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Peroxidase production and ligninolytic potentials of fresh water bacteria *Raoultella ornithinolytica* and *Ensifer adhaerens*

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ABSTRACT

Interest in novel ligninolytic bacteria has remained topical due to, in part, the maneuverability of the bacterial genome. Conversely, the fungal genome lacks the dexterity for similar maneuverability thus, posing challenges in the fungal enzyme yield optimization process. Some impact of this situation includes the inability to commercialize the bio-catalytic process of lignin degradation by fungi. Consequently, this study assessed some fresh water bacteria isolates for ligninolytic and peroxidase properties through the utilization and degradation of model lignin compounds (guaiacol and veratryl alcohol) and the decolourization of selected ligninolytic indicator dyes; Azure B (AZB), Remazol Brilliant Blue R (RBBR) and Congo Red (CR). Bacterial strains with appreciable ligninolytic and peroxidase production potentials were identified through 16S rDNA sequence analysis and the nucleotide sequences deposited in the GenBank. About 5 isolates were positive for the degradation of both guaiacol (GA) and veratryl alcohol (VA) thus, accounting for about 17% of the test isolates. Similarly, AZB, RBBR and CR were respectively decolorized by 3, 2 and 5 bacterial strains thus, accounting for 10%, 7% and 17% of the test isolates. Two of the test bacterial strains were able to decolourize AZB, RBBR and CR respectively and these bacterial strains were identified as *Raoultella ornithinolytica* OKOH-1 and *Ensifer adhaerens* NWODO-2 with respective accession numbers as KX640917 and KX640918. Upon quantitation of the peroxidase activities; 5250 ± 0.00 U/L was recorded against *Raoultella ornithinolytica* OKOH-1 and 5833 ± 0.00 U/L against *Ensifer adhaerens* NWODO-2. The ligninolytic and dye decolourization properties of *Raoultella ornithinolytica* OKOH-1 and *Ensifer adhaerens* NWODO-2 marks for novelty particularly, as dyes with arene substituents were decolourized. Consequently, the potentials for the industrial applicability of these test bacterial strains abound as there is a dearth of information on organisms with such potentials.

1. Introduction

Lignin; the aromatic, non-carbohydrate, component of lignocellulose is recalcitrant to degradation. Thus, effective degradation of lignin is of prime importance to the industrial sectors utilizing lignocellulose as raw materials for various value-added products [1]. More so, the recalcitrance of lignin to degradation constitutes an undesirable barrier to the efficient and optimum utilization of the abundant lignocellulosic materials. On the same note, the large amount of lignin generated during industrial production of ethanol, pulp and paper making processes, accumulates and, thus, constitutes serious environmental challenge hence, the need for effective and eco-friendly lignin degradation techniques [2].

The biological means of lignin degradation involves microbial or/and microbial enzymes degradative activities. This technique is advocated over the physical and chemical methods which are generally expensive and saddled with lots of other limitations [3,4].

Fungal degradation of lignin, particularly, the white-rot basidiomycetes have been studied extensively [5–7] and, white-rot fungi have been reported as the most effective microbial lignin-degrader. Effectiveness in lignin degradation has been attributed to some extracellular enzymes produced by the white-rot fungi [8]. These extracellular enzymes include laccases (EC 1.10.3.2), some heme-peroxidases such as lignin peroxidase (EC 1.11.1.14), manganese peroxidase (EC 1.11.1.13), versatile peroxidase (EC 1.11.1.16) and dye-decolourizing peroxidase (EC 1.11.1.19). Nonetheless, industrialization of white-rot fungal bio-catalytic/extracellular enzyme process for the de-polymerization of lignin is yet to be achieved. Perhaps, the insufficiency in

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the maneuverability of the white-rot fungal genome for optimum extracellular enzyme yield, as a function of production cost to commercial value quotient may have constituted an important factor impeding industrialization of the process [9,10].

Bacteria, on the other hand, hold very strong potential considering their striking resilience in diverse environments and, as well, their biotechnological significance following, faster growth rate and high dexterity in genome maneuverability [10,11]. Hence, the imperativeness in the exploration of bacteria species for lignin depolymerization potentials. Besides, the evolving significance of bacteria in the degradation of lignin has been severally documented [9]. Bacteria species classed into the actinomycetes, α-proteobacteria and γ-proteobacteria have been reported to possess lignin degrading ability [9,12,13]. Documented ligninolytic bacteria includes *Streptomyces viridosporus* T7A, *Rhodococcus* sp, *Nocardia autotrophica* [14], *Microbacterium* sp, *Brucella melitensis*, *Ochrobactrum* sp, *Sphingomonas* sp [15], *Streptomyces coelicolor, Arthrobacter globiformis, Rhodococcus jostii RHA1, Pseudomonas putida mt-2* [16], *Serratia* sp. JHT01, *Serratia liquefacien* PT01, *Pseudomonas chlororaphis* PT02, *Stenotrophomonas maltophilia* PT03 and *Mesorhizobium* sp. PT04 [11].

Ligninolytic bacteria similarly produce extracellular oxidative enzymes including peroxidases which have been implicated in lignin degradation. Besides the association of these extracellular peroxidases in lignin degradation, they have applications in the removal of phenolic pollutants [17], synthetic dye decolourization [18], and the synthesis of natural aromatic flavours [19,20]. Other applications have likewise included deodourization of manure [21], applications in peroxidase biosensors [22], analysis and diagnostic kits [23] and development of skin lightening agents [24,25]. Given, the diverse applications of peroxidases in different industrial sectors, the exploration of bacteria species with novel ligninolytic abilities and high potentials for peroxidase production is of prime importance. Consequently, the reported study evaluated bacterial isolates from fresh water milieu of the Raymond Mhlaba Municipality, Eastern Cape, South Africa for peroxidase production potentials and ligninolytic activities.

2. Materials and methods

2.1. Chemicals and reagents

Hydrogen peroxide, Pyrogallol, Magnesium sulphate, Manganese II sulphate, Sodium chloride, Ammonium nitrate, Potassium dihydrogen phosphate, Dipotassium hydrogen phosphate, Nystatin, Nalidixic acid, yeast extract, Agar bacteriological, Azure B, Remazol Brilliant Blue R and Congo red were sourced from Merck KGaA, Darmstadt, Germany while Guaiacol, Veratryl alcohol and Kraft lignin were sourced from Sigma-Aldrich, South Africa. Unless stated otherwise, all other chemicals are of analytical grade while the water was glass distilled.

2.2. Sample collection and sampling site

Water samples and sediments (Table 1) were collected from Tyhume river courses in Raymond Mhlaba Municipality, Eastern Cape, South Africa and transported on ice to Applied and Environmental Microbiology Research Group (AEMREG) Laboratory, University of Fort Hare, Alice, South Africa for analysis.

Tyhume River is situated in the Raymond Mhlaba Municipality of the Eastern Cape Province, South Africa. It originates from the Amathole mountains in Hogsback and flows through the lower coastal escarpment down to Alice, a small town with several suburbs. Sample collection was on the courses of Tyhume River, with reduced anthropogenic activities, in Alice.

2.3. Isolation and preliminary screening of ligninolytic bacteria

Ligninolytic bacteria were isolated by enrichment method as described by Sasikumar et al. [26] with minor modifications. Briefly, a phosphate buffered (pH 7) minimal salts medium (MSM) containing 4.55 g/L K_2HPO_4, 0.53 g/L KH_2PO_4, 0.5 g/L $MgSO_4$ and 5 g/L NH_4NO_3 [27] was supplemented with 1 g/L of commercially available kraft lignin (Sigma-Aldrich, South Africa) and 0.1 g/L yeast extract (MSM-L). The components were mixed and the resulting broth autoclaved at 121 °C for 15 min. Enrichment culture was performed in 250 mL Erlenmeyer flask by placing 5 g of sample in 95 mL MSM-L and the culture was incubated in an orbital shaking incubator at 30 °C and 140 rpm for 168 h [26]. Enriched sample of 1 mL was transferred to 9 mL of sterile normal saline and stirred vigorously at room temperature. Using 1 mL of the liquid mixture, serial dilutions was prepared. Thereafter, 100 µL of serially diluted sample was spread on MSM-L agar plate amended with 50 mg/L nystatin (to inhibit the growth of fungi) and the plates were incubated at 30 °C for 168 h until colonies developed. Subsequently, the cultures were purified and presumptive ligninolytic bacteria stored in 20% glycerol at −80 °C for further analysis.

Table 1
Isolated ligninolytic bacteria from fresh water milieu of Raymond Mhlaba Municipality, Eastern Cape, South Africa.

S/N	Isolate code	Isolation source	Guaiacol degradation	Veratryl alcohol degradation
1.	T1CS3[1]	Sediment	−	−
2.	T1CS3[2]	Sediment	−	+
3.	T1CS3[3]	Sediment	−	−
4.	T1CS3[4]	Sediment	−	−
5.	T1B2S3[1]	Sediment	−	+
6.	T1B2S3[2]	Sediment	−	+
7.	T1B2S3[3]	Sediment	−	+
8.	T1B1S3[1]	Sediment	+	+
9.	T1B1S3[2]	Sediment	−	−
10.	T1B1S3[3]	Sediment	−	−
11.	T1B1S3[4]	Sediment	+	+
12.	T1B1S3[5]	Sediment	+	−
13.	T1B1W3[1]	Water	+	+
14.	T1B1W3[2]	Water	−	+
15.	T1B2W3[1]	Water	−	−
16.	T1B2W3[2]	Water	−	−
17.	T1B2W3[3]	Water	−	−
18.	T1CW3[1]	Water	−	−
19.	T1CW3[2]	Water	−	−
20.	T1CW3[3]	Water	+	−
21.	T2BS2[1]	Sediment	−	+
22.	T2BS3[1]	Sediment	−	+
23.	T2BS3[2]	Sediment	−	−
24.	T2BW3[1]	Water	+	+
25.	T2BW3[2]	Water	−	+
26.	T2BW3[3]	Water	−	−
27.	T1CS3[B]	Sediment	−	−
28.	T1CS3[C]	Sediment	−	−
29.	T1CS3[D]	Sediment	+	+
30.	T1CS3[A]	Sediment	−	−

2.4. Evaluation of ligninolytic activity

The ligninolytic activities of isolates were assessed through the utilization and degradation of model lignin compounds (guaiacol and veratryl alcohol) using the modified method of Taylor et al. [28]. Briefly, 5 µL of standard inoculum of the bacterial suspension (O.D. 600 nm ≈ 1.0) was aseptically inoculated onto guaiacol and veratryl alcohol plates composed of the following: K_2HPO_4 (4.55 g/L), KH_2PO_4 (0.53 g/L), $MgSO_4$ (0.5 g/L), NH_4NO_3 (5 g/L), yeast extract (0.1 g/L), guaiacol or veratryl alcohol (0.1% v/v), and agar (15 g/L). The plates were incubated at 30 °C for 168 h and subsequently flooded with Gram's iodine solution to determine the zone of degradation.

2.5. Arene subsituent dye decolourization assay

Decolourization of selected dyes; Azure B (AZB), Remazol Brilliant Blue R (RBBR) and Congo Red (CR) is indicative of ligninolytic activity. Consequently, isolates with ligninolytic activity (Section 2.4) were assessed following the methods of Bandounas et al.[29]. In brief, 5 µL of 18 h culture was aseptically inoculated onto dye agar plate composed of: K_2HPO_4 (4.55 g/L), KH_2PO_4 (0.53 g/L), $MgSO_4$ (0.5 g/L), NH_4NO_3 (5 g/L), yeast extract (0.1 g/L), glycerol (40 mM), dye (100 mg/L AZB and RBBR; 50 mg/L CR), and agar (15 g/L). The plates were then incubated at 30 °C for 168 h and examined daily for growth and development of decolourization zones.

2.6. Qualitative determination of peroxidase activity

The peroxidase activity was determined qualitatively using the method proposed by Rayner and Boddy [30] as reported by López et al. [31]. Briefly, isolates were inoculated in nutrient agar and incubated at 30 °C for 48 h. Thereafter, 30 µL of 0.4% (v/v) hydrogen peroxide (H_2O_2) and 1% pyrogallol in water were added to colonies. Colonies with yellow-brown colour were recorded as positive.

2.7. Peroxidase production and crude enzyme preparation

Peroxidase was produced in a submerged fermentation; about 2% standard inoculum of bacterial suspension in normal saline (O.D. 600 nm ≈ 1.0) was aseptically inoculated in 250 mL Erlenmeyer flasks containing 100 mL of the fermentation media composed of the following: K_2HPO_4 (4.55 g/L), KH_2PO_4 (0.53 g/L), $MgSO_4$ (0.5 g/L), NH_4NO_3 (5 g/L), yeast extract (0.1 g/L) and 0.1% w/v kraft lignin (Sigma-Aldrich, South Africa) at pH 7. The culture was subsequently incubated in an orbital shaking incubator at 30 °C and 140 rpm for 48 h, the period initially used for the qualitative determination of peroxidase activity. Thereafter, cultures were aseptically withdrawn and centrifuged at 15000 rpm for 10 min at 4 °C using benchtop centrifuge (SIGMA 1–14 K) to remove the cells. Recovered supernatant was subsequently utilized as crude enzyme for peroxidase assays.

2.8. Peroxidase activity assay

Peroxidase activity was measured by the rate of hydrogen peroxide-dependent oxidation of pyrogallol to purpurogallin as described by Chance and Maehly [32] with slight modification. Reaction mixture (350 µL) contained 5% w/v pyrogallol in 100 mM potassium phosphate buffer (pH 6) and 25 µL of culture supernatant. The reaction mixture without the crude enzyme served as the blank. The reaction was activated through the addition of 0.5% v/v hydrogen peroxide (30% w/w) and the linear increase in absorbance at 420 nm was monitored per 34 s at 25 °C using SynergyMx 96-well microtitre plate reader (BioTeK Instruments). The peroxidase activity was subsequently calculated.

2.9. Bacterial identification using 16S rDNA sequence analysis

Bacterial isolates with the best ligninolytic and peroxidase production potentials were characterized by 16S rDNA sequence analysis. Briefly, bacterial DNA was extracted using the ZR Fungal/Bacterial DNA Kit™ (Zymo Research). Thereafter the 16S target region was amplified using DreamTaq™ DNA polymerase (Thermo Scientific™) and the following universal primers: 16S-27F (5′ AGAGTTTGATCCTGGCTCAG 3′) and 16S-1492R (5′ CGGTTACCTTGTTACGACTT 3′). Subsequently, the polymerase chain reaction (PCR) products were gel extracted using Zymoclean™ Gel DNA Recovery Kit (Zymo Research), and sequenced in the forward and reverse directions on the ABI PRISM™ 3500xl Genetic Analyser. The sequencing products which were further purified by ZR-96 DNA Sequencing Clean-up Kit™ (Zymo Research) were analysed using CLC Main Workbench 7 followed by a BLAST search in National Centre for Biotechnology Information (NCBI) [33]. Subsequently, the phylogenetic tree showing the evolutionary relationships among selected ligninolytic bacteria available at the NCBI database was constructed by neighbour-joining method using Molecular Evolutionary Genetics Analysis software, version 7.0.21 [34]. Similarly, the 16S rRNA gene sequences of the isolates were deposited in the NCBI GenBank as *Raoultella ornithinolytica* strain OKOH-1 (accession number KX640917) and E*nsifer adhaerens* strain NWODO-2 (accession number KX640918) respectively.

2.10. Data analysis

Results of replicates were pooled and expressed as mean ± standard deviation (STD) using Microsoft Excel Spreadsheet. Data were subsequently subjected to one-way analysis of variance (ANOVA) using GraphPad Prism 7 and the least significant difference was carried out [35]. Significance was accepted at $P \leq 0.05$.

3. Results and discussion

3.1. Ligninolytic bacteria isolation and identification

A total of thirty (30) potential ligninolytic bacteria were isolated from the samples collected from the Tyhume River (Table 1) and, the 16S rDNA sequence analysis of the two bacteria strains with the best ligninolytic and peroxidase production potentials revealed T1CS3^D as having 99% similarity to *Raoultella ornithinolytica* strain G.W-CD.10 (KP418804) while T2BW3^1 had 99% similarity to *Ensifer adhaerens* strain S4-6 (KY496256). The respective nucleotide sequences of the organisms were deposited in a GenBank as *Raoultella ornithinolytica* OKOH-1 (accession number KX640917) and *Ensifer adhaerens* NWODO-2 (accession number KX640918).

These ligninolytic bacteria are classified into the alpha-proteobacteria (*Ensifer adhaerens* NWODO-2) and gamma-proteobacteria (*Raoultella ornithinolytica* OKOH-1) respectively. This finding is consistent with earlier classification of ligninolytic bacteria into Actinomycetes, α-Proteobacteria and γ-Proteobacteria [9]. However, the ligninolytic potential of some *Bacillus* sp. has also been reported [29,36]. Some of the reported Proteobacteria with ligninolytic activity include but not limited to, *Sphingobium* sp. SYK-6 [37], *Pseudomonas putida* mt-2, *Acinetobacter* sp. [16] and *Raoultella ornithinolytica* S12 [38]. Genome sequencing analysis of *Raoultella ornithinolytica* strain S12 (CP010557) isolated in China has revealed many genes involved in aromatic compound degradation and other pathways implicated in lignin degradation mechanism [38,39]. This further confirms the lignin degradation potential of *Raoultella ornithinolytica* OKOH-1 as claimed in this study. Furthermore, Fig. 1 showed the phylogenetic relationships between the ligninolytic bacteria in this study and some of those previously reported. The ligninolytic bacteria in this study (indicated with green tips) are, perhaps, more closely related.

3.2. Ligninolytic activities

The utilization of lignin monomers; guaiacol (2-methoxyphenol) and veratryl alcohol (3, 4-Dimethoxybenzyl alcohol), was indicative of lignin utilization and degradation potentials of the isolates. Guaiacol and veratryl alcohol utilization serves as ligninolysis indicator and as well, lignin oxidation [29]. Only 17% (5 isolates) of the test isolates were able to degrade both guaiacol (phenolic substrate) and veratryl alcohol (non-phenolic substrate). However, all the test isolates grew either on guaiacol or on veratryl alcohol. Isolates substrates utilization intensity was determined by the zone of degradation (Table 2) which became visual after flooding with Grams' iodine. The reaction of hydrogen iodide (HI) with the substrates in the presence of oxygen resulted in a brown coloration of the un-degraded part of the medium while the degraded part was revealed as a clear halo zone around the

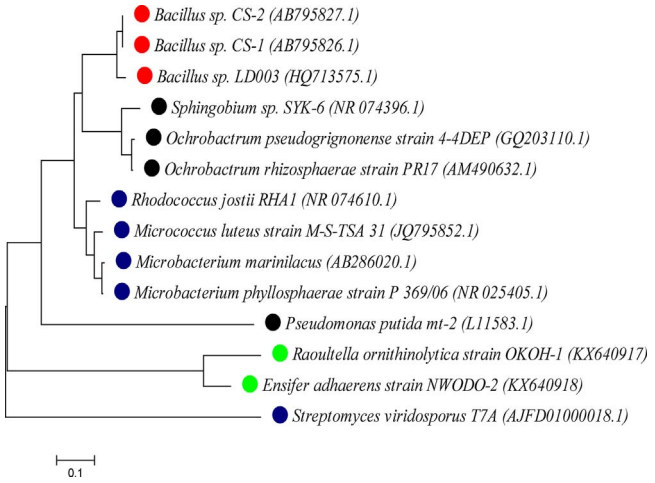


Fig. 1. Phylogenetic tree showing the relationship between ligninolytic bacteria in this study and some previously reported ligninolytic bacteria in the NCBI database. The tips shown in green represent the ligninolytic bacteria isolated and sequenced in this study while the tips with other colours (red, black and blue) indicate the previously reported ligninolytic bacteria. Red tips indicate Bacilli; blue tips represent Actinobacteria while the green and black tips indicate Proteobacteria.

Table 2
Degradation of guaiacol and veratryl alcohol by bacterial isolates.

S/N	Isolate code	Diameter of halo zone for GA (mm)	Diameter of halo zone for VA (mm)
1.	$T1B1S3^{1}$	26.00 ± 2.00^{a}	27.00 ± 1.00^{a}
2.	$T1B1S3^{4}$	27.00 ± 1.00^{a}	30.00 ± 0.00^{b}
3.	$T1B1W3^{1}$	25.00 ± 3.00^{a}	25.00 ± 1.00^{c}
4.	$T1CS3^{D}$	28.00 ± 0.00^{a}	31.00 ± 1.00^{d}
5.	$T2BW3^{1}$	32.00 ± 0.00^{b}	34.00 ± 0.00^{e}

GA:Guaiacol; VA:Veratryl Alcohol. Values represent mean ± standard deviation, number of replicate, n = 3. Values with the same superscript letter along the same column are not significantly different ($P > 0.05$).

colony. Isolate $T2BW3^{1}$ showed the highest halo zone on both substrates (32 mm against guaiacol and 34 mm against veratryl alcohol) while isolate $T1B1W3^{1}$ had the least (25 mm). However, about 80% of the positive isolates showed halo zones of over 25 mm.

3.3. Decolourization of dyes with different arene substituents

The structural complexity of dyes is somewhat similar to those of lignin and the recalcitrance of dyes to degradation has been variously documented [29]. The enzymatic decomposition of the phenolic compounds in lignin leads to effective degradation and this is only possible due to the hydrophilic attack at the arene substituents [40,41]. Consequently, application of such enzyme system in the decolourization of dye would only be effective if the arene substituents of the dye are susceptible to hydrolyzation [42].

Isolates showing ligninolytic activity on guaiacol and veratryl alcohol were evaluated for dye decolourization using Azure B (AZB), Remazol Brilliant Blue R (RBBR) and Congo Red (CR). Azure B, a thiazine dye, can only be decolourized by high redox potential agents, particularly lignin peroxidases [43–45]. On the other hand, manganese peroxidase and laccase alone cannot oxidize Azure B [43,46]. The inclusion of dyes with *ortho* and *para* arene (phenolic and non-phenolic) substituents (Fig. 2), was motivated by the quest to ascertain the broad spectrum of activity and specificity of the oxidative enzyme systems produced by these organisms. Congo red has two azo groups (−N═N−) which impacts the chromophore properties shown by the dye and, the azo groups are attached at the *ortho* position. Conversely, Remazol Brilliant Blue R is an anthraquinone dye with a *para* position arene substituent and this dye is recalcitrant to degradation. The carbonyl group (C═O), which constitutes the structural backbone of the dye, has been shown to impact the chromophore properties of the dye. The arene substituents position on the aromatic rings is the factor impacting degradation recalcitrant to the dyes. As such, the effective cleavage of the arene substituents at the *ortho, meta* and *para* positions marks for novelty. Thus, the natures of the enzymes produced by the organisms are, perhaps, novel and the kinetics as well as the properties shall be further investigated.

Dye decolourization (Table 3) showed 10% (3 bacteria strains) of the isolates as positive against AZB, 7% (2) against RBBR and 17% (5) against CR. Quite remarkable was isolates $T1CS3^{D}$ and $T2BW3^{1}$ which showed competence in the decolourization of dyes with the representative *ortho, meta* and *para* positions arene substituent. Perhaps, the extracellular oxidative enzymes produced by these organisms are novel or, are, known enzymes with a blend of properties including peroxidases and laccases. Nonetheless, extracellular oxidative enzyme decolourization of Azure B has been associated with lignin peroxidase [47], while decolourization of azo and anthraquinone dyes are linked with the activity of DyP-type peroxidases [48,49].

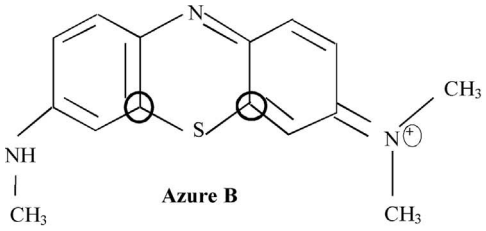
3.4. Peroxidase activity

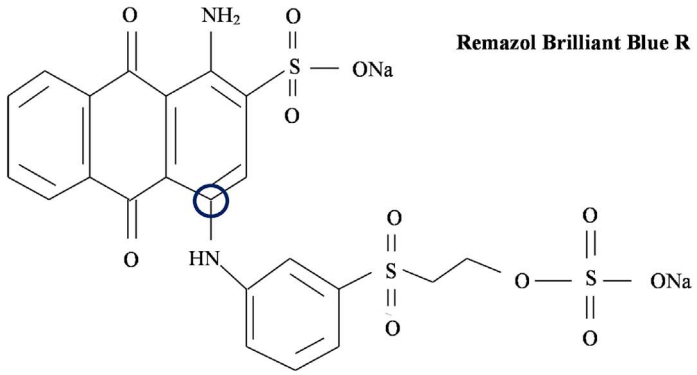
These isolates; $T1CS3^{D}$ and $T2BW3^{1}$, which respectively showed activity against representative dyes with *ortho*, *meta* and *para* substituents, similarly holds high potentials as peroxidase producers. These ligninolytic bacteria strains were qualitatively positive for peroxidase production (Fig. 3), as was reflected in the appearance of yellowish-brown colouration of the bacterial colony after interaction with 0.4% v/v hydrogen peroxide (H_2O_2) and 1% w/v pyrogallol [31].

Upon quantitation of peroxidase production (Table 4), $T1CS3^{D}$ showed activity of 5250 U/L and $T2BW3^{1}$ showed 5833 U/L activity at 48 h incubation timeline. Some related investigation reported similar result however, the peroxidase activity achieved with $T1CS3^{D}$ and $T2BW3^{1}$ were significantly higher than what has been reported in previous studies; *Streptomyces* strain EC22 had an extracellular peroxidase activity of 270 U/L [50] and *Streptomyces* sp. F6616 showed peroxidase activity of 535 U/L [51]. The reason for the marked difference in the peroxidase activity observed with these isolates in comparison with documented report is unclear however; it is a motivation for further investigation. Perhaps, it would be pertinent to note that the peroxidase activity shown by $T1CS3^{D}$ and $T2BW3^{1}$ are consistent with their ligninolytic activities as shown with model lignin compounds degradation (Table 2) and decolourization of dyes with varied arene substituent (Table 3). This, therefore, may be suggestive of the production of lignin modifying enzymes by the test bacterial strains including peroxidases.

4. Conclusions

The bacterial strains; $T1CS3^{D}$ and $T2BW3^{1}$, isolated from the fresh water milieu of the Raymond Mhlaba Municipality, Eastern Cape, South Africa which have shown novel ligninolytic activities were identified *Raoultella ornithinolytica* OKOH-1 and *Ensifer adhaerens* NWODO-2 with KX640917 and KX640918 as respective accession numbers. These Proteobacteria strains produced extracellular enzymes with the capacity to degrade dyes with *ortho*, *meta* and *para* arene substituent and as such, decolourize the model dyes. Consequently, *Raoultella ornithinolytica* OKOH-1 and *Ensifer adhaerens* NWODO-2 hold high potentials for industrial applications, particularly in the dye-wastewater management process. Besides their potential industrial relevance on industrial treatment, they may serve important purpose on the pretreatment of lignocellulosic biomass, a significant step in the bioconversion of lignocellulose to ethanol. Nevertheless, further study on the mechanism(s) of action of these novel bacterial strains for lignin-degradation is imperative as this is significant to their scalability and commercial





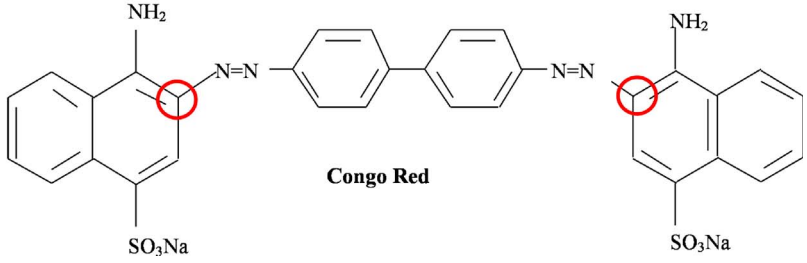


Fig. 2. Structures of dyes used in this study. *Ortho* positions are shown in red circles, *Meta* positions in black circles while *Para* position is indicated in blue circle.

Table 3
Decolourization of dyes with different arene substituent.

S/N	Isolate code	AZB	RBBR	CR
1.	T1B1S3[1]	−	−	+
2.	T1B1S3[4]	−	−	+
3.	T1B1W3[1]	+	−	+
4.	T1CS3[D]	+	+	+
5.	T2BW3[1]	+	+	+

+: positive; −: negative; AZB: Azure B; RBBR: Remazol Brilliant Blue R; CR: Congo Red.

Fig. 3. Qualitative peroxidase activity of ligninolytic bacteria.

Table 4
Evaluation of ligninolytic bacteria for peroxidase production.

S/N	Isolate Code	Peroxidase Activity (U/L)
1.	T1CS3[D]	5250.00 ± 0.00
2.	T2BW3[1]	5833.00 ± 0.00

Values represent mean ± standard deviation, number of replicate, n = 3.

application in the future.

Conflict of interest

Authors declare that there are no conflicts of interest.

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