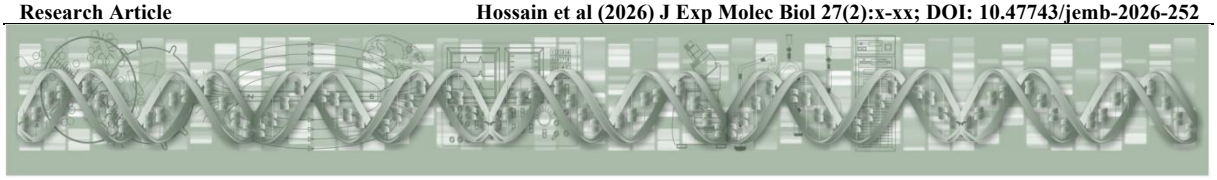


PRODUCTION OF EXTRACELLULAR RECOMBINANT PHYTASE IN YEAST AND ITS APPLICATION IN ANIMAL FEED AS ENZYME SUPPLEMENT

Sk Amir Hossain[1*], Sheikh Julfikar Hossain[1], Tonima Rahman Tuli[1], Rima Akter[1]

[1]Biotechnology and Genetic Engineering Discipline Khulna University, Khulna
[*] Corresponding author e-mail: skamir@bge.ku.ac.bd

Abstract

Phytase is a crucial enzyme widely employed in animal nutrition and agriculture for hydrolyzing phytic acid (phytate), the primary storage form of phosphorus in plant tissues, thereby releasing digestible inorganic phosphate. However, its commercial application is often constrained by the thermal instability of the enzyme, particularly under the elevated temperatures used in industrial feed processing. To address this limitation, the present study aimed to develop a robust and scalable yeast-based expression system capable of producing a thermostable form of phytase. A novel expression plasmid, pESC-TRP6HisHA, was constructed, featuring galactose-inducible bidirectional promoters, a 6×His-HA epitope tag for easy detection, and a secretion signal to facilitate extracellular expression. The *PhyA* gene from *Aspergillus niger*, encoding phytase, was cloned into this vector, resulting in the construct pESC-TRP6HisHAPhyA, which was subsequently transformed into *Saccharomyces cerevisiae*. Under galactose induction and phytin-supplemented medium the recombinant yeast successfully expressed and secreted active phytase, as evidenced by clear halo zones on phytin-containing agar plates. Biochemical assays demonstrated that the recombinant phytase exhibited significantly enhanced thermostability compared to the wild-type enzyme, retaining greater enzymatic activity after incubation at 30°C, 40°C, and 50°C for three hours. This improvement was supported by an aliphatic index of 83.2, suggesting moderate thermal resilience. The expression system offers both secreted and potentially surface-displayed phytase, which can facilitate purification, enhance stability, or enable direct use in feed processing. This study establishes a simple, efficient, and scalable platform for the production of thermostable phytase using genetically engineered *S. cerevisiae*. The system holds substantial promise for industrial applications, especially in animal feed manufacturing, where enzyme stability at high temperatures is critical. Future work will focus on optimizing yield, evaluating long-term stability, and conducting feed trials to validate its performance under commercial conditions.

Keywords
Enzyme stability, Genetic engineering, Phytase, *PhyA* gene, Surface display, Thermostability

Received 11 September 2025; Reviewed 25 February 2026 Accepted 22 May 2026; Published Online 22 May 2026; Published xx June 2026

Introduction

The production of recombinant proteins using microbial platforms represents a cornerstone of modern industrial biotechnology. These platforms provide scalable, cost-effective, and sustainable solutions across diverse sectors (Elazzazy et al., 2025). Among these platforms, microbial cell surface display systems have emerged as powerful tools for protein engineering, presenting functional proteins on the cell surface through fusion with specific cell wall-anchoring domains (Pham & Polakov, 2020). This approach is widely applied in biocatalysis, vaccine delivery, environmental biosensing, and bioremediation.

Compared to bacterial systems, yeast-based display platforms offer several notable advantages, including the ability to perform post-translational modifications, improved folding of heterologous proteins, and reduced degradation due to low endogenous protease activity (Petushkova & Zamyatnin, 2020). In addition to these technological advantages, yeast species such as *S. cerevisiae* are classified as generally recognized as safe (GRAS), possess robust cell wall structures for stable protein anchoring, and are already widely accepted in food and feed industries (Teparić & Mrša, 2016). Over the last few decades, several yeast species including *Hansenula polymorpha* (Kim et al., 2002), *Pichia pastoris* (Wang et al., 2008), *Yarrowia lipolytica* (Yuzbasheva et al., 2012), and *S. cerevisiae* (Sun et al., 2014) have been extensively studied for recombinant protein and extracellular enzyme expression. However, many existing expression vectors are designed for specific research applications and often lack modularity, industrial scalability, or optimization for high-efficiency protein secretion during fermentation (Long et al., 2024). These limitations restrict their use in large-scale enzyme production across various industrial applications (Hossain et al., 2019). Therefore, there is a critical need for a yeast-compatible expression system that enables efficient gene insertion, tightly regulated robust expression, and high-efficiency protein secretion under industrially relevant conditions (Otto, 2023).

To address this challenge, we constructed and validated a novel yeast expression vector, pESC-TRP6HisHA. This plasmid features, a bidirectional *GAL1*/*GAL10* promoter system for coordinated expression, a secretion signal sequence to direct proteins into the extracellular medium, and an HA epitope tag for immunodetection (Cevheroğlu, 2015). The vector allows straightforward insertion of target genes via restriction cloning, supporting both surface display and extracellular secretion of heterologous proteins in *S. cerevisiae* (Zhao et al, 2024). As a proof-of-concept, the *PhyA* gene from *Aspergillus niger*, encoding the enzyme phytase, was cloned into this vector to generate the recombinant construct pESC-TRP6HisHAPhyA (Liao et al., 2012).

Phytase is a phosphatase enzyme that catalyzes the hydrolysis of phytic acid (myo-inositol hexakisphosphate), the principal storage form of phosphorus in plant tissues, thereby releasing inorganic phosphorus that is bioavailable to monogastric animals such as poultry and swine (Gerke, 2015). The inclusion of phytase in animal feed significantly enhances phosphorus bioavailability, reduces the need for inorganic phosphate supplements, and lowers environmental phosphorus pollution (Abbasi et al., 2019). However, native microbial phytases often suffer from low thermal stability, which poses a major limitation during high-temperature feed pelleting processes (Kumar et al., 2015). Additionally, the high production and purification costs of phytase restrict its commercial application, particularly in resource-limited settings (Singh et al., 2018). In developing countries such as Bangladesh, where livestock and poultry farming are vital to the agricultural economy, thermostable and cost-effective phytase is essential (Islam et al., 2021). Using *S. cerevisiae* not only enables safe and efficient enzyme production but also provides additional nutritional benefits through its natural content of B-complex vitamins, trace minerals, and other growth-promoting factors (Hossain et al., 2019).

This study aims to develop a robust yeast-based recombinant platform for extracellular phytase production, combining enhanced enzyme thermostability with the nutritional benefits of the yeast host. This dual-functional approach holds significant promise for sustainable and cost-effective feed enzyme supplementation in the animal nutrition industry.

Materials and Methods

***In silico* Design and Construction of the pESC-TRP6HisHAPhyA Expression Vector.** A novel yeast expression plasmid, pESC-TRP6HisHAPhyA, was computationally designed using SnapGene 6.3.2 (GSL Biotech) to enable inducible and efficient extracellular expression of recombinant phytase in *S. cerevisiae*. The plasmid backbone features a *GAL1*/*GAL10* promoter, allowing tight transcriptional regulation in response to galactose induction (Elison et al., 2018). An N-terminal 6×His tag and a hemagglutinin (HA) epitope tag were included to facilitate downstream protein detection and purification (Motejadded & Altenbuchner, 2009). These were introduced into the pESC-TRP backbone by designing the forward PCR primer to include the sequences for the tags and signal peptide upstream of the *PhyA* coding sequence. The PCR-amplified fragment was then cloned into the multiple cloning site of the vector using NotI and EcoRI restriction sites, generating the final recombinant construct pESC-TRP6HisHAPhyA. To enable extracellular secretion, a secretion signal peptide sequence was placed upstream of the target gene. The coding sequence of the *A. niger* phytase gene (*PhyA*) was selected as the gene of interest (Li et al., 2009). Specific primers were designed to amplify *PhyA* together with its signal peptide, incorporating NotI and EcoRI restriction sites for directional cloning into the multiple cloning site of the pESC-TRP vector (Zhou et al., 2018). The resulting plasmid construct, named pESC-TRP6HisHAPhyA, was used for subsequent functional validation (Figure 1).

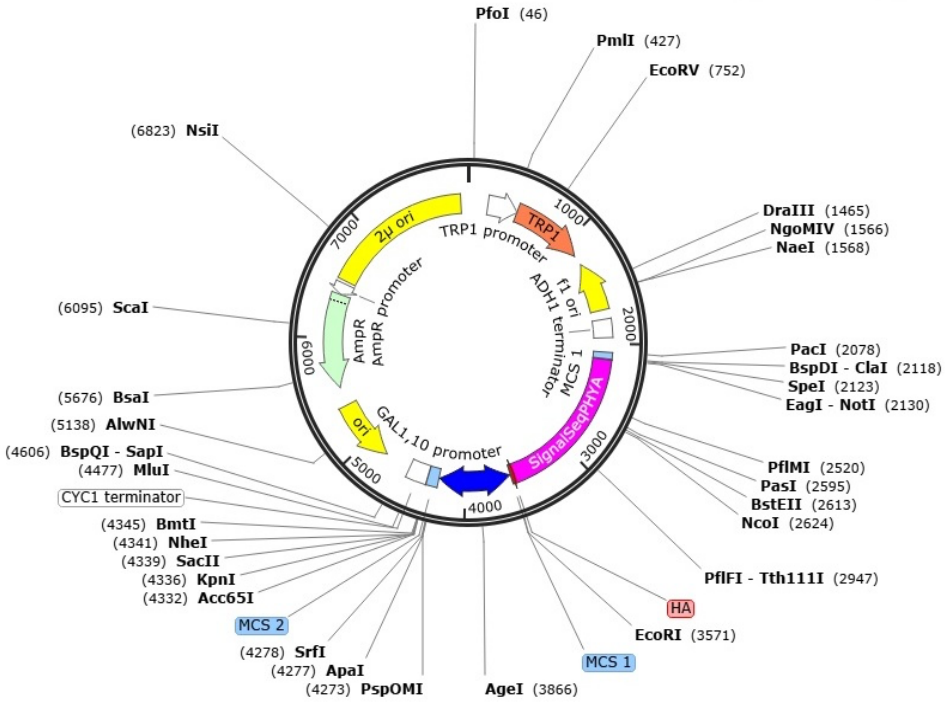


Figure 1. Genetic map of the pESC-TRP6HisHAPhyA final plasmid designed using the Snap Gene software.

Fungal Strain, Culture Conditions, and Genomic DNA Isolation. Grapes were surface-sterilized with 70% ethanol for 1 min, followed by 1% sodium hypochlorite for 3 min, and rinsed three times with sterile distilled water before fungal isolation. *A. niger* was isolated from these surface-sterilized grape samples and cultured on potato dextrose agar (PDA) plates at 28°C for 5–7 days. Mycelia were harvested from actively growing cultures, and approximately 100 mg of mycelium was used for genomic DNA extraction using a modified phenol-chloroform-isoamyl alcohol protocol (Kumar et al., 2018). The phenol–chloroform–isoamyl alcohol DNA extraction protocol was slightly modified by extending the lysis step to 30 min and including an RNase treatment for cleaner genomic DNA. DNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) and confirmed by agarose gel electrophoresis. Genomic DNA quality was assessed by electrophoresis on 1% agarose gel run at 100 V for 30 minutes.

Amplification and Cloning of the Phytase Expression Cassette. The *PhyA* (GenBank accession no. Z16414) gene along with its native secretion signal was PCR-amplified using high-fidelity DNA polymerase (Phusion, Thermo Fisher Scientific, USA). The *PhyA* gene was amplified using PhyA-NotI-F primer 5′-GCGGCCGCATGGGCGTCTCTGCCGTTCTC-3′ and PhyA-EcoRI-R primer 5′-GAATTCTCAAGCAAAACACTCCGCCCAATC-3′, incorporating NotI and EcoRI restriction sites, respectively. The PCR product was gel-purified, digested with NotI and EcoRI, and ligated into the similarly digested pESC-TRP plasmid using T4 DNA ligase. Recombinant plasmids were transformed into chemically competent *Escherichia coli* DH5α cells. Positive clones were screened by colony PCR and validated by plasmid restriction digestion and Sanger sequencing.

Transformation of *Saccharomyces cerevisiae*. The recombinant plasmid pESC-TRP6HisHAPhyA was introduced into *S. cerevisiae* strain BY4741 using the lithium acetate/polyethylene glycol (LiAc/PEG) transformation method (Bernardi et al., 2019). Transformed yeast cells were plated on synthetic defined (SD) medium lacking tryptophan (SD-Trp) and incubated at 30°C for 48–96 hours. Successful transformants were confirmed by colony PCR targeting the *PhyA* insert (Casado-del et al., 2019).

Isolation and Microscopic Identification of *Aspergillus niger* and *Saccharomyces cerevisiae*. *A. niger* was isolated from decomposing soil waste, while *S. cerevisiae* was obtained from grapes. Pure cultures were established by streaking samples on selective agar media. *A. niger* was cultured on potato dextrose agar (PDA) composed of potato extract (200 g/L), dextrose (20 g/L), and agar (20 g/L), and incubated at 28°C for 5–7 days. *S. cerevisiae* was maintained on yeast extract–peptone–dextrose (YPD) medium containing yeast extract (10 g/L), peptone (20 g/L), dextrose (20 g/L), and agar (20 g/L) at 30°C for 48–72 hours. Microscopic analysis of the cultures was performed using a light microscope to confirm morphological characteristics, including filamentous hyphae and conidial heads in *A. niger* and budding cells in *S. cerevisiae*.

Detection of Native Phytase Activity in *Aspergillus niger*. The phytase-producing ability of *A. niger* was evaluated using a phytin-containing screening medium, in which phytin served as the sole phosphate source. The medium was composed of glucose (15 g/L), NH_4NO_3 (5 g/L), KCl (0.5 g/L), $MgSO_4 \cdot 7H_2O$ (0.01 g/L), $MnSO_4 \cdot 4H_2O$ (0.01 g/L), agar (15 g/L), and phytin (2 g/L). Fungal cultures were spot-inoculated onto the medium and incubated at 28°C for 5 days.

Phytase activity was qualitatively assessed by observing clear halo zones around colonies, indicating hydrolysis of phytin and release of inorganic phosphate.

Qualitative Plate Assay for Phytase Activity. To evaluate extracellular phytase activity, both recombinant *S. cerevisiae* transformants and wild-type *A. niger* were cultured on PDA plates supplemented with 2% sodium phytate (phytin) as the sole phosphorus source (Upton, 2017). Plates were incubated at 30°C for up to 5 days. Enzymatic activity was qualitatively assessed by observing clear halo zones around colonies, indicative of phytate degradation and phosphate release (Sharma & Bansal, 2024).

Quantitative Thermal Stability Assay of Phytase. Thermal stability of the expressed phytase enzyme was assessed by incubating culture supernatants at 30°C, 40°C, and 50°C for 3 hours. Phytase activity was quantified using a standard colorimetric phosphate release assay, where liberated inorganic phosphate was detected using ammonium molybdate reagent, and absorbance was measured at 700 nm using a microplate reader (Qvirist et al., 2015). Enzyme activity was expressed as the percentage of residual activity relative to untreated controls. All assays were conducted in biological triplicates.

Bioinformatic Evaluation of Aliphatic Index. To predict thermal stability from a sequence-based perspective, the amino acid sequence of the expressed phytase was analyzed using the ProtParam tool available on the ExPASy Bioinformatics Resource Portal (Naqqash, 2023). The aliphatic index, a metric associated with protein thermostability, was calculated based on the relative molar content of aliphatic amino acids (alanine, valine, isoleucine, and leucine) using the formula:

$$AI = X(Ala) + aX(Val) + b[X(Ile) + X(Leu)]$$

where a and b are weighting coefficients reflecting volume contributions of respective residues.

Statistical Analysis. All experimental procedures were performed in triplicate (n = 3) unless otherwise specified. Data are presented as mean ± standard deviation (SD). Unpaired Student's t-tests were used for pairwise comparisons (e.g., wild-type vs. recombinant strains), while one-way ANOVA followed by Tukey's post hoc test was employed for comparisons across multiple temperatures. A significance threshold of $P < 0.05$ was considered statistically significant. All statistical analyses and graphical visualizations were carried out using GraphPad Prism version 9.0 (GraphPad Software, USA).

Results and discussions

Construction and Validation of pESC-TRP6HisHAPhyA Recombinant Plasmid. The recombinant expression vector pESC-TRP6HisHAPhyA was successfully constructed by inserting the PCR-amplified NotI-EcoRI flanked HA-tagged *PhyA* signal sequence into the pESC-TRP backbone via restriction-ligation. Following ligation, the plasmid was digested with NotI and EcoRI, generating two distinct bands of approximately 6503 bp and 1441 bp, consistent with the expected sizes of the vector and insert, respectively (Figure 2). This molecular confirmation was further substantiated by commercial DNA sequencing (GeneScript, China), which verified the correct orientation, frame, and integrity of the insert sequence.

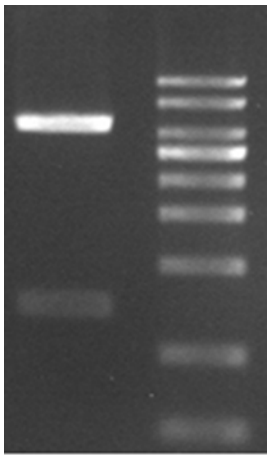


Figure 2. Restriction digestion of the pESC-TRP6HisHAPhyA plasmid with NotI and EcoRI, generating fragments of 6503 bp and 1441 bp. A 1 kb DNA ladder (Thermo Fisher Scientific, USA; 250 bp–10 kb) was used to estimate fragment sizes.

Isolation and Microscopic Identification of *Aspergillus niger* and *Saccharomyces cerevisiae*

Pure cultures of *A. niger* and *S. cerevisiae* were successfully obtained. Microscopic examination revealed filamentous hyphae and globular conidial heads in *A. niger*, while budding cells were observed in *S. cerevisiae* (Figure 3).

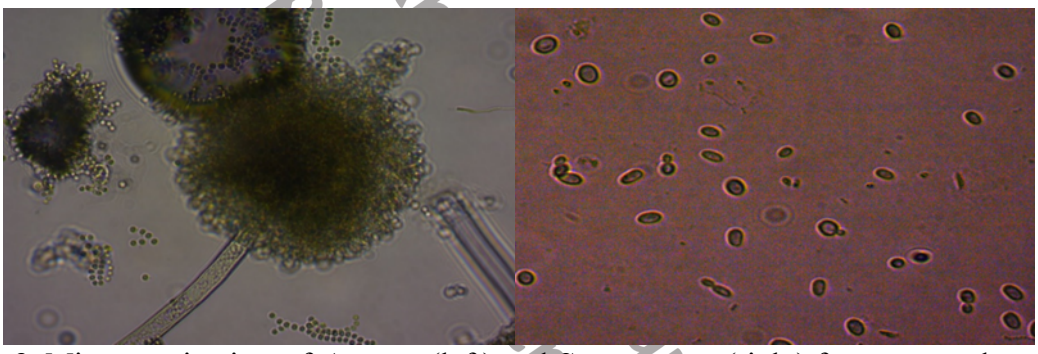


Figure 3. Microscopic view of *A. niger* (left) and *S. cerevisiae* (right) from pure culture.

Detection of Native Phytase Activity in *Aspergillus niger*

A. niger exhibited clear halo zones around colonies on phytin-containing plates, indicating active phytase secretion and hydrolysis of phytin (Figure 4).

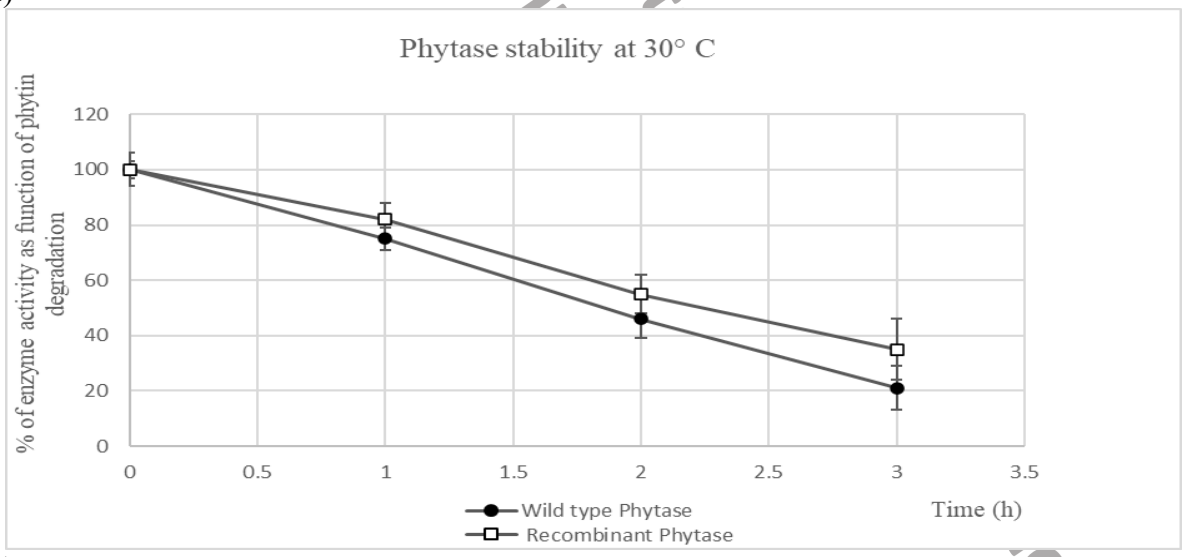
Figure 4. Clear zone around *A. niger* observed in PDA screening media containing phytin, indicating phytase activity.

Functional Expression of Recombinant Phytase in *S. cerevisiae*. To evaluate heterologous expression, *S. cerevisiae* cells harboring the pESC-TRP6HisHAPhyA plasmid were cultured on YPD medium supplemented with galactose (20 g/L) and 2% phytin. The appearance of a prominent clear zone surrounding transformed colonies confirmed the secretion of active recombinant phytase and efficient degradation of phytin (Figure 5). This observation highlights the functional success of the yeast expression system and the correct targeting of the recombinant protein to the extracellular environment.

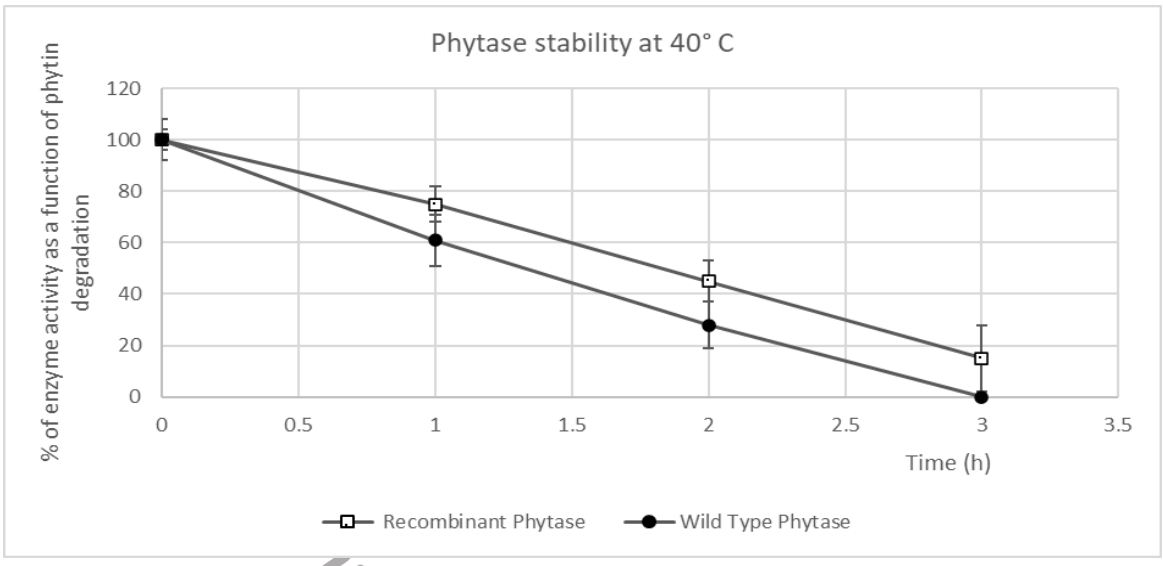
Figure 5. Clear zone around *S. cerevisiae* observed in YPD media containing phytin, confirming recombinant phytase activity.

Evaluation of Thermal Stability of Recombinant Phytase. Thermostability assays were conducted to compare the residual activity of recombinant phytase with that of wild-type enzyme after 3-hour incubations at 30°C, 40°C, and 50°C. At all tested temperatures, the recombinant enzyme retained significantly greater enzymatic activity, indicating improved thermal resilience (Figure 6a–c). The enhanced stability is likely attributable to yeast-mediated post-translational modifications, particularly glycosylation, which are known to confer structural integrity under heat stress.

a)



b)



c)

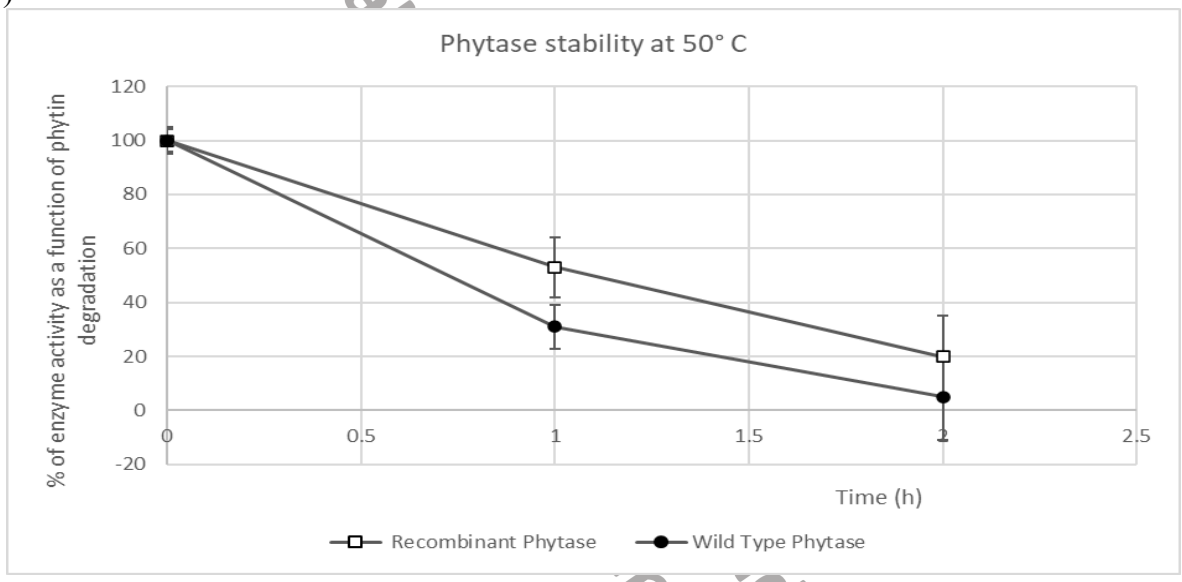


Figure 6. Temperature stability of phytase at: a) 30°C; b) 40°C; c) 50°C.

These results underscore the potential of yeast-based phytase production for applications requiring moderate thermal endurance, such as feed pelleting or processing in warm environments.

***In Silico* Assessment of Phytase Thermostability via Aliphatic Index.** To further evaluate thermostability from a structural perspective, the aliphatic index of the phytase protein was calculated using the ExPASy ProtParam tool, yielding a value of 83.2. This metric, which correlates with the proportion of aliphatic side chains (e.g., Ala, Val, Ile, Leu), is often predictive of thermostability, with values above 90 typically denoting high thermal tolerance. Although the recombinant enzyme demonstrated improved thermal stability *in vitro*, its aliphatic index suggests it remains moderately thermostable and may still require protein engineering or mutagenesis for high-temperature industrial applications.

Conclusions

This study successfully demonstrates the construction of an optimized genetic cassette enabling both surface display and extracellular secretion of recombinant phytase in *S. cerevisiae*. The engineered yeast strain exhibited enhanced thermal stability of the expressed enzyme relative to its wild-type counterpart, underscoring its potential for industrial applications, particularly in the animal feed industry, where high-temperature processes such as pelleting are routinely employed. These findings align with previous reports highlighting the advantages of yeast-based expression systems in enhancing the functional properties of heterologous proteins, including improved stability and secretion efficiency. Notably, the dual localization strategy employed here, represented by simultaneous surface anchoring and secretion, offers greater operational flexibility, facilitating both direct application in fermentation processes and efficient enzyme recovery for downstream use. Despite these promising outcomes, the study acknowledges the need for further validation under real-world industrial settings, including long-term stability assays, cost-benefit analyses, and performance evaluations within complex feed matrices. In addition, animal feed trials will be essential to assess bioavailability, digestibility, and overall nutritional benefits. Such investigations will be critical for transitioning this recombinant phytase system from laboratory development to commercial-scale implementation.

Acknowledgments: Authors are thankful to the Research and Innovation Center, Khulna University, Bangladesh for their financial support to carry out this research work.

References

Abbasi F, Fakhur-un-Nisa T, Liu J, Luo X, Abbasi IHR. 2019. Low digestibility of phytate phosphorus, their impacts on the environment, and phytase opportunity in the poultry industry. Environ Sci Pollut Res. 26:9469-9479.

Bielen A, Teparic R, Vujaklija D, Mrša V. 2014. Microbial anchoring systems for cell-surface display of lipolytic enzymes. Food Technol Biotechnol. 52(1):16-34.

Bernardi B, Kayacan Y, Akan M, Wendland J. 2019. Overexpression of RAD51 enables PCR-based gene targeting in lager yeast. Microorganisms. 7(7):192.

Casado-del Castillo V, MacCabe AP, Orejas M. 2021. Agrobacterium tumefaciens-mediated transformation of NHEJ mutant Aspergillus nidulans conidia: an efficient tool for targeted gene recombination using selectable nutritional markers. J Fungi. 7(11):961.

Cevheroğlu O. 2015. More than just a dimer: Detection of G protein-coupled receptor oligomers using fluorescent protein reassembly of Ste2p, a yeast pheromone receptor [dissertation]. Middle East Technical University (Turkey).

Elazzazy AM, Baeshen MN, Alasmi KM, Alqurashi SI, Desouky SE, Khattab SM. 2025. Where biology meets engineering: Scaling up microbial nutraceuticals to bridge nutrition, therapeutics, and global impact. Microorganisms. 13(3):566.

Elison GL, Xue Y, Song R, Acar M. 2018. Insights into bidirectional gene expression control using the canonical *GAL1/GAL10* promoter. Cell Rep. 25(3):737-748.

Gerke J. 2015. Phytate (inositol hexakisphosphate) in soil and phosphate acquisition from inositol phosphates by higher plants. A review. Plants. 4(2):253-266.

Hossain AS, Teparić R, Mrša V. 2019. Comparison of two models of surface display of xylose reductase in the *Saccharomyces cerevisiae* cell wall. Enzyme Microb Technol. 129:109353.

Islam MT, Habib M, Ferdaus AJM, Mollah MBR, Ali MS. 2021. Supplementation of diet with microbial and thermo-stable 6-phytase with recommended and deficient Ca and P level on broiler performance. SAARC J Agric. 19(1):211-222.

Kim SY, Sohn JH, Pyun TR, Choi ES. 2002. A cell surface display system using novel GPI-anchored proteins in Hansenula polymorpha. Yeast. 19(13):1153-1163.
Kumar M, Mugunthan M. 2018. Evaluation of three DNA extraction methods from fungal cultures. Med J Armed Forces India. 74(4):333-336.
Kumar V, Singh D, Sangwan P, Gill PK. 2015. Management of environmental phosphorus pollution using phytases: current challenges and future prospects. In: Applied environmental biotechnology: present scenario and future trends. p. 97-114.
Li G, Yang S, Li M, Qiao Y, Wang J. 2009. Functional analysis of an Aspergillus ficuum phytase gene in *Saccharomyces cerevisiae* and its root-specific, secretory expression in transgenic soybean plants. Biotechnol Lett. 31:1297-1303.
Liao Y, Zeng M, Wu ZF, Chen H, Wang HN, Wu Q, et al. 2012. Improving phytase enzyme activity in a recombinant phyA mutant phytase from Aspergillus niger N25 by error-prone PCR. Appl Biochem Biotechnol. 166:549-562.
Long B, Zhang F, Dai SY, Foston M, Tang YJ, Yuan JS. 2024. Engineering strategies to optimize lignocellulosic biorefineries. Nat Rev Bioeng. p. 1-15.
Motejadded H, Altenbuchner J. 2009. Construction of a dual-tag system for gene expression, protein affinity purification and fusion protein processing. Biotechnol Lett. 31:543-549.
Naqqash T, Shabbir G, Hanif MK, Kazmi MB, Qaisrani MM. 2023. In silico analysis and functional characterization of phytase enzyme from Bacillus amyloliquefaciens. J Microbiol Mol Genet. 4(3):42-55.
Otto M. 2023. Combining metabolic engineering and synthetic biology approaches for the production of abscisic acid in yeast [dissertation]. Chalmers Tekniska Hogskola (Sweden).
Petushkova AI, Zamyatnin AA Jr. 2020. Redox-mediated post-translational modifications of proteolytic enzymes and their role in protease functioning. Biomolecules. 10(4):650.
Pham ML, Polakovič M. 2020. Microbial cell surface display of oxidoreductases: concepts and applications. Int J Biol Macromol. 165:835-841.
Qvirist L, Carlsson NG, Andlid T. 2015. Assessing phytase activity–methods, definitions and pitfalls. J Biol Methods. 2(1).
Sharma M, Bansal S. Production, characterization of bacterial phytase and its applications.
Singh N, Kuhar S, Priya K, Jaryal R, Yadav R. 2018. Phytase: The feed enzyme, an overview. In: Gahlawat S, Duhan J, Salar R, Siwach P, Kumar S, Kaur P, editors. Adv Anim Biotechnol Appl. Singapore: Springer. p. 355-372.
Sun H, Wang T, Zhang J, Liu Q, Wang L, Chen P, et al. 2014. Display of heterologous proteins on the *Saccharomyces cerevisiae* surface display system using a single constitutive expression vector. Biotechnol Prog. 30(2):443-450.
Teparić R, Mrša V. 2016. Overview of systems and techniques for surface display of recombinant proteins in yeast S. cerevisiae. Appl Food Biotechnol. 3(1):3-14.
Upton D. 2017. In silico guided metabolic engineering of Aspergillus niger for sustainable organic acid production [dissertation]. University of York.
Wang X, Li L, Chen M, Qi Q, Wang PG. 2008. Construction of a novel Pichia pastoris cell-surface display system based on the cell wall protein Pir1. Curr Microbiol. 56(4):352-357.
Yuzbasheva E, Yuzbasheva TV, Gvilava IT, Sineoky SP. 2012. Protein display on the Yarrowia lipolytica yeast cell surface using the cell wall protein YIPir1. Appl Biochem Microbiol. 48(6):650-655.
Zhao M, Ma J, Zhang L, Qi H. 2024. Engineering strategies for enhanced heterologous protein production by Saccharomyces cerevisiae. Microb Cell Fact. 23(1):32.
Zhou Y, Yang L, Duan J, Cheng J, Shen Y, Wang X, et al. 2018. Hinge region of Arabidopsis phyA plays an important role in regulating phyA function. Proc Natl Acad Sci USA. 115(50):E11864-E11873.