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Research Article

Capacity of *Histoplasma capsulatum* to Survive the Composting Process

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Histoplasma capsulatum (H. capsulatum) is a thermal-dimorphic fungus, the causal agent of histoplasmosis. Its presence in the environment is related with chicken manure due to their high nitrogen and phosphorus content. In Colombia, chicken manure is the most used raw material in the composting process; however, there is no information about the capacity of *H. capsulatum* to survive and remain viable in a composted organic fertilizer. To address this question, this study shows three assays based on microbiological culture and the Hc100 nested PCR. First, a composting reactor system was designed to transform organic material under laboratory conditions, and the raw material was inoculated with the fungus. From these reactors, the fungus was not isolated, but its DNA was detected. In the second assay, samples from factories where the DNA of the fungus was previously detected by PCR were analyzed. In the raw material samples, 3 colonies of *H. capsulatum* were isolated and its DNA was detected. However, after the composting process, neither the fungus was recovered by culture nor DNA was detected. In the third assay, sterilized and nonsterilized organic composted samples were inoculated with *H. capsulatum* and then evaluated monthly during a year. In both types of samples, the fungus DNA was detected by Hc100 nested PCR during the whole year, but the fungus only grew from sterile samples during the first two months evaluated. In general, the results of the assays showed that *H. capsulatum* is not able to survive a well-done composting process.

1. Introduction

Histoplasma capsulatum (H. capsulatum) is a thermally dimorphic fungus and the causal agent of histoplasmosis. In its natural habitat or when cultured at approximately 28°C, it behaves like a slow-growing mold taking 3 to 6 weeks to be recovered by culture. The mold form of *H. capsulatum* has thin hyaline septate hyphae, tuberculate macroconidia measuring around 7 to 15 μm in diameter, and thin-walled microconidia measuring 2 to 6 μm. The microconidia and

hyphae fragments constitute the infectious particles. When the fungus is exposed to temperatures of approximately 37°C, as occurs inside a mammalian host or under laboratory conditions, the fungus transforms into a yeast measuring 2 to 4 μm in diameter [1–3].

H. capsulatum inhabits porous soils with high concentrations of phosphorus and nitrogen, with a large organic matter content and an acidic pH. It is favored by temperatures between 22 and 29°C, annual precipitation around 1000 to 1200 mm^3 and a relative humidity of between 67 and 87%. These characteristics occur especially in soils enriched with bird and bat excreta. When an environment contaminated with *H. capsulatum* is disturbed, infective particles are aerosolized, and then inhaled by a potential host [4–7]. Within the host, the fungus transforms into its yeast form. Development of histoplasmosis depends on several factors such as the inoculum size, the virulence of the fungus, as well as the immune status and lung condition of the host. Infections with *H. capsulatum* can be asymptomatic, acute (epidemic), chronic, or become lethal depending on the interaction of these factors. Furthermore, after exposure to the fungus, people may experience a latent infection that can be reactivated. People at risk of reactivation of latent histoplasmosis include transplant recipients, patients with hematologic malignancies, those undergoing corticosteroid therapy, or patients in the advanced stages of infection with human immunodeficiency virus-acquired immunodeficiency disease syndrome (HIV-AIDS) [1, 8, 9]. Histoplasmosis is often diagnosed in urban and rural areas and may affect farmers, gardeners, housekeepers, builders, explorers, speleologists, and archaeologists. People involved in these occupations are more predisposed to becoming infected with *H. capsulatum*; hence, histoplasmosis has been classified as an occupational and recreational disease [10–13].

The first isolation of *H. capsulatum* from soil was reported by Emmons in 1949, in the State of Virginia (United States). In that study, 387 samples were tested, and only two were positive for *H. capsulatum* [14]. Later, Ajello [15] studied the ecological relationship between *H. capsulatum* and different places where the patients diagnosed with histoplasmosis lived and found that 25% of the positive samples included soil containing poultry droppings [15]. In contrast, soil samples without poultry droppings were positive only in 7%. These results led to conclude that henhouses favor the presence of the fungus [15]. Furthermore, other studies describe the isolation of *H. capsulatum* from sites related to large amounts of bird and bat droppings in the near vicinity such as dirty floors, farms, old water tower, mines, tree holes, abandoned buildings, composted fertilizers, caves, and abandoned hangar [16–22].

Although numerous studies have been carried out to search *H. capsulatum* in soils from different geographical locations, little is known about the ability of *H. capsulatum* to survive the composting process. Furthermore, there is no information about how long the fungus can remain viable in a substrate rich in organic matter. While there is a lack of knowledge on this topic, a few *H. capsulatum* outbreaks have been documented, related to the handling of substrates rich in organic matter like chicken manure or organic fertilizers. Examples include the outbreaks reported in Medellin-Colombia and Acapulco-Mexico, where in both cases, the source of infection was houseplants containing a fertilizer enriched with chicken excreta [11, 23]. Other outbreaks have been associated with the removal of bird excreta, such as the outbreak that occurred at a Michigan paper company and the outbreak that occurred in Argentina that was linked to a hangar in an airbase [22, 24]. The characteristics of these outbreaks suggested that *H. capsulatum* has an affinity with enclosed and abandoned spaces where there are larger quantities of bird and bat excreta. These reports also demonstrate the ability of this fungus to infect mammals entering or living in these contaminated areas.

In Colombia, *H. capsulatum* has been also isolated from environmental samples. Moncada et al. isolated the fungus in 4% of soil samples from a cave inhabited by bats and birds in the Rio Claro region (Antioquia-Colombia). The samples were collected from a transitional area between humid tropical rainforest and very humid tropical rainforest with an average annual rainfall of 3,200 mm^3, relative humidity of 80%, and an average temperature of 24°C. These are the common characteristics described for *H. capsulatum* habitat [5].

Ordoñez et al. [12] gathered information about 18 outbreaks of histoplasmosis occurred in Colombia, between 1970 and 1997, and identified locations that represent the preferred habitat of the fungus. According to their results, activities related to demolition, cleaning of buildings, logging, and trips to caves were associated with outbreaks of histoplasmosis [12]. In 2002, Jiménez et al. reported one additional Colombian outbreak comprising a family which became infected after one family member handled an organic fertilizer containing contaminated chicken manure with *H. capsulatum* from a local nursery [11].

The Colombian poultry industry is experiencing sustained growth, this created the need to seek alternatives for the disposal of excrements. For example, during 2018, there was an increase of 6.7% in the production of broilers and table eggs [25]. This growing combined with the need to restore organic matter in agroecosystems [26] generated an intensification in the production of organic fertilizers made from poultry manure, which must be stabilized and sanitized. Composting is the most commonly used stabilization procedure [27].

Organic fertilizers in Colombia are primarily produced from poultry manure, and these fertilizers are used to restore fertility to nutrient-poor soils. These products are manufactured by the composting process which provides to the fertilizer a chemical and biological stability [26]. Composting is a spontaneous aerobic process that decomposes organic matter by the activity of decomposer microorganisms such as fungi and bacteria. This large population of saprophytic microorganisms compete with each other for space and nutrients and eventually transform the organic matter into a sanitized substrate free of toxins and pathogens [28–31]. After composting, the resulting material possesses important physicochemical characteristics for soil conditioning, including a stable pH, sufficient moisture, and nutrient (nitrogen, phosphorus, and potassium) concentrations above 1% [26].

Because of the boom in the production and use of composted organic fertilizers in Colombia, the Ministry of Agriculture and the ICA generated policies for handling of organic fertilizer, which were contained in the resolution 150 of 2003 [32]. This policy outlines the parameters of the technical regulation of fertilizers and soil conditioners and defines organic fertilizers in terms of the physical, chemical, and microbiological variables contained in the Colombian Technical Standard (NTC 5167) [33]. The goal of microbiological studies is to find *Salmonella* spp., facultative mesophiles, and total enterobacteria and fungi; culture media such as Sabouraud or potato dextrose agar (PDA) are used for the latter purpose. *H. capsulatum* is not searched and is very difficult to recover from these media because this fungus grows slowly (3 to 6 weeks); thus, fast-growing environmental bacteria and mold overcome *H. capsulatum* in most cases.

As poultry manure favors the growth of *H. capsulatum* due to its high content of phosphorus and nitrogen and because it is also the raw material for the production of many organic fertilizers, we propose that these fertilizers might represent an important source of *H. capsulatum* infection. However, the role of organic fertilizers based on poultry manure as a potential source of *H. capsulatum* infection for compost workers, housekeepers, gardeners, and those involved in the production and use of such products has not been sufficiently investigated. Similarly, the longevity of *H. capsulatum* in fertilizers manufactured from this material is unknown. This study was designed to determine the ability of *H. capsulatum* to survive the composting process and to investigate its longevity in composted organic fertilizers.

2. Materials and Methods

2.1. Inoculum of H. capsulatum. *Histoplasma capsulatum* isolate 13180 was obtained from a bronchoalveolar lavage sample provided by a patient who attended to the Medical and Experimental Mycology Unit, at the Corporación para Investigaciones Biológicas (CIB). The isolate was maintained at room temperature in the mycelial phase by passaging every three months in tubes containing fresh Mycosel agar (BBL™; ref. 211462. Franklin Lakes, NJ, USA).

For each experiment, the inoculum was produced by culturing the fungus on potato dextrose agar (PDA) (BBL™; ref. 213400. Franklin Lakes, NJ, USA) followed by incubation at 25°C for 14 days. After this incubation period, 3 ml of sterile 0.85% saline solution containing 0.01% Tween 80 (Sigma, ref. P4780. St. Louis, MO, USA) was added to the culture tubes. The aerial mycelia were scraped with an inoculation loop, and the product of this washing was deposited into a sterile beaker. The number of colony-forming units per milliliter (CFU/ml) was determined by plating in duplicate on Sabouraud dextrose aAgar (BBL™; ref. 211584. Franklin Lakes, NJ, USA), 200 μl of each serial dilutions 1 : 10, 1 : 100, and 1 : 1000. The compost samples were artificially contaminated by adding 5 ml of inoculum solution per 10 g of sample.

2.2. Composting Reactor System. The *H. capsulatum* survival ability to the composting process was evaluated in a small-scale composting system designed to allow the controlled conditions. A mesophilic-type composting process was performed within glass bottles that functioned as reactors, where the organic matter was spontaneously transformed throughout 45 days only by native microorganisms present within the substrate (Figure 1).

The system consists of two glass bottles (reactors) with 4 liter capacity. Each reactor has a rubber cap with two holes that allow a hose to pass through. One hose was connected to an air pump to allow aeration of the organic matter and the other hose permitted gas release into a disinfectant solution (hypochlorite 5000 ppm) to avoid the aerosolizing of infective particles into the environment. Each reactor had a grid at the base to prevent caking of the organic matter and to achieve homogenous aeration of the substrate.

2.3. The Composting Substrate. Chicken manure and sawdust were mixed in a 1 : 1 ratio. Six hundred grams of this mixture was deposited in each reactor (glass bottle). One reactor was inoculated with 300 ml of the inoculum of *H. capsulatum* and the second reactor was inoculated with the same volume of sterile 0.85% saline solution. Both reactors were located inside a biosafety cabinet level 3. Twenty milliliters of sterile 0.85% saline solution was added to each reactor every two days, with manual shaking to allow the aeration of the organic matter. After 45 days, the organic matter was considered to be a stable product, and the control reactor content was analyzed according to the NTC 5167 parameters to confirm the product was entirely transformed. The product within the reactor inoculated with *H. capsulatum* was analyzed as follows: the reactor bottle was demarcated according to the depth; surface, 5 cm, 10 cm, and 15 cm depth, and the substrate of each profundity was removed and separately placed into a container for downstream analysis using the Hc100 nested PCR, and Mycosel culture as it is described below.

2.4. DNA Extraction from Composted Material. The FastDNA SPIN Kit For Soil® (MP Biomedicals, Santa Ana, CA, USA) was used to extract DNA from composted fertilizers using the manufacturer's instructions with some modifications. Briefly, the extraction was performed using the supernatant obtained after suspending 10 g of composted sample in 30 ml of saline solution containing 0.001% Tween 80 and 0.1% antibiotics (gentamycin and penicillin). The suspension was stirred vigorously for 1 min and allowed to settle for 20 min. This procedure was repeated twice. After the last stirring, the suspension was allowed to sit until the largest particles were settled. Next, the supernatant was collected for DNA extraction. The other modification consisted of increased contact time between the sample and kit reagents.

2.5. Hc100 Nested PCR. The Hc100 nested PCR assay in composted fertilizers was performed as was described before by Gomez et al. [34].

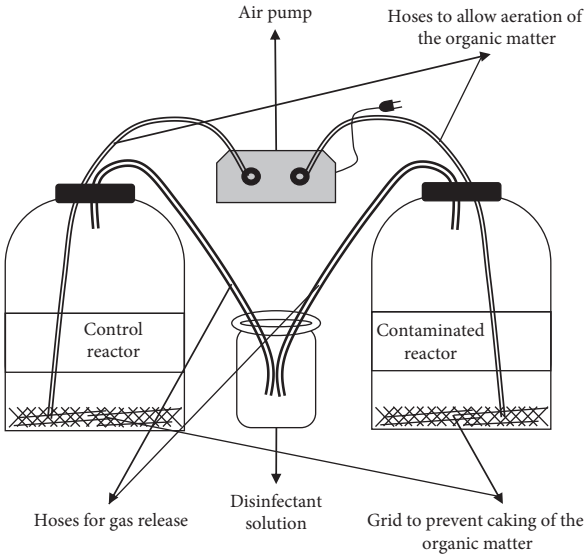

Figure 1: Composting reactor system.

Two sets of PCR primers specific for *H. capsulatum* were used (a pair of external primers Hc I ⟶ 5′-GCG TTC CGA GCC TTC CAC CTC AAC-3′ and Hc II ⟶ 5′-ATG TTC CAT CGG GCG CCG TGT AGT-3′ and a pair of internal primers Hc III ⟶ 5′-GAG ATC TAG TCG CGG CCA GGT TCA-3′ and Hc IV ⟶ 5′-AGG AGA GAA CTG TAT CGG TGG CTT G-3′) that were designed by previous investigators [35]. These primers amplify a fragment of a single-copy gene encoding a 100 kDa *H. capsulatum* protein [36]. The external primers amplified a 391-bp fragment, while the internal primers amplify a 210-bp fragment from within that. The presence of this amplification product was considered positive for *H. capsulatum*. The primers were synthesized by Integrated DNA Technologies (IDT, Coralville, IA, USA).

2.6. H. capsulatum Viability in Organic Fertilizer. The ability of *H. capsulatum* to survive in an organic composted fertilizer produced from chicken manure was evaluated in a composted chicken manure sample that was compliant with the conditions described in NTC 5167 [33], and with concentrations of nitrogen, phosphorus, and potassium (NPK) above 1%. The compost sample was divided into two portions, one was sterilized at 121°C for 15 minutes and the other was retained for downstream analysis. Both samples were aliquoted into 26 portions of 10 g in 50 ml conical tubes. Five ml of the *H. capsulatum* inoculum solution was added to each tube. The 52 contaminated tubes were used to evaluate the growth of the fungus within sterile and unsterilized samples. From days 0, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, and 300, DNA was extracted from a sample and tested for *H. capsulatum* using the Hc100 nested PCR, and a portion of the sample was subjected to Mycosel culture.

2.7. H. capsulatum Persistence in Compost Factories. The samples were send to the Grupo Interdisciplinario de Estudios Moleculares (GIEM), Chemistry Institute, Faculty of Exact and Natural Sciences, Universidad de Antioquia, who has the permission from Instituto Colombiano Agropecuario (ICA) through the resolution number 124 00005865 from May 19/2017 to perform the physical, chemical, and microbiological analysis of organic fertilizers, soil amendments, and nitrogen sources for organic fertilizers.

In an earlier study, the Hc100 nested PCR was used to detect *H. capsulatum* in composted organic fertilizers; the compost manufacturers that were positive for *H. capsulatum* DNA were newly contacted to be part of the present study to investigate the persistence of *H. capsulatum* in those factories [34]. The manufacturers were asked to provide two samples between 500 g and 1000 g of material collected from the same compost pile, representing different times: the first corresponded to day zero of the composting process (i.e., the mixture of raw materials before composting) and the second to the endpoint of the process (i.e., the transformed product). Those samples were tested for the presence of *H. capsulatum* using the Hc100 nested PCR and culture on Mycosel agar.

2.8. Detection of H. capsulatum by Cultivation in Mycosel Agar. The culture was performed using the supernatant obtained after suspending 10 g of sample in 30 ml of sterile saline solution (0.85%) containing 0.001% Tween 80 (Sigma, ref. P4780. St. Louis, MO, USA), 100 pg/ml oxytetracycline (MK. Cali, Valle, COL), and 0.1% antibiotics (gentamycin and penicillin). The suspension was stirred vigorously for 1 min and allowed to settle for 20 min. This procedure was repeated twice. After the last stirring, the suspension was allowed to settle. The resulting supernatant was collected, and serial dilutions were performed (1 : 10, 1 : 100, and 1 : 1000), and 200 μl of each dilution was plated on Mycosel agar in duplicate. The cultures were incubated at room temperature over two months, and the cultures were observed on days 5, 10, 15, 30, 45, and 60, to look for *H. capsulatum*–compatible colonies.

2.9. Confirmation of Colonies Compatible with H. capsulatum. Colonies consistent with *H. capsulatum* are cottony, raised, hard-edged, white, creamy or light coffee, and slow-growing. Each colony suspected as being *H. capsulatum* was subcultured on a fresh Mycosel agar plate for subsequent microscopic examination with Lactophenol blue to observe the characteristics compatible with *H. capsulatum,* i.e., thin septate hyaline hyphae, thin-walled microconidia, and tuberous macroconidia. Colonies with compatible features were subcultured in brain heart infusion agar (BHI Agar, BBL™; ref. 211065. Franklin Lakes, NJ, USA) supplemented with 1% glucose (Sigma, Ref. G5400. St. Louis, MO, USA), 0.001% L-cysteine (Sigma, ref. C-7755. St. Louis, MO, USA), and 5% anticoagulated blood, and then the cultures were incubated at 37°C in 5% CO_2 atmosphere to evidence the transformation into the yeast phase.

3. Results

3.1. Design and Assembly of the Reactor System. The efficiency of the reactor system at converting organic matter into a

stable product was evaluated before the inoculation of the raw material with the *H. capsulatum*. Once composting process in the reactor was completed, the product was analyzed according to the parameters set by the NTC 5167. Ultimately, the final product was found contain nitrogen (N), phosphorus (P) and potassium (K), at levels above 1%, which is consistent with the parameters provided in the NTC 5167.

Five subsequent assays were conducted spiking the raw material (sawdust + poultry) with a quantified *H. capsulatum* inoculum solution. In any of the trials, the fungus was not isolated at the culture in Mycosel medium; however, in all reactor products the genetic material of *H. capsulatum* was detected. Table 1 summarizes the results of each experiment.

3.2. H. capsulatum Viability in Organic Fertilizer. In this study, aliquots of both sterile and nonsterilized composted samples contaminated with *H. capsulatum* were analyzed at different time points. The microbiological study in the Mycosel culture medium was negative in all samples that were nonsterilized and positive with different CFUs on the sterile composted samples. In contrast, the results of the Hc100 nested PCR were positive in all samples, both sterile and nonsterilized. Table 2 illustrates the results of *H. capsulatum* recovery on different sample processing days.

3.3. H. capsulatum Persistence in Compost Factories. The five companies that were contacted for the *H. capsulatum* persistence study agreed to participate.

A total of 56 samples were analyzed by culture in Mycosel and by Hc100 nested PCR for *H. capsulatum* search. From those, 33 (59%) samples were raw material, and 23 (41%) samples were transformed product by the composting process. From samples of factories 1, 3, and 5 neither the *H. capsulatum* was isolated on Mycosel culture, and its DNA was not detected by the Hc100 nested PCR. Contrarily, the *H. capsulatum* DNA was detected by Hc100 nested PCR on raw samples from factories 2 and 4 and in one composted sample from Factory 4. Interestingly, only from one raw sample of the Factory 2 (F2), three colonies of the fungus were successfully grown in Mycosel culture (Table 3).

3.4. Confirmation of Colonies Compatible with H. capsulatum. Colonies compatible with *H. capsulatum* obtained from Mycosel culture were confirmed by microscopic observation. Additionally, all *H. capsulatum* colonies obtained were transformed into the yeast phase successfully.

Colonies from other fungi genera were identified and discarded. The genera most frequently encountered were *Chrysosporium* spp., *Sepedonium* spp., *Aspergillus* spp., *Penicillium* spp., *Acremonium* sp., *Chrysonilia* sp., and *Trichoderma* sp. among other molds without sporulation. Additionally, yeast colonies from different genera were also isolated. These included *Candida* spp., *Rhodotorula* spp., and *Geotrichum* spp. Some bacterial colonies were also cultured, although no attempts were made to identify these.

4. Discussion

The findings of this work suggest that a well-done composting process results in an organic fertilizer free of *H. capsulatum*. Despite *H. capsulatum* DNA could be detected using the Hc100 nested PCR, this DNA is the genetic fingerprint of the fungus.

To determine the ability of *H. capsulatum* to survive the composting process, three assays were performed. The first assay was conducted to obtain a composted product under laboratory conditions, which met Colombian regulations (NTC 5167) [33]. Once the well-done composting process was successfully obtained, five further experiments were performed on the resulting composted product, which involved inoculating it with a solution containing live *H. capsulatum*. Following the analysis of the final composted products, the viable *H. capsulatum* could not be reisolated in the Mycosel culture. However, analysis by Hc100 nested PCR showed positive results for the reactors in almost all depths analyzed. Therefore, the detection of *H. capsulatum* DNA at almost all depths of the laboratory reactor system suggests the heterogeneity of the fungal distribution in the environment.

Regarding the second experiment, five compost companies provided samples collected at the beginning and at the end of the composting process (Table 3). *H. capsulatum* DNA was detected in the raw material collected from factories 2 and 4 and from one composted sample from Factory 4. Additionally, a viable fungus was successfully isolated in culture from raw material provided by Factory 2. For samples sent by Factory 1, *H. capsulatum* DNA was not detected, and isolation of viable fungus was not achieved, despite the large quantity of material assayed and the fact that the fungus has been previously detected in their composted products. This result might be due to the control strategies that the company implemented since the initial detection of the fungus, to improve the quality and health security of the final product. In general, this assay has a behavior similar to that previously described; a higher positivity of raw materials in contrast with the composted ones [34]. Unfortunately, there are no other works similar to this to compare the results shown in this study.

It is important to notice that the detection of the *H. capsulatum* genetic material in the organic fertilizers both at the start and at the end of the process and, furthermore, the isolation of *H. capsulatum* on culture from a raw material led us to issue an alarm due to the presence of *H. capsulatum* at the beginning of the composting process. Consequently, it is important to encourage the use of protective measures by the people involved in the production of organic fertilizers and also to emphasize the incorporation of the protocol that we reported previously [34]; preferably by screening at regular intervals using the Hc100 nested PCR, for the presence of *H. capsulatum* in both the raw materials and the final composted product. Ultimately, routine screening during the composting process could help avoid infection of people with *H. capsulatum* and reduce the incidence of outbreaks [37].

Table 1: Trials of the composting reactors system leading to establish survival of *H. capsulatum* at the composting process after 45 days.

Assay no.	Contaminated reactor				Control reactor	Observations
	Inoculum (CFU/ml)†	Depth	Culture on mycosel	Hc100 nested PCR	NTC 5167	
1	4200	Not done	Negative	Positive	Approved	Flies grew inside culture media; this situation lead to an excessive grow of bacteria in culture, which did not allow to visualize the *H. capsulatum* growth.
2	3800	Not done	Negative	Positive	Approved	Bacteria and other fungi had an abundant growth, which did not allow to visualize the *H. capsulatum* growth.
3	5800	Surface 5 cm 10 cm 15 cm	Negative Negative Negative Negative	Positive Negative Positive Positive	Approved	
4	4050	Surface 5 cm 10 cm 15 cm	Negative Negative Negative Negative	Positive Positive Positive Negative	Approved	Microbiological study was carried out to the satisfaction, although the growth of *H. capsulatum* was not obtained.
5	3200	Surface 5 cm 10 cm 15 cm	Negative Negative Negative Negative	Positive Negative Positive Positive	Approved	

†Quantification of *H. capsulatum* inoculum solution which was used to contaminate each reactor.

Table 2: Recovery of colony-forming units (CFU/ml) of *H. capsulatum* from sterile and nonsterilized composted samples contaminated with the fungus.

Day of processing after sample contamination	*H. capsulatum* inoculum solution (CFU/ml)	Culture Mycosel		Hc100 nested PCR	
		Nonsterile soil (CFU/ml)	Sterilized soil (CFU/ml)	Nonsterile soil	Sterilized soil
0	3460	0	1960	Positive	Positive
15		0	44000	Positive	Positive
30		0	75200	Positive	Positive
45		0	15600	Positive	Positive
60		0	26200	Positive	Positive
90		Not done	Not done	Positive	Positive
120		Not done	Not done	Positive	Positive
150		Not done	Not done	Positive	Positive
180		Not done	Not done	Positive	Positive
210		Not done	Not done	Positive	Positive
240		Not done	Not done	Positive	Positive
270		Not done	Not done	Positive	Positive
300		Not done	Not done	Positive	Positive

Table 3: *H. capsulatum* persistence in composting factories.

Factory number	Composting process stage		Culture on Mycosel				Hc100 nested PCR positives			
	Raw materials ($n^{\dagger}$)	Final product (n)	Raw materials (n)	%	Final product (n)	%	Raw materials (n)	%	Final product (n)	%
Factory 1 (F1)	19	6	0	0	0	0	0	0	0	0
Factory 2 (F2)	9	7	1	11,1	0	0	2	22,2	0	0
Factory 3 (F3)	0	5	NA	NA	0	0	NA	NA	0	0
Factory 4 (F4)	4	4	0	0	0	0	2	50	1	25
Factory 5 (F5)	1	1	0	0	0	0	0	0	0	0
Total	33	23	1	11, 1	0	0	4	12,1	1	4,3

Raw material refers to the mixture of the organic material to be composted, i.e., at day zero. Final product refers to the transformed product after 45 days of composting process. $n^{\dagger}$: number of samples sent by each factory to search *H. capsulatum*.

The challenging isolation of *H. capsulatum* from environmental samples using direct culture is a feature which shares with other thermic dimorphic human fungi pathogens that form yeast-like *Blastomyces dermatitidis* and *Paracoccidioides* spp. Despite having data about the characteristics of their habitat, like the meteorological parameters and the chemical features of the soil that they inhabit or the possible sources of infection, all the attempts to isolate them from the environment have not been successful [38–41].

Another common difficulty linked to the isolation of *H. capsulatum* using culture media is the isolation of fungi with similar characteristics since the observation of a fungus with a cottony colony, color white to beige, with tuberous macroconidia do not guarantee *H. capsulatum* was recovered. *H. capsulatum* could be morphologically mistaken with other fungi-like *Sepedonium* spp. which has tuberculate macroconidia or *Chrysosporium* spp., which produce microconidia similar to *H. capsulatum*; in those cases, it is necessary to confirm by inducing transformation to the yeast phase [42, 43]. Considering that the isolation of *H. capsulatum* using Mycosel culture is time consuming and requires a skilled observer, the Hc100 nested PCR becomes an excellent alternative to the presumptive search for *H. capsulatum* in the environmental samples.

In the third assay, experiments were conducted to establish the viability of *H. capsulatum* in an organic fertilizer and it was observed that the recovery of *H. capsulatum* from sterile samples on days 0, 15, 30, and 45 postinfection was 2,400, 22,000, 37,600, and 7,800 CFU/ml, respectively. The quantity of CFU/ml of *H. capsulatum* suggests an increase compared with the initial inoculum. The higher growth may be due to the sterilization by autoclaving process that induces two different effects: one is the hydrolysis of the soil resulting in a highly significant increase in pH, NO_3-N, NH-N, extractable Mg, Mn, and Fe, and the other is the death of the organisms of the soil community. These two effects favor the nutrition and growth without competitors. Emmons described a similar result after conducting a study where *H. capsulatum* was inoculated in a sterile sample of soil rich in humus, and then he noted that the fungus proliferates freely producing macroconidia. Given that *H. capsulatum* has the ability to proliferate in sterile soil with high organic matter content and that we reached the isolation of the fungus from a raw chicken manure by using conventional culture in Mycosel, it would be interesting to develop a specific and enriched media culture based on the organic matter to improve the isolation of *H. capsulatum* from environmental sources [14, 44].

At the beginning of this study, experiments were performed to standardize the inoculum size (results not shown) to contaminate the environmental samples with *H. capsulatum*. During those trials, we observed that to recover the fungus on culture an inoculum higher than 40,000 CFU/ml of *H. capsulatum* is necessary, in contrast with the DNA from three cells of *H. capsulatum* that is needed to be detected by the Hc100 nested PCR [34]. Also, we observed on the culture of these samples the growth of numerous environmental molds like *Chrysosporium* spp., *Sepedonium* spp., *Penicillium* spp., *Aspergillus* spp., *Trichoderma* spp., and *Geotrichum* spp. These molds have also been reported in other studies. The isolation of these fungi suggests that they share the habitat with *H. capsulatum* and act as strong competitors for space and nutrients; this factor affects the *H. capsulatum* survival in the soil and moreover its recovery by conventional culture media [43]. Also, in relation with the composting process, since this is a decomposition process in which multiple microorganisms are involved such as fungi, bacteria, and actinomycetes, the isolation of *H. capsulatum* from the samples is very difficult given that those microorganisms grow faster and in more quantity than *H. capsulatum* [45, 46].

The results of this study point out the importance to use molecular tools such as Hc100 nested PCR due to the difficulty to isolate *H. capsulatum* from soil samples in culture [47]. This leads us to believe that most of the studies that have reported *H. capsulatum* at low percentages from soil samples do not reflect the real data of potentially infected places [6, 7, 10, 14, 48, 49], which have a significant impact on public health as the real sources of infection have not been certainty identified.

Moreover, in Colombia, the rate of histoplasmosis was calculated in 0. 6 cases per 100.000 inhabitants based on publications, disease reports, and an estimate of the Colombia population, but it is known that this rate is underestimated [50] because this disease has no mandatory report. In other countries as Cuba, through the Joint Resolution number 2 in 1996, they recognize the histoplasmosis as an occupational disease and it is mandatorily communicated to the health authority. It is important to appeal to health regulators about the need to implement in Colombia a model to the surveillance of histoplasmosis similar to the implemented by Cuba.

5. Conclusions

In conclusion, the substrates rich in organic matter give *H. capsulatum* the ideal conditions for its maintenance in nature. Thus, *H. capsulatum* is part of the community of organisms that are present in the raw material that would be transformed by the composting process, and it is undeniable that the presence of this fungus is since the beginning of the process. Therefore, to avoid outbreaks and cases by *H. capsulatum*, it is crucial promoting inside the compost factories the implementation of the preventive measures recommended by the Center for Disease Control and Prevention (CDC) to protect the workers and also adequately performing the composting process to ensure sanitization of the final product [37].

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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