Article

Potential use of prickly pear glochids as a substrate for the production of *Pleurotus* sp. mycelium.

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Abstract

The glochids of prickly pear fruits were valued as a substrate-improving component for the production of *Pleurotus* sp. mycelium, through indicators of macroscopic colonization and characterization of the mycelium at the stereoscopic level. Glochids of prickly pear produced in Nopaltepec, Mexico, were used. Mycelium (F2) from the collection of strains of the Institute of Ecology, AC, was integrated with mixtures in different proportions (% in weight) of sorghum (S) grains and ground (EM) or whole (EE) glochids according to the following treatments: control, 90S-10EM, 80S-20EM, 70S-30EM, 90S-10EE, 80S-20EE and 70S-30EE. The experimental units were incubated at 25 °C for 11 days. The degree of invasion was evaluated based on type of growth, texture, color, aerial mycelium and density, through hedonic scale by variable. The results were processed with non-parametric tests of analysis of means and contrasts with a confidence level of 95%. A complete and homogeneous colonization with mycelium from very dense to dense was observed in all treatments with ground or whole glochids, which indicated that the conditions were adequate to promote the branching of hyphae and biomass. The best treatments in terms of homogeneous growth and very high density corresponded to the mixtures 90S-10EM and 80S-20EE. Texture and color were significantly good in the treatment with the highest proportion of whole glochids (70S-30EE), while growth type and presence of aerial mycelium were favored with the highest proportion of ground glochids (70S-30EM). The results were conclusive, glochids are an excellent material that can be used for the production of mycelium.

Keywords: *Opuntia ficus indica*, characterization of *Pleurotus* sp. mycelium, degree of invasion.

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Introduction

The prickly pear *Opuntia ficus indica* is used in obtaining a wide variety of products. Within the process of benefiting of prickly pear, a large number of glochids known as '*ahuates*' are produced, according to Ulloa-Leitón *et al*. (2021), for each tonne of clean prickly pear, 65 kg of *ahuates* are generated, material that is useless for producers, which, given its difficult management, its resistance to natural degradation, even to burning, is discarded in plastic bags in landfills, generating a problem of environmental pollution. Studies of this byproduct indicate that its resistance to microbial decomposition is due to its high contents of cellulose (41.14%), hemicellulose (41.21%) and lignin (5.27%) in the crystallized state (Ulloa-Leitón *et al*., 2021).

Some authors mention that these types of structures can be weakened by the action of fungi such as *Pleurotus ostreatus*, widely known for its ability to convert lignocellulosic agricultural waste into foods (Nieto and Chegwin, 2010). In fact, the production of *Pleurotus ostreatus* (oyster mushroom) is based on using lignocellulosic agricultural residues as a substrate to produce food of high protein quality, valued between 17 and 25% depending on the characteristics of the substrate (García-Oduardo *et al*., 2011; Rodríguez *et al*., 2021).

The propagation and cultivation of *Pleurotus* sp., requires an inoculum developed mainly in cereal grains. Among the substrates most used to obtain mycelium, grains of sorghum, wheat, millet, corn and barley stand out, which have contents of cellulose between 23% and 35%, hemicellulose from 26% to 67% and lignin between 0.1% and 21% (Wilson and Godiño, 2000; Chuck-Hernández *et al*., 2011). This substrate must supply carbon (from cellulose, hemicellulose and lignin), nitrogen and inorganic compounds as nutrient sources, a particle size that allows the proper management of moisture and oxygen (Ríos *et al*., 2010). These last conditions could be met with prickly pear glochids because their composition and size can generate a greater contact surface, an indispensable quality for the development of the primary inoculum (Jennings and Lysek, 1999; Harris, 2008).

According to Suárez-Arango (2010), when the substrate provides the right conditions, the mycelium develops in a homogeneous dense way and in a short time (approximately two weeks), white in color, with a cottony texture, regular density with the presence of aerial hyphae. At the microscopic level, the hyphae are very branched, with generally thin walls and the presence of clamp connections (Pérez-Roldán, 2006). Based on the above, the present research aimed to a) assess prickly pear glochids as a substrate-improving component for the production of *Pleurotus* sp. mycelium; through indicators of macroscopic colonization and characteristics of the mycelium at the stereoscopic level. This research is important because it contributes to generating added value to a byproduct of the prickly pear production system, which, at present, constitutes a waste of difficult management with negative environmental impact on the cultivation areas.

Materials and methods

Characteristics of glochids

Prickly pear glochids obtained from the production of the locality of San Felipe Teotitlán, Municipality of Nopaltepec, State of Mexico, were used. According to Ulloa-Leitón *et al*. (2021), glochids contain 41% (±0.2) of cellulose, 41% (±0.2) of hemicellulose and 5.27% (±0.2) of lignin, measure 1 667 μm (±292) long and 67.3 μm (±9.56) in diameter, with a density of 0.1574 g ml^{-1}.

Mycelium (F2) from the collection of strains of the Institute of Ecology, AC (INECOL), was sown in mixtures of sorghum (S) grains and ground (EM) or whole (EE) glochids, according to the treatments indicated in Table 1. Prior to the preparation of the treatments, the material was cleaned, removing the large residues. The sorghum was washed with running water by immersion, to remove impurities and broken grains, drained and dried for 12 h, spreading the material on absorbent paper. Subsequently, the indicated treatments were prepared.

Table 1. Proportions by weight of the materials used in the preparation of the substrate by treatment.

Treatment	Sorghum (%)	Ground glochids (%)	Whole glochids (%)
Control	100	0	0
90S-10EM	90	10	0
80S-20EM	80	20	0
70S-30EM	70	30	0
Control	50	0	0
90S-10EE	40	0	10
80S-20EE	30	0	20
70S-30EE	20	0	30

S= sorghum; EM= ground glochid; EE= whole glochid.

Samples of each experimental unit were placed in bottles with a capacity of 200 and 450 ml for ground and whole glochid, respectively. Once the mixtures were made, the dry weights of each experimental unit were recorded, covered and homogenized. Subsequently, an alkaline solution composed of commercial calcium hydroxide $Ca(OH)_2$ at 1.8% and commercial sodium bicarbonate ($NaHCO_3$) at 1% was added to saturation, with those with ground glochid remaining for 12 h in this condition, and for 24 h those of whole glochid, since they take longer for hydration (Figure 1).

Figure 1. Experimental unit, a) weighed; b) homogenized; c) saturation with alkaline solution; and d) resting time.

Once the hydration was completed, the samples were filtered in an organza sieve, weighed and placed back in their bottle (previously washed with soap), finally, they were placed a lid of aluminum foil (Figure 2).

Figure 2. a) sample filtering; and b) filtered sample.

The units were sterilized in a pressure cooker at 20 pounds for 20 min, cooled to room temperature and inoculated with 3 g of *Pleurotus* sp. mycelium, in a laminar flow hood. They were incubated at 25 °C for 11 days, time in which they reached 100% colonization. In this period, the samples were homogenized every three days (Figure 3). Prior to the sowing of the mycelium, the wet weight of each experimental unit was recorded to assess this parameter in the substrate mixture.

Figure 3. a, b) samples in incubation; and c, d) colonized samples.

Variables evaluated

The degree of mycelium invasion was evaluated based on type of growth, texture, color, aerial mycelium and density, for which a hedonic scale by variable was constructed, Table 2, according to the proposal by (Rodríguez-Macías, 1996) modified for the purposes of this experiment (Figure 4).

Table 2. Hedonic scale parameters.

Type of growth	Texture	Color	Aerial mycelium	Density (pattern of development)
1= homogeneous	1= cottony	1= white	1= regular	1= very high
2= irregular	2= aborted	2= whitish	2= scarce	2= high
3= sparse	3= venous	3= yellowish	3= absent	3= medium
4= with rings	4= velvety			4= low
	5= waxy			

Figure 4. Parameters evaluated. a) type of growth; b) texture; c) color; d) aerial mycelium; and e) mycelium density.

From the point of view of the ideal growth of the mycelium of *Pleurotus* in the substrates, in the assessment of the parameter type of growth, this should tend to homogeneity, the texture must be cottony, the color preferably white, with the presence of aerial mycelium and high to very high density.

In order to confirm that the mycelium developed in the prickly pear glochids in the different mixtures, samples of the best treatments were taken, and observations were made in a stereoscope. The evaluated variables were processed with non-parametric tests of analysis of means and contrasts in the Statistical Analysis Software (SAS) package with license number 70074773 with a confidence level of 95%.

Results and discussion

Degree of mycelium invasion

The analysis of variance of the characteristics evaluated with the hedonic scale indicated that at least two treatments were significantly different in each of the variables (Table 3).

Table 3. Comparison of means of the parameters of degree of invasion evaluated obtained by hedonic scale.

Treatment	Type of growth	Texture	Color	Aerial mycelium	Density
Control1	31 a	25.75 ab	15.75 cd	20.33 bc	30.33 a
90S10EM	15.5 b	33.42 a	24.67 abc	8.83 d	10.58 c
80S-20EM	23.83 ab	20.75 bc	30.17 ab	13.17 cd	19.17 abc
70S-30EM	15.33 b	25 ab	31.58 a	14.5 cd	26.92 a
90S-10EE	28.18 a	21.67 abc	20.83 abc	34.33 a	25.83 a
80S-20EE	13.18 b	11.83 c	8.17 d	28.17 ab	13.08 bc
70S-30EE	23.5 ab	12.08 c	19.33 bcd	31.17 a	24.58 ab
Pr> F	0.036*	0.0109*	0.0033**	<0.0001**	0.0204*
MSD	12.39	12.14	11.65	9.75	12.48

S= sorghum; EM= ground glochid; EE= whole glochid; MSD= minimum significant difference, different letters in the same column indicate a significant difference with a confidence level of 95%.

Based on the variables evaluated, after 11 days of inoculation of the substrates with *Pleurotus* sp., the treatments 90S-10EM and 80S-20EE showed ideal characteristics expected in the development of the mycelium and superior to the control. The treatment 90S-10EM had homogeneous growth, velvety texture, yellowish color, aerial mycelium present and very high density, in 80S-20EE the growth was homogeneous, cottony texture, white in color and very high density, although without the presence of aerial mycelium (Figure 5).

The remaining treatments in ground (EM) and whole (EE) glochid reached complete and homogeneous colonization after 11 days, obtaining a quality close to ideal in attributes such as type of growth, texture and density, even better than the control (Figure 5). When the nutritional conditions of the substrate are favorable, the branching of the hyphae, as well as the amount of biomass produced benefit (Prosser and Tough, 1991; Arana-Gabriel *et al*., 2014), consequently, the results of the experiment show that ground glochids contribute to improving the conditions of the traditional substrate for the development of *Pleurotus* mycelium. Although the best mixtures were those mentioned above, qualitatively, in the six treatments with ground or whole glochids, a complete and homogeneous colonization with mycelium from very dense to dense was observed.

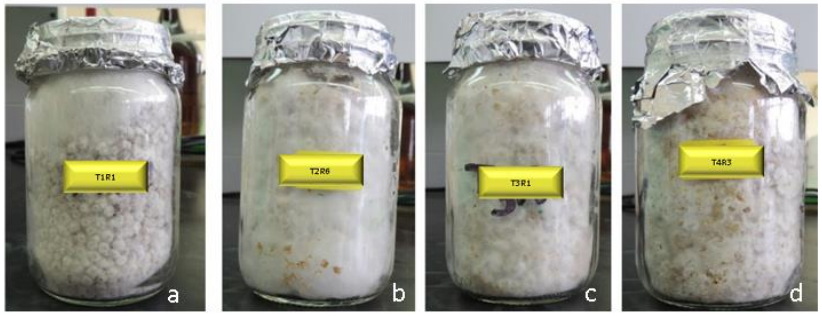

Figure 5. Degree of colonization of the mycelium at 11 days of incubation. a) control; b) 90S-10EM; c) 80S-20EM; and d) 70S-30EM.

The texture of the mycelium was cottony to velvety, according to López-Ramírez (2014), this type of texture is reached when the hyphae are well branched and compact. The color of the mycelium was yellowish to whitish, the yellowish color may be due to the fact that some glochids adhere to the walls of the bottle and show the pigmentation of these structures, since when hydrated they give off colors ranging from yellow to intense pink (Figure 6).

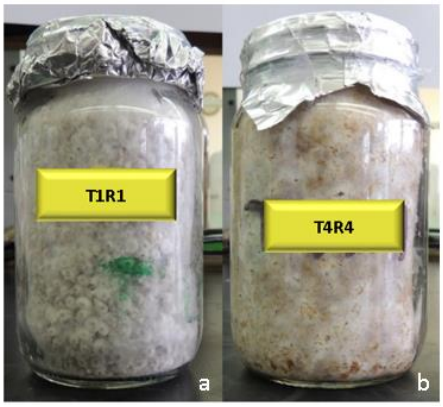

Figure 6. Color comparison, a) control; and b) 70S-30EM.

The parameter aerial mycelium is important because, according to Rahardjo *et al*. (2002), its presence is indicative of a favorable diffusion of oxygen for the respiratory process of the fungus. In this sense, all the treatments with EM, as well as the control, facilitated this type of growth and in particular the mixture 90S-10EM. In contrast, with EE its presence was lower and was not reflected in the comparison of means.

All the morphological characteristics of the mycelium assessed in this study agree with those reported by Gaitán-Hernández (2005), who indicates that the mycelium should be white, cottony and with a regular density, as well as that exposed by Bermúdez-Savón *et al*. (2007), describing the colonies as white, cottony of regular density and aerial mycelium present.

Likewise, Suárez-Arango and Holguín-Hernández (2011) achieved a homogeneous, dense, white development with abundant growth in two weeks of incubation in wheat grain. For *Flammulina mexicana* and *Lyophyllum* aff. *shimeji*, developments of dense mycelium are also reported, with

colonization percentage of 98.3% at 20 days of inoculation in wheat grains (Arana-Gabriel *et al*., 2014). From the point of view of the oyster mushroom producer, a homogeneous and high-density mycelium reflects that it has enough vigor to obtain a fast and abundant harvest.

As in the selection of seed for agricultural crops, the producer makes sure ensures that the 'seed' to be used is adequate and recognizes it as adequate when the structure of the mycelium is abundantly cottony and compact (López-Ramírez, 2014), this appreciation of the producer is accurate since it is an indicator that there is a greater amount of mycelium.

The test of contrasts with what was observed in Table 4, in C1, shows that the treatment 90S-10EM presented the best characteristics of mycelium development, since there are highly significant differences in three of the five variables evaluated. With this contrast, the result obtained in the mean test is confirmed. For the case of 70S-30EE (C3), it was also highly significant in terms of texture, color and aerial mycelium. The contrast that compares all treatments that contain ground glochids *vs* whole glochid shows that when the mixture contains EM, the development of mycelium is greatly favored through the qualitative variables type of growth, aerial mycelium and density, while with EE, it stands out in texture and color. These results are conclusive that glochids are an excellent material that can be used for the production of mycelium in either of the two forms of management, the choice will depend on what is most practical for the producer.

Table 4. Test of contrasts of the parameters to assess the degree of invasion of *Pleurotus* sp. mycelium.

Treatments	Type of growth	Texture	Color	Aerial mycelium	Density
90S-10EM *vs* 90S-10EE	0.0454**	0.0573	0.5085	<0.0001**	0.018**
C1: μ2 - μ5	-12.68	11.75	3.84	-25.5	-15.25
80S-20EM *vs* 80S-20EE	0.0893	0.1448	0.0005**	0.0036**	0.329
C2: μ3 - μ6	10.65	8.92	22	-15	6.09
70S-30EM *vs* 70S-30EE	0.1896	0.0377**	0.0398**	0.0014	0.7065
C3: μ4 - μ7	-8.17	12.92	12.25	-16.67	2.34
EM *vs* EE	0.3429	0.0026**	0.0005**	<0.0001**	0.5251
C4: μ2+ μ3+ μ4- μ5- μ6- μ7	-10.2	33.59	38.09	-57.17	-6.82
Control *vs* T2-T4 EM	0.0148**	0.8966	0.0085**	0.0447	0.0288**
C5: 3μ1 - μ2-μ3-μ4	38.34	-1.92	-39.17	24.49	34.32
Control *vs* T5-T7 EE	0.0679	0.0375**	0.939	0.0088**	0.0763
C6: 3μ1 - μ5-μ6-μ7	28.14	31.67	-1.08	-32.68	27.5

*= significance at 0.05; **= significance at 0.01. The sign of the means below the significance indicates the best contrast response. Negative sign best response in EM y positive sign best response in EE.

Moisture content of the substrate

The moisture of the substrate is a critical factor for the optimal development of the mycelium, the average of this parameter in the mixture with EM at 10% was 39.7 (±1.5%), for 20% it was 46.3% ±2.2 (M) and in that of 30% it was 50.9% ±1.1 (M), while that of sorghum was 33.7% ±0.4 (M). For mixtures with EE at 10%, M was 47.2 ±2.2%, for 20% it was 55.1 ±3.8 (M) and in 30% it was

62.3 ±1.9 (M). According to Sánchez and Royse (2001), moisture contents below 50% are not adequate and greater than 80% have a negative effect on the growth of *Pleurotus* sp. Garzón-Gómez and Cuervo-Andrade (2008) point out that the optimal moisture content depends on the species of fungus and the substrate selected, since each substrate has particular characteristics of water retention capacity, therefore, the optimal moisture in each substrate will be conditioned by this characteristic. Based on these references, the results obtained in the mixtures tested in the present research indicated that the glochids favor the retention of moisture of the substrate within the optimal conditions for the development of the mycelium.

Stereoscopic observations of mycelium in the substrate

The observations of the development of the mycelium in mixtures with a higher proportion of EM and EE show, on the one hand, that glochids tend to adhere to sorghum grains (Figure 7a and 7b) due to the presence of retrorse beards, on the other hand, it is also seen that the glochids grouped together forming granular aggregates (Figure 7b).

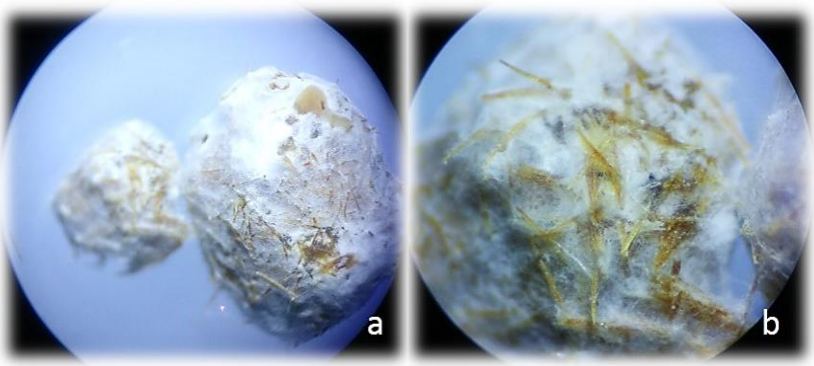

Figure 7. a) glochids adhered to the grain of sorghum invaded with mycelium; and b) whole glochid granule colonized by *Pleurotus* sp.

These images show that the mycelium developed by enveloping the glochids and this invasion was favored by the spaces within and between granules (Figure 8a and 8b) derived from the formation of aggregates by the structures, which favor aeration and the appropriate retention of moisture.

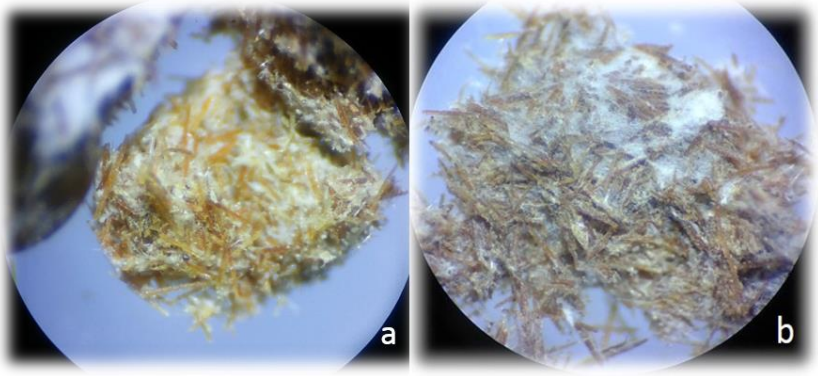

Figure 8. a) granule of ground glochids invaded by mycelium; and b) invasion of the mycelium within the granule of glochids.

According to Harris (2008), the particle size of the substrate is decisive for the development of the mycelium, with advantages in the degree of colonization when using materials that increase the contact surface. Therefore, it can be concluded that the glochids mixed with sorghum grains function as a substrate improver for the production of the 'seed' of oyster mushrooms.

On the other hand, an important aspect of the invasion of the mycelium to the glochids was to verify that the hyphae managed to break their crystalline organization. Figure 9 shows that the originally solid and rigid structure (Figure 9a) was fractured by the attack of the hyphae (Figure 9b) since spaces can be seen between the cellulose and hemicellulose microfibrils that make up the structure of the glochid.

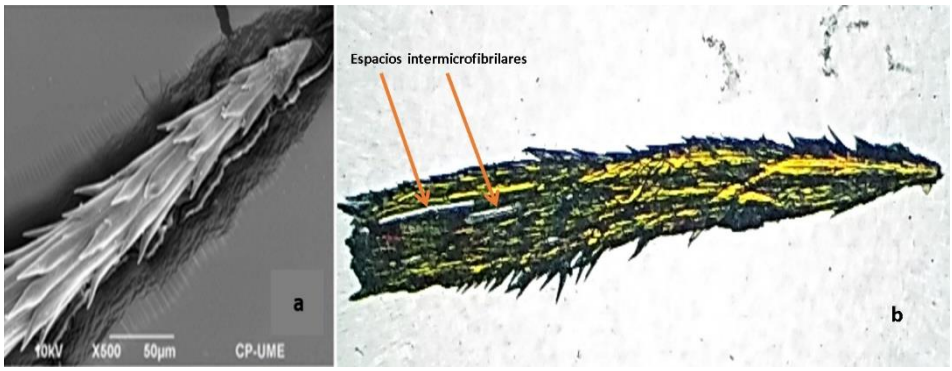

Figure 9. a) glochid structure prior to the invasion process; and b) glochid structure after the invasion process of *Pleurotus* sp., 4x.

Pleurotus sp., corresponds to the group of basidiomycete fungi typified as 'white rot', which cause a softening of organic tissues accompanied by significant weight loss. According to Salmones (2005), this rot is characterized by a preference for lignin of the lignocellulose fraction, which leads to the formation of microscopic cavities within the organic fiber and sometimes, it produces a discoloration and crack pattern similar to dark rot. Lucas *et al*. (2001) mention that the fungi of white rot, by degrading the different lignocellulosic polymers of wood, transform it into a material of spongy and fibrous appearance.

According to Deacon (2013), basidiomycetes can achieve the total degradation of lignin simultaneously; that is, cellulose, hemicellulose and lignin or selective rot are degraded more or less synchronously and in the same proportion, where lignin is degraded more rapidly at the beginning of the rotting process (Blanchette, 1984). *Pleurotus* species are considered to belong to type of selective white rot (Lucas *et al*., 2001). This would explain the presence of the interfibrillar spaces shown in Figure 9b.

Conclusions

The results of the present research indicated that glochids as a byproduct of the prickly pear benefiting, either in ground or whole form, can be used as a substrate improver for the production of *Pleurotus* sp. mycelium, their mixture with the traditional substrate for such purposes contributed to increasing the precocity of invasion, promoting a complete and homogeneous colonization, with very dense mycelium of cottony texture. Stereoscopic observations showed that hyphae break the crystalline structure of glochids.

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