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ORIGINAL ARTICLE

Peroxidase-producing actinobacteria from Algerian environments and insights from the genome sequence of peroxidase-producing *Streptomyces* sp. S19

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Abstract

Unique environments often serve as a source of novel microorganisms with novel chemistries. In this study, telluric samples collected from different regions of Algeria were processed for the isolation of novel peroxidase-producing actinobacterial strains. An agar-based screening identified 45 isolates with the ability to produce peroxidase. The 16S rRNA gene sequencing showed that most of the strains belong to the genus *Streptomyces*. Optimization of cultivation conditions was performed for the top five peroxidase-producing strains. Apart from strain 36 (optimal growth temperature of 30 °C) and strain 45 (optimal medium pH of 6.0), the strains exhibited optimal peroxidase production when cultivated for 5 days at 37 °C and in a medium at pH 7.0. Extracellular peroxidase production was induced by ferulic acid in three of the five strains, while the presence of canola lignocellulosic waste (CLW) induced peroxidase production in all strains. Strain 19 (S19) was selected for further optimization and the extracellular peroxidase purified using acid and acetone precipitation, followed by size exclusion chromatography. The purified fraction showed a single band on the polyacrylamide gel with an estimated molecular weight of 21.45 kDa. Genome analysis confirmed the assignment of S19 to the genus *Streptomyces*, the presence of genes encoding for peroxidases, and the presence of genes encoding for carbohydrate-active enzymes. The presence of biosynthetic gene clusters potentially encoding for biosurfactants further highlighted the great biotechnological potential of the strain.

Keywords Actinobacteria · Canola lignocellulosic waste · Genome sequencing · Optimization · Peroxidase · Purification

Introduction

The actinobacteria are a large group of mostly Gram-positive bacteria and are known to have a genomic G+C% of between 60 and 70%. They are widely distributed in nature and are found in almost all ecosystems including aquatic environments such as seawater (Subramani and Aalbersberg 2013), ocean sediments (Jensen et al. 2005; Solano et al. 2009), and in some plants and animals (Fiedler et al. 2005). However, soil remains the richest reservoir of actinobacteria. They are found in hot and dry desert soils, soils highly contaminated with heavy metals, frozen polar soils, highly alkaline lakes, and salt lakes (Kitouni 2007). Actinobacteria are known for their production of various metabolites and bioactive molecules such as antimicrobials, antidepressants, antitumor compounds, and immunosuppressants (Angehrn et al. 2004; Imada 2005) and are also able to degrade pesticides, herbicides, and hydrocarbons (Benimeli et al. 2003). This production and degradation potential give actinobacteria a wide range of potential industrial applications: food, pharmaceuticals, and agriculture. These bacteria are also widely and frequently exploited for their production of a variety of enzymes, such as peroxidases, that are increasingly sought after and applied in various industries.

Peroxidases represent a large family of oxidoreductases that catalyze the oxidation of a variety of organic and inorganic compounds using peroxides, such as hydrogen peroxide (H_2O_2) as a co-factor (Musengi et al. 2020). They have a molecular weight ranging from 30 to 150 kDa, and

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most contain heme as a co-factor (Raveendran et al. 2018). Peroxidases are widely distributed in plants, animals, and microorganisms, reflecting the important role they play in biological systems (El-Dein et al. 2014). Plant and fungal peroxidases dominate the market for peroxidases with the plant enzyme, horseradish peroxidase (HRP), being extensively studied. However, the low thermostability of the enzyme (Tams and Welinder 1998) as well as its low activity at low concentrations of hydrogen peroxide (Hiner et al. 2002) makes it difficult to apply on an industrial scale. It is therefore not surprising that bacterial peroxidases are increasingly sought after as alternatives, in particular because of their ease of production, better stability, and other biochemical properties (e.g., salt tolerance) making them suited to industrial applications. Different authors have described the production of extracellular peroxidases by actinobacteria (Gottschalk et al. 2008; Van Bloois et al. 2010; Fodil et al. 2011, 2012; El-Dein et al. 2014; Jaouadi et al. 2014; Musengi et al. 2014).

Algeria possesses an important climatic diversity: in the North are the snowy mountainous regions bordering the Mediterranean Sea and in the South is the Sahara Desert, one of the hottest deserts in the world. This diversity leads to a large biodiversity, rich and varied in actinobacteria, which corresponds to a large chemodiversity of metabolites (Djinni et al. 2019). Algerian soil is increasingly being explored for new species of actinobacteria, new antibacterial, and antifungal molecules as well as industrial enzymes. Nevertheless, very little work has been carried out: only selected studies on fungal peroxidases (Bouacem et al. 2017; Rekik et al. 2018) and peroxidases of the bacterial genus *Bacillus* (Khelil et al. 2015) have been reported, but no research has been carried out on peroxidases produced by actinobacteria. In this study, actinobacterial strains were isolated from soil samples collected from various sites in Algeria. These strains were screened for their ability to produce peroxidase, whereafter peroxidase production was optimized, and the genome of the top peroxidase producer sequenced to gain insight into its biotechnological ability.

Materials and methods

Substrates and chemicals

Unless specified, all substrates, chemicals, and reagents were of analytical grade or highest available purity and were purchased from the Merck Group (Darmstadt, Germany). Canola lignocellulosic waste (CLW; Fig. 1) was obtained from a canola edible oil processing facility, Swellendam, South Africa.

Fig. 1 Canola lignocellulosic waste material generated in the processing of canola (*Brassica napus* subsp. *napus*) during the production of canola edible oil

Sampling and soil pretreatment

All soil samples were collected from different Algerian sites (Fig. 2; Table 1). After discarding the first five centimeters of soil, a sufficient amount of soil was collected for each sample, placed in sterile plastic bags, and then transported directly to the laboratory and refrigerated at 4 °C until use. In total, 25 samples were collected, and each sample was pre-treated as a standalone sample (no pooling of samples) with $CaCO_3$ (1 g of $CaCO_3$ per 10 g of soil in sterile vials) (El-Nakeeb and Lechevalier 1963). The vials were then incubated at 30 °C for 1 month.

Isolation and cultivation of actinobacteria

During the sample incubation, 10 g were taken from each sample on a weekly basis. A 10% (w/v) stock solution was prepared in sterile distilled water, and ten-fold dilutions were carried out. The undiluted samples as well as the dilutions were spread-plated (200 µl) onto a modified R2A agar medium (R3A; $g.L^{-1}$): 1.0 yeast extract, 1.0 peptone, 1.0 tryptone, 1.0 glucose, 1.0 soluble starch, 0.6 potassium sodium tartrate tetrahydrate ($KNaC_4H_4O_6.4H_2O$), 0.6 dipotassium phosphate monohydrate (K_2HPO_4), 17.0 agar, and pH adjusted to 7. After the media was autoclaved, it was supplemented with a 0.04% (v/v) filter-sterilized (0.22 µm) guaiacol solution (Abd El Monssef et al. 2016). All plates were incubated at 37 °C for 7–15 days and evaluated for

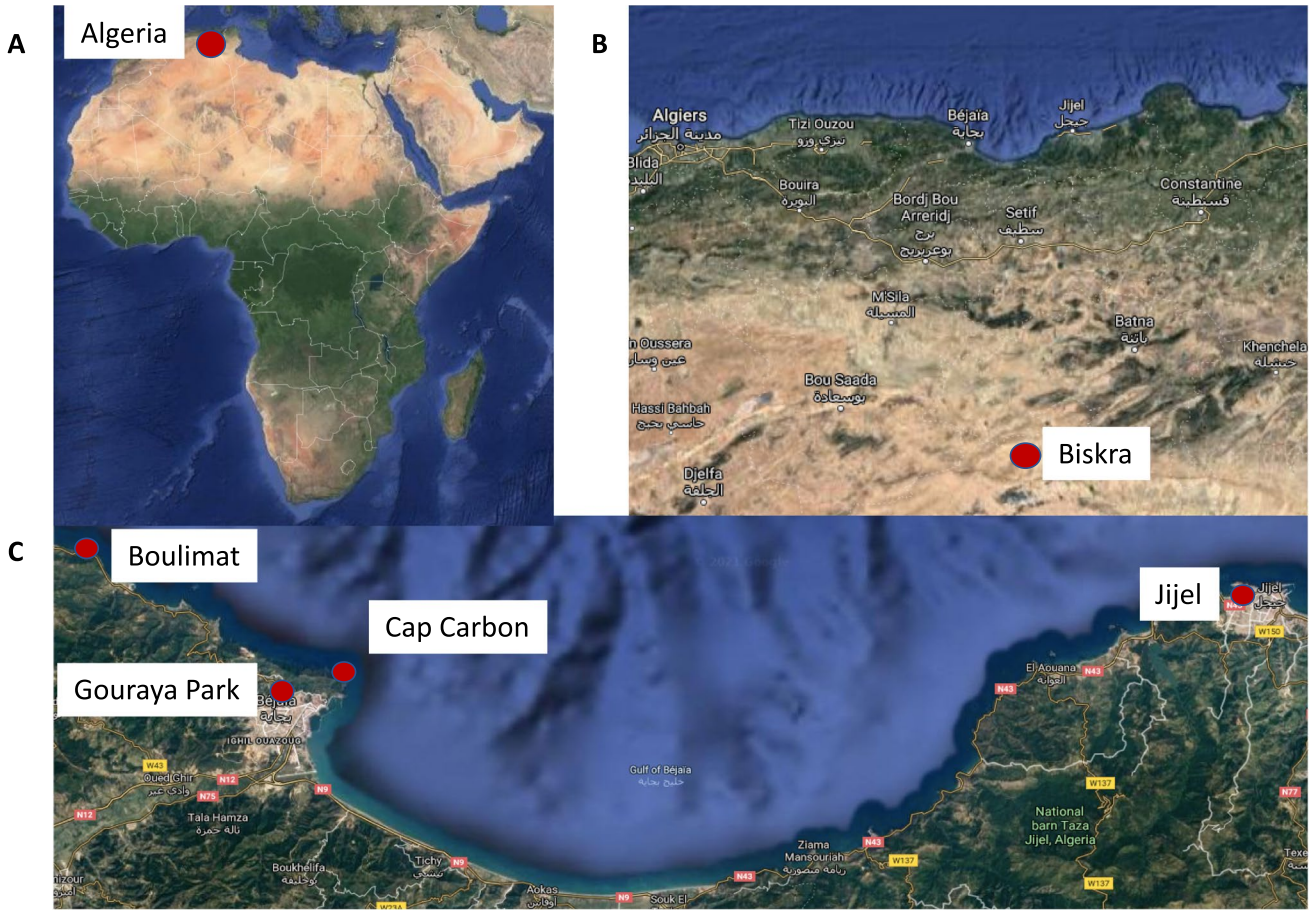

Fig. 2 Maps of the sampling sites for this study with (**A**) indicating the country where sampling took place, (**B**) indicating the Biskra sampling site, while (**C**) (from left to right) shows the Boulimat, Gouraya Park, Cap Carbon, and Jijel sampling sites. Maps were generated using Google Maps

Table 1 Geographical location of sampling sites in Algeria selected for the collection of soil samples. All samples collected were subjected to a pre-treatment method for the selective isolation of actinobacteria

Region	Latitude	Longitude	Number of samples
Biskra (desert region)	34°51′00″N	05°44′00″E	1
Boulimat (landfill site)	36°80′96″N	04°98′45″E	5
Cap Carbon (plant-rich environment)	36°46′34″N	05°06′20″E	8
Gouraya Park (plant-rich environment)	36°46′15″N	05°04′52″E	10
Jijel (soil containing effluent from a paper industry)	36°49′00″N	05°46′00″E	1

the presence of colonies with the typical morphological characteristics of actinobacteria and having a brown color/halo around the colony. Colonies of interest were streaked for pure culture and the selected strains maintained on R3A agar (pH 7.0) without guaiacol at room temperature (22 ± 3 °C) and as a suspension in 20% (v/v) glycerol at -20 °C.

Screening on solid media for peroxidase-producing strains

The initial screening for peroxidase-producing actinobacteria was performed on R3A agar supplemented with guaiacol (0.04%, v/v) as substrate, which is colorless in reduced form and brown in oxidized form. Actinobacteria were streaked

onto the agar plates and incubated at 37 °C for 7–15 days to confirm their ability to produce peroxidase. The intensity of product formation (brown color) was the criterion for the selection of strains for further analysis.

Screening in liquid media for peroxidase-producing strains

As a second screening, the selected strains were inoculated into a 10 ml of R3A broth and into a modified phenoxazinone production medium (MPPM) consisting of the following ($g.L^{-1}$): 10.0 glucose, 10.0 glycerol, 10.0 soya flour, 5.0 yeast extract, 5.0 casamino acids, 4.0 $CaCO_3$, 1 ml trace salts, and the pH adjusted to 7 before autoclaving (trace salts composition, $g.L^{-1}$: 1.0 $FeSO_4$, 0.9 $ZnSO_4$, 0.2 $MnSO_4.7H_2O$) (Graf et al. 2007). The cultures were grown at 30 °C with shaking at 160 rpm for 10 days, and 1-ml samples were taken after 3, 5, 7, and 10 days of incubation. The samples were centrifuged at 10,000 rpm for 10 min, and the obtained supernatant fluid was used in the peroxidase assay (Le Roes-Hill et al. 2011). All strains were cultivated in triplicate.

Peroxidase assay

Peroxidase activity was monitored using 2,4-dichlorophenol (2,4-DCP, Sigma-Aldrich) as a substrate. The reaction mixture contained equal volumes (50 µl each) of the following: 50 mM potassium phosphate buffer (pH 7.0), 25 mM 2,4-DCP, 16 mM 4-aminoantipyrine, 50 mM H_2O_2 and the sample containing the enzyme (Antonopoulous et al. 2001a). In addition, the peroxidase activity was also determined with 2,2′-azinobis-3-ethylbenzothiozolin-6-sulfonic acid (ABTS, Sigma-Aldrich). A final volume of 200 µl of the reaction mixture contained an equal volume (50 µl each) of the following: 50 mM sodium acetate buffer (pH 5.0), 3.6 mM ABTS solution, the sample containing the enzyme, and 50 mM H_2O_2. Substrate oxidation was initiated by the addition of H_2O_2 and the change in absorbance monitored for 5 min at 510 nm for 2,4-DCP ($\varepsilon = 21{,}647\ M^{-1}\ cm^{-1}$) and at 405 nm for ABTS ($\varepsilon = 36{,}000\ M^{-1}\ cm^{-1}$). One unit of enzyme activity is defined as the amount of enzyme required to convert 1 µmol of substrate per minute. In all of the enzyme activity tests, the uninoculated medium was used as a negative control and HRP as positive control. All assays were performed in triplicate.

Identification of peroxidase-producing strains and DNA sequence determination

The peroxidase-producing strains were inoculated into 10 ml of yeast extract-malt extract liquid media (YEME; Shirling and Gottlieb 1966) and incubated at 30 °C on a rotary shaker (160 rpm) for 7 days. Extraction of genomic DNA from isolates was performed using a modified PSC-B (phosphate, SDS, chloroform-BeadBeater) method (Miller et al. 1999). The cells were harvested by centrifugation at 12,000 rpm, washed with 1 ml of sodium phosphate buffer (100 mM, pH 8.0), resuspended in 300 µl of sodium phosphate buffer, and frozen overnight at -20 °C. The buffer and cell mixtures were thawed and transferred to a centrifuge tube containing glass beads (0.5 g each). A 300 µl SDS lysis buffer (100 mM NaCl; 0.5 M Tris–HCl, pH 8 and 10%, w/v, SDS) and 300 µl chloroform:isoamyl alcohol (24:1, v/v) were added, and the samples were incubated at room temperature for 2 h. The samples were vortexed for 2 min and centrifuged at 13,200 rpm for 5 min, and the supernatants were transferred to a clean centrifuge tube, and then 360 µl of 7 M ammonium acetate was added. After a second centrifugation (13,200 rpm for 5 min), the upper aqueous phase was transferred to a new tube. The DNA was precipitated with 315 µl isopropanol, and the pellet was washed with 1 ml 70% (v/v) ethanol, allowed to dry, and then resuspended in 10 mM Tris–HCl buffer (pH 8.0).

16S rRNA gene sequence analysis

The 16S rRNA gene was amplified by polymerase chain reaction (PCR) using two universal bacterial 16S rRNA primers, F1 and R5 (Cook and Meyers 2003). The PCR cycling conditions were as follows: 96 °C for 2 min; 30 cycles of 96 °C for 30 s, 56 °C for 30 s, and 72 °C for 2 min; and 72 °C for 5 min. PCR products were analyzed on a 1% (w/v) agarose gel containing ethidium bromide (10 µl of 10 mg/ml solution/100 ml of agarose solution). The amplified PCR products (~1.5 kb) were purified using the MSB Spin PCRapace PCR purification kit (Invitek) according to the manufacturer's instructions. The concentration of the purified DNA was determined using a Genova Nano Micro-Volume Spectrophotometer (Jenway, Staffordshire, UK), and the samples were submitted for sequencing at Inqaba Biotechnical Industries (Pretoria, South Africa). Sequence data were analyzed and edited using FinchTV version 1.4.0 (GeoSpiza) and then submitted to EzBioCloud (Yoon et al., 2017) to determine the identity of the isolates (genus level) and nearest phylogenetic neighbors.

Optimization of peroxidase production

The five highest peroxidase producers (S4, S19, S36, S44, and S45) were selected for further optimization. To determine the optimal incubation time for each strain, the isolates were inoculated into liquid MPPM (10 ml per flask), and activity was determined after 3, 5, and 7 days of incubation. The effect of temperature on peroxidase production was also determined by incubating the strains at 30 °C and 37 °C

(activity monitored over a 7-day period). Once the optimal temperature was determined, the pH of the liquid medium was adjusted to 5.0, 6.0, 7.0, and 8.0 using NaOH or HCl before medium sterilization, and strains were incubated at their optimal temperature. In order to confirm the pH of the production medium, the liquid MPPM was prepared as described above, and after autoclaving, the pH was determined. Peroxidase activity was monitored using the 2,4-DCP assay as described previously. All cultivation and enzymes assays were performed in triplicate.

Effect of inducers

Strains S4, S19, S36, S44, and S45 were grown at their respective optimal pH and temperature in MPPM in the presence of the following inducers: wood (1% w/v), paper (1% w/v), veratryl alcohol (10 mM), ferulic acid (10 mM), and different concentrations of CLW. The first four inducers were added to the cultures after 3 days of incubation, and the activity measured on the 4th and 5th days of incubation. For the CLW, 0.5, 1, and 2% (w/v) were added to the MPPM (prior to autoclaving), and peroxidase activity measured after 3, 5, 6, and 7 days of incubation. Cultures were also incubated in MPPM without CLW to determine whether the waste material had an effect on peroxidase production.

Characterization of canola lignocellulosic waste

To determine the composition of the CLW samples (autoclaved and non-autoclaved), treatment was performed according to the gravimetric method of Harper and Lynch (1981) as described in Sridevi et al. (2015) was applied. Briefly, 1 g of CLW was used as a main sample which was chemically treated to extract the different components of the CLW to obtain the net cellulose; 75 ml of purified water was added to the sample and then boiled at 100 °C for 2 h while stirring at 200 rpm. The CLW/water mixture was recovered and washed 3 times with 75 ml of cold-water using filter paper (House of Coffees filter bag, size 102) and placed at 60 °C overnight to dry. By subtracting the weight of the dried product from the initial weight (1 g) of the sample, the mass of soluble components was determined. The second step of the treatment consisted of heating the dried product at 80 °C with 100 ml ethanol, while stirring at 200 rpm. After 2 h, the product was washed twice with 100 ml ethanol and twice more with 100 ml water. After one night of drying at 60 °C, the difference in weight indicated the hot ethanol soluble fraction. Subsequently, a mixture of 30 ml water, 10 ml aqueous solution of 10% (v/v) acetic acid, and 0.6 g sodium hypochlorite were added to the dry sample and heated at 70 °C for 2 h while stirring at 200 rpm. After one hour of heating, a further 2 ml of the aqueous solution of 10% (v/v) acetic acid and 0.6 g of sodium hypochlorite were added. The mixture was captured and washed 5 times with 30 ml of water, twice with 30 ml of acetone, and once with 30 ml of ether and then placed at 60 °C overnight for drying. The difference in weight indicated the lignin content. The hemicellulose contained in the sample was extracted by incubating the sample at 25 °C (room temperature) with stirring at 200 rpm for 2 h in 20 ml of a 24% (w/v) KOH solution. Washing was carried out five times with 20 ml water, once with 20 ml 5% (v/v) aqueous acetic acid solution, once with 20 ml acetone, and finally once with 20 ml ether. The washed sample was left to dry overnight at 60 °C. The weight of the product corresponded to the cellulose content of the sample, and, consequently, the weight difference between the cellulose sample and the lignin-stripped sample indicated the hemicellulose content (Sridevi et al. 2015).

Degradation of canola lignocellulosic waste—release of sugars

The degradation of CLW can be verified by measuring the amount of reduced sugars released into the culture medium containing the optimal CLW concentration for S19. Release of reduced sugars was determined by using a 3,5-dinitrosalicylic acid (DNS) assay. Culture supernatants were used as samples (in triplicate). The DNS reagent was prepared according to Gonçalves et al. (2010). Briefly, 30 µl of DNS was added to 30 µl of sample in a 1.5-ml reaction vessel and heated to 100 °C for 5 min. The reaction was stopped by placing the reaction vessel directly onto ice. A 300 µl distilled water was added to the mixture. The absorbance measurement at 540 nm was performed using a 96-well microplate with each well containing 300 µl of each sample. The negative controls used for this test were MPPM without additives and the same medium plus CLW at different percentages (0.5, 1, and 2%, w/v), and the positive control was glucose solutions prepared at different concentrations (mM): 0.05, 0.1, 0.15, 0.2, 0.5, 0.8, 1.0, 2.0, 5.0, 7.5, 10, and 15. A standard curve was drawn by relating each glucose concentration to the corresponding absorbance.

Isolation and purification of the peroxidase of *Streptomyces* sp. S19

S19 was selected for further optimization and peroxidase purification. To determine the effect of aeration on peroxidase production, pre-cultures were prepared in 10 ml volume media (MPPM, pH 7.0). After 3 days of growth (37 °C, 160 rpm), two flasks of the 10 ml pre-cultures were used to inoculate 200 ml of MPPM (pH 7.0) in duplicate standard Erlenmeyer flasks and duplicate baffled flasks. The cultures were incubated at 37 °C, 160 rpm. Peroxidase activity was determined on days 3 and 5 of incubation. For enzyme purification, 400 ml of sterile MPPM was inoculated with a

10% (v/v) pre-culture and incubated as described previously. After 5 days of incubation, the cultures were centrifuged at 10 000 g for 5 min at 4 °C and the supernatant used as the crude enzyme fraction.

Peroxidase purification

For peroxidase purification from the S19 culture, the pH of the crude enzyme fraction was adjusted to pH 2.3 with HCl followed by a 15-min incubation at room temperature (22 ± 3 °C). The acid precipitate was centrifuged at 9500 g for 10 min. The pellet was resuspended in 100 mM Tris–HCl (pH 8.0)—"acid fraction." Ten volumes of ice-cold acetone were added to the acid fraction and incubated overnight at -20 °C. The mixture was centrifuged at 9500 g for 10 min, the supernatant was discarded, and the pellet was resuspended in 100 mM Tris–HCl (pH 8.0)—"acetone fraction" (Musengi et al. 2014). Enzyme samples obtained with the acetone precipitation ("acetone fractions"; 10 ml each) were applied to a Sephadex G-75 size-exclusion chromatography (SEC) column and was eluted in 100 mM Tris–HCl buffer, pH 8.0. Fractions (1 ml each) were collected and tested for peroxidase activity using the 2,4-DCP assay (as previously described). The fractions with positive results were combined and filtered (0.2 µm filter), and their protein concentration determined using the standard Bradford's assay (Bradford 1976). Commercial Bradford's reagent (Sigma-Aldrich) was used, and standard curves of known protein concentrations [0–100 µg/ml bovine serum albumin (BSA)] were constructed every time the assay was performed. The peroxidase-containing SEC fractions were concentrated by centrifugation using Amicon® Ultra-15 centrifugal filter tubes (10,000 MWCO; 5000 g for 30 min).

Electrophoresis analysis: SDS-PAGE

To analyze the semi-purified (acetone fraction) and the purified (SEC fraction) proteins, SDS-PAGE (sodium dodecyl sulphate–polyacrylamide gel electrophoresis; denaturing) was performed. A 5% (w/v) stacking gel and 15% (w/v) resolving gel were prepared as per the manufacturer's instructions for the PROTEAN® Mini-Gel system (Bio-Rad, CA, USA) and used for the separation of the proteins. A 20 µl of each peroxidase sample was mixed with 5 µl of 6 × denaturing sample buffer [375 mM Tris–HCl (pH 6.8), 48% (w/v) glycerol, 0.03% (w/v) bromophenol blue, 9% (w/v) 2-mercaptoethanol, and 6% (w/v) SDS]. The 25 µl samples were boiled for 15 min and loaded onto the gel and run at 180 V for 45 min in electrophoresis buffer [mix 3.0 g Tris (25 mM), 14.4 g glycine (192 mM) and 1 g SDS in 1 L water; the final pH should be 8.8] at room temperature. A protein ladder (Color Prestained Protein Standard P7719S, New England BioLabs) was used to determine the protein size. The gel was stained overnight with Coomassie blue staining solution (2.5 g Coomassie Brilliant Blue R-250 in 450 ml methanol, 100 ml acetic acid, and 450 ml distilled water) on a rsocking shaker. Destaining was performed using a destaining solution (same as staining solution, omitting the Coomassie blue dye) for 30 min, followed by washing in distilled water for 20 min.

Genome sequencing and analyses

Strain S19 was cultivated in 10 ml YEME liquid media (Shirling and Gottlieb 1966) and genomic DNA isolated using the DNA isolation method described by Mandel and Marmur (1968). Genomic DNA was submitted for genome sequencing to the Central Analytical Facility, Stellenbosch University, South Africa. After confirming the quality of the DNA, the genome was sequenced using the Ion Torrent S5 platform. The BAM output file was converted to FASTA format by using bedtools v2.27.1. The genome sequence was assembled using SPAdes version 3.14.1 (Bankevich et al. 2012) and the resultant FASTA file submitted to the Rapid Annotation using Subsystem Technology (RAST version 2) server for annotation (Aziz et al. 2008). Peroxidase sequences were extracted from the annotated genome and analyzed using BLASTp (https://blast.ncbi.nlm.nih.gov/; Altschul et al. 1990), SignalP-5.0 (https://services.healthtech.dtu.dk/service.php?SignalP; Armenteros et al. 2019), as well as PSORTb 3.0.3 (http://www.psort.org/psortb/; Yu et al. 2010). SnapGene viewer 5.1.7 was used for the determination of the molecular weight, amino acid content, and pI of the annotated DyP-type-peroxidase. In addition, 19 *Streptomyces rochei* genomes were downloaded from the EzBioCloud server (https://www.ezbiocloud.net/; accessed 27 November 2021) and were analyzed for the presence of DyP-type peroxidases. The properties of the DyP-type peroxidases were evaluated using SnapGene viewer 5.1.7, and a multiple sequence alignment performed using Kalign (https://www.ebi.ac.uk/Tools/msa/kalign/; accessed 27 November 2021) in order to determine DyP-type peroxidase amino acid sequence similarity.

The genome sequence was also submitted to antiSMASH 5.0 (Blin et al. 2019) to determine the presence of biosynthetic gene clusters that could potentially be involved in biosurfactant production and to dbCAN2 (Zhang et al. 2018) to determine whether the strain has the potential to degrade lignocellulosic materials. The genome sequence data was also uploaded to the type (strain) genome server (TYGS), a free bioinformatics platform (https://tygs.dsmz.de) that allows for a whole genome-based taxonomic analysis (Meier-Kolthoff and Göker 2019). The results were provided by the TYGS on 08 August 2021. The TYGS analysis was subdivided into the following steps: determination of closely related type strains, pairwise comparison of genome sequences,

phylogenetic inference, and type-based species and subspecies clustering. This whole genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JAIFOQ000000000. The version described in this paper is version JAIFOQ010000000.

Results

Isolation of actinobacteria

A total number of 257 strains were selected on solid medium based mainly on the morphological properties of actinobacteria, as well as the brown coloration on the guaiacol-containing agar plates. After selecting for pure colonies, a second selection was made based on the intensity of the brown color. By this selection, 45 strains were sub-cultured onto solid medium (R3A and YEME agar) and were tested for their ability to produce peroxidase in liquid media.

Screening of isolates: liquid media

The verification and quantification of the extracellular peroxidase production by the different strains were performed in MPPM and R3A liquid media, with activity being monitored using the substrates 2,4-DCP and ABTS. Enzyme activity detected with 2,4-DCP as substrate (Supplementary Table S1) and with ABTS as substrate (Supplementary Table S2) clearly highlights the difference in enzyme production in R3A vs MPPM. Oxidation of 2,4-DCP was positive for all strains grown in the MPPM, and production varied from strain to strain and according to the days of incubation. Maximum activity was recorded for S19 with 0.388 ± 0.083 U.ml^{-1} after 5 days of incubation. However, the results of the oxidation of ABTS by strains grown in MPPM were lower for the majority of strains, with a maximum activity of 0.413 ± 0.282 U.ml^{-1} for S19 after 7 days of incubation. The enzyme activities obtained by the strains cultivated in R3A were even lower, with only an activity of 0.036 ± 0.0016 U.ml^{-1} for S29 with 2,4-DCP as substrate and an activity of 0.053 ± 0.002 U.ml^{-1} for S45 with ABTS as substrate. Based on the activity profiles, the following five strains were selected for further optimization: S4 (isolated from sample 3, Cap Carbon), S19 (isolated from sample 7, Cap Carbon), S36 (isolated from sample 8, Gouraya Park), S44 (isolated from Jijel), and S45 (isolated from Biskra), with cultivation in MPPM and peroxidase activity monitoring with the substrate 2,4-DCP.

Identification of peroxidase-producing strains

Peroxidase-producing strains were identified by the amplification and sequencing of their 16S rRNA genes. EzBioCloud was used to determine the identity of the microorganisms and their most closely related phylogenetic neighbors (Supplementary Table S3). From the EzBioCloud analysis for the 19 strains identified, the majority of these belong to the genus *Streptomyces*, except for strains 3 and 15 (*Bacillus*), 27 (*Deinococcus*), 29 (*Paracoccus*), and 33 (*Massilia*).

Optimization of peroxidase production

Temperature optimization

Figure 3A–E summarize the peroxidase production of the five strains selected for this study. All strains were incubated under the same conditions at temperatures of 30 °C and 37 °C. The results showed that maximum peroxidase production for all strains was detected on the fifth day of incubation (U.ml^{-1}): 0.170 ± 0.07, 0.639 ± 0.358, 0.138 ± 0.008, and 0.291 ± 0.006 for S4, S19, S44, and S45 at 37 °C, respectively, and an activity of 0.124 ± 0.008 U.ml^{-1} for S36 at 30 °C.

pH optimization

Peroxidase production was determined in MPPM over a pH range of pH 5.0 to 8.0 and at the optimal production temperature for each strain. The results (Figs. 4A–E) indicate that pH 7.0 is the most favorable for peroxidase production by four of the five actinobacterial strains. Maximum activity was reached on the fifth day of incubation when the initial pH of the MPPM was 7.0 for strains S4 (0.170 ± 0.079 U.ml^{-1}), S19 (0.639 ± 0.358 U.ml^{-1}), S36 (0.124 ± 0.008 U.ml^{-1}), and S44 (0.138 ± 0.0008 U.ml^{-1}). However, peroxidase production for S45 was highest at pH 6.0 (0.506 ± 0.07 U.ml^{-1}). It should be noted that measuring the pH of the MPPM after autoclaving showed a clear change in medium pH (average of three preparations ± standard deviation): pH 5.47 ± 0.14 (original pH 5.0), pH 6.48 ± 0.01 (original pH 6.0), pH 7.05 ± 0.14 (original pH 7.0), and pH 7.53 ± 0.19 (original pH 8.0). Results reported in this study therefore reflect the pH of the media prior to autoclaving.

The effect of peroxidase inducers on peroxidase production

The effects of four different lignin model compounds on peroxidase production by the five strains (S4, S19, S36, S44, and S45) were determined (Table 2). For S4, the presence of paper and ferulic acid slightly increased peroxidase production after 24 h of their addition (from 0.25 ± 0.121 U.ml^{-1} to 0.37 ± 0.050 U.ml^{-1} and from 0.29 ± 0.10 U.ml^{-1} to 0.32 ± 0.060 U.ml^{-1}, respectively). However, there was a decrease in enzyme activity in the medium containing veratryl alcohol (from 0.96 ± 0.143 U.ml^{-1} to 0.61 ± 0.120

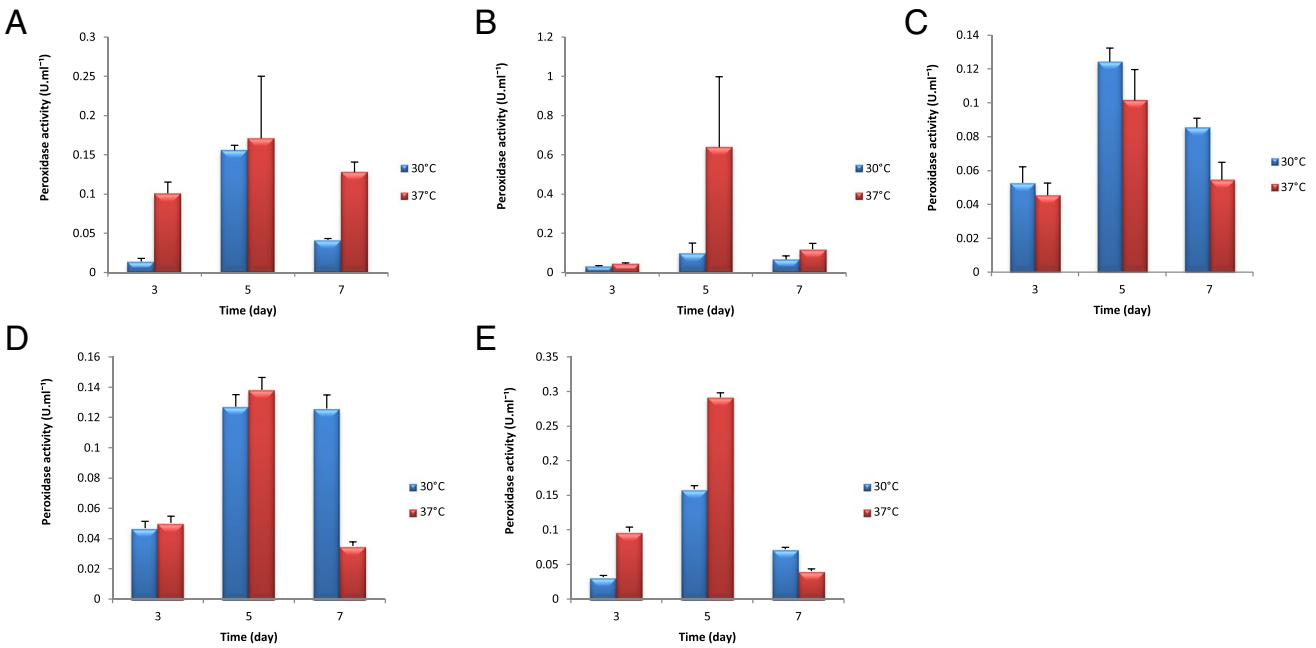

Fig. 3 Peroxidase production by **A** S4, **B** S19, **C** S36, **D** S44, and **E** S45 at 30 °C and 37 °C in modified phenoxazinone production medium (7 days, pH 7.0, 160 rpm). The error bars represent the standard deviation ($n = 3$)

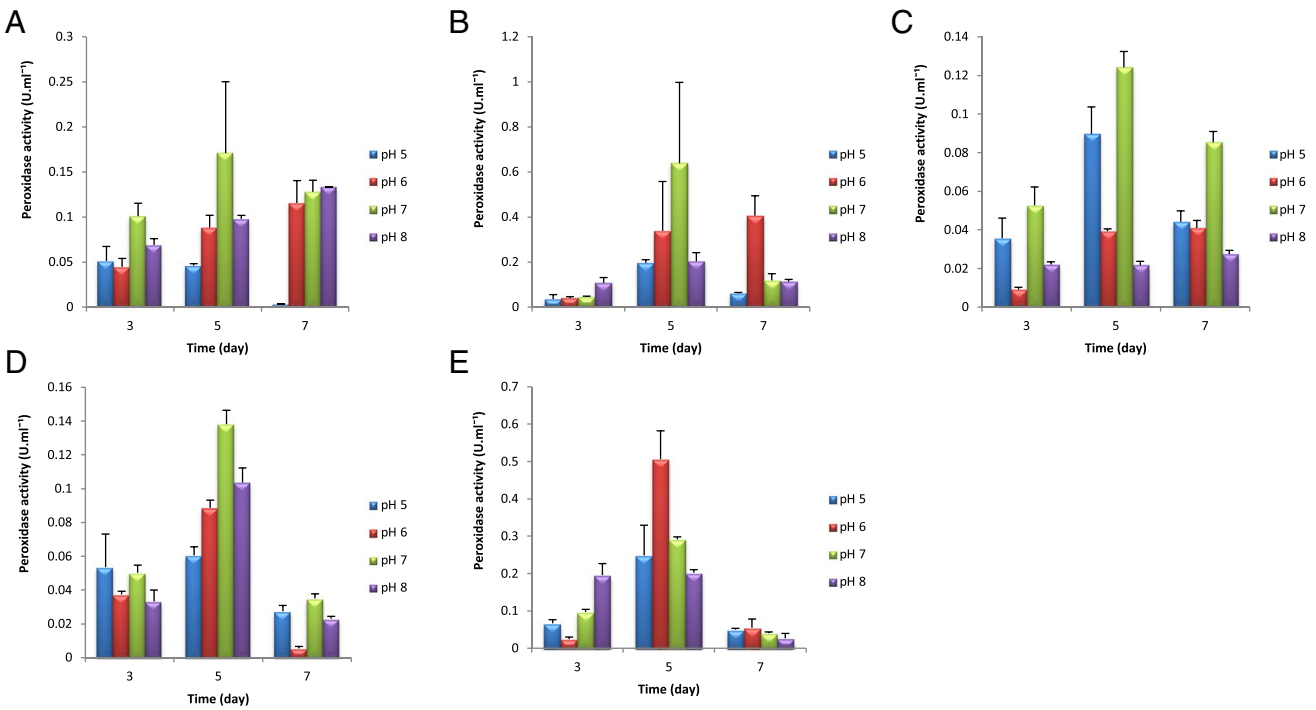

Fig. 4 Effect of pH on peroxidase production by S4 (**A**), S19 (**B**), S36 (**C**), S44 (**D**), and S45 (**E**) at 37 °C, 160 rpm. The error bars represent the standard deviation ($n = 3$)

U.ml^{-1}) when compared to the activity detected in the medium without inducer on days 4 and 5. The wood had no effect on the peroxidase production of S4. Compared to the control, a decrease in the peroxidase production of S19 was observed in the presence of all inducers except veratryl alcohol, which had no effect on the activity of the strain.

Table 2 Peroxidase activity (U.ml^{-1}) for the five top peroxidase-producing strains in the presence of inducers in modified phenoxazinone production medium. Values represent the average of triplicate readings and ± the standard error of the mean

		Strain 4	Strain 19	Strain 36	Strain 44	Strain 45
Type of inducer	Day of incubation					
Paper	Day 4	0.25 ± 0.120	0.62 ± 0.100	0.24 ± 0.012	0.064 ± 0.002	0.47 ± 0.098
	Day 5	0.37 ± 0.050	0.43 ± 0.100	0.12 ± 0.005	0.056 ± 0.004	0.36 ± 0.100
	Induced	48%	-31%	-49%	-14%	-23%
Wood	Day 4	0.14 ± 0.090	0.51 ± 0.090	0.20 ± 0.002	0.033 ± 0.010	0.50 ± 0.120
	Day 5	0.14 ± 0.012	0.37 ± 0.050	0.14 ± 0.007	0.03 ± 0.001	0.36 ± 0.070
	Induced	0.14%	-28%	-29%	-7%	-29%
Veratryl alcohol	Day 4	0.96 ± 0.144	0.48 ± 0.142	0.20 ± 0.009	0.009 ± 0.005	0.11 ± 0.012
	Day 5	0.61 ± 0.121	0.47 ± 0.125	0.18 ± 0.007	0.007 ± 0.005	0.292 ± 0.101
	Induced	-37%	-2%	-8%	-16%	174%
Ferulic acid	Day 4	0.29 ± 0.101	0.75 ± 0.280	0.28 ± 0.009	0.55 ± 0.019	0.14 ± 0.028
	Day 5	0.32 ± 0.060	0.43 ± 0.062	0.16 ± 0.008	0.62 ± 0.007	0.50 ± 0.040
	Induced	9%	-42%	-43%	12%	248%
No inducer	Day 4	0.34 ± 0.15	0.43 ± 0.100	0.26 ± 0.002	0.004 ± 0.001	0.09 ± 0.004
	Day 5	0.37 ± 0.031	0.48 ± 0.106	0.14 ± 0.002	0.01 ± 0.001	0.21 ± 0.067

A decrease in the activity of S36 was noted in the presence of all inducers, whereas a slight increase in activity was observed for S44 in the presence of ferulic acid (from 0.55 ± 0.018 U.ml^{-1} on day 4 of incubation to 0.62 ± 0.007 U.ml^{-1} on day 5). S45 had constant activity during the fourth and fifth day of incubation. An increase was recorded in the presence of veratryl alcohol (0.11 ± 0.011 U.ml^{-}1 to 0.29 ± 0.101 U.ml^{-1}) and ferulic acid (0.14 ± 0.028 U.ml^{-1} to 0.50 ± 0.039 U.ml^{-1}), in contrast to paper and wood which had a reduced effect on the enzymatic production of the strain.

The CLW was tested for its potential to induce peroxidase production by the five selected strains. The peroxidase production by S4 was increased by the addition of 0.5% (w/v) CLW in the culture medium (Fig. 5A), giving a maximum activity of 0.59 ± 0.055 U.ml^{-1} after 3 days of incubation, compared to 0.15 ± 0.033 U.ml^{-1} in the medium without CLW. S19 showed improved production with 2% (w/v) CLW in the culture medium after the seventh day of incubation (Fig. 5B), resulting in a maximum of 0.25 ± 0.043 U.ml^{-1} compared with 0.20 ± 0.002 U.ml^{-1} in the MPPM without CLW. With a concentration of 0.5% (w/v) CLW, S36 had maximum activity on the sixth day of incubation (Fig. 5C) (0.07 ± 0.003 U.ml^{-1}) versus activity in the control medium (0.01 ± 0.001 U.ml^{-1}). The best peroxidase production for S44 was in the presence of 1% (w/v) CLW (0.07 ± 0.003 U.ml^{-1}) on the seventh day of incubation (Fig. 5D). The peroxidase production of S45 in media with the different CLW concentrations were similar on the sixth and seventh day of incubation (Fig. 5E). However, the activity in the presence of 0.5% (w/v) CLW on day 6 (0.23 ± 0.073 U.ml^{-1}) was slightly higher than that obtained with the same concentration of CLW on day 7 (0.22 ± 0.124 U.ml^{-1}).

Effect of aeration on peroxidase production by S19

The effect of aeration on peroxidase production of S19 was studied. The difference in peroxidase production was negligible when comparing growth of the strain in Erlenmeyer flasks vs baffled flasks after the third day of incubation, with values of 0.014 ± 0.002 U.ml^{-1} and 0.019 ± 0.002 U.ml^{-1}, respectively. On the fifth day, the activity recorded in the baffled flasks (0.170 ± 0.007 U.ml^{-1}) was double that of the activity obtained in the Erlenmeyer flasks (0.085 ± 0.004 U.ml^{-1}).

Isolation and purification of S19 peroxidase

The culture of strain S19 was inoculated by a preculture and cultivated in triplicate under its optimal conditions, i.e., MPPM, pH 7.0, incubation temperature at 37 °C for 5 days and under agitation at 160 rpm. The purification profile for the peroxidase from S19 is summarized in Table 3. A color change was observed between the different fractions resulting from the different purification steps from a yellow/brown colored crude enzyme to the acid fraction which was a pinkish/light brown color and the acetone fraction which had a red/red-brown color. The specific activities in the supernatants of the three cultures increased from 0.511, 0.313, and 0.581 U.mg^{-1} to 0.811, 1.383, and 1.255 U.mg^{-1} respectively. Overall, an increase in enzyme purity was observed, with up to a 14.31-fold increase for culture 2.

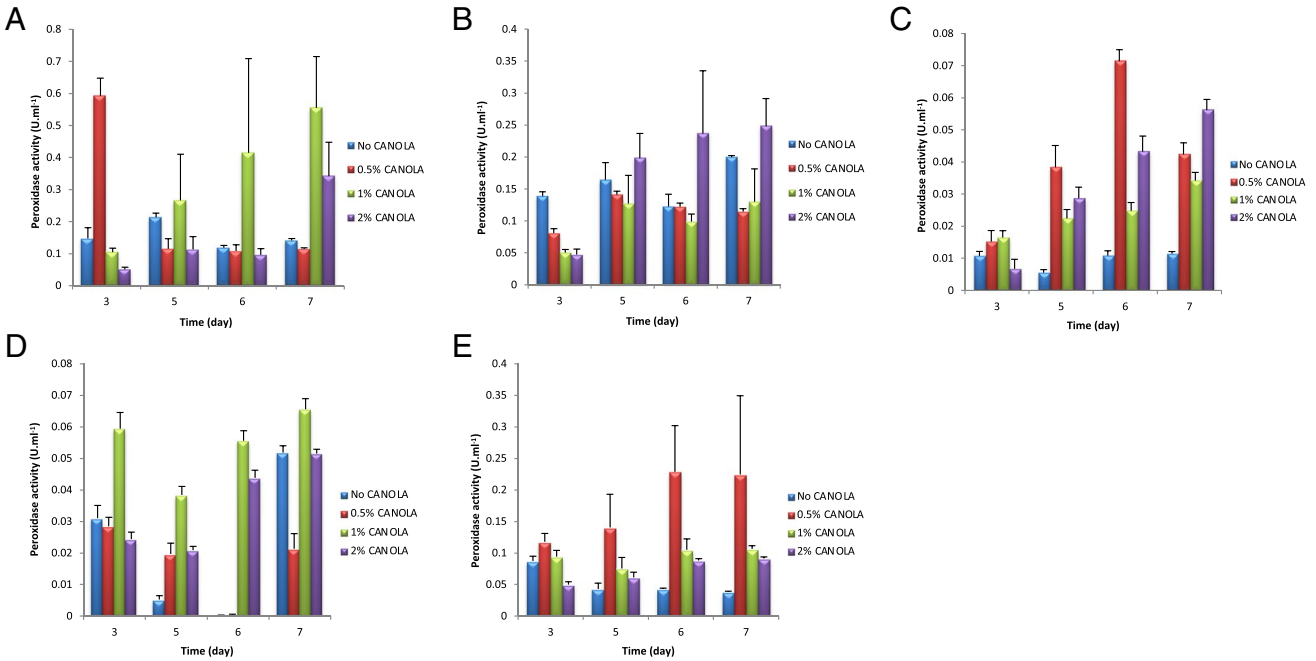

Fig. 5 Effect of different concentrations of canola lignocellulosic waste on peroxidase production by S4 (**A**), S19 (**B**), S36 (**C**), S44 (**D**), and S45 (**E**) in modified phenoxazinone production medium (pH 7.0, 37 °C, 160 rpm; pH 6.0 for S45)

SDS-PAGE

The partially purified and purified peroxidases produced by S19 were analyzed by SDS-PAGE (Supplementary Fig. S1). The SEC fractions (Lane 2 and Lane 3) resulted in individual and distinct protein bands, while the acetone fractions (Lanes 4–7) showed multiple bands indicating that the peroxidase was only partially purified at this stage. This reflects the efficiency of chromatography in the purification of the protein. The molecular weight of the pure protein was estimated by calculating the relative migration distance of the standard proteins in the protein marker and the S19 peroxidase. The denatured purified protein is approximately 21.45 kDa in size.

Characterization of canola lignocellulosic waste and its degradation by S19

The composition of the CLW was determined using a gravimetric method. The percentage values of hot water and ethanol soluble components, lignin, hemicellulose, and cellulose are summarized in Table 4. The lignin and cellulose content of pre-treated CLW is higher than that of untreated CLW, and both have the same hemicellulose content. However, the non-autoclaved CLW sample presents a higher content of hot water-soluble and hot ethanol-soluble components. S19 was tested for its ability to degrade the CLW by measuring the amount of reduced sugars released in the culture medium containing the optimal concentration of CLW. Treatment of CLW with S19 resulted in the release of 2.02 mM (364 mg/L) glucose equivalents, while a peroxidase activity of 0.543 ± 0.067 U.ml^{-1} was detected.

Genome analyses

The assembled S19 genome has a predicted size of 8.16 Mbp, a 72.4% GC content, and 8523 coding sequences. Genome mining revealed the presence of six peroxidase types: catalase-peroxidase KatG (EC 1.11.1.21); Thiol peroxidase, Bcp-Type (EC 1.11.1.15); 4-carboxymuconolactone decarboxylase domain/alkylhydroperoxidase AhpD family core domain protein; glutathione peroxidase (EC 1.11.1.9); thioredoxin peroxidase (EC 1.11.1.15); a putative DyP-type peroxidase associated with bacterial analog for Cox17 protein; and a non-heme chloroperoxidase (EC 1.11.1.10) (Supplementary information). Analysis with SignalP showed that only the DyP-type peroxidase contains the signal peptide for export via the twin arginine translocation (TAT) system. This was confirmed by a PSORTb analysis, which predicted that the DyP-type peroxidase is an extracellular enzyme (with a 97.2% probability). This putative DyP-type peroxidase is characterized by an estimated molecular weight of 46.72 kDa and is composed of 442 amino acids and a pI of 7.84.

dbCAN2 analyses showed the predicted presence of a vast array of carbohydrate-active enzymes, including lytic polysaccharide monooxygenases, various carbohydrate binding modules, carbohydrate esterases, glycoside hydrolases,

Table 3 Purification of an extracellular peroxidase produced by S19 through the use of an acid-acetone precipitation approach

		Volume (ml)	Protein concentration ($mg.ml^{-1}$)	Total protein (mg)	Sample activity ($U.ml^{-1}$)	Total activity (U)	Specific activity ($U.mg^{-1}$)	Yield (%)	Purification fold
Culture 1	Crude enzyme	193	0.0301	5.809	0.02 ± 0.001	2.972	0.511	100	1
	Acid fraction	20	0.0739	1.478	0.06 ± 0.003	1.2	0.811	40.37	1.59
	Acetone fraction	10	0.1823	1.823	0.53 ± 0.004	5.325	2.92	179.17	5.71
Culture 2	Crude enzyme	192	0.0341	6.547	0.01 ± 0.001	2.054	0.313	100	1
	Acid fraction	20	0.0741	1.482	0.10 ± 0.002	2.05	1.383	99.80	4.42
	Acetone fraction	10	0.1302	1.302	0.58 ± 0.055	5.844	4.48	284.51	14.31
Culture 3	Crude enzyme	195	0.0366	7.137	0.02 ± 0.002	4.153	0.581	100	1
	Acid fraction	20	0.963	1.926	0.12 ± 0.004	2.418	1.255	58.22	2.16
	Acetone fraction	10	0.1525	1.525	0.39 ± 0.015	3.923	2.572	94.46	4.42

glycosyltransferases, and polysaccharide lyases (Supplementary Table S4). In addition, antiSMASH predicted the presence of biosynthetic gene clusters (BGCs), including non-ribosomal peptide synthetase (NRPS), and polyketide synthase (PKS) BGCs that may be involved in the production of biosurfactants (Supplementary Table S5).

Pairwise comparisons of the S19 genome and type-strain genomes showed that strain 19 belongs to a known species, *Streptomyces vinaceusdrappus*, with a digital DNA-DNA hybridization (dDDH) value of > 70% (Supplementary Table S6). Similar dDDH values were also observed against the type strains of *Streptomyces plicatus* JCM4504 and *Streptomyces geyseriensis* JCM 4962. According to the "List of Prokaryotic Names with Standing in Nomenclature" (https://www.lpsn.dsmz.de; Parte et al. 2020), *S. vinaceusdrappus*, *S. plicatus*, and *S. geyseriensis* are all synonyms of *Streptomyces rochei*. Therefore, *Streptomyces* sp. strain S19 belongs to the known species, *S. rochei*, a fact which was also confirmed by 16S rRNA gene sequence analysis (Supplementary Table S3). A comparison of the DyP-type peroxidase from S19 to DyP-type peroxidases from *S. rochei* strains showed 100% amino acid sequence similarity to the DyP-type peroxidases from strains WAC06128 (isolated from Cancún, Mexico), WAC08401 (isolated from Enugu, Nigeria), WAC05458 (unknown source), and SGAir0924 (isolated from air) (Supplementary Table S7 and Fig. S2). The remainder of the sequences showed 99.09–99.87% similarity to the S19 DyP-type peroxidase, with 99.77% similarity to the DyP-type peroxidase from the *S. rochei* type strain.

Table 4 Composition of canola lignocellulosic waste samples as determined according to the Harper and Lynch (1981) method as described in Sridevi et al. (2015)

Canola sample	Hot water-soluble components (%)	Hot ethanol-soluble components (%)	Lignin (%)	Hemicellulose (%)	Cellulose (%)
Non-autoclaved canola	30	7	5	20	38
Autoclaved canola	28	6	6	20	40

Discussion

Since its discovery, the class actinobacteria has been attracting increasing attention, and its exploitation has been growing steadily. This group of bacteria is diverse and produces many interesting molecules, such as antibiotics and enzymes, for agriculture, biotechnology, and medicine. Peroxidases are oxidative enzymes that are known to have various applications, such as the degradation of phenolic compounds, the degradation of dyes, or the degradation of lignocellulosic compounds (Mielgo et al. 2003; Higuchi 2004; Datta et al. 2017). Peroxidases of plant and fungal origin have been utilized extensively. However, the peroxidases produced by actinobacteria have not been utilized to the same extent since there have been only a limited number of studies reported. Given the large surface area and diversified ecosystems of Algeria, there is huge potential to study peroxidases produced by actinobacterial strains isolated from Algerian environments.

In this study, most of the actinobacterial strains isolated from several soil samples from different regions of Algeria were identified as belonging to the genus *Streptomyces*. Actinobacteria are found universally in soil microflora and represent about 10–50% of the soil microbial community (Olanrewaju and Babalola 2019; Bawazir and Shantaram 2018; Sapkota et al. 2020). Since *Streptomyces* is the most

described group of actinobacteria, representing up to 20% of cultivable soil microbes, it is not surprising that the majority of strains isolated in this study belong to this genus (Olanrewaju and Babalola 2019).

Oxidation of the 2,4-DCP substrate confirmed peroxidase activity for most of the streptomycete isolates. However, low or no production was recorded when ABTS was used as a substrate to quantify peroxidase production. Antonopoulous et al. (2001b) reported that *Streptomyces albus* ATCC 3005 oxidized a range of phenolic compounds, including L-DOPA, 4-chlorophenol, 2,4-DCP, 2,6-dichlorophenol (2,6-DCP), 2,4,5-trichlorophenol, and 2,4,6-trichlorophenol, but not pentachlorophenol, with 2,4-DCP and 2,4,5-trichlorophenol being the most reactive substrates. *Streptomyces* sp. F6616 studied by Tuncer et al. (2009) produced a peroxidase also active against the test compounds L-DOPA, 4-chlorophenol, 2,4-DCP, 2,6-DCP, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, and the dye Azure B, but did not oxidize veratryl alcohol and guaiacol.

The production of extracellular peroxidases is directly linked to the growth of actinobacteria (Tuncer et al. 1999; Niladevi and Prema 2008). This means that all the factors capable of affecting bacterial growth, such as pH, temperature, composition of the medium, and aeration, have a direct impact and play an important role in the production of enzymes. The growth factors of selected peroxidase-producing strains were optimized in this study. Four selected strains (S4, S19, S44, and S45) showed improved production at 37 °C, while S36 showed optimum production at 30 °C. Rajkumar et al. (2013) reported optimum peroxidase production of *Bacillus* sp. at 30 °C, whereas Rao and Kavya (2014) and Musengi et al. (2014) reported that *Bacillus subtilis* and *Streptomyces* sp. BSII#1 had optimum peroxidase production at 37 °C. This variance in optimum temperature for peroxidase production in different species is still unclear (Falade et al. 2019a). All the peroxidases produced by the different microorganisms are optimally produced at mesophilic temperatures, and the results obtained in this study for the five strains tested corresponded to this observation. In addition, actinobacteria are generally terrigenous microorganisms that grow well within the mesophilic temperature range (Falade et al. 2019a). Furthermore, in this study, optimal peroxidase production was observed at pH 7.0 for strains 4, 19, 36, and 44 and at initial pH 6.0 for S45. These results are in accordance with those reported by Rob et al. (1997) and El-Dein et al. (2014), where maximum peroxidase production was noted at pH 7.0 and pH 7.5, respectively, for *Streptomyces avermitilis* UAH30 and *Streptomyces* sp. K37 and pH 6.0 for the production of the *Streptomyces viridosporus* T7A peroxidase (Lodha et al. 1991). However, other reports have shown improved activity at alkaline or acidic pH levels. Musengi et al. (2014) observed that pH 8.0 was most favorable for optimal peroxidase production of *Streptomyces* sp. BSII#1. Additionally, Tuncer et al. (2009) reported that *Streptomyces* sp. F6616 exhibited maximum peroxidase production at pH 8.0, while pH 5.0 was the optimal for peroxidase production by *Raoultella ornithinolytica* OKOH-1 (Falade et al. 2019b). It is essential to consider that for biotechnological applications, it would be more beneficial to produce peroxidases at neutral pH, which in turn saves large volumes of acids and bases required for pH adjustment, thus reducing the cost of peroxidase production (Xia et al. 2008).

A variety of compounds were evaluated for their potential induction of peroxidase production by the top five strains selected in this study. Among the inducers tested, ferulic acid was the prominent peroxidase production inducer for strains 4, 44, and 45 by increasing their production by 9% (from 0.29 ± 0.100 U.ml^{-1} to 0.32 ± 0.060 U.ml^{-1}); 12% (from 0.55 ± 0.018 U.ml^{-1} to 0.62 ± 0.007 U.ml^{-1}); and 248% (from 0.14 ± 0.028 U.ml^{-1} to 0.50 ± 0.039 U.ml^{-1}), respectively. In contrast to results reported in this study, Falade et al. (2019a) reported that ferulic acid was a repressor of peroxidase synthesis in the bacterial strain *Ensifer adhaerens* NWODO-2. The peroxidase production of strains 4 and 45 was also induced, respectively, by paper 48% (from 0.25 ± 0.121 U.ml^{-1} to 0.37 ± 0.050 U.ml^{-1}) and veratryl alcohol 174% (0.11 ± 0.011 U.ml^{-1} to 0.29 ± 0.101 U.ml^{-1}), while inhibition of peroxidase production by S19 and S36 was noted in the presence of all the inducers tested. Musengi et al. (2014) found that peroxidase production by *Streptomyces* sp. BSII#1 was significantly increased in the presence of veratryl alcohol in the culture medium. However, no further data could be found in the case of peroxidase induction in actinobacteria. The results of the different studies indicate that inducers play an important role in enhancing production of ligninolytic enzymes. However, their differences indicate that ligninolytic microorganisms may respond differently to the same inducer, suggesting that the inducers may be organism-specific and is most probably driven by the environment the organism was sourced from.

Since lignocellulose can act as an inducer for ligninolytic enzymes such as peroxidases, the effect of a lignocellulosic waste (canola) on peroxidase production was evaluated. In order to understand how CLW can act as an inducer, the composition of the CLW used in this study was determined. An analysis of the composition of steam exploded CLW (autoclaved) showed the presence of a low lignin content (6%) and a high cellulose content (40%), which is slightly higher than that of untreated CLW (5% and 38%, respectively); both samples had a similar hemicellulose content (20%), which is supported by the study performed by Adapa et al. (2011). Garmakhany et al. (2014) also found that, with the exception of hemicellulose, lignin and cellulose levels were higher in steam pre-treated canola. Pretreatment of biomass by steam explosion is described by Chornet and

Overend (1991) as a process with thermal, mechanical, and chemical effects. The thermal effect is due to the heat, which in the form of steam penetrates and wets the lignocellulosic material. This moisture in turn hydrolyzes the acetyl groups of the hemicellulosic fractions of the biomass, which is considered a mechanical effect. Organic acids such as acetic and uronic acids are formed during the hydrolysis of the biomass by the moisture. These resulting acids in turn catalyze the depolymerization reaction of the hemicellulose, releasing xylan and a limited amount of glucan. This action characterizes the chemical effect of the steam explosion. However, the levels of the components of CLW (treated and untreated) obtained in our present study and the studies conducted by Adapa et al. (2011) and Garmakhany et al. (2014) are different. This difference in the composition of the same substrate may be due to different analytical methods (Foyle et al. 2007), but also the composition may be influenced by the biomass harvest season and *Brassica* species analyzed (Wyman et al. 2011; Van Dyk and Pletschke 2012).

Besides the effect of pH, temperature, incubation period, and inducers, aeration also plays a key role in enzyme production. An improved enzyme activity was obtained in S19 cultures grown in baffled flasks, under agitation, with an activity of 0.170 ± 0.007 U.ml^{-1} after 5 days of incubation compared to an activity of 0.085 ± 0.004 U.ml^{-1} in standard Erlenmeyer flasks. The baffles in the flasks increase the agitation and turbulence of the cultures and thus increase the degree and efficiency of aeration (Musengi et al. 2014). As a result, improved growth of S19 was observed in the baffled flasks due to improved aeration, resulting in higher peroxidase production compared to the same strain grown in the same volume and conditions in the Erlenmeyer flasks. The same effect on peroxidase production by *Streptomyces* sp. BSII#1 was also observed by Musengi et al. (2014).

The acidification of the S19 supernatant by adding HCl proved to be very effective in the purification of the peroxidase as it significantly increased the purity of the enzyme. Musengi et al. (2020) also reported the efficiency of acidification in the purification of the DyP-type peroxidase produced by *Streptomyces* sp. BSII#1. However, this acidification step is not commonly used in the purification of peroxidases produced by actinobacteria (Musengi et al. 2020). The effect of acetone precipitation on the purification of the enzyme was also demonstrated in this study resulting in an increase in purity by a factor of between 2 and 3 for the acid fractions of the samples studied. These results are consistent with those obtained by Musengi et al. (2020) where acetone precipitation increased the purity of the peroxidase studied by a factor of 1.23. The red/red-brown color observed in the acetone fraction may be due to a chromogenic cofactor and presumably the heme element (Van Bloois et al. 2010; Fodil et al. 2012; Musengi et al. 2020). The purification yield noted an increase in the acetone fractions in all samples, exceeding 100%. This may be due to the presence of culture medium components, lipids, and other proteins that could interfere with enzyme activity by preventing direct access to the substrate, resulting in low total activity in the crude enzyme sample. After acid and acetone precipitation, enzyme access to the substrate is facilitated by removing inhibitors present in the crude enzyme preparation; therefore, total activity increases in the acetone fraction, resulting in yields greater than 100%. The SDS-PAGE analysis of the concentrated fraction obtained with size exclusion chromatography gave a distinct and singular visible band with an estimated molecular weight of ~ 22 kDa. Peroxidases produced by actinobacteria that have been studied by other researchers had different molecular weights such as peroxidases produced by *Streptomyces thermoviolaceus* with molecular weights of 82 and 60 kDa (Iqbal et al. 1994); *Streptomyces* sp. AM2 producing extracellular peroxidases of 40.3 and 25.2 kDa (Fodil et al. 2011); *Streptomyces* sp. AH4 which produces a peroxidase of 60.2 kDa (Fodil et al. 2012); *Streptomyces avermitilis* which produces a DyP-type peroxidase of 50 kDa (Sugawara et al. 2017); and *Streptomyces albidoflavus* BSII#1 which produces an extracellular peroxidase of 46 kDa (Musengi et al. 2020).

The genome study and analysis revealed that S19 produces six peroxidases, with different localizations. Five out of the six peroxidases are cytoplasmic peroxidases (catalase-peroxidase KatG; Thiol peroxidase, Bcp-type; 4-carboxymuconolactone decarboxylase domain/alkylhydroperoxidase AhpD family core domain protein; glutathione peroxidase; thioredoxin peroxidase; and a non-heme chloroperoxidase), while only the DyP-type peroxidase is secreted into the extracellular medium. In addition, the TAT signal peptide was only detected in the amino acid sequence of the predicted DyP-type peroxidase. Based on these predictions, it is proposed that the peroxidase studied in this work is a DyP-type peroxidase. The single band observed on the SDS-PAGE gel (~ 22 kDa) is most probably either a monomer or degradation product of the predicted DyP-type peroxidase, especially since RNA studies showed the expression of the gene encoding for the DyP-type peroxidase occurred when extracellular peroxidase activity was detected (Supplementary Information). A similar observation was made by Musengi et al. (2020). In addition, a multiple sequence alignment and pairwise analysis of the DyP-type peroxidase amino acid sequence from S19 and the DyP-type peroxidase amino acid sequences from available *S. rochei* genome sequences showed a low variability in the amino acid sequences. Since the strains were all isolated from diverse environments (Supplementary Table S7), the slight difference in amino acid sequence may be due to environmental pressures but may also be a result of sequencing error/the use of different sequencing platforms and genome assembly tools. Regardless, it is clear that strains representing the same species

have the potential to produce the same DyP-type peroxidase, which ultimately may exhibit the same (or very similar) biochemical properties.

Peroxidases are classified as enzymes that provide "auxiliary activities" in the degradation of lignocellulosic materials. Lignocellulose is mainly composed of cellulose, hemicellulose, and lignin (Andlar et al. 2018). The quantities of these constituents differ according to the origin of the material. The different bonds formed between cellulose, hemicellulose, and lignin give lignocellulose a compact structure, which makes this material a very complex substrate for enzymes (Stech et al. 2014). The degradation of lignocellulosic material in ecosystems such as soil is achieved by the multiple and synergetic action of a set of oxidative and hydrolytic enzymes that break the bonds within and between cellulose, hemicellulose, and lignin, with those targeting cellulose and hemicellulose being termed carbohydrate active enzymes (CAzymes) (Payne et al. 2015). An analysis of the S19 genome shows a great collection of glycosyltransferases (GT), carbohydrate esterases (CE), polysaccharide lyases (PL), glycoside hydrolases (GH), various carbohydrate binding modules in association with these CAzymes, and other auxiliary activity enzymes, including lytic polysaccharide monooxygenases. S19 therefore has the genetic toolkit required for the degradation of lignocellulosic material, as is evident in the utilization of the CLW for growth and peroxidase production.

Biosurfactants have many fields of application such as the detergent industry, environmental applications (bioremediation, oil dispersant), pharmaceutical industry, textile industry, petroleum industry, agricultural application (biocontrol), bioprocessing industry (microemulsions; biotransformation), and the cosmetic industry (Henkel et al. 2012; Marchant and Banat 2012; Lawniczak et al. 2013; Reis et al. 2013). Many authors have studied the involvement of surfactants in the degradation of lignocellulosic material and have reported the positive effect of surfactants in the hydrolysis of lignocellulosic material (Qing et al. 2010; Sipos et al. 2011; Parnthong and Kungsanant 2014; Hsieh et al. 2015; Min et al. 2015; Li et al. 2016; Mesquita et al. 2016). A significant increase in the enzymatic conversion of cellulose to soluble sugars has been reported in the presence of surfactants (Eriksson et al. 2002; Kristensen et al. 2007; Bardant et al. 2013; Hsieh et al. 2015; Min et al. 2015; Li et al. 2016). Based on the studies and observations of different authors, several mechanisms of action of surfactants in enzymatic hydrolysis of lignocellulosic biomass have been proposed. The nature of the substrate could be modified by the surfactant by eliminating the inhibitory lignin barrier or by increasing the available surface area of the cellulose (Helle et al. 1993). The presence of biosynthetic gene clusters in the S19 genome that could potentially encode for biosurfactant production further enhances the genetic toolkit of the strain for the degradation of lignocellulosic materials. However, the ability of the strain to produce these compounds would have to be confirmed by laboratory-based studies.

Conclusion and future work

In this study, a range of isolates was obtained from several telluric samples from Algeria. The analysis of the genome of *Streptomyces* sp. S19 showed that this strain has a promising involvement in the degradation of lignocellulosic material, through the presence of genes encoding for various families of CAzymes principally involved in the biodegradation of lignocellulosic material; as well as genes with the potential to code for the production of biosurfactants also involved in the hydrolysis of lignocellulosic material. Various streptomycete strains have the ability degrade lignocellulytic materials (Saini et al. 2015). Different *S. rochei* strains have been identified to have the ability to degrade lignocellulosic materials where an extracellular peroxidase played a key role in lignin solubilization (Pasti et al. 1990). This study is the first to report the production of an extracellular DyP-type peroxidase by a *S. rochei* strain. It is also important to highlight that even though strains 4, 19, 44, and 45 all represented *S. rochei* strains, they all exhibited different properties during peroxidase production optimization, an aspect also observed by Pasti et al. (1990). In total, six genes encoding for peroxidases were identified in the partial genome sequence of *Streptomyces* sp. S19. The DyP-type peroxidase was the only extracellular peroxidase produced by the strain and will be targeted in future studies for recombinant expression, as well as structural and biochemical characterization to determine the biotechnological potential of this peroxidase. In addition, in order to gain more insight into the DyP-type peroxidases produced by the other *S. rochei* strains targeted in this study, strains 4, 44, and 45 will also be subjected to genome sequencing and any DyP-type peroxidases present targeted for recombinant expression and biochemical characterization.

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Data availability All strains reported in this study are readily available at no cost to the requester. The Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JAIFOQ000000000. The version described in this paper is version JAIFOQ010000000.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no competing interests.

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