Summary

The assembly of an automatic turbidity recording device is described. Typical experiments and results are presented indicating the usefulness of this equipment in the study of growth rates in liquid culture and in microbiological assay procedures.

Antibiotics as Preservatives for Industrial Materials

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A wide variety of materials used in manufactured items are subject to microbiological deterioration. Those materials which are of chief concern include textiles and cordage, paper, leather, paints, plastics and plasticizers, rubber, waxes, and wood. The organisms responsible for microbiological deterioration, their mode of attack, and effects on materials, have been reviewed (Greathouse et al., 1951; Greathouse and Wessel, 1954).

The preservatives commonly in use today are chiefly organic compounds or metallo-organic compounds which are either incorporated into a material during its manufacture or applied as an after-treatment to manufactured material. Of the limited number of practical fungicides, there are instances when many of these compounds have undesirable properties such as deteriorating influences on rubber or other materials, color, toxicity to handlers, cost, or lack of permanence.

The chief nonclinical uses for antibiotics are for agricultural purposes (Leben and Keitt, 1954; Croxall, 1956; Lees, 1956; Rhodes, 1956) in preventing plant infections and in the field of food preservation (Wrenshall, 1957). There is almost no published literature on the use of antibiotics for the prevention of fungal deterioration of manufactured materials. The fact that certain antibiotics are effective against microorganisms at low concentrations, and may be safe for handling of treated materials, is of special interest in deterioration problems. Those antibiotics reported to have good antimicrobial properties, as well as some antibiotics available only recently, were selected in order to determine their effectiveness in fungusproofing several representative types of manufactured materials in preliminary tests.

REFERENCES


An evaluation of the relative fungitoxicity of the selected antibiotics was first made by a pure culture test using the shake flask method and a glucose-salts medium. For the tests on materials, several representative types of industrial materials were used: filter paper, representative of cellulosic materials; castor oil, representative of oils and plasticizers; and glue-bonded cork, whose binder is representative of proteinaceous materials.

Materials and Methods

Glucose-Salts Shake Culture Test

The antibiotics which were being considered as preservatives for industrial materials were first evaluated as inhibitors of fungus growth by means of the shake culture technique. Each of the test compounds was added directly to 50 ml of medium in 250 ml Erlenmeyer flasks.

Glucose-salts medium:

\[
\begin{align*}
\text{KH}_2\text{PO}_4 & : 2.0 \\
\text{MgSO}_4 \cdot 7\text{H}_2\text{O} & : 1.0 \\
\text{NH}_4\text{NO}_3 & : 3.0 \\
\text{KCl} & : 0.5 \\
\text{FeSO}_4 \cdot 7\text{H}_2\text{O} & : 0.02 \\
\text{Glucose} & : 30.0 \\
\text{Distilled water, 1000 ml. Sufficient KOH to make pH = 6.3 after sterilization.}
\end{align*}
\]

The antibiotics were used as supplied by the manufacturers or cooperating research laboratories without further purification. The compounds and their purity, wherever known, are listed in table 1, together with experimental results. Two of these compounds, \( \beta \)-chloroethyl mucochlorate and mucochloric acid, are not antibiotics but are of value because of previously demonstrated antifungal properties (Unpublished Observations, H. D. Brown). Four flasks were prepared...
for each compound evaluated and sufficient antibiotic was added to each flask to give a concentration of 250 μg per ml of the antibiotics, as received. More effective compounds were also evaluated at lower concentrations. The flasks were inoculated with 1 ml of a spore suspension prepared from a 10-day old slant culture of Aspergillus versicolor (Frankford Arsenal strain no. 483) previously grown on potato-dextrose agar. The spore suspension was prepared by scraping the surface of the agar slant to which 5 ml of sterile water was added previously. The spore suspension was poured off into a 30 ml vial containing a few 5 mm solid glass beads and shaken to break up the spore clumps. The spore suspension was then filtered through sterile glass wool, centrifuged, washed, centrifuged and resuspended in 10 ml of sterile distilled water. The spore count was adjusted with sterile water to make a suspension containing 10⁴ spores per ml. After inoculation, the flasks were incubated on a rotary shaking machine at 30 °C. A flask was removed at 2 to 4 day intervals for each test compound being evaluated. The dry weight of mycelium formed was determined by filtering the flask contents through a medium porosity fritted glass crucible, washing the mat with water, and weighing the crucible after drying for 3 hr at 105 °C.

**Table 1**

<table>
<thead>
<tr>
<th>Compound (Activity)</th>
<th>Antibiotic Concentration in Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>250 μg/ml</td>
</tr>
</tbody>
</table>

### A. Effective Inhibitors

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>mg</th>
<th>mg</th>
<th>mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-59</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Filipin</td>
<td>11</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Rimocidin</td>
<td>0</td>
<td>—</td>
<td>37</td>
</tr>
<tr>
<td>Mycostatin</td>
<td>8</td>
<td>—</td>
<td>146</td>
</tr>
<tr>
<td>Fungichromia</td>
<td>10</td>
<td>2</td>
<td>153</td>
</tr>
<tr>
<td>Nocardine</td>
<td>3</td>
<td>—</td>
<td>211</td>
</tr>
<tr>
<td>β-Chloroethyl mucochlorate</td>
<td>0</td>
<td>282</td>
<td>558</td>
</tr>
<tr>
<td>Endomycin (100%)</td>
<td>69</td>
<td>96</td>
<td>225</td>
</tr>
<tr>
<td>Ustilagine acid</td>
<td>66</td>
<td>—</td>
<td>538</td>
</tr>
<tr>
<td>Antibiotic C-160</td>
<td>191</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (No compound)</td>
<td>538</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### B. Ineffective Inhibitors at 250 μg/ml of Antibiotics

<table>
<thead>
<tr>
<th>Compound</th>
<th>mg</th>
<th>mg</th>
<th>mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycloserine</td>
<td>318</td>
<td>Mucochloric acid</td>
<td>464</td>
</tr>
<tr>
<td>Streptovarin</td>
<td>323</td>
<td>Penicillin (1666 units/mg)</td>
<td>494</td>
</tr>
<tr>
<td>Afactin</td>
<td>415</td>
<td>Mycolutein</td>
<td>508</td>
</tr>
<tr>
<td>Thiolutin (100 μg/mg)</td>
<td>431</td>
<td>Streptomycin</td>
<td>528</td>
</tr>
<tr>
<td>Neomycin</td>
<td>447</td>
<td>Acesine</td>
<td>553</td>
</tr>
<tr>
<td>Anisomycin (900 μg/mg)</td>
<td>439</td>
<td>Actidione (85-100%)</td>
<td>554</td>
</tr>
<tr>
<td>Oligomycin (1065 units/mg)</td>
<td>447</td>
<td>Eulicin sulfate (390)</td>
<td>591</td>
</tr>
<tr>
<td>Puromycin</td>
<td>452</td>
<td>Control (No Compound)</td>
<td>538</td>
</tr>
</tbody>
</table>

**Soil Burial Test of Filter Paper Treated with Antibiotics**

To determine the effectiveness of antifungal antibiotics as preservatives for cellulosic type materials, Whatman no. 1 filter paper was used as the substrate. The antibiotics were dissolved in appropriate solvents and 0.2 ml of antibiotic solution was pipetted onto 1 by 3 in. strips of filter paper. When the antibiotic was not sufficiently soluble, one or more applications were made of an appropriate lower concentration. The strength of the solutions was adjusted so that the treated papers contained 0.5, 1, or 2 per cent of antibiotic, based on the weight of the paper. The treated papers were air dried and evaluated for fungus resistance by means of a 1-week soil burial test. Three replicate specimens for each treatment were buried horizontally in biologically active soil in enamel pans and incubated at a temperature of 27 to 30 °C with the soil moisture kept at approximately 30 per cent. The soil beds were composed of equal parts of humus, topsoil, and sand. The cellulyotytic activity of the soil was such that 10-ounce cotton duck lost 100 per cent of its breaking strength after burial for 7 days in these soil beds. After 1 week, the antibiotic-treated filter paper specimens were removed from the soil and their breaking strengths were determined according to TAPPI Method T404m-50 (TAPPI, 1950).

**Fungus Resistance of Castor Oil Containing Antibiotics**

The antibiotics and fungicides used for comparison were incorporated into castor oil (USP) to provide three concentrations, 0.5, 1, and 2 per cent of antibiotic or fungicide by weight. The test compounds were ground to a fine particle size with a mortar and pestle and thoroughly mixed with the castor oil. Some of the compounds were insoluble in the oil, in which case dispersions were used. The fungicides copper-8-quinolinolate and phenyl mercury quinolinolate were also evaluated at two lower concentrations, namely 0.1 and 0.05 per cent. Hypodermic syringes were used to pipette 1 ml of the various oil formulations onto the center of the surface of sterile nutrient salts agar previously solidified in Petri dishes.

**Nutrient-salts agar medium:**

\[
\begin{align*}
\text{KH}_2\text{PO}_4 & \quad 0.7 \\
\text{K}_2\text{HPO}_4 & \quad 0.7 \\
\text{MgSO}_4 \cdot 7\text{H}_2\text{O} & \quad 0.7 \\
\text{NH}_4\text{NO}_3 & \quad 1.0 \\
\text{NaCl} & \quad 0.005 \\
\text{FeSO}_4 \cdot 7\text{H}_2\text{O} & \quad 0.002 \\
\text{ZnSO}_4 \cdot 7\text{H}_2\text{O} & \quad 0.002 \\
\text{MnSO}_4 \cdot \text{H}_2\text{O} & \quad 0.001 \\
\text{Agar (Difco)} & \quad 15.0 \\
\text{Distilled water, 1000 ml, pH after sterilization} & = 6.4.
\end{align*}
\]
Three replicate plates for each compound were inoculated with a mixed, washed spore suspension consisting of the following organisms: *Trichoderma* sp. (Frankford Arsenal strain no. 69), *Aspergillus niger* (no. 81), *Aspergillus flavus* (no. 70), and *Penicillium fumiculosum* (no. 71).

The agar surface and oil drop were inoculated by spraying with the mixed spore suspension, as described previously (Ross et al., 1956). The inoculated plates were incubated for 6 weeks in a constant temperature incubator maintained at 29 ± 1 C and 95 per cent relative humidity.

**Fungus Resistance of Antibiotic Treated Cork**

Specimens of 1/16 in. thick glue-bonded cork were cut into strips 1/2 by 2 in., and a hole was punched in one end of each specimen, enabling it to be suspended. The test compounds were dissolved in the appropriate solvent according to their solubility characteristics. The cork specimens were immersed in each solution for 5 min, removed, allowed to drain, and weighed to determine the per cent “addon” of antibiotic or fungicide based on the wet pickup. The specimens were then allowed to air dry for a week. They were inoculated by spraying with a spore suspension similar to that described for the castor oil test.

Three replicate inoculated specimens were incubated in individual incubation chambers, which consisted of 16 ounce French Square bottles with plastic screw caps, from which the cork specimens were suspended by means of chromel wire. The relative humidity in the jar was kept at a nominal 100 per cent by adding about a 1-in. layer of water to each jar before sealing it. Specimens were incubated at a temperature of 29 ± 1 C for a period of 6 weeks.

**Results and Discussion**

The results of the glucose-salts shake culture test are shown in table 1. The highest weights of the mycelial mats that formed during the 10-day incubation period are listed in order to show the relative inhibition of fungus growth of *A. versicolor* by 25 antibiotics. These antibiotics which were effective inhibitors (shown by lowest mat weights) are shown in part A of the table, and those ineffective are grouped in part B of the table. The most effective antibiotics based on the results of this test were antibiotic C-59, filipin, rimocidin, mycostatin, fungichromin, netropsin sulfate, and β-chloroethyl mucoclorate. Endomyein and ustilagic acid were only partially effective at 250 μg per ml. The remainder of the antibiotics were noninhibitory.

Table 2A lists the susceptibility to microbial attack of the antibiotic treated filter papers as measured by the effect on their breaking strengths after the 1 week soil burial test. Untreated filter paper and controls treated with the various solvents used were completely decomposed during the incubation period. The retentions of breaking strength in per cent of the exposed specimens, compared to unexposed specimens, are listed for the various antibiotic treatments. The most effective antibiotics were endomyein, filipin, fungichromin, and thiolutin. Table 2B lists the ineffective antibiotics. The soil burial test is a severe test which exposes the test specimens to actinomycetes and bacteria, as well as fungi, present in the microbial population of the soil, and does not limit the test to any particular organisms.

It appears that some of the antibiotics compare rather favorably with the fungicides included in the test (copper-8-quinolinolate, phenyl mercury quinolinolate, and Compound G-4). It should be noted, however, that in these tests on materials no attempt was made to determine the degree of permanence of the treated papers, such as would be shown by water leaching or heating prior to the soil burial test. An investigation of these properties is essential before any fungicide could find practical application.

Plain castor oil supports heavy fungus growth after a 1 week incubation period, and generally the fungus susceptible castor oil containing formulations developed moderate to heavy fungus growth after 1 or 2 weeks of incubation. None of the antibiotics were anywhere near as effective as the two quinolinolate compounds, copper-8-quinolinolate or phenyl mercury
quinolinolate, both of which inhibited fungus growth on castor oil completely at a concentration of 0.05 per cent. The best of the antibiotics was endomycin, which was inhibitory at 1 per cent and partially inhibitory at 0.5 per cent. Fungichromin was completely inhibitory at 2 per cent, but only partially inhibitory at the lower concentrations. Five of the compounds showed only very limited inhibition of growth at best. These were β-chloroethyl mucochlorate, filipin, mucochloric acid, netropsin sulfate, and rimocidin. All the rest of the antibiotics were completely ineffective in preventing the development of fungus growth on castor oil. Figure 1 shows the development of fungus growth on plain castor oil, on an ineffective antibiotic formulation containing actidione, and on an ineffective formulation containing the fungicide p-nitrophenol. Although p-nitrophenol was not very effective as a preservative for castor oil in this test, it is effective as a protectant for proteinaceous materials such as leather and glue-bonded cork, and is widely used. The oil specimens containing the antibiotic endomycin or the fungicide copper-8-quinolinolate were free of fungus growth. The opacity of the actidione-oil mixture is due to the insolubility of the antibiotic in the oil.

The extent of mold growth which developed on the glue-bonded cork specimens treated with the various effective antibiotics is shown in table 3A. Plain cork supports heavy fungus growth after 1 week and the entire surface of untreated control specimens are covered with mold after the second week of incubation. A series of control specimens was prepared with only the solvents used to dissolve the test compounds. After thorough drying to remove the solvents these specimens were exposed to fungi as were the antibiotic treated specimens. All the solvent treated controls supported heavy fungus growth after 2 weeks, except for cork treated with formamide alone which developed only a trace of growth after 4 weeks. Therefore, the absence of fungus growth in the case of the thiolutin treated cork may be due to the solvent, formamide, or to a combination of the antibiotic and solvent. Formamide was used since it was the only available solvent for thiolutin.

**Figure 1.** Fungus growth on castor oil containing antibiotics or fungicides after 6 weeks' incubation at 29 C and 95 per cent relative humidity on nutrient salts agar medium. Plain unincubated castor oil is shown for comparison.
Formamide is a very high boiling liquid, 210.5°C at 760 mm and it probably did not evaporate from the treated cork. Only one of the antibiotics, rimocidin, at 0.4 per cent, was completely inhibitory at the lowest level for 6 weeks and at 1 per cent was effective for 8 weeks. β-Chloroethyl mucochlorate and mucochloric acid were fairly effective. None of the antibiotics were as good as the quinolinolinate fungicides or p-nitrophenol, all of which were effective for 12 weeks at 0.5 per cent. Endomycin, filipin, fungichromin, and mycostatin were only partially inhibitory. The fungicide, o-phenylphenol was ineffective. The ineffective antibiotics are shown in table 3B.

**TABLE 3**

*Fungus susceptibility of protein-bonded cork treated with antibiotics or fungicides. Incubated with mixed, fungus spores and incubated 6 weeks in humid jars. (Avg of 3 replicates)*

<table>
<thead>
<tr>
<th>Growth</th>
<th>A. Effective Inhibitors</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.4% β-chloroethyl</td>
<td>1% Endomycin ..</td>
</tr>
<tr>
<td></td>
<td>mucochlorate ...</td>
<td>2% Endomycin ..</td>
</tr>
<tr>
<td></td>
<td>1% β-chloroethyl</td>
<td>2% Filipin ....</td>
</tr>
<tr>
<td></td>
<td>mucochlorate ...</td>
<td>2% Fungichromin..</td>
</tr>
<tr>
<td></td>
<td>0.5% Mucochloric acid</td>
<td>1% Mycostatin ..</td>
</tr>
<tr>
<td></td>
<td>1% Mucochloric acid</td>
<td>2% Mycostatin ..</td>
</tr>
<tr>
<td></td>
<td>0.4% Rimocidin</td>
<td>0% Mucochlorate</td>
</tr>
<tr>
<td></td>
<td>0.6% Thiolulin</td>
<td>0% Plain cork</td>
</tr>
<tr>
<td></td>
<td>0.5% Phenyl mercury</td>
<td>2% o-Phenylphenol</td>
</tr>
<tr>
<td></td>
<td>8-quinolinolate</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>0.5% Copper-8-quinolinolate</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>0.5% p-Nitrophenol</td>
<td>0%</td>
</tr>
</tbody>
</table>

B. Ineffective Inhibitors (Treated at Approximately 2% Antibiotic Level Unless Indicated Otherwise)

- Actidione
- Anisomycin
- Antibiotic C-59
- Antibiotic C-160
- Ascosin
- Ayafactin
- Eulicin SO₄

* Fungus Growth Code:
  - 0 = No growth on surface of specimen.
  - ++ = Trace of growth, up to 5% of surface moldy.
  - +++ = 5% to 25% of surface moldy (light growth).
  - ++++ = 25% to 50% of surface moldy (moderate growth).
  - +++++ = 50% to 75% of surface moldy (heavy growth).
  - ++++++ = 75% to 100% of surface moldy (very heavy growth).
  - a = 2% β-Chloroethyl mucochlorate was effective after 12 weeks.
  - b = 1% Mucochloric acid was effective after 12 weeks.
  - c = 1% Rimocidin was effective until 8 weeks.
  - d = 0.6% Thiolulin was effective after 12 weeks.
  - e = Still effective after 12 weeks.

**Over-all Effectiveness of Antibiotics**

There were five antibiotics among those evaluated which were good inhibitors of fungus growth in at least two of the four tests used, and fair inhibitors in the two remaining tests. These were fungichromin (a good inhibitor in three tests, fair in the cork test), filipin, endomycin, rimocidin, and β-chloroethyl mucochlorate. Thiolutin was a good inhibitor in two tests (filter paper and cork tests), but was ineffective in the other two tests (glucose-salts and castor oil tests). The remaining less effective antibiotics varied in their specificity as inhibitors of microbial attack on the several types of materials evaluated. This variability was not unexpected since many currently used fungicides find specific application in the preservation of one or more similar types of materials to the exclusion of other types. For example, p-nitrophenol is excellent as a preservative for leather and glue-bonded cork (proteinaceous materials), but is of little value as a preservative for paper (cellulosic material). Those antibiotics found to protect only one type of material and which meet the usual criteria for a good material preservative may be of definite value and merit further consideration.

The over-all effectiveness of the antibiotics cannot be determined from pure culture tests alone. Mycostatin and antibiotic C-59 both were good inhibitors in the pure culture test but were only fair or ineffective in tests with actual materials. Thiolutin and mucochloric acid caused little inhibition of fungus growth at a concentration of 250 µg per ml in the pure culture test, but proved to be of some value in the preservation of one or more of the materials tested. Thus, the choice of test methods is important in the evaluation of compounds as preservatives for materials.

The choice of test organisms should also be considered in the screening of antibiotics for possible use as materials preservatives. Most of the literature is concerned with the possible clinical applications of antibiotics, and thus in initial screening tests those organisms responsible for the deterioration of materials (Greathouse et al., 1951; Greathouse and Wessel, 1954) are usually not considered. Lately, however, more attention has been paid to the evaluation of antibiotics for phytopathology and the prevention of food spoilage, with the result that associated microorganisms have been used in these nonclinical type studies.

In considering the practical application of antibiotics as materials preservatives, it will be necessary to evaluate those which showed promise in more extensive tests. The qualities to be determined are resistance to heating and leaching, outdoor weathering properties, corrosiveness, resistance to soil microorganisms, and effects on materials.

With the continuing discovery of new types of anti-
biotics, a supply of interesting new compounds is becoming available which may become an important factor in the prevention of the microbial deterioration of materials.

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Summary

High antimicrobial activity and low toxicity for man upon handling suggest the use of antibiotics for preserving industrial materials. Twenty-five antibiotics, and five fungicides for comparison, were investigated as preservatives for representative industrial materials.

Antibiotics which inhibited fungus growth (Aspergillus versicolor) in glucose-salts shake flasks at 250 μg per ml were Antibiotic C-50, β-chloroethyl mucochlorate, filipin, fungichromin, mycostatin, metropsin sulfate, and rimocidin.

To study the prevention of deterioration of cellulosic materials, filter paper was used as a representative substrate. Endomyein, filipin, fungichromin, and thiolutin were effective at 0.5 per cent based on the weight of the paper. The antibiotic treated papers retained 60 to 70 per cent of original strength after a 1 week soil burial test, comparing favorably with several standard fungicides.

Only endomyein (1 per cent) or fungichromin (2 per cent) were capable of protecting castor oil, which is representative of oily type materials, from fungal attack after incubation for 6 weeks on salts-agar. Comparison fungicides found more effective were copper-8-quinolinate or phenyl mercury quinolinate at 0.5 per cent.

Glue-bonded cork, which is representative of proteinaceous materials, was free of fungus growth for 12 weeks in humid jar tests when treated with either β-chloroethyl mucochlorate (2 per cent), mucochloric acid (1 per cent), or thiolutin (0.6 per cent). Rimocidin (1 per cent) was effective for only 8 weeks, while comparison fungicides, phenyl mercury quinolinate and p-nitrophenol (at 0.5 per cent) were still effective at 12 weeks.

Further investigation is necessary to determine the permanence of antibiotics in treated materials.

References


