Soft Matter

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Soft Matter*, 2025, **21**, 9323

Strain, substrate, and matrix selection for controlled growth of wood-fungi

Sophie Hofer, Natalie Nussbaum, Peter Fischer * and Patrick A. Rühs *

Received 23rd July 2025,
Accepted 16th November 2025

DOI: 10.1039/d5sm00749f

rsc.li/soft-matter-journal

Fungal fermentation offers a promising approach for the development of engineered living materials (ELMs). The design of the substrate materials to support and enhance fungal growth in both 2D and 3D is essential to realize this potential. We evaluated the mycelium of seven edible mushroom-forming fungi for growth vigor as a function of various abiotic factors. Growth assays using standard malt agar, with varying concentrations of carbohydrates and proteins, revealed that the radial expansion of the fungal is affected by the carbohydrate concentration, showing a maximum expansion rate at mid-low concentrations and a diminishing expansion rate at higher concentrations. In contrast, higher carbohydrate concentrations increased mycelium density. Different plant-based proteins also significantly influenced growth vigor, *i.e.* the mycelium's thickness and expansion rate. Beyond chemical substrate conditions, we modified the substrate viscoelasticity by increasing agar concentration, which resulted in higher growth proliferation. This was further confirmed using non-standard gelling agents such as guar gum, corn starch, κ-carrageenan, and bacterial cellulose. In a final step to enhance growth for practical applications, we foamed an optimized substrate material for 3D growth, achieving successful growth throughout the entire matrix. This work provides a framework to aid the selection of edible substrate materials for fungal growth, *i.e.* the design of engineered living materials.

I. Introduction

Engineered living materials are partially or completely built by microorganisms to create materials with biological features such as biodegradability and adaptability.[1–7] Promising microorganisms, such as bacteria and fungi, have been shown to create materials that are inherently biodegradable and exhibit strong mechanical properties depending on the hybridization with other materials. In particular, mycelium-forming fungi, which colonize substrates have recently been explored to develop mycelium-based composite materials. Mycelia of edible fungi, for example *Pleurotus ostreatus* and *Ganoderma lucidum* have been investigated for their use as living glue and their potential application in packaging, insulation, construction, fashion, and architecture.[1,8–10] Recently, mycelium composites have been engineered by 3D printing to obtain thermally insulating materials or other functional materials for buildings,[9,11–13] by fusing large-scale structures by bio-welding of individual mycelium structures, to obtain packaging materials or flexible textile-like composites,[5] or to reinforce the mechanical properties of wood scaffolds and foams.[7,14] The mycelium-based composites possess a heterogeneous structure with the mycelium's hyphal network embedded in the initial substrate material. The physical properties of these mycelium-based composites have been reported to depend on various factors such as fungal strain, initial substrate composition, and substrate packing.[15–17] Regarding the mechanical properties of pure mycelium, Islam *et al.*[18] indicated that they are determined by the arrangement of the hyphae within the network and that mycelium can be treated as a foam material. The mechanical properties of pure mycelium depend on the growth substrate,[19–21] which could hold promise for controlled self-grown mycelium-based materials with various functionalities. Therefore, the physical and chemical environment must be controlled to promote fungal growth.

The growth of mycelium into materials has been explained as a combination of enzyme-catalyzed digestion of the material and subsequent pressure-driven penetration.[22,23] Furthermore, the mycelium can cross regions depleted with nutrients, which gives most fungi the ability to translocate nutrients within their mycelium networks.[24] Fungi explore the available substrate with rapidly growing sparse mycelium. Once expanding hyphae discover rich nutrient sources, the expanding rate declines, and hyphae branch to form an interwoven network and harness the resource. While biomass formation and hyphae branching occur throughout the mycelium,[25] only the hyphal tip continuously expands radially at a steady rate to reach new nutrient sources.[26,27] The rate of this linear extension has been shown to depend on various conditions such as the growth substrate

ETH Zurich, Institute of Food, Nutrition and Health, Schmelzbergstrasse 7, 8092 Zurich, Switzerland. E-mail: peter.fischer@hest.ethz.ch, patrick.ruehs@hest.ethz.ch

(carbon and nitrogen supply), temperature, humidity, pH, illumination, and gas composition of the atmosphere.[20,28–33] Although the growth conditions of fungi are well-studied on standard media such as malt extract and potato dextrose media,[34,35] substrate additives and the viscoelastic properties of the substrates are often overlooked, even though they show a significant morphological effect on fungal growth.[21]

Here, we study mycelium proliferation by modifying the chemical and physical properties of the substrate. We investigated how the substrate composition, such as carbohydrates and proteins, affects growth proliferation and mycelium thickness. The growth of fungi was monitored by measuring the fungal growth diameter and the height of mycelium. Since substrate alterations often lead to changes in the mechanical properties, we modified the mechanical properties by increasing the agar concentration or replacing the agar with other polysaccharides and protein sources. By consolidating all growth parameters, we demonstrate that we can grow fungi in a designed porous matrix with a fine-tuned substrate composition.

II. Materials and methods

A. Fungal strains

Strains of six mushroom-forming fungi were selected: *Pleurotus djamor* (*salmoneo-stramineus*) strain no. 101004 was obtained in a living cell culture flask from Tyroler Glückspilze (Innsbruck, Austria). The mycelium of *Ganoderma lucidum* was purchased from Grown.bio (Heerewaarden, Netherlands) on digested Grow-It-Yourself Mushroom® material. The four remaining fungi were obtained as liquid pure cultures from MycoGenetics (Münster, Germany): *Grifola frondosa* MG6110, *Laetiporus sulphurous* MG14800, *Lentinula edodes* MG2500, and *Pleurotus ostreatus* MG1005.

B. Growth media

The standard malt agar (SMA) consisted of 2 wt% malt extract from barley (Morga, Ebnat-Kappel, Switzerland), 0.2 wt% yeast extract (Sigma-Aldrich, Darmstadt, Germany), and 2 wt% agar (Morga). The influence of carbon supply, protein supply, gelling agents, and gel strength on the growth vigor of fungi was investigated by modifying the SMA accordingly.

The effect of malt extract, glucose and yeast extract in SMA was studied by varying their concentrations. The influence of carbon supply was studied by replacing the 2 wt% malt extract of the SMA with 0.1 or 5 wt% malt extract or 10 or 25 wt% glucose. The effect of increasing gel strength was investigated for SMA with 0.4, 2, 5 and 10 wt% agar. The role of alternative gelling agents was investigated by using 20 wt% corn starch, 3 wt% κ-carrageenan and 2.8 wt% guar gum (Unipektin, Vidogum G200 1, Switzerland) instead of agar. The influence of plant-based protein concentrates as nutrient source was investigated by adding 5 wt% protein concentrate to the SMA. Protein concentrates of faba bean (Vitessence Pulse CT36002, protein concentration c_p = 58%), pea (Vitessence Pulse CT 1552, c_p = 51%), and lentil (Vitessence Pulse 2550, c_p = 52%) were obtained from Ingredion (Germany). Protein concentrates of pumpkin seed (c_p = 60%), sunflower seed heliaflor (c_p = 55%), hempseed (c_p = 80%), oat (c_p = 55%), and rice (c_p = 80%) were obtained by Kündig (Switzerland). All protein samples were concentrates, *i.e.*, contain polysaccharides and lipids, and were used as received to study the influence of widely available raw materials. Polysaccharides were also used as received to simulate closely real-world conditions as possible. The influence of the medium pH on the mycelium growth was investigated by adjusting the pH of the SMA (pH = 6.7) by adding monohydrated crystalline citric acid (Amavita Pharmacy, Switzerland) and sodium bicarbonate (baking soda Betty Bossi, Coop, Switzerland).

C. Culture conditions

Only actively growing mycelium cultures without spores were used for the inoculation procedure. All axenic stock mycelium cultures were first inoculated and evenly spread on solid agar plates using a sterile loop. After five days of incubation of the stock mycelium cultures, single mycelium pre-colonies of each fungal strain were isolated by using a scalpel and transferred to the SMA using a spatula. The mycelium stock cultures were maintained by subculturing mycelium colonies every two weeks and incubating them at 24 °C and 85% RH. Mycelium pre-cultures were incubated at 24 (*G. frondosa*, *L. edodes*) and 30 °C (*P. ostreatus*, *P. djamor*, *G. lucidum*, *L. sulphureus*) with a relative humidity of 85% RH. All mycelium cultures were incubated in the absence of light since darkness promotes vegetative mycelium growth and prevents the initiation of the reproductive growth phase.[26,28,36]

D. Assessment of mycelium growth: radial extension

The growth kinetics of the mycelium cultures were determined by measuring the radial extension over time. Mycelium discs from the pre-cultures were obtained by punching out cylindrical discs with a truncated sterile pipette with a diameter of 7.0 mm (VWR International). Only mycelium discs obtained from the edge of the fungal pre-culture colony were used as inoculum. The mycelium discs were placed at the side of the Petri dishes. Radial extension was measured from the inoculum to the tips of the hyphal using a ruler. Measurements were performed every 24 h until the test series were completed. The mean radial extension was averaged from three independent colonies and plotted as a function of time and fitted with a linear model (Data Analysis Software, Origin2019). The extension rate was obtained from the slope of the linear function considering the time interval of at least three days.

E. pH development during incubation

SMA with pH 6.7 and SMA with adjusted pH of 4.5 and 9.2 were used to investigate transient changes in pH during fungal growth. The pH values of the media were measured after seven days of incubation at the bottom of the plates. The values at the center and the edge of the plate were averaged from three replicates. Non-inoculated plates operated as a negative control. The pH ranges of the autoclaved media were measured

by using a flat membrane glass electrode (780 pH Meter, Metrohm, Switzerland).

F. Rheological properties

The rheological measurements were carried out using an MCR702 twin drive rheometer with a rough plate geometry PP25/S of 25 mm (Anton Paar) at 22 °C. Before the measurement, a normal force of approximately 1 N was applied to avoid slipping, and samples were allowed to relax for a few minutes. Depending on the height of the sample, the gap ranged from 1.5 to 2.5 mm. Amplitude sweep tests were performed at a constant frequency of 1 Hz and a logarithmic strain increase from 0.01 to 100%. The frequency dependence of the samples was tested in the range of 0.1 to 30 Hz. A constant strain of 0.1% was fixed within the linear viscoelastic range. Measurements were carried out in duplicates or triplicates and the calculated standard deviations are in the size of the data points.

G. Foam medium, foaming, inoculation, and incubation

The formulation of the chickpea-based foam is based on 15 wt% chickpeas (Cirio, Coop), 5.5 wt% malt extract (Morga), and 3 wt% agar (Morga). This formulation provided a higher malt concentration than the standard SMA and resulted in a higher pH of 5.9. To minimize any detrimental chemical reactions during sterilization, chickpeas were autoclaved separately from the malt and agar. Both liquids were autoclaved at 121 °C for 20 min. Before sterilization, chickpeas were mixed in 200 ml of water until a smooth viscous liquid was reached (WMF, Cult Pro). Malt and agar as well as some droplets of red food dye (Patissier, Migros) were blended with the remaining water. In the foaming process, the 55 °C hot medium was pressure aerated by using a Kisag whipper (Kisag whipper professional, 1 L, Kisag Bellach, Switzerland). For inoculation, the foams were directly transferred to actively growing mycelium cultures of *P. ostreatus*. Round microboxes were filled with SMA, onto which subsequently mycelium discs were placed. The microboxes were incubated at 30 °C for one week before the constructed foam was placed onto the colony. After inoculation, the microboxes were closed again and incubated for another week.

H. Compression testing of the colonized foam

The mechanical properties of the pure foam matrix and the colonized foam were investigated in compression tests using a texture analyzer fitted with a 10 cm blade (Stable Micro Systems TA.XT2i, United Kingdom). The test settings were uniaxial compression with return to start, pre-test speed of 2.0 mm s^{-1}, test speed of 0.2 mm s^{-1}, and post-test speed of 10 mm s^{-1} with trigger force of 0.049 N and deformation to 50%. Mechanical parameters were obtained from stress–strain curves derived from the force–distance profiles.[37,38] The appropriate stress was calculated by dividing the applied force by the initial cross-sectional area. Young's modulus was obtained from the slope of the linear part of the stress–strain curve at low deformation (below 10%). Following the method of Tacer-Caba,[15] the compressive strength was obtained from the calculated stress at 35% strain. Values were averaged from five replicates.

III. Results and discussion

A. Fungal screening on malt agar as a function of temperature

Fungal mycelium proliferation across the surface largely depends on the strain type and incubation temperature. To screen for competitive and fast-growing fungi, we selected six strains of filamentous fungi (*P. djamor*, *P. ostreatus*, *G. lucidum*, *Laetiporus sulphureus*, *Lentinula edodes*, *Grifola frondosa*). By exploring mycelium growth on standard malt agar (SMA), various distinctive morphological features and considerably different growth rates were observed among the studied fungi (Fig. 1A). *P. djamor*, *P. ostreatus* and *G. lucidum* grew relatively fast and revealed a thick mycelium surface density. Their mycelium covered almost the whole plate of 9 cm diameter after four days of incubation (Fig. 1B). In contrast, *L. edodes* and *G. frondosa* showed rather slow and irregular mycelium growth. Their colony radii were less than 1 cm after four days of incubation. These findings align with previous work reporting relatively fast mycelium spawning by *Pleurotus* spp. and *G. lucidum* compared to the time needed for substrate colonization by *L. edodes* and *G. frondosa*.[28,36,39,40] To determine a suitable incubation temperature, *P. ostreatus*, *G. lucidum*, and *P. djamor* were grown at 22, 24, and 30 °C, while 85% RH and dark light conditions were kept constant. All three fungi grew well at these temperatures with the largest mycelium colony radii at 30 °C (Fig. 1C).[29,30,32,33] For the following experiments, the oyster mushrooms *P. djamor* and *P. ostreatus* were selected for their rapid radial expansion.

B. Influence of substrate composition on growth vigor

The impact of substrate composition on the growth vigor of *P. djamor* and *P. ostreatus* was investigated in SMA with varying concentrations of malt and sugar (Fig. 2A and B). With an increase in malt extract concentration from 0.1 to 5 wt%, the radial extension rate decreased while the growth height and mycelium surface density increased. This trend was observed for both fungi (Fig. 2C and F). Typically, fungi show rapid growth but sparse mycelium when nutrients are scarce (guerilla) and slower but denser growth when rich resources are available (phalanx).[23,26,41] An overall increase in growth vigor of both fungi could be achieved on the substrate with a malt extract content of 5 wt% (Fig. 2A, B, D, and E). Taking the example of *P. ostreatus*, the mycelium appeared about 1 mm thick and expanded at a rate of approximately 0.9 cm per day. Expressed as radial area, *P. ostreatus* occupied 22 cm^2 after only four days of incubation, supporting its widely recognized fast growth.[23,26,40] 10 or 25 wt% glucose resulted in reduced or inhibited growth vigor. At 10 wt% glucose concentrations, *P. djamor* showed a considerably reduced extension rate, growth height, and mycelium density while at 25 wt% glucose growth was completely inhibited. This limited growth could be related to a shift towards the phalanx growth strategy due to

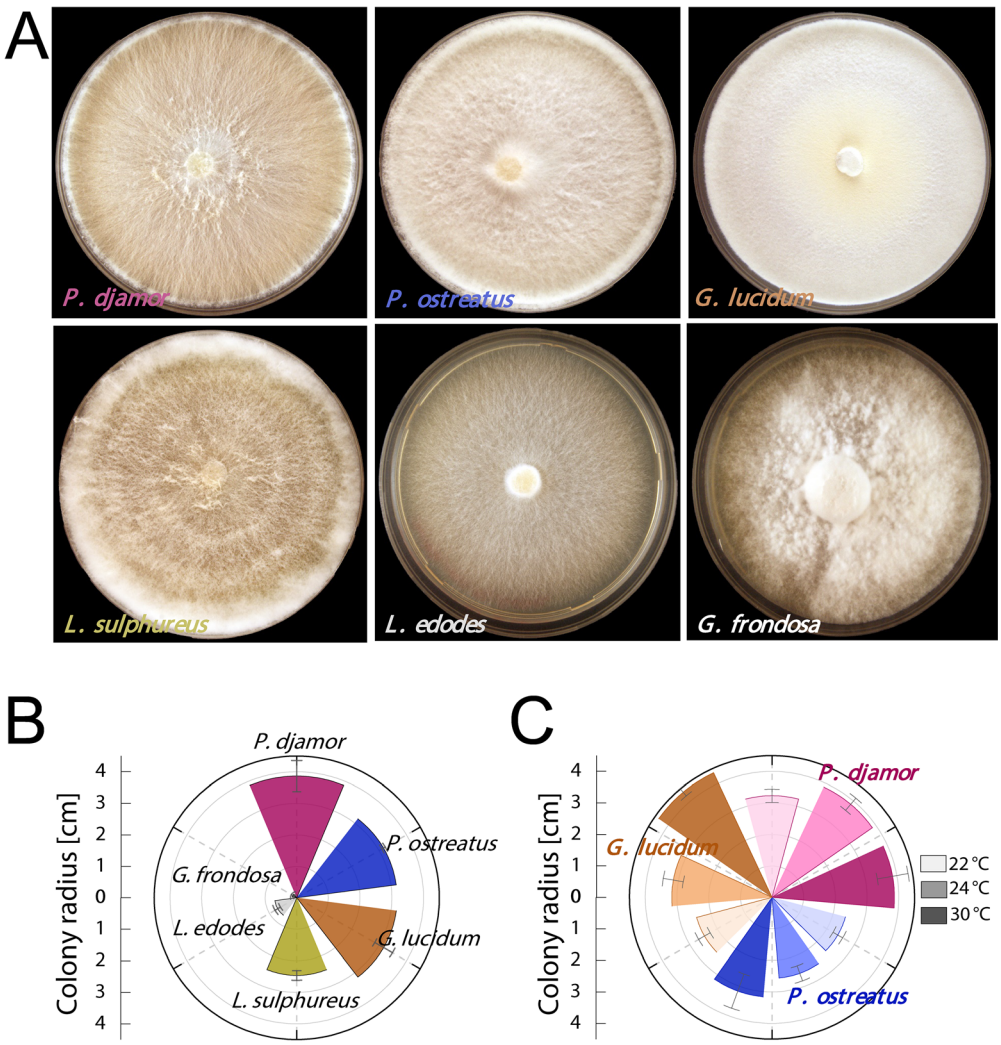

Fig. 1 Mycelium growth of selected fungi on SMA medium. (A) Morphological characteristics of fungal colonies once plate was fully covered by mycelium. Incubation time varied from five days to three weeks. Plate diameter = 9 cm. (B) Colony radii after 4 days of incubation expressed as mean ± standard deviation (n = 3). *P. djamor* and *P. ostreatus* were incubated at 30 °C, 85% RH. *G. lucidum*, *L. sulphureus*, *L. edodes* and *G. frondosa* were incubated at 24 °C, 85% RH. (C) Influence of temperature on mycelium growth of *P. djamor*, *P. ostreatus* and *G. lucidum*. Colony radii are expressed as mean ± standard deviation (n = 3). Fungi were incubated for four days at 22 °C with 55% RH and at 24 as well as 30 °C with 85% RH.

nutrient abundance or decreased water activity at 25 wt% glucose (0.96) compared to SMA (0.99), even though glucose, sucrose, and molasses are considered favorable carbon sources for *Pleurotus* spp.[32] Whereas sugar is often used to preserve food, much lower water activities (0.6–0.8) are needed to stop fungal growth. To conclude, SMA supplemented with 5 wt% malt extract increased the overall growth vigor of both *Pleurotus* spp., suggesting that malt extract could be used as a growth-promoting carbon source. Glucose concentrations at 10 wt% and above limited mycelium growth of *Pleurotus* spp. Therefore, substrate design, particularly when using high-sugar waste streams, must consider and balance carbohydrate and protein concentrations.

To go beyond the typical constituents of microbial culture medium, the growth of mycelium was investigated in medium with plant-based protein concentrates as a nutrient source. As shown in Fig. 3A and B, *P. ostreatus* grew on all media composed of 5 wt% plant-based protein concentrates. However, the extension rate, growth height, and growth density differed strongly among the protein sources. The highest growth vigor was observed in the concentrate of oat, sunflower seed and pea protein, which appeared even higher than in SMA. Consistent with these results, enhanced mycelium growth of *P. ostreatus* on medium supplemented with soybeans was reported by Hoa *et al.*[32] The results are also in good agreement with mushroom production guidelines that suggest an increased mushroom yield with nitrogen supplemented spawn substrates.[36,42,43] This effect seems plausible, as it is known that nitrogen is an essential nutrient for most fungi and is used for the synthesis of proteins, vitamins, and cell wall components.[28,44] In conclusion, the results revealed that plant-based protein concentrates can be used as the substrate, particularly pea, oat, and sunflower seed protein concentrates can promote mycelium growth. However, no group-specific growth pattern was

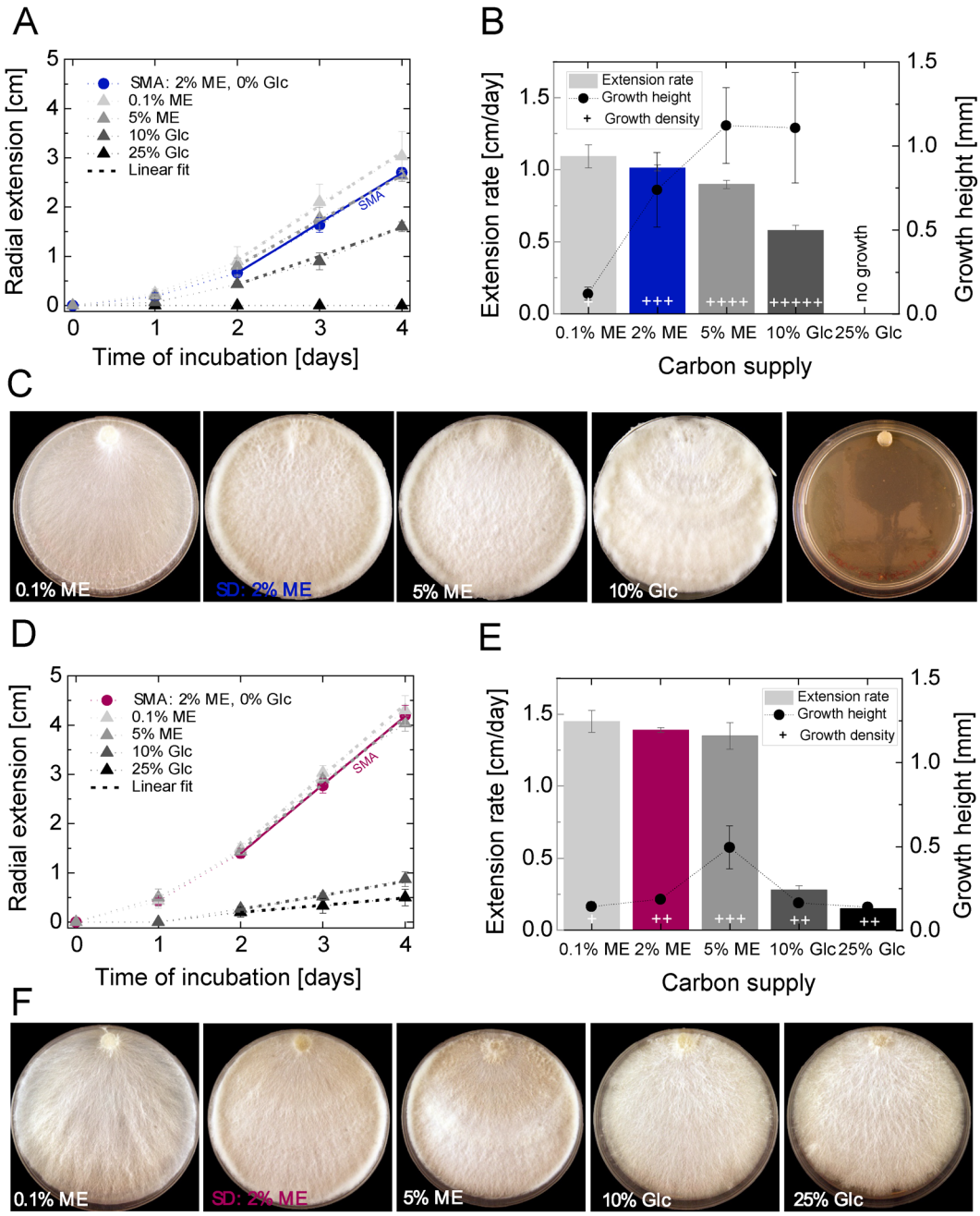

Fig. 2 Influence of malt extract and glucose on mycelium growth. *P. ostreatus* and *P. djamor* were incubated on SMA medium and media with varying malt extract (ME) and glucose (Glc) concentrations at 30 °C and 85% RH. Values are expressed as mean ± standard deviation (n = 3). (A) and (D) show radial extension over time. (B) and (E) show the extension rate in comparison to mycelium density and growth height. In (C) and (F) the morphological features of fungal colonies once the plates were fully covered by mycelium. Incubation time varied from seven days to three weeks. Plate diameter = 9 cm.

observed with respect to the common plant-based protein types of pulses, cereals, and seeds.

The impact of increasing protein concentrate content on the growth of *P. ostrateus* was assessed on media consisting of 5, 10, 15, and 20 wt% pea protein concentrate (Fig. 3C and D). While *P. ostreatus* grew comparably well on media with 5 and 10 wt%, a decline in the extension rate was observed on media with 15 and 20 wt% pea protein concentrate. Since the growth assays on gel strength showed a positive trend with increasing agar gel strength, it seems unlikely that the observed decline in growth rate was related to differences in the gel strength. The decline in growth rate was also unlikely associated with the water activity and the pH of the media, as both medium characteristics remained at the same level for all mentioned media.

C. Effect of pH on fungal proliferation

Natural substrates such as side streams and other plant-based sources that could be used for fungal mycelium formation

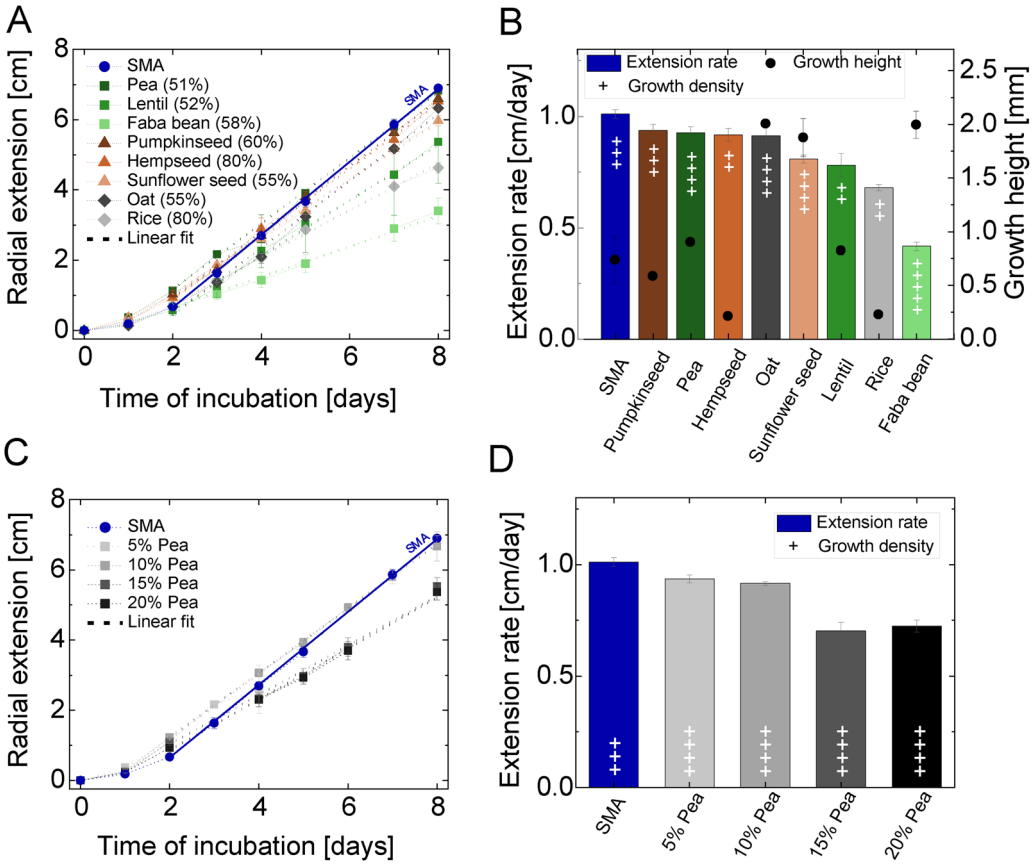

Fig. 3 Mycelium growth of *P. ostreatus* on selected pulse-, seed- and cereal-based protein concentrates. *P. ostreatus* was incubated for eight days at 30 °C. Growth on media containing 5 wt% protein concentrate and its effect on (A) radial extension, (B) extension rate, growth height and mycelium density. Effect of increasing weight percentage of pea protein concentrate on (C) radial extension and (D) extension rate and mycelium density. Values shown are means ± standard deviation (n = 3).

vary in pH and are often not close to the optimum pH. Therefore, we measured the influence of pH on mycelial growth and pH development during incubation (Fig. 4) for fungal growth on SMA with adjusted pH levels ranging from 3.1 to 9.2. Fig. 4A and B show images of growth after 11 days of incubation. Fig. 4C and D show that *P. ostreatus* exhibited the fastest radial extension at a pH of 6.7, closely followed by growth on substrates with pH of 5.2 and 4.5. Radial extension was slower at pH of 3.2 and pH of 9.2. Comparable results were obtained for mycelium growth of *P. djamor* (Fig. 4E and F). As previously observed, *Pleurotus* spp. showed maximal growth at a pH of 6 to 7.[29] As pH alters cellular functions such as gene expression and enzymatic activities. As a consequence, fungi can alter the environmental pH by secreting protons and organic acids to achieve pH stability.[44] This regulatory ability was also observed as regardless of the initial pH, all media showed a similar pH of around 5 to 6 after seven days of incubation (Fig. 4B). The observed pH development can clearly be attributed to fungal metabolism, as the non-inoculated control plates did not show pH changes. In summary, the growth of *Pleurotus* spp. is the highest at neutral pH, where the fungi are able to regulate the pH to neutral values around 7.

D. Effect of viscoelasticity on fungal proliferation

The influence of viscoelasticity of the substrate material on mycelium growth of *Pleurotus* spp. was investigated on SMA with 0.4, 2, 5, and 10 wt% agar (Fig. 5). Viscoelasticity was measured by small amplitude oscillatory shear rheology as a function of strain and frequency (Fig. 5A and B). With increasing agar concentration, the viscoelasticity increased with higher storage G' and loss G'' moduli and a slight reduction in the linear viscoelastic region. G' was predominantly higher than G'' over most of the strain and frequency range, indicating a gel-like behavior. The increasing gel strength G' increased the growth vigor of *P. ostreatus* and *P. djamor* (Fig. 5C–F). The growth assays on higher agar concentrations showed higher extension rates, growth heights, and mycelium densities. This positive trend was more pronounced for *P. djamor* than for *P. ostreatus*, which could also be verified through visual inspection of the morphology of the colonies. Larger growth vigor on stronger gels seems plausible as *Pleurotus* spp. naturally colonize solid materials such as wood.[26]

To verify the hypothesis that a stronger gel network G' increases growth vigor and to demonstrate material versatility, we investigated the impact of viscoelasticity by using different polysaccharides than agar (Fig. 6). Bacterial cellulose pellicles,

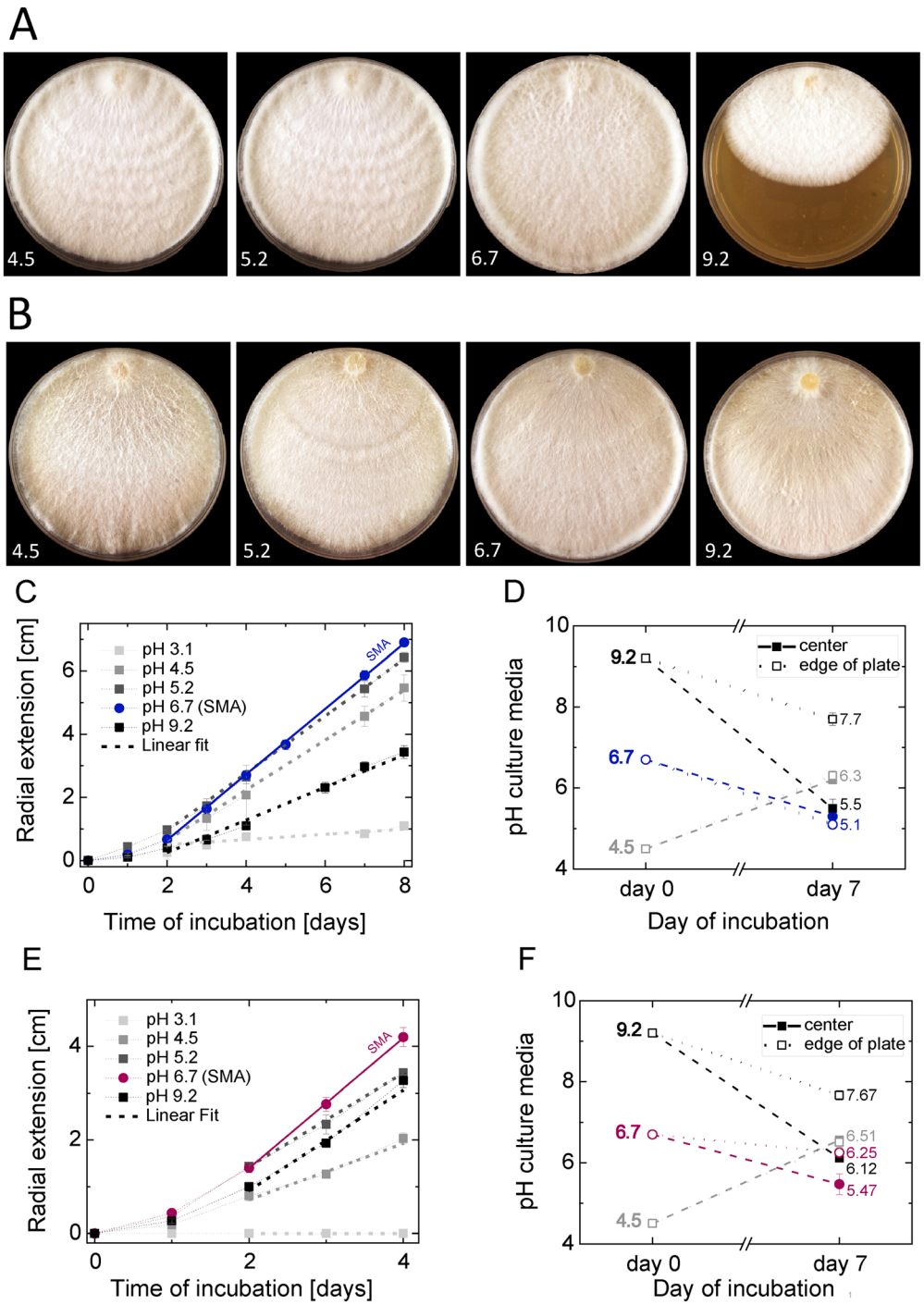

Fig. 4 Influence of pH on the morphology of the mycelium. (A) Mycelium of *P. ostreatus* and (B) *P. djamor* after 11 days of incubation at 30 °C and at 85% RH on SMA media with a adjusted pH to 4.5, 5.2, 6.7 and 9.2. (C) Radial extension of *P. ostreatus* on SMA with adjusted pH ranging from 3.1 to 9.2. (D) pH of the media at the bottom of the plate prior inoculation with *P. ostreatus* and after 7 days of incubation at 30 °C and 85% RH. (E) Radial extension of *P. djamor* on SMA with adjusted pH ranging from 3.1 to 9.2. (F) pH of the media at the bottom of the plate prior inoculation with *P. djamor* and after 7 days of incubation at 30 °C and 85% RH. Values shown are means ± standard deviation (n = 3).

guar gum, κ-carrageenan, and corn starch were used as materials with varying viscoelastic properties (Fig. 6A and B). These polysaccharides substitute agar, but the substrate composition was equal to SMA. The 20 wt% corn-starch-based medium and the bacterial cellulose pellicles exhibited similar strain and frequency-dependent behavior as the agar-based SMA. In contrast, the κ-carrageenan-based medium and the guar gum-based medium showed considerably lower viscoelastic moduli. Whereas 3 wt% κ-carrageenan appeared to form a weak gel with an almost uniform solid response over the frequency window,

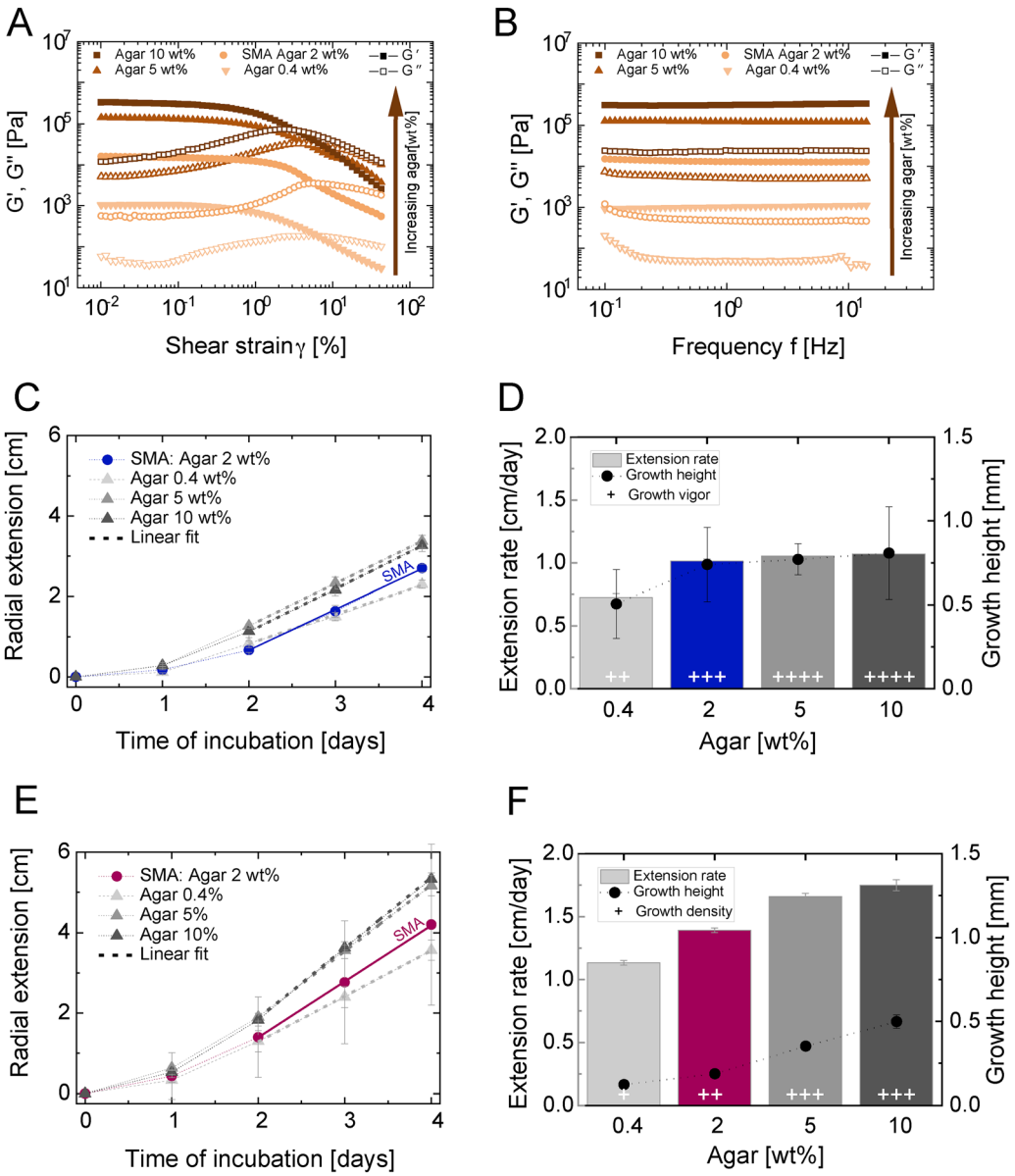

Fig. 5 Influence of agar gel strength on mycelium growth. Development of the storage modulus G' and loss G'' moduli of SMA with 0.4, 2, 5, and 10 wt% agar upon applied (A) strain and (B) frequency. (C) and (E) show the radial extension of *P. ostreatus* and *P. djamor* over time as a function of gel strength. (D) and (F) show the extension rate in comparison to growth height and mycelium density. Values are expressed as mean ± standard deviation (n = 3).

2.8 wt% guar gum seemed to yield an intermediate state between liquid- and solid-like behavior. In these two media, the mycelium morphology appears to be more branched and fluffier than in SMA, and the extension rate decreased by almost 50% compared to SMA, *i.e.*, show a declining extension rate with decreasing gel strength. Corn starch and bacterial cellulose with an elasticity G' between 10^4 and 10^5 Pa showed similar extension rates as observed for agar concentrations of 5 and 10 wt%. Radial extension, extension rate, and morphological properties are shown in Fig. 6C, D, and E, respectively. Although bacterial cellulose forms a morphological different network,[45] *P. ostreatus* also colonized the bacterial cellulose pellicles, indicating that the mycelium can use scaffolds with different physical properties to grow. Growth on alternative polysaccharides reveal and confirm the hypothesis that elastic gel-like networks increase the radial expansion of mycelium with increasing elasticity G'. However, radial expansion only assesses superficial mycelium formation and does not reveal growth into the substrate.

E. 3D growth on foamed substrate

By consolidating the growth factors mentioned above, we developed a foam substrate that enabled 3D growth of *P. ostreatus* (Fig. 7A). Processing conditions and the determined growth factors defined the formulation of the foam substrate. The foam was made of 15 wt% chickpea, 3 wt% agar, and

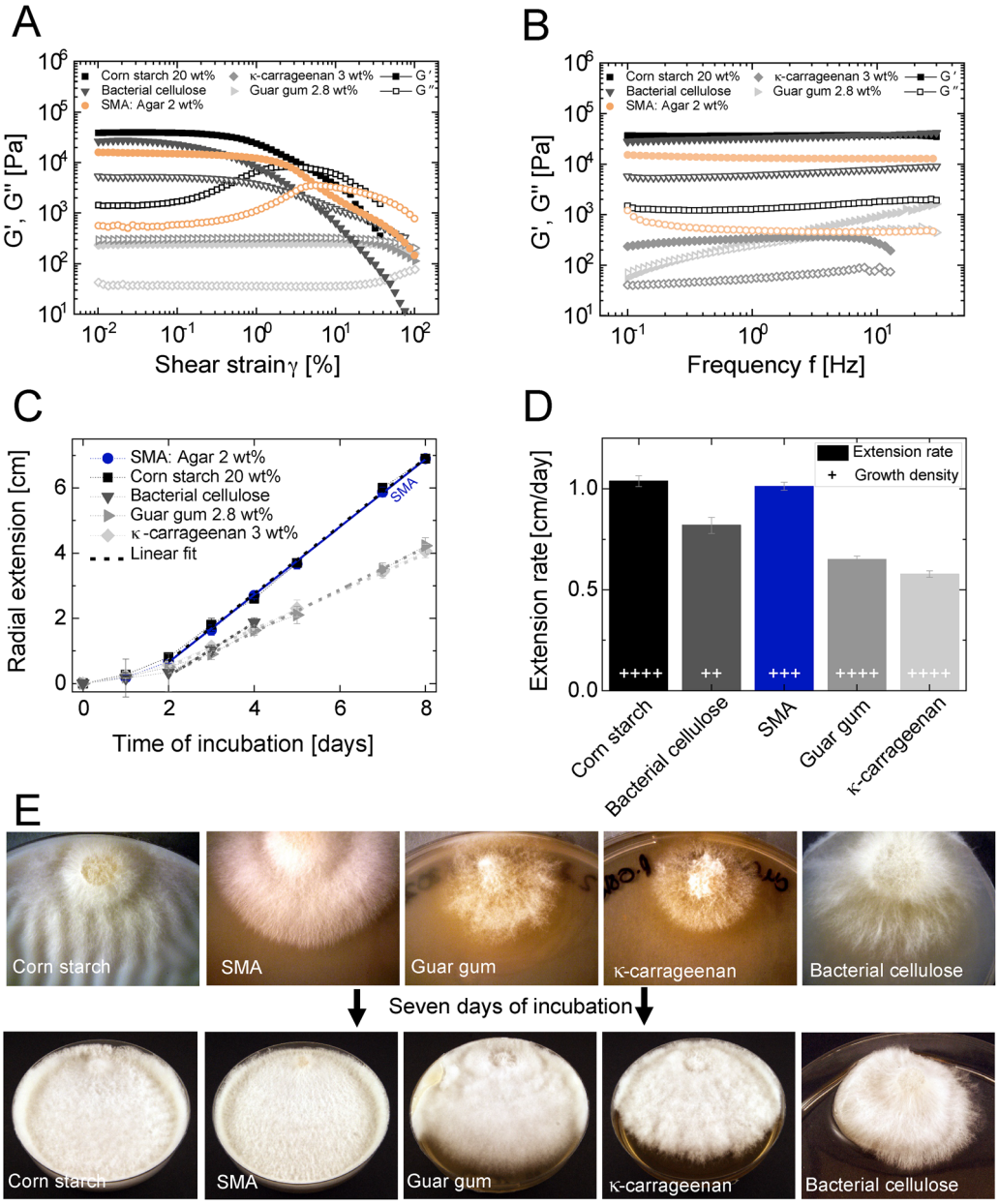

Fig. 6 Mycelium growth of *P. ostreatus* on gel and gel-like media. Storage modulus G' and loss modulus G'' of SMA and media composed of alternative polysaccharides upon applied (A) shear and (B) frequency. Effect of viscoleastic properties of the culture media on (C) radial extension over time and (D) extension rate and growth vigor. Values are expressed as mean ± standard deviation (n = 3). (E) Morphological characteristics of *P. ostreatus* colonies after four and eleven days of incubation at 30 °C and 85% RH. Plate diameter = 9 cm.

5.5 wt% malt extract resulting in a pH of 5.9. The viscoelasticity of the foam substrate is comparable to the gel strength of the SMA with 5 wt% agar and was expected to be favorable for mycelium growth. The growth assay revealed a slightly lower radial extension on the foam medium than on the SMA with 5 wt% agar (9.1 compared to 10.5 mm per day). However, the mycelium density appeared considerably larger and the height of the mycelium mat was twice as high on the foam medium (1.47 ± 0.12 mm) than on the SMA with 5 wt% agar (0.74 ± 0.22 mm). This increased mycelial growth is probably related to the nutrient-rich formulation of the foam compared to SMA. Thus, the growth assay showed that the foam medium provides the necessary nutrients for abundant mycelium growth of *P. ostreatus*.

The colonization of the foam substantially affected its mechanical behavior in compression (Fig. 7B and Table 1) shows, exemplarily, the stress–strain behavior only after the foam substrate was fully colonized by the fungi. Intermediate time points, as shown in Nussbaum *et al.*[14] were not recorded to not compromise the growth vigor by damaging the foam's structure or by contamination. Standard deviations (n = 5) are due to some variations in the foaming process and potential

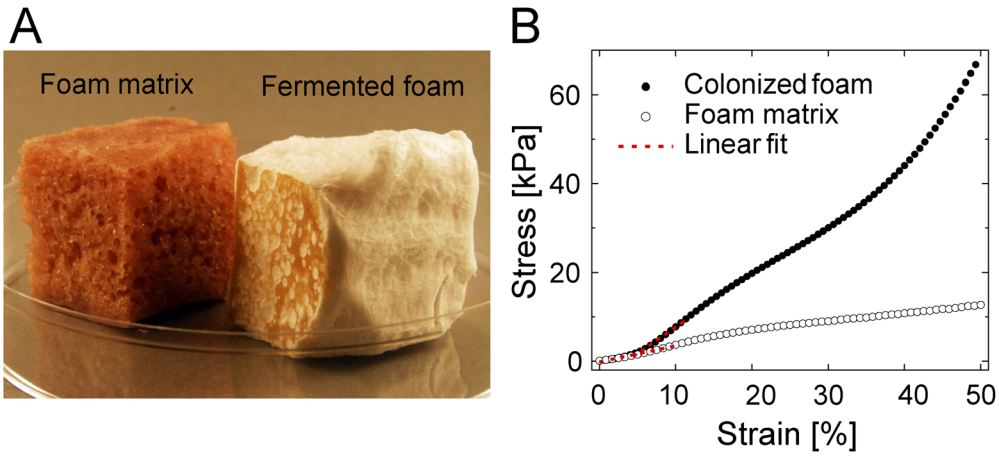

Fig. 7 Colonization and compression behavior of the fresh and colonized foam matrix. The foam matrix was composed of 15 wt% chickpeas, 5.5 wt% malt and 3 wt% agar. The foam matrix was incubated with the mycelium of *P. ostreatus* for one week at 30 °C and 85% RH. (A) Foam matrix and colonized foam matrix. (B) Typical experimental stress–strain behavior of the foam matrix and the colonized foam matrix. The stress–strain curve is obtained after the foam substrate was fully colonized by the fungi. Young's modulus and compressive strength as listed in Table 1 were obtained from the linear stress–strain regime indicated as linear fit.

Table 1 Compression testing of the foam matrix and the colonized foam. Young's modulus = E [kPa], compressive strength = σ [kPa] at 35% strain, water loss during compression = ΔW [%], and respective approximated densities prior compression = ρ [kg m^{-3}]. Values are expressed as mean ± standard deviation from 5 replicates. *P. ostreatus* was incubated for 1 week at 30 °C and 85% RH

Foam	E [kPa]	σ [kPa]	ΔW [%]	ρ [kg m^{-3}]
Control	28 ± 7	10 ± 3	4.2 ± 2.5	469 ± 60
Colonized	84 ± 33	37 ± 4	1.4 ± 1.4	608 ± 91

different fungal growth as summarized in Table 1. However, the reproducibility of the foam colonization can be guaranteed by well-maintained sterile conditions and no interference with the growth process. Before inoculation, the foam showed the expected stress–strain relationship of typical three-dimensional cellular solids.[46,47] An initial linear elastic regime in the stress–strain curve, which is attributed to the bending of the cell edges is indicated by the dotted line (linear fit) in Fig. 7B. The prolonged stress plateau after reaching a strain of about 15% can explained by progressive cell collapse. Typically, a third regime of densification of cellular solids should be observed at higher strains, which was not the case here. Contrary to the control foam without fungal growth, the colonized foam did not yield a typically expected compression curve for cellular solids. No pronounced compression plateau was observed. As the linear elastic region was followed by a steep increase in stress, it can be assumed that the mycelium growth in the foam pores substantially contributed to the mechanical response. Previous work suggested that the fungal cell wall polymer chitin provides mechanical strength to mycelium and mycelium compounds.[7,19,48,49]

In comparison, the colonized foam yielded a substantially larger Young's modulus and compressive strength than the control foam (Table 1). Similar to the literature values,[10,14] the compressive strength of the colonized foam was in the range of 10 to 30 kPa. Higher values of the compressive strength were reported for mycelium-colonized straw and sawdust within 150 kPa up to 4 MPa.[1,10,13] For example, *P. ostreatus* incubated on oat husk and rapeseed-cakes[15] revealed a compressive strength ranging from 17 to 300 kPa depending on the fungal strain, growth substrate, and mechanical post-processing. Mycelium-colonized wood hydrogel scaffolds[7] reach about 100 kPa in tensile strength depending on the fungi. The Young's modulus of the colonized foam in Fig. 7 is in the order of 90 kPa, substantially smaller as reported for substrates used for construction or any kind of load-bearing structures. Straw, sawdust, and wood substrates[1,8,13] are in the regime of several 100 kPa up to 2 MPa. However, direct comparison is sometimes hampered as boundary conditions for tensile and compression test might vary in literature. In conclusion, mycelium-based composites generally shown an increased stiffness and mechanical strength, but the complex interaction leading to the final mechanical properties are still to be studied. On the same note, oxygen availability is an important boundary condition for fungal growth and can be limited by reduced mass transport in foam structures. Influencing factors are bubble size, bubble size distribution, and interconnectivity of the bubbles. Generally, with increasing surface area, increased growth vigor can be observed,[14] but a detailed investigation of the parameter space is work in progress.

IV. Conclusion

Our study highlights the critical role of both chemical and physical growth environments to enhance the growth vigor of filamentous fungi, particularly *Pleurotus ostreatus* and *Pleurotus djamor*. We identified key factors that significantly influence fungal growth by systematically varying carbohydrate and protein concentrations, pH levels, and substrate viscoelasticity.

The successful transition from 2D to 3D growth using an optimized foamed substrate demonstrates the potential for practical applications of engineered living materials. These findings pave the way for the development of robust, biodegradable materials by strategically manipulating fungal growth conditions. The presented work discusses the use of different food materials such as proteins, polysaccharides, sugars, and lipids on fungal proliferation. All raw materials used for the alternative growth media were food-grade with a low purification grade (proteins) or used as received (polysaccharides, sugars, lipids) to reflect real-world application for versatile fungi-biocomposites. While the results indicate clear trends, several boundary conditions could be considered in future work. Gelling behavior from weak to brittle gels, gel strength, and water activity of the polysaccharides influences the growth vigor and could be monitored with a selection of different gel-forming polysaccharides or mixtures thereof. Higher purification levels of the proteins (*i.e.* protein isolates), optimization of the foaming process towards defined bubble sizes and an open foam structure, and faster growing fungi species will certainly also provide a more detailed picture of the influencing parameters.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The data supporting this article are included in this manuscript.

Acknowledgements

The authors thank Planted Foods AG for their scientific support and continuous discussions. NN, PR, and PF acknowledge the Gebert Rüf Stiftung (GRS-095/20 – Self-Grown Meat Analogues) for support.

References

1 M. Jones, A. Mautner, S. Luenco, A. Bismarck and S. John, Engineered mycelium composite construction materials from fungal biorefineries: A critical review, *Mater. Des.*, 2020, **187**, 108397.
2 M. R. Binelli, P. A. Rühs, G. Pisaturo, S. Leu, E. Trachsel and A. R. Studart, Living materials made by 3D printing cellulose-producing bacteria in granular gels, *Biomater. Adv.*, 2022, **141**, 213095.
3 E. Elsacker, M. Zhang and M. Dade-Robertson, Fungal engineered living materials: The viability of pure mycelium materials with self-healing functionalities, *Adv. Funct. Mater.*, 2023, **33**, 202301875.
4 J. H. Teoh, E. Soh and H. Le Ferrand, Manipulating fungal growth in engineered living materials through precise deposition of nutrients, *Int. J. Bioprint.*, 2024, **10**, 3939.
5 S. C. Shen, N. A. Lee, W. J. Lockett, A. D. Acuil, H. B. Gazdus, B. N. Spitzer and M. J. Buehler, Robust myco-composites: a biocomposite platform for versatile hybrid-living materials, *Mater. Horiz.*, 2024, **11**, 1689–1703.
6 A. Sinha, L. G. Greca, N. Kummer, C. Wobill, C. Reyes, P. Fischer, S. Campioni and G. Nyström, Living fiber dispersions from mycelium as a new sustainable platform for advanced materials, *Adv. Mater.*, 2025, **37**, 2418464.
7 C. Wobill, Z. Zhang, P. Fischer and P. A. Rühs, Anisotropic growth of filamentous fungi in wood hydrogel composites increases mechanical properties, *ACS Appl. Biomater.*, 2025, **8**, 5024–5031.
8 E. Elsacker, S. Vandelook, J. Brancart, E. Peeters and L. De Laet, Mechanical, physical and chemical characterisation of mycelium-based composites with different types of lignocellulosic substrates, *PLoS One*, 2019, **14**, e0213954.
9 E. Soh, Z. Y. Chew, N. Saeidi, A. Javadian, D. Hebel and H. Le Ferrand, Development of an extrudable paste to build mycelium-bound composites, *Mater. Des.*, 2020, **195**, 109058.
10 C. Girometta, A. M. Picco, R. M. Baiguera, D. Dondi, S. Babbini, M. Cartabia, M. Pellegrini and E. Savino, Physico-mechanical and thermodynamic properties of mycelium-based biocomposites: A review, *Sustainability*, 2019, **11**, 281.
11 D. Sharma and H. Le Ferrand, 3d printed gyroid scaffolds enabling strong and thermally insulating mycelium-bound composites for greener infrastructures, *Nat. Commun.*, 2025, **16**, 5775.
12 E. Soh, N. Loh, J. H. Teoh, A. Jain and H. Le Ferrand, Biodegradable mycelium tiles with elephant skin inspired texture for thermal regulation of buildings, *Energy Build.*, 2025, **328**, 115187.
13 D. Raslan, E. Elsacker, K. B. Debnath and M. Dade-Robertson, The role of surface interventions in bio-welding mycelium based-composites, *Sustainable Mater. Technol.*, 2025, **44**, 01326.
14 N. Nussbaum, N. Repond, A. Gandia, P. Fischer and P. A. Rühs, Mycelial growth of wood fungus ganoderma sessile in porous scaffolds, *Mater. Today Bio*, 2025, **35**, 102082.
15 Z. Tacer-Caba, J. J. Varis, P. Lankinen and K. S. Mikkonen, Comparison of novel fungal mycelia strains and sustainable growth substrates to produce humidity-resistant biocomposites, *Mater. Des.*, 2020, **192**, 108728.
16 Z. J. Yang, F. Zhang, B. Still, M. White and P. Amstislavski, Physical and mechanical properties of fungal mycelium-based biofoam, *J. Mater. Civ. Eng.*, 2017, **29**, 04017030.
17 F. V. Appels, S. Camere, M. Montalti, E. Karana, K. M. Jansen, J. Dijksterhuis, P. Krijgsheld and H. A. Wösten, Fabrication factors influencing mechanical, moisture- and water-related properties of mycelium-based composites, *Mater. Des.*, 2019, **161**, 64–71.
18 M. R. Islam, G. Tudryn, R. Bucinell, L. Schadler and R. C. Picu, Morphology and mechanics of fungal mycelium, *Sci. Rep.*, 2017, **7**, 13070.
19 M. Haneef, L. Ceseracciu, C. Canale, I. S. Bayer, J. A. Heredia-Guerrero and A. Athanassiou, Advanced materials

from fungal mycelium: Fabrication and tuning of physical properties, *Sci. Rep.*, 2017, **7**, 41292.
20 N. Nussbaum, T. von Wyl, A. Gandia, E. Romanens, P. A. Rühs and P. Fischer, Impact of malt concentration in solid substrate on mycelial growth and network connectivity in Ganoderma species, *Sci. Rep.*, 2023, **13**, 21051.
21 C. Wobill, P. Azzari, P. Fischer and P. A. Rühs, Host material viscoelasticity determines wrinkling of fungal films, *ACS Biomater. Sci. Eng.*, 2024, **10**, 6241–6249.
22 N. Money, Biomechanics of invasive hyphal growth, *Biology of the Fungal Cell wall*, Springer, 2007, pp. 237–247.
23 N. A. R. Gow and G. M. Gadd, *The growing fungus*, Chapman and Hall, 1995, p. 478.
24 L. Boddy, Saprotrophic cord-forming fungi: Warfare strategies and other ecological aspects, *Mycol. Res.*, 1993, **97**, 641–655.
25 N. Nussbaum, L. Balmelli, N. Steiger, L. Nyström, P. Fischer and P. A. Rühs, Quantifying fungal growth in 3D: An ergosterol-based method to distinguish growth modes, *Mater. Adv.*, 2025, **6**, 7261–7272.
26 D. Moore, G. D. Robson and A. P. J. Trinci, *21st Century Guidebook to Fungi*, Cambridge University Press, 2011.
27 A. P. Trinci, A study of the kinetics of hyphal extension and branch initiation of fungal mycelia, *J. Gen. Microbiol.*, 1974, **81**, 225–236.
28 S.-T. Chang and P. G. Miles, *Mushrooms: cultivation, nutritional value, medicinal effect, and environmental impact*, CRC Press, 2004.
29 H. Sardar, M. Ali, C. Ayyub and R. Ahmad, Effects of different culture media, temperature and pH levels on the growth of wild and exotic Pleurotus species, *Pak. J. Phytopathol.*, 2015, **27**, 139–145.
30 I. Fletcher, A. Freer, A. Ahmed and P. Fitzgerald, Effect of temperature and growth media on mycelium growth of Pleurotus ostreatus and Ganoderma lucidum strains, *Cohesive J. Microbiol. Infect. Dis. 2*, 2019, **2**(5), CJMI.000549.
31 P. C. Guadarrama-Mendoza, G. Valencia del Toro, R. Ramírez-Carrillo, F. Robles-Martínez, J. Yáñez-Fernández, M. E. Garín-Aguilar, C. G. Hernández and G. Bravo-Villa, Morphology and mycelial growth rate of Pleurotus spp. strains from the Mexican mixtec region, *Braz. J. Microbiol.*, 2014, **45**, 861–872.
32 H. T. Hoa and C. L. Wang, The effects of temperature and nutritional conditions on mycelium growth of two oyster mushrooms (Pleurotus ostreatus and Pleurotus cystidiosus), *Mycobiology*, 2015, **43**, 14–23.
33 G. E. Zharare, S. M. Kabanda and J. Z. Poku, Effects of temperature and hydrogen peroxide on mycelial growth of eight Pleurotus strains, *Sci. Hortic.*, 2010, **125**, 95–102.
34 T. Luangharn, S. C. Karunarathna, K. D. Hyde and E. Chukeatirote, Optimal conditions of mycelia growth of Laetiporus sulphureus sensu lato, *Mycology*, 2014, **5**, 221–227.
35 J. Le, S. Hu and M. Xu, Optimisation of submerged culture conditions for the production of mycelial biomass and exopolysaccharide by Pleurotus nebrodensis, *Ann. Microbiol.*, 2007, **57**, 389–393.
36 D. C. Zied and A. Pardo-Giménez, *Edible and medicinal mushrooms*, Wiley Blackwell, 2017, p. 592.
37 H. A. Kuhn and C. T. Corporation, Uniaxial compression testing, *Mech. Test. Eval.*, 2018, **8**, 143–151.
38 ASTM E111, Standard test method for young's modulus, tangent modulus, and chord modulus, ASTM International, 2017, DOI: **10.1520/E0111-04R10**.
39 S. Montoya, C. E. Orrego and L. Levin, Growth, fruiting and lignocellulolytic enzyme production by the edible mushroom Grifola frondosa (maitake), *World J. Microbiol. Biotechnol.*, 2012, **28**, 1533–1541.
40 M. J. Carlile, S. C. Watkinson and G. W. Gooday, The economic significance of fungi, *The Fungi*, Academic Press, 2001, pp. 461–542.
41 S. Gantenbein, R. Colucci, J. Käch, E. Trachsel, F. B. Coulter, P. A. Rühs, K. Masania and A. R. Studart, Three-dimensional printing of mycelium hydrogels into living complex materials, *Nat. Mater.*, 2023, **22**, 128–134.
42 N. R. Curvetto, D. Figlas, R. Devalis and S. Delmastro, Growth and productivity of different Pleurotus ostreatus strains on sunflower seed hulls supplemented with N-NH4+ and/or Mn(II), *Bioresour. Technol.*, 2002, **84**, 171–176.
43 R. Naraian, R. K. Sahu, S. Kumar, S. K. Garg, C. S. Singh and R. S. Kanaujia, Influence of different nitrogen rich supplements during cultivation of Pleurotus florida on corn cob substrate, *Environmentalist*, 2009, **29**, 1–7.
44 S. C. Watkinson, Physiology and adaptation, *The Fungi*, Academic Press, 3rd edn, 2015, pp. 141–187.
45 M. K. Hausmann, P. A. Rühs, G. Siqueira, J. Läuger, R. Libanori, T. Zimmermann and A. R. Studart, Dynamics of cellulose nanocrystal alignment during 3D printing, *ACS Nano*, 2018, **12**, 6926–6937.
46 L. Gibson and M. Ashby, *Cellular solids: Structure and properties*, Cambridge Universtiy Press, 1997.
47 L. J. Gibson, Biomechanics of cellular solids, *J. Biomech.*, 2005, **38**, 377–399.
48 N. A. R. Gow, J.-P. Latge and C. A. Munro, The fungal cell wall: Structure, biosynthesis, and function, *The Fungal Kingdom*, John Wiley & Sons, Ltd, 2017, pp. 267–292.
49 N. P. Money, Fungal Cell Biology and Development, *The Fungi*, Academic Press, 3rd edn, 2016, pp. 37–66.