Distinctive Protein Signatures Provide Molecular Markers and Evidence for the Monophyletic Nature of the Deinococcus-Thermus Phylum

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The Deinococcus-Thermus group of species is currently recognized as a distinct phylum solely on the basis of their branching in 16S rRNA trees. No unique biochemical or molecular characteristics that can distinguish this group from all other bacteria are known at present. In this work, we describe eight conserved indels (viz., inserts or deletions) in seven widely distributed proteins that are distinctive characteristics of the Deinococcus-Thermus phylum but are not found in any other group of bacteria. The identified signatures include a 7-amino-acid (aa) insert in threonyl-tRNA synthetase, 1- and 3-aa inserts in the RNA polymerase β' subunit, a 5-aa deletion in signal recognition particle (Ffh/SR54), a 2-aa insert in major sigma factor 70 (σ70), a 2-aa insert in seryl-tRNA synthetase (SerRS), a 1-aa insert in ribosomal protein L1, and a 2-aa insert in UvrA homologs. By using PCR primers for conserved regions, fragments of these genes were amplified from a number of Deinococcus-Thermus species, and all such fragments (except SerRS in Deinococcus proteolyticus) were found to contain the indicated signatures. The presence of these signatures in various species from all three known genera within this phylum, viz., Deinococcus, Thermus, and Meiothermus, provide evidence that they are likely distinctive characteristics of the entire phylum which were introduced in a common ancestor of this group. The signature in SerRS, which is absent in D. proteolyticus, was likely introduced after the branching of this species. Phylogenetic studies as well as the nature of the inserts in some of these proteins (viz., σ70 and SerRS) also support a sister group relationship between the Thermus and the Meiothermus genera. The identified signatures provide strong evidence for the monophyletic nature of the Deinococcus-Thermus phylum. These molecular markers should prove very useful in the identification of new species related to this group.

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lum. The identified signatures include a 7-amino-acid (aa) insert in Thr-tRNA synthetase (ThrRS), a 5-aa deletion in the signal recognition particle protein Ffh, a 1- and a 3-aa insert in the β subunit of RNA polymerase RpoC, a 1-aa insert in the ribosomal protein L1, and 2-aa inserts in major sigma factor 70 (σ70), seryl-tRNA synthetase (SerRS), and UvrA homologs. The sequence information for these proteins was previously available from only a limited number of Deinococcus and Thermus species. As the Methanothermus genus has only recently been established, there is little sequence information currently available for this group (33). We have tested the specificity of the identified signatures by PCR amplifying and sequencing fragments of these genes from additional Deinococcus and Methanothermus species for which no sequence information was available. The presence of these signatures in all of the species examined (with a single exception) provide evidence that they are likely distinctive characteristics of the entire phylum and might be used as molecular markers for this group of species.

MATERIALS AND METHODS

Identification of signature sequences. Deinococcus-Thermus-specific signatures were identified in global multiple sequence alignments by means of visual inspection. Alignments for different proteins were constructed by using the CLUSTALW PLUS 4 program (Scientific & Educational Software, Durham, N.C.) as described in earlier work (12, 14, 19). To qualify as a useful group-specific signature, any identified indel was required to be uniquely (or mainly) present in the Deinococcus-Thermus-Methanothermus group of species and to be flanked on both sides by conserved regions to ensure that the observed insertion or deletion was not a result of sequencing errors or alignment artifacts.

PCR amplification and sequencing. Cultures of Methanothermus ruber (ATCC 35948), Methanothermus silvanus (DSMZ 9946), and Deinococcus grandis (DSMZ 3963) were generously supplied by Peter Bogarten and Lorraine Olenzenski (36). Deinococcus proteolyticus (ATCC 35074) high-molecular-weight DNA was prepared as previously described (6, 16). Oligonucleotide primers, in opposite orientations, were designed for conserved regions in the protein sequences that flanked these signatures based on sequence information from available Deinococcus-Thermus and other species. Degeneracy was incorporated into the primers to account for differences in codon usage among different species. The primers were synthesized at the Molecular Biology Central Facility (MOBIX) of McMaster University, Hamilton, Ontario, Canada.

PCRs. PCR was performed in a Techne Techgene thermocycler. The PCRs had a final volume of 10 μl, and all primer sets were optimized for MgCl2 concentration (in the range of 1.5 to 4 mM) for each DNA strain tested. PCR amplification was carried out over 30 cycles (15 s at 94°C, 15 s at 55 or 45°C, 1 min at 72°C) with an initial 1-min hot start at 94°C and a final extension step (15 s at 94°C, 15 s at 55°C, 7 min at 72°C) (12). The reaction mix also contained 2% dimethyl sulfoxide, which improves PCR performance by lowering the melting temperature of DNA. DNA fragments of the expected size were purified from 0.8% (wt/vol) agarose gels (using a GENECLEAN kit) and subcloned into the plasmid pDRIVE by using a TU cloning kit (Invitrogen). Escherichia coli JM109 cells were transformed with the ligated vector and insert, and the inserts from a number of positive clones were sequenced at MOBIX. Sequences of all cloned fragments were run through a BLAST search to ensure that the amplified gene was from a novel source. The primer sequences used for the amplification of different genes are as follows.

(i) σ70. The following primers were successful in amplifying 504-bp inserts from D. grandis, D. proteolyticus, M. silvanus, and M. ruber: forward, 5'-ACNTA YGNCA/NCTGTGGTGCA-3'; reverse, 5'-GRCNGCTRTYTGTCAT-3'; where N represents A, G, C, or T; Y is C or T; R is A or G; and D is A, G, or T.

(ii) Threonyl tRNA synthetase. Fragments 432 bp in length were generated from D. grandis, M. silvanus, and M. ruber genomic DNA with the following primers: forward, 5'-TCTCCGCACCGCGTGATGCTAC-3'; reverse, 5'-CCNCKCCARTANGCNCC-3'; where S represents C or G, W is A or T; K is G or T; and M is A or C.

(iii) Signal recognition particle Ffh. The following primers were used to amplify a 264-bp fragment from D. grandis: forward, 5'-ATHTYNGNATGGNGA-3'; reverse, 5'-CKYTCTYTNACNGTCA-3'; where H represents A, C, or T.

(iv) SerRS. Fragments from M. silvanus and D. proteolyticus of 234 bp in length were successfully amplified by using forward primer 5'-CACSARTTYGCGAA GTCGGGARGCAG-3' and reverse primer 5'-GARCAGGARTGCGTTGTC GRTC-3'.

(v) RNA polymerase β subunit RpoC. RpoC gene fragments (645 bp) were amplified by PCR from M. silvanus, M. ruber, D. proteolyticus, and D. grandis by using the following primers: forward, 5'-GYAGGNGGMGNTTYGC-3'; reverse, 5'-CATYTGTRNCCTCRAARTC-3'.

(vi) Ribosomal protein L1. A 510-bp fragment was generated from M. silvanus and D. grandis by using the following primers: forward, 5'-ATGCTCTAACGGC GGCAGCGTTACC-3'; reverse, 5'-CCGGTCTGTGCTCGGAGAACC-3'.

(vii) Exonuclease ABC subunit A UvrA. A 639-bp fragment was amplified from M. silvanus by using forward primer 5'-TGGCYYTGACCACTTACGGC AGG-3' and reverse primer 5'-AGGGGAATTCTCAGWACAGCTCCTC-3'.

Phylogenetic analysis. Phylogenetic analysis on protein sequences was carried out by procedures described in earlier work (12, 20). Multiple alignment of protein homologs from different groups of bacteria was created by using the CLUSTALW program. The data for the newly sequenced fragments were added to the alignment, and the fragments were all trimmed to the same length as the amplified fragments. Phylogenetic analyses were performed in both the presence and the absence of the signature region to determine its influence on the branching pattern. The aligned sequences were used to generate 100 bootstrapped data sets with the SEQBOOT program, and genetic distances were calculated by PROTDIST by using Kimura’s method (23). Neighbor-joining trees from these distances were constructed by the NEIGHBOR program (40). A consensus tree for various bootstrapped sequences was obtained by using the CONSENSE program. All of these phylogenetic programs are part of the PHYLIP software package (version 3.5; J. Felsenstein, University of Washington, Seattle, Wash.).

Nucleotide sequence accession numbers. The sequence data for all of the gene fragments cloned and sequenced in this work have been deposited in the GenBank database under accession numbers AY450950, AY452779, AY453862, AY489057, and AY453858 for D. grandis; AY450951 and AY453857 for D. proteolyticus; AY450952, AY452780, AY455864, AY489058, AY489059, and AY452782 for M. silvanus; and AY452778, AY452781, and AY453861 for M. ruber.

RESULTS

Description of conserved indels that are distinctive of the Deinococcus-Thermus group. Conserved indels that are shared by all members of one particular group (group-specific signatures), or are commonly present in species belonging to more than one tax (main-line signatures), provide powerful means to identify individual taxa in molecular terms and to understand the interrelationships among them (13, 14, 17). Evolutionarily significant indels are generally of defined size, are present at a specific location, and are flanked by conserved regions to ensure their reliability. We describe below a number of conserved indels in widely distributed proteins that are distinctive characteristics of the Deinococcus-Thermus group (Deinococcus, Thermus, and Methanothermus) of species.

In σ70, which plays a central role in the transcription process by conferring promoter specificity to RNA polymerase (5), a 2-aa insert is present in a conserved region in various available Deinococcus-Thermus homologs (viz., Deinococcus radio- durans, Thermus aquaticus, and Thermus thermophilus) but not in any other bacteria. However, variable inserts are present in this region in Mycoplasma species (data not shown), which are likely of independent origin. The specificity of this insert for the Deinococcus-Thermus phylum was tested by PCR amplifying and sequencing fragments of the σ70 gene from four other members belonging to this group for which no sequence infor-
mation was available. Results of these studies, which are included in Fig. 1, show that all four species tested, which included two Deinococcus (D. grandis and D. proteolyticus) and two Meiothermus (M. ruber and M. silvanus) species contained the identified signature. The sequence region which flanked the identified insert (Fig. 1, boxed region) was also found to be distinctive for Deinococcus-Thermus-Meiothermus species. Since sequence information for this signature is now available for representatives from all three genera within the Deinococcus-Thermus phylum, the shared presence of this insert in all of

FIG. 1. Partial sequence alignment for eDNA proteins showing a 2-aa insert (boxed area) in a conserved region that is uniquely present in Deinococcus, Thermus, and Meiothermus homologs. Dashes in this and all other alignments indicate identity to the amino acid on the top line (E. coli protein). The accession numbers of different proteins are provided in the second column. Sequence information for only representative species from different bacterial groups is presented. The sequences marked with an asterisk were cloned and sequenced in the present work. Abbreviations for the species names are as follows: A., Agrobacterium; Aqu., Aquifex; Bac., Bacillus; Bact., Bacteroides; Bif., Bifidobacterium; Bor., Borrelia; Buch., Buchnera; C., Caulobacter; Camp., Campylobacter; Cfx., Chloroflexus; Chl., Chlamydia; Chlam., Chlamydophila; Cor., Corynebacterium; Des., Desulfovibrio; E., Escherichia; Ent., Enterococcus; Fus., Fusobacterium; Geo., Geobacter; H., Haemophilus; Hel., Helicobacter; Helio., Heliobacillus; L., Lactococcus; Lep., Leptospira; Lis., Listeria; M., Mycoplasma; Mei., Meiothermus; Myc., Mycobacterium; Nei., Neisseria; Nit., Nitrosomonas; Oce., Oceanobacillus; Pas., Pasteurella; Pse., Pseudomonas; Rat., Ralstonia; Rh., Rhodobacter; Rho., Rhodospirillum; R., Rickettsia; Sal., Salmonella; Sta., Staphylococcus; Str., Streptococcus; Sy., Synechocystis; Syn., Synechococcus; T., Thermotoga; Therm., Thermococcus; Thermosyn., Thermosynechococcus; Tre., Treponema; Tri., Trichodesmium; Troph., Tropheryma; V., Vibrio; X., Xylella. GNS, green nonsulfur bacteria; Gram(+)ve, gram-positive.
them strongly indicates that it is very likely a distinctive characteristic of the entire phylum.

We also performed phylogenetic analysis based on $\sigma^{70}$ sequences from different bacteria. For these purposes, 169 aa positions for which sequence information was available from different species were utilized. The sequence alignment data were used to generate 100 bootstrapped data sets, and a consensus neighbor-joining tree was obtained from these data. At the same time, a neighbor-joining distance tree showing branch lengths, shown in Fig. 2, was also constructed. The bootstrap scores for different nodes which were >50 are marked on this tree. As shown in Fig. 2, most bacterial groups are clearly distinguished from each other in the tree (as shown by their high bootstrap score), but their branching orders or interrelationships are not resolved, which is a common problem with phylogenetic trees (13, 25). Importantly, in the present context, all of the Deinococcus-Thermus-Meiothermus species formed a well-defined group, branching together 100% of the time. Within this group, different Deinococcus-Thermus genera (viz., Deinococcus, Thermus, and Meiothermus) formed distinct clusters. Of these genera, Deinococcus was found to be the earliest branching lineage, whereas a closer relationship was seen between the Thermus and Meiothermus genera. A similar relationship among these groups is seen in the 16S rRNA trees (2, 39). It is noteworthy that the insert sequence in Deinococcus species consists of two alanine residues, whereas in Thermus and Meiothermus species, the insert sequence is comprised of one alanine and one lysine residue (i.e., AK), again indicating a closer relationship between these two genera. Thus, the inference from signature sequences is in accordance with results from phylogenetic analysis (2, 6, 39, 42). We have also performed phylogenetic analysis on these sequences after omitting the insert region. The tree obtained in this case was very similar to that in Fig. 2 (results not shown), indicating that the observed relationship is not dependant upon or affected by the presence of the insert.

Aminoacyl-tRNA synthetases play an essential role in protein synthesis by catalyzing the attachment of correct amino acids to the 3'-terminal ends of their cognate tRNA to form the aminoacyl-tRNA, which provide the basic substrate for protein synthesis (21). We have identified a 7-aa insert in a conserved region of ThrRS which is uniquely present in all three available sequences from Deinococcus-Thermus species (D. radiodurans, T. thermophilus, and T. aquaticus) but is not found in any other bacterial homologs (Fig. 3). The specificity of this signature was tested by PCR amplifying fragments of

FIG. 2. A neighbor-joining distance tree with branch lengths based on $\sigma^{70}$ sequences. The tree is based on 169 aa positions for which sequence information was available for various species. The bootstrap scores (out of 100) of various nodes which were >50 are indicated. The arrow marks the suggested position where the identified insert in this gene was introduced.
the ThrRS gene from several additional species, viz., *D. grandis*, *M. silvanus*, and *M. ruber*. The sequences for these species are included in Fig. 3, and all of the sequences were found to contain the identified signature. These results strongly indicate that the identified indel in ThrRS is likely a group-specific signature for the *Deinococcus-Thermus* group. In a phylogenetic tree based on ThrRS sequences (data not shown), all of the *Deinococcus-Thermus* species were found to group together with high affinity (95% bootstrap score), supporting the inference that they form a monophyletic group.

The core subunits of the RNA polymerase (i.e., $\beta$H9251, $\beta$H9252, and $\beta$H9252/H11032) are evolutionarily conserved in sequence, structure, and function in all species ranging from bacteria to humans (24, 37). In the $\beta$H9252/H11032 subunit of RNA polymerase, which is encoded by the *rpoC* gene, we have identified a 1- and a 3-aa insert in conserved regions that are only present in *D. radiodurans*, *T. aquaticus*, and *T. thermophilus* but are not found in any other bacteria or species (Fig. 4). Further studies on this indel were carried out by cloning and sequencing fragments of the *rpoC* gene from three other *Deinococcus-Thermus* species (*D. grandis*, *M. ruber*, and *M. silvanus*). Results of these studies, which are included in Fig. 4, show that both of these inserts were present in all of these species, indicating that they are distinctive characteristics of the *Deinococcus-Thermus* phylum. Furthermore, as seen in the case of $\beta$H9268 homologs, the sequence of the 3-aa insert in various *Meiothermus* and *Thermus* species (i.e., KDE) was identical and differed from that seen in the *Deinococcus* species, pointing to a closer relationship between the *Meiothermus* and *Thermus* species.

The L1 protein of the 50S ribosomal subunit has been implicated in the release and removal of deacylated tRNA from the E site (32). Our studies have revealed a conserved 1-aa insert in all available *Deinococcus-Thermus* species (Fig. 5). We have amplified 510-bp fragments of the L1 protein gene from two other members of this group (*M. silvanus* and *D. grandis*), and both were found to contain the indel. The insert in all cases is a lysine residue.
indicating that it was introduced only once in a common ances-
tor of these species.

A 2-aa insert is also found in the exinuclease ABC subunit A
homologs (i.e., UvrA protein) of the Deinococcus-Thermus
group (Fig. 6). UvrA is one of the two subunits of the damage
recognition complex required for nucleotide excision during
repair of UV light-induced DNA damage (8). Previously, se-
quences were available only from two species belonging to this
phylum (D. radiodurans and T. aquaticus), and no information
existed for the Meiothermus group of species. To bridge this
gap, we have ampli
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ed a 639-bp fragment of the uvrA
gene from M. silvanus. The ampli
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insert, providing evidence that this signature is also a distinc-
tive characteristic of the Deinococcus-Thermus group. In addi-
tion to the Deinococcus-Thermus species, a 2-aa insert is also
present in this position in Borrelia burgdorferi, which may have
originated either independently or through lateral gene trans-
fer (LGT).

All sequenced organisms contain an Ffh/SRP54 family mem-
er, which forms part of the signal recognition particle and
coordinates the cotranslational targeting of secretory and
membrane proteins to either the membrane of the endoplas-
mic reticulum or the plasma membrane in bacteria (29). In
E. coli, the signal recognition particle is composed of Ffh pro-
tein and the 4.5S RNA. A 5-aa deletion is present in a con-
served region of the Ffh protein that is only seen in
Deinococcus-Thermus homologs but is not found in any other bacteria (Fig. 7). Since sequence information for
Deinococcus-Thermus was available only from D. radiodurans
and T. aquaticus, we have ampli
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ed and sequenced a fragment of the Ffh gene from D. grandis. The fragment from this species was also found to
contain the deletion (Fig. 7), indicating that this signature may
also be specifi
c for the entire
Deinococcus-Thermus phylum.

Due to DNA limitation, sequence information for this signa-
ture for Meiothermus species was not obtained.

Another signature for the Deinococcus-Thermus group is
present in the protein SerRS (21). The signature in this case
consists of a 2-aa insert in a conserved region that is commonly
present in the Deinococcus-Thermus group (Fig. 6). UvrA is one of the two subunits of the damage recognition complex required for nucleotide excision during repair of UV light-induced DNA damage (8). Previously, sequences were available only from two species belonging to this phylum (D. radiodurans and T. aquaticus), and no information existed for the Meiothermus group of species. To bridge this gap, we have amplified a 639-bp fragment of the uvrA gene from M. silvanus. The amplified fragment contained the 2-aa insert, providing evidence that this signature is also a distinctive characteristic of the Deinococcus-Thermus group. In addition to the Deinococcus-Thermus species, a 2-aa insert is also present in this position in Borrelia burgdorferi, which may have originated either independently or through lateral gene transfer (LGT).

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Deinococcus-Thermus homologs but is not found in any other bacteria (Fig. 7). Since sequence information for Deinococcus-Thermus was available only from D. radiodurans and T. aquaticus, we have amplified and sequenced a fragment of the Ffh gene from D. grandis. The fragment from this species was also found to contain the deletion (Fig. 7), indicating that this signature may also be specific for the entire Deinococcus-Thermus phylum. Due to DNA limitation, sequence information for this signature for Meiothermus species was not obtained.

Another signature for the Deinococcus-Thermus group is
present in the protein SerRS (21). The signature in this case
consists of a 2-aa insert in a conserved region that is commonly
present in the SerRS homologs from various available Deinococcus-Thermus species (D. radiodurans, T. aquaticus, and T. thermophilus) (Fig. 8). By means of PCR amplification, we have obtained sequence information for the SerRS gene from two additional species, viz., M. silvanus and D. proteolyticus. Interestingly, while the M. silvanus homolog contained the signature, this insert was not found in the fragment derived from D. proteolyticus (Fig. 8). The most parsimonious explanation for these results is that the insert was introduced in a common ancestor of D. radiodurans, T. aquaticus, T. thermophilus, and M. silvanus after the divergence of D. proteolyticus. However, the possibility that the insert has been lost from D. proteolyticus cannot be excluded.

**DISCUSSION**

In 16S rRNA and various protein trees, the Deinococcus-Thermus phylum represents one of the earliest branching groups within the Bacteria (10, 16, 19, 25, 38, 39, 42, 44). In the past, this phylum consisted of only two genera (Deinococcus and Thermus); however, a third genus (Meiothermus) has recently been established (34). According to branch patterns, species belonging to the genus Meiothermus form a sister lineage with Thermus species, forming the order Thermales (family Thermaceae), which clusters together with the distantly related Deinococcales in a single lineage (2, 34, 39). Although Deinococcales shows 77.5 to 81% 16S rRNA sequence similarity with the Thermus-Meiothermus group, and species of the Deinococcus-Thermus group share an A3/H9252 murein-type peptidoglycan (L-ornithine as the diamino acid and glycylglycine as the interpeptide bridge) and menaquinone-8 as their major respiratory quinone, these characteristics are not unique to these groups, and they share few other characteristics in common (2, 4, 30, 31). Although a few unique base pairs that appear limited to the genus Deinococcus have been identified in the 16S rDNA sequences (2), currently there is no molecular
or structural marker known that is distinctive to the entire Deinococcus-Thermus phylum which might be used to distinguish or define this group of bacteria from all others.

In the present work, we have identified eight conserved indels in seven widely distributed proteins that are distinctive characteristics of the Deinococcus-Thermus phylum. Based on the work reported here and information available in the databases, information for six of these proteins containing seven signatures (viz., SerRS, ThrRS, RpoC, UvrA, and ribosomal L1 protein) is available from all three genera within the Deinococcus-Thermus phylum. The sequence information for Ffh/SR54 is currently available from only Deinococcus and Thermus genera, but based on the observation that Meiothermus forms a sister lineage with Thermus species (9, 33, 39), it is expected that this signature will also be found in Meiothermus organisms. Except for the absence of the SerRS insert in D. proteolyticus, the identified signatures are present in all Deinococcus-Thermus species examined but not in other bacteria. These signatures thus provide molecular markers for distinguishing the Deinococcus-Thermus phylum from all other bacteria and for identifying new species related to them based simply on the presence or absence of these signatures. The presence of these distinctive signatures also provides strong evidence for the monophyletic nature of the Deinococcus-Thermus phylum as indicated by 16S rRNA trees (38, 42, 44). The most likely explanation for these signatures is that they were introduced in a common ancestor of this lineage and then were passed on to all descendants. This inference is also supported by phylogenetic analysis based on a number of these proteins.

The presence of the insert in SerRS in various Deinococcus-Thermus species, but not D. proteolyticus, might be accounted for by two different possibilities. First, it is possible that this insert was introduced in a common ancestor of the other Deinococcus-Thermus species after the branching of D. proteolyticus. Alternatively, this insert may have been introduced in a common ancestor of the entire phylum but then subsequently lost from D. proteolyticus. We favor the first of these possibilities, based on the observation that in phylogenetic trees...
rived from 16S rRNA sequences, a branch comprised of *D. proteolyticus* and *D. radiophilus* forms the deepest group within the *Deinococcus-Thermus* phylum (2, 39).

LGT is indicated to have played an important role in the evolution of the *Deinococcus-Thermus* group. These organisms are thought to have received genes from a number of other phyla such as the *Archaea*, *Eucarya*, and cyanobacteria (11, 26, 36, 43). However, for the various genes studied in the present work, which contain identified signatures, there is no evidence of lateral gene exchange between the *Deinococcus-Thermus* group and other bacterial phyla, except possibly the UvrA gene in *B. burgdorferi*. If these genes were subjects of LGTs, one would expect a more random distribution of these signature sequences in which these indels would have been present in other groups of bacteria and at the same time several *Deinococcus-Thermus* species would be lacking them, which is clearly not the case here. However, in contrast to these genes, a number of genes studied in earlier work contained signature sequences that were commonly shared by cyanobacteria and the *Deinococcus-Thermus* species, which may be the results of LGTs (13, 18).

We have also previously described many main-line signatures (i.e., indels commonly shared by a number of different bacterial phyla), which provide useful information concerning the phylogenetic placement of the *Deinococcus-Thermus* group within the bacterial domain (13, 15, 17). The distribution patterns of these signatures in bacterial sequences indicate that the *Deinococcus-Thermus* phylum has evolved after the divergence of various gram-positive phyla (viz., *Firmicutes*, *Actinobacteria*, *Clostridia*, and relatives) but before the emergence of *Aquifex*, Chloroflexi, cyanobacteria, spirochetes, the *Chloramydia-Cytophaga-Flavobacteria-Bacteroides-green sulfur bacteria* group, and proteobacteria (15, 17). The branching of the *Deinococcus-Thermus* phylum in between the gram-positive bacteria and gram-negative bacteria also accounts for a hitherto puzzling characteristic of *Deinococcus*. Although all *Deinococcus-Thermus* species are surrounded by an outer membrane, which is a distinguishing property of the gram-negative bacteria, most species belonging to the genus *Deinococcus* (all except *D. grandis*) exhibit positive Gram staining and contain a thick sacculus characteristic of gram-positive bacteria (2, 30, 31, 41). These seemingly contradictory properties are readily explained by the suggested placement of the *Deinococcus-Thermus* phylum between the gram-positive bacteria and gram-negative bacteria also accounts for a hitherto puzzling characteristic of *Deinococcus*. Although all *Deinococcus-Thermus* species are surrounded by an outer membrane, which is a distinguishing property of the gram-negative bacteria, most species belonging to the genus *Deinococcus* (all except *D. grandis*) exhibit positive Gram staining and contain a thick sacculus characteristic of gram-positive bacteria (2, 30, 31, 41). These seemingly contradictory properties are readily explained by the suggested placement of the *Deinococcus-Thermus* phylum between the gram-positive bac-

### FIG. 7. Partial sequence alignments of Ffh protein showing a 5-aa deletion (boxed area) that is a unique characteristic of the *Deinococcus-Thermus-Meiothermus* homologs. See the legends to Fig. 1 and 3 for abbreviations.
Proteobacteria

Aquifex, Chlamydiales, CFG

Spirochetes, Cyano bacteria, GNS Bacteria

Deinococcus-Thermus

Gram(+)/(-) Bacteria

\[ \text{FIG. 8. Excerpt from SerRS sequence alignment showing a 2-aa inserted area (boxed area) that is present in various Deinococcus-Thermus-Mei othermus species, except D. proteolyticus. This insert was likely introduced in a common ancestor of this group after the branching of D. proteolyticus.} \]

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