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.
2) To analyze the phylogenetic relationship among selected proteins.
3) 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.

Materials and Methods

Retrieval of protein sequence

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Selection of homologous proteins

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Physicochemical characterisation of the selected proteins

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Phylogenetic analysis of the protein sequences

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Virulence prediction of the selected proteins

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Prediction of disorders of these proteins under study

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Pathway analysis of the concerned protein

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Protein-protein interactions of the concerned protein

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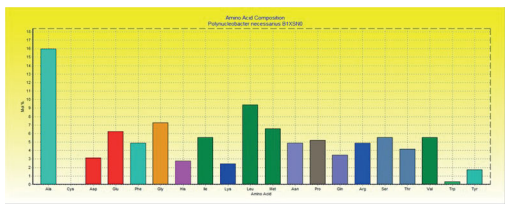
Estimation of the per-site evolutionary rate among homologues

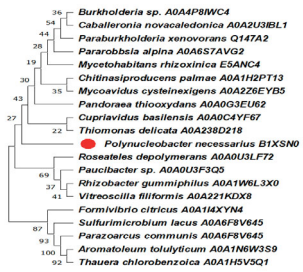
Results and Discussions

The desired protein sequences were retrieved and saved.

Physicochemical characterisation: The physicochemical properties such as number of amino acids, charged residues, molecular 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

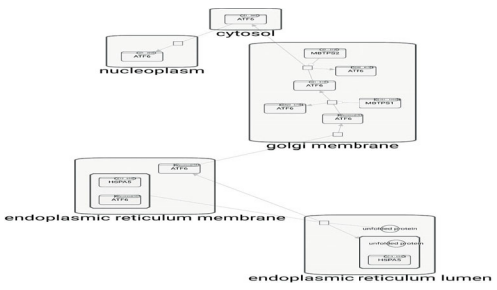


Virulence prediction: All the organisms were found to be non-virulent. However, the virulence prediction was done on the basis of SVM prediction scores. The prediction score ranged from -1.092 to 0.940. The organism having higher prediction scores are considered as more pathogenic. But more comprehensive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

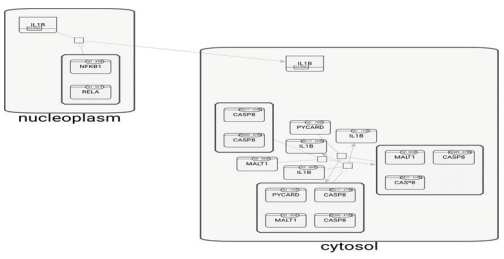
Protein disorder prediction: The overall percentage of protein disorder ranged from 18.53 to 43.69. The lowest percentage of disorder included 53 disordered residues from 286 residues and the highest percentage of disorder included 128 disordered residues from 293 residues. Disordered regions lack a 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:

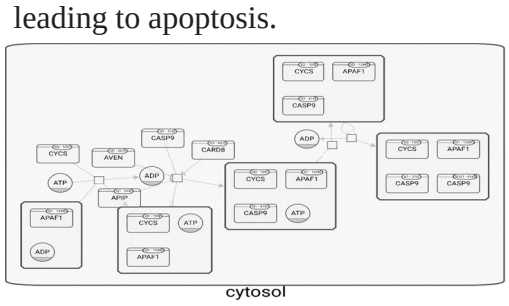
ATF6 (ATF6-alpha) activates chaperons: It is related to the maintenance of protein quality control. ATF6-alpha and HSP5 are two endoplasmic reticulum membrane protein which bind with unfolded proteins and convert them into folded. ATF6 was translocated into the Golgi due to endoplasmic reticulum stress 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.



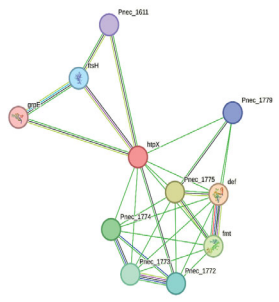
CLEC7A/inflammasome pathway: This pathway involves the formation of inflammasome which leads to inflammation. Il1B activated by NFKB1 and RELA complex entered into the cytosol from the nucleoplasm. In the cytosol CASP8 bound with MALT1, and later PYCARD bound to this complex. Then, CASP8 was activated which cleaved IL1B to its active form. ASC then bound to the CASP8-complex and formed the inflammasome.



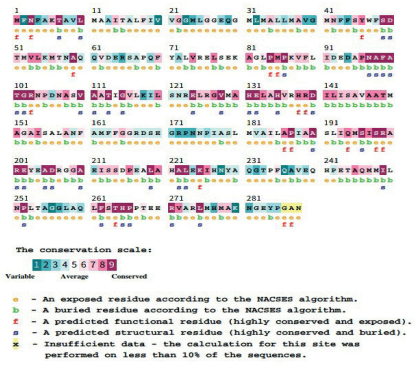
Formation of apoptosome: It occurs in the cytosol. CYCS attached to APAF1 in the presence of ATP. CYCS: APAF1 complex bound to inactivate CASP9 and activated it. Activated CASP9 activated CASP3, leading to apoptosis.



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. Protein-protein 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.



Conclusion

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 boost to the study.

References

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