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ORIGINAL ARTICLE

Enhanced endoxylanase production by *Myceliophthora thermophila* with applicability in saccharification of agricultural substrates

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Abstract

The production of enzymes by solid-state fermentation is an interesting process and currently used worldwide as it can be carried out in solid matrix in absence of free water. In present study, *Myceliophthora thermophila* BJTLRMDU3 produced high titres of endoxylanase (890.55 U/g DR, dry residue) using 5 g rice straw at pH 7.0 and at 45 °C with 1:7 (w/v) solid-to-moisture ratio with inoculum rate of 12×10^6 spores/ml after 4 days in solid-state fermentation. High enzyme titre was produced after moistening the rice straw with solution containing ammonium sulphate (0.4%), K_2HPO_4 (1.0%), $MgSO_4 \cdot 7H_2O$ (0.3%), $FeSO_4 \cdot 7H_2O$ (0.03%) and $CaCl_2$ (0.03%). Addition of sucrose (2% w/v) and ammonium nitrate (2% w/v) further enhanced the endoxylanase production. A high endoxylanase production was achieved at water activity (a_W) of 0.95 (1639.80 U/g DR) that declined drastically below this value. Among different surfactants, Tween 20 (3% v/v) enhanced the secretion of endoxylanase (2047.91 U/g DR). Furthermore, on optimization of K_2HPO_4 concentration, it was found that 0.5% K_2HPO_4 improved (2191.28 U/g DR) endoxylanase production and overall 4.35-folds increase in production of endoxylanase was achieved after optimization of culture conditions. The enzyme has potential to liberate monomeric (xylose) as well as oligomeric (xylotiose, xylotetrose, and xylopantose) sugars from xylan. On saccharification of rice straw and corncob with endoxylanase, maximum yield of reducing sugars was 135.61 and 132.61 mg/g of substrate recorded after 48, and 36 h, respectively.

Keywords Endoxylanase · *Myceliophthora thermophila* · Rice straw · Solid-state fermentation · Xylooligosaccharides · Saccharification

Introduction

The large quantity of agricultural residual biomass can be used for production of a variety of value-added commercial products such as, enzymes, chemicals, animal feed, and biofuels. For industrial point of view, lignocellulosic biomasses are considered as potent feed stocks (Goldman 2009). After crop harvesting this residual biomass is left to burn or rot in fields, but use of these residual biomass for industrial application not only deals with proper dumping but also becomes an additional source of income for farmers (Akpinar et al. 2010). Various biomasses are reported as perfect substrate for the growth of a variety of microorganisms that are industrially important, and also for nutraceutically and pharmaceutically important enzymes production. There is enough scope for use of these agricultural residues for feed and food application like, production of xylooligosaccharides, xylose, and xylitol (Aachary and Prapulla 2009; Bala and Singh 2017). Asian countries, utilized more than 1.2 million km^2 of land to cultivate rice, accounting for 60% of rice production world wide. Rice is a widely grown staple crop in which straw and husk generated as by-products of rice harvesting and processing, respectively, are important energy sources. Globally India ranks second in production of rice (Hiloidhari et al. 2014) and generate around 112 million metric tons (MMT) of rice straw and 22 MMT of rice husk,

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respectively. Every ton of paddy produces 1.1–1.3 MT of straw and 0.23–0.25 MT of husk. Being considered a renewable resource, rice straw can be converted into different valuable products through the application of biotechnology. Rice straw contains 39 ± 3% cellulose, 26 ± 0.3% hemicellulose and 9.22 ± 3% lignin content (Chang et al. 2011). Xylan is the important component of plant residues left in agricultural fields. In world it is the second most abundant hemicellulosic matter and economically important products, xylitol and xylose, can be formed from xylan along with xylooligosaccharides which the new emerging products are having various applications including use as prebiotics, in cancer treatment, in food and feed industries, etc. Xylanases catalyse the hydrolysis of xylan specifically endo-1,4-β-xylanase (EC 3.2.1.8) that breaks the glycosidic bonds in backbone of xylan, and reduced the substrate's degree of polymerization. Xylanases have been classified according to release of end product after xylan hydrolysis, e.g. arabinose, xylose, xylobiose, and xylotriose and from industrial point of view, for bioconversion of hemicellulose endoxylanases are more important. Fungi, particularly filamentous fungi, are the most promising producers of xylanases for industrial purposes because filamentous fungi release their xylan-degrading enzymes directly into medium, and there is no need for disruption of cell before purification of their enzymes (Polizeli et al. 2005). Currently, thermostable enzymes from thermophilic microorganisms of expanded origin are gaining more attention in biotechnological applications (Elleuche et al. 2014; Singh et al. 2016a; Singh 2016) as thermophilic enzymes showed fast hydrolytic rate, gave high product yield, and *Myceliophthora thermophila* is a thermophilic fungus that produces a variety of thermostable enzymes in solid-state as well as submerged fermentations such as lignocellulolytic enzymes including endoglucanase (Singh 2016; Bala and Singh 2016), β-glucosidase, exoglucanase (Bala and Singh 2016), laccase (Bobot et al. 2011; Lloret et al. 2012; Toledo-Nunez et al. 2012) endoxylanase (Katapodis et al. 2003; Bala and Singh 2016), cellobiose dehydrogenases and feruloyl esterase (Topakas et al. 2004). Among fermentation systems employed for production of enzyme, solid-state fermentation (SSF) is considered as a desired one because of many advantages over submerged fermentation (SmF), especially for fungal cultivation (Thomas et al. 2013). In SSF, the productivity per reactor volume is much more than that of SmF and also the costs of operation are lower because SSF required simple machinery, plant and energy which allowed a high operation temperature, reduced viscosity and increase in mass-transfer rates resulting into enhanced substrate solubility (Thomas et al. 2013). Microbial xylanases can also be used in saccharification of agricultural substrates for production of sugars which can be used for production of various commercial products including bioethanol. The objectives of this study were to optimize the culture conditions for higher production of endoxylanase in SSF using rice straw and its application in saccharification of different agricultural substrates.

Materials and methods

Raw materials

Rice straw of Pusa Basmati 1121 rice variety was used for this study and collected from local fields of Rohtak district of Haryana, India. Rice straw was washed first with tap water and then distilled water followed by drying at 50 °C. After this, the straw was cut into small pieces and ground with mixer grinder. The straw was used without any pretreatment in solid-state fermentation.

Microorganism and growth conditions

The thermophilic fungus, *M. thermophila* BJTLRMDU3 was isolated from compost soil and taken from culture collection of Laboratory of Bioprocess Technology, Department of Microbiology, Maharshi Dayanand University, Rohtak, Haryana, India and was grown routinely on Emerson's YpSs (Emerson 1941) agar medium [g/l yeast extract (4.0), starch (15.0), $MgSO_4 \cdot 7H_2O$ (0.5), agar (15.0) and pH 7.0 ± 0.2] at 45 °C and was maintained at 4 °C on Emerson's YpSs (Emerson 1941) slants and − 20 °C in glycerol stocks.

Inoculum preparation

The fungus *M. thermophila* BJTLRMDU3 was grown on Emerson's YpSs (Emerson 1941) agar slants for 4 days and used for inoculum preparation. Normal saline (25 ml) containing Tween 80 (0.1% w/v) and NaCl (0.85%) was used to remove the fungal spores from slant under aseptic conditions using sterilised loop and this suspension was filtered with a sterile filter assembly. Then spores were counted with the help of haemocytometer.

Endoxylanase production in SSF

Rice straw was mixed with the production medium employed for enzyme production in 250 ml Erlenmeyer flasks. The solid substrate-to-moistening agent ratio (w/v) was 1:3 initially. Then the flasks were autoclaved at 121 °C for 20 min. The flasks were weighted before and after autoclaving for checking any weight loss. After autoclaving the flasks were cooled at room temperature and inoculated with spore suspension (9×10^6 spores/ml) under sterile conditions. After that, flasks were incubated at 45 °C in static condition for 4 days and flasks were gently tapped against palm after regular time intervals for proper heat transfer and aeration.

Enzyme extraction

The solid substrate in flask after fermentation was harvested with normal saline solution adding 10 ml per gram of substrate and kept for 1 h in a shaking incubator at room temperature. After 1 h filtered the flask content and centrifuged it at 10,000 rpm for 15 min, the clear cell-free culture filtrate was utilized for enzyme assay.

Enzyme assay

Xylanase assay was performed using cell-free culture filtrate at 60 °C and pH 5.0 (Sodium Acetate buffer 0.1 M) and 0.5% beechwood xylan (Sigma-Aldrich chemicals Co, St Louis, MO, USA) as a substrate (Bala and Singh 2016). The amount of reducing sugars released was measured using dinitrosalicylic acid (DNS) method as described by Miller (1959). One enzyme unit of xylanase (IU) is defined as the amount of enzyme required to liberate 1 µmol of xylose per min under the assay conditions. However, the enzyme production is represented as unit per gram dry residue (U/g DR).

Optimization of culture conditions in SSF for endoxylanase production

'One variable at a time' (OVAT) approach was used for optimizing culture conditions for endoxylanase production in SSF. For this, one factor was varied by keeping all the other factor constants and for finding optimized conditions for endoxylanase production all the factors were optimized in a sequential manner. First, the effect of substrate concentration for maximum endoxylanase production was determined by growing the mould on different substrate concentrations (5–15 g) at 45 °C for 4 days and endoxylanase activity was determined in supernatant as described earlier. Thereafter, effect of different pH on the growth of mould for maximum production of endoxylanase was determined in pH range 4.0–8.0 at 45 °C. Similarly, effect of incubation temperature (35–50 °C) was studied by incubating the mould at different temperatures for 4 days. In addition to these parameters, incubation time (1–8 days) for highest endoxylanase production at optimum pH and temperature was also determined by recording endoxylanase activity from 1 to 8 days. Effect of substrate-to-moistening agent ratio was determined by varying ratio 1:3–1:7 (w/v) and measured endoxylanase activity in supernatant. Effect of water activity was studied by growing the fungus in flasks with water activity (a_w) values adjusted with glycerol according to Grajek and Gervais (1987). Different moistening media (Supplementary Table 1) were examined for maximum production of endoxylanase. Effect of different carbon (lactose, glucose, sucrose, starch, and xylose) and nitrogen (ammonium sulphate, ammonium nitrate, sodium nitrate, urea, yeast extract, and beef extract) sources and effect of di-potassium salt (K_2HPO_4) was also studied on production of endoxylanase. Different surfactants (Tween-20, Tween 60, Tween 80, SDS, and Triton X-100) were also examined to determine their effect on endoxylanase production.

End product analysis by thin layer chromatography (TLC)

To 5 ml beechwood xylan solution (1% beechwood xylan in 0.1 M sodium acetate buffer pH 5.0), 1 ml endoxylanase was added and incubated at 60 °C and samples were taken at different time intervals. The end products were analysed by TLC (Silica gel 60 F254, Merck chemicals, Germany) taking a running buffer having *n*-butanol:ethanol:water in 5:3:1 (v/v/v/) ratio. The TLC plate was heated at 100 °C for 20 min for detection of resulting sugars after spraying with a solution of methanol:sulphuric acid in 9:1 (v/v) ratio.

Applicability of endoxylanase in saccharification of agricultural residues

The potential of *M. thermophila* BJTLRMDU3 was examined for the saccharification of corncob and rice straw. For this both substrates were first washed with tap water and then with distilled water and dried at 50 °C for 3 days and then ground into powder form before use. In 250 ml Erlenmeyer flasks, corn cob and rice straw were mixed separately with required volume of buffer (sodium acetate 0.1 M, pH 5.0) and then 25 U/g of substrate partially purified endoxylanase was added. Then the flasks were incubated at 60 °C and 160 rpm for 48 h and required amount of sample was taken out from 0 to 48 h and reducing sugars were calculated by Miller's method as described earlier and further analysed by TLC.

Results and discussion

Optimization of culture conditions in SSF

For the production of endoxylanase, SSF is an attractive technique by cultivating fungus on solid substrate which acts as an energy source and also provide physical support for the growth of fungal mycelium (El-Bakry et al. 2015). A comparative study between SSF and SmF has proved that SSF yields higher production of enzymes and other advantages for products' formation. In addition to this, utilization of agro-industrial by-products in SSF is a notable way to reduce

the cost of production because these substrates are full of carbon and nitrogen sources supporting development and growth of microorganism and also act as natural habitat of microorganisms (Lizardi-Jiménez and Hernández-Martınez 2017). In this study, rice straw was used for production of endoxylanase by thermophilic mould *M. thermophila* BJTLRMDU3. The use of rice straw for endoxylanase production lowers the production cost as rice crops produces a large quantity of rice straw in fields because rice is the chief food in many of Asian countries (Trivedi et al. 2017; Singh et al. 2016b; http://eands.dacnet.nic.in/PDF/Agricultural_Statistics_At_Glance2015.pf). Gautam et al. (2018) and Bharti et al. (2017) used rice straw for xylanase production under SSF by *Schizophyllum commune* ARC-11 and *Talaromyces stipitatus* MTCC12687, respectively. Chaiyaso et al. (2011) also reported cellulase-free xylanase production from thermophilic *Streptomyces thermovulgaris* TISTR1948 using rice straw as a solitary source of carbon. *Myceliophthora thermophila* BJTLRMDU3 produced high endoxylanase (503.09 U/g DR) when the mould was grown on 5 g rice straw and with increase in concentration of rice straw production of enzyme was decreased. Similarly, Gautam et al. (2018) also reported 5 g rice straw for maximum (6721.9 IU/gds) xylanase production while, Addela et al. (2015) and Irfan et al. (2011) found maximum xylanase production with 10 g substrate. Maximum endoxylanase production (636.90 U/g DR) was observed at 45 °C by *M. thermophile* BJTLRMDU3 (Fig. 1a). The enzyme production was found to be decreased at higher and lower than 45 °C. The thermophilic fungus tends to grow better in 40–50 °C temperature range and *M. thermophila* BJTLRMDU3 has 45–50 °C optimum growth temperature (Singh et al. 2016a; Singh 2016). Bala and Singh (2017) reported production of high titres of xylanase from *Sporotrichum thermophile* BJAMDU5 at 45 °C. Moretti et al. (2012) and Sadaf and Khare (2014) reported maximum xylanase production at 45 °C from *Sporotrichum thermophile.* Singh et al. (2000) reported high xylanase production from *Thermomyces lanuginosus* SSBP at 50 °C.

Among different pH values, pH 7.0 supported highest (680.26 U/g DR) endoxylanase production followed by decline at higher pH value (Fig. 1b). Enzyme production from fungi is pH-specific as change in pH can influence the stability as well as production of enzymes (Ramanjaneyulu and Reddy 2016). During SSF, metabolic activities of microbes can change the medium pH and this may increase or decrease as a result of assimilation or production of organic acids, respectively (Gautam et al. 2018; Bharti et al. 2017). It was found that most of fungal origin enzymes have a broad range of pH stability (Kar et al. 2013) and during SSF it is very difficult to maintain pH because of insufficient mixing process while solid substrates on the other side have buffering effect due to their composite structure (Kumar et al. 2016). The filamentous fungi show optimal production of enzyme in acidic pH (Singh et al. 2016a; Singh 2016). Gautam et al. (2018)

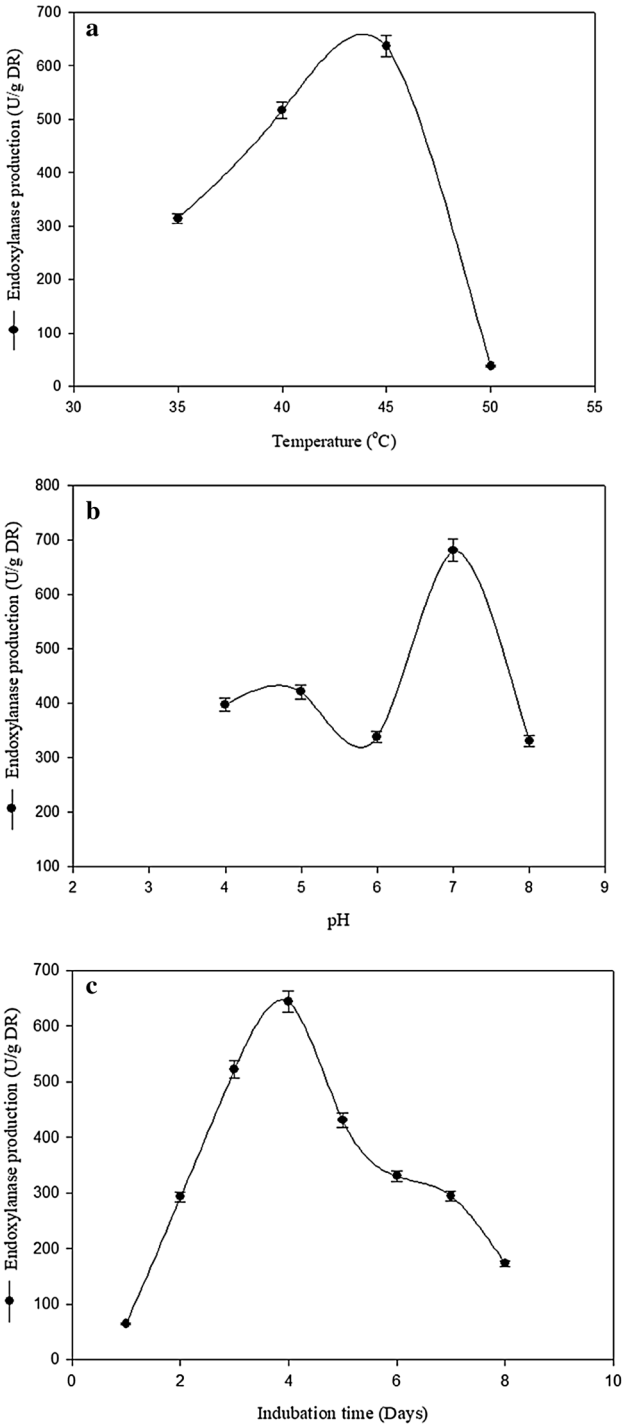

Fig. 1 **a** Effect of varied temperatures on endoxylanase production in SSF by *M. thermophila* BJTLRMDU3. **b** Effect of varied pH on endoxylanase production in SSF by *M. thermophila* BJTLRMDU3 at 45 °C. **c** Effect of different incubation time on endoxylanase production in SSF by *M. thermophila* BJTLRMDU3 at 45 °C and pH 7.0

attained maximal production of xylanase from *S. commune* ARC-11 at pH 7.0 and observed that beyond pH 7.0, productions reduced. Bala and Singh (2017) found pH 5.0 as optimum pH for production of xylanase from *S. thermophile* BJAMDU5. Kaur and Satyanarayana (2004) and Singh et al. (2000) found pH 4.0 and 6.5 as optimal pH for production of xylnase by *S. thermophile* and *T. lanuginosus* SSBP, respectively. Addela et al. (2015) found optimum xylanase production from *Pencillium* sp. at pH 5.0. The production of xylanase was reduced with decrease or increase in initial pH from optimal value. In view of this fact, enzymes are proteins and polymers of amino acids and ionic characters of the amino and carboxylic acid groups of amino acids are affected by the change in pH and the catalytic properties of the enzymes are strikingly influenced by the change in ionic characters of amino acids (Agnihotri et al. 2010).

In this study, maximum endoxylanase production (644.59 U/g DR) was found on the 4th day after incubation and after that enzyme production was decreased till 8th day of incubation (Fig. 1c). The reason behind this decrease in enzyme production might be the deficiency of micro- and macro-nutrients in production medium which altered the physiology of fungus leading to inactivation of enzyme's secretary machinery and also the formation of toxic substance that inhibit the growth of fungus and enzyme production (Kar et al. 2013; Irfan et al. 2011). Bala and Singh (2017) also reported highest production of xylanase on 4th day of incubation by *S. thermophile* BJAMDU5 and found declined enzyme production afterwards. Kaur and Satyanarayana (2004) observed maximum production of xylanase by *S. thermophile* after 4 days while Bharti et al. (2017) and Ghoshal et al. (2012) reported highest production of xylanase from *Talaromyces stipitatus* MTCC 12687 and *Penicillium citrinum* MTCC 2553 on 5th day of incubation. Among different inoculum size which was used for enzyme production, 12×10^6 spores/ml was found to be best for maximum production of endoxylanase (Table 1). The concentration of inoculum has great effect on enzyme production from fungi. The fungus might over grow at an increased inoculum level and led to the phenomena where the fungus face scarcity of nutrients for the enzyme production (Abd-Aziz et al. 2008) and it has also been observed that the size of inoculum influenced the production of exobiopolymer and mycelia growth. The small size of inoculum was found to control and reduce the initial lag phase while the larger size of inoculum increased the moisture level to a significant extent; the over abundance of free lipids creates an additional diffusion barrier together with that imposed by solid nature of the substrate which results into reduction in growth and production of enzyme (Abo-State et al. 2010). Irfan et al. (2011) reported maximum xylanase production from *Trichoderma viride*-IR05 with 10% inoculum size.

Table 1 Effect of various culture conditions on the production of endoxylanase in SSF

Culture conditions	Endoxylanase production (U/g DR)
Concentration of rice straw (g)	
5	503.09 ± 15.09
7.5	345.66 ± 10.36
10	242.30 ± 7.26
15	112.49 ± 3.37
Inoculum size (spores/ml)	
2×10^6	218.68 ± 6.56
4×10^6	368.51 ± 11.05
9×10^6	680.41 ± 20.41
10×10^6	714.02 ± 21.42
12×10^6	738.22 ± 22.14
Substrate-to-moisture ratio (w/v)	
1:3	432.43 ± 12.97
1:4	451.55 ± 13.54
1:5	541.25 ± 16.23
1:6	738.86 ± 22.16
1:7	890.55 ± 26.71
Moistening solutions	
Tap water	320.65 ± 9.61
Distilled water	316.65 ± 9.49
Salt solution 1	734.89 ± 22.04
Salt solution 2	832.75 ± 24.98
Salt solution 3	908.59 ± 27.25
Salt solution 4	599.36 ± 17.98
Carbon source (1%)	
Control	768.52 ± 23.05
Sucrose	935.75 ± 28.07
Lactose	910.04 ± 27.30
Xylose	878.39 ± 26.35
Starch	788.64 ± 23.65
Glucose	622.08 ± 18.66
Nitrogen source (0.5%)	
Ammonium nitrate	1232.45 ± 36.97
Urea	1193.97 ± 35.81
Ammonium sulphate	1100.20 ± 33.00
Yeast extract	1027.68 ± 30.83
Beef extract	867.48 ± 26.02
Sodium nitrate	859.35 ± 25.78
Water activity (a_w)	
Control (0.95)	1639.80 ± 49.17
0.92	66.17 ± 1.98
0.87	64.45 ± 1.93
0.85	52.59 ± 1.57
K_2HPO_4 (%)	
Control	980.07 ± 29.40
0.25	1836.06 ± 55.08
0.5	2191.28 ± 65.73

Table 1 (continued)

Culture conditions	Endoxylanase production (U/g DR)
1.0	1928.62 ± 57.85
1.5	1765.54 ± 52.96

Among different substrate-to-moistening medium ratio used, 1:7 (w/v) supported highest (890.55 U/g DR) endoxylanase production (Table 1). Water content is the most important factor which affects the efficiency of SSF process as high and low moisture ratio negatively affect the production of enzyme (Sanghvi et al. 2010; Kaur and Satyanarayana 2004). As the growth of fungus obtains nearby or on surface of solid substrate and during SSF moisture level plays significant role in enzymes' production (Kar et al. 2013). There is varied optimum moisture amount for a specific microbe which is grown on different solid substrates (Chugh et al. 2016). It has been reported that moisture content more than the optimal value decreased the porosity which results into decreased availability of required oxygen for the growth and development of aerobic microorganisms. On another side, lower moisture level than the optimal level reduced the swelling of the substrate and resulted into difficult nutrient transfer (Maan et al. 2016; Kar et al. 2013). Ghoshal et al. (2012, 2015) obtained higher endoxylanase production at 1:5 solid-to-moisture ratio on 5th day of incubation by *P. citrinum* MTCC 9620 and *P. citrinum* MTCC 2553, respectively. Out of all salt solutions used, salt solution three supported maximum production (908.59 U/g DR) of endoxylanase as compared to other salt solutions (Table 1). The composition and type of moistening medium is other most key factor that affects enzyme production in SSF (Sapna and Singh 2014). Bala and Singh (2017) also reported enhanced production of xylanase by *S. thermophile* BJAMDU5 on addition of salts in moistening medium. Irfan et al. (2011) found Vogel's medium suitable for maximum production of xylanase and Ghoshal et al. (2015) reported Czapek yeast extract media for maximum endoxylanase production from *P. citrinum* MTCC 9620. But in contrast to these reports, Kaur and Satyanarayana (2004) reported tap water as a good moistening medium in SSF. Jain et al. (2015) found highest level of xylanase production from *T. aurantiacus* using distilled water as moistening agent.

Supplementation of freely accessible form of carbon and nitrogen sources is beneficial for enhancing production of enzymes from filamentous fungus (Singh and Satay-narayana 2006). Among various carbon sources, sucrose supported high endoxylanase production as compared to others (Table 1). Addition of sucrose at 2% level enhanced the production of endoxylanase (Fig. 2a) followed by decline afterwards. Bala and Singh (2017) found highest

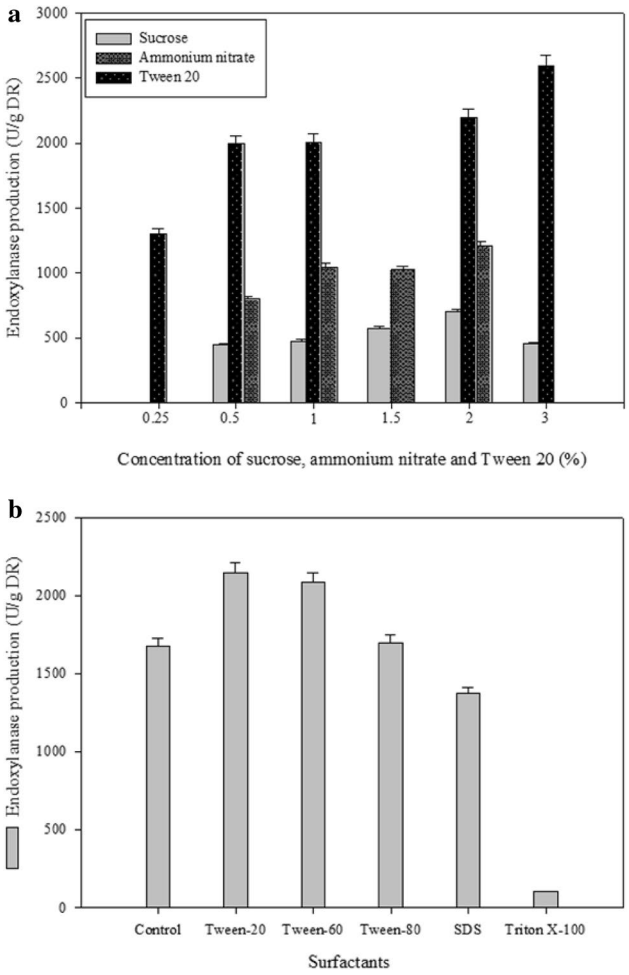

Fig. 2 **a** Effect of varied concentrations of sucrose, ammonium nitrate, and Tween 20 on endoxylanase production in SSF by *M. thermophila* BJTLRMDU3 at 45 °C and pH 7.0 after 4 days. **b** Effect of various surfactants (0.5%) on endoxylanase production in SSF by *M. thermophila* BJTLRMDU3 at 45 °C and pH 7.0 after 4 days

xylanase production from *S. thermophile* BJAMDU5 with 1% lactose supplementation in medium. Irfan et al. (2011) achieved maximum production of xylanase with addition of xylose in moistening medium from *T. viride*-IR05. Ho and Hood (2014) reported sucrose as best carbon source to be added in medium for enhanced production of xylanase from *Aspergillus brasiliensis*. Sukumaran et al. (2005) found cellobiose and lactose as preferred carbon source for increased cellulase production from *Trichoderma reesei*. Sapna and Singh (2014) reported addition of 1% sucrose for enhanced phytase production from *Aspergillus oryzae* SBS50 using wheat bran. Among varied nitrogen sources, simple or complex, those were tested; endoxylanase production was affected by ammonium nitrate (Table 1). Ammonium nitrate at 2% concentration was found to support high endoxylanase production (Fig. 2a). Irfan et al. (2011) and Goyal et al. (2008) reported sodium nitrate for maximum

production of xylanase by *T. viride* while Bharti et al. (2017) reported peptone (1% w/v) for increased xylanase production by *Talaromyces stipitatus* MTCC 12687. Gautam et al. (2018) and Bala and Singh (2017) found highest xylanase production from *S. commune* ARC-11 and *S. thermophile* BJAMDU5 with addition of ammonium sulphate (0.08% and 0.5%), respectively.

A high endoxylanase production (1639.80 U/g DR) was achieved at a_W of 0.95 and it declined drastically below these values (Table 1). In SSF, the production of enzymes and stability of protein influences the water activity of substrates (Sapna and Singh 2014). Similarly, Bala and Singh (2017) also reported highest xylanase production at a_w of 0.95. Sapna and Singh (2014) obtained high titre of phytase from *A. oryzae* SBS50 at a_w of 0.95. Singh and Satay-narayana (2006) and Kumar and Satyanarayana (2004) also found high production of phytase and glucoamylase from *S. thermophile* and *Thermomucorindicae seudaticae* at a_w of 0.95, respectively. The production of enzymes during SSF, probably increased by surfactants as they increase water penetration into matrix of solid substrate and increasing surface area for growth of microorganism (Chugh et al. 2016) and also it is well mentioned in literature that the permeability of microbial cell membrane is increased by surfactant and enhances the release of proteins into the medium. The stimulatory effect of surfactants may also be due to the release of cell-bound enzymes in the presence of surfactants (Ramanjaneyulu and Reddy 2016; Kumar et al. 2016). Among all surfactants which were tested for enhanced enzyme production, Tween-20 (3%) supported enhanced endoxylanase production (2147.91 U/g DR) followed by Tween 60 whereas Triton X 100 showed inhibitory effect on endoxylanase production (Fig. 2a, b). Bharti et al. (2017) also reported enhanced xylanase production from *T. stipitatus* MTCC 12687 by addition of Tween-20 (0.10% w/v). Similarly, Gautam et al. (2018) reported maximum xylanase from *S. commune* ARC-11 with Tween-20 (0.10% w/v) while, Bala and Singh (2016) and El-Gendy and El-Bondkly (2014) reported significant increase in xylanase production from *S. thermophile* and *Streptomyces chartreusis* L1105 with Tween 80, respectively. For microbial growth and enzyme production salts and their concentration play critical part by generating ideal osmotic pressure. The influence of di-potassium salt, K_2HPO_4, and its concentrations on endoxylanase production were determined and it was found that increased (2191.28 U/g DR) endoxylanase production was obtained by addition of 0.5% K_2HPO_4 in moistening medium (Table 1). Salihu et al. (2015) also reported that K_2HPO_4 was one of most important components for obtaining maximum xylanase production from *Aspergillus niger* AS-1. Jeya et al. (2005) also found that K_2HPO_4 significantly increased the production of endoxylanase from *Aspergillus versicolor* MKU3. Bibi et al. (2014) found 0.25% K_2HPO_4 as maximum endoxylanase production supporter. K_2HPO_4 has also been reported as most vital factor for growth of cell and for various microbial polysaccharides production (Gao et al. 2010).

Analysis of end products by TLC

The hydrolysis products of endoxylanase were analysed using TLC. The products released after hydrolysis of beechwood xylan by endoxylanase from *M. thermophila* BJTLRMDU3 (Suppl. Figure 1). The enzyme degrades the xylan and a number of intermediate products were obtained including xylose. Even after the 24 h, the xylose production along with other higher xylooligosaccharides such as xylotriose, xylotetrose, and xylopantose was observed. The analysis of end products of endoxylanase by TLC revealed that this enzyme generates a high amount of oligomers along with xylose, a monomeric sugar, and it makes this enzyme a potential candidate for biotechnological applications such as for biofuel production and prebiotics applications. It also confirms the enzyme as endoxylanase. Bala and Singh (2017) and Sadaf and Khare (2014) also reported xylooligosaccharides production by xylanase of *S. thermophil*e. Ribeiro et al. (2014) found that xylanase from *Penicillium grisefulvum* liberates xylose as a key product while, xylose was not liberated as the major end product from *Melaleuca pulchella*. Boonchuay et al. (2016) investigated and found xylooligosaccharides production from corncob by thermostable xylanase by *S. thermovulgaris* TISIR1948. Liu et al. (2018) reported 75% yield of xylooligosaccharides from recombinant xylanase of *Paenibacillus barengoltzii*.

Applicability of endoxylanase in saccharification of agricultural substrates

The structure of xylan is much complicated than that of cellulose and it needs various different kind of enzymes with distinct specification for thorough hydrolysis and the fermentable sugars thus procured have high value from market point of view. The application of enzymatic hydrolysis for obtaining sugars from agricultural waste is of significant interest in present biotechnology, mainly for bioethanol and xylooligosaccharides. In present study saccharification of agricultural substrates was carried out by endoxylanase and the maximum yields of reducing sugars were 135.61 and 132.61 mg/g of substrate obtained from rice straw, and corn cob after 48 and 36 h, respectively (Fig. 3). The hydrolysis products were also analysed by TLC (Suppl. Figures 2 and 3). Chapla et al. (2012) investigated saccharification of dilute NaOH treated wheat straw, rice straw and corncob by *Aspergillus foetidus* MTCC 4898 and obtained maximum reducing sugars 151.6, 163.06, and 172.66 mg/g, respectively. Endoxylanase of *Chaetomium globosum* showed

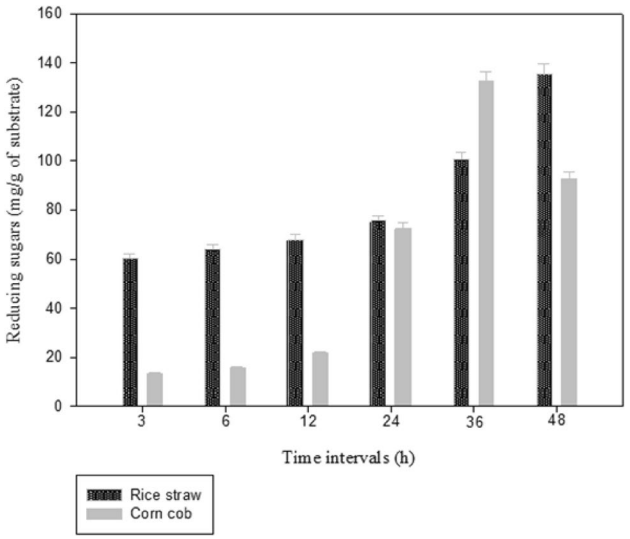

Fig. 3 Saccharification of rice straw and corncob with endoxylanase of *M. thermophila* BJTLRMDU3 at different time intervals

better for saccharification of rice straw compared to commercial endoxylanase Singhania et al. (2013). Zhang and Sang (2015) studied saccharification of corn cob by crude xylanase of *Penicillium chrysogenum* QML-2 and obtained maximum (553.94 mg/g) reducing sugars. Sharma and Bajaj (2017) obtained 76.04 mg/g of reducing sugars from *Aspergillus terreus* S9 xylanase from KOH-treated rice straw.

Conclusions

Myceliophthora thermophila BJTLRMDU3 produced enhanced amount of endoxylanase using rice straw at 45 °C in SSF. After optimization of culture conditions, an overall 4.35-fold increase in endoxylanase production was achieved as compared to initial production. This enzyme is capable of producing xylooligosaccharides for prebiotics production and also can be used for saccharification of agricultural substrates which generates reducing sugars which can be used for various industrial applications such as biofuel production. On the basis of these observations this mould can be used as a potential candidate for biotechnological applications.

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Compliance with ethical standards

Conflict of interest The authors have no conflict of interest.

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