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Mobile genetic elements in the bacterial phylum Acidobacteria

Jean F. Challacombe* and Cheryl R. Kuske

Bioscience Division; Los Alamos National Laboratory; Los Alamos, NM USA

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Analysis of the genome of "*Candidatus* Solibacter usitatus Ellin6076", a member of the phylum Acidobacteria, revealed a large number of genes associated with mobile genetic elements. These genes encoded transposases, insertion sequence elements and phage integrases. When the amino acid sequences of the mobile element-associated genes were compared, many of them had high (90–100%) amino acid sequence identities, suggesting that these genes may have recently duplicated and dispersed throughout the genome. Although phage integrase encoding genes were prevalent in the "*Can.* S. usitatus Ellin6076" genome, no intact prophage regions were found. This suggests that the "*Can.* S. usitatus Ellin6076" large genome arose by horizontal gene transfer via ancient bacteriophage and/or plasmid-mediated transduction, followed by widespread small-scale gene duplications, resulting in an increased number of paralogs encoding traits that could provide selective metabolic, defensive and regulatory advantages in the soil environment. Here we examine the mobile element repertoire of "*Can.* S. usitatus Ellin6076" in comparison to other genomes from the Acidobacteria phylum, reviewing published studies and contributing some new analyses. We also discuss the presence and potential roles of mobile elements in members of this phylum that inhabit a variety of environments.

The abundant and phylogenetically diverse set of bacteria present in soils play important roles in terrestrial ecosystems through their interactions with plants and their functions in nutrient cycling processes. Acidobacteria is one of the most widespread and abundant phyla found in soils and sediments worldwide.[1-3] In some soils, up to 50% of the rRNA gene sequences from bacterial clone libraries are from Acidobacteria members.[4] The Acidobacteria phylum is defined by a large collection of 16S rRNA gene sequences (> 11,589 in the ARB_SILVA Database (August 2012[5]) that fall into 26 major subdivisions.[6] In addition to soils and sediments, Acidobacteria members been found in aquatic,[7,8] extreme[9,10] and polluted environments,[6] as well as wastewater systems.[11]

Members of this phylum have been difficult to isolate and culture in vitro. This situation has precluded their biological and physiological characterization,[10,12-16] and is the reason for the current lack of whole genome sequence data for the Acidobacteria. Because known members are widely abundant and phylogenenetically diverse, the Acidobacteria may be important constituents of a variety of ecosystems and further genomic studies are warranted.

Mobile elements play important evolutionary roles in bacteria by facilitating genome plasticity.[17-21] Their abundance in bacterial genomes varies for reasons that are not yet completely clear.[21] The "*Candidatus* Solibacter usitatus Ellin6076" genome encodes multiple genes often associated with mobile elements (**Table 1**). Fifty nine of the 123 mobile element associated genes encode transposases. Of these, 42 genes are annotated as insertion sequence (IS) elements, representing the IS3, ISL3, IS66, and IS110 families (**Table 1**). The genome also includes genes encoding phage integrase family proteins from the lambda integrase family, and other proteins containing an integrase, catalytic region domain.[22]

An insertion sequence (IS) element is a short DNA sequence that functions as a simple transposable element in bacteria.[23] IS elements are small compared with other transposable elements, typically less than 2,500 bp in length, and encode only the proteins needed for their own mobility,[23] including the transposase that catalyzes the enzymatic reaction that confers IS mobility, and a regulatory protein that either stimulates or inhibits the transposition activity.[24] The coding region in an insertion sequence is usually flanked by inverted repeats.[23,24] IS elements have been classified into families and sub-groups within each family, based on specific structural features. These include size range and presence of terminal inverted or direct target repeats.[25]

The "*Can.* S. usitatus Ellin6076" genome contained 16 genes encoding members of the IS3 transposase family, specifically the IS3/IS911 subgroup. The IS3 family is represented in more than 40 bacterial species,[25] including at least three

*Correspondence to: Jean F. Challacombe; Email: jchalla@lanl.gov
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Table 1. *Can.* S. usitatus Ellin6076 genes associated with mobile elements and their presence in other acidobacteria genomes

Type	Function/ Domain	Family	Number	Family found in other acidobacteria genomes?					
				A. capsulatum	*"Can.* K. versatilis"	*G. mallensis*	*G. tundricola*	*T. saanensis*	*"Can. C. thermophilum"*
phage integrase family protein	COG4974 Site-specific recombinase XerD	lambda integrase	27	yes	yes	yes	yes	yes	yes
integrase catalytic region	pfam00665 rve	NA	37	yes	yes	yes	yes	yes	no
transposase IS3/IS911 family protein	Pfam01527 transposase_8	IS3	16	yes	no	no	yes	no	no
transposase IS204/ IS1001/ IS1096/ IS1165	COG3464 Transposase and inactivated derivatives pfam01610 Transposase_12	ISL3	2	no	no	no	yes	yes	no
putative transposase protein Y4bF	pfam01548 Transposase_9 pfam02371 Transposase_20	NA	4	yes	yes	yes	yes	yes	no
IS116/ IS110/ IS902		IS110	20	yes	yes	yes	yes	yes	no
transposase IS66	pfam03050 Transposase_25 COG2251 Predicted nuclease (RecB family)	IS66	4	no	no	no	no	no	no
transposase	transposase_11 pfam01609	NA	1	yes	no	yes	yes	no	no
transposase	NA	NA	4	no	no	no	no	no	no
putative transposase	NA	NA	7	no	no	no	yes	no	no
transposase-like	NA	NA	1	no	no	no	no	no	no

Data presented in this table were obtained from BLAST [45] analysis, the Integrated Microbial Genomes (IMG) System,[35] and the references that describe the genomes.[16, 22, 28, 34]

acidobacteria genomes ("*Can.* S. usitatus Ellin6076," *G. tundricola* MP5ACTX9 and *A. capsulatum*). The defining features of IS3 family transposition include a transposase encoded by OrfAB, where the resulting product is a fusion protein generated by translational frame shifting,[23] and excision and circularization mediated by the OrfAB transposase.[23,26]

Members of the ISL3 family generate 8-bp direct repeats upon insertion, but exhibit no obvious target sequence specificity, even though studies suggest that these elements may prefer AT-rich regions.[23] The most well-characterized member of the ISL3 family is IS31831 from *Corynebacterium glutamicum* (Phylum Actinobacteria).[27] Members

of this family have been found in other bacterial species,[23,25] including "*Can.* S. usitatus Ellin6076," which contained two genes annotated as members of this family. ISL3 is also represented in the acidobacteria *Granulicella tundricola* MP5ACTX9 and *Terriglobus saanensis* SP1PR4[28] (**Table 1**). The transposition mechanism of these elements has not yet been determined, but evidence suggests that IS1411 from the proteobacterium, *Psuedomonas putida,* forms a circular species.[29]

IS66 family members are widely distributed in the phylum Proteobacteria e.g., (Agrobacterium, Rhizobium Escherichia, Pseudomonas, and Vibrio spp).[30] Four copies of IS66 were found in "*Can.* S. usitatus Ellin6076," but not in the other acidobacteria genomes (**Table 1**). The mechanism of IS66 family transposition appears to be different from that of the IS3 family members. The IS66 family elements do not produce a transposase by translational frame-shift; instead they produce three proteins by a translational coupling mechanism, where the distal ORF is translated only after translation of the proximal ORF.[30]

Twenty genes encoding members of the IS110 family were identified in the "*Can.* S. usitatus Ellin6076 genome," and representatives of this family were also found in all of the other acidobacteria genomes, except "*Can.* C. thermophilum B" (**Table 1**). The IS110 family forms two distinct subgroups, IS110 and IS111, which could be classified as separate families.[23,25] The mechanism of transposition of IS110 family elements is unclear. However, the presence of a circular form of the element is supported by evidence in *Streptomyces coelicolor*[31] and *Pseudoalteromonas atlantica*.[32]

The presence of phage integrases in bacterial genomes can indicate past phage transduction events, even in the absence of intact prophage regions in the genome, which is the case for the soil acidobacteria genomes that we previously analyzed.[16,22] Phage integrases, also known as site-specific recombinases, catalyze site-specific recombination between short (30–40 bp) phage and bacterial DNA attachment sequences termed *attP* (phage) and *attB* (bacterial).[33] The catalytic domain acts as an endonuclease when two nucleotides are removed from the 3' ends of the blunt-ended viral DNA made by reverse transcription. This domain also catalyzes the DNA strand transfer reaction of the 3' ends of the viral DNA to the 5' ends of the integration site. There are two integrase families differentiated by the presence of a tyrosine or serine amino acid in the catalytic site. These families differ from each other with respect to the mechanism of recombination, characteristics of attachment sites, and requirements for bacterial host cofactors.[33] Phage integrase genes were present in all of the acidobacteria from soils or sediments [*A. capsulatum*, "*Can.* K. versatilis," *G. mallensis* MP5ACTX8, *G. tundricola* MP5ACTX9, and *T. saanensis* SP1PR4[16,22,28](**Table 1**)], as well as in the genome of "*Can.* C. thermophilum B" from an alkaline hot spring.[34] However, while the genomes of the acidobacteria from soils or sediments contained genes encoding proteins with integrase catalytic domains, "*Can.* C. thermophilum B" did not.[34] Other mobile element genes found in the "*Can.* C. thermophilum B" genome were unique to this species; they were not found in the other acidobacteria. The majority of these genes encoded IS605 family proteins.[34] A cursory examination of the arctic tundra genomes by searching for the "phage" keyword in IMG[35] revealed the presence of genes encoding phage terminase subunits, phage portal, phage prohead protease and phage major capsid proteins in *G. mallensis* MP5ACTX8 and *G. tundricola* MP5ACTX9, suggesting that these genomes may contain prophage regions. In contrast, the genomes of "*Can.* S. usitatus," *A. capsulatum* and "*Can.* K. versatilis" do not contain any identifiable prophage regions, but they do contain genes encoding phage integrase family proteins and other proteins containing integrase catalytic domains.[16] There were no prophage regions reported in the genome of "*Can.* C. thermophilum B."[34]

In summary, all of the sequenced Acidobacteria genomes contain multiple genes that are often associated with mobile elements (**Table 2**). Increasing evidence indicates that mobile element abundance correlates positively with the frequency of horizontal gene transfer between genomes or between replicons of the same genome (reviewed in refs. 18 and 21). Mobile elements can transfer adaptive traits, such as pathogenicity islands and virulence genes (reviewed in refs. 18 and 36), antibiotic resistance,[37-39] metabolic functions,[29,40] and also play a significant role in genome plasticity and evolution.[17-21]

The types and abundances of mobile element-associated genes present in particular organisms may be highly influenced by environmental conditions. Phage-mediated transduction events could occur within a relatively local population, among unrelated bacteria that live in close proximity (reviewed in refs. 41–43). One may speculate that particular families of mobile elements are common to the inhabitants of soil and sediment ecosystems, and may differ in composition from those in other environments (e.g., aquatic and hot springs). In support of this conjecture, the acidobacteria genomes from soils and sediments[16,22,28] contained some similar types of mobile element genes, in spite of the very different geographic regions and geochemical characteristics of the soils/sediments from which they were isolated. In contrast, the genome of the hot springs isolate, "*Can.* C. thermophilum B,"[34] contained a unique assortment of mobile element genes compared with the other acidobacteria. Significantly, the mobile elements found in the "*Can.* C. thermophilum B" genome were most similar to those found in the genomes of other, more distantly related bacterial inhabitants of the hot springs environment.[34]

The "*Can.* S. usitatus Ellin6076" and *G. tundricola* MP5ACTX9 genomes harbored increased numbers of mobile element genes compared with the other acidobacteria genomes. This could be due to a particular need for increased functional diversity in these species, which could aid them in coping with extremes of moisture, temperature, geochemical conditions, and potentially provide them with an enhanced competitive ability to exploit different environmental resources.[22] However, other isolates from the same environments as "*Can.* S. usitatus Ellin6076" and *G. tundricola* MP5ACTX9 did not contain similar increased numbers of mobile elements. The genomes of "*Can.* K. versatilis Ellin345," isolated from the same pasture as *Can.* S. usitatus Ellin6076;[44] and genomes of *G. mallensis*

Table 2. Mobile element-associated genes in acidobacteria genomes

Genome	Habitat	Number of mobile element genes
"Can. S. usitatus Ellin6076"	Ryegrass/clover pasture, mineral soil (pH ~5.5), Victoria, Australia [44, 46, 47]	123
"Can. K. versatilis Ellin345"	Ryegrass/clover pasture, mineral soil (pH ~5.5), Victoria, Australia [44, 46, 47]	29
"Can. C. thermophilum B"	Alkaline (pH ~8), silicious hot springs bacterial mat, Montana, USA [12]	31
A. capsulatum ATCC 51196	Acidic (pH 2.6 – 5.3) mineral sediments, pyrite mine, Japan [10, 48]	38
G. mallensis MP5ACTX8	Arctic tundra heath, organic layer (pH 4.5–5.2), Finland [49]	63
G. tundricola MP5ACTX9	Arctic tundra heath, organic layer (pH 4.5–5.2), Finland [49]	154
T. saanensis SP1PR4	Arctic tundra heath, organic layer (pH 4.5–5.2), Finland [50]	35

MP5ACTX8 and *T. saanensis* SP1PR4, isolated from the same arctic soil as *G. tundricola* MP5ACTX9,[28] all contained much lower numbers of mobile element genes. This situation underscores the need for isolation and study of additional acidobacteria and their genomes, from as many diverse environments as possible, to further explore the prevalence and functions of mobile genetic elements in members of this genetically and geographically diverse phylum.

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