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A Potential Screening of Peptide Deformylase Inhibitors Towards the Control of *Aeromonas hydrophila*

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Abstract

Currently, the prevalence of multidrug resistance *Aeromonas hydrophila* is one of the major issues and challenges for aquatic and terrestrial organisms. Therefore, an urgent need arises to control it using a potent and specific drug. Here, we identified a peptide deformylase (PDF) in *A. hydrophila*, which is a ubiquitous enzyme and one of the most attractive drug targets. We used the PDF protein sequences for generating a 3-D model using homology modeling. The 3-D model was validated and it was found 91% of the present amino acids in allowed regions of the Ramachandran plot. We used the 3-D model of PDF for the screening of drugs through molecular docking and found BB-3497, actinonin, and BBS-02 were more potent than other studied drugs based on binding energy. We have also generated a phylogenetic tree of PDF from *A. hydrophila* with other homologous bacteria, suggesting that similar drugs could also be applied to the control of those bacteria. These findings provide a new insight for the better understanding of PDF, which is a novel target for the development of more potent inhibitors towards the better control of multidrug resistant *A. hydrophila*.

Keywords: *Aeromonas hydrophila*; Peptide deformylase; Docking; Phylogeny; Drugs

Introduction

Aeromonas hydrophila causes a wide range of diseases including soft tissue infection and diarrhea in humans, while also causing hemorrhagic septicemia, and tail and fin rots in aquatic animals [1,2]. It secretes a number of enzymes and proteins (are also known as virulence factors) such as proteases, elastase, lecithinase, lipase, chitinase [3-5], cytotoxic enterotoxins [6], and hemolysins [7-10]. It plays a vital role in causing a high rate of mortality in aquaculture and great economical losses to fish farmers. *A. hydrophila* is also capable of contaminating food products due to its growth and the secretion of toxins material into the foods. A variety of foods used in our daily life as well as their derivatives can be affected, including fish, seafood, chicken, milk and dairy products, raw and cooked meats and others, all of which are not considered to be safe for eating following contamination. In 2009, a disease outbreak was reported concerning the infection of *A. hydrophila* in 48 farms of catfish in West Alabama with more than 3 million pounds of losses [11]. A recent outbreak has been reported in goldfish due to the infection of Cyprinid herpes virus-2 and multidrug resistant *A. hydrophila* in India [12]. Therefore, an urgent need arises to accurately detect and control the further spread of *A. hydrophila* into new areas. Over the past three decades, the polymerase chain reaction (PCR) has received a more attention for its use in the rapid, sensitive, and specific diagnosis of pathogens from a wide range of organisms. *A. hydrophila* was isolated from different organisms and confirmed by the PCR assay of variety of genes including *aerolysin*, *hemolysin*, *lipase,* and *protease* [13-18]. Nam and Joh [19] detected aerolysin (*aer*), GCAT (*gcat*), serine protease (*ser*), nuclease (*nuc*), lipase (*lip*), and lateral flagella (*laf*) genes in *Aeromonas* species. Even though accurate diagnostic can be made, we still need to control *A. hydrophila* within fish and water samples. Currently, antibiotic resistance is one of the major global issues for animal and human health [20,21]. There is currently an increasing use of antibiotics, which at the same time is also promoting the spread and evolution of antibiotic-resistant bacteria. In general, as we target to kill pathogenic bacteria, if they are not all killed, survivors can develop enhanced resistance over time and multiply, strengthening the pool of bacteria. Therefore, with the repeated uses of the same antibiotics, bacteria can potentially develop complete resistance against them [22]. In the previous study, 34 *A. hydrophila* isolates were collected from diverse locations and it was observed that all of the strains were resistant to ampicillin, carbenicillin, and rifampicin [23]. In addition, 234 isolates of *A. hydrophila* were tested in an antibiotics assay and it was found more than 90% of isolates were resistant to tetracycline, trimethoprim-sulfamethoxazole, and cephalosporins [24]. Furthermore, it was also found that *Aeromonas* isolates showed resistance to gentamicin, chloramphenicol, ciprofloxacin, and cotrimoxazole [25]. In addition, a total of 25 *A. hydrophila* isolates from fish and water samples have been tested for antibiotic sensitivity and it was found that all of these isolates were resistant to cephalothin, ampicillin, novobiocin and nitrofurazone [26]. Antibiotic resistance genes are commonly derived from plasmid, and so commonly have multiple copies and with the capability for autonomous transfer into microbial populations [27]. Potentially the build-up of resistance over time may have also occurred due to evolutionary adaptation and random mutations in genes within strains, as well as through excessive and poor use of antibiotics. There are more than 200 essential and conserved target proteins present within bacteria, but only a small proportion of these are currently exploited [28]. There is consistent need to identify a conserved and novel target for the control of *A. hydrophila*. The availability of the complete genome sequence of *A. hydrophila* has allowed us to identify a target for drug design and development [29]. The sensor histidine kinase identified in *A. hydrophila* has been suggested to be a good target and useful

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for novel drug discovery [30]. In addition, a DNA gyrase, an enzyme essential for DNA replication in *A. hydrophila* has been used for targeting using a number of antibiotics for control of cell growth [31]. Peptide deformylase (PDF) is a metalloenzyme and one of the most attractive targets for controlling of *A. hydrophila* growth [32]. Study of the sequence homology of *A. hydrophila* PDF shows that it is absent in eukaryotic life forms and therefore it is suitable for use as a target without the potential of biological cross talk [33,34]. The biosynthetic pathway can be blocked using potent antibiotics or drugs that could be a specific to the pathogen [35,36]. In order to target PDF, a need arises for either a 3-dimensional structure or a valid model. Homology modeling plays a key role in the generation of the 3-D model using the known 3-D crystal structure [37]. It requires protein sequence homology that should be higher than 25%; thereafter we would be able to generate a 3-D model. Currently, there is no 3-D structure of PDF of *A. hydrophila* available yet. The homology modeling was used to generate a 3-D model of hemolysin and aerolysin of *A. hydrophila* [10,38]. Recently, Fazil et al. [39] used homology modeling for generating a 3-D model of histidine kinase of *A. hydrophila*. In addition, DNA gyrase has also been modeled for targeting with several drugs and used for the design of novel drugs [31]. In order to control multidrug resistance and excessive use of antibiotics, phylogeny analysis can play a vital role in the identification of an evolutionary and genetic relationship of *A. hydrophila* with other bacteria. The phylogenetic relationship has been established for *Aeromonas* species based on the sequence of the β-subunit of DNA gyrase. In addition, they have also used 16S rDNA variable regions for genetic relationship analysis [39]. A number of proteins sequences such as hemolysin, aerolysin and DNA gyrase of *A. hydrophila* have been used for phylogenetic analysis [10,31,38]. The housekeeping genes *rpoD* and *gyrB* are important for cell activity, and can be used to construct a phylogeny [40]. In the present study, we construct a 3-D model for peptide deformylase of *A. hydrophila* that could be used for the screening of potent drugs using molecular docking. Additionally, we generated a PDF based phylogeny of *A. hydrophila,* along with other bacteria.

Materials and Methods

Homology modeling of peptide deformylase

We have retrieved the protein sequences of peptide deformylase (NCBI accession number: KER65290) of *A. hydrophila* and other bacteria from National Center for Biotechnology Information, NIH, US (www.ncbi.nlm.nih.gov). PDF was further used for finding structural similarity with deposited protein data bank 3-dimensional structures through BLAST, (basic local alignment search tool) [41]. It shows the structural similarity with 1N5N that was pair wise aligned using CLUSTALX [42]. We used the X-ray crystal structure of peptide deformylase of *Pseudomonas aeruginosa* as a template for generating the 3-D model of the PDF of *A. hydrophila*. The resolutions of X-ray crystal structure of peptide deformylase of *P. aeruginosa* was 1.80 Å. A total of five 3-D models of PDF of *A. hydrophila* have been generated using Modeller9v2, all of which have been further evaluated on the basis of lowest free energy of the models and the template [37]. These 3-D models were visualized using the PYMOL [43]. They were further evaluated using a PROCHECK that has also generated a Ramachandran plot [44]. The quality of the 3-D model shows maximum number of amino acids present in the allowed region of the Ramachandran plot.

Screening of potent drugs and phylogenetic analysis

We have taken drugs from NCBI PubChem compound in the SDF format, and these were further converted into 3-D models using OpenBabel. The 3-D model of PDF and the 3-D model of drugs were used for the screening of potent drug using the molecular docking tool AutoDock 4.2.3 [45]. We have considered docking parameters including random starting position and conformational translation step ranges of 1.5 Å, rotation step ranges of 35, 100 docking trials, a population size of 150, a cross over rate of 0.8, a mutation rate of 0.02, 25 million energy evaluations, and a local search rate of 0.06 [31,46]. Here we could choose correct docking sites based on the minimum docked energy between the PDF and the drug molecules. In order to construct a phylogenetic tree, the protein sequence of PDF from *A. hydrophila* has been used to find homologous protein sequences in other bacteria using BLASTP. We retrieved all homologous protein sequences and aligned them with the CLUSTALX [42]. Subsequently, we studied the aligned file within the MEGA4 software using passion correction equation [47]. We used the neighbor-joining method for construction of the phylogenetic tree with a total of 100 bootstrapped values, which was sampled to determine a measure of the support for each node on the consensus tree.

Results and Discussion

Risk assessment of multidrug resistance A. hydrophila

Currently, interest in the awareness of the health benefits and nutritional value of diets including fish consumption has been significantly increasing. Fish is an important source of low fats and is rich in proteins, which can be extensively assist immunity, and prevent infection and cardiac diseases [48,49]. However, *A. hydrophila* is a major threat for fish farming and stocking. It can spread rapidly into new areas and infect other fish and fish stocks. Therefore, aquaculture systems are facing a plethora of issues such as over-catching, disease outbreaks, exchanging of stock, preserving of stock, and the dumping of untreated industrial waste into freshwater water bodies [50,51]. In order to manage and control of *A. hydrophila* infection and spreading, we would need to use a modern approach to overcome the current issues of fish farming and stocking.

Antibiotics are currently used for the control of *A. hydrophila* infection in a wide range of organisms including fish, animals, amphibians, chicken, and mammals. In the previous study, *A. hydrophila* shows resistant to several antibiotics including novobiocin, ampicillin, cephalothin, and nitrofurazone while remaining sensitive to gentamicin (80%), co-trimoxazole (92%), chloramphenicol, and ciprofloxacin [26]. In addition, *A. hydrophila* isolated from rifampicin containing medium could help the development of attenuated vaccine for fish [52]. Very recently, a multidrug resistant *A. hydrophila* has been isolated from goldfish. It shows resistance against a number of antibiotics including amoxyclav, ampicillin, azetreonam, ceftazidime, cefuroxime, cephalothin, cephotaxime, cloxacillin, imipenem, lincomycin, methicillin, oxacillin, penicillin, sulphatriad, and vancomycin [12]. Multidrug resistance is currently an important global challenge and a major issue for the control of microbial infections. It is assisted through changes in the gene, over-dosing of antibiotics, or simply the misuse of antibiotics. Microbes are constantly adjusting within stressful condition and have survived by changing their physiological conditions [22]. Therefore, the current antibiotics are not potent and sufficient to treat *A. hydrophila* infections.

The 3-D modeling of peptide deformylase

We have identified a novel drug target peptide deformylase in the genome of *A. hydrophila* after the complete genome sequencing [29]. Currently, the 3-D structure of PDF is not determined. The protein

sequences of PDF were 51% homologous with 3-D structure similarity of deformylase of *P. aeruginosa* (PDB code 1N5N). Therefore, we used a homology modeling approach to build the 3-D model using the known 3-D crystal structure. Both the protein sequences of peptide deformylase of *A. hydrophila* and *P. aeruginosa* (PDB: 1N5N) were pair-wise aligned (Figure 1). The asterisk represents the identical amino acids. The 5 3-D models have been generated by Modeller9v6, and it was found that the Gibbs free energy of the peptide deformylase of *A. hydrophila* was very similar to its template. The 3-D model of peptide deformylase of *A. hydrophila* was shown in Figure 2a and it retains alpha-helix as well as beta-sheets in the model. The Ramachandran plot (RP) for peptide deformylase of *A. hydrophila* was also determined (Figure 2b), and represents in the allowed and disallowed regions of the amino acid residues 91% and 1.6%, respectively. All the above properties of peptide deformylase satisfied the need for good quality of the 3-D model. The previous study also supported our data regarding the quality of the 3-D model and the amino acids present in allowed region of the Ramachandran plot [10,31,38].

A number of drugs have been used for binding with PDF, however, mutation and low efficacy reduced the binding affinity, inhibiting better control of *A. hydrophila*. Single mutations in a gene can change the active amino acid residue, which may play a key role in binding with the drug. Therefore, a drug that has not been strongly bound with amino acids can lead to the appearance of drug resistance [53,54]. To address this issue, we used the 3-D model of PDF for the screening of potent drugs that can be further used for the control of *A. hydrophila* infection in different animals.

Screening of potent drugs and phylogenetic analysis

In the present study, we have identified six drugs and used these for molecular docking against the entire 3-D model of peptide deformylase. A total of 10 docking experiment have been performed with the entire 3-D model of peptide deformylase, which is considered the lowest free energy of the docked complex with hydrogen bonds. We found BB-3497, actinonin, and BBS-02 show the highest binding affinity as represented by docking energy. The docking energy of BB-3497, actinonin, and BBS-02 were -19.24, -19.85, and -19.24 kcal/mol, respectively (Table 1). A number of amino acids residue in the 3-D model of PDF during the interaction with the drug were given in Table 2. Amino acid residues including Glu36, Arg67, Ile60, Ile61, Tyr39, Gly44, Asn43, Asp62, Ala40, and Ile45 in the 3-D model of PDF of *A. hydrophila* were observed with the interaction of BB-3497 molecule. The drug was bound with these amino acids of PDF (Figure 3). In the case of actinonin, a number of amino acids such as Arg67, Glu36, Asp62, Ala40, Leu63, Ile61, Ile60, Gly44, and Tyr39 in PDF of *A. hydrophila* were observed (Figure 4a). It also formed a hydrogen bond between actinonin and amino acids, via the UNK0:H-Asp62:OD2 atoms with a distance 2.077 Å (Figure 4b). BB-3497 and actinonin are potent inhibitors of PDF and are able to control cell activity of *Escherichia coli* [36,55]. The effectiveness of actinonin against PDF of *E. coli* and *Staphylococcus aureus* has been further tested and it has been found that similar concentrations could control the growth of both bacteria [35]. Molecular docking has been used for the screening of potent drug molecules by targeting the aerolysin of *A. hydrophila* [38] and 3-oxoacyl-acyl carrier protein synthase II of *Mycobacterium tuberculosis* [56]. Sharma et al. [57] have tested BB-3497 and actinonin and both were found to be potent drugs against the PDF of *M. tuberculosis* at low concentrations. While in the case of interaction of a BBS-02 molecule with PDF, a number of amino acids including Tyr39, Ile45, Gly46, Gln51, Leu92, His137, Glu134, Cys91, Gly90, Ile120, and Leu92 were obtained (Figure 5a). This also showed three hydrogen bonds (HB) between UNK1:H-Gln51:OE1, UNK1:H-Gln51:NE2, and UNK1:H-Leu92:N atoms with 2.214 Å, 1.910 Å and 2.168 Å distances, respectively (Figure 5b). In a relevant study, a peptide deformylase in *M. tuberculosis* was identified and used in molecular docking for the screening of a wide range of inhibitors. They have found BB-3497, BBS-54, actinonin, and BBS-02 were potent inhibitors against PDF [58]. In our study, we tested *A. hydrophila* and found that BB-3497, actinonin, and BBS-02 are potent drugs, expanding beyond other studied drugs such as BBS-54, BBS-88, and BBS-52. These potent drugs can be applied to the control of *A. hydrophila* infections. The phylogenetic tree holds a key to solving some of the genetic relationship issues by building a phylogeny based on peptide deformylase of *A. hydrophila* and other bacteria those have homologous PDFs (Figure 6). Until now, there

Drugs	Binding energy (kcal.mol^{-1})	Docked energy (kcal.mol^{-1})	Inter molecular energy (kcal.mol^{-1})
BB-3497	-18.32	-19.24	-19.56
BBS-54	-14.36	-14.76	-14.98
Actinonin	-18.81	-19.85	-20.68
BBS-02	-13.63	-14.39	-14.87
BBS-88	-7.45	-8.16	-8.7
BBS-52	-13.16	-13.38	-14.71

Table 1: The interaction energy of peptide deformylase of *A. hydrophila* and drugs.

```
PDF             ------------MAMLDILTVPDPRLGIKAEQVEDIA-AVQGLIDDMLETLYATDNGIGL
1N5N            MGSDKIHHHHHHMAILNILEFPDPRLRTIAKPVEVVDDAVRQLIDDMFETMYEAP-GIGL
                           **:*:** .*****   *: ** :  **: *****:**:* :  ****

PDF             AATQVGRKEAIVIIDLSEKRDQPLVLINPQVTSGTEPALG-QEGCLSVPDYYADVERYGS
1N5N            AATQVNVHKRIVVMDLSEDKSEPRVFINPEFEPLTEEMDQYQEGCLSVPGFYENVDRPQK
                *****. :: **::****.:.:* *:***:. . **     ********.:* :*:*  .

PDF             VVVSALDRHGVPITVKSSDFLAIVMQHEIDHLQGKVFIDYLSPLKRKMALKKVKKHLKAT
1N5N            VRIKALDRDGNPFEEVAEGLLAVCIQHECDHLNGKLFVDYLSTLKRDRIRKKLEKQHRQQ
                * :.****.* *:   :..:**: :*** ***:**:*:****.***.   **::*: :

PDF             -
1N5N            A
```

Figure 1: Pairwise alignment of peptide deformylase (PDF) sequences of A. hydrophila with template crystal structure 1N5N sequence. The asterisk (*) indicates the identical amino acids.

Protein name	Amino acids in PDF	Drugs	Interaction of PDF and drugs	Distance of hydrogen bonds (Å)
PDF	Glu36, Arg67, Ile60, Ile61, Tyr39, Gly44, Asn43, Asp62, Ala40, Ile45	BB-3497	ND[a]	ND[a]
PDF	Arg67, Glu36, Asp62, Ala40, Leu63, Ile61, Ile60, Gly44, Tyr39	Actinonin	UNK0:H-Asp62:OD2	2.077
PDF	Tyr39, Ile45, Gly46, Gln51, Leu92, His137, Glu134, Cys91, Gly90, Ile120, Leu92	BBS-02	UNK1:H-Gln51:OE1 UNK1:H-Gln51:NE2 UNK1:H-Leu92:N	2.214 1.910 2.168

[a] Not detected

Table 2: The amino acid residues and hydrogen bond formed between the drugs and PDF.

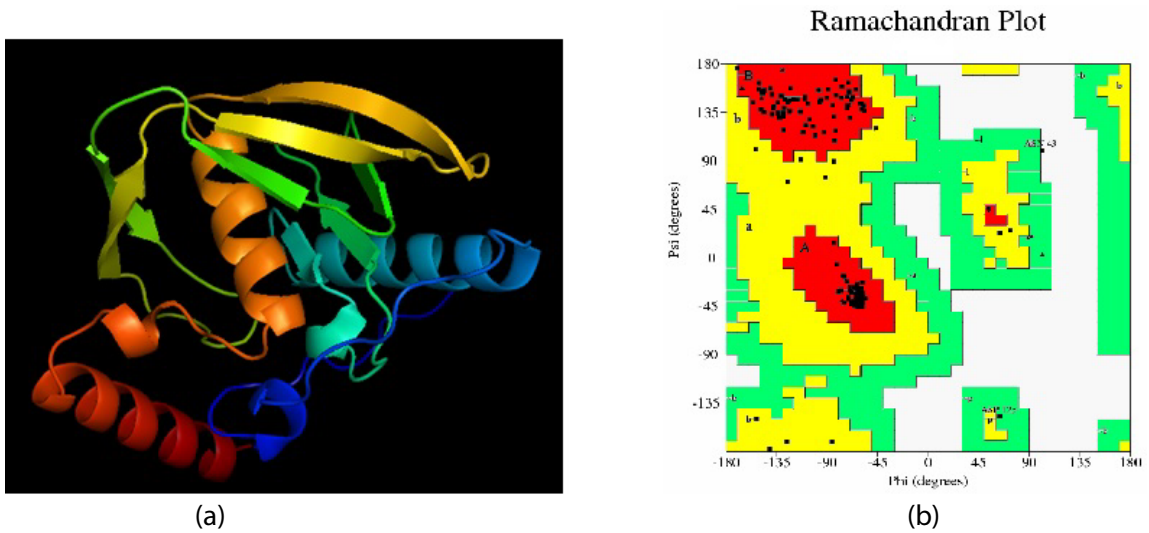

Figure 2: (a) The 3-D model of peptide deformylase of A. hydrophila. (b) Ramachandran plot for peptide deformylase and 91.0% amino acids in allowed region (red region).

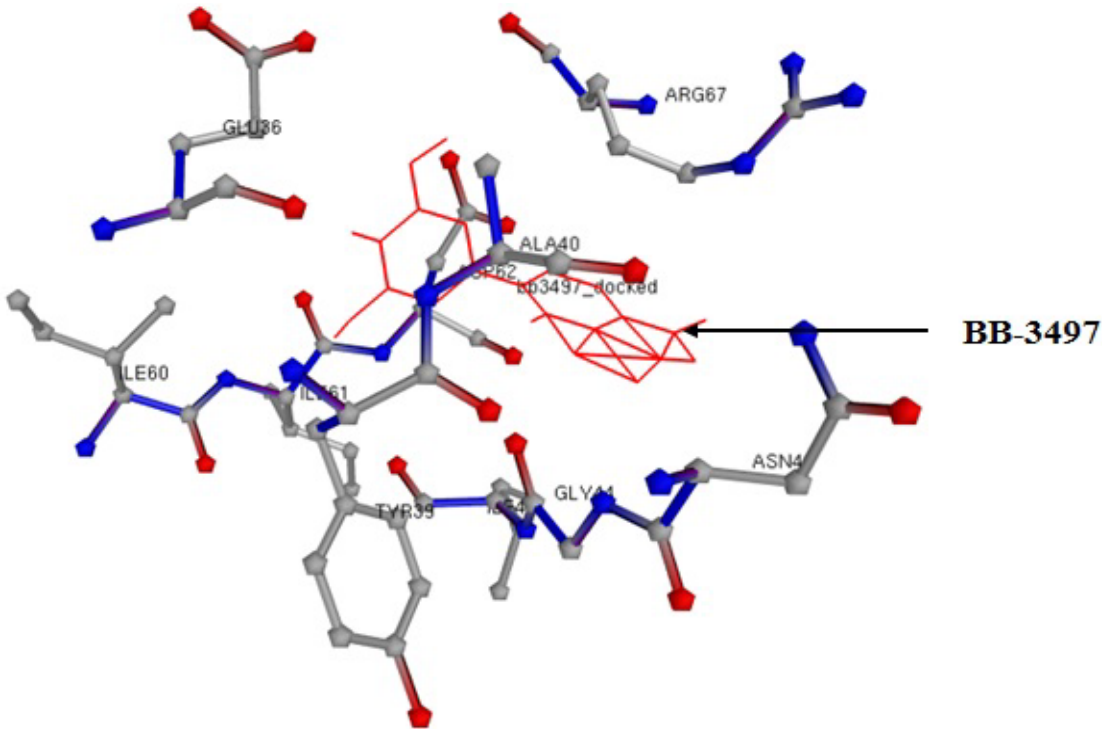

Figure 3: Interaction of BB-3497 drugs with PDF.

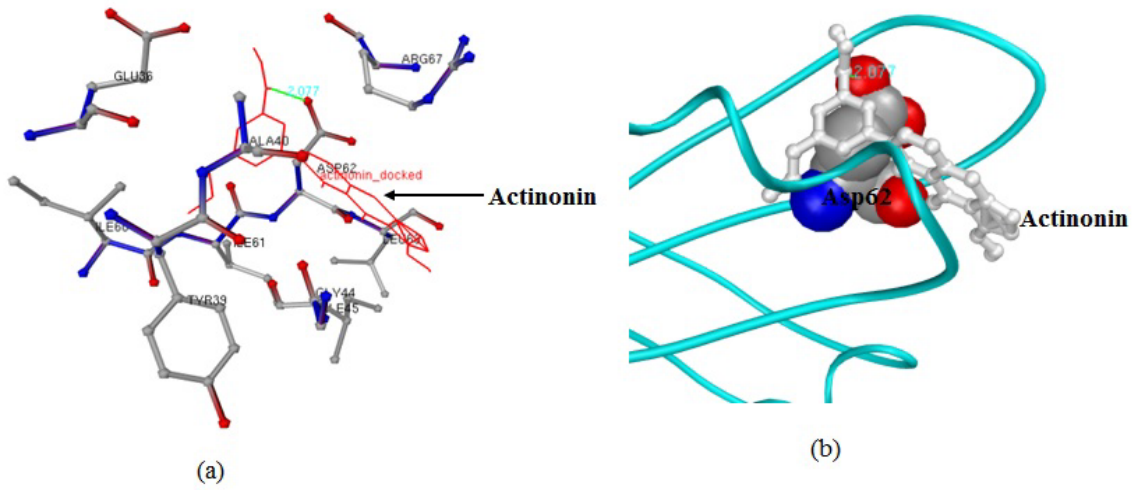

Figure 4: Interaction of actinonin with 3-D of PDF, showing a hydrogen bond. **(a)** Active pocket of amino acids interact with actinonin **(b)** Asp62 shows the HB with actinonin.

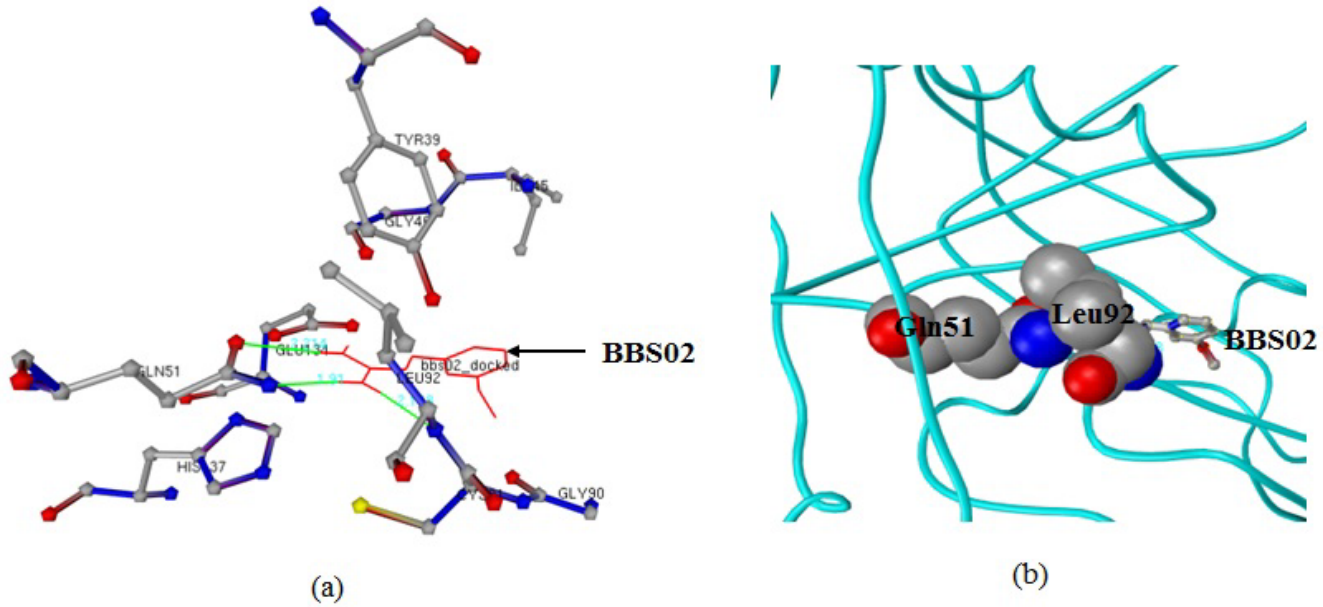

Figure 5: Interaction of BBS02 with 3-D of PDF, showing a hydrogen bond in green color. **(a)** Active pocket of amino acids interacts with BBS02 and **(b)** Gly51 and Leu92 shows the HB with BBS02.

has been no phylogenetic tree that has been constructed for the PDF of *A. hydrophila*. This is our approach to identify the PDF targets in other bacteria based on protein sequences homology that allows us to treat those bacteria with similar drugs towards better control of the misuse of drugs and/or avoiding the development of multidrug resistance. A total of 3 major clades were formed, clades A, B and C. *A. hydrophila* presented in clade B in between the phylogeny. It indicates that the similar antibacterial drugs such as BB-3497, actinonin, and BBS-02 can be used to inhibit the peptide deformylase activity. In clade A, it shows different species of *Vibrio* while in clade C, all other bacteria are present. *Vibrio* species were also close with *Aeromonas* species; the same drugs may be helpful for the inhibition of peptide deformylase function towards control of infection and spreading of these bacteria. The phylogeny indicates that the peptide deformylase is a stable and potential drug target for *A. hydrophila* and *V. cholerae*. The housekeeping genes such as *rpoD* and *gyrB of A. hydrophila* have been used for constructing a phylogenetic tree and they established the genetic relationship among *Aeromonas* species [40,59]. In addition, Küpfer et al. [60] have used *gyrB* and *rpoB* sequences for construction of phylogeny of *Aeromonas* species. In a relevant study, a phylogenetic tree has been constructed using the aerolysin and hemolysin protein sequences of *A. hydrophila* and they have identified a similarity among *Aeromonas* species and other pathogenic bacteria [10,38].

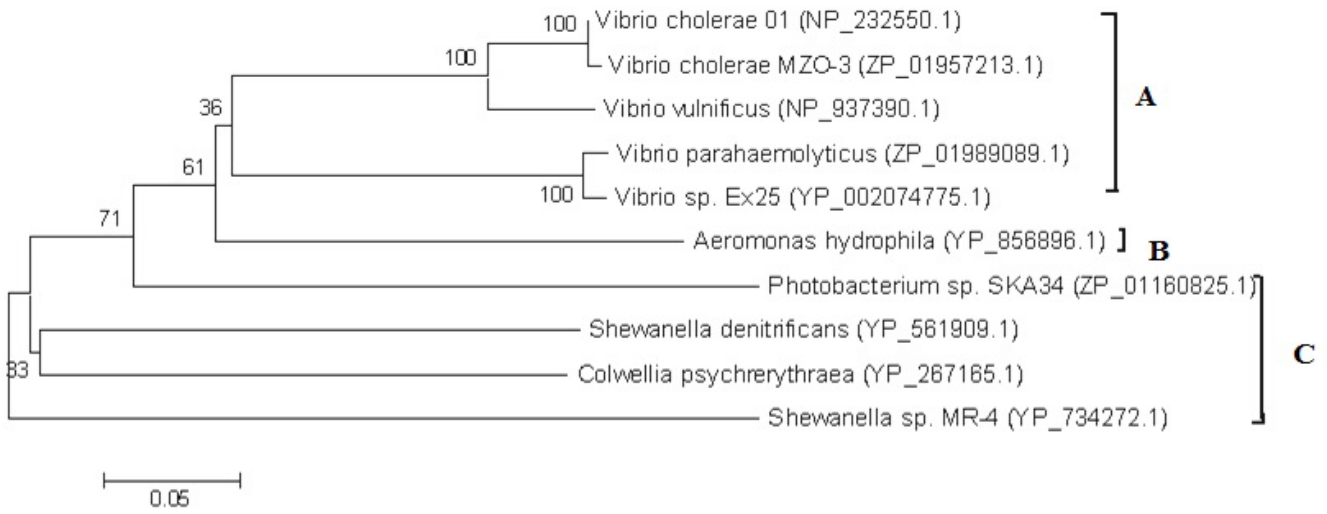

Figure 6: Unrooted phylogenetic tree based on peptide deformylase of *A. hydrophila* and other related bacteria showing the homology. Bar 0.05 represents amino acid changes per site.

Conclusions

We have built a 3-D model for peptide deformylase of *A. hydrophila* based on a known 3-D crystal structure. It was used for the screening of effective and potent drugs using a molecular docking approach and it was found that BB-3497, actinonin, and BBS-02 show a strong binding affinity towards PDF. It indicates that it can be further used for the control of *A. hydrophila* without further misuse or superfluous use of drugs to help avoid multidrug resistance. Phylogeny provides a better insight into the understanding of the evolutionary relationship and can be useful for the control of other bacteria with the same drug towards the control of infections. A need arises to use those drugs for *in vitro* test against *A. hydrophila* to validate efficacy and dose for future use against this important pathogen.

References

1. Janda JM, Abbott SL, Cheung WK, Hanson DF (1994) Biochemical identification of citrobacteria in the clinical laboratory. J Clin Microbiol 32:1850-1854.
2. Austin B, Austin DA (1999) Bacterial fish pathogens: Diseases in farmed and wild fish. Praxis Publishing, Chichester, UK.
3. Pemberton JM, Kidd SP, Schmidt R (1997) Secreted enzymes of Aeromonas. FEMS Microbiol Lett 152: 1-10.
4. Lan X, Zhang X, Hu J, Shimosaka M (2006) Cloning, expression and characterization of a chitinase from the chitinolytic bacterium *Aeromonas hydrophila* strain SUWA-9. Biosci Biotechnol Biochem 70: 2437-2442.
5. Lan X, Zhang X, Kodaira R, Zhou Z, Shimosaka M (2008) Gene cloning, expression and characterization of a second beta-N-acetylglucosaminidase from the chitinolytic bacterium *Aeromonas hydrophila* strain SUWA-9. Biosci Biotechnol Biochem 72: 492-498.
6. Chopra AK, Houston CW, Peterson JW, Jin GF (1993) Cloning, expression and sequence analysis of a cytolytic enterotoxin gene from *Aeromonas hydrophila*. Canadian J Microbiol 39: 513-523.
7. Howard SP, Buckley JT (1985) Activation of the hole- forming toxin aerolysin by extracellular processing. J Bacteriol 163: 336-340.
8. Howard SP, Garland WJ, Green JM, Buckley JT (1987) Nucleotide sequences of the gene for the hole forming toxin aerolysin of *Aeromonas hydrophila*. J Bacteriol 169: 2869-2871.
9. Singh V, Rathore G, Kapoor D, Mishra BN, Lakra WS (2008) Detection of aerolysin gene in *Aeromonas hydrophila* isolated from fish and pond water. Ind J Microbiol 48: 453-458.
10. Singh V, Somvanshi P, Rathore G, Kapoor D, Mishra BN (2009) Gene cloning, expression and homology modeling of hemolysin gene from *Aeromonas hydrophila*. Protein Expr Purif 65:1-7.
11. Pridgeon JW, Klesius PH (2011) Molecular identification and virulence of three *Aeromonas hydrophila* isolates cultured from infected channel catfish during a disease outbreak in west Alabama USA, in 2009. Dis Aquat Organ 94: 249-253.
12. Sahoo PK, Swaminathan TR, Abraham TJ, Kumar R, Pattanayak S, et al. (2016) Detection of goldfish haematopoietic necrosis herpes virus (Cyprinid herpesvirus-2) with multi-drug resistant *Aeromonas hydrophila* infection in goldfish: First evidence of any viral disease outbreak in ornamental freshwater aquaculture farms in India. Acta Tropica 161: 8-17.
13. Baloda SB, Krovacek K, Eriksson L, Linne T, Mansson I (1995) Detection of aerolysin gene in Aeromonas strains isolated from drinking water, fish and foods by the polymerase chain reaction. Comp Immunol Microbiol Infect Dis 18:17-26.
14. Wang G, Clark CG, Liu C, Pucknell C, Munro CK, et al. (2003) Detection and characterization of the hemolysin genes in *Aeromonas hydrophila* and *Aeromonas sobria* by multiplex PCR. J Clin Microbiol 41: 1048-1054.
15. Aguilera-Arreola MG, Hernandez-Rodriguez C, Zuniga G, Figueras MJ, Garduno RA, et al. (2007) Virulence potential and genetic diversity of *Aeromonas caviae*, *Aeromonas veronii,* and *Aeromonas hydrophila* clinical isolates from Mexico and Spain: A comparative study. Canadian J Microbiol 53:877-887.
16. Singh V, Rathore G, Kumar G, Swaminathan TR, Sood N, et al. (2007) Detection of the hole forming toxin hemolysin gene of *Aeromonas hydrophila* isolates. Ind Veter J 84: 900-902.
17. Singh V, Somvanshi P, Rathore G, Kapoor D, Mishra BN (2010) Gene Cloning, expression and characterization of recombinant aerolysin from *Aeromonas hydrophila*. Appl Biochem Biotechnol 160: 1985-1991.
18. Singh V, Mani I, Chaudhary DK, Somvanshi P (2011) Molecular detection and cloning of thermostable hemolysin gene from *Aeromonas hydrophila*. Mol Biol 45: 551-560.
19. Nam IY, Joh K (2007) Rapid detection of virulence factors of *Aeromonas* isolated from a trout farm by hexaplex-PCR. J Microbiol 45: 297-304.
20. Neu HC (1992) The crisis in antibiotic resistance. Science 257: 1064-1073.
21. Smith I (2003) Mycobacterium tuberculosis pathogenesis and molecular determinants of virulence. Clin Microbiol Rev 16: 463-496.
22. Kohanski MA, DePristo MA, Collins JJ (2010) Sublethal antibiotic treatment leads to multidrug resistance via radical-induced mutagenesis. Mol Cell 37: 311-320.

23. McNicol AL, Aziz MK, Huq I, Kaper BJ, Lockman AH, et al. (1980) Isolation of drug resistant *Aeromonas hydrophila* from aquatic environments. Antimicrob Agent Chemother 17: 477-483.

24. Nagar V, Shashidhar R, Bandekar JR (2011) Prevalence, characterization, and antimicrobial resistance of Aeromonas strains from various retail food products in Mumbai, India. J Food Sci 76: M486-M492.

25. Leitao MFDF, Silveira NFDA (1991) *Aeromonas* spp. and *Plesiomonas shigelloides* in water, seafood, freshwater fish and vegetables in Sao Paulo State, Brazil. Coletânea do Instituto de Técnologia de Alimentos 21: 90-99.

26. Rathore G, Singh V, Kumar G, Swaminathan TR, Mahanta PC (2006) Antibiotic sensitivity and characterization of *Aeromonas hydrophila* isolated from fish and water samples. J Ecophysiol Occup Health 6:41-43.

27. Nordmann P, Dortet L, Poirel L (2012) Carbapenem resistance in Enterobacteriaceae: Here is the storm. Trend Mol Med 18: 263-272.

28. Bugg TD, Braddick D, Dowson CG, Roper DI (2011) Bacterial cell wall assembly: Still an attractive antibacterial target. Trend Biotechnol 29: 167-173.

29. Seshadri R, Joseph SW, Chopra AK, Sha J, Shaw J, et al. (2006) Genome sequence of *Aeromonas hydrophila* ATCC 7966: Jack of All Trades. J Bacteriol 188: 8272-8282.

30. Fazil MH, Kumar S, Subbarao N, Pandey HP, Singh DV (2010) Homology modelling of a sensor histidine kinase from *Aeromonas hydrophila*. J Mol Mod 16: 1003-1009.

31. Singh V, Chaudhary DK, Mani I (2012) Gene network analysis of *Aeromonas hydrophila* for novel drug target discovery. Syst Synth Biol 6: 23-30.

32. Huntington KM, Yi T, Wei Y, Pei D (2000) Synthesis and antibacterial activity of peptide deformylase inhibitors. Biochemistry 39: 4543-4551.

33. Jain R, Chen D, White RJ, Patel DV, Yuan Z (2005) Bacterial peptide deformylase inhibitors: A new class of antimicrobials. Curr Med Chem 12: 1607-1621.

34. Jones RN, Sader HS, Fritsche TR (2005) Antimicrobial activity of LBM415 (NVP PDF-713) tested against pathogenic *Neisseria* spp. (*Neisseria gonorrhoeae* and *Neisseria meningitides*). Diagn Microbiol Infect Dis 51: 139-141.

35. Chen DZ1, Patel DV, Hackbarth CJ, Wang W, Dreyer G, et al. (2000) Actinonin, a naturally occurring antibacterial agent, is a potent deformylase inhibitor. Biochemistry 39: 1256-1262.

36. Clements JM, Beckett RP, Brown A, Catlin G, Lobell M, et al. (2001) Antibiotic activity and characterization of BB-3497, a novel peptide deformylase inhibitor. Antimicrob Agent Chemother 45: 563-570.

37. Sali A, Blundell TL (1993) Comparative protein modeling by satisfaction of spatial restrains. J Mol Biol 234: 779-815.

38. Singh V, Somvanshi P (2009) Inhibition of oligomerization of aerolysin from *Aeromonas hydrophila*: Homology modeling and docking approach for exploration of hemorrhagic septicemia. Lett Drug Des Discov 6: 215-223.

39. Yanez MA, Catalan V, Apraiz D, Figueras MJ, Martinez-Murcia AJ (2003) Phylogenetic analysis of members of the genus Aeromonas based on gryB gene sequences. Int J Syst Evol Microbiol 53: 875-883.

40. Soler L, Yáñez MA, Chacon MR, Aguilera-Arreola MG, Catalán V, et al. (2004) Phylogenetic analysis of the genus Aeromonas based on two housekeeping genes. Int J Syst Evol Microbiol 54: 1511-1519.

41. Altschul SF, Thomas LM, Alejandro AS, Jinghui Z, Zheng Z, et al. (1997) Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acid Res 25: 3389-3402.

42. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTALX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25: 4876-4882.

43. DeLano WL (2002) The PyMOL molecular graphics system. DeLano Scientific, Palo Alto, CA.

44. Laskowski RA, MacArthur MW, Moss DS, Thornton JM (1993) A program to check the stereochemical quality of protein structure. J Appl Crystallogr 26: 283-291.

45. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, et al. (1998) Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem 19:16-39.

46. Singh V, Mani I, Chaudhary DK (2013) *ATP4A* gene regulatory network for fine-tuning of proton pump and ion channels. Syst Synth Biol 7:23-32.

47. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 24: 1596-1599.

48. Din JN, Newby DE, Flapan AD (2004) Omega 3 fatty acids and cardiovascular disease-fishing for a natural treatment. Br Med J 328: 30-35.

49. Singh V, Chaudhary DK, Mani I, Jain R, Mishra BN (2013) Development of diagnostic and vaccine markers through cloning, expression and regulation of putative virulence proteins encoding genes of *Aeromonas hydrophila*. J Microbiol 51: 275-282.

50. Karl TR, Knight RW, Plummer N (1995) Trends in high-frequency climate variability in the twentieth century. Nature 377: 217-220.

51. Carr RM, Blumenthal UJ, Mara DD (2004) Guidelines for the safe use of wastewater in agriculture: Revisiting WHO guidelines. Water Science and Technology 50: 31-38.

52. Jiang X, Zhang C, Zhao Y, Kong X, Pei C, et al. (2016) Immune effects of the vaccine of live attenuated *Aeromonas hydrophila* screened by rifampicin on common carp (*Cyprinus carpio* L). Vaccine 34: 3087-3092.

53. Guay DR (2007) Drug forecast - the peptide deformylase inhibitors as antibacterial agents. Thera Clin Risk Manage 3: 513-525.

54. Duroc Y, Giglione C, Meinnel T (2009) Mutations in three distinct loci cause resistance to peptide deformylase inhibitors in *Bacillus subtilis*. Antimicrob Agent Chemother 53: 1673-1678.

55. Smith HK, Beckett RP, Clements JM, Doel S, East SP, et al. (2002). Structure-activity relationships of the peptide deformylase inhibitor BB-3497: Modification of the metal binding group. Bioorgan Med Chem Lett 12: 3595-3599.

56. Singh V, Somvanshi P (2009) Homology modeling of 3-oxoacyl-acyl carrier protein synthase II (KAS II) from Mycobacterium tuberculosis H37Rv and molecular docking for exploration of drugs. J Mol Modeling 15: 453-460.

57. Sharma A, Sharma S, Khuller GK, Kanwar AJ (2009) *In vitro* and *ex vivo* activity of peptide deformylase inhibitors against *Mycobacterium tuberculosis* H37Rv. Int J Antimicrob Agents 34: 226-230.

58. Singh V, Somvanshi P (2009) Targeting the peptide deformylase of *Mycobacterium tuberculosis* leads to drug discovery. Lett Drug Des Discov 6: 487-493.

59. Martínez-Murcia A, Monera A, Alperi A, Figueras MJ, Saavedra MJ (2009) Phylogenetic evidence suggests that strains of *Aeromonas hydrophila* subsp. dhakensis belong to the species *Aeromonas aquariorum* sp. nov. Curr Microbiol 58(1): 76-80.

60. Küpfer M, Kuhnert P, Korczak BM, Peduzzi R, Demarta A (2006) Genetic relationships of Aeromonas strains inferred from 16S rRNA, gyrB and rpoB gene sequences. Int J Syst Evol Microbiol 56: 2743-2751.