Research Article

Cross-Genome Comparisons of Newly Identified Domains in *Mycoplasma gallisepticum* and Domain Architectures with Other *Mycoplasma* species

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Accurate functional annotation of protein sequences is hampered by important factors such as the failure of sequence search methods to identify relationships and the inherent diversity in function of proteins related at low sequence similarities. Earlier, we had employed intermediate sequence search approach to establish new domain relationships in the unassigned regions of gene products at the whole genome level by taking *Mycoplasma gallisepticum* as a specific example and established new domain relationships. In this paper, we report a detailed comparison of the conservation status of the domain and domain architectures of the gene products that bear our newly predicted domains amongst 14 other *Mycoplasma* genomes and reported the probable implications for the organisms. Some of the domain associations, observed in *Mycoplasma* that afflict humans and other non-human primates, are involved in regulation of solute transport and DNA binding suggesting specific modes of host-pathogen interactions.

1. Introduction

Progress in DNA sequencing technology has produced the whole genomes of many important organisms including humans. The proper utilization of such sequence information requires understanding of the function of each protein in the database. The ever-increasing gap between the number of sequences deposited in databases and the numbers with accurate functional annotation is a big concern to the scientific community. The goal of functional genomics is to determine the function of proteins predicted from the sequencing projects [1, 2]. To reach this goal, computational approaches can assist in the classification of functional genomics targets. Functional and evolutionary relationships can be inferred from sequence comparisons, especially at high sequence identities. The established computational methods to function detection primarily depend on homology matching to genes with known functions by employing programs such as FASTA [3] and BLAST [4]. Nevertheless, establishing homology is not straightforward and provides limited coverage. Over the past few years, many new methods have emerged to organize the proteins; some of them are highly automated, and others are curated. Position-specific iterative BLAST (PSI-BLAST) can be used to extend the search to distantly related homologues [5]. Some of the other methods rely on the hierarchical classification of proteins into families such as the superfamilies/families in the PIR-PSD [6] protein groups in ProtoMap [7]. Few other methods organize proteins to families of domains such as Pfam [8] and SMART [9]. Others rely on sequence motifs or conserved regions, such as in PROSITE [10] and PRINTS [11]. Databases like CATH [12], SCOP [13], and FSSP [14] employ structural data to organize proteins into domains. Others are integrations of various family classifications, such as InterPro [15]. However, each of these databases is useful for particular needs, and most of them rely on high sequence similarity for accurate function annotation.
transfer, and no classification scheme is by itself adequate for addressing all genomic annotation needs [16]. The Gene Ontology (GO) consortium provides a controlled vocabulary to describe the function of a protein [17].

Identification of domains at the sequence level most often relies on the detection of global and local sequence alignments between a given target sequence and domain sequences found in databases such as Pfam [8] and SMART [9]. However, sequence-based methods often fail under low sequence identity conditions. Intermediate sequence approach has been shown to be more effective in enhancing the coverage in homology search and in connecting remotely related proteins of common function [18]. It was shown that about 70% improvement over direct search [18] is possible using this method. Using similar approach in the domain assignment to sequences, earlier, we showed that the domain assignment could be substantially enhanced in the family of genes containing adenylyl cyclases [19]. PURE, this computation-intensive search protocol, was further developed as a web tool [20]. Next, we had implemented our method at the whole genome level by taking smaller genome organism Mycoplasma gallisepticum as a specific example [21]. This paper reports the cross-genome comparisons of 14 Mycoplasma genomes to study the conservation of domains and domain architectures involving new domain associations identified by us in Mycoplasma gallisepticum.

As shown in the earlier paper, PURE approach is effective in establishing remote domain relationships [20, 21] and can be useful when the user fails to assign domains to the sequence by using direct search methods like Pfam [8]. We also showed, by comparing different versions of Pfam databases, that the PURE approach can give a good hint at the domains, which are going to be assigned in the updated Pfam database [19].

Mycoplasma constitutes a unique group of bacteria best characterized as lacking peptidoglycan and having one of the smallest genomes of all free-living prokaryotes. Members of this group also represent important pathogens of humans, animals, and plants. Over the last few years, the genomes of many Mycoplasma species were sequenced, reinforcing comparative genome studies which permit a better understanding of their metabolism and the relations with their hosts. Phylogenetic analyses indicate that Mycoplasmas have undergone a degenerative evolution from related, low G+C content, Gram-positive eubacteria [22, 23]. Mycoplasmas possess no complete routes for amino acids synthesis and degradation, implying that these monomers must be acquired either from their hosts or from a culture medium, depending upon membrane transporters [24]. Exogenous peptides are an important source of amino acids. Indeed, bacteria have evolved peptide transport systems that also assist in responses to environmental changes, mediating functions such as quorum sensing, sporulation, pheromone transport, and chemotaxis [25].

2. Materials and Methods

Complete protein sequences of 14 different Mycoplasma genomes were obtained from National Center for Biotechnology Information website [26]. The species we considered for our study were Mycoplasma gallisepticum strain R (total number of proteins in the genome 726), Mycoplasma genitalium strain G37 (477), Mycoplasma agalactiae strain PG2 (742), Mycoplasma arthritidis strain 158L3-1 (631), Mycoplasma capricolum subsp. capricolum (812), Mycoplasma hyopneumoniae strain 232 (691), Mycoplasma hyopneumoniae strain 7448 (657), Mycoplasma hyopneumoniae strain J (657), Mycoplasma mobile strain 163K (633), Mycoplasma mycoides subsp. mycoides SC str. PG1 (1016), Mycoplasma penetrans strain HF-2 (1037), Mycoplasma pneumoniae strain M129 (689), Mycoplasma pulmonis strain UAB CTIP (782), and Mycoplasma synoviae (659) (Table 1).

Mycoplasma species can be categorized into different groups based on motility and host specificity [27]. Mycoplasma gallisepticum, Mycoplasma genitalium, Mycoplasma mobile, Mycoplasma pneumonia, and Mycoplasma pulmonis were grouped as motile and the remaining species Mycoplasma agalactiae PG2, Mycoplasma arthritidis 158L3, Mycoplasma capricolum ATCC 27343, Mycoplasma hyopneumoniae 232, Mycoplasma hyopneumoniae 7448, Mycoplasma hyopneumoniae, Mycoplasma mycoides, Mycoplasma penetrans, and Mycoplasma synoviae 53 were grouped as non-motile. Mycoplasmas were also classified based on the host specificity. Mycoplasma genitalium, Mycoplasma penetrans, Mycoplasma pneumonia, and Mycoplasma pulmonis were primate specific, Mycoplasma synoviae 53 and Mycoplasma gallisepticum grouped as avian specific, Mycoplasma hyopneumoniae 232, Mycoplasma hyopneumoniae 7448, and Mycoplasma hyopneumoniae –I were grouped as swine-specific Mycoplasmas. Mycoplasma arthritidis 158L3 and Mycoplasma pulmonis are grouped as rodent specific, Mycoplasma agalactiae PG2, Mycoplasma capricolum ATCC 27343, and Mycoplasma mycoides are grouped as ovine specific, and lastly Mycoplasma mobile is fish-specific Mycoplasma in targeting its host for survival.

We assigned domain region to the Mycoplasma gallisepticum protein sequences by scanning the sequences against HMM profiles in the Pfam database (version 21.0) [8] which consists of 8957 families by using standalone version of Hmmpfam of the HMMER suite [28] with E-value cutoff 0.1.

HMMTOP [29] server was used for transmembrane helix prediction, and a standalone version of COILS [30] program was used for coiled-coil region prediction. We used PSI-BLAST [5] (with three iterations and expectation cutoff value of 0.001) for search for similar sequences. During the blast searches, low complexity filter was turned on. Non-redundant database [31] was used for sequence similarity searches. Standalone version of PSIPRED [32] was used for secondary structure prediction. Multiple sequence alignments were performed using CLUSTALW program [33].

3. Results and Discussion

Earlier analysis revealed 71 new domain relationships in the Mycoplasma gallisepticum genome which corresponds to 62 unassigned regions [21]. 22 domains, which are in the border
regions of cut-off expectation value, were excluded from the cross-genome analysis, and 49 domains which belong to 42 unassigned regions are used in the analysis. Detailed domain architectures, along with newly predicted domains, are shown in Table 2. 24 sequences out of 42 sequences picked up one or more domains, which were initially full-length unassigned sequences. Interestingly, some of the newly predicted domains such as Chase 3, DUF 1393, DUF 30, DUF 31, LMP, and HTH 12 are not present in the other Mycoplasma genomes. These domains could only be identified in Mycoplasma gallisepticum genome in the indirect searches. This could be because these domains may have species-specific functions or Mollicutes may have evolved by degenerative or reductive evolution, accompanied by significant losses of genomic sequences [34], wherein some of these domains might have lost their function and diverged beyond recognition by direct search methods. The intermediate sequences through which these domain relationships are established are predominantly of prokaryotic in origin and have relatively fewer hits in the PSI-BLAST search.

Our analysis also revealed the presence of extra copy of domains such as RMMBL, Lactamase_B, ABC_membrane, ABC_tran, Lipoprotein_X, SBP Bac5, ATP_syn, ab N, Helicase C, tRNA_anti, and GTP_EFTU in the Mycoplasma gallisepticum genome. Because of the limited coding capacity of their genome, Mycoplasmas lack many enzymatic pathways characteristic of most bacteria; consequently, Mycoplasma genes encode many proteins with functions related to catabolism and metabolite transport while encoding few anaerobic proteins [35]. Most of these newly predicted domains related to transportation function. Despite low sequence identities, these domains could have critical function in the nutrient transportation. Some of the interesting examples are explained below.

Protein NP_853190.1 was a completely unassigned protein. Our method predicted peptidase_M23 (Peptidase family M23) domain relationship in the protein. Members of this family are zinc metallopeptidases and have a characteristic HxH motif [36], and the current gene product also preserved this functional motif in the unassigned region. We found this domain in Mycoplasma gallisepticum only through indirect searches, and the unassigned sequence has less than 20% sequence identity with the typical peptidase_M23 members, albeit with few indels in the alignment (Figure 1). Perhaps, the low sequence identity could explain why this is not associated with domain in the direct searches. Peptidase_M23 domain is present in only two other Mycoplasma members (Mycoplasma mobile and Mycoplasma pulmonis). Interestingly, chaperonin (cpn60 or GroEL) domain is absent from these species but is present in Mycoplasma gallisepticum genome. Peptidases and chaperonins are components of protein homeostatic mechanisms. Molecular chaperones promote protein folding and prevent protein misfolding and aggregation, while certain proteases function primarily to degrade improperly folded proteins [37, 38]. It has been hypothesized that the protein homeostatic process in Mollicute organisms has shifted through evolution towards favoring protein degradation rather than protein folding [39]. Since peptidase_M23 is present only in M. mobile and M. pulmonis (Figure 2) along with other peptidases where GroEL is completely absent from the genomes, this may explain the need for higher peptidases to degrade improperly folded proteins. Whereas, in M. gallisepticum, the presence of GroEL reduces the pressure on peptidases like peptidase_M23 and sequences could have diverged substantially.

The full-length region of the sequence ID NP_852865.1 was unassigned; that is, no sequence domains were observed and recorded. Our method indirectly assigned amino terminal Lactamase_B and carboxy terminal RMMBL (RNA-metabolizing metallo-beta-lactamase) domains in the sequence. In the initial PSI-BLAST search against nonredundant database, it has picked up which belongs to more than 100 different species, including Homo sapiens, at very low expectation values. In the Hmmmpfam search, all the hits showed identical domain architectures in all the sequences with amino terminal Lactamase_B and carboxy terminal

Table 1: 14 Mycoplasma species considered in this study. Host-group specificity and motility information is provided with genome size and total number of proteins present in the individual species.

| Organism                   | Genome size (nt) | No. of proteins | Host-group specificity | Motility    |
|----------------------------|------------------|-----------------|------------------------|-------------|
| M. agalactiae_PG2          | 877,438          | 742             |                        |             |
| M. arthritidis_15L3_1      | 820,453          | 631             |                        |             |
| M. capricolum_ATCC_27343   | 1,010,023        | 812             |                        |             |
| M. gallisepticum           | 1,012,800        | 726             |                        |             |
| M. genitalium              | 580,076          | 477             |                        |             |
| M. hyopneumoniae_232       | 892,758          | 691             |                        |             |
| M. hyopneumoniae_7448      | 920,079          | 657             |                        |             |
| M. hyopneumoniae_−8        | 897,405          | 657             |                        |             |
| M. mobile                  | 777,079          | 633             |                        |             |
| M. mycoides                | 1,211,703        | 1016            |                        |             |
| M. penetrans               | 1,358,633        | 1037            |                        |             |
| M. pneumoniae              | 816,394          | 689             |                        |             |
| M. pulmonis                | 963,879          | 782             | Human/primates and Rodents | Motile     |
| M. synoviae_53             | 799,476          | 659             |                        |             |
Table 2: New domain architectures of 42 *Mycoplasma gallisepticum* proteins. For each protein reference ID is given in column two along with protein length. Fully associated domains are indicated with blue color, partially associated domains with brown color, and yellow color indicated the domains already associated with protein. Each domain is indicated by its name, starting and ending residues. In column four D represents Domain, Da indicates domain architectures. If particular domain or domain architecture present in the proteome it is indicated by P indicates present and domain/domain architecture not present in the existing proteome is indicated by NP meaning not present. Symbol * indicates that; this protein sequence is completely unassigned before out method, @ indicated unique domain/domain architecture and & indicated average sequence identities.

| S. no. | Protein ID & unassigned regions | Domain architectures | m. gallisepticum |
|--------|---------------------------------|----------------------|-----------------|
| 1     | NP_853387.1 1-582 &18%         | CHASE-3 55–582       | D: NP           |
|       |                                 |                      | DA: NP          |
| 2     | NP_853341.1 1-338 &68%          | DUF-1393 42–338      | D: NP           |
|       |                                 |                      | DA: NP          |
| 3     | NP_853479.1 1-810 &19%          | DUF-30 370–770       | D: P            |
|       |                                 |                      | DA: P            |
| 4     | NP_853440.1 1-613 &18%          | DUF-31 220–317       | D: P            |
|       |                                 |                      | DA: NP          |
| 5     | NP_853441.1 1-681 &17%          | DUF-31 233–337       | D: P            |
|       |                                 |                      | DA: NP          |
| 6     | NP_853488.1 1-809 &14%          | Lactamase_B 40–248   | D: P            |
|       |                                 |                      | DA: P          |
| 7     | NP_852865.1 1-598 &14%          | Peptidase_M23 484–657| D: NP          |
|       |                                 |                      | DA: NP          |
| 8     | NP_853190.1 1-760 &22%          | Sigma70_r4_2 120–170 | D: P            |
|       |                                 |                      | DA: NP          |
| 9     | NP_852863.1 1-182 &25%          | VapD_N 7–49          | D: P            |
|       |                                 |                      | DA: NP          |
| 10    | NP_853458.1 1-124 &31%          | SBP_Jac_6–181        | D: NP            |
|       |                                 | Lipoprotein_X 289–544| DA: NP          |
|       |                                 | Lipoprotein_10_600–731|                |
| 11    | NP_852814.1 Q7NC49_MYCGA 1-288 &18% | Lipoprotein_10_600–731 |                |
| S. no. | Protein ID & unassigned regions | Domain architectures | m. gallisepticum |
|--------|--------------------------------|----------------------|-----------------|
| 12*   | NP_852786.1 1-129 &30%         | ABC\_membrane 2–126 | D: P DA: NP     |
| 13*   | NP_853051.1 1-523 &22%          | ABC\_tran 317–467   | D: P DA: P      |
| 14*   | NP_852899.1 1-481 &18%          | Lipoprotein\_X 257–414 | D: P DA: NP     |
| 15*   | NP_853298.1 1-904 &21%          | SBP\_bac\_5 461–889 | D: NP DA: NP    |
| 16*   | NP_852891.1 1-130 &27%          | Transposase\_mut 6–108 | D: P DA: P     |
| 17*   | NP_853257.1 1-83 &98%           | Transposase\_mut 16–83 | D: P DA: P     |
| 18*   | NP_853068.1 1-369 &14%          | ABC\_2\_membrane 100–230 | D: P DA: P |
| 19*   | NP_853438.1 Q7NAJ4\_MYCGA 1-127 &17% | ATP\_synt\_ab\_N 4–126 | ATP\_synt\_ab 128–341 | 510 D: P DA: NP |
| 20*   | NP_853333.1 1-1976 &17%         | LMP 1260–1420–1600–1976 | D: NP DA: NP   |
| 21*   | NP_852801.1 1-275 &22%          | GMP\_synt\_C 220–275 | D: NP DA: NP    |
| 22*   | NP_852813.1 Q7NC50\_MYCGA 338–563 &53% | SecA\_DEAD 5–30 | SecA\_PP\_bind 226–337 | SecA\_PP\_SW 564–775 | 890 D: P DA: NP |
| S. no. | Protein ID & unassigned regions | Domain architectures | \( m. \) gallisepticum |
|-------|---------------------------------|---------------------|------------------|
| 23   | NP_853467.1 Q7NAH2_MYCGA 455-1051 &14% | 1 HSDR_N 6–217 ResIII 267–454 Helicase_C 660–730 1051 | D: P DA: NP |
| 24   | NP_853482.1 Q7NAF8_MYCGA 435-641 &17% | 1 DNA_ligase_aden 9–319 DNA_ligase_ZBD 321–402 HHH 589 717 | D: NP DA: NP |
| 25   | NP_853456.1 1-1274 &24% | 1 HNH 650–708 1274 | D: NP DA: NP |
| 26   | NP_853136.1 1-118 &15% | 1 HTH_11 28–73 HrcA 119–335 362 | D: NP DA: NP |
| 27   | NP_853240.1 Q7NB22_MYCGA 1-257 &14% | 1 HTH_12 38–89 RNB 257–586 S1 644–698 707 | D: NP DA: NP |
| 28   | NP_853425.1 GIDA_MYCGA 406-622 &30% | 1 GIDA 14–405 HTH_5 580–622 622 | D: NP DA: NP |
| 29   | NP_852895.1 Q7NBZ6_MYCGA 134-625 &21% | 1 NusA_N 8–133 S1 140–210 KH_1 333–393 625 | D: P DA: NP |
| 30   | NP_852906.1 1-190 &23% | 1 Methyltransfer_3 7–185 190 | D: NP DA: NP |
| 31   | NP_853364.1 Q7NAQ3_MYCGA 453-559 &35% | 1 PGM_PMM_JI 54–197 PGM_PMM_JH 330–452 PGM_PMM_IV 481–550 559 | D: NP DA: NP |
| 32   | NP_853326.1 1-125 &17% | 1 PTS_EIIIB 47–85 125 | D: P DA: NP |
| 33   | NP_853386.1 63-127 &18% | 1 RuvA_N 1–62 HHH 63–92 226 | D: NP DA: NP |
Table 2: Continued.

| S. no. | Protein ID & unassigned regions | Domain architectures | m. gallisepticum |
|--------|---------------------------------|----------------------|-----------------|
| 34     | NP_853171.1 Q7NB90_MYCGA 1-398 &18% | 1 Sigma70_r1_1 288-342 Sigma70_r1_2 357-398 Sigma70_r1_3 Sigma70_r1_4 573-627 | D: NP DA: NP |
| 35     | NP_852968.1 Q7NB87_MYCGA 360-723 &13% | 1 HD 53-157 RelA_SpoT 248-359 TGS 417-487 | D: NP DA: NP |
| 36     | NP_853282.1 Q7NA25_MYCGA 1-179 &22% | 1 THUMP 78-170 Thil 180-378 | D: NP DA: NP |
| 37     | NP_852812.1 Q7NC31_MYCGA 521-666 &20% | 1 Transketolase N Transketolase_C | D: P DA: NP |
| 38     | NP_852876.1 Q7NC15_MYCGA 1-319 &18% | 1 tRNA_anti_H3 230-310 PHF 329-393 Exonuclease 417-586 DNApol3_alph 815-1300 | D: P DA: NP |
| 39     | NP_853404.1 Q7NA44_MYCGA 1-118 &20% | 1 DUF258 7-104 MMR_HSR1 119-253 | D: P DA: NP |
| 40     | NP_853200.1 Q7NA45_MYCGA 1-158 &14% | 1 GTP_EFTU 68-151 MMR_HSR1 158-218 | D: P DA: NP |
| 41     | NP_853174.1 | 1 RecO 1-74 | D: NP DA: NP |
| 42     | NP_852793.1 | 1 GHMP_kinase_N 115-170 GHMP_kinase_C 200-255 | D: NP DA: NP |

RMMBL domains and with very good E values. The metallo-beta-lactamase fold contains five sequence motifs. The first four motifs are found in Lactamase_B (PF00753) and are common to all metallo-beta-lactamases. The fifth motif appears to be specific to function. RMMBL represents the fifth motif from metallo-beta-lactamases involved in RNA metabolism.

Multiple sequence alignment of predicted regions with typical Lactamase_B and RMMBL (Figures 3 and 4) revealed that the most residues that are typical to the family are not
Figure 1: Multiple sequence alignment between peptidase_M23 family representative sequences and unassigned protein sequence (NP_853190.1) from *M. gallisepticum* genome. Peptidase_M23 sequences obtained from Pfam database. Characteristic HxH motif is conserved and has few insertion regions in the unassigned sequence.

Figure 2: Peptidase domain presence in different *Mycoplasma* genomes. Domain represented by square box and species by circle. Lines connecting domain and species indicate the presence of domain in that species. Edge numbers indicate number of domains copies in genome.
required for its survival. We found that the two domains namely, HNH (endonuclease) and HTH_5 (helix turn helix motif containing transcription factor), are specific to M. mobile (found in fresh water Tench fish—Tinca tinca). Five domains namely, GMP_synth_C (GMP synthase CTD), HHH (helix-hairpin-helix motif involved in DNA binding), Methyltransferase_3 (O-methyltransferases), SBP_bac_1 (Bacterial extracellular solute-binding protein), and Transposase_mut (Transposase, Mutator family with DNA-based transposition activity), were found to be primarily in human-specific and primate group-specific pathogens. Most of these species-specific domains are involved in DNA binding and have transcription factor functions. One of them, GMP_synth_C (GMP synthase CTD), is associated with GATase (Glutamine amidotransferase class-I) and Peptidase_C26 domains to form a gene product in M. penetrans involved in GMP biosynthesis. Amongst the human- and primate-specific pathogens, M. penetrans has the largest genome (1,358,633 nt) and maximum number of proteins (1037) among all 14 Mycoplasma species analyzed in this study (Table 1), suggesting that this organism may possess additional genetic information involved in its unique infection process. This organism lacks pyrimidine biosynthetic machinery but using orotate-related metabolism (again unique to M. penetrans) circumvents this problem [33]. On the other hand, presence of purine biosynthesis (GMP synthase) related protein assists on the purine part of nucleotide biosynthesis. Also, the larger size of genome and number of proteins present underlines presence of GMP_synth_C domain specific to M. penetrans. Such an inspection of domain architectures in proteins containing these newly predicted domains was carried out for all host-group specific domains. It revealed that, except for GMP_synth_C, all other domains are present as single domains in complete protein sequences. Most of the newly predicted domains are transcription factors not only involved in nucleotide biosynthesis but also specifically involved in the regulation of solute transport. This fact emphasizes the importance of solute transfer across the membrane in conditions of minimal genomes. Host-group-wise comparative analysis revealed that the TGS domain is present in two groups, rodents and ovine/caprine. Even within rodent-specific pathogens, it is present in only M. arthritidis_158L3_1, whereas; it is present in all three species of the ovine/caprine host group. TGS domain is named after threonyl-tRNA synthetase (ThrRS), GTPase, and guanosine-3’,5’-bis(diphosphate) 3’-pyrophosphohydrolase (SpoT). Its presence in proteins like GTPases suggests its role in ligand (nucleotide) binding or some regulatory function, but it has no direct information about function [35]. However, in M. mycoides, it is present in association with other domains in two different proteins. One of them is GTP diphosphokinase involved in guanosine tetraphosphate metabolic process explaining the possible involvement of TGS domain in nucleotide biosynthetic machinery. Here, M. mycoides, which also infects cattle (causing contagious bovine pleuropneumonia (CBPP)), has the second largest genome (1,211,703 nt) and number of proteins (1016) in the 14 Mycoplasma species under consideration (Table 1), explaining the presence of additional genetic information [34]. Some of the domains are specific to motile group, for example, HHH, HNH, HTH_5, Peptidase_M23, and SBP_bac_1 are specific to motile group, whereas GMP_synth_C,
Methyltransferase, NusB, TGS, and Transposasemut domains are specific to nonmotile group (Supplementary Table S2). Inspecting the domain architectures for all the domains specific to the motility group, we found that they were not associated with any other domain in the complete protein sequence, except for the HHH domain in *M. pneumoniae*. Even in *M. pneumoniae*, HHH (helix-hairpin-helix motif—small DNA-binding motif) was associated with three different ligase domains involved in replication, repair, and recombination events. Therefore, although there is no obvious link between the presence and absence of these domains and motility function, these distant relationships perhaps acquired new function, which may be required for motility of the pathogens.

### 4. Conclusions

The investigation in the sequence information among closely related genomes helps in tracing of appearance, disappearance, and reappearance of genes or their close homologues...
in closely related bacterial genomes. Generally, functional annotation transfer is accomplished by phylogenomics-based methods that exploit strong phylogenetic relationship and based on the closest orthologue identified [42]. Apart from different sequence homology-based methods, microarray expression data along with machine learning techniques like Support Vector Machines (SVM) are integrated together for functional annotations [43]. Although use of such methods will be useful, GO annotations could be more comprehensive with regards to the biological process part or the cellular component part than for the exact molecular function [44]. Protein classification methods along with gene ontology terms are very useful tools in protein functional annotation. However, the best hit with respect to sequence identity may not be the correct protein to be used for annotation transfer since paralogous protein sequences from...
the same organism do share high identity but function may vary.

In this study, newly and indirectly identified domains in Mycoplasma gallisepticum have been compared across 14 Mycoplasma species. This study showed that some of the newly identified domains are specific to Mycoplasma gallisepticum genome. Such genome-specific domains will perhaps provide important clues to the physiological and pathogenic specificities of the genome.

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