Agroresidues enhanced peroxidase activity expression by *Bacillus* sp. MABINYA-1 under submerged fermentation

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**Abstract**

Agroresidues have continued to gain preference over conventional carbon sources for microbial enzyme production due to the low price and abundance in the environment. Therefore, this study aimed at improving peroxidase yield by *Bacillus* sp. MABINYA-1 (BMAB-1) using agroresidues under submerged fermentation. The culture parameters that support maximum peroxidase yield by BMAB-1 was initially determined and the results showed that peroxidase activity expression was optimum at pH 5, 30 °C and 150 rpm while veratryl alcohol and ammonium sulphate served as the best peroxidase-inducer and inorganic nitrogen source, respectively. BMAB-1 exhibited maximum peroxidase expression (17.50 ± 0.10 U/mg) at 72 h using kraft lignin liquid medium (KLLM) under the optimized culture conditions. Upon utilization of selected agroresidues (sawdust, wheat straw and maize stover) as sole carbon sources by BMAB-1 in the fermentation process, peroxidase activity was significantly enhanced when compared with glucose (14.91 ± 0.31 U/mg) and kraft lignin (17.50 ± 0.10 U/mg). Sawdust produced the highest peroxidase yield (47.14 ± 0.41 U/mg), followed by maize stover (37.09 ± 0.00 U/mg) while wheat straw yielded the lowest peroxidase specific activity (21.65 ± 0.35 U/mg). This indicates that utilization of sawdust by BMAB-1 resulted in 3.2- and 2.7-fold increase in peroxidase activity expression as compared to glucose and kraft lignin, respectively. The aptitude of BMAB-1 to utilize agroresidues would reduce the cost of peroxidase production by the bacteria since the substrates are cheaper than the conventional carbon sources and are, as well, more readily available.

**Keywords:** Culture conditions, Maize stover, Peroxidase, Sawdust, Submerged fermentation, Wheat straw

**Introduction**

Peroxidase has continued to gain attention, probably, due to the robust industrial application potentials; notably, the emerging role in bioremediation that has extensively been documented (Min et al. 2015; Taboada-Puig et al. 2015; Falade et al. 2018). Peroxidases are characterized by promising application potential in the bioenergy sector as members of class-II peroxidase-catalase superfamily (lignin peroxidase, manganese peroxidase and versatile peroxidase) are efficient lignin degraders. Hence, they are part of the suggested “lignocellulolytic enzyme system” for biological delignification of feedstock for biofuel production (Wang et al. 2013; Falade et al. 2017a). Peroxidase has also been applied for analytical purposes including development of diagnostic kits for determination of “uric acid and glucose” (Regalado et al. 2004). Other applications of peroxidase include but not limited to development of biosensor, skin-lightening cream and biobleaching of paper pulp (Saleem et al. 2018). Due to the enormous application potentials of peroxidase in different industrial sectors, increased demand is unavoidable. Hence, it is important to increase peroxidase yield and continue to explore novel sources with capacity for improved peroxidase production.

Enzyme yields have been improved over the years using two main approaches. The first one is genetic engineering, which involves “classical mutagenesis and screening”
and “rational design” (Arnau et al. 2020). Examples of rational design strategies include the use of “stronger promoters and multicopy strains” to improve enzyme production by overexpression of the target gene; “gene deletion”; and “gene fusions” for improved production (Arnau et al. 2020). The second approach, which is more or less preliminary, is the “optimization of culture and nutritional conditions” of microorganisms for improved enzyme yield through the conventional method of “one variable at a time” or response surface methodology (RSM). In the conventional approach, culture conditions that are important for microbial growth (pH, temperature and agitation) and metabolic activities are determined because the continuous production of enzymes has been linked to microbial growth (Niladevi and Prema 2008; Musengi et al. 2014). In addition, nutritional components of the fermentation medium including carbon and nitrogen sources are also significant to the growth of microorganisms. The choice of carbon source is vital because it provides the required energy for the microbial strain. Most bacteria utilize “simple sugars” as carbon sources, but simple sugars and other synthetic sources of carbon are expensive, hence, contributing to the high cost of enzyme production. Besides, simple sugars may not be the most appropriate carbon source for production of inducible enzymes including peroxidases, which may require the introduction of an “inducer” in the growth medium. It is, thus, necessary to explore cheap alternative carbon sources capable of inducing increased enzyme expression.

Waste biomass seems to be a promising alternative to the costly conventional carbon sources including glucose, fructose and xylose as they are readily available and abundant in the environment. Several thousands of tons of lignocellulosic wastes are generated from the agro-industrial sector annually, with no adequate provision for proper disposal. As such, they are burnt, thereby causing environmental pollution (Falade et al. 2017a). Therefore, bioprospecting of lignocellulosic wastes as raw materials for enzyme production becomes an imperative.

Recent studies have reported the utilization of agroresidues (sawdust, wheat straw, wheat bran, corn stover, corn cob, mandarin peels, etc.) for microbial enzyme production (Ijoma et al. 2018; Unuofin et al. 2019; Falade et al. 2019a). Unuofin et al. (2019) valorized maize stover for improved laccase production by two γ-proteobacteria strains. Also, Falade et al. (2019a) reported the usage of some agricultural residues by a Raoultella species for maximum peroxidase secretion. Recently, Kumar et al. (2020) valorized a wide range of agroresidues including “rice bran, wheat bran, sawdust, banana peel, orange peel, potato peel, pea peel and sugarcane bagasse” for enhanced laccase yield by Bacillus sp. AKRC01. There is, however, paucity of information on the utilization of lignocellulosic waste biomass for peroxidase production by Bacillus species. This present study, therefore, aimed at improving peroxidase yield of BMAB-1 using some agroresidues as carbon sources in a submerged fermentation.

Materials and methods

Source of microorganism

BMAB-1 was isolated from soil sample collected from Hogsback forest reserve in Eastern Cape Province, South Africa, as described in a previous study (Falade et al. 2017b). The bacteria was tested for ligninolytic and peroxidase-producing abilities as reported in an earlier study (Falade et al. 2019b). The ligninolytic potential was determined by its aptitude to metabolize “lignin model compounds: guaiacol and veratryl alcohol” (Taylor et al. 2012) while it was positive to a plate screening for peroxidase activity by “yellowish-brown” colouration after reacting with a mixture of “0.4% (v/v) hydrogen peroxide” and “1% pyrogallol” on nutrient agar (Falade et al. 2017b). Subsequently, the bacteria was identified using 16S rRNA gene sequence analysis (Falade et al. 2019b). Bioinformatics analysis involving the use of Basic Local Alignment Search Tool (BLAST) in the National Center for Biotechnology Information (NCBI) database revealed the identity of the bacteria as a Bacillus species as it had 99% similarity to Bacillus sp. NC62 (KY454505). Hence, it was identified as Bacillus sp. MABINYA-1, with the GenBank accession number, KX640920.

Submerged fermentation (SMF) for peroxidase production

The enzyme was produced in a SMF process using the method of Falade et al. (2017b). About 2% standardized bacterial suspension (OD 600 nm = 0.1) was inoculated in a kraft lignin liquid medium (KLLM) comprising the following components: K2HPO4, KH2PO4, MgSO4, NH4NO3, yeast extract and kraft lignin with 4.55, 0.53, 0.5, 5.0, 0.1 g/L and 0.1% w/v as the respective concentrations. Subsequently, the culture was incubated for 48 h using the conditions for isolation (temperature: 30 °C; pH: 7.0; and agitation rate: 140 rpm). The enzyme was prepared by removing the bacterial cells through centrifugation at 10,000 × g in a SIGMA 1–14 K cold centrifuge. The recovered supernatant, which served as crude enzyme, was subsequently, assayed for peroxidase activity.

Assessment of expressed peroxidase activity

The level of peroxidase produced by BMAB-1 was assessed by the exoperoxidase activity using the procedure of Chance and Maehly (1955) with slight changes. Details of the modifications have been reported earlier (Falade et al. 2017b).
Evaluation of culture conditions for optimum peroxidase activity expression
The various culture parameters favourable for maximum peroxidase expression by BMAB-1 were determined through the conventional one-variable-at-a-time (OVAT) approach (Falade et al. 2019b). BMAB-1 was cultured in KLLM with initial pH ranging from 3 to 11. The medium pH was adjusted using one molar hydrochloric acid and sodium hydroxide as appropriate. Subsequently, the temperature that promotes optimum peroxidase expression by BMAB-1 was determined by cultivating the bacteria at different temperatures (20–45 °C) for 48 h, whereas the optimal agitation rate was determined by incubating the culture in an orbital shaker at different agitation rates (0–200 rpm) under predetermined optimal temperature. The observed optimal culture parameters were subsequently, used for further fermentations.

Influence of lignols on peroxidase activity expression
The influence of augmenting KLLM with varied lignols on peroxidase activity expression by BMAB-1 was evaluated using the method of Musengi et al. (2014). The bacteria was cultured in KLLM supplemented with 1 mM of selected lignols including guaiacol, veratryl alcohol and vanillin under culture conditions for optimum peroxidase production predetermined in this study.

Influence of inorganic nitrogen supplementation on peroxidase activity expression
The influence of inorganic nitrogen supplementation on peroxidase activity expression was evaluated by cultivating the bacteria in KLLM augmented with various inorganic nitrogen including NH₄NO₃; NH₄Cl and (NH₄)₂SO₄ under optimized conditions for peroxidase production (30 °C, pH 5 and 150 rpm) with veratryl alcohol as the most effective lignol.

Time course assay
Exoperoxidase activity expression by BMAB-1 and the growth rate of the bacterial cells were assessed for 144 h (Tuncer et al. 1999). BMAB-1 was cultivated in KLLM under optimized process conditions predetermined in this study. Subsequently, culture was aseptically withdrawn every 24 h for peroxidase activity assay and determination of non-peroxide-dependent enzyme activity while the protein content was evaluated (Bradford 1976; Falade et al. 2019a) simultaneously with the bacterial cell growth, which was assessed by reading the absorbance of the culture at 600 nm. We expressed the enzyme specific activity as unit of enzyme per milligram of protein.

Exploitation of agroresidues for improved peroxidase activity expression
The following agroresidues: sawdust, wheat straw and maize stover, obtained from Alice area in South Africa, were processed as described by Falade et al. (2019a) and then, optimized as carbon sources for peroxidase secretion by BMAB-1 in a SMF. The different agroresidues (1% w/v) were used in place of kraft lignin in KLLM. Peroxidase activity expressed by the bacteria when grown on the agroresidues was then compared with the enzyme activity expressed upon glucose and kraft lignin utilization as carbon sources.

Data analysis
Data generated were analyzed using one-way ANOVA accompanied by Tukey’s and Dunnett’s post tests as appropriate, with the use of Graph Pad Prism 7.0. Significance was recognized at 95% confidence interval (p ≤ 0.05).

Results and discussion
Evaluation of culture conditions for maximum peroxidase activity expression
The various culture parameters (pH, temperature and agitation) that promote maximum peroxidase expression by BMAB-1 was initially determined because such microbial growth factors have been linked to persistent enzyme secretion.

Firstly, the initial medium pH that promotes optimum peroxidase secretion by BMAB-1 was assessed and the result is given in Fig. 1. The result showed that peroxidase activity expressed by the test bacteria across the pH range differs significantly (p < 0.05). It is remarkable that BMAB-1 expressed significant exoperoxidase activity over a broad pH range (3–11) with the highest activity recorded at pH 5 (6.65 ± 0.23 U/ml). The aptitude of BMAB-1 to exhibit significant peroxidase activity at very low pH (3 and 4) indicates that the bacteria is acidophilic in nature. The extremophilic attribute of this bacteria is notable as extremophiles are known for their uniqueness in tolerating harsh environmental conditions, which makes them promising biotechnological candidates. More so, enzymes produced by extremophiles tend to be remarkably stable under extreme conditions (Dumorne 2018). In other words, enzymes from acidophilic bacteria are stable and active at very low pH and as such hold great biotechnological potential (Jackson et al. 2007; Sharma et al. 2016; Dumorne 2018). Our result concurs with some prior studies, where peroxidase production was optimum at acidic pH region.
A recent study reported maximum peroxidase yield by a *Raoultella* species at pH 5 (Falade et al. 2019a). This is corroborated by Rao and Kavya (2014), whose findings revealed pH 6 as the most favourable for optimum peroxidase activity expression by a *Bacillus* species. These findings may be attributed to the electric charge on the bacterial cell. Since the cell wall of *Bacillus* species and other Gram-positive bacteria are negatively charged, the bacterial strain tends to thrive more in an acidic environment, made up of more hydrogen ions (H\(^+\)).

More so, the “electric charge on the bacterial cell” is imperative for absorption of nutrient with consequent effect on the microbial growth (Falade et al. 2019a).

The influence of temperature on peroxidase secretion by BMAB-1 is presented in Fig. 2. The result showed that peroxidase activity across the examined temperatures (25–45 °C) was significantly different \( (p < 0.05) \), with the highest activity expressed at 30 °C (6.70 ± 0.23 U/ml). However, the difference in peroxidase activity observed at 40 °C (2.33 ± 0.00 U/ml) and 45 °C (1.58 ± 0.06 U/ml) was not significant \( (p > 0.05) \). Our finding is comparable to what was reported by Rajkumar et al. (2013), where the optimal temperature for peroxidase secretion by a *Bacillus* species was 30 °C. More so, 30 °C has been reported as optimal temperature for peroxidase production by some other bacteria belonging to a different genus. One of such bacteria is *Ensifer adhaerens* NWODO-2, which similarly, expressed optimum peroxidase activity at 30 °C (Falade et al. 2019c). Nonetheless, 37 °C was the most favourable temperature for *Bacillus subtilis* with regard to peroxidase production (Rao and Kavya 2014). It is clear that peroxidase secretion by most of the reported bacteria was optimal at the mesophilic range. The decreased peroxidase activity observed at 25 °C and temperatures greater than 30 °C in this study is perhaps, consequent upon reduced metabolic activities with resultant effect on peroxidase biosynthesis by the test bacteria (Ray et al. 2007; Tandon and Sharma 2014).

Furthermore, we evaluated the effect of agitation on peroxidase activity expression by BMAB-1 and the result is presented in Fig. 3. The result showed that peroxidase activity across the agitation rates was significantly different \( (p < 0.05) \). However, Dunnett’s post test showed that the enzyme activity expressed by BMAB-1 at 50 and 100 rpm does not differ significantly \( (p > 0.05) \) when compared with static condition (0 rpm). Nevertheless, the bacteria expressed optimal peroxidase activity at 150 rpm (3.85 ± 0.82 U/ml), which significantly differs \( (p < 0.05) \) from the enzyme activity at static condition (0.58 ± 0.00 U/ml). It is obvious that high agitation rate was more advantageous towards expression of peroxidase activity by BMAB-1. This finding is justifiable because agitation is capable of affecting the degree of “aeration” and promotes efficient mixing of nutrients in the fermentation medium (McNeil et al. 2006). Therefore, increased agitation is likely to encourage nutrient accessibility to the bacteria thereby, affecting the growth and consequently, the enzyme production. On the other hand, an increase in agitation speed beyond 150 rpm caused a significant reduction in peroxidase activity at 200 rpm (2.33 ± 0.81 U/ml), which is attributable to “foaming or shearing stress” exerted on the bacterial cells through agitation (Falade et al. 2019c).
is consistent with some prior related studies, which also reported 150 rpm as the most favourable for bacterial peroxidase secretion (Falade et al. 2019a, 2019d). However, maximum peroxidase yield by Bacillus species was supported at 180 rpm in a different study (Patil 2014). Thus, the effect of agitation on enzyme production varies from individual bacteria.

**Influence of lignols on peroxidase activity expression**

Lignols are known inducers of ligninolytic enzymes including peroxidases in bacteria and fungi. Consequently, supplementation of the fermentation medium with the precise lignol may improve peroxidase yield in bacteria with intrinsic peroxidase-producing ability. Therefore, we augmented the carbon source (kraft lignin) in the production medium (KLLM) with selected lignols (Guaiacol-Gua, Veratryl alcohol-VAlc and Vanillin-Van). The influence of lignols on peroxidase activity expression by BMAB-1 is given in Fig. 4. The result showed that peroxidase activity expressed by BMAB-1 grown on lignol-supplemented medium increased significantly \( (p<0.05) \) when compared with the non-supplemented production medium, having kraft lignin alone (KLA) as the carbon source (control). While veratryl alcohol induced the highest peroxidase expression in BMAB-1 \( (2.57 \pm 0.04 \text{ U/ml}) \), the control medium induced the lowest peroxidase activity \( (0.47 \pm 0.01 \text{ U/ml}) \). In other words, veratryl alcohol increased peroxidase activity by over fivefold when compared with the control. This finding agrees with that of Musengi et al. (2014), in which veratryl alcohol was also reported as the best peroxidase-inducer in a Streptomyces species. In contrast, guaiacol gave the best inducing effect on peroxidase expression by Bacillus sp. in a study by Falade et al. (2019d).

**Influence of inorganic nitrogen supplementation on peroxidase activity expression**

The impact of nitrogen nature and concentration on the production of different lignocellulolytic enzymes (LCEs) including peroxidases are not constant as this can either be stimulatory or inhibitory (Pedri et al. 2015). Cultivation of bacteria in a medium with precise nitrogen source has been reported to improve enzyme production (Kaal et al. 1995). However, some microbes prefer fermentation medium with limited nitrogen for optimum enzyme production (Galhaup et al. 2002; Gao et al. 2005). This therefore, necessitated augmentation of the production medium with some inorganic sources of nitrogen \( (\text{NH}_4\text{NO}_3; \text{NH}_4\text{Cl and (NH}_4)_2\text{SO}_4) \). The effect of nitrogen supplementation on peroxidase activity expression by BMAB-1 is given in Fig. 5. The result showed a significant difference \( (p<0.05) \) in peroxidase activity expressed by BMAB-1 grown on inorganic nitrogen-augmented medium \( (\text{YE}+\text{NH}_4\text{NO}_3; \text{YE}+\text{NH}_4\text{Cl and YE}+(\text{NH}_4)_2\text{SO}_4) \) when compared with the non-supplemented medium (control): which had yeast extract alone (YEA) as the nitrogen source. While supplementation with \( \text{NH}_4\text{Cl and (NH}_4)_2\text{SO}_4 \) produced stimulatory effects on peroxidase activity expression by BMAB-1, with \( (\text{NH}_4)_2\text{SO}_4 \) as the best inorganic nitrogen source.
(13.07 ± 0.00 U/ml), addition of NH₄NO₃ to the medium repressed peroxidase secretion by BMAB-1. This finding agrees with what we previously reported (Falade et al. 2019d), where augmentation of production medium with (NH₄)₂SO₄ supported optimal peroxidase secretion by a Bacillus strain. Meanwhile, addition of both NH₄NO₃ and NH₄Cl was reported to have repressed peroxidase activity expression in the aforementioned study. However, supplementation of organic nitrogen source with NH₄Cl yielded maximum exoperoxidase activity expression by a Raoultella species (Falade et al. 2019a). These findings further validate the inconsistency in the influence of nitrogen sources on lignocellulolytic enzymes secretion as claimed by Niladevi and Prema (2008).

**Time course of peroxidase activity expression**

Expression of peroxidase and non-peroxide dependent enzyme activity by the Bacillus species as well as its cell growth were monitored for 144 h and the results are given in Fig. 6. The results revealed that BMAB-1 expressed the highest specific peroxidase activity at 72 h (17.50 ± 0.10 U/mg), representing the beginning of stationary growth phase of the bacteria, where production of secondary metabolites mostly occur. It is worthy of note that a non-peroxidase activity was simultaneously expressed by the bacteria, which was however, optimum at 48 h (10.43 ± 0.67 U/mg). It is obvious that the enzyme secretion was connected to the bacterial growth. The decline in peroxidase activity expressed after 72 h is perhaps, due to nutrient exhaustion, “denaturation” or “proteolysis” (Papagianni and Moo-Young 2002). This finding is comparable to some past studies, which also reported optimal peroxidase activity expression at 72 h (Nour El-Dein et al. 2014; Falade et al. 2019a). Nonetheless, other studies have observed optimum peroxidase activity at 48 h (Falade et al. 2019c, 2019d). It is remarkable that the maximum peroxidase activity expressed by BMAB-1 is higher than the enzyme yield from other bacteria (Ehiusun and Usman 2018). The optimum peroxidase yield by Raoultella ornithinolytica was 16.48 ± 0.89 U/mg at 72 h (Falade et al. 2019a) while Falade et al. (2019c) reported 12.76 ± 1.09 U/mg as the specific peroxidase activity expressed by E. adhaerens NWODO-2 at 48 h. More so, Streptomyces sp K37 achieved 0.537 U/mg as its optimal specific peroxidase activity (Nour El-Dein et al. 2014), whereas Bacillus sp. FALADE-1 attained optimum peroxidase activity at 48 h with 8.32 U/mg. Expression of a non-peroxide-dependent extracellular enzyme activity by BMAB-1 probably, suggests laccase activity, as laccase utilizes molecular oxygen as electron acceptor instead of hydrogen peroxide required by peroxidases.

**Exploitation of agroresidues for improved peroxidase activity expression**

The imperativeness of the search for new and cheap alternative carbon sources for peroxidase production cannot be overemphasized as the cost of conventional sources of carbon contributes in no small measure to the huge cost of enzyme production, which is still a main obstacle in industrial enzyme production. In a bid to finding a low-cost alternative substrate for sustainable peroxidase production,
production, we evaluated some agroresidues including sawdust, wheat straw and maize stover as substrates for peroxidase production by BMAB-1. The result, as given in Fig. 7, showed a significant increase \( (p<0.05) \) in peroxidase activity expressed by the bacteria cultivated on the agroresidues when compared with glucose (a standard carbon source) and kraft lignin (a synthetic peroxidase inducer), with sawdust inducing the highest peroxidase activity expression \( (47.14 \pm 0.41 \text{ U/mg}) \) while the lowest peroxidase activity was induced by glucose \( (14.91 \pm 0.31 \text{ U/mg}) \). This finding indicates that sawdust would be a suitable alternative to both glucose and kraft lignin as regards peroxidase production. Apart from being cheaper than glucose and other conventional carbon sources, sawdust is more readily available. Hence, utilization of sawdust as substrate by BMAB-1 would reduce the cost of peroxidase production by the bacteria.

Several other studies have also reported sawdust as an effective substrate for production of peroxidase and other ligninolytic enzymes by bacteria (Kamsani et al. 2016; Falade et al. 2019a, 2019c). Falade et al. (2019a) reported 15.21 \( \pm \) 2.48 U/mg as the peroxidase yield by \textit{R. orthinithinolytica} OKOH-1 upon utilization of sawdust, which was only comparable to peroxidase activity expressed by the bacteria when grown in a kraft lignin medium \( (16.48 \pm 0.89 \text{ U/mg}) \). In contrary, sawdust increased peroxidase activity expression in this current study by 2.7-fold when compared with kraft lignin. It is noteworthy that peroxidase yield by BMAB-1 grown on sawdust is significantly higher than what was previously reported. While Falade et al. (2019c) reported 37.50 U/mg as the specific peroxidase activity expressed by \textit{E. adhaerens} NWODO-2 when grown on sawdust under solid state fermentation, BMAB-1 expressed specific peroxidase activity of \( 47.14 \pm 0.41 \text{ U/mg} \) when cultivated on sawdust in a submerged fermentation. However, Ijoma et al. (2018) identified wheat straw as the best inducer of manganese peroxidase. The discrepancy in the enzyme titre produced by the test bacteria when cultivated on the different agroresidues as observed in this study may be due to the type and concentration of phenolic and non-phenolic constituents of the substrates.

**Conclusion**

BMAB-1 exhibited the characteristic of an acidophile as it expressed optimum exoperoxidase activity at pH 5 and considerable activity was expressed by the bacteria at extreme pH 3 and 4. Maximum peroxidase yield by the bacteria was supported at 30 °C and 150 rpm while augmentation of the production medium with veratryl alcohol and ammonium sulphate improved peroxidase activity expression by BMAB-1 significantly. This study showed that agricultural residues are excellent bioresources for enhanced peroxidase production because all the investigated agroresidues (sawdust, wheat straw and maize stover) improved peroxidase activity expression by BMAB-1. However, the bacteria showed the most remarkable peroxidase activity on sawdust because the substrate improved peroxidase yield by 3.2-fold and 2.7-fold when compared with glucose and kraft lignin, respectively. Thus, sawdust would be a suitable alternative to the conventional carbon sources and kraft lignin for peroxidase production by the bacteria. Furthermore, the ability of BMAB-1 to utilize agroresidues would reduce the cost of peroxidase production since agricultural residues are cheaper than the conventional carbon sources and as well are more readily available.

**Abbreviations**

ANOVA: Analysis of variance; BLAST: Basic local alignment search tool; BMAB-1: Bacillus sp. MABINYA-1; KLA: Kraft lignin alone; KLLM: Kraft lignin liquid medium; LCEs: Lignocellulolytic enzymes; NCBI: National Center for Biotechnology Information; OD: Optical density; OVAT: One-variable-at-a-time; SMF: Submerged fermentation; YEA: Yeast extract alone.

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**Authors’ contributions**

Conceptualization: AO, LV, AI and UU. Investigation: AO. Data curation and analysis: AO. Writing—original draft preparation: AO. Writing—review and editing: LV, AI and UU. Supervision: LV, AI and UU. All authors read and approved the final manuscript.
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Competing interests
Authors declare that they have no competing interests.

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