ELSEVIER

FEMS Microbiology Letters 143 (1996) 77–82

FEMS MICROBIOLOGY LETTERS

Comparative methodology to investigate the presence of *Escherichia coli* K-12 strains in environmental and human stool samples

Gabriele Blum [a,*], Inge Mühldorfer [a], Peter Kuhnert [b], Joachim Frey [b], Jörg Hacker [a]

[a] *Institut für Molekulare Infektionsbiologie, Röntgenring 11, 97070 Würzburg, Germany*
[b] *Institut für Veterinär-Bakteriologie, Länggass-Str. 122, 3001 Bern, Switzerland*

Received 21 May 1996; revised 24 July 1996; accepted 24 July 1996

Abstract

This study was undertaken to evaluate the specificity and efficiency of different methods to detect *Escherichia coli* K-12 strains. Another aim was to determine the frequency of *E. coli* K-12 strains among wild-type *E. coli* isolates from different sources. The detection of K-12 strains was performed both genotypically by K-12 specific polymerase chain reaction (PCR) and on the basis of phenotypical tests. In addition, the genome structures of *E. coli* strains were characterized by pulsed-field gel electrophoresis (PFGE). The most specific results could be obtained by the genotypical tests PCR and PFGE as well as by the K-12 specific phage assay. In total, 131 stool and 95 water isolates as well as 14 K-12 derivatives were examined by the different methods. No *E. coli* K-12 strains were detected among the wild-type isolates.

Keywords: Environmental isolate; Stool isolate; *Escherichia coli* K-12; K-12 specific primer; Pulsed-field gel electrophoresis

1. Introduction

Escherichia coli K-12 strains are variants of the species *Escherichia coli*, which are normal inhabitants of the intestinal flora of humans and animals. The original wild-type *E. coli* K-12 strain was isolated from the feces of a convalescent diphtheria patient in 1922 at Stanford University [1] and was recovered by Gray and Tatum [2] and soon after by Lederberg and Tatum [3] at the beginning of the 1940s. They used this original K-12 strain in order to study recombination in bacteria and constructed a broad spectrum of auxotrophic variants by treating this K-12 strain with X- and UV-irradiation [4]. Since then the wild-type *E. coli* K-12 strain and its derivatives have been extensively used in many laboratories. These strains have often been applied as host strains in recombinant DNA technology and in biotechnological research. Most of the *E. coli* K-12 strains show a mutation within the *rfb* gene cluster which affects the assembly of the O-antigen and therefore represent the prototype of a safe and non-pathogenic strain. This mutation is due to an IS5 element insertion into the *rfb* determinant, encoding the outer part of the lipopolysaccharide of

* Corresponding author. Tel.: +49 (931) 312581; Fax: +49 (931) 571954.

PII S0378-1097(96)00293-5

E. coli (*rfb*-50 mutation). Therefore, *E. coli* K-12 strains have a complete core structure but an incomplete O antigen and show a rough phenotype [5,6]. Recently, Kuhnert and co-workers [7] have developed a rapid and accurate PCR-based identification system for *E. coli* K-12 strains based on the insertion of the IS5 element into the *rfb* gene cluster.

The present study was undertaken to compare the efficiency and discriminatory abilities of different methods to identify and characterize *E. coli* K-12 strains. For this purpose, the microbial flora of healthy volunteers and of natural residential surface water habitats was investigated for the presence of *E. coli* K-12.

2. Materials and methods

2.1. Bacterial strains

In total, 226 wild-type *E. coli* strains, isolated from the feces of healthy volunteers (n=131) and from surface water samples (n=95), were analyzed following growth in LB media. The stool samples were obtained from students or from the technical and scientific staff of the University of Würzburg, Germany. The water samples were collected in Germany from surface water of the Elbe river (region of Wernigerode), surface water originating from the Harz mountains and from water ponds in Sachsen-Anhalt (region of Magdeburg). In addition, the following 14 *E. coli* K-12 strains were investigated: *E. coli* DH5α, Sm10λpir, C600, HB101, EN99, LE392, 5K, 35, K-12 wild-type, JM 109, WK6, DH1, J53, 678-54.

2.2. Physiological tests and agglutination reactions

The *E. coli* strains were tested for their ability to utilize lactose on MacConkey agar plates containing 1% lactose. To determine the presence of auxotrophic strains, growth on minimal agar plates enriched with 0.2% glucose and 0.004% thiamine was analyzed. Rough variants were detected by performing an agglutination assay in 3.5% NaCl [8].

2.3. ELISA and phage assays

A whole-cell ELISA based on the technique of Voller et al. [9] was carried out. The monoclonal antibody mAb 9G11 which has been raised against the K-12 core antigen and was generously provided by B. Jann, Institut für Immunologie, Freiburg, Germany was applied. K-12 specific phage U3 was used to identify K-12 specific core antigens [10].

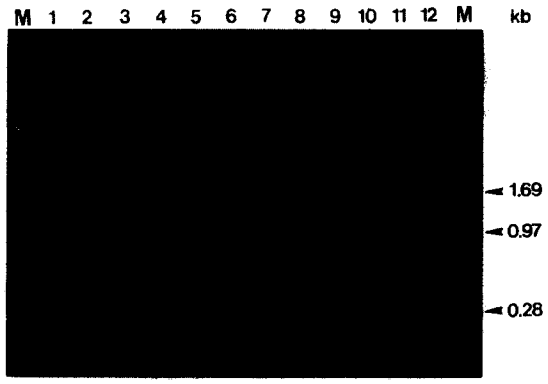

Fig. 1. PCR results for nine selected *E. coli* water and stool isolates and three *E. coli* K-12 control strains. Lanes 1–6, water isolates WT632, WT636, WT644, WT655, WT666, W7792. Lanes 7–9, stool isolate nos. 9, 83 and 86. Lanes 10–12, *E. coli* K-12 control strains K-12 WT, DH5α and HB101. Lanes M, SPPI-DNA cleaved with *Eco*RI was used as DNA size marker.

2.4. Oligonucleotide primers and polymerase chain reaction (PCR)

The presence of K-12 strains was examined using two oligonucleotide primer sets that amplify regions of *orf*264 specific for *E. coli* K-12 strains. One pair amplifies a 1.69 kb internal fragment of the *orf*264 (primers K12-R and K12-L). The second primer pair is dependent on the presence of the 3′ end of *orf*264 and an IS5 sequence (K12-R and K12IS-L) and the product is 0.97 kb in size. As an internal control, PCR amplification of a segment of the *pal* gene (0.28 kb) was used (primer pair ECPAL-R and ECPAL-L), which indicates the presence of *E. coli* strains. Conditions for the PCRs were chosen as described in [7]. PCR was performed with a thermocycler 60 apparatus from Biomed, Theres, Germany. Synthetic oligonucleotides were obtained from MWG Biotech, Ebersberg, Germany. PCR products were analyzed in 1% agarose gels. Aliquots of 5 μl of both K-12 specific PCRs and the control PCR were loaded on the same slot.

2.5. Pulsed-field gel electrophoresis (PFGE)

For the analysis by PFGE, genomic DNA was prepared in agarose plugs and cleaved with the restriction enzyme *Xba*I, was obtained from Pharmacia LKB (Freiburg, Germany). PFGE was carried out with the CHEF DrII system from Bio-Rad (München, Germany) in 0.5×TBE buffer. Conditions for running the gel were chosen as described in [11], and are indicated in the legend to Fig. 2.

3. Results

3.1. Phenotypical characterization of E. coli K-12 strains and of E. coli stool and environmental isolates

In order to compare the physiological properties of K-12 variants with those of wild-type isolates, 14 *E. coli* K-12 strains and a total of 226 stool and environmental strains isolated from healthy volunteers and from residential water habitats were investigated. As shown in Table 1, K-12 strains differ significantly from the stool and environmental isolates in their ability to utilize lactose. Only 28.6% of the K-12 strains but 93.1% of the stool isolates and 100% of the environmental isolates were able to ferment lactose. Concerning the presence of auxotrophic mutants, only 0.8% of the stool isolates and 1.1% of the environmental strains were auxotrophic. In contrast, 57.1% of the K-12 strains were auxotrophic mutants. While all of the *E. coli* K-12 control strains showed a positive agglutination reaction in 3.5% NaCl, indicative of the presence of rough variants, only 19.9% of the stool isolates and 8.4% of the environmental samples were identified as rough strains.

3.2. Detection of E. coli K-12 by ELISA and lysis with phage U3

The different isolates were also investigated for the presence of the K-12 specific core antigen. The monoclonal antibody mAb 9G11, which had been raised against the K-12 core antigen, was used. While all K-12 variants showed a positive reaction with mAb 9G11, 2.3% of the human stool samples and 6.3% of the water isolates were also positive (see Table 1). While all of the *E. coli* K-12 strains were lysed by the phage U3, none of the 226 stool and environmental strains showed a reaction following infection with phage U3.

3.3. Determination of E. coli K-12 specific sequences by PCR and pulsed-field gel electrophoresis

Following the preselection for K-12 specific sequences by phenotypical methods, genotypical screening was performed by using PCR and PFGE technology. All the 226 stool and environmental *E. coli* wild-type isolates as well as the 14 *E. coli*

Table 1
Phenotypical and genotypical investigation of *E. coli* K-12 and wild-type strains

Test systems used	K-12 strains n=14 (100%)	Human stool isolates n=131 (100%)	Environmental water isolates n=95 (100%)	K-12 specificity [b]	Efficiency[c]
Utilization of lactose on MacConkay agar plates	4 (28.6%)	122 (93.1%)	95 (100%)	+	+++
Growth on minimal agar plates	6 (42.9%)	130 (99.2%)	94 (98.9%)	+	+++
Agglutination in 3.5% NaCl	14 (100%)	26 (19.9%)	8 (8.4%)	+	+++
ELISA with mAb 9G11	14 (100%)	13 (2.3%)	6 (6.3%)	++	++
K-12 specific phage U3	14 (100%)	0 (0%)	0 (0%)	+++	++
K-12 specific primers	14 (100%)	0 (0%)	0 (0%)	+++	+++
K-12 specific PFGE pattern	14 (100%)	0[a] (0%)	0[a] (0%)	+++	+

[a]Only strains with a positive reaction in ELISA were analyzed.
[b]+, ++, +++ describe the degree of specificity of the various test systems for K-12 strains.
[c]The term efficiency qualifies the various test systems with respect to both fastness and practicability. The various degrees of efficiency are indicated by +, ++, +++.

K-12 control strains were analyzed for the presence of a 1.69 kb segment of *orf*264, located within the *rfb* gene cluster, which flanks the insertion locus of IS5 in *E. coli* K-12 strains. In contrast to the outcome of the phenotypical tests and in agreement with phage lysis, no K-12 specific sequences were detectable among the investigated stool and water isolates (see Table 1), while all of the *E. coli* K-12 control strains showed a positive reaction. The PCR products of nine stool and water isolates which reacted with the monoclonal antibody mAb 9G11 were compared to the PCR products of K-12 strains by using conventional agarose gel electrophoresis (Fig. 1). While the *E. coli* K-12 strains K-12 WT, HB101 and DH5α showed K-12 specific PCR products of 1.69 and 0.97 kb, no amplification products of the same sizes were detected in either of the stool or water isolates. A specific 0.28 kb segment of the *pal* gene, which is conserved in *E. coli* and closely related bacteria, and which encodes the peptidoglycan-associated lipoprotein, served as a control for the PCR reactions. The 0.28 kb fragment was amplified in all of the tested strains, indicating that the *pal* gene is present in the stool and water isolates.

In order to confirm the discriminatory ability of the PCR technique, the stool and water isolates which showed a positive reaction with the monoclonal antibody mAb 9G11 by ELISA, were further analyzed by PFGE. The *Xba*I restriction patterns of the genomic DNA of the nine stool and water samples were compared to K-12 specific *Xba*I macrorestriction patterns of three selected K-12 strains. As shown in Fig. 2, the PFGE patterns of the K-12 strains are very similar, especially in the size range of 20–360 kb, but no characteristic K-12 profiles could be detected in any of the wild-type isolates.

4. Discussion

In this study we aimed at investigating the discriminatory abilities of different methods to identify *E. coli* K-12 derivatives. As *E. coli* K-12 strains are so-called safety measurements, frequently used in molecular microbiology, another aim was to determine whether they can survive and spread in the environment and within the human population. Therefore, 226 *E. coli* strains of human and environmental origin were analyzed for the presence of *E. coli* K-12 variants. The strains were isolated from stool samples of healthy volunteers and from surface waters which were anthropologically influenced by communal sewage. Recently, these strains as well as 14 *E. coli* K-12 strains have already been tested

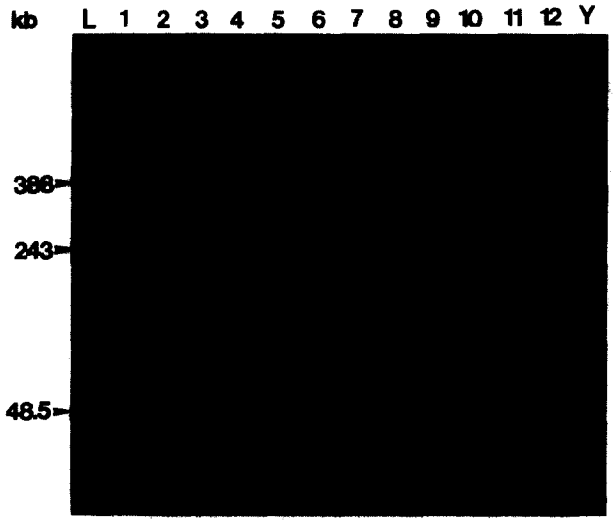

Fig. 2. *Xba*I restriction pattern of the genomic DNAs of nine selected *E. coli* water and stool isolates and three *E. coli* K-12 control strains. Lanes 1–6, water isolates WT632, WT636, WT644, WT655, WT666, W7792. Lanes 7–9, stool isolate nos. 9, 83 and 86. Lanes 8–10, *E. coli* K-12 control strains K-12 WT, HB101 and DH5α. λ-concatemers (L) and yeast chromosomes (Y) were used as DNA size markers. PFGE was carried out for 24 h with increasing pulse times from 5 to 50 s.

for the presence of extraintestinal and intestinal virulence factors [12]. In contrast to *E. coli* wild-type strains, K-12 strains are characterized by genetic defects which allow their detection. Therefore, the K-12 strains as well as the stool and water isolates were subjected to physiological tests like utilization of lactose and growth on minimal agar plates, to agglutination tests with 3.5% NaCl and to infection with the K-12 specific phage U3 [8,10]. The results indicate that the physiological test systems as well as the agglutination assay are useful tools to preselect for the presence of *E. coli* K-12 strains but that they are not sufficient to ensure the specific detection of *E. coli* K-12.

In contrast, K-12 strains could be specifically detected by phage U3. Another specific test system should be the ELISA using the monoclonal antibody mAb 9G11, which had been raised against the core antigen of *E. coli* K-12. While all of the 14 *E. coli* K-12 control strains were efficiently detected with this antibody, the positive results obtained for 2.3% of the stool and 6.3% of the environmental isolates could not be confirmed by PCR methodology and pulsed-field gel electrophoresis. The false positive results obtained by ELISA might have been due to crossreactions of the monoclonal antibody mAb 9G11 with the epitopes of other rough variants present among the stool and water isolates. The most specific results were obtained by the already mentioned assay using phage U3 and by PCR using K-12 specific primers [7]. This PCR is based on the insertion of an IS5 element in the *orf*264, which encodes the enzyme rhamnose transferase on the *rfb* gene cluster of *E. coli* K-12 strains. The PCR method is one of the easiest and fastest typing methods, because the template DNA does not have to be of high molecular mass or highly purified and only nanogram quantities of DNA are required. The advantage of PCR is that it can be used for an efficient and sensitive high-throughput typing. The discriminatory potential of the PCR method was concordant with macrorestriction typing by pulsed-field gel electrophoresis. Neither PCR nor pulsed-field gel electrophoresis characterized any of the stool and environmental isolates as K-12 strains. Macrorestriction typing is labor-intensive because it requires the preparation of high molecular mass DNA and its purification, however, it is highly discriminative. Whereas the PCR method can be used for identification of K-12 variants, PFGE is helpful for accurate strain typing and can be used for distinguishing between single K-12 strains (Fig. 2).

In summary, the study revealed that no *E. coli* K-12 strains were present among the investigated stool and environmental isolates. While the assay employing the K-12 specific phage U3 and the PCR-based method allowed a rapid and accurate identification of *E. coli* K-12 derivatives, the physiological test systems as well as the agglutination assay and the ELISA were shown to be only useful for preselecting for the presence of *E. coli* K-12 strains. Pulsed-field gel electrophoresis was demonstrated to be of great value for the macrorestriction examination of representative isolates and to be the most powerful tool to discriminate between single isolates.

Acknowledgments

The authors thank Uschi Wallner for expert technical assistance. This work was supported by grant BEO 21-0319458A from the BMFT and by a grant from the Bayerische Forschungsstiftung (FORBIOSICH, F15).

References

[1] Lederberg, J. (1951) Genetic studies with bacteria. In: Genetics in the 20th Century (Dunn, L.C., Ed.), pp. 263–289, Macmillan, New York.

[2] Gray, C.H. and Tatum, E.L. (1944) X-ray induced growth factors deficiencies in bacteria. Proc. Natl. Acad. Sci. USA 30, 404–410.

[3] Lederberg, J. and Tatum, E.L. (1946) Gene recombination in *Escherichia coli*. Nature 158, 558.

[4] Tatum, E.L. and Lederberg, J. (1947) Gene recombination in the bacterium *Escherichia coli*. J. Bacteriol. 53, 673–684.

[5] Prehm P., Schmidt, G., Jann, B. and Jann, K. (1976) The cell-wall lipopolysaccharide of *Escherichia coli* K-12. Eur. J. Biochem. 70, 171–177.

[6] Liu, D. and Reeves, R. (1994) *Escherichia coli* K12 regains its O antigen. Microbiology 140, 49–57.

[7] Kuhnert, P., Nicolet, J. and Frey, J. (1995) Rapid and accurate identification of *Escherichia coli* K-12 strains. Appl. Environ. Microbiol. 61, 4135–4139.

[8] Leinonen, M. (1985) Serological methods for the study of bacterial surface antigens. In: Enterobacterial Surface Antigens: Methods for Molecular Characterization (Korhonen,

T.K., Dawes, E.A. and Mäkelä, P.H., Eds.), pp. 179–206, Elsevier, Amsterdam.

[9] Voller, A., Bidwell, D. and Bartlett, A. (1980) ELISA. In: Manual of Clinical Immunology (Rose N.R. and Friedman H., Eds.), pp. 359–371, ASM, Washington, DC.

[10] Watson, E. and Paigen, K. (1971) Isolation and characterization of an *Escherichia coli* bacteriophage requiring cell wall galactose. J. Virol. 8, 669–674.

[11] Blum, G., Schmittroth, M. and Hacker, J. (1995) *Escherichia coli* K-12: Herkunft, Nachweiskriterien und Ausbreitung. Biospektrum 4, 11–16.

[12] Mühldorfer, I., Blum, G., Donohue-Rolfe, A., Heier, H., Ölschläger, T., Tschäpe, H., Wallner, U. and Hacker, J. (1996) Characterization of *Escherichia coli* strains isolated from environmental water habitats and from stool samples of healthy volunteers. Res. Microbiol., in press.