Full Length Research Article

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Research Journal of Agricultural Sciences
Volume 16; Issue 06 (Nov–Dec 2025); pp 617–623

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Isolation, Screening and Characterization of Ligninolytic Bacteria from Diverse Environmental Sources

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Received: 24 Oct 2025; Revised accepted: 03 Dec 2025

Abstract

Lignin, a major component of agricultural residues. It is highly resistant to biodegradation, posing significant challenges for sustainable residue management. The present study aimed to isolate and characterize ligninolytic bacteria from diverse lignin-rich environments in Thrissur, Kerala, and to evaluate their potential for lignin degradation. A total of 46 bacterial isolates were obtained from eight environmental sources, such as cow dung, coir pith, decayed wood, forest soil, marshy soil, rice straw, sawdust and termites. Screening with lignin-mimicking synthetic dyes (Congo Red, Azure B, and Methylene Blue) revealed 25 isolates capable of decolourising at least one of the tested dyes. Quantitative assays revealed that isolate S–4 exhibited the highest decolourisation of Congo Red (93.07%) and Methylene Blue (84.73%), while D–6 showed the highest decolourisation of Azure B (32.70%). Among the tested isolates, only two showed strong cellulose degradation, while others were weak or inactive. The study highlights the potential of these bacterial isolates as efficient lignin degraders, offering promising applications in lignocellulosic biomass valorisation and sustainable residue management strategies.

***Key words*:** Ligninolytic bacteria, Dye decolourisation, Alkali lignin, Congo red, Azure B, Methylene blue, Cellulose degradation

Lignin forms an essential structural component of agricultural residues such as straw, stalks, and husks [1]. Globally, it is estimated that over five billion tonnes of crop residues are generated every year [2]. The recalcitrant nature of lignin makes it one of the most challenging components of plant biomass to degrade. This characteristic poses a major challenge to effective crop residue management, particularly for rice straw and other cereal-based residues [3-4]. The continuous accumulation of large quantities of lignocellulosic residues from agriculture has led to widespread open-field burning, which contributes significantly to air pollution and other environmental constraints [5-6].

To address this issue, various residue management interventions have been developed. *In-situ* approaches, such as the use of Happy Seeder, Super Seeder, and mulchers, enable direct sowing without the need for residue removal [7]. On the other hand, *ex-situ* strategies involve the collection and utilization of residues for energy generation, bioethanol production, composting, paper manufacturing, and mushroom cultivation [8].

In recent years, there has been growing interest in biological methods, particularly those utilizing microbial decomposers to accelerate straw degradation under field conditions [9-11]. Among biological agents, white-rot species such as *Phanerochaete chrysosporium* and *Ceriporiopsis subvermispora* are well known for their lignin-degrading abilities [12-13]. However, large-scale commercial use of fungal enzymes is often limited by high production costs and reduced stability under extreme environmental conditions [11], [14]. In contrast, ligninolytic bacteria exhibit greater adaptability to environmental fluctuations, are easier to genetically manipulate, and show better compatibility with integrated residue management systems [15-17]. Bacterial lignin degradation is mediated by specific oxidative enzymes such as laccases, lignin peroxidases, manganese peroxidases, versatile peroxidases, dye-decolourising peroxidases, and catalase–peroxidases, which oxidise the aromatic structures of lignin and facilitate its breakdown [18-19].

The present study aimed to isolate ligninolytic bacteria from diverse lignin-rich environments and evaluated their ligninolytic potential through decolourisation of lignin-mimicking aromatic dyes. Additionally, the cellulolytic activity of the isolates was assessed using carboxymethyl cellulose. The findings of this study helped identify efficient bacterial candidates with potential applications in lignin valorisation and sustainable management of agricultural residues.

MATERIALS AND METHODS

Sample collection and isolation

Samples of coir pith, cow dung, decayed wood, forest soil, marshy soil, rice straw, sawdust, and termites were

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Citation: Vyshnav T, Unnikrishnan BV, Gopal SK, Panchami PS, Binitha NK. 2025. Isolation, screening and characterization of ligninolytic bacteria from diverse environmental sources. *Res. Jr. Agril. Sci*. 16(6): 617-623.

collected from various lignin-rich environments across the Thrissur district of Kerala. Each sample was enriched in a selective medium for seven days to promote the growth of ligninolytic microorganisms. Following enrichment, serial dilutions were prepared, and aliquots were spread onto selective agar plates [20]. The composition of the selective medium (per litre of distilled water, pH 7.0) was as follows: alkali lignin (1.00 g), K_2HPO_4 (4.55 g), KH_2PO_4 (0.53 g), $MgSO_4 \cdot 7H_2O$ (1.02 g), $NaNO_3$ (5.30 g), $(NH_4)_2SO_4$ (4.17 g), and yeast extract (0.10 g). The plates were incubated at 30 °C for 1-3 days, and morphologically distinct colonies were purified by repeated streaking on nutrient agar.

Screening for dye decolourisation

The ligninolytic potential of the purified bacterial isolates was qualitatively assessed using three aromatic dyes (Congo Red, Azure B, and Methylene Blue). The screening medium consisted of K_2HPO_4 (4.55 g), KH_2PO_4 (0.53 g), $MgSO_4 \cdot 7H_2O$ (1.02 g), $NaNO_3$ (5.30 g), $(NH_4)_2SO_4$ (4.17 g), yeast extract (0.10 g), and glycerol (40 mM), 17 g L^{-1} agar and supplemented with 100 mg L^{-1} of each dye (pH 7.0). Purified bacterial cultures were inoculated onto the dye plates and incubated at 30 °C for 3–7 days. The extent of decolourisation was visually graded as strong (+++), moderate (++), weak (+), or absent (–).

Based on the qualitative screening, the ten most efficient isolates were selected for quantitative dye decolourisation assays. Each isolate was inoculated into broth containing the same basal medium and 100 mg L^{-1} of the respective dye, and incubated at 30 °C under shaking conditions for 7 days. The absorbance of the culture supernatant was measured spectrophotometrically at 498 nm (Congo Red), 650 nm (Azure B), and 664 nm (Methylene Blue) (Haq *et al*. 2018, Wu *et al*. 2022, Sarkar *et al*. 2025). The percentage of dye decolorizations was calculated using the formula:

$$\textit{Dye decolourisation}\ (\%) = 1 - \frac{A_{sample}}{A_{control}} \times 100$$

Screening for cellulose degradation

The cellulolytic potential of the bacterial isolates was evaluated using carboxymethyl cellulose (CMC) agar medium [21]. The isolates were inoculated onto CMC agar plates and incubated at 28 °C for 48 hours. After incubation, the plates were flooded with Gram's iodine solution and observed for 3–5 minutes for clear zones.

Morphological and biochemical characterization

Morphological features, including Gram reaction and endospore formation, were examined using standard staining techniques. Biochemical characterization was carried out using a series of tests, including indole production, methyl red, Voges–Proskauer, catalase, citrate utilization, oxidase, and urease [221.

RESULTS AND DISCUSSION

Isolation of ligninolytic bacteria

A total of 46 bacterial isolates were successfully obtained from eight diverse environmental sources (Table 1). The highest number of isolates was obtained from decayed wood and sawdust, with nine isolates from each.

Table 1 Details of purified isolates from different sources

S. No.	Source	No. of isolates	Code for isolates
1.	Cow dung	3	CD-1, CD-2, CD-3
2.	Coir pith	4	CP-1, CP-2, CP-3, CP-4
3.	Decayed wood	9	D-1, D-2, D-3, D-4, D-5, D-6, D-7, D-8, D-9
4.	Forest soil	7	F-1, F-2, F-3, F-4, F-5, F-6, F-7
5.	Marshy soil	7	M-1, M-2, M-3, M-4, M-5, M-6, M-7
6.	Rice straw	4	R-1, R-2, R-3, R-4
7.	Sawdust	9	S-1, S-2, S-3, S-4, S-5, S-6, S-7, S-8, S-9
8.	Termite	3	T-1, T-2, T-3
	Total	46	

Screening for dye decolourisation

Out of 46 isolates, 25 exhibited visible decolourisations of at least one of the three test dyes (Table 2, Fig 1). Ten isolates exhibiting the strongest qualitative dye decolourisation were selected for quantitative assessment. Among them, isolate S–4 achieved the highest decolourisation of Congo Red (93.07%) and Methylene Blue (84.73%), whereas isolate D–6 showed maximum decolourisation of Azure B (32.70%) (Table 3, Fig 2-3). The observed variation in decolourisation efficiency among isolates highlights the substrate-dependent ligninolytic enzyme activity. Champagne and Ramsay [23] reported that in *Trametes versicolor*, manganese peroxidase efficiently decolourised azo dyes, while laccase was more effective against anthraquinone dyes, indicating enzyme–substrate specificity. Similarly, Wang *et al* [24] noted that the oxidative range of ligninolytic enzymes varies with substrate structure, with laccase and MnP preferring phenolic compounds, and LiP and VP acting on non-phenolic ones. These findings align with the observed variation in dye decolourisation among isolates in this study.

Table 2 Qualitative screening of bacterial isolates for dye decolourisation

S. No.	Isolates	Dye decolourisation		
		Congo red	Azure B	Methylene blue
1.	CD-1	+++	+++	-
2.	CP-2	+++	-	-
3.	T-3	+++	-	-
4.	D-6	++	+++	-
5.	S-5	++	++	-
6.	S-4	++	++	++
7.	R-1	++	++	++
8.	M-2	++	++	-

9.	D-1	-	++	+
10.	D-2	-	++	+
11.	F-5	+++	-	-
12.	M-7	++	-	-
13.	M-6	++	-	-
14.	F-2	++	-	-
15.	T-2	++	-	-
16.	D-3	-	-	++
17.	D-4	-	-	++
18.	D-5	-	-	++
19.	CD-2	+	-	-
20.	CP-1	+	-	-
21.	F-1	+	-	-
22.	M-5	+	-	-
23.	S-1	+	-	-
24.	S-2	+	-	-
25.	T-1	+	-	-

Clear zone: +++ (strong), ++ (moderate), + (weak), - (absent)

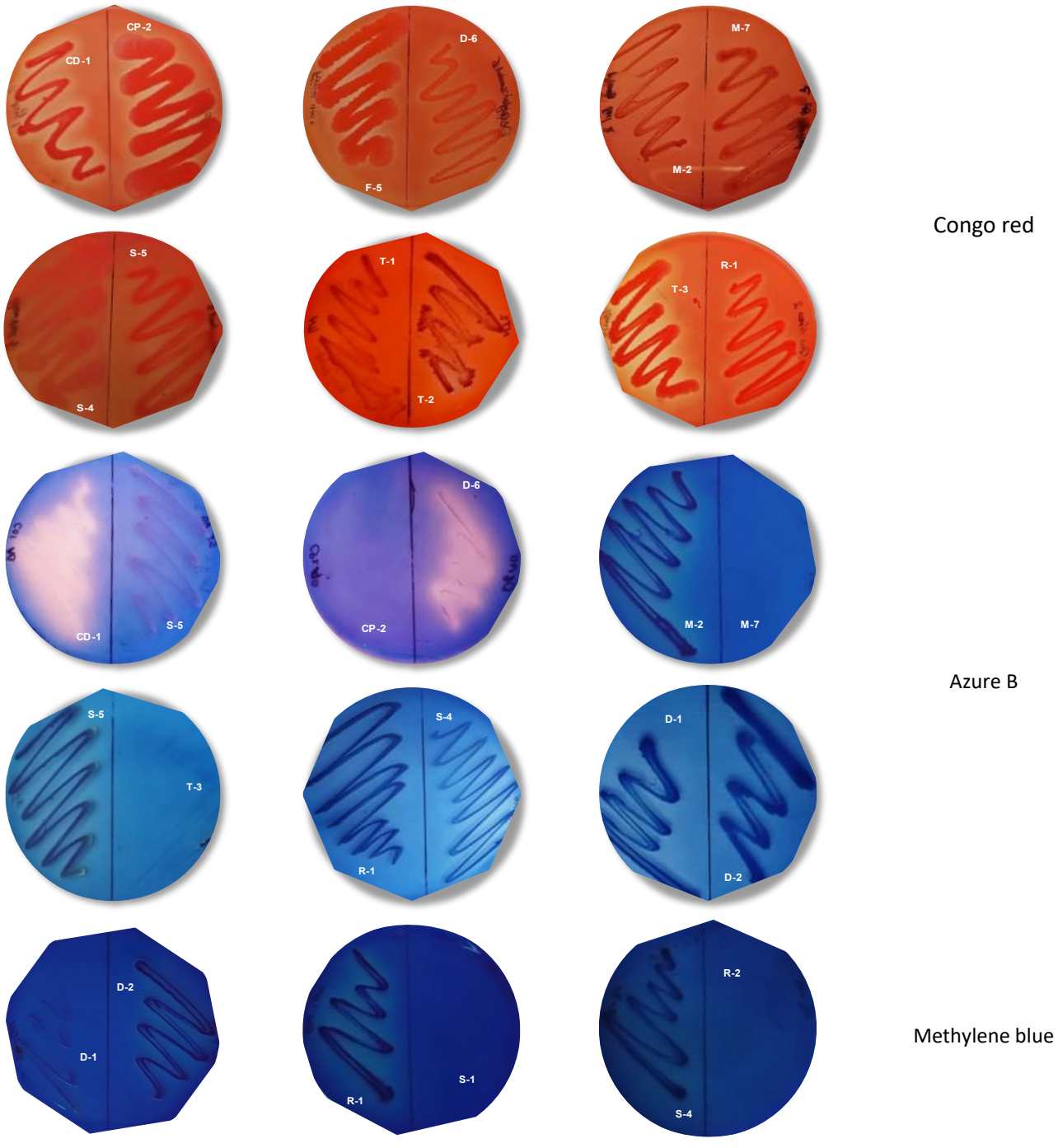

Fig 1 Screening for ligninolytic activity by dye decolourisation

Table 3 Quantitative dye decolourisation by selected isolates

Treatments	Dye decolourisation (%)		
	Congo red	Azure B	Methylene blue
CD–1	49.06 ± 3.06^{c}	26.33 ± 5.41^{b}	9.09 ± 1.12^{f}
CP–2	74.75 ± 12.84^{b}	4.73 ± 0.64^{f}	17.32 ± 0.28^{e}
D–1	17.08 ± 2.12^{e}	16.20 ± 0.97^{c}	81.10 ± 6.33^{a}
D–2	48.12 ± 2.88^{c}	12.74 ± 1.48^{cd}	33.62 ± 3.04^{c}
D–6	20.12 ± 2.63^{de}	32.70 ± 2.13^{a}	40.93 ± 1.40^{b}
M–2	25.91 ± 0.83^{d}	23.84 ± 1.93^{b}	18.07 ± 1.36^{de}
R–1	49.55 ± 0.94^{c}	9.96 ± 0.93^{de}	30.17 ± 1.03^{c}
S–4	93.07 ± 2.64^{a}	10.67 ± 1.27^{d}	84.73 ± 4.08^{a}
S–5	50.54 ± 0.73^{c}	26.32 ± 5.52^{b}	14.36 ± 0.94^{e}
T–3	74.73 ± 4.11^{b}	5.68 ± 1.54^{ef}	22.51 ± 2.07^{d}

Values are mean ± SD; Treatments with the same letter grouping are not significantly different according to DMRT

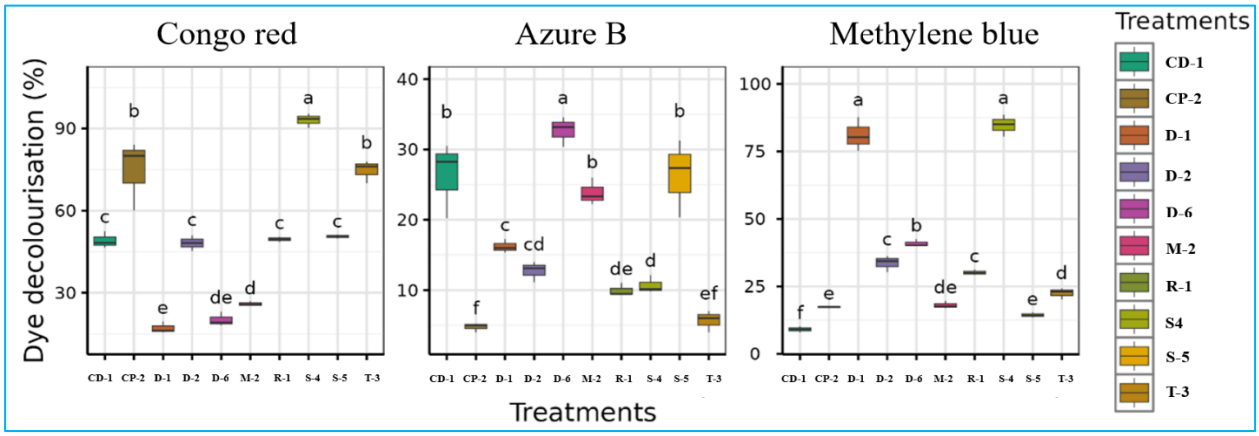

Fig 2 Decolourisation of aromatic dyes by selected isolates

Congo red

Control

S-4

CP-2

Azure B

Control

D-6

CD-1

Fig 3 Quantitative dye decolourisation assay in liquid medium

Screening for cellulose degradation

The cellulose-degrading ability of the selected bacterial isolates was assessed using carboxymethyl cellulose (CMC) agar medium (Fig 4). This suggests that these isolates possess robust cellulolytic enzyme systems capable of breaking down complex polysaccharides into simpler sugars. Similar observations have been reported in earlier studies, where bacterial isolates showing clear halos on carboxymethyl cellulose (CMC) agar were confirmed to produce high levels of cellulase activity [21], [25].

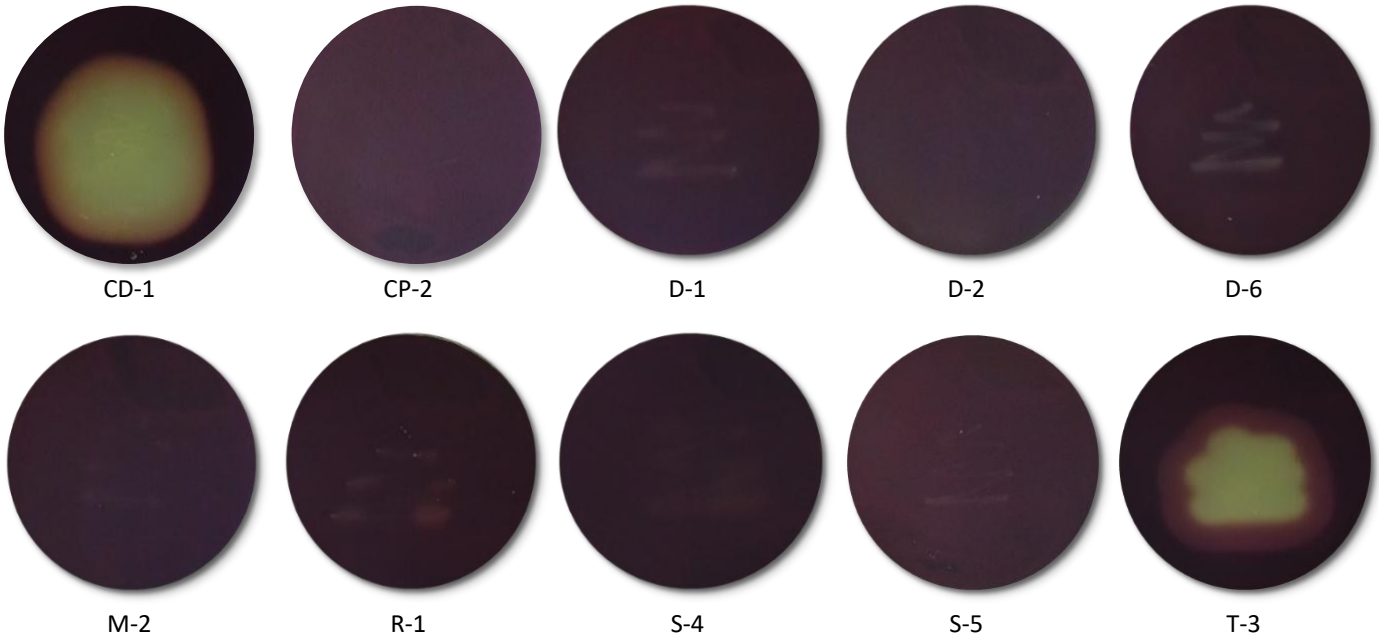

Fig 4 Cellulose-degrading activity of isolates on carboxymethyl cellulose (CMC) agar medium

Table 4 Morphological and biochemical characterization of selected isolates

S. No.	Isolate	Size	Shape	Gram reaction	Endospore	Indole	MR	VP	Catalase	Citrate	Oxidase	Urease
1.	CD–1	Large	Rods (single)	+	+	-	+	-	+	-	+	-
2.	CP–2	Large	Rods (single); elongated	+	+	-	-	-	+	-	+	-
3.	D–1	Small	Cocci (single)	-	-	-	-	-	+	-	weak +	-
4.	D–2	Small	Very short rod	+	-	-	weak +	+	+	+	weak +	-
5.	D–6	Large	Rods (Single)	+	+	-	+	weak +	+	-	+	-
6.	M–2	Small	Cocci (tetrad)	+	-	-	-	-	+	-	+	-
7.	R–1	Small	Very short rod	+	-	-	+	-	-	+	-	-
8.	S–4	Small	Rods (Single)	-	-	-	-	-	weak +	+	+	+
9.	S–5	Large	Rods (Single)	-	-	-	-	-	+	+	+	+
10.	T–3	Large	Rod in chains	+	+	-	-	-	+	-	-	-

*(+) Positive; (-) Negative

Characterization of bacterial isolates

Among the ten isolates, seven (CD–1, CP–2, D–2, D–6, M–2, R–1, and T–3) were Gram-positive, mostly rod-shaped with variations in size and arrangement (single rods, chains, or short rods). Endospore staining procedure identified CD–1, CP–2, D–6, and T–3 as endospore formers. Biochemical characterization demonstrated variations in metabolic activity. Most isolates were catalase-positive and indole-negative, while weak or variable responses were noted for MR, VP, and oxidase tests. Such diversity suggests metabolic versatility among the ligninolytic strains (Table 4).

CONCLUSION

The present study successfully isolated and characterized ligninolytic bacteria from diverse lignin-rich environments in Thrissur, Kerala. A total of 46 isolates were obtained, of which 25 demonstrated the ability to decolourise at least one of the lignin-mimicking dyes, indicating ligninolytic potential. Quantitative assays identified isolate S–4 as the most efficient, achieving 93.07% and 84.73% decolourisation of Congo Red and Methylene Blue, respectively, while isolate D–6 showed maximum decolourisation of Azure B (32.70%). Only two isolates exhibited strong cellulose degradation, whereas others showed weak or no activity. Morphological and biochemical characterization revealed considerable diversity and metabolic adaptability among the isolates. The findings of this study suggested that the selected bacterial isolates were effective lignin degraders with additional cellulose-degrading ability, making them promising candidates for developing microbial formulations for residue decomposition and lignocellulosic biomass valorisation. Future research could focus on the purification and characterization of ligninolytic enzymes, molecular identification of efficient isolates, and optimization of cultural conditions to enhance enzyme production. Consortium-based field studies could further improve the efficiency and applicability of biological residue management.

Conflict of interest

The authors declare that there is no conflict of interest.

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