findings may help to explain the rigid multistep regulation of this pathway in *E. coli* (9).

A number of lysine auxotrophs of *S. aureus* have previously been characterized as to the nature of the biochemical lesions (1). Coordinate repression by lysine of both early and late synthetic enzymes was demonstrated to be present. The biochemical evidence suggested that this pathway might function as an operon. Transductional analysis was performed to examine the genetic organization of the pathway. This paper presents the results of the genetic analysis and a probable map of the lysine region.

MATERIALS AND METHODS

Bacterial strains. The parental lysine prototroph was AB-HH69:*lys*$^+$, a strain of *S. aureus* from the Hahnemann Medical College stock culture collection. Lysine auxotrophs were isolated after chemical mutagenesis. The isolation and biochemical characterization of these mutants was described previously (1). The following designations were used to identify the various mutant groups: *lysA* mutants lacked DHP synthetase, *lysB* strains were deficient in DHP reductase, *lysF* mutants were deficient in DAP epimerase, *lysG* strains lacked DAP decarboxylase, and *lysO* was a mutant strain which lacked activity of all of the enzymes tested. Those mutants which were not designated by a letter possessed activity of all enzymes tested but required lysine for growth. These strains were thought to lack one of the enzymes responsible for the transformation of tetrahydrodipicolinic acid to succinyl-DAP.

Transduction procedure. The porcedure of Kasatiya and Baldwin (4) was generally followed. The transduction mixture was diluted 1:10, and 0.1-ml samples were surface-plated on the defined medium of Bondi et al. (2) supplemented with 0.05% phosphate and dextrose broth (8) to obtain the maximal recovery of the wild-type transductants. In some instances, the defined medium also contained 29 μM DAP. The plates were incubated at 35 C for 48 hr prior to counting. The transduction frequency was reported as the average number of wild-type colonies recovered from a 0.1-ml sample of a 1:10 dilution of the transduction suspension. Reciprocal crosses were done with selected mutants.

[1] This work was presented in part at the 69th Annual Meeting of the American Society for Microbiology, Miami Beach, Fla., May 1969.

Staphylococcal phage 53 of the International Typing Series was used as the transducing phage. It was passaged three times on each of the various donor strains prior to use in transduction studies. The high-titer donor phage preparations used in transduction were prepared as described by Patee and Baldwin (6), with Trypticase Soy Broth (BBL) supplemented with 400 μg of $CaCl_2$ per ml as the propagating medium. The phage preparations used in transduction contained a minimum of 10^{10} plaque-forming units per ml. Prior to use in transduction, the phage were ultraviolet-irradiated to give 99% killing.

RESULTS

Representative strains were selected from each biochemical group and subjected to genetic analysis by transduction. The order of the mutant sites and thereby the order of the structural genes controlling lysine biosynthesis was determined by the best-fit test. This refers to the order of mutant sites which best fits all frequencies of recombination in reciprocal transductions between all mutants. The results of this analysis are given in Table 1, which lists the mutants in the linear sequence determined. The order appeared to be *lysO-lysA-lysB-lysF-lysG-lys*. The finding that with any given recipient strain a higher transduction frequency occurred with wild-type donor phage than when any lysine mutant donor phage were utilized indicates that these genes are closely linked and probably are in a cluster. It can be noted that phage propagated on strains 21:*lysF* and 46:*lysB* did not act as efficient donors. This made it difficult to place these sites accurately. When mutant 17:*lysO* was used in crosses as either a donor or a recipient, it tended to map prior to the *lysA* site.

In an attempt to map the *lysF* and *lysB* sites more accurately, transduction experiments were done to see which locus was closer to the terminal *lys* locus. The donor phage were propagated on strains 20:*lysB*, 5:*lysB*, 21:*lysF*, and 41:*lys*, all of which were able to utilize DAP for growth. The post-transduction plating medium was supplemented with DAP in an attempt to recover both donor and wild-type recombinants. The results are shown in Table 2. Although no difference in size or morphology could be seen among the various colonies present, the presence of DAP did increase the number of transduced colonies recovered. The transduction frequencies indicated that the 21:*lysF* locus was located closer to the 41:*lys* region than to the various *lysB* mutants tested. This reconfirmed the previously derived order.

TABLE 1. *Transductional analysis of the linkage relationship of grouped lys auxotrophs*

Recipient	Reversion frequency	Transduction frequency[a] obtained with donor												
		69: *lys*+	17: *lysO*	20: *lysA*	24: *lysA*	12: *lysA*	46: *lysB*	21: *lysF*	31: *lysG*	7: *lysG*	57: *lysG*	22: *lysG*	49: *lys*	1: *lys*
17:*lysO*	0	37	0	—	0	5	19	2	16	28	—	—	6	—
20:*lysA*	11	80	—	0	0	8	1	0	1	9	27	33	24	36
24:*lysA*	0	72	—	0	0	3	0	0	2	8	26	19	—	17
12:*lysA*	1	78	221	111	49	0	13	1	4	26	48	45	48	44
46:*lysB*	2	90	43	15	2	15	1	3	12	18	—	29	2	40
21:*lysF*	6	59	39	53	13	9	0	0	4	0	0	18	0	7
31:*lysG*	2	87	—	11	4	0	0	0	0	0	0	1	4	3
7:*lysG*	7	92	—	70	51	18	1	0	0	0	0	4	34	34
57:*lysG*	0	221	45	75	4	4	2	1	0	1	2	7	17	47
22:*lysG*	0	20	—	29	14	5	8	3	11	3	22	3	24	19
49:*lys*	1	53	21	34	0	3	8	0	0	1	11	1	0	0
1:*lys*	6	114	—	59	39	10	11	1	1	13	24	6	4	3

[a] Average number of prototrophs recovered from a 0.1-ml sample of a 1:10 dilution of the transduction mixture. The transduction frequency was corrected for the spontaneous reversion frequency.

TABLE 2. *Transduction analysis of the lysB and lysF loci*

Recipient	Transduction frequency with donor[a]			
	20:*lysB*	5:*lysB*	21:*lysF*	41:*lys*
29:*lysB*	5	5	10	25
7:*lysG*	2	5	2	25
57:*lysG*	4	0	0	30

[a] Colonies in 0.1 ml of a 1:10 dilution of the transduction mixture plated on DAP-containing medium. Values are corrected for the spontaneous reversion frequency.

DISCUSSION

Transductional analysis has been used to study a number of amino acid biosynthetic pathways in *S. aureus* (5, 7, 8; Humbert and Baldwin, Bacteriol. Proc., p. 31, 1963). These systems have all been shown to be composed of closely linked gene clusters. We have presented evidence obtained by

transductional analysis that the genes governing lysine biosynthesis are located in a gene cluster. The linear gene order, however, did not follow the enzymatic sequence to the pathway. The structural loci occurred in the order: *lysA*, DHP synthetase; *lysB*, DHP reductase; *lysF*, DAP epimerase; *lysG*, DAP decarboxylase; and the *lys* region which was hypothesized to contain the sites for tetrahydrodipicolinic acid acylase, *N*-succinyl-DAP-glutamic acid transaminase, and *N*-succinyl-DAP deacylase. Thus, the order appears to be as follows: the sites governing the first two enzymes in the pathway, the terminal two enzymes, and then the intermediate enzymes. A similar situation was found in the *S. aureus* histidine region where the genes for the first and last enzymes were adjacent (5).

The mutant strain 17:*lysO* had previously been shown to be devoid of detectable lysine biosynthetic activity (1), although it had been obtained after treatment with ethylmethane sulfonate, an agent which induces point mutations. The *lysO* site maps prior to the *lysA* area. Phage propagated on this strain gave rise to wild-type recombinants when any of the other mutant groups acted as recipients. This indicates that this organism did not carry either a deletion for the entire lysine region or mutant structural genes, but rather was a single-site mutation which affected the expression of adjacent areas. This control of adjacent sites makes it very tempting to represent the 17:*lysO* region as a mutant control region. The mutation could be a polar mutation described as the 0° type by Jacob et al. (3), a mutant repressor of the *i* region, or a mutant promotor region. At present we are unable to distinguish among these possibilities.

The biochemical evidence (1) had previously indicated that DHP synthetase, DHP reductase, and DAP decarboxylase were coordinately repressed by lysine in *S. aureus*. In this paper, we have reported the occurrence of the genes governing lysine biosynthesis in a cluster and the identification of a control mutation in the *lysO* strain. The enzymatic data combined with the genetic evidence indicate that lysine biosynthesis in *S. aureus* is governed by the classically defined operon. This is the first amino acid biosynthetic operon to be described in *S. aureus*. It is interesting to note that the genetic organization of the lysine biosynthetic pathway is quite different in *E. coli* (10). The genes are scattered in three general regions of the *E. coli* chromosome. Various steps of the pathway are independently controlled by repression and others are controlled by end-product feedback inhibition (9). The genes coding for the lysine biosynthetic enzymes probably do not function as a classically defined operon in *E. coli* but apparently do in *S. aureus*.

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