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Cloning and restriction mapping of the L-sorbose utilization genes from a clinical isolate of *Escherichia coli* (1)

D. K. OLUKOYA

Genetics Division, National Institute for Medical Research, Yaba, Lagos.

Summary

About 30% of clinical isolates of *Escherichia coli* tested utilized L-sorbose as a carbon and energy source. *Escherichia coli* K-12 is naturally sorbose negative. The genes for L-sorbose utilization (*sor*$^+$) is being used as a prototype for studying variable genes amongst bacterial pathogens. The *sor*$^+$ genes from seven isolates were transferable to *E. coli* K-12. The (*sor*$^+$) region was cloned into plasmid pBR322 to give pDOK1. Plasmid pDOK1 is approximately 20kb in size. A restriction endonuclease map of pDOK1 is presented.

Résumé

Environ 30% des isolements cliniques de *Escherichia coli* examinés out utilisé la sorbose-L comme carbone et source d'énegie. L'*Escherichia coli* K-12 est naturellement negative a la sorbose. Nous utilisons les gènes pour elutilisation de sorbose-L (*sor*$^+$) comme un prototype dans l'etude de divers genes parmi les pathogènes bacteriels. Les genes *sor*$^+$ de sept isolements out ete transferables a *E. coli* K-12. La region *sor*$^+$ a ete clonée en plasmide pBR322 pour donner pDOK1. Une plasmide pDOK1 est approximativement de dimension 20kb. Un schéma de restriction endonuclease de pDOK1 est représentée.

Introduction

Escherichia coli is an important micro-organism in Medical Microbiology. The need for rapid identification of pathogens necessitates a system of classification in which particular traits are given importance. For example, the ability to use citrate as a sole source of carbon and energy, the ability to produce H_2S and the ability to ferment lactose, differentiate *Salmonella* from *Escherichia*. Traits commonly present or absent are valuable in the identification of different species while those showing variable expression may be used for differentiation. The variability *E. coli* strains to metabolize L-sorbose is well documented and this character is now routinely used in biotyping. A high proportion of sorbose-utilizing *E. coli* has been reported among isolates from clinical specimens[1,2]. Genetic studies of the variable traits of pathogenic bacteria will go a long way in improving their classification and identification.

The present study describes the cloning and mapping of a variable character, that is, L-sorbose utilization in clinical isolate of *E. coli*.

Materials and methods

Bacteriology

The procedure used for collection of specimens, identification, serotyping, test for enterotoxins and test for invasiveness have already been described[3].

Genetic methods

For example, transduction and conjugation followed[4] except when stated otherwise.

Growth media

MacConkey medium contained Difco Bacto MacConkey agar base and 1% L-sorbose or maltose instead of lactose, as necessary. Tetracycline and ampicillin were present at 25 μgml^{-1}, when required.

Bacterial strains

These are listed below.

* Correspondence: D. K. Olukoya, Genetics Division National Institute for Medical Research, P.M.B. 2013, Yaba, Lagos.

Strain	Genotype	Reference
DHI	F⁻ *recA1 endA1 gyrA96, thi-1 hsdR17* (r_k^-, m_k^+) *supE44 relA1*	[9]
HB 101	F⁻ *hsds20* (r_B^-, m_B^-) *recA13 ara14 pro A2 lacY1, galk2, rpsL20 xyl-s mtl supE44*	[10]
PA505MP11	F⁻ *argH metA malB rpsL*	[11]

Cloning techniques

Chromosomal DNA was prepared as described by Marmur [5] except that cells were lysed with lysozyme and Triton-X100 [6]. Plasmid DNA was prepared as described by [7] and [8]. Restriction enzymes and T4 DNA ligase were obtained from BRL and were used according to the recommendation of the manufacturers. Plasmid pBR322 DNA (BRL) and chromosomal DNA from the strain, clinical isolate DOK25 *sor*$^+$ were separately digested with Eco RI and then mixed at a plasmid DNA concentration of 20mgml^{-1} and about five times as much chromosomal DNA. Ligation with T4 DNA ligase was at 15°C for 18hr. Transformation was as described by [8]. The transformed cells were selected on sorbose-MacConkey medium containing ampicillin (25 μgml^{-1}) or tetracycline (25 μgml^{-1}).

Electrophoresis was carried out on 0.7 - 1% agarose horizontal slab gels in Tris/borate buffer. DNA fragments were visualised with ethidium bromide (0.5 μgml^{-1}) and compared with those of known sizes obtained from Hind III or Eco RI digestion of lambda DNA.

Results

Transfer of the genes for L-sorbose utilization

Of 397 enteric pathogens isolated, 92 were accepted as *E. coli* on the basis of bacteriological tests. Each was Gram-negative, methyl red positive, Voges-Proskauer negative, citrate negative, KCN negative, lactose positive, indole positive, H_2S negative and Eijkman positive. On streaking the *E. coli* strains on L-sorbose minimal medium and incubating for 21 days, 30 were found to use sorbose as a sole carbon and energy source. Ther region for L-sorbose utilization designated as *sor* region was transferred by transduction into an *E. coli* K12 strain PA505MPE11. Seven of the *E. coli* isolates transferred their *sor* genes. One of the K12 *sor*$^+$ hybrid designated DOK 25 was used in the cloning experiment.

Cloning and restriction mapping of the sor region

Chromosomal DNA from the clinical isolate DOK 25 and plasmid DNA of pBR322 were digested with Eco RI and after ligation, the mixture was used to transform DH1 and HB101. Selection was for sorbose fermenting colonies or sorbose- MacConkey plates containing either ampicillin or tetracycline. A single sorbose utilizing ampicillin and tetracycline resistant colony was purified and analysed. The strain (DHI) carried a plasmid of about 20kb now designated pDOK1. The plasmid was able to complement all point mutations in the *sor*$^+$ genes. A restriction map of pDOK1 is presented (Figure 1). The 15.4kb, Eco RI insert also has cleavage sites for PvuII (2) Pst I (1) Eco R V (4), Xmn I (6), Hpa I (4), Nru I (6), Sst I (1).

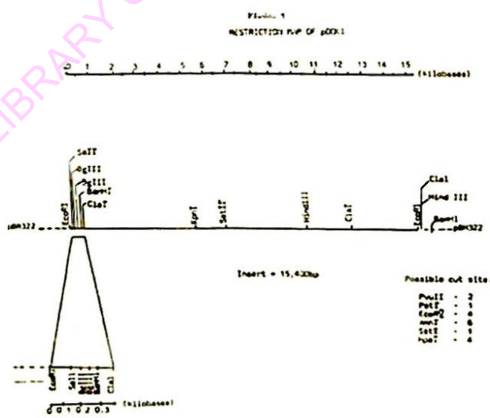

Fig. 1: Restriction map of pDOK 1

Discussion

The ability to utilize L-sorbose as a carbon and energy source is useful in the biotyping of *Escherichia coli.* Since biotyping is an epidemiological tool, genetic studies into biotyping markers is sure to yield dividends.

About 30% of the isolates of *E. coli* tested utilized L- sorbose as a source of carbon and energy. Certain questions are then pertinent. Why do strains vary in their ability to utilize certain substances as carbon and energy source?. Do negative strains for

particular characters possess defective genes or are they devoid of all sequences of those genes? Do negative strains possess alternative characters in nature? A necessary prelude to answering these questions is the cloning of a variable gene. L-sorbose utilization has been used as a prototype of a variable gene in this work. The precise methods of hybridization and ultimately DNA sequencing can now be used to investigate whether *sor⁻ E. coli* strains possess defective *sor* genes or lack them altogether. Such information is essential for evaluating the use of variable traits in the biotyping of pathogenic bacteria. Moreso since bacteria may undergo genetic variation during epidemic spread thereby affecting typing characters. It is also worth pointing out that studies of this nature led to the discovery that *Shigella dysenteriae* is phenotypically *Lac⁻* but contains functional *Lac I* and *Lac Z* genes [12].

Future work will concentrate on the development of a probe which would be useful in detecting *sor⁺ E. coli* from clinical specimen and would also be used to test whether *sor⁻ E. coli* possess defective *sor* genes or lack them altogether.

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