Analysis of Pilus Biogenesis Genes in Bacteria Expressing Type IV Pili

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Abstract
The structural and sequences similarities of type-IV pili proteins were analysed in Pseudomonas aeruginosa, Vibrio cholerae, Neisseria meningitidis and Neisseria gonorrhoeae. Pathogenic Neisseria species possess pili-genes that code structural and assembly proteins of type-IV pili. A collection of assembly proteins reveal homology amongst P. aeruginosa and Neisseria. While in V. cholerae, these proteins ensure functional counterparts. The pilus retraction and assembly ATPases, PilB/PilF and PilT, PilU are homologous in Neisseria and P. aeruginosa, whereas V. cholerae holds only one ATPase called TcpT. In this work only type-IV pilus machinery of P. aeruginosa shows high likeness with pathogenic Neisseria.

Keywords: Type IV pilus genes, Sequence similarity, Pilus machinery, Schematic overview

INTRODUCTION
Bacteria express hair-like appendages on their surface called pili or fimbriae, which facilitate the interaction between bacterial and the host cell surfaces [1]. One the most essential pili is Type IV, involved in adhesion to host cells, twitching and gliding motility, DNA uptake, formation of biofilm and microcolonies, signals transduction and escaping from the immune response [2]. Type IV pili has type IVa and type IVb subtypes differentiated on the basis of structure and function of their assembly system. Type IVa pili are expressed in Neisseria, Pseudomonas and Dichelobacter, found in plants, animals and humans. Type IVb pili are expressed in bacteria such as Salmonella enterica serovar Typhi, Enterotoxigenic Escherichia coli, V. cholerae and P. aeruginosa [2]. Type IV pili mediated migration of bacteria make a polysaccharide-like Psl-fibres and with the loss of Psl-fibre a reduction of biofilm biomass occur [3,4]. Neisserial Type IV pili play an important role in the pathogenesis of disease. The PiID, PiIF, PiIM, PiIN, PiIO, PiIP are the major proteins required for the functional assembly of type IV pili in this species [3]. PiIV and PiIX are minor pilin protein required for Neisseria type IV assembly and are involved in adherence of type IV pili to human cells. PiIV also mediate PiIC exposure and modification within the type IV pili [5]. The structure of Neisseria type IV pili consists of approximately 500-2000 major PiIE subunits [7].
Toxin-coregulated pilin (TCP) is the major subunit of *V. cholerae* biogenesis [8]. The tcpA gene is located in the cluster of genes required for the assembly and regulation of type IV pilus biogenesis. In this cluster of genes, downstream of the tcpA gene, are tcpB, tcpQ, tcpC, tcpR, tcpD, tcpS, tcpT, tcpE, tcpF and tcpJ, while the genes located upstream of tcpA are tcpH, tcpP, tcpI [9]. Among all the TcpA involved in TcpA processing, while tcpC gene plays an important role in the pilus biogenesis and involved in resistance to complement [10].

The Typ4 pili systems in *P. aeruginosa* include additional, non-core minor pilins [2] called FimU, PilV, PilW, and PilX, encoded in an operon with the large putative adhesin, PilY1, and the non-core minor pilin, is PilE [11]. PilVWX and PilY1 depend on one another for incorporation into pili and they form a sub-complex required for PilE to be recovered in the pilus fraction [12]. *P. aeruginosa* has only one non-core minor pilin (PilE), while *Neisseria meningitidis* has three, and are used for direct binding to DNA [13].

Few studies revealed the similarities among genes in different species. For instance Helaine et al. [14] reported that the PilXNm protein in *N. gonorrhoeae* is possible orthologous to *P. aeruginosa* PilE, based on same location at their respective minor pilin operons and their sequence identity. Similarly PilVNm, of Neisserial genome, is also the orthologue of *P. aeruginosa*-PilE indicating that *N. meningitidis* possibly encodes two PilE equivalents. A cluster of genes in *V. cholerae* shows homology to the pilus encoding genes of *P. aeruginosa* and *Neisseria* is the pil genes. The nomenclature of these genes is based on *P. aeruginosa* related genes, as *P. aeruginosa* was the first identified organism with type IV pili [15]. We, in the present study have analysed the similarity and homology of proteins involved in type IV pili assembly and functions in between *Neisseria meningitis*, *N. gonorrhoeae*, *P. aeruginosa* and *V. cholerae*. Moreover we have compared the type IV pilus machinery between bacterial species and summarized the function of type IV pili in biofilm formation.

**MATERIAL and METHODS**

**Bacterial Strains and Species**

The type IV pilus biogenesis machinery of bacterial strains used in this study is described in Table 1 and Table 2. KEGG (Kyoto Encyclopedia of Genes and Genome) database was used for the collection of protein sequences.

**Sequence Alignment and Structure and Function of Proteins**

KEGG database (koyoto encyclopedia of genes and genomes), ClustalX, ClustaW and Clustal Omega were used for multiple sequence alignment of proteins. All the protein sequences of type IV pilus biogenesis were aligned in pairwise between the strains and different species to observe similarity between proteins. String database is used for the function and structure of protein and their direct or indirect interactions.

**RESULTS**

**Structural Similarities Between Type IV Pili Biogenesis Pil Proteins**

Eleven pilus biogenesis proteins were analysed for similarity among *P. aeruginosa*, *V. cholerae* and *N. meningitidis*. The analysed proteins are: PilQ-an outer membrane pore, PilO-a pre-pilin peptidase, PilP-secretin dynamic associated protein, PilM, PilN, PilO-the pilus assembly proteins, PilT-pilus retraction ATPase, PilU-a pilus ATPase, PilC-a platform protein, PilF-a pilotin, and PilB-a pilus ATPase. The proteins are described below and the similarities demonstrated by schematic figures.

As shown in Table 1, the analysed sequence comparison of Pil genes within the Neisseria strain, the first column contains the strains name we have compared, and in upper first row Pil gene name has been mentioned. For this analysis we keeps Nmc (*N. meningitidis* FAM18) as a standard and compared with Pil gene of other 16 strain of Neisseria, After analysis the sequence similarity by Clustal X, W, and Omega we observed almost 100% sequence similarity within Pil genes (Nm, Nmp, Nnm, Nmd, Nmm, Nms, Nmq, Nmz) of these strains, Except PilD gene which was not present in some of the Neisseria strain of my study. The PilC gene (Nma, Nme, Nmm, Nmt, Nmw, Nmz, Ngo) between Nmc (Standard) shows only 18% sequence similarity. While PilE gene between Nmc, Nmt and Nmi shows 24% and 26% similarity, Table 1. The type IV pilin protein in *P. aeruginosa* which were compared are shown in Fig. 1A, which shows the overview of the genomic map of the Pil and their associated genes which are responsible for type IV pilin in *P. aeruginosa*.

In Table 1, we have mentioned the observed sequence similarity between Neisseria standard strain Nmc (*N. meningitidis* FAM18) and *V. cholerae* strain. The gene name of *V. cholerae* strains has been listed in first column of the table while the upper first column contain Pil genes name. After analysing the sequence similarity it has been investigated that there is no significant similarity between Nmc and *V. cholerae* strains. The highest similarity 62% has been shown in PilT gene between Nmc and *V. cholerae* strains. While a PilD gene does not exist in *V. cholerae* strains of Vcj and Vcl, Table 1. The position of type IV pili proteins and the genomic map of type IV pili and their associated genes in *V. cholerae* M66-2 are clear in Fig. 1B and Fig. 1C.

Table 2 contain the comparison of the Sequence similarities within the standard strain Nmc (*N. meningitidis* FAM18) and *Pseudomonas* strains. In the first column the Pseudomonas strains name while in the first row the Pil genes name has
been listed. From this analysis we observed that PilC gene has no similarity except 18% similarity has been shown within Pdk and Nmc, while PilC of Pae and Paf are pseudo gene. The highest sequence similarity is 66% and was observed in PilT and Nmc of 

| Table 2. The percentage of protein sequences similarities between Nmc (Neisseria meningitidis FAM18) key factors of type IV pilus machinery and TCP key factors of V. cholerae. (Nil) in the table below shows that these protein sequences are not found in strains of V. cholerae By using KEGG database |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Key Protein     | Vch  | Vce  | Vcj  | Vco  | Vcr  | Vcm  | Vci  | Vcl  |
| TcpA PilE       | Nil   | 24%  | 50%  | 24%  | 24%  | 24%  | Nil  | Nil  |
| TcpC PilQ       | Nil   | 17   | Nil  | 17   | 17   | 17   | Nil  | Nil  |
| TcpS PilP       | Nil   | 15   | Nil  | 15   | 15   | 15   | Nil  | Nil  |
| TcpQ PilW       | Nil   | 50   | Nil  | 50   | 50   | 50   | Nil  | Nil  |
| TcpJ PilD       | 28    | 28   | Nil  | 28   | 28   | 28   | Nil  | 28   |
| TcpE PilR       | Nil   | 17   | Nil  | 17   | 17   | 17   | Nil  | Nil  |
| TcpT PilF       | Nil   | 26   | Nil  | 26   | 26   | 26   | Nil  | Nil  |

Comparison of Pilus Machinery

The major subunits PilE of Neisseria sp. and PilA of P. aeruginosa have a conserved N-terminal region with a short signal peptide, which is not present in the major subunit TcpC of V. cholerae. We have shown the protein sequences similarities between the key factors of type IV pilus machinery of Nmc (Neisseria meningitidis FAM18) and TCP (key factors of Vibrio cholerae). We have found no significant similarity (Table 1). Similarly the protein sequences similarities between Pau (P. aeruginosa UCBPP-PA14) key factors of type IV pilus machinery and key factors of V. cholerae TCP shows no significant similarity (Table 2).

DISCUSSION

Many bacterial species express type IV pili. In the current study type IV pili of P. aeruginosa, V. cholerae, N. meningitidis and N. gonorrhoeae were discussed. Type IV pili are flexible and long filaments attached to the bacterial cell. The type
IV pilus system is located in the cell membranes/cell wall of bacteria. Many similarities between type IV pili and the type II secretion have been observed. Many proteins are involved in the biogenesis of type IV pili and many proteins play different roles in type IV pilus machinery within different bacteria. Type IV pilus biogenesis play a very important role in the pathogenicity of bacteria and it has been observed that type IV pilin and their assembly is the main target for vaccine and drugs. It is also noticed that type IV pili are able to evoke the immune system of the host. Type IV pili play many roles in causing the disease (i) attachment of bacteria to the surface of host cell. Attachment initiate micro colony formation and initiate host cell signal transduction. (ii) Type 4 pili expressing bacteria are able to move in a special type of movement called as twitching motility and twitching motility is the cause of rapid colonization on the new surfaces and it is also noticed that twitching motility lead to complex process of fruiting bodies and biofilm formation. Type II pili expression and its relationship to bacterial type IV pili and archaeal flagella. Microbiology, 149, 3051-3072, 2003. DOI: 10.1099/mic.0.26364-0

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