

Full Length Research Paper

Morphological characteristics of mycelia growth of two strains of the indigenous medicinal mushroom, *Lentinus squarrosulus* Mont. (Singer), on solid media

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Morphological characteristics of the mycelia growth of two wild strains of *Lentinus squarrosulus* Mont. (Singer), *Sqw* and *Lsf*, collected from the Volta and Greater Accra regions of Ghana respectively, were studied. Growth characteristics including mycelia growth rate and mycelia density and morphology by the 7th and 26th days of incubation on solid media formulated from four lignocellulosic wastes: elephant grass (EG), rice straw (RS), thatch (TH) and sawdust (SD) were classified. Tissue cultures of the fruit bodies were prepared on Malt Extract Agar (MEA). Eight-day old cultures of the subsequently prepared 1st generation cultures were inoculated on the media and incubated at 25°C. Strain *Sqw* recorded lower growth rates (between 0.47 and 0.64 cm/day) on the solid media than *Lsf* (between 0.69 and 0.93 cm/day). The maximum growth rate of strain *Sqw* was 0.64 cm/day on both SD and TH whereas that of *Lsf* was 0.93 cm/day on EG. *L. squarrosulus* mycelia density is not dependent on the growth rate and vice-versa, irrespective of the strain. Although both strains generally had the longitudinally radial morphology with concentric rings, with extended incubation, the culture morphology of both strains changed, usually into thick mats. Mycelia of both strains on all the media were white at the initial stages of incubation. Cultures of strain *Sqw* largely remained whitish and turned brownish only on EG, whereas strain *Lsf* turned into different shades of brown on all media with extended incubation. These colour changes were not uniform on the entire plate, appearing in undefined sectors. Mycelia growth characteristics were seen to be substrate and strain-dependent. Further investigations of these observations could uncover some behavior of *L. squarrosulus* such as changes in enzyme profile and the phenolic content, which could have applications in biotechnology.

Key words: Culture morphology, growth rate, mycelia density, indigenous, lignocellulosic waste, solid media, strains.

INTRODUCTION

The human diet is very diverse, and includes products ranging from plants to seafood to other animals. Mushrooms are fungi, which have occupied a part of this diversity in the human diet over centuries, due to their

nutritional, medicinal, and organoleptic properties including aroma, flavor and taste (Chang, 1998; Zawirska-Wojtasiak, 2004). Fruit bodies of mushrooms were mainly collected from the wild in their natural habitats in the past.

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However, due to increasing demand and problems such as hallucinations, allergic reactions and sometimes death caused by ingestion of inedible and/or poisonous mushrooms collected by inexperienced collectors, the need arose to develop technologies to ensure the production of edible and/or medicinal mushrooms. So far, *Agaricus* spp. are the most cultivated mushrooms worldwide (Chang, 1998). Other cultivated mushrooms include members of the genus *Auricularia* (Tang et al., 2010), *Pleurotus* (Cohen et al., 2002; Obodai et al., 2011; Royse, 1992) and, to a lesser extent, *Lentinus* (Adesina et al., 2011; Mhd Omar et al., 2011; Nwanze et al., 2006; Pukahuta et al., 2008).

Lentinus squarrosulus Mont. (Singer) is a white rot fungus of the order, Polyporales. The mushroom is usually found in the wild on dead or decaying wood (Karunarathna et al., 2011) of various trees. It is widely known as an indigenous mushroom in Nigeria (Adesina et al., 2011; Nwanze et al., 2005), having been reported to have various organoleptic, nutritional and medicinal attributes (Mhd Omar et al., 2011; Nwanze et al., 2006). *L. squarrosulus* has been reported to be rich in proteins, sugars, lipids, amino acids, vitamins B, C, and D, and minerals (Mhd Omar et al., 2011; Royse et al., 1990). According to Mhd Omar et al. (2011), liquid mycelia extract of the mushroom have ulcer prevention and healing capabilities in rats.

Despite the diverse research output on *L. squarrosulus* cultivation on various substrates under various conditions (Adesina et al., 2011; Mhd Omar et al., 2011; Nwanze et al., 2005; Okhuoya et al., 2005), nutritional composition and medicinal properties (Bhunja et al., 2011; Bhunja et al., 2010; Mhd Omar et al., 2011), toxicological evaluation (Kadiri, 2005), enzyme profile (Mtui, 2012; Tripathi et al., 2012; Wuyep et al., 2003), and effect of different media on the mycelial growth of submerged cultures (Subramonian et al., 2010), there is very little information on the mycelia morphological characterization on solid media. However, Atri and Lata (2013) have reported on the vegetative growth of *Lentinus cladopus* Lév on both commercial solid and liquid media including Malt Extract Agar, Potato Dextrose Agar, Pea Extract Agar, Potato Malt Agar, Malt Broth, Potato Dextrose Broth, Czapek Solution, Glucose Asparagine Medium, Glucose Peptone Medium, and Dimmick Medium.

According to Chang (1998) the important steps preceding the development of active spawn for mushroom cultivation include selection of an acceptable mushroom species and secreting a good quality fruiting culture. In addition, because of the known phenomenon of species-specificity of fruiting mycelium, the minimal information on the mycelia morphology of *L. squarrosulus* on solid media is considered a problem even though Stamets and Chilton (1983) have indicated that rhizomorphic and longitudinally radial mycelia are more likely to produce primordia than cottony mycelia. This is more so because the culture behavior of mushrooms has been reported (Sharma and Atri, 2013) to be directly

linked with cultivation and pharmaceutical aspects of the mushrooms.

This report therefore seeks to characterize and document the mycelial behavior of two strains of indigenous *L. squarrosulus* on various solid media formulated from four lignocellulosic wastes: elephant grass (EG), rice straw (RS), thatch (TH), and sawdust (SD), in terms of their mycelia growth rates, densities, and morphologies.

MATERIALS AND METHODS

Mushroom strain

L. squarrosulus strains from two sources were used; *L. squarrosulus* strain *Sqw* (Figure 1A) collected on a mango tree during a field trip at Wli Agumatsa waterfalls, a suburb at the Volta Region of Ghana, and strain *Lsf* (Figure 1B) collected on the log of a jack tree at the CSIR-Food Research Institute premises in Accra. Identification of the mushrooms was done with macro-morphological features. Samples of fresh fruit bodies of both strains were used in the preparation of tissue cultures.

Mushroom tissue culture preparation

Malt Extract Agar (MEA; OXOID™ Ltd., England) was prepared and sterilized according to the manufacturer's instructions but with supplementation of the carbon source with dextrose (20 g/L). Each poured plate of MEA was aseptically inoculated with an aliquot of the inside tissue from the region between the cap and stipe of fresh *L. squarrosulus* fruit bodies. Plates were incubated in the dark at 25°C in an incubator (Tuttlingen™ WTC Binder, Germany) to obtain the tissue cultures. Upon complete colonization of the MEA (8 days of incubation), the tissue cultures were sub-cultured on MEA plates and incubated under the same conditions as the tissue cultures. This culture, referred to as the 1st generation culture, was used for further studies.

Solid media preparation and inoculation

Solid media were prepared from dried and chopped straw-based lignocellulosic wastes (elephant grass (EG), rice straw (RS) and thatch (TH)), and fresh sawdust (SD) respectively. Substrates (75 g each) were separately soaked in 1.1 L of tap water for 1 hour (to allow leaching of nutrients from the substrate into the water), after which the supernatants were decanted and filtered through cotton wool into a measuring cylinder. To each of the filtrates, 17.5 g agar and 20 g glucose were added and stirred. The resulting solutions were separately brought to a boil on a hot plate while stirring (to avoid lump formation). The prepared solid media were distributed into clean conical flasks after ensuring the agar and glucose were well dissolved in the solution (about 5 mins of boiling). All solid media were sterilized at 121°C for 1h. Cooled solid media were aseptically poured into the required number of 9 cm diameter petri dishes and allowed to set. The various substrates were each inoculated with 1 cm² disks of 8-day old 1st generation cultures. The experiment was performed in triplicates.

Measurement of mycelia growth rates and mycelia densities

Radial mycelia growth was measured by taking daily readings of mycelia extensions from the center of the inocula on the plates in two perpendicular directions with a ruler. This was taken initially on



Figure 1A. Picture of *L. squarrosulus* strain Sqw in natural habitat



Figure 1B. Picture of *L. squarrosulus* strain Lsf in natural habitat.

the third day of incubation and consequently after every 24 h for 4 days. Readings were taken in the same perpendicular angle each time and the readings were taken following the same sequence each day. Recorded values per day are means of the two readings taken at the perpendicular angle.

Mycelia densities were recorded by direct observation of the plates based on the thickness of the mycelia as judged by the observers. The mycelia densities on the plates were rated from 1 to 5 with 1 being the least dense and 5 being highly dense. Least dense mycelia were defined as mycelia, which are barely visible on the solid media and are transparent such that the media can be observed through the mycelia when observed from the cover of the plate. On the other hand, highly dense mycelia were defined as mycelia, which are very thick and are opaque such that the solid media cannot be seen through them when the plate is observed from the cover. In case of plates with sectorized mycelia, the mycelia density was judged based on the density of the most prominent (with regards to size of sector) mycelia on the plate.

Statistical analysis

Data obtained from triplicates were analyzed with GenStat Discovery Software (4th Edition). The table of correlations was generated with the software. Mycelia growth rates were obtained from linear trendline equations from the plot of the incubation period versus the radial mycelia extension averaged from the triplicates of each setup.

RESULTS AND DISCUSSION

Mycelia growth characteristics

Wide variations of the radial mycelia extension of *L. squarrosulus* strain Sqw on the various substrates studied on the 3rd day of incubation were obtained among the triplicates for all the substrates (Figure 2A). The mean values recorded were negatively skewed on all the substrates for the strain and the mycelia extension on EG was significantly lower than that observed on RS and TH by the third day of incubation. Mycelia growth on SD did not differ significantly from that recorded on the other substrates (EG, RS and TH).

The radial mycelia extension of *L. squarrosulus* strain Lsf on the various substrates on the third day of incubation from the triplicates on EG and TH had minimal variation (Figure 2B). Mycelia growth of the strain was significantly fastest on EG followed by the growth on RS, whereas slower growth was observed on SD and TH (Figure 2B). *L. squarrosulus* strain Lsf was more vigorous under the study conditions, showing longer mycelia extensions than *L. squarrosulus* strain Sqw on the solid media by the third day of incubation (Figure 2A and B). No outliers were recorded among the data sets obtained for both strains (Figure 2A and B; outliers would be indicated by green or red asterisks on the affected box and whisker in the graphs).

The mycelia growth rates of *L. squarrosulus* strain Sqw (Figure 3A) on the solid media were 0.64, 0.64, 0.60 and 0.47 cm/day on SD, TH, RS and EG respectively. However, mycelia growth rates of *L. squarrosulus* strain Lsf on the substrates (Figure 3B) followed a different pattern and the growth rates on EG, RS, TH, and SD were 0.93, 0.91, 0.78, and 0.69 cm/day respectively.

The observed trend of different mycelia growth on various solid media has also been reported in another study on *L. cladopus* Lév (Atri and Guleria, 2013). According to the authors, taxonomically *L. cladopus* is quite close to *L. squarrosulus* Mont. in having a similar morphology and hyphal construction but differs from it in having thin pileus, lacking squamules and presence of broader spores. However, the recorded growth rates of both strains on all the solid media studied were lower than that recorded (1.8 cm/day) for indigenous Indian *L. squarrosulus* grown on Potatoes Dextrose Agar (PDA) at 27±1°C (Sharma and Atri, 2013). The discrepancy observed could be attributed to the growth media used, the incubation temperature or a combination of both factors.

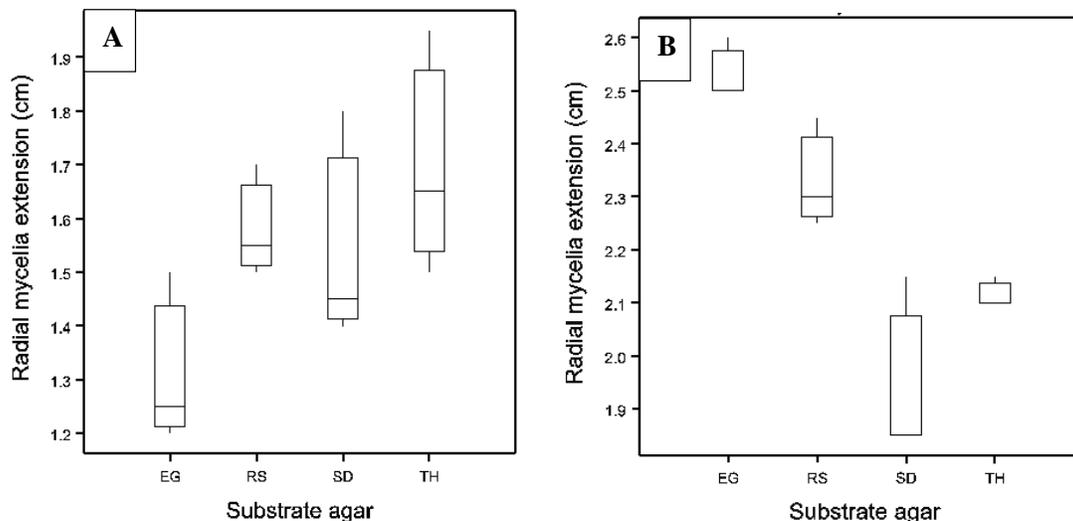


Figure 2. (a) Radial mycelia extension of *L. squarrosulus* strain Sqw on third day of incubation in relation to solid media. n=3. (b) Radial mycelia extension of *L. squarrosulus* strain Lsf on third day of incubation in relation to solid media. n=3.

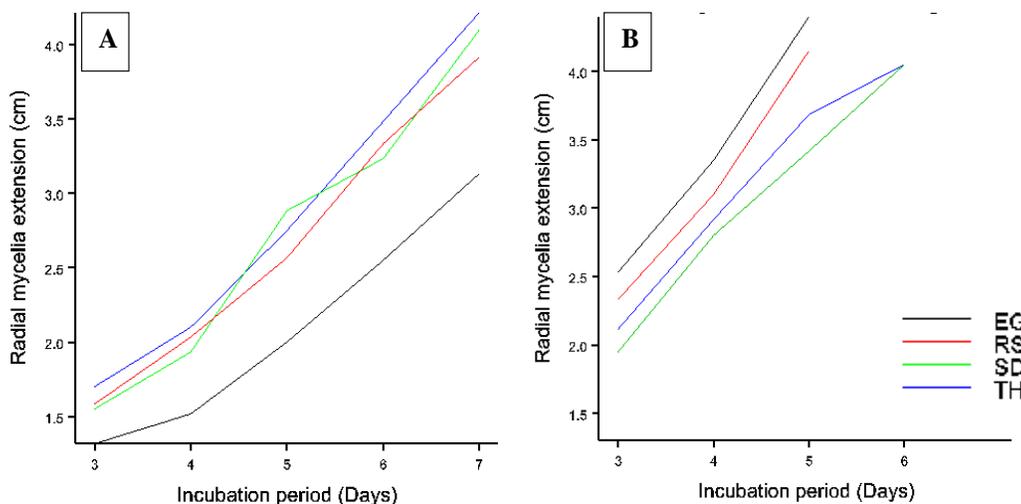


Figure 3. (a) Mycelia growth of *L. squarrosulus* strain Sqw within one week of incubation. n=3. and (b) Mycelia growth of *L. squarrosulus* strain Lsf within one week of incubation. n=3.

The results of this study demonstrate that *L. squarrosulus* media preference is strain specific.

Mycelia morphologies on solid media

The mycelia morphologies of the strains, presented in Figure 4 and Figure 5, varied on the various solid media under the conditions employed in this study.

Culture morphology of *L. squarrosulus* strain Sqw

On EG, the mycelia showed linear growth with clear

concentric rings and relatively dense mycelia (Figure 4A). While the linear growth with concentric morphology was also observed on RS and TH (Figure 4B and C respectively), the rings were not as pronounced as that observed on EG (Figure 4A). The prominence of the concentric rings on these solid media was in the order, EG>RS>TH. The culture morphology on RS was relatively uniform in comparison to that observed on TH (Figure 4B and C respectively). There were no concentric rings observed on SD (Figure 4D). On this solid media, a linear morphology with low mycelia density was observed (Figure 4D). The mycelia were white on all solid media from the beginning of incubation through to the 7th day of

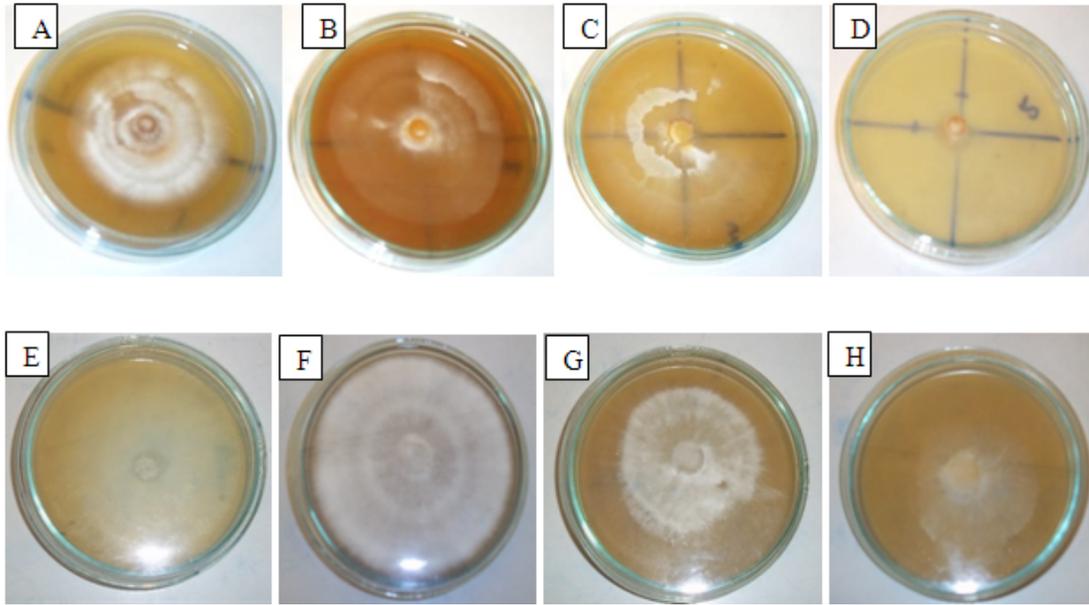


Figure 4. *L. squarrosulus* strains *Sqw* and *Lsf* (top and bottom rows respectively) colony morphology on the 7th day of incubation (young cultures) at 25°C on various solid media. A and E, B and F, C and G, and D and H represent the strains inoculated on EG, RS, TH, and SD respectively.

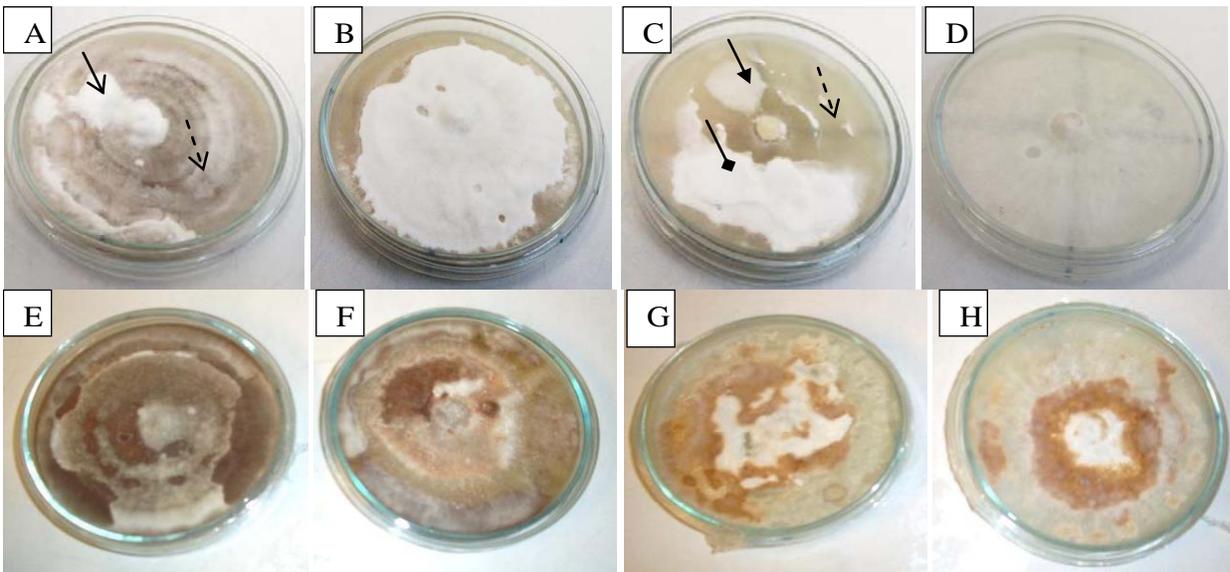


Figure 5. *Lentinus squarrosulus* strains *Sqw* and *Lsf* (top and bottom rows respectively) colony morphology and mycelia density on the 26th day of incubation (“aged” cultures) at 25 °C on various solid media. A and E, B and F, C and G, and D and H represent the strains inoculated on EG, RS, TH, and SD respectively. Arrows point to some observed sectors of mycelia colonies on the media.

incubation (Figure 4A, B, C and D) and throughout the period of substrate colonization (all solid media were fully colonized by the 10th day of incubation; data not shown). The culture morphologies on the various substrates changed upon further incubation (or with maturity) for

both strains. This was clearly apparent by the 26th day of incubation (Figure 5). Though the concentric rings observed for *L. squarrosulus* strain *Sqw* on the 7th day of incubation on EG remained prominently present with age (Figure 4A and 5A), that on RS and TH were not obvious

by the 26th day of incubation (Figure 4B and 4D and Figure 5B and 5D respectively). On the 26th day of incubation, the mycelia of *L. squarrosulus* strain *Sqw* on EG appeared polymorphic (Figure 5A). This polymorphism was in the form of sectoring and difference in mycelia coloration. For instance, the sectors with cottony mycelia were whitish (Figure 5A; solid arrow) whereas the longitudinally radial mycelia had brownish coloration. Furthermore, there were regions on this substrate (EG) where the brownish coloration had a layer of whitish mycelia over it (Figure 5A; dashed arrow). On RS, virtually the whole *L. squarrosulus* strain *Sqw* mycelia turned densely white, forming a dense mat (Figure 5B), rather than the longitudinally radial mycelia observed at the initial stages of incubation, by the 26th day of incubation. Strain *Sqw* also showed polymorphism with age on TH, on which the mycelia showed undefined sectors of densely white cottony mycelia, densely white mat of mycelia, and sectors with low mycelia density by the 26th day of incubation (Figure 5C; solid, diamond, and dashed arrows respectively). The only difference observed on SD by the 26th day of incubation was the improved mycelia density (Figure 5D). The mycelia remained uniformly white with longitudinally radial mycelia on this solid media (Figure 5D). It is worth noting that the strain only changed a colour change when cultured on EG.

Culture morphology of *L. squarrosulus* strain *Lsf*

Variations in culture morphology of the strain on the various solid media under the study conditions were eminent both at the initial incubation stage and with extended incubation (Figure 4 and 5E, F, G and H). While the strain showed longitudinally radial morphology on all the solid media studied by the 7th day of incubation (Figure 4E, F, G and H), concentric rings were absent on EG and SD (Figure 4E and H respectively) whereas there was the presence of concentric rings when cultured on RS and TH (Figure 4F and G respectively).

With extended incubation of the strain, polymorphisms were apparent on all the solid media (Figure 4E, F, G and H). As indicated for *Sqw*, the observed polymorphisms appeared in the form of sectoring and difference in mycelia coloration. The colours ranged from white to shades of brown (Figure 4E, F, G and H). Occurrence of different mycelia morphology in terms of presence or absence of concentric rings, and colour and density of mycelia has also been reported on *L. cladopus* Lév (Atri and Guleria, 2013). Comparing the culture morphologies of the 2 strains presently studied on the various solid media at both the initial and later stages of incubation, it appears reasonable to infer that *L. squarrosulus* culture morphology on solid media is strain dependent and that pigmentation of the mycelia or cultures of the strains occurs differently on the media with extended incubation.

However, this is also dependent on the strain inoculated. *Polyporus arcularius* mycelia also show a change from white, dense aerial mycelia to light brown crusts of mycelia with further incubation on Y2 solid media (Hibbett et al., 1993). Changes in colour of five *Lentinus* spp. including *L. squarrosulus* cultures from white to brown mycelia during incubation on PDA has also been reported (Sharma and Atri, 2013). Contrary to the report by Sharma and Atri (2013), the changes in mycelia colour on the solid media in this study did not occur from the middle of the plate, but rather, randomly. The authors of the said manuscript attributed the specific changes in the culture morphology to the species under study. However, based on the results of the present study, we infer that *Lentinus* spp. culture morphology also depends on the strain under study and on the growth media used.

According to Stamets and Chilton (1983), a mycelium is senescent when it grows old. The authors have indicated that signs of senescing include a change from rhizomorphic to cottony looking mycelia. Thus, it can be inferred that both sectoring and pigmentation could be signs of senescing in *L. squarrosulus* cultures.

However, based on these studies, it cannot be clearly deduced whether the pigmentation observed in the 26 day old cultures of *Lsf* is solely genetic and what the composition of the observed pigments are. It is also unclear whether this pigmentation is as a result of enzymatic browning caused by enzymes released during incubation (Tripathi et al., 2012; Wuyep et al., 2003) and the phenolic compounds present in the mycelia (Mhd Omar et al., 2011). Further studies are required to investigate these observations and to discover biotechnological applications of enzymes or compounds present in *Lsf* with extended incubation on solid media. The effect of the mycelia morphology on the quality of spawns produced with its consequent effect on fruit body yield will be investigated in further studies.

Relationship between mycelia growth rate and mycelia density

Various degrees of negative correlation were obtained between mycelia growth and the mycelia densities observed on the 7th and 26th days of incubation (Table 1) for strain *Sqw*. This was observed throughout the incubation period, regardless of the incubation time. This observation was obvious on EG, for instance, on which although the mycelia growth rate was lowest (0.47 cm/day), the mycelia densities of both the young and "aged" cultures (Figure 3 and 4 top rows respectively) were relatively high. Conversely, a strong positive correlation (0.96; Table 1) was obtained between the mycelia densities observed on the 7th and 26th days of incubation on the various solid media under the study conditions. This same trend was observed for strain *Lsf*. These results indicate that *L. squarrosulus* mycelia

Table 1. Table of correlations between mycelia extension during incubation and mycelia densities on days 7 and 26 of incubation for strain *Sqw*. A value closer to +1 means there is a high positive correlation, a value closer to -1 shows a strong negative correlation and a value of 0 means there is no correlation between compared parameters.

Correlation						
Day_3						
Day_4	0.8762					
Day_5	0.7434	0.8592				
Day_6	0.7429	0.8829	0.8772			
Day_7	0.7336	0.8414	0.9315	0.9149		
Mycelia_density_day_7	-0.5181	-0.6161	-0.8063	0.6919	-0.8482	
Mycelia_density_day_26	-0.4493	-0.4999	-0.7478	0.5774	-0.7677	0.9621
	Day_3	Day_4	Day_5	Day_6	Day_7	Mycelia_density_day_7

density is not dependent on the growth rate and vice-versa, irrespective of the strain. Thus, a strain that is growing fast on a given media does not necessarily have to have dense mycelia. However, mycelia having a high density at the beginning of incubation are more likely to have a corresponding high mycelia density at the end of incubation.

Conclusion

Mycelia growth rate, density and morphology of *L. squarrosulus* is media and strain-dependent. Cultures of the mushroom differ morphologically between the strains studied and on the different solid media. These differences are more pronounced with extended incubation. Irrespective of the strain, *L. squarrosulus* mycelia density is not dependent on the growth rate, and vice-versa.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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