

MOLECULAR ANALYSIS OF *STREPTOCOCCUS THERMOPHILUS* SUPPORTING PROBIOTIC ACTION TOWARDS DRUG TARGETS

DSVGK KALADHAR*, APPANA V S RAMAKRISHNA

For Author affiliations see end of the text
This paper is available online at www.jprhc.in

ABSTRACT

Streptococcus thermophilus is a Gram-positive facultative anaerobe with probiotic action. Nearly 2070 genes are predicted in complete genome out of which 566 genes are related to hypothetical and uncharacterized genes. The predicted gene percent obtained by characterization of complete genome of *S.thermophilus* is 72.67% with 25 functional categories. There is high number of transport, protein synthesis, binding and cell envelop proteins that don't have pathogen responses. The genes ptsK, glgP, malQ, lacZ, amyL, scrB, etc are related to energy metabolism (with biodegradation and synthesis of polysaccharides) are found in chromosome CNRZ 1066. The genes such as glgP, malQ, lacZ and amyL had shown direct interactions that has not found direct link with scrB gene. All the proteins are observed with more the 50 percent identities that are present in homology models.

Keywords

Keywords: Probiotics, *Streptococcus thermophilus*, Molecular analysis

INTRODUCTION

Traditional molecular dieting methods for the characterization of beneficial and pathogenic microorganisms can be manageable because of index variation that is difficult to compare in laboratories can be done through modeling approaches¹. In food regulations, the viability of bacteria emphasizes input and regulations, fermented milk have beneficial health, stimulation of the human immune system by oral administering fermented milks (or) probiotic cultures². Ancient people used to take the curd rice in the mornings and milk before sleep that improves probiotic action and provides good health. Due to change of culture from ancient to modern, this habit of intake has been lost and humans are losing the general nutritional values turning towards aging diseases.

Bioinformatics play key role in sequencing, storing, integrating and analyzing the field of “omics” data from few decades in reconstruction of metabolic potential using bioinformatics databases and tools^{3,4}. It provides regulatory network properties, a challenge to improve exploitation of versatile food bacteria⁵. A combination of functional studies with comparative genomic approaches provides effective strategies for improving the functionality and safety of foods⁶.

Genomics, proteomics and microbial analysis will be the driven force in disease marker development⁷ and their application's to the molecular characterization of probiotic bacteria and post-microbe interactions in core technique.

Investigating these organisms with new and potentially powerful tools facilitates the development of bacteria as therapeutic agents and provides the mechanisms to produce advanced probiotic strains.⁸

Probiotics aimed delivering living bacterial cells to the living ecosystem whereas prebiotics are non-digestible carbohydrates delivered in food to provide preventable substance for selected bacteria. Members of *Lactobacillus* and *Bifidobacterium*⁹ can be used as probiotic bacteria, using as a living sources with food products such as curd, buttermilk, yoghurt etc. The genomics of probiotic microorganisms provides an in-site to developing technologies for analysis of gut microorganism in evaluation of the potential prebiotic substances and the potential disease prevention in the host. Understanding the host microbe interaction and microbial community provides the cellular processes that would be used as novel anti-bacterial and pathogenic targets.

Nutritional Genomics and proteomics play vital role in understanding of metabolism and optimizing companion nutritional and health status of a multicellular organism. Various biological scientists in the “OMICS” era has continuous focus to evolve and shape the way science in the analysis of Protein expression, localization and interactions¹⁰. Different strains of probiotic organisms have much interest as commercial actors as well as from researchers from different discipline including microbiology, immunology and gastroenterology¹¹.

MATERIALS AND METHODS

Streptococcus thermophilus is a Gram-positive facultative anaerobe and an essential lactic acid bacterium used for commercial purposes in food and pharmaceutical industries. The species is being used as a biological indicator of sterilization of autoclave in Microbiology laboratory, GITAM University. As the species is useful in various purposes for humans, the comprehensive data analysis has been done using *in silico* methods.

Identification and Characterization

The whole genome (NC_017581.1) from NCBI has been retrieved for analysis. Genes from Genomic sequences can be identified and characterized using *in silico* methods. Finding of functions in the protein sequences can be done based on central dogma of life. Gene prediction from the genomic sequence of *Streptococcus thermophilus* can be done using GenMark. Each gene can be identified using BLAST Tool. The main criterion has been checked for Lac genes for further studies.

JCVI-CMR

One of the comprehensive and microbial resources is JCVI-CMR. The database contains the information of the microorganisms related to both pathogenic and non-pathogenic organisms. *Streptococcus thermophilus* has been selected to know the gene role category and the genes involved that perform the function.

Protein-Protein Interaction

The interaction of lac protein with another provides the better understanding of systems approaches in *Streptococcus thermophilus*. This microbial system can form a network of interactions which performs useful effects in humans (probiotic resource). Protein-Protein interactions from *Streptococcus thermophilus* can be analyzed using String server.

STRING

STRING is a database of known and predicted protein interactions. The interactions include direct (physical) and indirect (functional) associations that are derived from four sources: Genomic context, High-throughput experiments, Co-expression (Conserved) and previous knowledge. STRING provides quantitatively integrates

interaction data from these sources for a large number of organisms, and transfers information between these organisms where applicable. The genes such as *glgP*, *malQ*, *lacZ* and *amyl* (*Streptococcus thermophilus* 18311) had been submitted to the server to predict protein interactions.

RESULTS AND DISCUSSION

The metabolism and genetics of *Streptococcus thermophilus* have widely used in milk fermentation processes. The proteolytic activity is a good indicator of showing the ability of probiotic microorganisms used to improve the nutritional value of milk and milk products by the formation of bioactive peptides and free amino acids¹².

A complete genomic analysis of *S.thermophilus* from **JCVI CMR** has been presented in Figure 1. There is high number of transport, protein synthesis, binding and cell envelop proteins that don't have pathogen responses. The genes *ptsK*, *glgP*, *malQ*, *lacZ*, *amyL*, *scrB*, etc are related to energy metabolism (with biodegradation and synthesis of polysaccharides) are found in chromosome CNRZ 1066.

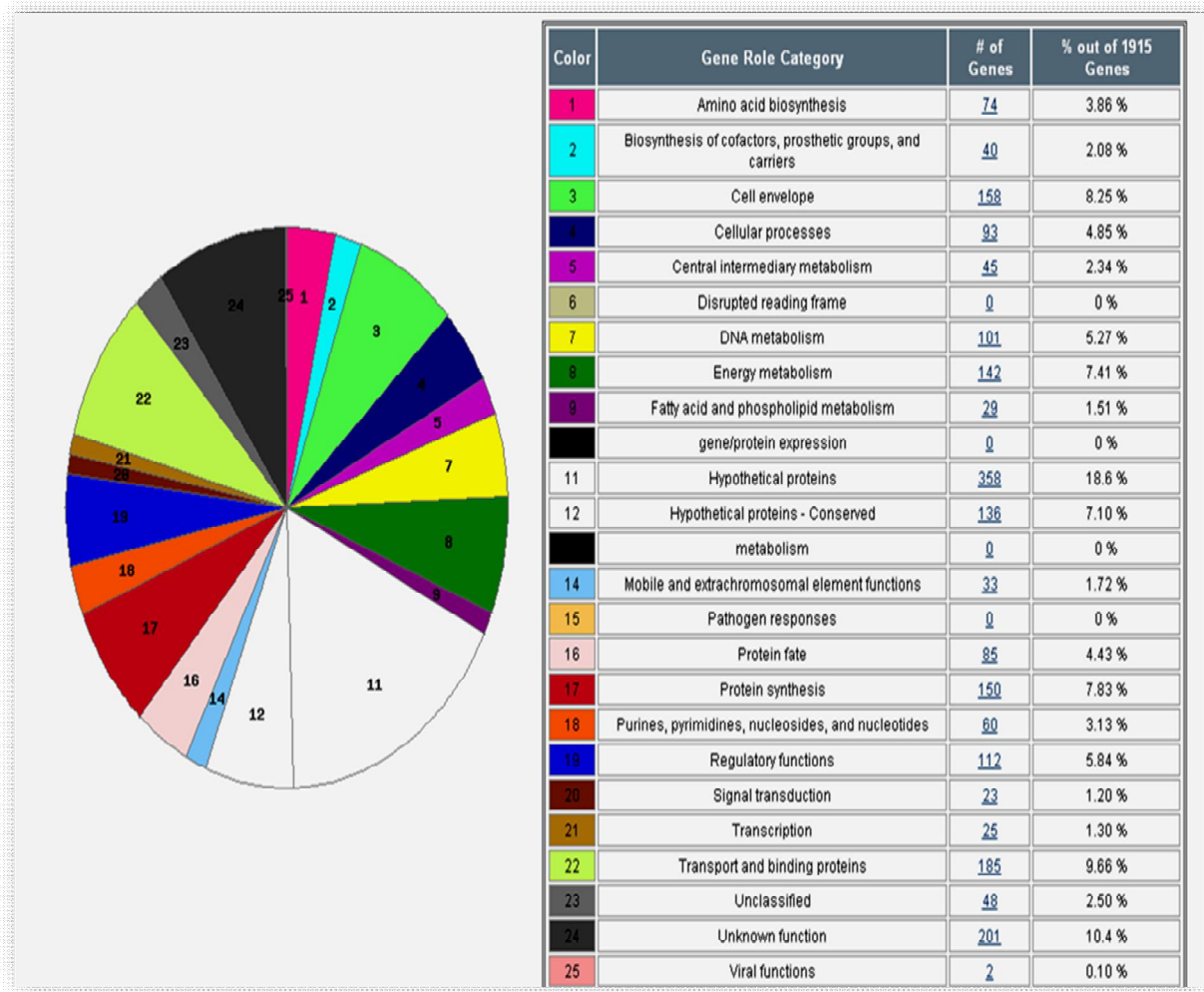


Figure 1: JCVI CMR Result for *S. thermophilus*

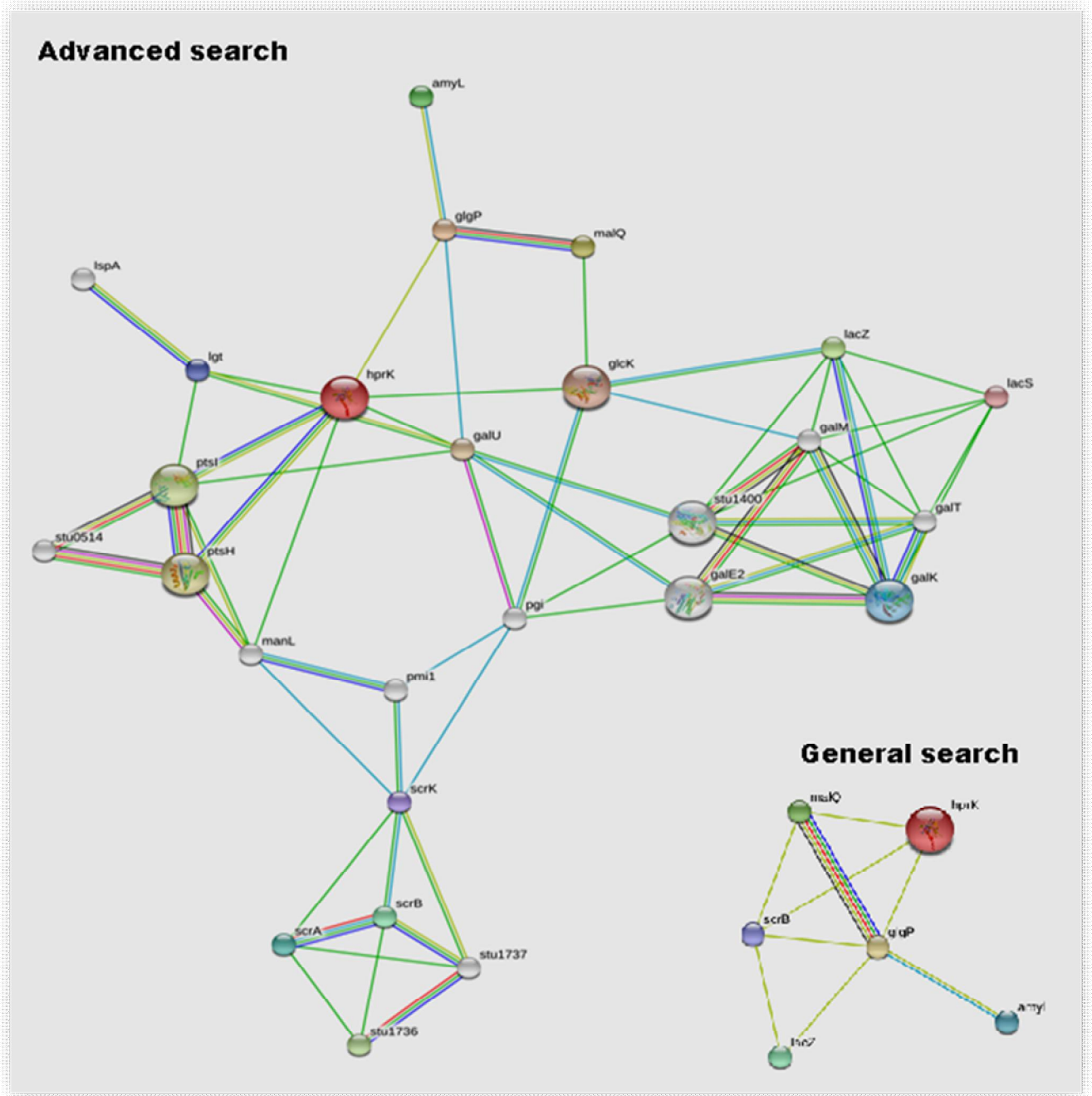


Figure 2: Protein interaction network in carbohydrate metabolism

The genes such as *glgP*, *malQ*, *lacZ* and *amyL* had shown direct interactions that has not found direct link with *scrB* gene (Figure 2). All the proteins are observed with more the 50 percent identities that are present in homology models (Figure 3).

There are 2070 genes predicted out of which 566 genes are related to hypothetical and uncharacterized genes. The predicted gene percent obtained in characterization is 72.67% (Figure 4).

Genomic and proteomic studies reveal effects of genes and proteins in probiotics on health, disease prevention and treatment, in clinical application and functional foods, can significantly increase the understanding mechanism in Genetics¹³. In the post-genomic era, genomics and proteomics provides powerful approaches in probiotics for the analysis of normal and mutated cells, for further identification of disease specific target and provide a novel evaluation of chemotherapeutic agents. The potential genomic information has greatly exploded genetic bases for proteomes to identify proteins present in cancer tissues¹⁴.

Genomic based studies have been reported on some probiotic strains and have found significant importance in the food and health products¹⁵. Functional genomics towards the investigation of these probiotic bacteria exploit recent progress in sequencing capacity to establish solid health benefits in physiology and genetics¹⁶.

High throughput DNA sequence analysis can make possible stream line and automation; have been a paramount in the advances made in genomic sequencing. The science of genomics in probiotics can provide systematic study of the structure, content and evolution of complete genomes. Nearly 25 percent of the genomes are unpredicted and can be analyzed through proteomic and systems biology approaches. They can also provide the interactions of gene-protein or protein-protein or protein-gene so that the communication within a cell or at physiological levels can be better understood. Major types of putative extracellular proteins have to be investigated, which are of interest because of their possible role in host-microbe interactions¹⁷.

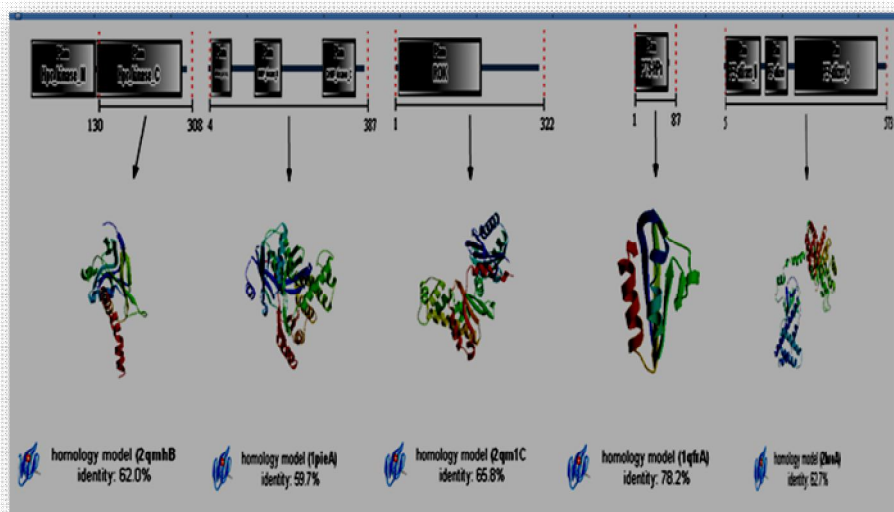


Figure 3: Modeled structures of genes in relevant to carbohydrate metabolism

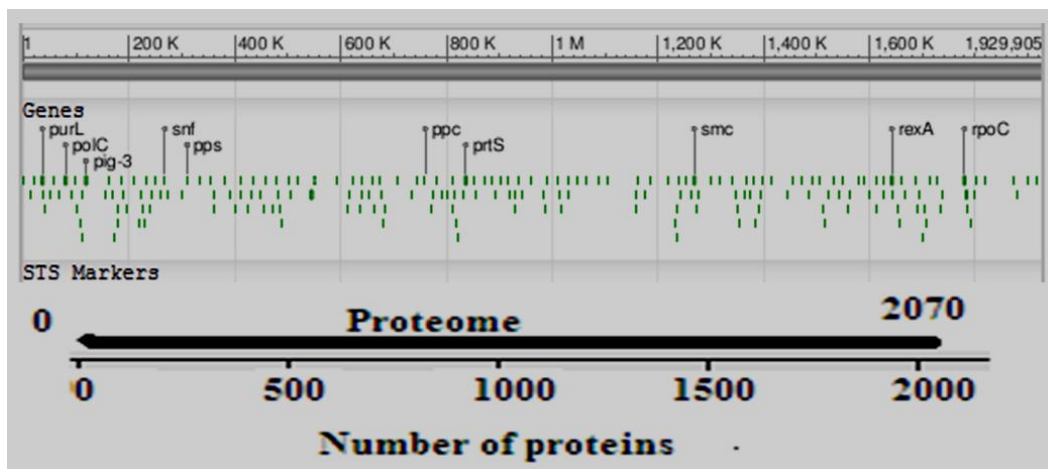


Figure 4: *Streptococcus thermophilus* JIM 8232 genome

CONCLUSION

Probiotic species such as *Streptococcus thermophilus* provide good intestinal health conditions that can control and coordinate with components in the alimentary system. The species along with other intestinal microbes such as *E.coli*, *Lactobacillus* and *Bifidobacterium* in normal conditions provide healthy environment in complex vertebrates such as humans due to production of beneficial metabolites inside living system.

Acknowledgement

Author would like to thank management and staff of GITAM University Visakhapatnam, India for their kind support in bringing out the above literature and providing lab facilities.

REFERENCES

1. Eisenbrandt G, Pool-Zobel B, Bakerc V, Ballsd M, Blaauboere BJ, Boobisf A, Carereg A, Kevekordesh S, Lhuguenoti JC, Pieterse R, Kleinerj J. Methods of in vitro toxicology. *Food and Chemical Toxicology* 2002;40(2):193-236
2. Dave RI, Shah NP. Viability of yoghurt and probiotic bacteria in yoghurts made from commercial starter cultures. *International Dairy Journal* 1997;7(1):31-41
3. Kaladhar DSVGK, Devi TU, Rao PN. An in silico genome wide identification, characterization and modeling of Human Papilloma Virus strain 92. *International Journal of Engineering Science and Technology* 2010;2(9):4288-91
4. Kaladhar DSVGK, Rayavarapu KA. *Basics in Bioinformatics: for Students and Researchers*. LAP Lambert Publishers, Germany 2012.
5. Roland JS, Frank HJE, Michiel K, Bas T. Genome data mining of lactic acid bacteria: the impact of bioinformatics. *Current Opinion in Biotechnology* 2004;15(2):105-15
6. Willem MV. Advances in genomics for microbial food fermentations and safety. *Current Opinion in Biotechnology* 2001;12(5):493-98
7. Bernhard GZ, Nohjin P, David TW. Genomic Targets in Saliva. *Annals of the New York Academy of Sciences* 2007;1098:184-91
8. Callanan M, Mining the Probiotic Genome: Advanced Strategies, Enhanced Benefits, Perceived Obstacles. *Current Pharmaceutical Design* 2005;11(1):25-36.
9. Gerald WT. *Probiotics and Prebiotics: Scientific Aspects*. Caister Academic Press, 2005.
10. Kelly SS, Lawrence BS, George CF. Nutritional Genomics: Implications for Companion Animals. *The American Society for Nutritional Sciences J. Nutr.* 2003;133:3033-40
11. Hans J, Elisabeth S, Stefan R. Addition of mucin to the growth medium triggers mucus-binding activity in different strains of *Lactobacillus reuteri* in vitro, *FEMS Microbiology Letters* 2001;204(1):19-22

12. Marvin M, Kayanush JA. Influence of “Mild” Sonication Conditions on the Characteristics of *Streptococcus thermophilus* ST-M5. *Advances in Microbiology* 2012;2:8-16
13. Fang Y, David BP. Probiotics: progress toward novel therapies for intestinal diseases. *Curr Opin Gastroenterol.* 2010;26(2):95-101
14. Verma M, Wright GL, Hanash SM, Gopal-srivastava R, Srivastava S. Proteomic Approaches within the NCI Early Detection Research Network for the Discovery and Identification of Cancer Biomarkers. *Annals of the New York, Academy of Sciences* 2001; 945:103-115
15. Maija S, Soile T, Tiina MS, Willem MV. Probiotic and other functional microbes: from markets to mechanisms. *Current opinion in biotechnology* 2005;16(2):204-11
16. Todd RK, Martin JK. Selection and design of probiotics. *International Journal of Food Microbiology* 1999;50(1-2):45-57
17. Jos B, Roland JS, Marie-Camille Z, David V, Raymond DP, Annick M, Michiel K, Willem MV, Harald B, Frank D. The complete genomes of *Lactobacillus plantarum* and *Lactobacillus johnsonii* reveal extensive differences in chromosome organization and gene content. *Microbiology* 2004;150:3601-11

AUTHORS AFFILIATION AND ADDRESS FOR CORRESPONDENCE

Department of Bioinformatics, GIS, GITAM University, Visakhapatnam-530045, India
Phone: +91-9885827025
email:dr.dowluru@gmail.com.