Induction of Dimorphism in the Basidiomycete

*Lenzites saepiaria*

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Sugars can serve as the germinant for basidiospores of the wood-rotting fungus *Lenzites saepiaria*. Hexoses sterilized by autoclaving were better germinants than hexoses that were sterilized by filtration. The degradation products in heated hexose which were responsible for the stimulation of germination were levulinic and formic acid. Another product of hexose degradation, hydroxymethyl furfural, had a marked effect on outgrowth of *L. saepiaria* basidiospores and on the development of mycelia. Basidiospores that germinated in the presence of hydroxymethyl furfural yielded large rounded bodies that, in some cases, developed as a chain of yeastlike cells. Addition of hydroxymethyl furfural to developing mycelia resulted in the production of chains of round yeastlike structures. Similar results were obtained by treating basidiospores or mycelia with phenethyl alcohol.

Basidiospores of the wood-rotting fungus *Lenzites saepiaria* utilize various carbohydrates as substrate for germination (9). Germination in this organism has been defined as the initiation of outgrowth (9), since there are no metabolic or physical changes observable other than an increase in length of the spore.

Continued experiments on basidiospore germination indicated that sugars autoclaved prior to testing supported an increased rate and percentage of outgrowth when compared with filter sterilized or unsterilized sugars (H. W. Scheld and J. J. Perry, Bacteriol. Proc., p. 34, 1969). When known products of hexose degradation (8), such as levulinic or formic acid, were added to spore suspensions, there was an increased rate of outgrowth. The addition of hydroxymethyl furfural, another product of hexose degradation, to germinating spores had a marked effect on subsequent outgrowth and morphogenesis. Hydroxymethyl furfural induced the spores to develop as rounded yeastlike structures and caused the mycelia to grow in chains of spherical and odd-shaped bodies.

Emerson (3) demonstrated that furfural effectively replaced heat activation in the germination of ascospores of *Neurospora crassa*. Ascospores of *N. tetrasperma* are activated by furfural, furfuryl alcohol, and other heterocyclic compounds (11, 12), but hydroxymethyl furfural was not tested in these studies. Spores of thermophilic spore-forming bacteria were also activated by furfural (7).

High concentrations of hexoses have been implicated as a cause of dimorphism in various strains of *Mucor* (1, 2). Under anaerobic culture, both hexose and *p*CO₂ have an effect on the growth form of this organism. Other workers (13) demonstrated that strains of *M. rouxii* were induced to form yeastlike, bud-producing spherical cells when phenethyl alcohol and hexose were added to the germination medium.

Lingappa and Lingappa (5) demonstrated that a diffusible substance produced by conidia of *Glomerella cingulata* acted as an auto-inhibitor of mycelial growth resulting in yeastlike development of this organism. *Candida albicans* (6) also produces an auto-inhibitor of the mycelial form, resulting in growth of yeastlike colonies particularly under crowded conditions.

This report is concerned with germination in *L. saepiaria* and the formation of yeastlike cells caused by the addition of sugar degradation products to the growth medium.

**MATERIALS AND METHODS**

**Materials.** *L. saepiaria* is a bracket fungus, and mature fruiting structures (basidiocarp) can be readily collected from decaying pine branches. The fruiting structures can also be grown in laboratory culture (Scheld and Perry, unpublished data). In all experiments involving germination on a solid medium, fruiting bodies were suspended over the agar surface, the basidiocarp was moistened, and spores were cast for a time sufficient to attain the desired inoculum.

Difco purified agar (0.3%), melted in a steam cabinet, was used as the solidifying agent. Furfural (2-furfuraldehyde) and hydroxymethyl furfural [5-(hydroxymethyl)-2-furfuraldehyde (HMF)] were pur-
Degradation of sugars. Sugar solutions (15%, w/v) in distilled water were autoclaved for 15 min at 121 C. These solutions were added to melted agar to give a final sugar concentration of 1%. Control plates were prepared in an identical fashion by using filter-sterilized sugar. The sugars were acid-degraded by acidifying a 15% sugar solution to 0.4 N with respect to H2SO4 and heating for 1 hr at 100 C in a steam cabinet. Degradation products were recovered from autoclaved or acid-treated sugar solutions by diethyl ether extraction. KOH was added to the reaction mixture, and neutral extractables were recovered by ether extraction, and then by acidification and recovery of the acidic materials. The concentrated extracts were streaked on 0.5-mm Silica Gel G. (E. Merck AG, Darmstadt, Germany) thin-layer chromatography (TLC) plates. Chloroform or benzene was used to separate furfural and related compounds, and n-butyl alcohol:acetic acid:water (5:4:1) was used to recover levulinic acid. Separated bands were visualized by spraying a part of the plate with phenylhydrazine or exposing the plate to iodine vapors. Component bands were scraped from the TLC plates and eluted with methanol. The methanol was evaporated in a stream of N2, and the residue was dissolved in hot water and added to 0.3% agar containing 1% maltose. The agar was poured in thin films on plates and seeded with basidiospores. Furfural, HMF, levulinic acid, and formic acid were chromatographed and tested in the same manner.

Effect on germination. The germination percentage was determined by examining the outgrowth of basidiospores on agar plates at 125x magnification with a light microscope. Three fields with 30 to 50 spores per field on six individual plates were examined for each test. Morphological changes were recorded by covering the spores on the agar surface with a cover slip and photographing through a Leitz microscope equipped with bright-field optics. The photographs were taken at 600x magnification.

RESULTS

The effect of heat treatment on the subsequent ability of sugars to support germination of *L. saepiaria* basidiospores is illustrated in Table 1. The extent of germination on maltose, fructose, glucose, or galactose was markedly increased when these substrates were autoclaved prior to testing. Ribose was not altered as a germinant by heat treatment. The factor(s) in heated sugar that affected this increase in germination was readily recovered from the reaction mixture by ethyl ether extraction. Addition of mineral acid to sugar solutions prior to heating resulted in an increased production of these factor(s).

Experiments were conducted to determine whether available products of acid-heat treatment of hexose (HMF, levulinate, and formate) or furfural would have a similar effect on germination. These results are presented in Table 2. Under the conditions of the test, maltose supported 25% germination of the basidiospores in 18 hr. Addition of furfural resulted in a complete inhibition of germination. HMF did not affect the rate or extent of germination under these conditions. The addition of levulinate or formate to the basal medium resulted in an increased rate of germination equal to that obtained with ethyl ether extracts of heat-treated sugars. Co-chromatography of ethyl ether extracts with levulinate demonstrated that the stimulatory compound from heated glucose migrated to the same area of the TLC plate as levulinate. When this material was scraped from the plate, extracted, and tested, the results were similar to those obtained with levulinic acid.

The effects on germination varied with the amount of ethyl ether extractable factor(s) added to the basal maltose germination medium. Addition of low levels of extract resulted in an increased amount and rate of basidiospore germina-

**Table 1. Comparison of autoclaved and unautoclaved sugars as germination substrates for basidiospores of *Lenzites saepiaria***

<table>
<thead>
<tr>
<th>Sugar added</th>
<th>Per cent germination (18 hr)</th>
<th>Autoclaved</th>
<th>Unautoclaved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltose</td>
<td>95</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>91</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>96</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
<td>60</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Ribose</td>
<td>71</td>
<td>65</td>
<td></td>
</tr>
</tbody>
</table>

*Sugars (1%) added to 0.3% purified agar. Incubation temperature was 30 C.*

**Table 2. Effect of addition of sugar degradation products to *Lenzites saepiaria* basidiospores germinating on maltose***

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Per cent germination (12 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltose</td>
<td>25</td>
</tr>
<tr>
<td>Maltose + furfural</td>
<td>0</td>
</tr>
<tr>
<td>Maltose + hydroxymethyl furfural</td>
<td>30</td>
</tr>
<tr>
<td>Maltose + levulinic acid</td>
<td>93</td>
</tr>
<tr>
<td>Maltose + formic acid</td>
<td>90</td>
</tr>
<tr>
<td>Maltose + acetate</td>
<td>95</td>
</tr>
</tbody>
</table>

*Maltose was added at 0.03 M, all other substrates were added at 1 mM. Levulinic and formic acid were neutralized with NaOH. Results are recorded for 100 spores per six replicate plates.*
The rate of outgrowth of these basidiospores approached that obtained on malt extract or a medium to which maltose and acetate were added (Fig. 1). However, when the concentration of diethyl ether-extractable material was increased, a number of effects on germination was evident. The results of an experiment with increased amounts of extract are presented in Fig. 2. The basidiospores slowly enlarged (Fig. 2A), developed a single slow-growing hypha (Fig. 2B), produced branched irregular mycelium (Fig. 2C and E), or lysed and extruded the cellular contents (Fig. 2D). The size and shape of the cell wall resulting from the extrusion of cell contents (Fig. 2D) suggested that considerable growth of the basidiospore had occurred during the incubation period and prior to lysis.

The effect of various combinations of HMF, levulinate, acetate, and PEA on the development of L. saepiaria basidiospores is illustrated in Fig. 3. When basidiospores were treated with 10 mM HMF added to the basal maltose medium, enlarged spores were produced similar to those seen in Fig. 2A. Levulinate added to the basal medium stimulated germination of mycelium but caused no marked change in cell structure. When HMF and levulinate were combined, enlarged rounded cells were produced that, in some instances, exhibited slow-growing hyphae as shown in Fig. 3A. Addition of HMF and acetate resulted in the growth of rounded bodies (Fig. 3B and C). After 10 days, many of the enlarged cells lysed (Fig. 3D). Comparable results were obtained when PEA and acetate were added to the basal medium (Fig. 3E).

Some additional effects of HMF and levulinate or acetate on the development of basidiospores are shown in Fig. 4A, B, and C. The developing mycelium in Fig. 4A has stunted and odd-shaped branches not associated with normal mycelial growth (see Fig. 1). The basidiospore in Fig. 4B was apparently forming a bud, whereas that shown in Fig. 4C developed a cross-wall between the two areas of the spore. Some basidiospores grew out as a chain of odd-shaped cells (Fig. 4D).

The effect of PEA or HMF on the mycelium that grew from a single spore isolate was tested by casting a small number of basidiospores onto

Fig. 1. Germination of L. saepiaria basidiospores. (A) Ungerminated basidiospores; (B–E) Germinated spores after 4, 8, 12, and 16 hr on 0.3% agar containing 1% maltose and 5 mM acetate (Na). Ungerminated spore (arrow). × 730.
FIG. 2. Effect of fructose degradation products on germination of L. saepiaria basidiospores. Spores after (A, B) 3 days, (C) 5 days, and (D, E) 10 days on agar containing 1% maltose and 2 mg of ethyl ether-extractable material per ml. × 800.
PEA or HMF was added in a well cut in the agar near the edge of the growing mycelium. The effect on the growing mycelium is illustrated in Fig. 4E and F. The mycelium that grew in the presence of HMF (Fig. 4E) developed as a chain of rounded cells. Similar growth occurred when PEA was added (Fig. 4F), except that the cells were smaller and longer.

**DISCUSSION**

These experiments demonstrated that the increased rate and level of germination in the presence of autoclaved hexoses resulted from the presence of small amounts of organic acids formed by the degradation of the sugar. Previous results (9) suggested that fatty acids stimulate germination and outgrowth of *L. saepiaria* basidiospores. This stimulation is most marked when hexose is also present.

Products formed when the sugars were heated also had an effect on the development of the fungal hyphae. The dimorphism observed in *L. saepiaria* on addition of HMF was similar to that reported for *M. rouxii* when higher levels (8%) of hexose were added to the growth medium (1). Terenzi and Storck (13) also observed that the concentration of glucose was important in the induction by PEA of a yeastlike morphology in *M. rouxii*. PEA causes the same morphological change in *L. saepiaria* as that induced by addition of HMF. Since PEA is effective in disrupting membrane organization (10), it seems reasonable to suggest that HMF has a similar effect on membranes of *L. saepiaria*.

**ACKNOWLEDGMENT**

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**LITERATURE CITED**


FIG. 4. Growth of L. saepiaria basidiospores and hyphae in the presence of HMF and PEA. (A) Hyphae from spore treated with HMF and levulinic acid showing unusual branching, 10 days. (B) Basidiospores after addition of HMF and levulinate, 5 days. (C) Basidiospores after addition of HMF and acetate, 5 days. (D) Basidiospores after addition of HMF and levulinate, 10 days. (E) Hyphal cells after treatment with HMF and acetate, 10 days. (F) Hyphal cells after treatment with PEA and acetate, 10 days. All were plated on agar with 1% maltose added. HMF was added at a concentration of 10 mM, PEA at 16 mM, levulinate at 5 mM, and acetate at 3 mM. × 750.


