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Isolation and screening of potential lignocellulolytic microbes from Phra Nakhon Si Ayutthaya Province

Sunisa Suwannaphan[1]*, Paweena Suksaard[2], Piyangkun Lueangjaroenkit[3], Suvichark Aroonluk[4], Phakhwan Thongrak[1]

[1]Department of Food Science and Technology, Faculty of Agricultural Technology and Agro-Industry, Rajamangala University of Technology Suvarnabhumi, Phra Nakhon Si Ayutthaya, Thailand.
[2]Department of Microbiology, Faculty of Science, Rajamangala University of Technology Suvarnabhumi, Phra Nakhon Si Ayutthaya, Thailand.
[3]Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok, Thailand.
[4]Program in Agricultural Science and Entrepreneurship, Faculty of Agriculture and Life Sciences, Chandrakasem Rajabhat University, Bangkok, Thailand.

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ABSTRACT

Phra Nakhon Si Ayutthaya Province is an important area for rice cultivation in Thailand. However, large amounts of rice straw generated at harvest time are often burnt in the fields, causing environmental degradation and air pollution. To address this, rice straw composting by degrading microorganisms has been identified as an effective alternative for sustainable waste management, with potential applications in environmental biotechnology. For this study, 28 microorganism samples were collected from various sources in Phra Nakhon Si Ayutthaya Province, including a corrugated paper manufacturer, plant-cultivated soil, compost from dung, black soldier flies, and vermicompost. Lignocellulolytic microorganisms were screened for cellulase, xylanase, and manganese peroxidase production on agar plates and broth media using commercial and rice straw substrates. The lignocellulolytic activities were compared among the isolated and non-pathogenic microorganisms. A microbial consortium with high potential to degrade rice straw was identified, consisting of two thermophilic bacteria, *Bacillus licheniformis* BKT1 and BOT4, two mesophilic actinomycetes, *Streptomyces ardesiacus* AQ4 and An6, two thermophilic actinomycetes, *Streptomyces thermoalcalitolerans* APT3 and *Streptomyces thermoviolaceus* subsp. *thermoviolaceus* AQT2, and two fungi, *Penicillium* sp. Fh11 and *Aspergillus* sp. Fj6. This microbial consortium induced rapid composting of rice straw, thereby supporting sustainable agricultural waste management and reducing PM25 air pollution.

1. INTRODUCTION

Thailand is a highly productive agricultural country, with 47% of the cultivated area comprising rice, rubber, cassava, sugarcane, and palm oil. In Phra Nakhon Si Ayutthaya Province, 89.55% of the land area is under rice cultivation, generating a large amount of rice straw as waste after harvesting [1,2]. Rice straw has high silica and lignin contents, and the C:N ratio (80:1) hampers degradation [3]. Many farmers burn rice straw in the fields as an easier and cheaper disposal option, but this negatively impacts air pollution and public health and reduces nutrients in the organic matter returned to the soil [4-6]. Rice straw is a complex natural polymer and a valuable source of lignocellulosic materials consisting of three major components: cellulose (40–50%), hemicellulose (25–30%), and lignin (15–20%) [7]. Composting rice straw is an effective alternative route for sustainable waste management in Thailand that ensures recycling of the nutrients contained in the residues and also has economic and ecological benefits [8]. Conventional composting requires a long processing period, while the introduction of potent microbial inoculum with specific functions into rice straw compost plays an important role in accelerating composting and improving the conversion of organic matter into nutrients [9,10]. The potential impact of inoculum is mostly generated by mesophilic and thermophilic lignocellulolytic microorganisms with high capacity to produce cellulase, xylanase, and ligninolytic enzymes that degrade cellulose, xylan, and lignin in the rice straw. Lignocellulolytic microorganisms with the capacity to degrade rice straw have been reported from bacteria in the genera *Actinobacteria*, *Bacillus*, *Clostridium*, *Cellulomonas*, and *Pseudomonas* with actinobacteria in *Actinomycosis bovis*, *Cellulomonas flavigena*, *Cellulomonas fimi*, *Thermobifida fusca*, and *Xylanimonas cellulosilytica* and fungi in *Aspergillus niger*, *Cladosporium cladosporioides*, *Fusarium* spp., *Pleurotus ostreatus*, *Phlebia radiata*, and *Trichoderma reesei* [11]. *Bacillus pumilus* B37 exhibited optimal lignocellulolytic activities and adaptation to rice straw amended medium [12]. *Pleurotus ostreatus* T1.1 and *Penicillium* sp. HC1 played a central role in cellulolytic enzyme production and the ability to use rice straw as a carbon source [13]. The microbial consortium LTF-27 composed of *Alcaligenes*, *Clostridium*, *Lysinibacillus*, *Parabacteroides*, *Sphingobacterium*, and uncultured bacteria efficiently degraded rice

*Corresponding Author:
Sunisa Suwannaphan,
Department of Food Science and Technology,
Faculty of Agricultural Technology and Agro-Industry,
Rajamangala University of Technology Suvarnabhumi,
Phra Nakhon Si Ayutthaya, Thailand.
E-mail: sns_mai@hotmail.com

straw [14], while Firmicutes showed high efficiency in hemicellulose degradation, and Proteobacteria and Bacteroidetes exhibited cellulose and lignin degradation [15]. This is the first study to isolate indigenous mesophilic and thermophilic lignocellulolytic microorganisms from Phra Nakhon Si Ayutthaya Province. Microorganisms with high potential for the production of lignocellulolytic enzymes were screened on agar plates and selected in broth media using specific commercial and rice straw substrates. These microorganisms, belonging to *Bacillus licheniformis*, *Streptomyces* sp., *Penicillium* sp., and *Aspergillus* sp. were combined into an indigenous microbial consortium to effectively speed up the rice straw composting process. However, efficiencies in rice straw composting need further investigation in field plot experiments. Farmers must be educated about the benefits of rice straw utilization through composting as a sustainable option to avoid environmental pollution through rice straw incineration in the field.

2. MATERIALS AND METHODS

2.1. Sources of Lignocellulolytic Microorganisms

Lignocellulose-degrading mesophilic and thermophilic microorganisms were isolated from water sourced from a corrugated paper manufacturer, dung from cow, chicken, pig, quail, and duck, compost from districts in Phra Nakhon Si Ayutthaya Province, black soldier flies, vermicompost, and soil from a bamboo garden, cornfield, and rice field.

2.2. Media for Isolation and Screening of Lignocellulolytic Microorganisms

Nutrient agar (NA) comprising (g/l) beef extract 3.0, peptone 5.0, yeast extract 1.0, and agar 15.0 was used to isolate the bacteria.

Starch casein agar (SCA) was used to isolate actinomycetes composed of (g/l) starch 10.0, casein 0.3, potassium nitrate 2.0, sodium chloride 2.0, dipotassium hydrogen phosphate 2.0, magnesium sulfate 0.02, calcium carbonate 0.01, ferrous sulfate 0.01, nalidixic acid 0.04, and agar 15.0.

Potato dextrose agar (PDA) containing chloramphenicol 0.05 g/l was used to isolate fungi.

International Streptomyces Project Medium 2 (ISP2) agar consisted of (g/l) yeast extract 4.0, malt extract 10.0, glucose 4.0, and agar 15.0.

Basal medium (BM) was composed of (g/l) ammonium sulfate 2.0, magnesium sulfate 0.5, dipotassium hydrogen phosphate 1.0, and agar 15.0.

Cellulolytic basal medium (CBM) consisted of 2% carboxymethyl cellulose (CMC) in BM, while xylanolytic basal medium (XBM) consisted of 2% xylan from corn core in BM.

NA/SCA/PDA consisted of 0.01% indigo carmine or Remazol brilliant blue (RBBR) was used for primary screening of the lignin-degrading microorganisms.

NAI and NAR consisted of 0.01% indigo carmine and 0.01% RBBR in NA, respectively.

SCI and SCR consisted of 0.01% indigo carmine and 0.01% RBBR in SCA, respectively.

Lignin-modifying basal medium (LBM) agar (g/l) composed of dipotassium hydrogen phosphate 1.6, potassium dihydrogen phosphate 0.5, magnesium sulfate 0.58, sodium chloride 0.25, ammonium sulfate 1.25, ferric chloride 0.0025, 2,6-dimethoxy phenol (DMP) 0.1, and agar 15.0 was used for primary screening of the lignin-degrading microorganisms.

Cellulolytic broth (CB) and xylanolytic broth (XB) (g/l) containing carboxymethyl cellulose or xylan from corn core 10.0, magnesium sulfate 0.2, potassium nitrate 0.75, dipotassium hydrogen phosphate 0.5, ferrous sulfate 0.02, calcium chloride 0.04, and yeast extract 2.0 was used to estimate the cellulase and xylanase activities, respectively.

Laccase broth (LB) (g/l) comprising peptone 5.0, beef extract 3.0, yeast extract 5.0, sodium chloride 10.0, copper sulfate 0.032, and manganese sulfate ($MnSO_4$) 0.27 was used to determine the manganese peroxidase (MnP) activity.

Rice straw broth (RSB) (g/l) comprising rice straw meal 10.0, magnesium sulfate 0.2, potassium nitrate 0.75, dipotassium hydrogen phosphate 0.5, ferrous sulfate 0.02, calcium chloride 0.04, and yeast extract 2.0 was used to estimate the lignocellulolytic enzyme content using rice straw as the substrate.

2.3. Isolation of Microorganisms

Samples were collected from different areas around Phra Nakhon Si Ayutthaya Province. Notably, 10 g of each sample were resuspended in 90 ml of sterile 0.85% NaCl and 1 ml of the suspension was serially diluted to the appropriate dilution. Then, 0.1 ml of the liquid mixture was spread on NA, SCA, and PDA plates to isolate bacteria, actinomycetes, and fungi, respectively. The plates were incubated at 30°C and 60°C to isolate mesophilic and thermophilic microbes, respectively.

2.4. Primary Screening of Lignocellulolytic Microorganisms

2.4.1. Screening of cellulase- and xylanase-producing microorganisms

The isolated microbes were spotted on CBM and XBM agar plates and incubated at 30°C or 60°C for 2 days (bacteria) and 3–7 days (actinomycetes and fungi), respectively. The plates were then flooded with iodine solution for 3–5 min. The clear zone and microbial colony diameters were then measured to calculate the potency index (PI) and indicate cellulase and xylanase activities, respectively, as follows:

$$\text{Potency index (PI)} = \frac{\text{Diameter of the formed clear zone (mm)}}{\text{Diameter of the microbial colony (mm)}}$$

2.4.2. Screening of ligninolytic microorganisms

The isolated bacteria and actinomycetes were spotted on NAI/NAR and SCI/SCR, respectively. The plates were incubated at 30°C or 60°C for 2 days (bacteria) and 3–7 days (actinomycetes), respectively. The decolorization zone and microbial colony diameters were then measured to calculate the PI values. The isolated fungi were screened for decolorization of indigo carmine and RBBR dyes using the tube overlay method. These tubes were then incubated at 30°C and dye decolorization was recorded every 5 days up to 30 days. Bacteria and actinomycetes that showed high PI values and fungi with rapid dye decolorization were further spotted on LBM agar plates and incubated at 30°C or 60°C for 2 days (bacteria) and 3–7 days (actinomycetes and fungi), respectively. The formation of a yellow zone indicated a positive reaction between MnP and lignin peroxidase, and the PI values were calculated.

2.5. Secondary Screening of Lignocellulolytic Microorganisms

2.5.1. Screening of lignocellulolytic microorganisms using a commercial substrate for cultivation

At least eight microbial isolates with the highest PI values on CBM, XBM, and LBM agar plates were inoculated in CB, XB, and LB medium

for secondary screening of potential cellulase, xylanase, and MnP-producing microorganisms using a commercial substrate for cultivation. Each flask was inoculated with 5% inoculum (10^8 CFU/ml of bacteria or actinomycetes and 10^8 spores/ml of fungi). The cultures were incubated at 30°C or 60°C with shaking at 200 rpm for 3 days (bacteria) and 7 days (actinomycetes and fungi), respectively. The supernatant was harvested daily by centrifugation at 10,000 rpm at 4°C for 10 min, and the lignocellulolytic enzyme activities were investigated.

2.5.2. Screening of lignocellulolytic microorganisms using rice straw for cultivation

Microbial isolates exhibiting at least three of the highest cellulase/xylanase/MnP activities were selected and cultured in RSB using rice straw as the substrate. Each flask was inoculated with 5% inoculum, as previously described. The supernatant was harvested daily by centrifugation at 10,000 rpm, 4°C for 10 min. The lignocellulolytic enzyme activities were evaluated for 1–3 days for bacteria and 1–7 days for actinomycetes and fungi, respectively.

2.6. Determination of Lignocellulolytic Enzyme Activities

2.6.1. Cellulase activity

Cellulase activity was determined following the method of Sadhu et al. [16]. A 0.5 ml aliquot of 1% CMC in 0.1 M phosphate buffer pH 7.0 was mixed with 0.5 ml of enzyme solution and incubated at 37°C or 60°C for 30 min. The reaction mixture was terminated by adding 1 ml of 3,5-dinitrosalicylic acid (DNS) reagent, boiling for 10 min, and then cooling in ice. The mixture was added with 5 ml of distilled water, with absorbance measured at 540 nm. One unit of cellulase activity was defined as the amount of enzyme that liberated reducing sugars equivalent to 1.0 µmol of glucose in a 1 min reaction time.

2.6.2. Xylanase activity

Xylanase activity was assayed following the cellulase assay procedure but using xylan from beechwood as the substrate. One unit of xylanase activity was defined as the amount of enzyme that liberated reducing sugars equivalent to 1.0 µmol of xylose in a 1 min reaction time.

2.6.3. MnP activity

MnP activity was evaluated following the method described by Lueangjaroenkit et al. [17]. The reaction mixture comprised 0.5 ml of 0.5 mM DMP, 0.25 ml of 0.1 M phosphate buffer pH 7.0, 0.25 ml of 1 mM $MnSO_4$, 0.75 ml of distilled water, and 0.5 ml of crude enzyme. The reaction was started by adding 0.05 ml of 1 mM hydrogen peroxide (H_2O_2) and incubated at 37°C or 60°C. Enzyme activity was recorded by the absorbance changes within 3 min at 469 nm. Enzyme denaturation at 100°C was performed as the control. One unit (U) of MnP was defined as the amount of enzyme necessary to oxidize 1 µmol of the substrate in a 1 min reaction time.

2.7. Compatibility Between Microorganisms

2.7.1. Compatibility between bacterial isolates

Compatibility between bacterial isolates was evaluated according to the modified method of Irabor and Ambaga [18]. A loopful of bacteria grown in NA was inoculated into 5 ml of nutrient broth and incubated for 24 h on an incubator shaker at 200 rpm at 30°C or 60°C. Cell concentrations were adjusted to an optical density (OD) value at 600 nm of 0.08–0.12 using a spectrophotometer. Then, 0.1 ml of bacterial suspension (bacteria 1) was spread on an NA plate and 0.01 ml (bacteria 2) was pipetted onto sterile 5-mm Whatman number 1 filter paper discs and air-dried for 30 min. Discs with cell suspensions of bacteria 2 were gently pressed onto the bacteria 1 plate agar surfaces using sterile forceps, and the plates were incubated at 30°C or 60°C for 24–48 h. The incompatibility between the bacterial isolates was evaluated by the inhibition zone between them.

2.7.2. Compatibility between actinomycete isolates

Compatibility between actinomycete isolates was performed by the perpendicular streak method of Singh et al. [19]. Actinomycetes 1 was single-streaked in the middle of an ISP2 agar plate. The plates were incubated at 30°C or 60°C for 72 h and then seeded with actinomycetes 2 by a single streak at a 90° angle to the streak of actinomycetes 1, followed by incubation at 30°C or 60°C for 72 h. The incompatibility between the actinomycete isolates was determined by the zone of inhibition between them.

2.7.3. Compatibility between fungal isolates

Compatibility between fungal isolates was determined following the method of Malviya et al. [20]. Fungus 1 was inoculated on the same PDA plates, 2.0–2.5 cm away from fungus 2. The plates were incubated at 30°C for 3–7 days, and the incompatibility was investigated by the inhibition zone between them.

2.8. Identification of Lignocellulolytic Microorganisms

The isolated microorganisms were identified using 16S rRNA gene sequencing (bacteria and actinomycetes) and the Internal Transcribed Spacer region (fungi), and sequenced by Macrogen Inc (Seoul, Republic of South Korea). A homology search was performed using the basic local alignment search tool, and the isolated sequences were deposited in GenBank.

2.9. Statistical Analysis

Three independent replicates were performed with mean values reported. Statistical analyses were conducted using SPSS Version 16.0 (SPSS Inc., USA). Duncan's multiple range test was used to evaluate the level of significant differences ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Isolation of Microorganisms

A total of 726 isolates were identified from 28 sources in Phra Nakhon Si Ayutthaya Province. These included 316 mesophilic bacterial isolates, 112 mesophilic actinomycete isolates, 148 mesophilic fungal isolates, 104 thermophilic bacterial isolates, and 46 thermophilic actinomycete isolates, with no thermophilic fungal isolates recorded. Compost, cow and goat dung, and soil from a bamboo garden were the major sources for isolating mesophilic and thermophilic microorganisms, with quail dung and vermicompost as minor sources. This result concurred with Jagadeesh and Muthuraju [21], who found potential lignocellulolytic microorganisms in farmyard manure, compost, rotten wood, vermicompost, and cow dung.

3.2. Primary Screening of Lignocellulolytic Microorganisms

3.2.1. Screening of cellulase-producing microorganisms

Results showed that 76 and 43 isolates had the potential to produce lignocellulolytic enzymes at 30°C and 60°C, respectively. A total of 12 mesophilic bacterial isolates exhibited the highest PI values, ranging from 10.50 to 22.25 on CBM [Table 1]. These values were higher than those of cellulolytic bacteria from herbivore manure (1.35–3.38) [22].

Table 1: PI values of microorganisms degrading cellulose, xylan, and lignin on CBM, XBM, and LBM agar plates after incubation at 30°C.

Isolate	PI Value				
	CBM	XBM	Indigo Carmine	RBBR	DMP
BB9	10.50 ± 0.00	14.00 ± 0.35	9.75 ± 0.35	6.33 ± 0.00	9.00 ± 0.12
BE4	4.43 ± 0.20	8.75 ± 0.35	6.20 ± 0.28	2.58 ± 0.12	10.00 ± 0.21
BW5	1.95 ± 0.07	11.00 ± 0.21	1.58 ± 0.12	1.80 ± 0.28	-
BW7	2.73 ± 0.09	24.50 ± 2.12	2.79 ± 0.10	3.00 ± 0.20	-
BK3	1.74 ± 0.00	11.25 ± 1.77	1.82 ± 0.00	2.75 ± 0.12	-
BQ1	11.00 ± 0.00	2.75 ± 0.35	1.42 ± 0.07	1.43 ± 0.00	-
BQ3	7.50 ± 0.71	7.50 ± 0.13	3.00 ± 0.11	6.00 ± 0.21	11.00 ± 0.31
BR3	22.25 ± 0.35	20.00 ± 0.41	2.69 ± 0.11	1.69 ± 0.09	-
BZ4	4.00 ± 0.02	3.80 ± 0.24	-	8.00 ± 0.22	22.00 ± 0.16
Ba6	13.00 ± 0.25	11.50 ± 0.13	2.47 ± 0.09	3.26 ± 0.21	-
Bb10	5.83 ± 0.24	-	6.60 ± 0.28	-	13.50 ± 0.21
Bc5	19.33 ± 0.51	8.25 ± 0.03	3.7 ± 0.06	4.93 ± 0.10	13.50 ± 0.18
Bc6	2.72 ± 0.05	12.00 ± 0.09	-	2.43 ± 0.20	-
Bd6	3.25 ± 0.45	8.50 ± 0.03	-	4.58 ± 1.30	2.30 ± 0.97
Bd7	14.75 ± 0.35	4.75 ± 0.35	3.25 ± 0.35	-	-
Be7	15.00 ± 0.78	18.00 ± 0.85	-	-	-
Be8	11.33 ± 0.78	15.00 ± 0.72	1.93 ± 0.11	2.00 ± 0.22	-
Bf7	20.00 ± 0.24	13.00 ± 0.25	2.43 ± 0.06	6.33 ± 0.23	18.70 ± 0.21
Bf10	-	7.50 ± 0.45	7.34 ± 0.12	6.10 ± 0.32	3.00 ± 0.16
Bf11	12.00 ± 1.05	13.00 ± 0.78	9.33 ± 0.22	5.00 ± 0.11	14.50 ± 0.15
Bg12	-	-	7.00 ± 0.34	-	9.00 ± 0.21
Bh6	12.00 ± 0.45	9.00 ± 0.81	6.31 ± 0.22	1.55 ± 0.03	3.00 ± 0.11
Bh10	2.14 ± 0.05	-	11.00 ± 0.34	9.00 ± 0.32	7.00 ± 0.18
Bh12	9.00 ± 0.15	9.00 ± 0.56	-	4.00 ± 0.08	3.00 ± 0.11
Bk1	12.00 ± 0.32	16.00 ± 0.25	2.78 ± 0.07	4.40 ± 0.22	14.00 ± 0.25
Bl16	5.67 ± 0.14	-	-	5.50 ± 0.14	2.90 ± 0.16
BI18	6.50 ± 0.23	5.00 ± 0.18	6.00 ± 0.16	3.60 ± 0.15	3.50 ± 0.21
AJ3	4.88 ± 0.18	4.00 ± 0.14	4.50 ± 0.13	2.88 ± 0.17	6.00 ± 0.32
AK7	5.38 ± 0.18	-	4.88 ± 0.18	2.17 ± 0.24	2.95 ± 0.22
AL3	4.38 ± 0.18	4.67 ± 0.14	4.50 ± 0.24	4.83 ± 0.24	5.90 ± 0.13
AM3	4.20 ± 0.07	8.00 ± 0.23	2.09 ± 0.13	1.20 ± 0.09	-
AO1	4.11 ± 0.16	-	6.25 ± 0.35	4.00 ± 0.21	3.00 ± 0.15
AO2	7.17 ± 0.24	-	5.33 ± 0.27	7.17 ± 0.24	6.70 ± 0.24
AQ4	3.17 ± 0.24	8.10 ± 0.22	3.00 ± 0.15	2.67 ± 0.18	-
AR5	5.75 ± 0.35	6.00 ± 0.24	4.60 ± 0.25	6.75 ± 0.23	2.00 ± 0.18
Af5	5.50 ± 0.12	10.50 ± 0.21	-	5.50 ± 0.21	-
Ag1	4.50 ± 0.21	10.00 ± 0.71	3.00 ± 0.12	5.50 ± 0.17	-
Ag2	2.50 ± 0.09	4.00 ± 0.09	4.00 ± 0.23	7.00 ± 0.22	-
Ah1	13.00 ± 0.21	14.00 ± 0.24	3.50 ± 0.15	5.00 ± 0.17	-
Ah3	3.00 ± 0.21	9.00 ± 0.06	1.50 ± 0.08	2.80 ± 0.09	-
Ah4	6.00 ± 0.19	6.50 ± 0.14	5.00 ± 0.18	-	6.00 ± 0.32
Ah6	4.67 ± 0.09	6.67 ± 0.03	4.50 ± 0.12	-	5.30 ± 0.21
Ah9	-	7.33 ± 0.14	3.00 ± 0.16	7.50 ± 0.18	6.70 ± 0.32
Ak2	5.80 ± 0.16	10.00 ± 0.21	2.75 ± 0.11	5.00 ± 0.16	-
Al1	7.50 ± 0.21	6.50 ± 0.11	-	6.00 ± 0.21	7.00 ± 0.23
Al2	7.20 ± 0.25	6.00 ± 0.19	-	-	-
Am2	-	-	-	5.00 ± 0.17	-
Am10	-	7.00 ± 0.03	-	7.00 ± 0.11	6.70 ± 0.16
An4	6.68 ± 0.22	2.00 ± 0.05	2.25 ± 0.07	2.00 ± 0.08	-
An5	6.67 ± 0.19	4.50 ± 0.15	-	3.50 ± 0.03	-
An6	3.20 ± 0.08	6.00 ± 0.21	1.00 ± 0.03	6.00 ± 0.04	9.50 ± 0.34
An9	6.98 ± 0.13	6.00 ± 0.11	1.50 ± 0.06	3.50 ± 0.11	-
Aq5	6.90 ± 0.18	6.00 ± 0.15	1.00 ± 0.02	3.00 ± 0.06	-
Aq6	3.33 ± 0.12	4.50 ± 0.12	1.00 ± 0.00	7.50 ± 0.13	8.00 ± 0.26
Aq7	5.50 ± 0.08	10.50 ± 0.13	2.00 ± 0.01	3.50 ± 0.08	-
Aq8	6.50 ± 0.15	6.33 ± 0.11	1.00 ± 0.01	4.50 ± 0.21	-
Aq12	8.33 ± 0.24	10.00 ± 0.21	1.20 ± 0.00	9.00 ± 0.23	2.00 ± 0.12
Aq13	-	7.20 ± 0.12	5.50 ± 0.56	6.00 ± 0.21	10.70 ± 0.26
Al1	8.25 ± 0.21	10.00 ± 0.23	5.80 ± 0.23	6.25 ± 0.26	8.30 ± 0.24
Al2	6.00 ± 0.31	8.33 ± 0.16	5.00 ± 0.24	4.75 ± 0.32	1.50 ± 0.07
FR11	2.03 ± 0.04	2.13 ± 0.03	11/D30	10/D30	-
FW1	2.77 ± 0.06	2.24 ± 0.03	13/D30	13/D30	-
FW3	1.30 ± 0.05	1.97 ± 0.03	35/D10	35/D30	2.75 ± 0.01
Fa5	2.05 ± 0.09	1.48 ± 0.02	35/D10	35/D10	2.09 ± 0.01
Fb2	2.73 ± 0.07	2.50 ± 0.04	14/D30	4/D30	-
Fb5	2.04 ± 0.08	-	35/D5	15/D30	2.25 ± 0.02
Fc3	-	2.00 ± 0.02	35/D10	12/D30	2.00 ± 0.02
Fe4	1.59 ± 0.02	1.88 ± 0.03	35/D15	7/D30	4.17 ± 0.02
Ff3	2.07 ± 0.03	2.33 ± 0.05	3/D30	2/D30	-
Fg1	1.53 ± 0.03	2.00 ± 0.04	25/D30	16/D30	-
Fg3	-	-	35/D15	11/D30	2.12 ± 0.02
Fh5	2.08 ± 0.02	3.38 ± 0.03	25/D30	15/D30	-
Fh11	4.00 ± 0.04	2.40 ± 0.04	35/D15	12/D30	3.33 ± 0.02
Fj4	1.47 ± 0.05	-	35/D5	17/D30	2.67 ± 0.02
Fj6	2.07 ± 0.01	2.20 ± 0.03	10/D30	12/D30	-
Fj8	3.13 ± 0.03	2.50 ± 0.03	25/D30	17/D30	-
Fl5	1.47 ± 0.02	1.18 ± 0.02	35/D5	12/D30	2.37 ± 0.01
Fl6	1.69 ± 0.03	1.50 ± 0.01	35/D15	12/D30	2.12 ± 0.02
Fl7	2.50 ± 0.01	1.87 ± 0.04	35/D10	35/D15	2.50 ± 0.03
Fn2	2.50 ± 0.03	2.13 ± 0.02	15/D30	25/D30	-
Fn3	3.33 ± 0.01	2.00 ± 0.01	27/D30	11/D30	-
FII2	2.56 ± 0.04	1.25 ± 0.04	12/D30	15/D30	-
FII5	1.79 ± 0.03	1.63 ± 0.01	35/D10	15/D30	2.83 ± 0.02
FXIII1	4.00 ± 0.03	1.12 ± 0.03	35/D10	15/D30	4.15 ± 0.01

(Continued)

Table 1: (Continued)

Isolate	CBM	XBM	Distance of Dye Discolorizing (mm)/day (D)		LBM
			Indigo	RBBR	

Eight thermophilic bacterial isolates had PI values ranging from 3.33 to 10.00 [Table 2]. These values were lower than those of cellulase-producing thermophilic bacteria in tropical dry deciduous forest soil (9.65–24.04) [23]. The PI values of cellulase obtained from thermophilic bacteria were lower than cellulase from mesophilic bacteria, conflicting with Sakpetch et al. [24], who reported lower PI values of mesophilic bacteria on media containing CMC (1.95–14.58) than thermophilic bacteria (2.88–20.13). A total of 11 isolates of mesophilic and thermophilic actinomycetes presented high PI cellulase values ranging from 6.50 to 13.00 and 8.70 to 30.00, respectively. The former PI values were higher than those of cellulase-producing mesophilic actinomycetes from termite-infested wood; soil from a garden, kitchen garden, and wasteland; and decaying vegetation (2.5–6.0) [25]. The latter PI values were slightly higher than those from thermophilic actinomycetes from rubber bark and other agricultural residues, with PI values of 1.98–20.20 [24]. Cellulase-producing mesophilic fungi had maximum PI values ranging from 2.50 to 4.00, which were lower than PI values from mesophilic actinomycetes and bacteria. These values were higher than those of fungi isolated from wood degradation (1.02–3.05) [26]. Generally, fungi and bacteria are good producers of cellulase, especially fungi due to their extracellular properties [27]. This study revealed that cow and goat dung, compost from dung and black soldier flies, and soil from a cornfield, rice field, and bamboo garden were major sources of potential cellulase-producing microorganisms. Lignocellulolytic microbes are significant contributors to composting, with cow and goat dung being rich sources of lignocellulosic fibers, and bamboo garden soil containing abundant lignocellulosic biomass. These findings aligned with Sakpetch et al. [24], Do et al. [28], and Huang et al. [29], who identified lignocellulolytic microorganisms from composting heaps, cow manure, soil from naturally occurring bamboo groves, and the rumen of Vietnamese native goats.

3.2.2. Screening of xylanase-producing microorganisms

The highest PI values of xylanase mesophilic bacteria were 11.00–24.50, which is higher than those of thermophilic bacteria (4.00–6.50) [Tables 1 and 2] and xylanase-producing bacteria isolated from a mixture of decayed wood and sand, water from a volcano crater, and sea sand (0.11–0.71) [30] because the mesophilic bacteria grew faster and produced more enzymes than the thermophilic bacteria. Eleven xylanase-producing mesophilic actinomyces and thermophilic actinomyces possessed maximum PI values ranging from 8.00 to 14.00 and 5.30 to 26.00, respectively. The former PI values were higher than those of xylanase-producing mesophilic actinomycetes from areas around New Delhi in India (1.5–9.0) [31]. The PI values of xylanase obtained from mesophilic fungi ranged from 2.00 to 3.38, lower than those of mesophilic actinomycetes and bacteria, respectively. These results differed from Sunna and Antranikian [32], who reported that fungi and bacteria were the best xylanase producers.

3.2.3. Screening of MnP-producing microorganisms

Mesophilic bacteria and thermophilic bacteria having PI values of 4.00 (NAI) and 2.00 (NAR) were spotted on LBM agar. Mesophilic bacteria exhibited the highest PI values of 7.00–22.00, slightly less than thermophilic bacteria at 8.65–32.00 [Tables 1 and 2]. Mesophilic actinomycetes showing PI values higher than 4.00 (SCI) and 6.00 (SCR), and thermophilic actinomycetes having PI values more than 5.00 on SCI and SCR, were spotted on DMP agar. Mesophilic actinomycetes had the highest PI values of 5.30–10.70, less than thermophilic actinomycetes at 17.00–36.00. Mesophilic fungi showed a greater capacity to decolorize RBBR than indigo carmine. Mesophilic

Table 2: PI values of microorganisms degrading cellulose, xylan, and lignin on CBM, XBM, and LBM agar plates after incubation at 60°C.

Isolate	PI Value				
	CBM	XBM	Indigo Carmine	RBBR	LBM
BKT1	-	5.00 ± 0.47	-	1.17 ± 0.05	
BOT4	1.93 ± 0.10	4.18 ± 0.15	1.73 ± 0.13	2.38 ± 0.18	9.00 ± 0.12
BPT3	6.25 ± 0.35	-	4.33 ± 0.47	3.63 ± 0.06	32.00 ± 0.32
BRT2	5.36 ± 0.12	-	1.30 ± 0.11	-	-
BTT1	-	6.50 ± 0.15	-	-	-
BTT4	-	4.00 ± 0.11	-	1.82 ± 0.08	-
BVT3	3.33 ± 0.21	-	-	-	-
BZT5	-	-	4.67 ± 0.21	4.91 ± 0.16	22.00 ± 0.31
BbT1	-	-	3.05 ± 0.19	3.79 ± 0.21	11.00 ± 0.14
BcT1	-	-	3.00 ± 0.23	4.08 ± 0.31	32.00 ± 0.31
BdT5	-	-	3.92 ± 0.16	-	8.65 ± 0.12
BfT2	-	4.10 ± 0.17	-	1.10 ± 0.11	-
BfT3	3.85 ± 0.09	4.20 ± 0.25	-	1.01 ± 0.12	-
BiT1	-	-	2.71 ± 0.19	1.67 ± 0.05	10.00 ± 0.14
BiT3	8.00 ± 0.32	-	1.67 ± 0.07	2.40 ± 0.02	11.00 ± 0.17
BnT1	3.64 ± 0.08	6.00 ± 0.21	-	3.17 ± 0.13	10.00 ± 0.09
BnT5	2.25 ± 0.11	4.01 ± 0.18	-	-	-
BnT7	-	-	0.33 ± 0.00	2.00 ± 0.09	12.00 ± 0.11
BnT10	-	5.00 ± 0.18	5.20 ± 0.11	1.50 ± 0.12	11.00 ± 0.21
BnT13	-	-	2.60 ± 0.15	3.00 ± 0.12	20.00 ± 0.24
BIT1	6.00 ± 0.23	-	-	-	-
BIXT2	10.00 ± 0.32	3.20 ± 0.14	2.70 ± 0.15	2.01 ± 0.08	20.00 ± 0.11
ALT5	-	26.00 ± 0.34	-	-	-
APT3	2.80 ± 0.23	-	6.30 ± 0.16	2.10 ± 0.09	25.00 ± 0.34
AQT2	4.20 ± 0.13	3.60 ± 0.13	6.00 ± 0.13	2.90 ± 0.04	32.00 ± 0.41
AQT4	20.00 ± 0.15	5.20 ± 0.17	23.00 ± 0.35	20.00 ± 0.23	26.00 ± 0.36
AQT6	2.20 ± 0.09	3.90 ± 0.14	8.10 ± 0.16	3.10 ± 0.07	36.00 ± 0.41
AQT9	12.00 ± 0.12	-	4.00 ± 0.21	-	-
AQT11	15.00 ± 0.21	-	3.40 ± 0.15	3.80 ± 0.12	-
AQT12	23.00 ± 0.17	6.50 ± 0.23	4.30 ± 0.13	4.40 ± 0.31	-
ART1	-	11.70 ± 0.36	-	25.00 ± 0.45	28.00 ± 0.43
ART7	20.50 ± 0.21	7.00 ± 0.15	4.40 ± 0.11	4.20 ± 0.13	-
ATT1	-	5.30 ± 0.21	3.50 ± 0.09	5.10 ± 0.21	28.00 ± 0.26
AWT2	12.00 ± 0.11	5.30 ± 0.19	3.82 ± 0.06	5.10 ± 0.16	9.00 ± 0.13
AXT5	13.0 ± 0.08	-	3.69 ± 0.04	5.10 ± 0.13	8.00 ± 0.31
AaT7	-	-	5.50 ± 0.04	3.60 ± 0.21	9.00 ± 0.25
AeT2	10.00 ± 0.16	-	3.50 ± 0.12	4.60 ± 0.13	-
AgT5	-	20.00 ± 0.34	-	-	-
AhT1	8.70 ± 0.21	7.00 ± 0.21	2.70 ± 0.12	3.40 ± 0.13	-
AhT2	30.00 ± 0.34	19.00 ± 0.32	7.30 ± 0.13	5.00 ± 0.13	23.00 ± 0.41
AhT4	5.00 ± 0.16	7.00 ± 0.13	3.10 ± 0.09	6.00 ± 0.16	17.00 ± 0.32
AhT5	6.00 ± 0.21	7.00 ± 0.17	-	4.80 ± 0.12	-
AiT2	11.00 ± 0.32	4.50 ± 0.23	2.46 ± 0.05	5.50 ± 0.22	8.00 ± 0.21

fungi that rapidly degraded indigo carmine, RBBR, or both dyes were spotted on LMP agar. The highest PI values ranged from 2.00 to 4.17. The PI values of thermophilic bacteria and actinomycetes were higher than those of mesophilic bacteria and actinomycetes, respectively. The PI values of mesophilic fungi were lower than those of mesophilic actinomycetes and bacteria. Major sources of MnP-producing microorganisms were similar to cellulase-producing microorganisms, except for the water sample from a corrugated paper manufacturer.

Based on the primary screening results, mesophilic bacteria had higher efficacy in producing lignocellulolytic enzymes than mesophilic actinomycetes and fungi on a solid medium. This result differed from Saini et al. [33], who reported that fungal enzymes could break down lignocellulosic biomass more efficiently than bacterial enzymes. Thermophilic actinomycetes showed a significant capacity to produce higher lignocellulolytic enzymes than thermophilic bacteria.

3.3. Secondary Screening of Lignocellulolytic Microorganisms Using a Commercial Substrate

3.3.1. Screening of lignocellulolytic bacteria

At least eight isolates showing high potential of lignocellulolytic enzyme-producing microorganisms in primary screening were selected to investigate lignocellulolytic enzyme-producing capacity in broth medium using a commercial substrate for cultivation. Mesophilic bacterial isolates BR3, Ba6, Bc5, Bd7, and Be8 had cellulase activities of 0.27–1.12 U/ml. Their cellulase activities were significantly higher than the other isolates (0–0.20 U/ml) [Figure 1A]. Isolate Ba6 exhibited the highest cellulase activity, higher than *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, and *Serratia marcescens* (0.4–0.9 U/ml) [34]. Isolates BB9, Ba6, Be8, Bf7, and Bk1 had xylanase activities ranging from 2.52 to 4.16 U/ml [Figure 1B]. These values were lower than those of the bacterial isolates XPB-CW01 (12.2 U/ml) and XPB-GS02 (18.3 U/ml) [35]. Only isolates Bf11 and Bk1 exhibited high MnP activities at 0.13 and 0.06 U/ml, respectively [Figure 1C]. These values were higher than those of *Paenibacillus* sp. (12.33 U/l) and *Bacillus pumilus* (31.66 U/l) [36]. Mesophilic bacterial isolates BR3, Ba6, Be7, and Be8 produced cellulase and xylanase enzymes, while isolates Bf11 and Bk1 produced three kinds of lignocellulolytic enzymes.

The thermophilic bacterial isolates BRT2, BiT3, and BIXT2 presented the highest cellulase activities (2.51–3.07 U/ml) [Figure 1D]. Their activities were lower than those of *Bacillus subtilis* K-18 (KX881940) (3.51 U/ml) [37]. The xylanase activities of isolates BKT1, BOT4, BfT2, and BnT10 ranged from 5.45 to 12.45 U/ml [Figure 1E], and were higher than those from *Bacillus licheniformis* KBFB4 (0.76 U/

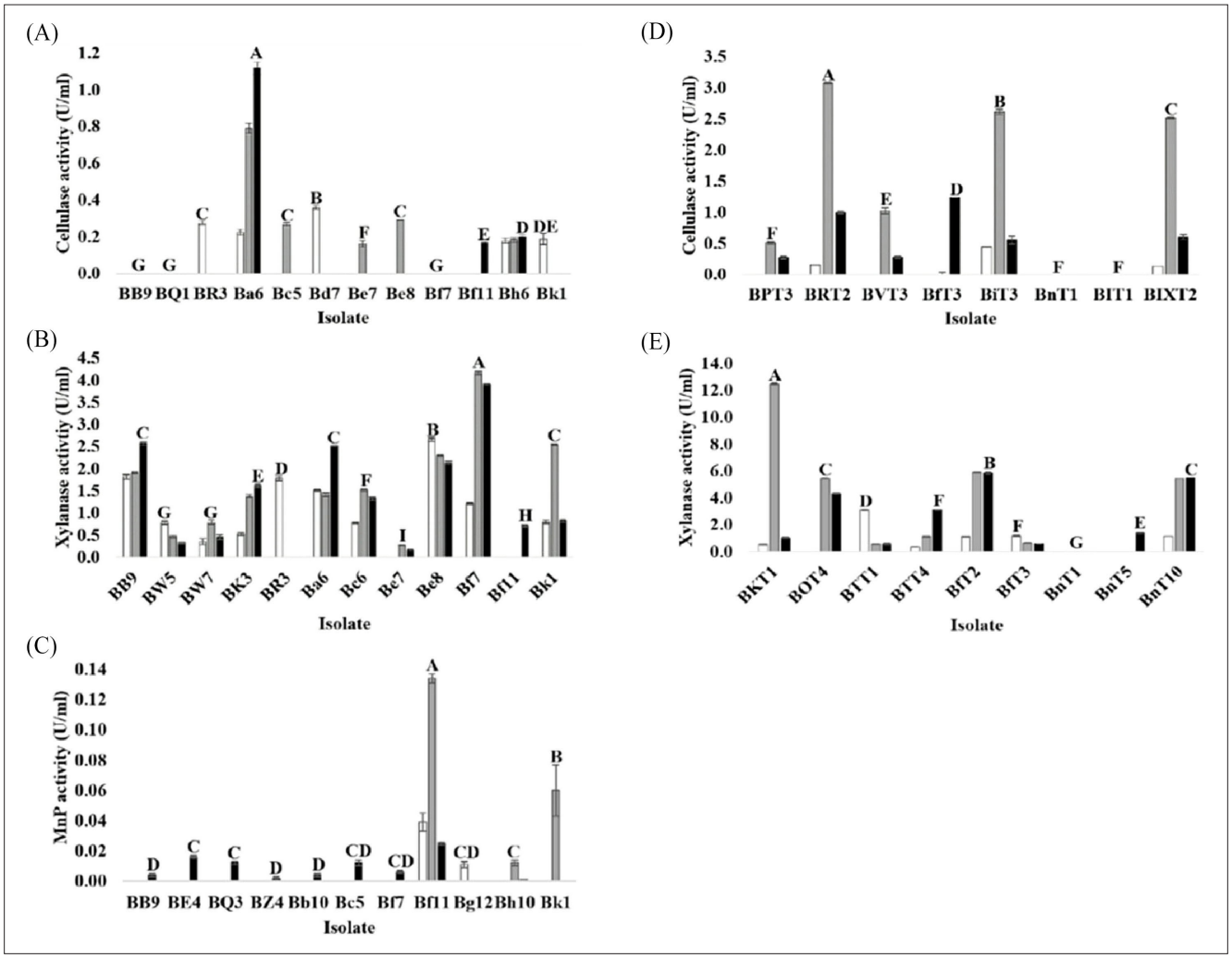

Figure 1: Activities of cellulase **(A–D)**, xylanase **(B–E)**, and MnP **(C)** of mesophilic and thermophilic bacteria at day 1 (□), 2 (■), and 3 (■) using a commercial substrate for cultivation.

ml) and *Bacillus subtilis* VSDB5 (1.00 U/ml) [38]. No MnP activity was found in thermophilic bacteria because the 2,6-DMP aromatic substrate structure was difficult to digest, and the enzyme activities of MnP were unstable after prolonged exposure to high temperatures.

Thermophilic bacteria had higher cellulase and xylanase enzyme activities than mesophilic bacteria. This observation conflicted with the primary screening results showing PI values of thermophilic bacteria lower than mesophilic bacteria. This result concurred with Mingardon et al. [39], who reported that cellulase activities of thermophilic bacteria outperformed their mesophilic counterparts at higher temperatures. Isolate BnT1 showed cellulase and xylanase activities on CBM and XBM agar plates but was not found in CB and XB broth media. Therefore, nine mesophilic bacteria (BB9, BR3, Ba6, Bc5, Bd7, Be8, Bf7, Bf11, and Bk1) and seven thermophilic bacteria (BKT1, BOT4, BRT2, BfT2, BiT3, BnT10, and BIXT2) were selected to further investigate the lignocellulolytic enzymes in RSB medium.

3.3.2. Screening of lignocellulolytic actinomycetes

Mesophilic actinomycete isolates Ah1, Al2, and An4 showed high cellulase activities ranging from 0.73 to 1.52 U/ml [Figure 2A], and higher than those of actinomycete isolates Ac1 (0.19 U/ml) and Ac6 (0.05 U/ml) [40]. High xylanase activities of isolates AM3, AQ4, Ah1, and Ak2 ranged from 1.49 to 2.29 U/ml [Figure 2B]. Isolates AJ3, Ah6, Ah9, and An6 showed similar MnP activities (0.027–0.029 U/ml) [Figure 2C]. Their activities were lower than those of *Streptomyces psammoticus* (3 U/ml) [41]. Activities of xylanase and MnP enzymes obtained from mesophilic actinomycetes were lower than the activities of mesophilic bacteria. The cellulase activities of thermophilic actinomycete isolates AWT2, AXT5, AhT1, and AiT2 were 0.18–0.48 U/ml [Figure 2D]. These values were higher than thermophilic actinomycetes Nos. 4–8 (0.07–0.13 U/ml) [42]. Isolates ART1, ART7, AWT2, and AhT1 had xylanase activities of 0.44–0.59 U/ml [Figure 2E]. Only APT3 and AQT2 displayed MnP activities at 0.09 and 0.06 U/ml, respectively [Figure 2F]. Isolates AWT2 and AhT1 produced both cellulase and xylanase. Thermophilic actinomycetes showed lower cellulase and xylanase activities than thermophilic bacteria. These results conflicted with the primary screening results, showing PI values of thermophilic actinomycetes higher than those of mesophilic bacteria because agar plate screening is a sensitive method with many factors (temperature, moisture, agar concentration, and media type) impacting clear zone size. Thus, deducing an obvious correlation between enzyme activity and clear zone size is difficult [43]. In total, 11 mesophilic actinomycetes (AJ3, AM3, AQ4, Ah1, Ah6, Ah9, Ak2, Al2, An4, An6, and Aq12) and 8 thermophilic actinomycetes (APT3, AQT2, ART1, ART7, AWT2, AXT5, AhT1, and AiT2) were selected to further evaluate the lignocellulolytic enzymes produced using RSB for cultivation.

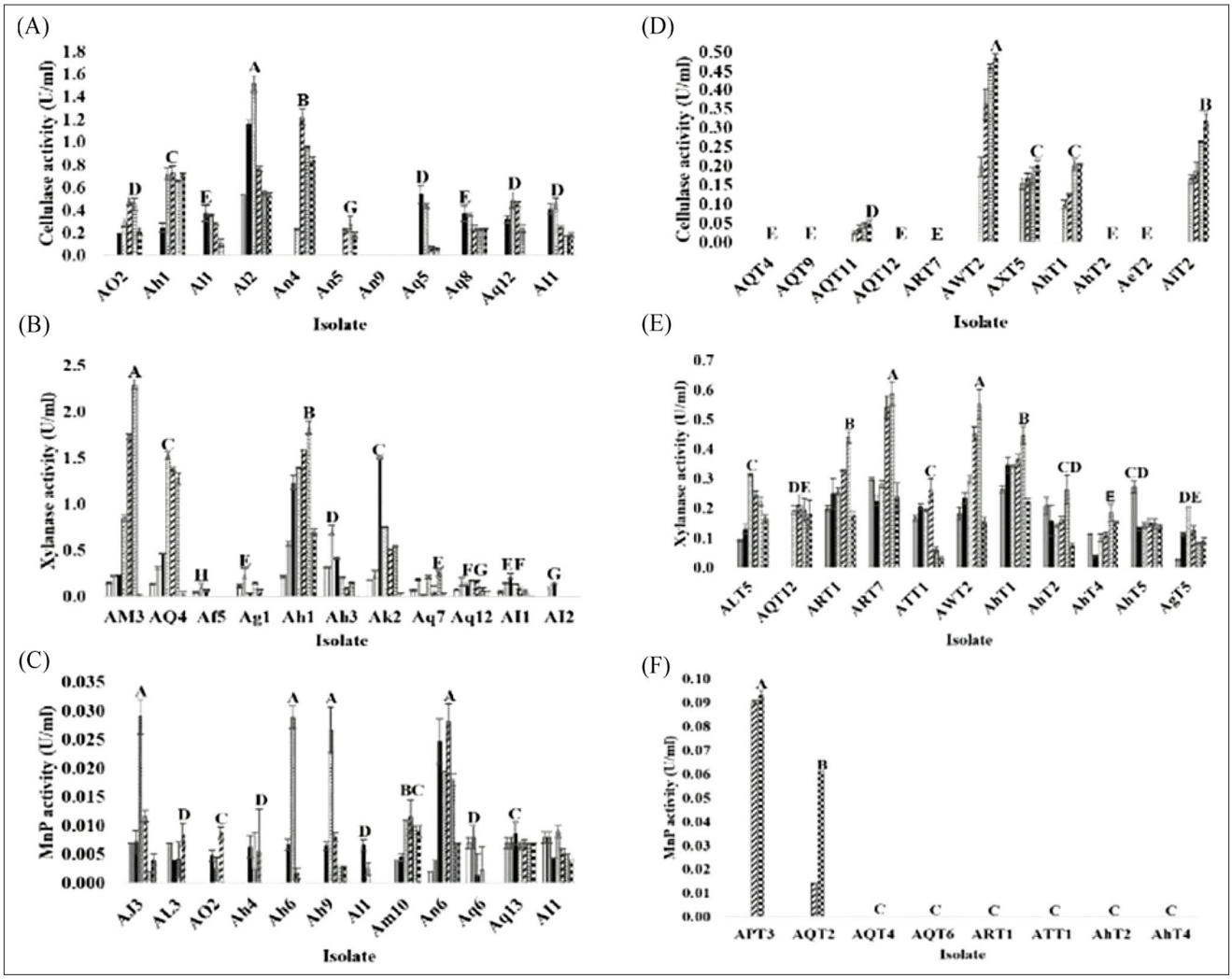

Figure 2: Activities of cellulase **(A-D)**, xylanase **(B-E)** and MnP **(C-F)** of mesophilic and thermophilic actinomycetes at day 1 (□), 2 (■), 3 (■), 4 (⊡), 5 (▨), 6 (▤), and 7 (⊠) using a commercial substrate for cultivation.

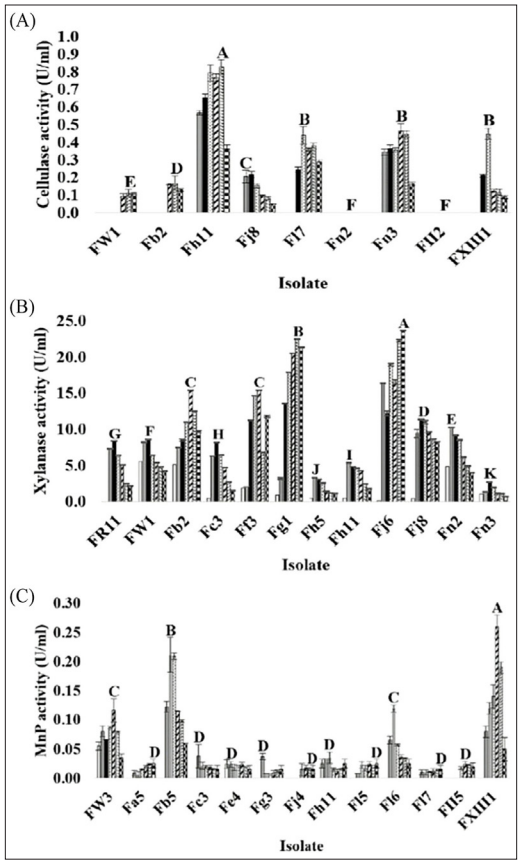

Figure 3: Activities of cellulase **(A)**, xylanase **(B)** and MnP **(C)** of mesophilic fungi at day 1 (□), 2 (■), 3 (■), 4 (▨), 5 (▨), 6 (▨), and 7 (▨) using a commercial substrate for cultivation.

3.3.3. Screening of lignocellulolytic fungi

Mesophilic fungi isolates Fh11, Fl7, Fn3, and FXIII1 had cellulase activities ranging from 0.44 to 0.83 U/ml [Figure 3A]. These values were higher than *Trichoderma* sp. isolates C03-11, C03-24, SC11-65, SC15-DY53, C01-L2, C01-L3, and C01-L4 (0.06–0.23 U/ml) but lower than *Trichoderma* sp. isolates C03-63c, SC56-113, SC13-114, and LZ117 (2.09–7.17 U/ml) [44]. The activities of xylanase isolates Fb2, Ff3, Fg1, and Fj6 ranged from 15.43 to 23.68 U/ml [Figure 3B]. These values were higher than those of *Penicillium* sp. SC3-DY9, SC3-48, SC3-49, and SC18-123 (0.74–4.28 U/ml) [44]. Mesophilic fungi isolates FW3, Fb5, Fl6, and FXIII1 had MnP activities ranging from 0.12 to 0.26 U/ml [Figure 3C], lower than *Podoscypha elegans* strain FTG4 (14.13 U/ml) [45]. The activities of xylanase and MnP from mesophilic fungi tended to be higher than those of mesophilic bacteria and mesophilic actinomycetes due to the lower capacity of bacteria to degrade lignin than that of fungal species [46]. Hence, 11 mesophilic fungi namely FW3, Fb2, Fb5, Ff3, Fg1, Fh11, Fj6, Fl6, Fl7, Fn3, and FXIII1 were selected to further examine the lignocellulolytic enzymes in RSB medium.

3.4. Secondary Screening of Lignocellulolytic Microorganisms Using Rice Straw

3.4.1. Secondary screening of lignocellulolytic bacteria

Mesophilic bacteria were investigated for lignocellulolytic activities when cultivated in RSB medium with rice straw as the substrate. Results showed that most mesophilic bacteria produced xylanase and MnP enzymes after incubation for 1–2 days by digesting xylan and lignin in the external structure of plant cell walls [47]. Cellulase enzymes were observed after incubation for 2–3 days after digesting cellulose in the cell walls. Mesophilic bacteria isolates Ba6, Be8, Bf7, and Bk1 had high xylanase activities in XB medium (2.52-4.16 U/ml), with high cellulase activities in RSB medium (0.27–0.75 U/ml) [Figure 4A]. These values were higher than cellulase activities from bacterial isolates B8 (0.014 U/ml),

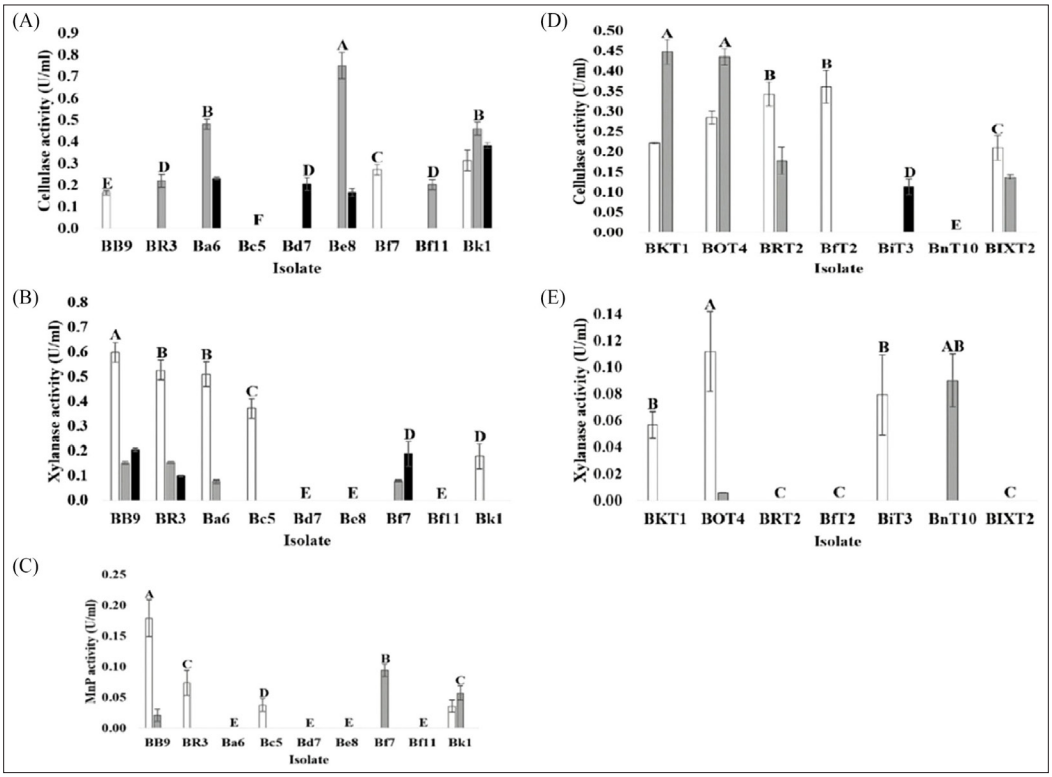

Figure 4: Activities of cellulase **(A–D)**, xylanase **(B–E)**, and MnP **(C)** of mesophilic and thermophilic bacteria at day 1 (□), 2 (■), and 3 (■) using a rice straw for cultivation.

B10 (0.17 U/ml), and B15 (0.21 U/ml) using decomposed rubber bark and rice straw as substrates for cultivation [24,48]. This result indicated that xylanase played an important role in using rice straw as a carbon source for bacteria. Microorganisms may produce enzymatic cocktails with several catalytic capabilities for degrading cellulose [49]. Isolates BR3, Bc5, and Bd7 had high cellulase activities in CB medium (0.27–0.36), with low cellulase activities in RSB medium (0.00–0.22 U/ml). CB medium contains soluble cellulose derivatives that increase the production of cellulase enzymes by the non-requirement of time for degrading lignin [50]. Five isolates (BB9, BR3, Ba6, Bf7, and Bk1) exhibited lower xylanase activities (0.18–0.60 U/ml) in RSB medium [Figure 4B] than in XB medium (2.52–4.16 U/ml). Their activities in RSB medium were higher than observed in bacterial isolate B8, which had xylanase activities of 0.136 and 0.151 U/ml using sugarcane bagasse and rice straw as the substrate, respectively [48]. These results were due to the structure of rice straw (RSB medium) being more complex than that of xylan (XB medium), resulting in more difficult digestion and lower activity. Isolates BB9, Bc5, and Bf7 had MnP activities of 0.04–0.18 U/ml in RSB medium [Figure 4C], higher than in LB medium (0.004–0.012 U/ml).

Activities of cellulase and xylanase enzymes from thermophilic bacteria in RSB medium were lower than in commercial media because the complex structure of rice straw led to difficult digestion and the enzymes were not stable at high temperatures. Cellulase activities of BRT2, BiT3, and BIXT2 and xylanase activities of BKT1, BOT4, and BnT10 were detected in both commercial and RSB media. Isolates BKT1 and BOT4 had lower cellulase activities (0.45 and 0.44 U/ml) [Figure 4D] and xylanase activities (0.06 and 0.11 U/ml) [Figure 4E] than the bacteria MAM-38 (248 U/ml) and *Aneurinibacillus thermoaerophilus* SSA2 (6,033 U/ml) using rice straw as the substrate, respectively [51,52]. These results suggested that thermophilic bacteria cultured in RSB medium produced low cellulase and xylanase enzyme activities with no MnP activity.

3.4.2. Secondary screening of lignocellulolytic actinomycetes

Higher cellulase activities of mesophilic actinomycetes were observed in CB medium than in RSB medium [Figure 5A]. The xylanase and MnP activities of mesophilic actinomycetes in the commercial medium were lower than in RSB medium [Figures 5B and 5C]. Five isolates, namely AQ4, Ah6, Al2, An4, and An6 had high activities of three lignocellulolytic enzymes. These results indicated that mesophilic actinomycetes can use rice straw as a carbon source to produce high xylanase and MnP activities.

Thermophilic actinomycete isolates AWT2 and AXT5 had higher cellulase activities in CB medium (0.48 and 0.20 U/ml) than in RSB medium (0.09 and 0.00 U/ml) [Figure 5D]. By contrast, thermophilic

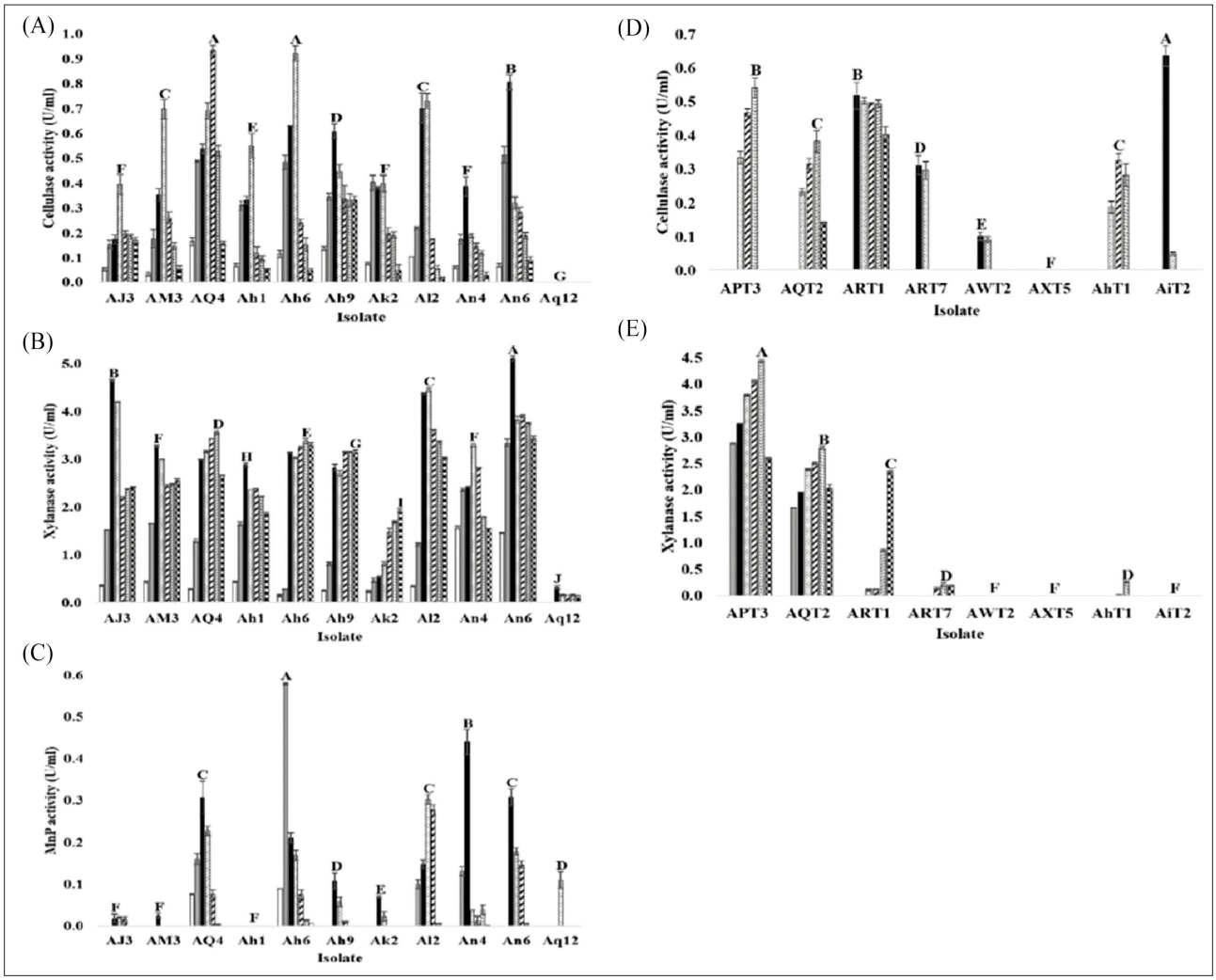

Figure 5: Activities of cellulase **(A-D)**, xylanase **(B-E)** and MnP **(C)** of mesophilic and thermophilic actinomycetes at day 1 (□), 2 (■), 3 (■), 4 (▨), 5 (▨), 6 (▨), and 7 (▨) using a rice straw for cultivation.

actinomycete isolates ART7, AhT1, and AiT2 had lower cellulase activities in CB medium (0.00–0.32 U/ml) than in RSB medium (0.31–0.64 U/ml). Most thermophilic actinomycetes (ART7, AWT2, and AhT1) had higher xylanase activities in XB medium (0.45–0.59 U/ml) than in RSB medium (0.00–0.25 U/ml) [Figure 5E]. No MnP activities were observed in thermophilic actinomycetes. The highest cellulase and xylanase activities were compared between thermophilic actinomycetes cultured in commercial and RSB media. Results showed that thermophilic actinomycetes had lower cellulase and xylanase activities in a commercial medium than in the RSB medium. Isolates APT3, AQT2, and ART1 had high cellulase and xylanase enzyme activities.

3.4.3. Secondary screening of lignocellulolytic fungi

The cellulase activities of mesophilic fungal isolates Fb2, Fh11, Fl7, Fn3, and FXIII1 in CB medium were 0.17–0.83 U/ml, and comparable to those observed in RSB medium (0.20–1.07 U/ml) [Figure 6A]. Their cellulase activities were higher than those produced from *Aspergillus terreus* (F14) (0.213 U/ml) and *Penicillium brevicompactum* (F16) (0.182 U/ml) using sugar bagasse and rice straw as substrates, respectively [48]. These results contrasted with Kumar et al. [53], who revealed that fungi showed lower cellulase activity using rice straw, rice husk, wheat straw, and sugarcane bagasse than when using carboxymethyl cellulose as the substrate for cultivation. Most isolated mesophilic fungi had lower xylanase activities in RSB medium (0.16–6.63 U/ml) [Figure 6B] than in XB (5.33–23.68 U/ml). Isolate Fl6 showed maximum xylanase activity of 9.94 U/ml, higher than produced by the fungal isolate F56 (4.16 U/ml) [50] but less than that produced by *Trichoderma reesei* Rut C-30 (92 U/ml) when using rice straw as the substrate. Most isolated mesophilic fungi showed higher MnP activities in LB medium (0.04–0.21 U/ml) than in RSB medium (0.00–0.14 U/ml) [Figure 6C] and higher than *Trametes villosa* (Sw.) Kreisel CCMB 651 (0.12 U/ml) when using sugar bagasse as the substrate for solid-state fermentation [54]. The mesophilic fungal isolate FXIII1 had the highest MnP activity. Five isolates, FW3, Fb5, Fg1, Fn3, and FXIII1, recorded three lignocellulolytic enzymes.

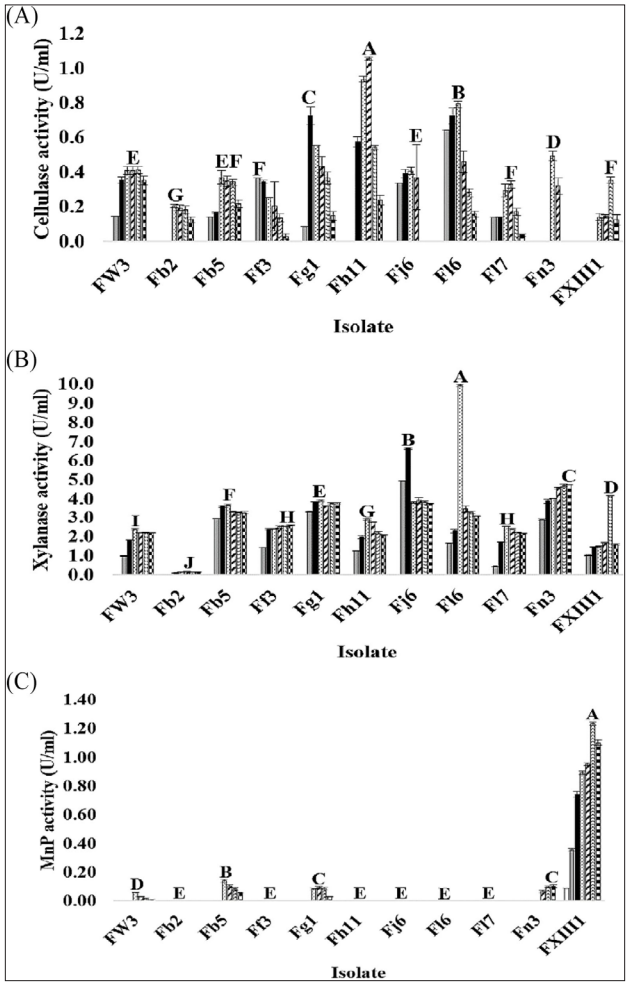

Figure 6: Activities of cellulase **(A)**, xylanase **(B)** and MnP **(C)** of mesophilic fungi at day 1 (□), 2 (■), 3 (■), 4 (▣), 5 (▨), 6 (▤), and 7 (▩) using a rice straw for cultivation.

The activities of lignocellulolytic enzymes from bacteria, actinomycetes, and fungi cultivated in RSB medium were compared. Results suggested that lignocellulolytic enzyme activities of mesophilic actinomycetes and mesophilic fungi were higher than those of mesophilic bacteria, while the xylanase activities of thermophilic actinomycetes were higher than those of thermophilic bacteria. Generally, bacteria and fungi are excellent producers of cellulase, especially fungi because they have extracellular properties [27]. Actinomycetes are better MnP producers than bacteria and fungi [55]. Mesophilic actinomycete isolates AQ4, Ah6, An6, and mesophilic fungal isolates Fh11 and Fl6 gave maximum cellulase activities in the top five. The two fungi isolates (Fl6 and Fj6) and actinomycete isolate An6 had high xylanase activity, while two actinomycete isolates (Ah6, An4) and fungi isolate FXIII1 showed high activity of MnP. The mesophilic actinomycetes An6 had the highest amount of lignocellulolytic enzymes. Among thermophilic microorganisms, bacterial isolates BKT1 and BOT4 and thermophilic actinomycete isolates APT3, ART1, and AiT2 had the highest cellulase activities. Thermophilic actinomycete isolates APT3, AQT2, and ART1 showed excellent xylanase activities.

3.5. Compatibility Between Microorganisms

3.5.1. Compatibility between bacterial isolates

Only the mesophilic bacterial isolate BB9 was compatible with mesophilic bacterial isolates BR3 and Bk1, while mesophilic bacterial isolates Ba6 and Bd7 were incompatible with mesophilic bacterial isolates BB9, Be8, and Bk1. The mesophilic bacterial isolate BR3 was compatible with all the isolates. Thermophilic bacterial isolates BKT1 and BOT4 were incompatible with thermophilic bacterial isolates BRT2 and BIXT2, whereas thermophilic bacterial isolates BRT2, Bft2, BiT3, and BIXT2 were compatible with the other two thermophilic bacterial isolates [Table 3].

3.5.2. Compatibility between actinomycete isolates

The mesophilic actinomycete isolate Aq12 was compatible with isolate An4. Mesophilic actinomycete isolates Ak2 and An6 were compatible with all mesophilic actinomycete isolates, except for Ah6 and Aq12. Furthermore, the mesophilic actinomycete isolates AQ4 and Ah1 were incompatible with mesophilic actinomycete isolates AM3, Ah6, Ah9, Aq12, and An4. The thermophilic actinomycete isolate AiT2 was compatible with all the isolates, except for APT3 and AQT2, while thermophilic actinomycete isolates AhT1 and AXT5 were compatible with all thermophilic actinomycete isolates, except for the thermophilic actinomycete isolate ART7 [Table 4].

3.5.3. Compatibility between fungal isolates

Only the mesophilic fungal isolate Ff3 was compatible with isolates Fb2, Ff3, and Fl7, while the mesophilic fungal isolate Fb2 was compatible with all mesophilic fungal isolates [Table 5]. The important

Table 3: Compatibility between mesophilic and thermophilic bacterial isolates

Isolate	BB9	BR3	Ba6	Bc5	Bd7	Be8	Bf7	Bf11	Bk1
BB9	■	■							■
BR3	■	■	■	■	■	■	■	■	■
Ba6		■	■	■	■		■	■	
Bc5		■	■	■	■	■	■	■	■
Bd7		■	■	■	■		■	■	
Be8		■		■		■	■		■
Bf7		■	■	■	■	■	■	■	■
Bf11		■	■	■	■		■	■	
Bk1	■	■		■		■	■		■

Isolate	BKT1	BOT4	BRT2	BfT2	BiT3	BnT10	BIXT2
BKT1	■	■		■	■	■	
BOT4	■	■		■	■	■	
BRT2			■			■	■
BfT2	■	■		■			
BiT3	■	■			■		
BnT10	■	■	■			■	■
BIXT2			■			■	■

Table 4: Compatibility between mesophilic and thermophilic actinomycete isolates.

Isolate	AJ3	AM3	AQ4	Ah1	Ah6	Ah9	Ak2	An6	Aq12	Al2	An4
AJ3	■		■	■			■	■			
AM3		■			■		■	■		■	
AQ4	■		■	■			■	■			
Ah1	■		■	■			■	■		■	
Ah6		■			■	■					
Ah9					■	■	■	■		■	■
Ak2	■	■	■	■		■	■	■		■	■
An6	■	■	■	■		■	■	■		■	■
Aq12									■		■
Al2		■	■	■		■	■	■		■	
An4						■	■	■	■		■

Isolate	APT3	AQT2	ART1	ART7	AhT1	AWT2	AXT5	AiT2
APT3	■	■	■	■	■	■	■	
AQT2	■	■	■	■	■	■	■	
ART1	■	■	■	■	■	■	■	■
ART7	■	■	■		■	■		■
AhT1	■	■	■	■	■	■	■	■
AWT2	■	■	■	■	■	■	■	■
AXT5	■	■	■	■	■	■	■	■
AiT2			■	■	■	■	■	■

criteria of a microbial consortium should be applied to rapid rice straw composting, based on the high potential of compatible lignocellulolytic properties. Two isolates of thermophilic bacteria (BKT1 and BOT4), two isolates of mesophilic actinomycetes (AQ4 and An6), three thermophilic actinomycetes (APT3, AQT2, and ART1), and four isolates of mesophilic fungi (Fh11, Fj6, Fl6, and FXIII1) were selected and further identified.

Table 5: Compatibility between mesophilic fungi.

Isolate	FW3	Fb2	Fb5	Ff3	Fg1	Fh11	Fj6	Fl6	Fl7	Fn3	FXIII1
FW3	■	■	■		■	■	■	■	■		
Fb2	■	■	■		■	■	■	■	■	■	■
Fb5	■	■	■		■		■	■	■		■
Ff3		■		■					■		
Fg1	■	■	■		■				■	■	■
Fh11	■	■				■	■		■	■	■
Fj6	■	■	■			■	■	■	■	■	■
Fl6	■	■	■			■	■	■	■		
Fl7	■	■	■				■	■	■	■	■
Fn3		■			■	■	■	■	■	■	■
FXIII1		■	■		■	■	■			■	■

Table 6: Identification of lignocellulolytic-degrading microorganisms

Isolate	Genus	Identities (%)	GenBank ID of Reference Strain	Accession Number
AQ4	*Streptomyces ardesiacus*	100.00	OK356613.1	PP053022
An6	*Streptomyces ardesiacus*	99.86	OK356613.1	PP053024
Fh11	*Penicillium* sp.	100.00	MW019429.1	PP053029
Fj6	*Aspergillus* sp.	100.00	MK450633.1	PP053030
Fl6	*Penicillium chrysogenum*	100.00	MT524448.1	PP053032
FXIII1	*Rhizopus delemar*	100.00	MT590597.1	PP077108
BKT1	*Bacillus licheniformis*	100.00	CP042252.1	PP053025
BOT4	*Bacillus licheniformis*	99.93	LC588561.1	PP053026
APT3	*Streptomyces thermoalcalitolerans*	99.79	NR_041408.1	PP053027
AQT2	*Streptomyces thermoviolaceus* subsp. *thermoviolaceus*	99.93	KC470043.1	PP053023
ART1	*Laceyella sacchari*	99.93	CP103866.1	PP053028

3.6. Identification of Lignocellulolytic Microorganisms

Thermophilic bacterial isolates BKT1 and BOT4 were identified as *Bacillus licheniformis* [Table 6], which expressed high cellulase activities. *Bacillus* is the main genus in agricultural waste composting because of its superior cellulase-secreting function, thermotolerant nature, and high adaptability to the environment. Most actinomycete isolates were identified in the genus *Streptomyces* including *Streptomyces ardesiacus*, *Streptomyces thermoalcalitolerans*, and *Streptomyces thermoviolaceus* subsp. *thermoviolaceus*, while isolate ART was identified as *Laceyella sacchari. Streptomyces thermoviolaceus* subsp. *thermoviolaceus* (AQT2) was an endophytic actinomycete isolated from compost. This result concurred with Bettache et al. [56], who reported that *Streptomyces thermoviolaceus* isolated from agricultural waste compost, chicken dung, and dust had high cellulase and lignin activities. The thermophilic actinomycete

isolate ART1 was isolated from compost and identified as *Laceyella sacchari* or *Thermoactinomyces sacchari*, concurring with Song et al. [57], who reported that *Laceyella sacchari* isolated from mushroom compost and animal manure caused bagassosis. The fungal isolate Fh11 was recorded in black soldier fly compost and identified as *Penicillium* sp. This result concurred with Hassine et al. [58], who isolated Fh11 from compost with high cellulase activity. The fungal isolate Fj6 was found in cow dung and identified as *Aspergillus* sp., concurring with Haas et al. [59], who isolated *Aspergillus* sp. from compost containing *A. fumigatus*, *A. niger*, and *A. neoellipticus* including *Acremonium*, *Alternaria*, *Aureobasidium*, *Cladosporium*, *Mortierella*, *Mucor*, *Paecilomyces*, *Penicillium*, *Scedoporium*, *Talaromyces*, and *Trichoderma*. The fungal isolate FXIII1 from rice straw compost was identified as *Rhizopus delemar.* This exhibited excellent MnP activity but has been identified as an invasive fungal pathogen that can cause fatal mucormycosis in immunodeficient individuals [60].

4. CONCLUSION

Major sources to isolate lignocellulolytic enzyme-producing microorganisms were compost, black soldier fly compost, rice straw compost, and cow manure. Potential microbial consortiums for the rapid composting of rice straw comprised *Bacillus licheniformis*, *Streptomyces* sp., *Aspergillus* sp., and *Penicillium* sp.

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6. AUTHORS' CONTRIBUTIONS

All authors contributed to the design of the research experiments. SS has been actively involved in the finalization and revision of the manuscript. Analysis and interpretation of the data are carried out by SS, PS and PL.

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8. CONFLICT OF INTEREST

The authors report no financial or other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All data underlying the results are available as part of the article and no additional source data are required.

11. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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