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Research Article

Ab Initio Whole Cell Kinetic Model of *Streptococcus thermophilus* STH_CIRM_65 (stheVS26)

Sragvi Verma[1,2], Diya Nanthakumarvani[1,2], Leesha Haarshiny Perumal[1,2], Atoshi Abirami RajKumar[1,2], Cheryl Kai Ning Kang[1,2], Shafeeqa Abul-Hasan[1,2] and Maurice HT Ling[2,3,4]*

[1]*Department of Applied Sciences, Northumbria University, United Kingdom*

[2]*Management Development Institute of Singapore, Singapore*

[3]*Newcastle Australia Institute of Higher Education, University of Newcastle, Australia*

[4]*HOHY PTE LTD, Singapore*

***Corresponding Author:** Maurice HT Ling, Management Development Institute of Singapore, Singapore.

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Abstract

Streptococcus thermophilus is a lactic acid bacterium, which is used in yogurt production, and known for its ability to produce folate, exopolysaccharides; thus, making it a highly valuable organism for food biotechnology and probiotic applications. Mathematical kinetic models, which extend beyond steady-state predictions in genome-scale models, are useful tools for directing metabolic engineering efforts. Although a genome-scale models of *S. thermophilus* is available, a comprehensive whole-cell kinetic model is lacking. In this study, we describe a simulatable kinetic model of *S. thermophilus* STH_CIRM_65, constructed in an *ab initio* fashion by locating enzymes from the genome sequence and mapping them to corresponding reactions in KEGG. The resulting model, stheVS26, encompasses 322 metabolites, 400 enzymes along with their transcription and translation processes, and 336 enzyme reactions. This model provides a foundational platform for the simulation and prediction of cellular behaviour, allowing for informed design decisions in metabolic engineering.

Keywords: Whole-cell Model; Kinetic Model; Differential Equations; AdvanceSyn Toolkit; Yoghurt; Exopolysaccharides

Introduction

Streptococcus thermophilus is widely used as a lactic starter culture in the dairy industry [1], and is known to produce folate [2]. As a starter culture, *S. thermophilus* produces beta-galactosidase [3], which digests lactose - beneficial for lactose-intolerant individuals. Its proteolysis of milk proteins generates bioactive peptides [4], which have demonstrated anti-hypertensive activities [5,6]. When *S. thermophilus* is consumed and digested, peptides often known as "postbiotics" [7] may demonstrate anti-inflammatory properties [8], which potentially helpful for managing conditions like irritable bowel syndrome (IBS) or antibiotic-associated diarrhoea [9]. During fermentation, *S. thermophilus* produces exopolysaccharides (EPS), which are long chains of carbohydrates that act like natural thickeners [10,11]. Hence, *S. thermophilus* has been engineered to improve the sweetness [12], and fragrance profile [13] of yoghurt; on top of optimizing its EPS production [14].

Mathematical modelling plays an essential role in guiding metabolic engineering efforts, helping identify viable modifications

before experimental work begins [15,16]. Two modelling approaches are typically used [17,18]: constraint-based genome-scale models (GSMs) and kinetic models (KMs). While GSMs are widely adopted, they primarily generate predictions of fluxes. KMs extend this by providing insights into both metabolic rates and yields [19], and they are generally more amenable to performing in silico gene knock-ins [20]. These advantages position KMs as an especially practical platform for comparing different engineering strategies computationally. As a result, the field is seeing a renewed push toward building and improving kinetic models to support more informed design decisions [21,22].

Although a GSM of *S. thermophilus* has been published [23], there is no KM of *S. thermophilus* published to-date. As such, this study aims to construct a KM of *S. thermophilus* STH_CIRM_65 using *ab initio* approach by identifying enzymes from its genome, and identifying the corresponding reaction from KEGG (Kyoto Encyclopedia of Genes and Genomes) [24]. The result is a whole cell KM of *S. thermophilus* STH_CIRM_65, named as stheVS26, using the nomenclature proposed by Cho and Ling [25], which consists of 322 metabolites, 400 enzymes with corresponding transcriptions and translations, and 336 enzymatic reactions.

Materials and Methods

Identification of Reactome

The genome of *Streptococcus thermophilus* STH_CIRM_65 (NCBI RefSeq assembly GCF_903886475.1; NCBI GenBank Accession NZ_LR822015.1) was used as source to identify enzymatic genes using the process previously described [20,26,27]. Briefly, each enzymatic gene was identified as a presence of complete Enzyme Commission (EC) number in the GenBank record and mapped into reaction IDs via KEGG Ligand Database for Enzyme Nomenclature [24]. For example, EC 1.1.1.23 (https://www.genome.jp/entry/1.1.1.23) catalyses reactions R01158, R01163, and R03012; where the substrates and products of each rection can be identified.

Model development

The model was developed using the principles described in Sim., *et al.* [28]. BioNumbers estimates indicate that an *E. coli* cell contains roughly 3000 RNA polymerase molecules (BioNumbers 106199) [29], with around one quarter in an active state (BioNumbers 111676) [30]. At a polymerization speed of 22 nucleotides per second (BioNumbers 104109) [31] and a nucleotide mass of 339.5 Da, this equates to an RNA output of about 5600 kDa per second or 9.3e-18 grams per second. Dividing this by a cell volume of 7e-16 litres [32] and 4225 coding genes (BioNumbers 105443) [33] yields a transcription rate of 2.92 micromolar per gene per second. Using an average stability of 107.56 seconds (BioNumbers 107666) [34] (0.93% decay per second), we obtain the differential equation: d[mRNA]/dt = 0.00292 - 0.0093[mRNA]. For translation, mammalian data suggest 0.278 peptides produced per transcript per second (BioNumbers 106382) [35], while protein turnover in *E. coli* occurs at 1% per hour (2.78×10^{-6}/s) (BioNumbers 109924) [36]. Thus: d[peptide]/dt = 0.278[mRNA] - 0.00000278[peptide]. The pathway network was then encoded as ODEs [26,37] with median enzymatic parameters from Bar-Even., *et al*. (kcat = 13.7 per second; Km = 1 mM) [38], following AdvanceSyn's model format [39].

Model simulation

The constructed model was tested for simulatability using AdvanceSyn Toolkit [39]. Initial concentrations of all mRNA and enzymes were set to 0 mM. Initial concentrations of all metabolites were set to 1 mM except the following which were set to 1000 mM: (I) C00001 (Water), (II) C00002 (ATP), (III) C00025 (L-Glutamate), (IV) C00031 (D-Glucose), (V) C00037 (Glycine), (VI) C00041 (L-Alanine), (VII) C00047 (L-Lysine), (VIII) C00049 (L-Asparate), (IX) C00051 (Glutathione), (X) C00064 (L-Glutamine), (XI) C00065 (L-Serine), (XII) C00073 (L-Methionine), (XIII) C00097 (L-Cysteine), (XIV) C00133 (D-Alanine), (XV) C00148 (L-Proline). The model was simulated using the fourth-order Runge-Kutta method [40,41] from time zero to 3600 seconds with timestep of 0.1 second, and the concentrations of metabolites were bounded between 0 millimolar and 1000 millimolar. The simulation results were sampled every 2 seconds.

Results and Discussion

The annotated genome of *Streptococcus thermophilus* STH_CIRM_65 consists of 2181 gene; of which, 2009 are protein coding sequences. 400 unique EC numbers consisting of 336 enzymatic reactions involving 322 metabolites were identified and developed into a model based on AdvanceSyn Model Specification [39]. In addition, 800 ODEs acting as placeholder for enzyme transcriptions and translations were added.

Using the AdvanceSyn Toolkit [39], we simulated the stheVS26 whole-cell model and confirmed successful execution, as documented in Figure 1. This confirms that the model is correctly assembled and free from syntax errors as previously argued [20,27,42-46], which may often derail large-scale kinetic constructions. The model outputs suggest that phosphatidyl-glycerophosphate is produced then used before stabilizing at a lower concentration; however, this stems directly from our use of median enzyme parameters across all reactions [47], which standardize behaviour and remove biological nuance. Rather than a biological conclusion, this discrepancy highlights the importance of future kinetic tuning. The contribution here is a fully functioning kinetic model of *S. thermophilus* STH_CIRM_65 that researchers may adopt as a platform for implementing organism-specific values, integrating downstream pathways, or examining global allocation strategies under different environmental conditions [48-50], or as a system to examine cellular resource allocations [51-54].

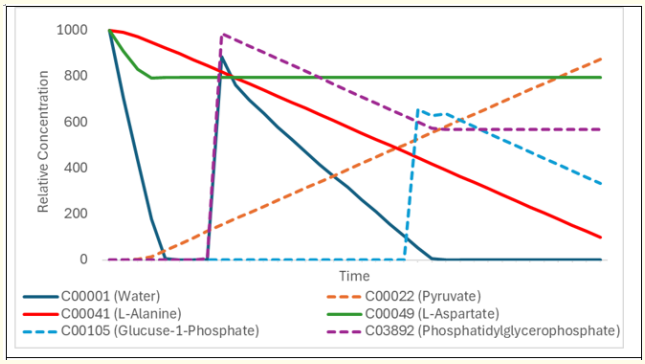

Figure 1: Selection of Simulation Results.

Conclusion

We present an *ab initio* whole cell kinetic model of *Streptococcus thermophilus* STH_CIRM_65 consisting 336 enzymatic reactions involving 322 metabolites.

Supplementary Materials

Reaction descriptions and model can be download from https://bit.ly/stheVS26.

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Bibliography

1. Roux E., *et al*. "The Genomic Basis of the Streptococcus thermophilus Health-Promoting Properties". *BMC Genomics* 23.1 (2022): 210.

2. Abubakr RAH., *et al*. "Genetic and Biotechnological Characterization of Folate-Producing Probiotics Isolated from Local Dairy Products". *Beni-Suef University Journal of Basic and Applied Sciences* 14.1 (2025): 62.

3. Yu P., *et al*. "Short Communication: Lactose Utilization of Streptococcus thermophilus and Correlations with β-Galactosidase and Urease". *Journal of Dairy Science* 103.1 (2020): 166-171.

4. Auestad N and Layman DK. "Dairy Bioactive Proteins and Peptides: A Narrative Review". *Nutrition Reviews* 79.2 (2021): 36-47.

5. Helal A., *et al*. "Effect of Fermentation with Streptococcus thermophilus Strains on In Vitro Gastro-Intestinal Digestion of Whey Protein Concentrates". *Microorganisms* 11.7 (2023): 1742.

6. Ayala-Niño A., *et al*. "Whey-Derived Antihypertensive Peptides Produced by Proteinase K Hydrolysis and Fermentation". *Waste and Biomass Valorization* (2025).

7. Vinderola G., *et al*. "The Concept of Postbiotics". *Foods (Basel, Switzerland)* 11.8 (2022): 1077.

8. Allouche R., *et al*. "Streptococcus thermophilus: A Source of Postbiotics Displaying Anti-Inflammatory Effects in THP 1 Macrophages". *MDPI Molecules* 29.7 (2024): 1552.

9. Pattapulavar V., *et al*. "Probiotic-Derived Postbiotics: A Perspective on Next-Generation Therapeutics". *Frontiers in Nutrition* 12 (2022): 1624539.

10. De Vuyst L., *et al*. "Exopolysaccharide-Producing Streptococcus thermophilus Strains as Functional Starter Cultures in the Production of Fermented Milks". *International Dairy Journal* 13.8 (2003): 707-717.

11. Broadbent JR., *et al*. "Biochemistry, Genetics, and Applications of Exopolysaccharide Production in Streptococcus thermophilus: A Review". *Journal of Dairy Science* 86.2 (2003): 407-423.

12. Sørensen KI., *et al*. "Enhancing the Sweetness of Yoghurt through Metabolic Remodeling of Carbohydrate Metabolism in Streptococcus thermophilus and Lactobacillus delbrueckii subsp. Bulgaricus". *Applied and Environmental Microbiology* 82.12 (2016): 3683-3692.

13. Chaves ACSD., *et al*. "Metabolic Engineering of Acetaldehyde Production by Streptococcus thermophilus". *Applied and Environmental Microbiology* 68.11 (2002): 5656-5662.

14. Kong L., *et al*. "CRISPR/dCas9-Based Metabolic Pathway Engineering for the Systematic Optimization of Exopolysaccharide Biosynthesis in Streptococcus thermophilus". *Journal of Dairy Science* 105.8 (2022): 6499-6512.

15. Khanijou JK., *et al*. "Metabolomics and Modelling Approaches for Systems Metabolic Engineering. *Metabolic Engineering Communications* 15 (2022): e00209.

16. Gudmundsson S., *et al*. "Recent Advances in Model-Assisted Metabolic Engineering". *Current Opinion in Systems Biology* 28 (2022): 100392.

17. Richelle A., *et al*. "Towards a Widespread Adoption of Metabolic Modeling Tools in Biopharmaceutical Industry: A Process Systems Biology Engineering Perspective". *npj Systems Biology and Applications* 6 (1 (2020): 6.

18. Lee YQ., *et al*. "Genome-scale metabolic model-guided systematic framework for designing customized live biotherapeutic products". *NPJ Systems Biology and Applications* 11.1 (2025): 73.

19. Prabhu S., *et al*. "Derivative-Free Domain-Informed Data-Driven Discovery of Sparse Kinetic Models. *Industrial and Engineering Chemistry Research* 64.5 (2025): 2601-2615.

20. Yeo KY., *et al*. "Ab Initio Whole Cell Kinetic Model of Yarrowia lipolytica CLIB122 (yliYKY24)". *Medicon Medical Sciences* 8.4 (2025): 01-06.

21. Foster CJ., *et al*. "Building Kinetic Models for Metabolic Engineering". *Current Opinion in Biotechnology* 67 (2021): 35-41.

22. Lázaro J., *et al*. "Enhancing genome-scale metabolic models with kinetic data: resolving growth and citramalate production trade-offs in Escherichia coli". *Bioinformatics Advances* 5 (1 (2025): vbaf166.

23. Rau MH., *et al*. "Genome-Scale Metabolic Modeling Combined with Transcriptome Profiling Provides Mechanistic Understanding of Streptococcus thermophilus CH8 Metabolism". *Applied and Environmental Microbiology* 88 (16 (2022): e0078022.

24. Okuda S., *et al*. "KEGG Atlas mapping for global analysis of metabolic pathways". *Nucleic Acids Research* 36 (2008): W423-W426.

25. Cho JL and Ling MH. "Adaptation of Whole Cell Kinetic Model Template, UniKin1, to Escherichia coli Whole Cell Kinetic Model, ecoJC20". *EC Microbiology* 17 (2 (2021): 254-260.

26. Kwan ZJ., *et al*. "Ab Initio Whole Cell Kinetic Model of Stutzerimonas balearica DSM 6083 (pbmKZJ23)". *Acta Scientific Microbiology* 7.2 (2024): 28-31.

27. Maiyappan S., *et al*. "Four Ab Initio Whole Cell Kinetic Models of Bacillus subtilis 168 (bsuLL25) 6051-HGW (bshSM25), N33 (bsuN33SS25), FUA2231 (bsuGR25)". *Journal of Clinical Immunology and Microbiology* 6.2 (2025): 1-6.

28. Sim BJH., *et al*. "Multilevel Metabolic Modelling Using Ordinary Differential Equations". Encyclopedia of Bioinformatics and Computational Biology (Second Edition), eds Ranganathan S, Cannataro M, Khan AM (Elsevier, Oxford) (2025): 491-498.

29. Müller-Hill B. "The lac Operon: A Short History of a Genetic Paradigm (Berlin, Germany)". (1996).

30. Churchward G., *et al*. "Transcription in Bacteria at Different DNA Concentrations". *Journal of Bacteriology* 150.2 (1982): 572-581.

31. Gray WJ and Midgley JE. "The Control of Ribonucleic Acid Synthesis in Bacteria. The Synthesis and Stability of Ribonucleic Acid in Rifampicin-Inhibited Cultures of Escherichia coli". *The Biochemical Journal* 122.2 (1971): 161-169.

32. Kubitschek HE. "Cell Volume Increase in Escherichia coli After Shifts to Richer Media". *Journal of Bacteriology* 172.1 (1990): 94-101.

33. Hu P., *et al*. "Global Functional Atlas of Escherichia coli Encompassing Previously Uncharacterized Proteins". *PLoS Biology* 7.4 (2009): e96.

34. So L-H., *et al*. "General Properties of Transcriptional Time Series in Escherichia coli". *Nature Genetics* 43.6 (2011): 554-560.

35. Schwanhäusser B., *et al*. "Corrigendum: Global Quantification of Mammalian Gene Expression Control". *Nature* 495.7439 (2013): 126-127.

36. Maurizi MR. "Proteases and Protein Degradation in Escherichia coli". *Experientia* 48.2 (1992): 178-201.

37. Murthy MV., *et al*. "UniKin1: A Universal, Non-Species-Specific Whole Cell Kinetic Model". *Acta Scientific Microbiology* 3.10 (2020): 04-08.

38. Bar-Even A., *et al*. "The Moderately Efficient Enzyme: Evolutionary and Physicochemical Trends Shaping Enzyme Parameters". *Biochemistry* 50.21 (2011): 4402-4410.

39. Ling MH. "AdvanceSyn Toolkit: An Open Source Suite for Model Development and Analysis in Biological Engineering". *MOJ Proteomics & Bioinformatics* 9.4 (2020): 83-86.

40. Yong B. "The Comparison of Fourth Order Runge-Kutta and Homotopy Analysis Method for Solving Three Basic Epidemic Models". *Journal of Physics: Conference Series* 1317 (2019): 012020.

41. Ling MH/ "COPADS IV: Fixed Time-Step ODE Solvers for a System of Equations Implemented as a Set of Python Functions". *Advances in Computer Science: An International Journal* 5.3 (2016): 5-11.

42. Saisudhanbabu T., *et al*. "Ab Initio Whole Cell Kinetic Model of Limosilactobacillus fermentum EFEL6800 (lfeTS24)". *EC Clinical and Medical Case Reports* 8.4 (2025): 01-04.

43. Arivazhagan M., *et al*. "Ab Initio Whole Cell Kinetic Model of Bifidobacterium bifidum BGN4 (bbfMA24). *Acta Scientific Nutritional Health* 9.1 (2025): 42-45.

44. Senthilkumar A., *et al*. "Ab Initio Whole Cell Kinetic Model of Lactobacillus acidophilus NCFM (lacAS24). *Journal of Clinical Immunology and Microbiology* 6.1 (2025): 1-5.

45. Wong TB., *et al*. "Ab Initio Whole Cell Kinetic Models of Escherichia coli BL21 (ebeTBSW25) and MG1655 (ecoMAL25)". *Scholastic Medical Sciences* 3.22 (2025): 01-04.

46. Ambel WB., *et al*. "UniKin2 - A Universal, Pan-Reactome Kinetic Model. *International Journal of Research in Medical and Clinical Science* 3.2 (2025): 77-80.

47. Bar-Even A., *et al*. "The Moderately Efficient Enzyme: Futile Encounters and Enzyme Floppiness". *Biochemistry* 54.32 (2015): 4969-4977.

48. Ahn-Horst TA., *et al*. "An Expanded Whole-Cell Model of E. coli Links Cellular Physiology with Mechanisms of Growth Rate Control". *npj Systems Biology and Applications* 8.1 (2022): 30.

49. Chagas M da S., *et al*. "Boolean Model of the Gene Regulatory Network of Pseudomonas aeruginosa CCBH4851". *Frontiers in Microbiology* 14 (2023): 1274740.

50. Hao T., *et al*. "Reconstruction of Metabolic-Protein Interaction Integrated Network of Eriocheir sinensis and Analysis of Ecdysone Synthesis". *Genes* 15.4 (2024): 410.

51. Thornburg ZR., *et al*. "Fundamental Behaviors Emerge From Simulations of a Living Minimal Cell". *Cell* 185.2 (2022): 345-360.e28.

52. Bianchi DM., *et al*. "Toward the Complete Functional Characterization of a Minimal Bacterial Proteome". *The Journal of Physical Chemistry B* 126.36 (2022): 6820-6834.

53. Sun G., *et al*. "Cross-Evaluation of E. coli's Operon Structures via a Whole-Cell Model Suggests Alternative Cellular Benefits for Low- Versus High-Expressing Operons". *Cell Systems* 15.32 (2024): 27-245.e7.

54. Choi H and Covert MW. "Whole-cell modeling of E. coli confirms that in vitro tRNA aminoacylation measurements are insufficient to support cell growth and predicts a positive feedback mechanism regulating arginine biosynthesis". *Nucleic Acids Research* 51.12 (2023): 5911-5930.