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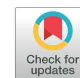
Research article

Studies on peroxidase production and detection of *Sporotrichum thermophile*-like catalase-peroxidase gene in a *Bacillus* species isolated from Hogsback forest reserve, South Africa



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ABSTRACT

This study sought to determine the process conditions for optimum peroxidase production by a *Bacillus* species (*Bacillus* sp. FALADE-1-KX640922) isolated from Hogsback forest reserve in South Africa and characterize the peroxidase gene in the bacteria. We optimized peroxidase production by manipulating the environmental and nutritional parameters under submerged fermentation. Subsequently, the gene encoding heme-peroxidase was determined through nested polymerase chain reaction and Sanger DNA sequencing. The studied bacteria had maximum peroxidase production at pH 8, 30 °C and 150 rpm. The addition of guaiacol to lignin fermentation medium enhanced peroxidase production by over 100 % in the studied bacteria. However, the other lignin monomers (veratryl alcohol, vanillin, vanillic acid and ferulic acid) repressed the enzyme activity. Modification of the fermentation medium with ammonium sulphate gave the maximum peroxidase yield (8.87 U mL^{-1}). Under the predetermined culture conditions, *Bacillus* sp. FALADE-1 expressed maximum specific peroxidase activity at 48 h (8.32 U mg^{-1}). Interestingly, a search of the sequenced gene in PeroxiBase showed 100% similarity to *Sporotrichum thermophile* catalase-peroxidase gene (*katG*), as well, the deduced protein sequence clustered with bacterial catalase-peroxidases and had a molecular weight of about 11.45 kDa with 7.01 as the estimated isoelectric point. Subsequently, the nucleotide sequence was deposited in the National Center for Biotechnology Information (NCBI) repository with the accession number MF407314. In conclusion, *Bacillus* sp. FALADE-1 is a promising candidate for improved peroxidase production.

1. Introduction

Peroxidases are oxidoreductases which have recently received increased attention, probably, owing to their high redox potentials and the capability to oxidize compounds with known resistance to degradation (Falade et al., 2017a). These characteristics have motivated for their application potentials in various industrial sectors (Jia et al., 2002; Agostini et al., 2002; Kalyani et al., 2011; Draelos, 2015; Falade et al. 2017a, 2018; Taboada-Puig et al., 2015). Nevertheless, the industrial applicability of peroxidases is being hampered by poor yield and high-priced production (Ferrer et al., 1991; Torres et al., 2003). More so, the commercially available peroxidases including horseradish peroxidase (HRP), *Bjerkandera adusta* peroxidase and peroxidase from *Streptomyces avidinii* (streptavidin) could not probably meet the increasing industrial demand for peroxidases. Hence, the imperativeness of new sources of peroxidase to satisfy the growing market demands.

Production of peroxidases from plant and fungi have been widely studied (Lavery et al., 2010; Kharatmol and Pandit, 2012; Rathnamsamy et al., 2014; Kong et al., 2016; Zhang et al., 2016). Peroxidase production by a number of bacteria, predominantly, actinobacteria (Tuncer et al., 2009; Nour El-Dein et al., 2014; Musengi et al., 2014) and very few belonging to *Bacillus* genus (Dawkar et al., 2009; Rajkumar et al., 2013; Patil, 2014; Rao and Kavya, 2014) have also been reported. Moreover, bacteria seem to be more promising for enhanced peroxidase production, perhaps, due to their high genetic maneuverability, which is difficult in fungi. More so, detection of the gene encoding peroxidase in bacteria is another step that could be explored for improved peroxidase production through genetic engineering.

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Bacillus species are described as the “major workhorse industrial microorganisms” (Schallmey et al., 2004), with enhanced enzyme production potential. They are characterized by high growth rate, ability to produce extracellular proteins in large quantity and general safety (Schallmey et al., 2004). Moreover, *Bacillus* species have shown great potential for production of various extracellular enzymes (Sevinc and Demirkan, 2011; Barros et al., 2013; Pant et al., 2015). Also, some *Bacillus* species have been employed for production of cellulolytic and pectinolytic enzymes (Soares et al., 2001; Dias et al., 2014; Padilha et al., 2015). Nonetheless, there is dearth of information on the production of ligninolytic enzymes, particularly, peroxidase by *Bacillus* species. Therefore, this study aimed at determining the culture conditions that support optimum peroxidase production by *Bacillus* sp. FALADE-1 (Accession number: KX640922) and characterize the peroxidase gene in the bacteria.

2. Materials and methods

2.1. Bacterial source

The bacteria (*Bacillus* sp. FALADE-1) was isolated from rock scrapings collected from Hogsback forest in Eastern Cape, South Africa using the modified method of Sasikumar et al. (2014) described elsewhere (Falade et al., 2017b). Then, the bacteria was screened for lignin-degradation and peroxidase production potentials. Subsequently, the bacteria was identified as a *Bacillus* sp. using 16S rDNA partial sequence analysis (Falade et al., 2019a).

2.2. Enzyme production

Peroxidase was produced through submerged fermentation as previously described by Falade et al. (2017b), where 100 mL of lignin fermentation medium (LFM): K_2HPO_4 (4.55 g L^{-1}), KH_2PO_4 (0.53 g L^{-1}), $MgSO_4$ (0.5 g L^{-1}), NH_4NO_3 (5 g L^{-1}), yeast extract (0.1 g L^{-1}) and 0.1 % w/v lignin (Sigma-Aldrich, SA) was inoculated with 2 % bacterial suspension ($A_{600\ nm} \approx 0.1$) at pH 7 using uninoculated media as control. The culture was subsequently, incubated for 48 h using the conditions for isolation (Falade et al., 2017b), afterwards, the crude enzyme was prepared as previously reported (Falade et al., 2019a).

2.3. Enzyme activity assay

Peroxidase production was assessed by determining the extracellular peroxidase activity using the method described in our previous study (Falade et al., 2017b).

2.4. Optimization studies on peroxidase production by Bacillus sp. FALADE-1

The culture conditions that support maximum peroxidase secretion by the *Bacillus* species were determined using the method reported by Falade et al. (2019b). Peroxidase production was optimized by adjusting the various conditions under which the bacteria was cultivated such as pH (3–11), temperature (20–45 °C) and agitation rate (0–200 rpm). The observed optimum culture parameters were then employed for subsequent fermentations. As well, the fermentation medium (LFM) composition was manipulated by supplementing the carbon source with one millimolar of selected “lignin monomers: guaiacol, veratryl alcohol, vanillin, vanillic acid and ferulic acid” (Falade et al., 2019b) while the fermentation medium was further modified using 5 g L^{-1} of different “inorganic nitrogen: ammonium nitrate, ammonium chloride and ammonium sulphate” (Falade et al., 2019b).

2.5. Kinetics of enzyme production and bacterial growth

The kinetics of peroxidase secretion by *Bacillus* sp. FALADE-1 and its cell growth were determined using the method of Tuncer et al. (1999) with slight modifications described elsewhere (Falade et al., 2019b). The bacteria was grown in LFM under predetermined optimized culture conditions (pH 8, 30 °C and 150 rpm). Bacterial culture was withdrawn at 24 h interval (Tuncer et al., 1999) for 120 h and assayed for peroxidase activity and protein concentration using Bradford method. As well, the cell growth was determined by monitoring the absorbance at 600 nm.

2.6. Peroxidase gene detection using nested polymerase chain reaction (PCR)

The target gene was amplified by employing the following set of primers (Baprx F1: 5′-GCAAAAAAGGGCAGTCACGCAA-3'; Baprx F2: 5′-AAAAGGGCAGTCACGCAATGTA-3′ and Baprx R: 5′-TTGAAGAA-CATCGTCAGCGAATAAT-3′) in a nested PCR. The primers were designed using the DNA sequence of *Bacillus* sp. ABP14 (CP017016). The reaction mixture comprising master mix (BioLabs, SA), Baprx F1 and Baprx R, nuclease-free water and the DNA template was used for the first PCR under the following conditions: initial denaturation at 95 °C for 5 min, 35 cycles [denaturation: 94 °C (1 min), annealing: 55 °C (1 min), extension: 72 °C (1 min)] and final extension at 72 °C for 5 min. The amplicon from the initial PCR was employed as the DNA template for the subsequent experiment using Baprx F2 and the reverse primer, Baprx R under the same PCR conditions in a thermal cycler apparatus (G-STORM, UK). Thereafter, the amplified product was visualized by electrophoresis using a 1.5 % agarose gel (Merck, SA) stained with ethidium bromide (Sigma-Aldrich, SA) at 100 V for 45 min in 0.5X TBE buffer through ultraviolet illumination (Alliance 4.7, France). The PCR product was subsequently analysed after being purified, using Sanger sequencing method. Thereafter, the sequenced nucleotides were searched in peroxidase database (PeroxiBase) using blastn.

2.7. Phylogenetic analysis

Phylogenetic analysis of the deduced protein sequence from *Bacillus* sp. FALADE-1 peroxidase gene and some heme-peroxidases of bacteria origin was conducted using neighbour-joining method (Saitou and Nei, 1987) in MEGA 7.0 software (Kumar et al., 2016) while the physicochemical properties were determined using geneious 10.2.2.

2.8. Data analysis

Data, where applicable, were subjected to ANOVA and Tukey's multiple comparison test. Significance was accepted at $P \leq 0.05$.

3. Results and discussion

Considering the varied application potentials of peroxidase in a wide range of industrial processes, it is highly imperative to improve the enzyme production. It has therefore become necessary to explore novel sources of peroxidase with increased production capability. In this study, we assessed the peroxidase production capacity of *Bacillus* sp. FALADE-1 under optimized culture conditions including pH, temperature and agitation rate. Also, the composition of the fermentation medium was amended for optimum peroxidase production by the bacteria. Subsequently, the kinetics of peroxidase production and bacterial growth were assessed as the peroxidase gene was also characterized. The peroxidase production was determined in this study by measuring the peroxidase activity of the supernatant recovered from the bacteria.

3.1. Optimization studies on peroxidase production by Bacillus sp. FALADE-1

The pH of the cultivation environment exerts a significant influence on microbial growth and metabolism (Saini et al., 2014) as nutrient absorption is determined by the charge on the microbial cells (Salehizadeh and Shojaosadati, 2001). It is therefore important to determine the medium pH that is most favourable for metabolic activities of bacteria. The results of initial medium pH for optimum peroxidase production by *Bacillus* sp. FALADE-1 as presented in Figure 1 showed that the bacteria produced peroxidase over a wide pH range (5.0–10.0) with optimum production observed at pH 8 (3.15 U mL^{-1}). Nevertheless, peroxidase activity was not observed at pH 3, 4 and 11. Even though peroxidase production by *Bacillus* sp. FALADE-1 at the optimal pH differs significantly ($P < 0.05$) when compared with other pH values, the difference in the enzyme production at pH 6, 7, 9, 10 was not significant ($P > 0.05$). This finding concurs with previous related study by Rajkumar et al. (2013), in which optimum peroxidase production by a *Bacillus* sp. was recorded at pH 8. However, Rao and Kavya (2014) reported that pH 6 supported maximum peroxidase production by *Bacillus subtilis*. This indicates that pH plays a significant role in peroxidase production (McCarthy, 1987; Niladevi and Prema, 2008).

Bacteria can grow only within certain limits of temperatures. This environmental factor tends to influence the growth rate, macromolecular composition, levels of intracellular metabolites and enzyme production. It is therefore expedient to determine the temperature that best supports the growth of bacteria and optimum enzyme production. The effect of temperature on peroxidase produced by the *Bacillus* strain as shown in Figure 2 revealed a significant difference ($P < 0.05$) in the enzyme production across all the studied temperatures, with optimum peroxidase production detected at 30 °C (3.15 U mL^{-1}). Nonetheless, the difference in peroxidase production at 20 °C, 35 °C and 45 °C was not significant ($P > 0.05$). However, there exist a significant ($P < 0.05$) decrease in peroxidase production at lower and higher temperatures, suggesting a reduction in metabolic activities of the bacteria (Tandon and Sharma, 2014), which may subsequently, inhibit its growth and enzyme biosynthesis (Ray et al., 2007). This finding concurs with that of Rajkumar et al. (2013), in which optimum peroxidase production by a *Bacillus* sp. was also attained at 30 °C. On the contrary, Rao and Kavya (2014) reported optimum peroxidase production by *Bacillus subtilis* at 37 °C. Moreover, optimum peroxidase production by other bacterial species at 37 °C and 40 °C had also been reported (Nour El-Dein et al., 2014; Musengi et al., 2014), indicating that incubation temperature for optimum peroxidase production may be strain-dependent (Gautam et al., 2011).

Agitation is an additional critical factor that influences microbial growth and secretion of extracellular enzymes, as it is accountable for aeration and even distribution of nutrients during fermentation (Satyanarayana and Adhikari, 2006). To improve peroxidase production by the test bacteria, it is therefore important to determine the agitation rate that is most suitable for optimum peroxidase production by the bacteria. The agitation speed that best support peroxidase production by *Bacillus* sp. FALADE-1 is shown in Figure 3. The results revealed that peroxidase production by the studied bacteria across all agitation speeds significantly ($P < 0.05$) differs from the enzyme production under static condition, with the optimum production detected at 150 rpm (3.04 U mL^{-1}). In other words, production of peroxidase by *Bacillus* sp. FALADE-1 was more favourable at a high agitation rate. In this case, optimum peroxidase production may be attributed to increased aeration of the cultivation medium, consequently, supplying adequate dissolved oxygen in the medium (Kumar and Takagi, 1999; Sepahy and Jabalameli, 2011) as well as increased nutrient uptake by the bacteria (Sepahy and Jabalameli, 2011; Beg et al., 2003). The decrease observed in peroxidase production at agitation rate higher than 150 rpm, may be due to foaming and shearing effects (Falade et al., 2019c) as high agitation may result in cell damage, consequently reducing the number of peroxidase producers. This finding is in agreement with our recent study (Falade et al., 2019b), where optimum exoperoxidase activity by *Raoultella ornithinolytica* OKOH-1 was observed at 150 rpm. In contrast, Patil (2014) reported 180 rpm as the optimal for peroxidase secretion by a *Bacillus* species. Moreover, Falade et al. (2019c) observed 100 rpm as the optimal agitation speed for peroxidase production by *Ensifer adhaerens* NWODO-2.

Some lignin monomers have been implicated in the induction of peroxidase production by bacteria (Falade et al. 2019b; c). In this study, the fermentation medium was augmented with 1 mmol L^{-1} of the various lignin monomers (guaiacol-GA, veratryl alcohol-VALC, vanillin-VAL, vanillic acid-VA and ferulic acid-FA) and the results are shown in Figure 4. The findings revealed a significant difference ($P < 0.05$) in peroxidase production by the bacteria when cultivated in the production medium modified with the varied lignin monomers as compared with the non-modified medium (LGO), which was used as the control. The

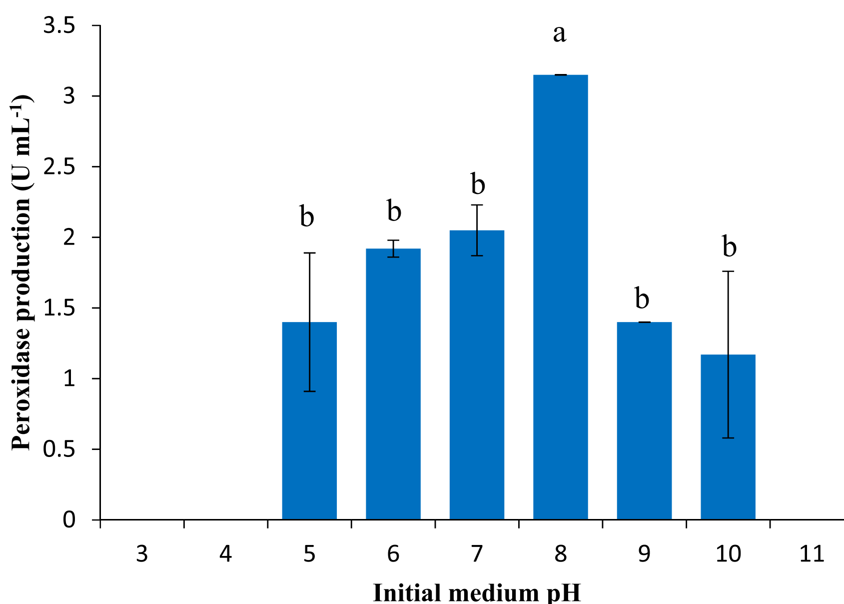

Figure 1. Determination of initial pH for optimum peroxidase production by *Bacillus* sp. FALADE-1. Each column represent mean ± standard deviation. Error bars with the same alphabet are not significantly different ($P > 0.05$).

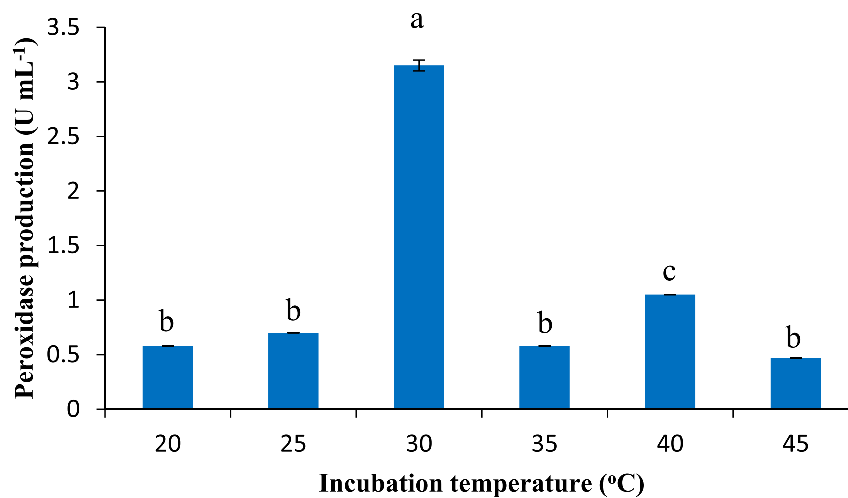

Figure 2. Determination of incubation temperature for optimum peroxidase production by *Bacillus* sp. FALADE-1. Each column represent mean ± standard deviation. Error bars with the same alphabet are not significantly different ($P > 0.05$).

addition of the different lignin model compounds except guaiacol, which increased peroxidase production by over 100 % in *Bacillus* sp. FALADE-1 (LGO: 3.15 U mL^{-1}, LG + GA: 6.42 U mL^{-1}), repressed peroxidase activity in the studied bacteria. This result is comparable to a recent study (Falade et al., 2019c), in which guaiacol supported maximum peroxidase secretion by *E. adhaerens* while vanillin, vanillic and ferulic acids repressed peroxidase production in the study. This is also corroborated by Falade et al. (2019b) who reported guaiacol as the best inducer of peroxidase in *R. ornithinolytica* OKOH-1. However, Musengi et al. (2014) reported effective induction of peroxidase production by 0.1 mmol L^{-1} veratryl alcohol in *Streptomyces* sp. BSII#1 but at higher concentration, veratryl alcohol repressed peroxidase production in the bacteria (Musengi et al., 2014).

The effects of nitrogen on the secretion of ligninolytic enzymes have been widely studied (Kachlishvili et al., 2005; Mikiashvili et al., 2006; Stajić et al., 2006; Falade et al., 2019b, c). However, only limited studies are available on the impacts of nature and concentration of nitrogen sources on production of peroxidase. More so, the results from these studies are not generally consistent (Niladevi and Prema, 2008). In some cases, cultivation medium with adequate nitrogen enhanced ligninolytic enzyme production (Kaal et al., 1995) while in others, production of lignin modifying enzymes was improved in nitrogen-limited conditions (Mester and Field, 1997; Gianfreda et al., 1999; Galhaup et al., 2002). Moreover, increase in the concentration of nitrogen may also limit the production of ligninolytic enzymes (Buswell, 1992). In this present study, we assessed the impact of supplementing the low organic nitrogen source, yeast extract (0.1 g L^{-1}) in the fermentation medium with different inorganic nitrogen sources (5 g L^{-1}) including ammonium nitrate (AN), ammonium chloride (AC) and ammonium sulphate (AS). The results, as presented in Figure 5 showed that the bacteria exhibited a significant difference ($P < 0.05$) in peroxidase production when cultivated in the fermentation medium augmented with inorganic nitrogen as compared with the control (fermentation medium without the supplements). Peroxidase production by *Bacillus* sp. FALADE-1 was enhanced by the addition of only ammonium sulphate (8.87 U mL^{-1}) while the other inorganic nitrogen sources studied seemed to repress peroxidase production by the organism (Figure 5). This finding agrees with that of Falade et al. (2019c), where modification of fermentation medium with

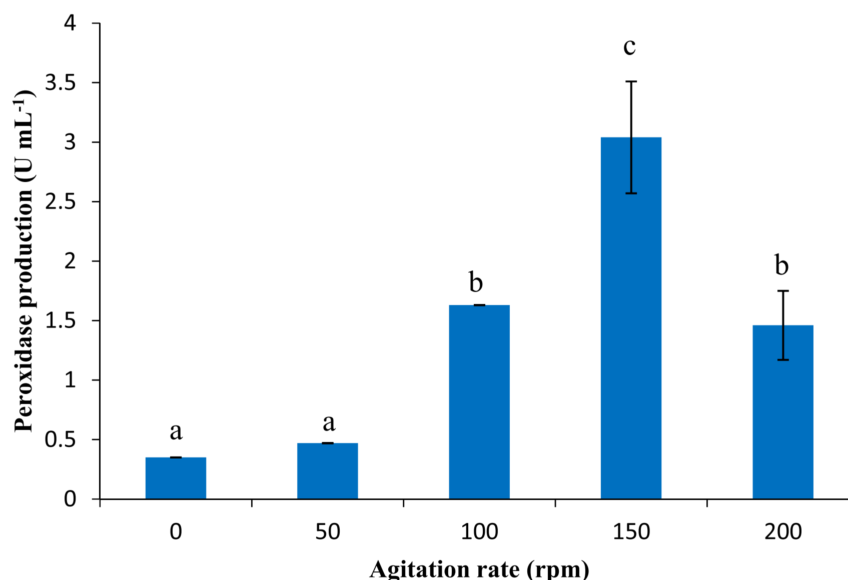

Figure 3. Determination of agitation rate for optimum peroxidase production by *Bacillus* sp. FALADE-1. Each column represent mean ± standard deviation. Error bars with the same alphabet are not significantly different ($P > 0.05$).

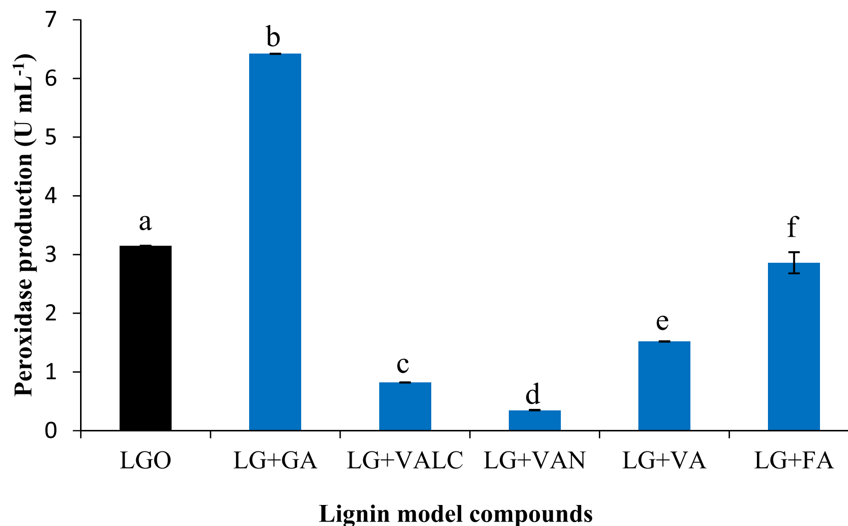

Figure 4. Effect of lignin model compounds on peroxidase production by *Bacillus* sp. FALADE-1. Each column represent mean ± standard deviation. LGO: lignin only (control), LG + GA: lignin and guaiacol, LG + VALC: lignin and veratryl alcohol, LG + VAN: lignin and vanillin, LG + VA: lignin and vanillic acid, LG + FA: lignin and ferulic acid. Error bars with different alphabets are significantly different ($P < 0.05$).

ammonium sulphate gave maximum peroxidase production by *E. adhaerens* NWODO-2. However, ammonium chloride was observed as the best nitrogen supplement for *R. ornithinolytica* OKOH-1 (Falade et al., 2019b).

3.2. Kinetics of enzyme production and bacterial growth

The kinetics of peroxidase production by *Bacillus* sp. FALADE-1 and its growth pattern were assessed in a 120 h incubation period. The results, as shown in Figure 6, revealed that *Bacillus* sp. FALADE-1 attained its optimum peroxidase production at 48 h (late logarithmic growth phase), with specific peroxidase activity of 8.32 U mg^{-1} protein. This indicates that production of peroxidase by the bacteria was growth associated. The decrease observed in the level of peroxidase produced by the bacteria after the optimal period of incubation may be as a result of denaturation or proteolysis (Fatokun et al., 2017). This finding contradicts a number of similar studies where the maximum peroxidase was produced at 72 h of incubation (Nour El-Dein et al., 2014; Falade et al., 2019b). However, this finding agrees with our recent study (Falade et al., 2019c) in which optimum exoperoxidase production by *E. adhaerens* NWODO-2 was observed at 48 h. It is worthy of note that *Bacillus* sp. FALADE-1 exhibited a higher optimum peroxidase production than most of the previously reported peroxidase-producing bacteria (Rob et al., 1997; Tuncer et al., 2009; Nour El-Dein et al., 2014; Musengi et al., 2014). This discrepancy has been attributed to bacterial growth, fermentation medium and inoculum size (Falade et al., 2019c). Attainment of maximum peroxidase production at a short incubation time (48 h) by *Bacillus* sp. FALADE-1 is quite remarkable as this augurs well for biotechnological applications.

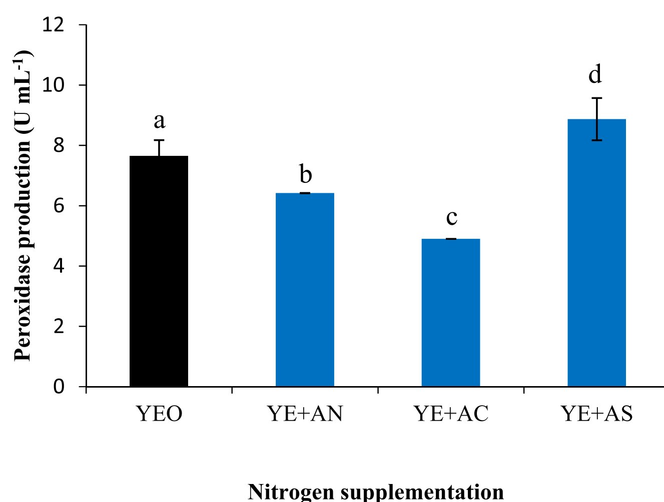

Figure 5. Effect of nitrogen supplementation on peroxidase production by *Bacillus* sp. FALADE-1. Each column represent mean ± standard deviation. YEO: yeast extract only (control), YE + AN: yeast extract and ammonium nitrate, YE + AC: yeast extract and ammonium chloride, YE + AS: yeast extract and ammonium sulphate. Error bars with different alphabets are significantly different ($P < 0.05$).

3.3. Detection of *Sporotrichum thermophile*-like catalase-peroxidase gene (*katG*)

The search result of the nucleotide sequence of the amplified gene from *Bacillus* sp. FALADE-1 showed 100 % similarity to a catalase-peroxidase gene from *Sporotrichum thermophile* (PeroxiBase ID: 10141) when searched in Peroxidase database (peroxibase.toulouse.inra.fr). More so, the deduced protein sequence formed a distinct cluster with bacterial catalase-peroxidases in the PeroxiBase (Figure 7) and had a molecular weight of approximately 11.45 kDa with isoelectric point of about 7.01. The nucleotide sequence was accessioned MF407314 and deposited in the NCBI gene repository. The similarity of *Bacillus* sp. FALADE-1 peroxidase gene to a fungal catalase-peroxidase gene (*katG*) may be as a result of horizontal gene transfer or evolution. This is corroborated by Passardi et al. (2007), in which the evolution of catalase-peroxidase genes in the PeroxiBase database was analyzed and observed that, occasionally, bacterial species that are closely related differ as they possess catalase-peroxidase genes of diverse origin or do not have any *katG*. Moreover, *katG* in eukaryotes (algae and fungi) have been suspected to originate from horizontal gene transfer of bacteria genome (Passardi et al., 2007; Zamocky et al., 2007).

Catalase-peroxidase (KatG) belongs to class I peroxidases of the peroxidase-catalase superfamily of heme-peroxidases (Zamocky and Obinger, 2010), also known as the superfamily of plant, bacterial and fungal heme-peroxidases (EC 1.11.1.7). The corresponding gene (*katG*) encodes a bifunctional enzyme with predominant catalase activity and significant peroxidase activity (Zamocky et al., 2008) in an organism. Thus, the peroxidase activity expressed by *Bacillus* sp. FALADE-1 may be

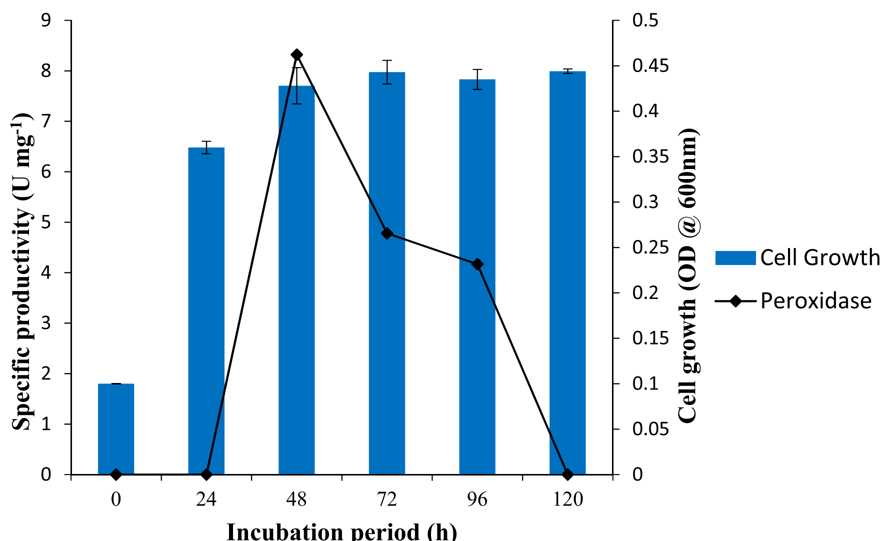

Figure 6. Growth pattern and kinetics of peroxidase production by *Bacillus* sp. FALADE-1.

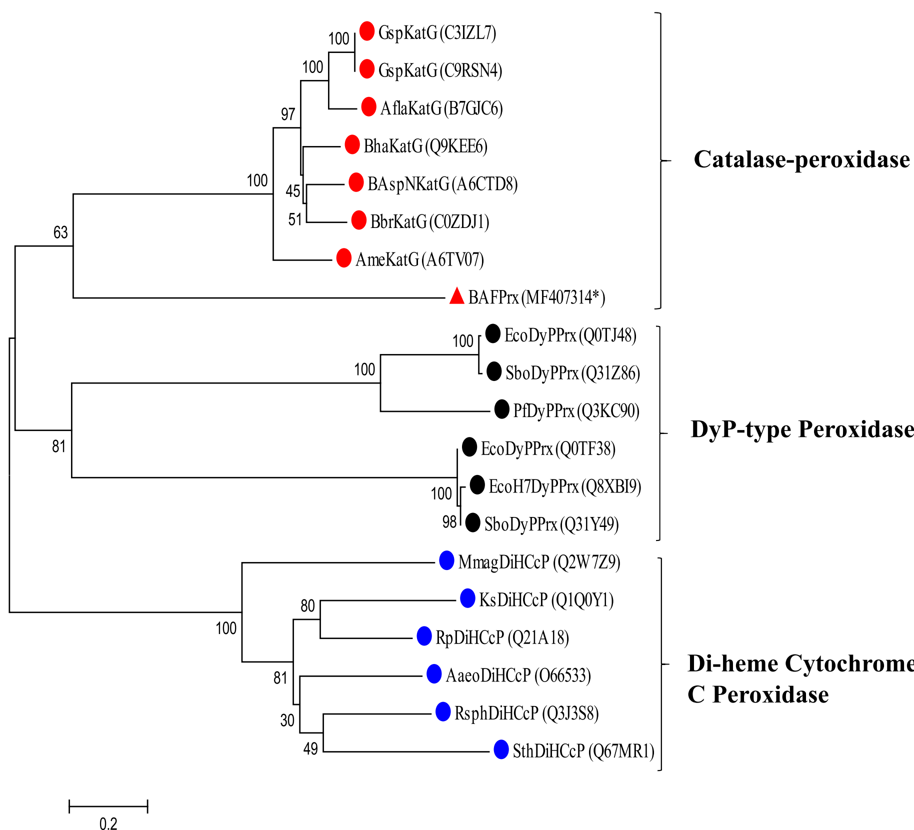

Figure 7. Phylogenetic tree showing the family of *Bacillus* sp. FALADE-1 peroxidase in the bacterial heme-peroxidases from PeroxiBase. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Red tips indicate Catalase-peroxidase, black tips indicate DyP-type Peroxidase while the blue tips represent Di-heme Cytochrome C Peroxidase (DiHCcP). The red triangular tip indicates the studied protein sequence (BAFPrx). The UniProtKB reference numbers/GenBank accession number* of the proteins are indicated in parentheses. Afla: *Anoxybacillus flavithermus*, Ame: *Alkaliphilus metalliredigenes*, BAspN: *Bacillus* sp. NRRL B-14911, Bbr: *Brevibacillus brevis*, Bha: *Bacillus halodurans*, Gsp: *Geobacillus* sp., Aaeo: *Aquifex aeolicus*, Ks: *Kuenenia stuttgartiensis*, Mmag: *Magnetospirillum magneticum*, Rp: *Rhodopseudomonas palustris*, Rsph: *Rhodobacter sphaeroides*, Sth: *Symbiobacterium thermophilum*, Eco: *Escherichia coli*, Pf: *Pseudomonas fluorescens*, Sbo: *Shigella boydii*, EcoH7: *Escherichia coli* 0157:H7, BAFPrx: *Bacillus* sp. FALADE-1 Peroxidase, KatG: Catalase-peroxide, DyPPrx: Dye Decolourizing Peroxidase.

due to *katG* identified in the bacteria. Hence, overexpression of the gene could be explored for enhanced peroxidase and catalase production through molecular optimization and genetic engineering, a process that is problematic in fungi (Bugg et al., 2011).

4. Conclusion

Bacillus sp. FALADE-1 exhibited optimum peroxidase production at an alkaline pH, mesophilic temperature and relatively high agitation speed. The bacteria showed excellent potential for improved peroxidase production as it had a higher optimum specific peroxidase activity (8.32 U mg^{-1}) than some previously reported producers. Also, the ability of the bacteria to attain optimum peroxidase production at a short incubation time (48 h) augurs well for biotechnological application. These indicate the dexterity of the bacteria for large scale peroxidase production. Nonetheless, identification of *katG* in *Bacillus* sp. FALADE-1 prompts the need for further study in gene expression in order to ascertain the involvement of the detected gene in peroxidase production by the bacteria.

Declarations

Author contribution statement

Ayodeji O. Falade: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Leonard V. Mabinya, Anthony I. Okoh, Uchechukwu U. Nwodo: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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