Fungi in biofilms of a drinking water network: occurrence, diversity and mycotoxins approach

ABSTRACT
Results showed that 75% of the analyzed samples in a drinking water network were positive for fungi, in a range of 1–3,000 CFU/mL. Identification resulted in nine species of fungi and four species of yeasts being listed: Bjerkandera, Penicillium, Paraconiothyrium, Paecilomyces, Debaryomyces, Rhodotorula and Cryptococcus. Although yeasts showed higher traceability than filamentous fungi, the fungal genus Penicillium had relevance by both traceability (six species) and its role in mycotoxin generation. From volatile organic compound (VOC) mycotoxins and extracts analysis from P. ochrochloron and P. purpurogenum water–M9 culture, six groups were identified: phenols, alcohols, alkenes, monoterpenes, aldehydes and alkanes, phenols being the predominant group (2,4-bis(1,1-dimethyl)phenol 40–88%). P. ochrochloron water culture and M9 culture reported signals of toxicity: the first one as genotoxic for 0.5 y 1 mg/mL mycotoxin extract and the second one as cytotoxic. M9 media promoted a higher number of compounds in both species and a decrease in phenol predominance in P. ochrochloron but not in P. Purpurogenum. The results showed Penicillium and Debaryomyces as prevalent filamentous fungi and yeast in assessed networks, suggesting that these could be indicators of fungi and yeast presence in drinking water systems.

Key words | biofilms, drinking water, fungi, yeast

INTRODUCTION
The occurrence of filamentous fungi and yeasts in biofilms of drinking water distribution systems has already been reported in other studies (Hageskal et al. 2009; Pereira et al. 2009). Classic and molecular techniques have been developed to recover and identify biofilm microorganisms of drinking water networks. This diversity of available procedures to identify biofilm microorganisms entails differences between isolation reports and their associated inferences (Pereira et al. 2010).

Some of these microorganisms produce secondary metabolites that modify organoleptic properties of water; also some fungal genera such as Penicillium spp., Aspergillus spp., Cladosporium spp. and Phialophora spp., have been reported as mycotoxin producers (Goncalves et al. 2006; Hageskal et al. 2009; Pereira et al. 2009). Penicillium spp. and Aspergillus spp. have widely been identified in food and beverages as potential producers of flavoring secondary metabolites (i.e. aldehydes, alcohols), or undesirable metabolites that alter the production process (i.e. aflatoxins, geosmin) (Moreau 1979; Pitt & Hocking 1999; Hageskal et al. 2009); geosmin, an earthy-muddy-smelling metabolite, also produced in drinking water, has principally been associated with taste-and-odor outbreaks (Hageskal et al. 2009).

From in vitro studies, in two different water distribution systems, Kelley (2005) concluded that fungi in water could produce mycotoxins and other secondary metabolites. Although mycotoxins in water would be under detection limits for dilution effects, these concentrations could be increased when water is stored during long periods...
(i.e. tanks, bottles). For example, Mata et al. (2015) detected frequently aflatoxin B2, and eventually aflatoxin B1, aflatoxin G1 and ochratoxin in bottled water, and also fungi of genders Cladosporium, Fusarium and Penicillium were isolated. For these cases, daily intake of water affected by fungi growth becomes a concern for human health (Hageskal et al. 2009).

Fungi as ubiquitous organisms are present in soil, air, food, and water as well as their metabolites. Fungal volatile organic compounds (VOC) are derived from both primary and secondary metabolism pathways. Approximately 250 VOC have been identified from fungi where they occur as mixtures of simple hydrocarbons, heterocycles, aldehydes, ketones, alcohols, phenols, thioalcohols, thioesters and their derivatives, including, among others, benzene derivatives, and cyclohexane (Morath et al. 2014). The analysis of fungal VOC in the environment and its relation with human health has been focused on indoor exposure of fungi isolated from water-damaged, moldy buildings (Institute of Medicine of the National Academies 2004; WHO 2009). Inamdar et al. (2014) tested VOC produced by three fungi species in Drosophila melanogaster to assess their toxicity; negative effects were observed for eight carbon volatile compounds exposure. Fungal secondary metabolites, volatile (VOC) and non-volatile, in water are still not well understood and limits for treated water are rare in drinking water rules. Additionally, in treated water these organic compounds react with disinfectants, forming disinfection by-products (DBP), some controlled by water rules for their probable health effects (Richardson et al. 2007). Taking into account this information, fungal presence and fungi metabolites production must be assessed in drinking water distribution systems, more specifically in household systems and tap water, where stagnation is common.

In order to determine the potential impact of filamentous fungi and yeasts on water quality, and to identify prevalent species, phenotypic and genotypic features were used to characterize fungal and yeast populations recovered from biofilm samples in drinking water distribution systems; also an identification was performed of mycotoxins extract from isolated species from the main pipeline and household plumbing, to make an approximation to principal compounds that could be being generated and their genotoxic effect in mammalian cells.

**MATERIAL AND METHODS**

**Fungi isolation**

**Biofilm sampling**

Twelve biofilm samples from two Colombian drinking water networks, which supply surface water treated by conventional processes (i.e. flocculation, settling, filtration and hypochlorite sodium chlorination), were analyzed. Distribution systems include both primary and secondary networks. The range of free chlorine was between 0.5 and 1.5 mg Cl/L. The number of samples was limited by the scheduled maintenance dates of distribution systems, according to the specifications of the public service operator. The samples were taken according to previous protocol (Zhang et al. 2009) from the water-surface interfaces, when sites were in not flow mode and water was removed by pumping: biofilms were scraped with sterile spatulas and stored in Ziploc® bags at 4°C until analysis. Sampling details are specified in Table 1.

**Samples pre-treatment**

In order to remove inorganic material that may inhibit microbial growth, the biomass of samples was extracted according to the established protocol (Zhang et al. 2009) and dissolved to obtain the relation 1:1 mL per cm², with an additional 30 minute-shaking period in M9 media (7.25 g Na₂HPO₄·2H₂O, 5 g KH₂PO₄, 0.71 g NaCl, 2 g NH₄Cl, 1 mL MgSO₄ 1 mol/L, 0.1 mL CaCl₂ 1 mol/L, 1 mL FeCl₃ 10 mmol/L, 2 g yeast extract), which allows sufficient sample aeration for fungal demands.

**Fungal recovery**

Fungal and yeast populations were recovered in enriched and fungal specific culture media Potato Dextrose Agar (PDA) (4 g potato infusion, 20 g dextrose, 15 g agar), Rose Bengal Agar (RBC) (5 g soy peptone, 0.1 g chloramphenicol, 0.05 g Rose Bengal, 10 g dextrose, 1 g monopotassium phosphate, 0.5 g magnesium sulphate, 15 g agar) and Malt Extract Agar (MEA) (1 g peptone, 20 g malt extract, 20 g
glucose, 20 g agar) from samples with and without pre-treatment. Each sample was serial diluted ($10^{-1}$ to $10^{-3}$), and 1 mL of each dilution was plated three times. An incubation period at 26.5°C (Deacon 2006; Cepero de Garcia et al. 2012) was adjusted to 8–15 days for fungi and 3–5 days for yeasts. Additionally, direct plating of samples with the same culture conditions was analyzed.

### Fungal counting

Colony forming units per millilitre (CFU/mL) of each described morphotype were visually counted (partial counts), and the sum of CFU/mL in all plates from each of the first two dilutions was calculated (total counts). In each case, the most representative counting per dilution triplicate was reported.

### Fungal isolation

Fungal morphotypes were isolated by puncture culture, while yeast morphotypes were isolated by streaking culture. Direct plating of predominant recovered morphotypes was done per duplicate for each sample, followed by an incubation period of 15–30 days for filamentous fungi and 3–5 days for yeasts, both at 26.5°C.

### Species identification

Isolated morphotypes were identified by their phenotypic (macroscopic and microscopic characterizations by Lactophenol Blue Staining) and genotypic characters. Macroscopic characters were registered at two different incubation periods: after the recovery process, and after the isolation procedure. Microscopic characters were registered only at the post-isolation period. DNA was extracted according to the protocol for fungal endophytes of the Laboratory of Mycology and Plant Pathology of Universidad de Los Andes. Polymerase chain reaction (PCR) reactions were carried on with ITS1 and ITS4 universal primers. Sequencing of PCR products was performed by the Macrogen Company. The resulting sequences were edited and assembled using Geneious Pro v 4.8.5 software (Geneious, 2011). Comparisons between these reports and results from phenotypic and genotypic identification allowed us to determine the final species of filamentous fungi and yeasts from biofilm samples.

### Mycotoxins

*Penicillum ochrochloron* and *P. purpurogenum* isolated from the main pipeline and household plumbing were cultured in M9 media and tap water for 7 days (30°C) in a...
glass Erlenmeyer flask. The system was fed daily with 72 mL of sterilized tap water or M9 until it reached the final volume of 500 mL and was continuously shaken at 300 rpm.

### Mycotoxins extraction

At the end, mycotoxins were extracted by liquid–liquid extraction with methanol, hexane and chloroform based on the García *et al.* (2009) protocol. In an Erlenmeyer flask 50 mL of media and 150 mL of methanol were mixed for 10 min. After this, the samples were filtered by Whatman No. 4 filter paper adding 50 mL of methanol. In a separating funnel, 40 mL of hexane were added to the filtered methanolic samples; after which they were mixed for 1 min and the phases allowed to separate. The hexane was discarded. Water under 8 °C was added and pH was adjusted to 2 with HCl. The process was repeated twice with 40 mL of chloroform at 2 min shaking. Then 30 mL of water were applied for washing. Finally, chloroform extracts were evaporated at 35 °C. The solid extracts were suspended in (deionized) DI water for total organic carbon (TOC) and VOC.

### VOC

The protocol used was ASTM 6520-06/EPA 8260B as follows: solid-phase microextraction with polydimethylsiloxane (PDMS) coated fiber was used. Sodium chloride (3 g) was added to 10 mL of extract suspension. Samples were shaken and heated at 35 °C for 20 min. After, the fiber was exposed into the head space for 10 min. VOC were detected by Agilent Technologies 6890 N® GC-MS (gas chromatography–mass spectrometry).

### Genotoxicity

DNA damage was determined by Comet Assay. Vero cells, cultured as for cytotoxicity assay, were exposed to 0.01, 0.1 and 1 mg TOC/mL of extracts for 3 h. Electrophoresis was run at 25 V and 290 m for 35 minutes. The sheet was washed with neutralizing solution, pH 7.5, stored with absolute methanol to be dyed subsequently with GelGreen® (Biotium) and read on a fluorescent microscope Zeiss® (excitation filter BP 450–496 nm, scan filter at 520 nm and ×100 magnification). One hundred cells were analyzed for each sample and DNA damage was determined by % DNA in the tail, using the CASP®1.2.3b1 program.

### RESULTS

#### Fungi isolation

Fungal and yeast species were present in 75% of the analyzed samples in a range of 1–3,000 CFU/mL. Initial recovery of 12 fungal and nine yeast morphotypes was phenotypically characterized, and then was expanded to 27 genetically characterized morphotypes. Additionally, the results supported higher diversity for secondary distribution systems (household showers), and higher predominance for the primary distribution systems (Table 1).

*Paraconiothyrium sporulosum* (*P*. *sporulosum*; 2935 CFU/mL), and *Paecilomyces lilacinus* (*P*. *lilacinus*; 108 CFU/mL) were the most numerically abundant fungi, according to the highest averaged count. Under the same criteria, the most predominant yeasts were *Debaryomyces hansenii* (*D*. *hansenii*; 143 CFU/mL), *Cryptococcus diffluens* (*C*. *diffluens*; 95 CFU/mL), and *Rhodotorula mucilaginosa* (*R*. *mucilaginosa*; 70 CFU/mL). Additionally and by sample origins, secondary distribution systems evidenced a wider variety of species (a total of six species) while primary water networks showed the highest total counts in M8 sample (maximum of 2,935 CFU/mL); however in general, the recoveries at both primary and secondary samples were similar in range (Figure 1). Differences in diversity in sample M12 could be associated with low residual chlorine at extreme points in the network, the higher surface area/volume ratio of water in household pipelines compared to a mains system or a material incidence (i.e. zinc vs concrete or iron).

*Debaryomyces hansenii* was recovered from both primary and secondary distribution systems. Similarly, *Penicillium* spp. were found in both types of networks, but with...
values of averaged counts less than those reported for yeast species. However, the wide variability of this genus supports possible complex mechanisms to repair mutations that could explain their persistence in distribution systems, even after water treatment.

**Mycotoxins**

Mycotoxin extracts would contribute TOC of 3.5–6.1 mg/L, depending on fungal culture medium, being that M9 promoted a higher quantity of organic compounds per litre of medium. Even when quantity differences between the two sources of carbon were relevant (water TOC: 1 ± 0.1 mg/L; M9 TOC 0.700 ± 0.07 g/L), extracts of organic metabolites were not dissimilar in the same order, according to TOC results. From VOC analysis, six groups were identified: phenols, alcohols, alkenes, monoterpenes, aldehydes and alkanes, phenols being the predominant group (40–88%) in mycotoxins extracts (Table 2).

M9 medium promoted a higher number of compounds in both species and a decrease in phenol predominance in *P. ochrochloron* but not in *P. purpurogenum*. The extract of *P. purpurogenum* was constituted by phenols, alkenes and aldehydes but these last compounds were not identified in *P. ochrochloron* mycotoxins. The results of genotoxicity showed an increase in DNA damage (percentage DNA in tail) for 0.1 and 1 mg/mL mycotoxin extract of *P. ochrochloron* cultured in water. The comet assay did not detect DNA damage for the other cultures. The compound phenol, 2,4-bis-(1,1-dimethylethyl), principal substance detected by VOC analysis in mycotoxins extract, could be important in toxicity due to genotoxic positive sample having the highest relative quantity over the total of detected compounds (87.9%).

**DISCUSSION**

Results for fungi in water depend on identification techniques: i.e. sampling, pretreatment, culturing: media, temperature; as a consequence, the first limitation of the analysis is how to compare with other studies. Having in mind that the recoveries were obtained from non-specific PDA, RBC and MEA media, which were selected for being widely used in identification of cultivable fungi, the results and the discussion must be interpreted.

Filamentous fungi and yeasts were identified in samples of both primary and secondary distribution systems, showing a relevant role in biofilm networks because of the amount of species as well as the probable implications of their presence on water quality, possibly affecting human health. An assessment of exposure to fungi in the
environment (i.e. water, air, food) would help understanding of the relevance of their occurrence in drinking water, which would be different, for example, in a water-damaged, moldy buildings scenario (Inamdar et al. 2014) than in new buildings. Additionally, mycotoxins, secondary metabolites and special by-products of disinfection would be dissimilar or specific in drinking water systems, so the health response would depend not only on the species but also on the environment in which it grows.

Six identified species of Penicillium corroborate their reported ubiquity, even in aquatic environments. Moreover, four of these species are potential producers of mycotoxins such as Penitrem A by P. crustosum, Rubratoxin by P. purpurogenum, Verruculogen by P. janthinellum and Citromycetin by P. glabrum (Pitt et al. 2013; Bial–Aristegui Pharmaceutical Laboratories 2015). In addition, the genus Paraconiothyrium that was identified in the highest count (2,935 CFU/mL) can produce mycosis infections (Quillet-Dye et al. 2015). Moreover, we also identified yeast pathogens for humans. For example, R. glutinis is another opportunistic pathogen associated with brain diseases such as meningitis in immunocompromised patients (Lanzafame et al. 2001). Cryptococcus difluenus causes subcutaneous cryptococcosis (Serda Kantarcioğlu et al. 2007) through skin colonization of patients with atopic dermatitis (Sugita et al. 2003), and D. hansenii has been reported as a participant in diseases such as extrinsic allergic alveolitis (Yamamoto et al. 2002). As a conclusion, 10 of 13 species identified by culturing in biofilm samples of analyzed drinking water distribution systems could have an impact on consumers’ health. In spite of these results, a total profile of fungi in water-biofilm by total DNA sequencing is needed to support the relevance of these organisms and specifically pathogen species in drinking water systems diversity, because only a few microorganisms grow in culture media.

Taking into account sample location, an increased risk of negative effects on consumer health is suggested by the recovering of P. chrysogenum (M12 sample), P. purpurogenum (M12 sample), and C. difluenus (M10 sample) from samples of the secondary networks. Some reports are consistent with these potential negative effects of microbial presence in distribution systems. For example, bacterial pathogens such as Mycobacterium avium, M. gordenae and Legionella pneumophila were identified in shower biofilm samples, which were partially volatilized by usage processes (Feazel et al. 2013). As a consequence, microbial aerosols may constitute a risk of illness because of possible inhalation of contaminated water droplets (Feazel et al. 2009).

In addition, the occurrence of the identified species could be associated in some cases with pipeline material or aggregates. For example, P. ochrochloron is commonly

### Table 2 | Results of the assessment of mycotoxins from filamentous fungi isolated from drinking water biofilm: VOC

<table>
<thead>
<tr>
<th>Filamentous fungi species</th>
<th>P. ochrochloron</th>
<th>P. purpurogenum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
<td><strong>Site of biofilm sampling</strong></td>
<td><strong>VOC</strong></td>
</tr>
<tr>
<td>Phenols</td>
<td>2,4-Bis(1,1-dimethyl)phenol (%)</td>
<td>87.9</td>
</tr>
<tr>
<td></td>
<td>3,5-Bis(1,1-dimethyl)-1,2-benzenediol (%)</td>
<td>2.07</td>
</tr>
<tr>
<td>Alcohol</td>
<td>1-Hexadecanol (%)</td>
<td>0.245</td>
</tr>
<tr>
<td>Alkenes</td>
<td>1-Tetradecene (%)</td>
<td>0.406</td>
</tr>
<tr>
<td></td>
<td>1-Hexadecene (%)</td>
<td>4.41</td>
</tr>
<tr>
<td></td>
<td>1-Octadecene (%)</td>
<td>6.32</td>
</tr>
<tr>
<td>Monoterpenes</td>
<td>Camphene (%)</td>
<td>0.106</td>
</tr>
<tr>
<td>Aldehydes</td>
<td>Octanal (%)</td>
<td>0.715</td>
</tr>
<tr>
<td></td>
<td>Decanal (%)</td>
<td>0.548</td>
</tr>
<tr>
<td>Alkanes</td>
<td>Tetradecane (%)</td>
<td>0.863</td>
</tr>
<tr>
<td></td>
<td>TOC extracts (mg C/L culture)</td>
<td>5.773</td>
</tr>
<tr>
<td></td>
<td>Genotoxicity extract: %DNA in tail respect to negative control 0–1 mg/mL TOC</td>
<td>6</td>
</tr>
</tbody>
</table>

NS: not significant.
isolated from copper environments (Mohapatra 2008). Its presence in a ductile iron pipeline (M2 sample) could be supported by the high affinity of iron with anionic copper (Moreno 2012). Nevertheless, this fungus was not recovered from the other ductile iron pipeline (M1 sample), suggesting that some spatial differences, related to water–pipeline interactions, are important to determine fungal traceability. Another example is P. janthinellum, which is able to solubilize phosphorus in terrestrial environments. Its presence in the same ductile iron pipeline (M2 sample) could be linked to the high affinity of iron with phosphorus (Moreno 2012). Also, the genus Paraconiothyrium was identified in biofilm from a pipeline (M8 sample) 4 km from DWTP output, where manganese concentration is high because KMnO₄ is used as the oxidant agent in treatment. This genus has been associated with Mn(II) oxidation, lacasse production and it has been found in Mn rich stream sediments and surface water (Miyata et al. 2007). Furthermore, as was mentioned, the highest variety of fungal species in the M12 sample could be explained by the low disinfectant concentrations in household systems provisioned by tanks, such as the secondary networks analyzed in this study. This condition and other physical factors may enhance microbial proliferation. Bacteria such as Bacillus cereus, which were detected in the same kind of network with similar characteristics (Rueda 2011), are an example of potential proliferating microorganisms. Even though some apparent relations between pipeline material and water characteristics with fungi diversity were observed, more samples and also inorganic analysis are needed to point at specific or relevant dependency in the complex system comprising biofilms and drinking water networks.

Although in culturing techniques the number of filamentous fungi is underestimated for their growth in hyphae form, from filamentous fungi and yeast counts it was possible to infer an opposite occurrence of these types of microorganisms in primary distribution network B. For example, in the M7 sample, the yeast D. hansenii had an averaged count of 143 CFU/mL while filamentous fungi P. crustosum and B. adusta had only 1 CFU/mL. In contrast, in the M8 sample the fungus P. sporulosum had the most averaged count of 2,935 CFU/mL while no yeasts were recovered (Figure 1). Based on this relationship between counts, the interspecific competition could be a determinant factor of microbial identification studies, especially those of bacterial species, which constitutes the 80% of biofilm diversity. Previous studies of the samples used reported bacterial species such as Citrobacter freundii (M4 and M3 samples), Klebsiella oxytoca (M1 and M2 samples), Aeromonas hydrophila (M7 and M8 samples), Micrococcus kristinae (Rueda 2011), and Herminiimonas sp. (M6 sample) (Viancha 2011). These species could inhibit fungal growth and permanence by competition, even more so in drinking water distribution systems, being consistent with the same phenomenon in mineral water environments (Fujikawa et al. 1999). Inhibition results from the effects of different factors such as disinfectant concentrations, which may influence the proliferation’s rate of competitive bacteria. Hence, a higher competition level between species recovered from the M6 sample could exist due to low disinfectant concentrations in the storage tank, located far away from the drinking water treatment plant. Nevertheless, correlation between fungi/yeasts and bacteria in biofilm samples and their impact on water quality are not totally understood because of the scarce knowledge of additional factors that determine the survival of particular species. Specifically for D. hansenii, biocontrol mechanisms could be considered. Transformation of this yeast with plasmids encoding toxins has been suggested to regulate the presence of other bacteria (Hernández-Montiel et al. 2010). Despite being unproved, this strategy is conceivable from average counts of this species (M1, M4, M7 and M12 samples) which were higher than the fungal ones. The biocontrol mechanisms could explain the prevalence of D. hansenii in primary and secondary networks. Biedunkiewicz et al. (2014) also found D. hansenii in tap water from two different cities in Poland and bottled water, Exophiala jeanselmei and Aspergillus fumigates being the only species, of 36 identified, recorded in the three types of samples. These tendencies of prevalence could be associated with D. hansenii’s capacity to oxidize a wide range of organic compounds such as alcohols, carbohydrates, amino acids and others (Arlyapov et al. 2013) that are present in treated water, and also in biofilms as secondary metabolites of other microorganisms such as those found from P. purpurogenum culture (Table 2). Even though the absence of enough reports of D. hansenii in biofilms from other distribution systems does not allow proposing it as a quality indicator, these results give preliminary information about constant D. hansenii.
presence in different points through the studied networks. More specific analysis in other systems based on temporal variation are suggested.

Yeast traceability in samples of both primary and secondary distribution systems (with an average count of 2 log CFU/mL; Figure 1) could support resistance mechanisms of species like D. hansenii which show a wide tolerance to saline and halogenated environments (Hernández-Montiel et al. 2010). Halogenated compounds are frequently detected in drinking water networks because of the presence of chlorine and/or disinfection by-products (DBPs) such as trihalomethanes and haloacetic acids (Rodríguez et al. 2004). Also, some crystalline and amorphous compounds of iron–manganese are found (Moreno 2012). Hence, these substances may not only be tolerated by yeasts at different disinfactant concentrations, but also may enhance yeast survival by selection pressure.

The production of secondary metabolites could meet bacterial nutritional demands, facilitating their survival. Volatile metabolites, phenols, alcohols, alkenes, and alkanes, produced in both P. ochrochloron and P. purpurogenum cultured in tap water and M9 medium respectively, and additionally aldehydes for P. purpurogenum in M9 and terpenes for P. ochrochloron in water (Table 1) are normally produced in fungal fermentation, principally alcohols (Larsen & Frisvad 1995). Those volatile compounds have been related to by-products of enzymatic reactions over polyunsaturated fatty acids, linoleic or linolenic acids by lipoygenase and hydroperoxide lyase converting to C8 volatile and C10 non-volatile compounds in the case of Penicillium. sp. (Kermasha et al. 2002). From specific compounds determined in our experiment, only decanal, octanal and tetradecane have been also recorded from Penicillium under other culture conditions (i.e. dry cured ham inoculated by P. chrysogenum; Martin et al. 2006); additionally, to our knowledge, the most relevant 2,4-bis(1,1-dimethyl)phenol has not been reported as a P. chrysogenum or P. ochrochloron and P. purpurogenum metabolite. According to Lattanzio et al. (2006), phenolic compounds are scarce in bacteria, fungi, and algae, but as common secondary metabolites in plants play a role in pigmentation, growth, reproduction and resistance to pathogens. Similar to plants, fungi metabolize bioactive substances with medical properties in pathogen control. Penicillium purpurogenum produced eight phenolic compounds: seven antraquinone derivates and one xanthone that exerted brine shrimp toxicity, moderate inhibition of G. saubinetti, and moderate phytotoxic activities against radish seedling growth (Li et al. 2014). Based on this information, the function of 2,4-bis(1,1-dimethyl)phenol could be linked to survival requirements of fungi in a hostile environment such as drinking water biofilm, where bacteria could represent 80% of total microorganisms and could have other toxicological effects. Even when a direct relationship between the volatile compounds and toxicity is difficult to make, it was observed that both extracts of P. ochrochloron water culture and M9 culture, reported signals of toxicity: the first one as genotoxic for 0.5 y 1 mg/mL mycotoxin extract and the second one as cytotoxic, having the highest relative quantity over the total of detected compounds (87.9%) of phenol, 2,4-bis(1,1-dimethyl), also presence of 3,5-bis(1-1 dimethyl)-1,2benzediol and 1-hexadecanol. Additionally, fungal antibiotics production such as penicillin by P. chrysogenum (Bial – Aristegui Pharmaceutical Laboratories 2002) could reduce populations of susceptible bacteria, modifying disinfectant action in the analyzed water networks. In addition, even low concentrations of antibiotics in drinking water could have unpredictable effects on consumer health related to bacterial resistance development.

According to specific parameters such as prevalence, D. hansenii and Penicillium spp. could be assessed as tracers of yeast and filamentous fungi presence in water networks, which compromise water quality by their occurrence (Holt & Miller 2011). Parameters that preliminary D. hansenii and Penicillium spp. accomplish and must be more deeply assessed in a possible bioindicator selection are: detection, ubiquity, and secondary metabolites. Other species such as R. mucilaginosa and P. lilacinus deserve attention in bioindicator studies because of their outstanding presence in secondary distribution systems.

CONCLUSIONS

From biofilm sampled in drinking water distribution systems, filamentous fungi and yeast were isolated and identified as being D. hansenii and Penicillium spp. found at different points within the network; more analyses are needed in order to identify if they are also prevalent in
other networks in primary and secondary systems. A higher diversity was detected in household systems and specifically \( P. \) purpurogenum isolated from a shower, which should generate Rubratoxin, as has been reported before, and also other metabolites such as phenols, alkenes and aldehydes that could produce other biological reactions. Similar \( P. \) ochrochloron, under water and M9 culture conditions, metabolized volatile compounds with unknown effects in biofilm dynamics and on water quality but signals of toxicity were observed on Vero cells as genotoxicity for 0.5 y 1 mg/mL mycotoxin extract of \( P. \) ochrochloron cultured in water. To our knowledge, \( P. \) purpurogenum and \( P. \) ochrochloron have not been assessed as secondary metabolite producers in drinking water systems either genotoxic assays of them have been made. It would be the first approach, so other deeper analyses, in water, biofilm, with higher number of samples are required to observe the relevance of fungi the presence in water.

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