

A computational proteomic study of Protease HtpX homolog in Polynucleobacter necessarius

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Abstract

Endodontic infections caused by endodontic bacteria such as primary apical periodontitis and posttreatment apical periodontitis are still higher even in the presence of advanced medical and healthcare systems. An extensive study should be done, including identification, mechanism of action, pathogenesis etc., so that therapeutic strategies can be developed. This study includes the study of physicochemical properties, evolutionary history, virulence, disorders and pathways of protease HtpX homolog in Polynucleobacter necessarius. This study may be helpful in better identification and effective drug design related to it.

Objectives

This study has the following objectives regarding interpreting properties and nature as well as mode of action and evolutionary significance of the metalloprotease:

1) To study the physicochemical properties of the concerned protein and its homologues.

To analyze the phylogenetic relationship among selected proteins.
In silico prediction and analysis of virulence of these proteins.

4) Prediction of protein disorders of these proteins.

5) Pathway analysis of the concerned protein.



Results and Discussions

The desired protein sequences were retrieved and saved.

Physicochemical characterisation: The physicochemical properties such as number of amino acids, charged molecular residues, weight, theoretical isoelectric point, atomic composition, aliphatic and instability index and GRAVY were explored. The number of amino acids, charged residues and atomic composition affect the protein structure and function. The number of amino acids ranged from 279 to 336. Similarly, 29905.90 to 36286.93 was the range of molecular weight. Theoretical isoelectric point ranged from 5.70 to 9.29. This indicates the selected proteins are slightly acidic to basic in nature. It is inversely related to solubility of proteins, which is an important factor in the biological environment. Aliphatic index had the range of 85.06 to 99. This is related to structural, thermal stability and hydrophobicity. Hence all the selected proteins are thermally stable. Similarly, instability index varied from 24.38 to 37.87. All the selected proteins are stable as they have instability index below 40. GRAVY value ranged from 0.122 to 0.266. This indicates all the selected proteins are hydrophobic and will reside in and interact with biological membranes.



Phylogenetic analysis: This analysis showed that *Polynucleobacter necessarius* was the ancestor organism and other related organisms were found to be more or less clustered together. Some organisms clustered together forming distinct clades. It may be possible due to extent of sequence similarities and less divergence in due course of evolution



Protein disorder prediction: The overall percentage of protein disorder ranged from 18.53 to 43.69. The lowest percentage of disorder included 53 disordered residues from 286 residues and the highest percentage of disorder included 128 disordered residues from 293 residues. Disordered regions lack a well-defined tertiary structure, but they provide functional flexibility. They can attach linkers and macromolecular complexes and increase the pathogenicity.

Pathway analysis: 3 pathways related to Protease HtpX homolog was interpreted. They are:

ATF6 (ATF6-alpha) activates chaperons: It is related to the maintenance of protein quality control. ATF6-alpha and HSP5 are endoplasmic two reticulum membrane protein which bind with unfolded proteins and convert them into folded. ATF6 was translocated into the Golgi due to endoplasmic reticulum stress where it was cleaved by MBTPS1 and MBTPS2 to an activated form. Then, it moved to the cytosol and then to the nucleoplasm, where it activated transcription of genes involved in unfolded protein response.



CLEC7A/inflammasome pathway: This pathway involves the formation of inflammasome which leads to inflammation. II1B activated by NFKB1 and RELA complex entered cytosol from into the the nucleoplasm. In the cytosol CASP8 bound with MALT1, and later PYCARD bound to this complex. Then, CASP8 was activated which cleaved IL1B to its active form. ASC then bound to the CASP8-complex and formed the inflammasome.

NPK81	16.3 B

ucleoplasm

Protein-protein interaction: def, Pnec_1775, Pnec_1774, Pnec_1773, Pnec_1772, ftsH, Pnec_1779, Pnec_1611 and grpE were the functional partners of htpX. Proteinprotein interaction plays a key role in predicting the protein function of target protein and drug ability of molecules.



Estimation of the per-site evolutionary rate among homologues: The result showed 19 residues as conserved & exposed and 38 residues as conserved & buried. It describes the extent to which amino acid residue is conserved. This conserved residue must have some functional significance due to which they are conserved in due course of evolution.

1	11	21	31	41
HPNPAKTAVL	MAAITALFIV	VGGMLGGEOG	MLMALLMAVG	MNFFSYWFSD
eeeeebeeb	ecebbeecee	• b • • • • • • • • •	ecebecebee	ebeebeeebb
ft ss				1 88
51	61	71	81	91
THVERMINAQ	OVDERSAPOF	YALVRELSEK	AGLEMEKVFL	IDEDAPNAFA
eebbebbeee f	ebeeeebeeb	eebbeebbee	ffe	Deeebbbbbbb
101	111	121	131	141
TGRNPDNASV	ATICVLEIL	SNRELRGVMA	RELARVENED	ILISAVAATN
bbeebeebbb	bbbebbeeeb	eeebbebbbb	bbbbbbeeeb	bbbbbbbbbb
sst s		8 8	ss s ffs	
151	161	171	181	191
AGAISALANF	AMFFGGRDSE	GRPNNPIASL	NVAILAPIAA	SLIQNSISRA
bbebeeebee	beeebeeeee	eeeeeebee	eeeebeebbb	ebbeebbeee
201	211	221	721	241
REYBADBOOA	RISSDPRAMA	BALBETBNYA	OGTPPOAVEO	HPETAONNIL
bbbebbebbb	ebbeebebbb	ebbeebeeeb	eeeebebbee	
		88 £		
251	261	271	281	
NPLTACGLAQ	LPSTHPPTEE	RVARENAK	NGEYPGAN	
ebbeeeeee	ebeebbebee	bbeebeebee	eeebeeee	
The conserva 123456 ariable Average • - An expon- b - A buries f - A predi- s - A predi- x - Insuffi	tion scale: 789 Conserved sed residue act d residue according cted functions cted structur. cient data -	coording to the ording to the al residue (h al residue (h the calculati	he NACSES algor NACSES algor ighly conserv on for this s	orithm. ithm. ad and exposed) ad and buried). ite was
perform	on less th	an 10% of the	sequences.	

Physicochemical properties described the nature of the protein while evolutionary history described the degree of relatedness among organisms. These results may be helpful in designing a drug that may become effective for the related ones. All the results particularly described the pathways that can be considered as a way to analyze more about the proteins involved in endodontic infection. A comprehensive study of endodontics and related therapeutics must be done. Molecular approaches and bioinformatics tools can be a

Virulence prediction of the selected proteins

Prediction of disorders of these proteins under study



Estimation of the per-site evolutionary rate among homologues

28	Paraburkholderia xenovorans Q147A2
	Pararobbsia alpina A0A6S7AVG2
19	Mycetohabitans rhizoxinica E5ANC4
30	Chitinasiproducens palmae A0A1H2PT13
35	Mycoavidus cysteinexigens A0A2Z6EYB5
43	Pandoraea thiooxydans A0A0G3EU62
27	Cupriavidus basilensis A0A0C4YF67
22	Thiomonas delicata A0A238D218
	Polynucleobacter necessarius B1XSN0
	Roseateles depolymerans A0A0U3LF72
	Paucibacter sp. A0A0U3F3Q5
37	Rhizobacter gummiphilus A0A1W6L3X0
41	Vitreoscilla filiformis A0A221KDX8
	Formivibrio citricus A0A1I4XYN4
	Sulfurimicrobium lacus A0A6F8V645
07	Parazoarcus communis A0A6F8V645
100	Aromatoleum tolulyticum A0A1N6W3S9

Virulence prediction: All the organisms were found to be nonvirulent. However, the virulence prediction was done on the basis of prediction scores. SVM The prediction score ranged from -1.092 to 0.940. The organism having higher prediction scores are considered as more pathogenic. But more comprehensive study with experimental validation should be done for evident conclusion.

MALTI CASPO PYCARD CASPS MALTI CASP8

Formation of apoptosome: It occurs

in the cytosol. CYCS attached to APAF1 in the presence of ATP. CYCS: APAF1 complex bound to inactivate CASP9 and activated it. Activated CASP9 activated CASP3, leading to apoptosis.



boost to the study.

References

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