



Glycosyl transferase-group-1 based computational proteomic study of *Sphaerobacter thermophilus*

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ABSTRACT

Sphaerobacter thermophilus, a Gram-positive, non-motile & non-spore forming bacteria is reported in the tooth producing glycosyltransferase and found to be responsible in endodontic infection. The enzyme is found in the human root canal and is involved in peptidoglycan biosynthesis, which is significant for bacterial adhesion and biofilm production. In the present study, relevant databases & web servers were accessed for *in silico* proteomic characterization, virulence and protein disorder prediction to support the metabolic pathway designing. Glycosyltransferase-1 family members were phylogenetically investigated, which are exclusively endodontic in origin. Subsequently, the enzyme was subjected to a subcellular localization-based pathogenicity study using computational algorithms & an already established pathway database. Afterwards, a putative metabolic pathway was engineered & presented that may further lead towards exploration of glycosyltransferases with a wider range of chemical configurations and competent drug designing.

INTRODUCTION

- ❑ *Sphaerobacter thermophilus* was first isolated from thermophilic sewage sludge plant in Germany in the years 1973-1980.
- ❑ It is a gram positive bacteria, coccus shaped, thermophilic with 66.3%-68.1% G-C content.
- ❑ It contains Glycosyltransferase having 397 amino acids.
- ❑ It has transferase, carbohydrate metabolism, adhesion and glyosidic bond formation activity.

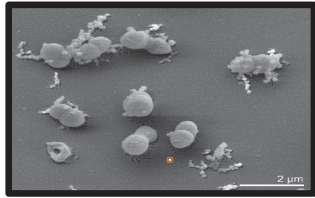


Fig 1: *Sphaerobacter thermophilus*

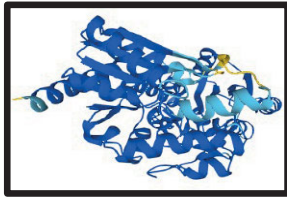


Fig 2: Glycosyltransferase 1

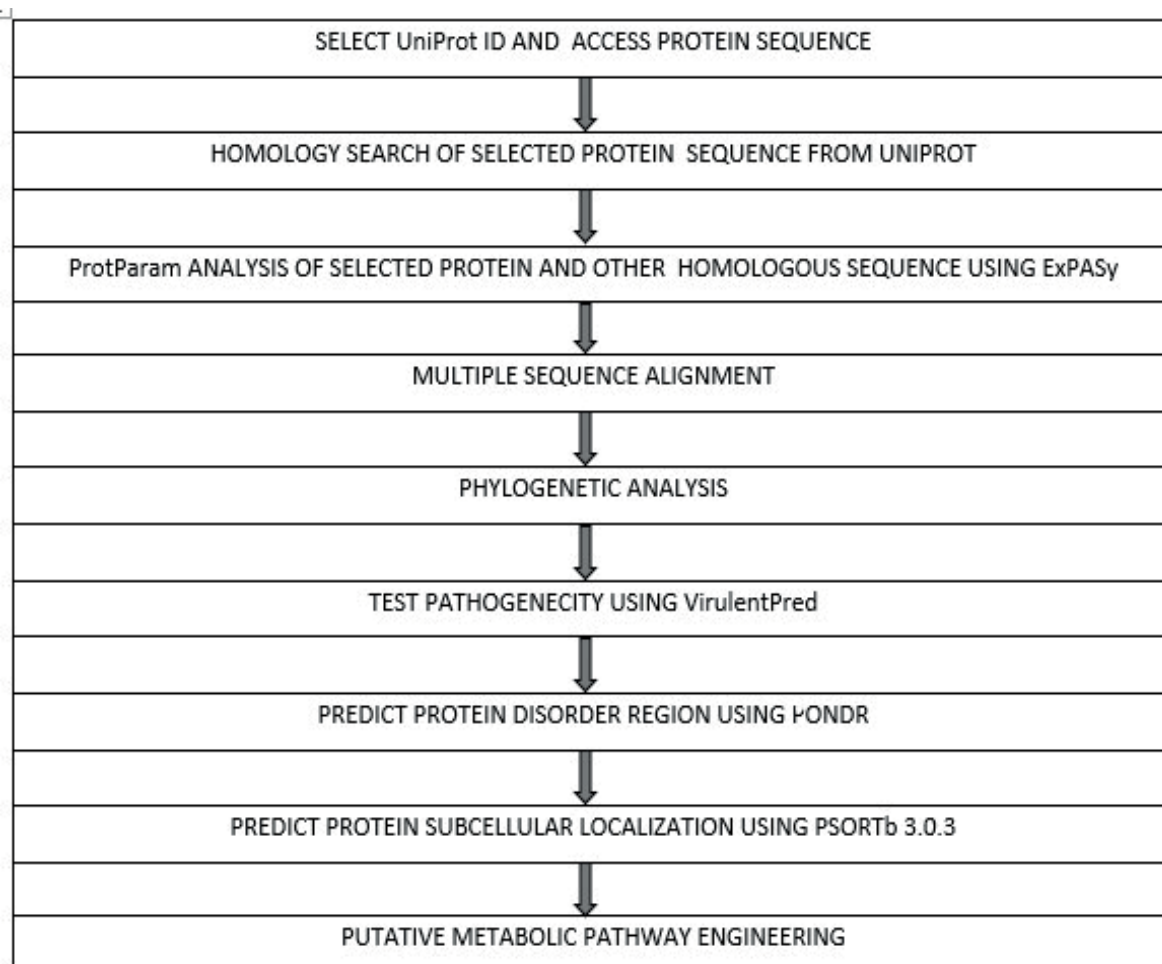


Fig 3: Periapical abscess

OBJECTIVES

- Evolutionary relationship among bacterial pathogens based on protein sequences.
- Virulence prediction of homologous sequence.
- Disordered region prediction of homologous proteins.
- Putative metabolic pathway engineering.

METHODOLOGY



RESULTS & DISCUSSION

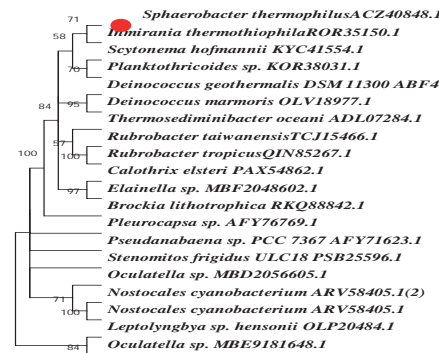


Fig 4: Phylogeny tree by maximum likelihood method

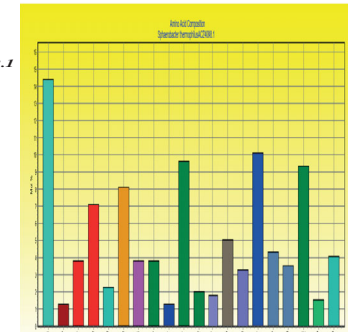


Fig 5: Sequence alignment by BioEdit

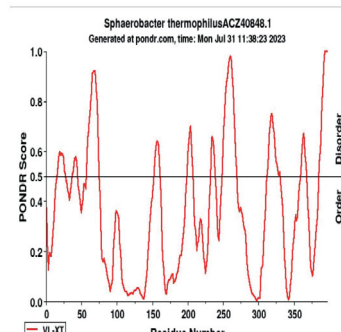


Fig 6: PONDR score vs residue number

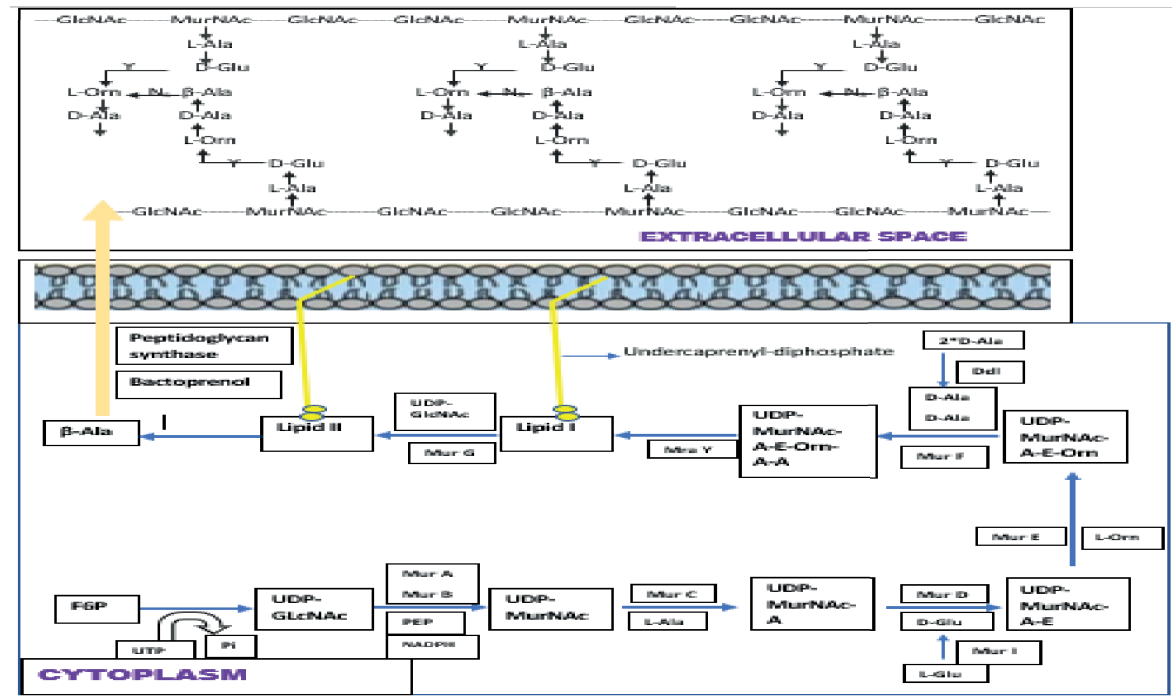


Fig 7: Putative metabolic pathway engineering : peptidoglycan biosynthesis

- Glycosyltransferase contains 397 amino acid residues. It contains 43 negatively charged amino acids and 45 positively charged amino acids. The elements carbon, nitrogen, hydrogen, oxygen and sulphur are 1944, 578, 3066, 551 and 13 in number respectively.
- The molecular weight of glycosyltransferase is found to be 43168.05.
- The theoretical Pi of glycosyltransferase is 8.25, which indicates it contains more basic amino acids.
- The aliphatic index is found to be 93.45, which indicates it is thermostable.
- The instability index obtained is 36.62, which indicates it is a stable protein.
- The GRAVY obtained is -0.150, which indicate it contains more non-polar amino acid.
- Sequence alignment by BioEdit of glycosyltransferase gave the highest composition of alanine (with 57 in number) and the lowest composition of lysine and cysteine (with 5 in number).
- The phylogeny result with *Sphaerobacter thermophilus* and *Inmirania thermothiophila* on the same clade shows more homology.
- *Sphaerobacter thermophilus* was found to be virulent with a 0.7972 virulent score.
- The PONDR residue was found to be 397, with 10 disorder regions and 124 disorder residues. The longest disorder region was 21. The overall disorder percentage was found to be -31.23. The average prediction score gave value of 0.3622.
- The sub cellular localization final prediction score is found to be cytoplasmic: 7.50.

DISCUSSION

- Peptidoglycan biosynthesis of *Sphaerobacter thermophilus* involves Lys-Ala linkage (Keggs database). The primary murein structure analysis show that peptides 6 and 8 have an interpeptide bridge consisting of beta-alanine cross-linking. The crosslinking connects the amino group of L-Ornithine with the carboxyl group of D-alanine of the adjacent peptide subunit (Demharter et al., 1989) . This study engineered a metabolic pathway demonstrating this process.
- *Sphaerobacter thermophilus* is a highly isolated bacterial species with the closest related organism being *Thermomicrobium roseum* (DSM 5159) based on a 16 S rRNA sequence recorded in GenBank . However they share only 87% sequence similarity (Jackson et al.,1973; Wu et al., 2009). In the present study, *Sphaerobacter thermophilus* was found to be the most closely related to *Inmirania thermothiophila* ,with sequence similarity of 71%, 87% and 89% determined using maximum likelihood, neighbour joining, and minimum likelihood respectively, with the bootstrapping method consisting of 500 replications.

CONCLUSION

After an analysis of available biological databases and subsequent phylogenetic analysis of 19 homologous proteins of glycosyltransferase group 1, the study investigated the metabolic pathway of GT1 based on *Sphaerobacter thermophilus*, which is responsible for endodontic infection. ProtParam analysis, PSORTb 3.0.3 tools followed by phylogenetic analysis of proteins that conventionally relies on the evaluation of amino acid sequence or coding sequence gave insights into the metabolic pathways of the protein concerned.

REFERENCES

1. Demharter W, Hensel R, Smida J, Stackebrandt E. (1989) *Sphaerobacter thermophilus*, A deeply rooting member of the actinomycetes subdivision isolated from thermophilically treated sewage sludge. Syst Appl Microbiology, Vol.11(3):261-266.
2. Gloster T.M. (2014) Advances in understanding glycosyltransferases from a structural perspective. Curr Opin Struct Biol. Vol.28:131-141.