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Received May 31, 1962. P.S.E.B.M., 1962, v110.

A Transduction Analysis of Complex Loci Governing the Synthesis of Tryptophan by *Staphylococcus aureus.** (27611)

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Transduction analysis of complex loci involved in the synthesis of tryptophan(1), adenine(2), histidine(3), and cysteine(4), in *Salmonella typhimurium* and tryptophan(5) and arabinose(6) in *E. coli* have been described. In each instance auxotrophic mutants lacking the ability to synthesize a given growth factor were divided into functional groups which correspond to the arrangement of a group of mutant sites on the chromosome. The order of sites on the chromosome was the same as the sequence of biochemical reactions that the sites controlled. Transduction technics make possible the genetic mapping of loci involved in the synthesis of a metabolite by strains of *S. aureus*(7,8,9,10). This report concerns an analysis of the genetic relationship among tryptophan-dependent mutants of *S. aureus*.

Methods. The strain of *S. aureus* employed, W-26, originally obtained from The Ohio State University Hospital, and the mutants derived from it, were maintained on brainheart infusion (BHI) agar slants at 4°C.

The minimal medium (M) contained Casamino acids, acid-hydrolyzed (Difco)—500 mg; l-cystine—10 mg; dextrose—1000 mg; sodium citrate $5H_2O$—500 mg; K_2HPO_4—500 mg; $MgSO_4$—10 mg; NaCl—4 mg; $FeSO_4 \cdot H_2O$—4 mg; $MnCl_2 \cdot 4H_2O$—15 mg; adenine sulfate—1 mg; guanine hydrochloride—1 mg; uracil—1 mg; xanthine—1 mg; thiamine hydrochloride—0.1 mg; nicotinic acid—0.5 mg; and distilled water to make 100 ml. For a solid medium 1.5% washed agar was added, citrate was omitted, and the dextrose was added aseptically after autoclaving. Both broth and solid media had a pH of 6.6 to 6.8 after autoclaving. When desired, 5 mg of tryptophan, 10 mg of anthranilic acid, or 10 mg of indole were added to 100 ml of M medium to make MT, MA, or MI medium, respectively.

Tryptophan-requiring mutants were selected by the penicillin screening method of Davis(11) and Lederberg(12) from a suspension of ultra-violet irradiated *S. aureus* W-26. One ml of the irradiated suspension was inoculated into 5 ml of MT broth and incubated at 37°C for 24 hrs; the culture was centrifuged and resuspended in 5 ml of double distilled deionized water. One ml of this suspension was then added to 4 ml of M broth containing 1.25 units of penicillin per ml.

* Presented in part as partial fulfillment of the degree Doctor of Philosophy at The Ohio State University. Supported by a grant from Nat. Science Foundation.

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After incubation for 30 to 36 hr 0.05 ml of the penicillin-treated culture was streaked onto MT agar plates. Plates showing less than 200 colonies after incubation for 24 hrs at 37°C were replicated to M agar plates. Colonies on the MT master plate which failed to show corresponding colonies on the M agar plates were inoculated onto MT agar. Growth from 40 to 50 colonies could be spotted on a single plate. After incubation for 6 hrs at 37°C these plates were replicated to M agar plates which were incubated at 37°C for 24 hrs. Growth on MT agar not matched by growth on M agar was transferred to BHI agar slants and stored for further study.

Growth responses of the mutants were determined by streaking 0.05 ml of a distilled water suspension of the mutants onto M, MT, MI and MA agar plates. Tests for syntrophism were done on M agar containing 0.5 μg of tryptophan. Reversion to tryptophan independence was determined by streaking 0.1 ml of a MT broth culture onto M agar plates which were incubated at 37°C for 3 days.

The methods of phage propagation and transduction were the same as previously described except that distilled water was used for washing and suspending transduction mixtures(10). Each mutant was exposed to phage 80 which had been propagated on each of the other mutants and on the parent strain. One ml of a phage suspension containing 1.1-1.3 $\times$ 10^{10} plaque forming units and 1 ml of a suspension of *S. aureus* containing 5.1-7.6 $\times$ 10^{10} cells were used in the transduction experiments.

Results. Ten tryptophan-dependent strains of *S. aureus* W-26 were obtained from 3 irradiation experiments. All of the mutants and the parent strain were sensitive to lysis by phages 29, 79 and 80. These strains were given designations similar to those used by Demerec(13) to identify biochemical mutants of *Salmonella typhimurium*. The designation includes the specific growth factor required by the mutant, the order in which it was isolated, and its response to metabolic intermediates. A listing of the mutants and their growth responses is shown in Table I.

TABLE I. Growth Responses of Tryptophan Mutants of *Staphylococcus aureus*.

Mutant	Degree of growth on minimal medium supplemented with: Anthranilic acid	Indol	Tryptophan
tryB-1	—	+	+
tryB-2	—	+	+
tryB-4	—	+	+
tryB-5	—	+	+
tryB-7	—	+	+
tryB-8	—	+	+
tryA-9	—	—	+
tryA-10	—	—	+
tryB-11	—	+	+
tryAB-12	—	—	+

+ = Confluent growth.
— = Faint or no growth.

The group A mutants required tryptophan while strains in group B grew well in the presence of either indole or tryptophan. Mutant, *tryAB-12*, grew only in the presence of tryptophan yet was different from the group A mutants as determined by syntrophism experiments. The group A mutants were capable of stimulating the growth of group B mutants, whereas *tryAB-12* was not. The group A mutants and *tryAB-12* were not stimulated by any other mutants, nor were group B mutants capable of stimulating any members of the B group.

The reversion rates of the different mutants are shown in Table II. The differences in rates are evident when one compares the high rate of *tryA-9*, the intermediate rates of 6 of the group B mutants, the extremely low rate of *tryB-11*, and the absence of reversion of *tryAB-12* and *tryA-10*.

TABLE II. Spontaneous Reversion to Tryptophan-independence by Mutants of *Staphylococcus aureus*.

Mutant	Reversion rate ($\times 10^{-8}$)	Mutant	Reversion rate ($\times 10^{-8}$)
tryB-1	2.1	*tryB-8*	1.2
tryB-2	1.4	*tryA-9*	23.1
tryB-4	1.3	*tryA-10*	0
tryB-5	3.1	*tryB-11*	.17
tryB-7	1.7	*tryAB-12*	0

Marked variation in the sizes of the revertant colonies on M agar was noted. The larger colonies represented true reverse mutation, while the smaller colonies probably reflected mutations of a suppressor gene. A

similar situation was observed in studies of purine-dependent mutants of *S. typhimurium* (14).

The results of reciprocal transductions between different pairs of mutants are shown in Table III. There was marked variation in as donors, a new colony type appeared on the transduction plates. These colonies were differentiated from the yellow, prototrophic colonies by their small size and white pigment. These colonies were donor type recombinants occurring as a result of the linked incorpora-

TABLE III. Frequency of Transduction among 10 Tryptophan-Requiring Mutants of *Staphylococcus aureus*.

	No. of prototrophic colonies from various recipients*									
Donor	*B–1*	*B–8*	*B–11*	*B–5*	*B–2*	*B–4*	*B–7*	*A–10*	*A–9*	*AB–12*
B–1	28	990	177	1320	530	460	492	550	2900	0
B–8	105	52	25	580	1350	1400	1120	900	3000	0
B–11	129	364	1	255	1140	1070	1060	372	4690	0
B–5	201	594	5	13	930	960	980	650	2040	0
B–2	480	1100	158	550	3	6	6	144	376	0
B–4	830	1690	186	1580	9	8	0	237	620	0
B–7	260	1040	140	400	3	5	5	120	256	0
A–10	510	800	71	540	210	200	221	0	236	0
A–9	1110	1430	401	1200	280	230	283	50	101	0
AB–12	10	16	1	22	6	5	3	0	29	0
W–26	3350	6395	4735	6880	3430	3260	2765	1980	8380	1880
Control	23	30	2	11	4	4	8	0	96	0

* Figures represent the number of prototrophic colonies obtained from 0.1 ml samples of the transduction mixture.

the numbers of prototrophic colonies obtained from the different crosses. The greatest number of tryptophan-independent colonies was obtained when the recipient strain was transduced by phage grown on the parent strain, W-26. Mutants *tryB-2, tryB-4* and *tryB-7* did not recombine with each other, behaved similarly in crosses with the other mutants, were transduced at similar rates by 80/W-26 and demonstrated similar rates of reversion. These 3 mutants were isolated from the same tube of MT broth and could have arisen as a consequence of one mutational event, since the irradiated cells were allowed to multiply for 24 hrs before exposure to penicillin.

Two of the mutants, *tryA-10* and *tryAB-12*, appeared to be deletion or multisite mutants since no colonies were detected on the control plates. In addition, both exhibited diminished rates of transduction when W-26 was used as the donor. *TryAB-12* was incapable of acting as a donor strain and could be transduced only by the prototrophic strain, W-26.

When *tryA-9* and *tryA-10* were utilized as recipients with any of the group B mutants tion into the recipient genome of both the mutant and wild type marker of the donor The limited growth of these colonies was due to the accumulation of small amounts of indole from the background growth of the recipient strains on the M agar plates.

Several observations were made which support the hypothesis of linked transduction. Transfers of these isolates were capable of growth on MI agar as well as on MT agar, a characteristic of the group B strains, the original donor strains in these experiments. In addition, rates of reversion of these isolates were similar to the rates of the group B donors, not of *tryA-9* or *tryA-10*. When small colony isolates were used as recipients they could be transduced only by the recipient of the cross in which they were obtained, not by the donor.

Once occurrence of linked transduction of tryptophan markers was established an attempt was made to map the chromosomal region. The number of independent transductions and the total number of transductions was determined. Theoretically, the closer together 2 markers are on the chromosome, the greater the probability of linked transduction

and the isolation of tryptophan-dependent donor type colonies. The greater the distance between the 2 markers, the greater is the chance that they will be separated with subsequent production of tryptophan-independent transductants. The results of experiments involving *tryA-9* as a recipient are seen in Table IV, *tryA-10* as a recipient in Table V.

TABLE IV. Frequency of Independent Transduction of Different Tryptophan Markers into the Recipient Strain, *tryA-9*.

Donor	Prototrophic colonies	Donor type colonies	Ratio of independent to total transduction
tryB-1	625	117	.842
tryB-8	2452	2007	.550
tryB-11	415	400	.509
tryB-5	1980	2168	.475
tryB-2	1190	3706	.243
tryB-7	578	2137	.213
tryB-4	589	2302	.204

TABLE V. Frequency of Independent Transduction of Different Tryptophan Markers into the Recipient Strain, *tryA-10*.

Donor	Prototrophic colonies	Donor type colonies	Ratio of independent to total transduction
tryB-1	379	372	.504
tryB-8	573	1133	.336
tryB-5	450	1352	.250
tryB-11	43	134	.243
tryB-7	197	1963	.091
tryB-4	240	2467	.089
tryB-2	305	3461	.088

Discussion. From the data obtained by reciprocal transductions among the various mutants and by the quantitative observations concerning linked transductions, it was possible to construct a linkage map of the sites represented by each of the mutants. The most logical arrangements of the mutant sites are represented in Fig. 1.

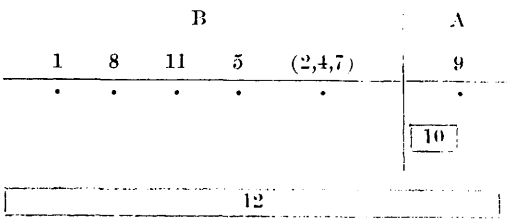

FIG. 1. Order of linkage of mutants sites on the chromosome of *Staphylococcus aureus* as determined by transduction analysis.

This map is divided into 2 functional gene loci within which it is possible for mutation and recombination to take place. The B locus apparently is concerned with production of indole, the A locus with conversion of indole to tryptophan. Mutations in either of the 2 loci are manifested in an alteration of the function in which the particular gene is involved.

TryB-2, tryB-4, and *tryB-7,* which appear to be similar in many respects, are represented as a single point on the map. *TryA-10* and *tryAB-12,* thought to be deletion or multiple site mutants, are represented on the map by solid bars. The map area encompassed by *tryAB-12* involves all the known sites of both the A and B loci, a fact indicated by the lack of recombination of *tryAB-12* with any of the other mutants. Thus, *tryAB-12* would be expected to be deficient in the enzymes necessary to bring about the formation of indole and conversion of indole to tryptophan. The failure of *tryAB-12* to feed the group B mutants is further evidence for involvement of 2 functional loci.

Of the 2 methods used to determine the order of linkage of the mutant sites, the test utilizing the ratio of independent to total transduction events has been reported to be best(3). Using the data from the reciprocal transduction experiments, it was difficult to locate the mutant sites of *tryB-1* and *tryB-8* on the map. However, the results of the ratio test were utilized to obtain what was considered to be the most probable sites of these 2 mutants.

Summary. Experiments involving ultraviolet irradiation of a tryptophan-independent strain of *S. aureus* and the exposure of the irradiated cells to penicillin, yielded 10 tryptophan-dependent mutants. Mutants were differentiated by growth responses, syntrophism, and reversion rates. Ten tryptophan-dependent mutants of *S. aureus* were subjected to a genetic analysis using the transduction technic. This allowed the construction of a chromosome map of 2 different functional linkage groups, closely linked to each other.

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Received June 4, 1962. P.S.E.B.M., 1962, v110.

Comparison of Renal Clearance of Calcium During Infusions of Calcium Chloride and Calcium Gluconate. (27612)

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Calcium infusions are frequently utilized in clinical and physiologic investigations of osseous and parathyroid metabolism. In general, it has not been considered necessary to control the type of calcium salt infused. In earlier work the authors suggested that renal clearance of calcium in man during a calcium infusion could be altered by the associated anion concomitantly administered(1). Subjects receiving calcium gluconate tended to have higher calcium clearances than those receiving calcium chloride. However, the design of that study did not permit a definitive conclusion to be made in this regard. The following investigation was undertaken to determine whether the two commonly used salts of calcium, calcium chloride and calcium gluconate, had comparable effects on renal clearance of calcium.

Methods. The studies were performed in the fasting state on 6 hospitalized males, ages 30 to 49 years, convalescing from acute self limited illnesses. After 3 days on a 1 g calcium, 1.5 g phosphorus diet they each received an infusion of 1 g of calcium ion as either the chloride or gluconate salt over a 4-hour period (8:30 a.m. to 12:30 p.m.). One week later each subject received an infusion of the other calcium salt. Three individuals received the calcium chloride infusion first followed by the calcium gluconate, and in the other 3 the order was reversed. Two successive 30-minute urine collections were obtained prior to the infusion. At 1½ and 3½ hours after initiating the infusion, and one hour after its completion, additional 30-minute collections were obtained. A mild water diuresis (3-5 cc/min) was continually maintained, and the subjects reclined throughout, standing only to void. Blood samples were obtained without stasis at the mid-point of each period, through an intravenous indwelling needle.

Calcium clearance was determined by dividing the quantity of calcium excreted per minute in the urine by the diffusible calcium in the serum. The latter figure was obtained by multiplying the total serum calcium by 0.65. This value represents the mean per cent diffusible calcium as previously determined in this laboratory(1). The "corrected clearance" of calcium was expressed as the ratio of the observed calcium clearance to the