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Heterologous Cellulase Genes Expression and Post-Translational Modifications Analysis of Recombinant *Saccharomyces cerevisiae*

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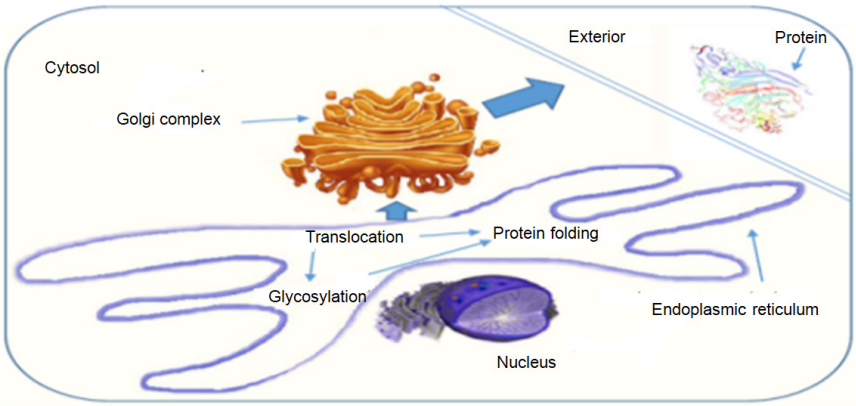
ABSTRACT: The recombinant *Saccharomyces cerevisiae* is a demanding and ideal consolidated bioprocessing (CBP) host for industrial bioethanol production. In the current study, the post-translational modifications in a recombinant *S. cerevisiae* strain PMP-Sc1 were observed. The recombinant PMP-Sc1 cultivation profile at 30°C, 200 rpm, with respect to galactose induction (Zero Gal, Min Gal and Max Gal) was observed for 96 hours. The PMP-Sc1 test strains entered the exponential growth phase as a little lag phase ended. The growth of PMP-Sc1 (Max Gal) marginally enhanced in comparison with PMP-Sc1 (Zero Gal) and PMP-Sc1 (Min Gal). Moreover, all the test strains exhibited slight impact of galactose induction on their growth. It is, however, important to note that during the stationary phase of cell growth at 72 hours, PMP-Sc1 (Zero Gal), PMP-Sc1 (Min Gal) and PMP-Sc1 (Max Gal) reached an eventual maximum OD 600 of 9.43 ± 0.15, 9.56 ± 0.15 and 10.03 ± 0.20, respectively. There was appearance of only one hyperglycosylated band on SDS-PAGE in all the test strains at 180 kDa which was greater than the theoretically calculated molecular weight of protein. Crude protein lyzates were examined for substrate specific zymography which revealed proteolytic cleavage of stitched genes. The protein modeling and amino acid analysis exhibited the presence of N-glycosylation sites NMS and NGT in the amino acid sequence and N-acetyl-d-glucosamine (NAG) in protein model indicating the post-translational modification in protein.

Keywords: Cellulase, Galactose, Hyperglycosylation, *Saccharomyces cerevisia*, SDS-PAGE, Zymogram.

Abbreviations: OD, Optical density; NAG, N-acetyl-d-glucosamine; PTM: Post-translational modification.

Graphical Abstract



I. INTRODUCTION

Saccharomyces cerevisiae is a preferred host for industrial bioethanol production. Numerous research findings have revealed that the resultant ethanol content acquired through fermenting capacity of *S. cerevisiae* is nearly 79.25–96.29 g/L [1]. The highest ethanol producing industrial *S. cerevisiae* strains in Brazil are CAT1 and PE2, with ethanol content of 79.25 g/L and 77.35 g/L, respectively [2]. Pretreatment of lignocellulosic biomass, enzymatic hydrolysis and ethanol fermentation are the basic processes involved in conversion of polysaccharides to disaccharides and subsequently to glucose monomers for ethanol production through mico-organisms, in general and

Saccharomyces cerevisiae, in particular [3]. The expenditure on the production of ethanol is affected most by performing hydrolysis and fermentation processes separately which may be lowered by using the alternative methods of simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-fermentation (SSCF), and consolidated bioprocessing (CBP) [4, 5]. It is estimated that commercial cellulase enzymes cover almost 25–50% of the overall bioethanol production expenditures [6]. Therefore it is desired to produce an ideal recombinant *Saccharomvces cerevisiae* carrying cellulase genes to reduce the cost of cellulase enzymes and increase ethanol production.

Saccharomyves cerevisiae is an industrial model micro-organism on account of its simplicity, fastidiousness and availability of the whole genome sequence [7]. Most of the commercial vectors for *Saccharomyces cerevisiae* have GAL as promoter such as pYES-DEST52 (7.6 kb) GAL 1 promoter, pYES2 (5.9 kb) GAL 1 promoter, pYES2-NT, pYES2-CT (6.0 kb) GAL 1 promoter (Invitrogen), pESC yeast epitope tagging vectors (pESC-HIS: 6.7 kb, pESC-LEU: 7.8 kb, pESC-TRP: 6.5 kb, pESC-URA: 6.6 kb) series with divergent promoters pGAL 1/ pGAL 10 which require galactose as carbon source to induce the promoter for gene expression, nonetheless galactose is not a preferable carbon source for *S. cerevisiae* in terms of growth [8].

In eukaryotic cells, the transcription level of gene expression must be regularized as nutritional shift and unfavorable environmental factors antagonize it the most [9]. Restrictions in the yeast secretory pathway such as translocation, endoplasmic reticulum (ER) based protein folding, glycosylation, protein arrangement and trafficking [10], often results in reduced secretion of protein [11]. In *S. cerevisiae*, the protein secretion is a multi-step mechanism, regulated by a number of proteins [12] physically influenced by the presence of the disulfide bonds, hydrophobicity of protein molecules and the molecular weight of it [13]. N-linked glycosylation, covalently linked to proteins, is one of the most significant, structurally complex, and pervasive post-translational modification in *S. cerevisiae* [14]. In addition, protein activity in recombinant *S.cerevisiae* is generally influenced by N-hyperglycosylation [10].

In this study, the recombinant *Saccharomyces cerevisiae* strain PMP-Sc1, carrying stitched cellulase genes including CMCase (*Trichoderma harzianum*), cellobiohydrolase (*Penicillium oxalicum*) and β-glucosidase (*Aspergillus niger*) [15]. The main purpose of this cohort study was to explore the posttranslational modifications in stitched cellulase genes with respect to galactose induction as in this study, galactose was used as an inducer of GAL promoter to induce protein expression.

II. MATERIALS AND METHODS

Strain, Media and Galactose Induction

The recombinant *Saccharomyces cerevisiae* INVSc1, named as PMP-Sc1 carrying stitched cellulase genes including CMCase (*Trichoderma harzianum*), cellobiohydrolase (*Penicillium oxalicum*) and β-glucosidase (*Aspergillus niger*) [15] prepared at Fermentation Research Technology Center (FTRC), Kasetsart University, Bangkok, Thailand, was used in this study. Synthetic complete media comprised of 0.67% yeast nitrogen base without amino acids, 0.13% uracil dropout media, 2% glucose/galactose (glucose was used for growing cultures and galactose was used for genes expression by inducing GAL promoter) and 2% agar (optional) was used as culture media and for protein expression.

Cellulase Cultivation Profile by PMP-Sc1

An analysis of growth curve was performed by following the protocol of Nel [16]. A series of experimental observations were made to assess the cellulose cultivation profile by PMP-Sc1 transformants. Cells of PMP-Sc1 (A 600 nm = 0.1) were inoculated in triplicate into 250 mL Erlenmeyer flasks containing 50 mL SC^{-URA} broth from cultures grown for 16 hours with galactose as sole carbon source. For inhibition of bacterial growth, the media was added with chloramphenicol (300 µg/mL) and streptomycin (500 µg/mL) as antibiotic source. The inoculated flasks were incubated at 30℃ and 200 rpm. A 400 µL sample was removed from each test flask at each interval and optical density was measured at A 600 nm. The samples were collected at 24 hours interval, up to 96 hours cultivation time.

Protein Expression in PMP-Sc1

To induce the expression of our desired protein of stitched EG-CBH-BGL genes through GAL1 promoter, galactose was added to the medium. With prior glucose based growth, the recombinant protein is ordinarily observed in 4 hours after galactose induction [17]. For galactose induction, the recombinant INVSc1 (PMP-Sc1) was tested for three experimental conditions;

i) No galactose induction: This test condition was named as PMP-Sc1 (Zero Gal).
ii) Minimum galactose: The recombinant INVSc1 grown in this condition was named as PMP-Sc1 (Min Gal). In this condition, the minimum galactose i.e. 0.02% was used.
iii) Maximum galactose: The recombinant PMP-Sc1 grown in this state was induced with 2% galactose [18]. This strain was named as PMP-Sc1 (Max Gal).

A marginal colony of recombinant INVSc1 constituting pESC-URA-EG-CBH-BGL (pESC-PMP) was inoculated into 15 mL of the synthetic complete selective medium comprising 2% glucose, grew overnight at 30℃ with 200 rpm and determined OD_{600} of it. An OD_{600} of 0.4 was calculated and pelleted at 1,500 × g (5 minutes) at room temperature. The pallet was resuspended in 50 mL induction medium and grew at 30℃ with 200 rpm shaking. Harvesting of cells was carried out at 4℃ by centrifugation at 1,500 × g for 5 min. The pelleted cells were again suspended in sterile deionized water (500 µL) and stored at –80℃ until ready to use [19].

Cell Lysate Preparation: To prepare a cell lysate from recombinant PMP-Sc1 transformants, a conventional acid-washed glass beads procedure was followed. This protocol was first published in the Current Protocols in Molecular Biology [20]. Moreover, this protocolis also recommended by Invitrogen (The host strain INVSc1, manipulated to form PMP-Sc1 by expression of cellulase genes, is a commercial *Saccharomyces cerevisiae* strain of Invitrogen). The method is as following; the cells were harvested from fresh cultures and suspended in 500 µL of lysis buffer which comprised of 1 mM EDTA, 1mL Triton X, 50 mM sodium phosphate (pH 7.4) and 5% glycerol. The suspension

was centrifuged at high speed for 5 minutes at 4℃ to separate out the supernatant and the pallet. After draining off the supernatant, the pallet was suspended in appropriate volume of lysis buffer to acquire an OD_{600}. An equivalent measure of acid-washed glass beads were added in the blend and vortex for four minutes to obtain the lysed cells. This lysate was used for protein assay on SDS-PAGE.

SDS-Polyacrylamide (PAGE) Gel Electrophoresis: Recombinant strain PMP-Sc1 (Zero Gal), PMP-Sc1 (Min Gal) and PMP-Sc1 (Max Gal) were investigated for protein production through SDS-PAGE analysis. The reducing sample buffer was prepared by adding 12% SDS (w/v), 30% Glycerol (w/v), 0.05% Coomassie blue G-250 (w/v) and 150mM Tris-HCl (pH7.0). The loading sample was prepared by adding the 5 µl reducing sample buffer to the 10µl sample and heated at 95℃ for 5 min. The Page Ruler Prestained Protein Ladder (10-180 kDa) was used for assessment of molecular weight of protein. Laemmli [21] protocol was used for separation of protein fractions through a 12% (v/v) SDS-PAGE. For Native PAGE (without SDS), the loading samples were loaded without heating [22]. Electrophoresis was conducted at 100 volts initially for 30 minutes, followed by 120 volts for 45 minutes. The bands were viewed on gel by using silver staining protocol as performed by Huang [23] for protein assessment in cellobiohydrolase, β-glucosidase and endoglucanase enzymes.

Zymogram Analysis: The zymogram analysis for endoglucanase and cellobiohydrolase were carried out by following the procedures performed by Grigorevski-Lima [24]. The enzyme supernatant was used in SDS-PAGE analysis. Polyacrylamide gel of 12% that contained 1% CMC (for endoglucanase) or 1% Avicel (forcellobiohydrolase) was used. As the electrophoresis ended, the gel was transferred to a 2.5% (v/v) solution of Triton X-100 and allowed to soak for 2 hours.The soaked gel was further incubated for 1 hour at room temperature in a 50 mM sodium acetate buffer (pH 5.0) so that the SDS may be removed. The gel was later washed with a 50 mM sodium acetate buffer (pH 5.0) at 45℃ for 30 min and staining of it was performed with Congo red dye [1% (w/v)] at room temperature for 30 minutes. 1N NaCl was used to destain the gel (about 30 min incubation at room temperature). The clear zones on PAGE exhibited the activity of either endoglucanase or cellobiohydrolase, subjected to specific substrate used.

For zymography of β-glucosidase, the protocol designed by Gao [25] was followed. An appropriately measured blend of enzyme extract and loading buffer were loaded onto a gel without heating up. The gel was washed thrice with deionized water after electrophoresis and a marginal column of it was cut with a sharp scalpel and stained with Coomassie, so that it may be used as a marker. The rest of gel was incubated in Tris–HCl buffer for SDS removal and later stained with pNPG and addition of Na_2CO_3. The bands exhibiting yellow coloration were cut out from the gel and used for zymography on Native-PAGE with silver staining.

Amino Acid Sequence Analysis

The sequence of open reading frames (ORFs) of the glycosyl hydrolases expressed in this study were the hyperglycosylation signals in amino acid sequence of PMP-Sc1; CLC Genomics Workbench v5.5.1 with the Pfam Protein Domain database was used in current study.

Protein Modeling: Proteins encoded by the ORFs were annotated using the Phyre2 software for protein modeling [26]. The SWISS-MODEL was used for predicted protein model analysis [27].

III. RESULTS AND DISCUSSION

Growth Profile of PMP-Sc1

The recombinant PMP-Sc1cultivation profile at 30℃, 200 rpm, with respect to galactose induction (Zero Gal, Min Gal and Max Gal) was monitored for 96 hours, exhibited in Fig. 1. After a short lag phase, the PMP-Sc1 strains entered the exponential growth phase. The growth of PMP-Sc1 (Max Gal) was observed a tad enhanced than the PMP-Sc1 (Zero Gal) and PMP-Sc1 (Min Gal). Moreover, all the test strains exhibited little impact of galactose induction on their growth. Heterologous genes expression may cause metabolic burden which exerts robust impact on cell growth of a micro-organism. Conversely, in this study, the growth rates of the PMP-Sc1 test strains were almost nearer to one another which reveals that no palpable metabolic burden was caused by heterologous cellulase genes expression [28] in PMP-Sc1. It is, however, important to note that the cell growth stationary phase of PMP-Sc1 (Zero Gal), PMP-Sc1 (Min Gal) and PMP-Sc1 (Max Gal) at 72 hours, reached an eventual maximum OD_{600} of 9.43 ± 0.15, 9.56 ± 0.15 and 10.03 ± 0.20, respectively which was similar to the findings of Davison [29] where transformants reached a maximum OD 600 nm in the range of 12.19 – 9.50. The growth competence of the PMP-Sc1 test strains had no significant ($p>0.05$) damaging impact exerted by the expressed genes in test strains [29, 30]. It was interesting to observe that the optical density of all the test strains at 48 h raised almost two folds greater than the values at 24 h which in contrast reduced to only 1.25 fold from 48 to 72 hour to reach the maximum optical density. Similar observations were reported by Davison [29] and van Zyl [31].

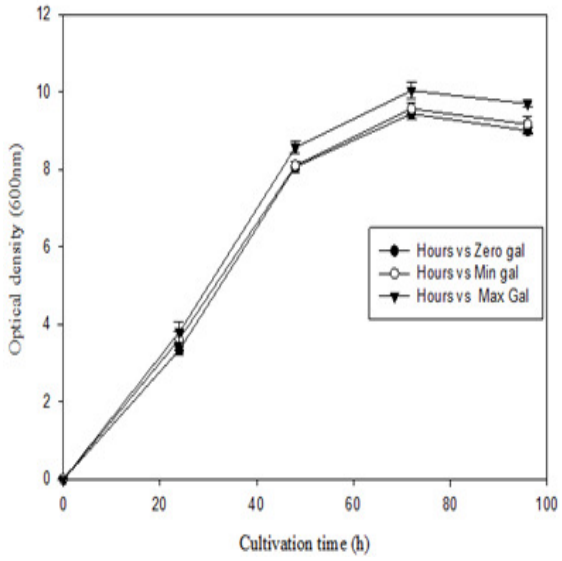
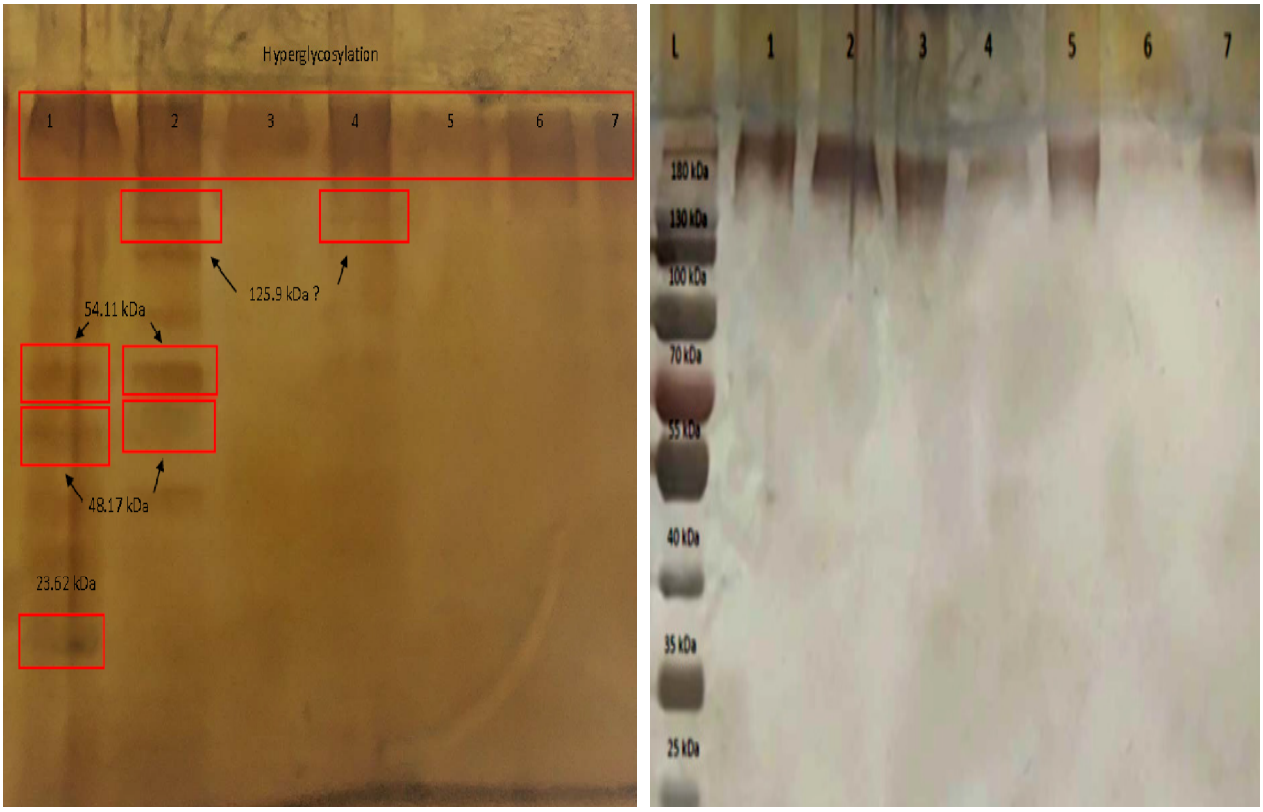


Fig. 1. Graphical comparison of the optical density (OD 600) of *Saccharomyces cerevisiae* PMP-Sc1 a) Zero Gal, b) Min Gal and c) Max Gal.

Protein Size Determination and Zymogram Analysis

SDS polyacrylamide gel electrophoresis of endoglucanase, cellobiohydrolase and β-glucosidase was performed after lysate preparation. The native-PAGE [22] without boiling of loading sample (lysate with loading buffer) displayed numerous bands which were approximately equal to theoretical molecular weights of endoglucanase, cellobiohydrolase, and β –glucosidase estimated as 23.62 kDa, 48.17 kDa, and 54.11 kDa, respectively. As, all the genes were fused and as theoretically, there should be a huge band around 130 kDa of prestained ladder, but instead of this, there were huge protein bands observed approximately equal to 180 kDa (Fig. 2. a and b) which indicated hyperglycosylation phenomenon. Hyperglycosylation has been observed by many researchers working on *Saccharomyces cerevisiae* or other eukaryotes like Aspergillus due to which there is a significant increase in the molecular weights of expressed genes. The N-glycosylation sites in amino acid sequence of assembled cellulase genes are shown in Fig. 4. Tang [10] observed hyperglycosylation in expressing CelA (cellulase gene) even though it was from a prokaryote. This indicates that a yeast has the potency of glycosylating a protein which carries glycosylation sites. The similar pattern of hyperglycosylation was observed in this research experiment when boiled samples (at 98℃) were loaded onto SDS-PAGE. There was only one hyperglycosylated band appeared in all the test samples at 180 kDa (Fig. 2.) band instead of appearing around 130 kDa protein ladder band. The zymogram activity was also performed for each cellulase gene by following the protocol of Garima [32] to additionally confirm, and directly ascribe, the observed activity to cellulase genes and not to any contaminant proteins. The crude protein lyzates were investigated for their ability to hydrolyze CMC, and Avicel added into the gel (using 12% Native PAGE) as substrates for EG, CBH, respectively. There were clear zones appeared on the Native-PAGE (Fig. 3.) indicating the activity of particular enzyme (endoglucanase and cellobiohydrolase) and pale colouration for β-glucosidase, instead of a single fused protein.



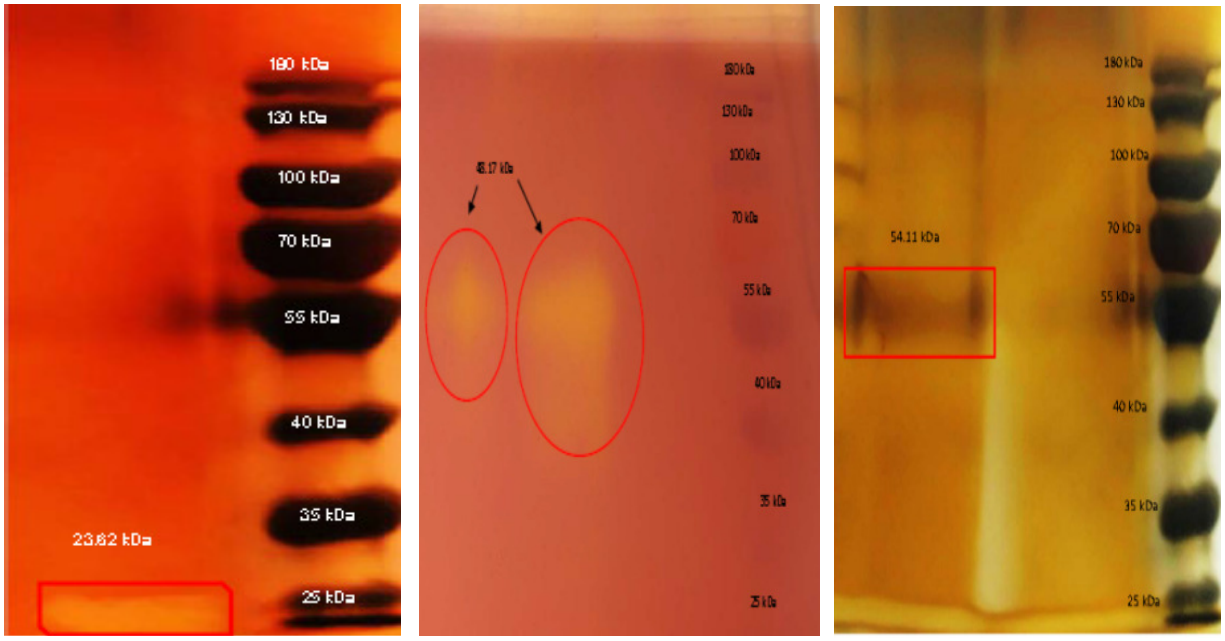
(a) (b)

Fig. 2. (a) SDS-PAGE analysis of the PMP-Sc1 lyzate (L: Prestained molecular mass marker (15-180 kDa); lane 1-7: protein lysate [1,2: PMP-Sc1 (Max Gal), 3,4: PMP-Sc1 (Min Gal), 5, 6: PMP-Sc1 (Zero Gal), 7: PMP-Sc1 (Max Gal) (b) Native-PAGE analysis of PMP-Sc1 lysate for molecular weight and expression assessment (lane 1-7: protein lysate) [1,2 PMP-Sc1 (Max Gal), 3,4: PMP-Sc1 (Min Gal); 5, 6: PMP-Sc1 (Zero Gal); 7: PMP-Sc1 (Max Gal)

For zymogram activity of endoglucanase and cellobiohydrolase, 1% CMCase, and 1% Avicel was used, respectively. It was interesting to find that similar to SDS and native PAGE, the zymogram also did not show the expected theoretical molecular weight of the fused genes. The activity of endoglucanase and cellobiohydrolase were exhibited at the position on zymogram identical to their respective molecular weight i.e. 23.62 kDa and 48.17 kDa. The zymogram of β-glucosidase (Fig. 3. b) also showed the parallel pattern of proteolytic cleavage as observed for cellobiohydrolase (Fig. 3. a) and CMCase and expressed the band at its specific molecular weight i.e. 54.11. Similar pattern of zymograms (Fig. 3. c) were

observed by Kurniasih [33] who found molecular mass of fused endoglucanase and β- glucosidase with an expactaion of 107 kDa protein molecular weight of fused gene to show on zymogram. Nevertheless, there were four bands exhibited of fused endoglucanase and β-glucosidase which were partially hydrolyzed and similar to the expression of individual endoglucanase and β-glucosidase genes. Kurniasih [33] related this band pattern to protein cleavage. Similarly, in the current study, there may also be a proteolytic cleavage in fused protein which avoided the expression of whole fused gene zymogram.

The zymograms in Fig. 3, representing the individual molecular weight protein bands of CBH, BGL and EG, respectively instead of a one big protein at around 130 kDa protein band for exhibiting the fused protein band of just about 126 kDa(125.9 kDa) protein band as theoretical molecular weight of whole fused protein.



(a) (b) (c)

Fig. 3. Zymogram of (a) cellobiohydrolase, (b) β-glucosidase and (c) endoglucanase.

The appearance of smear in Lane 1-7 of the Fig. 2 indicates the Hyperglycosilation of the stitched cellulase genes which is a sound reason for unexpected protein size, differing from the expected molecular weight. *S. cerevisiae*is well-known for hyperglycosilation phenomenon [16] on account of which, it usually affects the heterologous protein activity [10]. In a study on *Pichia pastoris*, Bernardi [34] observed that despite deglycosylation, the protein produced by endoglycosidase H (molecular weight: 60 kDa) was a little bit higher than the theoretically calculated size. He attributed this change in protein size to the hyperglycosylation mechanism, which is a general feature of yeast characteristics [34].

In order to investigate the hyperglycosylation signals in amino acid sequence of PMP-Sc1, The CLC Genomics Workbench v5.5.1 with the Pfam Protein Domain database was used. The analysis revealed two theoretical glycosylation sites, NMS and NGT in the protein sequence of PMP-Sc1 (Fig. 4) which causes the enhancement in molecular weight of the protein [35].

MKLFQILPVILPVAVAQTSCVQYAVFTGGNGYSVSNNLWGQSAGRGFGCILSTHSTRLPPGMQTGSGLAAKTTSS
PTPMFKLPFPKSELSTASEACLRLLPGATRAAIFALLPLIISSLHQIPTMGLIPEITSSQSGWQDTETLAPLGQLRARL
TSMVKAGHSTTASTELCKSIALWPPAPSPIGVEMARTSSTIYETTKDTRHQANMSSVTNLVLSLLQAAGRMYQRA
LLFSALMAGVPAQQVGTQKPETHPPLAWKECTSSGCTSKDGSLVIDANWRWVHSVDGYKNCYTGNEWDSTLCP
DDATCATNCAVEPADYAATYGATTEGDSLSINFVTGSNIGSRFYLMEDENKYQMFKLSNKEFTFDVDVSTLPCGL
NGALYFVSMDADGGMSKYETNPAGAKYGAGYCDPQCPRDLKFINGKGNVEGWKPSANDKNAGVGPHGSCCAE
MDIWEANSISTALTPHPCDTNGQTICEGDSCGGTYSTTRYAGTCDPDGCDFNPFRMGNESFYGPGKMVDTKSK
MTVVTQFITSDGTDTGSLKEIKRVYVQNGKVIANSASDVSGITGNSITSDFCTAQKKTFGDEDVFNKHGGLSGMGD
ALGEGMVLVMSLWDDHNSNMLWLDGEKYPTDAAASKAGVSRGTCSTDSGKPSTVESESGSAKVVFSNIKVGSI
GSTFSAMGSATASTLPPDFLWGFATASYQIEGAVTEDGRGPSIWDTFCKIPGKIAGGANGDVACDSYHRTAEDIA
LLKECGAQAYRFSISWSRIIPLGGRNDPINDKGVQHYVKFVDDLLAAGITPLVTLFHWDLPDALDKRYGGLLNKEEF
VADFANYARVMFRALGSKVKHWITFNEPWCSSVLGYNVGQFAPGRTSDRSKSAEGDSSRECWIVGHNILVAHGA
AVKIYREEFKSRDGGEIGITLNGDWAEPWDPENPADIEACDRKIEFAISWFADPIYHGRYPDSMIKQLGDRLPSWT
AEDIALVHGSNDFYGMNHYCANYIKAKTGEADPNDTAGNLEILLKNKKGEFIGPETQSASLRPYALGFRKLLKWLS
DRYGQPKIYVTENGTSLKGENDLPVDQLLKDEFRAQYFRDYIAAMADAYTPHGVNVRAYMAWSLMDNFEWAEG
YETRFGSTYALYEHGQKRIPKDSAKQIGQIFSQYIEKK

Fig. 4. N-glycosylation prediction in amino acid sequence of assembled cellulose (endoglucanase, cellobiohydrolase, and β-glucosidase) genes; the N-glycosylation sites NMS and NGT are shown in bold.

The Phyr 2 software was used to model the recombinant amino acid sequence of the assembled cellulase gene cassette, expressed in PMP-Sc1 [15]. The protein analysis was performed with SWISS-MODEL software. The model exhibited the post translational modification in the sequence consisting of N-acetyl-d-glucosamine (NAG). Moreover, there was the presence of two non-covalent zinc ions including Zn. 2: 5 residues within 4Å:

Chain A: I.104, D.375, H.377, P.411, E.415; 5 PLIP interactions (4 interactions with chain A, 1 Ligand-Water interactions and metal complexes: A: D.375, A:D.375, A:H.377, A:E.415, H_2O.17), and Zn. 3: 1 residues within 4Å: 4 PLIP interactions (1 interactions with chain A, 3 Ligand-Water interactions and metal complexes: A:D.46, H_2O.4, H_2O.4, H_2O.19). In Fig. 5, the predicted protein model (a) is exhibited with magnified parts of Zn. 2 (b), Zn. 3 (c), NAG (d) and the possible mutation prediction (e).

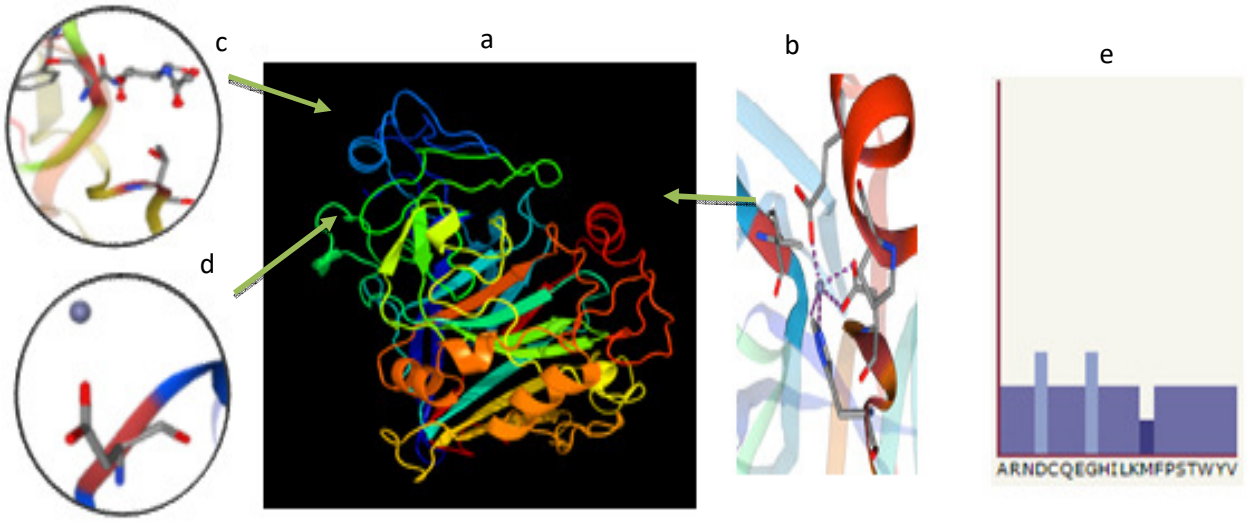


Fig. 5. Protein modeling of assembled cellulase genes cassette (a) Predicted protein modelof assembled genes; (b) NAG-Post translational modification; c) Zinc 2; d) Zinc 3; (e) Mutation tendency in PMP-Sc1 protein.

IV. CONCLUSION

The cellulase cocktail protein expression by *Saccharomyces cerevisiae* PMP-Sc1 was investigated through SDS-PAGE, zymgography and predicted protein modeling. The results revealed hyperglycosylation effect on SDS-PAGE and cleavage in stitched genes on zymogram. The amino acid sequence displayed the presence of N-glycosylation sites NMS and NGT. The protein model indicated the NAG-Post translational modification in the stitched cellulase protein.

V. FUTURE SCOPE

In *Saccharomyces cerevisiae*, the post-translational (PTMs) modifications involve the reversible addition of a chemical group like phosphate, carbohydrates in glycosylation, and polypeptides in ubiquitylation. Furthermore, proteolytic processes may occur during protein maturation or deamination processes is stimulated too. These alterations may influence the protein structure, composition, secretion or activity. A targeted analytical approach can be used for a comprehensive portrayal of N-glycosylation role in the in vivo protein maturation at a molecular level. A combination of various techniques such as stable isotope labeling by amino acids in cell culture (SILAC) strategy and a parallel reaction monitoring (PRM) based mass spectrometry (MS) can be used for the identification and quantification of N-linked glycoproteins In order to meet the upsurging demand of energy [36] such as bioethanol production through recombinant *Saccharomyces cerevisiae*, the investigation of the post translational modifications are necessarily required.

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Conflict of Interest. The Authors declare that there is no conflict of interest. This study is based on an analysis of heterologous genes expression and post-translational modifications in recombinant *Saccharomyces cerevisiae* PMP-Sc1.

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