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ORIGINAL ARTICLE

Cultivation methods, characterization, and biocatalytic potential of organic solid waste degrading bacteria isolated from sugarcane rhizospheric soil and compost

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Abstract

The study aimed to investigate organic waste-degrading microbial communities in compost and sugarcane rhizospheric soil from Kodinar, Gujarat, India and to assess their potential for producing novel bioactive compounds. Compost contained higher total culturable heterotrophic bacteria (1.3×10^8) and cellulolytic bacterial (4×10^6) populations compared to rhizospheric soil. A total of 96 isolates were obtained using various cultivation strategies with 35 isolates obtained through enrichment techniques, and the rest through direct plating on nutrient agar. Remarkably, four isolates CP2, CP4, CP9 and CP11 showed significant lignin hydrolysis. Further, morphological characterization revealed 26 Gram-positive isolates. Based on microscopic, cultural, and biochemical assessments, as well as 16 S rRNA gene sequencing, selective isolates with lignocellulolytic potential were identified as *Bacillus licheniformis* (SRS 11), *Priestia megaterium* (SRS 8, CP 5), *Bacillus subtilis* (CP11), and *Priestia flexa* (CP 2 & CP 9). Multiple enzyme secretion potential of the isolates was also investigated. Around 16.66% of isolates secreted at least one lytic enzyme such as cellulase, ligninase, amylase, protease, and pectinase, with variable percentages. The salt significantly affected growth and CMCase activity, with strain SRS8 being less tolerant (3% NaCl) compared to CP2, CP9, and CP11, which exhibited activity between 0 and 7% NaCl (w/v). Compost isolates preferred higher temperatures (40–45 °C), while most rhizospheric isolates thrived at lower temperatures (35 °C). Rhizospheric isolates preferred pH 7. Most compost isolates grew at pH 7, except CP9 and CP11, which showed best growth at pH 8. Overall, the study highlights microbial waste-degrading potential, enzyme production, and environmental preferences.

Keywords Lignocellulose · Agricultural waste degradation · Population · Diversity · Sugarcane rhizospheric soil and compost

Abbreviations

ABTS	2, 2- Azino-bis 3-ethylbenz-thiazoline-6-sulfonic acid
CMC-MSM	Carboxymethylcellulose - Minimal salt medium
CMC	Carboxymethylcellulose sodium
CP	Compost pits
CPCB	Central pollution control board
EC	Enzyme commission number
KL	Kraft lignin
L-MSM	Lignin - Minimal salt medium
LBM	Lignin basal medium
MSW	Municipal solid wastes
NCBI	National Center for Biotechnology Information
OSW	Organic solid waste
REA	Relative enzyme activity
SRS	Sugarcane rhizospheric soil
TCI	Tokyo chemical industry

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Introduction

Solid waste substances or materials containing organic constituents generated by human activities include municipal organic solid wastes such as kitchen waste, green waste, slaughterhouse wastes and sludge. Similarly, agricultural organic solid waste includes livestock, manure, and crop straw. These types of wastes are normally incinerated, disposed to landfill sites or utilised to feed animals and dung cake preparation. Unsafe disposal of organic fractions of municipal solid wastes (MSW) causes health issues, environmental pollution in terms of methane emissions, and leachate issues thereby contaminating groundwater, etc. According to the data displayed by CPCB (Central Pollution Control Board), India, 117,644 MT of total solid wastes were collected in 2018 and out of these only 49,401 MT of solid wastes were treated. Major constituents of organic agricultural waste are materials rich in lignocellulose, minerals, proteins, and other sugars. Organic solid waste (OSW) could be productively utilized as a substrate or raw materials for various processes like composting, anaerobic digestion, ex-situ green manuring etc.

Organic solid waste is a source of underutilized, renewable biomass on the earth. An enormous quantity of organic waste is generated annually which can be managed by composting. Heterotrophic bacterial and fungal species have the potential to convert this organic heteropolymer to a simple monomeric substance which has a potential application. The major fraction of agricultural waste is lignocellulose (35-50% cellulose and 11 -15% lignin) (Jiang et al. 2020). Other domestic food wastes like bread, savoury, fruits, vegetables, onion & potato pill wastes, restaurant wastes and slaughterhouse wastes contain starch, pectin and proteins.

Endoglucanase (EC 3.2.1.4), exoglucanase (EC 3.2.1.74), and β-glucosidase (EC 3.2.1.21) are the cellulases responsible for the conversion of cellulose to glucose with a different mechanism of action and substrate specificity. Lignin is the most degradation-resistant constituent found in plant residue. Lignin degradation is facilitated by three major complex enzymes namely laccases (EC 1.10.3.2), lignin peroxidases (EC 1.11.1.14), and manganese peroxidases (EC1.11.1.13) (Tao et al. 2022). Hydrolysis of pectin is catalyzed by pectin methylesterase (EC3.1.1.11) which acts as a de-methoxylating enzyme that removes the methyl group from pectin while pectin lyase (EC 4.2.2.10) breaks pectin in exo action pattern generating oligomers (Datta et al. 2020). Amylolytic enzymes are widely distributed in bacterial and fungal genera. Endo-acting α amylase (EC 3.2.1.1) and exo-acting β amylase (EC 3.2.1.2) hydrolyse polymeric starch to monomeric glucose (Paul et al. 2021). Extracellular proteases (EC 3.4) hydrolyze proteinaceous wastes by breaking peptide bonds present in the polypeptide chain of amino acids.

Trichoderma, Penicillium, Chaetomium, Aspergillus, Phanerochaete, and other white-rot basidiomycetes have been widely studied for lignocellulases production (Li et al. 2019; Xia et al. 2019; Machado et al. 2020). Fungus has a higher potential for lignocellulose degradation than bacteria but in contrast, bacteria have the expression of multiple enzyme complexes, rapid growth rate, and extreme environmental tolerance. Due to this versatility bacterial enzyme secretion has become the emphasis of production research (Chuanjiao et al. 2021). Bacterial lignocellulases production has been extensively reported in the literature. Actinomycetes genera like *Streptomyces, Arthrobacter, Micromonospora, Nocardia* and non-filamentous bacterial genera like *Acinetobacter, Flavobacterium, Cellulomonas, Micrococcus, Pseudomonas*, and *Xanthomonas* have strong lignocellulose degrading ability (Wu et al. 2023; Dube et al. 2023; Zhang et al. 2021). Pectin methylestrase and pectin lyase production have been reported in many microbes, including, *Pseudomonas solanacearum, Aspergillus niger, Neurospora crassa, Aspergillus sp*., *Bacillus sp. Lactobacillus lactis, Penicillium occitanis, A. japonicus* (Datta et al. 2020; Murad et al. 2011), *Erwinia carotovora, Pseudomonas syringae*, and *Bacillus sp.* (Murad et al. 2011). Bacterial amylolytic enzymes are widely distributed in bacterial and fungal genera including, *Bacillus, Proteus, Pseudomonas, Escherichia coli, Lactobacillus, Streptomyces, Talaromycesemersonii, Aspergillus ficuum*, and *Thermomyceslanuginosus* (Wu et al. 2018; Pokhrel et al. 2013; Shishtawy et al. 2014). Extracellular protease production was reported in bacterial strains including *Bacillus safensis, Bacillus lentus, Pseudomonas fuorescens, Geobacillus, Bacillus alkaloophilus, Bacillus pumilus, Bacillus halodurans, Pseudomonas aeruginosa, Bacillus licheniformis* and *Bacillus amyloliquefaciens* (Ahmad et al. 2020; Contesini et al. 2018; Majithiya and Gohel 2020). Fungus is the next microbes in protease production. *Aspergillus, Cephalosporium, Rhizopus, Fusarium, Penicillium, Mucor*, and *Trichoderma* are the fungal protease producers (Abu-Tahon et al. 2020; Naveed et al. 2021).

Metabolites from bacteria/actinobacteria are exceptional, novel, and occasionally intricate (Gohel and Singh 2018; Chauhan and Gohel 2020). They possess excellent enzymatic and antibacterial properties, while also showing low toxicity (Chauhan et al. 2021; Majithiya and Gohel 2022a, b; Vaghela and Gohel 2023). Therefore, the study aimed to cultivate lignocellulolytic bacteria from rhizospheric soil and compost samples using an enrichment and serial dilution process with diverse, nutrient-rich growth media. Moreover, the study covers morphological, cultural, biochemical, and

molecular characterization, as well as growth and enzymatic profile analysis of potent rhizospheric and compost isolates.

Materials and methods

Sample collection

Sampling sites were selected such that sufficient lignocellulose can be available naturally, whereby the inhabitant microbial population could primarily be lignocellulolytic by nature which could be easily isolated in large numbers. Samples collected from compost and rhizospheric soil of sugarcane plantations in different areas of Kodinar Taluka, Gujarat, India were screened for the isolation of organic waste-degrading bacteria. The isolation process was initiated within 8 h of collection to minimize saprophytic developments.

Enrichment of lignocellulolytic bacteria

A nutrient enrichment technique was used to enrich and isolate lignocellulolytic bacteria. A total of 05 g sample was added in 100 mL of minimal salt media (MSM-L), which contained, $NaNO_3$ 2.5 g; KH_2PO_4 2 g; $MgSO_4$ 0.2 g; NaCl 0.2 g; $CaCl_2.6H_2O$ 0.1 g; and lignin (TCI) 100 mg in a litre. Due to the higher molecular weight of lignin, bacterial strains could not grow by consuming lignin as a single carbon source. Therefore, glucose (w/v) 10 g; peptone (w/v) 5 g; and trace elements solution (1 mL L^{-1}) were added in an MSM- L broth as suggested earlier (Pfenning and Lippert 1966; Zainith et al. 2019). The medium pH was adjusted to 7.5 ± 0.1. The flasks were incubated at shaking conditions (120 RPM) for seven days. After 7 days, the 10 mL sample was retransferred in fresh 90 mL of the same culture medium and incubated for 72 h. At every 72 h, three more successive transfers were done to enrich lignolytic bacteria (Chandra et al. 2012). Similarly, cellulolytic bacteria were enriched by using minimal media (MSM-CMC) supplemented with 1% Na salt of carboxymethylcellulose (medium viscosity).

Isolation of bacteria

For each sample, dilution ratios of 10^{-4}, 10^{-5} and 10^{-6} were spread in duplicate in petri dishes containing L-MSM, CMC-MSM and nutrient agar. An isolation medium was prepared, having a similar composition to the enrichment medium. The abundance of bacteria was calculated from the counting data, as colony-forming units (CFU g^{-1}) of the sample. Representative colonies were purified by repeated streaking on NA plates. Purified colonies from L-MSM agar plates were further studied for utilisation of sole carbon source lignin (300 mg L^{-1}) by nutrient enrichment plate assay (Chandra et al. 2012).

Morphological and cultural characterization

Isolates recovered on L-MSM and CMC-MSM media plates were further studied for morphological and cultural characterizations by inoculating in sterile NA media plates. After 48 h of incubation at 37 °C, their morphological characteristics such as Grams staining, shape and arrangements were observed in a compound microscope under 100X magnification. Colony characteristics like colony size, form, margin and elevation were noted. The pure cultures were preserved on a nutrient agar slant at 4 °C temperature.

DNA extraction, PCR amplification and identification

DNA was extracted and amplified using universal primers (forward primer 27F, 5' AGAGTTTGATCCTGGCTCAG 3', and reverse primer 1492R, 5' TACGGTTACCTTGTTACGACTT 3') for 16 S rRNA gene amplification at 94 °C for 5 min initial denaturation, 94 °C for 20s denaturation, 58 °C for 20s annealing, 72 °C for 10 min extension. The size of the fragments was confirmed using agarose gel electrophoresis. The Sanger sequencing was performed for 16 S rRNA gene sequencing. The sequence was submitted to NCBI (National Center for Biotechnology Information). The phylogenetic tree was constructed using MEGA X software.

Qualitative screening of isolates for cellulase production

Purified strains were inoculated as a regular spot into media plates containing, Bushnell Haas agar supplemented with 1% carboxymethylcellulose Na salt (medium viscosity) and incubated for 48 h at 37 °C temperature. Clearance zone surrounding colonies were observed by flooding plates with Gram's iodine. The relative enzyme activity of isolates was calculated by following the formula:

REA = Diameter of clearance zone (mm)/ Diameter of bacterial colony (mm).

Qualitative screening of isolates for amylase production

The starch agar medium was used to screen extracellular amylase production. The bacterial suspension was inoculated for 24 h at 37 °C temperature. Gram's iodine was used as a differentiating stain to observe the zone of clearance. Above mentioned formula was used to calculate REA.

Qualitative screening of isolates for protease production

Gelatine agar medium was used to screen extracellular protease production. The bacterial suspension was inoculated in gelatin agar and incubated for 24 h at 37 °C temperature. Frazier's reagent [containing (g L^{-1}) concentrated HCl (200 mL); mercuric chloride (150 gm)] was used to flood the plate. The clearance zone indicated protease secretion. Above mentioned formula was used to calculate REA.

Qualitative screening of isolates for pectinase production

Qualitative screening of extracellular pectinase was carried out on the pectin agar medium. Bacterial strains were spot inoculated and incubated for 24 h at 37 °C temperature. Detection of extracellular pectinase was carried out by flooding the plates with Grams iodine. The clearance zone surrounding colonies was measured and REA was calculated by the above-mentioned formula.

Qualitative screening of isolates for ligninases production

Lignin degrading ability was measured using the ABTS agar method. Bacterial strains were grown on Lignin Basal Medium (LBM) containing (g L^{-1}) (KH_2PO_4; 1, $C_4H_{12}N_2O_6$; 0.5, $MgSO_4$. $7H_2O$; 0.5, $CaCl_2.2H_2O$; 0.01, yeast extract; 0.01, $CuSO_4.H_2O$; 0.001, Fe_2 $(SO_4)_3$; 0.001, $MnSO_4.H_2O$; 0.001, agar; 16) supplemented with 0.1% w/v ABTS. 1 mL of 20% glucose (separately sterilized) was aseptically added to 100 mL of LBM. Cells were inoculated on LBM and plates were incubated at 28 °C temperature for 24 h. ABTS (2, 2- Azino-bis 3-ethylbenz-thiazoline-6-sulfonic acid) was converted to ABTS- azine a green colour substance by laccase-producing isolates. Thus, colonies with a green colour indicate ligninase-secreting isolates (Neethu et al. 2017).

Biochemical characterization

Strains producing the mentioned enzymes were further studied for biochemical characterization. Positive and negative results were noted for biochemical tests viz. indole test, MR test, VP test, citrate utilization test, catalase test, oxidase test, urea hydrolysis test, gelatine hydrolysis test, ammonia production test, nitrate reduction test, H_2S production test, dehydrogenase test and haemolysis production test.

Salt profile

The impact of NaCl on extracellular cellulase secretion was examined at NaCl concentrations ranging from 0 to 15% w/v. The study used an agar medium containing 1% carboxy-methylcellulose Na salt (medium viscosity) as the sole carbon source, supplemented with Bushnell Haas agar. After 48 h of incubation at 37 °C, the plates were flooded with Gram's iodine, and the ratio of the zone of clearance to the colony diameter was calculated.

Temperature growth profile

The optimal growth temperature of bacterial isolates was determined by inoculating uniform inoculums (1 absorbance) in nutrient broth and incubating at temperatures ranging from 30 to 50 °C for 48 h. The medium pH was adjusted to 7 using 1 N HCl and 1 N NaOH. Bacterial growth was periodically estimated spectrophotometrically at 600 nm wavelength after 0, 24, and 48 h of incubation using 5 mL harvested suspensions.

pH growth profile

The effects of pH on the growth behaviour of bacterial isolates were determined in nutrient broth. The pH of the medium was adjusted in the range of acidic to alkaline (4, 5, 6, 7, 8, 9, 10, and 11) by 1 N HCl and 1 N NaOH. The inoculum (1 absorbance) was added to media broth of various pH values and incubated for 48 h at 35 °C temperature. Aliquots of 5 mL inoculated suspension were periodically harvested at 0, 24 and 48 h of incubation and turbidity of bacterial growth was estimated spectrophotometrically at 600 nm wavelength. Growth assessment involved a comparison of the turbidity in the growth medium with that of the controls.

Statistical analysis

The physiological characteristics along with the enzymatic profile were examined in triplicate and both standard deviation and standard error were calculated to estimate variability within a sample and across the samples respectively. The statistical analysis was performed using SPSS software. Moreover, the ANOVA (Analysis of Variance) was performed for physiological characteristics and enzymatic profile.

Results

The synergistic action of endoglucanases, exoglucanases and β-glucosidase is required to break cellulose polysaccharides. However, some fungal cellulase systems lack β-glucosidase, causing feedback inhibition and repression due to cellobiose accumulation. Bacteria have rapid growth rates and multienzyme expression potential; therefore, emphasis has been given to the isolation of bacterial genera having highly active and specific secretion systems (Petre et al. 2000).

Enrichment and isolation of bacteria

Cellulolytic and lignolytic bacteria were enriched in CMC-MSM and L-MSM enrichment broth. CMC was used as the sole carbon and energy source in the CMC-MSM enrichment broth. However, in addition to lignin, glucose and peptone were used as additional carbon and nitrogen sources in L-MSM enrichment broth, which worked as a co-substrate to enhance lignolytic bacteria (Zainithet al. 2019). Variations in the total culturable heterotrophic bacterial populations (CFU g^{-1}) were observed in these samples. The highest population density of total culturable heterotrophic bacteria was observed as 1.3×10^8 and 1.2×10^8 CFU g^{-1}on L-MSM and NA plates respectively, while the culturable cellulolytic bacterial population displayed 4×10^6 CFU g^{-1} population densities in the compost sample. However, culturable heterotrophic bacterial population density of sugarcane rhizospheric samples on L-MSM and NA plates were observed as 1.2×10^7 CFU $g^{-1}$and 1.7×10^7 CFU g^{-1}respectively, while culturable cellulolytic bacterial population density was 2.5×10^5 CFU g^{-1}. The proportion of the population of cellulose-degrading bacteria to the total culturable heterotrophic bacteria in compost and rhizospheric soil was 3.33% and 1.47% respectively.

In the present investigation, cultivation strategies resulted in 96 bacterial isolates from compost and sugarcane rhizospheric soil. Enrichment techniques yielded 36.45% isolates (L-MSM; n = 21, 21.87% and CMC-MSM; n = 14, 14.58%) whereas, the rest of the n = 61, 63.54% isolates by direct plating on NA medium. Results are shown in Fig. 1. A total of 21 isolates were recovered on an L-MSM isolation medium containing supporting carbon and nitrogen sources. These isolates were further streak plated on a minimal medium supplemented with lignin (300 mg L^{-1}) as a sole carbon and energy source to study lignin utilisation. Remarkable growth was observed in four isolates CP2, CP4, CP9 and CP11 showing the indication of lignin hydrolysis. Additionally, 14 isolates in this study, recovered from CMC-MSM plates were considered cellulolytic bacterial isolates.

Morphological and cultural characterization of isolates

With the aim of morphological and cultural characterization in the present investigation, 35 isolates recovered on L-MSM and CMC-MSM mediums were selected and cultivated on nutrient agar for 24 h at 37 °C temperature. The morphological and cultural characterization of these isolates recovered on CMC-MSM and L-MSM media plates are shown in Table 1. Microscopic observations revealed that 75% of isolates were Gram-positive. Furthermore, rod shape was observed in 82.85% of isolates while 22% of isolates were arranged in chains and 48% of isolates displayed isolated cell arrangements. Colony characteristics revealed that 45% of colonies have intermediate size whereas, 31% large and 15% small colonies were observed. The irregular

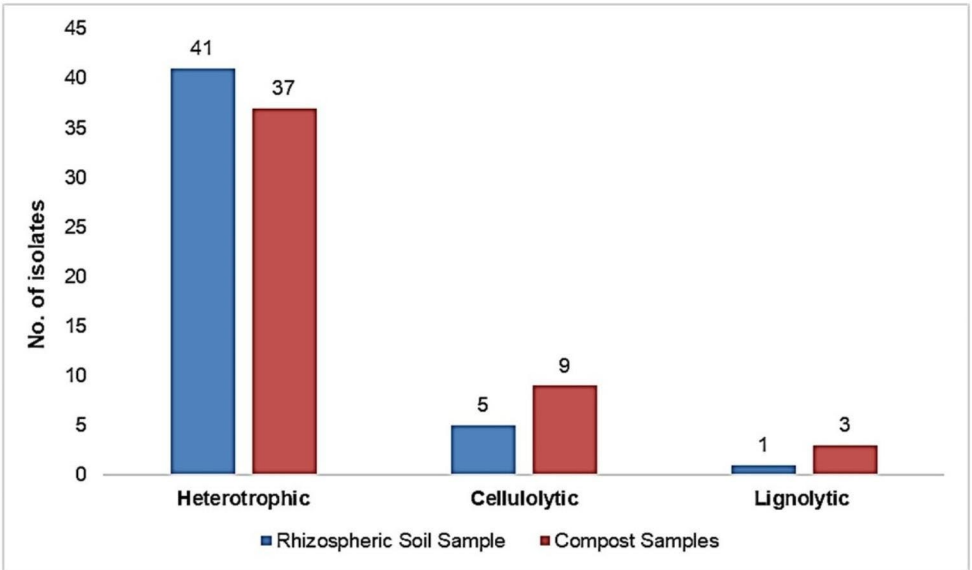

Fig. 1 Total number of heterotrophic, cellulolytic and lignolytic isolates recovered from rhizospheric soil and compost sample from Kodinar, Gujarat

Table 1 Morphological and cultural characterization of isolates

Sr. No.	Isolates	Gram's staining	Shape	Arrangement	Colony size	Shape/ Form	Margin/Edge	Elevation
1	CP2	G (+)	Rod	Isolated	Small	Irregular	Entire	Convex
2	CP4	G (-)	Rod	Isolated	Intermediate	Irregular	Undulate	Flat
3	CP5	G (+)	Rod	Chain	Large	Round	Undulate	Flat
4	CP9	G (+)	Rod	Isolated	Large	Round	Undulate	Convex
5	CP11	G (+)	Rod	Chain	Intermediate	Round	Erose	Flat
6	CP17	G (+)	Rod	Isolated	Intermediate	Round	Erose	Flat
7	CP18	G (-)	Cocci	Cluster	Intermediate	Round	Entire	Convex
8	CP21	G (+)	Rod	Chain	Intermediate	Round	Erose	Umbonate
9	CP22	G (+)	Oval to Round	Isolated	Punctiform	Round	Entire	Raised
10	CP26	G (-)	Cocci	Cluster	Punctiform	Round	Erose	Raised
11	CP30	G (+)	Rod	Chain and Pair	Large	Round	Undulate	Flat
12	CP32	G (+)	Rod	Chain and Pair	Large	Round	Undulate	Flat
13	CP35	G (-)	Cocci	Cluster	Small	Round	Entire	Raised
14	CP36	G (+)	Rod	Isolated	Intermediate	Irregular	Curled	Flat
15	CP38	G (+)	Rod	Chain and Pair	Intermediate	Round	Entire	Flat
16	CP39	G (+)	Rod	Isolated	Intermediate	Irregular	Undulate	Flat
17	CP42	G (+)	Rod	Chain	Large	Round	Entire	Convex
18	CP44	G (+)	Rod	Isolated	Small	Irregular	Lobate	Flat
19	CP47	G (+)	Rod	Chain	Large	Irregular	Undulate	Flat
20	CP48	G (-)	Rod	Pair	Intermediate	Round	Entire	Pulvinate
21	SRS3	G (+)	Rod	Isolated	Intermediate	Irregular	Erose	Raised
22	SRS8	G (+)	Rod	Chain	Large	Round	Lobate	Convex
23	SRS11	G (+)	Rod	Isolated	Intermediate	Round	Entire	Flat
24	SRS17	G (+)	Rod	Chain	Large	Round	Undulate	Flat
25	SRS18	G (-)	Rod	Chain and Pair	Punctiform	Round	Entire	Raised
26	SRS21	G (+)	Rod	Isolated	Large	Round	Lobate	Convex
27	SRS22	G (+)	Rod	Chain and Pair	Intermediate	Irregular	Entire	Flat
28	SRS25	G (+)	Rod	Isolated	Intermediate	Irregular	Erose	Flat
29	SRS30	G (-)	Cocci	Chain	Intermediate	Round	Entire	Raised
30	SRS34	G (-)	Cocci	Cluster	Small	Round	Entire	Raised
31	SRS35	G (+)	Rod	Isolated	Large	Round	Entire	Convex
32	SRS39	G (+)	Rod	Isolated	Large	Irregular	Undulate	Flat
33	SRS43	G (-)	Rod	Isolated	Small	Round	Entire	Raised
34	SRS44	G (+)	Rod	Isolated	Intermediate	Irregular	Curled	Flat
35	SRS46	G (+)	Rod	Isolated	Intermediate	Irregular	Erose	Raised

and round shape was observed in 34% and 65% of colonies respectively.

Identification of isolates

In order to identify the potent isolates SRS11, SRS8, CP11, CP9, CP5, and CP2, the 16S rRNA gene was first amplified using the universal primers 1492R (5' TACGGTTACCTTGTTACGACTT 3') and 27F (5' AGAGTTTGATCCTGGCTCAG 3'). Subsequently, sequencing of the 16 S rRNA gene amplified product of the isolates SRS11, SRS8, CP11, CP9, CP5, and CP2 resulted in 882 bp, 1516 bp, 1510 bp, 1510 bp, 1507 bp, 1504 bp sequences respectively, which was closely related to the sequences of *Bacillus licheniformis, Priestia megaterium, Bacillus subtilis, Priestiaflexa, Priestia megaterium*, and *Priestia flexa* respectively with the sequence similarity of 98.61%, 99.97%, 99.60%, 99.54%, 99.53%, and 99.73% respectively. Further, the 16 S rRNA gene sequences of potent isolates were submitted to NCBI and the assigned Genbank accession numbers to the isolated SRS11, SRS8, CP11, CP9, CP5, and CP2 strains were OR856070, OR856075, OR856071, OR856072, OR856073, and OR856074 respectively. Moreover, the 16 S rRNA gene sequences were used to construct a phylogenetic tree by a neighbour-joining approach in Mega X software which represents three clusters of closely related microbial isolates (Fig. 2). The isolates' sharing ancestry and evolutionary divergence were distinguished in the clusters. The main three clusters observed in the phylogeny tree contained *Priestia* sp. and *Bacillus* sp. In the first cluster,

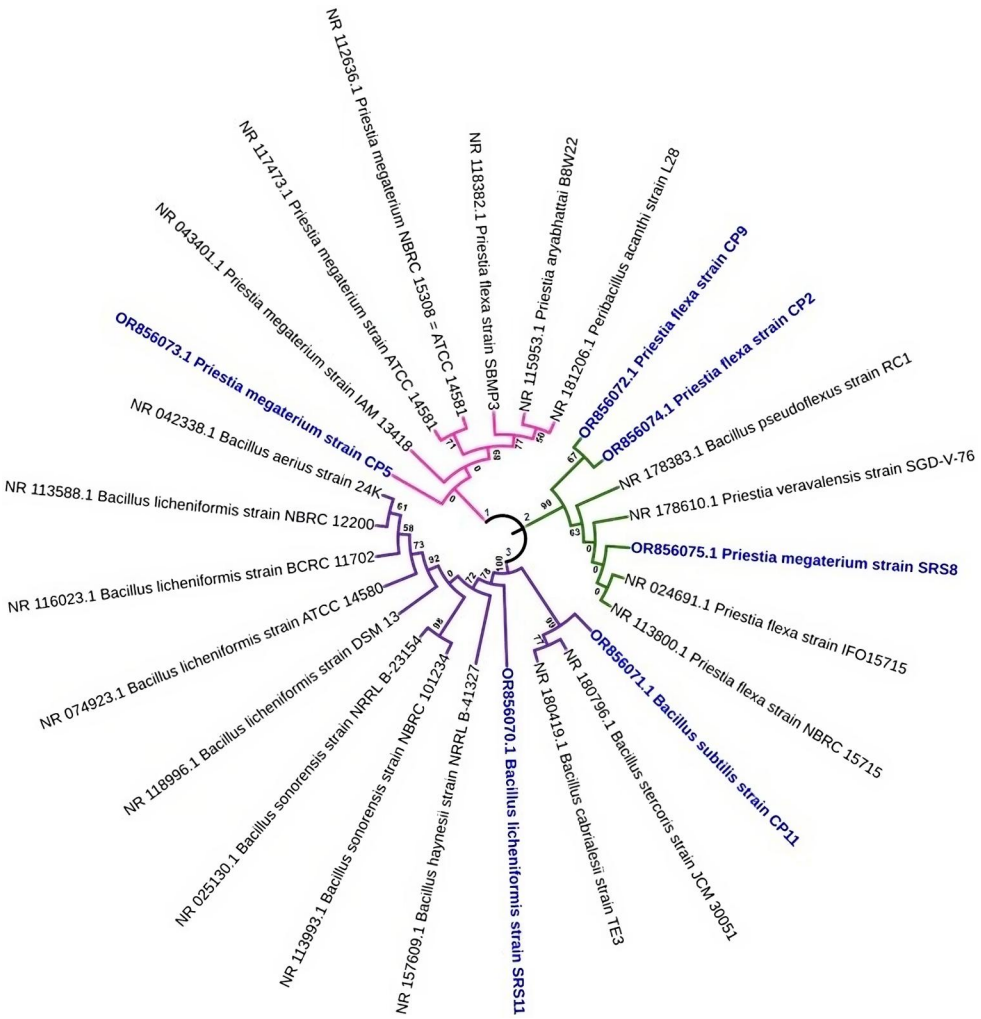

Fig. 2 Phylogenetic tree of rhizospheric soil and compost isolates constructed in MEGA X sofwere using neighbour joining method

Priesta sp. and in the third cluster *Bacillus* sp. were predominant, while both genera were observed in the second cluster which exhibited a higher diversity of microbial species. The genetic diversity seen in the microbial communities was revealed by this analysis, which also provided insights into the taxonomy and evolutionary background of the microbial strains isolated from the sugarcane rhizospheric soil and compost.

Qualitative screening of isolates for hydrolytic enzyme secretion

Organic solid wastes are varied in composition and are made up of heterogeneous organic polymers. Multiple enzyme systems are required for the saccharification of these wastes. Morphologically distinct isolates were further studied for *in-vitro* qualitative screening of enzymes to study waste saccharification. 1% CMC, starch and pectin were used as a substrate; differentiating stain iodine has a binding ability with these sugars and produces a bluish-black complex but not with hydrolyzed sugars. The details of isolates having multiple enzyme secretion potentials are shown in Table 2. Out of the total isolates, 16.66% of isolates were found positive for at least one lytic enzyme. Cellulase, ligninase, amylase, protease and pectinase production were observed in 14.53%, 4.16%, 9.37%, 7.29% and 5.20% isolates respectively. The results revealed that the compost isolates CP2 secreted all five lytic enzymes, whereas isolates CP4 and CP11 secreted all lytic enzymes except pectinase. Moreover, isolate CP9 secreted all enzymes except protease. The least relative enzyme secretion was observed in the isolates of sugarcane rhizosphere SRS3, which displayed positive results for cellulase and protease, and SRS11, which showed positive results for cellulase and pectinase, while, SRS8 was observed positive for cellulase, amylase and protease. Multiple enzyme secretion was recorded optimum in isolates of compost samples. The analysis of cellulase, amylase, protease, and pectinase secretion as variables in a Completely Randomized Design (CRD) revealed significant results with a p-value < 0.01 (Table 3). The highest levels of cellulase, amylase, and pectinase secretion were observed in isolates CP2, SRS8, and CP9, respectively, and none of the other

Table 2 Qualitative screening of isolates for enzyme production

Sr. No.	Isolates	Cellulase	Amylase	Pectinase	Protease	Ligninase
1	SRS3	1.40 ± 0.100^{e}	0.00 ± 0.000^{e}	0.00 ± 0.000^{d}	1.65 ± 0.040^{b}	
2	SRS8	1.46 ± 0.057^{e}	3.00 ± 0.000^{a}	0.00 ± 0.000^{d}	1.19 ± 0.135^{d}	
3	SRS11	2.03 ± 0.057^{c}	0.00 ± 0.000^{e}	1.82 ± 0.011^{b}	0.00 ± 0.000^{e}	
6	CP2	3.56 ± 0.057^{a}	2.00 ± 0.000^{c}	1.28 ± 0.075^{c}	2.28 ± 0.075^{a}	+
7	CP4	1.67 ± 0.075^{d}	1.22 ± 0.110^{d}	0.00 ± 0.000^{d}	1.29 ± 0.040^{d}	+
8	CP5	2.00 ± 0.000^{c}	2.00 ± 0.000^{c}	0.00 ± 0.000^{d}	1.47 ± 0.030^{c}	
9	CP9	2.53 ± 0.057^{b}	1.90 ± 0.100^{c}	2.32 ± 0.086^{a}	0.00 ± 0.000^{e}	+
10	CP11	2.56 ± 0.057^{b}	2.36 ± 0.152^{b}	0.00 ± 0.000^{d}	2.31 ± 0.017^{a}	+
Sem		0.0367	0.0435	0.0235	0.0344	
CD (LSD) 5%		0.1101	0.1304	0.0705	0.103	
CD (LSD) 1%		0.1517	0.1797	0.0971	0.1419	
C.V.		2.9522	4.8282	5.9997	4.663	

Table 3 Analysis of Variance forcellulase, amylase, protease and pectinase

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F value	*P* value	Result
Cellulase						
Treatment	7	10.8699	1.552	383.8115	< 0.01	** Sig. at 1% level
Residual	16	0.0647	0.004			
Total	23	10.9346				
Amylase						
Treatment	7	24.6297	3.5185	619.5506	< 0.01	** Sig. at 1% level
Residual	16	0.0909	0.0057			
Total	23	24.7206				
Protease						
Treatment	7	16.6007	2.3715	669.6079	< 0.01	** Sig. at 1% level
Residual	16	0.0567	0.0035			
Total	23	16.6574				
Pectinase						
Treatment	7	20.0305	2.8615	1725.5302	< 0.01	** Sig. at 1% level
Residual	16	0.0265	0.0017			
Total	23	20.0571				

isolates were at par with it. However, the highest levels of protease were secreted by isolates CP11 and CP2 and were found statistically at par with it. The mean values along with standard error and Tukey test results are shown in Table 3.

Biochemical characterization of isolates

Biochemical characterization of isolates having lytic activity was carried out on various mediums and results are reflected in Fig. 3. Biochemical tests following the protocol outlined by Cowan and Steel (1974), were conducted to identify the unknown genus. Microscopic observation and cultural characteristics were also considered for the identification of isolates. Isolate CP4 was included in Gram-negative rods, catalase and oxidase-positive category. It was found catalase, oxidase, MR, VP, and citrate were positive, while, indole, urease, nitrate reduction, ammonia production, H_2S production, dehydrogenase and hemolysis production were negative. According to Cowan and Steel's manual, isolate CP4 was tentatively identified as *Pseudomonas* spp. Isolate CP5 was included in Gram-positive rods, catalase-negative; oxidase-positive category. Bacilli was observed in chain arrangement and found indole, VP, citrate, urease, nitrate reduction, ammonia production, H_2S production, dehydrogenase, and hemolysis production negative while, MR test and starch hydrolysis positive. All other isolates are categorised in Gram-positive rods, catalase and oxidase-positive category. They were found oxidase and catalase positive, whereas, indole, MR, ammonia production, H_2S production, and dehydrogenase negative. A haemolysis pattern was observed in the CP11 isolate. Nitrate reduction was observed in isolates SRS3, SRS8, and CP9. Citrate utilization was displayed by SRS3, SRS8, CP2 and CP11, whereas, VP negative was displayed by CP2 and CP9 isolates. As per the Cowan and Steel manual, isolates SRS3, SRS8, SRS11, CP2, CP9 and CP11 were tentatively identified as *Bacillus* spp.

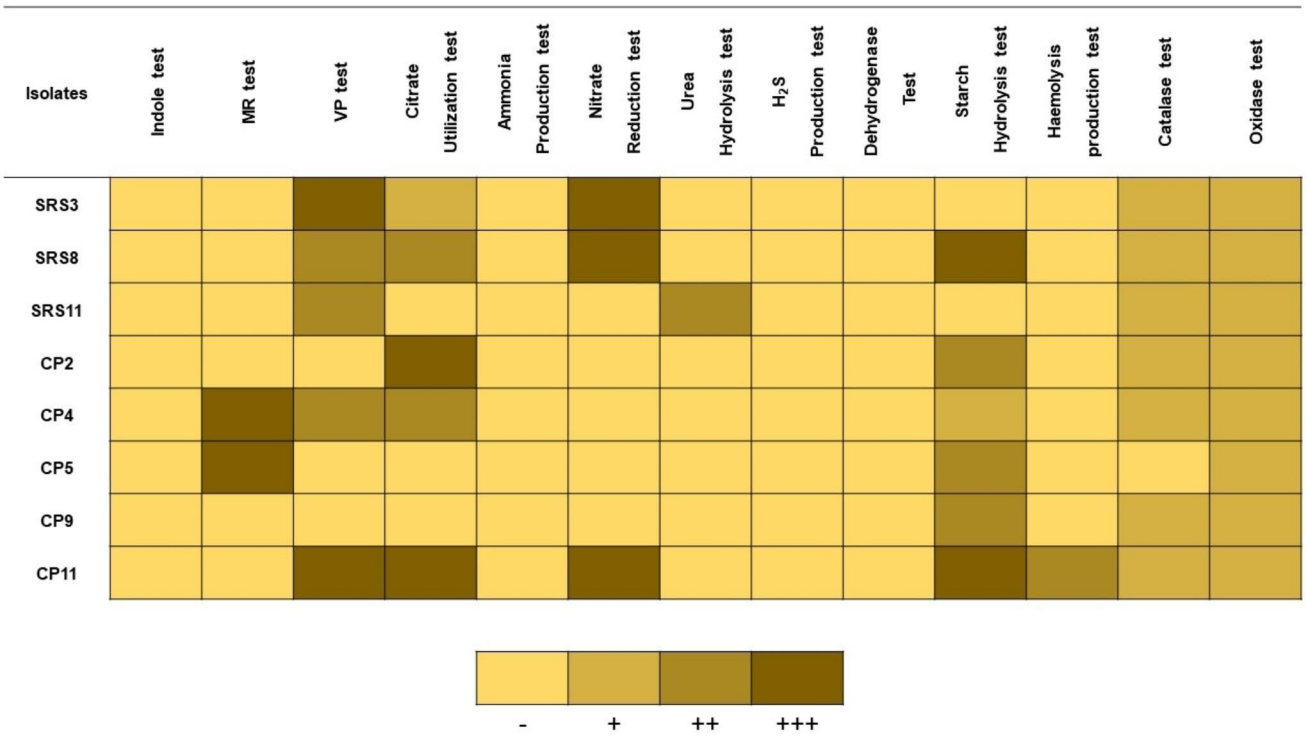

Fig. 3 Heat map of biochemical characteristics of isolates of rhizospheric soil and compost

Table 4 Effect of NaCl on relative activity and growth of potent isolates

Sr. No.	Isolates	Zone Ratio at 0% NaCl (w/v)	Zone Ratio at 3% NaCl (w/v)	Zone Ratio at 5% NaCl (w/v)	Zone Ratio at 7% NaCl (w/v)
1	SRS3	1.56 ± 0.057^{ef}	1.50 ± 0.250^{ef}	1.23 ± 0.028^{f}	0.00 ± 0.000^{g}
2	SRS8	1.76 ± 0.028^{ef}	1.23 ± 0.000^{f}	0.00 ± 0.000^{g}	0.00 ± 0.000^{g}
3	SRS11	2.00 ± 0.000^{de}	2.00 ± 0.000^{de}	1.60 ± 0.190^{ef}	0.00 ± 0.000^{g}
6	CP2	3.56 ± 0.055^{a}	3.33 ± 0.330^{ab}	2.82 ± 0.170^{bc}	1.80 ± 0.000^{ef}
7	CP4	1.80 ± 0.200^{ef}	1.66 ± 0.729^{ef}	1.23 ± 0.028^{f}	0.00 ± 0.000^{g}
8	CP5	3.40 ± 0.200^{a}	2.00 ± 0.000^{de}	1.33 ± 0.170^{f}	0.00 ± 0.000^{g}
9	CP9	2.60 ± 0.000^{c}	2.44 ± 0.046^{cd}	1.8 ± 0.200^{ef}	1.60 ± 0.053^{ef}
10	CP11	2.48 ± 0.057^{cd}	2.62 ± 0.203^{c}	1.47 ± 0.046^{ef}	1.44 ± 0.140^{ef}
Sem		0.1017			
CD (LSD) 5%		0.2873			
CD (LSD) 1%		0.3818			
C.V.		10.7582			

Salt profile

The effect of NaCl concentration on enzyme activity was carried out at the range of 0 to 15% by using a CMC-BH medium and relative enzyme activity was calculated after 48 h in terms of zone ratio displayed in Table 4. The results revealed that the sugarcane rhizospheric isolate SRS8 displayed the lowest tolerance limit of up to 3% NaCl concentration, with its optimum activity (zone ratio 1.76 ± 0.028) observed at 0% NaCl concentration. Additionally, colony growth (4 mm) was observed at 5% NaCl, but a hydrolytic zone was not observed. Similarly, isolates SRS3 and SRS11 exhibited relative activity up to 5% NaCl concentration. However, SRS11 showed optimum activity (zone ratio 2.00 ± 0.000) at 0% and 3% NaCl (w/v), while SRS3 displayed optimum activity (zone ratio 1.56 ± 0.057) at 0% NaCl. Colony growth (3 mm) was observed at 7% NaCl, but no hydrolysis zone was formed by SRS3. A similar pattern to SRS3 was also observed in compost sample isolate CP4, with a 5% NaCl tolerance limit. The optimum activity (zone ratio 1.80 ± 0.200) was observed at 0% NaCl, while growth without activity was observed at 7% NaCl. Isolate CP5 displayed optimum activity at 0% NaCl (zone ratio 3.40 ± 0.200), which was decreased up to 5% NaCl. Compost isolates CP2, CP9, and CP11 showed activity across a wide range of NaCl concentrations (0 to 7%), with

optimum activity at 0%, 0%, and 3% NaCl with zone ratios of 3.56 ± 0.055, 2.60 ± 0.000, and 2.62 ± 0.203 respectively. In the presence of 9%, 11%, and 15% NaCl, none of the isolates exhibited any growth, indicating their limited tolerance to high salinity conditions. Among all the isolates, CP2 displayed the highest relative activity, followed closely by CP5 and CP9, suggesting their superior enzymatic capabilities in response to the tested conditions. Moreover, the compost isolates demonstrated a greater relative activity compared to the isolates from sugarcane rhizospheric soil, indicating their better adaptability and efficiency in enzyme secretion under the given salinity levels. The analysis of the isolate factor component, NaCl factor component and interaction component variables in a Completely Randomized Design (CRD) revealed significant results with a p-value < 0.01 (Table 5). For the isolate variable, the highest zone ratio was observed for CP2 and none of the treatment combinations were at par with it. Additionally, for the NaCl variable, the highest zone ratio was observed at 0% NaCl (w/v) and none of the treatment combinations were at par with it. However, for interaction component variables (Isolate x NaCl) highest zone ratio was observed for CP2 × 0% NaCl and CP5 × 0% NaCl, CP2 × 3% NaCl, were found statistically at par with it. The mean values along with standard error and Tukey test results are shown in Table 5.

Temperature growth profile

The isolates displaying multiple enzyme secretion potentials were further examined to determine their temperature growth optima. The results revealed that sugarcane rhizospheric isolates SRS3 exhibited rapid growth with an absorbance of 1.15 ± 0.028 at 35 °C within 48 h, followed by SRS8 with an absorbance of 0.936 ± 0.003 at 40 °C. However, the growth of SRS11 was comparatively slower, indicated by an absorbance of 0.36 ± 0.010 at 35 °C within 48 h. Additionally, compost sample isolates CP5 exhibited rapid growth with an absorbance of 1.34 ± 0.032 at 40 °C within 24 h, followed by CP9 and CP2, which showed absorbance of 1.29 ± 0.023 and 0.87 ± 0.003 respectively at 45 °C within 48 h. On the other hand, CP11 and CP4 exhibited slower growth, indicated by absorbance of 0.71 ± 0.019 and 0.86 ± 0.003 at 40 °C and 35 °C respectively within 48 h. The analysis of the isolate factor component, time factor component, temperature factor component and interaction component variables of rhizospheric and compost isolates in a Completely Randomized Design (CRD) revealed significant results with a p-value < 0.01. The highest absorbance values for the isolate variable, time variable, and temperature variable were observed for SRS3, 48 h, and 40 °C, respectively, for rhizospheric isolates. For compost isolates, the highest absorbance values were observed for CP5 at 48 h and 40 °C temperature, and none of the treatment combinations were at par with it. Additionally, for isolates x time, isolate x temperature and time x temperature interaction component variables, the highest absorbance values were observed for SRS3 × 48 h, SRS3 × 35 °C and 48 h x 40 °C respectively for rhizospheric isolates and none of the treatment combinations were at par with it. For compost isolates the highest absorbance values were observed for CP5 × 48 h, CP 5 × 40 °C and 48 × 40 °C respectively, and none of the treatment combinations were at par with it. However, for the isolates x time x temperature interaction component variables, the highest absorbance value was observed for SRS3 × 48hx35°C, and none of the treatment combinations in rhizospheric isolates were found to be at par with it. Regarding compost isolates, the highest absorbance values were observed for CP5 × 48 × 40 °C and CP5 × 24 × 40 °C, CP9 × 48 × 45 °C, CP5 × 48 × 30 °C, CP5 × 48 × 35 °C, CP5 × 24 × 35 °C were statistically at par with the highest values. The absorbance values along with standard error and Tukey test results of rhizospheric and compost isolates are shown in Figs. 4a and b and 5.

The comparative discussion on temperature growth optima of bacterial isolates from compost and sugarcane rhizospheric soil revealed interesting findings. The results indicated that the optimal growth temperature for most bacterial isolates from compost was higher, with 45 °C being the optimum for CP2 and CP9. In contrast, some isolates showed optimal growth at 40 °C, such as CP5 and CP11, while CP4 exhibited optimal growth at 35 °C as indicated in Fig. 4a,b.

On the other hand, the bacterial isolates from sugarcane rhizospheric soil displayed their highest growth rate at lower temperatures. SRS3 and SRS11 exhibited optimal growth at 35 °C, and SRS8 showed the highest growth at 40 °C as shown in Fig. 5.

Table 5 Analysis of Variance for the interaction of isolates and NaCl

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F value	P value	Result
Isolates	7	39.4427	5.635	181.7742	< 0.01	** Sig. at 1% level
NaCl	3	45.6943	15.231	491.3226	< 0.01	** Sig. at 1% level
Isolatesx NaCl	21	9.9074	0.472	15.2258	< 0.01	** Sig. at 1% level
Residual	64	2.0141	0.031			
Total	95	97.0585				

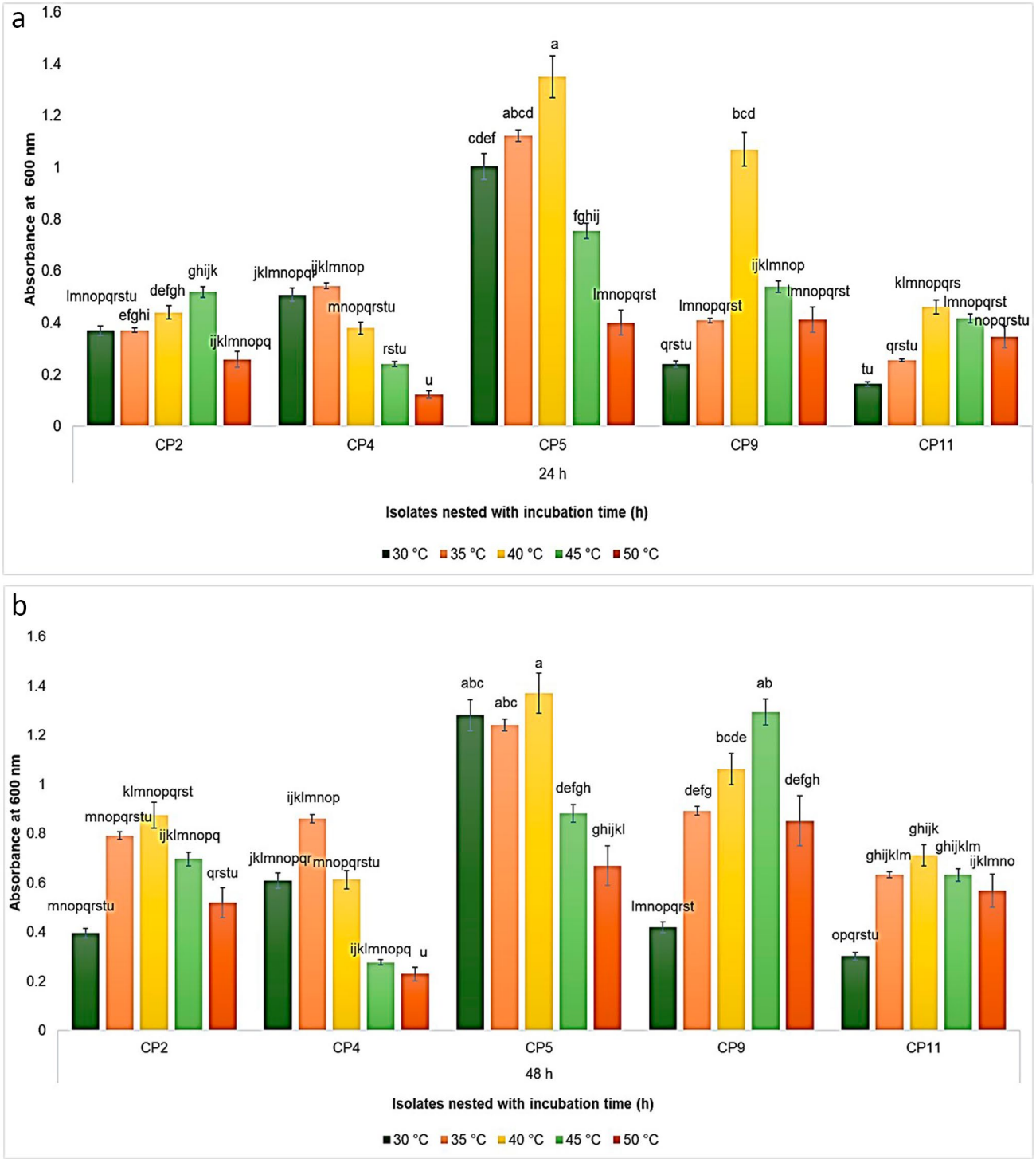

Fig. 4 Temperature growth profile at (**a**) 24 h and (**b**) 48 h of compost isolates across a temperature range of 30 to 50 °C

pH growth profile

The pH growth optima of multiple enzyme-secreting isolates were determined. Potent isolates from sugarcane rhizospheric soil (SRS3, SRS8, and SRS11) exhibited their optimum growth at pH 7. Isolate SRS3 displayed rapid growth with an absorbance of 1.14 ± 0.015 within 48 h, while SRS8 and SRS11 showed delayed growth with an absorbance of 1.00 ± 0.057 and 0.78 ± 0.002, respectively, within the same time frame as shown in Fig. 6. In

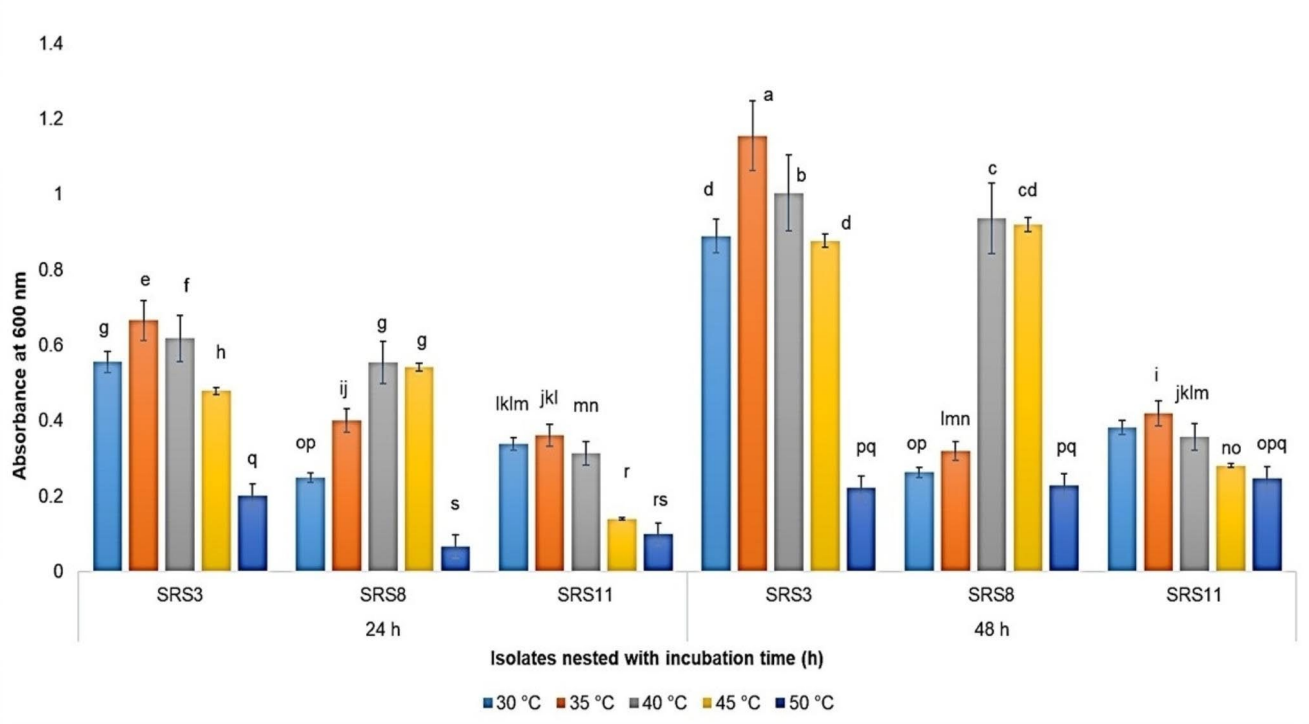

Fig. 5 Temperature growth profile of sugarcane rhizospheric isolates across a temperature range of 30 to 50 °C

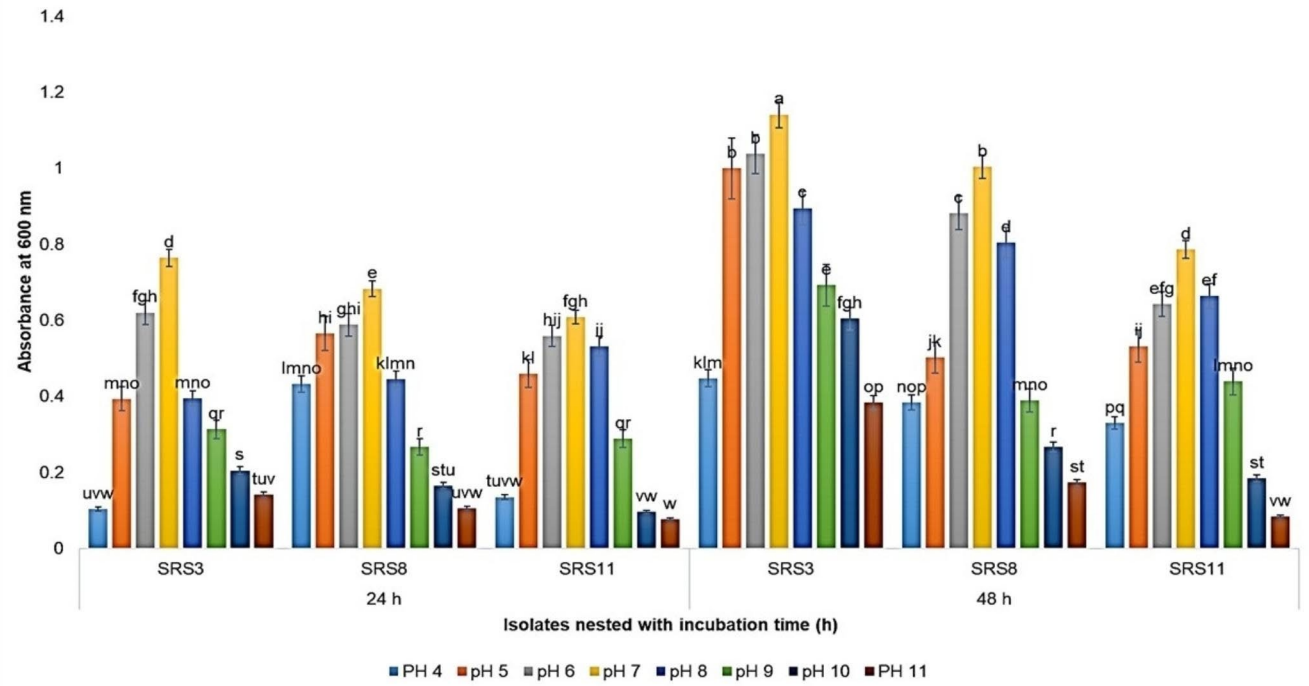

Fig. 6 pH growth profile of sugarcane rhizospheric isolates across a pH range of 4 to 11

contrast, potent isolates from compost samples CP2, CP4, and CP5 showed optimum growth at pH 7, while isolates CP9 and CP11 exhibited their best growth at pH 8. The isolate CP5 demonstrated rapid growth with an absorbance of 1.25 ± 0.037 within 24 h at pH 7, while CP2 and CP4 showed delayed growth with absorbance of 1.018 ± 0.045 and 1.01 ± 0.026, respectively, within 48 h at pH 7. Similarly, CP11 and CP9 displayed delayed growth, with absorbance of 1.27 ± 0.031 and 0.85 ± 0.020, respectively, within 48 h at pH 8 as indicated in Fig. 7a,b. The analysis of the isolate factor component, time factor component, pH factor component and interaction component variables of rhizospheric

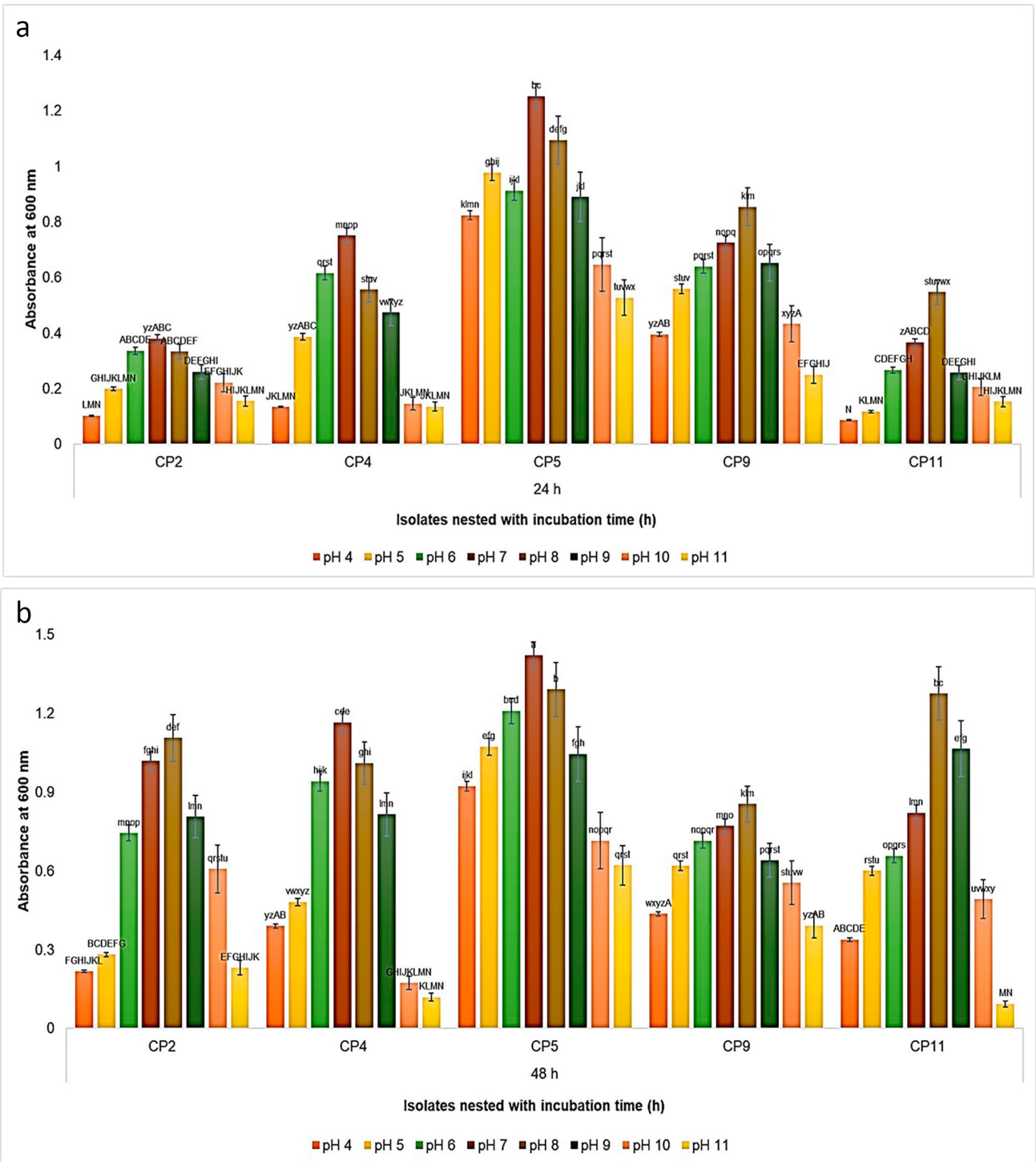

Fig. 7 pH growth profile at (**a**) 24 h and (**b**) 48 of compost isolates across a pH range of 4 to 11

and compost isolates in a Completely Randomized Design (CRD) revealed significant results with a p-value < 0.01. The highest absorbance values for the isolate variable, time variable, and pH variable were observed for SRS3, 48 h, and 7 pH, respectively, for rhizospheric isolates. For compost isolates, the highest absorbance values were observed for CP5, 48 h, and none of the treatment combinations were at par with it. For the pH factor, the highest absorbance value was observed for pH 8 and pH 7, which were found statistically at par with it. Additionally, for isolates x time, isolate x pH and time x pH interaction component variables, the highest absorbance values were observed for SRS3 × 48 h, SRS3 × 7 pH and 48 h x 7 pH, respectively, for rhizospheric isolates. The highest absorbance values were observed for CP5 × 48 h, CP5 × 7pH and 48 × 8pH respectively for compost isolates and none of the treatment combinations were at par with it. However, for the isolates x time x pH interaction component variables, the highest absorbance value was observed for SRS3 × 48 × 7pH and CP5 × 48 × 7pH respectively, for rhizospheric and compost isolates and none of the treatment combinations were found to be at par with it. The mean values along with standard error and Tukey test results of rhizospheric and compost isolates are shown in Figs. 5 and 6a and b. Optimum physiological conditions for potent isolates are summarized in Table 6.

Discussion

The present investigation showed that densities of cellulolytic bacteria were lower in rhizospheric soil and higher in compost than those reported by Saini et al. (2012) who detected 6.6×10^4 and 2.8×10^7 CFU g^{-1} in rhizospheric soil and compost respectively. Additionally, in support to present investigation, they have reported low cellulolytic as well as total bacterial population density in rhizospheric soil compared to the compost. Previously, Kim et al. (2013) stated that the high organic matter composition of compost was responsible for the elevated population of lignocellulolytic bacteria in compost than other samples. More recently, Rinady et al. (2023) reported a lower population of 1.71×10^5 and 4.24×10^4CFU g^{-1} of heterotrophic and cellulolytic bacteria, respectively at the pine-coffee agroforestry system of the plantation. In another study, Cabrera et al. (2013) reported a higher abundance of cellulolytic bacteria (10^6 CFU g^{-1}) and total culturable bacteria (10^7 CFU g^{-1}) in the alfalfa rhizosphere. They also conclude that the cellulolytic bacterial density in the rhizosphere enhanced with the growth of plants, and different plant *spp.* have different rhizospheric effects at various stages of growth according to the composition of root exudates. Recently, Mohammadipour et al. (2021) reported 3×10^9 CFU mL^{-1} heterotrophic and 94×10^4 CFU mL^{-1} cellulolytic population densities in compost leachate samples. Temperature, methods of composting, raw material and decomposition steps consisting of mesophilic and thermophilic microorganisms affect the population density and diversity of microbes in compost (Roy et al. 2018). More recently, Breza-Boruta et al. (2023) reported that soil treated with manure exhibited the highest abundance of heterotrophic bacteria (165.1×10^6 CFU g^{-1}) and cellulolytic bacteria (17.2×10^6 CFU g^{-1}). Additionally, using the CMC-MSM enrichment medium, Udume et al. (2023) obtained an average value of $2.92 \pm 0.10 \times 10^6$ CFU g^{-1} of cellulolytic organisms from water hyacinth compost enriched samples. In a microbial population study of various waste organic materials, Dąbrowska et al. (2022) reported that spent mushroom compost contained 1.47×10^{11} CFU g^{-1} heterotrophic and 4.46×10^9 CFU g^{-1} cellulolytic bacteria, while composted sawdust contained 2.38×10^7 CFU g^{-1} heterotrophic and 2.17×10^6 CFU g^{-1}cellulolytic bacteria. Moreover, raw sawdust and wood chips contained 8.20×10^3 CFU g^{-1} and 7.02×10^4 CFU g^{-1} heterotrophic bacteria, as well as 1.17×10^2 CFU g^{-1} and 1.76×10^3 CFU g^{-1} cellulolytic bacteria.

In the present investigation, enrichment techniques yielded 36.45% isolates on an L-MSM isolation medium containing supporting carbon and nitrogen sources. In a similar finding Verma et al. (2020) conducted a study on bacterial decolourization of Kraft lignin using glucose and peptone as supplementary carbon and nitrogen sources. The initial stage exhibited limited decolourization of KL, attributed to the utilization of peptone and glucose as nitrogen and carbon sources present in L-MSM. However, as these nutritional sources were gradually depleted in L-MSM, the bacterial culture shifted towards utilizing lignin as a co-substrate for further decolourization. In the current finding, these isolates were further screened for lignin utilization on a minimal medium supplemented with lignin (300 mg L^{-1}) as a sole carbon and energy source and remarkable growth was observed in 4 isolates. A similar study reported very fast growth of *Bacillus spp. Ochrobactrum sp.* and *Leucobacter sp.* on agar plates containing lignin (500 mg L^{-1})

Table 6 Optimum physiological conditions for potent isolates

Sr. No.	Isolates	Optimum growth pH	Optimum %NaCl for REA	Optimum growth Temperature
1	SRS3	7	0%	35
2	SRS8	7	0%	40
3	SRS11	7	0–3%	35
4	CP2	7	0%	45
5	CP4	7	0%	35
6	CP5	7	0%	40
7	CP9	8	0%	45
8	CP11	8	0%	40

without the addition of glucose and peptone (Rahman et al. 2013). In support to present investigation, Chen et al. (2012) used a maximum lignin concentration (300 mg L^{-1}) for *Comomonas sp.* due to inhibition of bacterial growth at high lignin concentration. Moreover, bacterial isolates have the competency to efficiently assimilate and degrade sole carbon source lignin as confirmed by the findings of Dube et al. (2023) who reported ten bacterial species isolated from compost with lignin utilization ability, whereas optimum growth was observed in *Pseudomonas aeruginosa* (CP031449.2) and *E. coli* (LR025096.1). Additionally, Radhika et al. (2023) conducted a study involving the selective enrichment of lignin-degrading bacterial consortia through a forced laboratory adaptation method. This method utilized Kraft lignin as the sole carbon source and involved samples from compost and soil. They identified lignin utilization potential in various bacterial genera, including *Bacillus*, *Pseudomonas*, *Brevibacillus*, *Paenibacillus*, and *Aneurini bacillus*. Moreover, in accordance with the current findings, Azman et al. (2019) studied the depolymerization of alkali lignin to demonstrate the capacity of thermophilic bacterial strains for utilizing and breaking down alkali lignin beyond a monomeric form within a 7 days incubation period. Findings revealed that *Stenotrophomonas* sp. S2 and *Bacillus subtilis* S11Y achieved around 50% and 20% reduction in alkali lignin over 7 days of incubation without the need for supplementary carbon sources. Additionally, 14 isolates in this study, recovered from CMC-MSM plates were considered cellulolytic bacterial isolates. Similar to the present finding, Mohammadipour et al. (2021) purified 15 isolates from compost leachates sample on CMC media plates according to differences in morphological characteristics. Nevita et al. (2018) also reported the isolation and identification of *Stenotrophomonas maltophilia RSD6* from the rhizospheric region of *O. sativa* L by CMC and Lignin–MSM nutrient enrichment techniques. Moreover, Sekhar et al. (2022) screened 24 cellulase-positive isolates from sugarcane rhizosphere on a CMC-MSM medium and identified two potential isolates *Bacillus amyloliquefaciens* and *Pseudomonas putida.* Moreover, Nur et al. (2020) reported positive screening of 125 cellulolytic bacteria on CMC agar medium using decomposing rice straw with varying C/N ratios. They found that the total number of isolates from rice straw with a higher degree of decomposition exceeded those from rice straw with a lower level of decomposition.

Findings of microscopic, cultural and biochemical characteristics revealed that 75% of isolates were Gram-positive and the majority of them were tentatively identified as a *Bacillus* spp. Similar to the present study, Ojo-Omoniyi et al. (2016) reported the optimum occurrence of *Bacillus* (42.5%) and related Gram-positive genera (22.5%) in dump site waste and compost compared to other isolates because they can thrive in harsh environments and they are indigenous to soil environments. In addition, Dube et al. (2023) reported the abundance of the *Bacillus* genus commonly found in plant litter, soil as well as compost samples and they were known for lignocellulolytic activity and responsible for the bioconversion of macromolecules. Henry et al. (2020) conducted a metagenomics analysis of compost samples and revealed the optimum occurrence of firmicutes, chloroflexi, bacteroidetes, actinobacteria, and acidobacteria. The highest abundance (59%) of firmicutes in the heating phase of tomato stalk composting was also reported by Zhang et al. (2021). In another study, Grenier et al. (2023) reported seventy-two lignocellulolytic species from compost samples, out of them 34 spp. belonging to the phylum Firmicutes were dominated by the order Bacillales with 22 spp. Lignocellulolytic bacteria, including *Arthrobacter, Brevibacterium, Bacillus, Chryseobacterium, Pseudomonas, Xanthomonas, Paenibacillus, Stenotrophomonas*, and *Streptomyces*, were reported in the rhizosphere of *Phleum pretense* (Saleh et al. 2019), *Streptomyces* in the rhizosphere of *Zea mays* (Adegboye et al. 2018), *Arthrobacter* and *Pseudomonas* in the rhizosphere of *Quercus sp*. (Lasa et al. 2019), and *Bacillus*, *Pseudomonas*, and *Kocuria*in the rhizosphere of *Salsola stocksii* and *Atriplex amnicola* (Mukhtar et al. 2019). It has long been known that *Bacillus* species produce large amounts of different enzymes with a wide range of catalytic activity. The *Bacillus* sp. produces enzymes that are used extensively in industry and aid in the development of economical and environmentally friendly procedures. The versatility of *Bacillus* enzymes, encompassing their stability and activity under diverse situations, renders them indispensable instruments in a multitude of sectors, encompassing bioremediation and the manufacturing of bio-based commodities. The *Bacillus* species demonstrate a noteworthy ability to decompose solid waste, underscoring its potential for use in waste management techniques. These bacteria are important decomposers of a variety of organic materials in both natural and artificial contexts because of their adaptability, enzymatic activity, and versatility.

Phylogenetic analysis is essential for the taxonomy classification of microorganisms (Gohel et al. 2023). Organisms can be arranged and classified according to their evolutionary relatedness with the assistance of a phylogenetic tree. It is essential to preserve a uniform and widely recognized system of naming and categorizing living things. The phylogenetic tree derived from genomic data provides a more accurate depiction of the branching patterns, making it easier to distinguish between closely related species and strains (Pearson et al. 2009; Patwardhan et al. 2014). In this study, the species belonging to the genera *Bacillus* were predominantly observed in the phylogenetic tree, constructed using isolates obtained from the sugarcane

rhizosphere and compost. Numerous bioactive chemicals are known to be produced by *Bacillus* species (Kaspar et al. 2019). The variety of secondary metabolite biosynthesis gene clusters found in *Bacillus* genomes are shown by genomic investigations (Belbahri et al. 2017). The diversity of *Bacillus* species enhances their capacity to generate new bioactive chemicals that find use in industry, agriculture, and medicine (Mondol et al. 2013; Kaspar et al. 2019). The adaptation of *Bacillus* to a variety of ecological habitats, such as compost and rhizospheric soil, was reflected by variations of genomic content, such as the presence of certain transporters or stress response genes (Zhang et al. 2016; Iqbal et al. 2021). The taxonomic categorization of *Bacillus* species is improved and species boundaries are drawn using genomic data. Numerous details, including functional features and evolutionary links, can be obtained from genomic and molecular insights into the phylogeny of *Bacillus* species (Alcaraz et al. 2010; Khurana et al. 2020). These discoveries are significant due to their implications in environmental science, agriculture, and medicine as well as for understanding microbial ecology and the biotechnology potential of microorganisms.

The present investigation unveiled that cellulase, lignase, amylase, protease, and pectinase production of isolates were observed in varying percentages. In a more or less similar outcome, Mukhtar et al. (2019) conducted enzymatic screening of bacterial isolates from the rhizosphere of *Salsola stocksii*. They observed protease, lipase, cellulase, amylase, urease, gelatinase, and catalase activity in 53%, 68%, 40%, 33%, 29%, 26%, and 34% of the isolates, respectively. Similarly, in a study published by Semira et al. (2021), 31% amylase-positive isolates were observed to give a zone of clearance from various rhizospheric soil samples. The rhizospheric microbes exhibited a preferential utilization of D-galacturonic acid and its reduced form, D-galactonic acid (Lopes et al. 2016). D-galacturonic acid, the most abundant component of pectin and a major constituent of plant cell walls is consequently released in the rhizosphere (Zhang et al. 2011). Furthermore, D-galacturonic acid has been identified as a component of root exudates by Tawaraya et al. (2015). Additionally, in sugarcane field soils, the abundance of hemicellulose is notably higher due to the continuous deposition of leaves on the surface (Chandel et al. 2012). Hemicellulose is a significant component of lignocellulose, which constitutes the most recalcitrant part of the plant cell wall and requires the participation of complex lignocellulase enzymes for degradation (Mansour et al. 2016). In support of the present investigation, Neethu et al. (2017) reported positive qualitative screening in 15 cellulolytic (14.56%) and 10 lignolytic (9.70%) bacterial isolates from various compost samples on CMC and ABTS plates. Similarly, Bambharolia et al. (2021) reported that among a total of 56 bacterial isolates, 73% exhibited distinct clear zones around their colonies on CMC agar, while only 8% isolates displayed coloured zones around their colonies on ABTS agar. Contradictory to the described trends, 38% protease and 28% cellulase-positive screening from various enriched samples was published by Admassie et al. (2022). Additionally, Jiang et al. (2020) reported that a commensal relationship between lignocellulolytic bacteria occurs through the enzymatic removal of lignin from lignocellulosic biomass. This process facilitates the availability of cellulose for degradation by cellulase-producing organisms. More recently, Chukwuma et al. (2023) reported that landfill bacteria, such as *Bacillus proteolyticus, Bacillus Sanguinis, Bacillus spizizenii, Bacillus paramycoides, Bacillus paranthracis*, and *Neobacillus fumarioli*, exhibited multienzymatic activity, including amylase, cellulase, xylanase, and protease. Earlier, Priyanka et al. (2021) also reported positive screening of 22.47% cellulolytic and 13% lignolytic bacterial isolates from rhizospheric soil and compost-enriched samples. In a metagenomic analysis conducted by Martins et al. (2013) on São Paulo Zoo compost samples, 56% of genomes were found to contain genes associated with β-glucosides, and 24% of the total genomes contained cellulases and β-glucosides. This finding suggests that the chemical nature and availability of carbohydrates in the habitat influence microbial adaptation and their carbohydrate-active enzyme composition. Soil and compost ecosystems have been extensively studied in this regard (López-Mondéjar et al. 2020). In a study by Mironov et al. (2021), abundant proteolytic microorganisms were reported throughout composting. During the initial 14 days, active growth of amylolytic microorganisms was observed, while cellulolytic microorganisms thrived during the thermophilic period, reaching their peak at the beginning of maturation. Similarly, in a study conducted by Chroni et al. (2009), a significant increase in amylolytic, cellulolytic, and proteolytic microorganisms was observed during the thermophilic stage. Similarly, López-González et al. (2014) reported distinct variations in activities related to the degradation of lignocellulosic and starchy polymers. Approximately 33% of the total isolates exhibited both amylolytic and hemicellulolytic activities. However, the percentage of microorganisms capable of hydrolyzing cellulose, lignin, and pectin did not exceed 4% in the composting process. Identification of cellulolytic *Pseudomonas* sp. was also performed by Poly et al. (2022) using the Cowan and Steel manual. Also, Pan et al. (2021) characterized *Thermohalobaculum Xanthum* Gen. Nov., Sp. Nov. using the method described by Cowan and Steel.

NaCl significantly affected CMCase activity, except SRS8 all the isolates exhibited activity between 0 and 7% NaCl (w/v), and no activity at higher NaCl concentrations.

A similar study reported supporting evidence as they identified 98 cellulolytic bacterial isolates from the soil (Cabrera et al. 2013). CMCase hydrolytic zone was observed in 100%, 82.6%, 50%, 24%, and 1% of the isolates at 1%, 3%, 7%, and 9% NaCl concentration, respectively, while growth was not observed at 12%. Balla et al. (2022) examined the influence of NaCl on 12 cellulolytic bacterial isolates from the rhizosphere. The results revealed that 58%, 34%, and 8% of the isolates displayed optimum growth at 0 mM, 400 mM, and 800 mM NaCl concentrations, respectively. Furthermore, Mukhtar et al. (2019) also reported that rhizospheric bacterial strains HL1RS13, AT2RP4, NRS4HaP9, and LK3HaP7 exhibited stable cellulase activity in the presence of NaCl concentrations ranging from 0.5 to 2.5 M. Moreover, Vijayalaxmi et al. (2013) demonstrated the ability of lignocellulolytic compost-isolated *Exiguobacterium sp.* strain VSG-1 to grow across a wide range of NaCl concentrations (1–16%), with the best growth observed at 1% NaCl (w/v).

Compost isolates such as CP5 and CP11 showed optimum growth at 40 °C, CP9 and CP2 showed optimum growth at 45 °C, while CP4 showed optimum growth at 35 °C. Rhizospheric isolates on the other hand such as SRS3 and SRS11 thrived at 35 °C. Previously, Buraimoh et al. (2015) reported the identification of three strains from a composting site such as *Bacillus sp.* (KF977555), *Bacillus megaterium* (KF977554) and *Streptomyces aureus* (KF977549). These strains were found to grow within a temperature range of 28 to 60 °C, with optimum growth occurring at 37 °C, 60 °C, and 50 °C respectively. López et al. (2021) reported the lignocellulolytic bacterial strains *Bacillus thermoamylovorans* 1141 and *Geobacillusthermodenitrificans* 3781 from compost samples, which displayed a temperature growth range of 30 to 60 °C and 20 °C to 60 °C, respectively, with optimum growth temperatures of 40 and 50 °C. Additionally, during the heating phase, *Bacillus sp*. has been investigated as the predominant genus in the compost, which can form endospores and has growth optima in the thermophilic range. In contrast, the rhizospheric region was moisturized due to root exudates, and mesophiles were the prominent flora (Mohammadipour et al. 2021; Villar et al. 2016). Furthermore, Mukhtar et al. (2019) reported that more than 90% of bacterial isolates from the rhizosphere of *Salsola stocksii* and *Atriplex amnicola* grew well at temperatures ranging from 25 to 40 °C, with some also capable of growing at 4 and 42 °C. Moreover, Balla et al. (2022) also revealed that 58%, 17%, and 25% of rhizospheric isolates exhibited optimum growth at temperatures 30 °C, 45 °C, and 50 °C, respectively.

According to the present study, sugarcane rhizospheric isolates preferred pH 7. Most compost isolates favoured pH 7, except CP9 and CP11, which showed best growth at pH 8. In support of the present finding, Zhang et al. (2021) reported an increase in pH from 4.5 to 9 during the composting process due to microbial activities, production of lactic acid, and release of ammonia. Studies have indicated that lignocellulose mineralization is more favourable in neutral or slightly acidic environments. However, the solubilization of lignin and the release of lignin by-products are most pronounced in alkaline pH conditions (Verma et al. 2017). Furthermore, several carbohydrate-active enzymes exhibited alkaliphilic behaviour and are most active in higher pH ranges (Mello et al.2017). Mironov et al. (2021) in their study reported the optimum occurrence of waste-degrading *Sphingobacterium, Bacillus, Planifilum*, and *Novibacillus* at pH 8 and *Leuconostoc* and *Lactococcus* at pH7 in different stages of composting. Kulkarni et al. (2019) reported growth of lignocellulolytic *Sphingobacterium sp.* ksn-11 in the range of pH 4 to pH 9 while optimum growth was observed within 12 h at pH7 and 40 °C temperature. Moreover, Vijayalaxmi et al. (2013) identified a lignocellulolytic *Exiguobacterium* sp. strain VSG-1, which displayed optimal growth at alkaline pH 9.0. The strain exhibited maximum growth between 30 and 50 °C, with the optimum temperature at 37 °C. Balla et al. (2022) unveiled that a higher proportion of rhizospheric isolates, specifically 66%, exhibited optimal growth at pH 7, whereas 44% demonstrated their optimal growth at pH 9. Some of the major lignocellulolytic bacteria with their substrates and enzymes are presented in Table 7.

Conclusion

In conclusion, this study demonstrated successful cultivation strategies for lignocellulolytic bacterial strains from compost and sugarcane rhizospheric soil using selective enrichment media. The use of CMC-MSM and L-MSM facilitated the growth of bacteria capable of lignin and cellulose degradation. Compost samples showed higher lignocellulolytic and heterotrophic bacterial density compared to rhizospheric soil. The majority of isolates from compost were identified as *Bacillus* species due to their potential to survive in diverse and dynamic habitats by producing various hydrolytic enzymes. The isolates displayed significant variations in enzyme secretion potential, NaCl tolerance, temperature preference, and pH growth optima. Cellulase production was prevalent among the isolates. The findings suggest that the potential of these isolated bacteria can be screened for the identification of valuable bioactive compounds, including enzymes and antibiotics, with potential commercial applications. This research contributes to the understanding of lignocellulolytic microbial communities

Table 7 Some of the major lignocellulolytic bacteria with their substrates and enzymes

Genus	Species	Morphology	Substrates	Enzymes	Reference
Thermobifida	*T. fusca,T.alba*, *T. ellulosilytica*, *T. halotolerans*,	Gram +	Agricultural Residue, Compost	Endoglucanase, Beta-glucosidase, Cellulose 1,4-beta-cellobiosidase, Beta-xylanase,	(Zhang et al. 2021)
Paenibacillus	*P. polymyxa*	Gram +	Lignin, Hemicellulose, Cellulose, CMC,	Cellulases, xylanases, lignin peroxidases, laccases,	(Weselowski et al. 2016)
Stenotrophomonas	*S. maltophilia RSD6*	Gram -	Wheat straw, H_2O_2, phenol red, Guaiacol, CMC	Manganese peroxidase, Lignin peroxidase, Laccase, Endoglucanase, Exoglucanase, Cellolbiase	(Nevita et al. 2018)
Bacillus	*B. subtilis*, *B. pumilus*, *B. cereus*, *B. sonorensis*, *B. licheniformis*, *B. safensis*, *B. megatarium* *B. subtilis K1*	Gram +	CMC, xylan, Cellulose, H2O2, Veratryl alcohol	Cellulases, Xylanases, Manganese peroxidase (MnP), Laccase	(López et al. 2021); (Mohammadipour et al. 2021) (Dube et al. 2023); (Abdel-Rahman et al. 2016); (Chandra et al. 2012)
Acidothermus	*A. cellulolyticus*	Gram +	Cellulose, Xylan, CMC, Avicel	Endoglucanases, Cellulases,	Sellstedt and Richau, (2013)
Clostridium	*C. hungatei*	Gram +	Lignocellulose, Crystalline cellulose	Cellulases, xylanases,	(Poehlein et al. 2017)
Streptomyces	*S. coelicolor*, *S.griseorubens*, *S. rubrolavendulae* *S. vietnamensis*	Gram +	Xylan, Avicel, Cellulose	Xylanases, cellulases, Endoglucanase	(Book et al. 2016); (Dahal et al. 2017); (Zhang et al. 2021)
Pseudomonas	*P. aeruginosa*, *P. putida* mt-2 *P. mendocina* strain fsznc-01, *P. stutzeri*	Gram -	Lignin extract from the plant, Milled wood Lignin from pine. CMC, Kraft lignin	lignin peroxidases, laccases, Manganese peroxidase, Cellulase	(Dube et al. 2023); (Ahmad et al. 2010)
Azomonas	*A. agilis*	Gram –	Cellulose, CMC, Filter paper	Cellulases, endoglucanases	(Latt et al. 2018)
Cellulosimicrobium	*C. cellulans*	Gram +	Agricultural Residue, Compost	Cellulases, Lignin peroxidases, Laccases,	(Mohammadipour et al. 2021)
Cellulomonas	*C. cellulans*, *C. gelida*, *C. iranensis ZJW-6*	Gram +	agro waste, Wheat, Flax, Sisal, Cassava, and Cotton fibres	Lignin peroxidases, Manganese peroxidase, Laccases, Endoglucanase, CMCase	(Wu et al. 2023), (Sugumaranet al.2013)
Rhodococcus *Arthrobacter*	*R, erythropolis*, *A. globiformis*	Gram +	Lignin extract from wheat straw and miscanthus	Manganese peroxidase, Laccase	(Ahmad et al. 2010)

and their biotechnological prospects in diverse environmental habitats.

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Declarations

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