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Actinobacteria-Enhanced Straw Recycling for Crop Yield, Soil Fertility, and Aquatic Ecosystem Protection

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ABSTRACT

The burning of paddy straw is linked to significant resource waste, environmental pollution, and adverse health effects. Additionally, the release of toxic pollutants from straw burning contaminates aquatic ecosystems, disrupting water quality and biodiversity. This study explored eco-friendly alternatives to straw burning depending on the lignocellulolytic activity of actinobacterial strains. It was hypothesized that specific actinobacterial isolates could effectively degrade lignocellulosic materials, thereby enhancing soil fertility. Semi-solid fermentation was conducted with the promising candidate, *Streptomyces coelicolor* strain W21.24, followed by a greenhouse experiment comparing soil properties in control pots versus those treated with residual straw from the fermentation process. Significant improvements in soil properties were observed for the treated group, including pH increase from 5.1 to 5.7 ($P< 0.001$), phosphorus from 0.681% to 0.729% ($P= 0.021$), potassium from 0.438% to 0.8% ($P< 0.001$), nitrogen from 3.58% to 0.4% ($P= 0.005$), and carbon content from 41.63% to 44.22% ($P< 0.001$), indicating enhanced soil quality. Additionally, the growth of *Vigna unguiculata* L. Walp was significantly improved, with average shoot heights of 114.15cm in the treated group versus 84.15cm in the control ($P= 0.044$). The fruit count showed a 55.7% increase ($P= 0.002$), while the flower count increased by 47.3% ($P= 0.041$). These results demonstrated positive effects on the above-ground growth. These findings show the benefits of returning organic matter to the soil and introduce an innovative method for utilizing actinobacteria in sustainable agriculture.

INTRODUCTION

Cereals are a crucial global food crop, serving as a primary source of food and energy for the majority of the world's population, particularly through staples such as rice, wheat, and maize. In Egypt, rice stands out as the most significant agricultural crop, largely due to its lower cultivation costs and the favorable soil characteristics of northern Egyptian lands, which support rice cultivation over many other crops **(Negm & Abu-hashim, 2019)**. The dependence on different types of fertilizers, including nitrogen and phosphate fertilizers, continues to increase each year due to the need to sustain high agricultural productivity **(Cui *et al.*, 2021)**. However, the abundant production of agricultural straws poses an environmental challenge, often overlooked as a potential

resource. The common practice of burning paddy straw results in resource wastage, environmental pollution, and negative impacts on human health **(Chanana *et al.*, 2023)**.

In 2018, Egypt produced approximately 6.1 million tons of rice straw, of which around 21% was incinerated creating the black cloud over the Nile Delta. This phenomenon releases harmful pollutants, including fine particulates matter and toxic gases, which degrade air and water quality and pose health risks to local populations. Additionally, it exacerbates environmental issues such as climate change and acid rain harming aquatic ecosystem **(Marey *et al.*, 2010)**. This practice is particularly concerning due to the straw's predominant lignocellulosic composition, which requires a lengthy decomposition period **(FOW, 2018)**. Reintroducing straw into the soil represents a promising solution to mitigate the issues associated with straw burning and the overuse of chemical fertilizers **(Wan *et al.*, 2020)**. Incorporating straw into the soil enriches it with valuable nutrients and organic matter, potentially reducing the need for chemical fertilizers while promoting sustainable soil productivity **(Marzouk *et al.*, 2024)**. Addressing suitable methods for straw decomposition is critical for the successful reintegration of straw into agricultural practices. The return of straw can significantly enhance soil fertility, increase carbon sequestration, and maintain soil productivity **(Li *et al.*, 2024)**. When reintroduced, straw gradually decomposes into organic matter (OM), offering various benefits. Higher OM content is associated with the increased levels of both water-soluble and organically-bound heavy metals in the soil **(Jin ShuLan *et al.*, 2018)**. Furthemore, the functional groups in OM, including carboxyl, hydroxyl, and carbonyl, allow it to bind with heavy metals, which in turn affects soil function and quality **(Ding *et al.*, 2018)**. A higher OM content can enhance nutrient availability, improve soil physical and biological properties, and bolster soil buffering capacity **(Singh *et al.*, 2024)**.

Using straw decomposition agents can speed up the process of straw breakdown, facilitating nutrient release **(Gao *et al.*, 2023)**. They support the Sustainable Development Goal 12 by promoting the efficient use of agricultural waste, reducing reliance on chemical fertilizers, and encouraging sustainable farming practices. Moreover, it addresses the Sustainable Development Goal 13 by helping to reduce emissions from straw burning and by enhancing soil health through an improved carbon storage. Specific microorganisms metabolize straw, converting it into OM and essential nutrients like nitrogen (N), phosphorus (P), and potassium (K), which are vital for plant health **(Wang *et al.*, 2022)**. Natural decomposition by local soil microorganisms is often slow, underscoring the importance of selecting effective microbial strains to enhance the breakdown process. Straw mainly consists of complex lignocellulosic biomass, featuring cellulose microfibers interconnected with hemicellulose networks and shielded by lignin **(Pérez *et al.*, 2002)**. Therefore, effective microorganisms must be capable of degrading all three components of this biomass. Actinobacteria, one of the most extensive bacterial phyla, are widely distributed across various habitats, including soil, aquatic ecosystems,

and extreme environments **(Alam *et al.*, 2021; Tistechok *et al.*, 2021)**. Actinobacteria are well-known for their unique characteristics and biotechnological applications, particularly their complex enzymatic systems and their ability to produce a diverse range of hydrolytic enzymes and natural products known as secondary metabolites. These secondary metabolites include antibiotics and other bioactive compounds used in the pharmaceutical, industrial, medical, and agricultural sectors **(Harir *et al.*, 2018)**. In this study, actinobacteria are isolated and screened to evaluate their lignocellulolytic activity and biodegradation of rice straw under semi-solid fermentation conditions. The effect of returning straw to the soil composition is also explored, along with its potential to enhance plant growth and crop yield. This research addressed a critical gap in understanding how specific microbial interventions can transform agricultural waste into valuable resources, improving soil health and promoting sustainability in agricultural practices. The innovative approach of using actinobacteria offers a promising solution for enhancing both environmental and agricultural outcomes.

MATERIALS AND METHODS

Collection of sample and isolation

A soil sample was obtained from the rhizosphere of sugar beet plants in Sindanhour village, located in Qalyubia Governorate, Egypt. The soil sample was dried at room temperature for 3 to 5 days, and then underwent serial dilution following the method described by **Hayakawa and Nonomura (1987)**. Starch casein agar (SCA) amended with cycloheximide (500 µg/ml) was used to isolate actinobacteria. The plates were kept at 30°C for incubation for two weeks, after which the purified actinobacterial isolates were transferred to SCA slants for preservation.

Screening lignocellulolytic activities

The cellulolytic activity of all actinobacterial isolates was assessed using a carboxymethyl cellulose (CMC) agar medium. After incubating the actinobacteria at 30°C for seven days, iodine was applied to the medium as an indicator of cellulose hydrolysis. A positive result was identified by the presence of a clear zone around the actinobacterial growth **(Kasana *et al.*, 2008).** The cellulolytic activity index was determined using the formula below (1) **(Ferbiyanto *et al.*, 2015)**:

$$\text{Cellulolytic Index} = \frac{\text{Clear Zone Diameter (mm)} - \text{Colony Diameter (mm)}}{\text{Colony Diameter (mm)}} \quad (1)$$

Where:

- Clear zone diameter (mm) is the diameter of the area of hydrolysis surrounding the actinobacterial colony;
- Colony diameter (mm) is the diameter of the actinobacterial colony.

Amylase enzyme production by actinobacteria was evaluated using a starch agar medium. The actinobacteria were plated on starch agar and were kept at 30°C for a duration of seven days. After incubation, an iodine solution was applied to the plate for a

few minutes. A clear zone around the actinobacterial growth indicated a positive result for starch hydrolysis **(Kausar *et al.*, 2011)**. The amylolytic activity index was calculated using the following formula (2):

$$\text{Amylolytic index} = \frac{\text{clear zone diameter (mm)} - \text{colony diameter (mm)}}{\text{colony diameter (mm)}} \quad (2)$$

Where:

- Clear zone diameter (mm) is the diameter of the area of hydrolysis surrounding the actinobacterial colony;
- Colony diameter (mm) is the diameter of the actinobacterial colony.

Lignin degradation by actinobacterial isolates was tested using a medium containing the following components (g/L): Ammonium phosphate dibasic (Sigma-Aldrich)1.0, potassium chloride (Sigma-Aldrich) 0.2, magnesium sulfate heptahydrate (Sigma-Aldrich) 0.2, yeast extract (Sigma-Aldrich) 2.0, glucose (Sigma-Aldrich) 2.0, azure B (Sigma-Aldrich) (0.01% w/v) 0.1, agar (Sigma-Aldrich) 15.0 and 1.0 l distilled water (pH 7.5 ± 0.2). The prepared plates were inoculated with actinobacteria and incubated at 30°C for 10 days. After incubation, growth on the media indicated the isolate's ability to degrade lignin **(Kausar *et al.*, 2011)**.

Decomposition of straw using selected actinobacteria

The most efficient actinobacterial isolate was chosen for semi-solid fermentation. Eight percent (w/v) rice straw was employed in the semi-solid fermentation. To prepare the fermentation broth, 20 grams of straw were mixed with 250mL of minimal broth media and sterilized by autoclaving at 121°C for 15 minutes. Once cooled, 5% (v/v) of the chosen isolate, with an optical density of 0.8 at 600nm, was added to the broth. The mixture was then incubated at 30°C for four weeks.

After the incubation period, the straw was collected for compositional analysis **(Gupta & Jana, 2018)**. The cellulose and soluble lignin content of the straw were determined. The leftover straw samples were placed in an oven and dried at 105°C for 24 hours. The cellulose percentage in each residual straw sample, along with the control, was determined according to the method described by **Van Soest and Robertson (1979)**, while soluble lignin was quantified using the method described by **Gupta and Jana (2018)**.

Bioassay

The experiment was conducted as a potted planting trial in a greenhouse with two groups of carefully prepared soil pots. One group served as the control, while the other was pre-treated with residual straw from the semi-solid fermentation process. After three weeks, soil samples from both groups were collected for chemical analysis. The chemical analysis included several parameters, including pH, phosphorus, potassium, nitrogen, and carbon content. The pH of soil was determined using a water-soil suspension at a 2.5:1 ratio **(Lu, 2000)**. Soil carbon, total nitrogen, total potassium, and total phosphorus (TP) were analyzed using established conventional methods **(Sparks *et al.*, 2020**).

Seeds of *Vigna unguiculata* L. Walp were utilized in the bioassay. The seeds were collected from the ASUEG Herbarium, sown in two groups of pots and watered. After Ten weeks, the shoot length, root length, number of flowers, and number of fruits were recorded. The data were analyzed statistically using one-way ANOVA with SPSS software.

Molecular identification

DNA extraction from the actinobacterial isolate was performed using the QIAamp DNA mini kit (Qiagen), following the manufacturer's protocol, and the samples were kept at −20 °C until use. The PCR was performed to amplify the 16S rDNA gene. The primers were 16S8FWD (5'-AGAGTTTGATCCTGGCTCAG-3') and 16S1510RVS (5'-GGTTACCTTGTTACGACTT-3'). The DNA amplification was carried out under the following conditions: 5min at 94°C for initial denaturation, then 30 cycles of 40sec at 94 °C (denaturation), 50sec at 55°C (annealing), and 50sec at 72°C(extension) and finally 7min at 72°C (Final extension).

The PCR product was examined using a 1% agarose gel with 0.4µg/ mL of ethidium bromide. The amplified fragment was analyzed for sequencing using the Big TriDye sequencing kit (ABI Applied Biosystems) at the Macrogen facility in Korea. The 16S rDNA gene sequence was analyzed using BioEdit version 7.2.5. It was then compared to all available sequences in the National Center for Biotechnology Information (NCBI) database using the basic local alignment search tool (BLAST). The nucleotide sequences were submitted to GenBank, and then aligned and compared with closely related sequences retrieved from the GenBank database. The phylogenetic analysis of sequences was performed using MEGA 11 software implementing the neighbor-joining method with 1000 bootstrap replications **(Tamura *et al*., 2021)**.

RESULTS

Isolation and screening of lignocellulolytic activities

A total of 17 actinobacterial isolates were obtained from the rhizospheric soil of a sugar beet plant. The isolates were assessed for their hydrolytic activities in degrading lignocellulosic materials, as summarized in Table (1). All isolates demonstrated significant potential to hydrolyze cellulose on a carboxymethyl cellulose (CMC) medium, forming clear zones around their growth upon the addition of iodine. Of the seventeen isolates, three isolates (I.10, I.15, and I.17) exhibited relatively higher cellulolytic activity, producing a cellulolytic index greater than 2. In contrast, the remaining isolates produced lower cellulolytic indices, with values below 2. The highest cellulolytic index (2.8) was observed in isolate I.17, followed by I.10 and I.15, with indices of 2.33 and 2.14, respectively.

All actinobacterial isolates demonstrated the ability to hydrolyze starch, as indicated by the formation of clear zones on starch agar following the addition of iodine. Out of the seventeen isolates, six (I.1, I.3, I.7, I.8, I.11, and I.17) demonstrated high amylolytic activity (amylolytic index ≥ 2), whereas the others displayed moderate to low activity (amylolytic index < 2). Isolate I.11 displayed the highest amylolytic index (4.6), followed by I.1 (3.8), I.3 (3.2), I.7 (2.6), I.8 (2.3), and I.17 (2.0). In terms of lignin degradation, only three out of the 20 tested isolates (I.9, I.12, and I.17) were capable of growing on the medium with Azure-B

Table 1. The ability of isolated actinobacteria to degrade cellulose, starch, and lignin

Isolates code	Cellulolytic enzyme	Cellulolytic index	Amylolytic enzymes	Amylolytic index	Lignin degradation
I.1	+	1.8	+	3.8	-
I.2	+	1.5	+	0.8	-
I.3	+	1	+	3.2	-
I.4	+	1	+	1.8	-
I.5	+	1	+	0.7	-
I.6	+	1.5	+	1.4	-
I.7	+	1.36	+	2.6	-
I.8	+	0.46	+	2.3	-
I.9	+	0.7	+	1.1	+
I.10	+	2.33	+	1.44	-
I.11	+	1	+	4.6	-
I.12	+	1	+	0.7	+
I.13	+	1.4	+	1	-
I.14	+	1.33	+	1	-
I.15	+	2.14	+	1	-
I.16	+	1.5	+	1.1	-
I.17	+	2.8	+	2	+

Decomposition of straw using selected actinobacteria

Out of all the isolates, three actinobacterial strains demonstrated the ability to degrade cellulose, starch, and lignin simultaneously. Isolate I.17 was selected for semi-solid fermentation using rice straw, as it exhibited the highest potency in degrading both cellulose and starch, as shown in Fig. (1). Following fermentation, the cellulose and soluble lignin content of both the residual and control straw samples were determined. The cellulose content of the straw treated with I.17 was 28.63%, significantly lower than the control, which had a cellulose content of 47.1%. For soluble lignin determination, the optical density at 270nm for the residual straw utilized with I.17 was 0.88, compared to 1.674 for the control straw, indicating a reduction in soluble lignin content after treatment with isolate I.17.

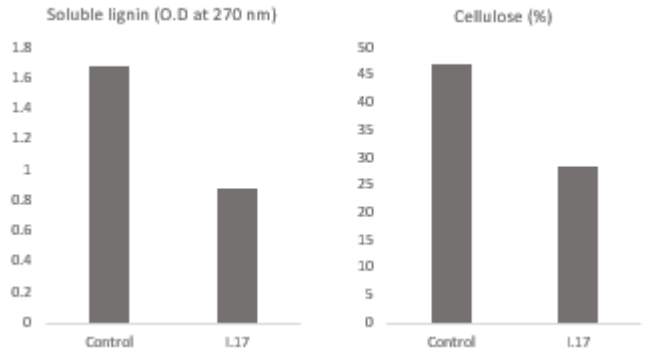

Fig. 1. Determination of cellulose and soluble lignin of residual treated straw of I.17 and the control

Bioassay

The experiment involved a greenhouse trial with two groups of soil pots: One serving as the control and the other treated with residual straw from semi-solid fermentation. After three weeks, soil samples from both groups were collected for chemical analysis. A comparison of soil properties between the control and treated groups revealed significant differences in several parameters, including pH, phosphorus, potassium, nitrogen, and carbon content, as shown in Fig. (2).

The pH level in the control group was 5.1, while the treated group had a higher pH of 5.7, with a *P*-value of 0.000, indicating a statistically significant difference. Phosphorus content (%) increased from 0.681 in the control to 0.729 in the treated group, with a *P*-value of 0.021, reflecting a significant effect of the treatment. Besides, potassium content (%) differed significantly, with the control measuring 0.438 and the

treated group 0.8, supported by a *P*-value of 0.000. Additionally, nitrogen content (%) showed a marked increase from 3.58 in the control to 4.0 in the treated group, with a *P*-value of 0.005.

Finally, the carbon content (%) in the control group was 41.63, while the treated group exhibited a higher carbon content of 44.22, with *P*-value of 0.000. These results demonstrate that the treatment improved soil pH, phosphorus, potassium, nitrogen, and carbon levels significantly, indicating enhanced soil quality and nutrient availability.

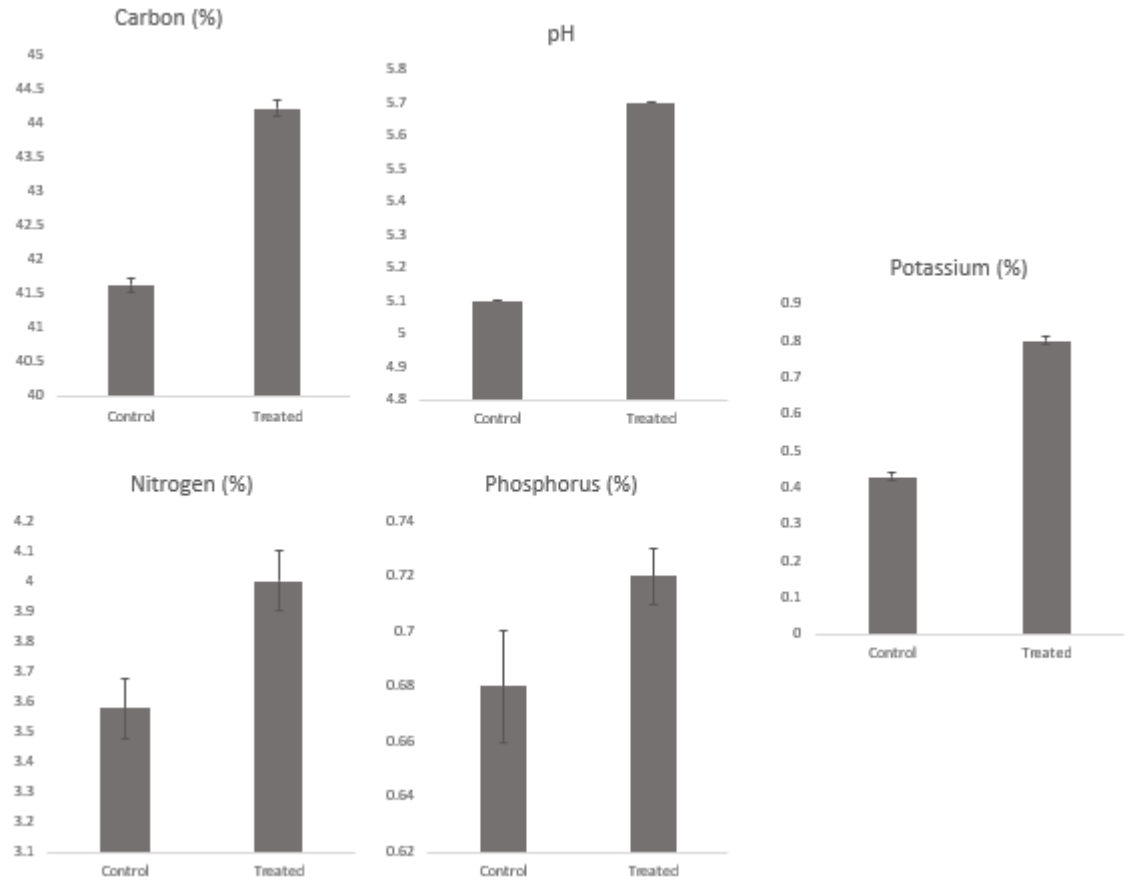

Fig. 2. Comparative analysis of soil properties between control and treated soil groups

A bioassay was conducted using seeds of *Vigna unguiculata* L. Walp, which were planted in the previously established groups of pots. The analysis of growth parameters provides a compelling evidence of the positive impact of straw return on plant development, as detailed in Table (2).

For shoot growth, the treated group outperformed the control significantly, with a mean shoot length of 114.15cm compared to 84.15cm in the control (*P*= 0.044), indicating a significant enhancement in shoot elongation as a result of the treatment.

Similarly, the number of fruits was significantly influenced by straw return, with a 55.7% increase, supported by a highly significant *P*-value of 0.002. This result highlights the strong positive impact of straw return on fruit production.

Flower growth benefitted from the treatment, with a 47.3% increase (*P*= 0.041). However, in contrast to these observations, root growth did not show a notable difference between the control and treated groups (*P*= 0.438). This suggests that while the straw return treatment was highly effective in promoting shoot, flower, and fruit development, its impact on root growth was minimal.

Table 2. Statistical analysis of the impact of straw returning on growth parameters of *Vigna unguiculata* L. Walp

Growth parameter	Group	Mean	95% Confidence Interval for Mean		*P*-value
			Lower bound	Upper bound	
Shoot	Control	84.15±33.56	63.87	137.24	0.044
	Treated	114.15±38.21	91.06	104.43	
Root	Control	17.69±7.02	13.44	21.93	0.438
	Treated	19.73±6.12	16.02	23.43	
Flower	Control	17.07±9.36	11.41	22.73	0.041
	Treated	25.15±9.65	19.31	30.99	
Fruit	Control	4.15±1.46	3.269	5.03	0.002
	Treated	6.46±1.808	5.368	7.55	

Molecular identification

The nucleotide sequence of the 16S rRNA gene from actinobacterial isolate I.17 was analyzed using nucleotide BLAST through the NCBI database. Isolate I.17 showed a close relationship to *Streptomyces coelicolor*, with a similarity index of 99.71%. The sequence of I.17 was submitted to GenBank as *Streptomyces coelicolor* strain W21.24, with the accession number PP 515638.1. These findings were corroborated by phylogenetic analysis conducted using the neighbor-joining method with 1,000 bootstrap

replications. The phylogenetic analysis revealed that the isolate was positioned within the *Streptomyces* clade, supported by a maximum bootstrap value of 100%, as illustrated in Fig. (3).

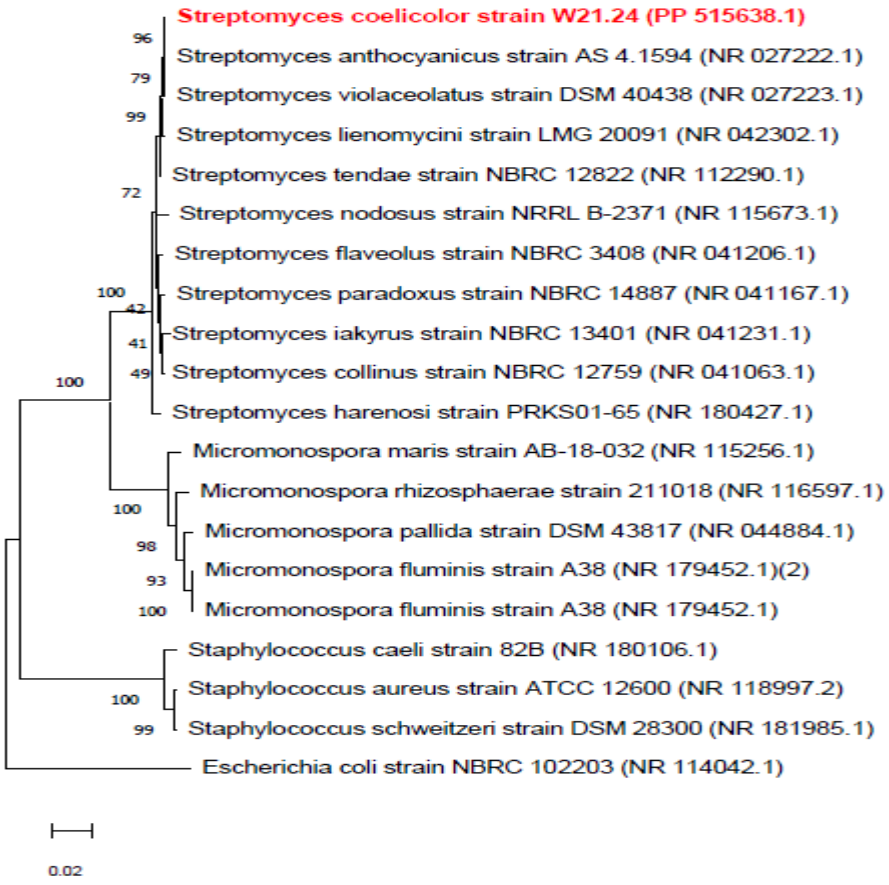

Fig. 3. Phylogenetic analysis of the 16S rRNA gene sequence from isolate I.17

DISCUSSION

Seventeen unique actinobacterial isolates were collected from the rhizosphere of a sugar beet plant. This finding aligns with previous research, which indicates that actinobacteria are the predominant phylum of bacteria in the rhizosphere of various crops, including wheat, rice, sugarcane, and medicinal plants **(Yadav *et al.*, 2018)**. All isolates demonstrated the capability to hydrolyze starch, suggesting that these actinobacteria may produce glucoamylase and α-amylase, consistent with findings from prior studies **(Dobariya *et al.*, 2023)**. Additionally, all isolated actinobacteria exhibited the remarkable ability to produce clear halo zones in a CMC medium as a result of producing cellulase enzymes that degrade cellulose. Notably, several strains of actinobacteria are known to produce hydrolytic enzymes while growing on cellulose substrates **(Korsa *et al.*, 2023)**. Of the seventeen isolates tested, only three were capable of thriving in media supplied with Azure-B, indicating their potential to produce lignin peroxidase, which corroborates

previous research **(Virmani *et al*., 2024)**. Lignin, a complex polymer, forms an irregular, non-crystalline structure in plant cell walls, providing protection for cellulose and hemicelluloses, which are inherently resistant to biodegradation. Therefore, the generation of ligninolytic enzymes is crucial for disrupting the lignin barrier, thereby exposing cellulose and hemicelluloses to subsequent biodegradation **(Kausar *et al*., 2011)**.

Straw is an abundant and valuable source of carbohydrates utilized in industrial and agricultural production. It is mainly made up of cellulose, hemicellulose, and lignin. However, current methods of improper straw disposal or direct burning result in substantial resource waste and lead to significant environmental consequences **(Gong *et al*., 2022)**. Therefore, the actinobacterial isolate exhibiting the highest lignocellulolytic activity was selected for semi-solid fermentation. This process utilized straw as the main carbon source and substrate for evaluating the lignocellulolytic activity of the actinobacteria. The cellulose content in the residual straw utilized with isolate I.17 was measured at 28.63% compared to 47.1% in the control. For soluble lignin determination, the optical density of the straw treated with I.17 was 0.88, while the control exhibited a value of 1.674. These findings are in agreement with earlier research **(Gong *et al.*, 2022)**, which reported the efficiency of three straw-degrading strains from the *Streptomyces* genus in straw return. These strains demonstrated notable degradation rates, effectively decomposing straw components. Actinobacteria are particularly beneficial for this degradation process, as they are highly efficient at breaking down large and complex molecules through the release of a diverse array of hydrolytic enzymes **(Abdulla & El-Shatoury, 2007)**.

Soil fertility has become a significant concern as the world's population continues to grow. Traditional agricultural practices often rely heavily on harmful chemicals, which contribute to the decline in soil fertility. An alternative approach is needed to enhance soil fertility and crop yield **(Ayub *et al*., 2020)**. Therefore, a recent study investigated the application of actinobacteria to demonstrate and accelerate the impact of short-term straw return on soil quality. The soil samples were analyzed, revealing significant differences in pH, phosphorus, potassium, nitrogen, and carbon content. Our findings align with previous studies which assessed the capability of straw to improve soil fertility, nutrient availability, and crop production **(Zhang *et al.*, 2016; Guan *et al.*, 2020)**. This approach holds the potential to revolutionize sustainable agriculture through advanced farming management practices that enhance soil fertility and nutrient levels.

Increasing crop yields is essential to meet the demands of a growing population, but it must be achieved sustainably **(Vitousek *et al*., 2009)**. The practice of straw returning presents a promising opportunity to enhance crop productivity without reliance on chemical fertilizers **(Wan *et al*., 2020)**. A bioassay utilizing *Vigna unguiculata* seeds demonstrated that straw returning, in conjunction with actinobacteria, significantly improved plant growth across several parameters. The treated group exhibited notable

enhancements in shoot length, flower number, and fruit number compared to the control group. These results coincide with those of a recent study **(Guo *et al*., 2024)**, which found that replacing potassium fertilizer with straw return significantly enhances maize and wheat yields in a dryland maize-wheat rotation system. This innovative approach not only increases crop productivity but also enhances the overall quality of the harvested wheat. Embracing straw returning holds great promise for sustainable and productive agricultural practices.

CONCLUSION

The traditional practice of burning paddy straw has long been a significant contributor to resource wastage, environmental degradation, and public health crises. This study reveals the untapped potential of rice straw as a valuable resource, emphasizing the transformative role of innovative and eco-friendly solutions. Our findings demonstrate the efficacy of *Streptomyces coelicolor* strain W21.24 (isolate I.17) in breaking down rice straw into nutrient-rich organic matter. This process not only reduces dependency on chemical fertilizers but also enhances soil health, plant growth, and crop productivity. Such applications underscore the promise of leveraging biological agents for sustainable agricultural practices.

We recommend the adoption of Actinobacteria-based bioconversion methods, such as the one employed in this study, as a scalable and environmentally sound strategy to recycle agricultural waste. This approach aligns with global efforts to reduce pollution, minimize reliance on chemical inputs, and support sustainable farming systems.

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