

FUNGAL BIODEGRADATION POTENTIAL OF CELLULOSE WASTE GENERATED DURING MANNED SPACEFLIGHTS

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Abstract

Efficient decomposition of lignocellulosic biomass composed of cellulose, hemicellulose, and lignin is vital for sustainable life-support systems in long-duration space missions. Fungi offer a promising solution due to their adaptability, low maintenance, and enzymatic ability to break down complex plant materials. Their enzymes, such as cellulases and lignin-degrading enzymes, convert biomass into fermentable sugars and aromatic compounds, which can be used to produce biofuels, bioplastics, or serve as inputs in closed-loop systems. Fungal strains isolated from woody species, fungal isolates from sawdust and wood surface, and five fungal consortia were tested for cellulase and lignolytic enzyme production. Three fungal strains and the natural consortia were selected as the most promising for the biodegradation of lignocellulosic wastes generated during manned spaceflights.

Key words: fungi, cellulose-degrading enzymes, lignin-degrading enzymes

Introduction. The efficient decomposition of lignocellulosic biomass is a crucial step in developing sustainable life-support systems for long-duration space missions. As space agencies explore possibilities for bioregenerative life support

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and in-situ resource utilization (ISRU) on the Moon, Mars, and beyond, biological systems capable of recycling organic waste become essential [1, 2]. Fungi, with their innate ability to degrade complex plant materials, present a promising solution due to their adaptability, low maintenance requirements, and enzymatic versatility [3]. Lignocellulosic biomass consists mainly of cellulose, hemicellulose, and lignin. These polymers require specialized enzyme systems for complete degradation. Fungal cellulose-degrading and lignin-degrading enzymes, including endoglucanases, exoglucanases, β -glucosidases, laccases, and peroxidases, act synergistically to break down these polymers into fermentable sugars and smaller aromatic compounds. These products can be used for the production of biofuels, bioplastics, or as precursors in closed life support systems [4].

The adaptation of fungal metabolic processes, growth morphology, and enzyme secretion in space environments could significantly impact their efficiency and reliability as biocatalysts during missions. Therefore, understanding these dynamics is critical for designing robust biological systems in extraterrestrial contexts.

Cellulosic materials, such as space debris, are believed to contain significant concentrations of soluble inducers that increase the production of cellulose-degrading enzymes. Therefore, a strategy of adding filter paper (FP) discs and carboxymethylcellulose (CMC) as a carbon source to the culture medium was tested.

This study aims to investigate the potential of selected filamentous fungi for the production of cellulolytic and lignocellulolytic enzymes using different carbon sources. By studying enzyme activity and the dynamics of enzyme secretion, this research contributes to the accumulation of knowledge necessary for the integration of fungi into future biotechnological applications in space exploration and extraterrestrial habitation.

Materials and methods. Microorganisms. The experiment included fungal strains *Lenzites warnieri* and *Schizophyllum commune* (isolated from woody species); fungal isolates Sp1; Sp2; Sp2tr; Sp4; Sp5; Sp6; Sp7; Sp8; Sp9; Sp10; Sp11; Sp12; Sp13; Sp15; Sp20 (isolated from wood chips); C7, C7, C9, C9, C10, C10, C11, C11 (isolated from a wooden surface), and Consortium 1 included: *L. warnieri* + *Trichoderma* sp. + Sp20; Consortium 2 included: Garden4 + Sp6 + Sp4, and Consortium 3 included: Sp2 + Sp13 + Sp.tr. The experiment also included two natural consortia – Gradina and Compost, isolated from soil and compost. All strains belong to the Mycological collection at the Stephan Angeloff Institute of Microbiology, Sofia.

Long-term preservation of fungal strains was performed using the Microbank system (Pro-Lab Diagnostics, Richmond Hill, ON, Canada), which consists of sterile vials containing 25 porous, colour-coded beads suspended in a cryopreservative fluid. The vials were stored at -80°C . Prior to use, fungal conidiospores were cultured on Beer agar at 28°C for 7 days.

Nutrient medium and culture conditions. Submerged cultivation was carried out in 500 mL Erlenmeyer flasks with 80 mL nutrient medium containing (g/l): MgSO_4 0.05/0.1; NaCl 0.23/0.1; $\text{NaHPO}_4 \cdot 2\text{H}_2\text{O}$ 0.05/0.1; yeast extract 0.2/0.1. The carbon source FP (1 g/100 mL) or CMC (68 mg/100 mL) was used. Each flask was inoculated with three mycelial plugs (diameter = 10 mm) from a 7-day-old fungal culture and incubated at 28 °C on a rotary shaker (220 rpm) for 120 h. Every 24 h, biomass was harvested, filtered through a Whatman (Clifton, USA) No. 4 filter, and the supernatants were used for enzyme activity assay.

Reducing sugars concentration and enzyme activity assays. Residual reducing sugars concentration (RSC) (e.g., glucose) were quantified using the Somogyi–Nelson method [5]. The activities of the extracellular enzymes laccase (Lac), lignin peroxidase (LiP), and manganese peroxidase (MnP) were determined using the methods described by BOURBONNAIS and PAICE [6], HOFRICHTER et al. [7], and AITKEN et al. [8], respectively. All spectrophotometric measurements were performed using a Shimadzu spectrophotometer.

All the experiments were performed in triplicate. The statistical comparison between the individual repetitions of the experiments is performed by Student's *t*-test and by one-way analysis (ANOVA), followed by an F-test with a significance level of 95% ($p = 0.05$).

Results. All fungal strains and isolates were screened for cellulase enzyme production. The amount of RSC was used as an indicator of the cellulolytic activity of the tested isolates. The cellulolytic activity assay was conducted using nutrient media containing either filter paper (FP) or carboxymethyl cellulose (CMC) as the sole carbon source. The results obtained are presented in Table 1.

The results indicated that the time required to reach peak RSC production varied depending on the isolate. The data in Table 2 clearly showed that CMC promoted the release of RSC more effectively over time in most isolates, particularly in Sp2, Sp4, Sp9, Sp13, Sp2tr, Sp15, and Sp1, which exhibited a late peak in RSC production after 120 h. Among the isolates cultivated in the presence of FP, higher RSC levels were observed in Sp2, Sp6, and Sp4 (at 96 h), as well as Sp1, which showed an early peak at 72 h.

The isolates Sp2tr, Sp5, Sp7, Sp9, Sp13, Sp15, and fungal strain demonstrated a preference for CMC as a carbon source. Among them, Sp13 exhibited the strongest preference, reaching a significantly higher peak RSC value on CMC (3.086 mg/mL) compared to filter paper (1.353 mg/mL). The isolate Sp2tr also showed a notable and early response to CMC. In contrast, isolates Sp1, Sp2, Sp4, and Sp6 favoured FP as the carbon source. Notably, Sp2 reached its peak activity at 96 h, with substantially higher RSC production on FP (4.616 mg/mL) compared to CMC (3.300 mg/mL).

The isolate Sp1 exhibited elevated enzymatic activity on filter paper (FP), reaching its peak at 72 h; however, a higher RSC value was observed on CMC at 120 h. Isolates Sp1, Sp5, Sp6, and Sp7 showed comparable results across both

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Cellulase activity based on RSC after cultivation of the fungal strains and isolates in media containing cellulase inducer. Values are means of three replicates. Cellulase showed statistically significant effect on the enzyme activity ($p \leq 0.05$) in dependence of fungal species

Isolate	Substrate	RSC [mg/mL]	Peak time [h]	Higher performing substrate
<i>L. warnieri</i>	FP	6.164±0.09	96	FP
<i>L. warnieri</i>	CMC	6.983±0.1	96	CMC
<i>S. commune</i>	FP	0.253±0.01	96	CMC (slightly)
<i>S. commune</i>	CMC	0.261±0.01	120	CMC
Sp 1	FP	2.706±0.08	72	FP (early peak)
Sp 1	CMC	2.703±0.05	120	CMC (higher late yield)
Sp 2	FP	4.616±0.07	96	FP
Sp 2	CMC	3.300±0.02	96	FP
Sp 2 tr	FP	1.496±0.07	72	FP
Sp 2 tr	CMC	2.346±0.05	72	CMC
Sp 4	FP	1.336±0.01	96	FP
Sp 4	CMC	1.050±0.008	72	CMC
Sp 5	FP	0.255±0.01	96	CMC (slightly)
Sp 5	CMC	0.287±0.01	120	CMC
Sp 6	FP	1.566±0.05	96	FP
Sp 6	CMC	1.150±0.03	96	CMC
Sp 7	FP	0.200±0.009	72	CMC
Sp 7	CMC	0.396±0.01	96	CMC
Sp 9	FP	1.208±0.02	72	CMC
Sp 9	CMC	1.542±0.04	72	CMC
Sp 13	FP	1.353/1.346	72 & 120	CMC
Sp 13	CMC	3.086±0.06	72	CMC
Sp 15	FP	1.330±0.01	120	CMC
Sp 15	CMC	1.543±0.01	120	CMC
Sp 20	FP	2.900±0.02	96	FP
Sp 20	CMC	2.824±0.02	96	CMC
C7	FP	2.712±0.02	96	Nearly equal
C7	CMC	2.702±0.01	96	Nearly equal
C9	FP	2.557±0.01	96	CMC
C9	CMC	2.612±0.03	96	CMC
C10	FP	2.710±0.02	96	FP
C10	CMC	2.612±0.02	96	FP
C11	FP	2.624±0.02	96	FP
C11	CMC	2.421±0.01	96	FP

substrates, making it difficult to determine a clear substrate preference. The isolate Sp6 demonstrated strong early activity on CMC, with a slightly higher overall peak compared to FP.

At the 96th hour, most isolates cultivated on filter paper (FP) released slightly higher amounts of reducing sugars into the medium, suggesting an ini-

T a b l e 2

Comparative analysis of ligninolytic enzyme activity in fungal isolates grown on paper and CMC. Values are means of three replicates. Cellulase showed statistically significant effect on the enzyme activity ($p \leq 0.05$) in dependence of fungal species and consortia

Strain/Isolate/ Consortium	Substrate	Lac (U/mL)	LiP (U/mL)	MnP (U/mL)	Total enzyme activity (U/mL)
<i>L. warnieri</i>	CMC	0.000	17.510±0.4	0.000	17.510±0.04
	Paper	0.000	8.460±0.41	0.000	8.460±0.23
<i>S. commune</i>	CMC	3.156±0.15	0.000	0.000	3.156±0.15
	Paper	2.777±0.09	0.000	0.000	2.777±0.09
Sp2	CMC	0.417±0.03	7.970±0.3	4.950±0.07	13.337±0.42
	Paper	0.396±0.03	8.550±0.43	0.000	8.946±0.4
Compost	CMC	3.665±0.11	0.000	0.000	3.665±0.11
	Paper	10.466±0.31	0.000	0.000	10.466±0.31
Garden	CMC	0.728±0.01	11.796±0.4	0.000	12.524±0.41
	Paper	1.932±0.03	22.690±0.43	7.153±0.2	31.775±0.36

tial preference for crystalline cellulose. For example, isolates Sp20 and C11 released 2.900 mg/mL and 2.624 mg/mL of reducing sugars on FP, respectively, compared to 2.824 mg/mL and 2.421 mg/mL on CMC. Similarly, isolate C10 showed a slightly higher sugar release on FP (2.710 mg/mL) than on CMC (2.612 mg/mL). In contrast, isolate C7 produced nearly identical amounts of reducing sugars on both substrates, with 2.712 mg/mL on FP and 2.702 mg/mL on CMC.

The fungal isolate C9 exhibited a predilection for CMC at this stage, with a release of 2.612 mg/mL in comparison to 2.557 mg/mL on FP. After 96 h, a clear trend emerged, with fungal isolates Sp20, C11, C10, C7, and C9 releasing more reducing sugars in the presence of CMC, thereby highlighting their preference for amorphous cellulose.

Three fungal consortia were assembled from the isolates that exhibited the highest cellulolytic enzyme activity, with each consortium comprising three fungal strains. All the consortia formed were cultured on liquid medium with CMC or FP as the carbon source. This investigation aimed to assess the cellulose degradation potential of these consortia by measuring their capacity to release RSC from cellulosic materials. The results obtained are presented in Fig. 1.

The fungal consortium 1, cultivated on FP medium, demonstrated the highest total RSC release (0.040 mg/mL), indicating robust enzymatic activity on crystalline cellulose. The RSC values for both the control (0.0268 mg/mL) and the CMC-containing medium (0.027 mg/mL) were comparable, suggesting low activity on amorphous cellulose. The fungal Consortium 2 exhibited consistent but modest RSC production across all substrates, with a slight increase on FP (0.027 mg/mL) compared to the control (0.025 mg/mL) and CMC (0.024 mg/mL). The minimal

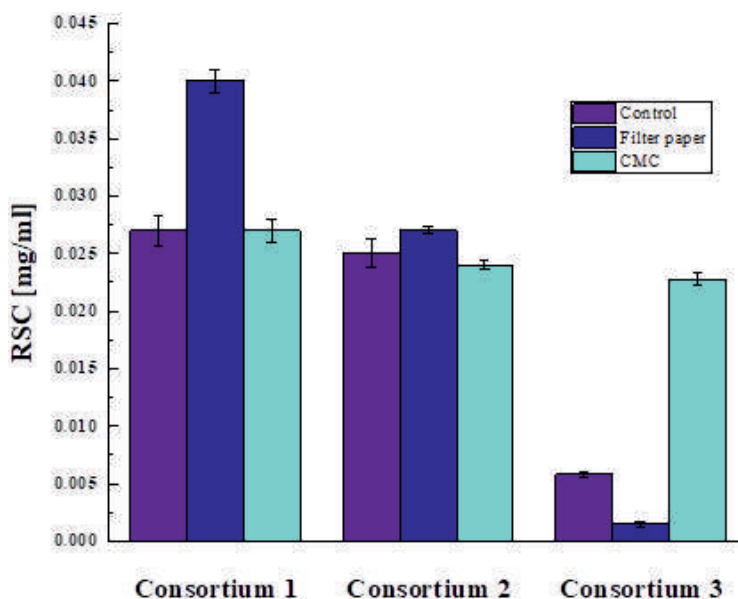


Fig. 1. Cellulase activity of the assembled consortia. Values are means of three replicates; error bars represent the standard deviation. Cellulase showed statistically significant effect on the enzyme activity ($p \leq 0.05$) in dependence of fungal consortia

variation suggests a relatively low level of cellulose degradation activity or limited substrate specificity within this consortium. Fungal Consortium 3 showed a clear trend, with minimal activity observed in the control (0.0058 mg/mL) and on FP medium (0.0015 mg/mL). However, a notably higher RSC concentration was measured in the medium containing CMC (0.0228 mg/mL). These results suggest that this consortium primarily exhibits cellulase activity targeting amorphous cellulose (represented by CMC) and has a limited ability to degrade crystalline cellulose.

Our assembled consortia did not show significant potential of cellulose degradation. For this reason, we included in our observation two natural consortia. The two fungal strains with the highest RSC values – *L. warnieri* and *S. commune*, as well as isolate Sp2, were tested together with two natural fungal consortia Garden and Compost for enzymatic lignolytic activity. The inclusion of the two natural consortia was aimed at comparing their efficiency in degrading lignocellulosic substrates. The activity of lignin peroxidase (LiP), laccase (Lac), and manganese peroxidase (MnP) was assessed on both nutrient media (Table 2).

The fungal strain *L. warnieri* revealed an augmentation in lignin peroxidase activity, particularly when cultivated in the presence of CMC as a carbon source (17.51 U/mL). Conversely, no Lac or MnP activity was detected. This finding suggests the presence of a specialized enzyme pathway that is induced by soluble cellulose analogues. The *S. commune* demonstrated notable laccase production, particularly when cultivated with CMC (3.156 U/mL), yet exhibited

no peroxidase activity. The enzyme system of strain *S. commune* was found to be predominantly laccase-based. The isolate Sp2 demonstrates a comprehensive enzyme profile, exhibiting moderate activity across all enzymes. It is noteworthy that the presence of CMC results in the highest MnP activity (4.95 U/mL), while FP maintains elevated Lac and LiP activity. This balanced activity makes the isolate Sp2 a potential candidate for the simultaneous degradation of lignin and cellulose. The Compost consortium exhibited elevated levels of Lac production, with peak activity observed on FP substrate (10.466 U/mL). It is noteworthy that LiP exhibited activity exclusively under control conditions (7.43 U/mL), indicating a potential for substrate-specific repression or enzyme regulation. The Garden consortium exhibited the most diverse array of enzyme potential. The FP substrate induced maximal activity for all enzymes: Lac, LiP and MnP levels were 1.932 U/mL, 22.690 U/mL and 7.153 U/mL, respectively. This consortium demonstrated a high degree of sensitivity to lignocellulosic materials and has the potential to contribute to the efficiency of composting or bioconversion processes.

Discussion. The present study investigated the cellulolytic activity of fungal strains, isolates and consortia cultivated on two distinct carbon sources: FP and CMC. The findings indicate that the type of substrate, duration of incubation, and the specificity of each fungal strain or isolate significantly affect the levels and dynamics of reducing sugar compound (RSC) release, serving as indicators of cellulolytic activity. A comparative analysis of the data reveals notable differences in cellulose-degrading capabilities depending on the carbon source. Most of the examined strains and isolates exhibited enhanced cellulase activity when grown on CMC, a soluble and amorphous cellulose derivative [9]. For example, isolate Sp13 showed a marked preference for CMC, with a peak RSC release of 3086 mg/mL at 120 h, compared to 1353 mg/mL on FP. Similarly, isolates Sp2tr and Sp15 demonstrated sustained and elevated activity on CMC-containing media.

The observation that the majority of isolates released higher amounts of RSC in CMC-containing media suggests that CMC is the preferred carbon source for these organisms. Comparable results have been reported by MAKI et al. [10], who observed up to a 5.6-fold increase in cellulase activity in bacterial isolates E2 and E4 when cultivated on CMC rather than FP. DAS et al. [11] also documented superior cellulolytic activity in *Aspergillus fumigatus* cultured on CMC. These findings support previous reports indicating that CMC acts as a more effective inducer of endoglucanase activity, owing to its higher solubility and accessibility compared to crystalline cellulose substrates such as FP [9].

Among the isolates analyzed, Sp13 displayed the highest affinity for CMC, with a pronounced increase in RSC release of +1733 mg/mL, whereas Sp2 exhibited the highest absolute cellulolytic activity on FP (4616 mg/mL), suggesting efficient degradation of crystalline cellulose. FP, although generally less preferred, provided higher peaks in RSC levels in the culture medium for certain isolates, indicating, in some cases, better induction of exocellulase activity. The temporal

dynamics of peak enzyme activity are subject to variation, with certain isolates demonstrating peak activity at 72 h (Sp1, Sp2tr, Sp7, Sp9, Sp13) and others at 96 h (*L. warnieri*, *S. Commune*, Sp2, Sp4, Sp5, Sp6, Sp20, C7, C9, C10, C11) or 120 h (Sp13 and Sp15). This observation signifies the presence of disparate metabolic processes. EL BERGADI et al. [12] reported on fungal isolates grown on filter paper as the sole carbon source. *Penicillium funiculosum* demonstrated higher cellulase activity on filter paper [13].

Fungal Consortium 1 exhibited the highest cellulose-degrading activity, particularly with regard to crystalline cellulose, rendering it a promising candidate for biomass degradation applications. Consortium 2 exhibited minimal activity, indicating restricted industrial viability under the prevailing experimental conditions. Fungal Consortium 3 exhibited substrate-specific activity, demonstrating a preference for the presence of CMC as a substrate. Concurrent findings have been documented by ZHANG and DONG [14]. Some consortia exhibit a specific behaviour towards the substrate, preferring CMC over crystalline cellulose. This property can be used in processes requiring the selective degradation of amorphous cellulose.

The *S. commune* strain exhibited robust laccase production, particularly on CMC (3.156 U/m), with no detected peroxidase activity. The predominance of Lac over MnP/LiP indicates that this strain utilizes a phenol oxidase-centred lignin degradation system, a feature that is characteristic of white-rot fungi adapted to softwood degradation [15, 16]. The induction of LiP by soluble cellulose analogues (as in *L. warnieri*) is less frequently documented, but it may reflect cross-regulation between cellulolytic and ligninolytic systems. Some fungi exhibit differential expression of peroxidases and laccases depending on carbon source, redox stress, and the presence of phenolic inducers (e.g., induction by copper complexes). The elevated laccase production observed in the *S. commune* strain is consistent with the findings in the study of JANUSZ et al. [17], which reported that laccase frequently acted as the predominant ligninolytic enzyme in the absence of strong peroxidase induction.

These findings underscore the significance of substrate composition in modulating enzyme induction and performance in fungal consortia, thereby corroborating prior reports that substrate structure (crystalline vs. amorphous) exerts a substantial influence on fungal metabolism [18]. The Garden consortium exhibited the most robust and diverse enzyme potential. When cultivated on filter paper, all three enzymes demonstrated a peak response: the levels of Lac, LiP, and MnP were found to be 1.932 U/mL, 22.690 U/mL, and 7.153 U/mL, respectively. This robust reaction to crystalline cellulose indicates that this consortium is highly responsive to lignocellulosic materials and may orchestrate a coordinated enzymatic attack on lignin and cellulose. This broad-spectrum capability is advantageous for composting or bioconversion applications, where simultaneous breakdown of lignin and cellulose is required.

These results are consistent with those obtained from studies on fungal consortia and synergistic enzyme induction. For instance, consortium cultures of white-rot fungi (e.g., *Lenzites betulina* and *Trametes versicolor*) have been shown to exhibit enhanced laccase and MnP activities in comparison to monocultures, with increases exceeding 40% in enzymatic yields (18.06 U/mL Lac and 13.58 U/mL MnP) under optimal lignin-amended conditions [18]. In a similar vein, as evidenced in the research by GILL et al. [19], the application of engineered fungal consortia for the purpose of biodelignification has been shown to yield elevated levels of enzyme synergies when compared to the results obtained from the use of individual isolates.

Conclusion. The results of this study indicate that CMC functions as a more effective inducer of cellulolytic activity in the majority of fungal strains and isolates, although certain strains also exhibit early or pronounced activity on FP. The inclusion of compost-derived microbial consortia and the analysis of ligninolytic enzymes further enrich the study, highlighting the potential of selected strains and isolates for integrated lignocellulose degradation.

These findings align with recent research trends that emphasize the importance of isolate selection, substrate-specific adaptation, and enzymatic diversity in the efficient bioconversion of lignocellulosic biomass. The comparative enzyme analysis underscores the substrate-dependent modulation of ligninolytic enzyme expression among distinct fungal strains and isolates. Notably, the two consortia – designated “Garden” and “Compost” – demonstrated superior lignocellulolytic potential, particularly on lignocellulosic substrates such as paper. This was evidenced by their capacity to co-produce laccase (Lac), lignin peroxidase (LiP), and manganese peroxidase (MnP) at functionally significant levels.

These results underscore the critical influence of both fungal identity and substrate composition on ligninolytic enzyme production. The Garden consortium cultivated on a paper-based substrate, along with the Sp2 isolate grown on CMC, emerged as particularly promising candidates for space biotechnology applications. Their potential lies in the bioconversion of biomass and organic waste into fermentable sugars or biomaterials. Incorporating such organisms into bioregenerative life support systems (BLSS) may support key functions including resource recycling, carbon recovery, and bioplastic production during extended space missions.

Furthermore, the observed induction of substrate-specific enzyme expression highlights the potential for targeted substrate formulation to optimize enzyme yields in microgravity-adapted bioreactors.

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