ShiBASE: an integrated database for comparative genomics of Shigella

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ABSTRACT

Among the major enteric bacterial pathogens, Shigella is found to display extreme genome diversity and dynamics, which imposes a challenge in comparative genomic studies. To facilitate further studies in this area, we have constructed an integrated online database, ShiBASE (http://www.mgc.ac.cn/ShiBASE/), which contains Shigella genomic sequences of four species and additional comparative genomic hybridization (CGH) data of 43 serotypes. ShiBASE offers online comparative analysis on DNA sequences, gene orders, metabolic pathways and virulence factors. In addition, ShiBASE has a newly developed online comparative visualization service, Shi-align, which enables the alignment of any query sequence with the reference genome sequences.

INTRODUCTION

Shigella species are the causative agents of bacillary dysentery or shigellosis, which remains a threat to public health worldwide, particularly in developing countries. Estimated shigellosis episodes are 160 million per annum, with 1.1 million deaths, the majority of which are children younger than 5 years of age (1). The clinical manifestation of shigellosis range from mild diarrhoea to severe forms of the disease which includes fever, abdominal cramps and blood, and pus and mucus in the stools (2).

Much of Shigella pathogenesis seems to be the result of multiple effects of its plasmid-borne type III secretion system (TTSS) encoded by mxi and spa genes. The TTSS secretes many effector proteins, including IpaA, IpaB, IpaC and IpgD, which can mediate epithelial signalling events, cytoskeletal rearrangements and other actions (3). The plasmid also encodes a 120 kDa outermembrane protein called IcsA, responsible for actin-based motility leading to intra- and intercellular spread of the bacteria by the binding of N-WASP (4). Furthermore, in addition to the virulence plasmid, many chromosomal loci, such as Shigella pathogenicity islands SHI-1 and SHI-2 also contribute to virulence (5,6). For a better understanding of the pathogenesis and epidemiology of Shigella infection, comparative genomics has emerged to be an important area of research.

Based on their somatic O-antigen, Shigella is divided into four species: S. dysenteriae, S. flexneri, S. boydii and S. sonnei. Our group and others have determined the complete genome sequences of five Shigella strains that cover all four species (7–9). In addition, comparative genomic hybridization (CGH) microarray analysis has been performed on 43 Shigella strains of different serotypes (J. Peng, X. Zhang, J. Yang, J. Wang, E. Yang, W. Bin, C. Wei, M. Sun and Q. Jin, manuscript in preparation), which revealed extensive diversity and dynamics between Shigella genomes. Hence, an integrated database capable of dealing with the complex genome comparison is urgently needed in addition to currently available online database, etal.oliBASE (10), which is limited within pairwise genome comparison.

We present here ShiBASE (http://www.mgc.ac.cn/ShiBASE/), a freely accessible online resource that is focused on the comparative genomics of Shigella. ShiBASE is able to summarize large volumes of genomic sequence and comparative genomic data in a visually intuitive format. ShiBASE also provides a novel Shi-align service for visualizing BLAST hits between query sequences and known Shigella genomes, thus allowing rapid examination of synteny, genomic rearrangements and putative genomic islands.

DATABASE CONTENT AND CONSTRUCTION

There are five completed Shigella genomes available. The S. flexneri 2a strain 2457T was excluded from the dataset of ShiBASE owing to its genome being highly similar to another serotype 2a strain, 301 (Sf301). Variation between the genomes of 2457T and Sf301 is limited to the quantity of IS elements and some single-nucleotide differences (8). The four Shigella genomes included in the current release of ShiBASE are S. dysenteriae 1 strain 197 (Sd197), S. boydii

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4 strain 227 (Sh227), S. sonnei strain 046 (Ss046) and S. flexneri 2a strain 301.

The genome sequences and annotation files from GenBank (11) were converted to tabular files by using the Bioperl toolkit (12). Genes from Escherichia coli K12 strain MG1655 (accession no. U00096) were used as references for defining orthologue groups. Pairwise orthologues were identified by INPARANOID program with default parameters (13), and were further inspected manually. Multi-copy genes related to mobile DNA, such as IS elements and bacteriophage were excluded. Protein sequences from each of the orthologue groups were processed in ClustalW (14) allowing for alignments and guide trees. Whole genome sequence comparisons were performed by BLASTn (15) and the information for each maximal segment pair (MSP) was deposited into the database for web illustration. The enzyme commission information of each genome as well as metabolic pathway maps were retrieved from the KEGG ftp server (16), and then revised by manual curation for dynamic web presentation.

Shi/BASE was built on a RedHat Linux 9.0 operation system, and MySQL was used to construct the database for storage of information, including genome annotations, orthologue groups, sequence comparisons and CGH results. The Perl programming language and several modules (DBI, GD and CGI) were used to generate dynamic web pages. Client-side JavaScript and Java applet were also applied in some cases for data presentation purposes.

Comparative Genomics in Shi/BASE

The interspecies comparison of Shigella genomes may be carried out at several different levels within Shi/BASE. One may compare basic genome features, such as genome size, IS elements, genome structure (inversions and deletions) and order of orthologous genes. One can also compare metabolic pathways and virulence factors among Shigella genomes. Moreover, the CGH data produced from analyses on 43 different serotypes of Shigella strains are also integrated within Shi/BASE. By using the Shi-align visualization platform one can also perform comparative analysis on any given query sequences.

Sequence-based comparison

All Shigella and E. coli strains sequenced to date are closely related and share an essentially collinear common ‘backbone’ genome sequence. However, there are unique DNA sequences present in each of the Shigella and E. coli chromosomes (7). For graphically viewing a selected region across all genomes, we have set a clickable web page in the Shi/BASE interface. Figure 1A shows a comparison of a 20 kb segment involved in flagella biogenesis from all Shigella genomes. The reference genome (from Sb227 in this case) is presented on the top and the query genome sequences are aligned below. The pre-computed MSPs from BLASTN are indicated by red bars between each pair, and additionally are equipped with popup messages that provide details of the sequence alignment. Genome annotations, including genes, stable RNAs and IS elements are also graphically illustrated for each region of synteny. The comparison range can be increased in size (up to 100 kb) and the window can slide along each genome for simple browsing. If necessary users can reverse and display the complementary strand (see SF301 in Figure 1A).

As shown in Figure 1A, there are different mutations in the fli genes across all Shigella strains, which explain genetically why members of the Shigella species are non-motile. An additional surface structure, namely fimbiae, is also eliminated from the members of the Shigella species by a different gene inactivation (data not shown). These are both examples of the genome interrogation that one may perform using Shi/BASE, thus adding insight into the biology and the evolutionary history of the Shigella species.

Shi/BASE also allows investigation of variation in genome structure at the amino acid level by gene order comparison, which is particularly useful for detecting orthologous genes. Orthologues are genes that are evolved vertically from a single ancestral gene in different genomes, and they often retain similar biological functions in the present-day organisms (13). Generally, protein sequence comparison is more sensitive because codon usage often leads to DNA sequence variations. For example, although the coding sequences of flIC show little similarity among Shigella genomes (Figure 1A) their protein sequences bare 46–62% in overall identity. In order to provide a uniform interface, the comparison of orthologues order was presented in a similar style as the comparison of DNA sequence described above, except that red bars were used here to link orthologue pairs. Multi-alignment and guide tree created by ClustalW for each orthologue group are directly linked from the individual graphic comparison pages.

Function-based comparison

Analysis of metabolic pathways is useful in illuminating the complexity of the Shigella metabolic patterns for the explanation of biochemical characteristics that distinguish Shigella from other enteric bacteria. By adopting the KEGG pathway maps, which offer graphic representations for most metabolic pathways (16), we have generated a comparative pathway map viewer. It combines individual pathways of E. coli K12 MG1655 and Shigella strains and displays the results in one map. For instance, Shigella strains do not synthesize lysine decarboxylase since the absence of lysine decarboxylase activity is important to their virulent lifestyle (17). These data can be easily accessed and browsed on our web page.

Another example is given in Supplementary Figure 1, which is the comparative view of the tyrosine metabolism pathway. It shows that Sf301, Sh227 and Ss046 possess the hpo operon, which offers them the ability to catabolize the aromatic compound 4-hydroxyphenylacetate (4-HPA), whereas Sd197 is lacking the hpo operon (18).

Although the four species of Shigella were originally divided on the basis of O-specific polysaccharide of the LPS, they additionally demonstrate distinctive differences in their pathological and epidemiological features. Furthermore, S. dysenteriae serotype 1 solely possesses the cytotoxic Shiga toxin and causes disease with neurological and renal complications (19). To highlight the diversity of virulence factors presented in each genome, we collated all known and putative virulence factors in a table, which links each virulence gene to detailed pages, allowing for further analysis or DNA/protein sequence retrieval.
Extended genome content comparison

In order to extend genomic comparison beyond genome sequences, we have integrated the newly available CGH data into ShiBASE. The CGH analysis includes 43 Shigella strains of different serotypes. The DNA microarray used to generate these data contains 5051 non-redundant genes from E.coli K12 MG1655 and all known Shigella genomes. Users may browse or search the CGH data and display the results in serological or clustering order.

Recent phylogenic analyses on eight housekeeping genes have grouped all Shigella strains into three main clusters with five outliers (20). Since use of the CGH results has generated a similar clustering order, the information is beneficial in demonstrating a correlation between genome structure and phylogenetic relationship. In order to facilitate further comparative analysis on the CGH results, an auto filter was set up to offer users the potential to rapidly examine commonly shared or lost genes within each cluster or serogroup.

For example, by screening the CGH results, a total of 228 genes present in the other S.flexneri strains are absent from S.flexneri 6. Most of the absent genes are related to LPS biosynthesis (such as rfbEFGIJ), outer membrane proteins (such as ompG, fhuA and nmpC) or enzymes (such as cai operon necessary for carnitine metabolism). Whereas S.flexneri 6 solely possesses the gsp genes encoding a novel type II secretion system. These findings may offer some reasoning to why S.flexneri 6 behaves differently from other S.flexneri strains and lies within a different cluster.

Online sequence comparison visualization service Shi-align

BLAST is a very powerful tool for finding sequence similarities and it has been widely used in database interrogation and sequence comparisons. However, when performing long sequence alignments, the voluminous and complex BLAST textual output is often difficult for biologists to read and analysis. Hence, several stand-alone programs, such as ACT (http://www.sanger.ac.uk/Software/ACT/) and GenomeComp (21), have been generated to tackle these issues.

In line with these programs, we have developed an online tool named Shi-align. Shi-align permits users to compare any sequence fragments with known Shigella genomes and in turn generate a graphic alignment view (Figure 1B) as an alternative to the textual output of BLAST. This tool is particularly useful for viewing the overall organization between query sequences and known Shigella genome sequences. Figure 1B shows a ~17 kb segment from enterohaemorrhagic E.coli (EHEC) encoding a putative adhesion aligned with corresponding sequences from various Shigella genomes in Shi-align. This graphical alignment demonstrates that sequences from Sb227 and Sf301 with the exception of IS insertions are essentially collinear with that of EHEC. Whereas the alignment sequences from Ss046 and Sd197 have obvious DNA rearrangements with respect to the EHEC sequence.
CONCLUSIONS AND FUTURE DIRECTIONS

As the developments of sequencing techniques and ubiquity of fast computers have led microbial genomics to the genus scale (22), resources at genus scale are also on demand. ShiBASE as well as other recently developed databases, such as coliBASE (10) and MolliGen (23), are all examples that aim to fulfil the requirement.

The current release of ShiBASE is dedicated to be a comprehensive online resource of Shigella and offers a platform for further comparative genomics studies on this important human pathogen. Nevertheless, the close genetic relationship between Shigella and E.coli is already well established. Moreover, an E.coli pathovar, named enteroinvasive E.coli (EIEC), is biochemically, genetically and pathogenically closely related to Shigella spp. EIEC is considered as an intermediate type in evolution between E.coli and Shigella. So, in the future, ShiBASE will not only include more data of Shigella (such as proteomics data) but also collect genomes of all types of pathogenic E.coli, especially EIEC.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR online.

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