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Comparative Optimization of Culture Media for Recombinant XynBTN63D Expression in *Saccharomyces cerevisiae* BJ1824 Toward Sustainable XOS Production

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Abstract

Comparative optimization of culture media for recombinant XynBTN63D expression in *Saccharomyces cerevisiae* BJ1824 was performed to identify an efficient and low-cost cultivation medium for sustainable xylooligosaccharide (XOS) production. The gene encoding endo-β-1,4-D-xylanase (XynBTN63D) from *Bacillus* sp. was cloned into pESC and pYHM1 expression vectors and heterologously expressed in *S. cerevisiae* BJ1824. Recombinant enzyme production is commonly carried out using Yeast Extract Peptone Galactose (YPG) medium; however, its relatively high cost limits its industrial applicability. Therefore, this study evaluated alternative minimal media to support yeast growth and recombinant XynBTN63D production at a lower cost. Three culture media, namely YPG, MTE, and YMin, were comparatively assessed based on cell growth, protein expression, and enzyme activity. Recombinant protein expression was analyzed by SDS-PAGE, enzyme activity was determined by UV spectrophotometry, and hydrolysis products were characterized by thin-layer chromatography (TLC). Growth analysis demonstrated that the optimal growth of *S. cerevisiae* BJ1824 was achieved after 72 hrs in YPG and YMin media and after 84 hrs in MTE medium. Among the tested media, YMin showed promising performance, with enzyme activities of 0.930 U/mL and 0.634 U/mL in *S. cerevisiae* BJ1824 harboring *pESC-xynBTN63D* and *pYHM1-xynBTN63D*, respectively. SDS-PAGE analysis revealed a recombinant XynBTN63D protein band at approximately 35 kDa, showing strong expression in YPG medium, weaker expression in Y-Min medium, and no detectable expression in MTE medium. TLC analysis identified xylotriose (X3) as the major hydrolysis product generated by the recombinant enzyme. Overall, these findings demonstrate that YMin medium has strong potential as a cost-effective alternative for recombinant XynBTN63D production in *S. cerevisiae* BJ1824, thereby supporting more sustainable and economically viable XOS production processes.

Keywords: Endo-β-D-1,4-xylanase, *Saccharomyces cerevisiae* BJ1824, Media Production, Xylooligosaccharide

INTRODUCTION

Xylooligosaccharides (XOS) are oligomeric sugars composed of 2-7 xylose units linked by β-1,4 glycosidic bonds.[1] XOS has attracted considerable attention as a potent prebiotic due to its physicochemical properties, including low viscosity, high hydrophilicity, thermal stability, and resistance to acidic conditions. These characteristics enable XOS to resist hydrolysis by gastrointestinal enzymes and gastric acid, allowing it to reach the intestine intact and selectively modulate beneficial gut microbiota.[2,3] Furthermore, XOS has been reported to possess several health-promoting effects, such as reducing neurotoxicity, diabetes, and colon inflammation; lowering blood cholesterol levels; enhancing calcium and mineral absorption; and exhibiting antioxidant activity.[4]

XOS is mainly produced by endo-β-1,4-D-xylanase (EC 3.2.1.8), which hydrolyzes β-1,4-glycosidic linkages on the substrate.[5] Based on the amino acid sequences and structural characteristics of their catalytic domains, this enzyme belongs to the glycoside hydrolase (GH) family.[6] Generally, endo-β-1,4-xylanase is produced by various microorganisms, including bacteria (*Streptomyces, Bacillus, Halomonas*, and *Paenibacillus*) and fungi (*Trichoderma, Aspergillus, Thielavia, Fusarium,* and *Rhizomucor*).[7] These enzymes have been applied in many industries, including food and beverage, animal feed, paper and pulp, pharmaceuticals, and bioethanol production.[8,9]

Currently, the enzyme market is experiencing rapid growth in both developed and developing countries.[10] This highlights the need to develop new microbial strains capable of producing enzymes in large quantities. Microbial engineering via genetic modification has transformed the production of valuable products.[11] Yeast strains, such as *Saccharomyces cerevisiae*, provide several practical advantages for industrial enzyme production. This microorganism is suitable for large-scale cultivation in bioreactors due to its high growth rate, tolerance to acidic environments, and "Generally Recognized as Safe" (GRAS) status.[12-14] Furthermore, *S. cerevisiae* efficiently performs post-translational modifications and

secretes recombinant proteins extracellularly.[13] These characteristics can simplify the purification process, leading to more cost-effective industrial production.

The isolation of the gene encoding endo-β-1,4-D-xylanase from *Bacillus* sp. and the development of its mutant variant, XynBTN63D, were successfully conducted in our previous study.[15] Expression of this variant in *Saccharomyces cerevisiae* BJ1824 through pYHMI and pESC plasmids successfully produced XynBTN63D, which can hydrolyze cassava pulp xylan into XOS. The prebiotic potential of XOS has also been proven to promote the growth of probiotic bacteria and enhance the production of short-chain fatty acids (SCFAs).[15-17]

The expression of XynBTN63D in *S. cerevisiae* BJ1824 is regulated by GAL promoters, which require galactose induction. Therefore, medium design must balance the need for sufficient carbon and nitrogen sources, essential minerals, and galactose to support both yeast growth and strong promoter induction. Recent studies emphasize that carbon source regulation, nutrient balance, and metabolic efficiency are critical factors for recombinant protein expression in yeast, particularly under inducible systems.[18,19] Developing a cost-effective minimal medium that sustains yeast growth and supports high-level XynBTN63D expression is essential for scalable, sustainable, and economically viable XOS production. Optimization of medium composition can minimize flocculation, enhance metabolic efficiency, and reduce costs while maintaining functional enzyme activity.

This study aims to comparatively evaluate the performance of YPG (rich medium), MTE (semi-defined medium), and YMin (minimal medium) for endo-β-1,4-D-xylanase (XynBTN63D) expression in *S. cerevisiae* BJ1824. The primary goal is to identify a minimal medium that balances enzyme productivity with production cost, providing a practical approach for industrial-scale XOS production.

MATERIALS AND METHODS

Cell culture and chemicals

Two *S. cerevisiae* BJ1824 isolates harboring *pESC-xynBTN63D* and *pYHM1-xynBTN63D*, respectively, were derived from the laboratory's stock collection. Beechwood xylan, YPD (yeast extract-peptone-dextrose) agar, yeast extract, and peptone were purchased from Himedia (Mumbai, India). p-Nitrophenyl-β-D-xylopyranoside (p-NP-X), p-Nitrophenyl-α-L-arabinofuranoside (p-NP-A), and galactose were obtained from Sigma-Aldrich (St. Louis, MO, USA). $MgSO_4 \cdot 7H_2O$, KH_2PO_4, and $(NH_4)_2SO_4$ were purchased from Merck (Darmstadt, Germany). In addition, this research used MTE medium (Indonesia, Patent No: IDP00004503).

Media preparation

This study used three distinct media: YPG, YPG Minimal (YMin), and MTE. The YPG medium was prepared by dissolving yeast extract (1% (w/v)), peptone (2% (w/v)), and galactose (2% (w/v)) in distilled water. The YMin medium was obtained by mixing 2% (w/v) galactose, 0.5% (w/v) yeast extract, 0.04% (w/v) $MgSO_4 \cdot 7H_2O$, 0.5% (w/v) KH_2PO_4, and 0.2% (w/v) $(NH_4)_2SO_4$ in distilled water. Meanwhile, the MTE medium was prepared using the patent formulations, containing K1 (2% galactose as a carbon source), K2 (Source of P and S), K3 (Source of Mg), and Trace elements (micronutrients). All media were sterilized using an autoclave for 15 minutes at 121 °C before further use.

Cell culture preparation

Saccharomyces cerevisiae BJ1824 isolates, each harboring *pESC-xynBTN63D* and *pYHM1-xynBTN63D*, were maintained at -20 °C. Prior to use, the cell culture was rejuvenated on solid YPD medium and incubated for 3 days. Then, the inoculum was prepared by transferring a loopful of each yeast culture into 5 mL of YPG broth. This mixture was then incubated in a shaker incubator at 150 rpm at 30 °C for 20 hours.

Determination of the growth curve of *S. cerevisiae* BJ1824

A 1% (v/v) inoculum of each *S. cerevisiae* strain was cultured in 100 mL of YPG broth and incubated at 30 °C in a shaking incubator at 150 rpm. Cell growth was monitored for 108 hours. Samples were collected at 2 hour intervals, and the optical density at 600 nm (OD_{600}) was measured using a UV-Vis spectrophotometer, with sterile medium used as a blank. Each measurement

was performed in triplicate. OD_{600} values were measured directly without dilution, and the same measurement conditions were applied consistently across all samples to allow reliable comparison of growth trends among different media.

Production of XynBTN63D

To produce XynBTN63D, the bacterial culture was grown in YPG, YPG Minimal (YMin), and MTE media (Indonesia, Patent No: IDP00004503). All cultures across the different media were incubated under the same aeration and continuous agitation (150 rpm) at 30 °C for 20 hours. XynBTN63D production was done by incubating each medium in a shaker incubator at 150 rpm at 30 °C for 20 hours. Then, the extracellular enzymes were harvested by centrifugation under specific conditions (20 minutes, 4,000 rpm, and 4 °C). The crude enzyme contained in the supernatant was kept in the refrigerator for further assay. The relative molecular mass of XynBTN63D was determined by SDS-PAGE using low-weight protein markers (GE Healthcare Life Sciences) as a standard. Protein concentration was determined by the Bradford method using bovine serum albumin as a standard.

Determination of enzyme activity

The enzyme activity was evaluated using spectrophotometric methods. For each sample, the test was conducted using three different substrates: Beechwood xylan, p-NP-X, and p-NP-A, for evaluating endo-β-1,4-D-xylanase, exo-β-1,4-D-xylosidase, and α-L-arabinofuranosidase activity, respectively.

Endo-β-1,4-D-xylanase activity

A 125 µL enzyme solution was added to 125 µL of 0.8% (w/v) Beechwood xylan substrate suspension in phosphate-citrate buffer (50 mM), pH 5.5 (1:1). The mixture was incubated for 60 minutes at 40 °C. This solution was then combined with the DNS reagent and heated for 5 minutes at 95 °C. The absorbance of each sample was measured by UV-Vis spectrophotometry at 540 nm. One unit (U) of xylanase activity was defined as the amount of enzyme required to release 1 µmol of reducing sugar equivalents per minute under the specified assay conditions.

Exo-β-1,4-D-xylosidase activity

A total of 50 µL enzyme sample was added to 450 µL of 0.9 M p-NP-X substrate in phosphate-citrate buffer (50 mM), pH 5.5, and incubated for 60 minutes at 40 °C. Then, an aliquot of 50 µL of 0.4 M Na_2CO_3 was added. The sample was measured spectrophotometrically at 405 nm. One unit (U) of xylosidase activity was defined as the amount of enzyme required to release 1 µmol of reducing sugar equivalents per minute under the specified testing conditions.

α-L arabinofuranosidase activity

A total of 5 µL of enzyme sample was added to 450 µL of 0.9 M p-NP-A substrate in phosphate-citrate buffer (50 mM), pH 5.5, and incubated with the control for 60 minutes at 40 °C. Then 50 µL of 0.4 M Na_2CO_3 was added. The samples were measured at 405 nm. One unit (U) of enzyme activity was defined as the amount of enzyme required to release 1 µmol of p-nitrophenol from the substrate per minute under the specified assay conditions.

Analysis of hydrolysis product using thin layer chromatography

The products derived from the hydrolysis of Beechwood xylan with XynBTN63D were analyzed by TLC. Each sample was spotted on TLC silica plates. This plate was incubated using the mobile phase consisting n-butanol: acetic acid: aquadest with ratio 2:1:1. Then, the spots were visualized by spraying the stained solution consisting α-naphthol: sulfuric acid: ethanol in a ratio of 1:10:200. The retention factor (R_f) was calculated against xylooligosaccharide (XOS) standard solution 1% (w/v) xylose, 1% (w/v) xylobiose, 1% (w/v) xylotriose.

RESULTS AND DISCUSSION

Growth curve study of *S. Cerevisiae* BJ1824 in various media

The growth of *S. cerevisiae* BJ1824 harboring the *pESC-xynBTN63D* plasmid (*S. cerevisiae* BJ1824/*pESC-xynBTN63D*) and *pYHM1-xynBTN63D* plasmid (*S. cerevisiae* BJ1824/*pYHM1-xynBTN63D*) was investigated in three production media: YPG, MTE, and YMin. Cell growth was monitored over 108 hours using OD_{600}

measurements with a UV-Vis spectrophotometer. The resulting growth curves for both isolates are provided in Figure 1.

All characteristic phases of microbial proliferation were observed in both growth curves, including adaptation, exponential, stationary, and death phases.[20] In YPG medium, the growth of *S. cerevisiae* BJ1824/*pESC-xynBTN63D* embarked on the exponential growth earlier and achieved the optimum phase sooner than in MTE or YMin media (Figure 1a). In contrast, in the stationary phase, these two media showed longer time to adaptation, indicating slower metabolic adjustment to their nutrient composition.[21] The similar pattern was demonstrated by the growth of *S. cerevisiae* BJ1824/*pYHM1-xynBTN63D*, which YPG again supports the fastest transition to exponential growth and highest cell densities (Figure 1b). Conversely, MTE and YMin delayed adaptation and prolonged the stationary phase before cell death began, occurring approximately 72-96 hours after inoculation across all media.

The growth of *S. cerevisiae* BJ1824 harboring the plasmids in MTE and YMin media showed slower adaptation than in YPG, suggesting differences in nutrient mixtures and inoculum conditions. As a rich nutrient, YPG facilitated faster metabolic activation and was utilized to promote rapid cell growth.[22] During the exponential phase, yeast metabolized swiftly and consumed great amounts of nutrients, resulting in significant enhancements in OD_{600} values, followed by a darker medium color. Conversely, the stationary phase persisted longer in MTE and YMin due to the formation of coagulants existing from minerals and salts during elongated agitation.[23,24] The death phase was found after approximately 72 hours across all media, marked by diminishing OD_{600} as nutrients became depleted and metabolic capacity reached its limit. Comparison with the findings of Simair et al. shows that *S. cerevisiae* has a longer growth cycle, up to 108 hours, while *Bacillus* sp. completes its growth curve in 5-60 hours.[25] The *S. cerevisiae* has held complex eukaryotic cell structures like a nucleus and vacuoles. This complex, large size required more time to reach the necessary division volume.[26]

To quantitatively evaluate growth performance, the specific growth rate and doubling time for each culture were calculated from optical density (OD_{600}) measurements during the exponential growth phase (Table 1). The specific growth rate is a measure of an

Table 1. Specific growth rate (μ) and doubling time (t) of recombinant *S. cerevisiae* in various media

		Specific Growth rate (h^{-1})	Doubling time
S. cerevisiae BJ1824/*pESC-xynBTN63D*	YPG	0.11	6.41
	MTE	0.09	7.91
	YMin	0.14	4.98
S. cerevisiae BJ1824/*pYHM1-xynBTN63D*	YPG	0.16	4.20
	MTE	0.07	10.06
	YMin	0.11	6.50

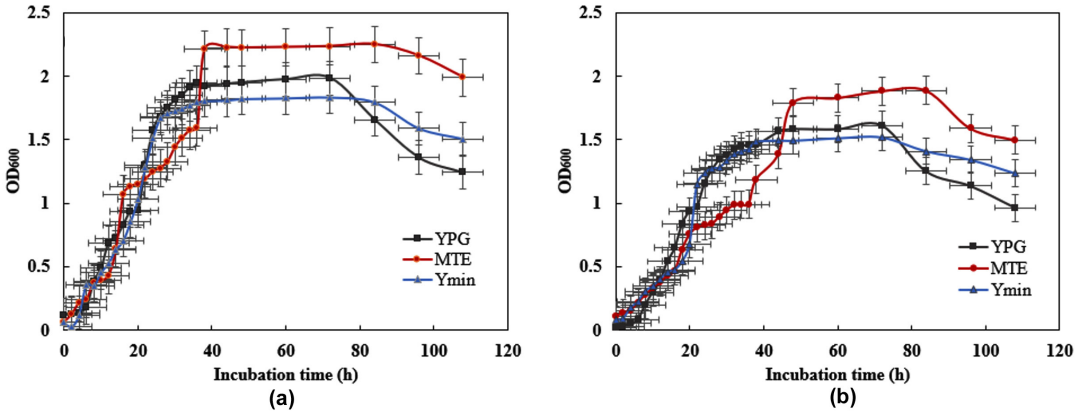

Figure 1. Optical density at 600 nm (OD_{600}) growth curves of *S. cerevisiae* BJ1824 (a) *S. cerevisiae* BJ1824/*pESC-xynBTN63D* and (b) *S. cerevisiae* BJ1824/*pYHM1-xynBTN63D*. Data are presented as mean ± standard deviation (SD) of three biological replicates

organism's growth rate. This parameter can then be used to determine the doubling time, which is the time required for a cell to divide. A higher double-time value indicated slower growth of microorganisms.[27]

Based on the data, the two recombinant *S. cerevisiae* isolates showed distinct growth patterns across different media compositions. The *S. cerevisiae* BJ1824/*pYHM1-xynBTN63D* isolate demonstrated the lowest doubling time in YPG media (4.20). As mentioned before, YPG contains abundant nutrients, allowing cells to direct their metabolic energy toward rapid cellular division. This is consistent with Figure 1b, which demonstrates that the isolate underwent a rapid extracellular division. However, a different pattern was observed in *S. cerevisiae* BJ1824/*pESC-xynBTN63D*, with the lowest doubling time in YMin medium (4.98). This difference suggests that *S. cerevisiae* BJ1824/*pESC-xynBTN63D* adapts more efficiently to nutrient-limited conditions.

Under certain conditions, minimal media can enhance yeast cell division by promoting more efficient metabolic adaptation under nutrient-limited environments. During glucose limitation, *Saccharomyces cerevisiae* upregulates high-affinity glucose transporters and activates alternative carbon metabolic pathways, thereby improving energy utilization efficiency. This metabolic reprogramming supports biomass synthesis and may contribute to increased cellular proliferation rates despite limited nutrient availability.[28-31]

Specific activity of XynBTN63D

The specific activity of XynBTN63D is expressed as micromoles of product formed by the enzyme per minute under quantified conditions, normalized to per milligram of total protein.[32] This assay was determined by combining enzyme activity (DNS/Miller method) and protein content (Bradford method). The resulting values were plotted to identify the optimal time for protein and enzyme production by the isolates, which correlated with their growth profiles determined from OD_{600} measurements.

As shown in Figure 2, the specific activity of XynBTN63D was highest in YPG medium for both *S. cerevisiae* isolates. This result reflected the nutrient-rich ingredients in YPG, which promote optimal enzyme expression.[33] Unlike YPG, MTE and YMin media yielded lower specific activities, with the lowest observed in MTE medium at 84 hrs (1.279 U/mg and 0.721 U/mg, respectively). This drop is consistent with the lengthened adaptation and stationary phases observed in these media, which led to flocculation during agitation. Flocculation can suppress extracellular enzyme secretion and yield OD_{600} readings from non-cellular particles, leading to an overestimation of cell density and an underestimation of enzymatic activity.[34,35]

Interestingly, the optimal activity time (72 hrs in YPG medium) was shorter than that reported by Mert et al., who observed peak activity at 84 hrs. This difference may be related

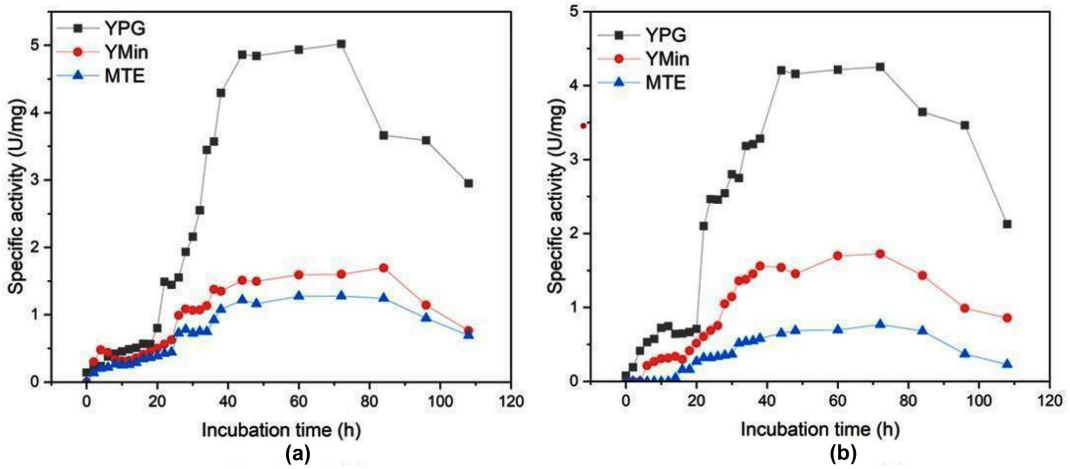

Figure 2. Specific activity curve of XynBTN63D: (a) in *S. cerevisiae* BJ1824/*pESC-xynBTN63D*; (b) in *S. cerevisiae* BJ1824/*pYHM1-xynBTN63D*

to the greater nutrient availability in YPG medium, which accelerates gene expression for protein and enzyme production.[36] These findings suggest that medium composition and flocculation properties significantly affect the enzyme production and its specific activity.[33,35]

The presence of minerals and salts in the culture medium can influence optical density (OD) measurements of *Saccharomyces cerevisiae* by affecting both cellular growth and the suspension's light-scattering properties. Essential ions such as Mg^{2+}, K^{+}, and phosphate contribute to enzymatic activity, membrane stability, and biomass production, thereby increasing cell density and, consequently, elevating OD values. In addition, changes in ionic strength and osmolarity can modify cell morphology and medium turbidity, potentially affecting OD readings independently of actual cell number.[37-39]

Table 2. Protein content XynBTN63D in various production media

Media	Protein content (mg/mL)	
	in *S. cerevisiae* BJ1824/*pESC-xynBTN63D*	in *S. cerevisiae* BJ1824/*pYHM1-xynBTN63D*
YPG	0.945	0.917
MTE	0.470	0.480
YMin	0.712	0.683

Analysis of molecular weight and protein content of XynBTN63D

The molecular weight and protein content of the XynBTN63D expressed in *S. cerevisiae* BJ1824/*pESC-xynBTN63D* and BJ1824/*pYHM1-xynBTN63D* cultivated in various production media were determined using the Bradford method (1976) and SDS-PAGE, respectively. Protein content (mg/mL) was used to determine the amount of protein present in each sample, whereas SDS-PAGE separated proteins in accordance with their molecular weight by using polyacrylamide gel electrophoresis.[40,41] The appearance of a clear protein band at the expected molecular weight indicates that XynBTN63D was successfully expressed in both systems. The protein content measured in this study corresponds to the actual amount of recombinant XynBTN63D produced in each culture medium. The resulting values are shown in Table 2, and the SDS-PAGE profile is presented in Figure 3.

As evident from Table 1, the highest XynBTN63D protein levels were detected in

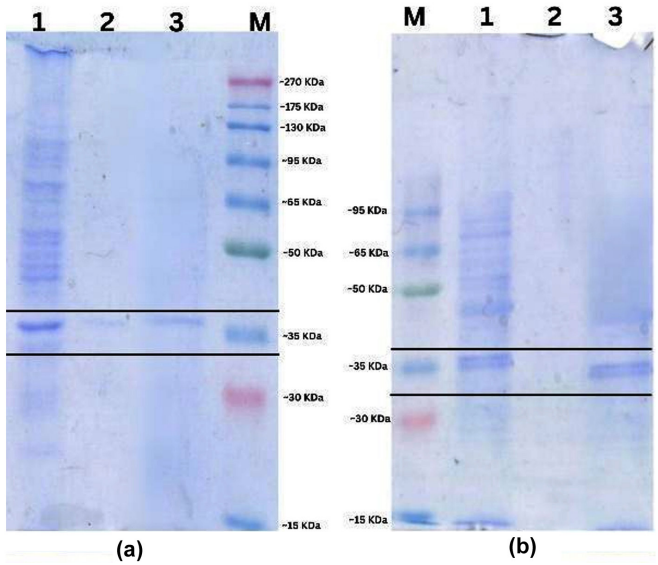

Figure 3. The SDS-PAGE profiles of XynBTN63D on various production media: (a) in *S. cerevisiae* BJ1824/*pESC-xynBTN63D*; (b) in *S. cerevisiae* BJ1824/*pYHM1-xynBTN63D*. Lane M: protein marker; lane 1: YPG medium; lane 2: MTE medium; lane 3: YMin medium

YPG medium for both isolates, which indicates that the rich media composition supports the enhancing of protein yield.[42] Alternatively, MTE medium produced the lowest protein content (0.470 and 0.480 mg/mL, respectively) due to low availability of nutrients and the existence of salts and minerals that can influence protein stability and extraction efficiency.[23] The YMin medium produced intermediate protein content for both isolates.

These results are supported by the observed electrophoretic band thickness (Figure 3). According to Jumrah et al. the band thickness aligned with the protein concentration.[43] The more intense band observed in the protein band in YPG medium, proving higher protein expression in both isolates. In contrast, MTE medium had the thinnest band in the gel electrophoresis, indicating the lowest protein expression. In addition, the results showed that the molecular weight of the XynBTN63D protein in YPG medium was 35 kDa. Previous research conducted by Safitri et al. stated that the XynBT expressed in *E. coli* has a molecular weight of around 30 kDa.[15] The higher molecular weight of the XynBTN63D protein in this study is expected due to post-translational modifications glycosylation in *S. cerevisiae*. Specifically, recombinant proteins derived from *S. cerevisiae* often undergo hypermannosylation (Man>50GlcNAc2).[44] The glycosylation in *S. cerevisiae* BJ1824 (xynBTN63D) is influenced by fermentation metabolism and high sugar media

Table 3. Activity of xylanolytic enzyme

Sample	Enzyme activity (U/mL)		
	endo-β-1,4-D-xylanase	exo-β-1,4-D-xylosidase	α-L arabino-furanosidase
S. cerevisiae BJ1824/pESC-YPG	4.387	0.769	0.256
S. cerevisiae BJ1824/pESC-MTE	0.472	0.237	0.146
S. cerevisiae BJ1824/pESC-YMin	0.930	0.511	0.236
S. cerevisiae BJ1824/pYHM1-YPG	3.735	0.621	0.135
S. cerevisiae BJ1824/pYHM1-MTE	0.265	0.201	0.188
S. cerevisiae BJ1824/pYHM1-YMin	0.634	0.475	0.129

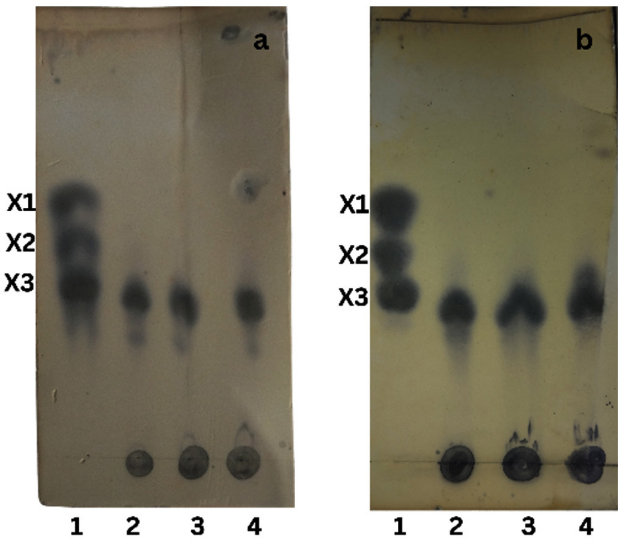

Figure 4. Thin-layer chromatography (TLC) profiles of xylooligosaccharides produced from the hydrolysis of beechwood xylan by XynBTN63D: (a) in *S. cerevisiae BJ1824/pESC-xynBTN63D*; (b) in *S. cerevisiae* BJ1824/*pYHM1-xynBTN63D*. Lane 1: XOS standard; lane 2: YPG medium; lane 3: MTE medium; lane 4: YMin medium

Table 4. R_f Value of Thin Layer Chromatography on various media

Sample	Eluent (cm)	Sample distance (cm)	R_f
X1		5.6	0.622
X2		4.9	0.544
X3		4	0.444
S. cerevisiae/pESC-YPG		3.5	0.388
S. cerevisiae/pESC-MTE		3.6	0.400
S. cerevisiae/pESC-YMin	9	3.5	0.388
S. cerevisiae/pYHM1-YPG		3.4	0.377
S. cerevisiae/pYHM1-MTE		3.7	0.411
S. cerevisiae/pYHM1-YMin		3.7	0.411

The chromatogram and R_f values indicate that the XOS produced by the hydrolysis of XynBTN63D in both isolates and three production media are entirely xylotriose (X3) or XOS containing three xylose monomers. The TLC results have established that the isolates *pESC-xynBTN63D* and *pYHM1-xynBTN63D* could express XynBTN63D in the three production media

such as galactose.[45] In silico prediction using the NetNGlyc 1.0 once more attested to the existence of potential glycosylation sites within the expressed protein sequence, consistent with the observed gain in molecular weight.

Determination of xylanolytic enzyme activity

Enzyme activity refers to the measurement of enzymes' functional proficiency in biological systems. This is quantified in units per mL (U/mL), where the unit of enzyme is the amount of enzyme required to hydrolyze 1 micromole of substrate to generate 1 micromole of product in 1 unit of time.[46] The activity of endo-β-1,4-D-xylanase was determined using the Miller method (DNS) with beechwood xylan substrate. To detect the existence of additional activities of other enzymes, such as exo-β-1,4-D-xylosidase and α-L-arabinofuranosidase, the p-NP-X and p-NP-A substrates were used.[47] The results of the enzyme activity assay are presented in Table 3.

Among all conditions tested, the highest enzyme activities were invariably present in YPG medium for both isolates. The *S. cerevisiae* BJ1824/*pYHM1-xynBTN63D* showed comparatively good xylanolytic activity in the rich YPG medium, though it had slightly lower activity than the pESC construct. However, both constructs consistently exhibited much lower endo-β-1,4-D-xylanase activities in MTE and YMin media. This pattern suggests that the rich nutrient content of YPG medium would support greater enzyme expression and secretion, whereas the more confined and salted conditions of MTE and YMin media would likely restrict enzyme stability, in accordance with the lower protein content in Table 1. Furthermore, the overall trend across these three enzymes indicates that *pESC-xynBTN63D* consistently yields higher activity than *pYHM1-xynBTN63D* under the same conditions, suggesting more efficient expression or greater stability of the recombinant protein under the pESC system.[23,48]

Analysis of thin layer chromatography profile

Xylooligosaccharides (XOS) are the result of XynBTN63D hydrolysis with xylan substrate. XOS is a type of oligosaccharide whose monomers consist of D-xylose (X1).[49] The type of XOS yielded in this study is anticipated to be a group of XOS containing two or more monomers. XOS analysis was also conducted to ensure that the isolates *S. cerevisiae* BJ1824/*pESC-xynBTN63D* and *pYHM1-xynBTN63D*, in various media components, were able to express XynBTN63D. The XOS analysis employed the thin-layer chromatography method developed by Ratnadewi et al., based on the principle of "like dissolves like" and molecular migration potential. The qualitative analysis was conducted by comparing the R_f (retardation factor) values of the samples with the standards. The standards used were xylose (X1), xylobiose (X2), and xylotriose (X3).[17] The results of the TLC are presented in Figure 4 and Table 4.

CONCLUSION

Recombinant XynBTN63D is an endo-β-1,4-D-xylanase with the ability to degrade biomass for xylooligosaccharide (XOS) production. XynBTN63D cloned into pYHM1 and pESC plasmids was successfully expressed in *Saccharomyces cerevisiae* BJ1824, a food-safe host capable of extracellular recombinant protein production. Since industrial-scale enzyme production requires cost efficiency, this study comparatively evaluated three media compositions, namely YPG (rich medium), YMin (minimal medium), and MTE (semi-defined medium), for recombinant XynBTN63D

production. All media supported the growth of recombinant *S. cerevisiae* BJ1824, as confirmed by OD_{600} measurements. Interestingly, YMin medium, containing galactose as the carbon source and yeast extract as the nitrogen source, promoted faster cell division than YPG. This result indicates that its nutrient composition supports efficient metabolic adaptation, although the resulting enzyme activity remained lower than that of YPG. SDS-PAGE analysis revealed recombinant XynBTN63D bands at approximately 35 kDa in YPG and YMin media, while weaker expression was observed in MTE medium. Among the detected xylanolytic enzymes, endo-β-1,4-D-xylanase activity was dominant, followed by exo-β-1,4-D-xylosidase and α-L-arabinofuranosidase activities. TLC analysis further confirmed that the major hydrolysis product was xylotriose (X3). Overall, YMin medium demonstrated promising potential as a more economical alternative medium for sustainable recombinant XynBTN63D production and XOS bioprocess development.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

AUTHORS' CONTRIBUTION

AAIR conceptualized the study. NNTP supervised the study. MAB performed validation. RT performed data curation and wrote the original draft. AKP and TDA wrote, reviewed and revised the manuscript. All authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies on human participants or animals performed by any of the authors.

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