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Analysis and comparative genomics of R997, the first SXT/R391 integrative and conjugative element (ICE) of the Indian Sub-Continent

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The aim of this study was to analyse R997, the first integrative and conjugative element (ICE) isolated from the Indian Sub-Continent, and to determine its relationship to the SXT/R391 family of ICEs. WGS of *Escherichia coli* isolate AB1157 (which contains R997) was performed using Illumina sequencing technology. R997 context was assessed by *de novo* assembly, gene prediction and annotation tools, and compared to other SXT/R391 ICEs. R997 has a size of 85 Kb and harbours 85 ORFs. Within one of the variable regions a HMS-1 β -lactamase resistance gene is located. The Hotspot regions of the element contains restriction digestion systems and insertion sequences. R997 is very closely related to the SXT-like elements from widely dispersed geographic areas. The sequencing of R997 increases the knowledge of the earliest isolated SXT/R391 elements and may provide insight on the emergence of these elements on the Indian sub-continent.

Integrative conjugative elements (ICEs) are a class of diverse bacterial mobile elements that are characterized by their ability to mediate and encode all determinants for their own integration, excision, and transfer from one host genome to another by a mechanism of site-specific recombination, self-circularisation, and conjugative transfer¹. They are a major factor in the evolution of bacterial genomes allowing bacteria to rapidly acquire new phenotypic traits and adaptive functions such as resistance to antimicrobial compounds and heavy metals, virulence mechanisms, metabolic pathways (such as pathways for the degradation of xenobiotic pollutants) and the ability to resist bacteriophage infection¹⁻⁴. SXT/R391 ICEs are chromosomal mobile genetic elements that consist of a conserved integrase that mediates site-specific integration into the 5' end of the *prfC* gene^{5,6}. The SXT/R391 family of ICEs is one of the largest of the ICE families with >100 elements being identified experimentally or bioinformatically to date⁷. R391 was the first element of the family discovered, in 1967; in a *Providencia rettgeri* clinical isolate from South Africa⁸. The R391 ICE mediates resistance to kanamycin and the heavy metal Hg⁹. In late 1992, SXT in MO10 was first discovered in one of the initial *Vibrio cholerae* O139 clinical isolates from Madras. This *V. cholerae* serogroup was the first non-O1 *V. cholerae* serogroup to give rise to epidemic cholera^{10,11}. SXT^{MO10} is an ~100 kb ICE that carries genes encoding resistance to sulfamethoxazole, trimethoprim, chloramphenicol, and streptomycin¹². Since then, SXT/R391 like elements have been found in a variety of *Vibrio* species as well as in other Gammaproteobacteria species including *Shewanella*, *Proteus* and *Photobacterium* species (11, Suppl Data).

This family of ICEs contains 51 near identical core genes, many of which are involved in integration/excision, conjugative transfer and regulation of the ICEs¹²⁻¹⁵. In addition, the elements contain five hotspots (called HS1-5) and five variable regions (called VRI-V) where accessory genes, such as antibiotic resistance genes, heavy metal resistant and DNA repair genes, can be found inserted^{1,2,16}. These elements can also promote the mobilisation of non-transmissible genomic islands and virulence plasmids between hosts¹⁷.

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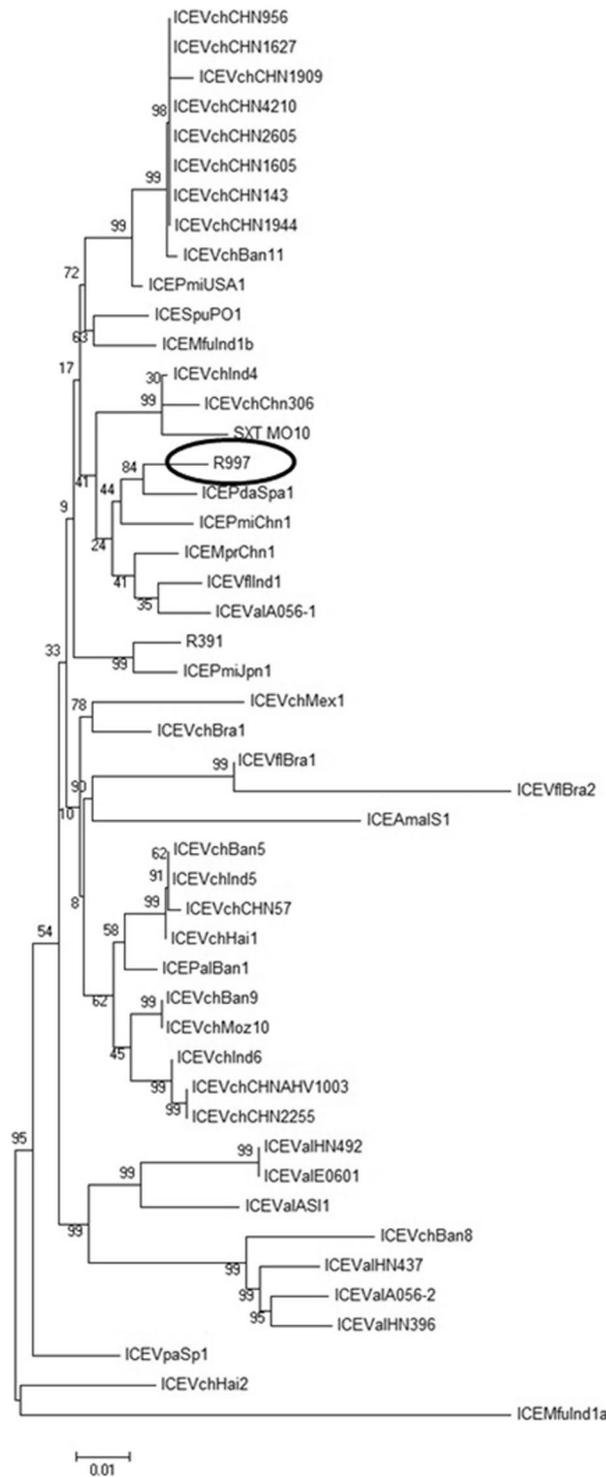


Figure 2. Phylogenetic tree from the maximum-likelihood analysis of the core concatenated proteins of 48 SXT/R391 ICEs.

Methods

Genome Sequencing and Annotation. The genome of *Escherichia coli* isolate AB1157 (which contains R997) was sequenced by Genospec Inc. (Houston, TX, USA) using paired-end (insert size between the ends 200–500 bp) HiSeq. 2000 Illumina technology giving approximately 300-fold coverage. The resulting reads were processed with Seqprep before being assembled using Newbler v2.5.3. The R997 genome was identified amongst 413 contigs by using the BLAST tool to investigate the presence of several different R391 (AY090559) and SXT (AY055428) core scaffold genes (*int*, *jef*, *traLEKBVA*, *setCD*). The R997 sequence was then annotated using the RAST Server (Rapid Annotation using Subsystem Technology) and the Basic Local Alignment Search Tool

(BLAST) programme at NCBI^{31, 32}. Any gaps among the sequence were filled in by PCR-linkage and Sanger sequencing. Primers can be seen in Supplementary Table 2. Putative functions for all proteins were inferred using the Basic Local Alignment Search Tool (BLAST) (<http://ncbi.nlm.nih.gov/BLAST>).

GenBank Accession Number. R997 was submitted to GenBank under accession number KY433363.

Phylogenetic Analysis of Core ICE genes. Phylogenetic analysis was performed based on the concatenated amino acid sequences of 48 SXT/R391 core genes encoded proteins on all 47 previously sequenced whole SXT/R391 elements. These elements are listed in Supplementary Table 1. An unrooted phylogenetic tree was constructed by maximum-likelihood method based on the Poisson correction model using the MEGA6³³. Bootstrap analysis with 1000 replications was performed to test the reliability of the tree.

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Author Contributions

M.P.R., P.A., J.O'H. and J.T.P. designed and performed the experiments, analysed the data, and drafted the article.

Additional Information

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