Multifunctional microbial strains for spent mushroom substrate treatment: from Cellulase production to plant growth promotion

Ngoc An Nguyen, Tan Viet Pham, Dung Vo Anh Truong, Hanh Thi-Dieu Nguyen*
Institute of Biotechnology and Food technology, Industrial University of Ho Chi Minh City, 12 Nguyen Van Bao Street, Go Vap District, Ho Chi Minh City, VIETNAM
*nguyenthidieuhanh@iuh.edu.vn

Abstract
This study investigated the potential of cellulose-degrading microorganisms for treating agricultural by-products, specifically spent mushroom substrate (SMS). From a pile of SMS, 48 thermotolerant microbes were isolated, comprising of 24 bacterial strains, 20 actinomycete strains and 4 mold strains. Following cellulase production and co-culture compatibility screening, seven strains were selected for further analysis. Phylogenetic analysis based on 16S rRNA and ITS sequences identified three strains (DVK16, DVK27, DVK33) as Bacillus spp., two (DXK61, DXK98) as Streptomyces spp. and two (DM1, DM2) as Rasamsonia spp.

Notably, the two strains Streptomyces sp. DXK98 and Rasamsonia sp. DM1 exhibited strong indole-3-acetic acid biosynthesis, while Bacillus sp. DVK33 demonstrated phosphorus solubilizing ability, suggesting potential plant growth-promoting properties. Treatment of SMS with 10% microbial inoculant resulted in a decreased C:N ratio and increased total nitrogen, potassium and phosphorus content. Furthermore, plants grown on treated SMS exhibited improved height and root length. These findings suggest that applying these microbes in SMS treatment not only aids in environmental protection but also offers economic benefits through the reuse of SMS in crop production.

Keywords: Spent mushroom substrate, cellulase, microbial consortium, plant growth-promoting, *Bacillus*, *Streptomyces*, *Rasamsonia*.

Introduction

Population growth and the reduction of arable land pose significant challenges to global food security. Concurrently, the overproduction of agricultural products intensifies the issue of agricultural waste which has become a pressing global concern. In 2020, the global agricultural sector consumed 201.7 million tons of fertilizers containing nitrogen, phosphorus and potassium. While chemical fertilizers enhance crop growth, they have adverse effects on both the environment and human health[10]. In 2018, approximately 64 million tons of spent mushroom substrate (SMS) were discarded worldwide, with projections suggesting that this figure will exceed 100 million tons by 2026[5]. Each ton of SMS contains 0.8 kg of nitrogen, 3.9 kg of phosphorus, 7.9 kg of potassium and other minerals.

Despite its high nutrient content, the direct use of SMS is limited due to its high salinity and electrical conductivity which can negatively impact crop quality. Fortunately, composting can improve the organic matter content and can enhance the utilization efficiency of SMS[17]. Consequently, reusing SMS as a fertilizer or growing substrate for plants could conserve non-renewable resources to reduce waste[24]. Thermotolerant microorganisms play a crucial role in the composting process. As the pile temperature increases over several days, it can reach up to 80°C at the center. These high temperatures may inhibit the growth of many microorganisms, thereby slowing the decomposition of organic matter. Therefore, thermotolerant microbial strains isolated from compost piles represent potential candidates for the production of microbial inoculants, which can effectively treat organic waste and create nutrient-rich substrates for planting[11].

Different groups of microorganisms contribute to various stages of the composting process. During the thermogenic phase, bacteria dominate, particularly genera such as *Bacillus*, *Thermus* and *Actinomycetes*, which produce hydrolytic enzymes like cellulases, hemicellulases and lignin-modifying enzymes to break down cellulose and lignin[11,32]. Additionally, these bacteria can generate valuable compounds that can inhibit pathogenic microorganisms and can promote plant growth by several mechanisms such as nitrogen fixation, indole-3-acetic acid (IAA) production and phosphate solubilization[23]. Molds, which decompose cellulose and lignin more effectively than bacteria, typically thrive at temperatures between 25-30°C and are thus more active during the cooling and maturation stages of the compost pile. Identifying thermotolerant molds capable of functioning during the heat-generating phase of the composting process thus provides an advantage for the effective decomposition of organic matter[3].

Studies have demonstrated the effectiveness and feasibility of incorporating microbes into the treatment of agricultural by-products for use as fertilizers or planting substrates. The treated compost meets high standards for total organic carbon and C:N ratio and it can stimulate seed germination and root length. As such, the treated compost is considered a potential candidate for replacing common soil[3,19]. In this study, thermotolerant microbial strains were isolated from

compost piles and were applied in the short-term treatment of SMS to create a planting substrate. This process offers an environmentally friendly and cost-effective method for converting SMS into a useful resource.

Material and Methods

Materials: Samples of spent mushroom substrate (SMS) and sawdust waste were collected from compost piles at Nam Viet Mushroom Farm, Tay Ninh Province, Vietnam. The samples were taken from the top, middle (~50 cm depth) and bottom layers of the compost piles. The samples were stored in sterilized bags and were immediately transported to the laboratory for further analysis.

Isolation of thermotolerant microorganisms: To isolate thermotolerant microorganisms, 100 g of each sample was mixed with 200 ml of sterile distilled water and incubated at 60°C. After 12 hours, the sample was diluted with 0.9% NaCl solution and spread on Luria-Bertani (LB) Agar, Gause I Agar and Potato Glucose Agar (PGA) to isolate bacteria, actinomycetes and molds respectively. After 24 to 96 hours of incubation at 50°C, microbial strains exhibiting distinct macroscopic and microscopic morphologies were purified on the corresponding nutrient media. The isolates were stored in 25% glycerol at -70°C for further experiments[37].

Selection of thermotolerant microorganisms producing extracellular cellulose: Microbial strains were grown on media supplemented with 1.0% carboxymethyl cellulose (CMC) to evaluate their ability to produce extracellular cellulase. After 48 to 96 hours of incubation at 50°C, the degradation zone was identified using 0.1% Congo Red reagent[18]. The ability to biosynthesize cellulase was assessed based on the size of the halo zone A = D - d, where D is the diameter of the halo zone in millimeters and d is the diameter of the colony in millimeters.

Determination of microbial strain compatibility: The compatibility of microbial strains was evaluated by inoculating microbial streaks perpendicularly on the corresponding nutrient media. After 24 to 96 hours of incubation at 50ºC, microbial strains that did not exhibit antagonistic interactions, were selected for the creation of microbial inoculants to treat SMS and sawdust waste[34].

Identification of candidate strains: For bacterial isolates, the 16S rRNA gene region was amplified and sequenced using primers 27F: 5'-AGAGTTTGATCMTGCTCAG-3' and 1540R: 5'-AAGGAGGTGATCCAACCGCA-3'. For fungal isolates, the ITS region was amplified and sequenced using primers V9D: 5'-TTAAGTCCCTGCCCTTTGTA-3' and LS266: 5'-GCATTCCCAAACAACTCGACTC-3'. PCR products were sequenced by Nam Khoa Biotek (Vietnam). Homologous gene sequences were retrieved from NCBI GenBank and aligned using Clustal X2.1. A phylogenetic tree was constructed by Mega 11 software using the Neighbor-Joining algorithm with 1000 bootstrap repetitions[7,37].

Effect of inducers on cellulase biosynthesis: Five raw materials used as inducers, including rice straw, rice husk, coconut fiber, peanut shell and rice bran, were ground and added to spent mushroom sawdust waste at a ratio of 5.0% (w/w) to create induced media. A control without inducers was also prepared. After bacterial, actinomycetes and mold strains were grown on LB broth, Gause I broth and Potato Glucose broth (PGB) respectively 10% of the microbial suspension was transferred to the prepared induced media and incubated at 50°C. The crude cellulase was collected every 24 hours by dissolving the induced culture in sterile distilled water and centrifuging to obtain the supernatant. Cellulase activity was determined using the Bernfeld method and one unit of activity (U) was defined as the amount of enzyme required to release 1.0 µmol of glucose per minute at 50°C[15,29].

Biosynthesis of IAA and insoluble phosphate solubilization of candidate strains: To evaluate the ability to synthesize indole acetic acid (IAA), candidate bacterial strains, actinomycetes and molds were respectively cultured on Mueller-Hinton Broth (MHB), Gause I broth and Potato Glucose broth supplemented with 0.1% L-tryptophan with shaking at 150 rpm. After 72 hours of incubation, culture supernatants were collected by centrifugation at 10,000 rpm and were reacted with Salkowski reagent in a ratio of 1:4. Microorganism strains capable of synthesizing IAA were identified based on a color change to red upon reaction with Salkowski reagent[2,9].

Pikovskaya's agar, containing insoluble $Ca_3(PO_4)_2$, was used to assess the insoluble phosphate solubilization capability of candidate microbial strains. After 72 hours of incubation at 37°C, strains capable of solubilizing phosphate were identified based on the presence of a halo zone around the colony[2,9].

Treatment of spent mushroom sawdust waste: Bacterial strains, actinomycetes and molds were cultured in LB broth, Gause I broth and PG broth for 12, 72 and 96 hours at 50°C respectively. 10% of the microbial suspension was transferred to culture media with or without 5% of the best inducer and incubated at 50°C. After appropriate incubation periods, microbial inoculants were prepared by mixing the microbial strains in equal proportions (w/w) and were subsequently used to treat SMS. SMS was sterilized at 121°C, 1 atm for 3 hours, cooled and mixed with the microbial inoculant at a ratio of 10:1 (w/w). The composting process occurred at room temperature. Untreated SMS served as the negative control while commercial growing soil served as the positive control. Indicators such as C/N ratio, total nitrogen content, total potassium content and total phosphorus content were analyzed after 20 days of composting by Hai Dang Chromatography Center (Vietnam).

Planting test on treated spent mushroom substrate: Mung bean seeds were soaked in warm water (~40°C) for 2 hours and then sown into prepared pots containing treated SMS, untreated SMS (negative control) and commercial growing soil (positive control). Germination rate, plant height and root length were evaluated after 3 days for germination and 10 days for plant height and root length[16].

Data analysis: All experiments were conducted in triplicate. Data were visualized using Microsoft Excel 2013 and ANOVA tests ($\alpha = 0.05$) were performed using Statgraphics Centurion 18 software (Statgraphics Technologies).

Results and Discussion

Isolation of thermotolerant microorganisms: From spent mushroom substrate sawdust piles, 48 microbial strains were isolated based on colony morphology, consisting of 24 bacterial strains, 20 actinomycetes strains and 4 mold strains, named DVK1-24, DXK1-20 and DM1-4 respectively. After 48 hours of culture on LB agar, bacterial colonies varied in size from 2.0 to 5.0 mm. Notably, strain DVK16 formed round colonies with serrated edges, DVK27 had regular edges and a concave center and DVK33 displayed a wrinkled membrane with a deeply concave center (Figure 1A). Actinomycete strains typically exhibited a dry, hard, radiating colony surface with an earthy smell. For example, strain DXK61 had regular edges with a concave center and light brown pigment secretion, DXK63 was pinkish white without pigment secretion and DXK98 had serrated edges without pigment secretion when grown on Gause I agar after 72 hours (Figure 1B).

After 72 hours of culture on PGA, mold strains DM1 and DM2 had concentric circles with pale orange and white colors respectively, while DM3 and DM4 showed a cottony appearance with strongly developed aerial mycelia in dark green and white (Figure 1C). Gram staining identified all 24 bacterial strains as Gram-positive, endospore-forming bacilli. Microscopic examination revealed that actinomycete strains DXK61 and DXK98 had spring-like helical aerial hyphae while mold strains DM1 and DM2 exhibited branched mycelium with septa and conidiophores (Figure 2).

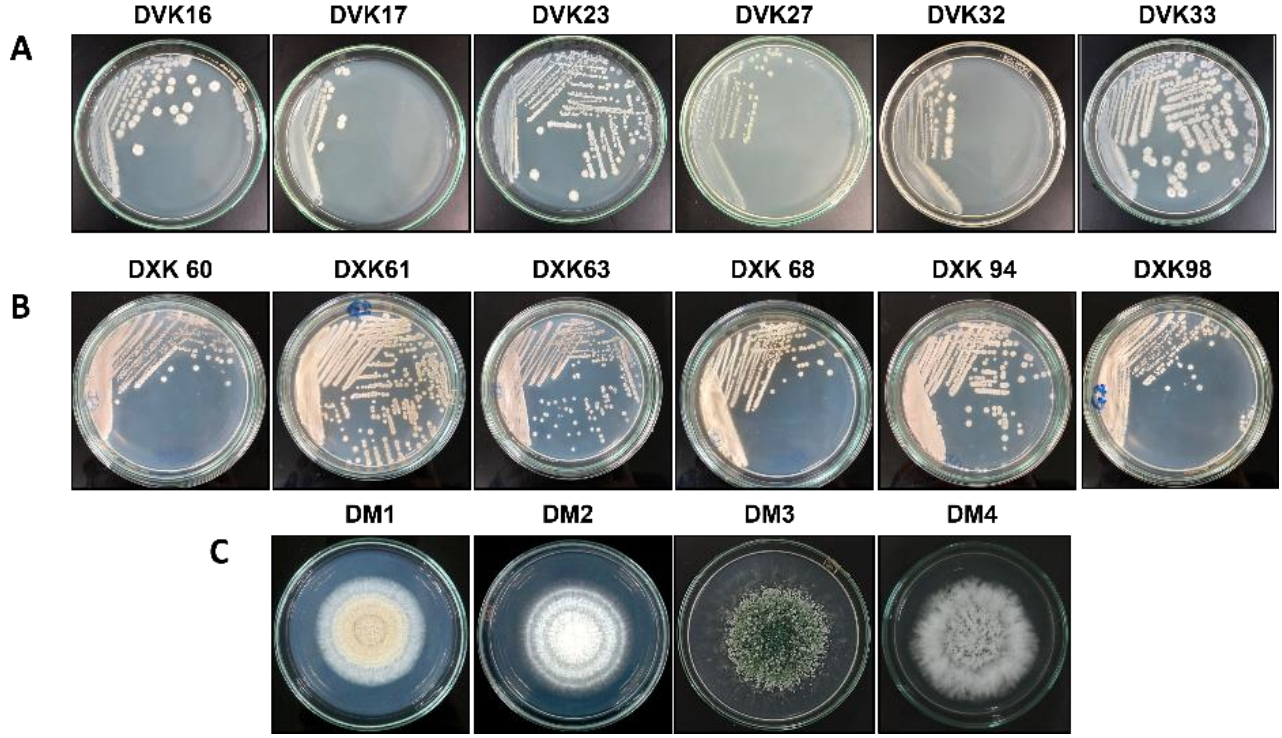

Figure 1: Colony morphology of some presentative isolates. (A) Bacterial strains, (B) Actinomycetes strains and (C) Mold strains

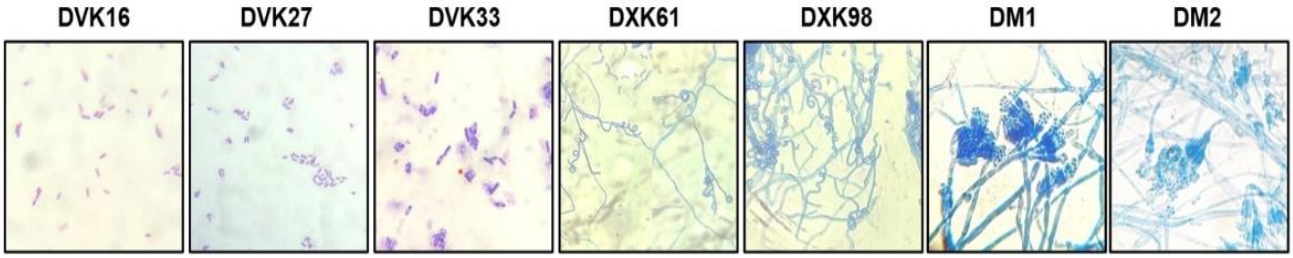

Figure 2: Microscopic morphology of some presentative isolates

Selection of thermotolerant strains producing cellulose: The cellulase production capacity of isolated strains was evaluated by inoculating them on nutrient medium supplemented with CMC, followed by detection using Congo Red 0.1% reagent. The results, illustrated in figure 3, indicated that 9/24 bacterial strains, 6/20 actinomycete strains and 2/4 mold strains were capable of extracellular cellulase production. Six strains displayed degradation zones greater than 20 mm (DVK33, DVK16, DVK27, DXK98, DXK63, DXK63), while seven strains had zones between 10-20 mm. Although mold strains DM1 and DM2 exhibited slower CMC degradation, they showed rapid mycelial growth and respective cellulase production. Comparatively, previous studies reported lower degradation zones such as 4-13 mm from vermicompost isolates[32] and 5-14.5 mm from chicken manure compost isolates[26]. Therefore, strains DVK33, DVK16, DVK27, DXK98, DXK63, DXK61, DM1 and DM2 were selected for further experiments.

The compatibility of microbial strains: To evaluate the potential application of the selected eight microbial strains, strain compatibility tests were conducted (Figure 4). Except for strain DXK63, which inhibited the growth of DM1 and DM2 mycelia (Figure 4D), no significant mutual inhibition was observed. Consequently, 3 bacterial strains (DVK16, DVK27, DVK33), 2 actinomycete strains (DXK61, DXK98) and 2 mold strains (DM1, DM2) were chosen for further experiments on SMS treatment.

Compatibility testing is essential before producing microbial inoculants. For example, Singh et al[32] demonstrated that *Trichoderma harzianum* was not antagonistic towards rhizosphere bacteria *Stenotrophomonas* spp., *Bacillus flexus and Brevibacterium halotolerans*. Similarly, Raja et al[26] showed that *Azospirillum, Phosphobacteria, Azotobacter and Pseudomonas* could coexist on the same medium. However, *Pseudomonas fluorescens* was reported by Haritha et al[13] to inhibit the mycelial growth of *Trichoderma viride*, preventing their coexistence. Compatible strains can be co-cultured for research into developing effective products that enhance plant growth, nutrient absorption and drought tolerance[26].

Identification of selected microbial strains: The 16S-rRNA gene amplification and sequencing (~1400 base pairs) were performed, followed by sequence alignment against *Bacillus* and *Streptomyces* species in GenBank for selected bacteria and actinomycetes. BLAST analysis and phylogenetic tree construction showed that DVK16, DVK27 and DVK33 had 98% similarity with *Bacillus subtilis* DSM 10 and NBRC 13719, while DXK61 and DXK98 showed 100% similarity with *Streptomyces enissocaesilis* KCa1 and *Streptomyces thermocarboxydus* AS13Y (Figure 5A and B). Hence, the bacterial strains were identified as *Bacillus* sp. DVK16, *Bacillus* sp. DVK27, *Bacillus* sp. DVK33, *Streptomyces* sp. DXK61 and *Streptomyces* sp. DXK98.

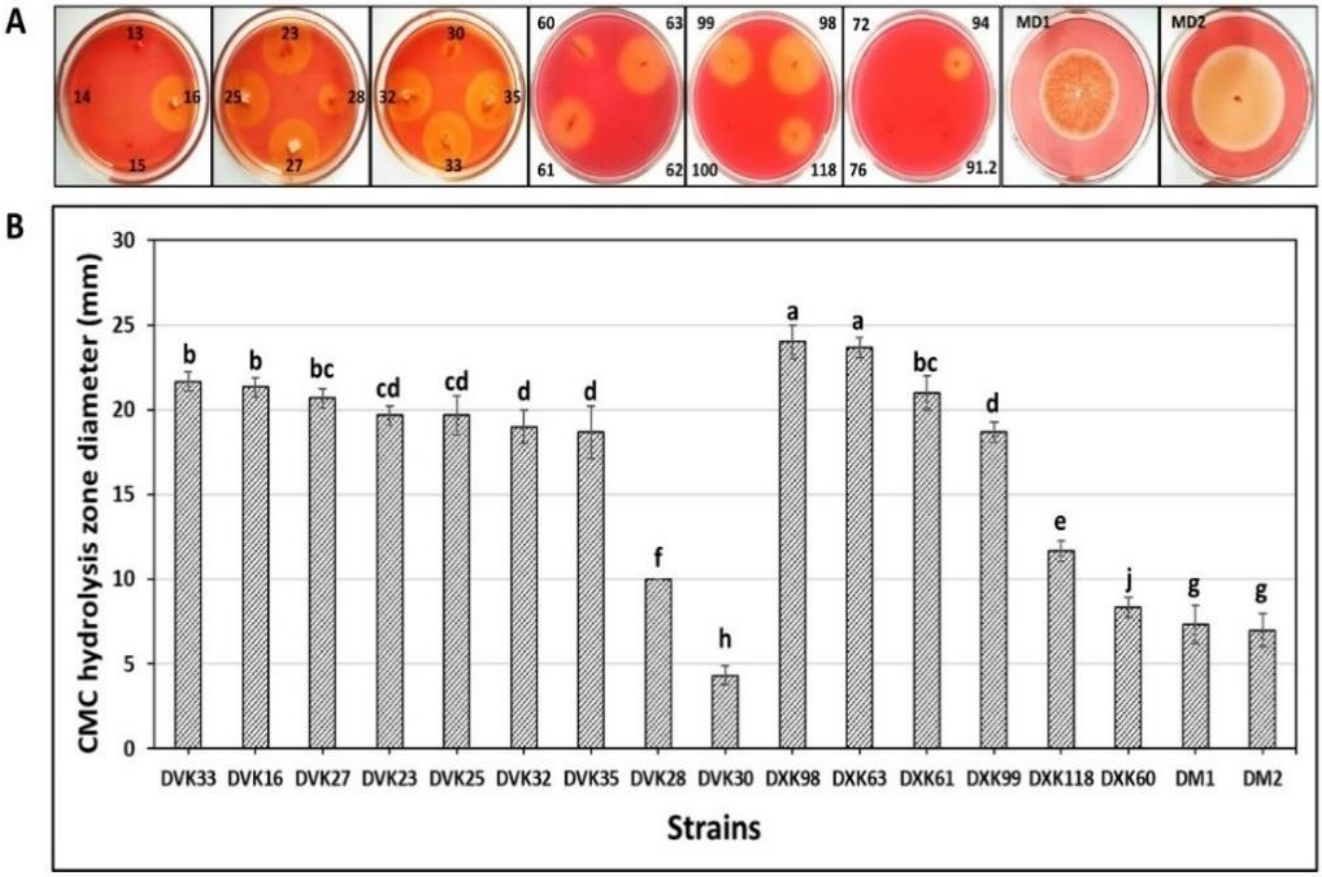

Figure 3: CMC degradation of microbial isolates. (A) Colonies with degradation zone and (B) Size of degradation zone. Different letters indicate significant differences ($p < 0.05$)

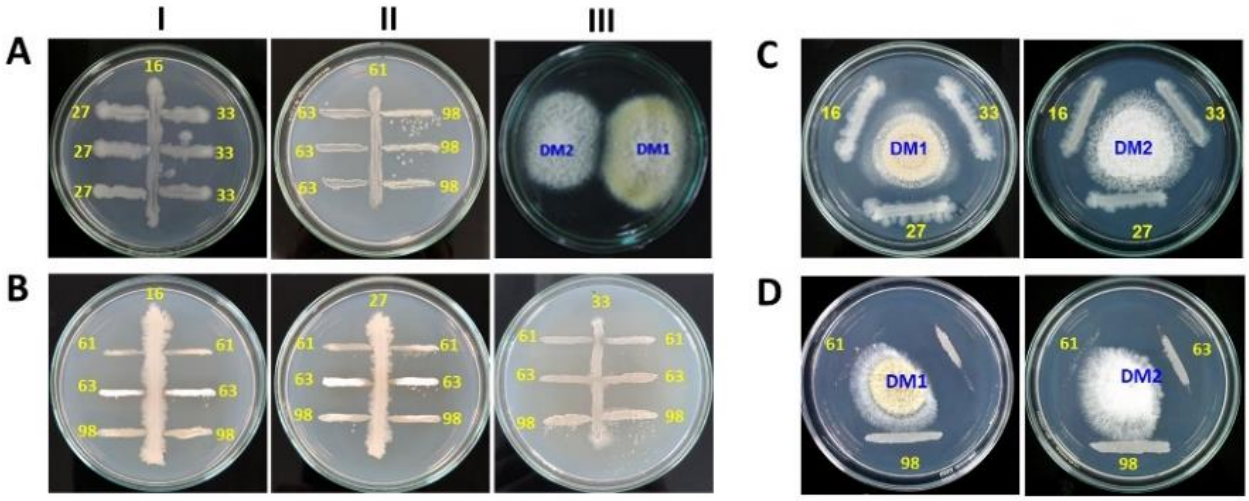

Figure 4: Strain compatibility of microbial isolates. (AI) Co-culture of bacteria; (A-II) Co-culture of actinomycetes; (A-III) Co-culture of molds; (B) Co-culture of bacteria and actinomycetes; (C) Co-culture of bacteria and mold; (D) Co-culture of actinomycetes and mold

For mold strains DM1 and DM2, PCR amplification of the ITS region (~900 base pairs) followed by sequencing and comparison with GenBank sequences identified these strains as belonging to the genus *Rasamsonia*, with 100% homology to *Rasamsonia emersonii* species. The phylogenetic tree analysis using the Neighbor-joining method further confirmed that DM1 and DM2 are closely related to *Rasamsonia emersonii* CBS 396.64, with 100% similarity (Figure 5C). Hence, the mold strains were named *Rasamsonia* sp. DM1 and *Rasamsonia* sp. DM2.

Previous studies have shown that *Bacillus* species are thermotolerant and are capable of decomposing lignocellulose, cellulose and hemicellulose, with potential applications in producing rice straw fertilizer[8] and palm waste compost[27]. Similarly, Ventorino et al[35] demonstrated that 24 *Streptomyces* strains isolated from naturally decomposing plant biomass possessed enzymatic activities specific to the degradation of lignocellulosic materials. Among these isolates, *Streptomyces argenteolus* strain AE58P exhibited not only the highest cellulase activity but also maintained this activity at elevated temperatures of 45-55°C[35]. In addition, species of the genus *Rasamsonia* are generally characterized as thermotolerant molds. Notably, *Rasamsonia emersonii* exhibits optimal growth at temperatures between 45-55°C and is recognized for its cellulase, amylase and lipase production capabilities[25,30,36].

Effect of inducers on cellulase biosynthesis: The impact of various inducers including rice husk, straw, coconut fiber, rice bran and peanut shell, on cellulase biosynthesis by selected microbial strains was shown in figure 6. Strains *Bacillus* sp. DVK16, *Streptomyces* sp. DXK98, *Rasamsonia* sp. DM1 and *Rasamsonia* sp. DM2 produced the highest cellulase activity in control medium without inducers after 3 days, with values of 312±15 U/g, 289±29 U/g, 262±13 U/g and 210±5 U/g respectively (Figure 6A, E, F, G). *Bacillus* sp. DVK27 and *Bacillus* sp. DVK33 showed the highest cellulase activity on medium supplemented with coconut fiber after 5 days, recording 245±7 U/g and 460±13 U/g respectively (Figure 6B, C). *Streptomyces* sp. DXK61 synthesized cellulase with high activity on medium supplemented with straw, with a value of 246±55 U/g (Figure 6D).

Different substrates impact cellulase biosynthesis due to variations in polysaccharide composition (cellulose, hemicellulose, lignocellulose) and other plant cell wall components (lignin, fats, waxes, tannins, resins)[28]. For example, several substrate inducers including millet husks, rice husks, maize cobs, guinea corn straws, rice straws and sawdust were used to stimulate cellulase biosynthesis from *Aspergillus niger*[1,21,28]. In addition, a 1:1 ratio of wheat bran and rice straw was employed to optimize cellulase production in the case of *Bacillus subtilis* subsp. *subtilis* JJBS300[4]. Statistical analysis of the obtained results indicated that the control medium was optimal for cellulase biosynthesis in *Bacillus* sp. DVK16, *Streptomyces* sp. DXK98 and *Rasamsonia* sp. strains DM1 and DM2.

Conversely, a medium supplemented with 5% coconut fiber proved suitable for *Bacillus* sp. strains DVK27 and DVK33. For *Streptomyces* sp. DXK61, a medium containing 5% straw was determined to be most conducive to cellulase production. These findings highlight the strain-specific nature of optimal growth conditions for cellulase biosynthesis among the tested microorganisms.

Biosynthesis of IAA and insoluble phosphate solubilization: Seven selected microbial strains were tested for their ability to biosynthesize indole-3-acetic acid (IAA) and solubilize insoluble phosphate, as shown in figures 7 and 8. In culture media supplemented with L-tryptophan, *Streptomyces* sp. DXK98 and *Rasamsonia* sp. DM1 demonstrated IAA biosynthesis, indicated by a color change in the Salkowski reagent from light yellow to pink and dark

red (Figure 7). While the ability of *Streptomyces* strains to produce IAA has been widely reported in previous studies[6,22], the capacity of *Rasamsonia* to produce IAA has not been previously documented.

In the presence of insoluble phosphate $Ca_3(PO_4)_2$ in Pikovskaya's medium, *Bacillus* sp. DVK33 exhibited the ability to solubilize insoluble phosphate, as evidenced by the clear zone surrounding the colony (Figure 8). This finding aligns with the study by Joshi et al[14] who identified *Bacillus* as a genus capable of solubilizing insoluble phosphate and promoting plant growth[14]. With these characteristics, *Bacillus* sp. DVK33, *Streptomyces* sp. DXK98 and *Rasamsonia* sp. DM1 are likely to play important roles in promoting plant growth, in addition to their potential for effectively treating spent mushroom substrate for use as planting material.

Treating spent mushroom sawdust waste: The results, presented in table 1, show that the C:N ratio of treated spent mushroom substrate (SMS) decreased from 33.4 to 29 compared to untreated SMS, indicating microbial consumption of the carbon fraction. Notably, commercial growing soil exhibited a higher C:N ratio of 49.5, reflecting its low nitrogen content (1.93 g/kg) compared to untreated SMS (7.40 g/kg) and treated SMS (8.75 g/kg). The total nutrient content (sum of N, P_2O_5 and K_2O) in treated SMS increased by 2.64 g/kg compared to untreated SMS and exceeded that of commercial growing soil by 6.14 g/kg. These results align with previous studies and highlight the efficacy of microbial treatment in enhancing the nutritional profile of SMS. Indeed, Saffari et al[27] reported that cellulase-producing microorganisms accelerated organic matter conversion into mature compost by reducing the C:N ratio during palm waste composting.

Similarly, Sun et al[34] observed a decrease in C:N ratio, total organic carbon (TOC) and organic matter content, accompanied by an increase in total nitrogen content (0.34%) after 60 days of post-mushroom sawdust treatment. Mung bean germination rates in untreated control, commercial soil and microbially treated SMS ranged from 88.89±1.57% to 91.11±1.57%, with no statistically significant differences observed between treatments (ANOVA, n=3, p>0.05) (Table 1). However, plants grown in treated SMS exhibited significantly greater average root length (29.63±3.23 cm) and height (11.32±0.46 cm) compared to other treatments (Table 1 and figure 9). This enhanced plant growth may be attributed to the higher total nutrient content in treated SMS and the IAA production capability of the microbial strains present (Figure 7).

These findings suggest that the microbial inoculant not only facilitates the decomposition of organic matter from agricultural waste but also promotes plant growth through the secretion of phytohormones. Similar plant growth-promoting effects of microbial inoculants have been reported in previous studies. For instance, Meng et al[20] observed that compost derived from biogas residue and spent mushroom sawdust waste improved tomato and pepper seedling quality without affecting germination rates. Singh et al[32] reported that co-culture of *Trichoderma harzianum* with plant growth-promoting rhizobacteria enhanced plant weight and oil content of *Mentha arvensis* on soil and vermicompost substrates, although no significant increase in plant height was observed.

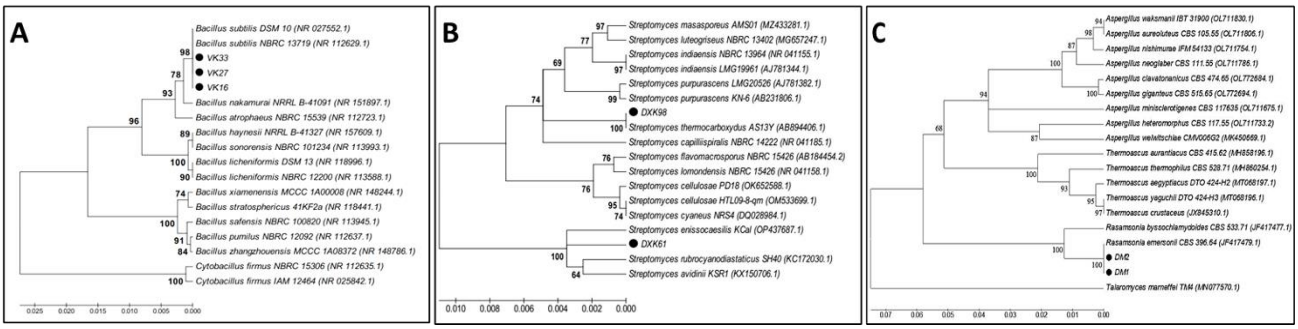

Figure 5: Neighbor joining phylogenetic tree of selected strains. (A) Bacteria; (B) Actinomycetes; (C) Molds

Table 1
Chemical properties and plant growth parameters of untreated and treated spent mushroom substrate (SMS) compared to commercial growing soil

	Untreated SMS	Positive control	Treated SMS
C:N ratio	33.4	49.5	29
Total Nitrogen (g/kg)	7.40	1.93	8.75
K_2O (g/kg)	6.62	6.5	7.48
P_2O_5 (g/kg)	2.44	1.89	2.87
Germination (%)	88.89±3.14^a	91.11±1.57^a	88.89±1.57^a
Average root length (cm)	20.75±1.92^a	27.88±2.07^b	29.63±3.23^b
Average height (cm)	4.94±0.4^a	7.72±0.46^b	11.32±0.46^c

*Different letters in the same row indicate significant differences ($p < 0.05$)

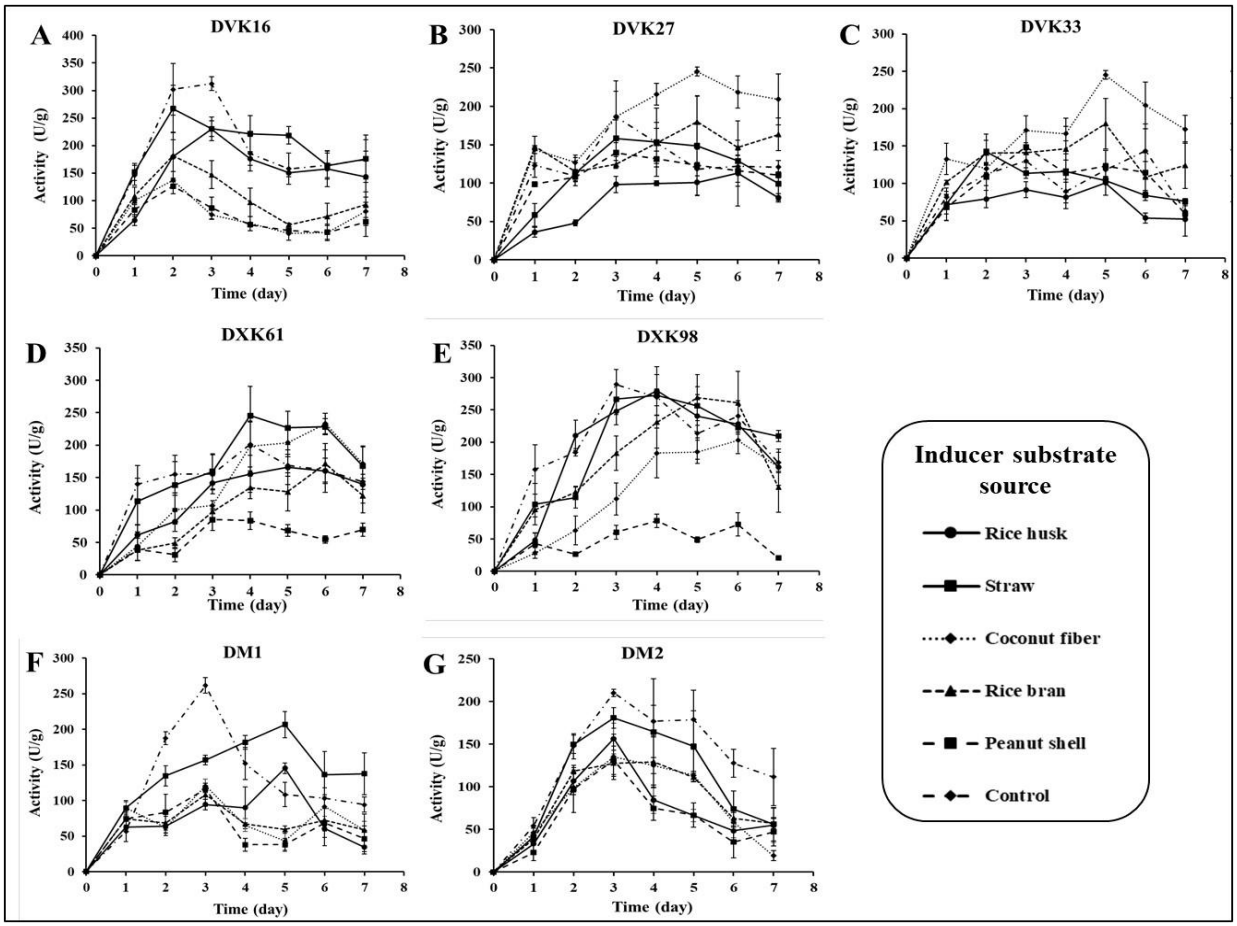

Figure 6: Effect of inducers on extracellular cellulase production of selected strains

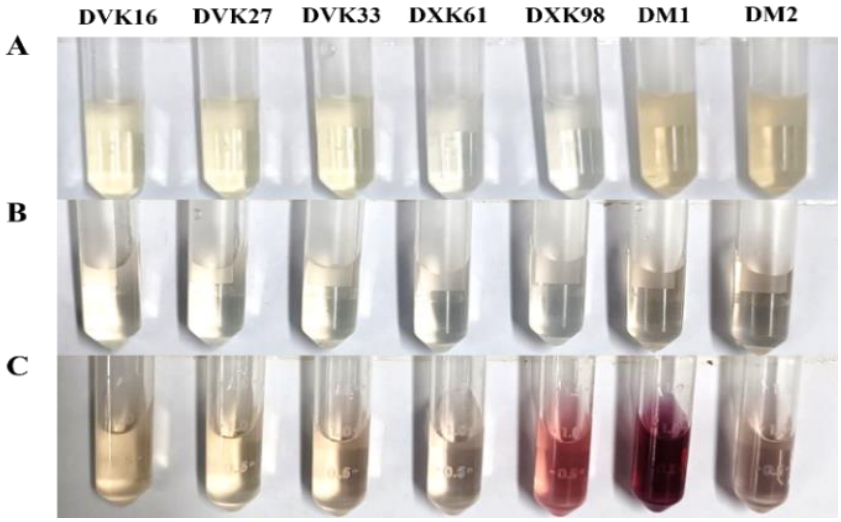

Figure 7: IAA biosynthesis of microbial strains after 72 hours of culture. (A) Microbial culture suspension, (B) Pure media + Salkowski reagent, (C) Microbial culture suspension + Salkowski reagent

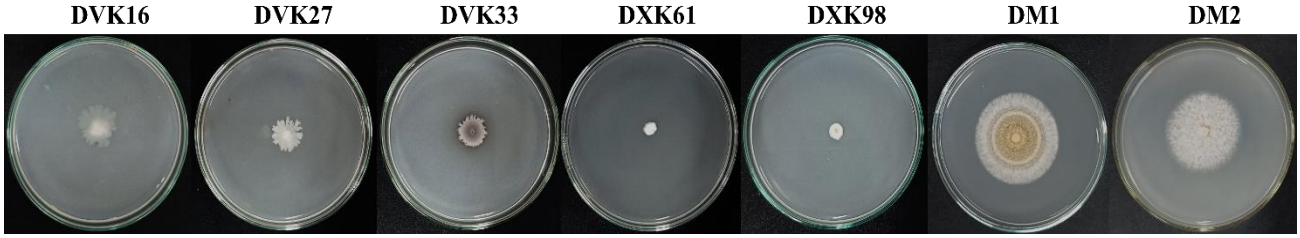

Figure 8: Insoluble phosphate solubilization of microbial strains

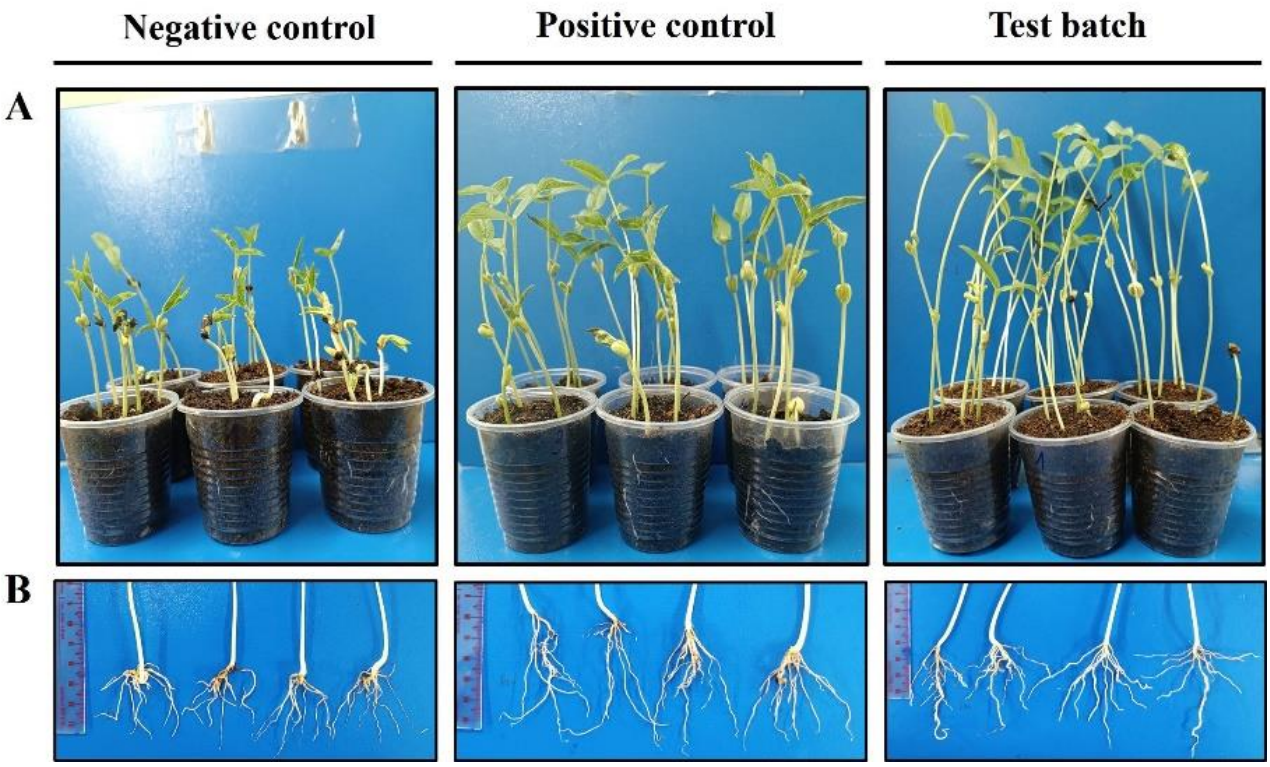

Figure 9: Comparative growth of mung beans in different substrates.
(A) Whole plant development in pots, (B) Root morphology across treatments

Additionally, a microbiological inoculant containing *Azospirillum lipoferum, Bacillus megaterium, Bacillus sporothermodurans, Trichoderma viride and Pseudomonas fluorescens* promoted height growth and tiller number in ginger when applied to vermicompost[13]. These studies collectively demonstrate that microbial preparations not only enhance biodegradation efficiency and promote maturation and humus formation during composting but also stimulate plant growth[12].

Conclusion

The study successfully isolated and characterized thermotolerant microorganisms from spent mushroom substrate (SMS) with potential for agricultural waste management and plant growth promotion. Seven strains were selected based on their cellulase production and compatibility: three *Bacillus* sp., two *Streptomyces* sp. and two *Rasamsonia* sp. The selected strains also exhibited plant growth-promoting characteristics. Some were capable of producing indole-3-acetic acid (IAA) and solubilizing insoluble phosphate. When applied to treat SMS, the microbial consortium improved its nutritional profile, reducing the C:N ratio compared to untreated SMS and commercial growing soil.

In addition, mung bean growth experiments demonstrated better efficacy in root length and plant height of the treated SMS as a growth substrate compared to untreated SMS and commercial growing soil. These findings suggest that the application of the selected microbial strains in treating SMS offers an environmentally friendly and cost-effective solution for SMS utilization. This process not only addresses waste disposal issues but also produces a valuable plant growth substrate with enhanced nutritional properties and growth-promoting effects.

References

1. Acharya P.B., Acharya D.K. and Modi H.A., Optimization for cellulase production by *Aspergillus niger* using saw dust as substrate, *Afr. J. Biotechnol.*, **7(22)**, 4147-4152 **(2008)**

2. Adeleke R. and Dames J. F., Kalaharituber pfeilii and associated bacterial interactions, *S. Afr. J. Bot.*, **90**, 68-73 **(2014)**

3. Aguilar-Paredes A., Valdés G., Araneda N., Valdebenito E., Hansen F. and Nuti M., Microbial Community in the Composting Process and Its Positive Impact on the Soil Biota in Sustainable Agriculture, *Agronomy*, **13(2)**, 542 **(2023)**

4. Anu Kumar S., Kumar A., Kumar V. and Singh B., Optimization of cellulase production by *Bacillus subtilis* subsp. *subtilis* JJBS300 and biocatalytic potential in saccharification of alkaline-pretreated rice straw, *Prep. Biochem. Biotechnol.*, **51(7)**, 697-704 **(2021)**

5. Atallah E., Zeaiter J., Ahmad M.N., Leahy J.J. and Kwapinski W., Hydrothermal carbonization of spent mushroom compost waste compared against torrefaction and pyrolysis, *Fuel Process. Technol*., **216**, 106795 **(2021)**

6. Boondaeng A., Vaithanomsat P., Apiwatanapiwat W., Trakunjae C., Janchai P., Suriyachai N., Kreetacgat T., Wongcharee S. and Imman S., Bological conversion of agricultural wastes into indole-3-acetic acid by *Streptomyces lavenduligriseus* BS50-1 using a response surface methodology (RSM), *ACS Omega*, **8(43)**, 40433-40441 **(2023)**

7. Cheng Y., Liu G., Li Z., Zhou Y. and Gao N., Screening saikosaponin d (SSd)-producing endophytic fungi from *Bupleurum scorzonerifolium* Willd, *World J. Microbiol. Biotechnol.*, **38(12)**, 242 **(2022)**

8. Chukwuma O.B., Rafatullah M., Tajarudin H.A. and Ismail N., A review on bacterial contribution to lignocellulose breakdown into useful bio-products, *Int. J. Environ. Res. Public Health*, **18(11)**, 6001 **(2021)**

9. Du Y., Wang T., Jiang J., Wang Y., Lv C., Sun K., Sun J., Yan B., Kang C., Gou L. and Huang L., Biological control and plant growth promotion properties of *Streptomyces albidoflavus* St-220 isolated from *Salvia miltiorrhiza* rhizosphere, *Front. Plant Sci.*, **13**, 976813 **(2022)**

10. FAO, World fertilizer trends and outlook to 2022 **(2019)**

11. Finore I., Feola A., Russo L., Cattaneo A., Di Donato P., Nicolaus B., Poli A. and Romano I., Thermophilic bacteria and their thermozymes in composting processes: A review, *Chem. Biol. Technol. Agric.*, **10(1)**, 7 **(2023)**

12. Greff B., Szigeti J., Nagy Á., Lakatos E. and Varga L., Influence of microbial inoculants on co-composting of lignocellulosic crop residues with farm animal manure: A review, *J. Environ. Manage*., **302**, 114088 **(2022)**

13. Haritha T.R. and Gopal K.S., Microbial inoculants and Trichoderma viride consortia for growth promotion and disease management in ginger, *JOSAC*, **30(1)**, 58 **(2021)**

14. Joshi G., Kumar V. and Brahmachari S.K., Screening and identification of novel halotolerant bacterial strains and assessment for insoluble phosphate solubilization and IAA production, *Bull. Natl. Res. Cent.*, **45(1)**, 83 **(2021)**

15. Kanti A. and Sudiana I.M., Production of phytase, amylase and cellulase by *Aspergillus*, *Rhizophus* and *Neurospora* on mixed rice straw powder and soybean curd residue, IOP Conference Series: Earth and Environmental Science, **166**, 012010 **(2018)**

16. Kim M.J., Shim C.K., Kim Y.K., Hong S.J., Park J.H., Han E.J., Kim J.H. and Kim S.C., Effect of aerated compost tea on the growth promotion of lettuce, soybean and sweet corn in organic cultivation, *Plant Pathol. J.*, **31(3)**, 259 **(2015)**

17. Maher M.J., Magette W.L., Smyth S., Duggan J., Dodd V.A., Hennerty M.J. and McCabe T., Managing spent mushroom compost, End of Project Reports, Teagasc, 34 **(2000)**

18. Mahmood R., Afrin N., Jolly S.N. and Shilpi R.Y., Isolation and identification of cellulose-degrading bacteria from different types of samples, *World J. Environ. Res.*, **9(2)**, 8-13 **(2020)**

19. Martín C., Zervakis G.I., Xiong S., Koutrotsios G. and Strætkvern K.O., Spent substrate from mushroom cultivation: Exploitation potential toward various applications and value-added products, *Bioengineered*, **14(1)**, 2252138 **(2023)**

20. Meng X., Dai J., Zhang Y., Wang X., Zhu W., Yuan X., Yuan H. and Cui Z., Composted biogas residue and spent mushroom substrate as a growth medium for tomato and pepper seedlings, *J. Environ. Manage*., **216**, 62-69 **(2018)**

21. Milala M.A., Shugaba A., Gidado A., Ene A.C. and Wafar J.A., Studies on the use of agricultural wastes for cellulase enzyme production by *Aspergillus niger*, *Res. J. Agric. Biol. Sci.*, **1(4)**, 325-328 **(2005)**

22. Myo E.M., Ge B., Ma J., Cui H., Liu B., Shi L., Jiang M. and Zhang K., Indole-3-acetic acid production by *Streptomyces fradiae* NKZ-259 and its formulation to enhance plant growth, *BMC Microbiol.*, **19**, 1-14 **(2019)**

23. Ngalimat M.S., Mohd Hata E., Zulperi D., Ismail S.I., Ismail M.R., Mohd Zainudin N.A.I., Saidi N.B. and Yusof M.T., Plant growth-promoting bacteria as an emerging tool to manage bacterial rice pathogens, *Microorganisms*, **9(4)**, 682 **(2021)**

24. Paula F.S., Tatti E., Abram F., Wilson J. and O'Flaherty V., Stabilisation of spent mushroom substrate for application as a plant growth-promoting organic amendment, *J. Environ. Manage.*, **196**, 476-486 **(2017)**

25. Rade L.L., Da Silva M.N., Vieira P.S., Milan N., De Souza C.M., De Melo R.R., Klein B.C., Bonomi A., Castro-de H.F., Murakami M. and Zanphorlin L.M., A novel fungal lipase with methanol tolerance and preference for macaw palm oil, *Front. Bioeng. Biotechnol.*, **8**, 304 **(2020)**

26. Raja P. and Santhi V.P., Comparative study of microbial inoculants of cultivated and virgin soils of *Nilgiri Biosphere* for plant growth promotion, *Int. J. Agric. Sci.*, **17(2)**, 293-298 **(2021)**

27. Saffari H., Pourbabaee A.A., Asgharzadeh A. and Besharati H., Isolation and identification of effective cellulolytic bacteria in composting process from different sources, *Arch. Agron. Soil Sci.*, **63(3)**, 297-307 **(2017)**

28. Sakthi S.S., Saranraj P. and Rajasekar M., Optimization for cellulase production by *Aspergillus niger* using paddy straw as substrate, *Int. J. Adv. Sci. Tech. Res.*, **1**, 68-85 **(2011)**

29. Saqib A.A.N. and Whitney P.J., Differential behaviour of the dinitrosalicylic acid (DNS) reagent towards mono-and di-saccharide sugars, *Biomass Bioenerg.*, **35(11)**, 4748-4750 **(2011)**

30. Schiano-di-Cola C., Kołaczkowski B., Sørensen T.H., Christensen S.J., Cavaleiro A.M., Windahl M.S., Borch K., Morth J.P. and Westh P., Structural and biochemical characterization of a family 7 highly thermostable endoglucanase from the fungus *Rasamsonia emersonii*, *FEBS J.*, **287(12)**, 2577-2596 **(2020)**

31. Siles J.A., García-Sánchez M. and Gómez-Brandón M., Studying microbial communities through co-occurrence network analyses during processes of waste treatment and in organically amended soils: A Review, *Microorganisms*, **9(6)**, 1165 **(2021)**

32. Singh S., Tripathi A., Maji D., Awasthi A., Vajpayee P. and Kalra A., Evaluating the potential of combined inoculation of *Trichoderma harzianum* and *Brevibacterium halotolerans* for increased growth and oil yield in Mentha arvensis under greenhouse and field conditions, *Ind. Crops Prod.*, **131**, 173-181 **(2019)**

33. Sonashia V.P. and Kamat N., Screening of actinobacteria for antimicrobial activities by a modified CrossStreak method, *Indian J. Pharm. Sci.*, **73**, 223-228 **(2011)**

34. Sun C., Wei Y., Kou J., Han Z., Shi Q., Liu L. and Sun Z., Improve spent mushroom substrate decomposition, bacterial community and mature compost quality by adding cellulase during composting, *J. Clean. Prod.*, **299**, 126928 **(2021)**

35. Ventorino V., Ionata E., Birolo L., Montella S., Marcolongo L., De Chiaro A., Espresso F., Faraco V. and Pepe O., Lignocellulose-adapted endo-cellulase producing *Streptomyces* strains for bioconversion of cellulose-based materials, *Front. Microbiol.*, **7**, 2061 **(2016)**

36. Witfeld F., Begerow D. and Guerreiro M.A., Improved strategies to efficiently isolate thermophilic, thermotolerant and heat-resistant fungi from compost and soil, *Mycol. Prog.*, **20(3)**, 325-339 **(2021)**

37. Zalma S.A. and El-Sharoud W.M., Diverse thermophilic *Bacillus* species with multiple biotechnological activities are associated within the Egyptian soil and compost samples, *Sci. Prog.*, **104(4)**, 1-12 **(2021)**.

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