クエン酸陽性大腸菌由来のクエン酸利用能の性状

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Characterization of Citrate-Utilizing (Cit) Ability of Citrate-Positive *Escherichia coli* Variants: Stability and Transferability of Citrate Utilization among *Escherichia coli, Shigella* and *Salmonella* Strains

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Abstract. Genetical investigation was carried out on the stability or transferability of the citrate-utilizing (Cit) character of 3 citrate-positive *Escherichia coli* strains isolated from a pigeon, a pig and a cow, respectively. The Cit character was less stable in Cit^+ *E. coli* strains at an incubation temperature of 43°C than at 25 or 37°C. Its spontaneous loss increased by successive cultures. A Cit-ability stable clone (A) and an unstable clone (B) were obtained from each of Cit^+ *E. coli* strains KE10 (pig origin) and C53 (cow origin). Each clone was further investigated for the stability or transferability of the Cit character. This character and drug resistance markers were more efficiently cotransferred to the *E. coli* K-12 strain at 25°C than 37°C. Cit^+R^- transconjugants were also obtained from Cit^+R^- donor strain. The Cit character of the Cit^+R^+ *E. coli* strains tested was transferred not only to the *E. coli* K-12 strain, but also to *Shigella sonnei, Shigella flexneri, Salmonella typhi, Salmonella abortusequi* and *Salmonella pullorum* with drug resistance markers at 25°C. The importance of the potential transmission of Cit character among the enterobacterial strains was discussed from a taxonomic point of view.

The citrate reaction on Simmons citrate agar is considered to be a key characteristic for identification of the family *Enterobacteriaceae,* especially coliforms. Citrate-positive variants of *Escherichia coli,* however, were isolated from clinical materials of human [15], domestic-pigeon and mammalian origin [2]. Sato et al. [8] reported that the citrate-utilizing (Cit) ability of citrate-positive *E. coli* strains of domestic animals was plasmid-mediated. Subsequently, Ishiguro and Sato [3] recognized the incidence of citrate-positive variants of *E. coli* in various specimens from human beings, domestic animals, birds and environments.

Smith et al. [12] also reported that 15 thermosensitive H1 plasmids derived from 12 *Salmonella typhi* strains and 3 enterobacterial strains mediated citrate utilization in *E. coli* K-12 strains. The incidence of plasmid-mediated character of citrate utilization is not very important from a taxonomical point of view, but also suggests

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the potential transmission of the Cit character among the members of the family *Enterobacteriaceae*. It is necessary to determine whether citrate utilization plasmid is stable enough to be inherited by *E. coli* strains, or transferable to *Shigella* spp. and *Salmonella* spp. lacking citrate utilization.

This paper deals with stability or transferability of the citrate-utilizing ability of the *E. coli* K-12 strain, transferability of the Cit character from citrate-positive *E. coli* strains to *Shigella* or *Salmonella* strains.

Materials and Methods

Bacterial strains: The citrate-positive *E. coli* strains tested, HT58, KE10 and C53, were isolated from a domestic pigeon, a pig and a cow, respectively [2, 8]. They were epizootiologically unrelated with one another. The HT58 and C53 strains were resistant to tetracycline (Tc), streptomycin (Sm), sulfadimethoxine (Su) and chloramphenicol (Cm). The KE10 strain was resistant to Tc, Sm, Su, Cm and kanamycin (Km) [8]. The three strains carried thermosensitive R plasmids classified into incompatibility group H [14]. Biochemical tests [2] revealed that they had all the properties, except citrate utilization, of *E. coli*. The initial recipient of the Cit character of the three strains was *E. coli* ML1410, the nalidixic acid-resistant K12 strain requiring methionine. The subsequent recipients of the Cit character were *E. coli* K-12 strain ML1410 Rif (F^-, *met*, nal^r, rif^r) and W3630 (F^-, *mal*). Two *S. abortusequi* strains (Manko and Horonobe) were supplied by Dr. K. Hashimoto, of the Hokkaido Branch Laboratory, National Institute of Animal Health. Nalidixic acid-resistant mutant strains, SG20, SG21 and SG22 were obtained from *S. abortusequi* Manko, *S. abortusequi* Horonobe and *S. pullorum* 17, respectively, stored at the authors' laboratory by the method of Smith and Gyles [9]. These *Salmonella* strains were used as recipient strains for transfer experiments on citrate-utilizing ability. Rifampin-resistant mutant strains, *S. typhi* TD, *S. sonnei* JS13389 and *S. flexneri* YSH-1, were also used [13].

Media: Penassay broth (Difco) was used for stability and transfer experiments of the Cit character. The selective medium used for citrate utilization was Simmons citrate agar (Eiken) plates [8], which had been supplemented with methionine (Met, 50 μ/m*l*) and either nalidixic acid (Nal, 50 μg/m*l*) or rifampin (Rif, 50 μg/m*l*) when necessary. Deoxycholate hydrogen sulfide-lactose agar (DHL, Eiken) was also used as selective medium for Tc (25 μg/m*l*), Sm (12.5 μg/m*l*), Cm (25 μg/m*l*) and Km (25 μg/m*l*). When the strains of *Salmonella* spp. or *Shigella* spp. were used as recipient strains in the transfer experiments of citrate utilization, Simmons citrate agar was supplemented with Met (50 μg/m*l*), tryptophan (Try, 50 μg/m*l*), histidine (His, 50 μg/m*l*), cysteine (Cys, 50 μg/m*l*), adenine hydrochloride (10 μg/m*l*), calcium pantothenate (0.2 μg/m*l*), pyridoxine hydrochloride (0.2 μg/m*l*), folic acid (0.001 μg/m*l*), riboflavine (0.2 μg/m*l*), nicotinic acid (0.2 μg/m*l*) and sodium thiosulfate (1 mg/m*l*), if necessary [5, 7]. To examine the drug resistance of a strain or the resistance patterns of transconjugants, heart infusion agar (Eiken) was used as basal medium for the susceptibility testing of Tc (25 μg/m*l*), Cm (25 μg/m*l*), Sm (12.5 μg/m*l*) and Km (25 μg/m*l*), and Mueller-Hinton agar (Eiken) for the same testing of sulfadimethoxine (Su, 800 μg/m*l*) [2].

Genetic stability experiments of Cit character and drug-resistance markers: The stability of Cit character or drug-resistance marker was obtained by the method of Sato et al. [8]. The effect of passage of Cit^+ strains in broth at 25, 37 and 43°C was investigated. Each strain from Cit^+ parent cells and transconjugant cells grown on Simmons citrate agar was inoculated into 10 m*l* of broth and incubated at each temperature with gentle shaking. It was subcultured in the same volume of fresh broth every day. After the first, third and fifth passage, the subcultures were diluted with saline, plated onto DHL agar, and incubated overnight at 37°C. More than 100 colonies grown on the plate were examined for Cit character and susceptibility to drugs by the replica-plating method [8]. No resistance to Su was tested.

Determination of drug resistance and transfer experiments: The methods used to determine the drug resistance and citrate-utilizing ability of the strains were described previously [2]. The methods employed for transfer experiments on citrate-utilizing ability were those described by Sato et al. [8]. Donor culture (0.1 m*l*) and recipient (1.0 m*l*) were added to 10 m*l* of fresh broth. After 6 hours' incubation at 37 or 25°C, 0.1 m*l* of the mixture was inoculated into each selective medium. Transfer frequency was determined from 6 hours' mating at 37 or 25°C and expressed as the number of transconjugants per donor cell. The colonies of transconjugants on each selective medium were purified by successive single-colony isolations on the same selective medium and examined for the Cit charac-

ter and drug resistance patterns by the agar dilution method [2].

Results

Stability of citrate-utilizing (Cit) character and drug resistance marker in *E. coli* strains

Three citrate-positive *E. coli* strains (HT-58, KE10 and C53) were allowed to multiply on alkalized Simmons citrate agar by incubation at 37°C within 2 days. Colonies grown on this agar were visible. Table 1 shows the stability of the Cit ability of 3 Cit$^+$ *E. coli* strains in broth cultures for passage at 25, 37 or 43°C. This ability was

Table 1. Stability of Cit character in parent Cit$^+$ *E. coli* strains (HT58, KE10A, KE10B, C53A and C53B) or in Cit$^+$ transconjugants (ML1410) at various temperatures

Incubation temperature	Subculture*	Percent of loss of Cit character							
		Parent strain						Transconjugant	
		HT58	KE10		C53		HT58-2	KE10	
			KE10A	KE10B	C53A	C53B		KE10A-1096	KE10B-133
25°C	1	16.8	0	0	0	0	23.3	0	2.9
	3	54.3	0.3	1.0	0	0	52.5	0	15.0
	5	84.9	0	1.1	0	0	81.1	0	14.2
37°C	1	38.8	0	3.6	0	0	41.8	0.4	1.8
	3	77.9	0	8.3	0	0	85.7	0	5.7
	5	94.9	0	12.6	0	3.8	91.1	0.5	8.5
43°C	1	30.1	0	11.3	0	15.6	63.9	13.0	13.8
	3	84.0	1.7	45.5	0	76.4	91.9	52.2	53.7
	5	94.6	0.3	57.8	0	91.7	99.3	94.9	75.6

* In each experiment, 0.1 m*l* of 10^{-6} dilution of culture was inoculated into 10 m*l* of penassay broth and inocubated with gentle shaking at various temperatures.

Table 2. Summary of characters of colonies tested in stability experiments

Bacterial strain	Total number of colonies tested	Percent of carrying characters*					
		Cit$^+$	Tc^r	Sm^r	Cm^r	Km^r	(Cit$^-$R$^-$)**
HT58 (Cit, Tc Sm Su Cm)	2252	34.9	98.3	98.7	98.3	—***	1.3
KE10A (Cit, Tc Sm Su Cm Km)	2293	99.6	100	100	100	100	0
KE10B (Cit, Tc Sm Su Cm Km)	1008	72.0	100	100	99.2	100	0
C53A (Cit, Tc Sm Su Cm)	2469	100	90.8	98.2	91.7	—	0
C53B (Cit, Tc Sm Su Cm)	2412	86.6	99.0	94.7	98.5	—	0.9
KE10A-1096 (Cit, Tc Sm Su Cm Km)	2889	84.1	84.6	84.3	84.1	84.2	15.7
KE10B-133 (Cit, Sm Su Cm Km)	1871	84.8	—	85.4	85.4	95.9	3.5

* Cit$^+$, citrate-utilizing ability; Tc, tetracycline; Sm, streptomycin; Cm, chloramphenicol; Km, kanamycin.

** Colonies were susceptible to drugs tested and carried no citrate-utilizing ability on Simmons citrate agar.

*** Not tested.

easily lost from the HT58 strain at each temperature. The colonies tested in the stability experiments were derived from a single ancestral organism, but there was a difference in the loss frequency of Cit character between Cit$^+$ *E. coli* strains KE10 and C53 in these stability experiments.

In the present investigation, 2 clones were selected from each of these strains. The one clone with stable Cit ability and the other with unstable Cit ability were designated A and B, respectively. Further investigation was done with them, as shown in Table 1. *E. coli* substrain KE10A presented more stable Cit ability in the parent cell than *E. coli* substrain KE10B. The spontaneous loss of Cit character increased in substrains KE10B and C53B by successive cultures. In contrast, the Cit ability of substrain C53A was quite stable at each temperature (Table 1).

The stability of Cit character was also studied in transconjugants (ML1410) (Table 1). The transconjugants tested were HT58-2, KE10A-1096 and KE10B-133, which had been obtained in transfer experiments on Cit character, as described below. The Cit character of transconjugant ML1410 (HT58-2) was lost at a high frequency, as well as that of the parent strain. However, the Cit character of transconjugant KE10A-1096, derived from *E. coli* substrain KE10A, was much more stable than that of KE10B-133 of Cit$^+$ *E. coli* strain ML1410 at each temperature (Table 1). The Cit character of a transconjugant derived from Cit$^+$ *E. coli* substrain C53A was also remarkably stable, but that of a transconjugant derived from Cit$^+$ *E. coli* substrain C53B was unstable (unpublished data).

The relationship between the Cit character and drug resistance determinants (R$^+$) in both parent and transconjugant cells tested in stability experiments is summarized in Table 2. The drug resistance markers were much more stable than the Cit character in all parent Cit$^+$ strains, except substrain C53A. Cit$^-$R$^-$ cells carrying neither drug resistance markers nor Cit character were obtained from Cit$^+$ *E. coli* strain HT58 and C53B in stability experiments. A strain designated C53A-1097 was obtained in this study. It had Cit character, but not drug resistance markers. In general, both drug resistance markers and Cit character of transconjugants KE10A-1096 and KE10B-133 were eliminated remarkably, as compared with those of the parent Cit$^+$ *E. coli* strains. Transconjugant ML1410 (KE10B-133) was resistant to Sm, Su, Cm and Km, but susceptible to Tc. Many of the Cit$^-$R$^-$ cells were obtained from Cit$^+$R$^+$ transconjugants (Table 2). Both Cit character and drug resistance markers controlled by conjugative plasmids may be unstable in the *E. coli* K-12 strains.

Transfer experiments on Cit character and drug resistance markers

The transfer frequencies of the Cit character or drug resistance markers between naturally occurring Cit$^+$ *E. coli* and *E. coli* ML1410 are shown in Table 3. Cit character and drug resistance determinants were transferred more efficiently to *E. coli* ML-1410 at 25°C than at 37°C, indicating that the Cit character was as thermosensitive as the R determinants. Therefore, subsequent mating experiments were carried out at 25°C. When selection was made for ability to utilize citrate on Simmons citrate agar, the Cit character was transferred from each Cit$^+$R$^+$ strain to *E. coli* ML1410 at a frequency ranging from $<10^{-2}$ to $>10^{-6}$ per donor cell. When the selection of drug resistance marker was made for the Cm marker in each mating, the Cit$^+$R$^+$ parent

Table 3. Transfer frequencies of Cit character or drug resistance markers among *E. coli* strains at 25 or 37°C

Donor strain	Recipient strain	Marker selected*	Transfer frequency**	
			At 25°C	At 37°C
HT58	ML1410	Cit	2×10^{-2}	8×10^{-8}
(Cit, Tc Sm Su Cm)		Cm	2×10^{-2}	4×10^{-8}
KE10A	ML1410	Cit	3×10^{-5}	$<10^{-9}$
(Cit, Tc Sm Su Cm Km)		Cm	8×10^{-5}	$<10^{-9}$
KE10B	ML1410	Cit	3×10^{-6}	$<10^{-9}$
(Cit, Tc Sm Su Cm Km)		Cm	9×10^{-5}	$<10^{-9}$
C53A	ML1410	Cit	9×10^{-5}	$<10^{-9}$
(Cit, Tc Sm Su Cm)		Cm	6×10^{-6}	$<10^{-9}$
C53B	ML1410	Cit	7×10^{-4}	$<10^{-9}$
(Cit, Tc Sm Su Cm)		Cm	6×10^{-4}	$<10^{-9}$
C53A–1097 (Cit)	ML1410	Cit	2×10^{-6}	$<10^{-9}$
KE10A–1096	ML1410Rif	Cit	5×10^{-2}	$<10^{-9}$
(Cit, Tc Sm Su Cm Km)		Cm	5×10^{-2}	$<10^{-9}$
KE10B–133	ML1410Rif	Cit	3×10^{-4}	$<10^{-9}$
(Cit, Sm Su Cm Km)		Cm	3×10^{-4}	$<10^{-9}$

* Cit, Simmons citrate agar containing methionine (50 $\mu g/ml$) and either Nal (50 $\mu g/ml$) or Rif (50 $\mu g/ml$) was used as selective medium for Cit character in each experiment.

** Transfer frequencies were determined from 6 hr mating at 25 or 37°C and measured as the number of transconjugants per donor cell.

Table 4. Characters of transconjugants obtained from KE10 in transfer experiments at 25°C

Donor strain	Recipient strain	Marker selected	Characters of transconjugants	
KE10A	ML1410	Cit	867/874	Cit^+, Tc Sm Cm Km*
(Cit, Tc Sm Su Cm Km)			6/874	Cit^+, Tc Sm Cm
			1/874	Cit^+, Tc Sm Km
		Cm	634/648	Cit^+, Tc Sm Cm Km
			14/648	Cit^+, Tc Sm Cm
KE10B	ML1410	Cit	44/44	Cit^+, Tc Sm Su Cm Km
(Cit, Tc Sm Su Cm Km)		Cm	14/47	Cit^+, Tc Sm Su Cm Km
			32/47	Tc Sm Su Cm Km
			1/47	Tc Sm Su Cm
KE10A–1096	ML1410Rif	Cit	40/40	Cit^+, Tc Sm Su Cm Km
(Cit, Tc Sm Su Cm Km)		Cm	30/30	Cit^+, Tc Sm Su Cm Km
KE10B–133	ML1410Rif	Cit	77/79	Cit^+, Sm Su Cm Km
(Cit, Sm Su Cm Km)			2/79	Cit^+, Su Cm Km
		Cm	74/91	Cit^+, Sm Su Cm Km
			2/91	Cit^+, Sm Cm Km
			1/91	Cit^+, Sm Su Cm
			11/91	Sm Su Cm Km
			2/91	Sm Su Cm
			1/91	Sm Cm Km

* Replica-plating method was used. No resistance to Su was determined.

Table 5. Transfer frequencies of Cit character from citrate-positive *E. coli* (KE10A, C53B and C53A-1097) to *Shigella* or *Salmonella* strains at 25°C

Donor strain	Marker selected**	Transfer frequencies to recipient strain*					
		S. sonnei[a] JS13389	*S. flexneri*[a] YSH-1	*S. typhi*[b] TD	*S. abortusequi*[c] SG20	*S. abortusequi*[c] SG21	*S. pullorum*[d] SG22
KE10A	Cit	ND***	3×10^{-7}	ND	ND	2×10^{-7}	ND
	Cm	5×10^{-5}	8×10^{-7}	3×10^{-7}	ND	ND	1×10^{-5}
C53B	Cit	1×10^{-6}	3×10^{-7}	ND	ND	1×10^{-6}	ND
	Cm	2×10^{-3}	3×10^{-5}	3×10^{-5}	4×10^{-6}	6×10^{-3}	9×10^{-4}
C53A-1097	Cit	ND	ND	ND	ND	ND	ND

* Transfer frequencies were the same as described in the footnote of Table 4.

** Cit, Simmons citrate agar containing amino acids or vitamins such as, [a] Met and nicotinic acid; [b] Met, Try, His, adenine hydrochloride, calcium pantothenate, pridoxine hydrochloride and folic acid; [c] Met, Try, His, Cys and riboflavin; [d] Met, Try, His, Cys, riboflavin and sodium thiosulfate, and either Nal (50 $\mu g/ml$) or Rif (50 $\mu g/ml$) was used as selective medium for Cit character.

*** Not detected.

donor strain transferred the Cm resistance marker at the same frequency. There was no remarkable difference in transfer frequency of the Cit character or drug resistance markers between selection made for ability to utilize citrate on Simmons citrate agar and selection made for resistance to antibiotics (Table 3). The Cit character of the Cit^+R^- (C53A-1097) cells obtained in stability experiments was self-conjugative and thermosensitive. Table 3 shows the results of the further transfer of the Cit character and drug resistance markers from the Cit^+R^+ ML1410 transconjugants (KE10A-1096 and KE10B-133) to *E. coli* ML1410Rif.

Table 4 indicates the results of transfer experiments on the character of transconjugants derived from the representative Cit^+R^+ *E. coli* strain, KE10. When a mating experiment was carried out between Cit^+R^+ *E. coli* strains KE10A and ML1410, the Cit character was found to be accompanied with drug resistance markers. However, of the transconjugants obtained from Cit^+R^+ *E. coli* substrain KE10B, those carrying drug resistance markers without the Cit character were found at a high rate (Table 4).

Two transconjugants, KE10A-1096 and KE10B-133, of *E. coli* ML1410 were obtained on Cm-selective medium from Cit^+ R^+ *E. coli* substrain KE10A and KE10B, respectively. They were used in further studies.

Table 4 shows the results of a further transfer of the Cit charactr and drug resistance markers from the Cit^+R^+ transconjugants to *E. coli* ML1410Rif. The Cit character was always transmitted together with resistance markers in substrain KE10A-1096. When selection was made for Cm marker, Cit^-R^+ transconjugants were obtained in a mating experiment with substrain KE10B-133. These results suggest that the versatile behavior of the *E. coli* strains with citrate-utilizing ability may have brought about different clones, as shown in stability experiments on the Cit character.

Transfer experiments on Cit character from Cit^+R^+ *E. coli* strains to *Shigella* or *Salmonella* strains

To examine the potential transmission of the Cit character to the genera, except

Escherichia, of the family *Enterobacteriaceae,* mating experiments were carried out between Cit$^+$R$^+$ *E. coli* parent strains and Simmons-negative *Shigella* or *Salmonella* strains. Table 5 shows the transfer frequencies of the Cit character or drug resistance marker from Cit$^+$R$^+$ *E. coli* strains to *Shigella* spp. or *Salmonella* spp. Simmons citrate agar containing appropriate amino acids and growth factors was used as selective medium. It allowed *Shigella* and *Salmonella* strains to grow when glucose was added to it as a sole carbon source.

The Cit character was transferred from Cit$^+$R$^+$ *E. coli* substrain KE10A to *S. flexneri* YSH-1 or *S. abortusequi* SG21 at a low frequency. When the Cit character or drug resistance marker was tested for transfer from Cit$^+$R$^+$ *E. coli* substrain C53B to *S. sonnei, S. flexneri* or *S. abortusequi,* the transconjugant selected for the Cit character were obtained. In contrast, when mating experiments were made between Cit$^+$R$^-$ *E. coli* substrain C53A-1097 and *Shigella* or *Salmonella* recipient strains, no transconjugants were found on Simmons citrate agar plates.

In general, when selection of drug resistance marker was made to determine a Cm marker for each mating, the drug resistance marker was transmitted more efficiently from the donor to the recipient strain than the Cit character. This difference in transfer frequency between the Cit character and drug resistance marker may have been caused by the difference in selection medium used. Simmons citrate agar was severe for the growth of *Shigella* and *Salmonella* strains, even when it was supplemented with appropriate growth factors. The transconjugants selected for the Cit character or Cm resistance marker were purified on the same medium. This result was confirmed by further experiments on transfer of the Cit character or drug resistance markers to *E. coli* W3630. All the transconjugant cells of *Shigella* spp. and *Salmonella* spp. selected for the Cit character carried both Cit character and drug resistance markers (unpublished data). From these results, the transfer frequencies of the Cit character from the Cit$^+$R$^+$ *E. coli* substrains to *Shigella* and *Salmonella* strains were lower than those from these substrains to the *E. coli* K-12 strains.

Discussion

The transferable substrate-utilizing characters, such as fermentation of lactose, sucrose and raffinose, and production of hydrogen sulfide, of the family *Enterobacteriaceae* are controlled by conjugative plasmids [4, 6, 10, 11]. The citrate-utilizing ability of citrate-positive *E. coli* strains isolated from domestic pigeons and animals was also controlled by conjugative plasmid [8]. Therefore, the Cit character among the unusual biochemical characteristics is considered to be one of the plasmid-mediated characters.

The present study suggests that the Cit character of Cit$^+$R$^+$ *E. coli* strains of a parent or transconjugant ML1410 may be relatively unstable at a temperature higher than 37°C. Ishiguro et al. [2] reported that an incubation at 43°C evidently hindered citrate utilization by Cit$^+$ *E. coli* strains isolated from pigeons and pigs. The growth feature of these strains on Simmons citrate agar incubated at 43°C may be due to the unstability of citrate-utilizing plasmids in Cit$^+$ *E. coli* cells.

Moreover, the potential change of elimination rate of the Cit character in different clones derived from a single ancestral Cit$^+$ R$^+$ cell was also observed in stability experiments. In particular, there was a marked difference in the loss frequency of

the Cit character between the Cit^+R^+ *E. coli* substrains of C53A and those of C53B. This difference in the loss frequency of the Cit character may be due to the maintenance of the citrate-utilizing plasmids in the Cit^+ *E. coli* cells.

Datta and Barth [1] reported that a certain known R plasmid could be integrated at least transiently into *E. coli* chromosome. If the citrate-utilizing plasmids could be integrated into the chromosome, the citrate-utilizing ability would be exactly stable in the *E. coli* cells at each temperature. The citrate-utilizing ability of the Cit^+R^+ *E. coli* substrain of C53A was found to have citrate-utilizing plasmid integrated into the chromosome. The chromosome transfer into suitable recipient strain was indicated in various experiments (Ishiguro et al., unpublished data). Detailed investigation is now under way on it by the authors.

From the results of mating experiments, the Cit character was always transmitted together with the drug resistance marker, although Cit^-R^+ transconjugants were obtained on Cm selective medium. It is not yet clear whether the Cit character and drug resistance markers are located on a conjugative plasmid or on any other plasmid. To study the physical independence of both characters, their genetic properties should be investigated.

The citrate-utilizing ability can be transferred from the Cit^+R^+ *E. coli* strains tested not only to *E. coli* K-12 strains, but to *S. sonnei, S. flexneri, S. typhi, S. abortusequi* and *S. pullorum* at rather low rates.

Stability of the Cit character in the host cell, such as *Shigella* spp. and *Salmonella* spp., is not investigated. Smith et al. [12] also reported that 12 thermosensitive H1 plasmids determining citrate utilization were isolated from strains of *S. typhi,* and that their citrate utilization was transferred to one strain each of *S. flexneri* and *S. sonnei.* The transmission of citrate utilization to *Shigella* spp. or *Salmonella* spp. originally lacking citrate utilization is an important problem for the identification of *Enterobacteriaceae.* Transferable substrate-utilizing character is always accompanied with the acquisition of drug resistance markers [6, 12], like the conjugative Cit character in the present study. Therefore, the conjugative R plasmid may play an important role in the spread of plasmid-mediated biochemical characters.

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References

[1] Datta, N., and Barth, P. T. (1976). Hfr formation by I pilus-determining plasmids in *Escherichia coli* K-12. *J. Bacteriol.* **125**, 811–817.

[2] Ishiguro, N., Oka, C., and Sato, G. (1978). Isolation of citrate-positive variants of *Escherichia coli* from domestic pigeons, pigs, cattle, and horses. *Appl. Environ. Microbiol.* **36**, 217–222.

[3] Ishiguro, N., and Sato, G. (1979). The distribution of plasmids determining citrate utilization in citrate-positive variants of *Escherichia coli* from humans, domestic animals, feral birds and environments. *J. Hyg. (Camb.)* **83**, 331–344.

[4] Lautrop, H., Ørskov, I., and Gaarslev, K. (1971). Hydrogensulphide producing variants of *Escherichia coli. Acta Pathol. Microbiol. Scand. B* **79**, 641–650.

[5] Meynell, G. G., and Meynell, E. (1965). Bacteriological culture media. *In* Theory and Practice in Experimental Bacteriology, The Syndics of Cambridge University Press, London, 36–37.

[6] Ørskov, I., and Ørskov, F. (1973). Plasmid-determined H_2S character in *Escherichia coli* and its relation to plasmid-carried raffinose fermentation and tetracycline resistance char-

acters. *J. Gen. Microbiol.* **77**, 487–499.
[7] Sakazaki, R. (1978). Bacterial Culture and Media, Sakazaki, R., editor, Kindai Shuppan, Tokyo, Japan (text in Japanese).
[8] Sato, G., Asagi, M., Oka, C., Ishiguro, N., and Terakado, N. (1978). Transmissible citrate-utilizing ability in *Escherichia coli* isolated from pigeons, pigs and cattle. *Microbiol. Immunol.* **22**, 357–360.
[9] Smith, H. W., and Gyles, C. L. (1970). The relationship between different transmissible plasmids introduced by F into same strain of *Escherichia coli* K-12. *J. Gen. Microbiol.* **62**, 277–285.
[10] Smith, H. W., and Parsell, Z. (1975). Transmissible substrate-utilizing ability in Enterobacteria. *J. Gen. Microbiol.* **87**, 129–140.
[11] Smith, H. W., and Parsell, Z. (1976). A transmissible plasmid determining lactose fermentation and multiple antibiotic resistance in a strain of *Klebsiella pneumoniae*. *J. Med. Microbiol.* **9**, 359–362.
[12] Smith, H. W., Parsell, Z., and Green, P. (1978). Thermosensitive Hl plasmids determining citrate utilization. *J. Gen. Microbiol.* **109**, 305–311.
[13] Terakado, N., Azechi, H., Koyama, N., Sato, G., and Mitsuhashi, S. (1975). Demonstration of R factors with chloramphenicol resistance in *Salmonella* strains isolated from domestic animals. *In* Microbiol Drug Resistance, Mitsuhashi, S., editor, University of Tokyo Press, Tokyo, 253–260.
[14] Terakodo, N., and Sato, G. (1978). Demonstration of the so-called Mexican type R plasmids in *Escherichia coli* isolated from domestic animals and pigeons. *Microbiol. Immunol.* **22**, 227–229.
[15] Washington, J. A. II, and Timm, J. A. (1976). Unclassified, citrate-positive member of the family *Enterobacteriaceae* resembling *Escherichia coli*. *J. Clin. Microbiol.* **4**, 165–167.

要　約

クエン酸陽性大腸菌由来のクエン酸利用能の性状—大腸菌，赤痢菌およびサルモネラにおけるクエン酸利用能の安定性と伝達性：浅木正義・石黒直隆・岡　千晶・佐藤儀平（帯広畜産大学獣医公衆衛生学教室），寺門誠致（農林水産省家畜衛生試験場）——鳩，豚，牛から分離したクエン酸陽性大腸菌由来のクエン酸利用性状について，安定性および伝達試験により遺伝学的に検索を行った．クエン酸陽性大腸菌のクエン酸利用性状は，25℃ および 37℃ に比べ，43℃ で不安定であり培養の継代数を追うごとに脱落の傾向を示した．しかし豚および牛由来株においてクエン酸の安定なクローン（A）と不安定なクローン（B）がそれぞれ別個に得られ，それらのクローンの安定性性状および伝達性状を検索した．クエン酸利用能と薬剤耐性は，37℃ に比べ 25℃ で良好に大腸菌 K-12 株に伝達された．また，クエン酸利用のみを保有する伝達株も得られた．また，クエン酸利用性状は，大腸菌ばかりでなく，*Shigella sonnei*, *Shigella flexneri*, *Salmonella typhi*, *Salmonella abortusequi* および *Salmonella pullorum* に薬剤耐性と共に 25℃ で伝達された．