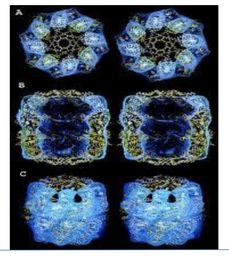

Proteomic analysis of stress proteins expressed in response to stress in haloarchaea

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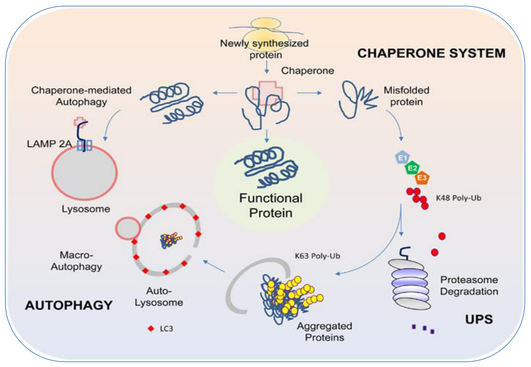
Introduction

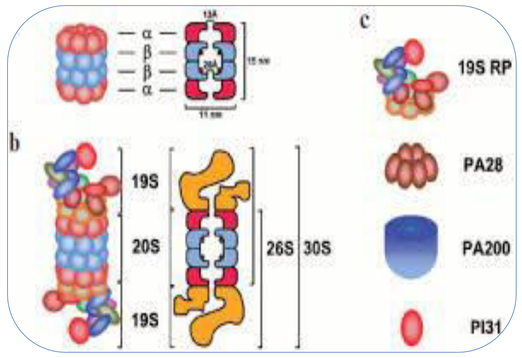
Halophilic archaea, the organisms living in extreme salt conditions have to face several challenges like temperature, chemical and salinity stresses.

These stress conditions induces production of certain stress proteins in them which work in association with molecular chaperonins and protect the cells from damage.

These proteins expressed in response to chemical stress are mainly from the Universal Proteasomal System (UPS) which helps in accumulation of abnormal proteins and maintains protein homeostasis in stress.



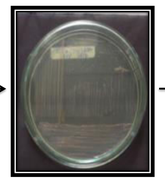
Interaction between Chaperones and Proteasomes in stress conditions and to maintain homeostasis (Lim and Zhang, 2013)



Structure of Proteasomal proteins (Sorokin *et al.,* 2009)

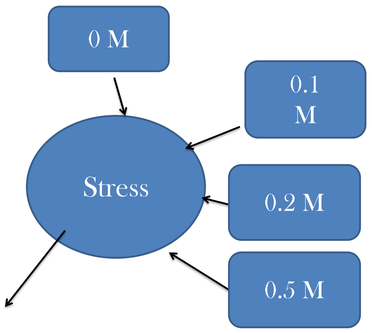
Materials and Methods

Ten haloarchaea were isolated from different sources like water, soil and rocks from shores of Western coast of Maharashtra, India.

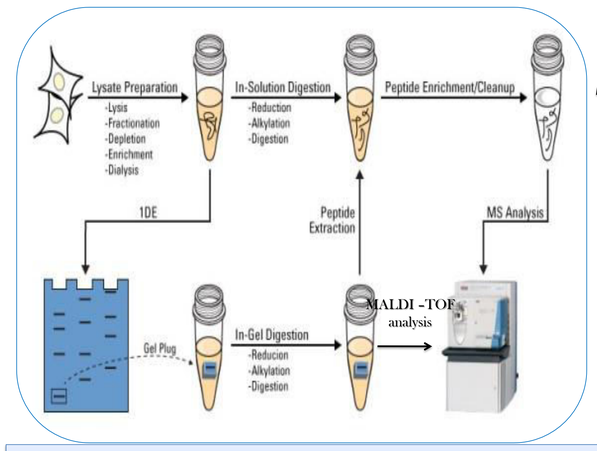


Identification of the organism using 16S rRNA gene sequencing and its morphological and biochemical studies

Growth of culture in SG medium and Enrichment of the ***H. argentinensis*** isolate on SG plate (with 15% NaCl)



Perchlorate stress to the ***Haloarcula argentinensis***

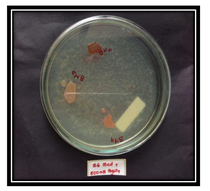


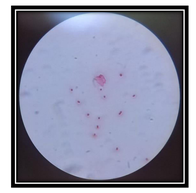
MASCOT analysis for protein identification

Peptide Extraction, SDS-PAGE and MALDI-TOF MS analysis of the stress proteins (Mass spectrometry sample preparation handbook)

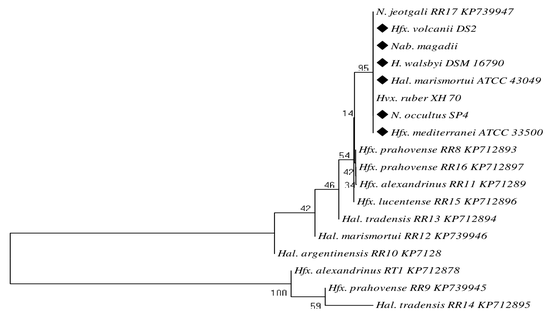
Results and Discussion

Isolation of haloarchaea: Haloarchaea was isolated and enriched on 15% SG medium as described by Digaskar *et al.,* 2015.





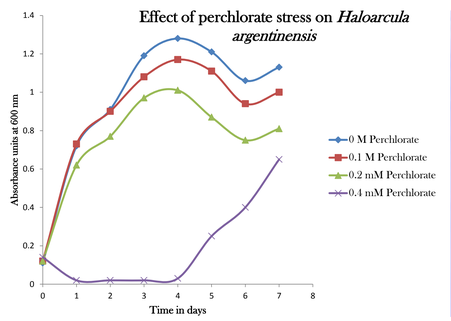
Isolation of an aerobic, orange red pigmented, gram negative isolate

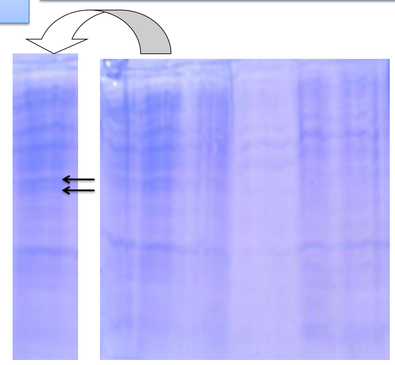


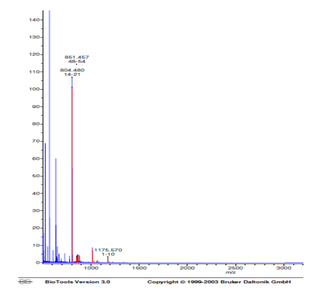
Phylogeny was drawn by MEGA 6 Neighbor Joining method.The evolutionary distances are in the units of the number of base substitutions per site. Phylogenetic analysis suggests that the isolated strain RR10 from sea water of Mumbai is ***Haloarcula argentinensis*** [Genbank Accession No. **KP712898**]

Effect of perchlorate stress on haloarchaea

SDS-PAGE and MALDI-TOF MS analysis of protein lysate of the ***Haloarcula argentinensis*** exposed to different temperature



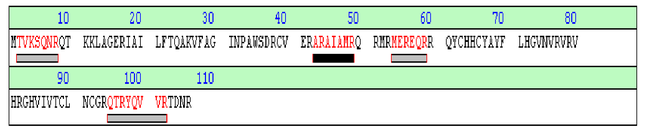




Band Description	Intensity coverage	Digest Match Score Mascot	Putative molecular weight	Putative pI Isoelectric point	Matched Sequence	Comments	Gene
Band 1 expressed in response to perchlorate stress to *Haloferax*	82 %	44	22.91kDA	8.7	Proteasome subunit beta 2 *OS=Sulfolobus solfataricus* (strain ATCC 35092 / DSM 1617 / JCM 11322 / P2) GN=psm	Three Threonine residues at site 15-17	*psm B*
Band 2 expressed in response to perchlorate stress to *Haloarcula*	83.0 %	45	22.9 kDA	8.1	Subunit beta of 20 S proteasome	Three Threonine residues at site 15-17	*psm B*

SDS -PAGE of the protein lysate of haloarchaea exposed to perchlorate stress (lane 5, 6: Perchlorate stress samples)

MALDI-TOF MS analysis of proteins expressed in response to stress.



Summary of the proteins expressed in *H. argentinensis* in response to chemical stress

Significance of perchlorate stress in Astrobiology

- Extreme environment persists on martian surface characterized due to presence of perchlorate, high salinity, high concentration of minerals like magnesium sulphate and radiation.
- Recent evidences suggest that tolerance to perchlorate, salt and magnesium would be a characteristic requirement for survival in martian soil. In the current investigation, we have studied the effect of perchlorate stress on haloarchaea.
- Results indicate that haloarchaea have the ability to tolerate multiple stress conditions of salinity, perchlorate and $MgSO_4$ concentrations that are a hallmark of Martian environment.
- The possibility of survival of haloarchaea in Martian conditions or the application of haloarchaea as 'models' for studying survival response during (Lithospermia) as well as the possibility of extreme halophilic life on Mars cannot be precluded.

Conclusion

Ten haloarchaea were isolated successfully from Western Coast of Maharashtra, Mumbai and Sindhudurg regions. They were subjected to various biochemical and morphological tests and then identified using 16S rRNA gene sequencing method. Wide range of diversity was seen in the isolated organisms. The organisms were subjected to chemical stress followed by the expression of stress proteins using SDS-PAGE and then its identification using MALDI-TOF MS MS analysis. The identified protein was a beta proteasomal subunit, which is a part of UPS that works in conjugation with UPS and chaperones. 2-DGE may be needed to study proteins expressed in response to stress in more details. The study suggests strong cooperative linkage between chaperones and UPS pathways in *Haloarcula* sps. Stress physiology studies can provide useful information about molecular mechanisms of repair and survival of haloarchaea in stress conditions.

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