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# MODELLING PRECISION CHARACTERISATION OF FIMBRIAL PROTEIN (*fimA*) OF *Edwardsiella tarda* AND *Edwardsiella piscicida*

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**ABSTRACT**

Edwardsiellosis caused by *Edwardsiella tarda* and *Edwardsiella piscicida* is one of the most common catfish diseases. For any bacterium to cause an infection, it has to adhere to and invade the host. Major fimbrial protein (*fimA*) plays an important role in the adherence of *Edwarsiella* spp. using an *in-silico* approach, physicochemical properties, secondary structure, functional properties and homology modelling of *fimA* protein from *E. tarda* and *E. piscicida* were analysed in this study. The primary structural analysis indicated that all proteins are soluble, stable and hydrophobic. Secondary structures analysed using the SOPMA tool revealed that extended strands, random coils and alpha helices are predominant amidst secondary structural elements. The homology models of these proteins were built using SWISS-MODEL and their qualities were validated using ProSA, ProQ, PROCHECK and RAMPAGE analysis. The validated models were verified as good structures. The results suggested that the *fimA* can be a potential target for diagnosis and drug development to combat edwardsiellosis infection.

**Keywords:** *In-silico*, 3D Modelling, Computational characterisation, *Edwardsiella* spp., Fimbrial protein

## INTRODUCTION

The aquaculture sector plays a vital role in the income and wealth of the nation's economy. The aquaculture industry has been adversely affected by the frequent occurrence of diseases, primarily due to intensive cultural practices for higher economic gain **(Walker and Winton, 2010).** The fish disease is one of the primary constraints to sustainable aquaculture production. Antibiotics are used to tackle pathogens causing deadly diseases, which became vulnerable due to the development of antibiotic resistance **(Watts *et al.*, 2017).** The study on novel potential targets plays a major role to come up with new drug discoveries to overcome already-developed antibiotic resistance. Edwardsiellosis caused by *Edwardsiella tarda* and *E. piscicida* is a significant threat to finfish aquaculture, especially to the catfish industry. *Edwardsiella* spp. are responsible for substantial losses in important wild and cultured fish species globally **(Abdelhamed *et al.*, 2019).** *Edwardsiella* spp. possess important virulence factors that supplement bacterial survival and pathogenesis in hosts **(Park *et al.*, 2012),** such as *katB, esrB, mukF, fimA, gadB, pstS, pstC, astA, isor, ompS2, ssrB, citC, hylA* and *gyrB* **(Moustafa *et al.*, 2016).** Adhesion of bacteria to the surface of their host is a crucial step in bacterial infection and fimbriae are known to be involved in adherence to the host **(Sakai *et al.*, 2007). Sakai *et al.* (2004)** found that a fimbrial gene cluster mediated the hemagglutination of *E. tarda*. The hemagglutination activity is also observed to be correlated with adherence to an epithelial cell line, HEp-2 **(Mahmoud, 2006).** The fimbrial gene cluster (*fimA*) of *E. tarda* constitutes of the fimbrial genes such as *etfA, etfB, etfC,* and *etfD* and are used to distinguish among fish pathogenic and non-pathogenic strains of *E. tarda* **(Sakai *et al.*, 2007).** The fimbriae are considered a drug target since it is an essential factor in the pathogenicity of *Edwardsiella* spp., which requires a study of its structure and function. Computational characterisation of proteins made the job simple to analyse the target virulent targets instead of tedious experimental analysis **(Cristobal et al., 2001, Rodríguez-Ruiz *et al.*, 2019)**. The *in-silico* approach study on various pathogens and hosts is gaining momentum in recent years. In this study, *in-silico* analysis was performed for the fimbrial proteins of *E. tarda* and *E. piscicida* to highlight their physicochemical characteristics, functional properties, secondary structure elements and homology modelling of proteins to identify the potential drug target to combat edwardsiellosis infection.

## MATERIALS AND METHODS

### Retrieval of protein sequences

Fimbrial protein's *(fimA)* amino acid sequences were recovered from the Universal Protein Resource (UniProt) database. Two *fimA* sequences namely major fimbrial subunit protein with accession number A0SQV7 of *Edwardsiella tarda* and type 1 fimbrial protein with accession number A0A034SUG7 of *Edwardsiella piscicida* were retrieved in FASTA format.

### Physicochemical properties characterisation

The ExPASy-Prot Param prediction server [https://web.expasy.org/protparam/] was used to evaluate the physicochemical properties of virulent fimbrial proteins **(Gasteiger *et al.*, 2005).** The physicochemical properties include amino acid composition, number of amino acids, molecular weight, extinction coefficient [EC] **(Gill and Hippel, 1989),** aliphatic index [AI] **(Ikai, 1980),** instability index [II] **(Guruprasad *et al.*, 1990),** grand average hydropathy [GRAVY] **(Kyte and Doolittle, 1982),** isoelectric point (pI), the total number of positive (Arg+Lys) and negative (Asp+Glu) residues (+R/-R).

### Functional analysis

The type of protein, i.e., soluble or transmembrane, was identified by the SOSUI server **(Hirokawa *et al.*, 1998)** [http://harrier.nagahamai-bio.ac.jp/sosui/sosui_submit.Html]. The CYS_REC tool [http://linux1.softberry.com] was used to predict the presence or absence of disulfide bonds, which defines the functional linkage and the stability of a protein, bonding pattern and the number of cysteine residues.

### Secondary structure characterisation

SOPMA (self-optimized prediction method with alignment) tool was used to predict the elements of the secondary structure of fimbrial proteins **(Combet *et al.*, 2000)** [https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html]. It anticipated the secondary structure of all the proteins in the database through a similarity algorithm and identified the parameters that maximize predictive accuracy.

### Homology modelling and validation of the model quality

Using a template from Protein Data Bank (PDB), the SWISS-MODEL a fully automated freely accessible server carried out the homology modelling of the 3D structure of the fimbrial proteins **(Waterhouse *et al.*, 2018)** [https://swissmodel.expasy.org/]. The built models were validated for their accuracy and quality using RAMPAGE **(Lovell *et al.*, 2003),** ProQ **(Cristobal *et al.*, 2001),** PROCHECK **(Laskowski *et al.*, 1993)** and ProSA-web **(Sippl, 1993; Wiederstein and Sippl, 2007).**

## RESULTS AND DISCUSSION

### Physicochemical properties characterisation

The physicochemical properties of the fimbrial proteins of *E. tarda* and *E. piscicida* analyzed by the ExPASy server's Prot Param Tool are summarised in Table 1. The amino acid compositions of the proteins are tabulated in Table 2. As tabulated, the *fimA* proteins of *E. tarda* and *E. piscicida* had 177 and 175 amino acids, respectively. The computed pI values of *fimA* proteins of *E. tarda* (6.72) and *E. piscicida* (5.72) suggested that these proteins are acidic, which would be helpful for protein purification by isoelectric focusing on a 2D gel **(Łapińska *et al.*, 2017).** The total number of positive (Arg+Lys) and negative charge residues (Asp+Glu) (+R/-R) of the fimbrial proteins were 16/16 for *E. tarda* and 11/12 for *E. piscicida*. The extinction coefficients of the fimbrial proteins measured at 280 nm were 9065 $M^{-1}$ $cm^{-1}$ for *E. tarda* and 8605 $M^{-1}$ $cm^{-1}$ for *E. piscicida*. At 280 nm, the maximum absorbance of proteins in the UV spectra occurred because of aromatic residues of tryptophan, tyrosine and phenylalanine **(Lakowicz, 2013).** The computed extinction coefficient values may be useful in studying the protein-protein and protein-ligand interaction in solution quantitatively.

**Table 1** Physicochemical properties of *fimA* proteins of *Edwardsiella tarda* and *E. piscicida* computed using ExPASyProtParam

| Physicochemical properties | *Edwardsiella tarda* *fimA* protein A0SQV7 | *Edwardsiella piscicida* *fimA* protein A0A034SUG7 |
|---|---|---|
| Number of amino acids | 177 | 175 |
| Molecular weight | 18531.92 | 17549.79 |
| Isoelectric point | 6.72 | 5.72 |
| Number of positive (Arg+Lys) and negative (Asp+Glu) residues (+R/-R) | 16/16 | 11/12 |
| Extinction coefficient, $M^{-1}$ $cm^{-1}$ | 9065 | 8605 |
| Instability index | 25.64 | 18.14 |
| Aliphatic index | 81.07 | 85.94 |
| Grand average hydropathy (GRAVY) | -0.084 | -0.269 |

The stability of the protein in a test tube was indicated by the instability index **(Ghosh *et al.*, 2017).** A stable protein has an instability index of <40 **(Guruprasad *et al.*, 1990, Rodríguez-Ruiz *et al.*, 2019).** The instability index of the *fimA* of *E. tarda* was 25.64 and those of *E. piscicida* was 18.14, which indicated that both proteins are stable. The aliphatic index (AI) is the thermostability of the proteins based on the relative volume occupied by aliphatic side chains such as alanine, valine, isoleucine and leucine. The higher the aliphatic index, the better the thermostability **(Ikai, 1980, Juibari *et al.*, 2019).** The AI values of the *fimA* of *E. tarda* and *E. piscicida* were 81.07 and 85.94, respectively, thus indicating that both are thermally stable. The grand average of hydropathy (GRAVY) provided the interaction of a particular protein with water. The GRAVY values of *fimA* of *E. tarda* and *E. piscicida* were -0.084 and -0.269, indicating the hydrophobic and non-polar nature of the fimbrial proteins. Also, the lower GRAVY index values indicated a better interaction **(Gangadhar *et al.*, 2016).**

**Table 2** Amino acid composition of *fimA* proteins of *Edwardsiella tarda* and *E. piscicida* computed using Expasy's ProtParam

| Amino acid | *Edwardsiella tarda* *fimA* protein A0SQV7 | | *Edwardsiella piscicida* *fimA* protein A0A034SUG7 | |
|---|---|---|---|---|
| | Number | % | Number | % |
| Alanine | 21 | 11.9 | 23 | 13.1 |
| Arginine | 3 | 1.7 | 1 | 0.6 |
| Asparagine | 10 | 5.6 | 6 | 3.4 |
| Aspartic acid | 9 | 5.1 | 7 | 4.0 |
| Cysteine | 2 | 1.1 | 2 | 1.1 |
| Glutamine | 6 | 3.4 | 7 | 4.0 |
| Glutamic acid | 7 | 4.0 | 5 | 2.9 |
| Glycine | 17 | 9.6 | 23 | 13.1 |
| Histidine | 2 | 1.1 | 1 | 0.6 |
| Isoleucine | 11 | 6.2 | 13 | 7.4 |
| Leucine | 10 | 5.6 | 10 | 5.7 |
| Lysine | 13 | 7.3 | 10 | 5.7 |
| Methionine | 3 | 1.7 | 2 | 1.1 |
| Phenylalanine | 7 | 4.0 | 9 | 5.1 |
| Proline | 6 | 3.4 | 3 | 1.7 |
| Serine | 14 | 7.9 | 18 | 10.3 |
| Threonine | 16 | 9.0 | 19 | 10.9 |
| Tryptophan | 0 | 0.0 | 1 | 0.6 |
| Tyrosine | 6 | 3.4 | 2 | 1.1 |
| Valine | 14 | 7.9 | 13 | 7.4 |

### Functional analysis and secondary structure characterisation

The SOSUI categorized the *fimA* of *E. tarda* and *E. piscicida* as soluble proteins. As they were soluble, the transmembrane region of the proteins could not be detected. The CYS_REC tool revealed that both proteins have only 2 cysteines in their amino acid sequences. None of the cysteines formed a disulphide bond, a measure of protein stability. The results indicated that these proteins are not structurally stronger to a great extent. The secondary structure is an intermediate before the protein folds into its three-dimensional tertiary structure. The calculated secondary structure elements of the proteins using SOPMA are tabulated in Table 3. The results showed that extended strands and random coils predominated among the secondary structure elements, followed by alpha-helices and beta-turn. The other secondary structure elements such as 310 helix, Pi helix (Ti), beta bridge, beta region and ambiguous states were not found in the fimbrial proteins of both strains.

### Homology modelling and validation of the model quality

Based on the similarity between the identified template and the sequence, the 3D model was built for fimbrial proteins of *E. tarda* and *E. piscicida* using the Swiss Model server. The models obtained with the best sequence identity are given in Figure 1a,b. The results of homology modelling are tabulated in Table 4. The *fimA* proteins were built with the template 5n2b.1.A described as putative fimbrial subunit type 1 at a resolution of 1.90Å. The Global Model Quality Estimation score (GMQE) of the *fimA* protein of *E. tarda* and *E. piscicida* were 0.57 and 0.56, respectively. The QMEAN Z-scores were -2.86 and -3.35 for the *fimA* proteins of *E. tarda* and *E. piscicida*, respectively.

**Table 3** Secondary structure elements of *fimA* proteins of *Edwardsiella tarda* and *E. piscicida* using the SOPMA tool

| Secondary structure elements | *Edwardsiella tarda* *fimA* protein A0SQV7 | | *Edwardsiella piscicida* *fimA* protein A0A034SUG7 | |
|---|---|---|---|---|
| | Number of amino acids | % | Number of amino acids | % |
| Alpha helix | 44 | 24.86 | 47 | 26.86 |
| Beta turn | 9 | 5.08 | 10 | 5.71 |
| Extended strand | 53 | 29.94 | 45 | 25.71 |
| Random coil | 71 | 40.11 | 73 | 41.71 |

**Table 4** Homology modelling data of *fimA* proteins of *Edwardsiella tarda* and *E. piscicida* using SWISS-MODEL

| Parameters | *Edwardsiella tarda* *fimA* protein A0SQV7 | *Edwardsiella piscicida* *fimA* protein A0A034SUG7 |
|---|---|---|
| Template ID | 5n2b.1.A | 5n2b.1.A |
| Sequence identity (%) | 30.87 | 26.35 |
| Global Model Quality Estimation score | 0.57 | 0.56 |
| QMEAN Z-score | -2.86 | -3.35 |

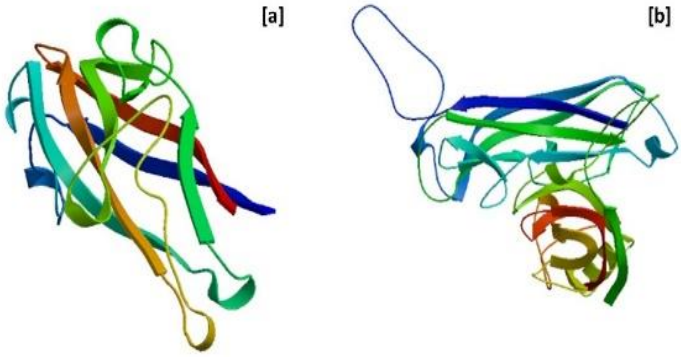


**Figure 1** Homology modelled 3D structures of *fimA* protein from (a) *Edwardsiella tarda* (A0SQV7) and (b) *E. piscicida* (A0A034SUG7) computed using SWISS-MODEL

Further, the stereochemical quality and accuracy of the predicted protein models were analysed by the RAMPAGE tool. The expected percentage of residues in the favoured and allowed region was ~98.0% and ~2.0%, respectively **(Lovell *et al.*, 2003).** The results showed that 92.90% of *E. tarda* and 94.60% of *E. piscicida fimA* residues lie in the favoured region whereas the minimum percentage contributed in the allowed and outlier region, thus indicating that the models are acceptable. ProSA and ProQ were used for further validation of the predicted models. The LG score values of the models predicted using SWISS-MODEL indicated that they are of very good quality (>2.5). MaxSub values indicated pretty good quality (>0.1) of both models. The Z scores of *fimA* of *E. tarda* and *E. piscicida* computed using ProSA were -4.36 and -4.49, which fell almost within the range of scores typically found for native proteins of similar size. Positive values generally correspond to problematic or erroneous parts of the input structure **(Wiederstein and Sippl, 2007).** The Ramachandran plot is a plot of the torsional angles - phi (φ) and psi (ψ) - of the amino acids (residues) contained in a peptide **(Hollingsworth and Karplus, 2010).** The Ramachandran plots of both fimbrial proteins were determined with the help of the PROCHECK server, which proved the values of RAMPAGE analysis (Figure 2a1, b1). The plot mainly showed negative values, indicating the good quality of the predicted model (Figure 2a2, b2). The Z-scores of the ProSA web is given in the form of an image (Figs. 2a3, a3) as determined by the X-ray crystallography (light blue) and nuclear magnetic resonance spectroscopy [NMR] (dark blue) by their length **(Pathak *et al.*, 2014).** The ProSA-web server also gave the plot of residue scores.

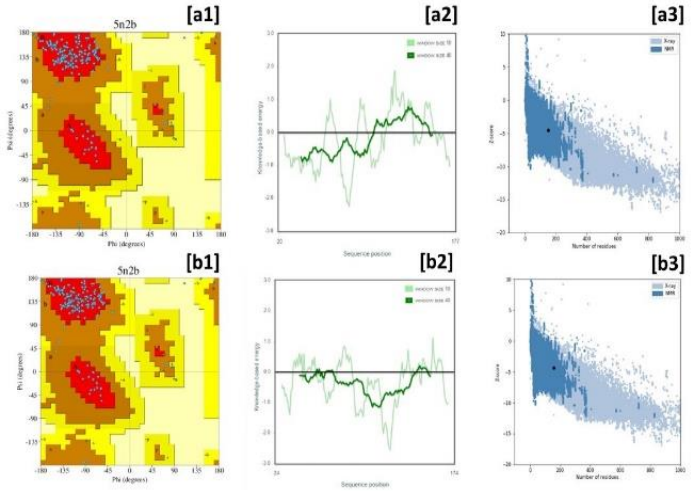


**Figure 2** Ramachandran plot predicted using PROCHECK of *fimA* protein from (a1) *Edwardsiella tarda* (A0SQV7), (b1) *E. piscicida* (A0A034SUG7); ProSA web Z-scores of protein chain in PDB determined by X-ray crystallography (light blue) NMR spectroscopy (dark blue) by their length of *fimA* protein from (a2) *E. tarda* (A0SQV7), (b2) *E. piscicida* (A0A034SUG7); Plot of residue scores of *fimA* protein from (a3) *E. tarda* (A0SQV7), (b3) *E. piscicida* (A0A034SUG7)

## CONCLUSION

Overall, this study provided insight into the differences in the fimbrial proteins of two closely related species of the genus *Edwardsiella*. Further, the *in-silico* characterisation and 3D structure prediction provided the baseline data on the fimbrial proteins of *E. tarda* and *E. piscicida* that would help initiate further research on the potential drug design against edwardsiellosis disease. The results of the present study provided a foundation upon which we can understand the role of bioinformatics in fish disease management. The adherence protein *fimA* can be a potential drug target to combat edwadsiellosis. The future scope can target natural compounds and drugs as an alternative to antibiotics by computer-aided drug designing to overcome huge economic losses due to disease.

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