Mysore J. Agric. Sci., 59 (4) : 414-424 (2025)

Cloning, Sequencing and Structure Prediction of *tHMG1* Gene from Auxotrophic Mutant Yeast *Saccharomyces cerevisiae* Strain BY4741

S. Vimanth[1], T. M. Ningaraju[2], C. N. Lakshminarayana Reddy[3], K. N. Chandrashekhara[4], Anitha Peter[5] and S. Shyamalamma[6]

[1,2,5&6]Department of Plant Biotechnology, [3]Department of Plant Pathology, College of Agriculture, UAS, GKVK, Bengaluru - 560 065, [4]Shriram Institute for Industrial Research, Whitefield, Bengaluru - 560 048

e-Mail : vimanth98@gmail.com

Authors Contribution

S. Vimanth :
Conceptualization, carried out experiment and draft preparation

T. M. Ningaraju;
C. N. Lakshminarayana Reddy &
K. N. Chandrashekhara :
Conceptualization, technical, assistance, methodology, draft correction and supervision

Anitha Peter &
S. Shyamalamma :
Editing and guidance

Corresponding Author **:**
S. Vimanth

Received : March 2025
Accepted : April 2025

Abstract

tHMG1 gene, a truncated variant of *HMG1* was successfully cloned, sequenced and its structure was predicted from *Saccharomyces cerevisiae* BY4741. The *HMG1* gene encodes 3-hydroxy-3-methylglutaryl-coenzyme. A reductase (HMG-CoA reductase), a key enzyme in the mevalonate pathway, crucial for synthesizing essential is oprenoids. The full-length *HMG1* gene, comprising 3165 base pairs with distinct regulatory and catalytic domains is subject to feedback inhibition. The truncated *tHMG1* lacks the regulatory domain, potentially enhancing mevalonate synthesis, which is vital for producing high-value terpenes. The *tHMG1* gene was amplified using PCR, cloned into the pTZ57R/T vector and transformed into *E. coli DH5į* cells. Successful cloning was confirmed by restriction digestion and sequencing, revealing a 100 per cent sequence identity with the *HMG1* gene available at NCBI database. Further analysis using InterPro identified conserved domains and functional motifs, including the core HMG-CoA reductase domain. The 3D structure of *tHMG1* was predicted using the Swiss-Model server, showcasing a structure with *tHMG1* specific architecture comprising of a prominent alpha-helical domain connected to a mixed alpha/beta domain. The predicted structure was assessed using Ramachandran plot, with 91.71 per cent residues in favoured regions, confirming the reliability of the model. This study lays the foundation for further functional aspects of *tHMG1* gene and its potential industrial applications in enhancing terpene production.

Keywords : *Saccharomyces cerevisiae*, tHMG1 (truncated 3-Hydroxy-3-Methylglutaryl-Coenzyme A reductase 1), Auxotrophic mutant, Ramachandran plot

3 - Hydroxy - 3 - Methylglutaryl - Coenzyme A Reductase (HMGR, EC 1.1.1.34), also known as Hydroxy-Methylglutaryl-Coenzyme A Reductase (NADPH), is the key enzyme controlling the rate of the sterol biosynthetic pathway (Ma *et al*., 2011). This pathway is responsible for producing all isoprene-based compounds, including sterols, ubiquinone, dolichol and isopentenylated adenosine. Since these end products play vital roles in processes like membrane formation, electron transport, glycoprotein synthesis, translation and DNA replication, HMG-CoA reductase activity levels can regulate a variety of cellular functions (Lu *et al*., 2022). Wadhwa *et al.* (2019) reported that the yeast cells have two HMGRs encoded by a pair of paralogous genes *HMG1* and *HMG2*, Even though they can substitute for each other in supplying sufficient HMGR activity when the other gene is deleted, there are significant differences in their regulation. *HMG1* is a stable protein controlled at the transcriptional and

translational levels, whereas *HMG2* is controlled post translationally. *HMG1* is the primary HMGR during aerobic growth. Such conditions stimulate synthesis of heme, which activates HMG1 transcription *via.,* the transcription factor Hap1p. On the other hand, transcription of *HMG2* is repressed during aerobic growth by an unknown mechanism. The activity of Hmg1p is also controlled by a negative feedback mechanism at the level of translation. Depletion of mevalonate leads to accumulation of *HMG1* and an increased HMGR activity without an increase in *HMG1* transcription. This effect depends on 5'-untranslated region of HMG1 mRNA, but the exact mechanisms remain unknown. HMGRs are highly conserved and the human homolog HMGR can complement both hmg1 and hmg2 mutations in yeast. In humans, the HMGR activity catalyzes a rate-limiting step in biosynthesis of cholesterol, an equivalent of ergosterol in yeast and is a target of cholesterol-lowering drugs such as statins (Su *et al.,* 2024).

Shalu *et al.* (2024) stated that the microbial production of terpenes has garnered significant attention due to their diverse applications. While various organisms, including *E. coli*, *Bacillus subtilis*, *Corynebacterium glutamicum* and even algae like *Phormidium autumnale*, have been explored. *Saccharomyces cerevisiae* stands out as a particularly promising host. Among the reported production levels, *S. cerevisiae* demonstrates the highest squalene production, reaching up to 10 g/L through optimized metabolic engineering and fermentation strategies, significantly exceeding the yields observed in other organisms. While bacteria like *E. coli* and *Bacillus subtilis* have been employed for terpene production, their yields are generally lower compared to yeast, often due to differences in cellular machinery and tolerance to terpene accumulation. Yeast, particularly *S. cerevisiae* and *Yarrowia lipolytica*, offer several advantages over prokaryotic hosts for terpene production, including robust eukaryotic metabolism, inherent compatibility with complex metabolic pathways and higher tolerance to product accumulation, enabling significantly greater yields (Moser *et al.,* 2019). The ability of yeast to perform complex post-translational modifications, coupled with their robust fermentation capabilities, makes them superior hosts for the production of valuable terpenes when compared to prokaryotes, which lack these features. Specifically, the native *tHMG1* gene, encoding a key enzyme in the mevalonate pathway, is crucial for efficient terpene biosynthesis in yeast. This is why isolation and optimization of native *tHMG1* from yeast is a vital aspect of research aimed at enhancing terpene production. Therefore, the focus on yeast, and particularly leveraging its native *tHMG1* gene, as a production platform is justified by its potential to achieve industrially relevant terpene titers.

The mevalonate pathway is a crucial metabolic route responsible for the biosynthesis of essential isoprenoids, including sterols like ergosterol in yeast, as well as a diverse array of other compounds such as terpenes, which hold significant industrial value. A key regulatory point within this pathway is 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), encoded by the *HMG1* gene. In its full-length form, *HMG1* (3-hydroxy-3-methyl-glutaryl CoA reductase) gene from *S. cerevisiae* is 3165 bp in length. It contains N-terminal regulatory region (corresponds to 1-1590 bp) and C-terminal catalytic region (corresponds to 1591-3165 bp) (Wadhwa *et al.*, 2019). *HMG1* catalyzes the conversion of HMG-CoA to mevalonate, a rate-limiting step subject to feedback inhibition by mevalonate itself. This feedback mechanism ensures tight control over mevalonate production, preventing over accumulation of this crucial intermediate. However, for applications such as the production of high-value terpenes, a sustained and elevated flux of mevalonate is often desired. This limitation posed by the feedback-regulated full-length HMG1 can be overcome by utilizing a truncated version of the enzyme, *tHMG1*. This truncated form, typically generated through genetic engineering, lacks the regulatory domain responsible for mevalonate feedback inhibition. Consequently, *tHMG1* allows for increased

mevalonate synthesis, even in the presence of high mevalonate concentrations, providing a crucial advantage for metabolic engineering strategies aimed at enhancing terpene production. Considering the importance of the *tHMG1* gene this study focuses on the cloning, sequencing and structural prediction of the *tHMG1* gene from *Saccharomyces cerevisiae* BY4741.

Material and Methods

Strains and Growth Medium

The strains and plasmids used in this study are listed in Table 1 and the primers are shown in Table 2 and Cloning vector details are mentioned in Table 3. The initial strain used in this study was BY4741 (MATa his3Δ1 leua2Δ0 met15Δ0 ura3Δ0), which was cultivated in YPD (1% yeast extract, 2% peptone (Himedia) and 2% glucose) medium and kept for incubation at 30° C, media preparation for yeast was done as per Bharti *et al.* (2019). *E. coli I* strain *DH5α* was used for plasmid propagation, maintenance and the transformed cells were grown at 37°C in Luria-Bertani (LB) medium (5 g/L yeast extract, 10 g/L tryptone and 10 g/L NaCl; 20 g/L agar was added for solid medium) with 50 µg/ml ampicillin.

Reagents and Chemicals

All the reagents and chemicals used in the present study were of analytical and molecular grade and purchased either from Sigma-Aldrich, USA or HiMedia Laboratories, India, Ethidium bromide, isopropanol were from Sigma chemicals. Agar, Luria bertani (LB) agar, LB broth, sodium chloride (NaCl), ethylene diamine tetra acetic acid (EDTA), magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$), tris-hydrochloric acid (HCl), tris-base, β-Mercaptoethanol, yeast nitrogen base, tryptone, yeast extract powder, lyticase, dextrose, glycerol were from Hi-media labs. chloroform, absolute Ethanol. The DNA ladder and nuclease free water were from Bangalore Genie Pvt. Ltd. Antibiotic used in the present study was ampicillin from Sigma-Aldrich.

Cloning of Truncated HMG-CoA Reductase Gene (*tHMG1*) Gene

In order to clone the gene encoding *tHMG1*, the genomic DNA of *Saccharomyces cerevisiae* S288C-derivative laboratory strain BY4741 was used as template for PCR. The *tHMG1* was amplified using Taq DNA polymerase in a reaction mixture containing template Genomic DNA, deoxy nucleotides, reaction buffer and the pair of forward with *BamH1* restriction sites and reverse primers with *EcoRI* restriction sites *viz.,* 52-CGGGATCCATGGACCAATTGGTGAAAA CTGAAG-32 and 52-CGGAATTCGTTAGGAT TTAATGCAGGTGACG-32. The thermal profile for carrying out PCR was as follows: 1 cycle at 94 °C for 5 min; 35 cycles at 94°C for 30 min; 55.8 °C for 30 sec; 72 °C for 1.10 min and 1 cycle at 72 °C for 7 min and PCR product was subjected to electrophoresis using 0.8 per cent agarose gel, gel extracted and amplified PCR product was cloned in pTZ57R/T vector by T/A cloning and mobilized into *E. coliDH5α*, by Heat shock method

Table 1

Strains and plasmids used in this study

Strain	Host Strain	Description	Source
E. Coli DH5a	*E. Coli*	recA1, endA1,recA1 mutations	This Study
BY4741	S288C-derivative laboratory strain	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	This Study

Table 2

Primers used for cloning

Primer	Sequence	Source
tHMG1-fwd	CGGGATCCATGGACCAATTGGTGAAAACTGAAG	This study
tHMG1-rws	CGGAATTCGTTAGGATTTAATGCAGGTGACG	This study

Table 3
Vector used for cloning

Plasmid	Description	Source
pTZ57R/T	Cloning Vector	This Study

of transformation. The transformed cells were dispensed on LB (Luria-Bertani) agar plate containing ampicillin (50 mg/mL), Xgal (20 mM) and IPTG (200 mg/ml). White colonies containing recombinant plasmid were selected and extracted. The recombinant cloning vector (pTZ57R/T + *tHMG1*) was isolated by using Qiagen plasmid isolation kit (QIAprep® Spin Miniprep Kit). The plasmid DNA concentration and purity (A260/A280) were analysed using multi-mode microplate spectrophotometer (Synergy-HTX. BioTek).

Restriction Digestion Analysis of the Purified pTZ57R/T + *tHMG1* Vector

PCR Amplified t*HMG1* ligated with pTZ57R/T was double digested with *BamH1* and *EcoRI* restriction enzymes. Accordingly, Double digestion was performed with the compatible buffers (10 x cut smart buffer) yielding 100 per cent activity of the enzymes at 37°C for 60 min and heat-inactivated at 65°C for 20 min.

Sequencing and Alignment

The isolated recombinant construct (pTZ57R/T + *tHMG1*) of concentration 100ng/µL was sent to Barcode Biosciences, (An ISO 9001:2015 Certified Company), for Sanger sequencing and sequenced *tHMG1* was submitted to NCBI data base (https://www.ncbi.nlm.nih.gov/search/) with accession No. (PQ144891). The obtained *tHMG1* gene sequence was made to multiple sequence alignment (MSA) using Clustal Omega (https://www.ebi.ac.uk/jdispatcher/msa/clustalo) with previously reported *HMG1* gene sequences available in the NCBI database. This comparative analysis included sequences submitted by various researchers, representing different *Saccharomyces cerevisiae* strains and related yeast species. The MSA aimed to assess the sequence conservation of *tHMG1* within the broader *HMG1* family and to identify any variations present in the sequenced *tHMG1* gene and potentially reveal regions of interest, such as conserved domains or motifs that might be relevant to its function or truncated nature. This comparison facilitated by Clustal Omega, provided valuable context for interpreting the *tHMG1* sequence and its potential functional implications.

Domain Search of *tHMG1*

Following sequencing, the *tHMG1* gene sequence was rigorously assessed for quality using *e.g.,* Phred scores, base calling accuracy visualization. High-quality sequence reads were then assembled and the resulting *tHMG1* gene sequence was translated *insilico* into its corresponding amino acid sequence using ExPASy Translate tool (https://web.expasy.org/translate/). To identify conserved domains and functional motifs within the predicted *tHMG1* protein, the amino acid sequence was analysed using the InterPro online software (https://www.ebi.ac.uk/interpro/). This analysis allowed for the identification of known domains characteristic of HMG-CoA reductases, providing insights into the potential functional roles of *tHMG1* in the mevalonate pathway.

Insilco Structure Prediction of *tHMG1*

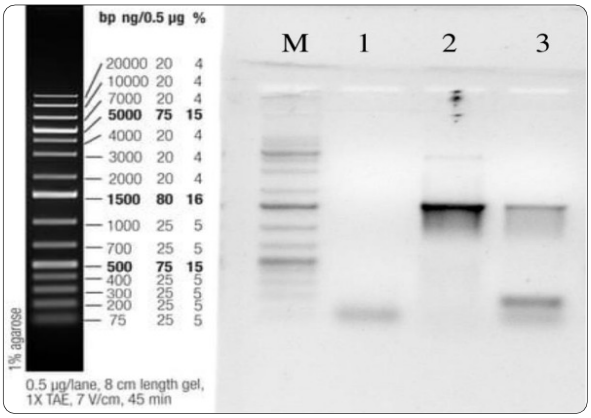
To gain insights into the three-dimensional structure of the tHMG1 protein, *insilico* modeling was performed using the Swiss-Model server (https://swissmodel.expasy.org/). This approach leverages homology modeling principles, utilizing known protein structures as templates to predict the structure of the target protein. The amino acid sequence of *tHMG1* was submitted to Swiss-Model, which identified suitable templates from the Protein Data Bank (PDB) based on sequence similarity. The resulting predicted protein structure was then evaluated using Ramachandran plot also generated by Swiss-Model. This plot, which visualizes the distribution of phi (φ) and psi (ψ) dihedral angles in the protein's backbone, served to assess the stereochemical quality and overall reliability of the

predicted model. Analysis of the Ramachandran plot allowed for the identification of residues falling within energetically favourable regions, providing confidence in the accuracy and validity of the generated *tHMG1* structure for further analysis and interpretation.

Results and Discussion

Amplification of *tHMG1* Gene by PCR and Gel Electrophoresis

The *tHMG1* gene was successfully amplified from genomic DNA of *Saccharomyces cerevisiae* strain BY4741 using gene-specific primers designed to target the truncated region. Agarose gel electrophoresis (0.8%) confirmed the amplification, revealing a distinct band of approximately 1578 base pairs and it was consistent with the expected size of the *tHMG1* gene (Fig. 1). This result indicates the successful isolation and amplification of the target gene, suggesting that the truncated *tHMG1* sequence is present in the studied *Saccharomyces cerevisiae* BY4741 strain. The clear band observed at the expected size validates the specificity of the primers and the integrity of the amplified product, providing a solid foundation for subsequent analyses, such as sequencing and downstream characterization of the *tHMG1* gene (Karaca *et al.,* 2024).



Lane M : 1 kb + DNA ladder, Lane 1: Non-template control, Lane 2; 3 PCR amplified *tHMG1* gene (1578bp)

Fig. 1 : Agarose gel electrophoresis of PCR amplified *tHMG1* gene

Transformation and Confirmation of *tHMG1* through Restriction Digestion

The *tHMG1* gene, amplified as a 1578 bp fragment, was successfully cloned into the pTZ57R/T vector using TA cloning. Following overnight ligation with T_4 DNA ligase at a 1:3 molar ratio of vector to insert. Fig. 2 showing *E. coli* DH5α cells which were transformed using the heat-shock method as described in the literature by Kiran and Ningaraju (2022). Colony PCR was performed on both blue and white colonies to screen for the presence of the *tHMG1* insert (Fig. 3). White colonies, presumptive positives from the blue/ white screening were further analysed. Plasmid DNA was extracted from selected white colonies and the presence of the *tHMG1* insert was confirmed through double digestion with *BamHI* and *EcoRI*. Gel electrophoresis of the digested plasmids revealed distinct bands at approximately 2887 bp corresponding to the linearized pTZ57R/T vector and 1578 bp consistent with the size of the *tHMG1* insert (Fig. 4). The appearance of these bands confirmed the successful ligation of the *tHMG1* gene into the pTZ57R/T vector and the release of the insert from the recombinant plasmid, validating the cloning procedure, similar results was reported by Chaturvedi *et al.* (2021).

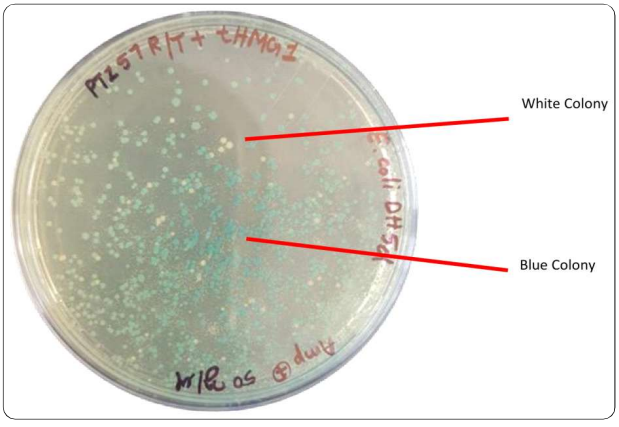
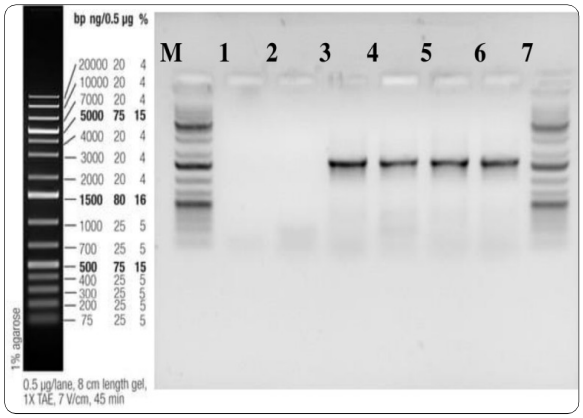
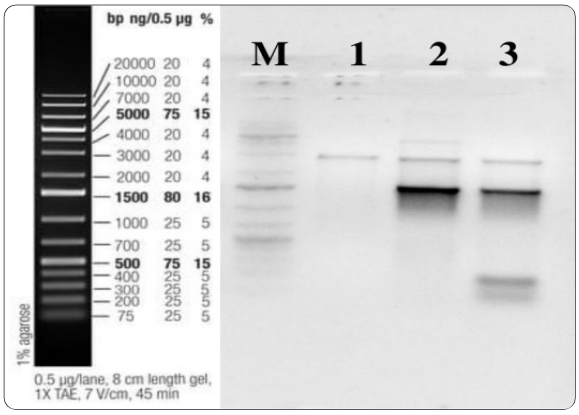


Fig. 2 : Blue-white colonies after transformation of pTZ57R/T + *tHMG1* gene



Lane M : 1 kb + DNA ladder, Lane 1 : Non-Template Control, Lane 2 : Blue Colony (Negative transformed colony), Lane 3, 4, 5, 6, 7 : Confirmed *tHMG1* colony PCR amplified *tHMG1* bands (1578bp)

Fig. 3 : Agarose gel electrophoresis of colony PCR of transformed colonies.



Lane M: 1 kb + DNA ladder, Lane 1: pTZ57R/T+ *tHMG1*(Supercoiled), Lane 2;3: Restriction digestion of pTZ57R/T+ *tHMG1* with *BamH1*and *EcoRI*

Fig. 4 : Restriction digestion of the recombinant pTZ57R/T+ *tHMG1* plasmid

Sequencing and Multiple Sequence Alignment of *tHMG1*.

Sequencing results indicates that the sequences of *tHMG1* gene were fine and no mutation occurred during the process of amplification, cloning and transformation.

ATGGACCAATTGGTGAAAACTGAAGTCAC
CAAGAAGTCTTTTACTGCTCCTGTACA
AAAGGCTTCTACACCAGTTTTAACCAAT
AAAACAGTCATTTCTGGATCGAAAGT
CAAAAGTTTATCATCTGCGCAATCGA
GCTCATCAGGACCTTCATCATCTAGTGA
GGAAGATGATTCCCGCGATATTGAAAGCT
TGGATAAGAAAATACGTCCTTTAGAAGAAT
TAGAAGCATTATTAAGTAGTGGAAATAC
AAAACAATTGAAGAACAAAGAGGTCGC
TGCCTTGGTTATTCACGGTAAGTTAC
CTTTGTACGCTTTGGAGAAAAAATTAGGTGAT
ACTACGAGAGCGGTTGCGGTACGTAGGAAG
GCTCTTTCAATTTTGGCAGAAGCTCCTGTAT
TAGCATCTGATCGTTTACCATATAAAAATTA
TGACTACGACCGCGTATTTGGCGCTTGTTGTGA
AAATGT TATAGGTTACATGCCTTTGCCCGTT
GGTGTTATAGGCCCCTTGGTTATCGATGGT
ACATCTTATCATATACCAATGGCAACTACAG
AGGGTTGTTTGGTAGCTTCTGCCATGCGTGG
CTGTAAGGCAATCAATGCTGGCGGTGGTGCA
ACAACTGTTTTAACTAAGGATGGTATGACA
AGAGGCCCAGTAGTCCGTTTCCCAACTTTGA
AAAGATCTGGTGCCTGTAAGATATGGTTAGAC
TCAGAAGAGGGACAAAACGCAATTAAAAAA
GCTTTTAACTCTACATCAAGATTTGCACGTCT
GCAACATATTCAAACTTGTCTAGCAGGAG A
TTTACTCTTCATGAGATTTAGAACAACTACTG
GTGACGCAATGGGTATGAATATGATTTCTAAA
GGTGTCGAATACTCATTAAAGCAAATGGTAG
AAGAGTATGGCTGGGAAGATATGGAGGTTGT
CTCCGTTTCTGGTAACTACTGTACCGACAAA
AAACCAGCTGCCATCAACTGGATCGAAGGTC
GTGGTAAGAGTGTCGTCGCAGAAGCTACTATT
CCTGGTGATGTTGTCAGAAAAGTGTTAAAAAG
TGATGTTTCCGCATTGGTTGAGTTGAACATTG
CTAAGAATTTGGTTGGATCTGCAATGGCTGGG
TCTGTTGGTGGATTTAACGCACATGCAGCTAAT
TTAGTGACAGCTGTTTTCTTGGCATTAGGACA
AGATCCTGCACAAAATGTTGAAAGTTCCAAC
TGTATAACATTGATGAAAGAAGTGGACGGT
GATTTGAGAATTTCCGTATCCATGCCATCCAT
CGAAGTAGGTACCATCGGTGGTGGTACTGTT
CTAGAACCACAAGGTGCCATGTTGGACTTATT
AGGTGTAAGAGGCCCGCATGCTACCGCTCCT
GGTACCAACGCACGTCAATTAGCAAGAATAG
TTGCCTGTGCCGTCTTGGCAGGTGAATTATCC
TTATGTGCTGCCCTAGCAGCCGGCCATTTGGT
TCAAAGTCATATGACCCACAACAGGAAACTG

CTGAACCAACAAAACCTAACAATTTGGACGC
CACTGATATAAATCGTTTGAAAGATGGGTCC
GTCACCTGCATTAAATCCTAA

Multiple sequence alignment (MSA) was performed between the *tHMG1* sequence obtained from *Saccharomyces cerevisiae* strain BY4741 and the corresponding *HMG1* sequences available in the NCBI database. Fig. 5 showing alignment conducted using Clustal Omega, revealed a 100 per cent sequence identity between the cloned *tHMG1* and the NCBI *HMG1* sequence, This findings of ours were similar to the results reported by Xu *et al.* (2021). The absence of any sequence variations or point mutations indicates that the *tHMG1* gene cloned into the pTZ57R/T vector perfectly matched the reference sequence, confirming the fidelity of the cloning process and suggesting that the amplified *tHMG1* gene represents the expected target sequence without any introduced errors.

Computational Analysis of *tHMG1* : Domain Architecture and Structure Prediction

Analysis of the full length *HMG1* protein sequence using InterPro software revealed a variety of conserved domains and features, providing insights into its function and structure (Blum *et al.,* 2025). As shown in the Fig. 6, the most prominent feature is the core HMG-CoA reductase domain, which spans a significant portion of the protein. This domain is characteristic of HMG-CoA reductases and contains the active site responsible for catalysing the conversion of HMG-CoA to mevalonate. In addition to the catalytic domain, several other notable features are present *e.g.,* 'A sterol-sensing domain (SSD) is located near the N-terminus, suggesting a regulatory role in response to sterol levels'. The presence of transmembrane helices, particularly in the N-terminal region, indicates that *HMG1* is likely an integral membrane protein, consistent with its known localization to the endoplasmic reticulum. InterPro also identified regions of low complexity and potential coiled-coil domains, which may be involved in protein-protein interactions or structural stabilization. These findings corroborate the known features of *HMG1* and provide a comprehensive overview of its domain architecture, contributing to a deeper understanding of its catalytic mechanism and regulatory control within the mevalonate pathway (Kress *et al.,* 2023; Deng *et al*., 2015).

Fig. 5 : Multiple Sequence Alignment of *HMG1* gene sequence retrieved from NCBI and *tHMG1* gene sequenced from BY4741 strain Yeast

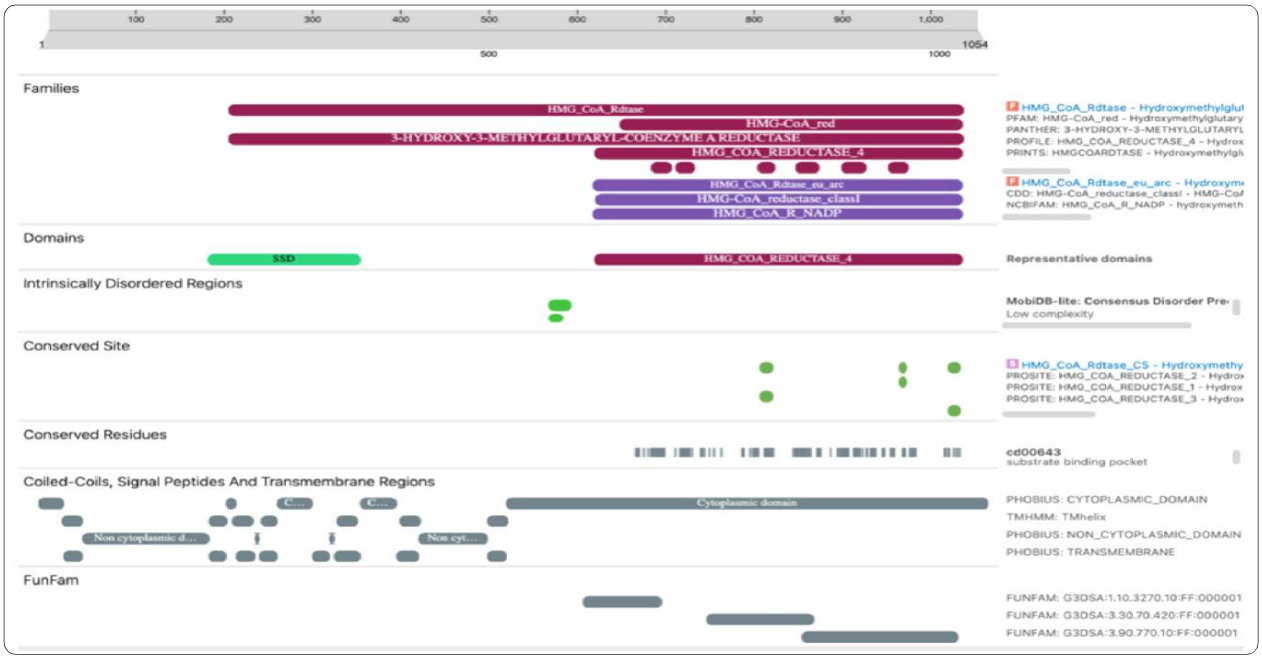


Fig. 6 : Domain architecture of the *tHMG1* protein, as identified by InterPro. This figure illustrates the various domains and functional motifs present in the HMG1 protein, including the catalytic domain and other relevant features

The three-dimensional structure of the *tHMG1* protein was predicted according to Sasidharan *et al*. (2022) using the Swiss-Model homology modeling server. Based on the amino acid sequence of *tHMG1*, Swiss-Model identified suitable templates from the Protein Data Bank (PDB) with similar sequences, enabling the construction of a reliable structural model. The predicted *tHMG1* structure exhibits a distinct architecture comprised of the secondary structure elements visible in the Fig. 7, *e.g.,* a prominent alpha-helical domain (shown in red and orange) connected to a mixed alpha/beta domain (shown in light blue and green). The specific features like the long red alpha helix or the cluster of orange helices, likely plays a crucial role in a potential function based on the structure, *e.g.,* substrate binding or enzymatic activity. Earlier reports by Azmi *et al.* (2023) supports our results that the model provides a valuable framework for understanding the structural determinants of *tHMG1* function and for designing future experiments to probe its catalytic mechanism and regulatory properties within the mevalonate pathway. Further analysis of the predicted structure, including assessment of its stereochemical quality using Ramachandran plots, strengthens the reliability of this model for subsequent structure-based investigations.

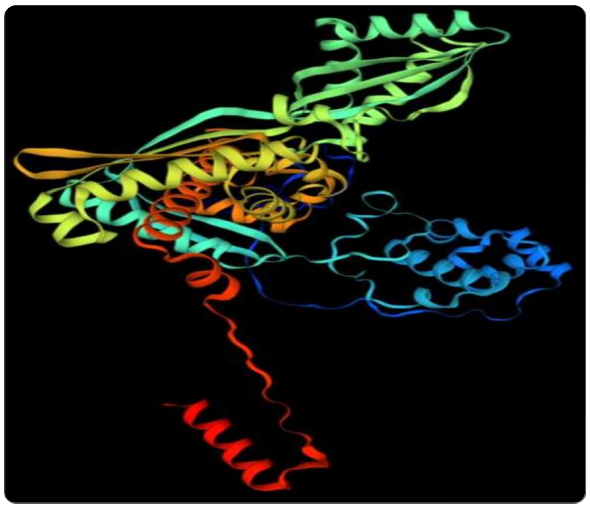


Fig. 7 : Predicted 3D structure of the *tHMG1* protein generated by Swiss-Model server. This figure shows the predicted three-dimensional structure of the *tHMG1* protein, highlighting its overall architecture and secondary structure elements

The predicted *tHMG1* structure was assessed using several metrics, revealing a high-quality model. The Global Model Quality Estimation (GMQE) score of 0.84 indicates a high level of confidence in the overall accuracy of the predicted structure. Homology

modeling was performed using the AlphaFold DB model of A0A5P2UBI4 as a template, which shares 76.69 per cent sequence identity with tHMG1 across 99 per cent of its sequence, spanning residues 5-525. This high sequence identity and coverage suggest a strong structural similarity between *tHMG1* and the template available at PDB, further supporting the reliability of the predicted model. The predicted *tHMG1* protein is likely a monomer in its native state, as indicated by the 'Monomer' designation for its oligomeric state, this findings were aligned with the previously reported results of Belachew *et al.* (2021). These assessments collectively indicate that the generated model is a robust representation of the *tHMG1* structure, suitable for further analysis and interpretation.

The stereochemical quality of the predicted *tHMG1* protein structure was assessed using a Ramachandran plot, which visualizes the distribution of φ (phi) and ψ (psi) dihedral angles for each amino acid residue in the protein backbone (Hayward *et al.,* 2021). As depicted in the Fig. 8, the majority of residues (91.71%) are clustered within the energetically favoured regions of the plot, indicative of a well-defined and stable protein conformation. A smaller number of residues (3.08%) are observed in

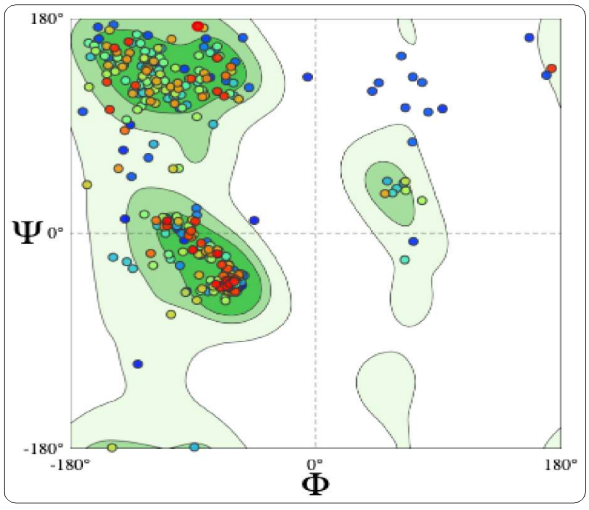


Fig. 8: Ramachandran plot of the predicted *tHMG1* protein structure. This figure assesses the stereochemical quality of the predicted *tHMG1* structure, showing the distribution of ö and ř angles and the percentage of residues in favoured and outlier regions

the outlier regions. These outliers, specifically ALANINE 63 GLUTAMIC ACID, ALANINE 51 SERINE, ALANINE 380 HISTIDINE, ALANINE 499 ALANINE, ALANINE 55 GLUTAMIC ACID, ALANINE 56 GLUTAMIC ACID, ALANINE 48 SERINE, ALANINE 33 SERINE, ALANINE 498 PROLINE, ALANINE 31 VALINE, ALANINE 32 ISOLEUCINE, ALANINE 19 GLUTAMINE, ALANINE 52 SERINE, ALANINE 54 SERINE, ALANINE 50 PROLINE and ALANINE 501 PROLINE, may indicate regions of less defined structure, such as flexible loops or termini or could potentially highlight areas of interest, such as active sites or binding interfaces. The MolProbity score of 1.09 and a low clash score of 0.26 (with the clash occurring between ALANINE 18 VALINE and ALANINE 19 GLUTAMINE) further support the overall good quality of the model in which our results were on par with results of Madrigal *et al.* (2022). Other quality metrics, including rotamer outliers (0.24% at ALANINE 18 VALINE), C-beta deviations (7 involving several SERINE and GLUTAMIC ACID residues) and a small number of bad bonds (0) and angles (34, involving several SERINE, GLUTAMIC ACID and other residues), were also evaluated. Analysis of cis non-prolines, cis prolines, twisted non-prolines and twisted prolines provided additional insights into the protein's conformational details. Overall, while a small percentage of residues fall within outlier regions of the Ramachandran plot, the favorable MolProbity score and high percentage of residues in favored regions, combined with analysis of other stereochemical parameters, suggest that the predicted *tHMG1* structure is energetically favourable and structurally sound, lending confidence to its reliability for further structure-based functional predictions and analyses (Azmi *et al.,* 2023).

In this study, we have successfully cloned, sequenced, and predicted the 3D structure of the truncated *HMG1* gene (*tHMG1*) from the auxotrophic yeast *Saccharomyces cerevisiae* BY4741. By confirming the 100 per cent sequence identity and identifying key functional domains, this research provides a comprehensive understanding of the *tHMG1* gene. Furthermore, the reliable 3D structural model,

validated through Ramachandran plot analysis, offers insights into the unique architecture of *tHMG1*. This work lays a solid foundation for future investigations into the functional characteristics of *tHMG1* and its potential application in enhancing terpene production, highlighting its relevance for industrial biotechnology.

Acknowledgment : The author would gratefully acknowledge the Department of Biotechnology, UAS, GKVK, Dr. C.N. Lakshminarayana Reddy, Department of Pathology, UAS, GKVK and Dr. Chandrashekara K.N. Sriram Institute for Industrial Research for their invaluable support throughout this research.

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