FUNGI FROM POTABLE WATER: INTERACTION WITH CHLORINE AND ENGINEERING EFFECTS

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ABSTRACT

In recent years various types of imperfect fungi have been isolated from water systems. Fungal spores and mycelia can be inactivated by low concentrations of chlorine in the laboratory but survive in some habitats in water distribution systems. This report describes a field study which provides evidence that some types of fungi are able to grow in water distribution systems. Replicate samples from private residences were used to demonstrate that fungal densities are sometimes much greater than the levels which could be explained by adventitious spores.

The microbiological content of water samples from fire hydrants was often significantly different from that of water samples from nearby private residences. The treated water input to distribution systems was found to be significantly lower in fungus content than water from private residences. Elevated storage tanks open to the atmosphere appear to be significant sources of fungal input to some systems.

KEYWORDS

Drinking water, imperfect fungi, water distribution systems, chlorination, fire hydrants, storage tanks, yeast, molds.

INTRODUCTION

In recent years fungi have been found in a number of water systems in Europe and North America. Bays et al. (1970) in the United Kingdom, Niemi et al. (1982) in Finland, Hinzelin and Block (1985) in France, Nagy and Olson (1982, 1985) in California, West (1986) in Nevada and Rosenzweig et al. (1986) in Pennsylvania and New Jersey all have made surveys demonstrating the presence and documenting the genera of fungi in distribution networks. The great majority of the fungi which have been isolated from water systems are soil hypomycetes (imperfect fungi) which are found in many different habitats. Since the spores of soil fungi are air-borne, it is only to be expected that these organisms will be found in water distribution systems. The mere isolation of fungi from a distribution system does not demonstrate that they are growing therein.

The chlorine demand of fungal conidia has been measured to be in the range of $3.6 \times 10^{-9}$ to $3.2 \times 10^{-8}$ mg per coccidium (spore) while vegetative yeast cells have a chlorine demand between $1.2$ and $8.0 \times 10^{-8}$ mg per cell (Rosenzweig et al. 1983). The use of chlorine for disinfection of water during treatment and to provide a disinfectant residual in the distribution system usually provides initial concentrations high enough (around $1 \text{ mg/L}$) to satisfy the chlorine demand of and inactivate any spores which might be introduced into the water distribution.
system. However, many materials in a distribution system exert a chlorine demand, there are locations where fungal mycelia may be protected from exposure to the chlorine residual and larger bits of mycelia might be able to survive the usual chlorine doses.

A field study was undertaken to find out if evidence of fungal growth in distribution systems could be obtained from water samples. Also, information on the input of fungi in the treated water and from elevated storage tanks was sought. Data on residual chlorine concentrations was collected along with the fungal population densities so that the effect of chlorine on fungal spores in actual distribution systems could be investigated.

**MATERIALS AND METHODS**

Sets of samples were obtained from five different water distribution systems and examined for fungi. Three of the systems are groundwater supplies which had no treatment other than disinfection with chlorine. These include: SR, chlorination dose = 1.0 mg/L; BG, chlorination dose = 2.0 mg/L; and FD, chlorination dose = 2.0 mg/L. Systems FV and WC use surface water supplies treated by coagulation, settling, filtration through rapid sand filters and disinfection with chlorine. The postchlorination dose for system FV is 1.5 mg/l and for system WC it is 0.8 mg/l.

Distribution system samples were obtained from residential taps in all systems and sometimes from fire hydrants and elevated storage tanks. Four replicate samples were obtained at each site for the groundwater systems and 8 replicate samples were obtained at each site in the surface water systems. Replicate sampling was essential to demonstrate the variations in fungal densities in the water systems (see Table 1).

Water was allowed to flow from the sampling tap for at least three minutes before the sample was collected and temperature and residual chlorine concentrations were measured. Samples were collected in sterile bottles to which thiosulfate solution had been added to neutralize any residual chlorine. The samples were protected from exposure to sunlight and stored in insulated chests with artificial coolant until return to the laboratory.

Fungi were isolated from 50 mL portions of the sample by the membrane filter (ML) procedure (Buck and Bubacis 1978). The filters were incubated on plates of Sabouraud dextrose agar that had been supplemented with 33.3 mg/L of rose bengal and 80.0 mg/L of streptomycin. Daily colony counts were made over 2 weeks of incubation. Fungal colonies were isolated by transferring to slants of Sabouraud dextrose agar. Other tests made on the samples in the laboratory were pH, total coliform count and heterotrophic plate count.

<table>
<thead>
<tr>
<th>System</th>
<th>Number of Samples Examined</th>
<th>Population Served</th>
<th>Water Source</th>
<th>Treatment</th>
<th>Daily Pumpage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Residences</td>
<td>Hydrants</td>
<td>Tanks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SR</td>
<td>136</td>
<td>12</td>
<td>none</td>
<td>550</td>
<td>Well</td>
</tr>
<tr>
<td>BG</td>
<td>120</td>
<td>12</td>
<td>48</td>
<td>1000</td>
<td>Well</td>
</tr>
<tr>
<td>FD</td>
<td>88</td>
<td>none</td>
<td>none</td>
<td>250</td>
<td>Well</td>
</tr>
<tr>
<td>FV</td>
<td>64</td>
<td>none</td>
<td>64</td>
<td>300</td>
<td>Surface</td>
</tr>
<tr>
<td>WC</td>
<td>432</td>
<td>96</td>
<td>8</td>
<td>25,000</td>
<td>Surface</td>
</tr>
</tbody>
</table>

b = chlorination, F = filtration, S = sedimentation

**RESULTS AND DISCUSSION**

More than 1200 samples were collected and examined for fungi over a two year period. Due to space limitation the results reported herein are examples of what has been found rather than comprehensive data presentations.

**Growth in Distribution Systems**

Is there evidence from these samples that fungi are actually growing in some places in the distribution systems? The answer to this question can only come from the variations in the types and numbers of fungi in distribution system samples. Only the samples from private residences are used in the following analysis.
A detailed examination of the results from each system revealed that most of the fungus colonies were obtained from only a few locations. In order to demonstrate this the sets of replicate samples from a particular location on one sampling day were classified into those which had no fungi, few fungi, and many fungi. The numbers of samples sets in each category are given in Table 2. For groundwater systems, the rule used to distinguish between samples sets with few fungi and those with many fungi was that all four replicates had to have colonies of the same fungus present and the total number of colonies of that fungus had to be more than 20 in order for a sample set to be placed in the "many fungi" category. The same rule was also used for the surface water systems although 8 replicate samples were collected.

### Table 2. Samples from Private Residences

<table>
<thead>
<tr>
<th>System</th>
<th>No. of Locations Sampled</th>
<th>Total No</th>
<th>Few Fungi</th>
<th>Many Fungi</th>
<th>Total Positive</th>
<th>Positive per 50 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR</td>
<td>34</td>
<td>9</td>
<td>21</td>
<td>4</td>
<td>136</td>
<td>66</td>
</tr>
<tr>
<td>BG</td>
<td>30</td>
<td>10</td>
<td>18</td>
<td>2</td>
<td>120</td>
<td>50</td>
</tr>
<tr>
<td>FD</td>
<td>21</td>
<td>7</td>
<td>13</td>
<td>1</td>
<td>84</td>
<td>37</td>
</tr>
<tr>
<td>FV</td>
<td>8</td>
<td>2</td>
<td>5</td>
<td>1</td>
<td>64</td>
<td>28</td>
</tr>
<tr>
<td>WC</td>
<td>54</td>
<td>20</td>
<td>31</td>
<td>3</td>
<td>432</td>
<td>96</td>
</tr>
</tbody>
</table>

*Four replicates per location were examined for the ground water systems and eight replicates per location for the surface water systems. In order for a set of replicates to be classified as having many fungi, the same fungus had to be present in at least 4 replicates and the total colony count for that fungus had to be at least twenty.*

In system SR, of the 25 locations which had fungi present, 21 were classified as having few fungi and 4 as having many fungi (Table 3). *Phialophora* was responsible for 2 cases of many fungi (the same residence sampled on different days) and the yeasts, *Phaeococcus* and *Cryptococcus*, were responsible for the other two. The numbers of fungus colonies found in the samples from these locations cannot be explained by incidental contamination of the water with spores but supports the hypothesis that fungi were growing in the distribution systems near the sampling locations. Locations 217 and 216 are dead end mains at the extreme end of the distribution system from the well and pump house. The two samples sets from system BG and the one from system FD which were classified as having many fungi were also from dead end locations.

### Table 3. Examples of Locations with Few and Many Fungi - System SR

<table>
<thead>
<tr>
<th>Date Code</th>
<th>Location</th>
<th>Genus</th>
<th>Count in Replicate Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>6a2</td>
<td>209</td>
<td><em>Cladosporium</em></td>
<td>1 0 0 1</td>
</tr>
<tr>
<td>6e1</td>
<td>217</td>
<td>sterile mycelium</td>
<td>1 0 0 1</td>
</tr>
<tr>
<td>6h1</td>
<td>202</td>
<td><em>Cephalosporium</em></td>
<td>0 1 0 1</td>
</tr>
<tr>
<td>6j1</td>
<td>213</td>
<td><em>Cladosporium</em></td>
<td>4 0 0 0</td>
</tr>
<tr>
<td>6i1</td>
<td>218</td>
<td><em>Alternaria</em></td>
<td>0 0 1 1</td>
</tr>
<tr>
<td>6j2</td>
<td>218</td>
<td><em>Penicillium</em></td>
<td>3 1 0 1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date Code</th>
<th>Location</th>
<th>Genus</th>
<th>Count in Replicate Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>6b2</td>
<td>218</td>
<td><em>Phialophora</em></td>
<td>&gt;200 &gt;200 &gt;200 &gt;200</td>
</tr>
<tr>
<td>6d1</td>
<td>217</td>
<td><em>Phaeococcus</em></td>
<td>3 5 22 26</td>
</tr>
<tr>
<td>6h1</td>
<td>218</td>
<td><em>Phialophora</em></td>
<td>2 2 5 19</td>
</tr>
<tr>
<td>6j1</td>
<td>218</td>
<td><em>Cryptococcus</em></td>
<td>72 60 70 29</td>
</tr>
</tbody>
</table>
Examples of the results from sampling locations classified as few fungi and many fungi for system WC are given in Table 4. In this case, 3 of the 34 sample sets with fungi were classified as having many fungi. Phaeococcus and Cryptococcus, two yeasts, were responsible for two of these and Cladosporium for the other. There were three cases in which Aspergillus or Phaeococcus or Rhodotorula colonies were present in numbers almost large enough to cause classification of the sample sets as having many fungi. These are listed in Table 5 as "in between." Yeasts seem to be well represented in samples with more than a few fungi. The distinction between sample sets with few fungi and those with many fungi is not sharp but the two categories intergrade with each other.

<table>
<thead>
<tr>
<th>Date</th>
<th>Location Code</th>
<th>Genus</th>
<th>Count in Replicate Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Few</td>
<td>703 307</td>
<td>Aspergillus</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>712 206</td>
<td>Cladosporium</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cryptococcus</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phaeococcus</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>721 151</td>
<td>Aspergillus</td>
<td>0</td>
</tr>
<tr>
<td>Many</td>
<td>707 911</td>
<td>Cladosporium</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Penicillium</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phaeococcus</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sterile mycelium</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>713 306</td>
<td>Candida</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cryptococcus</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhodotorula</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cladosporium</td>
<td>7</td>
</tr>
<tr>
<td>In Between</td>
<td>705 919</td>
<td>Aspergillus</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>707 205</td>
<td>Cladosporium</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phaeococcus</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhodotorula</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>732 308</td>
<td>Penicillium</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhodotorula</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sterile mycelium</td>
<td>0</td>
</tr>
</tbody>
</table>

For each system the sample sets in the three categories were compared to determine if there were any significant differences in the pH, residual chlorine levels, the total coliform count or the heterotrophic plate counts. No consistent significant differences were found. The occurrence of high fungus counts does not seem to be correlated with either low chlorine residuals or high bacterial counts.

The overall picture from the samples from residential locations is that many, usually less than half, of the 50 mL samples have fungi present. Most of the samples have no fungi or counts low enough that they could be explained by an occasional spore being introduced into the system. However, there are sets of samples with so many fungi present that the numbers are credible only if it is hypothesized that fungi are growing in the distribution systems. The genera of fungi found in the various systems in small numbers and in large numbers are summarized in Table 5. Since this table is based on relatively few samples from relatively few systems, it probably gives only a small part of the total picture.
Table 5. Genera of Fungi Isolated from Distribution System Samples

<table>
<thead>
<tr>
<th>Genus</th>
<th>Ground Water Supplies</th>
<th>Surface Supplies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small Numbers</td>
<td>Large Numbers</td>
</tr>
<tr>
<td></td>
<td>SR, BG, FD</td>
<td>FV, WC</td>
</tr>
<tr>
<td>Alternaria</td>
<td>SR</td>
<td></td>
</tr>
<tr>
<td>Aspergillus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candida (yeast)</td>
<td>SR, BG, FD</td>
<td>FV, WC</td>
</tr>
<tr>
<td>Cephalosporium</td>
<td>SR, BG, FD</td>
<td>FV, WC</td>
</tr>
<tr>
<td>Cladosporum</td>
<td>SR, BG, FD</td>
<td>FV, WC</td>
</tr>
<tr>
<td>Cryptococcus (yeast)</td>
<td>SR, BG, FD</td>
<td></td>
</tr>
<tr>
<td>Cunninghamella</td>
<td>SR, BG</td>
<td></td>
</tr>
<tr>
<td>Epicoccum</td>
<td>SR, BG</td>
<td></td>
</tr>
<tr>
<td>Geotrichum</td>
<td>BG</td>
<td></td>
</tr>
<tr>
<td>Mucor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paeilomyces</td>
<td>SR, BG</td>
<td></td>
</tr>
<tr>
<td>Penicillium</td>
<td>SR, BG</td>
<td></td>
</tr>
<tr>
<td>Peyronellaea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phaeococcus (yeast)</td>
<td>SR, FD</td>
<td></td>
</tr>
<tr>
<td>Philalophora</td>
<td>SR, BG, FD</td>
<td></td>
</tr>
<tr>
<td>Rhodotorula (yeast)</td>
<td>BG, FD</td>
<td></td>
</tr>
<tr>
<td>sterile mycelium</td>
<td>SR, BG, FD</td>
<td></td>
</tr>
<tr>
<td>Trichoderma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verticillium</td>
<td>SR</td>
<td></td>
</tr>
</tbody>
</table>

Fire Hydrant Samples

We have had little success attempting to obtain water samples from fire hydrants which are representative of microbiological water quality in the distribution systems. During the sampling of system WC, twelve pairs of sample sets were obtained from a single location on one day, one set from a fire hydrant and one set from a private residence. The fire hydrants were not flushed but they were allowed to run at a low rate until the residual chlorine level was the same as that obtained at the residence. In 11 of the 12 cases, the fungus colony count was higher in the fire hydrant set and the average count per 50 mL sample was significantly higher by the t tests in three of the paired sample comparisons. Half of the fire hydrant sample sets were classified as having many fungi although none of the comparable residential samples were. One species of Penicillium was responsible for the sample sets having many fungi and Rhodotorula was responsible for the other two (see Table 6).

Table 6. Comparison of Paired Fire Hydrant/Residence Samples

<table>
<thead>
<tr>
<th>Type of Sample</th>
<th>No. of Samples</th>
<th>Fraction</th>
<th>No. of Sets with Many</th>
<th>Avg. Count per 50 mL</th>
<th>Avg. Cl2 (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fire Hydrants</td>
<td>96</td>
<td>0.75</td>
<td>3</td>
<td>3.56</td>
<td>0.74</td>
</tr>
<tr>
<td>Private Residences</td>
<td>96</td>
<td>0.20</td>
<td>1</td>
<td>0.60</td>
<td>0.76</td>
</tr>
</tbody>
</table>

Sources of Fungi

The sampling of system FV was designed to provide a comparison of the fungal content of samples from private residences with those of the input water from a pumping station and water samples collected at the base of an elevated storage tank. The numerical results are summarized in Table 7. Eight sets of samples were collected at the elevated storage tank and at private
Table 7. Comparison of Types of Samples - System FV

<table>
<thead>
<tr>
<th>Type of Sample</th>
<th>No. of Samples</th>
<th>Fraction Positive</th>
<th>No. of Sets with Many per 50mL</th>
<th>Avg. Count (mg/L)</th>
<th>Avg. Cl₂ (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pumping Station 72 11 0.15 0 0.18 1.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Private Residences 64 28 0.44 1 1.56 1.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Storage Tank 64 43 0.67 3 5.45 0.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

residences while 9 sets of samples were collected at the pumping station which provides the input water to the distribution system. The samples from the pumping station were significantly lower in fungus content both in terms of the fraction of the samples with fungi present and the average colony count per sample than the samples from either the private residences or the storage tank. The samples from the storage tank had a significantly higher average fungus count than the samples from private residence and a higher fraction of samples with fungi present although that difference was not significant.

The genera of fungi found in the three types of sampling locations were quite similar except for those present in the lowest numbers. The fungus responsible for the one sample set with many fungi from the residences was a Cladosporium. The fungi which were responsible for the three sample sets with many fungi from the storage tank were a Penicillium and a Phialophia, neither of which were seen in residential samples.

CONCLUSIONS

The numbers of fungi found in some samples collected from water distribution systems are too high to be explained by adventitious spores surviving in the system. There is clear evidence that some fungi grow in distribution systems. The fungus content of water samples collected from fire hydrants was significantly different from the fungus content of water samples collected from private residences in the same block. Chlorinated water input to a distribution system had very low numbers of fungi compared to the numbers of fungi found in samples from private residences. In at least one case, an elevated storage tank appeared to be a significant source of fungus input.

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REFERENCES


