

Enhanced Endoglucanase Production by *Bacillus aerius* on Mixed Lignocellulosic Substrates

Mushafau Adebayo Oke,^{a,b} Mohamad Suffian Mohamad Annuar,^a and Khanom Simarani^{a,*}

Selected carbon sources including soluble carboxymethyl cellulose (CMC), insoluble microcrystalline cellulose (MCC), and single (SS)/mixed lignocellulosic substrates (MS), were evaluated for endoglucanase production by *B. aerius* S5.2. The lignocellulosic substrates of oil palm empty fruit bunch (EFB), oil palm frond (OPF), rice husk (RH), and their mixture (MS) supported growth of the strain better than CMC and MCC. The maximum endoglucanase activity on MS was 7.3-, 2.6-, 1.7-, and 1.2-fold higher than those recorded on MCC, CMC, EFB/OPF, and RH, respectively. While the reducing sugar concentration of the CMC medium was comparable to that of the EFB and MS media, wide variability was observed in the reducing sugar concentrations among the lignocellulosic substrates. Extremely low levels of sugar were detected in the MCC medium, reflecting its poor digestibility and hence unsuitability for growth and endoglucanase production. Endoglucanase production was predominantly extracellular when the strain was grown on CMC and MS. After seven days of fermentation, there was an approximately 25% reduction in MS dry weight. These findings show that the use of mixed lignocellulosics could potentially reduce the cost of cellulase production. Certain novel aspects of the cellulase system of *B. aerius* are reported in this study.

Keywords: Cellulase; Endoglucanase; Mixed lignocellulosic substrate; *Bacillus aerius*; Bioprocessing

Contact information: a: Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia; b: Department of Microbiology, Faculty of Life Sciences, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria; *Corresponding author: hanom_ss@um.edu.my; hanomks@gmail.com

INTRODUCTION

Cellulases, a group of synergistic enzymes that hydrolyze β -glycosidic bonds in cellulose, are crucial in the valorization of lignocellulosic biomass into value-added products. Cellulases are categorized into three major groups: endoglucanases, exoglucanases, and β -glucosidases (Lynd *et al.* 2002). Although the concerted action of these three cellulase types enhance cellulose hydrolysis, this feature may be undesirable in some situations because of the varied activities and substrate specificities of the individual cellulase components (Puranen *et al.* 2014). Hence, it may be necessary to focus on the production of certain cellulase types that exhibit properties more suitable for specific applications. Endoglucanases are of special interest because of their ability to initiate cellulose hydrolysis, and their action on the amorphous regions of crystalline cellulose matrix is considered the rate-limiting step of cellulose utilization (Malherbe and Cloete 2002). Furthermore, endoglucanases have special applications in textile and food processing industries (Juturu and Wu 2014; Puranen *et al.* 2014).

The commercial production of cellulases has been carried out predominantly using fungi because fungi produce higher titers than bacteria. Bacterial cellulases may be a competitive commercial product for the following reasons: high growth rate of bacteria, high stability, resistance to relatively harsh conditions, genetic amenability, and production of efficient multi-enzyme complexes (cellulosomes) (Maki *et al.* 2009, 2011).

The quantity and quality of cellulase produced by microorganisms is strongly dependent on the carbon source (Evans *et al.* 1992; Dashtban *et al.* 2011) and is controlled by repression and induction mechanisms (Bisaria and Mishra 1989; James and Ming 1991). The carbon source also influences the location of cellulases in the cell. Cellulases are expressed differentially in various cell locations when grown on different substrates (Berg 1975; Lo *et al.* 2009).

Various carbon sources such as disaccharides (*e.g.*, cellobiose, lactose, and sophorose) and pure soluble/insoluble cellulosics (carboxymethyl cellulose -CMC, Avicel, Solka floc, *etc.*) are good inducers of cellulase synthesis; however, the use of these substrates on a commercial scale is uneconomical because of their high cost (Chen and Wayman 1991). In fact, sensitivity analysis has shown that the carbon source is the major cost-factor in cellulase production (Ryu and Mandels 1980). Hence, researchers have explored the use of lignocellulosic biomass as the substrate for cellulase production because it is inexpensive and abundant.

Studies on the use of lignocellulosics for bacterial cellulase production have focused mainly on the use of single substrates (Assareh *et al.* 2012; Da Vinha *et al.* 2011; Harun *et al.* 2013; Krishna 1999). However, this approach might be unsustainable in a real biorefinery situation because the feedstock supply is subject to seasonal variation and other logistical problems (Allen *et al.* 1998; Rentizelas *et al.* 2009; Sokhansanj and Hess 2009). The use of mixed substrates in biorefineries can lower logistic costs as well as eliminate the need for extraneous nutrient supplementation (Martín *et al.* 2008; Thomsen and Haugaard-Nielsen 2008; Sultana and Kumar 2011). Consequently, the use of mixed substrates is an interesting option for lowering bacterial cellulase production costs.

Some members of the genus *Bacillus* are good cellulase producers (Acharya and Chaudhary 2012; Asha and Sakthivel 2014; Balasubramanian and Simões 2014; Gaur and Tiwari 2015), while some *Bacillus* spp. produce cellulases that are stable under extreme conditions (Rastogi *et al.* 2010; Annamalai *et al.* 2011; Trivedi *et al.* 2011). However, cellulase production has not been reported previously for *B. aerius*. This species was first classified in 2006, after Shivaji *et al.* (2006) isolated the bacterium from cryogenic tubes used for sampling air at high altitudes. The most commonly reported enzyme of biotechnological interest is lipase (Saun *et al.* 2014a; Saun *et al.* 2014b; Narwal *et al.* 2015), although Oliveira *et al.* (2016) recently reported the isolation of a protease-producing strain from waste feathers.

B. aerius S5.2 used in this study was isolated from decomposing oil palm empty fruit bunch (EFB) samples in Malaysia. This strain showed the unique ability to produce endoglucanase from mixed rice and oil palm residues (unpublished). Thus, the strain was selected as a model organism to study bacterial endoglucanase production from mixed lignocellulosic substrates. Furthermore, little is known about the cellulolytic system of *B. aerius*, and this study investigated the effects of various single and mixed carbon sources on endoglucanase production, as well as the location(s) of endoglucanase, when the bacterium was grown on different substrates. The utilization of the mixed substrate by *B. aerius* was determined from the extent of degradation after seven days of cultivation.

EXPERIMENTAL

Bacterial Strain

Bacillus aerius S5.2 was isolated from decomposing EFB residues collected from an oil palm plantation in Kuala Selangor, Malaysia. This strain produced high titers of endoglucanase on a mixed substrate (MS) medium comprised of EFB, oil palm frond (OPF), and rice husk (RH). The bacterium was identified as *B. aerius* following sequencing of the 16S rRNA gene and a sequence similarity check using the BLAST tool on the NCBI database (<http://www.ncbi.nlm.nih.gov>). The sequence was submitted to GenBank, and the accession number (KP178216) was obtained. The cell morphology was observed using field emission scanning electron microscopy (FESEM) (JSM-7001F, JOEL, Tokyo, Japan). Gram staining and the non-staining KOH methods (Buck 1982) were used to determine the Gram reaction of the strain. Stock cultures of the strain were stored on nutrient agar slants and maintained at 4 °C with regular sub-culturing. This strain was deposited at the Microbial Culture Collection Unit (UNiCC) of the Institute of Bioscience, Universiti Putra Malaysia, under accession number UPMC 1179.

Carbon Sources for Endoglucanase Production

The carbon sources used for the induction of endoglucanase production are presented in Table 1. Medium without cellulosic carbon sources was used as the control in the experiments.

Table 1. Carbon Sources for Endoglucanase Production by *B. aerius* S5.2

Pure Cellulosic Substrate	Lignocellulosic Substrate
CMC	-
MCC	EFB, OPF, RH, MS

CMC: soluble carboxymethyl cellulose; MCC: insoluble microcrystalline cellulose; EFB: oil palm empty fruit bunch; OPF; oil palm frond; RH: rice husk; MS: mixed substrate.

Fresh OPF samples were obtained from the Malaysian Palm Oil Board (MPOB), Bangi, Malaysia. The leaflets were removed, and the fresh petioles were cut into smaller pieces and dried under sunlight. Only the petioles were used in this study because OPF leaflets have other important uses in an oil palm plantation. The petiole has high sugar levels, which makes it more desirable for use as a feedstock in biofuels and other bioproducts (Zahari *et al.* 2012).

Dried and shredded EFB fibers were obtained from the Biocomposting Pilot Facility, Universiti Putra Malaysia. Rice husks were collected from a paddy field in Kedah, Malaysia. The three biomass samples were reduced to small particles (300 to 425 µm) using a Rapid granulator (GK 205-K, Terramar GmbH, Hamburg, Germany). The MS carbon source was prepared by mixing EFB, OPF, and RH in equal proportions (1:1:1). The mixture was stored in a dry airtight container.

The lignocellulosic substrates used in this study were pretreated sequentially with NaOH and autoclaved, according to the method in Umikalsom *et al.* (1997), with a slight extension of the autoclaving duration to 15 min. The washed and pretreated solids were dried in an oven at 60 °C for at least 12 h.

Culture Media

A modified version of the medium for the cultivation of cellulolytic bacteria, described by Dickerman and Starr (1951) and containing 2% (w/v) of the respective carbon sources, was used for endoglucanase production. Other components of the medium were (w/v): K_2HPO_4 (0.08%), KH_2PO_4 (0.02%), $MgSO_4 \cdot 7H_2O$ (0.02%), NaCl (0.02%), $NaNO_3$ (0.1%), $CaCO_3$ (0.001%), and yeast extract (0.05%). The medium pH was adjusted to 7.0 using 2 M NaOH or HCl. The media was sterilized at 121 °C for 15 min in an autoclave.

Growth and Endoglucanase Production

B. aerius S5.2 was cultivated in nutrient broth until the late log phase (12 h) was reached. Aliquots (approximately 10^7 cfu/mL) from this culture were used as inocula in the experiments. Five percent inoculum (v/v) was inoculated into 250-mL Erlenmeyer flasks containing the culture media with the respective carbon source. Each flask was incubated at 30 °C with 200 rpm agitation for 72 h. Triplicate flasks were used for each carbon source. Aliquots of the culture samples were initially collected after 6 h and then at 12-h intervals. Collected samples were centrifuged at 6000 rpm for 10 min at 4 °C. The cell-free supernatant was used as the crude enzyme preparation in the endoglucanase assay. Growth of the bacterium at each sampling period was monitored by estimating the total colony-forming units (cfu) in the culture supernatant using the drop plate technique (Herigstad *et al.* 2001).

Reducing Sugar Production

In order to evaluate the general digestibility and suitability of each of the substrates for growth and utilization by *B. aerius* S5.2, the amount of reducing sugar in the culture supernatant at each sampling period was monitored. Reducing sugar concentrations were determined using the 3,5-dinitrosalicylic acid (DNS) method (Miller 1959).

Localization of Endoglucanase

To determine the localization of endoglucanase in the cell, *B. aerius* S5.2 was grown in the culture media with 2% (w/v) of either CMC or pre-treated MS as the carbon source at 30 °C for 36 h with 200 rpm agitation. The culture broth (30 mL) was centrifuged at 8000 rpm for 10 min at 4 °C, and the supernatant was used as the extracellular enzyme sample. The cell pellet was washed twice with 10 mL of 0.05 M phosphate buffer (pH 7.0) and later re-suspended in 15 mL of the same buffer. The suspension was kept on ice to preserve enzyme activity. The intracellular and membrane-bound fractions of the enzyme were prepared by sonication (Lo *et al.* 2009). The cell pellet suspension was sonicated at varying 30% amplitude using a probe type sonicator (Branson Ultrasonics, Danbury, CT, USA). The sample was placed on ice and pulse-sonicated for 10 min for 30/10 sec pulse intervals. This was followed by centrifugation at 8000 rpm for 10 min at 4 °C. The supernatant was used as the intracellular enzyme sample. The cell pellet was resuspended in 5 mL of buffer (to concentrate the sample) and used as the membrane-bound enzyme sample. All enzyme fractions were analysed for endoglucanase activity and protein concentration. Enzyme activity in the various fractions was expressed as enzyme units per μ g protein to account for the different volumes of buffer in the re-suspensions.

Protein Concentration Measurement

Protein concentration was determined using the Bradford assay (Bradford 1976). The enzyme suspension (100 μ L) was mixed with 5 mL of Bradford reagent (Sigma-Aldrich, St. Louis, MO, USA). The mixture was maintained at room temperature for 10 min, and the absorbance was read against the reagent blank (100 μ L buffer plus 5 mL reagent) at 595 nm.

The protein concentration was determined by extrapolation from a standard calibration, which was computed using different concentrations (μ g/mL) of bovine serum albumin suspended in 0.05 M phosphate buffer vs. absorbance at 595 nm.

Endoglucanase Assay

The endoglucanase activity was determined by measuring the reducing sugars released after 200 μ L of the enzyme was reacted with 200 μ L of 2% CMC in 0.05 M phosphate buffer at pH 7.0 (Zhang *et al.* 2009). The mixture was incubated for 30 min at 50 $^{\circ}$ C, and the reaction was stopped by adding 800 μ L of DNS reagent, followed by immersion in boiling water for 5 min. The released sugars were measured as glucose equivalents using the DNS reagent (Miller 1959). One unit (U) of enzyme activity was defined as the amount of enzyme that liberated 1 μ mol of reducing sugar per mL per minute from the substrate.

Degradation of MS by *B. aerius* S5.2

Five millilitres of *B. aerius* S5.2 inoculum from the late log phase was transferred into a 250-mL conical flask containing 50 mL of medium with 1 g of MS as the carbon source. The flask was incubated for seven days at 30 $^{\circ}$ C with 200 rpm agitation. A control was prepared in a separate flask containing the same amount of substrate without inoculum. All experiments were conducted in triplicate. The extent of the degradation of MS was determined by calculating the dry weight loss of the substrate. After the cultivation period, the entire content of each flask was filtered through dry Whatman No. 1 filter paper (initial weight; W_0).

The liquid was allowed to drain completely, and the residue with the filter paper was dried in an oven at 70 $^{\circ}$ C until a constant weight (W_1) was obtained. The extent of the degradation of MS was expressed as the percentage of dry weight loss of the substrate as follows:

$$1.0 - (W_1 - W_0) \times 100\% \quad (1)$$

The numerical value 1.0 represents the initial amount of MS (1 g) for the degradation experiment. The mean of three replicates was recorded as the final dry weight loss.

RESULTS AND DISCUSSION

Morphology of *B. aerius* S5.2

The morphology of *B. aerius* S5.2 was observed using FESEM. The Gram reaction was also confirmed using normal Gram staining as well as the non-staining KOH method. *B. aerius* S5.2 cells were Gram-positive and rod-shaped. Micrograph images of the cell morphology are presented in Fig. 1. The cell size was 0.40 to 0.50 μ m by 1.22 to 2.49 μ m, while the endospores ranged from 0.23 to 0.26 μ m in size.

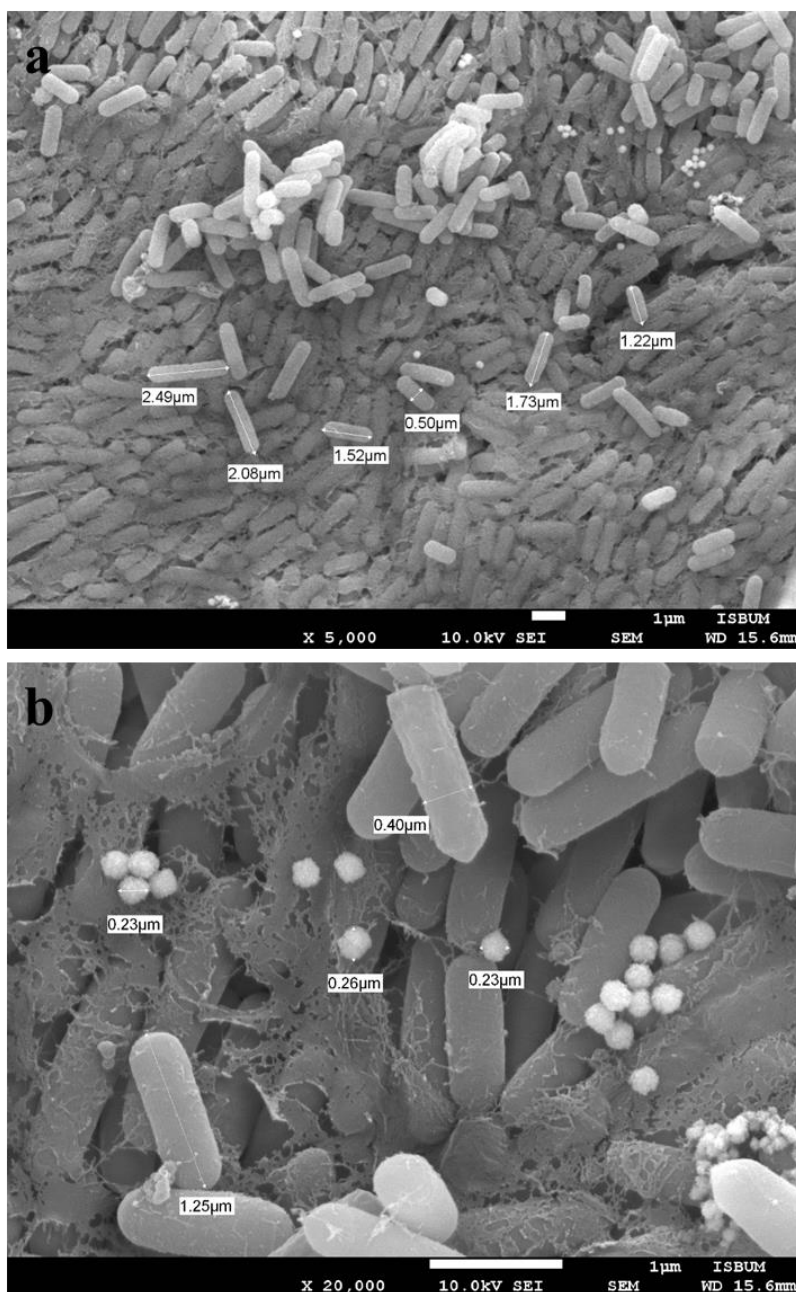


Fig. 1. Field emission scanning electron microscopy imaging of *B. aerius* S5.2. (a) Cells ($\times 5,000$); (b) cells and endospores ($\times 20,000$)

Growth and Endoglucanase Production on Various Cellulosic Carbon Sources

The growth curve and endoglucanase production profile of *B. aerius* S5.2 during growth on various cellulosic carbon sources are presented in Figs. 2 and 3, respectively. The bacterium showed better growth on the lignocellulosic substrates (single and mixed) than on pure cellulosic substrates (CMC and MCC). The least suitable substrate for growth was MCC (9.07 log cfu), followed by CMC (11.52 log cfu), because the maximum growth rates were inferior. The highest growth was recorded on EFB (max. 16.38 log cfu) and OPF

(max. 15.46 log cfu). The maximum growth value recorded on MS (12.96 log cfu) was comparable to that recorded on RH (12.92 log cfu), although the growth rate of *B. aerius* S5.2 was higher on the latter.

A similar trend was also observed for endoglucanase production (Fig. 3). The MCC and CMC produced the lowest enzyme titers, while higher titers were recorded for lignocellulosic substrates. The maximum endoglucanase activity recorded on MCC was 0.108 ± 0.050 U/mL, while on CMC, it was 0.305 ± 0.063 U/mL. However, the maximum endoglucanase titers recorded on MS (0.787 ± 0.062 U/mL) and RH (0.658 ± 0.019 U/mL) were the highest among all of the carbon sources investigated. Endoglucanase production on EFB and OPF was similar, with maximum titers of 0.470 ± 0.056 U/mL and 0.463 ± 0.007 U/mL, respectively. Based on the maximum enzyme titers obtained on each substrate, it was observed that the MS supported endoglucanase production 7.3-, 2.6-, and 1.2- times better than MCC, CMC, and RH respectively. Also, MS supported 1.7-fold higher endoglucanase titers compared with OPF and EFB.

Figures 2 and 3 show that endoglucanase production was growth-related as the strain's growth and endoglucanase production showed similar profiles. Similar observation was previously reported for *Bacillus* spp. (Ariffin *et al.* 2006). The higher cell growth and endoglucanase production recorded on the lignocellulosic substrates could be attributed to the availability of more growth-promoting substances. Lignocellulosic biomass materials have various proteins in addition to cellulose, hemicellulose, lignin, other components (Sluiter *et al.* 2008). These substances may improve the metabolism of the bacterium in comparison to the pure cellulosic carbon sources. Yang *et al.* (2014) compared CMCase production on CMC, MCC, rice hull, and wheat bran by *B. subtilis* BY-2. The authors reported that the highest enzyme production was obtained with wheat bran, while rice hull and CMC yielded lower enzyme values. A very low CMCase titer was recorded on MCC. Similar observations were reported by Chan and Au (1987), where *B. subtilis* AU-1 produced lower cell yields and CMCase when grown on pure cellulose, such as Sigmacell 20 and filter paper, compared with other carbon sources. In contrast, Harun *et al.* (2013) reported that MCC supported higher cellulase production by *Thermobifida fusca* than pretreated EFB. However, this observation could be attributed to strain differences and the fact that the authors dried their substrates at 105 °C after the pretreatment, while the substrates used in this study were dried at 60 °C. The high temperature employed for drying the EFB may have destroyed protein components of the substrate, thereby reducing the nutrients available for growth and enzyme production.

One interesting observation from these results is that the maximum endoglucanase titer of MS was significantly higher ($P < 0.05$) than the pure cellulose, or with EFB and OPF. The only exception was RH, which showed comparable titers with MS. This result was possibly due to the combination of favourable characteristics (*e.g.*, nutrients, cellulose accessibility, *etc.*) for each individual lignocellulosic material in the mixture. The strategy of using mixed substrates could reduce the cost of cellulase production, which is currently based on expensive synthetic substrates. Furthermore, the use of mixed lignocellulosics facilitates the management of feedstock supply fluctuations (Nilsson and Hansson 2001). This approach also reduces delivery costs to the biorefinery compared with the use of single type feedstocks (Sultana and Kumar 2011). Studies on other applications of lignocellulosic mixtures have demonstrated that combining substrates usually has no negative impacts on product yields; more often than not, higher yields were obtained on mixtures than on the single substrates. Such observations have been reported with respect to the pretreatment

and hydrolysis of mixed substrates (Jensen *et al.* 2008; Moutta *et al.* 2013; Moutta *et al.* 2014; Pereira *et al.* 2015), bioethanol production (Erdei *et al.* 2010; Pereira *et al.* 2015), and fungal cellulase production (Olsson *et al.* 2003).

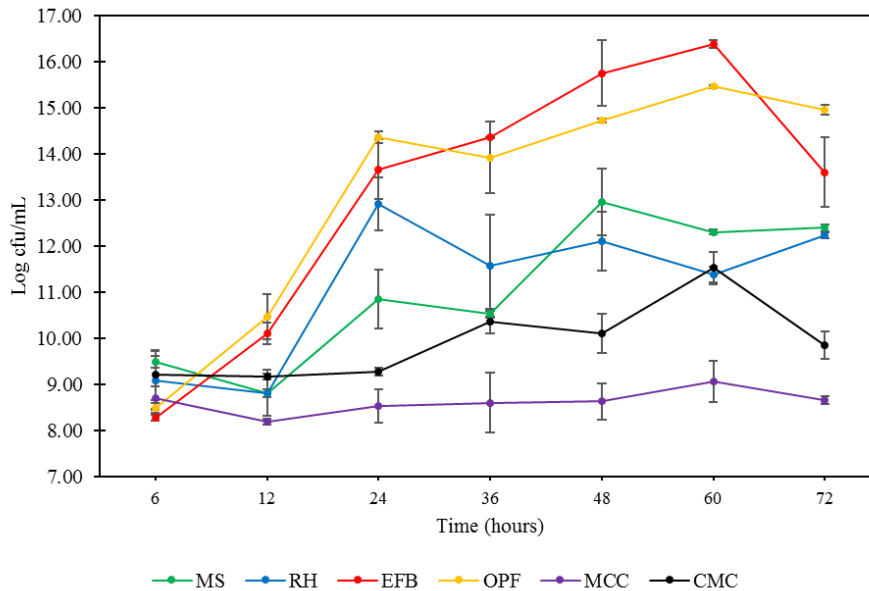


Fig. 2. Growth curve of *B. aerius* S5.2 on various pure cellulosic and lignocellulosic substrates. Error bars represent standard deviations.

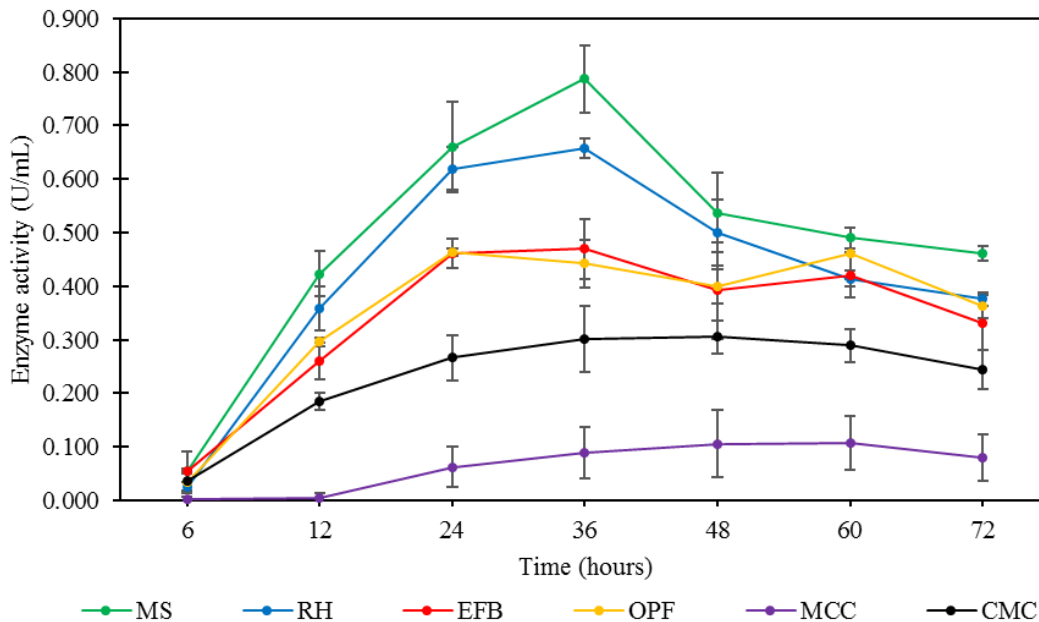


Fig. 3. Endoglucanase production by *B. aerius* S5.2 on various pure cellulosic and lignocellulosic substrates. Error bars represent standard deviations.

Reducing Sugar Production

The reducing sugar profile of the strain was monitored in the culture supernatants during growth on the carbon sources (Fig. 4). The amount of reducing sugar released into the medium was an indication of the relative digestibility of a substrate, and it also was an

adequate indication of the bacterial cellulolytic ability (Han and Callihan 1974). Hence, reducing sugars in the culture medium were generated as a result of the hydrolytic activity of the enzymes secreted by the bacterium. The maximum amounts of reducing sugars detected in the medium of each of the substrates during the entire fermentation period were compared. These were generally higher than the initial reducing sugar concentrations (0.00 to 0.01 mg/mL) in the media prior to fermentation. Extremely low levels of reducing sugar were detected in the MCC medium because it was a poor carbon source for growth and enzyme production (Figs. 2 and 3). Following MCC, the OPF medium had the lowest concentration of sugar (0.07 mg/mL). Interestingly, the highest amount of sugar was detected in the CMC medium (0.61 mg/mL), although this was not significantly higher ($P > 0.05$) than the highest amount detected in the EFB (0.56 ± 0.10 mg/mL) and MS (0.42 ± 0.05 mg/mL) media. The maximum reducing sugar concentration of the RH medium (0.33 ± 0.08 mg/mL) was comparable to that of the MS medium. This value was significantly lower ($P < 0.05$) than those of CMC and EFB, but significantly higher ($P < 0.05$) than OPF.

A relatively high amount of reducing sugar was detected in the CMC medium, despite the lower endoglucanase production. This result was attributed to the higher level of hydrolysis of CMC compared with the other substrates. Endoglucanase has high substrate specificity for CMC and lower specificity for crystalline forms of cellulose (Kim 1995; Dobrev and Zhekova 2012; Miotto *et al.* 2014). Hence, endoglucanase, produced by the medium, must have hydrolysed the CMC better, thus producing higher amounts of reducing sugar than the other substrates. The differences in the levels of reducing sugars produced by the media of the lignocellulosic substrates are probably related to differences in the structural and physicochemical characteristics of the substrates, as a result of the pretreatment. Pretreatment causes changes in the properties (*e.g.*, chemical composition, crystallinity, accessible surface area, porosity, *etc.*) of lignocellulosic substrates, which consequently results in varying degrees of digestibility with cellulase (Meng and Ragauskas 2014). Although the chemical composition of the lignocellulosic substrates used in this study was not determined, it is possible that the pretreatment caused the retention of higher amounts of lignin by the OPF, which may have resulted in the low digestibility on contact with endoglucanase. Lignin exerts an inhibitory effect on cellulases (Rahikainen *et al.* 2013; Gao *et al.* 2014).

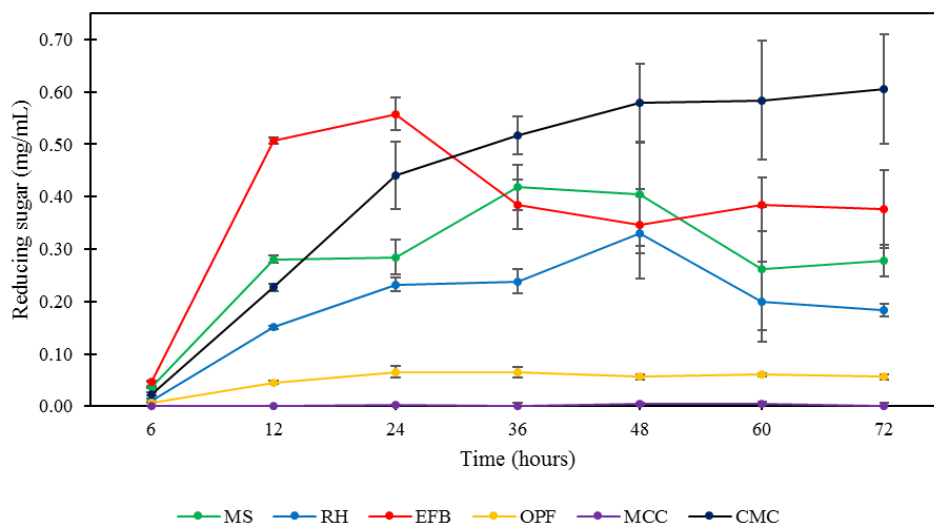


Fig. 4. Reducing sugar profile of the culture supernatants of *B. aerius* S5.2 during its growth on pure cellulosic and lignocellulosic substrates. Error bars represent standard deviations.

Localization of Endoglucanase on CMC and MS

The localization of the endoglucanase activities of *B. aerius* S5.2 grown on a soluble pure cellulosic carbon source and a lignocellulosic substrate was investigated. CMC was chosen as the pure cellulosic source because it resulted in higher endoglucanase activity than the MCC. Likewise, MS was chosen as the lignocellulosic substrate because it supported the highest endoglucanase production. This was done to investigate the expression pattern of the enzyme in relation to the cellular location when the bacterial strain was grown on different substrates (Fig. 5).

Endoglucanase production was predominantly extracellular, irrespective of the substrate solubility. As observed in the earlier carbon source experiments, endoglucanase production on MS was significantly higher ($P < 0.05$) than on CMC extracellularly. These observations were in agreement with results by Kricke *et al.* (1994), who found that CMCase was produced both extracellularly and intracellularly by a *Bacillus* spp. isolated from termite mount soils. The extracellular production of enzymes among *Bacillus* spp. is common (Priest 1977; Molva *et al.* 2009). Some endoglucanase activity was also detected as intracellular and membrane-bound when *B. aerius* S5.2 was grown on both substrates; however, this expression was minimal compared with extracellular production. Cell-bound (intracellular and membrane-bound) cellulase activity in bacteria is believed to represent a basal level of enzyme expression, reflecting the synthesis of cellulase by induction, transcription, and translation within the cell. Hence, cellulases are initially cell-bound before secretion into the medium (Gong and Tsao 1979). The higher extracellular expression of endoglucanase on MS and CMC is a reflection of the nature of both substrates because they both contain amorphous regions for which endoglucanase has high specificity. Furthermore, cellulases that are required for the hydrolysis of a particular substrate are usually expressed extracellularly (Ramasamy and Verachert 1980).

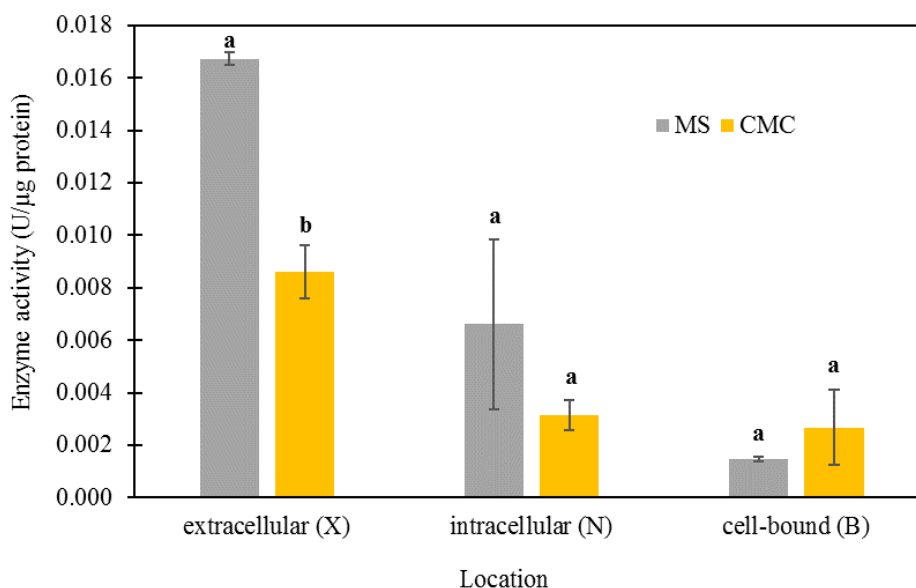


Fig. 5. Cellular location of *B. aerius* S5.2 endoglucanase during its growth on MS and CMC. Error bars represent standard deviations. Within the same location, bars that share the same letters are not significantly different ($P > 0.05$).

The extracellular production of enzymes by microorganisms has promising implications because such enzymes are easier to purify and can be less prone to proteolytic

attack compared with intracellular enzymes (Gao *et al.* 2015). Furthermore, extracellular secretion of cellulase by any strain is useful in developing consolidated bioprocessing (CBP) systems where cellulase production, cellulose hydrolysis, and fermentation occur simultaneously (Lynd *et al.* 2002; Gao *et al.* 2015). Because extracellular cellulase is available for the liberation of fermentable sugars, they could be immediately utilized by the fermenting microorganism.

Degradation of MS

Following the higher expression of endoglucanase by *B. aerius* S5.2 on MS, the ability to utilize MS was later assessed by substrate gravimetric dry weight loss after some period of cultivation. The gravimetric method evaluates the cellulolytic ability of bacteria on different lignocellulosic substrates (Gupta *et al.* 2012; Maki *et al.* 2014).

A total of $25.3 \pm 2.5\%$ of substrate dry weight loss occurred with MS compared with the control flask (19% dry weight loss). This difference was statistically significant ($P = 0.049$). The weight loss recorded from the control was primarily attributed to the dissolution of part of the substrate into the medium due to continuous agitation. Table 2 shows that the culture attained high cell growth on the MS after 72 h, with a cell yield reaching 12.96 log cfu/mL. However, at the end of the 7 d cultivation, the growth had declined to 9.88 log cfu/mL. It is likely that the bacterium was supported almost entirely by the soluble compounds of the MS during the period of active growth, thus resulting in the slight difference between the experimental and the control samples. Thus, it appears that the nature of lignocellulosic biomass affected the utility of the gravimetric method to assess cellulolytic ability of bacterial culture on certain substrates.

CONCLUSIONS

1. Cheap and abundant mixed lignocellulosics are promising for the commercial production of endoglucanase, where the cost of production might be reduced.
2. Higher amounts of extracellular endoglucanase were produced on mixed lignocellulosics by *B. aerius* S5.2 and could be advantageous in terms of enzyme recovery and use in CBP.
3. This study also provided insights into the less explored aspects of *B. aerius* cellulolytic system.
4. The *B. aerius* strain is promising and worth investigating further for possible application in the commercial production of endoglucanase production from lignocellulosics.

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