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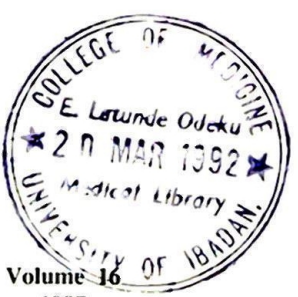

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Transduction of citrate-utilization genes into *Escherichia coli* K12

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Summary

Genes for citrate utilization were transferred by transduction into *Escherichia coli* from *Salmonella* and *Klebsiella* strains. The genes appear to take chromosomal positions at 6–10 min on the K12 linkage map. Growth on citrate medium by Cit^+ K12 hybrids was poor. Incorporate of glycine or L-serine into citrate minimal media improved growth of K12 Cit^+ hybrids on citrate. The significance of these findings is discussed.

Résumé

Les gènes pour la utilisation de citrate ont été transférées de strains *Salmonella* et *Klebsiella* à *Escherichia coli* par la méthode transduction. Les gènes se montrent de prendre les positions chromosomes entre 6 et 10 minutes sur position K12 de la carte des liens. La croissance sur la médium citrate par les hybrides Cit^+ K12 était médiocre. L'incorporation de glycine ou L-sérine dans la médium citrate minime avait augmenté la croissance des hybrides K12 Cit^+ dans citrate. La signification de ces constatations est discuté.

Introduction

The indole, methyl red, Voges-Proskauer, citrate utilization (IMViC) tests are most important and useful for differentiation of coliforms into species and varieties. It is generally accepted that most of the species of the tribes Salmonelleae, Klebsiellae and Proteeae possess the citrate utilizing ability, but other tribes e.g. Eschericheae and Edwardsielleae do not possess it.

The inability of *Escherichia coli* to utilize citrate is very important and valuable for identification in the family Enterobacteriaceae. The incidence of citrate-positive *E. coli* variants is reported to be very low (Edwards & Ewing, 1972). A number of investigators have reported the isolation of citrate utilizing *E. coli* from a variety of birds and mammals, but this phenotype in most cases was found to be mediated by conjugative plasmids (Ishiguro, Oka & Sato, 1978; Sato *et al.*, 1978; Ishiguro & Sato, 1979).

However, *E. coli* may utilize citrate under anaerobic conditions if a second substrate such as glucose or lactate is available (Lutgens & Gottschalk, 1980). The failure of *E. coli* to utilize citrate aerobically has been attributed to the absence of a citrate transport system (Lara & Stokes, 1952). It is, therefore, possible that *E. coli* possesses defective citrate-utilization genes.

As part of a study on the genetic basis of natural variation within the Enterobacteriaceae, genes responsible for variable characters are transferred to the Laboratory strain of *E. coli* K12 by transduction and the consequence of transfer studied (Alaeddinoglu & Charles, 1979; Woodward & Charles, 1983). This paper describes the transduction of the genes for citrate utilization from other members of the Enterobacteriaceae into *E. coli* K12.

Materials and methods

The methods used followed Alaeddinoglu and Charles (1979) except when stated otherwise.

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Bacterial strains

These, together with their designations and genotypes, are listed in Table 1. Sources refer to workers from whose laboratories these strains were obtained.

Abbreviations and symbols

Cit$^+$:	Genes for citrate utilization.
Escherichia coli K12:	A laboratory strain of *E. coli*, which is commonly used in microbial genetics.
Plclr100 km:	A bacteriophage, which is commonly used to transfer genes amongst enteric bacteria.
gal:	A mutation in one of the galactose genes resulting in failure to grow on galactose.
lac:	A mutation in one of the genes for lactose utilization.
xyl:	A mutation in one of the xylose genes.
rbtc mtl:	a mutation in one of the genes for ribitol and mannitol utilization.
lpcR: (lipopolysaccharide)	A mutation in the genes for the synthesis of the bacterial cell wall.
F^1:	F prime plasmid (*E. coli* sex factors).
bio:	A mutation causing a requirement for biotin.
(PI^s):	Sensitivity or resistance to the phage PI.
tonA:	Sensitivity of resistance to the phage TI.
thr:	A mutation in threonine biosynthesis causing a requirement of threonine for growth.
ara:	A mutation in one of the genes for arabinose utilization.
leu:	A mutation in leucine biosynthesis causing a requirement of leucine for growth.
met:	A mutation in methionine biosynthesis causing a requirement of methionine for growth.
thi:	Thiamine requirement.
rpsL:	Resistance to streptomycin.
hsd or *hspRl:*	Gene for host restriction and modification of DNA.
tsx:	Resistance to phage T_6.
glpD:	Gene for L- and D-glycerophosphate dehydrogenase.
rel:	Regulation of RNA synthesis.
F$^-$	'Female' strain of *E. coli*.
Hfr:	'Male' strain of *E. coli* able to transfer chromosomal markers with high frequency.

Refer to Bachmann (1983) for a comprehensive list of the gene symbols used in *E. coli* K12 genetics.

Minimal medium

This was mineral Base E of Owens and Keddie (1969). Substances used as carbon and energy sources were added to the minimal medium at a concentration of 0.2% (w/v). Selective media were devised so that the parent strains or recipient strains did not grow but the recombinants did. For example, selection against Hfr donors was often made by adding streptomycin (200 mg/l) to the medium because Hfr strains were usually sensitive to streptomycin, or by omitting amino acids that the donors required. Selection against recipient bacteria was usually done by providing, as the only source of carbon and energy, a sugar, which the recipient strain did not use but which the donor strain did. These were done on solid media.

Chemicals

Tri-sodium citrate, citric acid, streptomycin, kanamycin, threonine, leucine, sodium lauryl sulphate, acridine orange, casamino acids, proteose peptone, glycine, L-serine, and all 120 auxanographic substances were obtained from Sigma (London), Surbiton Station Yard, Kingston, Surrey, U.K.

Lysogenization and transduction by Plclr100 km

These were as described by Goldberg, Render and Streicher (1974).

Preparation of suspensions of phage Plclr100 km

The phage confers kanamycin resistance on its lysogens, and this offers a means of selecting for lysogens. The lysogens grow well at 30°C but the prophage enters the lytic cycle when the lysogens are incubated at 42°C.

Strains lysogenic for *Plclr100 km* were streaked onto T_2 nutrient agar containing kanamycin (12.5 mg/l), and single colonies were then used to inoculate T_2 broth, containing kanamycin (12.5 mg/l). Incubation was at 30°C overnight. The bacteria were resuspended in T_2 broth and incubated at 30°C to a density of about 5×10^8 bacteria per ml. The culture was then transferred to a 100 ml Ehrlenmeyer flask with side arm and incubated with vigorous shaking at 42°C for 40 min to induce lysis. The flask was then transferred to a shaking water bath at 37°C and incubation continued untill lysis was complete (usually 1–1.5 h). The lysate was centrifuged at room temperature for 20 min to remove debris and unlysed bacteria, and the supernatant was then transferred to a McCartney bottle and kept at 4°C over a few drops of chloroform.

Selection of Plclr100 km *lysogens*

The recipient strain to be lysogenized was streaked on complete medium and single colonies were inoculated into 10 ml volumes of complete broth and incubated overnight. A 2 ml sample was pipetted into a 19 × 150 mm test-tube to which was added 0.1 ml $CaCl_2$ (0.1 M). Separate 0.1 ml samples of recipient bacteria and phage suspension were then spread separately on T_2 nutrient agar containing kanamycin (12.5 mg/l) as control. A 0.1 ml sample of phage *Plclr100 km* suspension was added to the remaining bacterial suspension to give a multiplicity of exposure of five. Incubation was at 30°C for 30 min. Samples of the heated and diluted suspension were spread on T_2 nutrient agar containing kanamycin (12.5 mg/l) and incubated at 30°C overnight. The colonies which developed on the plates were usually lysogens and this was confirmed by growing the bacteria at 42°C to see if they lysed.

Generalized transduction by phage Plclr100 km

Two-millilitre volumes, containing 5×10^8 bacteria/ml of recipient bacteria were distributed between four 19 × 100 mm centrifuge tubes. $CaCl_2$ (0.05 ml 0.1 M) was added. Phage suspension (1 ml) was added to the tubes. The suspension was incubated at 37°C for 20 min to allow phage adsorption and then the bacteria were resuspended in 1 ml volumes of minimal medium. Ten 0.1 ml samples from each tube were spread separately onto selective media in petri-dishes. Two sets of controls were plated out: phage-only controls to check that the phage suspension was not contaminated with bacteria; and phage-free bacterial suspension to check that the recipient bacteria did not give colonies on the selective medium.

Conjugation (interrupted mating experiments)

F^- and Hfr strains were separately grown in complete broth to a density of $2–3 \times 10^8$ cells/ml. A 0.5 ml aliquot of the Hfr culture was then added to the F^- culture to give a ratio of Hfr to F^- bacteria of 1 : 20 in the mating mixture. The mating mixture was incubated at 37°C and with gentle shaking. Samples were removed at intervals, agitated violently to separate the mating bacteria and plated on selective media.

Complete medium contains the following in g/l; Tryptone (oxoid) 10 g, yeast extract (DIFCO) 5 g, K_2HPO_4 3 g, KH_2PO_4 1 g, Glucose 5 g; T_2 nutrient agar contains the following in g/l: nutrient broth base (DIFCO) 8 g, NaCl 4 g, Agar 12 g.

Table 1. Bacterial strains used

Bacterial strains	Genotype	Source or reference
Salmonella typhimurium (SL3684)	*gal* E	Mojica-A
Klebsiella aerogenes (FG9)	*rbtc 101 1PcB*	Knott
Klebsiella pneumoniae (KP5007)	*gal bio hsp R1* (PI^s)	Dixon
Klebsiella aerogenes (16G)	Wild strain	Departmental strain
Enterobacter aerogenes (16B)	Wild strain	Departmental strain
Citrobacter freundii (16H)	Wild strain	Departmental strain
Escherichia coli K12	F$^-$ *thrl leu6 tonA21*	
5K	*rpsl thi-l hsdR514 rpsL thi*-1	Glover
WA802	F$^-$ *LacY galk2 galT22 hsdRs metB*	Wood
AB1621	F$^-$ *ara lac tsx gal rpsl xyl-mtl glpD thiA*	Adelberg *et al.* (1965)
KG1673	Hfr *thi-l*	
KL208	Hfr *relA1*	

Results

Enteric bacteria from the departmental culture collection and those obtained from other workers were streaked on base E medium with tri-sodium citrate as the sole carbon and energy source. The bacteria were SL3684, FG9, KP5007, 16G, 16B, 16H, 5K and WA802. All grew on citrate minimal medium with the exception of 5K and WA802. FG9, KP5007 and SL3684 were already sensisitive to the phage PI. Attempts made to lysogenize 16G, 16B and 16H with phage *Plclr100 km* (Goldberg *et al.*, 1974) as a preliminary to transduction were unsuccessful. PI lysates of SL3684, FG9, and KP5007 were prepared by the confluent lysis technique. The phage lysates obtained ($1.2–5 \times 10^{10}$ phage/ml) were used to transduce 5K and WA802. Strains 5K and WA802 were used because they are restrictionless mutants and would, therefore, not degrade incoming foreign DNA. Selection was made for Cit$^+$ transductants. As a positive control to measure the transducibility of a gene common to K12 and the donors, selection was made for Thr$^+$ and Met$^+$ for 5K and WA802, respectively. Thr$^+$ and Met$^+$ transductants occurred with a frequency of 1 per 10^6–10^8 phage and were scoreable after 3 days. SL3684 and FG9 gave six Cit$^+$ transductants each from 6×10^9 phage, while KP5007 gave two transductants from 1×10^{10} phage. The transductants were scoreable after 5 days. Apart from being Cit$^+$, transductants retained characters typical of 5K and WA802 showing that the Cit$^+$ genes were transferable to K12. Transductants were smallish in size: 2 mm although a normal Cit$^+$ colony is 4 mm in diameter after 3 days.

Ability to utilize citrate was not due to the presence of plasmids

Tests were made to see whether ability to utilize citrate was due to presence of plasmids. Cells of *E. coli* K12 were incubated with those of SL3684, FG9 and KP5007. The K12 recipients in the different experiments were AB1621, 5K and WA802, selection being made for Cit$^+$ transconjugants resistant to streptomycin.

The Cit$^+$ character was not transferred in these tests, nor was it lost from the donor strains and the K12 Cit$^+$ hybrids on treatment with sodium lauryl sulphate (Tomoeda *et al.*, 1968) or acridine orange (Salisbury *et al.*, 1972).

Tests to reveal whether K12 Cit$^+$ strains needed extra growth factors

Transduction of the Cit$^+$ character into K12 was unsatisfactory in two ways. Firstly, Cit$^+$ transductants of K12 grew less well than the three Cit$^+$ donors when streaked on citrate minimal medium. Transductants from FG9 exhibited the poorest rate or growth. Secondly, the frequency of transduction of the Cit$^+$ character was very low.

Auxanographic tests (Lederberg, 1946) were made to see whether any single substance

improved growth on citrate. About 120 substances were tested. Casamino acids, proteose peptone, glycine and L-serine improved growth on citrate minimal medium. Incorporation of glycine and L-serine (40 mg/l) led to vigorous growth of K12 Cit^+ hybrids on citrate minimal medium. Incubation of Cit^+ strains on citrate minimal medium containing Na^+ (10 mM), added as sodium sulphate was done, since O'Brien and Stern (1969) found that sodium was required for the anaerobic growth of *Aerobacter aerogenes* on citrate. Incorporation with Na^+ had no effect on the growth of Cit^+ strains.

Preliminary mapping with Hfr strains

Using one each of the K12 Cit^+ hybrids as donor, Hfr strains KL208 and KG1673 were transduced to Cit^+. In time-of-transfer experiments KG1673 with point of origin around 6 min did not transfer its Cit^+ region, whereas KL208 with point of origin around 3 min transferred its Cit^+ region after 20 min giving a map position of 6–10 min.

Citrate utilization is temperature sensitive

In the course of this investigation, it was found that citrate utilization by donor strains and their K12 Cit^+ hybrids does not occur at 42°C. Strains grew better on citrate at 30°C instead of 37°C. Best results for transduction were also obtained at 30°C.

Discussion

Results from the experiments described show that the genes for citrate utilization can be transduced into *E. coli* from other members of the Enterobacteriaceae. The Cit genes from the three donors used all took chromosomal positions in *E. coli* K12. This probably reflects the presence of some homology between the Cit region in the donor strains and the corresponding region in *E. coli*.

Growth of K12 Cit^+ hybrids was poor suggesting that the acquisition of the Cit genes might have caused some physiological or genetical disruption in *E. coli* resulting in the requirement for extra growth factors. The finding that the incorporation of glycine and L-serine (which are related amino acids) improved on citrate is interesting, but the reason for this effect is still unknown. It is possible that the acquisition of the citrate genes affects the biosynthesis of both amino acids.

Citrate utilization is a very important criterion in the laboratory diagnosis of many enteric infections. Plasmids conferring Cit^+ ability have been reported in *E. coli* (Sato *et al.*, 1978). Hall (1982) discovered a mutant strain of *E. coli* K12 that utilizes citrate as a source of carbon and energy. Apart from mutating to Cit^+ or acquiring Cit^+ plasmids, the present study shows *E. coli* can acquire chromosomal genes, which confer the Cit^+ phenotype extraneously. All these go on to prove that some caution is necessary before dismissing Cit^+ bacteria as non-*E. coli*.

References

Adelberg, E.A., Mandel, M. & Chein Ching Chen, G. (1965) Optimal conditions for mutagenesis by N-methyl – – N′ – nitro – Nitrosoguanidine in *E. coli* K12. *Biochem. Biophys. Res. Commun.* **18**, 788–795.

Alaeddinoglu, N.G. & Charles, H.P. (1979) Transfer of a gene for sucrose utilization into *Escherichia coli* K12, and consequent failure of expression of genes for D-serine utilization. *J. Gen. Microbiol.* **110**, 47–59.

Bachmann, B.J. (1983) Linkage map of *Escherichia coli* K-12. Edition VII. *Bact. Rev.* **40**, 116–167.

Edwards, P.R. & Ewing, W.H. (1972) *Identification of Enterobacteriaceae* 3rd edn. Burgess Publishing, Minneapolis.

Goldberg, R.B., Render, R.A. & Streicher, S.L. (1974) Direct selection for Pl-sensitive mutants of enteric bacteria. *J. Bacteriol.* **118**, 810–814.

Hall, B.G. (1982) Chromosomal mutation for citrate utilization by *Escherichia coli* K12. *J. Bacteriol.* **151**, 269–273.

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

Ishiguro, N. & 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. Hygiene* **83**, 331–344.

Lara, F.J.S., & Stokes, J.L. (1952) Oxidation of citrate by *Escherichia coli*. *J. Bacteriol.* **63**, 415–420.

Lederberg, J. (1946) Studies in bacterial genetics. *J. Bacteriol.* **52**, 503.

Lutgens, M. & Gottschalk, G., (1980) Why a co-substrate is required for anaerobic growth of *Escherichia coli* on citrate. *J. Gen. Microbiol.* **119**, 63–70.

O'Brien, R.W. & Stern, J.R. (1969) Requirement for sodium in the anaerobic growth of Aerobacter aerogenes on citrate. *J. Bacteriol.* **98**, 388–393.

Owens, J.D. & Keddie, R.M. (1969) The nitrogen nutrition of soil and herbage coryneform bacteria. *J. Appl. Bacteriol.* **32**, 338–347.

Salisbury, V., Hedges, R.W. & Datta, N. (1972) Two modes of curing transmissible bacterial plasmids. *J. Gen. Microbiol.* **70**, 443–452.

Sato, G., Asagi, M., Oka, C., Ishiguro, N. & Terakado, N. (1978) Transmissible citrate-utilizing ability in *Escherichia coli* isolated from pigeons, pigs and cattle. *Microbiol. Immunol.* **26**, 357–360.

Tomoeda, M., Inzuka, M., Kubo, N. & Nakamura, S. (1968) Effective elimination of drug resistance and sex factors in *E. coli* by sodium dodecyl sulphate. *J. Bacteriol.* **95**, 1078–1089.

Woodward, M.J. & Charles, H.P. (1983) Polymorphism in *Escherichia coli*: *rtl* and *gat* regions behave as chromosomal alternatives. *J. Gen. Microbiol.* **129**, 75–84.

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