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Sodium polyacrylate: a component of disposable diapers that delays mycelial growth of edible mushrooms during culture *in vitro*

Poliacrilato de sodio: un componente de los pañales desechables que retrasa el crecimiento micelial de hongos comestibles durante su cultivo *in vitro*

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Abstract

The use of edible fungi represents a promising strategy for degrading lignocellulosic residues. Due to their cellulose content, disposable diapers are susceptible to degradation by fungi; however, an unknown component of disposable diapers delays or inhibits the growth of mycelia. In this study, we analyze the effect of the different constituents of classic and biodegradable diapers on the mycelial growth of five edible fungi strains (*Pleurotus eryngii*, *Pleurotus djamor,* and *Lentinula edodes*). Kinetic parameters were used to describe the mycelium extension rate by using primary models. The combination of cellulose within the diaper's sodium polyacrylate, a type of superabsorbent polymer (SPA), delayed the lag phase (λ = 1.44 days). Cellulose contained in diapers was a factor that enhanced mycelium extension rate (μ_{max}=5.66-8.82 mm/day), while sodium polyacrylate delayed it (μ_{max} =3.20-6.35 mm/day). Based on the findings, a 5% proportion of sodium polyacrylate (superabsorbent polymer) is proposed to guarantee mycelial growth.

Keywords: sodium polyacrylate, edible mushrooms, mycelial growth, disposable diaper, biodegradation.

Resumen

El uso de hongos comestibles representa una estrategia prometedora para la degradación de residuos lignocelulósicos. Debido a su contenido de celulosa, los pañales desechables pueden ser degradados por hongos comestibles; sin embargo, un componente desconocido de los pañales desechables retrasa o inhibe su crecimiento. En este estudio se analizó el efecto de los diferentes constituyentes de los pañales clásicos y biodegradables sobre el crecimiento micelial de cinco cepas de hongos comestibles (*Pleurotus eryngii*, *Pleurotus djamor* y *Lentinula edodes*). Se utilizaron parámetros cinéticos para determinar la tasa de extensión del micelio mediante el uso de modelos primarios de crecimiento. La combinación de celulosa con el poliacrilato de sodio, un tipo de polímero superabsorbente (SPA), del pañal retrasó la fase de latencia (λ=1,62 días). La celulosa contenida en los pañales fue un factor que incrementó la tasa de extensión del micelio (μ_{max}= 5.66-8.82 mm/día), mientras que el poliacrilato de sodio lo retrasó (μ_{max} =3.92-6.12 mm/día). Se propone una proporción máxima del 5% de polímero poliacrilato de sodio para garantizar el crecimiento del micelio.

Palabras clave: poliacrilato de sodio, hongos comestibles, crecimiento micelial, pañales desechables, biodegradación.

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1 Introduction

A diaper is a personal care product originally manufactured from cloth but now crafted from various materials to allow disposability after use. Disposable diapers are considered an essential product during early childhood. Due to their easy handling, they are also utilized by elderly or ill individuals (Kawai *et al.,* 2023). In countries such as Japan, an increase in the use of disposable diapers by the elderly population has been observed (Kawai *et al*., 2023). Despite this increase, studies on adult diapers are more complex than those on diapers for young children, as they may contain medications or antibiotics. The design of a disposable diaper is intended to contain feces and efficiently absorb significant quantities of liquid. While the composition of disposable baby diapers can vary according to the brand, the EDANA Sustainability Report (2011; 2015) disclosed the typical diaper composition as follows: 25-40% cellulose $C_6H_{10}O_5$ (fluff pulp); 30-35% sodium polyacrylate -$C_3H_3NaO_2$ or superabsorbent polymer (SPA), which provides absorbency; 5-6% low-density polyethylene -C_2H_4 (LDPE) and 16-20% polypropylene -C_3H_6 (PP), as anti-spill film; and 10-17% of straps, rubber elastics, adhesive and other materials (Figure 1). It has been estimated that the annual consumption of diapers per child is approximately 2,190 units (Dey *et al.*, 2016; Khoo *et al.,* 2019). In Mexico, the most recent data on disposable diaper waste reveals a generation of 8,108 tons per day (SEMARNAT, 2020). Although alternatives like cloth diapers exist to reduce waste, disposable diapers are currently the most popular choice due to modern lifestyles. Furthermore, water scarcity in various areas of the country is one of the reasons why people prefer disposable diapers over cloth ones. This situation leads to the continuous consumption and generation of disposable diaper waste, which poses a significant challenge for proper management.

To address the concern about the environment and the excessive generation of waste materials, the diaper industry developed a biodegradable diaper based on oxo-biodegradable materials, made of chlorine-free wood pulp, non-toxic SPA, latex-free, and partially biodegradable materials (Spurrier, 2018). However, due to its variable composition, the degradation of a disposable diaper is not simple. Regardless of the diaper brand, its main component is cellulose (Bachra *et al.*, 2020), susceptible to degradation by microorganisms and filamentous fungi (Khoo *et al.*, 2019; Pathak y Navneet, 2017; Gutierrez-Rojas *et al*., 2015). Among edible fungi, those of the genus *Ganoderma* and *Pleurotus spp*. can degrade the cellulose (Martinez *et al.*, 2005; Baldrian and Valášková, 2008) contained in the diapers.

The ability of edible fungi to grow on various substrates is related to their efficient enzymatic systems and is the basis for their biotechnological applications. The potential use of cultivable fungi as an alternative treatment for degrading disposable diapers has also been previously explored by authors such as Delfín and Durán (2003) and Espinosa *et al*. (2015; 2011). These studies tested several strains of *Pleurotus ostreatus* to degrade various mixtures of lignocellulosic substrates, including pineapple crowns, coffee residues, pruning waste, and wheat straw, with different proportions of disposable diapers as a co-

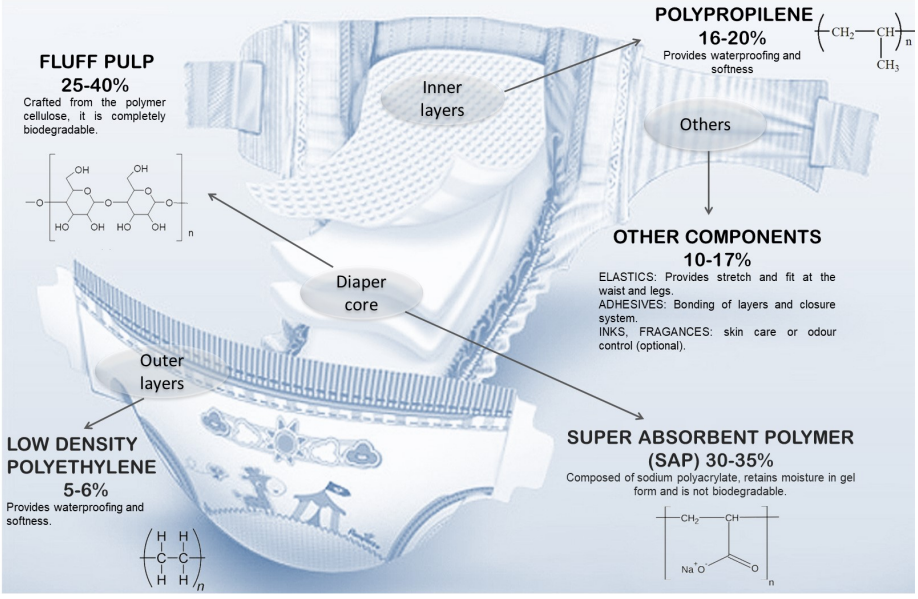

Figure 1. Typical composition of a disposable diaper according to the EDANA Sustainability Report (2011; 2015).

substrate (35% to 65%). Results were encouraging in terms of successful degradation of the cellulosic material and reduction in weight and total volume of the material. Nevertheless, low biological efficiencies (BE) were observed (1.6%–19.3%) after the fungal fruiting. According to the authors, those studies were focused on treating waste with high cellulose content rather than increasing the productivity of edible fungi. They proposed conducting further tests to optimize the process (Espinosa *et al*., 2015).

Studies such as those by Gaitán (2005) and Salmones *et al*. (1997) demonstrated that higher growth rates during *in vitro* cultivation of strains like *Pleurotus eryngii* and *Pleurotus djamor* are associated with greater biological efficiency during cultivation.

Due to the relationship between mycelial growth rate and biological efficiency —and considering the lack of information on treating disposable diaper waste using other fungal species or the effects of diaper components on mycelial growth rates— this study aimed to evaluate the response of five different edible fungal strains to *in vitro* cultivation with the main components of diapers. The study focused on the effect of each diaper constituent on the kinetic growth parameters, specifically the lag phase duration (λ) and the mycelium extension rate (μ_{max}). Generated data may allow the identification of the diaper constituent that delays or inhibits mycelial growth. This information could help determine the maximum proportion of diaper waste in substrates that allows fungal growth.

2 Materials and methods

2.1 *Edible fungi strains*

Five fungal strains were used in the present study. A commercial strain: *Pleurotus eryngii* (EMB); a wild strain isolated from the Mixtec region of Oaxaca, Mexico: *Pleurotus djamor* (DMR); and three strains donated by the Faculty of Chemistry at Universidad Nacional Autónoma de México (UNAM): *Pleurotus eryngii* (EFQ) and two strains of *Lentinula edodes* (LEN and L21). All the strains were selected for comparative purposes due to their differences in productivity parameters, specifically biological efficiency (BE) reported in previous studies (Alpuche *et al.*, 2022; Valenzuela *et al.*, 2019).

2.2 *Tester culture media*

Strains were reactivated in sterile 80-mm diameter Petri dishes containing malt extract agar (MEA, 15%) for their subsequent use in the experiments. Nine growth media were tested (Table 1), consisting of 10 mL of malt extract agar (MEA) as the basal ingredient and 5% of the main components of disposable diapers (cellulose, sodium polyacrylate, polyethylene- polypropylene, and cellulose-sodium polyacrylate mixture). To simulate realistic scenery for a diaper treatment, the experiment included diapers soaked with urine from young children. Moisture levels were replicated using distilled water in a 1:15 ratio to unused diapers. The materials were separated manually using scissors to reach the diaper's core. The core consisted of a mixture of cellulose and sodium polyacrylate. These components were carefully separated by hand. Sodium polyacrylate was removed from the cotton (cellulose) by shaking the core. Since sodium polyacrylate could not be fully detached from cotton, clean cotton was used to prepare the control cultures. The plastic material (polyethylene- polypropylene) from the outer and inner layers of the diaper (Figure 1) was cut into small pieces (0.5 cm in size). In the case of wet disposable diapers containing either urine or distilled water, the core components (sodium polyacrylate and cellulose) were incorporated into the experiment as a mixed composition due to the impossibility of separating them.

Table 1. Composition of tested culture media.

	GROWING MEDIA COMPONENT	**ABBREVIATION**
	Malt extract agar	MEA
	Cellulose *	CEL
	Sodium polyacrylate *	SPA
Disposable diaper	Cellulose/Sodium polyacrylate (clean) + MEA *	CSL
	Cellulose/Sodium polyacrylate (used) + MEA *	CSU
	Polyethylene and polypropylene + MEA *	POL
Biodegradable disposable diaper	Cellulose/Sodium Polyacrylate (clean) + MEA *	CSLB
	Cellulose/Sodium polyacrylate (used) + MEA *	CSUB
	Polyethylene and polypropylene + MEA *	POLB

*Growing media contains 5% of the indicated diaper component in 10 mL of MEA.

The experiment comprised ten replicates for each growth medium. The media were sterilized in an autoclave at 121°C and 15 psi for 20 minutes and incubated at 28°C for 48 hours to perform a sterility test before inoculating the five strains.

2.3 *Mycelial growth*

Petri dishes of 80 mm diameter, containing 10 ml of sterile media, were inoculated with an 8 mm disc of mycelium placed in the center of the dish. The cultures were incubated at 28°C, and the growth diameter was measured daily from 8 to 15 days according to the growth of every strain, using a digital Vernier.

2.4 *Statistical analysis and growth modelling*

A repeated measures analysis of variance was conducted to assess the impact of the diaper constituents added to the culture media and the strains on the average mycelial growth diameter.

To have a comprehensive knowledge of the strain's behavior and to determine the effect of the diaper components on the mycelial growth, kinetic growth parameters, such as the mycelium extension rate (μmax) and the adaptation time (λ), were calculated by using primary modeling. Three primary models were tested (data not shown): the Linear model, Baranyi's biphasic model and the modified Logistic model (Marín *et al.*, 2008; Baty and Delignette-Muller, 2004; Zwietering *et al.*, 1990). The modeling strategy employed the Levenberg–Marquardt method reported by Evangelista *et al.* (2021). The optimal model was selected based on an analysis of quadratic errors (Ross, 1996). The biphasic model developed by Baranyi was selected and is described as follows:

$$y(t_{max}) = y_{\max} + \ln \frac{-1 + \exp^{\mu_{\max}\lambda} + \exp^{\mu_{\max}t}}{(-1 + exp^{\mu_{\max}t}) + \exp^{(\mu_{\max}\lambda + y_{\max} - yo)}}$$

Where: $y0$ = inoculum diameter at day 0 (mm), $y_{\max}$ = maximum diameter of mycelial growth (mm), t = time (day), $\mu_{\max}$ = maximum extension rate (mm/day), λ = latency time or lag phase (day).

The kinetic values obtained from primary modeling were analyzed using a repeated measures analysis of variance (ANOVA). When statistical differences were detected, Duncan's Test was conducted with $\alpha = 0.05$. All analyses were performed using IBM SPSS V22 statistical software.

3 Results and discussion

3.1 *Mycelial growth according to diaper component*

To evaluate the effect of each diaper component on mycelial growth, fungal strains of *Pleurotus eryngii* (EMB and EFQ), *Pleurotus djamor* (DMR) and *Lentinula edodes* (LEN and L21) were cultivated in nine different culture media, which included each component of the diaper separately. Statistical analysis indicated significant differences in growth regarding culture media composition; five subgroups were identified according to Duncan's test. A considerable delay in mycelial growth ($p<0.05$) was found in the treatments containing SPA, reaching a diameter reduction of up to 23% compared to the control cultures (MEA) (Figure 2). These differences in growth diameter (Figure 2) were observed clearly between the control medium MEA (subgroup 5, with a diameter of 50.66 mm) and all the treatments containing SPA included in the subgroups 1, 2, and 3 (CSL, CSLB, CSU, SPA, CSUB, with diameters ranging from 38.74 to 42.66 mm). Although synthetic polymers were supposed to inhibit mycelial growth (Espinosa *et al.* 2011; 2015), the analyses grouped the synthetic polymer treatments (POL and POLB) in the same subgroup as cellulose (subgroup 4), a natural biodegradable polymer (CEL), indicating that synthetic polymers do not affect mycelial growth.

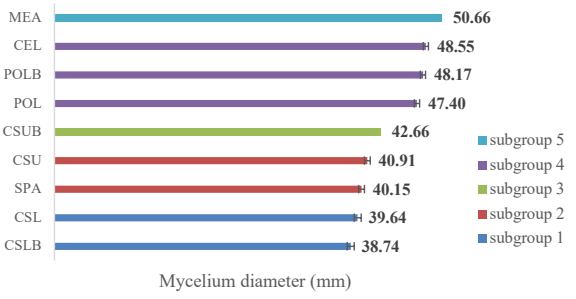

Figure 2. Mycelium diameter (mm) in different culture media after 15 days of cultivation. Homogeneous subgroups according to Duncan's test ($p< 0.05$, n=50) are indicated by the colours of the bars. MEA: malt extract agar, CEL: cellulose, CSL: classical disposable diaper's cellulose-superabsorbent polymer (clean: moistened with distilled water), CSLB: biodegradable diaper's cellulose-superabsorbent polymer (clean: moistened with distilled water), CSU: classical disposable diaper's cellulose-superabsorbent polymer (urine-wet), CSUB: biodegradable diaper's cellulose-superabsorbent polymer (urine-wet), POL: classical disposable diaper's polyethylene and polypropylene, POLB: biodegradable diaper's polyethylene and polypropylene, SPA: sodium polyacrylate.

Super absorbent polymers as sodium polyacrylate have specific features, including the capacity to absorb and trap various biological materials and inhibit microbial growth (Linn and Metters, 2006; Li *et al.*, 2021; Laftah *et al.,* 2011). Specifically, the disposable diaper industry included sodium polyacrylate because it efficiently absorbs urine within its polymeric structure and keeps it away from the baby's skin, helping to maintain dryness (Kosemund *et al.,* 2009; Braihi, 2017). The distinctive feature of hydrogels to absorb up to 1000 times their weight can vary according to the ions present in the liquid. For example, in the case of urine, ions such as nitrogen, sodium, potassium, and calcium can decrease its absorption capacity by as much as 10 times (Orzeszyna *et al.,* 2006). In the case of treatments containing urine (CSU and CSUB), no significant differences in mycelial growth were observed. Regardless, urine ions may affect the absorption capability of SPA; liquids and ions become trapped within the gelled core. Once encapsulated, trapped liquids do not alter the culture media, as they remain confined within the polymer, and no ions or liquids are released into the culture media, causing no effect on treatments.

The antimicrobial properties of SPA are extensively employed in the food packaging industry (Klein and Povorenov, 2020). However, this beneficial characteristic poses significant challenges during waste biodegradation processes. Studies conducted under both aerobic and anaerobic conditions have shown that the presence of acrylate, the monomer of polyacrylate, can alter the growth of microorganisms, such as *Escherichia coli* (Arya *et al.,* 2013). It is unclear whether acrylate affects the growth of *E. coli* by inhibiting the enzyme pyruvate formate lyase, repressing the expression of its gene (*pflB*), or interfering with its transcription (Arya *et al.,* 2013). The influence of acrylate on *E. coli* growth clearly demonstrates its critical role inhibiting essential enzymes or genes necessary for bacterial development. Sotelo et al. (2020) reported a decrease in hydrogen production during the fermentation process aimed at producing hydrogen as a biofuel. Although the specific mechanism by which SPA interferes with the fermentation process remains unknown, composting diapers to produce biofuels is currently not a viable option in the presence of SPA. In our experiment, the specific characteristics of SPA may interfere with the mycelium growth. This interference could occur through the inhibition of exogenous enzymes, altering gene expression or affecting the absorption and desorption of ions in the media by trapping nutrients from the agar (Gibas and Janik, 2010).

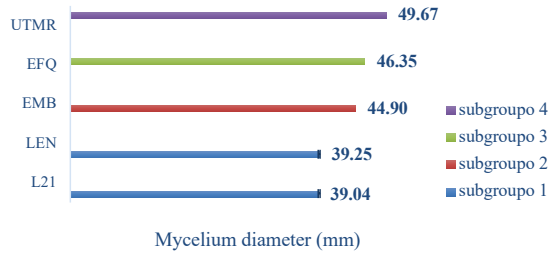

Figure 3. Mycelium diameter (mm) according to strain type after 15 days of cultivation. Homogeneous subgroups according to Duncan's test ($p< 0.05$, n=90) are indicated by the colours of the bars. DMR: *Pleurotus djamor*, EFQ and EMB: *Pleurotus eryngii* and LEN and L21: *Lentinula edodes*.

Consequently, this could limit hyphal growth and development.

3.2 Mycelial growth according to the fungi strain

Significant differences in hyphal growth diameter were found according to the strain type. Duncan's test grouped the strains into four homogenous subgroups (Figure 3). The Strains of subgroup 1 (L21 and LEN) showed the lowest growth by reaching less than 40 mm in diameter and subsequently stopped growing, even the ones cultivated on MEA (control group). Subgroups 2 and 3 included the EMB and the EFQ strains and exhibited a growth of 44.9 mm and 46.35 mm in diameter. Finally, subgroup 4, which included the DMR strain, showed the highest diameter growth (50 mm).

The growth of the *Lentinula* strains (subgroup 1) evaluated in this experiment (L21 and LEN) was lower than reported in previous studies. For example, Nogueira *et al*. (2008) assessed different *Lentinula* strains cultivated in PDA (potato dextrose agar) enriched with seven extracts of eucalyptus sawdust. They registered a growth between 45.13 and 61.4 mm after 10 days of *in vitro* culture. Villegas *et al.* (2007) also tested enriched cultures with eucalyptus sawdust, reporting 90 mm in diameter growth after 14 days. Siwulsky and Sobieralsky (2009) reported an interval growth between 28-65 mm in 14 days for 20 *Lentinula edodes* strains cultivated in wheat agar medium. Concerning growth experiments conducted with *Pleurotus,* Gaitán (2005) reported a growth diameter of 68.3 and 44.9 mm for two strains of *P. eryngii* in MEA media. The strain with the lower growth coincides with the EMB strain evaluated in this study. In contrast, our *P. djamor* strain (DMR) exhibited the highest growth (50 mm in diameter). This result coincides with the data obtained by Salmones *et al.* (2004), who documented diameters varying from 44.2 to 59.9 mm in MEA cultures.

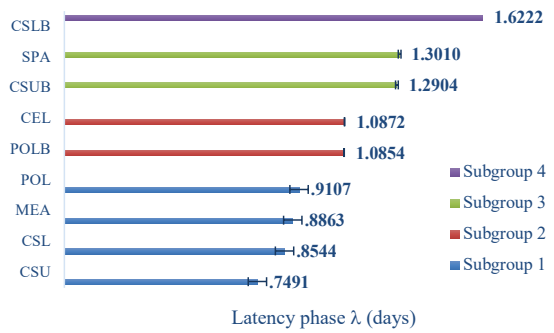

Figure 4. Latency phase values (days) of tested culture media. Different color bars indicate homogeneous sub-groups according to Duncan's tests ($p< 0.05$, n=50). MEA: malt extract agar, CEL: cellulose, CSL: classical disposable diaper's cellulose-superabsorbent polymer (clean: moistened with distilled water), CSLB: biodegradable diaper's cellulose-superabsorbent polymer (clean: moistened with distilled water), CSU: classical disposable diaper's cellulose-superabsorbent polymer (urine-wet), CSUB: biodegradable diaper's cellulose-superabsorbent polymer (urine-wet), POL: classical disposable diaper's polyethylene and polypropylene, POLB: biodegradable diaper's polyethylene and polypropylene, SPA: sodium polyacrylate.

3.3 *Influence of the culture media on kinetic parameters*

The statistical analysis (ANOVA, $p<0.05$) confirmed variations in kinetic parameters (lag phase λ and mycelium extension rate μ_{max}) of the strains depending on the specific diaper component incorporated into the culture medium.

3.3.1 *Latency phase (λ)*

The latency phase (λ) is a short adaptation period for microorganisms in a new culture medium. This period indicates the strain's aptitude to adapt to new environments or conditions. Our results showed that for each strain, there are significant variations in the lag phase according to the diaper component added to the culture medium (Table 2). Based on Duncan's test, the culture media were grouped into four homogeneous subgroups (Figure 4). The first subgroup comprised the treatments MEA (control), POL, CSL and CSU with lower values than one day. The second subgroup comprised the media POLB and CEL with λ values of 1.08 days. A significant delay in the lag phase was observed in subgroups three and four (CSLB, SPA and CSUB treatments), with λ values of 1.29 to 1.62 days.

The λ values (Table 2) for the EMB strain varied between 0.49 ± 0.31 to 1.39 ± 0.57 days, whereas the values for the EFQ strain were 0.19 ± 0.13 to 1.02 ± 0.50 days. These values were lower than those obtained by Alpuche *et al.* (2022) for the same strains (1.5 days and 2.5 days) cultivated in MEA and coincide with the adaptation phase of the hybrid reconstructed strains (HEMBXEFQ: 0.2 to 0.4 days and REFQ: 1.0 to 1.7 days) reported by the same authors.

Table 2. Latency phase values (days) of five edible mushroom strains cultivated in nine culture media. Different letters indicate significant differences according to Duncan's test ($p<0.05$, n=10). Capital letters indicate comparisons between different strains and lowercase letters indicate differences of culture media.

CULTURE MEDIA	EMB	EFQ	L21	LEN	DMR
MEA	0.94 ± 0.23^{Aa}	1.00 ± 0.27^{Aa}	0.66 ± 0.24^{Ba}	1.21 ± 0.34^{Da}	0.63 ± 0.17^{Ca}
CEL	1.39 ± 0.57^{Ab}	0.89 ± 0.19^{Ab}	0.47 ± 0.53^{Bb}	0.95 ± 0.21^{Db}	1.53 ± 0.23^{Cb}
CSL	0.75 ± 0.31^{Aa}	0.19 ± 0.13^{Aa}		0.65 ± 0.40^{Da}	1.82 ± 0.42^{Ca}
CSLB	0.85 ± 0.34^{Ad}	0.99 ± 0.19^{Ad}	1.93 ± 0.79^{Bd}	2.79 ± 0.62^{Dd}	1.23 ± 0.55^{Cd}
CSU	0.49 ± 0.31^{Aa}	0.34 ± 0.18^{Aa}			1.40 ± 0.36^{Ca}
CSUB	0.81 ± 0.54^{Ac}	0.69 ± 0.16^{Ac}		2.77 ± 0.33^{Dc}	0.96 ± 0.64^{Cc}
POL	0.72 ± 0.16^{Aa}	1.02 ± 0.50^{Aa}	1.07 ± 0.31^{Ba}	0.91 ± 0.38^{Da}	0.70 ± 0.35^{Ca}
POLB	0.95 ± 0.17^{Ab}	0.89 ± 0.40^{Ab}	0.94 ± 0.47^{Bb}	0.95 ± 0.36^{Db}	1.57 ± 0.25^{Cb}
SPA	0.77 ± 0.21^{Ac}	0.54 ± 0.30^{Ac}		2.46 ± 0.24^{Dc}	1.31 ± 0.35^{Cc}

Abbreviations: EMB: *P. eryngii*, EFQ: *P. eryngii*, L21: *L. edodes*, LEN: *L. edodes*, DMR: *P.djamor.* MEA: malt extract agar, CEL: cellulose, CSL: classical disposable diaper's cellulose-superabsorbent polymer (clean: moistened with distilled water), CSLB: biodegradable diaper's cellulose-superabsorbent polymer (clean: moistened with distilled water), CSU: classical disposable diaper's cellulose-superabsorbent polymer (urine-wet), CSUB: biodegradable diaper's cellulose-superabsorbent polymer (urine-wet), P.OL: classical disposable diaper's polyethylene and polypropylene, POLB: biodegradable diaper's polyethylene and polypropylene, SPA: sodium polyacrylate.

Table 3. Mycelium extension rate (μ_{max}, mm/day, n = 10) of five edible mushroom strains cultivated in nine culture media. Different letters indicate significant differences according to Duncan's test ($p< 0.05$). Capital letters indicate comparisons between different strains and lowercase letters indicate differences in culture media.

CULTURE MEDIA	EMB	EFQ	L21	LEN	DMR
MEA	8.09 ± 0.28^{Cc}	6.73 ± 2.4^{Bc}	7.34 ± 0.95^{Ac}	5.30 ± 1.09^{Bc}	11.91 ± 1.19^{Dc}
CEL	5.66 ± 0.46^{Cb}	6.43 ± 0.28^{Bb}	5.74 ± 0.29^{Ab}	6.61 ± 0.43^{Bb}	8.82 ± 1.19^{Db}
CSL	4.67 ± 0.65^{Ca}	4.42 ± 0.70^{Ba}		4.16 ± 0.40^{Ba}	6.35 ± 0.63^{Da}
CSLB	5.45 ± 0.74^{Ca}	5.36 ± 0.14^{Ba}	3.20 ± 0.41^{Aa}	4.95 ± 0.27^{Ba}	5.46 ± 0.41^{Da}
CSU	3.98 ± 0.41^{Ca}	4.70 ± 0.45^{Ba}			6.34 ± 0.58^{Da}
CSUB	3.69 ± 0.15^{Ca}	4.45 ± 0.55^{Ba}		5.56 ± 0.59^{Ba}	5.13 ± 0.61^{Da}
POL	7.30 ± 0.52^{Cb}	5.40 ± 0.56^{Bb}	4.86 ± 0.31^{Ab}	5.26 ± 0.33^{Bb}	9.09 ± 0.83^{Db}
POLB	6.31 ± 0.35^{Cb}	6.91 ± 1.20^{Bb}	3.81 ± 0.38^{Ab}	5.79 ± 0.40^{Bb}	8.75 ± 0.32^{Db}
SPA	3.92 ± 0.28^{Ca}	4.47 ± 0.27^{Ba}		4.95 ± 0.14^{Ba}	6.12 ± 0.27^{Da}

Abbreviations: EMB: *P. eryngii*, EFQ: *P. eryngii*, L21: *L. edodes*, LEN: *L. edodes*, DMR: *P.djamor.* MEA: malt extract agar, CEL: cellulose, CSL: classical disposable diaper's cellulose-superabsorbent polymer (clean: moistened with distilled water), CSLB: biodegradable diaper's cellulose-superabsorbent polymer (clean: moistened with distilled water), CSU: classical disposable diaper's cellulose-superabsorbent polymer (urine-wet), CSUB: biodegradable diaper's cellulose-superabsorbent polymer (urine-wet), POL: classical disposable diaper's polyethylene and polypropylene, POLB: biodegradable diaper's polyethylene and polypropylene, SPA: Sodium polyacrylate.

Our λ values for the L21 strain (0.47±0.53 to 1.93±0.79 days) and the LEN strain (0.65±0.40 to 2.79+0.62 days) were consistent with those reported by Valenzuela *et al.* (2020), who reported λ values of 1.04±0.68 days for the L21 strain and 1.31±0.42 days for the LEN strain in MEA. In contrast, our values for DMR (0.63±0.17 to 1.82±0.42 days) were lower than those reported by Valenzuela *et al.* (2020) for *P. djamor* (2.02±0.58 days). These findings indicate that in the case of *P. eryngii* and *P. djamor*, the adaptation phase and the early mycelial growth into a new culture medium do not have an impact mediated by the diaper compounds. Conversely, the strain LEN was severely affected by SPA polymer as it did not grow in cultures containing this polymer, regardless of whether they contained urine or only water.

3.3.2 *Mycelium extension rate (μ_{max})*

Using primary models to calculate kinetic parameters, such as the mycelium extension rate, is an efficient tool that facilitates the evaluation of different strains under specific cultivation conditions. The use of this methodology allows the identification of strains that exhibit superior growth characteristics for subsequent biotechnological processes. The μ_{max} values obtained in the present study are summarized in Table 3. Multivariate statistical analysis of the mycelium extension rate revealed significant differences among the five strains, depending on the diaper component added to the culture media. The Duncan test ($p<0.05$) identified three homogeneous subgroups based on the average μ_{max} in each culture medium (Fig. 5). The first subgroup (CSUB, CSLB, SPA, CSL, CSU) exhibited the lowest μ_{max} values (4.76–4.98 mm/day). Notably, all media in this group contained SPA alone

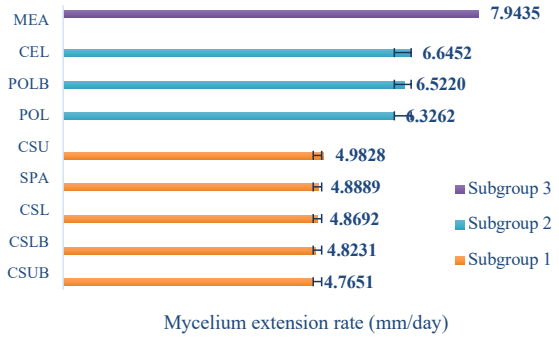

Figure 5. Mycelium extension rate (μ_{max}) of tested culture media. Different color bars indicate homogeneous subgroups according to Duncan's tests ($p< 0.05$, n = 50). MEA: malt extract agar, CEL: cellulose, CSL: classical disposable diaper's cellulose-superabsorbent polymer (clean: moistened with distilled water), CSLB: biodegradable diaper's cellulose-superabsorbent polymer (clean: moistened with distilled water), CSU: classical disposable diaper's cellulose-superabsorbent polymer (urine-wet), CSUB: biodegradable diaper's cellulose-superabsorbent polymer (urine-wet), POL: classical disposable diaper's polyethylene and polypropylene, POLB: biodegradable diaper's polyethylene and polypropylene, SPA: sodium polyacrylate.

or in combination with cellulose. The second subgroup included CEL, POLB, and POL media (6.37–6.65 mm/day). The third subgroup consisted solely of the control group (MEA: 7.94 mm/day). These results confirm that the presence of SPA in the culture medium delays mycelial growth and, in some cases, completely inhibits it.

The values of μ_{max} for *P. eryngii* in MEA, CEL, and POL media were consistent with those reported

by Andrino *et al.* (2011) for three strains of *P. eryngii* (7.43–7.81 mm/day) and were higher than those obtained by Gaitán (2005) (5.06 mm/day) in MEA. Furthermore, the treatments that included SPA resulted in a reduction in the growth rate. Thus, despite this delay, and since no medium completely inhibited mycelial growth, it is expected that these strains could fructify if cultivated in mixtures containing disposable diapers as a co-substrate.

The growth data for L21 in the control MEA medium (7.34 mm/day) were similar to those reported by Martínez-Guerrero *et al.* (2012), who obtained μ_{max} values of 7.5 and 8.5 mm/day for two strains of *L. edodes* cultivated in a substrate mixture containing oak sawdust, wheat bran, corn stover, and rice flour. Meanwhile, LEN cultivated in the CEL medium exhibited μ_{max} values (6.61 mm/day), which are consistent with those reported by Valenzuela *et al.* (2020), in MEA (6.48±0.53 mm/day).

The LEN and L21 strains are not viable alternatives for treating disposable diapers because their growth in these treatments was significantly slower compared to *Pleurotus eryngii* (EMB and EFQ) and *Pleurotus djamor* (DMR). The LEN and L21 strains showed the lowest growth rates when exposed to cellulose or other synthetic polymers. In the case of CSLB treatment, the growth rate was very slow, averaging only 3.20 ± 0.41 mm/day. Additionally, in the presence of SPA, both strains stopped growing. Hatvani *et al.* (2003) tested the effect of certain inorganic salts containing Na^+ ions on *L. edodes* mycelium and concluded that these salts inhibited mycelial growth by up to 70%. In the case of disposable diapers, their SPA polymer (sodium polyacrylate) is designed to release Na^+ ions upon contact with a liquid (Gómez-Crespo and Cañamero-Lancha, 2011). Therefore, the presence of Na^+ in SPA could also contribute to mycelial growth inhibition.

The DMR strain (*P. djamor*) exhibited the highest μ_{max} values of all tested media. Growth in MEA (11.91 mm/day) exceeded the reported values by Salmones *et al.* (2004) (1.96–8.2 mm/day) for the same medium. However, as observed with the other strains, a growth delay occurred in media containing SPA (5.17–6.34 mm/day) compared to CEL, POL, and POLB (8.75–9.09 mm/day). These findings suggest that the DMR strain is a promising candidate for cultivation in substrates containing disposable diapers; nevertheless, this hypothesis requires further validation through additional testing.

Based on these findings, the SPA content in a culture medium should not exceed 5%. Since SPA constitutes approximately one-third of a disposable diaper and due to the difficulty of separating it from other diaper components, our recommendation is to use a maximum of 15–16% of fragmented disposable diapers as a co-substrate during cultivation trials of straw-based substrates. Adding this proportion will ensure adequate mycelial growth and its subsequent fruiting.

Conclusions

The results confirmed that the SPA polymer delays or inhibits the mycelial growth of *Pleurotus eryngii*, *Pleurotus djamor*, and *Lentinula edodes*. This growth inhibition occurs independently of the source of the polymer, whether it comes from clean diapers hydrated with distilled water, used diapers containing urine, or from conventional or biodegradable diaper materials. Therefore, to ensure appropriate mycelial growth during the co-processing of disposable diaper waste, it is essential to monitor the SPA content. Here, it was established that the SPA proportion during *in vitro* culture should not exceed 5%. Since SPA constitutes about one-third of the composition of disposable diapers, the proportion of diapers during waste treatment with wheat straw should not exceed 15-16% to ensure optimal mycelial growth and fruiting. Additionally, the growth data suggests that the DMR strain is a promising option for the biodegradation of disposable diaper waste.

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