Isolation and molecular identification of lignin peroxidase-producing bacterial isolates from Jeddah City

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Background
The identification of naturally occurring bacteria with lignin-oxidizing enzymes would be significant. Several species of filamentous bacteria belonging to the genus *Streptomyces* (Actinomycetes) have been identified as degraders of lignin. Such species play the most important role in biodegradation of lignin.
Objective
This study aimed to isolate and discover promising isolates and ideal conditions for lignin peroxidase (LiP) production as well as 16S-rRNA identification of the ligninolytic bacterial strains.
Materials and methods
Lignin was isolated and purified from black wood liquor. The ligninolytic bacterial colonies were isolated from three types of soil farms (F1, F2, and F3) from Jeddah, KSA. Fermentation medium (FM) was used for screening of lignin-degrading bacteria after dilution of the soil sample using lignin (0.1% v/v). The FM medium was supplemented with 50 mg/l of Azure B and toluidine dyes and 100 mg/l of tannic acid. FM was used without any supplements and agar for isolation of lignin-degrading bacteria after dilution of the soil samples. Different concentrations of lignin (0.1–0.9%) were applied to optimize LiP production by the selected strains under different temperatures (30, 35, 40, and 45°C); different pH values (7, 7.5, 8.0, and 8.5); eight different carbon sources (0.1%, w/v), such as glucose, fructose, xylose, lactose, sucrose, carboxymethyl cellulose, and xylan; and four organic sources (0.1%, w/v), such as peptone, meat extract, sodium nitrate, and potassium nitrate. The enzyme productivity was evaluated in the culture supernatant. The bacterial strain genomic DNA was extracted from pure culture isolated from soil and subjected to amplification and sequencing of 16 S ribosomal RNA gene.
Results and discussion
Nine ligninolytic bacterial colonies that excrete peroxidases based on the use of lignin (as sole carbon source) were isolated from three types of soil farms (F1, F2, and F3) from Jeddah, KSA, and the promising isolates and the optimum conditions for LiP production using FM under three incubation periods were evaluated. Two most active isolates for production of LiP belonging to Actinomycetes and Bacilli designated (**R-St-1** and **R-B-1**) were identified using 16S-rRNA. Results showed that the highest LiP producer was ***Streptomyces* R-St-1** isolate (3.8 U/ml) followed by **Bacilli R-B-1** isolate (2.4 U/ml) after 3 days of fermentation. Different concentrations of lignin (0.1–0.9%) were tested for their effect on LiP production by ***Streptomyces* R-St-1**. As lignin concentration increased, LiP production increased, and the maximum productivity of 4.9 $U.mL^{-1}$ was observed at 0.5% lignin after which the LiP production was decreased. At the ideal temperature recorded of 35°C and at the optimum pH of 7.5, the production of LiP rose significantly (4.6 U.mL-1 and 4.0 U.mL-1).Various carbon sources were examined for LiP production, and glucose was shown to be the best option for producing a high yield of LiP by ***Streptomyces* R-St-1**, followed by lactose (4.6 and 4.0 U/ml, respectively). However, neither organic nor inorganic nitrogen sources were shown to be suited for high LiP output. As lignin concentration increased, LiP production increased, and the maximum productivity of 4.4 U/ml from **Bacilli R-B-1** isolate was observed at 0.7% lignin, after which LiP production was decreased. The optimum temperature was 40°C, where LiP production showed a significant increase (4.5 U/ml), whereas the optimum pH was 8.0, and sucrose was found to be more suitable for high yield of LiP production followed by glucose (4.3 and 3.3 U/ml, respectively). The identified bacterial DNA sequences were conserved in the GenBank under two accession numbers **OL697233.1** (*Streptomyces lavendulae* R-St-1) and *Priestia aryabhattai* **R-B-1**(OL697234.1) (formerly known as *Bacillus aryabhattai*).

Keywords:
lignin peroxidase, molecular identification, phylogenetic analysis, *Priestia*, *Streptomyces*

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Introduction

Lignin, along with cellulose, is a key component of plant materials and is by far the most prevalent aromatic compound in the biosphere. It is a plant cell wall component that gives strength and resilience [1] and is tenaciously resistant to degradation owing to its relatively random structure [2]. The development of a process for lignin degradation has significant potential in a variety of chemical production activities, which could replace a variety of petroleum-derived chemicals. As a result, effective lignin degradation is critical for industrial sectors that use lignocellulose as a raw material for a variety of value-added products [3]. Different authors have reported that a number of bacterial species, as well as certain fungi, degrade lignin [4–6]. Bugg *et al.* [5] and Huang *et al.* [6] reported that α-Proteobacteria, Actinomycetes, and γ-Proteobacteria are the three primary classes of lignin-degrading bacteria. Tian *et al.* [7] reported that lignin is decomposed by several species of *Ochrobactrum*, *Brucella*, *Sphingomonas* genera, and *Sphingobium* (class α-Proteobacteria). *Pseudomonas fluorescens* that produce lignin peroxidase (LiP), *Pseudomonas putida* that produce dye-decolorizing peroxidase and manganese peroxidase (MnP), *Escherichia coli* (laccase), and *Enterobacter lignolyticus* that produce dye-decolorizing peroxidase and catalase/peroxidase HPI are among the best γ-Proteobacteria. Abd-Elsalam and El-Hanafy [8] and Bondounas *et al.* [9] reported *Bacillus* sp. as a promising lignin-degrading bacterium. *Nitrobacter* and *Nitrosomonas*, among other nitrifying bacteria, have been found to break down lignin [10]. *Streptomyces viridosporus*, *Streptomyces paucinobilis*, and *Rhodococcus jostii* are actinomycetes that have been shown to produce catabolic enzymes [7]. Fernandes *et al.* [11] reported that several species of filamentous bacteria belonging to the genus *Streptomyces* have been identified as degraders of lignin, although only about ten lignin-degrading enzymes have been identified for them. Several actinomycetes species, such as *Streptomyces coelicolor*, *Streptomyces psammoticus*, and *Streptomyces griseus*, produce three enzymes (laccase, LiP, and MnP), which are thought to play the most important role in biodegradation of lignin [12,13]. Laccase and LiP activities were found in *Streptomyces cinnamomeus* [14]. Several extracellular peroxidases and laccase are produced by the best-studied strain, *S. viridosporus* [5,7]. *S*treptomyces *ipomoea* showed the activity of laccase as well [11,15]. These studies suggested that lignin-degrading bacteria are more essential than previously considered, especially in soil. Therefore, the identification of naturally occurring bacteria with lignin-oxidizing enzymes would be significant [9,16,17]. There is high potential of bacteria that degrading lignin, and the characterization enzymes that degrading lignin may give several potential benefits for textile, pulp and paper industries, bioconversion, food processing, feed, chemical industry, biosensors, and biofuel production from lignocellulosic material.

Materials and methods

Preparations of lignin: lignin was isolated and purified from black wood liquor. Purification was accomplished by adjusting the pH of pulping black fluid with aqueous sulfuric acid to 3 (20%). To encourage lignin precipitation, the suspension was agitated for 2 h at 50°C. The precipitates were collected with a Buchner funnel and rinsed with distilled water multiple times. Finally, the precipitated lignin was collected and dried overnight in an oven at 70°C.

Sources of isolates: the ligninolytic bacterial colonies were isolated from three types of soil farms (F1, F2, and F3) from Jeddah, KSA.

Primary screening of isolates for their ligninolytic potential

A fermentation medium (FM) (g/l) was used for screening of lignin-degrading bacteria after dilution of the soil samples: K_2HPO_4 (4.55 g/l), $MgSO_4$ (0.5 g/l), KH_2PO_4 (0.53 g/l), NH_4NO_3 (0.1 g/l), lignin (0.1% v/v), yeast extract (0.1 g/l), and agar (15 g/l), with pH adjusted to 7.0 [18]. The aforementioned FM was supplemented with 50 mg/l of Azure B and toluidine dyes and 100 mg/l of tannic acid.

Cultural conditions and fermentation

FM without any supplements and agar was used for isolation of lignin-degrading bacteria after dilution of the soil samples. The inoculum medium for actinomycetes strains consisted of 1% glucose, 0.2% yeast extract, 0.2% beef extract, 0.1% $CaCO_3$, and 0.2% polypeptone, with pH adjusted to 7.3 before

autoclaving [19]. The other bacterial strains were also grown aerobically in tryptone–yeast extract (TY) broth [6] for preparing the inoculum used in the fermentation. FM (g/l) was used for LiP production according to Taylor *et al*. [18]. The fermentation was continued for 3 days with 1% (v/v) inoculums medium before determination of LiP.

Cultural optimization of lignin peroxidase production

Substrate concentration: different concentrations (0.1–0.9%) of lignin were applied to study the effect on LiP production by the selected strains.

Cultivation temperature: different degrees of temperatures (30, 35, 40, and 45°C) were tested for their effect on LiP production by selected isolates.

Initial medium pH: growth in FM was carried out at four different pH values (7, 7.5, 8.0, and 8.5), and LiP production was monitored at the end of fermentation.

Carbon sources: growth was carried out using eight carbon sources (0.1%, w/v): glucose, fructose, xylose, lactose, sucrose, carboxymethyl cellulose, and xylan.

Nitrogen sources: organic sources (0.1%, w/v) such as peptone and meat extract and inorganic sources such as sodium nitrate and potassium nitrate were evaluated for their effect on LiP synthesis.

Assay of LiP: the enzyme productivity was evaluated in the culture supernatant collected by centrifugation in an Eppendorf tube under cooling at 5000 rpm for 5 min. The enzyme activity was measured using 0.5 ml of the clear supernatant. According to Tien and Kirk [20], LiP activity was measured at 310 nm, with 1 U representing 1 μmol veratryl alcohol oxidized to veratryl aldehyde per minute at 30°C.

Molecular identification of the bacterial isolates

DNA extraction from bacterial strains

The bacterial strain genomic DNA was extracted from pure culture, after grown overnight on nutrient broth medium using i-genomic BYF DNA extraction Mini Kit (iNtRON Biotechnology Inc., South Korea), and then the quantity and purity of extracted DNA was estimated using a spectrophotometer (Shimadzu model UV-240) at the UV-absorbance at 260 and 280 nm as reported by Sambrook [21].

PCR amplification and sequencing of 16S ribosomal RNA gene

Maxima Hot Start PCR Master Mix (Thermo K1051) was used for 16 S ribosomal gene PCR amplification, and two 16 S primers were used as 27 F primer-5'-AGAGTTTGATCCTGGCTCAG-3' and 1492 R primer-5' TACGGTTACCTTGTTACGACTT-3'. To each PCR vial containing 2 μl of each used primer (10 pmol/μl), 10 μl of 2× PCR Master Mix and 2 μl of the purified DNA sample (40 ng/μl) were added. Then, sterile distilled water was added to complete total volume of the amplification reaction to 20 μl. Then, the amplification protocol was carried out as follows: denaturation at 95°C for 5 min. Each 35 cycles consists of the following segments: denaturation at 95°C for 1 min, primer annealing for 2 min at 52°C, polymerization at 72°C for 2 min, and finally, hold the PCR at 4°C. Then, electrophoresis with 1.0% agarose gel and 1× TBE (Tris-borate-EDTA) buffer was used for 2 h at a constant 100 V. Then, different band sizes were determined using a 100-bp ladder (iNtRON Biotechnology Inc.).

Bacterial DNA purification: after PCR amplification, bands responsible for 16 S RNA genes were purified by MEGAquick-spin Plus Total Fragment DNA Purification Kit; iNtRON Biotechnology Inc..

Isolate's identification by DNA sequencing

DNA from purified PCR product was sequenced using forward primer and ABI 3730xl DNA sequencer (GATC Company, Germany).

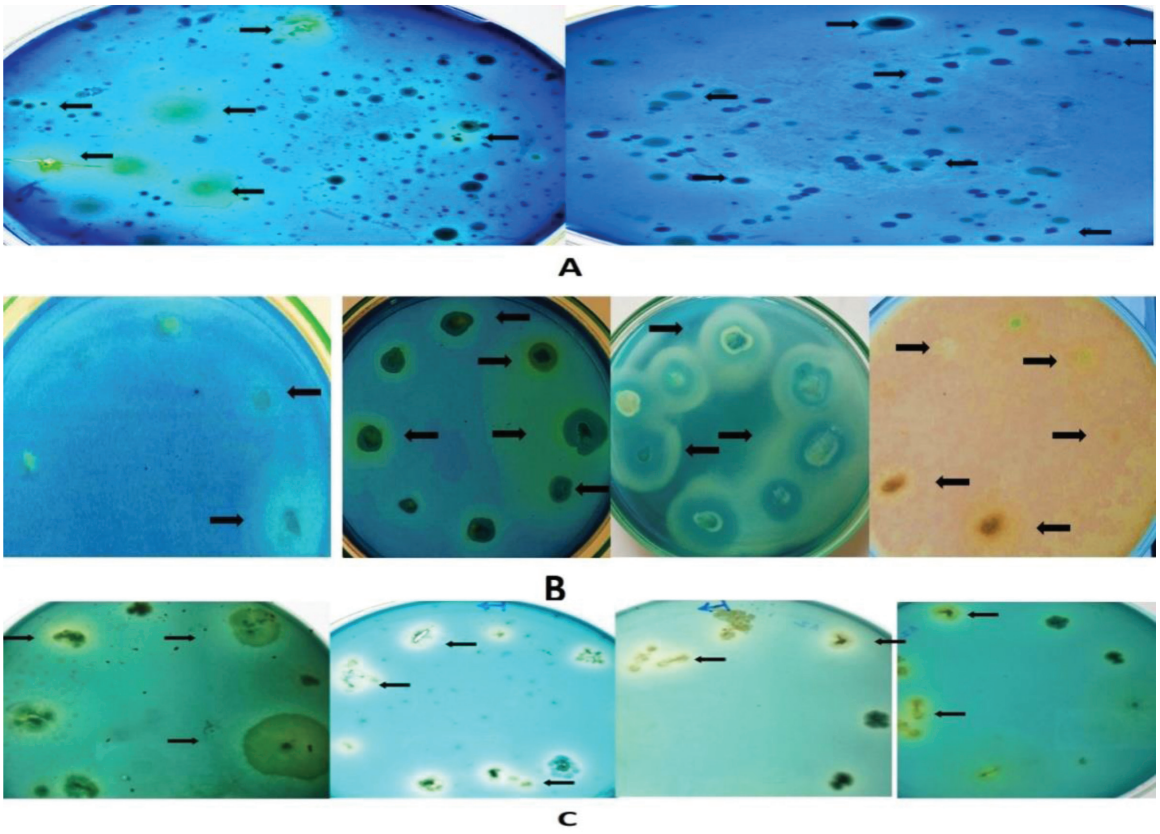
Phylogenetic analysis

The DNA sequences of the bacterial isolates were compared with sequences in the NCBI GenBank database (http://www.ncbi.nlm.nih.gov) using the Basic Local Alignment Search Tool (BLAST). The sequence was compared with those of reference taxa found in public databases, and the evolutionary distance was calculated using NCBI Neighbor Joining.

Results and discussion

The identification of naturally occurring bacteria with lignin-oxidizing enzymes would be significant. Several species of filamentous bacteria belonging to the genus *Streptomyces* (Actinomycetes) have been identified as degraders of lignin, and such species play the most important role in biodegradation of lignin. This study aimed to isolate and discover promising isolates and ideal conditions for LiP production as well as 16S-rRNA identification of the ligninolytic bacterial strains. Lignin was isolated and purified from black wood liquor. The ligninolytic bacterial colonies were isolated from three types of soil farms (F1, F2, and F3) from Jeddah, KSA. FM was used for screening of lignin-degrading bacteria after dilution of the soil sample using lignin (0.1% v/v).

Figure 1



Detection of lignin peroxidase-producing isolates using FM medium, from soil (a) and screening assay (b and c). FM, fermentation medium.

Screening for lignin peroxidase bacteria

Initially, several isolates from soil farms showed clear zones on FM medium supplemented with 50 mg/l of Azure B and toluidine dyes and 100 mg/l of tannic acid. The clear zone of these isolates was evaluated, and the highly clear zone isolates were selected, as shown in Fig. 1, and screened as in Fig. 1b, c. Furthermore, two most active isolates for production of LiP belonging to Actinomycetes and Bacilli species designated as **R-St-1** and **R-B-1**, respectively, were selected. Abd-Elsalam and El-Hanafy [8] and Bondounas *et al.* [9] reported *Bacillus* sp. as a promising lignin-degrading bacterium. Nitrobacter and Nitrosomonas, among other nitrifying bacteria, have been found to break down lignin [10]. *S. viridosporus*, *S. paucinobilis*, and *R. jostii* belonging to Actinomycetes have been shown to produce catabolic enzymes [7]. Several filamentous Streptomyces bacterial species have been found as lignin degraders [11]. Several Actinomycetes species, such as *S. coelicolor*, *S. psammoticus*, and *S. griseus*, produce three enzymes (laccase, LiP, and MnP), which are thought to play the most important role in lignin biodegradation [12,13]. Laccase and LiP activities were found in *S. cinnamomeus* [14]. Several extracellular peroxidases and laccase were produced by the best-studied strain (*S. viridosporus*) [5,7]. Therefore, identification of bacteria from nature with lignin-oxidizing enzymes would be significant [9,16,17].

Evaluation of the collected isolates and fermentation medium

Nine ligninolytic bacterial colonies that excrete peroxidases based on lignin use (as sole carbon source) were isolated from three types of soil farms (F1, F2, and F3) from Jeddah, KSA, and the most promising isolates and the optimum conditions for LiP production using FM under three incubation periods were selected. Two most active isolates for production of LiP belonging to Actinomycetes and Bacilli designated as **R-St-1** and **R-B-1**, respectively, were selected. Results of evaluation of the productivity of two groups of bacteria of LiP showed that the highest LiP producer was ***Streptomyces* R-St-1** isolate (3.8 U/ml) followed by **Bacilli R-B-1** isolate (2.4 U/ml) after 3 days of fermentation. Moreover, ***Streptomyces* R-St-4** and **Bacilli R-B-4** isolate produced 1.9 and 1.5 U/ml of LiP after 3 days of fermentation (Tables 1 and 2). These results are consistent with different studies [7–9]. The use of simultaneous fermentation of LiP

enzyme production is a feasible method as the enzyme production of ligninolytic enzymes is highly regulated by the composition or ingredients of the media [22,23], or more specifically by the levels of nutrients in the FM [24,25]. For maximal LiP synthesis, various bacteria require varied incubation periods. It ranges from 48 h in *Citrobacter* sp. [26] to 12 days in *Streptomyces* sp. [27].

Lignin peroxidase optimization by Streptomyces R-St-1 isolate

Different concentrations of lignin: different concentrations of lignin (0.1–0.9%) were tested for their effect on LiP production by ***Streptomyces* R-St-1** (Table 2). As lignin concentration increased, LiP production increased, and the maximum productivity of 4.9 U/ml was observed at 0.5% lignin after which the LiP production was decreased.

Temperature degrees: Table 3 shows the effect of different incubation temperatures on LiP production. The ideal temperature recorded for *Streptomyces* R-St-1 was 35°C, where the production of LiP rose significantly (4.6 U/ml). CLMT29 strain requires 50°C for enzyme production [28], and the maximal yield of LiP in bacteria was typically at 30°C [29]. According to a complete evaluation of the properties of bacterial LiP, LiP enzyme may function in a wide range of temperatures (25–60°C) [30,31].

Table 1 Lignin peroxidase production of the original isolates cultured in fermentation medium using three different incubation times

Isolates code No.	FM (U/ml)		
	1d	3d	5d
R-St-1	2.5	3.8	3.7
R-St-2	2.3	2.4	2.5
R-St-3	1.3	2.4	2.5
R-St-4	1.4	1.9	1.4
R-St-5	2.1	2.5	2.3
R-B-1	2.0	2.4	2.4
R-B-2	1.8	2.1	2.4
R-B-3	1.4	1.6	1.5
R-B-4	1.0	1.5	1.7

d, days; FM, fermentation medium.

Table 2 Effect of substrate concentration on lignin peroxidase production by R-St-1 isolate

Lignin concentration (%)	Lignin peroxidase (U/ml)
0.1	3.8
0.3	4.5
0.5	4.9
0.7	4.4
0.9	3.2

Different pH values: Table 4 shows the effect of different pH values on LiP production. LiP production showed a significant increase (4.0 U/ml) for *Streptomyces* R-St-1 when pH value was increased to 7.5. On the contrary, previous results have indicated that the LiP activity requires a pH range of 3.0–8.0. [32]. Conversely, LiP from *Thermomonospora* sp., *Leucobacter* sp. SCH3, and *Ochrobactrum* sp. SCH2 showed the highest enzyme activity at pH 2.7 [28,33].

Different carbon substrates: carbon is a critical substrate in the FM for LiP production. Therefore, various carbon sources were tested for LiP production, as shown in Table 5. Various carbon sources were examined for LiP production, and glucose was shown to be the best option for producing a high yield of LiP by ***Streptomyces* R-St-1**, followed by lactose (4.6 and 4.0 U/ml, respectively). According to Zhu *et al.* [25], microbial lignin break down requires an extra carbon source. Tian *et al.* [34] reported an increase in activity of LiP when cellulose was used as a carbon source.

Different nitrogen sources: Neither organic nor inorganic nitrogen sources were shown to be suited for high LiP output (Table 6). In comparison with the

Table 3 Effect of temperature on lignin peroxidase production by R-St-1 isolate

Temperature (°C)	Lignin peroxidase (U/ml)
30	3.8
35	4.6
40	4.2
45	0.5

Table 4 Effect of pH on lignin peroxidase production by R-St-1 isolate

pH	Lignin peroxidase (U/ml)
7	3.8
7.5	4.0
8.0	2.7
8.5	1.4

Table 5 Effect of addition of carbon source on lignin peroxidase production *by Streptomyces* R-St-1 isolate

Carbon source (0.1%, w/v)	Lignin peroxidase (U/ml)
Without	3.8
Glucose	4.6
Fructose	3.1
Xylose	3.0
Sucrose	3.2
Lactose	4.0
Carboxymethyl cellulose	3.2
Xylan	3.1

use of inorganic nitrogen sources such as urea and ammonium tartrate, organic nitrogen sources such as yeast extract and peptone might greatly enhance the synthesis of ligninolytic enzymes [35]. *Streptomyces psammomatous* used yeast extract as a nitrogen source for synthesis of laccase [36].

Lignin peroxidase optimization by Bacilli R-B-1 isolate

Different concentrations of lignin: lignin was used in different concentrations (0.1–0.9%) for studying their effect on LiP production by **Bacilli R-B-1** isolate (Table 7). As lignin concentration increased, LiP production increased, and the maximum productivity of 4.4 U/ml from the **Bacilli R-B-1** isolate was observed at 0.7% lignin after which LiP production was decreased.

Temperature degrees: Table 8 shows the effect of different incubation temperatures on LiP production of the **Bacilli R-B-1** isolate. The optimum temperature was 40°C, where LiP production showed a significant increase (4.5 U/ml). Rajkumar *et al.* [37] found that the optimum temperature was 30°C for peroxidase production in *Bacillus* species and in *Ensifer adhaerens* NWODO-2 [38], and 37°C was found for peroxidase production in *Bacillus subtilis* [39].

Table 6 Effect of nitrogen source on lignin peroxidase by Streptomyces R-St-1 isolate

Nitrogen source (0.1%, w/v)	Lignin peroxidase (U/ml)
Control (FM)	3.8
Peptone	2.7
Meat extract	2.5
Sodium nitrate	2.0
Potassium nitrate	1.7

FM, fermentation medium.

Table 7 Effect of substrate concentration on lignin peroxidase production by R-B-1 isolate

Lignin concentration (%)	Lignin peroxidase (U/ml)
0.1	2.4
0.3	3.7
0.5	4.3
0.7	4.4
0.9	3.1

Table 8 Effect of temperature on lignin peroxidase production by R-B-1 isolate

Temperature (°C)	Lignin peroxidase (U/ml)
30	2.4
35	3.5
40	4.0
45	3.0

Different pH values: results in Table 9 show the effect of different pH values on LiP production in the **Bacilli R-B-1** isolate. LiP production showed a significant increase (4.5 U/ml), when pH value was increased to 8.0. A species of *Raoultella* produces the greatest amount of peroxidase at pH 5 [40]. According to Rao and Kavya [39], pH 6 was the optimum pH level for certain *Bacillus* species to express the maximal peroxidase activity. pH values from 9 and 10 have been found to be ideal for dye decolorization by *Bacillus* sp. [41].

Different carbon substrates: as shown in Table 10, different sources of carbon were tested for LiP production in the **Bacilli R-B-1** isolate. Sucrose was more suitable for high yield of LiP production by the **Bacilli R-B-1** isolate, followed by glucose (4.3 and 3.3 U/ml, respectively). *Bacillus mycoides* produces the greatest amount of peroxidase using cellulose as the carbon source [42]. According to Pham *et al.* [43], glucose is the optimum carbon source for *Bacillus* sp., which reacts to express the maximal peroxidase activity.

Different nitrogen sources: as shown in Table 11, from the tested organic and inorganic nitrogen sources, peptone was found to be more suitable for high yield of LiP production (2.8 U/ml) in the **Bacilli R-B-1** isolate. Several lignocellulolytic enzymes, including peroxidases, are influenced by nitrogen nature and concentration in a variable manner, as it can either be stimulatory or inhibitory [44]. It has been shown that the bacteria grown in a nitrogen-rich medium show improved enzyme synthesis [45]. Some bacteria, however, require a low-nitrogen FM

Table 9 Effect of pH on lignin peroxidase production by R-B-1 isolate

pH	Lignin peroxidase (U/ml)
7	2.4
7.5	3.0
8.0	4.5
8.5	3.6

Table 10 Effect of additional carbon sources on lignin peroxidase production by R-B-1 isolate

Additional carbon sources (0.1%, w/v)	Lignin peroxidase (U/ml)
Without	2.4
Glucose	3.3
Fructose	2.1
Xylose	2.0
Sucrose	4.3
Lactose	3.1
Carboxymethylcellulose	3.0
Xylan	3.2

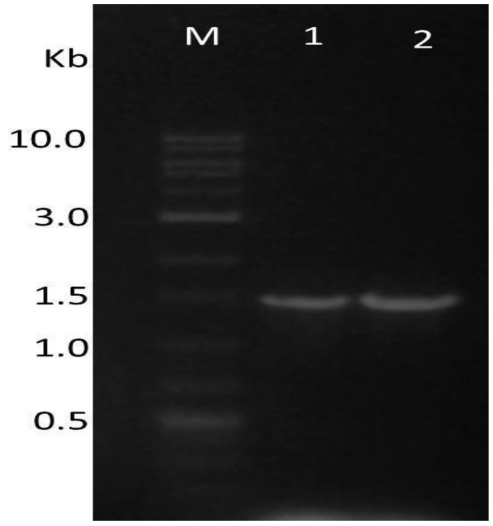
for optimal enzyme synthesis [46].Molecular identification of two isolates (*Streptomyces* R-St-1 and Bacilli R-B-1) based on 16S-rRNA gene

Table 11 Effect of nitrogen sources on lignin peroxidase production by R-B-1 isolate

Nitrogen sources (0.1%, w/v)	Lignin peroxidase (U/ml)
Control (FM)	2.4
Peptone	3.5
Meat extract	2.2
Sodium nitrate	2.6
Potassium nitrate	2.8

FM, fermentation medium.

Figure 2



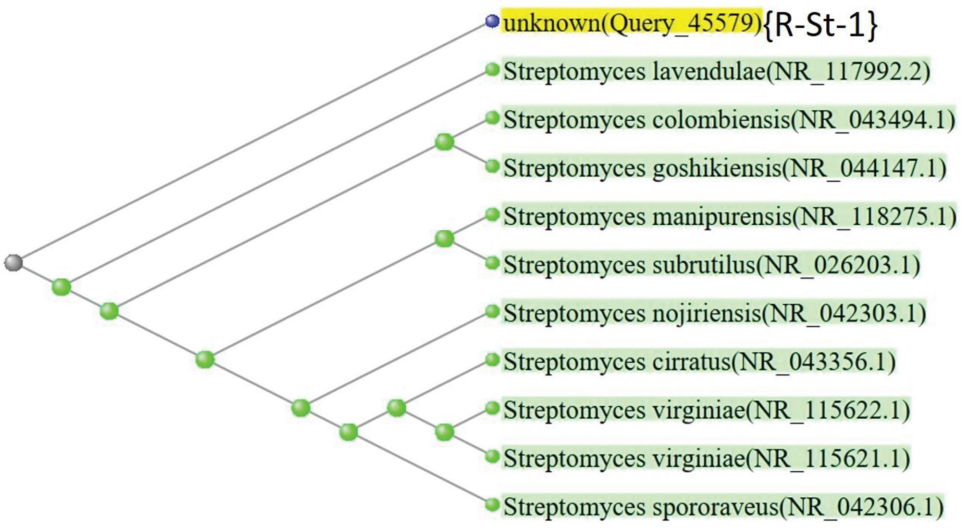
The 16Sr-DNA amplified band for two bacterial isolates (Lanes 1 and 2) against 100-bp DNA ladder marker (iNtRON Biotechnology Inc.), Lane M.

To study16S rRNA gene, the forward and reverse primers were used to amplify the region of the 16S ribosomal (rRNA) gene of the genomic DNA of the two (***Streptomyces* R-St-1** and **Bacilli R-B-1**) isolates, and products of nearly 1500 bp were obtained after the amplification by PCR (Fig. 2). The DNA sequence was submitted in the GenBank under the following accession numbers: *Streptomyces lavendulae* R-St-1 (OL697233.1) and *Priestia aryabhattai* R-B-1 (OL697234.1), as shown in Table 12. An important research topic among aerobic cellulolytic bacteria is whether Streptomyces produces enzymes that can break down lignin, so that they can be employed in different environments. For example, *S. psammoticus* can produce all three key ligninolytic enzymes, LiP, MnP, and laccase [47], as well as laccase in an alkaline environment (pH 7.5–10.0) [36]. S. *lavendulae* produces LiP and laccase [48]. On the contrary, the molecular identification helps to identify many strains of *Bacillus* that produce the enzyme ligninase and are important in the breakdown of lignin [43,49,50].

Table 12 GenBank accession numbers, closest phylogenetic relative, and identity percent of the isolated bacterial isolates (R-St-1 and R-B-1)

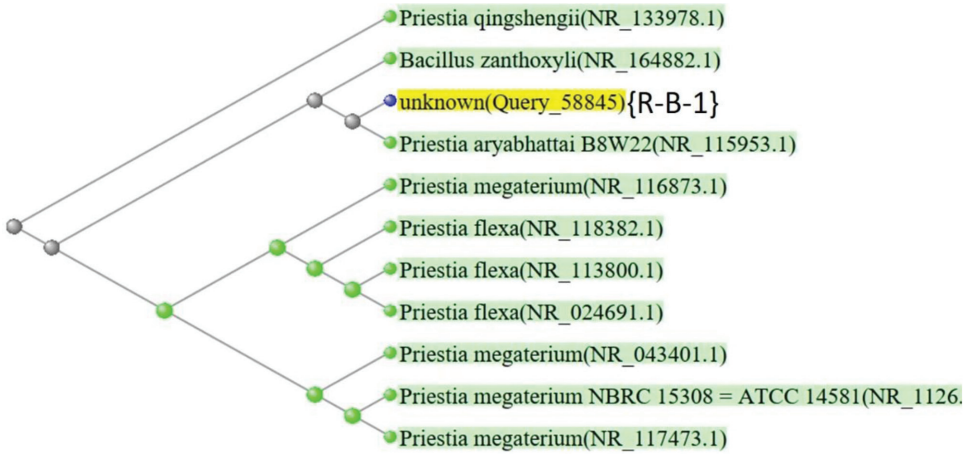
Isolates	Accession number	Closest phylogenetic relative and accession number	Identity %
Streptomyces lavendulae R-St-1	OL697233.1	*Streptomyces lavendulae* (NR_117992.1)	99.14
Priestia aryabhattai R-B-1	OL697234.1	*Priestia aryabhattai* (NR_115953.1)	99.00

Figure 3



Phylogenetic tree of the isolate (R-St-1) in contrast to the most closely related bacterial strain in the NCBI database [*Streptomyces lavendulae* (NR_117992.1)].

Figure 4



Phylogenetic tree of the isolate (R-B-1) in contrast to the most closely related bacterial strain in the NCBI database [*Priestia aryabhattai* (NR_115953.1)].

There is great need for lignin-degrading bacteria, and the characterization of lignin-degrading enzymes may give several potential benefits for textile industry, pulp and paper industry, bioconversion, food processing, feed, chemical industry, biosensors, and biofuel production from lignocellulosic materials. The extracellular LiP secreted by bacterial isolates is the key enzyme in lignin degradation. Random mutagenesis of the best-selected bacterial isolates in the next studies using UV rays and EMS-mutagen is required to improve its LiP production (Figs 3 and 4).

Conclusion

Different previous studies suggested that lignin-degrading bacteria is more essential than previously considered, especially in soil. Therefore, identification of bacteria from nature with lignin-oxidizing enzymes would be significant, and there is high potential of bacteria that degrading lignin, and the characterization of enzymes that degrade lignin may give several potential benefits for textile, pulp and paper industry, bioconversion, food processing, feed, chemical industry, biosensors, and biofuel production from lignocellulosic materials. This study aimed to isolate different bacteria that degrade lignin. Nine ligninolytic bacterial colonies that excreted peroxidases were isolated from three types of soil farms (F1, F2, and F3) from Jeddah, KSA and the best isolates and conditions for LiP production using FM under three incubation periods were evaluated. Two most active isolates for production of LiP (R-St-1 and R-B-1, as *Actinomycetes* and *Bacilli* spp., respectively) were identified using 16S-rRNA (Figs. 3 and 4). The results showed that the highest LiP producer was *Streptomyces* R-St-1 isolate (3.8 U/ml) followed by Bacilli R-B-1 isolate (2.4 U/ml). Different concentrations of lignin, temperature, various carbon source, nitrogen sources, and pH were tested for their effect on LiP production. The DNA sequence of the identified bacterial isolates was conserved in the GenBank under two accession numbers OL697233.1 (*S. lavendulae* R-St-1) and OL697234.1 (*P. aryabhattai*R-B-1, formerly known as *B. aryabhattai*).

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Conflicts of interest

There are no conflicts of interest.

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