Structural And Functional Sequence Analysis Of Amylase - Producing Halophilic Actinomycetes Using Bioinformatics Approaches

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Abstract

Halophilic actinomycetes are valuable sources of extremozymes with significant industrial potential due to their Sustain ability and function under high salinity and other harsh conditions. In this study, we conducted comprehensive bioinformatics-based structural and functional analysis on four amylase sequences from the halophilic actinomycetes Actinopolyspora erythraea, including α-glucosidase, glycosidase and two hypothetical proteins. We retrieved four amino acid sequences from the NCBI database. Sequences were analysed by using ProtParam to determine physicochemical properties associated with halophilic adaptation. Three-dimensional structures were predicted using SWISS-MODEL and validated through Global Model Quality Estimation (GMQE), QMEAN Z-score, MolProbity and Ramachandran plot to capture the novelty of amylases.

All analysed proteins exhibited hallmark features of halophilic enzymes, including low theoretical isoelectric points, enrichment of acidic amino acids, negative GRAVY values indicating enhanced hydrophilicity, and moderate to high aliphatic indices suggestive of thermostability. The α-glucosidase and glycosidase proteins demonstrated favourable stability indices and structural robustness, supporting their primary roles in carbohydrate metabolism under hypersaline conditions.

Overall, this study highlights key molecular determinants underlying halophilic adaptation in amylase-related proteins from Actinopolyspora erythraea and underscores the biotechnological potential of halophilic actinomycetes as promising sources of salt-tolerant enzymes for applications in the food, detergent, and pharmaceutical industries.

Keywords : Halophilic actinomycetes; Actinopolyspora erythraea; Amylases; Bioinformatics analysis; Protein structure prediction

Introduction

To date, millions of Actinomycetes species belong to order Actinomycetales have been discovered. The diversity of the species highlights along with their significance and biotechnological potential has captured the new attention among the researchers. Actinomycetes inhabit a diverse range of environments, including extreme habitats such as Antarctic soil, hot springs, and saline areas. Microorganisms that grow and survive at high salt concentrations (1–20% NaCl) are termed halophilic or halotolerant (Al-Tai and Ruan 1994). These organisms produce economically important amylases with diverse industrial applications, including starch processing, food and beverage production, textile desizing, detergents, pharmaceuticals, and bioethanol industries. (Pandey et al., 2000; Gupta et al., 2003; Souza & Magalhães, 2010).

Halophilic amylases exhibit higher acidic amino acid content and lower isoelectric point's than their non-halophilic counterparts. These acidic residues stabilise enzyme activity and contribute to structure and functional stability under high salt conditions. This bioinformatics study examines amylase production in halophilic actinomycetes through data mining of homologous features, unique traits, amino acid profiles, and

3D structures (Madern, Ebel and Zaccaï, 2000). In this study, we retrieved four amylases producing halophilic actinomycetes sequences of Actinopolyspora erythraea from the NCBI database and analysed using ProtParam and SWISS - MODEL. We observed distinctive primary structures; functional features, conserved motifs, and hydropathy profiles support adaptation to high salt and pH. Collectively these traits captured the potential of halophiles in the domain of the biotechnological, food, and pharmaceutical applications (Vaisht, and Bolhuis, 2005).

Materials and Methods

Retrieval of Amylase Sequencing

Four amylase producing sequences from Halophilic Actinomycetes were retrieved in FASTA format from the NCBI Protein database (Coordinators, 2015). The proteins analyzed included α-glucosidase (KGI81297.1), glycosidase (KGI81207.1), Hypothetical protein IL38_20700 (KGI79873.1) and Hypothetical protein IL38_20695 (KGI79872.1) all derived from Actinopolyspora erythraea.

Analysis of amino acid frequency among the selected actinomycetes

Four amylase sequences from halophilic actinomycetes were retrieved from the NCBI database .These sequences were compared to assess amino acid composition and its role in protein structural stability. Amino acid frequencies and other physicochemical properties were computed using the ProtParam tool (https://web.expasy.org/protparam/) is a widely used bioinformatics tool that enables the computation of various physicochemical properties of proteins based on sequences retrieved from the NCBI. The parameters calculated include molecular weight, theoretical isoelectric point (pI), amino acid and atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index, providing insights into protein stability, solubility, and functional characteristics (Gasteiger et al., 2005).

Protein 3D structure prediction and analysis

SWISS-MODEL is a fully automated protein structure homology-modelling server accessible via the ExPASy web platform, designed to facilitate three-dimensional protein structure prediction for life science researchers. This process further involved the use of the Global Model Quality Estimation (GMQE) score and QMEAN Z-score to assess model reliability, while structural validation was performed using MolProbity and Ramachandran plot analyses to evaluate stereochemical quality and backbone conformational integrity of the predicted three-dimensional structures.

Result

The amino acid sequences of four amylases derived from halophilic actinomycetes were analysed using the ProtParam tool available on the ExPASy server to evaluate their physicochemical properties, including amino acid composition and parameters associated with protein stability (Gasteiger et al., 2005).

Amylases from Halophilic Actinopolyspora erythraea

Bioinformatics analysis examined four amylase-related proteins from the halophilic actinomycete Actinopolyspora erythraea — α-glucosidase, glycosidase and two hypothetical proteins — for sequence length, physicochemical properties, amino acid composition, and stability indices to reveal high-salinity adaptations.

α-Glucosidase (Actinopolyspora erythraea)

The α-Glucosidase comprises 551 amino acids, with a predicted molecular mass of 62.30 kDa and a theoretical isoelectric point (pI) of 4.98, as estimated from the sequence. Acidic residues outnumber basic ones, aiding salt tolerance. The protein is stable (instability index), hydrophilic (negative GRAVY), and moderately aliphatic, supporting saline stability.

Glycosidase (Actinopolyspora erythraea)

The glycosidase consists of 464 amino acids with excess acidic residues, characteristic of halophilic adaptation. It is stable (instability index) and hydrophilic (negative GRAVY), enabling activity in high salt.

Hypothetical Protein IL38_20700 (Actinopolyspora erythraea)

The hypothetical protein IL38_20700 is composed of 433 amino acids, with a predicted molecular mass of 47.02 kDa and a low theoretical isoelectric point (pI) of 4.49 as estimated from the sequence. Acidic amino acids (Asp + Glu = 69) outnumber basic residues. A high aliphatic index indicates thermostability, but the instability index suggests lower stability under standard conditions. The GRAVY score indicates hydrophilicity, aiding halophilic adaptation.

Hypothetical Protein IL38_20695 (Actinopolyspora erythraea)

The hypothetical protein IL38_20695 comprises 409 amino acids, with a predicted molecular mass of 44.47 kDa and a theoretical isoelectric point (pI) of 4.89 as estimated from the sequence. Acidic residues outnumber basic residues. The instability index indicates stability, a high aliphatic index suggests robustness in extreme conditions, and the negative GRAVY score confirms hydrophilicity and saline adaptation.

Protein annotation	Molecular weight (Da)	Theoretical Pi	C	H	N	O	S	Instability index	Aliphatic index
α-Glucosidase	62304.80	4.98	2784	4165	797	826	9	35.34	71.03
Glycosidase	50610.44	4.51	2221	3337	613	721	14	30.22	69.01
Hypothetical protein IL38_20700	47021.50	4.49	2060	3235	589	653	10	43.50	87.92
Hypothetical protein IL38_20695	44473.06	4.89	1969	3077	565	595	9	32.69	87.31

Table 1. Comparative physicochemical analysis of amylases.

Physicochemical parameters were determined using ProtParam, where proteins exhibiting an instability index below 40 were classified as stable; elevated aliphatic index values indicated enhanced thermostability, while low theoretical isoelectric points (pI) reflected enrichment of acidic residues, a hallmark of halophilic enzymes.

Comparative Insights

All four proteins exhibit low isoelectric points, high acidic amino acid content and negative GRAVY values—hallmarks of halophilic enzymes. These traits enhance solubility, structural stability and catalytic efficiency in high-salt conditions, aiding starch and glycoside metabolism by Actinopolyspora erythraea in saline environments.

Protein	Length (aa)	Molecular Weight (kDa)	Theoretical Pi	Asp + Glu	Arg + Lys	Instability Index	Stability Class	Aliphatic Index	GRAVY
α-Glucosidase	551	62.30	4.98	92	57	35.34	Stable	71.03	-0.622
Glycosidase	464	50.61	4.51	66	33	30.22	Stable	69.01	-0.404
Hypothetical protein IL38_20700	433	47.02	4.49	69	40	43.50	Unstable	87.92	-0.281
Hypothetical protein IL38_20695	409	44.47	4.89	57	39	32.69	Stable	87.31	-0.209

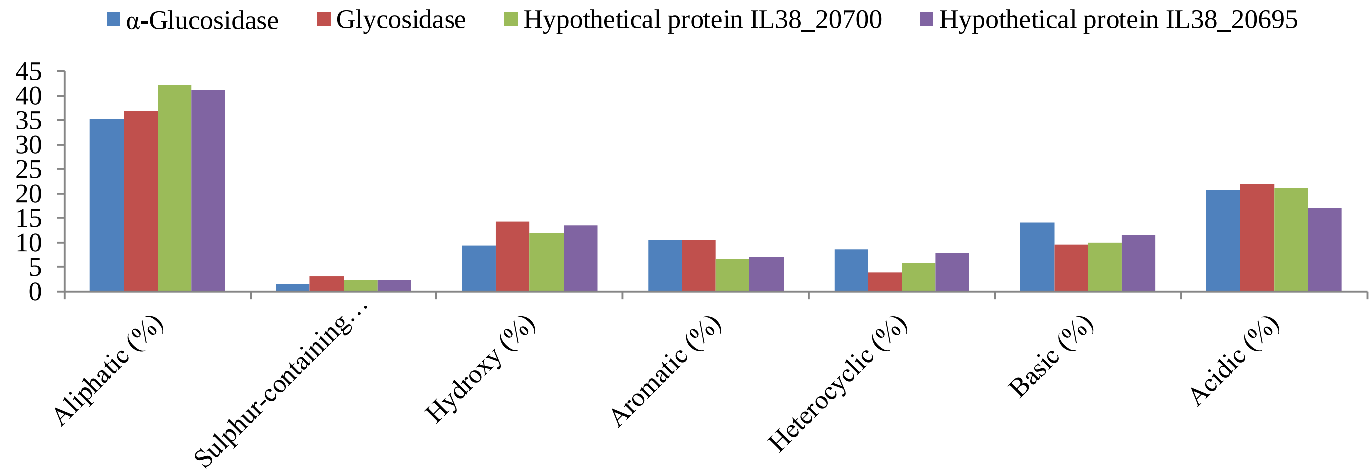
Table 2. Comparative Physicochemical Properties of Amylase-Related Proteins.

Protein	Key Physicochemical Features	Halophilic Adaptation Significance	Functional Implication
α-Glucosidase	Low pI (4.98), high acidic residues (Asp+Glu = 92), stable instability index, strongly negative GRAVY	Enhanced solubility and electrostatic stabilisation in high salt	Primary amylolytic enzyme involved in starch degradation
Glycosidase	Very low pI (4.51), high acidic residue content, stable structure, hydrophilic nature	Salt tolerance and maintained catalytic efficiency	Glycosidic bond hydrolysis under saline conditions
Hypothetical protein IL38_20700	Low pI (4.49), high aliphatic index (87.92), acidic residue dominance, unstable index	Possible salt- and temperature-adapted auxiliary protein	Putative amylase-related or regulatory role
Hypothetical protein IL38_20695	Low pI (4.89), stable instability index, high aliphatic index (87.31)	Structural robustness and halophilic compatibility	Potential novel amylolytic or carbohydrate-associated protein

Table 3. Comparative Interpretation of Amylases - Related Proteins.

Protein annotation	Aliphatic (%)	Sulphur-containing (%)	Hydroxy (%)	Aromatic (%)	Heterocyclic (%)	Basic (%)	Acidic (%)
α-Glucosidase	35.2	1.6	9.3	10.5	8.5	14.1	20.7
Glycosidase	36.9	3.0	14.2	10.5	3.9	9.6	21.9
Hypothetical protein IL38_20700	42.1	2.3	12.0	6.7	5.8	9.9	21.2
Hypothetical protein IL38_20695	41.1	2.2	13.4	7.1	7.8	11.5	17.0

Table 4. Comparative amino acid class distribution (%) of amylase-producing halophilic actinomycetes



Graph 5. Amino acid class distribution (%) in amylase-producing halophilic actinomycetes.

The values represent the percentage composition of amino acid classes, with elevated acidic and aliphatic residue content reflecting halophilic enzyme adaptations that enhance solubility, structural stability, and catalytic efficiency under high-salt conditions.

The Prediction and analysis of the Protein 3D Structure

The selected proteins were homology-modelled using SWISS-MODEL and assessed with the Swiss-Model workspace's structural evaluation tools. Details of the structures and evaluation metrics are shown in the table. The predicted α-amylase structures do not require metal ions for optimal activity. Scores from GMQE, QMEAN, MolProbity, and Ramachandran favored regions confirm their high quality.

Protein annotation	SWISS-MODEL template	Sequence identity (%)	Query coverage (aa range)	GMQE	QMEAN Z-score	MolProbity score	Ramachandran favoured (%)
α-Glucosidase	3wy1.1.A	39.10	9–549	0.68	-3.08	2.13	88.31
Glycosidase	1kxh.1.A	50.35	34–461	0.78	-1.81	1.55	94.37
Hypothetical protein IL38_20700	6aav.1.A	28.82	17–431	0.61	-3.66	2.31	89.83
Hypothetical protein IL38_20695	6aav.1.A	27.82	9–409	0.67	-2.59	2.06	93.98

Table 5. Comparative homology modelling and structural validation of amylase-producing halophilic actinomycetes.

GMQE values approaching 1 indicated higher model reliability, QMEAN Z-scores within the expected range for proteins of comparable size confirmed acceptable model quality, and MolProbity scores below 2.5 together with more than 88% residues in Ramachandran-favoured regions validated the good stereochemical quality of the predicted structures.

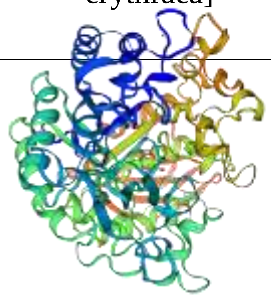
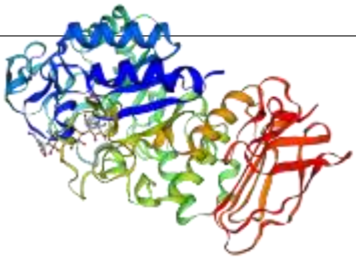
Alpha-glucosidase [Actinopolyspora erythraea]	Glycosidase [Actinopolyspora erythraea]	Hypothetical Protein IL38_20700 [Actinopolyspora erythraea]	Hypothetical Protein IL38_20695 [Actinopolyspora erythraea]
			

Image 1. Protein 3D Structure of Amylase Producing Halophilic Actinomycetes.

Discussion

Halophilic actinomycetes are well-recognized sources of industrially important enzymes due to their ability to functional attributes under high-salt conditions (Hutcheon et al., 2005; Madern et al., 2000). In the present bioinformatics study, four amylase-related proteins from Actinopolysporaerythraea were studiedto explore their physicochemical adaptations, functional stability to hypersaline environments.

All four proteins exhibited low theoretical isoelectric point (pI) values, important characteristic adopted by of halophilic enzymes (Madern et al., 2000). This feature arises from the enrichment of acidic amino acids (Asp and Glu), which was evident across all analysed sequences. The predominance of negatively charged residues over positively charged residues facilitates the formation of a stable surface, preventing protein aggregation and maintaining solubility under high-salt conditions which maintain catalytical efficiency of enzymes (Hutcheon et al., 2005).

The α-glucosidase and glycosidase proteins established marked halophilic adaptations, including high acidic amino acid content, low GRAVY values, and favourable instability indices, indicating overall structural stability. Negative GRAVY scores reflect enhanced hydrophilicity, promoting preferential interactions with water molecules rather than salt ions, thereby preserving enzymatic activity under osmotic stress (Madern et al., 2000). These physicochemical characteristics support their functional roles in carbohydrate metabolism within saline environments and highlight their suitability for industrial applications.

The hypothetical proteins IL38_20700 and IL38_20695 exhibited characteristic halophilic features, notably low theoretical isoelectric points (pI) and a high abundance of acidic amino acid residues. Both proteins exhibited high aliphatic indices, suggesting enhanced thermostability and structural rigidity, which are advantageous under extreme environmental conditions. However, IL38_20700 showed a high instability index, indicating predicted instability under standard conditions, which may be mitigated in vivo by high salinity or protein–protein interactions. In contrast, IL38_20695 was predicted to be stable, indicating a more robust structural framework.

Comparative analysis suggests that α-glucosidase and glycosidase represent well-characterized amylolytic enzymes, whereas the hypothetical proteins may function as novel or auxiliary amylase-related proteins involved in starch or oligosaccharide metabolism under extreme saline conditions. Despite the lack of experimental validation, their shared physicochemical features strongly support a role in halophilic adaptation and metabolic functionality.

Overall, the enrichment of acidic amino acids, low theoretical isoelectric points, enhanced hydrophilicity, and high aliphatic indices emerge as key determinants underlying the structural stability and functional adaptation of amylase-related proteins in Actinopolyspora erythraea under hypersaline conditions. These findings reinforce the potential of halophilic actinomycetes as promising reservoirs of extremozymes for applications in biotechnology, food processing, and pharmaceutical industries operating under high salinity and other harsh conditions (Hutcheon et al., 2005).

Conclusion

This bioinformatics analysis of four amylase-related protein sequences from the halophilic actinomycete Actinopolyspora erythraea indicated key salt-adaptive characteristics , including low isoelectric points, high acidic amino acid content, negative GRAVY values, and moderate to high aliphatic indices. These properties revealed about its protein solubility, structural stability and functional properties under high-salt conditions,.

SWISS-MODEL homology modelling defined high-quality protein structures without metal cofactor dependence. The α-glucosidase and glycosidase showed high stability, indicating crucial roles in saline carbohydrate metabolism, while the hypothetical proteins exhibited amylolytic-like properties. Overall, Actinopolyspora erythraea rising source of salt-tolerant extremozymes for food, detergent, and pharmaceutical applications.

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