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## Isolation, screening, and molecular identification of lignocellulolytic bacteria from various spent Mushroom substrates and consortium preparation

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## Abstract

Spent Mushroom Substrate (SMS) disposal poses significant environmental challenges due to its high lignocellulosic content. This study aimed to isolate and characterize lignocellulolytic bacteria from SMS derived from paddy straw, wheat straw, and coconut coir. Thirty-four bacterial strains were isolated and screened for hydrolysis capacity, cellulolytic, and ligninolytic activities. The most effective isolates, CDB 2, CDB 7, CDB 11 and CDB 14, were further characterized using cultural, morphological, and molecular methods, revealing them to be *Bacillus* spp. These strains demonstrated compatibility and formed a stable consortium named AAU PG24. The findings highlight the potential of utilizing lignocellulolytic bacteria to address the environmental challenges posed by SMS.

## 1. Introduction

Mushroom cultivation has been a long-standing tradition in East Asian countries, particularly in China, where it began around 600 A.D (Kues and Liu, 2000). According to Chang (1999), there are over 12,000 species of fungi that can be classified as Mushrooms, with at least 2,000 of these being edible. The growing consumer health consciousness coupled with the escalating need for nutrient-rich and cholesterol-free food products is primarily driving the Mushroom

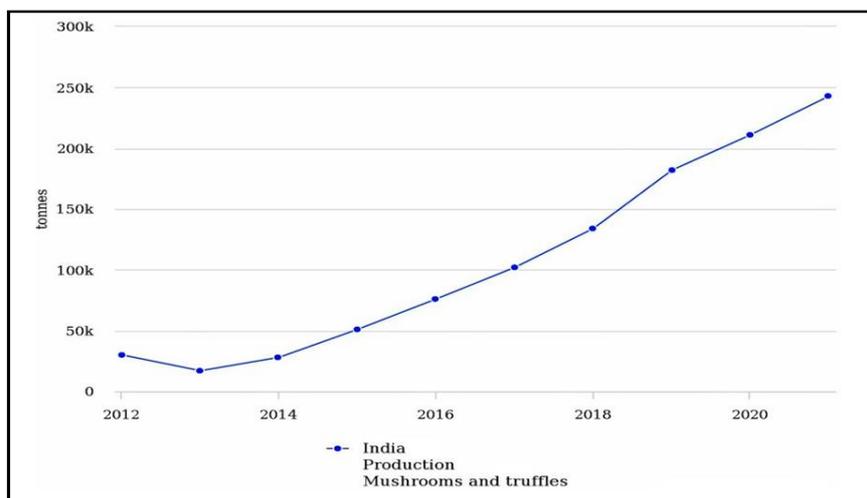
market. The emerging trend of veganism and the widespread adoption of Mushrooms as a substitute for meat due to their rich umami taste and ideal texture (Sun *et al.*, 2020) are also propelling market growth. Additionally, the utilization of Mushrooms in dietary supplements, owing to their rich content of fiber and digestive enzymes that promote gut and immunological health (Cheung, 2013), is catalyzing product demand. Straw from traditional paddy cultivars contains more cellulose and hemicellulose (Vellaiyan *et al.*, 2024).

Figure 1: Mushroom production quantity in India (2012-2021): FAOSTAT, May, 2023.

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Email: [ukaaz@yahoo.com](mailto:ukaaz@yahoo.com); Website: [www.ukaazpublications.com](http://www.ukaazpublications.com)In Mushroom production, a significant by-product known as spent Mushroom substrates (SMS) is generated. For every 1 kg of final Mushroom products, approximately 5 kg of SMS is produced (around 2 kg in dry weight) (Finney *et al.*, 2009). India's Mushroom production in 2021 resulted in the generation of approximately 1.2 MMT of SMS (FAOSTAT, 2023). Improper and careless dumping of

SMS creates serious environmental pollution and groundwater contamination (He *et al.*, 2021). Therefore, there is a pressing need for efficient degradation strategies to maximize its utilization while minimizing environmental impacts. Kumar *et al.* (2023) used wheat straw as substrate material with different doses of micronutrients for enhancing the *P. florida* production. Lignocellulolytic bacteria are microorganisms that possess the ability to degrade lignin, cellulose, and hemicellulose. These bacteria play a crucial role in the decomposition of plant biomass and have potential applications in various industries, such as biofuel production, agriculture, and waste management. The specifications of lignocellulolytic bacteria include: - Ability to degrade lignin, cellulose, and hemicellulose - production of enzymes such as cellulase, xylanase, and laccase - ability to grow on lignocellulosic substrates.

Different substrate materials have varying degrees of effectiveness in promoting biological efficiency SMS, a type of biomass made up of lignocellulose, consists mainly of cellulose (30-50%), hemicellulose (15-35%) and lignin (10-20%) (Mielenz, 2001; Girio *et al.*, 2010). Lignocellulolytic bacteria produce a variety of cellulases that work together to break down cellulose by hydrolyzing the  $\beta$ -1,4-glycosyl linkages of the cellulose chain, producing cello-oligosaccharides. Lignin, the second most abundant renewable biopolymer in nature, serves a vital role in plant cell walls by providing rigidity and protecting cellulose from pathogens. Due to its complex structure and non-hydrolysable bonds, lignin is more challenging to break down compared to cellulose or hemicellulose. Therefore, the identification of bacteria having lignin-oxidizing enzymes is significant (Raghukumar *et al.*, 2008). To address these challenges, the present study aims to isolate, screen and characterize lignocellulolytic bacteria from SMS and develop a bacterial consortium that can offer a viable solution for SMS disposal.

## 2. Materials and Methods

### 2.1 Sample collection

Various samples of SMS, composed of paddy straw, wheat straw and coconut coir, were collected from the Mushroom laboratory at the Department of Plant Pathology, B. A. College of Agriculture, Anand. To ensure the integrity of the samples and prevent contamination, sterile sampling bags were used for collection. Each bag was tightly sealed before transportation to the Department of Agricultural Microbiology for isolation and characterization.

### 2.2 Isolation of lignocellulolytic bacteria

Lignocellulolytic bacteria were isolated using the method described by Buswell *et al.* (1996). SMS samples were first serially diluted and then spread onto congo red carboxymethyl cellulose (CMC) Agar plates, which were prepared with the following composition:  $(\text{NH}_4)_2\text{SO}_4$  (1.0 g/l),  $\text{K}_2\text{HPO}_4$  (0.5 g/l),  $\text{KH}_2\text{PO}_4$  (0.5 g/l), NaCl (6.0 g/l),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (0.1 g/l),  $\text{CaCl}_2$  (0.1 g/l), yeast extract (0.1 g/l) and CMC (10 g/l) in distilled water (1000 ml). This medium supports the growth of bacteria capable of producing cellulase enzymes by using cellulose as the sole carbon source. After incubation at 30°C for up to 5 days, colonies exhibiting clear zones around them were identified as potential lignocellulolytic isolates. To obtain pure cultures, selected colonies with clear zones were streaked onto nutrient agar plates and incubated to isolate distinct bacterial strains free from contamination.

### 2.3 In vitro screening of lignocellulolytic isolates

The screening of lignocellulolytic isolates involved the use of two media types. Isolates were first inoculated onto carboxy methyl cellulose (CMC) agar plates, with cellulose as the sole carbon source to detect cellulase enzyme production. After incubation at 30°C, the plates were flooded with 1% Congo Red dye and 1N NaCl solution to highlight clear zones around colonies, indicating cellulase activity. Additionally, isolates were streaked onto lignin minimal salt media (L-MSM) plates, which contained  $\text{Na}_2\text{HPO}_4$  (2.4 g/l),  $\text{K}_2\text{HPO}_4$  (2.0 g/l),  $\text{NH}_4\text{NO}_3$  (0.1 g/l),  $\text{MgSO}_4$  (0.01 g/l),  $\text{CaCl}_2$  (0.01 g/l) and kraft lignin (1.0 g/l) in distilled water. The zigzag streaking method was used and plates were monitored for the speed of colony formation, with faster growth indicating effective lignin degradation (Rahman *et al.*, 2013).

#### 2.3.1 Cellulose degrading potential (Qualitative)

To determine the cellulose-degrading potential, isolates were further tested using the hydrolysis capacity (HC) method. Positive isolates were spot inoculated onto CMC Agar plates and incubated. After incubation, the plates were stained with 1% congo red dye and followed by a 1N NaCl solution. The hydrolysis capacity was calculated as the ratio of the total diameter of the clear zone plus colony to the diameter of the colony itself, using the formula:

$$\text{HC} = \frac{\text{Total diameter (colony + halo zone) (mm)}}{\text{Diameter of colony (mm)}}$$

#### 2.3.2 Cellulose degrading potential (in vitro filter paper assay)

The cellulose degradation ability of the isolates was further assessed using the filter paper assay. Each isolate was inoculated into 50 ml of cellulolysis basal broth in 250 ml Erlenmeyer flasks, containing a 4 x 4 cm strip of sterile filter paper. The cellulolysis basal broth was prepared with the following composition: ammonium tartrate (5.0 g/l), yeast extract (0.1 g/l),  $\text{KH}_2\text{PO}_4$  (1.0 g/l),  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (0.01 g/l),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (0.5 g/l) in distilled water with a pH of 7. The flasks were incubated at 30°C with aeration. The control flask was uninoculated. The duration required to degrade the filter paper, which is nearly 100% cellulose, was recorded (Mandels *et al.*, 1976).

#### 2.3.3 Lignin degradation assay (qualitative)

The lignin-degrading activity of the isolates was evaluated using methylene blue dye as an indicator. Lignolytic microbes possess enzymes that oxidize the dye, leading to decolorization. Selected isolates were streaked onto luria agar plates, which contained tryptone (10.0 g/l), yeast extract (5.0 g/l) and NaCl (5.0 g/l) in distilled water. The plates were supplemented with methylene blue dye at a concentration of 0.25 g/l and incubated at 30°C for 72 h. The decolorization of the dye was observed, indicating lignin degradation (Rahman *et al.*, 2013).

#### 2.3.4 Lignolytic activity (liquid assay)

To quantify lignolytic activity, 15 ml of Luria Broth was added to 20 ml borosilicate culture tubes, which were inoculated with a loop full of positive isolates. After 24 h. of incubation, methylene blue dye (25 mg/l) was added. The tubes were incubated at 30°C with shaking at 200 rpm for 7 days. A control tube without inoculation was included. Post-incubation, cultures were centrifuged at 10,000 rpm for 10 min. The percentage of decolorization was calculated using absorbance values at  $\lambda_{\text{max}}$  (610 nm) with the formula (Bandounas *et al.*, 2011):

$$\text{Decolouration \%} = \frac{(\text{A of control} - \text{A of isolate}) \times 100}{(\text{A of control})}$$

Based on the highest hydrolysis capacity, maximum methylene blue dye decolorization and shortest duration to degrade filter paper, four to five isolates with the best performance were selected for further characterization.

## 2.4 Characterization of selected isolates

### 2.4.1 Cultural characterization

Isolates were cultured on nutrient agar plates using the four-sector method. Colony characteristics, including size, shape, margin, elevation, texture, opacity and pigmentation, were recorded. Spent Mushroom substrates were collected at 30, 60, and 90 days after harvesting of the fruiting body.

### 2.4.2 Morphological characterization

Gram staining was performed to determine cell wall composition. Motility and the presence of spores or capsules were also assessed.

### 2.4.3 Molecular characterization

Molecular characterization of the isolates was carried out using 16S rRNA sequencing. Genomic DNA was extracted from isolates cultured on nutrient agar and grown in nutrient broth at 28-30°C with shaking, following the method described by Sambrook *et al.* (1989). DNA extraction was performed using the HiMedia Kit and the purity was assessed by agarose gel electrophoresis (0.8% agarose in 1X TAE buffer, stained with ethidium bromide) as per Sambrook *et al.* (1989). PCR amplification of the 16S rRNA gene was conducted with universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1392R (5'-GGTTACCTGTACGACTT-3') in a 25 µl reaction mixture. The amplification conditions included an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 58.6°C for 30 sec, extension at 72°C for 1 min and a final extension at 72°C for 5 min. The PCR products were analyzed by gel electrophoresis on a 1% agarose gel. The purified PCR products were sequenced using the big dye terminator 3.1 kit and ABI 3500 genetic analyzer (SLS Labs, Surat). Sequence assembly was performed with MEGA 4 software and sequences were compared with other strains using NCBI BLAST for identification and homology analysis (Yin *et al.*, 2017).

## 2.5 In vitro compatibility assessment

The compatibility of selected isolates was evaluated using a cross-streaking method on nutrient agar plates. Isolates were streaked perpendicular to each other and interactions or inhibition at the intersection points were observed (Liu, 2019).

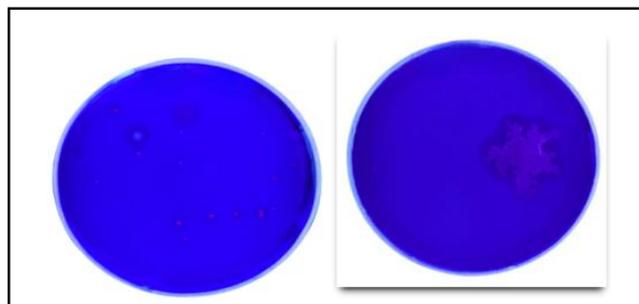
## 2.6 Consortium preparation

Selected isolates were grown in nutrient broth at 30°C. After reaching optimal growth, equal volumes of broth from each isolate were mixed under aseptic conditions to prepare a consortium formulation with a population of  $5 \times 10^8$  CFU/ml.

## 3. Results

### 3.1 Isolation

A total of 34 lignocellulolytic bacterial isolates were identified based on clear zones around colonies on congo red carboxymethyl cellulose (CMC) agar plates (Figure 2). Among these isolates, 16 were obtained from paddy straw SMS, 12 from wheat straw SMS and 6 from coconut coir SMS.



**Figure 2: Colonies of potential lignocellulolytic bacteria on congo red CMC agar plates.**

**Note:** Plate was visualized using a UV stimulator.

### 3.2 In vitro screening for efficient lignocellulolytic isolates

All 34 isolates were screened by spot inoculation on CMC agar plates and streaking onto lignin minimal salt media (L-MSM) plates. After incubation, 15 isolates were identified with prominent clear zones on CMC plates and rapid colony formation on L-MSM plates (Figure 2). These isolates, detailed in Table 1, include 9 from paddy straw SMS, 4 from wheat straw SMS and 2 from coconut coir. These 15 isolates were selected as the most effective cellulose and lignin degraders for further screening.

**Table 1: Cellulose and lignin degradation screening results**

S. No.	Source (SMS)	Zone on CMC plate	Colonization on L-MSM
1	Paddy straw	-	+
2	Paddy straw	++	++
3	Paddy straw	++	-
4	Paddy straw	+++	++
5	Paddy straw	+	-
6	Paddy straw	+++	+++
7	Paddy straw	++	++

8	Paddy straw	-	-
9	Paddy straw	++	++
10	Paddy straw	+++	+
11	Paddy straw	+++	+
12	Paddy straw	+++	+++
13	Paddy straw	-	+
14	Paddy straw	+	-
15	Paddy straw	++	-
16	Paddy straw	+++	-
17	Wheat straw	++	++
18	Wheat straw	-	-
19	Wheat straw	+	-
20	Wheat straw	+++	+
21	Wheat straw	+	+
22	Wheat straw	++	+
23	Wheat straw	+++	++
24	Wheat straw	-	-
25	Wheat straw	+++	+++
26	Wheat straw	+	+
27	Wheat straw	++	-
28	Wheat straw	++	+
29	Coconut coir	+	++
30	Coconut coir	+	-
31	Coconut coir	++	+++
32	Coconut coir	+	+
33	Coconut coir	+++	+
34	Coconut coir	+	-

Note: “+” (low activity on CMC plates/colonization on L-MSM), “++” (moderate level of activity on CMC plates/colonization on L-MSM), “+++” (high activity on CMC plates/colonization on L-MSM), “-” (no detectable activity on CMC plates/colonization on L-MSM). The highlighted isolates were selected for further screening.



Figure 3: Colonization of selected isolates on lignin minimal salt media plates.

### 3.2.1 Cellulose degrading potential (qualitative)

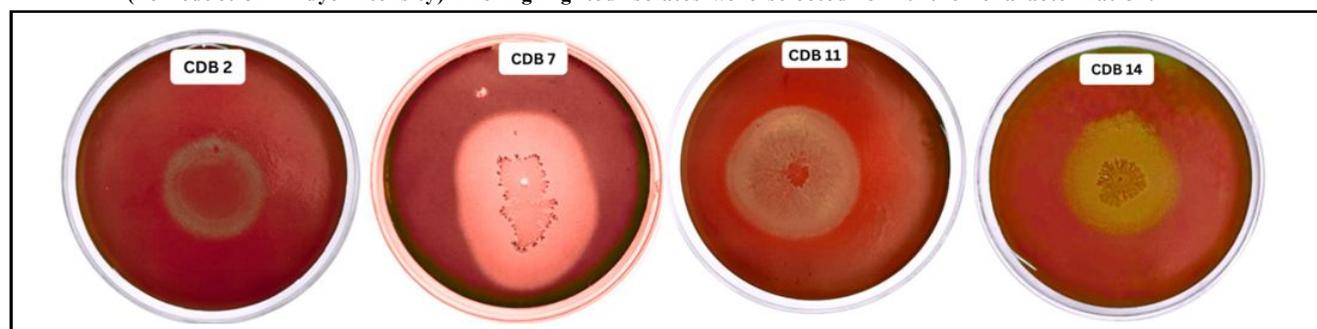
Cellulose degradation potential was assessed using CMC agar plates. All 15 selected isolates produced clear zones, indicating cellulolytic

activity. The hydrolysis capacity (HC) values varied between 1.2 and 2.5 (Table 2), with isolate CDB 14 showing the highest HC value of 2.5, followed by CDB 7 (2.2), CDB 5 (1.8) and CDB 2 (1.6) (Figure 3).

**Table 2: Screening of isolates for cellulose and lignin degradation**

Isolate	Source of isolation	HC	Days to degrade filter paper	MB decolourisation on luria agar	Percentage MB decolourisation in liquid media
CDB 1	Paddy straw	1.4	11	+	41.6
CDB 2	Paddy straw	1.6	9	+++	60.7
CDB 3	Paddy straw	1.3	12	++	51.2
CDB 4	Paddy straw	1.5	12	+	52.8
CDB 5	Paddy straw	1.8	12	-	28.7
CDB 6	Paddy straw	1.5	14	-	17.4
CDB 7	Paddy straw	2.2	9	+++	58.1
CDB 8	Paddy straw	1.2	13	-	19.6
CDB 9	Paddy straw	1.5	11	+	48.2
CDB 10	Wheat straw	1.4	12	-	25.9
CDB 11	Wheat straw	1.5	10	++	53.9
CDB 12	Wheat straw	1.2	13	-	18.6
CDB 13	Wheat straw	1.5	13	-	26.7
CDB 14	Coconut coir	2.5	10	++	52.9
CDB 15	Coconut coir	1.2	13	-	20.8

Note: "+" (low reduction in dye intensity), "++" (moderate reduction in dye intensity), "+++" (high reduction in dye intensity), "-" (no reduction in dye intensity) The highlighted isolates were selected for further characterization.



**Figure 3: Cellulolytic activity of selected isolates on CMC agar plates.**

Note: Plates were flooded with 1% Congo Red dye, followed by a 1N NaCl solution.



**Figure 4: Degradation of filter paper by selected isolates in *In vitro* filter paper assay**

Note: Gradual degradation of filter paper by isolate CDB 2, with control showing intact filter paper.

### 3.2.2 Cellulose degrading potential (*In vitro* filter paper assay)

The cellulose degrading potential was further assessed using an *In vitro* filter paper assay. All 15 isolates, except the control, successfully degraded the filter paper within 14 days, indicating their cellulolytic activity. Isolate CDB 2 (Figure 4) demonstrated the fastest degradation, followed by CDB 7, CDB 11 and CDB 14 (Table 2). The control flask maintained intact filter paper after 14 days.

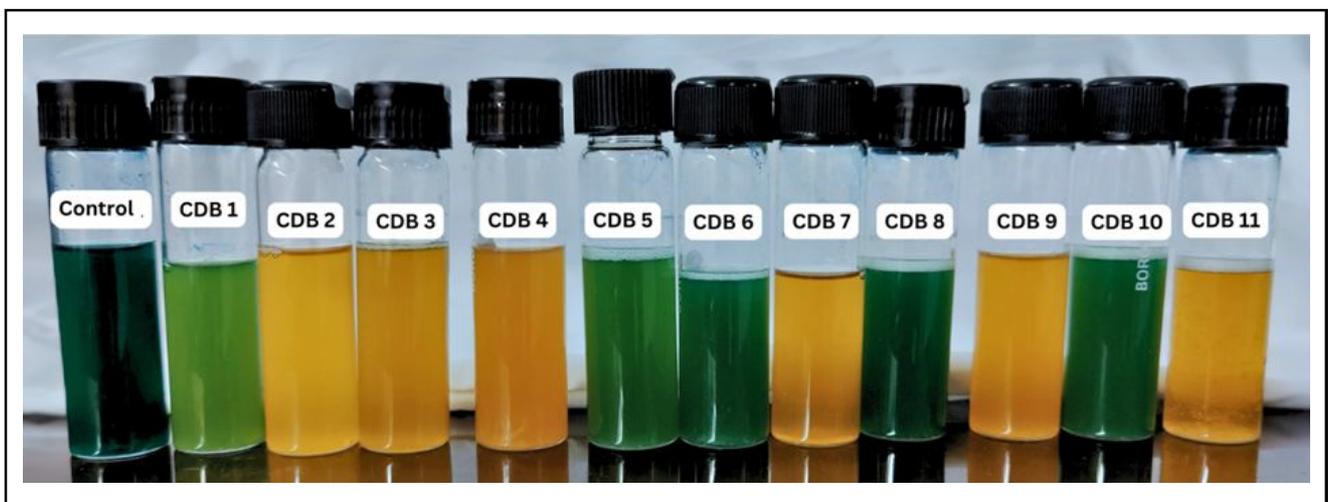
### 3.2.3 Lignin degradation assay (qualitative)

Lignin degradation potential was qualitatively assessed using methylene blue dye as an indicator. All 15 isolates were inoculated onto luria agar plates supplemented with methylene blue dye and clear zones around the colonies indicated lignin peroxidase activity. Among these, 8 isolates (CDB 1, 2, 3, 4, 7, 9, 11, 14) showed distinct

clear zones (Table 2), with CDB 2, 7, 11, and 14 displaying the most pronounced decolorization, indicating strong lignin-degrading capacity.

### 3.2.4 Lignolytic activity (liquid assay)

In the liquid assay, all 15 isolates were tested in Luria broth with methylene blue dye and decolorization was measured after 7 days of incubation. Decolorization percentages ranged from 18.6% to 60.7% (Table 1), with isolate CDB 2 showing the highest decolorization, followed by CDB 7, 11 and 14 (Figure 5). Based on the highest hydrolysis capacity (HC), maximum decolorization of methylene blue dye and the shortest duration to degrade filter paper, isolates CDB 2, CDB 7, CDB 11 and CDB 14 were selected for further characterization. Notably, CDB 2 and CDB 7 were isolated from paddy straw SMS, CDB 11 from wheat straw SMS and CDB 14 from coconut coir SMS.



**Figure 5: Decolorization of methylene blue in liquid assay.**

## 3.3 Characterization of selected isolates

### 3.3.1 Cultural characterization

On nutrient agar plates, the isolates exhibited distinct colony characteristics. Isolate CDB 2 formed small, round colonies with entire margins, flat elevation, a dry texture and a translucent appearance. Isolate CDB 7 produced moderate, irregular colonies with serrate margins, flat elevation, a slimy texture and a translucent appearance. Isolate CDB 11 displayed moderate, irregular colonies with lobate margins, flat elevation, a slimy texture and a translucent appearance. Finally, Isolate CDB 14 developed small, round colonies with entire margins, flat elevation, a dry texture and a translucent appearance. All isolates exhibited creamy white pigmentation.

**Table 3: Molecular characterization details of selected isolates**

Original code	Assigned code	Query coverage	Percentage identity	Organism	NCBI accession
CDB 2	AAU24-Cd1	99	97.82	<i>Bacillus stercoris</i> strain SMSI1	PP702053
CDB 7	AAU24-Cd2	98	96.47	<i>Bacillus licheniformis</i> strain SJMBL	PP737885
CDB 11	AAU24-Cd3	95	94.23	<i>Bacillus paralicheniformis</i> strain SJMBP	PP737886
CDB 14	AAU24-Cd4	99	97.84	<i>Bacillus rugosus</i> strain SMSI2	PP702055

### 3.3.2 Morphological characterization

All isolates (CDB 2, CDB 7, CDB 11 and CDB 14) were rod-shaped, motile, Gram-positive and spore formers.

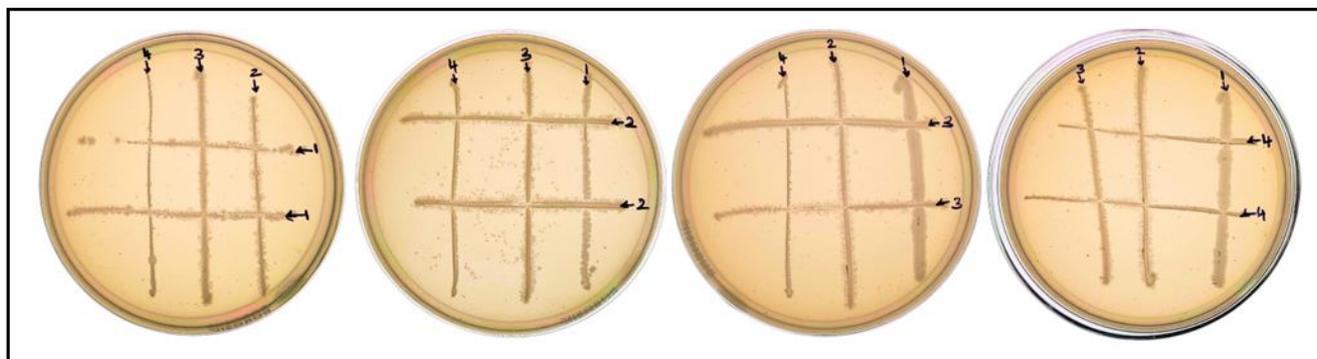
### 3.3.3 Molecular characterization

Molecular analysis revealed the following identities (Table 3), isolate CDB 2 showed 97.82% similarity to *Bacillus stercoris* (NCBI accession: PP702053); Isolate CDB 7 exhibited 96.47% similarity to *Bacillus licheniformis* (NCBI Accession: PP737885); Isolate CDB 11 matched 94.23% with *Bacillus paralicheniformis* (NCBI accession: PP737886); and Isolate CDB 14 showed 97.84% similarity to *Bacillus rugosus* (NCBI accession: PP702055).

### 3.4 *In vitro* compatibility assessment and consortium preparation

The selected isolates CDB 2, CDB 7, CDB 11 and CDB 14 were tested for compatibility using a cross-streaking method on nutrient agar plates, revealing no interactions or inhibition zones at the points of intersection, which confirmed their compatibility (Figure 6). For consortium

preparation, each isolate was grown in nutrient broth at 30°C until reaching optimal growth and equal volumes of these broths were aseptically mixed to create a consortium, designated AAU PG24, with a final concentration of  $5 \times 10^8$  CFU/ml. Similarly, Zhang *et al.*, (2022) found no antagonism among cellulose-degrading strains tested on CMC agar plates, leading to the formation of a high-activity cellulase consortium.



**Figure 6:** *In vitro* compatibility assessment of selected isolates on nutrient agar plates.

Note: Isolates 1, 2, 3, and 4 correspond to AAU24-Cd1 (CDB2), AAU24-Cd2 (CDB7), AAU24-Cd3 (CDB11), and AAU24-Cd4 (CDB14), respectively.

## 4. Discussion

Shamseldin and Abdelkhalek (2015) isolated 38 bacterial strains from soil using minimal salt agar with cellulose sources like filter paper, carboxymethyl cellulose (CMC), lignin and treated paddy straw, based on clearing zones around colonies, indicating their lignocellulolytic activity. Arundath *et al.* (2024) gave information about lignocellulosic wastes are abundant renewable resources that can be utilized sustainably. Evaluating the qualities of these wastes for Mushroom cultivation by assessing their chemical composition, particularly cellulose, hemicellulose, and lignin. Optimizing the potential of lignocellulosic waste materials as substrate needs a deeper understanding of lignocellulolytic enzyme and its activity modification based on the substrate materia. Bhuvanewari *et al.* (2022) isolated and characterize bioactive flavonoids from *A. pinnata* ethanolic leaf extract (EEAZ) and to test their analgesic and anti-inflammatory activities. *Azolla pinnata* R.Br. is a free-floating fresh water fern belonging to the family *Azollaceae* and the order of Pteridophyta.

The flavonoids derived from ferns have been shown to possess anticancer and antiinflammatory properties. Suman *et al.*, (2022) investigated bark extracts of *Prosopis cineraria* (L.) Druce (Khejri) resulted the isolation of 24-methylenecycloartan-3-one, lupeol, 5, 7, 4'-trihydroxy-3'-methoxy flavanone. Maryam *et al.* (2018) similarly found variability in cellulase enzyme activity, with PC-BC6 exhibiting the largest clear zone and a cellulolytic index of 7.75, while other isolates had indices ranging from 3.13 to 5.40. Similarly, Guder *et al.* (2019) performed a filter paper degradation test and found that eight out of twenty isolates were capable of degrading filter paper, evidenced by the formation of a turbid solution after 10 days of incubation.

Similarly, Sharma *et al.* (2018) used MSM agar medium with 0.25% methylene blue dye and observed clear halo zones around colonies of only five out of 22 isolates after one week of incubation. Similarly, Sharma *et al.* (2018) conducted a dye decolorization assay where

isolates in a methylene blue-containing medium achieved maximum decolorization efficiencies of 52.2% and 48.4% for isolates PI and T-1-A, respectively, after incubation at 50°C for 7 days.

## 5. Conclusion

This study provides a foundation for the sustainable management of SMS through microbial degradation. Thirty-four potential lignocellulolytic bacterial strains were isolated from SMS derived from paddy straw, wheat straw and coconut coir and were pure cultured. Through *in vitro* screening, isolates CDB 2, CDB 7, CDB 11 and CDB 14 were selected based on their superior hydrolysis capacity, cellulolytic and ligninolytic activities. These isolates were further characterized culturally, morphologically and molecularly, revealing them to be *Bacillus* spp. They demonstrated compatibility and formed a stable consortium named AAU PG24. The key findings of this research underscore the potential of utilizing lignocellulolytic bacteria to address the environmental challenges posed by SMS. The developed consortium not only offers a viable solution for SMS disposal but also holds promise for producing biofertilizer-enriched compost that could enhance soil fertility and crop productivity.

## 6. Limitations and future research

While the study successfully isolated and characterized lignocellulolytic bacteria from various SMS and developed a promising bacterial consortium, several limitations and potential areas for further research should be acknowledged. The current study was limited to assessments, so future studies should evaluate the efficacy of the bacterial consortium in decomposing different SMS under laboratory and net house conditions to validate its practical application. Additionally, evaluating the impact of biofertilizer-enriched compost derived from SMS on crop growth and yield, including assessing agronomic benefits and potential phytotoxic effects, is crucial for optimizing the use of these bacterial consortia in sustainable agriculture.

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## Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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