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PRODUCTION AND CHARACTERIZATION OF ENDOGLUCANASE FROM AN INDIGENOUS YEAST STRAIN

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Abstract

Cellulases, group of industrially important enzymes, are commercially obtained from mold or bacterial strains. Yeasts strains have scarcely been reported for their cellulolytic potential and are not applied on industrial scale. This study was aimed at the isolation of a promising cellulolytic yeast strain with the possible biotechnological application. The strain, MK-157, was identified as *Saccharomyces cerevisiae* on traditional and molecular basis. The gene encoding 1,3-endoglucanase was also amplified by Nested PCR and sequenced. The activity of endoglucanase (EG) band corresponding to 30 kDa was observed in zymogram. The data showed that at 30°C and pH 7.0, in presence of 1% Carboxymethyl cellulose (CMC), the strain produced more than 15 IU/ml of EG. The activity of EG was also characterized in crude preparation. The strain was able to tolerate up to 12% alcohol and produced 5.45 % ethanol when cultivated in a complex medium. The potential of the strain to grow and produce EG on untreated and alkali-treated wheat-bran (WB) and sugarcane-bagasse (SCB) was also evaluated. The results showed the ability of the strain MK-157 to produce EG in presence of CMC, SCB and WB. The study provides some insights on less explored cellulolytic yeasts. The strain MK-157 can find its application in consolidated bioprocessing of cellulosic mass.

Key words: Endoglucanase, *S. cerevisiae*, Sugarcane-bagasse, Wheat-bran, Nested PCR

Introduction

Lignocellulose (LC) is a matrix of cross-linked polysaccharides' network, glycosylated proteins and lignin, found in plant cell wall. Depending on the source of LC the amount of most abundant component, cellulose varies from 17-32% (Ritter, 2008). Although, cellulose is also produced by some bacteria (Lynd *et al.,* 2002), however, plant biomass remains the primary reservoir. It is estimated that this mass is increased by approximately 4×10^7 tons per year, making it an attractive renewable source of chemicals and energy. It is a linear homopolymer of D-glucopyranose units linked by 1, 4-glycosidic bonds with a varying degree of polymerization (Ritter, 2008). Cellulose is degraded by an enzyme system, cellulases, comprised of three components, endoglucanases (EG; EC. 3.2.1.4), cellobiohydrolases (CBH; EC. 3.2.1.91) and β-glucosidases (BGL; EC.3.2.1.21). These glycoside hydrolases (GH) hydrolyse the glyosidic linkages of cellulose to fermentable sugars. EG effectively cleaves glycosidic bonds of soluble substituted celluloses such as carboxymethyl cellulose (CMC) internally and randomly but cannot degrade crystalline cellulose efficiently. Crystalline celluloses are degraded by CBH into cellobiose. Ultimately, cellobiose and other short cello-oligosaccharides are hydrolyzed by BGL (Ritter, 2008). Cellulases, particularly, EGs are widely used in various industrial and commercial processes, for instance, in textile industry these are used to treat cellulose-containing textile materials during manufacture, finishing and for biopolishing of fabrics (Menendez *et al.,* 2015).

Considering the significance of EGs in the textile and other industries, a large number of EGs from filamentous fungi and bacteria have been purified and characterized (Lynd *et al.,* 2002). Although, yeasts reportedly produce different extracellular plant cell wall degrading enzymes, however, very few workers have described EGs from yeasts (Lara *et al.,* 2014). The production of plant cell-wall degrading enzymes from yeasts offer some novel prospects, as yeasts have shorter generation time than molds and their growth does not cause rheological problems in fermentation media. Additionally, cellulolytic yeasts can conveniently be co-cultivated with ethanologenic yeast thus the cost for biofuel production can be reduced substantially (Sahoo & Shirnalli, 2015). Furthermore, yeasts have been employed as a model eukaryotic system for heterologous protein expression (Rensburg *et al.,* 1998).

Limited distribution of EG genes amongst yeasts genera is evident from many studies, for instance, only 19% of the yeast strains out of 350 strains were found to be cellulolytic by Gomes *et al.,* (2015); the major genera included *Cryptococcus, Fellomyces, Myriangiale* and *Ocultifer.* In another study, strains of *Cryptococcus, Mrakia* and *Rhodotorula* isolated from Arctic region were found to be cellulolytic (Pathan *et al.,* 2010). Whereas, in most of the studies, the recombinant strains of *Saccharomyces cerevisiae* have been used displaying heterologous EG, CBH and/ or BGL to convert LC biomass directly to ethanol (Rensburg *et al.,* 1998). Recently, Adelabu *et al.,* (2017) has reported about the production of cellulases from a wild-type strain of *S. cerevisiae.* Moreover, plant cell wall degrading enzymes from yeasts have also been studied for their synergistic effect on bacterial EG to hydrolyze crude substrates such as sugarcane bagasse (Tariq *et al.,* 2018). In this context, the present study describes isolation and screening of indigenously isolated yeast strains for EG production. One of the most promising strains, MK-157 was identified as *S. cerevisiae* on genetic basis and EG production from that strain was optimized.

Materials and Methods

Isolation and screening of yeast strains: Yeast strains were isolated from various samples including garden soil, different plant parts, yogurt, chilies, grapes, banana juice and lemons. One of the soil samples was enriched with CMC for 2 weeks and was processed for the isolation along with the other samples. Each sample was diluted to appropriate level and diluted aliquots were spread on various mycological media as described by Shariq and Sohail (2018). Plates were incubated at 25°C for 3-4 days until yeast-like colonies appeared. Colonial characteristics were noted and isolates were maintained on Sabouraud Dextrose Agar (SDA) and preserved with 60% (v/v) glycerol (Shariq & Sohail, 2018). Along with the isolates, some strains were also retrieved from the lab culture collection.

The isolated and retrieved strains (221) were screened for the production of EG by cultivating on plates containing carboxymethyl cellulose (CMC) supplemented mineral salt medium (MSM; KH_2PO_4 20g/L, $(NH_4)_2SO_4$ 14 g/L, $MgSO_4.7H_2O$ 3g/L, $CaCl_2$ 3 g/L, protease peptone 10g/L, $FeSO_4.7H_2O$ 0.5g/L, $MnSO_4.H_2O$ 0.16g/L, $ZnSO_4.7H_2O$ 0.29g/L, $CoCl_2.6H_2O$ 0.29 g/L) agar plates. EG production was detected by following the method given by Jahangeer *et al.,* (2005). The EG titers were quantified by growing the strains in MSM broth containing CMC and extracting cell-free culture supernatant (CFCS) to perform EG assay (Sohail *et al.,* 2011). An endoglucanase unit was defined as the micromoles of reducing sugar produced by one ml of enzyme in one minute under standard assay conditions. On the basis of EG titers and stability, the strain MK-157 was selected for further studies.

Identification of the strain MK-157: The yeast strain, MK-157, was identified on the basis of morphological, cultural and biochemical tests as mentioned by Kurtzman & Fell (1998) and Guimaraes *et al.,* (2006). It was tested for various characteristics including acid production in chalk media, hydrogen sulfide production and growth at varying temperatures and in presence of acetic acid and ethanol (stress test). Identification on molecular basis was also carried out by using various combinations of ITS primers (Chen *et al.,* 2000; Leaw *et al.,* 2006). The chromosomal DNA was extracted according to Miller *et al.,* (1988) with some modifications. Briefly, an isolated colony from 24 h old culture was suspended in distilled water and washed twice by centrifugation at 4500×g with distilled water and then with TE buffer (50mM Tris Base, 1mM EDTA, 0.5% Triton X-100, pH 8.0). After that, cell lysate was digested by incubating overnight with 100 µl 1% SDS and 200 µl of Proteinase K (20 µg/ml or 0.6 units/ml) at 65°C. Proteins were precipitated by vortexing the mixture with 250 µl of 6M NaCl. Supernatant was collected by centrifugation at 13000×g for 5 min and re-centrifuged for 20 min at 4°C. Supernatant was mixed with isopropanol (in a ratio of 0.8:1) by inverting the tubes multiple times and briefly centrifuged. The pellet was dried by washing with 70% ethanol and suspended in 50 µl DEPC-treated water. It was either used immediately or stored at -20°C until used. The ratio of OD 260/280 was considered to assess the quality and quantity of DNA. The PCR reaction volume was 25 µl containing 1.5 mM $MgCl_2$, 200 nM dNTP, 0.5µM each primer, 100 ng DNA. The PCR products were analyzed by electrophoresis on 1.5% agarose gels stained with ethidium bromide and visualized under UV. Species specific primers for *S. cerevisiae* (MK-157-F and MK-157-R) were also used for identification as described by Pereira *et al.,* (2010). The phylogenetic tree of MK-157 was constructed with MEGA 5.0 software to compare with different *Saccharomyces* species amplified with ITS primers. All the sequences were aligned by Clustal W and neighbour joining phylogenetic tree was constructed with 1000 bootstrapping value.

Sequence determination of the gene encoding EG: The sequences of chromosome XII (330443681:c432016-429677) and XIV (330443715:c759099-755746) of *S. cerevisiae* obtained from NCBI nucleotide data base were used to design outer and inner primers for nested PCR by using FAST PCR software (Table 1). Initially, genomic DNA was used to amplify the target genes using outer primers. Then the products obtained were used as template to amplify the genes by using inner primer as described by Gundersen & Lee (1996). PCR conditions including annealing temperature, concentration of template and $MgCl_2$ were optimized. Amplicons were analyzed by performing agarose gel electrophoresis (1.5%) and observing the bands under UV light. Purified PCR product was shipped for sequencing by Macrogen (South Korea).

Table 1. Primer sequences of Chromosome XII and XIV used for the identification of gene encoding endoglucanase from *S. cerevisiae* MK-157.

Orientation of Primer	Primer sequences	Product size (bp)
IF-12	GTCACGGCTATTTACCACGATT	569
IR-12	TCTTGAACAGCAGCTTCCCGAAT	
OF-12	CTGGCCGCCAATCATACTTTAG	1753
OR-12	TCCAACCGTCATTCACTTGATC	
IF-14	GCTACCGGCGAAGATTCCTT	687
IR-14	CTCGAGGTGTACTTCCACAATG	
OF-14	CTACGCAAGCCAAGTATCTCA	1752
OR-14	GCTACCTGATGCCGACTCA	

OF1: Outer Forward, OR: Outer Reverse, IF: Inner Forward, IR: Inner Reverse

Gel electrophoresis and Zymogram: Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed in 10% (w/v) polyacrylamide gel under constant voltage of 80 V for 2 h in a Bio-Rad (mini protean II) system. Sample for gel analysis was prepared from CFCS by cold ethanol precipitation method as reported by van Oss (1989). Briefly, 9 volumes of absolute ethanol were added to 1 volume of the sample, placed at -20°C for 30 min and pellet (for molecular weight analysis) was obtained by centrifugation at 13000×g for 15 min. The gel was silver stained and observed for the molecular weight of the bands. For zymography, ethanol precipitated samples was not mixed with sample loading buffer. Sample were loaded in 10 µl, 15 µl, 20 µl and 25 µl in separate wells. After electrophoresis the gel was washed three times with 20% isopropanol for 10 min and then with 50mM sodium citrate buffer (pH 5). The EG activity in gel was detected by incubating the gel in activity buffer, followed by immersion in 0.1% Congo red for 1 h and washing with 1 M NaCl until the visualization of clearing around the band.

Optimization of factors affecting EG production and activity: Different factors affecting EG production by the strain MK-157 were optimized by adopting one-variable-at-a-time (OVAT) approach as given by Shariq and Sohail (2018). Initially, the effect of medium composition on the EG production was investigated by cultivating the strain MK-157, separately, on MSM, Yeast extract-peptone medium (YEPS) and Peptone medium (PM). CFCS was collected and EG activity was determined. The influence of cultivation temperature on the EG production was determined by incubating MK-157 in the most suitable medium at varying temperatures (25°, 30°, 35° and 40°C) followed by performing EG assay in CFCS. Similarly, to determine the optimum pH for the EG production by MK-157, the strain was grown in the medium with pH adjusted to 4.0, 5.0, 6.0 or 7.0 at the optimum temperature. Likewise, few other factors including, the effect of carbon and nitrogen source, concentration of substrate and inoculum size on the EG production were studied, one after another. After optimizing the factors affecting the EG production, the strain MK-157 was cultivated in 250 ml Erlenmeyer flask containing 100 ml suitable medium for 72 h under optimum conditions to investigate the growth and enzyme production kinetics. Aliquots (2 ml) were taken intermittently and OD_{600} was noted. CFCS obtained from aliquots were assayed for the EG activity. OVAT strategy was adopted to characterize EG produced by the strain MK-157 for the effect of temperature, pH, substrate concentration and presence of metallic ions (Sohail *et al.,* 2011).

Fermentation of Sugarcane-bagasse and Wheat-bran: The ability of the strain MK157 to produce EG on crude substrates was investigated by using Sugarcane-bagasse (SB) and Wheat-bran (WB) as substrates. SB and WB obtained locally, were dried and ground to 100 mesh size and pretreated using alkali or Hydrogen peroxide. Briefly, the substrates were loaded with 1% KOH (in solid liquid ratio 1:4) and with 2% solution of hydrogen peroxide (0.5 gm H_2O_2/gm biomass) and kept at room temperature for 1 h. The slurry was filtered and washed thoroughly with distilled water until pH of the wash-through became neutral. The substrates were dried in an oven at 60°C until constant mass. The pretreated SB or WB (1%) were added separately in the production medium and sterilized by autoclavingat 121°C for 30 min. MK-157 was inoculated and cultivated under previously known optimized conditions for 1 week. CFCS were obtained and assayed for the EG activity.

Alcohol production and dye-absorption: Initially, alcohol tolerance of the strain was studied by cultivating it in alcohol containing medium. Concentration of alcohol varied from 0 to 16% and growth was monitored spectrophotometrically at 600 nm. Alcohol production by the strain MK-157 was evaluated by growing it in complex medium and presence of alcohol was detected and assayed (Shukla *et al.,* 2011).

Dye absorption ability of the yeast cells was studied using various concentration of Congo red solution (20-100 ppm). The cells of MK-157 grown in SDB (with an OD_{600} 1.0) were transferred to Congo red solution in 100 ml Erlenmeyer flask with a working volume of 50 ml. The suspension was kept at room temperature under shaking at 150 rpm. Dye absorption was investigated by taking OD_{497} of the aliquots drawn intermittently.

Results

The current study was initiated to isolate cellulolytic yeast strains from the environmental and food samples as mentioned earlier. From 11 samples, 104 yeast strains were isolated. Additionally, 117 yeast strains were retrieved from the culture collection available in the lab. All the strains (221) were screened for cellulases activity by using plate method and only 73 strains showed zones of clearance around their colonies. The isolated strains were cultivated in mineral salt medium containing CMC and enzyme assays were performed for EG and BGL activities. The results showed that 30 strains produced EG with titers of more than 0.8 IU/ml (data not shown).

On the basis of EG titers and stability of the enzyme, the strain MK-157 was selected for further studies. The strain was originally isolated from grapes. It was identified on the basis of biochemical tests as well as on molecular basis. It showed ability to ferment various sugars including glucose, maltose, fructose, sucrose, galactose, refinose and mannose except lactose, trehalose and mannose and produced acid in chalk medium. It could not grow in presence of acetic acid and did not produce H_2S and urease. According to biochemical and physiological tests, the strain MK-157 was identified as *S. cerevisiae*. Furthermore, it was identified on molecular basis by using PCR primers (ITS 3 and ITS 4) based on 18S rDNA and 5.8S rDNA gene together with species specific HO gene (Pereira *et al.,* 2010). Annealing temperature of PCR reaction was optimized as 62°C; the product DNA band corresponding to 500 bp was obtained (Fig. 1) and sequence was submitted to GenBank with an accession number KY433852 (for HO gene primer) and KY748281 (for ITS region). The former sequence was having similarity to HO gene as determined by BLAST analysis.

Moreover, the sequence from the strain MK-157 was aligned perfectly with the sequences of *S. cerevisiae* (Fig. 2) that was in accordance to Montrocher *et al.,* (1998) for phylogenetic relationship of *Saccharomyces sensu stricto* group. MK-157 was found distinct from other species of *Saccharomyces*.

Since, literature reports about the EG production from *S. cerevisiae* are not available, therefore, the presence of the gene encoding EG was detected using nested PCR. PCR conditions were optimized for inner primers as 64°C annealing temperature, 1.5mM $MgCl_2$ concentration 50 ng/µl template concentration and for outer primer as 62°C annealing temperature, 2.5 mM $MgCl_2$ concentration 50 ng/µl template concentration. The band corresponding to the size, 1752 bp, 1753 bp (using outer primers) and 687 bp, 569 bp (using inner primer) for chromosome XIV and chromosome XII, respectively, were obtained (Fig. 1). The products were sequenced and submitted to GenBank with accession numbers MF871644 and KY660547. The results obtained from the SDS-PAGE showed the apparent molecular weight of EG from *S. cerevisiae* MK-157 as 30 kDa. After zymographic analysis, a single band of EG activity was detected (Fig. 3).

Subsequently, the parameters affecting production of EG by the strain MK-157 of *S. cerevisiae* were investigated by one factor at a time strategy. Amongst media tested, the highest level of EG (5.9 IU ml^{-1}) was obtained when *S. cerevisiae* MK-157 was cultivated in YEP medium followed by MSM (5.0 IU ml^{-1}). To study the effect of carbon or nitrogen source, MSM was used by replacing the source prescribed in the recipe. Interestingly, the strain was able to produce up to 2 IU ml^{-1} of EG in presence of glucose, however, CMC appeared as an inducer of the enzyme. Among nitrogen sources tested, yeast extract proved to be a better source of organic nitrogen.

When the effect of temperature on EG production by the strain MK-157 was studied, it was found that there was not much variation in the titers of EG when the strain was cultivated at temperature 25, 30, 35 or 40°C, however, the optimal activity was noted at 30°C. An increase in EG production by *S. cerevisiae* MK-157 was observed with the increment in the initial pH of the medium from 5 and the optimum activity was observed at pH 7. The optimum inoculum size for MK-157 for production of EG was estimated as 3%.

The effect of CMC concentration was investigated on EG production and it was found that 1% substrate was optimum, whereas, a drastic decrease in the enzyme titers was observed when the amount of CMC was increased to 1.5 or 2%. Generally, the rate of hydrolysis increases with substrate concentration but it lowers at high substrate loadings.

Nonetheless, after optimization of various factors (Table 2), the EG titers as high as 15 IU ml^{-1} were obtained from the strain that showed a 5-fold increase from initial experiments.

Table 2. Optimized condition for the production of endoglucanase from *S. cerevisiae* MK-157.

Parametrs	Optimized condition
Production media	YP Media
Temperature	30 ˚C
pH	7 pH
Carbon source	CMC
Nitrogen source	Yeast extract
Substrate concentration (CMC)	1%
Inoculum size	3%

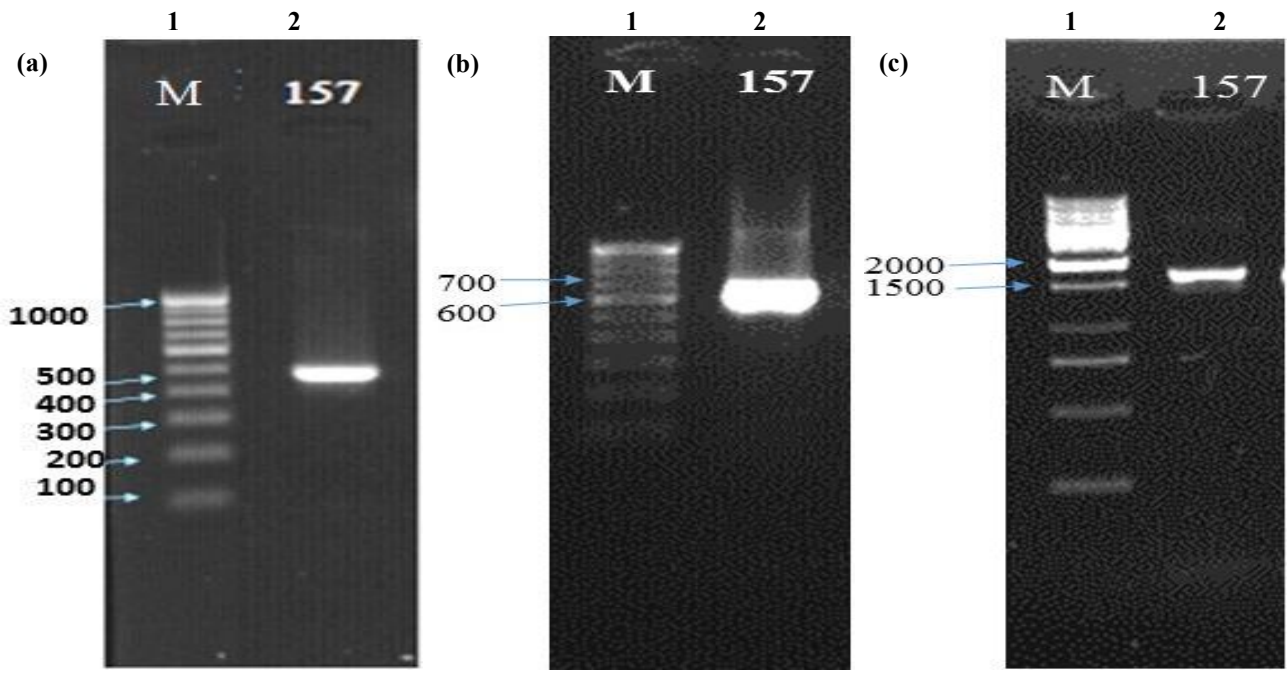

Fig. 1. (a) Agarose gel 1.5% of the product amplified by PCR. Specific primers of HO gene of *Saccharomyces cerevisiae* were used. Lane 1: Ladder marker (100 bp DNA ladder) Lane 2. *S. cerevisiae* MK-157 (b) Inner primer product from nested PCR (chromosome XII): Lane 1 Ladder marker (100 bp DNA ladder) Lane 2 amplified product of MK-157 with inner primer by using the outer primer as template (c) Outer primer PCR product from nested PCR: Lane 1 Ladder marker (1 kb DNA ladder) Lane 2: 1700 product with outer primers.

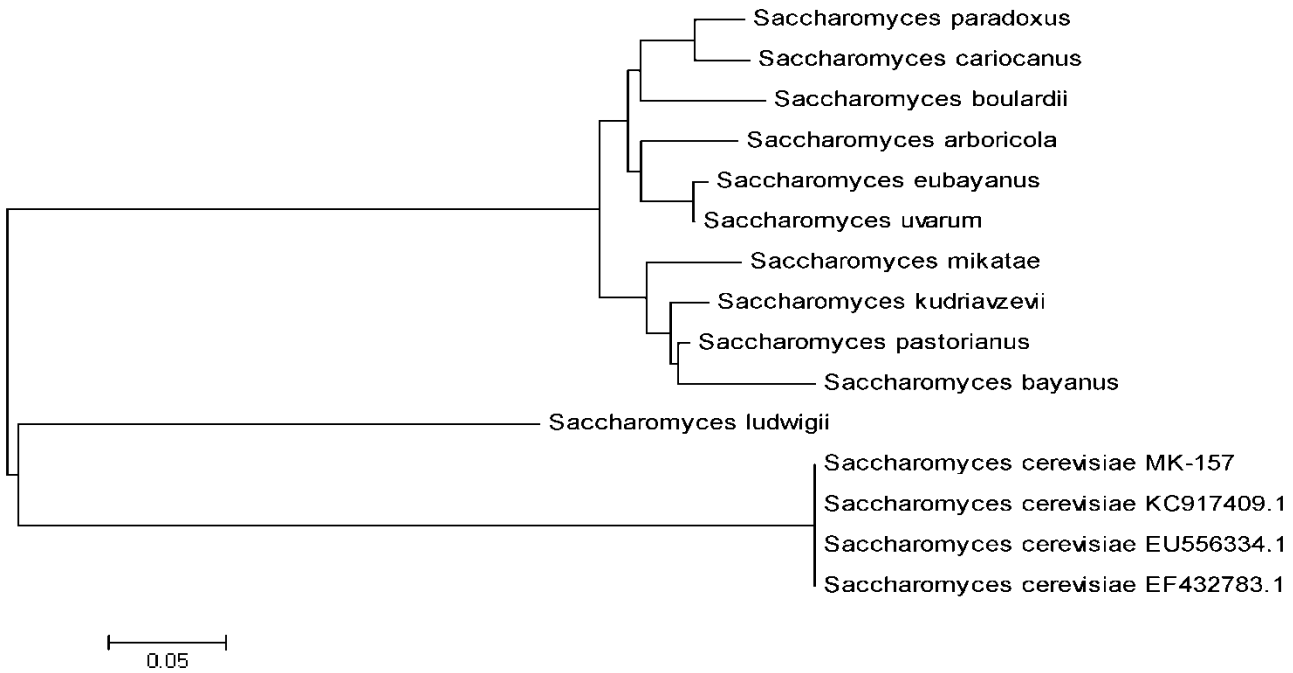

Fig. 2. Phylogenetic tree representation of *Saccharomyces cerevisiae* MK-157.

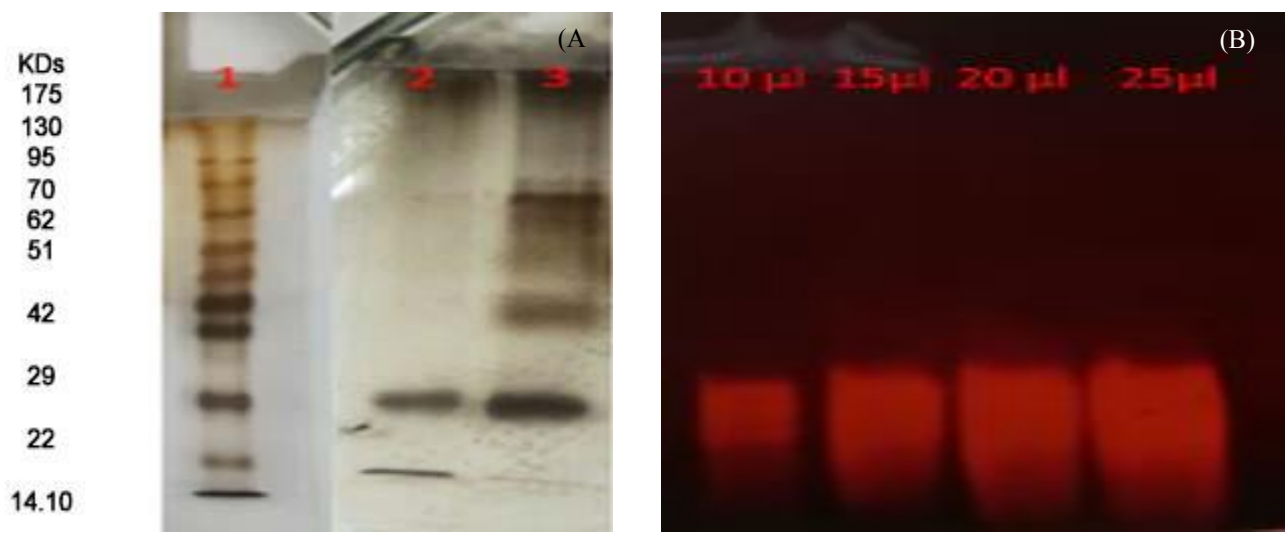

Fig. 3. Electrophoretic analysis of partially precipitated EG enzyme produced from *Saccharomyces cerevisiae* MK-157. (a) Lane 1: molecular weight marker in KDa, Lane 2: Silver stained bands of crude enzyme, Lane 3: partially precipitated enzyme from ethanol. (b) Zymogram of EG enzyme fromMK-157.

The growth and EG production kinetics was studied in YEP-CMC medium by taking aliquots intermittently. The data revealed that the OD_{600} of the medium increased rapidly between 24-48 h indicating the log-phase of the growth that was accompanied with the increase in the titers of EG (Fig. 4). With a slight decrease in the titers in early stationary-phase until 72 h, the production of the EG was again increased. The amount of reducing sugars (RS) in the medium was increased when the stationary phase was ensued that indicated utilization of RS by the cells in exponential phase of the growth.

After studying parameters affecting EG production by MK-157, EG activity in CFCS was characterized for its optimum temperature and pH. It was found that the enzyme can retain most of its activity over a wide range of these two factors. The optimum temperature was found to be 30°C. Whereas, the enzyme had a broad pH range with two activity peaks at pH 5 and 8.5 (data not shown). Although, the enzyme worked optimally in presence of 1% CMC, however, it exhibit >80% of its activity in presence of 0.1% CMC (data not shown). It can be attributed to high affinity of the enzyme. During the course of reaction in presence of 1% CMC, the EG could reach its maximum activity within 5 min (data not shown).

The activity of EG from *S. cerevisiae* MK-157 was determined in presence of various metal ions and chemicals at two different concentration i.e. 1mM and 5mM (Table 3). There was complete inhibition of EG activity in presence of Urea and EDTA, regardless of the concentration used. An enhancement in EG activity in presence of some divalent ions (Ca^{2+}, Mg^{2+} and Mn^{2+}) was also observed.

The ability of the strain MK-157 to ferment crude natural substrates was investigated under submerged conditions (Smf). Considering the abundance of the natural waste materials in Pakistan, the ability of *S. cerevisiae* MK-157 to produce EG was evaluated under Smf of pretreated and untreated sugarcane-bagasse (SB) and wheat-bran (WB). Significant difference in the production of EG was noted when Smf was carried out in MSM containing SB and WB, either untreated or pretreated with alkali or hydrogen peroxide (Table 4). Although, alkali pretreatment of SB proved better as its fermentation yielded 15.54 IU ml^{-1}, however, the strain produced 11.59 IU ml^{-1} and 13.32 IUml^{-1} in untreated and H_2O_2 pretreated SB, respectively. Whereas, the pretreatment given to the WB found to be unsuitable as fermentation of pretreated WB resulted in very low titers of EG.

The strain MK-157 was studied for its possible biotechnological applications including for ethanol production and for dye removal. The data showed that *S. cerevisiae* MK-157 exhibit good growth (with an OD of >1) in presence of 5% alcohol; the growth was, however, reduced to 0.189 OD in presence of 12% alcohol. Consequently, ethanol production ability of *S. cerevisiae* MK-157 was evaluated in YPG broth under static conditions. An amount equivalent to 5.45% (v/v) ethanol was obtained.

The strain MK-157 of *S. cerevisiae* was also studied for its ability to absorb a test (azo) dye, Congo red. It showed a noteworthy ability to absorb up to 94% of the dye in aqueous solution within 29 h at 30˚C and 150 rpm (data not shown). However, further experiments are required to ascertain the role of MK-157 in the remediation of dye-ridden environment.

Table 3. Effect of chemicals on the activity of endoglucanase (EG) from *S. cerevisiae* MK-157.

Chemical	EG activity (%) in presence of chemicals at concentration	
	1 mM	5 mM
Control	100	100
NaCl	129	79.79
$MgSO_4.7H_2O$	128	110
KH_2PO_4	100.98	114.33
$CaCl_2$	77.77	133.33
$MnSO_4.5H_2O$	122.22	139.39
KCl	103	100
$CoCl_2$	122	136.36
$CuCl_2$	128.78	121.21
UREA	0	0
EDTA	0	0

Table 4. Endoglucanase production (IU/ml) from *S. cerevisiae* MK-157 under submerged fermentation of sugarcane bagasse and wheat bran.

Substrates	EG Production (IU/ml) under fermentation of		
	Untreated	Alkali pretreated	H_2O_2 pretreated
Sugarcane-bagasse	11.592 ± 0.091652	15.54 ± 0.0543	13.32 ± 0.0432
Wheat-bran	16.24 ± 0.0313	2.366 ± 0.0287	2.294 ± 0.0631

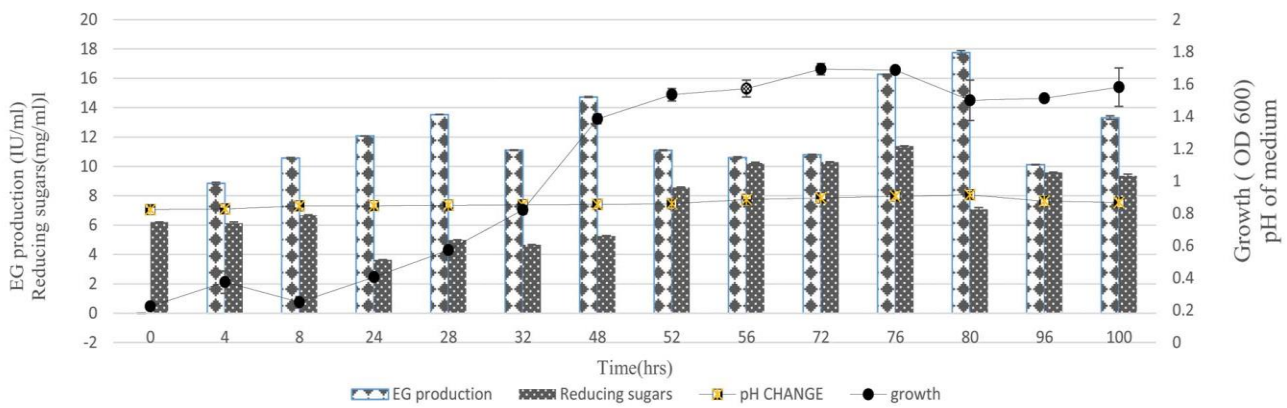

Fig. 4. The strain MK-157 was cultivated in MSM-CMC broth and aliquots were collected intermittently. Growth, reducing sugars, endoglucanase activity and pH were monitored.

Discussion

Exploring biodiversity in the search for new biocatalysts by screening indigenous microorganism may permit the development of biocatalysis on an industrial scale. In spite of the availability of high throughput techniques of gene-cloning, the isolation of the native strains with the ability to overproduce new or known enzymes has remained at the core of industrial Microbiology. Voluminous reports describe the production of cellulases from mold or bacterial strains, however, yeasts have scarcely been reported for this activity. For instance, Goldbeck *et al.,* (2012) obtained only 16 cellulase producing yeast strains out of 360 strains. In contrast cellulolytic molds were predominantly isolated by Jahangeer *et al.,* (2005) where 67% of the isolated strains were found cellulase producers. In the present study, out of 220, 73 strains were found to be positive on plate screen method, however, only 30 produced extracellular EG in broth medium.

The identification of the most promising strain, MK-157, as *S. cerevisiae* was confirmed by sequencing HO gene, which is responsible for switching mating cells of the yeast between a and α types (Russell *et al.,* 1986). It is unique to the strains of the *S. cerevisiae* and hence can be used as a valid marker for the molecular identification of

this yeast (Pereira *et al.,* 2010). The molecular weight (30 kDa) of EG from MK-157 was similar to the EGs from *Trichosporon Japonicum* AB001749 (Korish, 2003) and *Cryptococcus flavus* (Hatano *et al.,* 1994).

Although, EG activity in *S. cerevisiae* has not been described frequently (Adelabu *et al.,*2017), however, the production of cellulases in previously well characterized non-cellulolytic organisms has been reported for *M. tuberculosis* (Varrot *et al.,* 2005) and for *E. coli* (Pang *et al.,* 2017). It indicates the significance of screening of new strains of the same species for the detection of industrially important enzymes. It also signifies the various roles that cellulase component enzymes perform during cell growth and adaptation (Medie *et al.,* 2012).

The data related to optimization of factors affecting production of EG from MK-157showed that it was induced by CMC in the medium that has been reported as a good inducer for the production of cellulases by *Alternaria* sp. (Sohail *et al.,* 2011) and by *A. niger* (Gautam *et al.,* 2010). However, in these studies, glucose appeared as potent inhibitor for cellulase production. Whereas, the ability of MK-157 to produce EG even in presence of glucose can be an important prospect in designing production medium. Contrary to the finding of yeast extract as best nitrogen source for the EG production from MK-157, Malik *et al.,* (2010) described an inorganic source, $(NH_4)_2SO_4$, as the best nitrogen source for cellulase production by *Trichoderma viride* GCBT-11. It could be related to less nitrogen requirements by the strain MK-157, as organic sources contains less amount of nitrogen compared to the inorganic sources.

The strain MK-157 produced highest titers of the EG at 30°C which was in line with Malik *et al.,* (2010). The decrease in the enzyme yield at higher temperatures may be attributed to the denaturation of the enzymes or due to the inhibition of microbial growth. Slightly acidic pH of the medium favored the EG production from MK-157 that was in agreement with Acharya & Chaudhary (2012) that reported maximum cellulase production from *B. licheniformis* MVS1 at slightly acidic side of pH i.e. 6.5.

The enzyme production is greatly influenced by the inoculum size as smaller inoculum takes more time to achieve appropriate density, while a large inoculum may result in aggregation of the cells and depletion of nutrients. A relatively lower inoculum size (3%) was found appropriate for the EG production by the strain MK-157 of *S. cerevisiae* compared to reported by Singh *et al.,* (2014) for *Bacillus amyloliquefaciens*(6.96%). It could be attributed to rapid growth and expression of cellulases by the yeast.

The data about kinetics of growth of MK-157 and the EG production showed that EG titers increased with the increase in growth. The growth associated cellulase produciton has already been reported in an earlier study by Sohail *et al.,* (2009) where cellulase production from *A. niger* MS82 increased with an increase in fungal mass until early stationary-phase.

The EG from MK-157 was characterized for the effect of temperature and pH on its activity and it was found that EG activity attained its peaks at pH 5 and 8.5 indicating presence of isozymes or subunit of EG. Previosuly, multiple activity peaks at pH 3.5, 5.5 and 7.0 were reported for the EG activity from *A. niger* ANL301 (Chinedu *et al.,* 2011).

The cellulases and xylanases preparation are applied for the saccharification of agro-industrial wastes that may contain different metal ions and other chemicals (Sohail *et al.,* 2011). Equilibrium constant of the reaction mixture is markedly affected by metallic ions as enzyme substrate can form complexes with them (Blair, 1968) and may not remain available for the reaction. The influence of EDTA and metallic ions can be related to the metalloprotein nature of the EG which is in agreement with Singh *et al.,* (1990). EDTA and different metallic ions have been reported as activator or inhibitor for the EG from various organisms. For instance, EDTA activated EG by 1.5 fold from *Aspergillus* (Naika & Tiku, 2011), whereas, it exerted an inhibitory effect to EG by *Bacillus* sp. (Sadhu *et al.,* 2013). Chinedu *et al.,* (2008) studied EG from *Penicillium chrysogemum* that remained unaffected in presence of Ca^{+2} while the presence of Mn^{+2} resulted in increase in activity by 3-folds**.** These observations show the structural diversity among EGs from various organisms.

The production of cellulases was studied under Smf of pretreated or untreated sugarcane-bagasse (SB) and Wheat-bran (WB). The organism produced EG in high titers with untreated SB as compare to treated substrate. This reduced productivity may be related to the release of inhibitors during pretreatment processes. Nonetheless, the cost of pretreatment of SB can be avoided using this strain and a slightly low productivity can be compensated. It is also imperative to note that MK-157 produced more amount of EG than reported in some literature reports, such as, De Castro *et al.,* (2010) obtained 559 U L^{-1} (equivalent to 0.559 U ml^{-1}) of EG from *T. harzianum* IOC-4038 under Smf of SB. However, the role of EG in saccharification of SB needs to be studied, particularly when Tariq *et al.,* (2018) has recently reported about the synergistic effect of yeast xylanase and pectinase on the EG from *B. licheniformis* in the saccharification of SB.

Interestingly, the strain MK-157 that can grow on natural substrates was found to produce more than 5% of ethanol on commercial medium. Although the yield of ethanol from MK-157 was not comparable to Prasertwasu *et al.,* (2014) where 16 g L^{-1} of ethanol was yielded within 24 h by *S. cerevisiae* TISTR 5596, however, further experiments on fermentation of crude LC substrates into ethanol can explore the possible future application of the strain MK-157.

The textile effluent contains various dyes and hence is difficult to treat and pose significant environmental problem. Bioadsorption of dyes using microbial cells offers an attractive alternative as it is an environmental friendly technique. Microorganism with an ability to produce ligninolytic enzymes are of particular interest owing to their ability to break the ring of the chromophore (Boer *et al.,* 2004). The ability of the strain MK-157 to decolorize Congo-red solution to 94% was comparable with the report by Chen *et al.,* (1999). The removal of Congo red by dead hyphae of *A. niger* was previously reported by Fu & Viraraghavan (2002). Therefore, the strain MK-157 of *S. cerevisiae* offers prospects to ferment LC mass to ethanol with the concomitant production of EG and for subsequent utilization of the cells for dye absorption.

Conclusion

From the finding of present study, it can be concluded that indigenous yeast microflora can be a source of cell wall degrading enzymes. The strain MK-157 of *S. cerevisiae* produced more than 15 IU/ml of EG in optimized medium and is able to ferment sugarcane-bagasse effectively. The strain also yielded more than 5% ethanol in a complex medium. It is expected that the present study will provide an opportunity to explore new arena in this area.

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