

Construction of 3D Model of Protein Drug Targets for Renibacterium Salmoninarum - A Bacterial Pathogen Causing Bacterial Kidney Disease in Young Salmonid Fish

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The aim of this study is to construct 3D models of potential drug targets for the Bacterial Kidney Disease (BKD) causing pathogen *Renibacterium salmoninarum*. The bacterial pathogen *Renibacterium salmoninarum* was selected for homology modeling studies since there were no known protein structures of the organism reported in the NCBI database. The reported protein sequences were run through DrugBank to pick out drug-targets. Online databases and web tools such as PMDB, UniProt, Drug Bank, and SwissModel were employed in this analysis. An aggregate of 412 protein sequences were identified as potential drug targets and were retrieved from the UniProt. Homology models of the protein sequences were constructed using the SwissModel database for all 412 proteins. These were then refined through a protein blast and Ramachandran plot analysis. Out of the 412 constructed models, 143 models were of reliable quality. These were then submitted to the PMDB database for further reference. To demonstrate the application of these constructed models, protein-ligand docking analysis using Auto Dock Vina was performed. Among the antibiotics that were tested against their known drug targets, trimethoprim demonstrated significant potential for the inhibition of *R. salmoninarum*'s dihydrofolate reductase protein, with a binding energy of -9.06 Kcal/mol and with the formation of 3 hydrogen bonds. Therefore through protein-ligand docking studies and the construction of 3D models of protein drug targets, Trimethoprim is proposed as a solution to the Bacterial Kidney Disease (BKD) problem in salmonid fishes. Further in-vitro evidences are in demand to prove this hypothesis.

Keywords: Bacterial Kidney Disease; Homology Modelling; Protein Drug Targets; Protein Model Database; *Renibacterium salmoninarum*.

Salmon is the common name used to refer to the various species of the Salmonidae family. There are nine commercially important species of salmon and these are widespread across the world. Most are found in the Pacific and Atlantic oceans, contributing significantly to the economic and cultural value of the nations situated near these oceans. For example, *Rawas*, also known as the Indian salmon, the only species that can be found

in India, (and primarily spotted in the waters of the Indian states of Gujarat and Maharashtra) is a huge contributor to the income generated here. In 2010, the harvesting, processing, and retailing of the Bristol Bay Salmon (*Oncorhynchus kisutch*) created \$1.5 billion in sales value across the United States.¹ Norway is thought to be the largest contributor to the Atlantic Salmon Industry. The country accounted for a total of 63% (2.3 million

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tonnes) of the total production of Salmon in 2016.

Farmed salmon is now a global commodity which has seen an exponential rise in various markets.² Salmon aquaculture has expanded rapidly within the last two decades. It is predicted that salmon aquaculture will continue to expand in order to meet growing seafood demand, since wild capture fisheries have stagnated in terms of production. In Norway, this is a government backed industry with high levels of innovation.³ The production of the farmed species of salmon is restricted to a few countries like Norway, Chile, the UK and Canada. Nevertheless, this contributes a whopping 85% to worldwide production. Salmon aquaculture is responsible for 15% of the United Kingdom's agricultural economy.

Salmon fishes are a favourite with culinary chefs, and are liked for their orange-coloured meat. The meat is believed to have several health benefits, including reducing obesity, making it extremely valuable as seafood.

Renibacterium salmoninarum is responsible for causing disease in young Salmonid fish. The infection is commonly known as Bacterial Kidney Disease (BKD), Dee Disease, White Boil Disease or the Corynebacterial Kidney Disease. It is of substantial ecological importance due to its effect on both farmed and wild Salmonids. It was first described under the name of Dee disease (1930), and was identified in the Atlantic salmon (*Salmo salar*) species in Scotland. A gram positive diplobacillus bacterium that did not grow on any available media was recognized in the kidneys of the diseased fish. This pathogen was found to be exclusive to Salmonids. Ordal and Earp initially cultured the bacteria and identified it as a species of *Corynebacterium* based on its morphological appearance. Smith concluded that the Dee disease of salmonids in Scotland and the Bacterial Kidney Disease were caused by the same bacterium.^{4,5}

Government agencies for statutory fish health programmes in the UK (FRS Marine Laboratory, Aberdeen and Cefas, Weymouth) participated in a comparative study for the detection of *Renibacterium salmoninarum*. FRS conducted additional tests - Indirect fluorescent antibody (IFAT), Gram staining of tissue sections and H&E, a quantitative real-time PCR (qPCR) assay, to detect the elongation factor alpha 1 (ELF) gene of salmonids as well as the *Msa2* genes of *R.*

salmoninarum. Isolation using Mueller Hinton with added cysteine (MHCA) resulted in the fish testing to be culture positive.⁶

Renibacterium salmoninarum is an intracellular pathogen which multiplies within phagocytes. It is slow growing and is described as a coherent genus. The properties associated with the cell envelope demonstrate the intracellular survival and multiplication characteristics in phagocytic cells.

The cell surface hydrophobicity of *R. salmoninarum* strains, examined using a salt aggregation method showed the strains to be sticky, auto-agglutinating, and were found to possess a hydrophobic cell surface. Strains with low virulence were found to be non-agglutinating and non-sticky. The adherence of the bacteria to host tissues plays a significant role in their ability to colonize and cause infection.⁷

Renibacterium salmoninarum is a sessile, strongly Gram-positive, non acid fast, non spore forming, rod shaped bacterium that usually occurs in pairs (diplococcus). It is approximately 0.3-1.0 $\mu\text{m} \times$ 1.0-1.5 μm in length.⁸ A slow growing organism, it is one of the earliest known bacterial pathogens of fish (especially salmonids). It is a facultative intracellular parasite, and because of this intracellular nature of infection, the bacterium has the ability to evade the immune response of the host. This makes BKD an especially challenging disease to control.⁹

The cell wall of *R. salmoninarum* is composed primarily of peptidoglycan. The major sugar component is galactose, in addition to N-acetylfucosamine, rhamnose and N-acetylglucosamine. Major amino acids present are alanine, glutamic acid, lysine, and glycine, where the third position of the peptide subunit of the peptidoglycan contains lysine. There is an interpeptide bridge between the lysine and the D-alanine of adjacent peptide subunits that is composed of glycylalanine.

R. salmoninarum is very similar to the Coryneform group of bacteria.^{8,10} The bacterium lives inside the pronephric kidneys of salmonid fish. It spreads in two ways - vertically inside the ova, and horizontally in shared water between cohabiting fish. A general consensus is that *R. salmoninarum* has co-evolved along with its host, the salmonids.^{10,11} Diagnostic tools for BKD are

PCRs, especially real time quantitative PCRs (RT qPCRs).¹¹

Control of infection of *R. salmoninarum* is an arduous process since there are no vaccines available that are sufficiently effective. Antibiotic resistance is also a significant complication with this pathogen.^{9,12}

In spite of being a potent pathogen causing significant economic damage to the fish industry, specifically to salmon and trout⁸, very little information is available about this bacterial pathogen. It has been studied very little mainly due to the punctilious and slow growing nature of the disease. This makes researching *R. salmoninarum* difficult. A more thorough study of *R. salmoninarum* remains necessary to combat the evasive Bacterial Kidney Disease.

Homology Modelling is a computational technique that predicts the structure of a protein sequence by comparing it to the structure of a homologous protein sequence, on the basis of degree of similarity. In this study, the 3D structures of the proteins of *Renibacterium salmoninarum* were developed using the technique of homology modelling. The protein structures are essential in order to design and develop drugs that can be used to curb the spread of this disease. The lack of availability of protein structures has hindered the understanding of binding specificities of proteins and ligands, which are prerequisites for drug design and development^{13,14}. Well Established and recognized databases and tools were utilized for computing structures of potential drug target proteins. This data is important to curb the spread of this disease and also reduce the financial burdens incurred on the fisheries and the pisciculture industry on account of this disease. Homology modelling was carried out using preexisting FASTA format sequences of the proteins involved, along with similar preexisting templates that closely resembled the query sequence, in order to predict the structure of the proteins. It is absolutely necessary for the protein sequences to be available in order to understand the binding specificities of the protein and ligand to yield higher amounts of drugs to curb the spread of the disease. The structures developed in this study can be further exploited to develop drugs, which will be a boon to the fisheries involved in the rearing of salmonoid fishes¹³.

MATERIALS AND METHODS

NCBI Database

The pre-existing information available on the organism was extracted from the NCBI database. The database compiles data about the organism from multiple databases (<https://www.ncbi.nlm.nih.gov/>).

Sequence Retrieval

The amino acid sequences required for this study were collected from the Uniprot Knowledge Database. The database is centralized, reliable and publicly accessible. The sequences with the best match were sorted based on the length of the amino acid chain. The required amino acid chain files were downloaded in the FASTA file format. The downloaded sequences were then saved with their respective accession IDs. This was for enabling easier accessibility in the future. The database can be accessed at: www.uniprot.org.

Sequence Alignment

The UNIPROT database enabled the compilation of amino acid sequences. The sequences were compared using the BLASTp server. This permits the comparison of the query sequence (also known as the amino acid sequence) with pre-existing protein sequences in the Protein Data Bank (for gauging percentage similarity). The first BLAST was performed for getting values similar to that of the pre-existing sequences of the non-redundant database. This was followed by a second BLAST which was conducted to obtain similar values with the sequences present in the Protein Data Bank (www.rcsb.org). The percentage of similarity was documented for further reference.

Structure Prediction

The Homology modelling technique was performed using the SWISS MODEL tool (<https://swissmodel.expasy.org/>). This online tool helped predict the three-dimensional structures of the selected drug-target proteins. The respective amino acid sequences of the proteins, as well as the templates available in the protein data bank were used to predict the 3D structure of the protein. The sequences obtained from UNIPROT were uploaded as FASTA files and the output (3D structures) were stored in PDB format. The availability and percentage similarity of the templates were the important factors that influenced the developed models. Ramachandran plots were used to analyze

the structures. These are graphical plots that help in confirming the predicted structure's accuracy. The most accurate of models were downloaded in PDB format.

Model Analysis

Qualitative analysis of the accuracy of the predicted model was conducted using the built-in Ramachandran plot feature in SWISSMODEL. It was expected that the degree angles of all the residues would be found within the Most Favored regions of the Ramachandran plot, as this is what determines the quality of the predicted structure. The residues that were observed outside of this favored region were considered to be unfavorable. These outliers affected the confidence score of the predicted model.

Model Submission

The 3D models of the proteins that exhibited good quality Ramachandran plot (confidence score) were submitted to the Protein Model Database (PMDB) (<http://srv00.recas.ba.infn.it/PMDB/>). The Protein Model Database is a resource that harbors manually built protein models that have been published in research based journals. All the models were uploaded in pdb file format and each entry was given a unique PMDB ID for future reference.

RESULTS AND DISCUSSION

Pathogen & Drug Target Selection

The pathogen *Renibacterium salmoninarum* was identified as a potential target for homology modeling studies since there were no known protein structures of the organism present in the NCBI database (although there were 1309 reported protein sequences available). Thus, *R. salmoninarum* was ideal for computational protein model creation. Among the 1309 protein sequences, non-enzymatic proteins, subunits as well as duplicates were eliminated, leaving a final count of 1249 proteins. These 1249 proteins were then verified on the DrugBank website (www.drugbank.ca) in order to check if these were potential drug targets. Among the 1249 proteins, a total of 412 protein sequences were identified as drug targets. Homology model construction was carried out for these 412 protein sequences. Many similar studies have seen a rise due to the coronavirus outbreak, homology modelling and molecular docking have been key in identifying drug targets and potential medicines. A homology modelling study of TMPRSS2(spike entry priming) was done using a similar pathway.

Constructing Homology Models

The 412 selected protein sequences were retrieved from the UniProt database (www.uniprot).

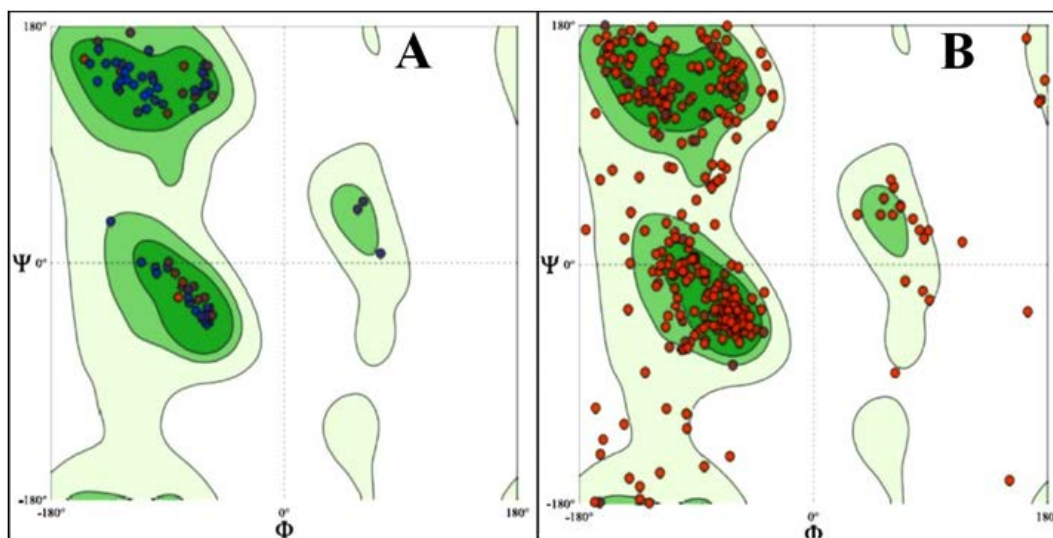


Fig.1. Ramachandran plot analysis for the created protein models. A: Most preferred protein model with 100% score in the favoured region; B: Lesser preferred protein model with 80% score in the favoured region

Table 1. PMDB entries for *Renibacterium salmoninarum*. The following drug targets and their respective UniProt Ids, the Ramachandran favoured region and the PMDB IDs have been used in this study to determine the potential drug targets for *R. salmoninarum*. The Ramachandran scores above 95% were considered to obtain the potential structure of proteins.

S. No.	Drug Target Name	UniProt ID	Ramachandran Favourable Region	PMDB ID
1	DHNA-CoA synthase	A9WLY2	95.96%	PM0083536
2	DXP reductoisomerase	A9WML8	95.49%	PM0083539
3	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase	A9WTJ3	96.76%	PM0083543
4	BPG-dependent PGAM	A9WSB8	97.00%	PM0083530
5	2,5-diketo-D-gluconic acid reductase	A9WQX7	95.27%	PM0083548
6	MECDP-synthase	A9WLY3	96.54%	PM0083556
7	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase	A9WLY2	96.32%	PM0083557
8	2-isopropylmalate synthase	A9WML8	95.80%	PM0083559
9	3-dehydroquinase	A9WTJ3	97.65%	PM0083560
10	3-dehydroquinase synthase	A9WSB8	95.34%	PM0083564
11	3-hydroxyisobutyryl-CoA hydrolase	A9WQX7	96.10%	PM0083565
12	Ketopantoate hydroxymethyltransferase	A9WS61	95.38%	PM0083567
13	HMBPP reductase	A9WPD7	96.38%	PM0083586
14	HTPA reductase	A9WQ18	96.28%	PM0083591
15	HTPA synthase	A9WNM3	95.76%	PM0083593
16	DMRL synthase	A9WR65	96.34%	PM0083596
17	6-phosphogluconate dehydrogenase	A9WV17	95.97%	PM0083597
18	Acetyl-CoA acetyltransferase	A9WSQ3	95.17%	PM0083598
19	Adenosylhomocysteinase	A9WU61	96.19%	PM0083599
20	Adenylate kinase	A9WST7	97.86%	PM0083600
21	Adenylosuccinate synthetase	A9WV79	96.69%	PM0083605
22	Aldo/keto reductase	A9WQQ7	96.21%	PM0083606
23	Alpha-methylacyl-CoA racemase	A9WST4	96.23%	PM0083608
24	Amino acid transporter	A9WNN1	95.42%	PM0083610
25	Ammonium transporter	A9WP34	95.55%	PM0083612
26	Anthranilate phosphoribosyltransferase	A9WR05	96.02%	PM0083614
27	Argininosuccinate synthase	A9WQ90	96.82%	PM0083616
28	Aspartate-semialdehyde dehydrogenase	A9WNNH0	95.58%	PM0083618
29	Aspartate—tRNA(Asp/Asn) ligase	A9WSD4	97.22%	PM0083620
30	ATP phosphoribosyltransferase	A9WR63	98.56%	PM0083622
31	ATP-dependent dethiobiotin synthetase	A9WL42	96.13%	PM0083623
32	ATP-dependent RNA helicase	A9WPJ4	95.90%	PM0083625
33	ATP-dependent RNA helicase	A9WNNH6	96.08%	PM0083629
34	Bacterial regulatory protein	A9WTC5	96.49%	PM0083631
35	Beta-thionase	A9WQJ7	95.36%	PM0083632
36	Bifunctional protein	A9WU39	96.16%	PM0083633
37	Bifunctional purine biosynthesis	A9WLT2	96.24%	PM0083635

	protein			
38	Branched-chain amino acid aminotransferase	A9WNW7	95.43%	PM0083637
39	Carbonic anhydrase	A9WME9	96.56%	PM0083642
40	Chaperone protein	A9WQP7	100.00%	PM0083705
41	Chitosanase	A9WUI6	96.17%	PM0083708
42	Chorismate mutase	A9WMQ5	100.00%	PM0083966
43	Chorismate synthase	A9WSC0	96.93%	PM0083710
44	Citrate synthase	A9WQB8	96.17%	PM0083890
45	Cystathionine gamma-lyase	A9WQJ8	96.05%	PM0083891
46	Cytidylate kinase	A9WRN3	97.27%	PM0083892
47	dCTP deaminase	A9WT08	97.33%	PM0083893
48	Delta-aminolevulinic acid dehydratase	A9WPH8	95.65%	PM0083955
49	Deoxyribodipyrimidine photolyase	A9WN66	97.86%	PM0083934
50	Deoxyribose-phosphate aldolase	A9WU17	97.02%	PM0083935
51	Diaminopimelate decarboxylase	A9WNA8	95.31%	PM0083936
52	Dihydrofolate reductase	A9WNH1	96.45%	PM0083938
53	Dihydroorotate dehydrogenase	A9WML3	95.38%	PM0083939
54	Dihydropteroate synthase	A9WUQ9	97.19%	PM0083940
55	DNA ligase	A9WTZ3	97.53%	PM0083941
56	DNA-3-methyladenine glycosylase	A9WPF0	97.71%	PM0083942
57	Enoyl-CoA hydratase	A9WVC8	96.19%	PM0083943
58	Farnesyl pyrophosphate synthetase	A9WRS8	95.31%	PM0083944
59	Fibrillar surface protein	A9WSZ8	95.33%	PM0083945
60	Fumarylacetoacetate	A9WLG1	95.00%	PM0083946
61	Gamma-glutamyltranspeptidase	A9WL74	99.38%	PM0083947
62	GDP-mannose 4,6 dehydratase	A9WN78	95.16%	PM0083948
63	Glucan endo-1,3-beta-glucosidase	A9WSK0	98.51%	PM0083949
64	Glucosamine-6-phosphate deaminase	A9WNE3	96.90%	PM0083950
65	Glucose-6-phosphate 1-dehydrogenase	A9WT42	95.49%	PM0083951
66	Glutamate racemase	A9WNP4	97.28%	PM0083952
67	Glutamate-1-semialdehyde aminotransferase	A9WPI0	96.02%	PM0083953
68	Glutamyl-tRNA synthetase	A9WP26	97.11%	PM0083954
69	D-fructose-6-phosphate amidotransferase	A9WMF4	95.19%	PM0083956
70	Glutaryl-CoA dehydrogenase (EC 1.3.8.6)	A9WQK3	96.55%	PM0083957
71	Glycerol kinase	A9WS93	96.39%	PM0083958
72	Glycerol-3-phosphate dehydrogenase	A9WMG6	95.28%	PM0083959
73	Glycine dehydrogenase	A9WME4	95.47%	PM0083960
74	Glutamine amidotransferase	A9WN01	95.46%	PM0083961
75	GTP cyclohydrolase 1	A9WUR0	96.47%	PM0083962
76	Guanylate kinase	A9WSA4	97.77%	PM0083963
77	Guanyl-specific ribonuclease	A9WLY7	100.00%	PM0083964
78	Haloacid dehalogenase-like hydrolase	A9WV91	95.93%	PM0083965
79	Histidine ammonia-lyase	A9WN95	96.09%	PM0083652
80	ATP-dependent DNA helicase	A9WSE4	97.76%	PM0083578
81	Homoserine O-acetyltransferase	A9WMJ0	95.67%	PM0083581
82	Hypoxanthine phosphoribosyltransferase	A9WUR5	96.87%	PM0083583
83	Lipoyl synthase	A9WS40	97.67%	PM0083585
84	L-serine dehydratase	A9WME8	95.68%	PM0083587
85	Lysine-tRNA ligase	A9WUP4	95.84%	PM0083590
86	Malate synthase	A9WLG6	96.88%	PM0083595
87	Malonyl-CoA-[acyl-carrier-protein] transacylase	A9WP65	95.36%	PM0083603
88	Methionine aminopeptidase	A9WM52	95.69%	PM0083607
89	Methionine-tRNA ligase	A9WP14	98.03%	PM0083617

90	Methylisocitrate lyase	A9WQB9	96.63%	PM0083626
91	Molybdopterin biosynthesis enzyme	A9WQ16	96.05%	PM0083653
92	NH(3)-dependent NAD(+) synthetase	A9WUC0	98.70%	PM0083654
93	Non-specific serine/threonine protein kinase	A9WTU4	97.76%	PM0083655
94	Nucleoside diphosphate kinase	A9WUV0	97.24%	PM0083656
95	Organic hydroperoxide resistance protein	A9WMD8	95.54%	PM0083657
96	Orotate phosphoribosyltransferase	A9WV95	96.33%	PM0083658
97	o-succinylbenzoate synthase	A9WRT7	95.71%	PM0083659
98	Pantothenate kinase	A9WMF5	96.41%	PM0083660
99	Phospho-2-dehydro-3-deoxyheptonate aldolase	A9WRF8	96.00%	PM0083661
100	Phosphoenolpyruvate carboxykinase	A9WL73	95.17%	PM0083662
101	Phosphoenolpyruvate-protein phosphotransferase	A9WUJ2	98.81%	PM0083663
102	Phosphoglycerate mutase family protein	A9WVN2	95.08%	PM0083664
103	Phosphopantetheine adenylyltransferase	A9WMZ3	96.08%	PM0083665
104	Phosphoribosylformylglycinamide cyclo-ligase	A9WQZ0	96.24%	PM0083666
105	Phosphoribosylglycinamide formyltransferase	A9WLR9	95.97%	PM0083667
106	Predicted secreted protein	A9WPE9	100.00%	PM0083668
107	Probable nicotinate-nucleotide adenylyltransferase	A9WUT8	96.83%	PM0083669
108	Recombinase A	A9WQ43	95.40%	PM0083969
109	Purine nucleoside phosphorylase	A9WU15	95.91%	PM0083970
110	Putative quinone oxidoreductase	A9WLL0	95.49%	PM0083971
111	Pyridoxamine 5'-phosphate oxidase	A9WQ92	95.89%	PM0083974
112	Pyruvate kinase	A9WR48	95.51%	PM0083973
113	Quinone oxidoreductase	A9WQ80	96.91%	PM0083975
114	Regulatory protein	A9WQ45	100.00%	PM0083976
115	Ribokinase	A9WUQ1	95.65%	PM0083977
116	Ribose 5-phosphate isomerase	A9WUW8	97.70%	PM0083978
117	Ribulose-phosphate 3-epimerase	A9WR72	95.39%	PM0083979
118	S-adenosylmethionine synthase	A9WSA1	96.28%	PM0083980
119	Serine hydroxymethyltransferase	A9WM39	96.39%	PM0083981
120	Serine-tRNA ligase	A9WUS1	97.40%	PM0083982
122	Signal recognition particle receptor	A9WP32	95.72%	PM0083983
123	Sodium/proline transporter	A9WL16	96.94%	PM0083984
124	Spermidine synthase	A9WUF9	96.15%	PM0083985
125	SURF1-like protein	A9WSP5	100.00%	PM0083986
126	Thioredoxin peroxidase	A9WSN6	98.65%	PM0083987
127	Thiosulfate sulfurtransferase	A9WPZ8	96.15%	PM0083988
128	Thymidine phosphorylase	A9WKS1	97.85%	PM0083989
129	Thymidylate synthase	A9WNH2	96.12%	PM0084073
130	Transaldolase	A9WT40	97.24%	PM0083990
131	Transcriptional regulator	A9WT73	96.32%	PM0084022
132	Transcriptional regulator	A9WMH1	95.85%	PM0084021
133	Tryptophan 2,3-dioxygenase	A9WT09	98.70%	PM0084023
134	Tryptophan synthase alpha chain	A9WR53	96.92%	PM0084024
135	Tyrosine-tRNA ligase	A9WQH9	96.14%	PM0084062
136	UDP-galactopyranose mutase	A9WMM1	95.45%	PM0084063
137	UDP-galactose 4-epimerase	A9WN76	95.46%	PM0084064

138	UDP-N-acetylglucosamine 2-epimerase	A9WMK3	96.44%	PM0084065
139	Undecaprenyl-PP-MurNAc-pentapeptide-UDPGlcNAc GlcNAc transferase	A9WRD8	95.40%	PM0084066
140	UDP-N-acetylmuramate—L-alanine ligase	A9WRD7	95.82%	PM0084067
141	Uracil-DNA glycosylase	A9WM93	96.93%	PM0084068
142	Urocanate hydratase	A9WN96	95.44%	PM0084069

org) and were stored in the FASTA file format (.fasta). These sequences were then subjected to a BLASTp (also called as Protein Blast) (<https://www.blast.ncbi.nlm.nih.gov/>) to find within the PDB website (www.rcsb.org), suitable templates for homology modelling for the protein structures that had more than 80% similarity match. All of the 412 sequences showed a match of greater than 80% with the available crystal structures in the PDB website. Therefore, all the 412 sequences were subjected to computational homology modelling using SWISS Model (<https://swissmodel.expasy.org/>). The tool created different models for each protein sequence. Among the created protein sequences modelled, the optimal model was selected using the Ramachandran plot analysis.

Ramachandran Plot Analysis

Ramachandran plot analysis was carried out on all the protein sequence models constructed using SWISSModel, via inbuilt options in the website. The protein models which portrayed more than 95% of residues in the favoured regions of the Ramachandran plot were considered to be eligible for structural application. Among the 412 constructed proteins, 143 protein models contained more than 95% of residues in the Ramachandran favoured region and were thus considered for further applications. Fig.1 shows the graphical representation of the most preferred protein model with 100% score in the favoured region and a least preferred model with 80% score within the favoured region.

PMDB ID Submission

Constructed protein models of all the 143 proteins were then submitted to a public database (PMDB) (<http://srv00.recas.ba.infn.it/PMDB/>) for public access. A catalogue of the constructed protein models that were submitted to the PMDB database is listed in Table.1. This allows researchers

working in this field of study to retrieve and execute further structural bioinformatics analyses specific to this pathogen.

Protein Ligand Docking

To demonstrate the application of the constructed homology models, protein-ligand study (to screen for an effective antibiotic against *R. salmoninarum*) was performed. Three known antibacterial drugs i.e., Isoniazid, Trimethoprim and Sulfadiazine, were subjected to protein ligand docking with their reported drug targets from *R. salmoninarum* (constructed in this study) as well as from other template organisms (as crystal structures from the PDB website, belonging to *Mycobacterium tuberculosis*, *Streptococcus pneumoniae*, & *Burkholderia cenocepacia* respectively).^{15,16} As shown in Table.2, the results of the docking study demonstrated that among the three antibiotics, trimethoprim exhibited the highest potential to be an effective inhibitor of the dihydrofolate reductase protein, with a binding energy of -9.06 Kcal/mol, while, Isoniazid exhibited an inconsequential binding energy of -5.36 Kcal/mol and sulfadiazine exhibited a binding energy of -6.65 Kcal/mol. The graphical representation of the protein-ligand interactions between the antibiotic (drug) and its protein (drug target) is shown in Fig.2. Among the three antibiotics, on account of it exhibiting a high binding energy of -9.06 Kcal/mol with the formation of 3 hydrogen bonds (Thr-84, Arg-85, Asn-136) and having 13 hydrophobic interactions, Trimethoprim may be proposed as a drug of choice against *R. salmoninarum* and the Bacterial Kidney Disease (BKD). The protein models built in this study could be applied to other docking studies as well as advanced molecular dynamic simulation studies for the purpose of recognition of effective antibiotic drugs against this specific pathogen/disease.

Table 2. Binding energies of the drug targets from *R. salmoninarum* and template organisms, docked against established antibiotic agents. Trimethoprim exhibited the highest potential and thus, is an effective inhibitor of the dihydrofolate reductase protein, with a binding energy of -9.06 Kcal/mol. Isoniazid exhibited an insignificant binding energy of -5.36 Kcal/mol and sulfadiazine exhibited a binding energy of -6.65 Kcal/mol

Antibiotic	Protein	Organism	Binding Energy (in Kcal/mol)
Isoniazid	Dihydrofolate reductase	<i>Mycobacterium tuberculosis</i>	-5.1
		<i>Renibacterium salmoninarum</i>	-5.36
Trimethoprim	Dihydrofolate reductase	<i>Streptococcus pneumoniae</i>	-8.01
		<i>Renibacterium salmoninarum</i>	-9.06
Sulfadiazine	Dihydropteroate synthetase	<i>Burkholderia cenocepacia</i>	-4.77
		<i>Renibacterium salmoninarum</i>	-6.65

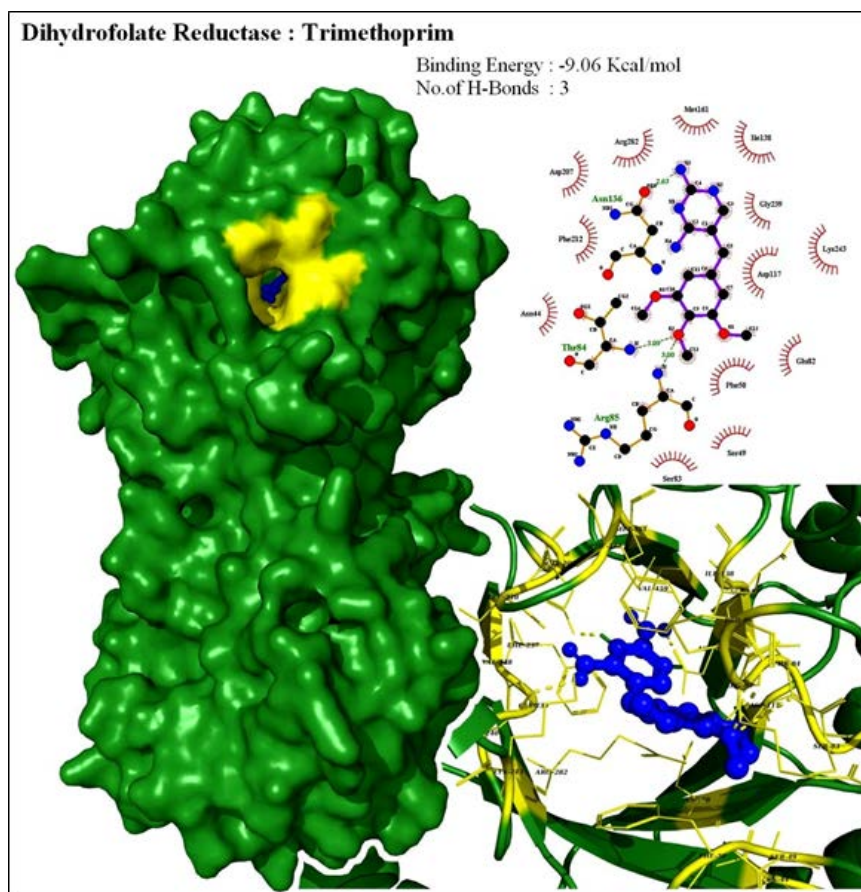


Fig. 2. Protein Ligand Docking of Trimethoprim with the constructed model protein (Dihydrofolate reductase), to understand the specificity of drug accuracy. It Represents the protein-ligand interactions between the antibiotic (drug) and its protein (drug target) There are 3 H-Bonds present and the binding energy is -9.06Kcal/mol

CONCLUSION

This study purported to build 3D computational protein structures of the under-exploited *R. salmoninarum* which is responsible for the Bacterial Kidney Disease (BKD) of the commercially viable Salmonid fishes. The results of the study also suggests that among the test antibiotics screened, since trimethoprim demonstrated significant affinity against its drug target, it can be a potent antibiotic effective against *R. salmoninarum*. This proposal can be investigated further via computational approaches such as MD simulation and/or in-vitro screening of antibiotics against the said pathogen.

The structures thus developed in this study can also be helpful in understanding the pathogenesis of this disease. These can also be used to lay out a roadmap for discerning the effect of inhibition of selective proteins. Those proteins might be efficient antibiotic drug targets, and they might lead to the construction of other antibiotic chemicals that may be better than currently available drugs. This docking study offers a novel grassroots level platform for eventual in-silico as well as wet lab work on *Renibacterium salmoninarum* specific drug discovery, development and design.

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Conflict Of Interest

The authors declare no conflict of interest.

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