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Comparative analysis of the genomes of *Shigella dysenteriae* type 2 & type 7 isolates

Partha Pal[*,†], Arunima Pal[*], Swapan Kumar Niyogi[**], T. Ramamurthy[**] & Rupak K. Bhadra[*]

[*]*CSIR-Indian Institute of Chemical Biology &* [**]*National Institute of Cholera & Enteric Diseases (ICMR), Kolkata, India*

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***Background & objectives*: The four species of the genus *Shigella*, namely, *S. dysenteriae, S. flexneri, S. boydii* and *S. sonnei* cause a wide spectrum of illness from watery diarrhoea to severe dysentery. Genomes of these four species show great diversity. In this study, *Not*I, *Xba*I or I-*Ceu*I restriction enzyme digested genomes of two *Shigella dysenteriae* isolates belonging to the serotypes 2 and 7 were extensively analyzed to find their relatedness, if any, with the whole genome sequenced strains of *S. dysenteriae* type 1 and *S. flexneri* type 2a.**

***Methods*: Pulsed-field gel electrophoresis (PFGE) technique was used to determine the diversity of *Shigella* genomes by rapid construction of physical maps. DNA end labelling, Southern hybridization and PCR techniques were also applied for mapping purposes.**

***Results*: The intron-coded enzyme I-*Ceu*I cuts the bacterial genome specifically at its *rrn* operon. PFGE of I-*Ceu*I digested *S. dysenteriae* genomes were found to carry seven *rrn* operons. However, I-*Ceu*I profiles showed distinct restriction fragment polymorphism (RFLP) between the isolates as well as with the whole genome sequenced isolates. Further studies revealed that the genome sizes and I-*Ceu*I linkage maps of the *S. dysenteriae* type 7 and type 2 isolates were similar to that of *S. dysenteriae* type 1 and *S. flexneri* type 2a genomes, respectively.**

***Interpretation & conclusions*: Our findings indicate that the type 7 and type 1 isolates of *S. dysenteriae* were probably evolved from a same precursor, while the type 2 and *S. flexneri* type 2a were probably evolved and diversified from a common progenitor.**

Key words PFGE - *rrn* operon - RFLP - *Shigella dysenteriae* - *S. flexneri*

Shigellosis or bacillary dysentery is a severe diarrhoeal disease caused by the Gram-negative bacteria *Shigella* spp[1-3]. The genus *Shigella* is comprised of four species, namely, *S. flexneri, S. dysenteriae, S. boydii* and *S. sonnei*, and each of these species is further classified into 6, 15, 18 and 1 serogroups[4,5], respectively, based on the 'O' antigen component of the lipopolysaccharide moieties present on the outer membrane of the cell wall.

[†]*Present address*: Department of Zoology, Scottish Church College, Kolkata 700 006, India

Among the four species, *S. dysenteriae* and *S. flexneri* are the major pathogen in developing countries[6]. It is believed that the manifestation of *S. dysenteriae* type 1 infection is more severe because of its exclusive property to produce shiga toxin, a potent enterotoxin[1]. The *Shigella* organisms invade and multiply within colonic epithelial cells causing mucosal ulcers but the organism rarely invades into the blood stream[1]. *Shigella* entry into susceptible host cells depends upon the coordinated actions of numerous genes that are activated in response to environmental cues[7,8]. Another important feature of *Shigella* spp. is that its virulence is absolutely dependent upon a large plasmid, which carries several genes needed for invasion of host epithelial cells[8]. However, chromosomal genes present in the 'pathogenicity islands' also participate in virulence processes directly or contribute to survival in the environment encountered during infection[9].

Although various aspects of *S. dysenteriae* type 1 and *S. flexneri* type 2a including whole genome sequences are known, but very little information is currently available on *S. dysenteriae* non-type 1 strains. In India, it was observed that dominant serotypes of *Shigella* spp. changed over time[10]. In Bangladesh, a significant number of *Shigella* strains isolated between 1999 and 2002 from hospitalized diarrhoeal patients could not be serotyped using the existing serotyping scheme[6]. These isolates exhibited very similar biochemical traits of *Shigella* and were *S. dysenteriae* type 2 isolates.

In this study, pulsed-field gel electrophoresis (PFGE)-based macrorestriction mapping (MRM) of two non-type 1 *S. dysenteriae* isolates, one belonging to type 2 and the other type 7 was performed to get insight about their genomic structures and compare with the genomes of well-studied *S. dysenteriae* type 1 and *S. flexneri* type 2a isolates. This technique has now been commonly used for demonstrating intra-species genome size variation[11,12]. Because of high conservation of intron coded I-*Ceu*I megarestriction sites in the bacterial 23S rRNA genes so far examined and conservation of the number and locations of *rrn* genes, the I-*Ceu*I MRM provides an excellent tool for rapid examination of genomes of related species of bacteria. In this study, we have also compared the I-*Ceu*I profiles of *S. dysenteriae* type 2 and type 7 isolates with reported whole genome sequenced *S. dysenteriae* type 1 and *S. flexneri* type 2a isolates. Further, we have constructed the I-*Ceu*I linkage maps of the genomes of type 2 (F23659) and type 7 (SH89) isolates.

Material & Methods

The study was conducted in the laboratory of Infectious Diseases and Immunology Division, Indian Institute of Chemical Biology, Kolkata, India, during 2004 and 2009.

Bacterial strains, *plasmids and growth conditions*: *Shigella* isolates obtained from stool samples of dysentery patients between 1999 and 2000 were included in this study. Two *S. dysenteriae* type 1 isolates, (283 and SH6), one each of *S. dysenteriae* type 2 (F23659), type 7(SH89) and *S. flexneri* type 2a (SH1) were also included in this study. The plasmids pKP31 (carries *dnaK* gene of *Escherichia coli*[13]), pOF12 (carries *groEL* genes of *E. coli*[14]), pKK3535 (carries *rrn* operon of *E. coli*[15]) and pALS10 (carries *relA* gene of *E. coli*[16]) were used for gene probing. Before preparation of *Shigella* genomic DNA, the serotypes of the strains were reconfirmed using the commercially available antisera kit (Denka Seiken, Tokyo, Japan). *Shigella* cells were grown in a gyratory shaker (200 rpm) at 37°C in Luria broth (LB; Difco, Detroit, USA) or in LB containing agar (1.5%) and were maintained at -70°C in 20 per cent glycerol stocks.

Preparation of high-molecular-weight genomic DNA and enzyme digestion: Intact genomic DNA of bacterial cells in agarose blocks was prepared essentially as described previously[17]. Agarose blocks were stored in a solution containing 0.5 M EDTA (*p*H 9.0), 1 per cent sarkosyl (Sigma-Aldrich, USA), and 1 mg/ml of proteinase K (Sigma-Aldrich) at 4°C for future use. Prior to restriction enzyme digestion, the blocks were washed with 1 mM phenylmethylsulphonyl fluoride (Sigma-Aldrich) in TE buffer [10 mM Tris-HCl, 1 mM ethylenediamine tetraacetic acid (EDTA), *p*H 8.0]. Finally, blocks were washed four times with TE buffer and used directly for restriction digestion. All restriction enzymes used in this study were obtained from New England Biolabs, USA and digestion of DNA was done essentially as directed by the manufacturer.

Pulsed-field gel electrophoresis (PFGE): PFGE was carried out in a Pulsaphor Plus System with a hexagonal electrode array (Pharmacia, UK) essentially as described previously[17]. Gels were usually run for 24 h at 5-10 V/cm and various pulse times. As molecular mass markers phage lambda multimeric DNA and yeast chromosomal DNA (New England Biolabs) were used.

End labelling: End labelling of DNA fragments following enzyme digestion was done by incubating

the agarose slices in a buffer containing Klenow enzyme and [α-^{32}P]dCTP, and the preparations were subjected to PFGE followed by drying of the gel and autoradiography[17].

Molecular biological techniques and PCR: Standard molecular biological methods[18] were followed throughout the study unless otherwise mentioned. Bacterial chromosomal DNA was prepared by cetyl trimethyl ammonium bromide (CTAB, Sigma-Aldrich)/NaCl method as described by Ausubel *et al*[19]. For hybridization experiment, 0.7 kb DNA fragment of the *ompR* gene was PCR amplified using 10 pmoles each of primers [*ompR*-F (5′-CTTTAGAGCCGTCCGGTACA-3′) and *ompR*-R (5′-CAAGATTCTGGTGGTCGATG-3′)][20] and genomic DNA of the *S. dysenteriae* type 2 isolates F23659 as template and used as a probe. PCR amplification conditions were as follows: initial denaturation at 94°C for 3 min followed by 30 cycles for amplification, each cycle comprises denaturation at 94°C for 1 min followed by annealing for 1 min at 55°C and extension at 72°C for 1 min. Final extension of DNA was carried out at 72°C for 7 min. PCR amplification was carried out by *Taq* DNA polymerase (Invitrogen, USA). PCR assays were performed using the GeneAmp PCR system (Model 9700; Applied Biosystems, USA). PCR amplified DNA was purified by electroelution method[18]. For labelling of DNA, about 50 ng of DNA was labelled with [α-^{32}P]dCTP (Amersham Bioscience, UK) by the random-priming method using NEBlot kit (New England Biolabs). For Southern blot hybridization, restriction enzyme digested bacterial genomic DNA was separated by electrophoresis, transferred to the Hybond-N+ membrane (Amersham Biosciences) and hybridized with a labelled DNA probe at 60°C as described before[21,22]. The membranes were washed under stringent conditions[21], dried, and exposed to Kodak X-OMAT AR5 films.

Results

Comparison of NotI, XbaI and I-CeuI genomic profiles of S. dysenteriae isolates: It was previously reported that *Not*I digestion of genomes of *Shigella* spp., produces small number of large fragments that could be separated by PFGE[20,23,24]. The enzyme *Not*I was also used earlier to construct the physical map of *S. flexneri* type 2a strain YSH600 by Okada *et al*[20]. Therefore, this enzyme was selected in this study to examine the extent of RFLP in the genomes of *S. dysenteriae* serotypes. Comparative analysis of macrorestriction patterns generated with *Not*I revealed distinct RFLP among the *S. dysenteriae* isolates (Fig. 1). The number of resolvable *Not*I fragments obtained from *S. dysenteriae* type 2 or 7 genome was found to be higher compared to that of *S. dysenteriae* type 1 (Fig. 1A). Talukder *et al* showed that digestion with the *Not*I enzyme of the genome of *S. dysenteriae* type 1 could generate about 12 fragments with top *Not*I fragment of about 1300 kb in size[24]. Similar *Not*I profile of the *S. dysenteriae* type 1 was obtained here. However, pulse conditions used here did not allow separation of that size of DNA fragment. Therefore, the top *Not*I fragment of *S. dysenteriae* type 1 failed to resolve properly and it appeared just above the largest lambda concatamer separated at about 630.5 kb region as shown in Fig. 1A. Hgher pulse conditions were used to separate the large *Not*I fragments of *S. dysenteriae* type 1 and non-type 1 (type 2 and 7) isolates and in that case the top *Not*I fragments were easily separated with their expected molecular sizes (data not shown). Similarly, PFGE analysis of the *Xba*I digested chromosomal DNA of the *S. dysenteriae* isolates revealed distinct RFLPs (Fig. 1B). Only

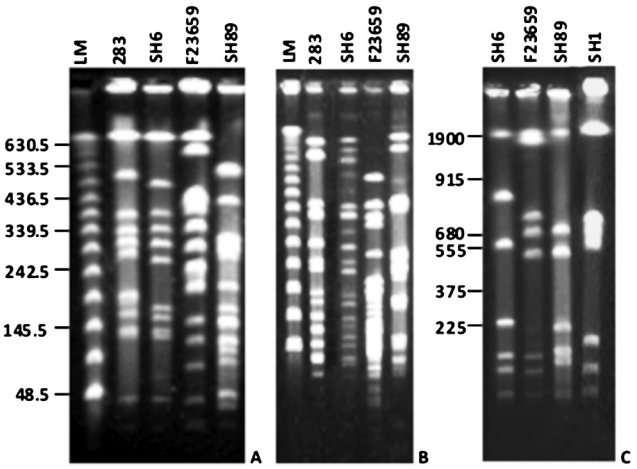

Fig. 1. A. PFGE profiles of *Not*I-digested chromosomal DNAs of different *Shigella* isolates. Enzyme digested genomic DNAs were separated by PFGE with pulse times ramping from 5 to 50 sec for 24 h using 10V/cm at 4°C. *Shigella* isolates used were 283 (*S. dysenteriae* type 1), SH6 (*S. dysenteriae* type 1), F23659 (*S. dysenteriae* type 2) and SH89 (*S. dysenteriae* type 7) as indicated above each lane. LM is the lambda concatameric DNA used as molecular size markers and their sizes (in kb) are shown in the left margin. **B.** PFGE separation of *Xba*I-digested genomic DNAs. Lanes and pulse conditions used are as indicated in panel 'A'. **C.** PFGE patterns of I-*Ceu*I-digested chromosomal DNAs. Enzyme-digested DNAs were separated by PFGE with pulse time ramping from 20 to 150 sec for 24 h using 10V/cm at 4°C. *Shigella* isolates used were SH6, F23659, SH89 and SH1 (*S. flexneri* type 2a) as indicated above each lane. YM denotes yeast intact chromosomes used as molecular size markers. Numbers in the left margin denotes sizes (in kb) of the yeast chromosomes.

Table I. Sizes of restriction fragments[a] observed by PFGE after digestion of genomic DNA of *S. dysenteriae* isolates F23659 and SH89 with restriction enzymes as indicated

F23659				SH89			
Fragment	Size (in kb) after digestion with			Fragment	Size (in kb) after digestion with		
	*Xba*I	*Not*I	I-*Ceu*I		*Xba*I	*Not*I	I-*Ceu*I
1	450	745	2500	1	720	550	2510
2	350	610	745	2	670	550	690
3	350	430	690	3	355	450	555
4	330	430	550	4	345	330	225
5	330	385	135	5	340	300	145
6	290	340	90	6	230	300	120
7	290	290	45	7	230	290	45
8	230	240		8	185	290	
9	175	240		9	180	225	
10	175	225		10	180	180	
11	160	210		11	175	160	
12	160	210		12	130	155	
13	145	150		13	130	125	
14	140	120		14	120	100	
15	130	90		15	75	90	
16	115	30		16	55	75	
17	100	20		17	40	45	
18	90	18		18	40	40	
19	80			19	37	30	
20	80			20	30	20	
21	80			21	28	10	
22	75						
23	70						
24	60						
25	60						
26	50						
27	50						
28	40						
29	37						
30	28						
Total	4720	4783	4755	Total	4295	4315	4290

[a]Individual fragment size was calculated as an average of the values obtained from at least three different gels

seven fragments were generated from the genomes of *Shigella* spp. when the intron coded enzyme I-*Ceu*I was used. Since, I-*Ceu*I cuts specifically at 23S *rrn* gene of prokaryotes, the number of fragments generated from a circular genome indicates the number of *rrn* operons present. Thus, like other *Shigella* strains the type 2 and 7 strains also possess 7 *rrn* operons. Like *Not*I and *Xba*I, distinct RFLP with respect to I-*Ceu*I was also observed among the *S. dysenteriae* isolates (Fig. 1C). The copy number of the *rrn* operons was determined by end-labelling assay, which also confirmed presence of 7 *rrn* operons in *Shigella* strains (data not shown). The major variations detected among I-*Ceu*I fragments 2, 3 and 4 suggested genomic rearrangements in *S.*

Table II. I-*Ceu*I partials of the genomes of *S. dysenteriae* isolates

Partials fragments (kb)	Composition of I-*Ceu*I fragments (kb)
S. dysenteriae type 2 isolate F23659	
P1 (1590)	C3+C4+C5+C6+C7 (1510)
P2 (1400)	C2+C4+C6 (1385)
P3 (1250)	C2+C4 (1295)
P4 (880)	C3+C5+C7 (870)
P5 (825)	C4+C5+C6+C7 (820)
P6 (290)	C5+C6+C7 (270)
P7 (225)	C5+C6 (225)
P8 (170)	C5+C7 (180)
S. dysenteriae type 7 isolate SH89	
P1 (2400)	ND[a]
P2 (980)	C2+C5+C6 (955)
P3 (835)	C2+C6 (810)
P4 (770)	C3+C4 (780)
P5 (580)	C4+C5+C6+C7 (535)
P6 (300)	C4+C7 (270)
P7 (250)	C5+C6 (265)
P8 (200)	C5+C7 (190)

[a]ND, not done

dysenteriae isolates examined. The sizes of I-*Ceu*I fragments obtained from the genomes of *S. dysenteriae* type 2 and 7 isolates were determined and the data gave an estimate of genome size of each serotype. *S. dysenteriae* type 1 strain SH6 was used as control. The genome sizes of *S. dysenteriae* non-type 1 isolates examined in this study were ranged from 4.29 to 4.75 Mb (Table I). It should be noted that the genome size of *S. dysenteriae* type 2 (4.75 Mb) was found to be much higher than either type 1 SH6 (4.26 Mb) or type 7 (4.29 Mb). Since, I-*Ceu*I was used to construct the macrorestriction maps of *S. dysenteriae* isolates, the restriction fragments were named on the basis of the enzyme used (C for I-*Ceu*I) and was numbered on the basis of size in descending order.

Construction of I-CeuI macrorestriction maps of S. dysenteriae type 2 and 7 isolates: Similarity in restriction digestion profiles and genome sizes between the *S. dysenteriae* type 2 and *S. flexneri* type 2a isolates or between *S. dysenteriae* type 7 and *S. dysenteriae* type 1 isolates prompted us to determine the linkage maps of non-type 1 strains for further confirmation. The I-*Ceu*I physical maps of *S. dysenteriae* type 2 (F23659) and type 7 (SH89) were constructed primarily from analysis of partial fragments generated by the enzyme. The partial fragments (designated as P1, P2, *etc.*) obtained from

Table III. Clubbing of *Not*I fragments within an I-*Ceu*I band and the size of end fragments generated from each I-*Ceu*I fragment of *S. dysenteriae* type 2 F23659 and the type 7 isolate SH89

I-*Ceu*I band	*Not*I fragments or site(s) present in a particular I-*Ceu*I band	End fragment generated by *Not*I digestion (approximate size in kb)	Name of the isolate
C1	N3,N4,N7,N8,N9,N11,N12,N13, N17, N18 and N19	200,190	SH89
C1	N5,N8,N9,N10,N11,N12,N13, N14,N15,N16 and N17	380, 205	F23659
C2	N14 and N18	360,190	SH89
C2	Only one *Not*I site	540, 215	F23659
C3	Only one *Not*I site	350,200	SH89
C3	N6	215, 110	F23659
C4	N17	110,80	SH89
C4	Only one *Not*I site	360, 180	F23659
C5	Only one *Not*I site	130, 28	SH89
C5	Only one *Not*I site	125, 20	F23659
C6	Only one *Not*I site	95, 35	SH89
C6	Only one *Not*I site	60, 40	F23659
C7	No *Not*I site	50	SH89
C7	No *Not*I site	50	F23659

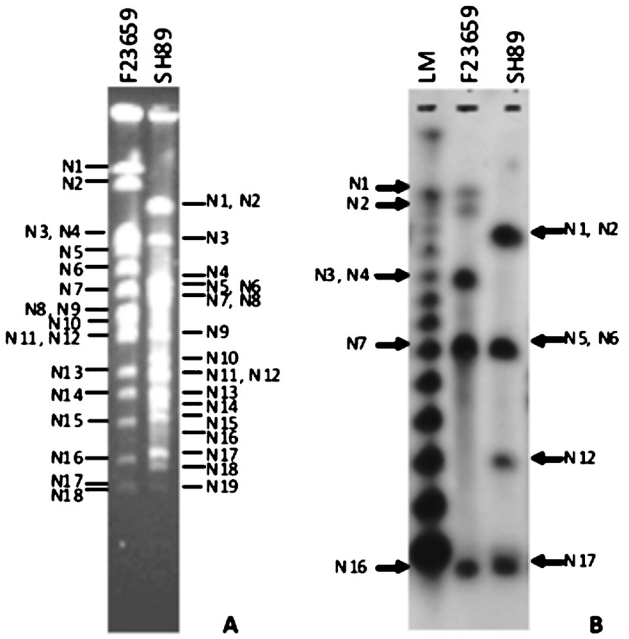

Fig. 2. Identification of *Not*I fragments carrying *rrn* operon(s). **A.** Ethidium bromide-stained gel containing the *Not*I-digested genomic DNAs of *S. dysenteriae* type 2 (F23659) and type 7 (SH89) isolates. *Not*I fragments of the isolates are as indicated. **B.** The gel shown in 'A' was transferred to a nylon membrane, hybridized with the *rrn* gene and autoradiographed. *Not*I fragments carrying *rrn* operon were marked, for F23659 (type 2) and SH89 (type 7) in the left and right margins, respectively. LM is the lambda concatameric DNA used as molecular size markers.

each isolate using the enzyme I-*Ceu*I and the possible linkages are described in Table II. However, linkage could not be established only for the P1 fragment (2400 kb) of the isolate SH89 (Table II) and this could be the C1 fragment of SH89. To confirm the linkages of I-*Ceu*I fragments, individual PFGE separated fragments were excised from an agarose gel; each of these was digested separately with *Not*I, end-labelled and subjected again to PFGE and autoradiography. Intact fragments as well as end fragments generated by the digestion of each I-*Ceu*I fragment by *Not*I are given in Table III. The end fragments were generated due to overlapping *Not*I fragment on an I-*Ceu*I junction. This approach allowed in determining the linkages between I-*Ceu*I fragments, identification of I-*Ceu*I fragments carrying a single or no *Not*I site and clubbing of *Not*I fragments that are linked and overlapping with a particular I-*Ceu*I fragment. For example, the gel-excised I-*Ceu*I fragment C1 (2510 kb) of *S. dysenteriae* type 7 isolate SH89 (Table III) digested with *Not*I gave eleven fragments (N3, N4, N7, N8, N9, N11, N12, N13, N17, N18 and N19) plus two end fragments (200 and 190 kb) as detected in the autoradiogram (data not shown). Similarly, C2 contains only two *Not*I fragments (N14 and N18) and two end-fragments (360 and 190 kb). C1-C2 linkage was confirmed by adding the end-fragments 190 and 360 kb, which corresponded to the size of N1 (550 kb). Absence of N1 fragment in a *Not*I/I-*Ceu*I double digests indicated that the N1 fragment contains I-*Ceu*I site(s) (data not shown). Southern hybridization of *Not*I digested genomic DNA of isolate SH89 using *rrn* probe[15] also proved the presence of I-*Ceu*I sites in N1, N2, N5, N6, N12 and N17 fragments (Fig. 2). With similar analysis, the linkages between the fragments C1-C2, C1-C3, C2-C6, C3-C4, C4-C7, C5-C6 and C5-C7 of isolate SH89 were determined. Using the same approach, the linkages among the I-*Ceu*I fragments of the isolate F23659 were established, which links C1-C2, C1-C3, C2-C4, C3-C7, C4-C6, C5-C6 and C5-C7 (Table III). The I-*Ceu*I linkage maps were thus constructed for F23659 and SH89 isolates (Fig. 4).

Comparative genome analysis of S. dysenteriae: For this, I-*Ceu*I-digested genomic DNAs of *S. dysenteriae* isolates belonging to different serotypes were subjected to Southern blot hybridization experiments. The extent of RFLP could be detected in greater details by positioning various genes on the I-*Ceu*I profiles of the isolates F23659 and SH89. The *dnaK*[13] and the *groEL*[14] genes, which code for the heat-shock protein Hsp70 and Hsp60, respectively, hybridized with the C3 fragment of the isolate F23659, but in the case of SH89 the same probes hybridized with the C2 fragment (Fig. 3). The *relA* gene[16], which codes for the (p)ppGpp synthetase enzyme, hybridized with the C2 and C4 fragments of the isolates F23659 and SH89, respectively. The *ompR* gene which codes for a response regulator, positioned in the C4 and C3 fragments of the isolates F23659 and SH89, respectively (data not shown). The positions of all these genes in the genomes of isolates 301 and 197 were determined mainly from their whole genome sequence information (*www.tigr.org*) and shown in Fig. 4. All these position specific genes indicate that during evolution some rearrangement had occurred among C2, C3 and C4 fragments of *S. dysenteriae* type 1, type 2 and type 7 isolates. Moreover, the order of the I-*Ceu*I fragments of F23659 matched with that of 301, while SH89 showed similarity (except *relA* and *ompR*) with that of 197 (Fig. 4). Considering the similarities between the genomes of *S. dysenteriae* type 2 isolate F23659 and *S. flexneri* type 2a isolate 301, it may be hypothesized that although SH89 (type 7) and F23659 (type 2) are taxonomically identical up to the species level, their evolutionary origins might be different. The

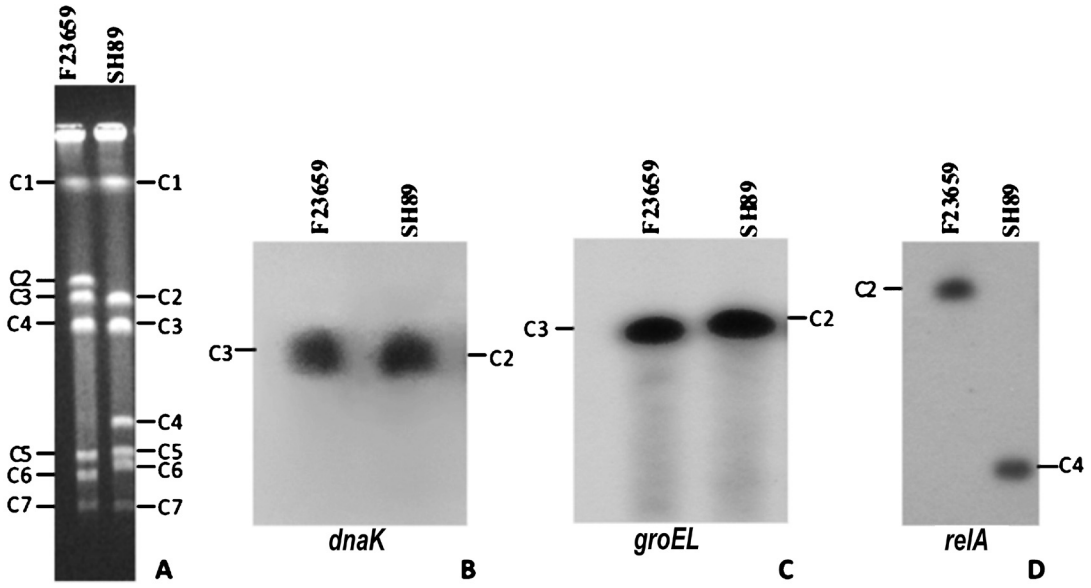

Fig. 3. RFLP in the genomes of *S. dysenteriae* isolates. **A.** Ethidium bromide stained PFGE gel containing I-*Ceu*I-digested chromosomal DNAs of *S. dysenteriae* type 2 isolate F23659 and the type 7 isolate SH89. Electrophoresis conditions were same as described in the legend of Fig. 1C. Seven I-*Ceu*I fragments (C1 to C7) of each isolate were marked. **B-D.** I-*Ceu*I-digested chromosomal DNAs of the isolates F23659 and SH89 shown in 'A' were transferred to a membrane and hybridized with *dnaK* (B), *groEL* (C) or *relA* (D) gene as a probe. The same membrane was successively used after deprobing each time. Both *dnaK* and *groEL* genes hybridized with the I-*Ceu*I fragment C3 of the F23659, but in the case of the SH89 the genes were hybridized with the I-*Ceu*I fragment C2. The *relA* gene hybridized with the C2 and C4 fragments of the F23659 and SH89, respectively.

gene probing data also reflect that *S. dysenteriae* type 2 may have originated from an ancestor closely linked with *S. flexneri* type 2a (Fig. 4).

Discussion

Recent clinical studies in Bangladesh[5] indicated that the prevalence of *S. dysenteriae* serotypes has been changed. Previously *S. dysenteriae* type 1 was the most frequently isolated serotype from clinical samples, which was gradually replaced by serotypes 2 and 4[6]. Recently, *Shigella* like strains were identified in Dhaka, Bangladesh, which are biochemically typical *Shigella* but could not be serotyped by using the commercially available serotyping kit[5]. The *S. dysenteriae* type 2 strains in Bangladesh may be emerging from a common ancestral clone[25]. Analysis of the serotyping results for the *S. dysenteriae* strains isolated between 1999 and 2000 at Dhaka had also shown an abrupt decrease of serotype 1 from 76.4 to 6.5 per cent, while the isolation rate of other *S. dysenteriae* types (types 2 to 12) was increased from 19 to 87 per cent[6]. This observation reflects that there is a temporal shift in the dominance of *S. dysenteriae* serotypes in recent years[6].

PFGE has been a valuable typing method for epidemiological investigation of several bacterial pathogens and clonality studies[26]. The current genomic RFLP data among the *S. dysenteriae* isolates belonging to serotypes 1, 2 and 7 indicate usefulness of the genomic RFLP method in identification of *Shigella* strains at the molecular level. We constructed the combined I-*Ceu*I physical and genetic maps of two *S. dysenteriae* isolates, F23659 and SH89, belonging to serotypes 2 and 7, respectively. In this study, rDNA was detected on the basis of the number of fragments obtained in the I-*Ceu*I digested chromosomal DNA of the strains. The number of the rDNA loci is seven in all the *Shigella* isolates, which suggest that the numbers of *rrn* loci are well conserved among the genus. It is noted that the genome size of *S. dysenteriae* type 2 isolate was larger than both type 1 and type 7 and similar to that of the genome size of *S. flexneri* type 2a strain. Moreover, comparative genome analysis revealed that the type 2 isolate F23659 was closely related with that of *S. flexneri* type 2a and the genome of type 7 isolate SH89 matched with that of *S. dysenteriae* type 1 isolate 197. Considering the facts that the *S. dysenteriae* type 2 isolate F23659 was similar to the whole genome sequenced strain[27] of *S. flexneri* type 2a (isolate 301) with respect to genome size, number of *rrn* operons and positions of genetic loci specific for house-keeping genes studied in this study, it could be possible that

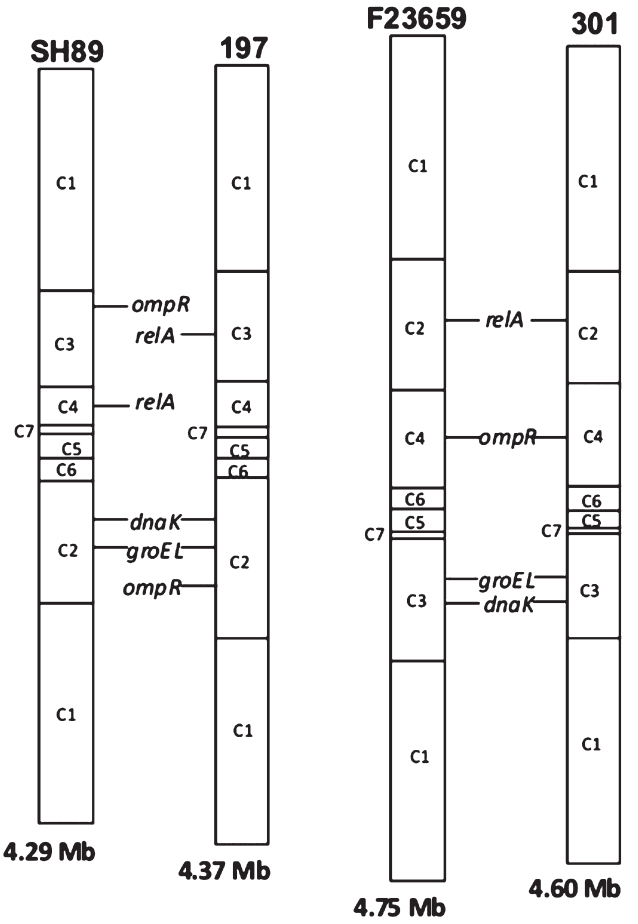

Fig. 4. Comparisons of the I-*Ceu*I maps of *S. dysenteriae* type 2 isolate F23659, type 7 isolate SH89, *S. dysenteriae* type 1 isolate 197 and *S. flexneri* type 2a isolate 301. The I-*Ceu*I map information for the whole genome sequenced isolates 197 and 301 were obtained from public database *(www.tigr.org)*. For simplicity, the circular maps of *Shigella* genomes were shown as linear maps at an arbitrarily chosen point. The order of the I-*Ceu*I fragments as well as positions of some selected genes, namely, *dnaK*, *groEL*, *relA* and *ompR* were also shown. Genome size of each *Shigella* isolate is indicated at the bottom of each linear map.

the *S. dysenteriae* type 2 and the *S. flexneri* type 2a isolate 301 are genetically similar. The ancestry of the *S. dysenteriae* type 2 may largely account for the differences in its genome with that of serotypes 1 and 7 studied here. The *S. dysenteriae* type 2 strains may have evolved from a primitive strain that was genetically linked with the ancestor of *S. flexneri* type 2a. Recent study involving O-antigen modification of *S. flexneri* serotype 1c identified a *gtrlC* gene cluster. Analysis of the sequences surrounding this cluster revealed that it was carried by a novel bacteriophage[28]. This finding provided a possible clue about the evolution of serotypes within *S. flexneri*. Similar mechanism(s) could be associated in the evolution of *S. dysenteriae* type 2. However, further genetic analysis is required to get an insight into the genome structure of isolates belonging to *S. dysenteriae* type 2. The emergence of *S. dysenteriae* type 2 with epidemic potential is of high concern and should be carefully monitored in countries of Indian subcontinent, which is believed to be an epicenter for the spread of *Shigella* infection.

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Reprint requests: Dr Rupak K. Bhadra, Infectious Diseases & Immunology Division, CSIR-Indian Institute of Chemical Biology
4, Raja S.C. Mullick Road, Kolkata 700 032, India
e-mail: rupakbhadra@iicb.res.in