SHORT COMMUNICATION

Incompatibility of Citrate Utilization Plasmids Isolated from *Escherichia coli*

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The 57 conjugative Cit plasmids isolated from 72 citrate-utilizing (Cit⁺) *Escherichia coli* strains from various sources were classified into four groups on the basis of their genetic properties. *Escherichia coli* K12 strains carrying these Cit plasmids could utilize *cis*-aconitate or tricarballoylate, in addition to citrate.

INTRODUCTION

Citrate-utilizing (Cit⁺) variants of *Escherichia coli* have been isolated from humans, domestic animals, birds and natural environments, and in most of them the ability to utilize citrate was mediated by conjugative plasmids (Ishiguro *et al.*, 1978; Ishiguro & Sato, 1979, 1980; Sato *et al.*, 1978). Smith *et al.* (1978) reported that 15 thermosensitive IncH1 plasmids derived from *Salmonella typhi* and enterobacterial strains mediated citrate utilization in an *E. coli* K12 strain. However, no attempt has been made to carry out a comparative study of the genetic properties of the various Cit plasmids. This paper describes the genetic properties and organic acid utilization of the Cit plasmids isolated from various sources.

METHODS

Bacterial strains. The 72 Cit⁺ *E. coli* variants used in this study were those isolated from humans, domestic animals and birds as described previously (Ishiguro *et al.*, 1978; Ishiguro & Sato, 1979). The derivatives of *E. coli* K12 were ML1410 (F⁻, *met nal*), SG1 (Hfr, *met rif*) and SG3 (*thr thi leu met hsdS rif*) (Ishiguro *et al.*, 1979). *Escherichia coli* ATCC 11775, *Citrobacter freundii* 10018-68, *Enterobacter aerogenes* 2A, *Proteus vulgaris* 1-1, *Salmonella typhimurium* LT-2 and *Serratia marcescens* E8-3 were used as reference strains for testing the utilization of organic acids.

Plasmids and phages. The following reference R plasmids of different incompatibility groups were used in incompatibility tests (Novick *et al.*, 1976): R40a (IncA), F'*lac-tet* (IncF1), R100 (IncFII), ColB-K98 (IncFIII), R124 (IncFIV), R27 (IncH1), R478 (IncH2), R144 (IncI α), Tp114 (IncI2), R621a (IncI γ), R391 (IncJ), R387 (IncK), R446b (IncM), RN3 (IncN), R14 (IncO), RP4 (IncP), Rts1 (IncT), R753 (IncV), Rs-a (IncW) and R6k (IncX). In the test of incompatibility with group IncH1, some of the test plasmids had selective markers in common with the reference IncH1 plasmid. In these cases plasmid pOH1477, conferring resistance to ampicillin and chloramphenicol, and identified as IncH1 in our laboratory, was also used. The male-specific phages used were f1 and f2. Phages λ vir, T4, T7 and ϕ 80 were also used for phage inhibition tests (Ishiguro *et al.*, 1979).

Media. Nutrient broth and selective media used for genetic experiments were as described by Ishiguro *et al.* (1979). Antimicrobial drugs used were ampicillin (Ap, 25 μ g ml⁻¹), chloramphenicol (Cm, 25 μ g ml⁻¹), kanamycin (Km, 25 μ g ml⁻¹), nalidixic acid (Nal, 50 μ g ml⁻¹), rifampin (Rif, 50 μ g ml⁻¹), streptomycin (Sm, 12.5 μ g ml⁻¹), sulphonamides (Su, 800 μ g ml⁻¹) and tetracycline (Tc, 25 μ g ml⁻¹). The organic acids tested were acetate, citrate, isocitrate, *cis*-aconitate, tricarballoylate, succinate, fumarate, *trans*-aconitate and L-malate, and the basal agar media were described by Imai *et al.* (1973).

Table 1. *Genetic properties of 57 conjugative Cit plasmids derived from 72 Cit⁺ E. coli strains from various sources*

Host	No. of Cit ⁺ <i>E. coli</i> strains	No. of strains with conjugative Cit plasmids	Plasmid-mediated characters				
			Drug resistance* or Cit character	No. of plasmids	Fi character†	Ts character‡	Inc group§
Humans	24	24	Cit, Km Tc	2	—	—	UT
			Cit	2	—	—	W
			Cit	20	+	—	UT
Cattle	13	7	Cit, Cm Sm Su Tc	1	—	+	H1
			Cit	6	+	—	UT
Pigs	12	10	Cit, Cm Km Sm Su Tc	2	—	+	H1
			Cit, Cm Sm Su Tc	8	—	+	H1
Horses	6	0					
Domestic pigeons	13	12	Cit, Cm Sm Su Tc	12	—	+	H1
Feral pigeons	2	2	Cit	2	+	—	UT
Crows	2	2	Cit, Cm Km Sm Su Tc	2	—	+	H1
Total	72	57 (79%)		57			

* Abbreviations for plasmid-mediated drug resistance: Cm, chloramphenicol; Km, kanamycin; Sm, streptomycin; Su, sulphonamides; Tc, tetracycline.

† Fi, Fertility inhibition.

‡ Ts, Cit plasmid showing thermosensitive transfer.

§ UT, Untypable.

Fertility inhibition (Fi) tests, phage inhibition tests and incompatibility tests. These were done as described by Ishiguro *et al.* (1979).

Utilization of organic acids in Cit⁺ E. coli strains. Utilization of organic acids in naturally occurring Cit⁺ *E. coli* strains or in *E. coli* ML1410 carrying the Cit plasmid was examined by the method of Imai (1977).

RESULTS

Genetic properties of conjugative Cit plasmids

Table 1 shows the characteristics of the conjugative Cit plasmids derived from the 72 Cit⁺ *E. coli* strains from various sources. Of the 57 conjugative Cit plasmids, 25 (44%) exhibited thermosensitive transfer. Conjugative citrate-utilizing ability carried by the Cit plasmids derived from pigs, domestic pigeons and crows was always transmitted to *E. coli* K12 strains together with drug resistance markers. None of the 25 thermosensitive Cit plasmids showed fertility inhibition, i.e. they were Fi⁻; four Cit plasmids obtained from human source were also Fi⁻. However, all 28 Cit plasmids exhibiting non-thermosensitive transfer and conferring Cit character alone (derived from humans, cattle and feral pigeons) were Fi⁺. Although all the Cit plasmids showing the Fi⁺ character supported the multiplication of male-specific phage f1 (data not shown), they were not only compatible with the standard F-like plasmids (F'*lac-tet*, R100, ColB-K98 and R124), but also with the other 16 reference R plasmids tested, indicating that they are untypable. Two Cit plasmids showing the Fi⁻ character (from humans) were incompatible with Rs-a, indicating that they belong to incompatibility group W, whereas two Fi⁻ plasmids conferring Cit, Km and Tc were untypable (Table 1). All thermosensitive Cit plasmids isolated were incompatible with pOH1477, and were also incompatible with F'*lac-tet*, so they were classified as belonging to incompatibility group H1. Moreover, all Cit plasmids but one from pig did not inhibit development of phages λ vir, T4, T7 and ϕ 80.

Table 2. Utilization of organic acids by Cit⁺ *E. coli* strains from various sources

Host	No. of strains tested	No. of strains showing each pattern	Utilization*								
			Acetate	Citrate	Isocitrate	cis-Aconitate	trans-Aconitate	Tricarballoylate	Succinate	Fumarate	L-Malate
Humans	24	23	+	+	—	+	—	+	+	+	+
		1	+	+	—	+	—	—	+	+	+
Cattle	13	11	+	+	—	+	—	+	+	+	+
		2	+	+	—	+	—	—	+	+	+
Pigs	12	12	+	+	—	+	—	+	+	+	+
Horses	6	2	+	+	—	+	—	+	+	+	+
		4	+	+	w+	+	w+	w+	+	+	+
Domestic pigeons	13	13	+	+	—	+	—	+	+	+	+
Feral pigeons	2	2	+	+	—	+	—	+	+	+	+
Crows	2	2	+	+	—	+	—	+	+	+	+
<i>E. coli</i> ATCC 11775			+	—	—	—	—	—	+	+	+
<i>E. coli</i> ML1410			+	—	—	—	—	—	+	+	+
<i>Citrobacter freundii</i> 10018-68			+	+	+	+	—	—	+	+	+
<i>Enterobacter aerogenes</i> 2A			+	+	+	+	+	+	+	+	+
<i>Proteus vulgaris</i> I-1			+	+	—	—	—	—	+	+	+
<i>Salmonella typhimurium</i> LT-2			+	+	+	+	—	+	+	+	+
<i>Serratia marcescens</i> E8-3			+	+	+	+	+	—	+	+	+

* +, Utilized; w+, weakly utilized; —, not utilized.

Utilization of organic acids in Cit⁺ *E. coli* strains

The results of tests for utilization of organic acids by the 72 Cit⁺ *E. coli* strains and by other members of the family *Enterobacteriaceae* are shown in Table 2. Though *E. coli* ATCC 11775 or *E. coli* ML1410 did not utilize citrate, isocitrate, cis-aconitate, trans-aconitate and tricarballoylate as a sole source of carbon in the basal medium, Cit⁺ *E. coli* strains could utilize these tricarboxylic acids. Three different utilization patterns for tricarboxylic acids in Cit⁺ *E. coli* strains were observed, but no identical utilization patterns of organic acids to those of Cit⁺ *E. coli* strains were found among the reference enteric bacteria tested (Table 2). In addition, transconjugants of *E. coli* ML1410 could utilize citrate, cis-aconitate or tricarballoylate due to carriage of the Cit plasmids, and no difference in utilization patterns was found between the Cit⁺ transconjugants and the naturally occurring Cit⁺ *E. coli* strains (data not shown). These results indicate that the utilization of tricarboxylic acids in Cit⁺ *E. coli* ML1410 is due to tricarboxylic acid-utilizing ability encoded on the Cit plasmid.

DISCUSSION

The 57 conjugative Cit plasmids isolated were classified into four groups: (i) plasmids belonging to incompatibility group W, (ii) F-like plasmids that were untypable, (iii) plasmids that carried Fi⁻ character and were untypable, and (iv) thermosensitive plasmids belonging to incompatibility group H1. Smith *et al.* (1978) reported that plasmid-mediated citrate utilization is relatively rare and may be principally confined to IncH1 plasmids. However, the present study indicates that citrate-utilizing ability is not always mediated by thermosensitive IncH1 plasmids and is widely distributed on plasmids showing different properties.

Cit⁺ *E. coli* K12 strains carrying conjugative Cit plasmids from various sources could utilize cis-aconitate or tricarballoylate, as well as citrate; the same was true for Cit⁺ strains in

which conjugative plasmids have not been demonstrated. However, there was no significant relationship between the utilization patterns of Cit⁺ *E. coli* K12 strains and the incompatibility groups of their Cit plasmids. The utilization pattern of tricarboxylic acids in *E. coli* K12 strains carrying the Cit plasmids was not identical to those in the originally Cit⁺ enteric bacteria tested. These results suggest that plasmid-borne ability to utilize citrate may have originated from limited sources, and this leads to speculation on the origin of the Cit plasmids among enterobacterial strains.

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