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First report on IS elements of *Shigella flexneri* 1a— A common Indian isolate

Suvidya Ranade[1]*, P S Khandekar[2], B A Chopade[3] and D N Deobagkar[4]

[1]Department of Chemistry, [3]Department of Microbiology and [4]Bioinformatics Center, University of Pune, Pune 411 007, India

[2]Malkolak Institute of Life Sciences, Hyderabad 500 038, India

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Shigella species are non-sporulating, Gram negative, facultative anaerobes. IS (insertion sequence) elements are the major cause of the dynamics of *Sf301* chromosome and due to IS-mediated DNA rearrangements and formation of pseudogenes, the *Shigella* spp. became highly specific human pathogens with variable epidemiological and pathological features. Nucleotide sequence analysis of *S. flexneri* 1a genomic DNA was performed through Big dye terminator chemistry using ABI 3730 48 capillary DNA analyzer. In total, 60 kb data in form of contigs were analyzed by homology search using various bioinformatics tools. IS elements were identified using nucleotide blast at NCBI as well as the Is finder. Eight different IS elements were identified, which were present in different copy number. A new IS element ISEhe3 was identified, which belonged to family IS3. ISEhe3 was found absent in *S. flexneri* 2a 301, but it was present in 2457T strain. Amongst the IS elements identified, IS2, IS3 and IS600 elements showed identity with SHI2 S*higella* pathogenicity island. The presence of large numbers of IS-elements in the *Shigella* genomes is likely the major cause of many of the genome rearrangements. Further investigations on IS elements will help to study genome dynamics and rearrangement of *S. flexneri* 1a strain.

Keywords: ISEhe3, IS elements, *S. flexneri* 1a, sequencing, shotgun cloning

Introduction

Shigella species (Family: Enterobacteriaceae) are Gram negative, non motile, lactose-nonfermenting rods. They are divided into four sub groups, *viz.*, *S. dysenteriae, S. flexneri, S. boydii* and *S. sonnei.* The global annual incidence of *Shigella* infection has been estimated as 80-165 million. Around 99% of the episodes occur in developing world and in children aged less than 5 yr[1,2]. *S. dysenteriae* type 1 produces Shiga toxin, the most potent one, and can cause large out breaks of dysentery under conditions like over crowding, poor water and hygiene infrastructure[3,4]. IS elements are the mobile genetic elements of bacteria and are considered to be the genomic parasites. Bacterial mobile genetic elements fall into two types, elements that can move from one bacterial cell to another (resistance plasmids, conjugative resistance transposons) and elements that can move from one genetic location to another in the same cell (resistance transposons, gene cassettes, ISCR-promoted gene mobilization). The acquisition of new genetic material is largely responsible for antibiotic resistance[5]. The simplest mobile elements are bacterial IS (insertion sequence) elements. The IS elements play important role in evolution of host genome due to their capacity to generate DNA rearrangements and influence the expression of neighbouring genes. Bacterial IS elements harbour one or two ORFs coding transposase. More than 2400 IS elements have been described and classified into families (IS finder http://www.is.biotoul.fr)[6].

The detrimental effect of mobile elements has been studied by population genetics and phylogenetic data[7]. It has been experimentally shown that mobile elements create adaptive deletions and duplications of the regions of genomes[8,9]. Frequently encountered mutation in bacteria due to transposable elements is the one which activate critical genes/operons when inserted into appropriate chromosomal loci. Mobile elements have also been found to cause beneficial mutations that change the pattern of gene expression[10]. Transposon-mediated mutations occurring under stress condition are beneficial to host organism. Mechanism of the mutation mediated by transposon IS5 in *Escherichia coli* has been

*Author for correspondence:
E-mail: suvidya@chem.unipune.ac.in

reported[11]. IS elements may also play important role in genome reduction process. In *Burkholderia pseudomallei*, IS-mediated deletions and rearrangement has been observed[12].

IS elements are the major cause of the dynamics of *Sf301* chromosome. IS1 and other IS elements have been shown to be able to mediate various genetic rearrangements, and IS1 in particular can cause inversions and deletions[13]. IS1 dominates in *Sf301, Ss046* and *Sb227*, and is associated with DNA rearrangements at many different loci in these three genomes. Due to IS-mediated DNA rearrangements and formation of pseudogenes, the *Shigella* spp. became highly specific human pathogens with variable epidemiological and pathological features. In the present study, authors have reported the sequence of IS elements in *S. flexneri* 1a for the first time along with the presence of new IS element in the chromosome.

Materials and Methods

S. flexneri 1a strain was obtained from the Department of Microbiology, Armed Forces Medical College, Pune, India. The strain was characterized microbiologically and biochemically by standard methods according to Bergey's manual[14]. Enzymes Sau3A, BamH1 and T4 DNA ligase as well as other biochemicals like IPTG (isopropyl β-D-1-thiogalactopyranoside), X-gal and M13 sequencing primers were procured from Banglore Genie, Bangalore, India. ABI Big dye terminator chemistry kit was obtained from Lab India.

The cells of *S. flexneri* 1a strain were grown in Luria broth at 37°C for 18 h on an orbital shaker. Genomic DNA library was prepared as follows. Genomic DNA of *S. flexneri* was isolated according to method of Chen and Kuo[15]. Plasmid isolation was carried out according to the method of Birnboim and Doly[16]. Genomic DNA of *S. flexneri* 1a was subjected to partial digestion with restriction endonuclease Sau3A and DNA fragments with average size between 1-2 kb were cloned into Bam H1 site of plasmid pUC 18. Recombinants were selected using IPTG and X-gal selection method and were further confirmed for the presence of insert by agarose gel electrophoresis[17].

Nucleotide sequence analysis of *S. flexneri* 1a genomic DNA was performed by Big Dye terminator chemistry using ABI 3730 48 capillary DNA analyzer. Each sample was sequenced three times from both the ends using M13 sequencing primers; 48 samples were processed in one plate using forward and reverse primers within 120 min by this machine. The best sequence of all 3 runs was selected depending on Q value. The software available at Staden package Gap4 was used for vector removal, repeat masking and partial assembly[18]. Nucleotide sequence analysis was done on 170 recombinants.

Results and Discussion

In total, 99.4 kb sequence data were generated from both ends (49.7 kb with either forward/reverse) of genomic DNA of *S. flexneri* 1a; the average length of sequence was 550 bases. Further, 60 kb data was obtained in form of contigs using Gap4 software. The reduction in the data size was due to removal of overlapping sequences from different clones and also by vector sequence removal. This data was analyzed through homology search using various bionformatic tools. IS elements were identified using nucleotide blast at NCBI as well as the Is finder. Amongst 170 recombinants sequenced and analyzed, 8 diffenent IS elements were obtained. Length and location of each (partial/complete) IS element was identified using BLASTN and ShiBASE, a newly developed software used for comparative genomics of *Shigella* genomes[19]. The criteria for BLASTN search were the significant e value (<0.01) and the 95% sequence identity, indicating close homology. Highly homologous sequence with that of the query sequence was considered for identification of IS elements. Fig.1 shows the sequence based comparison using ShiBASE. The entire assembled contig was aligned with the sequences of various strains of *Shigella* to identify IS elements of *S. flexneri* 1a.

The details of IS elements identified are shown in Table 1. It gives the information about their copy number, length, reported family length, per cent identity and the strain with which it is highly homologous. The sequence analyzed so far showed 99% identity with *S. flexneri* 2a 301 and thus taken as reference strain for localization of the various DNA fragments on genome. For the total sequenced data, AT and GC contents recorded were 48.2 and 51.8%, respectively. Eight different IS elements were identified, which were present in different copy number. These were IS1, IS2, IS3, IS4, ISEhe3, IS600, ISSf13 and ISSf14. About 7 copies of ISSf13, 3 copies of ISSf14, 6 copies of IS2, 2 copies of IS3, and 1 copy each of IS1, ISEhe3, IS4 and IS600 were

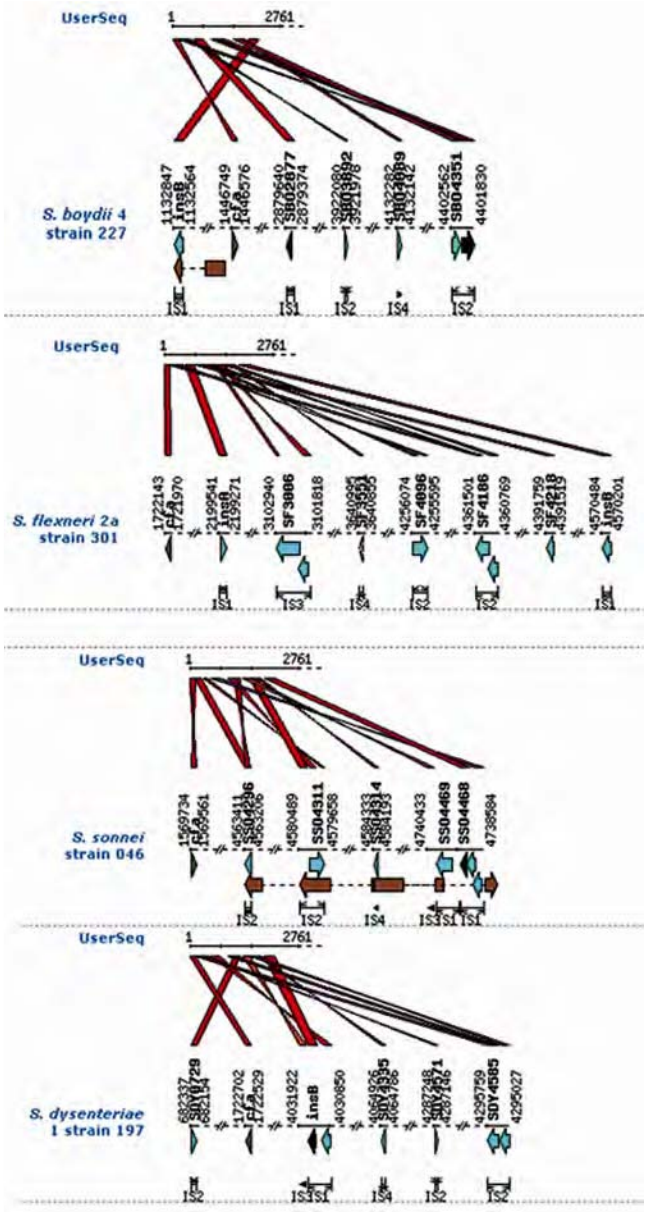

Fig. 1—Sequence based comparison in ShiBASE: Alignment of sequence of *S. flexneri* 1a with other strains of *Shigella* using ShiBASE that shows newly identified IS elements. The user sequence indicates the query sequence. The name of *shigella* strain is mentioned on left hand side and the name of IS element identified is mentioned below each alignment.

identified as partial sequences in *S. flexneri* 1a. The sequences have been submitted to GenBank (acc. nos DQ855070, DQ855071, DQ855072, DQ855073 & DQ855074).

In the present study, presence of a new IS element ISEhe3 from family IS3 was reported in the sequenced *S. flexneri* 1a. ISEhe3 has been also reported in 2457T strain at 5 different loci with length of 813 bp. ISEhe3 element of *S. flexneri* 1a sequence showed 100% identity with ISEhe3 element of 2457T strain but not with *S. flexneri* 2a 301, as per the BLASTN results. The sequence was also 99% identical to the transposase insF for insertion sequence IS3A/B/C/D/E/fA of *E. coli* K12. Thus, it shows that ISEhe3 must be having the functional role of transposase. Moreover, IS2, IS3 and IS600 elements showed maximum identity with *Shigella* pathogenicity island 2 (SHI-2). Therefore, they must also be the part of pathogenicity island (PAI) of *S. flxneri* 1a and must involved in imparting the pathogenicity to the strain. The differential matching in terms of identity indicated the closeness of sequences with respect to homology and evolutionary relatedness. *S. flexneri* 1a must have acquired this IS element during the strain evolution. Since the query sequence for new IS element has matched 100% with ISEhe3 of *S. flexneri* 2457T, it was named the same. The IS finder has the set of instruction that more than 95% identity be indicated the presence of the same IS element as reported in the database and so the same name was given to query IS element. Although the association of mobile genetic elements with PAIs is well established, the abundance and clustering of these elements in SHI-2 is unique. It has been shown that SHI-2 has remnants of multiple mobile genetic elements that show sequence identity to the transposase genes of IS3, IS629 and IS2, and there are no intact insertion sequences in the island. SHI-2 may allow *Shigella* survival in stressful environments, such as, those encountered during infection[13,20].

IS elements have strong impact on the genetic variability of microbial populations. The most striking feature of the *Shigella* genomes is their highly dynamic nature due to the presence of hundreds of IS elements in each of the genomes[21]. The presence of large numbers of IS elements in the *Shigella* genomes is likely the major cause of many of the genome rearrangements. As more and more sequence data would be available, more IS elements could be identified in *S. flexneri* 1a genome.

In the present study, a total of 8 IS elements were identified in *S. flexneri* 1a genome, which belong to the families IS3, IS4 and IS66. These were the partial sequences and were present in multiple copies. Amongst IS elements of *S. flexneri* 1a genome, a new IS element IsEhe3 belonging to family IS3 was identified, which has the transposase function. Other IS elements, *viz.*, IS2, IS3 and IS600, have also been

Table 1—Showing details of the IS elements identified in *S. flexneri* 1a, a common Indian isolate

IS element	Contig number	No. of copies	IS family	Origin	Length of IS element identified (bp)	Reported length in database (bp)	% identity
ISSf13	1. b11, 12-18	7	IS66	*S. flexneri* 2a 301	71	2729	100
	2. A11, 12-87				43		
	3. D5,6-87				43		
	4. D7,8-87				43		
	5. F6-87				43		
	6. G10-87				43		
	7. H8-87				43		
ISSf14	1. E5, 6-75	3	IS66	*S. flexneri* 2a 301	43	1602	100
	2. C3-75			*S. flexneri* 2a 2457T	43		
	3. D3,4-75			*S. boydii*	31		
				S. flexneri 2a 301			
IS600	1. F3-87	1	IS3	*S. flexneri* 2a 301	43	819	100
				S. sonnei			
IS2	1. FDEAB73377	6	IS3	*S. flexneri* 2a 301	205	1331	99
	2. B824			*S. flexneri* 2a 301	94	906	100
	3. B824			*S. flexneri* 2a 301	61	1331	91
	4. G255			*S. flexneri* 2a 301	237	411	99
	5.G155			*S. flexneri* 2a 301	179	1331	100
	6. H1-55			*S. flexneri* 2a 301	180	906	98
IS3	1. E6-55	2	IS3	*S. flexneri* 2a 301,	232	1258	100
	2. E5-55			*E. coli* K12			
				S. flexneri 301	104	867	100
				E. coli K12			
				MG1655			
ISEhe3	1. D11, 12-24	1	IS3	*S. flexneri* 2457T	241	813	100
IS1	1. B4-10	1		*S. flexneri* 2a 301	283	396	100
IS4	1. E7-55	1	IS4	*S. flexneri* 2a 301	140	1347	98
				E. coli K12			

found to be the part of the S*higella* pathogenicity island. Further investigations on IS elements would be helpful to study the genome dynamics and its rearrangement in *S. flexneri* 1a, a strain more common in India. The data on IS elements would also help in understanding the pathogenicity of *S. flexneri* 1a and its vaccine development. This is important for a developing country like India.

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