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A Tn*7*-Like Transposon Is Present in the *glmUS* Region of the Obligately Chemoautolithotrophic Bacterium *Thiobacillus ferrooxidans*

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The region downstream of the *Thiobacillus ferrooxidans* ATCC 33020 *atp* operon was examined, and the genes encoding *N*-acetylglucosamine-1-uridyltransferase (*glmU*) and glucosamine synthetase (*glmS*) were found. This *atpEFHAGDC-glmUS* gene order is identical to that of *Escherichia coli*. The *T. ferrooxidans glmS* gene was shown to complement *E. coli glmS* mutants for growth on minimal medium lacking glucosamine. A Tn*7*-like transposon, Tn*5468*, was found inserted into the region immediately downstream of the *glmS* gene in a manner similar to the site-specific insertion of transposon Tn*7* within the termination region of the *E. coli glmS* gene. Tn*5468* was sequenced, and Tn*7*-like terminal repeat sequences as well as several open reading frames which are related to the Tn*7* transposition genes *tnsA*, *tnsB*, *tnsC*, and *tnsD* were found. Tn*5468* is the closest relative of Tn*7* to have been characterized to date. Southern blot hybridization indicated that a similar or identical transposon was present in three *T. ferrooxidans* strains isolated from different parts of the world but not in two *Thiobacillus thiooxidans* strains or a *Leptospirillum ferrooxidans* strain. Since *T. ferrooxidans* is an obligately acidophilic autotroph and *E. coli* is a heterotroph, ancestors of the Tn*7*-like transposons must have been active in a variety of physiologically different bacteria so that their descendants are now found in bacteria that occupy very different ecological niches.

Thiobacillus ferrooxidans is an acidophilic (optimum pH, 1.6 to 2.2), obligately chemoautolithotroph and is a member of a consortium of physiologically related bacteria used in processes to recover metals such as copper, uranium, and gold from ores (4, 17, 21). Because of its interesting physiology and industrial use there has been considerable interest in this bacterium. The *T. ferrooxidans atp* genes had previously been cloned and sequenced (5). Limited sequencing of the region downstream of the *atp* operon (unpublished) had indicated that the gene order *atpEFHAGDC-glmUS* as found in *Escherichia coli* (19, 29) was the same for *T. ferrooxidans*. In *E. coli* the *pst* operon, which encodes a phosphate-specific transport system, lies downstream of the *glmUS* genes. Phosphate nutrition of *T. ferrooxidans* is of particular interest, as phosphate is one of the few nutrients added to industrial gold-bearing arsenopyrite ore treatment processes (21). Since the gene order of *E. coli* and *T. ferrooxidans* was conserved in this region, we wished to determine whether this conservation extended to the *pst* operon.

Isolation of *T. ferrooxidans* genomic DNA downstream of the *atp* operon. Cosmid p818.1 (Fig. 1) was isolated from a cosmid gene library of *T. ferrooxidans* ATCC 33020 by its ability to complement an *E. coli uncD* mutant for growth on minimal medium plus succinate according to the procedure reported by Brown et al. (5). The cosmid was mapped for restriction endonuclease sites and appeared to extend approximately 35 kb downstream of the *T. ferrooxidans atp* operon. In order to demonstrate that the insert in cosmid p818.1 represented unrearranged chromosomal DNA from *T. ferrooxidans*, the insert from subclone p818.52 (Fig. 1) was labelled by using a digoxigenin (DIG) DNA labelling kit (Boehringer Mannheim) and used in a Southern blot hybridization experiment (Fig. 2). Hybridizations were performed at 68°C for 18 h followed by two washings at 22°C in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) plus 0.1% sodium dodecyl sulfate and two washings at 65°C in 0.1× SSC plus 0.1% sodium dodecyl sulfate. Cosmid p818.1 and *T. ferrooxidans* genomic DNA gave positive hybridization signals to fragments of identical size when digested with the same restriction enzyme. *Bam*HI digests gave signals at 2.5 and 2.8 kb (Fig. 2, lanes 1 and 2), *Hin*dIII digests gave signals at about 10 kb (lanes 3 and 4), and *Bgl*II digests gave signals at 5.7 kb (lanes 5 and 6). The additional weak signals in the p818.1 lanes arose because the *Kpn*I-*Sal*I probe from p818.52 had a small quantity of contaminating vector (pBluescript KS) which has regions of homology to the cosmid vector (pHC79). This confirmed that the region extending from the *Bgl*II site shown at 37 kb to the *Hin*dIII site at 25.5 kb of p818.1 was unrearranged chromosomal DNA from *T. ferrooxidans* ATCC 33020.

Location of *glmUS* genes and *glmS* gene complementation. The 3.5-kb *Bam*HI-*Bam*HI fragment downstream of the *T. ferrooxidans* operon was cloned into vector pUCBM21 to give p818.16 (Fig. 1). This fragment was sequenced in both directions by the dideoxy chain termination method by using a Sequenase DNA sequencing kit (U.S. Biochemical Corporation, Cleveland, Ohio). This region contained a partial open reading frame (ORF) of 182 amino acids and complete ORFs of 711 and 277 amino acids. The partial ORF had a high percent amino acid sequence identity to the C terminus of the *glmU* gene products of *E. coli* (59.1%; SWISS-PROT accession no. P17114), *Haemophilus influenzae* (58.0%; SWISS-PROT accession no. P43889), *Neisseria meningitidis* (50.8% [27]), and *Bacillus subtilis* (43.5%; SWISS-PROT accession no. P14192).

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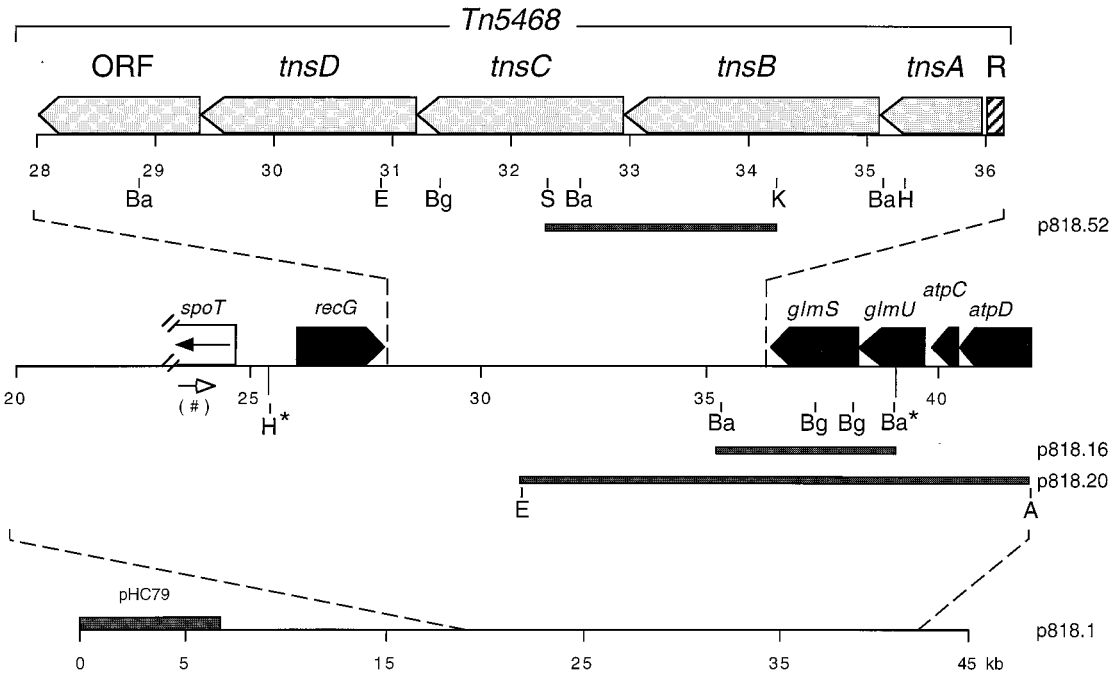

FIG. 1. A map of cosmid p818.1 and subclones showing the location of Tn*5468* as well as the direction of the genes and ORFs downstream of the *atp* operon. Abbreviations for restriction endonuclease sites are as follows: A, *Apa*I; Ba, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; and S, *Sal*I. The region sequenced in both directions extended from H* at 25.5 kb to Ba* at 38.5 kb. The pound sign and open arrow denote the direction of limited single-strand sequencing which identified the position and direction of the *spoT* gene. R, right.

The first complete ORF had amino acid sequence identity with the *glmS* gene products of *E. coli* (47.8%; SWISS-PROT accession no. P17169), *H. influenzae* (51.9%; SWISS-PROT accession no. P44708), *B. subtilis* (39.1%; SWISS-PROT accession no. P39754), *Saccharomyces cerevisiae* (39.4%; SWISS-PROT accession no. P14742), and *Mycobacterium leprae* (42.2%; SWISS-PROT accession no. P40831) as well as to the nodulation protein M sequences of *Rhizobium meliloti* (43.6%; SWISS-PROT accession no. P25195) and *Rhizobium leguminosarum* (44.0%; SWISS-PROT accession no. P08633).

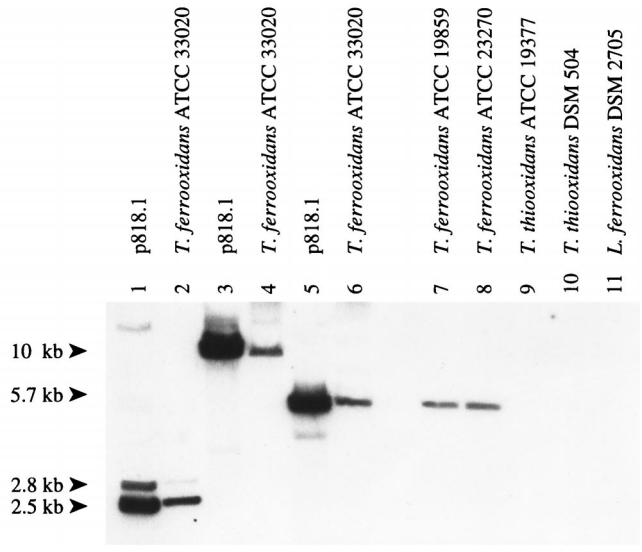

FIG. 2. Hybridization of DIG-labelled p818.52 to cosmid p818.1 and total DNA from strains of *T. ferrooxidans*, *T. thiooxidans*, and *L. ferrooxidans*. DNA in lanes 1 and 2 was digested with *Bam*HI; that in lanes 3 and 4 was digested with *Hin*dIII, and that in lanes 5 to 11 was digested with *Bgl*II.

Glucosamine-6-phosphate synthase (encoded by the *glmS* gene) and *N*-acetylglucosamine-1-uridyltransferase (encoded by the *glmU* gene) are two of the enzymes required for the synthesis of UDP-*N*-acetylglucosamine, a major precursor of the bacterial cell wall peptidoglycan. *E. coli glmS* mutants such as strain CGSC5392 (provided by B. Bachmann; originally isolated as E110 by Wu and Wu [30]) are not capable of growth in the absence of added glucosamine. To test whether the *T. ferrooxidans glmS* gene was functional in *E. coli*, plasmid p818.16 (Fig. 1), which has the *T. ferrooxidans glmS* gene cloned behind a pUCBM21 vector promoter, was transformed into competent *E. coli* CGSC5392 cells and plated onto Luria agar plus ampicillin (100 μg/ml). *E. coli* CGSC5392(p818.16) cells produced medium-sized colonies on Luria agar, whereas *E. coli* CGSC5392(pUCBM21) cells, which contained vector but no insert, produced pinprick-sized colonies. Large colonies were produced by both types of cells when D-glucosamine (200 μg/ml) was added to the plates. *E. coli*(p818.20) cells, which contained 10 kb of *T. ferrooxidans* DNA, including the *glmU* and *glmS* genes (Fig. 1), failed to produce large colonies in the absence of added D-glucosamine. This suggests that the *T. ferrooxidans glmS* gene is not expressed from its own promoter in *E. coli.*

Location of Tn*5468*. The region from *Bam*HI (35.2 kb) to *Hin*dIII (25.5 kb) (Fig. 1) was determined by double-stranded DNA automated sequencing using fluorescently labelled nucleotides; all regions were sequenced multiple times from both strands. The region from *Sal*I (near *tnsA*) to *Eco*RI (in the N terminus of *tnsD*) was dissected by using a primer walking strategy. The region from *Eco*RI (*tnsD*) to *Hin*dIII was determined by generating a number of mini-Tn*7* element insertions in this area by using an in vitro transposition system (23). After

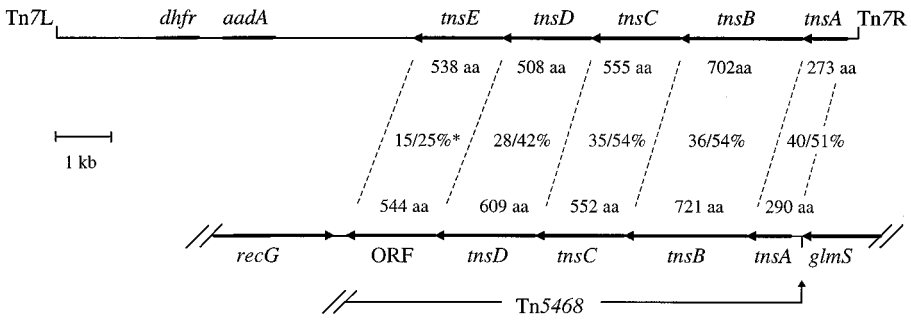

FIG. 3. Comparison of Tn*5468* and Tn*7*, indicating the sizes and relatedness of the predicted gene products. Tn*5468* is shown in its position within the *T. ferrooxidans* ATCC 33020 chromosome, with the right border of Tn*5468* indicated by an arrow and the truncated, left-hand region indicated by oblique parallel lines. Percent amino acid (aa) identities and similarities were calculated according to the same criteria as those used by the BLAST subroutine (NCBI database), with the length of the smaller protein as the denominator. *, the similarities between *tnsE* of Tn*7* and the unidentified ORF of Tn*5468* were apparent only within the N-terminal halves of the proteins.

transformation, individual mini-Tn*7* insertions were isolated and the sequences of the Tn*5468* DNA flanking the newly inserted mini-Tn*7* was determined with primers specific for the ends of Tn*7*.

Analysis of the Tn*7*-like transposon, Tn*5468*. Analysis of the newly sequenced region also revealed a DNA segment, now designated Tn*5468* (Fig. 1), immediately downstream of the *T. ferrooxidans glmS* gene, that has considerable similarity to Tn*7*. Tn*7* is a bacterial transposon (6, 7) which inserts site specifically downstream of *glmS* in other bacteria such as *E. coli* that are physiologically very distantly related to *T. ferrooxidans*. The finding of closely related transposable elements in the distinct ecological niches of *T. ferrooxidans* and *E. coli* raises interesting questions about the evolution and dispersal of transposable elements (see below).

Site-specific insertion of Tn*7* downstream of *glmS* proceeds via recognition of a particular DNA sequence in the COOH region of *glmS* (3, 11); the actual point of element insertion is 23 bp downstream of the *glmS* termination codon. Such a target selection strategy allows the transposon to use information within the highly conserved *glmS* gene to identify a particularly favorable target site; however, this strategy also avoids disruption of *glmS* through the insertion of Tn*7* of that gene itself. Such a site-specific insertion provides a safe haven for Tn*7* in bacterial chromosomes (6, 7). The biochemical roles of the Tn*7* transposition proteins (Tns proteins) have been established. TnsB specifically recognizes the ends of Tn*7* by binding to multiple repeats of its recognition sequence that are present in each end (1, 25). TnsA and TnsB then collaborate in an interdependent fashion to form the transposase, which executes the chemical steps of strand exchange (18, 22). The DNA breakage and joining activity of the Tn*7* TnsA+B transposase is not constitutive; rather, transposase activity depends upon activation of TnsA+B by TnsC, an ATP-dependent DNA binding protein (9, 24). The transposase activation activity of TnsC is, in turn, modulated by other Tns proteins that interact with target DNA (3, 23, 24). For example, the interaction of TnsC with TnsD, a protein that binds specifically to DNA sequences in *glmS*, mediates the site-specific insertion of Tn*7* just downstream of this gene (3).

Tn*5468* closely resembles Tn*7* in structure and organization (Fig. 3), encoding four ORFs that are clearly similar to the Tn*7* transposition proteins TnsA, TnsB, TnsC, and TnsD; also present in Tn*5468* is another ORF that is not obviously related to sequences currently present in databases. In the right end of both Tn*7* and Tn*5468*, there are four closely juxtaposed repeats that are separated by 8 bp from the extreme tip of the transposon (Fig. 4); these repeats in Tn*7* have been shown to be DNA sequences necessary in *cis* for Tn*7* transposition (2, 8). It has also been shown that in Tn*7* the identity of the terminal 5′-ACA-3′ is a key to breakage and joining activities (9a, 10). There is a very similar pattern of repeats and terminal sequences in the right end of Tn*5468*, providing evidence that Tn*5468* is indeed a Tn*7*-like transposon (Fig. 4). In Tn*7*, the left end also contains this 8-bp terminal sequence and multiple repeats (2); however, no such sequences are evident in the region between the *T. ferrooxidans recG* and the Tn*5468* ORF, suggesting that this element may have become truncated in some way. The repeat sequences at the ends of Tn*7* are binding sites for TnsB protein (1, 25). The sequence similarity of repeats (Fig. 4) and of Tn*7* and Tn*5468* TnsB's (Fig. 3) suggest that these *T. ferrooxidans* repeats are binding sites for *T. ferrooxidans* TnsB and thereby define the functional terminus of Tn*5468*. Tn*5468* TnsB also conserves several acidic residues which have been shown to be essential to the transposase activity of Tn*7* TnsB (22). In Tn*7*, TnsA and TnsB collaborate interdependently to form the transposase (18, 22); these TnsB proteins are also related to the transposases of a variety of mobile elements, as exemplified by comparison to the transposase of Tn*5053*, another element distantly related to Tn*7*, which is found in some species of *Xanthomonas* and *Klebsiella* (14, 20). Tn*5468* also encodes a protein highly related to Tn*7* TnsA, in which key residues (D134 and E169 [*E. coli* numbering]) that have been shown to be essential to transposase activity are conserved (18, 22). These TnsB's and TnsA's are all likely members of the human immunodeficiency virus integrase superfamily (14, 20, 22). Tn*5468* also contains ORFs that are related to TsnC, containing conserved purine nucleotide binding motifs (9), and to TnsD, which binds specifically to particular sequences in *glmS* (3) and thus mediates site-specific insertion (Fig. 5). The similarities support the view that Tn*5468* is a Tn*7*-like element that at some time transposed site specifically to a position just downstream of *T. ferrooxidans glmS* by a mechanism highly related to the Tn*7* transposition mechanism.

It should be noted that Tn*7* can also use a different class of target sites when transposition is mediated by a slightly different ensemble of Tns proteins, TnsABC+E, TnsE being an alternative target selector (15, 28). Inspection of the Tn*5468* sequences has not revealed a gene like that encoding TnsE. An interesting hypothesis is that the unidentified ORF of Tn*5468* provides yet another target selection protein that could promote insertion into other types of sites.

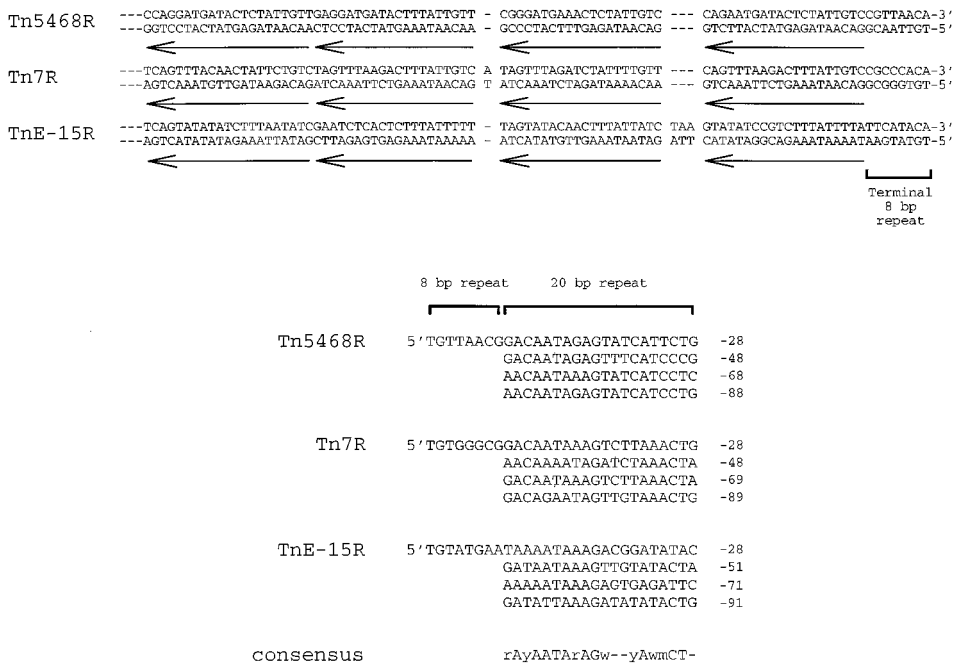

FIG. 4. The sequences at the right (R) ends of several Tn*7*-like elements, Tn*5468* from *T. ferrooxidans*, Tn*7* from *E. coli*, and TnE-15, a partial element also found in *T. ferrooxidans*, are shown. Inspection of the sequences reveals the presence of a 20-bp sequence present in four closely juxtaposed copies in the right end of each element. In Tn*7*, these right-end repeats and similar repeats in the left end of Tn*7* are required for transposition; the Tn*7* 20-bp repeat is a binding site for the element-encoded TnsB protein. The terminus of each element consists of an 8-bp segment ending in ACA-3′; the terminal A is the 3′ end of the transposon. Analysis of this segment in Tn*7* suggests that the ACA sequence is critical to provoke DNA breakage and joining; the other 5-bp segment likely serves as a spacer between the B binding site and the ACA sequence at the terminus (9a). Lowercase letters in the consensus sequence represent the following: r, purine; y, pyrimidine; w, A or T; m, A or C.

Distribution of Tn*7*-like transposons among other acidophilic chemoautolithotrophs. Evidence of the activity of another Tn*7*-like transposon has been found in *T. ferrooxidans* E-15 (12). In this strain there appears to have been a duplication of the *merC* and *merR* genes, which are separated by a region with Tn*7*-like terminal repeat sequences and a partial ORF with strong similarity to the Tn*7* *tnsA* gene. The insertion of the Tn*7*-like transposon in *T. ferrooxidans* E-15 is not associated with the *glmS* termination region. Although both the *T. ferrooxidans* ATCC 33020 and E-15 strains have Tn*7*-like transposon sequences, they are clearly different from each other and Tn*7*. The four repeated sequences of the three transposons are compared in Fig. 4.

We wished to determine whether transposons related to Tn*5468* were present in other *T. ferrooxidans* isolates and in other bacteria which grow in a similar environment. *Thiobacillus thiooxidans* and *Leptospirillum ferrooxidans* are physiologically similar to *T. ferrooxidans* in that they are all gram-negative, obligately autotrophic, acidophilic, chemolithotrophic bacteria (21). Chromosomal DNA was prepared from three strains of *T. ferrooxidans*, ATCC 33020, ATCC 19859, and ATCC 22370 (type strain); two strains of *T. thiooxidans*, DSM 504 and ATCC 19377 (type strain); and *L. ferrooxidans* DSM 2705 (type strain). This genomic DNA was digested with *Bgl*II, and the fragments were separated on an agarose gel, Southern blotted onto a Hybond N^+ nylon membrane, and hybridized to DIG-labelled p818.52 probe DNA as described earlier. The probe hybridized to a 5.7-kb *Bgl*II fragment from all three of the *T. ferrooxidans* isolates (Fig. 2, lanes 6 to 8) but not to either of the *T. thiooxidans* or the *L. ferrooxidans* strains (Fig.

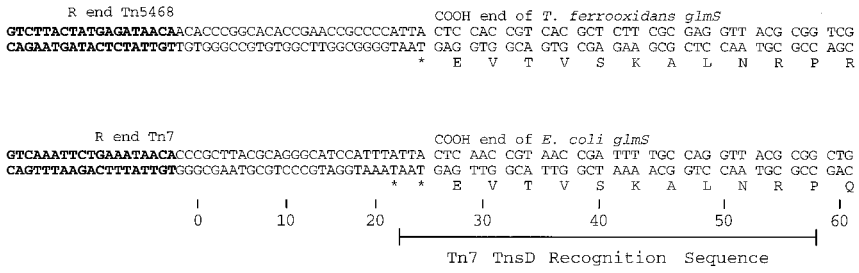

FIG. 5. The locations of the right (R) ends of Tn*5468* and Tn*7* with respect to the chromosomal *glmS* genes are shown. In both cases, the element has been inserted about 20 bp downstream from the COOH end of the *glmS* gene. The *E. coli* sequence (lower line) is numbered, with the center of the 5 bp duplicated upon Tn*7* insertion designated 0. Also shown is the *E. coli* sequence necessary for TnsD binding to attTn*7* (3). Although the *glmS* sequences, i.e., the TnsD binding sites, are highly conserved between *E. coli* and *T. ferrooxidans*, the sequences between the point of element insertion and the end of *glmS* are not. Other analysis of attTn*7* in *E. coli* has shown that sequence alterations in this region, even at the point of Tn*7* insertion itself, have little effect on attTn*7* target activity (3).

2, lanes 9 to 11). The *T. ferrooxidans* ATCC 33020 strain was isolated in Japan and genomic DNA was prepared from cells grown in iron medium in a laboratory in South Africa, while the ATCC 19859 and ATCC 22370 strains originated from Canada and the United States, respectively, and DNA was prepared from cells grown on tetrathionate medium in a laboratory in Sweden. This eliminates the possibility that the *T. ferrooxidans* strains or chromosomal DNA had become mixed during growth or preparation. The *glmS* region of the *T. ferrooxidans* chromosome appears to be highly conserved and a transposon closely related to Tn*5468* appears to be widely distributed as a single-copy insert in the genome of *T. ferrooxidans*. No sequences with similarity to the Tn*5468 tnsBC* sequence were detected in the isolates of *T. thiooxidans* or *L. ferrooxidans* tested.

Region downstream of Tn*5468*. An ORF of 652 amino acids was present in the region downstream of Tn*5468* in the opposite orientation to the Tn5468 genes (Fig. 1). Comparison of the predicted amino sequence with the GenBank database by using the BLAST subroutine (NCBI database) indicated the highest sequence similarities, 34 and 32% sequence identity, to the *recG* gene products of *E. coli* and *H. influenzae*, respectively. Limited sequencing further downstream indicated a region of high similarity, 64 and 59% amino acid identity (over 85 amino acids), to the *spoT* gene products from *E. coli* and *H. influenzae*, respectively. In both *E. coli* and *H. influenzae*, the *recG* (RecG is an ATP-dependent DNA helicase) and *spoT* (SpoT is a guanosine-3′,5′-bis diphosphate 3′-pyrophosphatase) genes are linked and together with *spoU* and *spoS* form the *spo* operon (13, 16). In *T. ferrooxidans* the situation appears different as the *spoT* and *recG* genes, although physically linked, are orientated in opposite directions and are therefore divergently translated.

No region with sequence similarity to the *pst* operon of *E. coli* was found, and there was insufficient space between the apparent end of Tn*5468* and the RecG-like ORF of *T. ferrooxidans* to accommodate the *spo* genes.

Comparison of *T. ferrooxidans atp-glm*-Tn*5468-spo* region with other bacteria. Given very different physiologies and ecological niches, *E. coli* and *T. ferrooxidans* show remarkable similarity in this region of their chromosome. The *glmU* and *glmS* genes are linked and located immediately downstream of the *atp* (*unc*) operon. In contrast, in *H. influenzae* and *Helicobacter pylori* (the only other bacteria in which the location of both *atp* and *glmS* genes have been reported), these genes are not physically linked on the chromosome (http://www.tigr.org).

The similarity between *E. coli* and *T. ferrooxidans* is further extended by the discovery of the Tn*7*-like transposon in the region downstream of the *T. ferrooxidans glmS* gene. Although specific Tn*7* insertion sites have been identified in the chromosomes of a variety of bacteria as divergent as *Agrobacterium tumefaciens*, *Caulobacter crescentus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Rhizobium meliloti*, *Rhodopseudomonas capsulata*, and *Xanthomonas campestris* (6), all these bacteria were deliberately exposed to Tn*7* in the laboratory. In contrast, a transposon that is Tn*7*-like but clearly different from Tn*7* has been active in natural isolates of *T. ferrooxidans* from different parts of the world. *T. ferrooxidans* is an obligately autotrophic bacterium whose growth is inhibited by low concentrations of most organic compounds, such as small amounts of free galactose in agar (26). As a result, much of the evolutionary development of *T. ferrooxidans* would be expected to have taken place with little physical contact or genetic exchange between it and typical heterotrophic bacteria such as those listed above. Tn*7*, Tn*5468*, and the Tn*7*-like transposon of *T. ferrooxidans* E-15 (12) must have originated from a common ancestor which was active in bacteria long before they became as physiologically divergent as *T. ferrooxidans* and *E. coli* are today.

Nucleotide sequence accession number. The 13-kb sequence between the *Bam*HI and *Hin*dIII sites indicated by asterisks (Fig. 1) has been assigned GenBank accession no. AFO32884.

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REFERENCES

1. **Arciszewska, L. K., and N. L. Craig.** 1991. Interaction of the Tn*7*-encoded transposition protein TnsB with the ends of the transposon. Nucleic Acids Res. **19:**5021–5029.
2. **Arciszewska, L. K., D. Drake, and N. L. Craig.** 1989. Transposon Tn*7* *cis*-acting sequences in transposition and transposition immunity. J. Mol. Biol. **207:**35–52.
3. **Bainton, R. J., K. M. Kubo, J.-N. Feng, and N. L. Craig.** 1993. Tn*7* transposition: target DNA recognition is mediated by multiple Tn*7*-encoded proteins in a purified in vitro system. Cell **72:**931–943.
4. **Brierley, C. L.** 1982. Microbiological mining. Sci. Am. **247**(2)**:**42–51.
5. **Brown, L. D., M. E. Dennehy, and D. E. Rawlings.** 1994. The F_1 genes of the F_1F_0 ATP synthase from the acidophilic bacterium *Thiobacillus ferrooxidans* complement *Escherichia coli* F_1 *unc* mutants. FEMS Microbiol. Lett. **122:** 19–26.
6. **Craig, N. L.** 1989. Transposon Tn*7*, p. 211–225. *In* D. E. Berg and M. M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
7. **Craig, N. L.** 1995. Transposon Tn*7*. Curr. Top. Microbiol. Immunol. **204:** 27–48.
8. **DeBoy, R. T., and N. L. Craig.** 1996. Tn*7* transposition as a probe of *cis* interactions between widely separated (190 kilobases apart) DNA sites in the *Escherichia coli* chromosome. J. Bacteriol. **178:**6184–6191.
9. **Gamas, P., and N. L. Craig.** 1992. Purification and characterization of TnsC, a Tn*7* transposition protein that binds ATP and DNA. Nucleic Acids Res. **20:**2525–2532.

9a. **Gary, P., and N. L. Craig.** Unpublished observations.

10. **Gary, P. A., M. C. Biery, R. J. Bainton, and N. L. Craig.** 1996. Multiple DNA processing reactions underlie Tn*7* transposition. J. Mol. Biol. **257:**301–316.
11. **Gringauz, E. K., A. Orle, C. S. Waddel, and N. L. Craig.** 1988. Recognition of *Escherichia coli attTn7* by transposon Tn*7*: lack of specific requirements at the point of Tn*7* insertion. J. Bacteriol. **170:**2832–2840.
12. **Inoue, C., K. Sugawara, and T. Kusano.** 1991. The *merR* regulatory gene in *Thiobacillus ferrooxidans* is spaced apart from the *mer* structural genes. Mol. Microbiol. **5:**2707–2718.
13. **Kalman, M., H. Murphy, and M. Cashel.** 1992. The nucleotide sequence of *recG*, the distal *spo* operon gene in *Escherichia coli* K12. Gene **110:**95–99.
14. **Kholodii, G., S. Mindlin, I. Bass, O. Yurieva, S. Minakhina, and V. Nikiforov.** 1995. Four genes, two ends, and a *res* region are involved in transposition of Tn*5053*: a paradigm for a novel family of transposons carrying either a *mer* operon or an integron. Mol. Microbiol. **17:**1189–1200.
15. **Kubo, K. M., and N. L. Craig.** 1990. Bacterial transposon Tn*7* utilizes two classes of target sites. J. Bacteriol. **172:**2774–2778.
16. **Lloyd, R. G., and G. J. Sharples.** 1991. Molecular organization and nucleotide sequence of the *recG* locus of *Escherichia coli* K-12. J. Bacteriol. **173:** 6837–6843.
17. **Lundgren, D. G., and M. Silver.** 1980. Ore leaching by bacteria. Annu. Rev. Microbiol. **34:**263–283.
18. **May, E. W., and N. L. Craig.** 1996. Switching from cut and paste to replicative Tn*7* transposition. Science **272:**401–404.
19. **Mengin-Lecreulx, D., and J. van Heijenoort.** 1993. Identification of the *glmU* gene encoding *N*-acetylglucosamine-1-phosphate uridyltransferase in *Escherichia coli*. J. Bacteriol. **175:**6150–6157.
20. **Rådström, P., O. Sköld, G. Swedberg, F. Flensburg, P. H. Roy, and L. Sundström.** 1994. Transposon Tn*5090* of plasmid R751, which carries an integron, is related to Tn*7*, Mu, and the retroelements. J. Bacteriol. **176:** 3257–3268.
21. **Rawlings, D. E., and S. Silver.** 1995. Mining with microbes. Bio/Technology **13:**773–778.
22. **Sarnovsky, R., E. W. May, and N. L. Craig.** 1996. The Tn*7* transposase is a heteromeric complex in which DNA breakage and joining activities are distributed between different gene products EMBO J. **15:**6348–6361.
23. **Stellwagen, A., and N. L. Craig.** 1997. Avoiding self: two Tn*7*-encoded proteins mediate target immunity in Tn*7* transposition. EMBO J. **16:**6348–6361.
24. **Stellwagen, A., and N. L. Craig.** 1997. Gain-of-function mutations in TnsC,

an ATP-dependent transposition protein which activates the bacterial transposon Tn7. Genetics **145:**573–585.
25. **Tang, Y., C. Lichtenstein, and S. Cotterill.** 1991. Purification and characterization of the TnsB protein of Tn7, a transposition protein that binds to the ends of Tn7. Nucleic Acids Res. **19:**3395–3402.
26. **Tuovinen, O. H., and D. P. Kelly.** 1973. Studies on the growth of *Thiobacillus ferrooxidans*. Arch. Mikrobiol. **88:**285–298.
27. **Ullrich, J., and J. P. M. van Putten.** 1995. Identification of the gonococcal *glmU* gene encoding the enzyme *N*-acetylglucosamine 1-phosphate uridyltransferase involved in the synthesis of UDP-GlcNac. J. Bacteriol. **177:**6902–6909.
28. **Waddel, C. S., and N. L. Craig.** 1988. Tn7 transposition, two pathways directed by five Tn7-encoded genes. Genes Dev. **2:**137–149.
29. **Walker, J. E., N. J. Gay, M. Saraste, and A. N. Eberle.** 1984. DNA sequence around the *Escherichia coli unc* operon. Biochem. J. **224:**799–815.
30. **Wu, H. C., and T. C. Wu.** 1971. Isolation and characterization of a glucosamine-requiring mutant of *Escherichia coli* K-12 defective in glucosamine-6-phosphate synthetase. J. Bacteriol. **105:**455–466.