Indian Journal of Biotechnology
Vol 9, July 2010, pp 298-301

Genotyping by ERIC-PCR of *Escherichia coli* isolated from bovine mastitis cases

Vivek Prabhu[1], S Isloor[1]*, M Balu[2], V V S Suryanarayana[3] and D Rathnamma[1]

[1]Department of Microbiology, Veterinary College, KVAFSU, Hebbal, Bangalore 560 024, India
[2]Project Directorate on Animal Disease Monitoring and Surveillance, Hebbal, Bangalore 560 024, India
[3]Molecular Virology Laboratory, Indian Veterinary Research Institute, Hebbal, Bangalore 560 024, India

Received 1 September 2009; revised 24 December 2009; accepted 25 February 2010

Mastitis is an important problem in dairy farms and pathogen *Escherichia coli* has a world-wide importance. In the present study, authors have shown that *E. coli* strains isolated from bovine mastitis cases could be differentiated using a PCR with enterobacterial repetitive intergenic consensus sequences (ERIC) primers. In all, 40 strains of *E. coli* from bovine mastitis cases were subjected for ERIC-PCR. Of these, 37 showed amplicons ranging from 350 to >3000 bp. The PCR profile generated showed polymorphism in 37 strains. An intense amplicon of 1300 bp was seen in all the strains, except *E. coli* O27 (code M10) and O69 (M33). Based on ERIC-PCR profiles, of 37 *E. coli* strains, 22 were found to be distributed among 4 genotypes, whereas each of the remaining 15 strains showed unique genotypic pattern. The study emphasizes the utility of ERIC-PCR in intraserotype differentiation of strains based on their genotype and, thus, it is complimentary to serotyping. Furthermore, it was possible to differentiate strains of the same serotype into different genotypes. PCR amplification with ERIC primers was a fast and reliable method for differentiation and identification of *E. coli* strains. The advantage of this method compared to serotyping is the fact that different genotypes could be found even in strains within the same serotype or in untypable strains

Keywords: Bovine mastitis, *Escherichia coli*, ERIC-PCR, strain differentiation

Introduction

Mastitis is an important problem in dairy farms and pathogen *Escherichia coli* has a world-wild importance. Conventionally, several methods, such as, serotyping, phage typing, biotyping and colicin typing, have been developed for characterization of *E. coli.* A PCR with primers on repetitive sequences in the DNA can be performed to identify strains[1,2]. ERIC-PCR uses any combination of primers designed to the conserved enterobacterial repetitive intergenic consensus (ERIC) region in order to generate an electrophoretic banding pattern based on the frequency and orientation of ERIC sequences in a bacterial genome. The specific band pattern of amplified PCR products obtained using these sequences can be used to genotype the bacteria. This method to differentiate between *E. coli* strains is simple, fast and less expensive than serotyping/other typing methods.

In the present study, 40 *E. coli* isolates from bovine mastitis cases were subjected to ERIC-PCR. Out of 40, 37 strains showed amplicons ranging from 350 to >3000 bp and the profile revealed polymorphic DNA fragments. Thus, ERIC-PCR could be used to differentiate strains intra-serotypically based on their genotype and is complimentary to serotyping.

*Author for correspondence:
Tel: 91-80-23411483 (202); Fax: 91-80-23410509
E-mail: kisloor@rediffmail.com

Materials and Methods

Cultures

For the study, *E. coli* isolates from cases of bovine mastitis were used. These isolates were typed at Central Research Institute, Kasauli and maintained in the Department of Microbiology, Veterinary College, Bangalore as per the standard procedures.

Extraction of Genomic DNA and Genotyping by ERIC-PCR

The isolate cultures grown for 18 h in Tryptose soya broth (TSB) were used for extraction. The DNA was extracted using Spin genomic DNA extraction kit (M/s Bangalore Genie). PCR using ERIC primers was used to identify strains[2]. Two primers with the sequences ERIC-IR, CATTAGGGGTCCTCGA ATGTA, and ERIC-2, AGTAAGTGACTGGGGT GAGCG, were used to amplify repetitive sequences contained in the chromosomal DNA of *E. coli* isolates.

ERIC-PCR was carried with 25 µL of the PCR mixture comprised of 300 ng of *E. coli* DNA, 1 µL

(50 pmol) of each ERIC-IR and ERIC-2 primers (Bioserve Biotechnologies Pvt. Ltd., Hyderabad) and 0.5 µL (50 µ*M*) of each dNTPs, 2.5 µ L of 10× PCR assay buffer and 1 µL (3U) of *Taq* DNA polymerase (M/s Bangalore Genie). Filtered quartz water was added to the mixture to make a final volume of 25 µL. The reactions were carried out in 0.2 mL microcentrifuge tubes using a programmable thermalcycler (M/s Corbett Research, Germany) according to the following cycles: Initial denaturation at 94°C for 3 min, 40 cycles of denaturation at 94°C for 1min, annealing at 55°C for 1min and extension at 72°C for 5min. An additional cycle with an extension step of 10 min was included to complete the synthesis of unfinished products.

The amplicons were electrophoresed on 2% agarose gel, alongwith 500 and 100 bp DNA ladders, and then 6× gel loading dye containing 0.5 mg/mL of ethidium bromide was added. The images were captured using gel documentation system (Alphaimager, M/s Alpha Innotech Corp., USA) for further analysis.

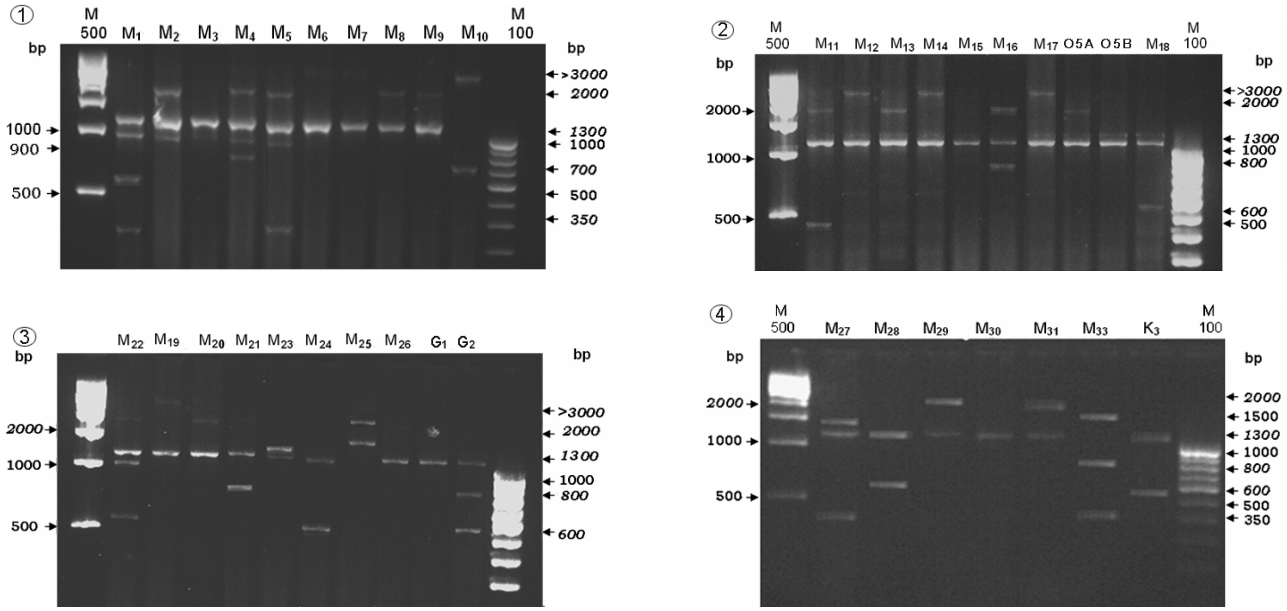
Analysis of ERIC Data

The ERIC banding patterns of 40 *E. coli* mastitis isolates were subjected to evaluation. The banding information was coded as 1 (band present) and 0 (band absent), and the binary data obtained was subjected for statistical analysis by squared Euclidean distance (SED) (Wards method) using the software STATISTICA. This enabled the plotting of dendrogram showing the level of genetic similarity among the strains.

Results and Discussion

Of 40 *E. coli* isolates subjected to ERIC-PCR, 37 isolates have amplicons ranging from 350 to >3000bp (Figs 1-4; Table 1). The generated banding profile showed highly polymorphic DNA fragments in these 37 isolates; 1, 2, 3, 4 and 5 amplicons were present in 8, 17, 9, 2 and 1 of isolates, respectively.

Binary data regarding DNA band profile was analyzed by SED using the software Statistica. This enabled the plotting of dendrogram showing the level of genetic similarity among the strains. Phylogenetic analysis of 37 *E. coli* isolates based on ERIC-PCR generated clusters A and B, and an unclustered group (Fig. 5). Cluster A contained 7 isolates and cluster B 17 isolates, while unclustered group contained 13 isolated. Cluster analysis further indicated wide variability among the isolates affiliated to the same serotype as they are included under different groups in phylogenetic tree. For example, isolates O59 (33), O59 (14), O59 (16) belong to cluster A, unclustered group and cluster B, respectively; O9 (36) belongs to cluster A and O9 isolate to unclustered group, whereas isolates O9 (13), O9 (11), O9 (3), O9 (4) and O9 (5) belong to cluster B (Fig. 5; Table 1). Thus,



Figs (1-4)—Agarose gel electrophoresis showing ERIC-PCR fingerprint of *E. coli* isolates: 1. Isolates M_1 to M_{10}; 2. Isolates M_{11}-M_{18}; Isolates K_1 to G_2; & 4. Isolates M_{27} to K_3 from bovine mastitis. (Lane M 100: 100 bp DNA ladder & lane M 500: 500 bp DNA ladder)

Table 1—*E.coli* serotypes v/s ERIC-PCR profile

Cluster	Code	Serotypes	Amplicons generated in ERIC-PCR (bp)											
			>3000	2000	1500	1400	1300	1000	950	800	750	600	450	350
A (7 isolates)	K3	O86 (40)	-	-	-	-	+	-	-	-	-	+	-	-
	M28	O59 (33)	-	-	-	-	+	-	-	-	-	+	-	-
	G2	UT6	-	-	-	-	+	-	-	+	-	+	-	-
	M24	UT7	-	-	-	-	+	-	-	-	-	+	-	-
	M10	O27	+	-	-	-	-	-	-	-	+	-	-	-
	M30	O9 (36)	-	-	-	-	+	-	-	-	-	-	-	-
	M29	UT8	-	+	-	-	+	-	-	-	-	-	-	-
Unclustered (13 isolates)	G1	O9	-	-	-	-	+	-	-	-	-	-	-	-
	M26	O120	-	-	-	-	+	-	-	-	-	-	-	-
	M23	UT5	-	-	-	+	+	-	-	-	-	-	-	-
	M22	UT4 (25)	-	+	-	-	+	+	-	-	-	+	-	-
	M20	O117	-	+	-	-	+	-	-	-	-	-	-	-
	M18	O59	-	-	-	-	+	-	-	-	-	+	-	-
	O5-B	O5	-	+	-	-	+	-	-	-	-	-	-	-
	M17	O147	+	-	-	-	+	-	-	-	-	-	-	-
	M15	O69	-	-	-	-	+	-	-	-	-	-	-	-
	M14	O59 (14)	+	-	-	-	+	-	-	-	-	-	-	-
	M7	UT2	-	-	-	-	+	-	-	-	-	-	-	-
	M6	O120	-	-	-	-	+	-	-	-	-	-	-	-
	M31	UT10	-	+	-	-	+	-	-	-	-	-	-	-
B (17 isolates)	M25	O_{155}	-	+	+	-	-	-	-	-	-	-	-	+
	M21	Rough	-	-	-	-	+	-	-	+	-	-	-	+
	O5-A	O5	-	+	-	-	+	-	-	-	-	-	-	-
	M16	O59 (16)	-	+	-	-	+	-	-	+	-	-	-	-
	M13	O9 (13)	-	+	-	-	+	-	-	-	-	-	-	-
	M12	O147	+	-	-	-	+	-	-	-	-	-	-	-
	M33	O69	-	-	+	-	-	-	-	+	-	-	-	+
	M27	O132	-	-	-	+	+	-	-	-	-	-	-	+
	M11	O9 (11)	-	+	-	-	+	-	-	-	-	-	+	-
	M9	O155	-	+	-	-	+	-	-	-	-	-	-	-
	M8	O132	-	+	-	-	+	-	-	-	-	-	-	-
	M3	O9 (3)	-	-	-	-	+	-	+	-	-	-	-	-
	M19	O147	+	-	-	-	+	-	-	-	-	-	-	-
	M4	O9 (4)	-	+	-	-	+	-	+	+	-	-	-	-
	M2	UT1	-	+	-	-	+	-	+	-	-	-	-	-
	M5	O9 (5)	-	+	-	-	+	-	+	+	-	-	-	+
	M1	O101	-	-	-	-	+	-	+	-	-	+	-	-

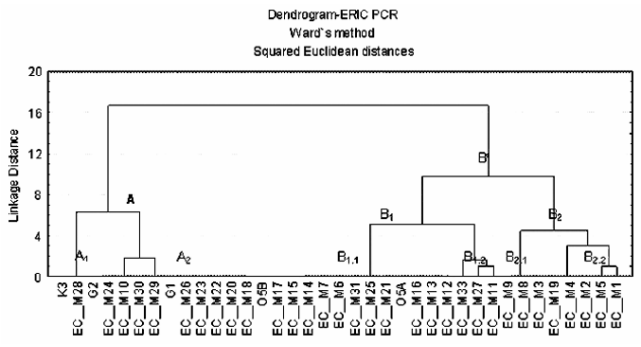


Fig. 5—Dendrogram based on ERIC-PCR profile of *E. coli*

ERIC-PCR differentiated strains of the same serotype in different genotypic patterns. Further, more than 83% (33/40) of isolates, used in the present study, were isolated from bovine mastitis cases of a single dairy farm. The diversity observed among these strains with respect to their serotype and genotype confirmed that cows in this dairy farm were infected with several strains. Thus, PCR amplification with ERIC primers in such cases was found to be a fast and reliable method for differentiation and identification of *E. coli* strains. The added advantage of this method compared to serotyping is the fact that different genotypes could be differentiated even in isolates within the same serotype or in untypable strains.

Phylogenetic analysis is a powerful tool to monitor the molecular epidemiology of bacteria and their

distribution in the geographical region. A PCR with primers on repetitive sequences in the DNA can be performed to identify the strains[1]. Chromosomal DNA from *Enterobacteriaceae* contains several repetitive sequences, such as, ERIC sequence[3,4]. These sequences have been used for fingerprinting *E. coli* strains from bovine mastitis cases for identification of isolates as well as for epidemiological investigations, and it is complimentary to serotyping[2]. Therefore, a combination of serotyping with genotyping is the best way to identify *E. coli* mastitis strains and is recommended for epidemiological studies.

Conclusion

In the present study, a comparison of DNA fingerprints of isolates of *E. coli* from episodes of mastitis was made in order to seek genotypes existing within herds. The identical or different genotypic patterns of *E. coli* strains observed could be used to determine the occurrence of recurrent clinical episodes of mastitis and to distinguish between such recurrent episodes of mastitis. The study emphasizes the utility of ERIC-PCR on intraseroptypic differentiation of strains based on their genotype and it is complimentary to serotyping. The dendrogram analysis indicated that *E. coli* strains were definable into clusters and sub-clusters, based on ERIC-PCR products.

Acknowledgement

The authors thank to the Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi for financial support to carry out the work.

References

1 Jayarao B M, Keane K A, Gillespie B E, Luther D A & Oliver S P, DNA fingerprinting: A new technique for identifying mastitis pathogens, *Proc Natl Mastitis Counc*, 32 (1993) 167-176.

2 Lipmann L J A, Nijs A, Lam T J G M & Gaastra W, Identification of *E. coli* from cows with clinical mastitis by serotyping and DNA polymorphism patterns with REP and ERIC primers, *Vet Microbiol,* 43 (1995) 13-19.

3 Versalovic J, Koelults T & Lupski J R, Distribution of repetitive DNA sequences in eubaacteria and application of fingerprinting in bacterial genomes, *Nucleic Acids Res,* 19 (1991) 6823-6831.

4 Williams J G K, Kubrlik A R, Livak K J, Pafalski J A & Tingy S V, DNA polymorphism amplified by arbitrary primers are useful as genetic markers, *Nucleic Acids Res,* 18 (1991) 6531-6535.