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Short Communication

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*

Tip IV Pili Eksprese Eden Bakterilerde Pilus Biogenez Genlerinin Analizi

Öz

Pseudomonas aeruginosa, Vibrio cholerae, Neisseria meningitidis ve *Neisseria gonorrhoeae* bakterilerinde Tip IV pili proteinlerinin yapısal ve sekans benzerlikleri incelendi. Patojenik *Neisseria* türleri tip IV pilinin yapısal ve montaj proteinlerini kodlayan pili genlerine sahiptir. Bir grup montaj proteinleri *P. aeruginosa* ve *Neisseria* arasında homoloji göstermektedir. *V. cholerae*'da ise bu proteinler fonksiyonel karşılıkları sağlamaktadır. Pilus retraksiyon ve montaj ATPaz'ları, PilB/PilF ve PilT, PilU *Neisseria* ve *P. aeruginosa*'da homolog olup *V. cholera* sadece TcpT isimli bir ATPaz'a sahiptir. Bu çalışmada sadece *P. aeruginosa*'nın tip-IV pilus organeli patojenik *Neisseria* ile yüksek benzerlik göstermiştir.

Anahtar sözcükler: *Tip IV pilus genleri, Sekans benzerliği, Pilus organeli, Şematik bakış*

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, Enterotoxic Escherichia coli*, *V. cholerae* and *P. aeruginosa* [2]. Type IV pilus 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 PilD, PilF, PilM, PilN, PilO, PilP are the major proteins required for the functional assembly of type IV pili in this species [5]. PilV and PilX are minor pilin protein required for *Neisseria* type IV assembly and are involved in adherence of type IV pili to human cells. PilV also mediates PilC exposure and modification within the type IV pili [6]. The structure of *Neisseria* type IV pili consists of approximately 500-2000 major PilE subunits [7].

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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 *TcpJ* are 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. aeuroginosa* 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, ClustalW 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. cholera* and *N. meningitides*. The analysed proteins are: PilQ-an outer membrane pore, PilD-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 (Nma, Nmp, Nmh, 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, Nmn, 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 pili 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 pili 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 *Pseudmonas* strains. In the first column the Pseudomonas strains name while in the first row the Pil genes name has

Table 1. *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	*V. cholerae* Strain							
	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	28	Nil
TcpE PilG	Nil	17	Nil	17	17	17	Nil	Nil
TcpT PilF	Nil	26	Nil	26	26	26	Nil	Nil

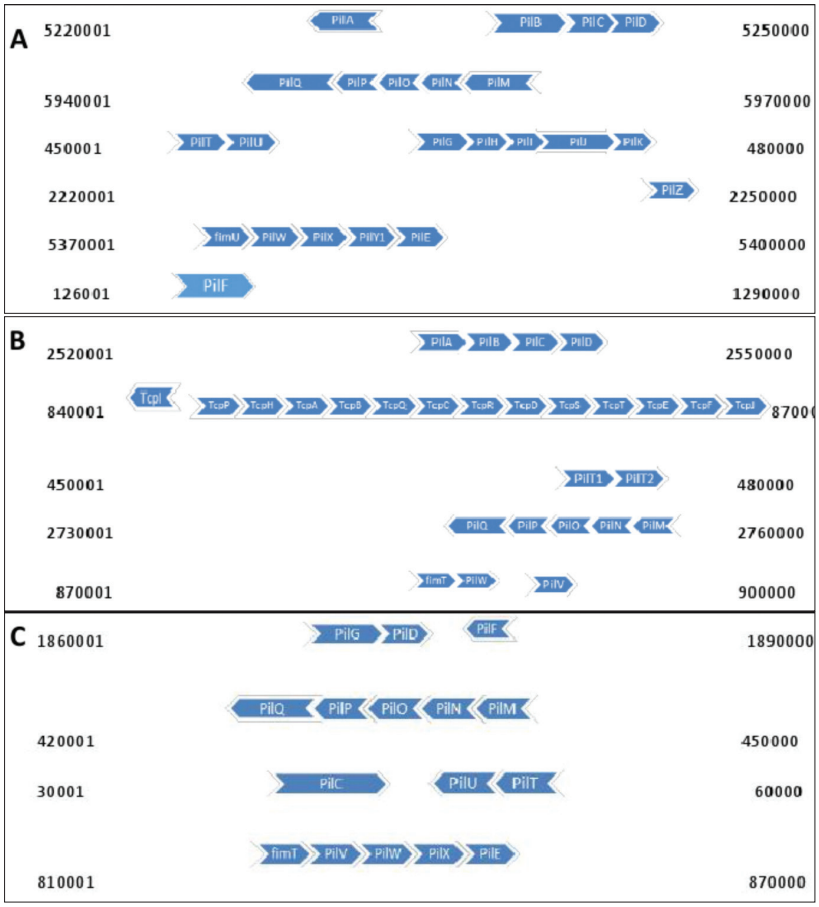

Fig 1. Schematic overview of type IV pili protein in *P. aeruginosa*. A- The figure shows the overview of the genomic map of the Pil and their associated genes which are responsible for type IV pili in *P. aeruginosa* UCBPP-PA14. B). Diagram has been originated from KEGGs database (koyoto encyclopaedia of gene and genome); B- Figure shows Schematic overview of type IV pili proteins in Vibrio cholera and the genomic map of type IV pili and their associated genes in *V. cholerae* M66-2. The position and location of Pil genes has been mentioned. Diagram has been originated from KEGGs database (koyoto encyclopaedia of gene and genome); C- The Schematic overview of type IV pili protein in Neisseria. The schematic overview of genomic map of the type IV pili responsible genes in Neisseria meningitidis (FAM18) are shown with respect to its position and location has been mentioned. Diagram has been originated from KEGGs database

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 *Pseudmonas* strains, while the rest Pil gene sequence similarity is in the range of 18% to 50%, *Table 2*.

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* [3,10]. 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 cholera)*. 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

Table 2. *This table shows the percentage of protein sequences similarities between Pau (P. aeruginosa UCBPP-PA14) key factors of type IV pilus machinery Pil proteins and key factors of V. cholerae TCP. (Nil) in the table below shows that these protein sequences are not found in these strains of V. cholerae By using KEGG database Bacterial strains are listed in Table 1*

Protein Names	Strains of *V. cholerae*							
	Vch	Vce	Vcj	Vco	Vcr	Vcm	Vci	Vcl
TcpA PilA	Nil	18%	30%	24%	24%	18%	Nil	Nil
TcpC PilQ	Nil	20	Nil	20	20	20	Nil	Nil
TcpS PilP	Nil	22	Nil	14	14	22	Nil	Nil
TcpQ PilF	Nil	50	Nil	50	50	50	Nil	Nil
TcpJ PilD	26	26	Nil	26	26	26	26	Nil
TcpE PilC	Nil	22	Nil	22	22	22	Nil	Nil
TcpT PilB	Nil	25	Nil	25	25	25	Nil	Nil

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 [3,5,15]. 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 [3,5,15]. Many proteins have the same function in type IV pilus biogenesis in different bacterial species. It is important to identify similarities between structures of those proteins which are involved in the type 4 pili biogenesis, since it will help in finding novel treatment against severe bacterial diseases caused by type pili expressing bacteria.

For the investigation of type IV pili and its associated functions in *P. aeruginosa* primary model systems has been important. Interestingly, in a single strain of *P. aeruginosa* three types of type IV pili have been identified; Type IVa, Type IVb and Tad. The Type IVb pili have been reported in *P. aeruginosa* strains with Pathogenicity Island PAPI-1 or PkLC102, while Tad and Type IVa pili are common to all *P. aeruginosa* strains. In *P. aeruginosa,* the Type IVa pili and Type IVb pili systems depend on the PilD prepilin peptidase, while it has been observed that Tad has its own prepilin peptidase called FppA [15].

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REFERENCES

1. Lory S, Strom MS: Structure-function relationship of type-IV prepilin peptidase of *P. aeruginosa*- A review. *Gene,* 192, 117-121, 1997. DOI: 10.1016/S0378-1119(96)00830-X

2. Giltner CL, Nguyen Y, Burrows LL: Type IV pilin proteins: Versatile molecular modules. *Microbiol Mol Biol Rev,* 76, 740-772, 2012. DOI: 10.1128/MMBR.00035-12

3. Peabody CR, Chung YJ, Yen MR, Vidal-Ingigliardi D, Pugsley AP, Saier MH: Type II protein secretion and its relationship to bacterial type IV pili and archaeal flagella. *Microbiology,* 149, 3051-3072, 2003. DOI: 10.1099/mic.0.26364-0

4. Yildiz FH, Visick KL: Vibrio biofilms: So much the same yet so different. *Trends Microbiol,* 17, 109-118, 2009. DOI: 10.1016/j.tim.2008.12.004

5. Carbonnelle E, Helaine S, Nassif X, Pelicic V: A systematic genetic analysis in *Neisseria meningitidis* defines the Pil proteins required for assembly, functionality, stabilization and export of type IV pili. *Mol Microbiol,* 61, 1510-1522, 2006. DOI: 10.1111/j.1365-2958.2006.05341.x

6. Brissac T, Mikaty G, Dumenil G, Coureuil M, Nassif X: The meningococcal minor pilin pilX is responsible for type IV pilus conformational changes associated with signaling to endothelial cells. *Infect Immun*, 80, 3297-3306, 2012. DOI: 10.1128/IAI.00369-12

7. Jain S, Moscicka KB, Bos MP, Pachulec E, Stuart MCA, Keegstra W, Boekema, EJ, van der Does C: Structural characterization of outer membrane components of the type IV pili system in pathogenic Neisseria. *PLoS One,* 6(1):e16624, 2011. DOI: 10.1371/journal.pone.0016624

8. Chattopadhyaya R, Ghose AC: Model of *V. cholerae* toxin coregulated pilin capable of filament formation. *Protein Eng,* 15, 297-304, 2002.

9. Strom MS, Lory S: Structure-function and biogenesis of the type IV pili. *Annu Rev Microbiol*, 47, 565-596, 1993. DOI: 10.1146/annurev.mi.47.100193.003025

10. Bose N, Taylor RK: Identification of a TcpC-TcpQ outer membrane complex involved in the biogenesis of the toxin-coregulated pilus of *Vibrio cholerae*. *J Bacteriol,* 187, 2225-2232, 2005. DOI: 10.1128/JB.187.7.2225-2232.2005

11. Belete B, Lu H, Wozniak DJ: *Pseudomonas aeruginosa* AlgR regulates type IV pilus biosynthesis by activating transcription of the *fimU-pilVWXY1Y2E* operon. *J Bacteriol,* 190, 2023-2030, 2008. DOI: 10.1128/JB.01623-07

12. Nguyen Y, Sugiman-Marangos S, Harvey H, Bell SD, Charlton CL, Junop MS, Burrows LL: *Pseudomonas aeruginosa* minor pilins prime type IVa pilus assembly and promote surface display of the PilY1 adhesin. *J Biol Chem,* 290, 601-611, 2005. DOI: 10.1074/jbc.M114.616904

13. Cehovin A, Simpson PJ, McDowell MA, Brown DR, Noschese R, Pallett M, Brady J, Baldwin GS, Lea SM, Matthews SJ, Pelicic V: Specific DNA recognition mediated by a type IV pilin. *Proc Natl Acad Sci USA,* 110, 3065-3070, 2013. DOI: 10.1073/pnas.1218832110

14. Hélaine S, Carbonnelle E, Prouvensier L, Beretti JL, Nassif X, Pelicic V: PilX, a pilus-associated protein essential for bacterial aggregation, is a key to pilus-facilitated attachment of *Neisseria meningitidis* to human cells. *Mol Microbiol,* 55, 65-77, 2005. DOI: 10.1111/j.1365-2958.2004.04372.x

15. Fullner KJ, Mekalanos JJ: Genetic characterization of a new type IV-A pilus gene cluster found in both classical and el tor biotypes of *Vibrio cholerae*. *Infect Immun,* 67, 1393-1404, 1999.