SHORT COMMUNICATION

Properties of a Transmissible Plasmid Conferring Citrate-utilizing Ability in *Escherichia coli* of Human Origin

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Transfer of citrate utilization (Cit⁺) was achieved with a plasmid (pCIT354) which is Fi⁺, has F-like pili and fails to inhibit phage propagation. Transduction of Cit⁺ was achieved with P1 phage. Results of incompatibility tests with R plasmids indicated that pCIT354 is a self-repressed F-like plasmid.

INTRODUCTION

In 27 citrate-positive strains of *Escherichia coli*, isolated from domestic pigeons, pigs, cattle and horses, the citrate-utilizing (Cit) ability was controlled by plasmids showing thermosensitive transfer with or without co-transfer of resistance markers including chloramphenicol (Ishiguro *et al.*, 1978; Sato *et al.*, 1978). Recently, Smith *et al.* (1978) reported that 15 thermosensitive H1 plasmids derived from 12 strains of *Salmonella typhi* and three enterobacterial strains mediated citrate utilization in *E. coli* K12. These H1 plasmids from *S. typhi* also conferred resistance to chloramphenicol and showed thermosensitive transfer.

We have detected 21 citrate-positive *E. coli* strains not carrying conjugative R plasmids from six human stool samples (Ishiguro & Sato, 1979). This paper describes some properties of the citrate-utilizing character derived from one of these human strains of *E. coli*.

METHODS

Bacterial strains. *Escherichia coli* Hu354 was isolated from a stool sample from a volunteer on non-selective media in 1977 in Obihiro, Japan. This strain was identified as typical *E. coli* by 34 biochemical reactions, with the exception of citrate utilization; it was sensitive to antibiotics and none of characters determined by other conjugative plasmids was demonstrated. The initial recipient of the Cit character of this strain was *E. coli* ML1410, a nalidixic acid-resistant K12 strain requiring methionine. Subsequent recipients of the Cit character were *E. coli* K12 strains ML1410Rif (F⁻, *met*, *nal*, *rif*), 1100 (F⁻, *nal*, *r*⁻), W1895 (Hfr, *met*, *rif*) and W4573 (F⁻, *str*, *ara*, *mal*, *xyl*, *mtl*, *gal*, *lac*-85).

Plasmids and phages. The standard R plasmids of different incompatibility groups (F'-*lac*-tet, R386, R100, R124, RA1, R40a, R391, R387, R144, RP4, Rs-a, R27, R446-b, R14, RN3, Rts1, R6k, R471a and R478) were used for incompatibility tests. In this study, the male-specific phages used were f1 and f2, and phages λ , ϕ 80, T1, T3, T6, W31 and P1 were also used.

Media. Penassay broth (Difco) was used for conjugative experiments. The selective media used for citrate utilization were Simmons citrate agar (Eiken) plates supplemented with methionine (50 μ g ml⁻¹) and either nalidixic acid (50 μ g ml⁻¹) or rifampin (50 μ g ml⁻¹). BTB/lactose agar and deoxycholate/hydrogen sulphide/lactose agar (DHL; Eiken) were used as the selective media for tetracycline (Tc, 25 μ g ml⁻¹) and chloramphenicol (Cm, 25 μ g ml⁻¹), respectively (Ishiguro *et al.*, 1978). L broth (LB) (Lennox, 1955), LB agar and soft agar were used for growth and titration of phages. In this study, CaCl₂ was added to LB and LB agar at a final concentration of 2.5 mM.

Transfer experiment on citrate-utilizing ability. The methods were as described by Sato *et al.* (1978) and used

Simmons citrate agar plates incubated for 4 d at 37 °C. To determine transconjugant recipients and their Cit character, 20 colonies of transconjugants on each selective medium were purified on DHL agar plates and examined for citrate-utilizing ability on the same selective medium.

The effect of passaging Cit⁺ strain in broth at different temperatures. The effects of passaging the Cit⁺ strain in broth at 37 and 43 °C were investigated as described by Sato *et al.* (1978).

Sensitivity to sex phages and phage inhibition tests. The Cit character was introduced into *E. coli* W1895Hfr, and its ability to repress production of F pili was studied by the spot test with phages f1 and f2. The Cit⁺ transconjugants of *E. coli* 1100 were also tested for the production of F-like pili by assaying their ability to support the multiplication of phages f1 and f2 according to the method described by Grindley & Anderson (1971). Phage inhibition experiments were as described by Taylor & Grant (1976). The Cit character was tested for its ability to reduce both the number of plaques and the plaque size of λ , ϕ 80, T1, T3, W31 and P1 phages, using *E. coli* 1100 as indicator strain.

Transduction experiments. Transduction (Lennox, 1955) was carried out following the propagation of phage P1 on ML1410Rif harbouring the Cit character (Mise, 1976). *Escherichia coli* W4573 was used as the recipient strain.

Incompatibility tests. The compatibility property of the Cit character was examined by the method of Datta (1977). *Escherichia coli* ML1410 was used as the donor strain and the rifampin-resistant mutant of this strain was used as the recipient.

RESULTS AND DISCUSSION

The citrate-utilizing character was transferred from *E. coli* Hu354 to *E. coli* ML1410 at a frequency of about 2×10^{-4} only at 37 °C, and the resulting Cit⁺ clones were also able to transfer the character at a similar frequency. Transconjugants were found on Simmons citrate agar plates used as selective media within 2 d of incubation at 37 °C. No Cit⁺ bacteria were found after 10 daily passages of *E. coli* Hu354 and transconjugant ML1410 in broth at 37 °C. However, after passage at 43 °C, 0.3% of ML1410 (Cit⁺) bacteria lost the Cit character, indicating that the element conferring the Cit character is fairly stable in the *E. coli* strain, in contrast to the unstable Cit character associated with H plasmids in the cells (Sato *et al.*, 1978). Citrate-utilizing ability has always been found in association with the thermosensitive H plasmids (Sato *et al.*, 1978; Smith *et al.*, 1978). There has been no report on the isolation of conjugative plasmids conferring citrate utilization alone from naturally occurring *E. coli* strains.

A culture of strain W1895Hfr (Cit⁺) was not lysed by phage f1 or f2, indicating that the Fi character of the Cit plasmid was Fi⁺, as has been described for the plasmids determining hydrogen sulphide production (Ørskov & Ørskov, 1973; Magalhães & Vêras, 1977) and metabolic characters such as lactose or sucrose fermentation (Le Minor *et al.*, 1976). Moreover, phage f1 multiplied about 100-fold in *E. coli* 1100 strains carrying the Cit character. These results suggest that the strains carrying the Cit plasmid produce F-like pili. No differences in the efficiency of plating of the phages employed between Cit⁺ and Cit⁻ bacteria was demonstrated. The Cit determinant did not confer the property of phage inhibition on its host bacteria.

Phage P1 propagated on *E. coli* ML1410Rif (Cit⁺) could transduce the Cit⁺ genes to *E. coli* W4573 at a frequency of 3×10^{-6} per plaque-forming unit, and the resulting transductants could transfer the Cit character to ML1410 (Cit⁻) at similar frequency when incubated in mixed culture. Henceforth, the element conferring the citrate-utilizing ability was designated as pCIT354.

Incompatibility of pCIT354 with representative F-like R plasmids is shown in Table 1. pCIT354 was transferred to a recipient carrying each of the R plasmids without reduction of its transfer frequencies, but when the strain carrying pCIT354 was used as a recipient, the transfer frequencies of some F-like R plasmids were reduced by about 10- to 10000-fold. In particular, a strong exclusion between pCIT354 and R100 was demonstrated. However, many purified transconjugant clones contained both characters, whether selection was made for the Cit character or for drug resistance (Table 1). Both sets of characters were stably inherited during subsequent growth in non-selective media. To examine the separate

Table 1. *Incompatibility of pCIT354 and standard R plasmids F'-lac-tet, R386, R100 and R124*

Incoming plasmid* (group)	Resident plasmid† (group)	Selection‡	Transfer frequency§	Phenotype of purified transconjugants			
				No. of colonies tested	Cit ⁺ R ⁺	Cit ⁺ only	R ⁺ only
pCIT354	—	Sim(met+ Rif)	3×10^{-4}	20	0	20	0
pCIT354	R386 (FI)	Sim(met+ Rif)	6×10^{-5}	20	20	0	0
pCIT354	R100 (FII)	Sim(met+ Rif)	2×10^{-5}	9	8	1	0
pCIT354	R124 (FIV)	Sim(met+ Rif)	8×10^{-5}	10	9	1	0
F'-lac-tet (FI)	—	Tc+ Rif	6×10^{-1}	20	0	0	20
	pCIT354	Tc+ Rif	3×10^{-1}	20	20	0	0
R386 (FI)	—	Tc+ Rif	2×10^{-2}	20	0	0	20
	pCIT354	Tc+ Rif	4×10^{-4}	20	20	0	0
R100 (FII)	—	Cm+ Rif	3×10^{-2}	20	0	0	20
	pCIT354	Cm+ Rif	3×10^{-6}	20	20	0	0
R124 (FIV)	—	Tc+ Rif	6×10^{-3}	20	0	0	20
	pCIT354	Tc+ Rif	6×10^{-4}	20	20	0	0

* Host was *E. coli* ML1410.† Host was *E. coli* ML1410Rif.‡ Sim(met+ Rif), Simmons citrate agar containing methionine ($50 \mu\text{g ml}^{-1}$) and rifampin ($50 \mu\text{g ml}^{-1}$); Cm, chloramphenicol; Tc, tetracycline; Rif, rifampin.

§ Determined from 2 h mating at 37 °C, measured as transconjugants per donor.

existence of the two plasmids (pCIT354 and an R plasmid), a transconjugant was used as a donor, separate selection plates being used to test transfer of each plasmid. Each plasmid was transferred separately to *E. coli* W4573 at a similar frequency. Furthermore, the incompatibility tests with pCIT354 and the other 15 standard R plasmids showed that both the Cit character of pCIT354 and the drug resistance of the R plasmids always co-existed stably. It could therefore be concluded that the pCIT354 was a self-repressed F-like plasmid not belonging to the incompatibility groups employed in this study, because it was compatible with the standard R plasmids tested. Smith *et al.* (1978) reported that 12 H1 plasmids from *S. typhi* strains mediated citrate utilization in *E. coli* K12 and the Cit character might be principally confined to H1 plasmids. However, since the transfer of the Cit character of pCIT354 was not thermosensitive and was compatible with R27 (H1 plasmid), this Cit plasmid is distinct from the H1 plasmid.

There is potential for spread of citrate utilization plasmids in the *Enterobacteriaceae*. It is not yet clear from experiments in progress whether the Cit character in all of the citrate-positive variants of *E. coli* isolated from various sources is located on a conjugative plasmid such as pCIT354. One citrate-positive *E. coli* strain isolated from a horse (Ishiguro *et al.*, 1978) could not transfer its Cit character to *E. coli* K12, although the character was lost following exposure to acridine orange (unpublished results).

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