JOURNAL OF BACTERIOLOGY, Nov. 1978, p. 714-722
0021-9193/78/0136-0714$02.00/0

Vol. 136, No. 2
Printed in U.S.A.

Genetic Mapping of Tyramine Oxidase and Arylsulfatase Genes and Their Regulation in Intergeneric Hybrids of Enteric Bacteria

YOSHIKATSU MUROOKA,* TADASHI HIGASHIURA, AND TOKUYA HARADA

The Institute of Scientific and Industrial Research, Osaka University, Yamadakami, Suita-shi, Osaka (565), Japan

Received for publication 11 August 1978

The genes for arylsulfatase (*atsA*) and tyramine oxidase (*tynA*) have been mapped in *Klebsiella aerogenes* by P1 transduction. They are linked to *gdhD* and *trp* in the order *atsA-tynA-gdhD-trp-pyrF*. Complementation analysis using F′ episomes from *Escherichia coli* suggested an analogous location of these genes in *E. coli*, although arylsulfatase activity was not detected in *E. coli*. P1 phage and F′ episomes were used to create intergeneric hybrid strains of enteric bacteria by transfer of the *ats* and *tyn* genes between *K. aerogenes*, *E. coli*, and *Salmonella typhimurium*. Intergeneric transduction of the tyn_K gene from *K. aerogenes* to an *E. coli* restrictionless strain was one to two orders less frequent than that of the leu_K gene. The tyramine oxidases of *E. coli* and *S. typhimurium* in regulatory activity resemble very closely the enzyme of *K. aerogenes*. The ats_E gene from *E. coli* was expressed, and latent arylsulfatase protein was formed in *K. aerogenes* and *S. typhimurium*. The results of tyramine oxidase and arylsulfatase synthesis in intergeneric hybrids of enteric bacteria suggest that the system for regulation of enzyme synthesis is conserved more than the structure or function of enzyme protein during evolution.

Tyramine oxidase synthesis in bacteria is of interest because it is controlled by carbon, nitrogen, and aromatic compounds (12). In particular, the enzyme has been implicated in the regulation of arylsulfatase synthesis (13). Immunological studies and studies on the genetic control of the *ats* and *tyn* genes have been reported (10, 11). Arylsulfate ester is hydrolyzed to an aryl compound and to inorganic sulfate by *atsA*-specified arylsulfatase, which is located in the periplasmic space of the cells (11). Tyramine oxidase is bound to the cell membrane and is specified by *tynA* and induced by specific monoamine compounds such as tyramine and catecholamine, which could be products of the action of arylsulfatase on arylsulfate esters (14). Monoamine compounds are oxidized to hydroxyphenylacetaldehyde compounds and ammonium ions. The effect of ammonium ions on tyramine oxidase synthesis is independent of the function of cyclic AMP and glutamine synthetase (12). Expression of the *tynA* gene results in derepression of arylsulfatase synthesis (11, 13). To clarify the derepression mechanism, useful genetic tools must be introduced from *Escherichia coli* because only limited genetic information can be obtained by analysis with PW52 phage. The present paper describes the mapping of *atsA* and *tynA* on the chromosome of *Klebsiella aerogenes*. This mapping was accomplished by use of F′ episomes from *E. coli* and the transducing phage P1, which were developed by Streicher et al. (17) and Goldberg et al. (6), respectively.

Recently we obtained evidence by an immunological technique of the occurrence of latent arylsulfatase protein regulated by sulfur compounds and tyramine in some enteric bacteria such as *E. coli*, *Salmonella typhimurium*, and *Citrobacter freundii*, although the enzyme activity was not detected in these strains (21). It seemed interesting to determine whether *E. coli* could obtain arylsulfatase activity by transduction and whether the gene for latent arylsulfatase protein of *E. coli* could be introduced into *K. aerogenes* or *S. typhimurium*. The tyramine oxidase gene acts as a positive regulator for derepressing synthesis of arylsulfatase in these enteric bacteria. Therefore, as described in this paper, we examined the conditions necessary for moving these genes from *K. aerogenes* to *E. coli* and from *E. coli* to *K. aerogenes* or *S. typhimurium* by using P1 phage and F′ episome, respectively.

MATERIALS AND METHODS

Bacterial strains and phages. The bacterial strains in this study are listed in Table 1. The *K. aerogenes* strains used were PW52 and/or P1-sensitive

TABLE 1. *List of bacterial strains and their characteristics*

Strain	Relevant characteristics or genotype	Source or reference
K. aerogenes		
W70	Wild type, PW52^s	MacPhee (9)
K13	*tynA13*	This laboratory (1)
K601	*tynA13 atsA1*	This laboratory (1)
K604	*tynA13 atsA4*	This laboratory (10)
KT1	as K601 but F'ats_E tyn_E[a]	F'123 mated with K601
KT2	as K604 but F'ats_E tyn_E	F'123 mated with K604
MK9000	P1^s PW52^s	Bender and Magasanik (17)
MK9672	*asm-200 gdhG1 trp-1*	Bender and Magasanik (5)
MK9678	*asm-200 pyrF1*	Bender and Magasanik (5)
MKN63	*tynA63*	Mutagenesis of MK9000
MKN202	*tynA63 atsA202*	Mutagenesis of MKN63
MKN211	*tynA63 trp-1*	Mutagenesis of MKN63
E. coli		
B	Prototroph	H. Ogawa
C	Prototroph	H. Ogawa
K12	Prototroph	A. Nakata
C600	r_K^-, m_K^-, *thr-1, leu-6, thi-1*	H. Ogawa (3)
CT1	r_K^-, m_K^-, *thr-1, leu-6, thi-1, tyn-1*	Mutagenesis of C600
CTT1	r_K^-, m_K^-, *thr-1, leu-6, thi-1,* tyn_K^{+a}	P1 from MK9000 to CT1
CTT3	r_K^-, m_K^-, *thr-1, leu-6, thi-1,* tyn_K^+	P1 from MK9000 to CT3[b]
CTT5	r_K^-, m_K^-, *thr-1, leu-6, thi-1,* tyn_K^+	P1 from MK9000 to CT5[b]
CTT6	r_K^-, m_K^-, *thr-1, leu-6, thi-1,* tyn_K^+	P1 from MK9000 to CT6[b]
CTT7	r_K^-, m_K^-, *thr-1, leu-6, thi-1,* tyn_K^+	P1 from MK9000 to CT7[b]
CTT8	r_K^-, m_K^-, *thr-1, leu-6, thi-1,* tyn_K^+	P1 from MK9000 to CT8[b]
KLF23/KL181	F'123 (extends from *rac* to *trp*)/*thi-1, pyrD34, his-68, trp-45, recA1, mtl-2, xyl-7, malA1, galK35, strA118*	B. Bachmann (8)
KLF25/KL181	F'125 (extends from *rac* to *pyrD*)/KL181	B. Bachmann (8)
KLF26/KL181	F'126 (extends from *rac* to *nadA*)/KL181	B. Bachmann (8)
W3350	*gal*, T6^r	H. Ogawa
S. typhimurium		
LT2	Prototroph	T. Fukazawa
LT2 (*trpD*)	*trpD10*	T. Fukazawa (15)
LTT1	*trpD10*/F'trp_E tyn_E	F'123 mated with LT2 (*trpD*)

[a] E, *E. coli*; K, *K. aerogenes*.
[b] Strains CT3 to CT8, like CT1, were isolated independently as *tyn* strains by mutagenesis of C600.

derivatives of *K. aerogenes* W70. Strains MK9000, MK9672, and MK9678 were kindly provided by R. A. Bender and B. Magasanik, Massachusetts Institute of Technology. Most of the *E. coli* strains used were derivatives of *E. coli* K-12 and were provided by B. Bachmann, Yale University, but strains C600, B, C, and W3350, were obtained from H. Ogawa, Osaka University, and strain K-12 (prototroph) was obtained from A. Nakata, Osaka University. *S. typhimurium* LT2 and its derivative were obtained from T. Fukazawa, Keio University. Transducing phage P1clr100KM, isolated by Goldberg et al. (6), was obtained from R. A. Bender and B. Magasanik.

Media. The rich media used were LB and LBC (6). LBKM medium was LB supplemented with 25 μg of kanamycin sulfate per ml. The minimal medium used for *K. aerogenes* strains was K medium, consisting of 0.5% carbon source, 0.1% nitrogen source, 0.05 M potassium phosphate buffer (pH 7.2), 0.01% $MgCl_2 \cdot 6H_2O$, and 1 mM sulfur compounds. The media used for the growth of *E. coli* strains were supplemented with 1 mM $CaCl_2 \cdot 2H_2O$ and vitamin B_1 (10 μg/ml). Unless otherwise mentioned, xylose, NH_4Cl, and Na_2SO_4 were used as carbon, nitrogen, and sulfur sources, respectively. Amino acids were added when necessary at a final concentration of 0.1% glutamine and 0.005% other compounds.

Cultivation of bacteria and preparation of phage lysates. *K. aerogenes* strains were grown aerobically in LB or LBC medium at 30°C; *E. coli* strains used as episome donors were grown without shaking in LBC medium at 37°C to a density of 2×10^8 cells/ml. The isolation of strains lysogenic for phage P1clr100KM (called P1) and the preparation of P1 lysates by thermal induction were done as described by Streicher et al. (17).

Transducton. PW 52 phage-mediated transductions have been described (10). P1 transduction was performed as described by Streicher et al. (17). P1 phages were used at a multiplicity of infection of 0.1 to 0.5 for all transductions except that from *K. aerogenes* into *E. coli*, which was done at a multiplicity of 5 to 10. Transductant colonies were purified by single-colony isolation on selective plates before scoring co-transduction of negative markers. The transfer of positive markers from the donor was tested directly with

unpurified transductant colonies. Scoring of the Ats and Tyn phenotypes was done as described previously (10), except that, when the transductants required glutamine, the Tyn phenotype was scored by assay of tyramine oxidase in test tubes. Gdh and Pyr phenotypes were scored as described by Bender et al. (5).

Episome transfer. *E. coli* episomes were transferred to *K. aerogenes* and *S. typhimurium* strains by plate matings as described by Streicher et al. (17).

Isolation of mutants. Induction of mutations with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine was done as described by Adelberg et al. (2). The isolations of tyramine oxidase and arylsulfatase negative mutants were described previously (10).

Enzyme assays. Arylsulfatase activity was assayed as described previously (1). One unit of activity was defined as the amount causing formation of 1 nmol of *p*-nitrophenol per min at 30°C. Tyramine oxidase was assayed as described previously (12), except that cells were collected on Whatman glass fiber paper (GF/F) with an aspirator and washed with 5 ml of 0.05 M potassium phosphate buffer (pH 7.4). The filter was then mixed with 1 ml of the same buffer, and the reaction mixtures were preincubated for 5 min at 30°C before 2 μmol of [^{3}H]tyramine hydrochloride (1 μCi/μmol) was added. One unit of enzyme activity was defined as the amount metabolizing 1 pmol of tyramine per min at 30°C.

Immunodiffusion and immunoelectrophoresis. The Ouchterlony double-diffusion test and immunoelectrophoresis were carried out as described previously (11).

RESULTS

Complementation of *K. aerogenes tynA* with *E. coli* episomes. Previously, we showed by transduction with PW52 phage that the arylsulfatase gene (*atsA*) in *K. aerogenes* is more than 78% cotransducible with the tyramine oxidase gene (*tynA*) (10). There are some reports that the map positions of genes in *E. coli* are similar to those of analogous genes in *K. aerogenes* (5, 17). Complementation of a mutation in *K. aerogenes* by an episome derived from the *E. coli* chromosome ought, therefore, to be a useful method for locating the mutation on the *K. aerogenes* chromosome, although no genetic study on arylsulfatase and tyramine oxidase in *E. coli* has been reported. The levels of tyramine oxidase activity in different strains of *E. coli*, even those derived from *E. coli* K-12, were found to vary considerably (Table 2), possibly due to differences in enzyme structure or in the mechanisms of regulation of the enzyme synthesis. No arylsulfatase activity was detected in any strain of *E. coli* (Table 2). *K. aerogenes* strains carrying various mutations in *tynA* were used as recipients for a variety of *E. coli* episomes representing almost the entire *E. coli* chromosome. Complementation of *tynA* was determined by direct selection of Tyn$^+$ exconjugants, which were able to grow with tyramine as the sole source of nitrogen. The results of these experiments suggested that the *tynA* gene may be located near *gal* and *trp*. We then isolated derivatives of *tynA* mutants carrying additional mutations in *trp*. Table 3 shows the complementation pattern for *E. coli* episomes carrying the chromosomal region corresponding to 15 to 30 min on the Taylor map (8, 18). Three of the episomes complemented *tynA*, although F'125 gave only a few small Tyn$^+$ exconjugant colonies. However, no Ats$^+$ exconjugant was selected using Ats$^-$ Tyn$^-$ mutant strains as recipients. This pattern of complementation suggests that *tynA* is located on the *E. coli* linkage map between 25 and 30 min.

Mapping of *tynA* and *atsA* in *K. aerogenes* by transduction with phage P1. We used phage P1 to determine the linkage of *tynA* or

TABLE 2. *Levels of tyramine oxidase and arylsulfatase in various strains of enteric bacteria*[a]

Strain	Enzyme activity (U/mg of cells)	
	Tyramine oxidase	Arylsulfatase
K. aerogenes MK9000	54.2	39.3
E. coli		
B	<0.1	<0.1
C	<0.1	<0.1
K-12	34.6	<0.1
C600	44.3	<0.1
KLF23/KL181	18.5	<0.1
KLF25/KL181	2.0	<0.1
KLF26/KL181	14.2	<0.1
W3350	2.1	<0.1
S. typhimurium LT2	95.7	<0.1

[a] Cells were grown in xylose (0.5%)-ammonium chloride (0.1%) medium containing tyramine (1 mM), harvested, and assayed when the density of the culture had reached about 100 Klett units.

TABLE 3. *Complementation of K. aerogenes mutations with E. coli episomes*[a]

Episome	*Klebsiella* markers[b]				
	gal	*atsA*	*tynA*	*trp*	*pyrF*
F'123	−	−	+	+	+
F'125	−	−	+	+	ND
F'126	+	−	+	+	ND
F'152	+	−	−	−	−

[a] Exconjugants were purified and scored for complementation of the indicated markers. The episomes complemented the indicated markers in several different recipient strains with allelic mutations.

[b] +, Episome complements the indicated marker; −, no complementation; ND, not done. Arylsulfatase activity was not detected in any donor strains derived from *E. coli* K-12.

atsA to genes located in the chromosomal region corresponding to about 27 min on the Taylor map. The results of a series of transduction experiments are summarized in Table 4. Cross 1 shows that *tynA* was linked approximately 6% with *trp* and approximately 1% with *pyrF*. Additional crosses were carried out to determine the order of the *tynA* gene in relation to *gdhD* and *trp*. When strains MKN202 and MK9672 were used as the donor and recipient, respectively (crosses 2 and 3), the *tynA* mutation was shown to be linked 15% with *gdhD* and 8% with *trp*. The cotransduction frequencies obtained in crosses 1 to 3 are consistent with the mapping order *gdhD-trp-pyrF* previously reported by Bender et al. (5). The data in Table 4 can also be analyzed as a three-factor cross, where the frequency of double crossover events should be much higher than that of quadruple crossover events. Of the three possible orders, cross 2 eliminated *tynA-trp-gdhD* because the Gdh^+ TynA transductants sorted about equally into Trp^+ and Trp^- recombinants. In cross 3, if the order *trp-tynA-gdhD* were correct then most of the Trp^+ Gdh^+ recombinants would have been *tynA*, not $TynA^+$. However, a large percentage (about 70%) of the Trp^+ transductants were Gdh^+ Tyn^+. Although there was high linkage of *gdhD* to *trp* and low linkage of *tynA* to *gdhD*, the data in crosses 2 and 3 were consistent with only the order *tynA-gdhD-trp*.

A similar procedure was used to establish the order of *atsA* with respect to *tynA* and *gdhD*. In this case, strain MKN202 (*atsA2 tynA1*) was also crossed with strain MK9672 (*gdhD typ-1*). Cross 4 eliminated the order *tynA-atsA-gdhD*, since the value of about 10% for the gdh^+ transductants was too high for a quadruple crossover. Crosses 4 and 6 showed that *atsA* was more closely linked to *tynA* than to *gdhD*. Therefore, the order *atsA-gdhD-tynA* could be eliminated. The data strongly suggest the order *atsA-tynA-gdhD*. Cross 5 also supported this order. Thus, on the basis of the cotransduction frequencies and a three-factor analysis, we propose that the order is *atsA-tynA-gdhD-trp*. Although the co-

TABLE 4. *Mapping of tyn and ats*[a]

Cross no.	Donor (relevant genotype)	Recipient (relevent genotype)	Selected phenotype (no. analyzed)	Unselected phenotype	No. of transductants with unselected phenotype	%
1.	MK9678 (*pyrF1*)	MKN211 (*trp-2 tynA2*)	Tyn^+ (200)	Trp^+ Pyr^+	10	5
				Trp^+ Pyr^-	2	1
				Trp^- Pyr^+	188	94
				Trp^- Pyr^-	0	0
2.	MKN202 (*atsA2 tynA1*)	MK9672 (*gdhD1 trp-1*)	Gdh^+ (400)	Trp^+ Tyn^+	235	59
				Trp^+ Tyn^-	26	7
				Trp^- Tyn^+	105	26
				Trp^- Tyn^-	34	9
3.[b]	MKN202 (*atsA2 tynA1*)	MK9672 (*gdhD1 trp-1*)	Trp^+ (112)	Gdh^+ Tyn^+	78	70
				Gdh^+ Tyn^-	9	8
				Gdh^- Tyn^+	24	21
				Gdh^- Tyn^-	0	0
4.	MKN202 (*atsA2 tynA1*)	MK9672 (*gdhD1 trp-1*)	Gdh^+ (380)	Ats^+ Tyn^+	317	83
				Ats^+ Tyn^-	36	9
				Ats^- Tyn^+	3	1
				Ats^- Tyn^-	24	6
5.	MKN202 (*atsA2 tynA1*)	MK9672 (*gdhD1 trp-1*)	Trp^+ (602)	Ats^+ Tyn^+	542	90
				Ats^+ Tyn^-	21	3
				Ats^- Tyn^+	6	1
				Ats^- Tyn^-	33	5
6.	MK9000 (+)	MKN202 (*atsA2 tynA1*)	Tyn^+ (400) (400)[c]	Ats^+	345 331[c]	86 83[c]
7.	MK9000 (+)	MKN211 (*trp-2 tynA2*)	Tyn^+ (718)[c]	Trp^+	57[c]	8[c]

[a] Recipients were transduced with P1 grown on the donors. Tyn^+ selection was done on X-Tyn-Cyt-Trp plates; Gdh^+ selection was done on G-N-Trp plates; Trp^+ selection was done on G-N-Gln plates (X, 0.5% xylose; G, 0.5% glucose; N, 0.1% NH_4Cl; Tyn, 0.1% tyramine; Gln, 0.1% glutamine; Cyt, 0.01% cytidine; Trp, 0.005% tryptophan).

[b] Tyn phenotype of transductants was scored by the assay of tyramine oxidase activity in a test tube because transductants (Gdh^-) required glutamine (0.1%) as a nitrogen source.

[c] Recipients were transduced with PW52 phage grown on the donors.

transduction frequency between *trp* and *tynA* with P1 was much higher than that with PW52 phage (Table 4, cross 7), the frequency between *atsA* and *tynA* with P1 was not much higher than that with PW52 (Table 4, cross 6).

Intergeneric transduction of *leu*, *tynA*, and *atsA*. Although mutations in the *tynA* gene of *K. aerogenes* ($tynA_K$) were complemented by episomes carrying the *tynA* gene of *E. coli* ($tynA_E$), *K. aerogenes* could not act as a male to donate genetic material in conjugation. Therefore, we tried to transfer tyn_K and ats_K to *E. coli* by P1-mediated transduction. Anticipating that the frequency of such intergeneric transduction would be low (6, 19), we used *E. coli* C600, a restrictionless strain, as the recipient. Since we needed to use a recipient strain with a very low reversion frequency for the selected marker, we isolated some $tynA_E$ mutants of *E. coli* C600 which revert to the Tyn^+ phenotype at a frequency of less than 10^{-10} cells. By using one of these strains (CT1) as a recipient for P1-mediated transduction, we obtained Leu^+ transductants at a frequency of less than 10^{-8}, 1×10^{-8}, and 1×10^{-6} infected cells at multiplicities of infection of 0.1, 1, and 5, respectively, with phage grown on Leu^+ *K. aerogenes* (Table 5). All the transductants were Ats^-, Tyn^-, Thr^-, and Thi^-, as was the recipient strain, whereas the donor strains were Tyn^+, Thr^+, and Thi^+. In a similar experiment, P1 lysates were prepared from *E. coli* K-12 and *S. typhimurium* LT2 and used to transduce the *leu* gene to *E. coli* CT1. Table 5 shows that Leu^+ recombinants were obtained at a frequency of about 1×10^{-6} infected cells with phage grown on Leu^+ *E. coli* K-12 at a multiplicity of infection of 0.1 and with phage grown on Leu^+ *S. typhimurium* LT2 at a multiplicity of infection of 5. The optimal multiplicity of infection was 0.1 to 0.5 for transduction between the same strains and 5 to 10 for intergeneric transduction. In the latter case, co-infection of P1 phage might be required. In addition, by using P1 grown on Tyn^+ *K. aerogenes* MK9000, we succeeded in introducing the $tynA_K$ gene into *E. coli*. However, the frequency of transduction was one to two orders less than the leu_K gene transfer from *K. aerogenes* to *E. coli*. Although we have no information on the peptide sequence of tyramine oxidase of *E. coli* and *K. aerogenes*, the low frequency presumably reflects lower DNA homology of the *tynA* region in *K. aerogenes* and *E. coli* than that of the *leu* region in *K. aerogenes*, *E. coli*, and *S. typhimurium*.

TABLE 5. *Effect of DNA homology on the frequencies of intergeneric transduction of leu, tyn, and ats genes*

Donor	MOI[a]	Frequency of transductants per infected cells[b]		
		Leu^+	Tyn^+	Ats^+
E. coli K-12	0.1	$<10^{-8}$	$<10^{-10}$	$<10^{-12}$
S. typhimurium LT2	5.0	2×10^{-6}		
K. aerogenes MK9000	0.1	1×10^{-6}		
	1.0	$<10^{-8}$		
	5.0	1×10^{-6}	1×10^{-6}	
		1×10^{-6}	6×10^{-8}	$<10^{-12}$

[a] MOI, Multiplicity of infection.
[b] *E. coli* CT1 (*tyn*) derived from strain C600 (*ats*, *leu*, *thr*, and *thi*) was used as a recipient strain.
[c] Reversion frequency for the selected marker.

Previously, we found no arylsulfatase activity in *E. coli* K-12, but we detected latent arylsulfatase by an immunological technique (21). However, no active arylsulfatase mutants, formed spontaneously or by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine or UV irradiation, were detected when about 10^{11} *E. coli* K-12 cells were analyzed on plates containing indoxylsulfate as an indicator of arylsulfatase. The homology of the peptide sequence of arylsulfatase protein in *K. aerogenes* and *E. coli* was estimated by Rocha's method (16) as about 70 to 85% from the index of dissimilarity, calculated from an immunological precipitation test (21). For the transduction of ats_K^+, *E. coli* strains CT1, CT3, CT5, CT6, CT7, and CT8, a restrictionless strain and *tynA* strains isolated independently from strain C600, were used as recipients, and *K. aerogenes* strain MK9000 was used as the donor. Transductants were selected on minimal medium plates with xylose and tyramine as the sole sources of carbon and nitrogen, respectively, in the presence of indoxylsulfate. Since the mutation of the *atsA* gene does not show any auxotrophic characteristics, we selected tyn_K^+ in which the cotransduction frequency with the *ats* gene was more than 86% in *K. aerogenes* (Table 4). However, in several experiments using several stocks of phages and recipient strains derived from C600, we could not find any Ats^+ transductant of *E. coli*, even at a multiplicity of infection of 10. Therefore, we conclude that the intergeneric transduction frequency between nonhomologous nucleotide sequences is less than 10^{-11} per infected cell or less than 10^{-12} per plaque-forming unit. The DNA structure of the ats_K and ats_E genes may have diverged somewhat during evolution.

Regulation of tyramine oxidase synthesis in intergeneric recombinants of enteric bacteria. Expression of the tyn_K gene and regulation of tyramine oxidase in *E. coli* were investigated. Donor strain *K. aerogenes* MK9000, recipient strain *E. coli* CT1, and the resultant intergeneric recombinant strains were grown in different media containing tyramine as an inducer or source of nitrogen. Strains CTT1,

CTT3, CTT5, CTT6, CTT7, and CTT8 were derived from *E. coli* strains CT1, CT3, CT5, CT6, CT7, and CT8, respectively. Xylose or glucose was added as a source of carbon, and ammonia was added to the media when growth was not limited by tyramine as a source of nitrogen. The cells were harvested during the exponential phase of growth and assayed for tyramine oxidase. Table 6 shows that the cells grown with xylose as a source of carbon in medium containing ammonia had high levels of the enzyme; cells grown with glucose as a source of carbon in medium containing ammonia had lower levels of the enzyme. Thus, like the enzyme in *K. aerogenes*, the enzyme in *E. coli* C600 and the recombinants containing the tyn_K gene was subject to catabolite repression. The recombinants can be divided into two groups on the basis of the degree of catabolite repression: one group (e.g., strains CTT3, CTT6, and CTT8) showed the same enzyme level as that in *K. aerogenes*; the other group (e.g., CTT1, CTT5, and CTT7) showed the same degree of catabolite repression as that in *E. coli*. The levels of tyramine oxidase in *E. coli* C600 and in recombinants in *E. coli* were much higher and more variable in cells grown on glucose with tyramine as the sole source of nitrogen than in those grown in glucose-ammonium medium. Thus, the regulation system for tyramine oxidase synthesis in *E. coli* is similar to that in *K. aerogenes*. This observation confirms that limitation of nitrogen relieves the catabolite repression of tyramine oxidase (12, 13).

Isolation of intergeneric conjugants and the regulation of tyramine oxidase in these hybrid strains. To transfer the genes of trp_E, tyn_E, and the latent arylsulfatase gene (ats_E) to *S. typhimurium* and *K. aerogenes*, *E. coli* strain KLF23/KL181 containing F'123 was mated with *S. typhimurium* LT2 (*trpD*) and *K. aerogenes* strains K13 (*tynA*), K604 (*tynA atsA*), and MKN211 (*tyn trp*). Trp^+ and/or Tyn^+ exconjugants were selected on appropriate plates. All the conjugants in *S. typhimurium* LT2 had the Trp^+Tyn^+ phenotype but were not Ats^+ since *S. typhimurium* and *E. coli* have no arylsulfatase activity. In *K. aerogenes* strain K13, all the conjugants showed $Tyn^+Trp^+Ats^+$ because this strain has arylsulfatase activity, whereas the conjugants in strain K604 did not have the Ats^+ phenotype. All the conjugants in MKN211 selected with Trp^+ and Tyn^+ phenotypes had the Ats^+ phenotype. The resultant conjugants recovered the original recipient markers when they were grown on nonselective medium (LB). Segregation was observed for all exconjugants and ranged from 5 to 25% varying with the *K. aerogenes* or *S. typhimurium* background.

TABLE 6. *Regulation of tyramine oxidase synthesis in intergeneric recombinants of enteric bacteria*

Organism (relevant genotype)	Tyramine oxidase (U/mg of cells)[a]		
	XNTyn	GNTyn	GTyn(N)[b]
K. aerogenes MK9000 (tyn_K^+)	54 (±10)	18 (±5)	100 (±20)
E. coli			
C600 (tyn_E^+)	44 (± 10)	6 (±5)	127 (±20)
CT1 (*tyn*)	0	0	ND[c]
CTT1 (tyn_K)	36	4	24
CTT3 (tyn_K)	87	21	63
CTT5 (tyn_K)	56	9	56
CTT6 (tyn_K)[d]	104	34	149
CTT7 (tyn_K)	62	5	27
CTT8 (tyn_K)	46	23	143

[a] The cells were grown in the following media: XNTyn, xylose (0.5%), NH_4Cl (0.1%), and tyramine (1 mM); GNTyn, glucose (0.5%), NH_4Cl (0.1%), and tyramine (1 mM); GTyn(N), glucose (0.5%) and tyramine (0.1%) as a sole source of nitrogen. The cells were harvested and assayed when the density of the culture had reached about 100 Klett units. It took about 4 h in XNTyn and GNTyn media (doubling times were about 75 to 90 min), except strain CTT6. Values are averages of three independent experiments. Values in parentheses represent approximate variations in the enzyme levels of the cultures.

[b] 18-h cultured cells (about 70 to 120 Klett units) were assayed because these strains grew slowly with tyramine as a sole source of nitrogen (doubling times were about 280 to 320 min).

[c] ND, Not determined because the strain was not able to grow in GTyn(N) medium.

[d] This strain was cultured for 18 h in XNTyn and GNTyn media and for 48 h in GTyn(N) medium because the strain did not grow well in these media.

Treatment with acridine orange by Hirota's method (7) resulted in loss of episome in more than 95% of the isolated colonies. Thus, we isolated hybrid strains containing the trp_E, tyn_E, and probably ats_E in *S. typhimurium* and *K. aerogenes*.

We next compared the expression and regulation of tyn_E in *S. typhimurium and K. aerogenes* under various culture conditions. Table 7 shows that the cells of hybrid strains in *K. aerogenes* grown with xylose as a carbon source in medium containing ammonia had a high level of tyramine oxidase, that the enzyme synthesis was subject to catabolite repression, and that repression was relieved by growing the cells with tyramine as the sole source of nitrogen (Table 6). The levels of tyramine oxidase in KT2 were much higher than those in KT1 when the cells were grown with tyramine as a nitrogen source, although we do not know the reason at present. However, three other exconjugants isolated from

K604 as a recipient gave enzyme levels similar to that of strain W70 under derepressing conditions. In *S. typhimurium*, tyramine oxidase was regulated by a similar mechanism to that in *K. aerogenes* or *E. coli*. Catabolite repression of the enzyme synthesis in *E. coli* and *S. typhimurium* was stronger than that in *K. aerogenes* (Tables 6 and 7). The degree of catabolite repression of tyn_E in *K. aerogenes* was similar to that in *K. aerogenes*. This shows that catabolite repression of tyramine oxidase in *K. aerogenes* is dominant to that in *E. coli*.

Expression of ats_E in *K. aerogenes* and *S. typhimurium*. These results suggest that the intergeneric exconjugants probably possess the ats_E gene together with trp_E. We examined whether formation of arylsulfatase protein occurs in these strains under derepressing conditions. Although we could not detect any arylsulfatase activity in *E. coli* KLF23/KL181 (F'123) or *S. typhimurium* LT2 (Table 2), we expected to be able to detect immunological cross-reacting protein against arylsulfatase antiserum. Therefore, we used strain KT2, a *K. aerogenes* strain derived from K604 (*tynA atsA*) carrying F'123 that is capable of producing tyramine oxidase but no arylsulfatase activity in xylose-tyramine medium. Double-diffusion reactions of crude extracts from the strains cultured under derepressing conditions against antiserum prepared against the arylsulfatase of *K. aerogenes* W70 were tested. Extracts from cells of *E. coli* KLF23/KL181 (F'123), *K. aerogenes* KT2 (F'tyn_E), and *S. typhimurium* LTT1 (F'trp_E) gave distinct lines which fused completely with each other but fused only partially with the homologous line (given by extracts from *K. aerogenes* W70). However, we could not distinguish the cross-reacting protein of *E. coli* from that of the recipient strains *S. typhimurium* LT2 and *K. aerogenes* K604, although the latter contained one-tenth as much of this cross-reacting protein. Therefore, immunoelectrophoretic differences between the cross-reacting protein of *E. coli* and that of the intergeneric exconjugant strains were examined. Figure 1 shows that the precipitin line from cells of *S. typhimurium* LTT1 was similar in mobility to that from *E. coli* KLF23/KL181 cells but slightly different from that of the parent strain LT2. *K. aerogenes* KT2 gave two precipitin lines: one was similar to that of *E. coli* and the other appeared similar to that of the parent strain K604. Thus, expression of the ats_E gene in *K. aerogenes* and *S. typhimurium* is under the same regulation systems as the expression in *K. aerogenes*. However, we could not find any arylsulfatase activity toward *p*-nitrophenylsulfate, *p*-nitrocatecholsulfate, indoxylsulfate, or ascorbate 2-sulfate (provided by F. Egami, Mitsubishi-Kasei Institute for Life Sciences, Tokyo) in crude extracts from these hybrid strains KT1, KT2, and LTT1. These findings confirm that the *ats* gene is located near *trp* and *tyn* on the chromosome of *E. coli* and that the arylsulfatase protein has lost enzyme function for the usual substrate.

TABLE 7. *Regulation of tyramine oxidase synthesis in intergeneric hybrids of enteric bacteria*

Organism (genotype)	Tyramine oxidase (U/mg of cells)[a]		
	XNTyn	GNTyn	GTyn(N)[b]
K. aerogenes			
W70 (tyn_K^+)	75 (±10)	20 (±5)	110 (±20)
K604 (*tynA*)	0	0	ND[c]
KT1 (tyn_E)	47	25	80
KT2 (tyn_E)	55	28	306
S. typhimurium			
LT2 (tyn_S^+)	96 (±10)	9 (±5)	135 (±20)
LTT1 (tyn_E)	51	2	78

[a] The cells were grown in the following media: XNTyn, xylose (0.5%), NH_4Cl (0.1%), and tyramine (1 mM); GNTyn, glucose (0.5%), NH_4Cl (0.1%), and tyramine (1 mM); GTyn(N), glucose (0.5%) and tyramine (0.1%). The cells were harvested and analyzed when the density of the culture had reached about 100 Klett units. It took about 4 h in XNTyn and GNTyn media. Values are averages of three independent experiments. Values in parentheses represent approximate variations in the enzyme levels of the cultures.

[b] 18-h cultured cells were assayed.

[c] ND, Not determined because the strain is not able to grow in GTyn(N) medium.

DISCUSSION

Recently, the transfers to *K. aerogenes* of chromosomal genes from *E. coli* using F factor and P1 phage have been reported (5, 6, 17). However, there have been no reports about the *tyn* gene in enteric bacteria other than *K. aerogenes*. In this work we showed that the tyramine oxidases of *E. coli* and *S. typhimurium* closely resemble the enzyme from *K. aerogenes* in regulatory activity; this finding made it possible for us to determine the location of the *tyn* gene on the chromosome of *K. aerogenes*. The map position of the *tynA* gene, and probably the *atsA* gene also, is the same in *K. aerogenes* and *E. coli*. P1 transductions in *K. aerogenes* suggest that *tynA* and *atsA* lie near 26 min (according to the revised genetic map of *E. coli* designations). This value was calculated by Wu's equation (20); the numerical value of the length of the transducing fragment was set at 2.0 min for phage P1 as recommended by Bachmann et al. (4). The distance between *trp* and *tynA* was calculated to be 1.14 min (Table 4). In addition, we estimated the genetic map length for PW52 phage as 1.5

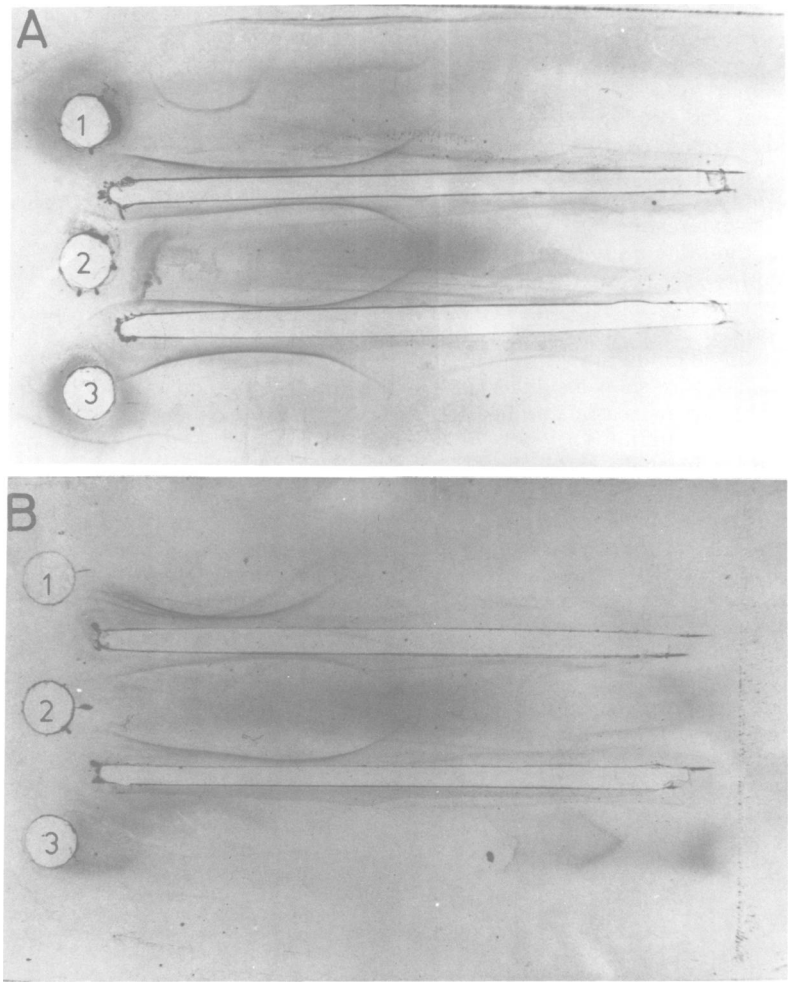

FIG. 1. *Immunoelectrophoresis of cell extracts from E. coli KLF23/KL181, S. typhimurium LT2, K. aerogenes K604, and hybrid strains. Crude cell extracts (about 70 μg of protein) were placed in the left wells. After electrophoresis from left to right, 150 μl of antiserum was placed in the center trough. Precipitin arcs were stained with 0.5% Amido black. Wells contained cell extracts from the following strains. A (1) S. typhimurium LTT1; (2) E. coli KLF23/KL181; and (3) S. typhimurium LT2. B (1) K. aerogenes KT2, (2) E. coli KLF23/KL181, and (3) K. aerogenes K604.*

min from data on the cotransduction frequency between *trp* and *tynA* (Table 4). However, the cotransduction frequency between *atsA* and *tynA* with P1 phage was not very much higher than that with PW52 phage. A similar finding, that a map interval calculated for a long distance is less than the value obtained when the distance is calculated for shorter intervals, has been noted in many other cases (4). Thus, P1 transduction is more useful than PW52 transduction in the genetic assay of a long distance.

Recently Tyler and Goldberg reported the transfer of the *glnA* and *hut* genes from *S. typhimurium* and *K. aerogenes* to *E. coli* and from *E. coli* and *S. typhimurium* to *K. aerogenes* (19). We have been able to transfer the *leu* gene from *S. typhimurium* and *K. aerogenes* to *K. coli* and the *tyn* gene from *K. aerogenes* to *E. coli* by P1-mediated transfer (Table 5). Since the frequency of intergeneric transduction is quite low, we used restrictionless recipients such as *E. coli* strain C600. The experiments reported here also support the notion that the low transduction frequency observed when the donor and recipient organisms are from different bacterial genera is caused by a lack of DNA homology between the selected genes (19). It is, however, somewhat unexpected that arylsulfatase gene ats_K could not be transferred from *K. aerogenes* to *E. coli* C600 and that the transduction fre-

quency was 10^5-fold lower than that of leu_K, although the peptide sequence of the arylsulfatase proteins in *K. aerogenes* and *E. coli* have a homology of about 70 to 85%, estimated by immunological analysis. The low transduction frequency of the tyramine oxidase gene also suggests that the structures of the tyn_K and tyn_E genes have diverged during evolution. This finding is particularly interesting since we previously suggested that the system for regulation of arylsulfatase synthesis is conserved better than the surface structure of enzymatic function of arylsulfatase protein itself in the family *Enterobacteriaceae* (21). The data for tyramine oxidase (Tables 2, 5–7) also show a similar pattern of evolution. This seems reasonable because a bacterial strain may maintain a selective advantage by keeping a mechanism to repress the production of an enzyme when it is not required or to induce (or to derepress) it only when it is needed. Since arylsulfatase and tyramine oxidase are not usually required for growth of the cells, the structural genes for these enzymes may show much more mutation than their control genes during evolution.

In this work, we succeeded in using F′episome to transfer the gene for latent arylsulfatase protein from *E. coli* to *K. aerogenes* and to *S. typhimurium*. The formation of the arylsulfatase protein in intergeneric hybrids also supports the evolution hypothesis. From these experiments we conclude that ats_E is the gene for latent arylsulfatase protein, which has lost the enzymatic function of acting on the usual substrates for arylsulfatase in *K. aerogenes* or other arylsulfatase-positive microorganisms. We do not know whether the latent arylsulfatase protein in *E. coli* and *S. typhimurium* has acquired some other enzymatic activity.

ACKNOWLEDGMENTS

We are grateful to R. A. Bender and B. Magasanik of the Massachusetts Institute of Technology for several mutant strains of *K. aerogenes* and P1clr100KM phage, and also to R. A. Bender for helpful information about the genetics of *K. aerogenes*. We are also indebted to H. Ogawa of Osaka University for valuable advice and critical reading of this manuscript and to A. Nakata for advice on the mating technique.

LITERATURE CITED

1. **Adachi, T., H. Okamura, Y. Murooka, and T. Harada.** 1974. Catabolite repression and derepression of arylsulfatase synthesis in *Klebsiella aerogenes*. J. Bacteriol. **120**:880–885.
2. **Adelberg, E. A., M. Mandel, and G. C. C. Chen.** 1965. Optimal conditions for mutagenesis by N-methyl-N′-nitro-N-nitrosoguanidine in *Escherichia coli* K12. Biochem. Biophys. Res. Commun. **18**:788–795.
3. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36**:525–557.
4. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *E. coli* K-12. Bacteriol. Rev. **40**:116–167.
5. **Bender, R. A., A. Macaluso, and B. Magasanik.** 1976. Glutamate dehydrogenase: genetic mapping and isolation of regulatory mutants of *Klebsiella aerogenes*. J. Bacteriol. **127**:141–148.
6. **Goldberg, R. B., R. A. Bender, and S. L. Streicher.** 1974. Direct selection for P1-sensitive mutants of enteric bacteria. J. Bacteriol. **118**:810–814.
7. **Hirota, Y.** 1960. The effect of acridine dyes on mating type factors in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **46**:57–64.
8. **Low, K. B.** 1973. *Escherichia coli* K-12 F-prime factors, old and new. Bacteriol. Rev. **36**:587–607.
9. **MacPhee, D. G., I. W. Sutherland, and J. F. Wilkinson.** 1969. Transduction in *Klebsiella*. Nature (London) **221**:475–476.
10. **Murooka, Y., T. Adachi, H. Okamura, and T. Harada.** 1977. Genetic control of arylsulfatase synthesis in *Klebsiella aerogenes*. J. Bacteriol. **130**:74–81.
11. **Murooka, Y., T. Yamada, S. Tanabe, and T. Harada.** 1977. Immunological study of the regulation of cellular arylsulfatase synthesis in *Klebsiella aerogenes*. J. Bacteriol. **132**:247–253.
12. **Okamura, H., Y. Murooka, and T. Harada.** 1976. Regulation of tyramine oxidase synthesis in *Klebsiella aerogenes*. J. Bacteriol. **127**:24–31.
13. **Okamura, H., Y. Murooka, and T. Harada.** 1977. Tyramine oxidase and regulation of arylsulfatase synthesis in *Klebsiella aerogenes*. J. Bacteriol. **129**:59–65.
14. **Okamura, H., T. Yamada, Y. Murooka, and T. Harada.** 1976. Purification and properties of arylsulfatase of *Klebsiella aerogenes*: identity of the enzymes formed by non-repressed and de-repressed synthesis. Agric. Biol. Chem. **40**:2071–2076.
15. **Ozeki, H.** 1959. Chromosome fragments participating in transduction in *Salmonella typhimurium*. Genetics **44**:457–470.
16. **Rocha, V., I. P. Crawford, and S. E. Mills.** 1972. Comparative immunological and enzymatic study of the tryptophan synthetase β_2 subunit in the *Enterobacteriaceae*. J. Bacteriol. **111**:163–168.
17. **Streicher, S. L., R. A. Bender, and B. Magasanik.** 1975. Genetic control of glutamine synthetase in *Klebsiella aerogenes*. J. Bacteriol. **121**:320–331.
18. **Taylor, A. L., and C. D. Trotter.** 1972. Linkage map of *Escherichia coli* strain K-12. Bacteriol. Rev. **36**:504–524.
19. **Tyler, B. M., and R. B. Goldberg.** 1976. Transduction of chromosomal genes between enteric bacteria by bacteriophage P1. J. Bacteriol. **125**:1105–1111.
20. **Wu, T. T.** 1966. A model for three-point analysis of random general transduction. Genetics **54**:405–410.
21. **Yamada, T., Y. Murooka, and T. Harada.** 1978. Comparative immunological studies on arylsufatase in bacteria of the family *Enterobacteriaceae*: occurrence of latent arylsulfatase protein regulated by sulfur compounds and tyramine. J. Bacteriol. **133**:536–541.