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SHORT COMMUNICATION

Molecular evolution of cell division proteins FtsA, FtsL, and FtsZ in bacteria: A phylogenetic analysis

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ABSTRACT

In the past 16s rRNA gene sequencing has been used to find out the evolutionary pattern and the phylogenetic relationship among bacteria. Despite its accuracy, 16S rRNA gene sequence analysis lacks widespread use beyond the large reference laboratories because of technical and cost considerations. The rapid development of the field of proteomics has now been very helpful in finding the phylogenetic relationship of microorganisms. An attempt has been made in this study to find out the molecular evolution of three cell division proteins FtsZ, FtsA and FtsL among certain bacteria and possibility of studying their phylogenetic relationship using various proteomics databases and tools. For the present study various economically and medically important bacteria were selected. The amino acid residue sequence of the three cell division proteins FtsZ, FtsA and FtsL were retrieved from UniprotKB database. The sequences thus obtained for each cell division protein were subjected to Multiple Sequence analysis in ClustalW database. The molecular evolution and phylogenetic study has been performed using TreeDomViwer. The study clearly revealed that the cell division proteins do follow a definitive evolutionary pattern and is based on gram staining character rather than the morphology. The present study has also clearly shown that the important conserved protein sequences can be very useful to study the phylogeny of bacteria.

Keywords: Cell division protein, bacteria, phylogeny, proteomics, molecular evolution

INTRODUCTION

The recent advances in molecular biology and bioinformatics have paved the way for the understanding of genetic diversity/similarity and evolutionary relationships in microorganisms. In the past 16srRNA gene sequencing has been used to find out the evolutionary pattern and the phylogenetic relationship among bacteria (Kiratisin *et al*., 2003). Despite its accuracy, 16S rRNA gene sequence analysis lacks widespread use beyond the large reference laboratories because of technical and cost considerations (Clarridge, 2004). The rapid development of the field of proteomics has now been very helpful in finding the phylogenetic relationship of microorganisms. Presently most of the bacteria's proteome has been deciphered and has been available in various protein databases.

The study on the molecular aspects of bacterial cell division has shown that it involves few important proteins and as complicated as that of eukaryotic cell division (Khattar, 2007). Most of the proteins are identified when studying the conditional lethal mutants that were unable to divide when grown under non-permissive conditions, typically an increase in temperature. Most of the cell division proteins are named as "Fts", filamentous temperature sensitive, a terminology which is based on the fact that mutants of some of these proteins formed filamentous phenotypes at non-permissive temperatures (Khattar, 2007).

In *Escherichia coli*, at least 9 proteins appear to be essential for cell division under most laboratory conditions for example, FtsZ, FtsA, ZipA, FtsK, FtsQ, FtsL, FtsW, FtsI and FtsN (Maggi *et al*. 2008). During cell division in Gram-negative bacteria, the cell envelope invaginates and constricts at the septum, eventually severing the cell into two compartments and separating the replicated genetic materials. All nine proteins have been localized to the septal ring, an equatorial ring structure at the division site (Chen and Beckwith, 2001).

FtsZ is a cytosolic tubulin-like protein that polymerizes into an oligomeric structure that forms the initial ring at midcell. FtsA is another cytosolic protein that is related to actin, but its precise function is unclear (Errington et al. 2003). However recently they have found that the actin-like FtsA protein interacts with the tubulin-like FtsZ protein, helping to assemble the cytokinetic Z ring, anchor it to the cytoplasmic membrane and recruit other essential division proteins (Daisuk and William 2007). FtsL is a 13-kDa bitopic membrane protein with a short cytoplasmic N-terminal domain, a membrane-

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spanning segment and a periplasmic domain that has a repeated heptad motif characteristic of leucine zippers (Ghigo and Beckwith 2000). All these cell division proteins are found to be widely distributed among various bacteria performing almost the same function. Interestingly these proteins also have undergone the evolutionary process along with the bacteria. Hence an attempt has been made in this study to find out the molecular evolution of three cell division proteins FtsZ, FtsA and FtsL among certain bacteria and possibility of studying their phylogenetic relationship using various proteomics databases and tools.

MATERIALS AND METHODS

For the present study various economically and medically important bacteria were selected (Table 1). They include both gram positive and gram negative bacteria. Apart from these, *Spirochaetes* were also included in the study.

Table 1: List of bacteria that are subjected for the phylogenetic analysis

Gram staining property	Morphology	Bacteria
Gram positive	Cocci	*Staphylococcus epidermidis*
		Staphylococcus haemolyticus
		Staphylococcus epidermidis
		Staphylococcus haemolyticus
		Staphylococcus aureus
		Streptococcus pneumoniae
		Streptococcus agalactiae
		Streptococcus pyogenes
		Enterococcus faecalis
	Rod-shaped	*Bacillus subtilis*
		Bacillus anthracis
		Bacillus thuringiensis
		Clostridium botulinum
		Clostridium perfringens
Gram negative	Cocci	*Neisseria meningitidis*
	Rod-shaped	*Yersinia pestis*
		Yersinia pseudotuberculosis
		Pseudomonas aeruginosa
		Salmonella typhi
		Salmonella paratyphi A
		Salmonella typhimurium
		Shigella flexneri
		Shigella boydii
		Shigella dysenteriae
		Klebsiella pneumoniae
		Escherichia coli
	Coccbacilli	*Haemophilus influenzae*
		Haemophilus ducreyi
	Pleomorphic	*Legionella pneumophila*
	Curved rod	*Vibrio mimicus*
		Vibrio cholerae
		Vibrio fischeri
		Vibrio parahaemolyticus
Nil	Spirochaete	*Borrelia burgdorferi*
		Leptospira interrogans
		Leptospira biflexa
		Treponema denticola

Retrieval of protein sequences

The amino acid residue sequence of the three cell division proteins, FtsZ, FtsA and FtsL were retrieved from UniprotKB database. The database was accessed through the website www.uniprot.org. The protein name was entered into the query box and the search was requested in Uniprot Knowledgebase (UniprotKB). The protein sequences were retrieved in FastA format.

Multiple sequence analysis and phylogenetic tree construction

The sequences thus obtained for each cell division protein were subjected to Multiple Sequence analysis in ClustalW

database. The ClustalW database was accessed through the website www.ebi.ac.uk/clustalw (Thompson *et al.*, 1994). The Ktup value was set to '5' for better accuracy of alignment. The Blossum was selected as the scoring matrix. For the construction of phylogeneitc tree the Neibour-joining method was selected. After selecting all the important parameters the sequences were submitted as FastA format and ClustalW alignment was performed.

Motif identification

To identify the region of motif and its types in the cell division protein, bacterial protein sequence was submitted and analyzed in Pfam database. The Pfam database was accessed through the website http://pfam.sanger.ac.uk (Bateman *et al*., 2002). The motif search was initiated both in PfamA and PfamB.

Phylogenetic analysis

The phylogenetic analysis and protein domain alignment were done in "TreeDomViewer" a visualization tool available as web based interface that combines phylogenetic tree description, multiple sequence alignment and InterProScan (Alako *et al.*, 2006). It is a multipurpose tool that can generate a phylogenetic tree projecting the corresponding protein domain information onto the multiple sequence alignment. The TreeDomViewer was accessed at http://www.bioinformatics.nl/tools/treedom/. The sequences were submitted into the tool with selecting all the required parameters. The image output was requested in JPEG format.

RESULTS AND DISCUSSION

The amino acid residue sequences of three cell division proteins FtsZ, FtsA and FtsL for various bacteria were successfully retrieved from UniprotKB as FastA format. However the sequence of FtsL for few bacteria that was selected for this study was not available in the database.

The sequences thus obtained were subjected to multiple sequence alignment analyses by ClustalW tool. A phylogram was obtained for each cell division protein after the multiple sequence analysis.

After the preliminary multiple sequence analysis in ClustalW, the sequences were submitted in TreeDomViewer for its phylogenetic analysis. Apart from the phylogeny, the results also showed the aligned domain structure thereby allowing the visualization of structurally conserved domain throughout evolution.

Figure 1 depicts the phylogram that was obtained for FtsA cell division protein. The result clearly shows that FtsA has evolved in an orderly manner along the lineage of the same genus. The result further shows that the molecular evolution of the FtsA is interestingly on the line of Gram staining property of the bacteria. The evolutionary pattern could not be formed in terms of the bacterial morphology (i.e., cocci and bacilli).

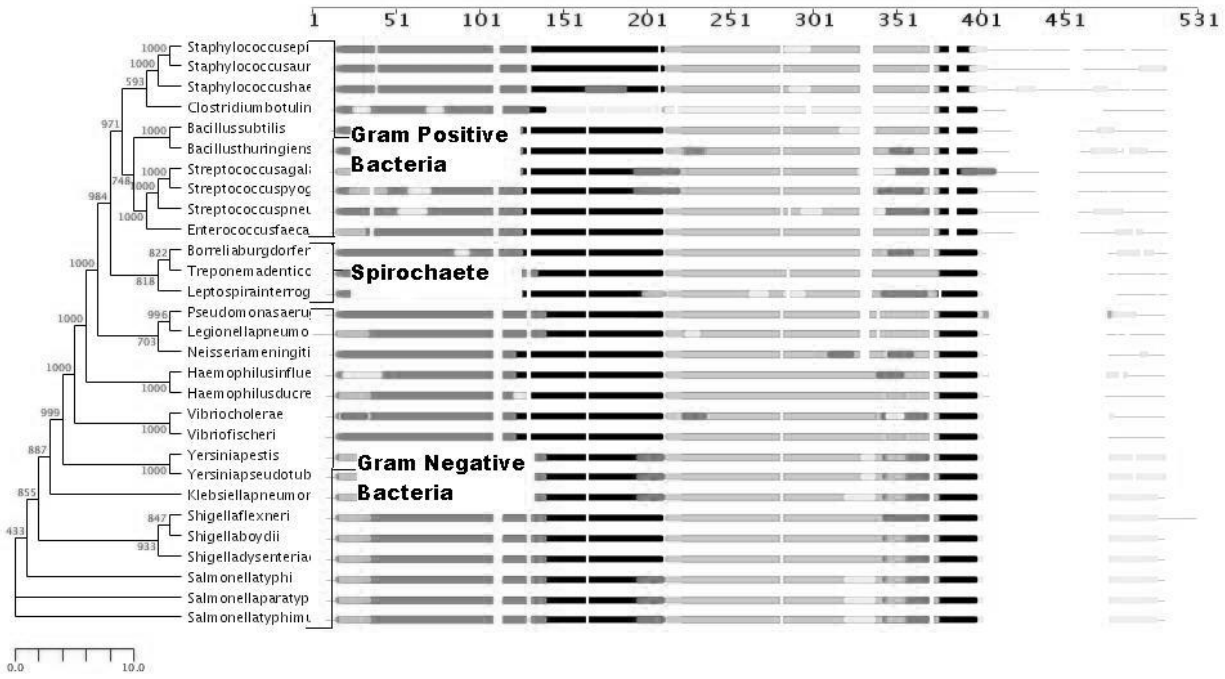

Figure 1: The phylogram showing the molecular evolution of FtsA protein along with the domain alignment and bootstrap values.

The phylograms of the FtsL and FtsZ are shown in Figure 2 and Figure 3 respectively. The phylogram confirms the same results. The FtsL and FtsZ protein have also been evolved on the same lineage for Gram positive and Gram negative bacteria. However, interestingly the FtsL protein of the *Haemophilus influenzae* and *Haemophilus ducreyi* have evolved along the line of Gram positive bacteria. Similarly FtsL protein of the *Clostridium perfringes* and *Clostridium botulinum* has evolved along the line of Gram negative bacteria.

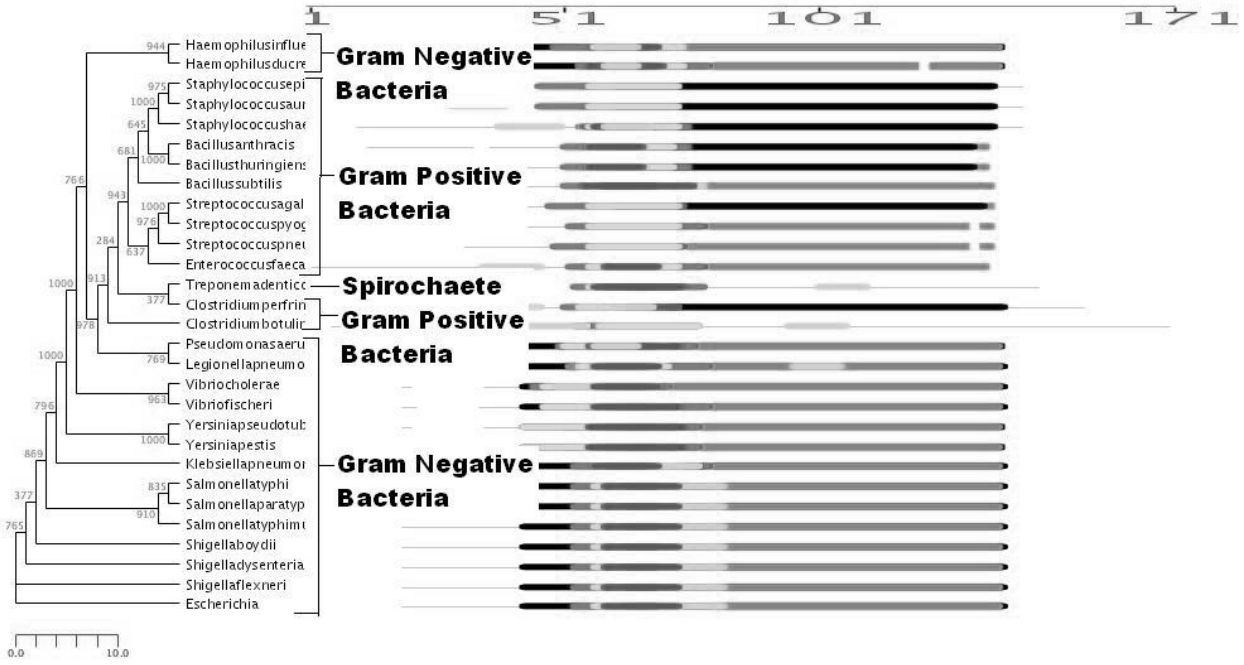

Figure 2: The phylogram showing the molecular evolution of FtsL protein along with the domain alignment and bootstrap values.

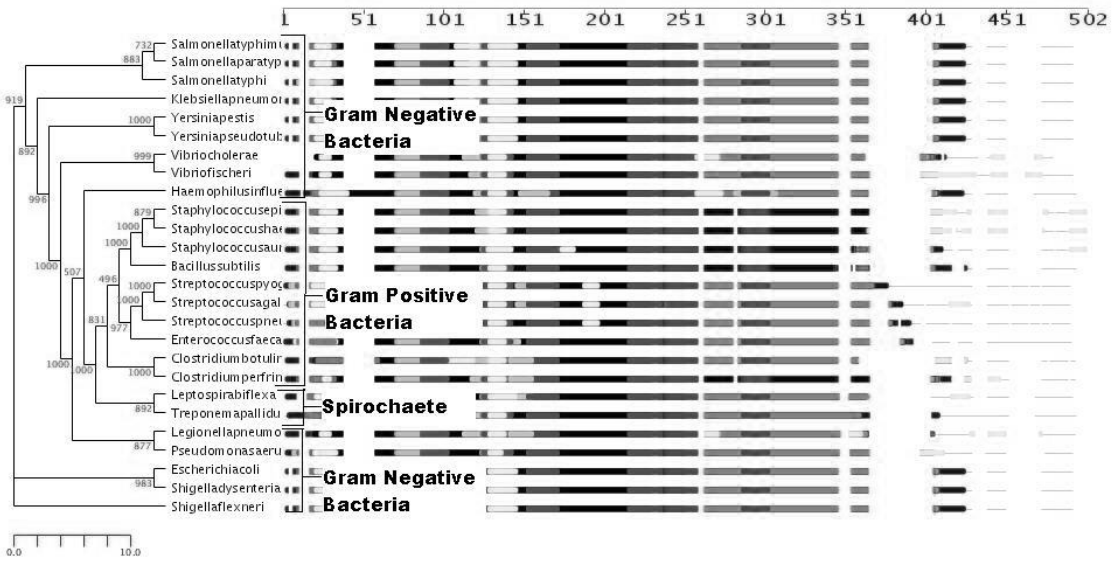

Figure 3: The phylogram showing the molecular evolution of FtsZ protein along with the domain alignment and bootstrap values.

The FtsA protein showed one significant motif and is found between amino acid residues 178 to 338. The FtsL protein too showed only one domain which is found between amino acid residues 24 to 124. The FtsZ protein showed two significant motifs. They are GTPase domain and C terminal domain. They both lay between the amino acid residues 13 to 204 and 207 to 331 respectively.

Another important feature that can be interpreted from the result as far as the domain alignment is concerned is the domains are conserved throughout evolution.

The availability of a meaningful molecular phylogeny for bacteria provides a context for examining the historical significance of various developments in bacterial evolution (Siefert and Fox, 1998). From the present study it is clear that bacterial morphology may not be the useful criterion for deriving the phylogenetic relationship. The same was observed in the study conducted by Siefert and Fox (1998).

Heyer *et al.* in 2002 conducted a study on methane oxidizing bacteria and showed that molecular evolution of important genes and their products are able to give the phylogenetic relationship in addition to 16SrRNA gene sequence. Studies have proved that bacterial evolution can be studied with conserved protein sequences (Woese, 1987). In 1990, DuBose and Hartl did a work on evolutionary significance of alkaline phosphatase of enteric bacteria in relation to *E. coli.*

This study clearly revealed that the cell division proteins do follow a definitive evolutionary pattern. The present study has also clearly shown that the important conserved protein sequences can be very useful to study the phylogeny of bacteria. For the study of phylogeny of bacteria, it is very important to look into only those proteins which are essential for bacteria. Jordon *et al.* in 2002 studied on essential and non essential genes of bacteria and concluded that the essential genes are more conserved than the non essential genes and are evolutionarily significant. Hence it is evident from the present study that the study of molecular evolution of certain proteins of bacteria will be highly significant in providing the taxonomic relationship of various bacteria.

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