Fundación Miguel Lillo
Tucumán
Argentina

Comparison and characterization of mycelium-based composites (MBCs) produced by the fungus *Trametes coccinea* and softwoods and hardwood sawdust

Comparación y caracterización de un compósito en base a micelio (MBCs) obtenido por el hongo *Trametes coccinea* y aserrín de maderas blandas y duras

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Abstract

There is a growing demand for materials of biological origin, particularly those derived from organic waste and filamentous fungi, known as mycelium-based composites (MBCs). This study evaluates the production of MBCs using sawdust from *Pinus radiata*, *Nothofagus obliqua*, and *Fitzroya cupressoides* in combination with a filamentous fungus identified as *Trametes coccinea* based on its rDNA ITS region. Microstructure, chemical composition, and mechanical and physical properties of the composites were assessed using scanning electron microscopy (SEM), compressive strength testing, standardized hardness testing, and FTIR spectroscopy. The results confirmed mycelial penetration at different structural levels within the substrates. The highest-performing MBC was obtained from *F. cupressoides*, with a Janka hardness of 0.111

➤ Ref. bibliográfica: Galleguillos, F.; Basuñán-Heredia, A.; Riquelme, C.; Latco, L.; Radic-Schilling, S. 2026. Comparison and characterization of mycelium-based composites (MBCs) produced by the fungus *Trametes coccinea* and softwoods and hardwood sawdust. *Lilloa 63* (2): 417-429. doi: https://doi.org/10.30550/j.lil/2421
➤ Recibido: 11 de mayo 2026 – Aceptado: 14 de agosto 2026 – Publicado: 17 de septiembre 2026.
➤ URL de la revista: http://lilloa.lillo.org.ar

kN and a compressive strength of 0.271 MPa. *T. coccinea* exhibited enhanced lignocellulolytic degradation efficiency in softwoods, likely associated with its enzymatic adaptability to guaiacyl-rich lignin and substrate anatomical features that favored colonization. The resulting biocomposites display properties comparable to synthetic materials and demonstrate strong potential for scalability.

Keywords: Composites; materials; mycelium.

Resumen

Existe una creciente demanda de materiales de origen biológico, particularmente aquellos producidos a partir de residuos orgánicos y hongos filamentosos, conocidos como compuestos basados en micelio (MBCs). Este estudio evaluó la producción de MBCs utilizando aserrín de *Pinus radiata*, *Nothofagus obliqua* y *Fitzroya cupressoides* en combinación con un hongo filamentoso identificado como *Trametes coccinea* en base a su región ITS del rDNA. La microestructura, composición química y las propiedades mecánicas y físicas fueron evaluadas mediante microscopía electrónica de barrido (SEM), ensayos de resistencia a la compresión, pruebas estandarizadas de dureza y espectroscopía FTIR. Los resultados confirmaron la penetración del micelio en distintos niveles estructurales dentro de los sustratos. El MBC con mejor desempeño se obtuvo a partir de *F. cupressoides*, con una dureza Janka de 0.111 kN y una resistencia a la compresión de 0.271 MPa. *T. coccinea* mostró una mayor eficiencia en la degradación lignocelulítica en maderas blandas, probablemente asociada a su adaptabilidad enzimática y las características anatómicas del sustrato que favorecieron la colonización. Los biocompositos resultantes presentan propiedades comparables a materiales sintéticos y demuestran un alto potencial de escalamiento.

Palabras clave: Compositos; materiales; micelio.

INTRODUCTION

Synthetic polymers derived from petroleum have generated global environmental concern due to their non-biodegradable nature, which results in persistent waste that threatens ecosystems (Pilapitiya & Ratnayake, 2024). This has driven the search for new, economically viable materials with reduced environmental and human health impacts. Biotechnological innovations play a key role in the transition toward a circular and sustainable economy (Lange *et al.*, 2020).

Fungi have emerged as a promising alternative for material production because of their metabolic versatility and ability to grow on diverse substrates (Sharma *et al.*, 2024). Rot fungi (RF) degrade lignocellulosic substrates by producing carbohydrate-active enzymes (CAZymes) capable of breaking down cellulose, hemicellulose, and lignin (Reyre *et al.*, 2023).

RF are classified into three groups based on the structural components they decompose: white-rot fungi (WRF), brown-rot fungi (BRF), and soft-rot fungi (SRF) (Fukasawa *et al*., 2011; Fukasawa, 2018).

Wood from different species varies in cellular structure, chemical composition, and anatomical features, largely according to taxonomic grouping. Gymnosperms (softwoods) and angiosperms (hardwoods) can be distinguished by their xylem anatomy: tracheids in gymnosperms provide both mechanical support and water transport, whereas angiosperms contain vessels for conduction and fibers for support (Li *et al*., 2021). The relative proportions of cellulose, hemicellulose, and lignin differ among species and reflect evolutionary patterns (Weng *et al*., 2008; Nawawi *et al*., 2016). Wood is used widely in the construction and paper industries, generating substantial residues such as sawdust.

Sawdust can serve as an appropriate substrate for rot fungi to produce mycelium-based composites (MBCs). These composites form through fungal colonization and partial biodegradation of lignocellulosic particles, generating a foam-like material with a continuous and dispersed phase (Elsacker *et al*., 2019; Rigobello & Ayres, 2023). MBCs are lightweight, inexpensive, and hold potential for applications in construction, packaging, and advanced textiles (Appels *et al*., 2019; Sun *et al*., 2019; Manan *et al*., 2022). Studies report favorable compressive strength, thermal and acoustic insulation, and hydrophobicity, positioning MBCs as environmentally responsible substitutes for plastics and other synthetic materials (Elsacker *et al*., 2019; Sydor *et al*., 2022).

White-rot species including *Trametes*, *Pleurotus*, and *Pycnoporus* are among the most widely used fungi for producing MBCs (Cerimi *et al*., 2019). Previous research demonstrates that MBCs produced with *Pycnoporus* spp. and pine sawdust exhibit properties comparable to expanded polystyrene (EPS), with superior compressive strength and increased thermal stability up to 350°C (Bruscato *et al*., 2019; Santos *et al*., 2021). Similarly, Saez *et al*. (2022) used *T. sanguinea* with beech and spruce chips to fabricate MBC blocks with higher density and strength for potential use in construction systems.

Here, we employed a white-rot fungus locally isolated, *Trametes coccinea* (Fr.) Hai J.Li & S.H.He, to manufacture MBCs using both softwoods (*P. radiata* and *F. cupressoides*) and a hardwood species (*N. obliqua*). We evaluated the resulting composites for their physical, mechanical, and chemical properties.

MATERIALS AND METHODS

Sampling and isolation

Fungi were collected from *Pinus radiata* in Concepción, Biobío Region, Chile (-36.830304, -73.030299), and processed the same day. A 2×2 cm internal tissue fragment was placed onto Petri dishes containing malt extract agar (MEA; 20 g malt extract, 20 g agar per liter) and incubated at 21°C for 10 days. Monocultures were obtained through repeated subculturing until a single species remained. Isolates with colored, filamentous mycelium were selected.

DNA extraction, PCR amplification, and sequencing

DNA extraction was carried out using the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1990) followed by rDNA ITS region was amplified using primers ITS1 and ITS4 (White *et al*., 1990). PCR conditions consisted of an initial denaturation at 94°C for 4 min; 30 cycles of 94°C for 1 min, 58 °C for 1 min, 72°C for 1 min; and a final extension at 72°C for 7 min. Reactions were performed in 25μL volume. PCR products were sequenced in Austral-omics, Universidad Austral, using the same primers. Sequences were compared using BLAST (Altschul *et al*., 1990).

Culture conditions

Trametes coccinea was first grown for 14 days on MEA and then transferred to liquid culture (20 g L^{-1} malt extract, 200 mgL^{-1} streptomycin). Glass flasks (1L) containing 500 mL of liquid medium were autoclaved (121°C, 21 min) and inoculated with four 5×5 mm agar plugs. Cultures were incubated at 25°C and 130 rpm for 14 days. Mycelium from liquid culture was transferred to sterilized rice grains (500 g brown rice per 25 mL inoculum) and incubated for 14 days at 25°C.

Fungal composites

Three types of sawdust—*P. radiata*, *N. obliqua*, and *F. cupressoides*—were combined with coconut fiber, vermiculite, and mycelium (10% w/w). Mixtures included 80% sawdust, 10% vermiculite, and 10% coconut fiber for all species.

Molding and drying

Mixtures were placed into 90×15 mm plastic molds and incubated at 25°C until full colonization. Samples were dried in a convection oven at 60°C for 24 hours. Mechanical test specimens measured 2.5×2.5 cm.

Scanning electron microscope (SEM)

Composite microstructure was analyzed using a JEOL JSM 1200EX-II electron microscope (JEOL ltd, Japan). Mycelial density were documented after fungal colonization.

Physical and mechanical properties

The physical properties were evaluated using different international standards. Density was measured following ASTM C303-21 (C16 Committee, 2021). Moisture content was calculated using ISO 16979:2003 (ISO, 2003). Compressive strength was tested according to ASTM D3501-24 (D07 Committee, 2024) using an Instron 4468 machine (50 kN load cell) at 0.75 mm min^{-1}. Deformation (Δ thickness) was calculated before and after testing. Janka hardness followed ASTM D143-22 (D07 Committee, 2023), using an 11.3 mm steel ball at 7.5 mm min^{-1}.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR-ATR spectra were obtained using a Jasco FT/IR-4X spectrometer (JASCO, Tokyo) (500–4000 cm^{-1}, 32 scans). Samples were analyzed in triplicate; spectra were converted to absorbance. OriginPro 2018 (OriginLab Corporation, 2018) was used to quantify peak intensities. Peaks between 1800 and 400 cm^{-1} were normalized. Ratios were calculated for lignin (1510 cm^{-1}), hemicellulose (1733 cm^{-1}), and carbohydrate (1143 cm^{-1}) signals.

Statistical Analysis

Compression tests were performed in triplicate and data were analyzed using a one-way analysis of variance (ANOVA) with wood species as the independent variables and compression as dependent variables. The Tukey test was run to determine the location of the significant difference ($p \leq 0.05$). The statistical procedures were perfomed using SigmaPlot 12.0 (Systat Software Inc., 201). Before analysis, data were verified to a normal distribution.

RESULTS AND DISCUSSION

Isolation and identification

Results indicate that the amplified section of the ribosomal DNA ITS region of isolate was 99,84% similar, according to the identity blast percentage, to 621 base pair (bp) sequence reported as *Trametes coccinea*, PV108643 a known decomposer white rot fungi. The new sequence was uploaded with the genbank accesion number PZ585405.

Mycelium-based composites

All composites developed a uniform mycelial surface layer with the characteristic orange color (Fig. 1). Growth density varied by substrate, with *F. cupressoides* showing the densest surface mycelium, *P. radiata* forming a smooth layer, and *N. obliqua* producing lighter coverage.

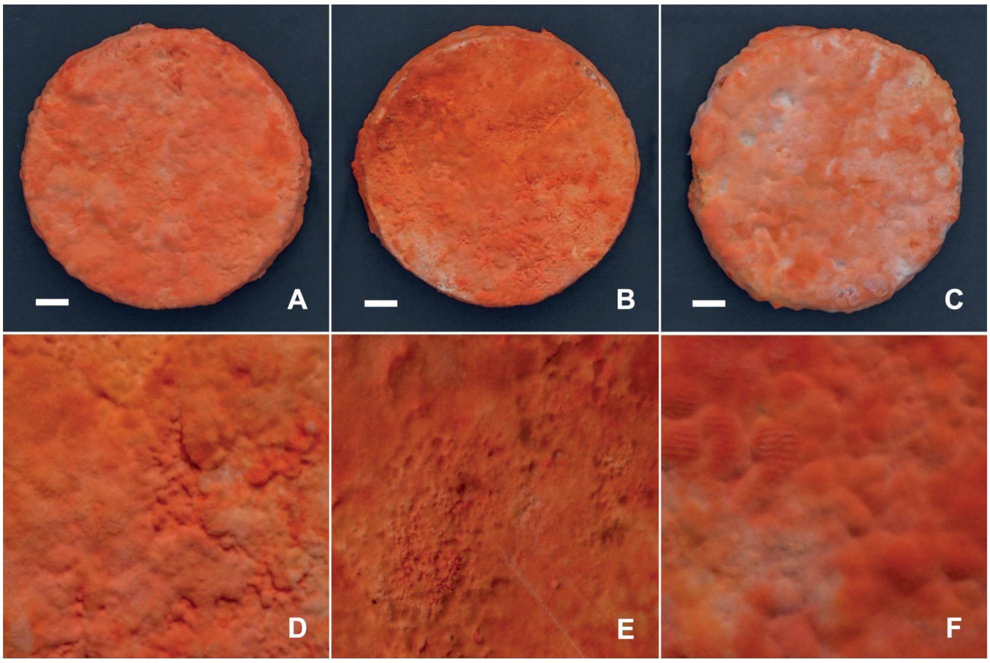

Fig. 1. MBCs obtained after incubation with *Trametes coccinea*. A and D) *Pinus radiata* composite and surface. B and E) *Fitzroya cupressoides* composite and surface. C and F) *Nothofagus obliqua* composite and surface. Scale bar: A-C = 1 cm.

Fig. 1. MBCs obtenidos después de la inoculación con *Trametes coccinea.* A y D) Compósito y superficie de *Pinus radiata.* B y E) Compósito y superficie de *Fitzroya cupressoides.* C y F) Compósito y superficie de *Nothofagus obliqua*. Escala: A-C = 1 cm.

Scanning electron microscope (SEM) analyses

Scanning electron microscopy is used to characterize mycelium-based composites, allowing visualization of the microstructure of the material consisting of mycelium and substrate particles (Fig. 2) revealed hyphal penetration at the surface, interface, and center of each composite separately. *F. cupressoides* MBC exhibited fewer voids and a denser microstructure than *N. obliqua* and *P. radiata*. Higher cavity abundance corresponded with lower density and reduced mechanical performance, consistent with previous findings (Islam, 2018). Substrate particle size has minor effect on fungal growth patterns (van Kuijk *et al*., 2016).

Physical and mechanical properties

Table 1 and Fig. 2 summarizes MBCs density, moisture content, hardness, deformation, and compressive strength. *F. cupressoides* displayed the highest density (358.77 kg m^{-3}), Janka hardness (0.111 kN), and compressive strength (0.271 MPa), attributed to its denser microstructure and smaller particle size. Similar to Sachs *et al*. (1991) and van Kuijk *et al*. (2016), substrate particle size alone does not dictate fungal growth but may influence mechanical performance. Compression analysis showed no significant differences between *N. obliqua* and *P. radiata* ($p>0.05$) and revealed significant differences between *F. cupressoides* and the other two woods ($p<0.05$). Fig. 3.

Table 1. Physical and mechanical properties of MBCs.

Tabla 1. Propiedades físicas y mecánicas del MBCs.

Wood type	Density (kg m^{-3})	Moisture content (%)	Δ thickness (mm)	Janka hardness (kN)	Compressive strength (MPa)
P.r-MBC	264.78 ± 27.21	10.21 ± 0.27	5.29 ± 0.485	0.0571 ± 0.042	0.175 ± 0.029
N.o-MBC	297.79 ± 18.09	11.29 ± 0.82	5.39 ± 0.571	0.0417 ± 0.009	0.184 ± 0.040
F.c-MBC	358.77 ± 10.76	11.36 ± 0.33	5.26 ± 0.303	0.111 ± 0.020	0.271 ± 0.046

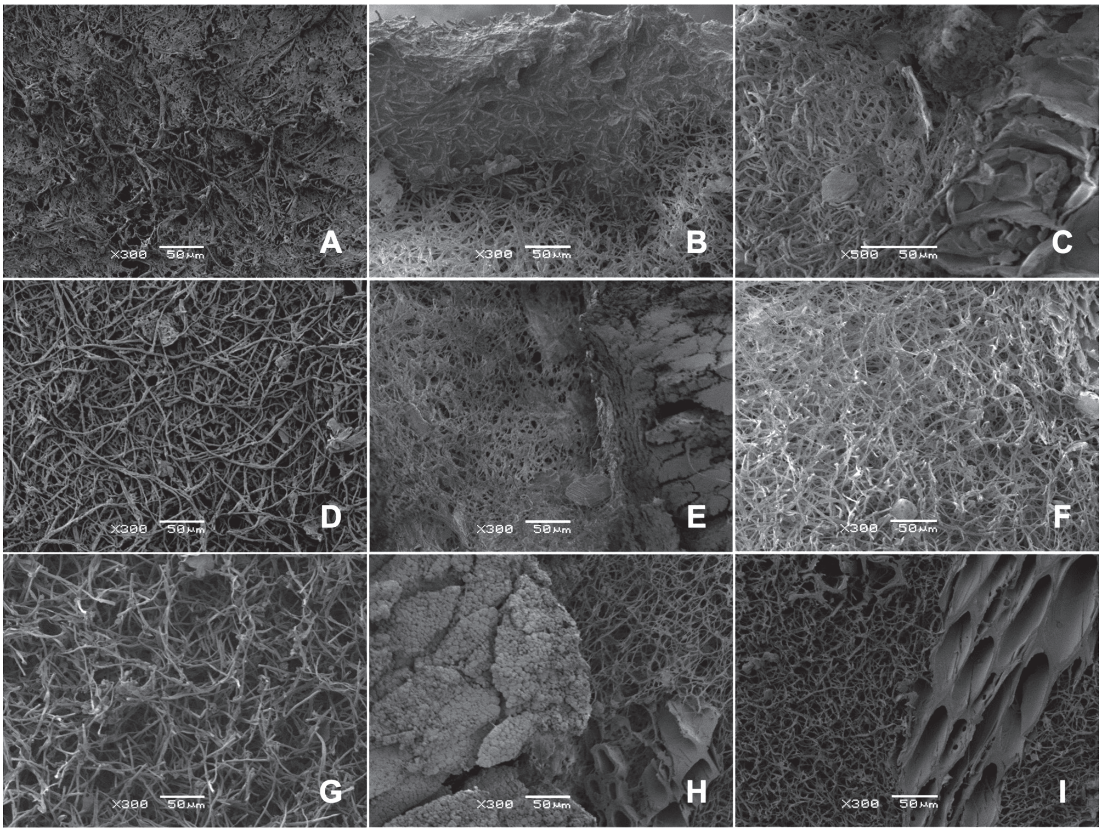

Fig. 2. SEM Microscopy of MBCs layered structures. *Fitzroya cupressoides*: A) Surface. B) Surface-center interface. C) Center of the composite. *Nothofagus obliqua*: D) Surface. E) Surface-center interface. F) Center of the composite. *Pinus radiata*: G) Surface. H) Surface-center interface. I) Center of the composite.

Fig. 2. Microscopía SEM estructura en capas de MBCs. *Fitzroya cupressoides:* A) Superficie. B) Interface superficie-centro. C) Centro del compósito. *Nothofagus obliqua*: D) Superficie. E) Interface superficie-centro. F) Centro del compósito. *Pinus radiata*: G) Superficie. H) Interface superficie-centro. I) Centro del compósito.

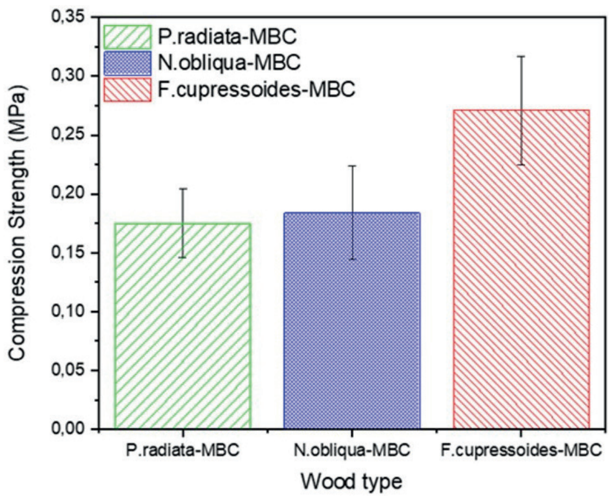

Fig. 3. Compressive strength of MBCs.

Fig. 3. Fuerza de compresión de MBCs.

FTIR analysis

FTIR provides information on chemical composition, revealing chemical changes associated with the colonization of the mycelium and the substrate, allows identification of vibrational functional groups on the material. The analysis of intensity and shape of peaks provides information about the functional groups and structure. Since white rot influences the chemical composition of fibers, especially lignin. FTIR analysis was performed to understand the chemical composition of the different types of wood focus in compounds of Table 2.

FTIR spectra (Fig. 4) showed the wood degradation patterns for each plant species, the mycelium-based composites, and the fungus-specific mycelium separately. Each sample showed characteristic degradation patterns: (a) carbohydrates (1143 cm^{-1}): *N. obliqua* and *F. cupressoides* MBCs displayed increased intensity relative to pure wood, indicating partial degradation. *P. radiata* showed minimal change. (b) lignin (1510 cm^{-1}): all MBCs exhibited decreased intensity, with softwoods (*F. cupressoides* and *P. radiata*) showing the strongest reductions, suggesting enhanced guaiacyl lignin degradation. (c) hemicellulose (1733 cm^{-1}): reductions were greater in softwoods, again reflecting higher enzymatic activity toward these polymers.

These results align with previous studies demonstrating strong white-rot activity in softwoods (Reyre *et al*., 2023) despite *T. coccinea* also colonizing hardwoods efficiently suggesting adaptability (Couturier *et al*., 2015). However, the increase in the bands attributed to carbohydrates cannot be interpreted on their own as evidence of low wood degradation in MBCs, since those bands also include the contribution of β-glucans and other polysaccharides from the mycelium's cell wall.

Table 2. Relative FTIR intensity.
Tabla 2. Intensidad relativa FTIR.

Wavelenght (cm^{-1})	Peak (cm^{-1})	Assignment	Reference
1770–1710	1733	C=O vibration of carbonyl stretching of acetyl group in hemicelluloses (xylans).	Peng et al. (2023)
1520–1500	1510	C=C stretching of aromatic ring (guaiacyl) in lignin.	Mátyás Báder et al., 2020
11621125	1143	C-O-C vibration in cellulose and hemicellulose.	Schwanninger et al., 2004

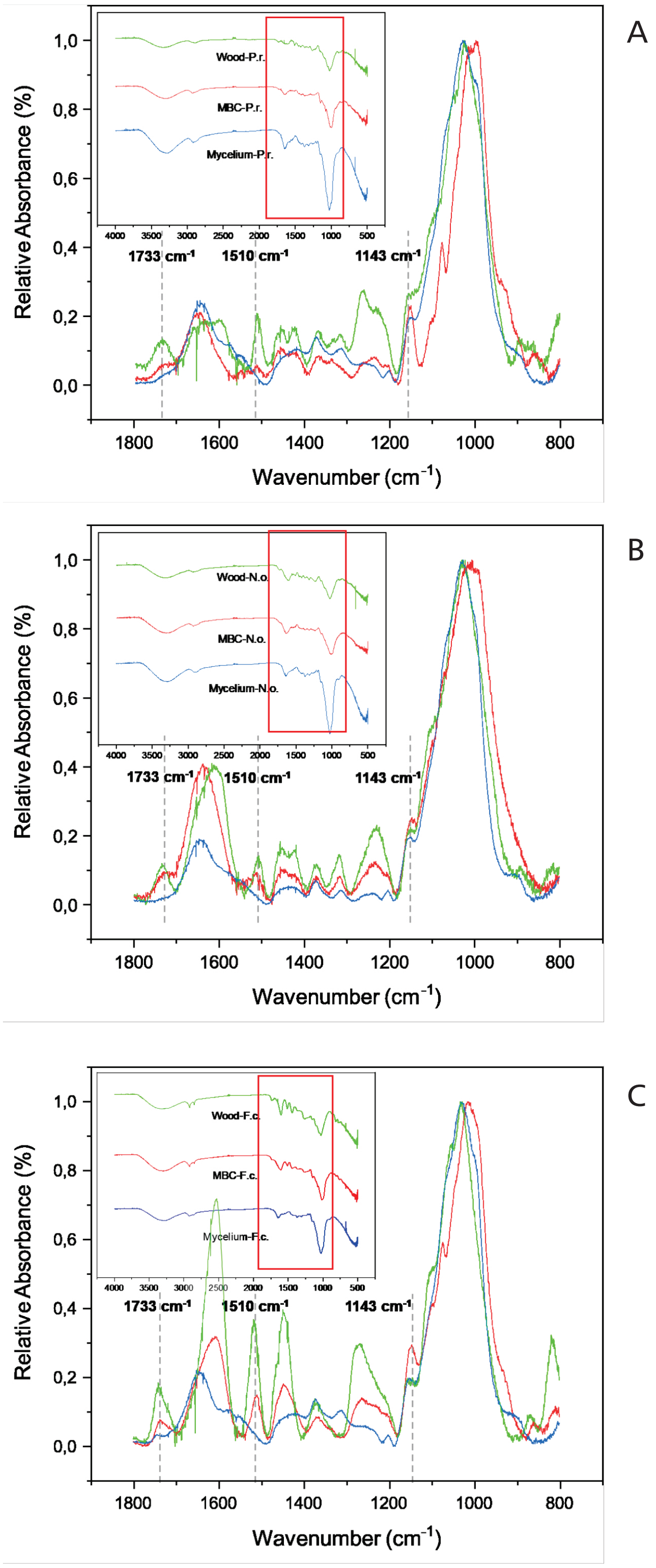

Fig. 4. FTIR analysis of mycelium (blue), wood (green) and MBC (red). Each letter indicates the type of wood species used: A) *Pinus radiata.* B) *Nothofagus obliqua.* C) *Fitzroya cupressoides*.

Fig. 4. FTIR análisis de micelio (azul), madera (verde) y MBC (rojo). Cada letra indica el tipo de madera usada: A) *Pinus radiata.* B) *Nothofagus obliqua.* C) *Fitzroya cupressoides.*

CONCLUSIONS

MBCs produced using *Trametes coccinea* and sawdust from three wood species resulted in materials with dense mycelial surfaces and controlled internal biodegradation. Softwood-based composites exhibited greater lignin and hemicellulose degradation, leading to higher mechanical performance, particularly in *F. cupressoides*. These findings highlight the influence of substrate type on fungal biodegradation mechanisms and support the potential of MBCs as sustainable alternatives to synthetic materials.

ACKNOWLEDGEMENTS

This work was supported by the Chilean National Agency for research and development (ANID), Fondecyt iniciación N° 11230014.

CONFLICTS OF INTEREST

Authors declare no conflicts of interest.

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