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RESEARCH ARTICLE OPEN ACCESS

Sawdust Biodegradation: Cellulase and Ligninase Production via Submerged Fermentation and Glucose Production by *Streptomyces lazureus*

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Abstract

Forty streptomycetes were isolated from the soils of farms in Riyadh. Only three isolates (St-2, St-9, and St-25) exhibited cellulolytic-ligninolytic activity, with the St-9 isolate exhibiting the highest activity and identified as *Streptomyces lazureus.* The optimum environmental and nutritional conditions for maximum cellulolytic-ligninolytic activity were determined as fermentation batch of pH of 7.5, inoculum size of 200 µL of bacterial suspension, incubation period of 7 d, and incubation temperature of 30°C. In addition, the fermentation batch contained peptone and yeast extract as the best nitrogen and carbon sources, respectively. Cellulase and ligninase were purified via gel filtration column chromatography. The accumulated end-product of the fermentation process was glucose powder, which was subjected to a partial characterization process. The glucose powder appeared white, melted at 146°C, was highly soluble in water, slightly soluble in ethanol, and insoluble in ethyl ether. The glucose solution appeared clear without precipitates and had a low electric conductivity of 15 µS.cm^{-1}.

Keywords: Biodegradation, Cellulose, Glucose, Ligninase, Streptomycetes

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INTRODUCTION

Sawdust is a waste material generated from the timber industry and is composed of fine wood particles. Sawdust may be an environmental hazard with misuse; some countries burn huge quantities of sawdust, causing considerable air pollution. Moreover, in tropical regions there is a danger of sawdust piles igniting at high temperatures.[1] Sawdust is also a common waste product of conifers and in Mexico and USA, is used as biomass during bioremediation in biofuel production.[2,3] Sawdust bioremediation and briquette production reduce air pollution and provide a useful and popular commodity.[4] Sawdust is converted to biofertilizers by composting, which ties up nitrogen for 180 d in soil.[5]

Sawdust is composed of carbon (60.8%), hydrogen (5.2%), oxygen (33.8%), nitrogen (0.9%), and polysaccharides (5%–10%).[6] It is a non-degradable organic waste because of its low nitrogen content and high lignin and cellulose content. Some microorganisms, especially Actinobacteria, effectively decompose sawdust with nitrogen supplementation.[7,8] Sawdust decomposition depends on the complete hydrolysis of lignin and cellulose by ligninase and cellulase, respectively. Lignin and cellulose are more complex polysaccharides with a high molecular weight, and cellulose has approximately 2×10^3 to 10^4 glucose monomers and a molecular weight ranging from 2×105 to 2.4×106 mole.[9,10]

Actinobacteria are gram-positive bacteria, with high guanine and cytosine content in DNA. They are widely prevalent in diverse habitats, even in extreme habitats, owing to their effective metabolites, including hydrolyzing enzymes that degrade complex organic substances. Actinobacteria are saprophytes that decompose recalcitrant organic substances, such as plant and animal debris. They are high producers of bioactive metabolites, including hydrolyzing enzymes.[11,12] According to Bergey's Manual of Systematic Bacteriology,[13,14] *Streptomyces* is an Actinobacteria genus belonging to the family Actinobacteriaceae. Different species of *Streptomyces*, such as *S. purpureus, S. albidoflavus, S. antibioticus, S. albus,* and *S. cyaneus* can grow on potato dextrose agar and Sabouraud agar containing different concentrations of sawdust (0.5, 1.0, and 1.5 mg/100 mL) as the sole carbon source.[15] Sawdust biodegradation depends on the release of a wide array of hydrolyzing enzymes, including cellulase and ligninase, which hydrolyze cellulose and lignin, respectively.[16,17] Environments rich in polysaccharides, such as soil, are a favorable source of sawdust-decomposing microorganisms, where effective hydrolyzing enzymes are continually being released.[18] This research aimed to convert sawdust to glucose during fermentation using *Streptomyces*, a high producer of cellulase and ligninase, which were subjected to extraction and purification.

MATERIALS AND METHODS

Collection of Soil Samples

Only five samples were collected from farm soils in Riyadh city. The soil was collected at a depth of 30 cm after the superficial layer was removed. Soil samples were placed in sterile polyethylene bags, transported to the laboratory, and left in air overnight at room temperature to dry. Dry soil (100 g) was added to 1 L of sterile distilled water, and the mixture was filtered through a sterile gauze to remove soil particles. The soil extract was diluted in sterile distilled water using the serial dilution method (10^{-1}–10^{-5}).

Isolation of *Streptomyces* Bacteria

Streptomycetes were isolated using inorganic salt starch agar medium (International *Streptomyces* Project medium 4 [ISP 4]) containing nystatin (50 µg.mL^{-1}) to prevent fungal contamination. The agar plates were inoculated with diluted fractions of soil extract (10^{-1} to 10^{-5}) using the spreading method. The plates were incubated at 30°C for 14 d, and each colony was streaked on the same medium, followed by incubation at 30°C for 7 d.

Screening Test of Cellulolytic-ligninolytic *Streptomyces* Bacteria

Pretreatment of Sawdust Waste

Sawdust heaps were collected from various timber industry sites in Riyadh. Metals were eliminated from sawdust heaps using a magnetic separator.[19] The sawdust heap was thoroughly ground to form a soft powder capable of acid hydrolysis,[20] and then left exposed for 7

d to be completely dried. It was then stored in polyethylene bags away from humidity, which was not allowed to exceed 14%.[21]

Acid Hydrolysis

The treated sawdust heap was hydrolyzed using 100 mL of absolute HCl and H2SO4 (0.6, 6, and 11 M) at 25°C. The treated sawdust (100 g) was added to 100 mL of different concentrations of absolute HCl and H2SO4 and left for 48 h.[21-23] The mixtures were then placed in a conical flask (1 L) and supplemented with distilled water to neutralize the acidity, after which they were filtered through filter paper (Whatman 1; Sigma-Aldrich). Glucose was detected in each filtrate using Benedict's reagent. Activated charcoal (50 g) was added to the filtrate containing glucose and left for 24 h to detoxify the toxic compounds formed during the reaction and to inhibit microbial activity.[24] The pH of each filtrate was adjusted to be in the range of 4.8–6 using 9 M NaOH.[25]

Cellulolytic Activity

The liquid medium (ISP 4) was inoculated with 1 mL of spore suspension of *Streptomyces* isolate, and incubated accompanied by shaking at 120 rpm at 30°C for 7 d. Each bacterial suspension (10 mL) was centrifuged at 5,000 × g for 20 min at 4°C. A clear upper layer containing the enzyme was used for enzymatic activity assays. Carboxymethylcellulose activity was assayed according to the method described by Mandels et al.[26] The reducing sugars produced were assayed using a standard, 3,4 dinitrosalicylic acid with glucose (Sigma-Aldrich). Hydrogen peroxide (50 mmol, 200 µL) was added to initiate the reaction, which was assayed at 510 nm per minute using a UV spectrophotometer (Beckman Coulter). The absorption coefficient 21.647 $M^{-1}.cm^{-1}$ was used to calculate the enzyme activity.[27]

Lignolytic Activity

Ligninase activity was assayed according to the method detailed in Kizhekkedathu et al.[28] using a standard, 2,4-dichlorophenol (Sigma-Aldrich). The reaction mixture (1 mL) was supplemented with 200 µL potassium phosphate buffer (0.1 M, pH 7), 25 mmol 2,4- dichlorophenol, 16 mmol 4-amino-antipyrine, and culture filtrate. Hydrogen peroxide (50 mmol, 200 µL) was added to initiate the reaction, which was assayed at 510 nm per minute using a UV spectrophotometer (Beckman Coulter). The absorption coefficient 21.647 $M^{-1}.cm^{-1}$ was used to calculate enzyme activity.

Identification of the most Potent *Streptomyces* Isolate (St-9)

Classical Method

The most potent cellulolytic-ligninolytic *Streptomyces* isolate (St-9) was identified according to the method described by Shirling and Gottlieb.[29] The isolate was cultured on agar medium (ISP 4) for 7 d in preparation for scanning electron microscopy. The medium was streaked with bacterial spores and incubated for 7 d at 35°C. Bacterial growth was collected, fixed in glutaraldehyde (2.5% v/v), washed with water, and refixed in osmium tetraoxide (1% w/v) for an hour. The specimen was washed twice with water, dehydrated in ethanol, dried in a dryer (Polaron E3000), coated with gold, and examined using a JEOLISM 541OLV scanning electron microscope at 15 kV.

Genetic Method

The 16S rRNA sequencing gene was carried out using a thermocycler (Perkin Elmer Cetus Model 480; PerkinElmer, Inc.) and universal primers 27f (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1525r (5'-AAG GAG GTG ATC CAG CC-3'). The reaction conditions were as follows: 94°C for 5 min; 35 cycles of 94°C for 60 s, 55°C for 60 s, 72°C for 90 s; and 72°C for 5 min. The product was sequenced using a BigDye Terminator Cycle Sequencing Kit (PE Applied Biosystems, USA) on an ABI 310 automated DNA sequencer (Applied Biosystems). The homology of the 16S rRNA sequence of the isolate was analyzed using the Basic Local Alignment Search Tool program of the GenBank database.[30]

Optimization of Maximum Activity of Cellulase and Ligninase

The environmental conditions were optimized according to the method described by Isenberg[31] and Van Derzant and Splittstoesser[32] to yield the maximum activity of cellulase and ligninase enzymes.

Effect of Inoculum Size

The ISP 4 liquid medium was prepared, dispensed into conical flasks (100 mL/flask), and autoclaved. Each autoclaved conical flask was inoculated with a specific inoculum (20 to 220 µL) of the filtrate of the most potent *Streptomyces* isolate (St-9). The flasks were incubated with shaking at 120 rpm and 30°C for 7 d. Cellulase and ligninase activities were assayed for each inoculum size.

Effect of Incubation Period

ISP 4 liquid medium was prepared (100 mL), autoclaved, inoculated with a filtrate of *Streptomyces* isolate St-9, and incubated with shaking at 120 rpm and 30°C for different periods (5–21 d). Cellulase and ligninase activities were assayed for each incubation period.

Effect of Incubation Temperature

ISP 4 liquid medium was prepared and dispensed into conical flasks (100 mL/flask), autoclaved, inoculated with a filtrate of *Streptomyces* isolate St-9, and incubated with shaking at 120 rpm and different temperatures (20–45°C) for 7 d. Cellulase and ligninase activities were assayed at each incubation temperature.

Effect of pH Value

ISP 4 liquid medium was prepared and dispensed into conical flasks (100 mL/flask), and the pH of each flask was adjusted to a specified value ranging from 6.5–9, after which the flasks were autoclaved. Each autoclaved conical flask was inoculated with the best inoculum size of the filtrate of the most potent *Streptomyces* isolate St-9, placed in a shaker 120 rpm, and incubated at the best incubation temperature for the best incubation period. Cellulase and ligninase activities were assayed as described above for each pH value.

Effect of Nitrogen Source

Potassium nitrate-free ISP 4 liquid medium was prepared and dispensed into conical flasks (100 mL/flask). Potassium nitrate was substituted with a specific nitrogenous substance in each flask, such as peptone, urea, casein, and beef extract. The amount of the alternative nitrogenous substance was calculated using the following equations:

$$M = \frac{W \times \%\text{ of N in an alternative nitrogenous substance}}{100}$$

$$\%\text{ of N in an alternative nitrogenous substance} = \frac{\text{Molecular mass}}{\text{Molecular weight}} \text{ X } 100$$

where M is the required amount of an alternative nitrogenous substance and W is the weight of the original nitrogenous substance.

The optimal pH value was adjusted for all flasks, which were then autoclaved. Autoclaved flasks were inoculated with the best inoculum size of the filtrate of *Streptomyces* isolate St-9, placed in a shaker at 120 rpm, and incubated at the best incubation temperature for the best incubation period. Cellulase and ligninase activities were assayed as described above for each nitrogenous substance.

Effect of Carbon Source

Soluble starch-free ISP 4 liquid medium was prepared and dispensed into conical flasks (100 mL/flask). Soluble starch was substituted with a specific carbonaceous substance in each flask, such as yeast extract, molasse, rice straw powder, and oat powder. The amount of the alternative carbonaceous substance was calculated using the following equations:

$$M = \frac{W \times \%\text{ of N in an alternative carbonaceous substance}}{100}$$

$$\%\text{ of N in an alternative carbonaceous substance} = \frac{\text{Molecular mass}}{\text{Molecular weight}} \text{ X } 100$$

where M is the required amount of an alternative carbonaceous substance and W is the weight of the original carbonaceous substance.

The optimal pH value was adjusted for all flasks, which were then autoclaved, after which they were inoculated with an appropriate inoculum size of *Streptomyces* isolate St-9 filterate, and placed in a shaker at 120 rpm, for the appropriate time at the appropriate temperature. Cellulase and ligninase activities were assayed as described above for each carbonaceous substance.

Submerged Fermentation of Sawdust Wastes Process

Sawdust heap (50 g) was placed in a conical flask (1 L) containing 500 mL of tap water. Equivalent weights of peptone and yeast extract were added, and the pH was adjusted to 7.5. The fermentation batch was inoculated with 200 $\mu L.mL^{-1}$ of bacterial suspension and incubated with shaking at 120 rpm and 30°C for 7 d.

Preparation of Cell Free Extract

The fermentation batch was filtered through a sterile gauze. The filtrate was centrifuged at 5,000 × g for 10 min, and the supernatant containing cellulase and ligninase was collected in a sterile glass test tube and stored at 4°C.

Extraction and Purification of Cellulase and ligninase

Enzyme Precipitation

A wide range of saturated ammonium sulfate (10%–90%) was used to precipitate cellulase and ligninase. The fraction was incubated for 2 h at 4°C and centrifuged at 5,000 × g for 20 min at 4°C. The precipitate was then dissolved in phosphate buffer (10 mL; pH 7.5) and centrifuged. The pellet was dissolved in phosphate buffer (10 mL, pH 7.5). Cellulolytic and ligninolytic activities were assayed for each fraction. The active fraction was dialyzed overnight in distilled water.

Estimation of Total Protein Content

One milliliter of fraction containing the enzyme was placed in a sterile glass test tube supplemented with 5 mL of alkaline copper reagent, and incubated at room temperature for 15 min.[33] The reaction fraction was mixed with diluted folin reagent (0.5 mL) and incubated at room temperature for 30 min. The optical density of the reaction was measured at 280 nm using a UV-spectrophotometer (Beckman Coulter) with a mixture of a copper reagent (5 mL) and diluted folin reagent (0.5 mL) as the blank.

Gel Filtration

Cellulase and ligninase enzymes were purified using the gel filtration method, wherein Sephadex G-200 (10 g) was dissolved in of phosphate buffer pH 7.5 (400 mL), boiled in a water bath for 6 h, cooled to 50°C, and packed

Table 1. Screening test for releasing cellulolytic-ligninolytic activity

Isolate	Activity (mg/ml)		Color series	Isolate	Activity ($mg.ml^{-1}$)		Color series
	Cellulase	Ligninase			Cellulase	Ligninase	
St-1	0.412	None	Grey	St-21	0.311	None	Grey
St-2	0.325	0.274	Grey	St-22	0.305	None	Grey
St-3	0.357	None	Grey	St-23	0.408	None	Green
St-4	None	None	Grey	St-24	0.125	None	Green
St-5	0.215	None	Grey	St-25	0.415	0.342	Green
St-6	0.294	None	Grey	St-26	None	0.102	Green
St-7	0.354	None	Grey	St-27	None	0.147	Green
St-8	0.425	None	Grey	St-28	None	0.271	Green
St-9	0564	0.482	Grey	St-29	None	0.232	Green
St-10	None	0.211	Grey	St-30	None	None	Green
St-11	None	0.384	Grey	St-31	None	None	Green
St-12	None	None	Grey	St-32	0.353	None	Green
St-13	None	None	Grey	St-33	None	0.211	Green
St-14	None	0.388	Grey	St-34	None	0.202	Green
St-15	None	0.247	Grey	St-35	None	None	Yellow
St-16	0.335	None	Grey	St-36	None	None	Yellow
St-17	0.247	None	Grey	St-37	None	None	Yellow
St-18	0.415	None	Grey	St-38	0.348	None	Yellow
St-19	0.259	None	Grey	St-39	0.314	None	Yellow
St-20	0.349	None	Grey	St-40	0.254	None	Yellow

in a column (2.5×50 cm). The active fractions were pooled, dialyzed, and gently poured into a Sephadex G-200 chromatography column, which was eluted with phosphate buffer (pH 7.5) at a flow rate of 5 mL/25 min.[34]

Partial Characterization of Purified Glucose

The glucose powder was partially characterized according to the method described by Shendurse and Khedkar.[35]

Detection of Glucose

Only two or three drops of an extract containing glucose were added to 5 mL of boiling Fehling's reagent, and a red precipitate indicated a positive result.

Appearance Test

A small amount of glucose was examined to discriminate the color of the solid substance, and consistency was detected.

Clarity and Color Test

Test solution: Glucose (10 g) was dissolved in 15 mL of water by heating in a water bath, and the solution was cooled to room temperature. Glucose was completely dissolved in water, and a clear solution was observed. The optical density of the glucose solution was measured and found to be equal to that of the control solution. Control solution: Di-cobalt chloride (2.5 mL), tri-iron chloride (6 mL), and di-copper sulfate (1 mL) were thoroughly mixed and diluted to 1000 mL with diluted hydrochloride solution (1:10).

Melting Point Test

A small amount of glucose was introduced into a capillary tube, which was attached to the

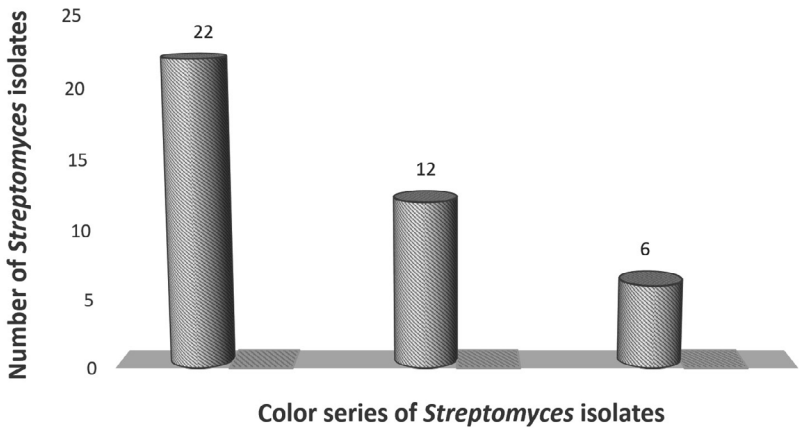

Figure 1. The color series of *Streptomyces* isolates

Table 2. Discriminative cultural characteristics of *Streptomyces* isolate St-9

Medium	Growth rate	Color		
		Aerial mycelia	Substrate mycelia	Diffusible Pigment
TYEB	Good	Light grey	Yellowish brown	Yellowish brown
YMEA	Poor	Light grey	Grayish yellow	None
OMEA	Good	Light grey	Grayish brown	Grayish brown
ISSA	Good	Pinkish grey	Yellowish brown	None
GAA	Good	Light grey	Yellowish brown	None
PYEIA	Moderate	White	Yellow	Light grey
TA	Good	White	Yellowish brown	Yellowish brown

TYEB; Tryptone Yeast Extract Broth, YMEA; Yeast-Malt Extract Agar, OMEA; Oat-Meal Extract Agar, ISSA; Inorganic Salt Starch Agar, GAA; Glycerol Asparagine Agar, PYEIA; Peptone Yeast Extract Iron Agar, TA; Tyrosine Agar.

stem of a thermometer centered in a heating bath that was heated slowly, and the melting point was observed at the beginning and completion of melting.

Solubility Test in Water

Glucose powder (1 g) was added to 10 mL of water. The mixture was then stirred at room temperature. A positive result was indicated by complete and easy dissolution of glucose in water, that is, a very clear solution with no precipitate.

Solubility Test in Ethanol

Glucose powder (1 g) was supplemented with 20 mL of ethanol, and the solution was boiled in a reflux condenser. A positive result was indicated by a clear solution.

Solubility Test in Ethyl Ether

Glucose powder (1 g) was supplemented with 20 mL of ethyl ether and boiled in a reflux condenser. A positive result was indicated by a clear solution.

Conductivity Test

Glucose powder (20 g) was dissolved in freshly boiled and cooled distilled water to a final volume of 100 mL. The conductivity of the solution was measured using a conductivity meter at 25°C with gentle stirring using a magnetic stirrer. The acceptable value of conductivity should not exceed 20 $\mu S.cm^{-1}$.

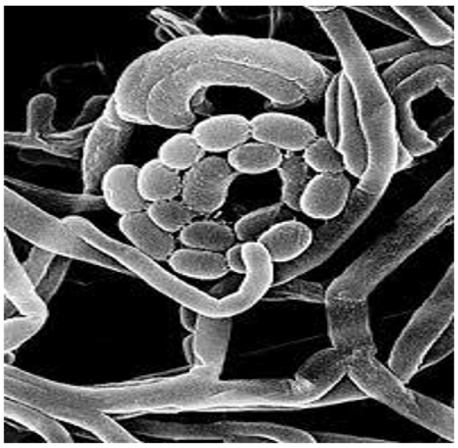

Figure 2. Scanning electron micrograph of *Streptomyces* isolate St-9 (X = 25000)

RESULTS

Forty unrepeated *Streptomyces* isolates were obtained from five soil samples. Three color series of *Streptomyces* isolates with discriminative cultural characteristics were observed (Figure 1). These isolates were subcultured on agar plates and agar slopes and stored at 4°C.

Forty streptomycetes were screened for production cellulolytic-ligninolytic activities. Only three isolates exhibited cellulolytic-ligninolytic activity: two had gray aerial mycelia indicating St-2 and St-9, and one had green spore mass indicating St-25. The St-9 isolate was the most potent isolate. Eight isolates did not exhibit cellulolytic or ligninolytic activity. Cellulolytic activity was produced by 19 isolates, while ligninolytic activity was produced by 10 isolates (Table 1).

The most potent isolate, St-9, was identified to be *S. lazureus*. The isolate was cultivated on seven recommended culture media according to the ISP to determine its cultural characteristics (Table 2). It had approximately the same cultural characteristics on both tryptone yeast extract broth and glycerol asparagine agar media, in which good growth of a light grey spore mass and yellowish-brown substrate mycelia were observed; however, a yellowish-brown diffusible pigment was observed only on tryptone yeast extract broth medium, and no diffusible pigment was observed on glycerol asparagine agar medium. Poor growth of a light grey spore mass, greyish-yellow substrate mycelia, and no diffusible pigment were observed on yeast–malt extract agar medium. Good growth of light grey spore mass, greyish-brown substrate mycelia, and diffusible pigment were observed on oat-meal extract agar medium. Good growth of pinkish-grey spore mass, yellowish-brown substrate mycelia, and no diffusible pigment were observed on inorganic salt starch agar medium. Moderate growth of a white spore mass, yellow substrate mycelia, and light grey diffusible pigment were observed on peptone yeast extract iron agar medium. Good growth of white spore mass, yellowish-brown substrate mycelia, and yellowish-brown diffusible pigment were observed on tyrosine agar medium.

A spiral spore chain composed of smooth-surfaced ellipsoidal spores was observed on the scanning electron micrograph (Figure 2). Grey

spore masses of the nonmotile isolates were observed (Table 3). The cell wall contained LL-diaminopimelic acid, and no sugar pattern was detected. Amylase, protease, lipase, cellulase, ligninase, pectinase, and catalase were produced, whereas lecithinase and nitrate reductase were not. Melanoid pigment was produced on tryptone yeast extract broth, peptone yeast extract iron agar, and tyrosine agar media. Hydrogen sulfide was not produced, and neither xanthin nor esculin was degraded. The isolate was sensitive to both streptomycin and amoxicillin. Rhamnose, xylose,

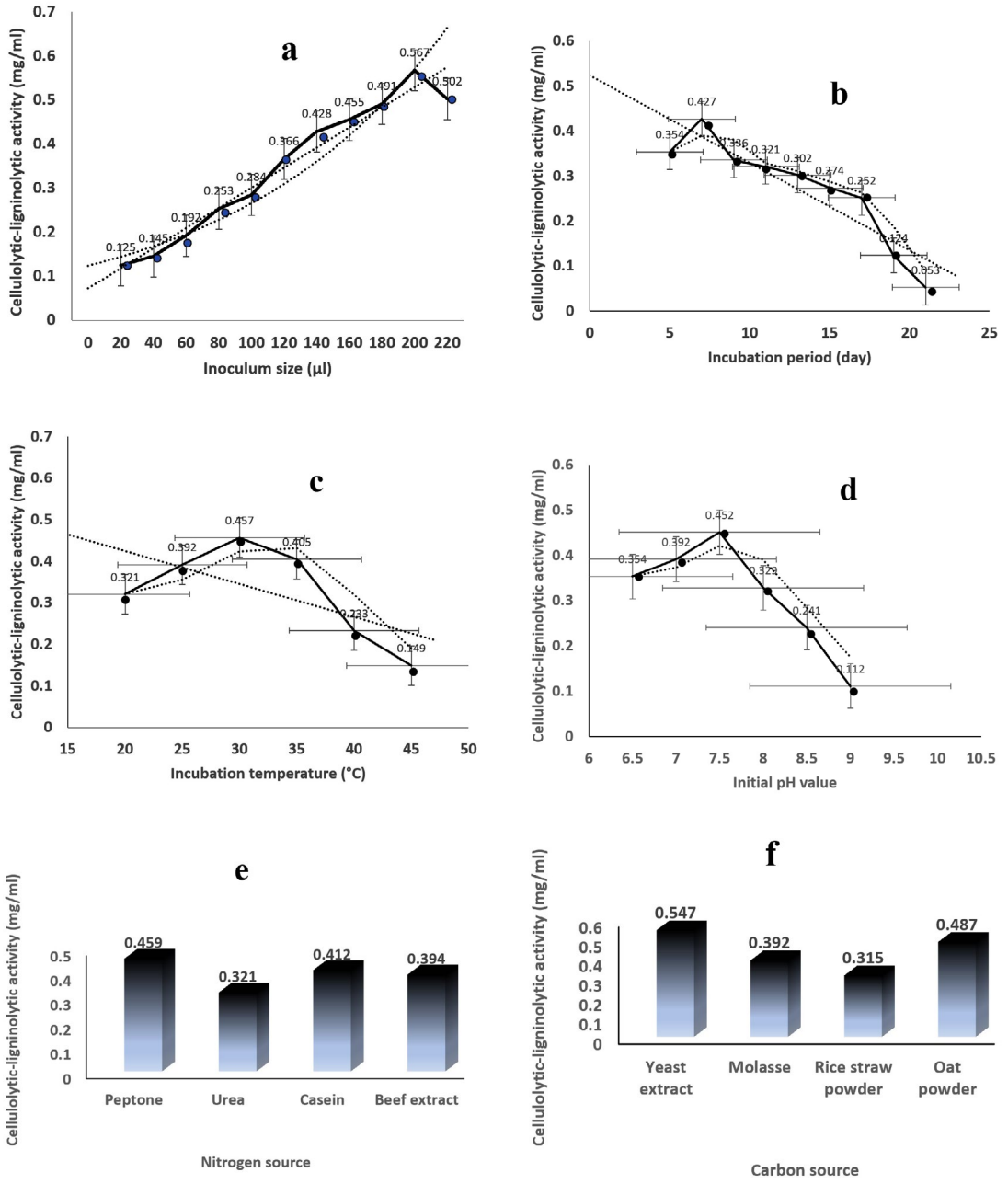

Figure 3. Determination the optimum environmental and nutritional factors to yield a maximum cellulolytic-ligninolytic activity by *Streptomyces* isolate St-9

arabinose, and inositol were not fermented, whereas glucose, fructose, galactose, sucrose, lactose, mannose, mannitol, raffinose, and starch were. In addition, cysteine, proline, valine, alanine, lysine, leucine, tyrosine, and phenylalanine were fermented. Good growth of the isolate was observed over a wide range of NaCl concentrations (1%–10%). For genetic identification, the sequence of the 16S rRNA gene was determined via polymerase chain reaction, which showed that the *Streptomyces* isolate St-9 and *S. lazureus* with accession number NBRC13384/AB184368 in GenBank are 97% similar.

Environmental and nutritional factors were optimized to yield the maximum cellulolytic-ligninolytic activity of *Streptomyces* isolate St-9. Different inoculum sizes (20–220 µL.mL^{-1}) of the bacterial filtrate were screened, and the best inoculum size was recorded at 200 µL, at which maximum activity (0.567 µL.mL^{-1}) was measured (Figure 3a). The best incubation period was recorded on day 7, at which the highest activity (0.427 µL.mL^{-1}) was measured (Figure 3b). The best incubation temperature was found to be 30°C, at which the highest activity (0.457 µL.mL^{-1}) was measured (Figure 3c). Meanwhile, the best pH value was 7.5, at which the highest activity (0.452 µL.mL^{-1}) was measured (Figure 3d). Peptone was found to be the best nitrogen source, at which maximum activity (0.459 µL.mL^{-1}) was measured (Figure 3e), while yeast extract was found to be the best carbon source, at which maximum activity (0.547 µL.mL^{-1}) was measured (Figure 3f).

The fermentation liquid medium containing cellulase and ligninase was filtered and centrifuged to obtain a clear supernatant. The enzyme was precipitated using different concentrations of saturated ammonium sulfate (10–90%). Total protein content (mg), enzymatic activity (mm), and total activity (u) were measured for each fraction. The active fractions were pooled and dialyzed overnight at 4°C. Cellulase was precipitated at 40% and 50% saturated ammonium sulfate (Table 4), where a specific activity of 43.9 and 35.5 u.mg^{-1}, purification fold of 39.1 and 31.6, and yield percentage of 97.2 and 86.4, respectively, were observed. Meanwhile, ligninase was precipitated at 40% and 50% saturated ammonium sulfate (Table 5), where a specific activity of 44.4 and 39.4, purification fold of 36.5 and 32.4, and the yield percentage of 94.1 and 90.5, respectively, were observed. Accordingly, cellulase and ligninase were abundantly produced with high activity by *Streptomyces* isolate St-9,

Table 3. Morphological and physiological characteristics of *Streptomyces* isolate St-9

Item	Character	Result
Morphological characteristics	Spore mass	Grey
	Motility	Non-Motile
Cell wall hydrolysis	Diaminopimelic acid (DAP)	LL-DAP
	Sugar pattern	Not detected
Physiological characteristics	Amylase, protease, lipase, cellulase, ligninase, pectinase and catalase	Positive
	Lecithinase and nitrate reductase	Negative
	Melanoid pigment	Positive
	H2S production	Negative
	Degradation of xanthin and esculin	Negative
	Streptomycin and amoxicillin resistance	Negative
	Utilization of glucose, fructose, galactose, sucrose, lactose, mannose, mannitol, raffinose and starch	Positive
	Utilization of rhamnose, xylose, arabinose and inositol	Negative
	Utilization of cysteine, proline, valine, alanine, lysine, leucine, tyrosine and phenylalanine	Positive
	NaCl tolerance (1 – 10%)	Positive

Table 4. Purification parameters of cellulase produced by *Streptomyces* isolate St-9

% $(NH_4)_2SO_4$	Activity (mm)	TA (u)	TPC (mg)	SA (u/mg)	PF	% of yield
Filtrate	40.0	185	165	1.121	1.0	100
10%	0.0	0.0	2.4	0.0	0.0	0.0
20%	0.0	0.0	2.9	0.0	0.0	0.0
30%	0.0	0.0	3.6	0.0	0.0	0.0
40%	35.0	180	4.1	43.9	39.1	97.2
50%	25.0	160	4.5	35.5	31.6	86.4
60%	0.0	0.0	4.9	0.0	0.0	0.0
70%	0.0	0.0	5.1	0.0	0.0	0.0
80%	0.0	0.0	5.4	0.0	0.0	0.0
90%	0.0	0.0	5.9	0.0	0.0	0.0

TA; Total Activity, TPC; Total Protein Content, SA; Specific Activity, PF; Purification Fold

Table 5. Purification parameters of ligninase produced by *Streptomyces* isolate St-9

% $(NH_4)_2SO_4$	Activity (mm)	TA (u)	TPC (mg)	SA (u/mg)	PF	% of yield
Filtrate	32.0	170	140	1.214	1.0	100
10%	0.0	0.0	2.0	0.0	0.0	0.0
20%	0.0	0.0	2.3	0.0	0.0	0.0
30%	0.0	0.0	2.8	0.0	0.0	0.0
40%	25.0	160	3.6	44.4	36.5	94.1
50%	20.0	154	3.9	39.4	32.4	90.5
60%	0.0	0.0	4.6	0.0	0.0	0.0
70%	0.0	0.0	4.8	0.0	0.0	0.0
80%	0.0	0.0	5.4	0.0	0.0	0.0
90%	0.0	0.0	5.7	0.0	0.0	0.0

TA; Total Activity, TPC; Total Protein Content, SA; Specific Activity, PF; Purification Fold

enabling the degradation of sawdust waste and liberation of glucose monomers as end products.

The solid-released glucose was partially characterized (Table 6). A white powder was observed, which melted at 146°C, and the solution was clear with no precipitates. The powder was highly solubilized in water owing to the high polarity and formation of hydrogen bonds and was slightly solubilized in ethanol owing to its low polarity; however, it was insoluble in ethyl ether owing to the lack of polarity and formation of hydrogen bonds. A low electrical conductivity (15 $\mu S.cm^{-1}$) of the glucose solution was measured because of the inability of glucose to ionize during dissolution in water; therefore, the glucose solution was considered to be a nonelectrolyte.

DISCUSSION

Cellulose, hemicellulose, and lignin are the main components of the plant cell wall. The two polysaccharides are nondegradable owing to their rigidity and complexity. Some microorganisms, particularly actinobacteria and *Streptomyces*, effectively degrade these recalcitrant substances in vitro and in vivo.[36] Biodegradation of these polysaccharides by *Streptomyces* and others enhances the maintenance of the ecosystem and the carbon cycle.[37] In particular, Actinobacteria and *Streptomyces* completely degrade recalcitrant substances due to the presence of encoded genes (CAZy) for hydrolyzing enzymes.[38,39]

Table 6. Partial characterization of purified glucose

Characters	Results
Appearance	White powder
Clarity of solution	Clear
Color of solution	Colorless
Melting point	146°C
Solubility in water	Very soluble
Solubility in ethanol	Slightly soluble
Solubility in ethyl ether	Insoluble
Conductivity	15 µS.cm^{-1}

Lignin is more resistant to biodegradation than other polysaccharides of the plant cell wall because its structure contains polyphenols, which inhibit the biodegradation effect.[40] Some microorganisms other than *Streptomyces* effectively degrade polysaccharides, such as *Sphingomonas paucimobilis* SYK-6, *Serratia, Citrobacter, Klebsiella, Paenibacillus, Aneurinibacillus*, and *Bacillus* spp.[41,42] Soil aromatic-degrading bacteria, such as *Pseudomonas putida* and *Rhodococcus* sp. RHA1, efficiently degrade lignin.[43] Air pollution resulting from sawdust burning was the main motivation to eliminate it through the biodegradation process.[44] Lignin biodegradation leads to the release of sugars, biofuels, and other economic products.[45] Identification of *Streptomyces* isolates was based on the determination of cultural, morphological, physiological, biochemical, and genetic characteristics. The cultural characteristics were represented by the growth rate and colors of spore mass, substrate mycelia, and diffusible pigment on different culture media recommended in ISP29 and by Pridham et al.[46] *Streptomyces* was differentiated from other similar anaerobic Actinobacteria and aerobic *Nocardia* and *Rhodococcus*; it was documented as aerobic filamentous, gram positive, and partially acid-fast stain negative.

The removal of sawdust from the environment must be carried out safely using saprophytic microorganisms. Owing to the high cellulose and lignin content in sawdust biomass, its degradation depends on the production of cellulase and ligninase. Actinobacteria, especially *Streptomyces* spp., are distributed in different polysaccharide-rich habitats, such as wood processing plants because of the secretion of hydrolyzing enzymes. Lignocellulosic biomass represents a complex ecosystem in which environmental conditions influence living organisms. Thus, it is very urgent to achieve the strategic goals of prevention of environmental pollution and production of valuable products, especially pharmaceuticals, intended to meet medical field requirements.[47] The lignocellulose-*Streptomyces* bacteria efficiently released sugars. Ventorino et al.[48] reported that the most potent lignocellulosic hydrolyzing strains were *S. argenteus* because they are highly adapted to lignocellulosic ecosystems. Different studies have shown that Actinobacteria, such as *Cellulomonas, Thermobifida,* and *Microbispora,* are high producers of cellulase.[49] A set of cellulases (endoglucanases, exoglucanases, and cellobiases), hemicellulase, and ligninase are produced by *Streptomyces* bacteria, making them the most efficient and commonly used enzymes in lignocellulose biomass degradation.[50,51] *Streptomyces* producing cellulases saccharify different lignocellulose biomasses, such as rice straw and cellulose-rich organic materials.[52]

Alam et al.[53] reported that *Streptomyces omiyaensis* exhibited maximum activity for lignocellulose biomass degradation after 5 d of incubation, while George et al.[54] showed that *Streptomyces noboritoensis* SPKC1 produced the highest lignocellulolytic activity after 8 d of incubation. Jang and Chen[55] revealed that *Streptomyces* T3^{-1} produced the highest lignocellulolytic activity at 50°C. The best nitrogen source for producing lignocellulolytic activity by *Streptomyces* sp. BRC2 was found to be ammonium phosphate. The highest lignocellulolytic activity of *Streptomyces* sp. was observed at pH 6.5–7.5.[56] Kunamneni et al.[57] showed that a high inoculum size lead to a reduction in enzyme yield due to nutrient limitations. Therefore, a low inoculum size may require a longer time to produce the desired enzyme. Conversely, a high inoculum size provided rapid proliferation of microbial biomass. Therefore, the balance between proliferating biomass and substrate utilization yielded maximum enzyme activity.[58]

Abdulkhair et al.[59] reported that seven marine *Streptomyces* isolates exhibited antidiabetic activity due to the production of α-glucosidase inhibitory protein, which was measured spectrophotometrically at 400 nm. The most potent isolate was identified as *Streptomyces*

coelicolor, which showed stable activity for 5 d at 37°C. The highest activity and stability of the antidiabetic compound were determined under optimum environmental conditions, including inoculum size (10 cfu.mL^{-1}.300 µL^{-1}), incubation period (14 d), agitation speed (160 rpm), incubation temperature (30°C), and pH (8.5). Actinobacteria can grow on a sawdust waste and produce different bioactive compounds.[60] Although the useful properties of sawdust and other lignocellulose wastes, they have a hazard effect on the environment and public health.[61] Different microorganisms including actinobacteria inhabit all environments especially the soil, where they degrade the organic polymers during fermentation process.[62]

CONCLUSION

This study showed that *S. lazureus* biodegraded sawdust waste rich in cellulose and lignin and released glucose monomers. *S. lazureus* exhibited the highest lignocellulolytic activity under optimum environmental and nutritional conditions. Sawdust biodegradation and glucose production are considered strategic and economic goals, with the prevention of environmental pollution resulting from sawdust burning and the production of glucose as a valuable pharmaceutical product at a low cost.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript and/or in the supplementary files.

ETHICS STATEMENT

This article does not contain any studies on human participants or animals performed by any of the authors.

REFERENCES

1. Fregoso-Madueno JN, Goche-Telles JR, Rutiaga-Quinones JG, Gonzalez-Laredo RF, Bocanegra-Salazar M, Chavez-Simental JA. Alternative uses of sawmill industry waste. *Rev Chapingo Ser Ciencias For Ambient.* 2017;23(2):243-260. doi: 10.5154/r.rchscfa.2016.06.040
2. Arroyo-Vinueza JS, Reina-Guzman WS. Exploitation of biomass resources in the form of wood waste for steam boiler. *Ingenius Rev Ciencias Tec.* 2016;16:20-29. doi: 10.17163/ings.n16.2016.03
3. Gwenzi W, Ncube RS, Rukuni T. Development, properties and potential applications of high-energy fuel briquettes incorporating coal dust, biowastes and post-consumer plastics. *SN Appl Sci.* 2020;2(1006):1-14. doi: 10.1007/s42452-020-2799-8
4. Diaz-Artigas IJ, Diaz-Concepcinn A, Rodriguez-Pinero AJ, Alfonso-Alvarez A, Tamayo-Mendoza JE. Energy briquettes with sawdust and pine bark. *Ing Energetica.* 2020;41:1-6.
5. Olayinka A, Adebayo A. Effect of pre-incubated sawdust-based cow dung on the growth and nutrient uptake of *Zea mays* L. and on soil chemical properties. *Biol Fert Soil.* 1989;7:176-179. doi: 10.1007/BF00292579
6. Horisawa S, Sunagawa M, Tamai Y, Masuoka Y, Miura T, Terazawa M. Biodegradation of non-lignocellulosic substances II: physical and chemical properties of sawdust before and after use as artificial soil. *J Wood Sci.* 1999;45(6):492-497. doi: 10.1007/BF00538959
7. Erikson KE, Blanchette RA, Ander P. Microbial and enzymatic degradation of wood and wood components. In Timell TE (eds.), Wood Decomposition, 1st Ed. Springer: Berlin, Heidelberg, New York. 1990:41-62. doi: 10.1007/978-3-642-46687-8_1
8. Adeline SY, Ka LP. Indigenous actinomycetes from empty fruit bunch compost of oil palm: Evaluation of enzymatic and antagonistic properties. *Biocat Agr Biotechnol.* 2014;3(4):310-315. doi: 10.1016/j.bcab.2014.03.004
9. Ververis C, Georghiou K, Danielidis D, et al. Cellulose, hemicelluloses, lignin and ash content of some organic materials and their suitability for use as paper pulp supplements. *Biores Technol.* 2007;98:296-301. doi: 10.1016/j.biortech.2006.01.007
10. Kulic GJ, Radojicic VB. Analysis of cellulose content in stalks and leaves of large leaf tobacco. *J Agr Sci.* 2011;56(3):207-215. doi: 10.2298/JAS1103207K
11. Ul-Hassan A, Wellington EM. Encyclopedia of Microbiology, 3rd Ed. Washington, D.C., American Society for Microbiology, USA. 2009.
12. Dhakal D, Pokhrel AR, Shrestha B, Sohng JK. Marine rare actinobacteria: Isolation, characterization, and strategies for harnessing bioactive compounds. *Front Microbiol.* 2017;8(1106):1-13. doi: 10.3389/fmicb.2017.01106
13. Boone DR, Castenholz RW, Garrity GM. The archaea and the deeply branching and phototrophic bacteria. In David RB, Richard W (eds.), Bergey's Manual of Systematic Bacteriology, 2nd Ed. Springer-Verlag, New York. 2001:21-166. doi: 10.1007/978-0-387-21609-6
14. Garrity GM, Bell JA, Lilburn TG. Taxonomic outline of

the prokaryotes. In George MG, Julia AB, Timothy GL (eds.), Bergey's Manual of Systematic Bacteriology, 2nd Ed. Springer, New York. 2004:1-401.
15. Bassima AA, Essra GA, Anmar AA. Test the inhibition activity of local Streptomyces spp. by used sawdust as a carbon source. Conf Pap Sci Coll Mosul Univ. 2019;4:301-309.
16. Sukumaran RK, Singhania RR, Pandey A. Microbial cellulases production, applications and challenges. *J Sci Ind Res.* 2005;64(11):832-844.
17. Wen Z, Liao W, Chen S. Production of cellulase/β-glucosidase by the mixed fungi culture Trichoderma reesei and *Aspergillus phoenicis* on dairy manure. *Proc Biochem.* 2005;40(9):3087-3094. doi: 10.1016/j.procbio.2005.03.044
18. Maki M, Leung KT, Qin W. The prospects of cellulase-producing bacteria for the bioconversion of lignocellulosic biomass. *Int J Biol Sci.* 2009;5(5):500-516. doi: 10.7150/ijbs.5.500
19. Kumar P, Barrett DM, Delwiche MJ, Stroeve P. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Ind Eng Chem Res.* 2009;48(8):3713-3729. doi: 10.1021/ie801542g
20. Leustean I. Bioethanol from lignocellulosic materials. *J Agro-aliment Proc Technol.* 2009;15:94-101.
21. Hayes DJ. An examination of bioreining processes, catalysts, and challenges. *Catal Today.* 2009;145:138-151. doi: 10.1016/j.cattod.2008.04.017
22. Nwakaire JN, Ezeoha SL, Ugwushiwu BO. Production of cellulosic ethanol from wood sawdust. *Agric Eng Int CIGR J.* 2013;15(3):136-140.
23. Olayemi S, Ibikunle A, Olayemi J. Production of ethanol from cassava and yam peels using acid hydrolysis. *Am Sci Res J Eng Technol Sci.* 2019;52(1):67-78.
24. Cao G, Ximenes E, Nichols NN, Zhang L, Ladisch M. Biological abatement of cellulase inhibitors. *Bioresour Technol.* 2013;146:604-610. doi: 10.1016/j.biortech.2013.07.112
25. Mishra A, Ghosh S. Bioethanol production from various lignocellulosic feedstocks by a novel "fractional hydrolysis" technique with different inorganic acids and co-culture fermentation. *Fuel.* 2019;236:544-553. doi: 10.1016/j.fuel.2018.09.024
26. Eveleigh DE, Mandels M, Andreotti R, Roche C. Measurement of saccharifying cellulase. *Biotechnol Biofuels,* 2009;2: 21. doi: 10.1186/1754-6834-2-21
27. Miller GL. Use of dinitro-salicylic acid reagent for determination of reducing sugar. *Anal Chem.* 1959;31(3):426-428. doi: 10.1021/ac60147a030
28. Kizhekkedathu NN, Parukuttyyamma P. Mangrove actinomycetes as the source of lignolytic enzymes. *Actinomycetologica.* 2005;19(2):40-47. doi: 10.3209/saj.19.40
29. Shirling EB, Gottlieb D. Methods for characterization of Streptomyces species. *Int Syst Bact J.* 1966;16:313-340. doi: 10.1099/00207713-16-3-313
30. Prapagdee B, Kuekulvong C, Mongkolsuk S. Antifungal potential of extracellular metabolites produced by Streptomyces hygroscopicus against phytopathogenic fungi. *Int Boil Sci J.* 2008;4(5):330-337. doi: 10.7150/ijbs.4.330
31. Isenberg HD. Clinical Microbiology Procedures Handbook, 3rd Ed. Washington, D.C., American Society for Microbiology, USA. 1992.
32. Van derzant C, Splittstoesser D. Compendium of Methods for the Microbiological Examination of Foods, 3rd Ed. Washington, D.C., American Public Health Association, USA. 1992.
33. Lowry OH, Rosebrough J, Farr AL, Randall RJ. Protein measurements with the folin phenol reagent. *Biol Chem J.* 1951;193(1):265-275. doi: 10.1016/S0021-9258(19)52451-6
34. Andrews P. Estimation of the molecular weight of proteins by sephadex gel filtration. *Biochem J.* 1964;91(2):222-223. doi: 10.1042/bj0910222
35. Shendurse AM, Khedkar CD. Glucose: properties and analysis. In Caballero B, Finglas P, Toldra F (eds.), Encyclopedia of Food and Health, 1st Ed. Elsevier, Academic Press, Oxford. 2015:239-247. doi: 10.1016/B978-0-12-384947-2.00353-6
36. Matulich KL, Martiny JBH. Microbial composition alters the response of litter decomposition to environmental change. *Ecology.* 2015;96(1):154-163. doi: 10.1890/14-0357.1
37. Chater KF, Biro S, Lee KJ, Palmer T, Schrempf H. The complex extracellular biology of *Streptomyces*. *FEMS Microbiol Rev.* 2010;34(2):171-198. doi: 10.1111/j.1574-6976.2009.00206.x
38. Berlemont R, Martiny AC. Phylogenetic distribution of potential cellulases in bacteria. *Appl Environ Microbiol.* 2013;79(5):1545-1554. doi: 10.1128/AEM.03305-12
39. Doroghazi JR, Metcalf WW. Comparative genomics of actinomycetes with a focus on natural product biosynthetic genes. *BMC genomics.* 2013;14(1):6-11. doi: 10.1186/1471-2164-14-611
40. Chandra R, Singh R. Decolorization and detoxification of rayon grade pulp paper mill effluent by mixed bacterial culture isolated from pulp paper mill effluent polluted site. *Biochem Engin J.* 2012;61:49-58. doi: 10.1016/j.bej.2011.12.004
41. Masai E, Katayama Y, Fukuda M. Genetic and biochemical investigations of bacterial catabolic pathways for lignin-derived aromatic compounds. *Biosci Biotechnol Biochem.* 2007;71(1):1-15. doi: 10.1271/bbb.60437
42. Chandra R, Singh S, Reddy MMK, Patel DK, Purohit HJ, Kapley A. Isolation and characterization of bacterial strains *Paenibacillus* sp. and *Bacillus* sp. for Kraft lignin decolorization from pulp paper mill waste. *J Gen Appl Microbiol.* 2008;54(6):399-407. doi: 10.2323/jgam.54.399
43. Ahmad M, Taylor CR, Pink D, et al. Development of novel assays for lignin degradation: Comparative analysis of bacterial and fungal lignin degraders. *Mol Bios.* 2010;6(5):815-821. doi: 10.1039/b908966g
44. Buraimoh OM, Ilori MO, Amund OO. Characterization of lignocellulolytic bacterial strains associated with decomposing wood residues in the Lagos lagoon, Nigeria. *Malays J Microbiol.* 2015;11(2015):273-283. doi: 10.21161/mjm.68814
45. Chen F, Dixion RA. Lignin modification improves fermentable sugar yields for biofuel production. *Nat Biotechnol.* 2007;25(7):759-761. doi: 10.1038/nbt1316
46. Pridham TG, Anderson PE, Foley LA, Lindenfelser E,

Hesseltine W, Benedict RG. A selection of media for maintenance and taxonomic study of Streptomyces. *Antibiot Ann.* 1956;1957:947-953.

47. Dawkins K, Esiobu N. Emerging insights on Brazilian pepper tree (*Schinus terebinthifolius*) invasion: the potential role of soil microorganisms. *Front Plant Sci.* 2016;7:712. doi: 10.3389/fpls.2016.00712
48. Ventorino V, Ionata E, Birolo L, et al. Lignocellulose-adapted endo-cellulase producing Streptomyces strains for bioconversion of cellulose-based materials. *Front Microbiol*. 2016;7:2061. doi: 10.3389/fmicb.2016.02061
49. Saini A, Aggarwal NK, Sharma A, Yadav A. Actinomycetes: a source of lignocellulolytic enzymes. *Enzym Res.* 2015;2015:279-381. doi: 10.1155/2015/279381
50. Amore A, Pepe O, Ventorino V, Birolo L, Giangrande C, Faraco V. Cloning and recombinant expression of a cellulase from the cellulolytic strain *Streptomyces* sp. G12 isolated from compost. *Microb Cell Fact.* 2012;11(1):164. doi: 10.1186/1475-2859-11-164
51. Raweesri P, Riangrungrojana P, Pinphanichakarn P. Alpha-L-arabinofuranosidase from *Streptomyces* sp. PC22: purification, characterization and its synergistic action with xylanolytic enzymes in the degradation of xylan and agricultural residues. *Bioresour Technol.* 2008;99(18):8981-8986. doi: 10.1016/j.biortech.2008.05.016
52. Prasad P, Bedi S, Singh T. *In vitro* cellulose rich organic material degradation by cellulolytic *Streptomyces albospinus* (M0TCC8768). *Malays J Microbiol.* 2012;8(3):164-169. doi: 10.21161/mjm.04312
53. Alam MZ, Manchur MA, Anwar MN. Isolation, purification, characterization of cellulolytic enzymes produced by the isolate *Streptomyces omiyaensis*. *Biol Sci.* 2004;7(10):1647-1653. doi: 10.3923/pjbs.2004.1647.1653
54. George J, Arunachalam R, Paulkumar K, Wesely EG, Shiburaj S, Annadurai G. Characterization and phylogenetic analysis of cellulase producing *Streptomyces noboritoensis* SPKC1. *Interdiscip Sci. Comput Life Sci.* 2010;2(2):205-212. doi: 10.1007/s12539-010-0069-y
55. Jang HD, Chen KS. Production and characterization of thermostable cellulases from *Streptomyces transformant* T3-1. *World J Microbiol Biotechnol.* 2003;19(3): 263-268. doi: 10.1023/A:1023641806194
56. Hossain FMM, Rahman MM, Choudhury N, Malek MA. Extracellular carboxy methyl cellulase and cellobiase of some aerobic bacterial isolates. *Bangladesh J Microbiol.* 1998;15:17-26.
57. Kunamneni A, Permaul K, Singh S. Amylase production in solid state fermentation by the thermophilic fungus *Thermomyces lanuginosus. J Biosci Bioeng.* 2005;100(2):168-171. doi: 10.1263/jbb.100.168
58. Ramachandran S, Patel AK, Nampoothiri KM, et al. Coconut oil cake-a potential raw material for the production of α-amylase. *Bioresour Technol*. 2004;93(2):169-174. doi: 10.1016/j.biortech.2003.10.021
59. Abdulkhair WM, Walaa SA, Bahy RH. Genetic improvement of antidiabetic alpha-glucosidase inhibitor producing *Streptomyces* sp. *Int J Pharm Pharma Sci.* 2018;10(5):77-84. doi: 10.22159/ijpps.2018v10i5.25338
60. Ekaterina VP, Maria ED, Maria MM, et al. The Use of Baikal psychrophilic actinobacteria for synthesis of biologically active natural products from sawdust waste. *Fermentation.* 2022;8(213):1-20. doi: 10.3390/fermentation8050213
61. Hajam ME, Plavan GI, Kandri NI, et al. Evaluation of softwood and hardwood sawmill wastes impact on the common carp "Cyprinus Carpio" and its aquatic environment: An oxidative stress study. *Environ Toxicol Pharmacol.* 2020;75:103327. doi: 10.1016/j.etap.2020.103327
62. Amin DH, Abdallah NA, Abolmaaty A, Tolba S, Wellington EMH. Microbiological and molecular insights on rare actinobacteria harboring bioactive prospective. *Bull Natl Res Cent.* 2020;44:5. doi: 10.1186/s42269-019-0266-8