CHEMICAL COMPOSITION OF OIDIODENDRON KALRAI

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Abstract

The chemical composition of yeast cells of Oidiiodendron kalrai was analyzed and is expressed as percent dry weight. Cultures were grown in tryptone broth and in a liquid synthetic medium containing ammonium salts as a nitrogen source. After 48 h, carbohydrate levels were higher in the synthetic medium, but lipid levels were lower. Deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein contents did not differ significantly in the two media. The chemical components were also studied at different stages of growth. DNA remained relatively constant, but other components varied with the age of culture. The RNA was 6.6% at 18 h and declined rapidly to 5% by 24 h and remained constant. An initial protein content of 23% at 18 h increased rapidly to 37% by 48 h and gradually declined to 30% by day 10. The lipid content of 33% at 18 h decreased over the entire growth period to 10% by day 10. An initial carbohydrate level of 30% increased to a maximum of 54% by day 5 and then declined.

Introduction

Oidiiodendron kalrai, classified in the form – genus Oidiiodendron of the Hyphomycetes, was described by Tewari & Macpherson (25). The dimorphic organism has been shown to demonstrate neuropathogenic properties in mice, and while the mycelial phase was the invasive form of the organism, both phases could be recovered from infected animals (22). Since Oidiiodendron kalrai has been shown to share common antigens with Histoplasma capsulatum as demonstrated by cross protection (23) and serological and skin test cross reactions (24), the present investigation was undertaken to study the RNA, DNA, protein, carbohydrate and lipid of yeast phase cells of O. kalrai grown in tryptone broth and a liquid synthetic medium. The chemical components of the yeast phase cells during different stages of the growth cycle were also studied.

Materials and methods

Organism

The strain of Oidiiodendron kalrai (y4-d) used in this study was obtained from our culture collection. Stock cultures of the yeast phase were maintained on Difco Brain Heart Infusion Agar slants supplemented with 1% dextrose and 0.1% L-cysteine hydrochloride (BHIA). Cultures were stored at 4°C and transferred to fresh slants every 2 weeks.

Inoculum

Twenty-four h growth on BHIA slants was harvested, washed twice with cold, sterile saline and centrifuged in a refrigerated Sorvall RC-2B centrifuge at 500 rpm for 1 minute to remove yeast cell aggregates. The suspension was standardized turbidimetrically to 10% light transmittance at 550 nm on a Bausch and Lomb spectronic-20 spectrophotometer.

Media

Cells were grown in a liquid synthetic medium (21) having the following composition (g/liter) with a final pH of 7.2: K$_2$HPO$_4$, 2.5; NH$_4$Cl, 0.5; (NH$_4$)$_2$SO$_4$, 0.5; glucose, 5; MgSO$_4$ · 7H$_2$O, 0.1; FeCl$_3$, 0.001; L-cysteine hydrochloride, 0.1. A protein enriched medium containing 1%
tryptone in place of the nitrogen salts was also used. Both media were dispensed in 250 ml Erlenmeyer flasks. Two ml of the standardized yeast cell suspension was inoculated into each flask. The flasks were incubated at 37 °C with continuous shaking on a gyrotory shaker (New Brunswick Scientific, New Brunswick, N.J.) set at 150 rpm.

**Fractionation scheme**

The extraction procedure as previously described (7) was used for the chemical analysis of the yeast cells. Twenty mg quantities of dried culture powder preparations were treated with 10 ml of cold 5 % trichloroacetic acid (TCA) for 1 h at 4 °C and centrifuged. The supernate containing only a small fraction of the extractable polysaccharides was discarded. The pellet was treated with 10 ml of an ethanol : ether (3:1) mixture for 1 h at room temperature. After centrifugation, the lipid containing supernate was discarded, leaving a pellet with contained DNA, RNA and protein. Nucleic acids were removed by boiling in 10% TCA for 30 minutes and centrifuging. The pellet after digestion by autoclaving for 20 minutes in 10 ml of 1 N NaOH was used for protein determinations. Separate samples of 20 and 100 mg powder, respectively, were used for the determination of carbohydrate and lipid.

**Estimation of components**

DNA was determined by the modified Dische diphenylamine color reaction (17) using deoxyribose as a standard. RNA was determined spectrophotometrically and was corrected for DNA (1). Protein was determined by the procedure of Lowry, Rosebrough, Farr & Randall (12) using crystalline bovine serum albumin fraction V as a standard. Total carbohydrate was determined by the anthrone reaction of Morris (13) using glucose as a standard.

Total lipid was determined as previously described (7) by 3, 3 h extractions with chloroform : methanol (1 : 1) followed by an additional 2 h extraction with HCl : chloroform : methanol (1 : 162 : 83). The pooled fractions were evaporated under nitrogen at 45 °C in a tared flask, redissolved in 5 ml of chloroform, and washed with an equal volume of cold 0.7 M potassium chloride. The chloroform phase containing the lipids was reevaporated to dryness under nitrogen at 45 °C in a tared flask.

**Dry weight determination**

For dry weight determinations, duplicate culture samples were filtered through cellulose paper pads with a pore diameter of 0.2-0.3 μ (Millipore Co., New Bedford, Mass.) and washed twice with deionized distilled water. The pad was transferred to a tared 57 mm aluminum weighing dish and the pre-weighed filter pad and dish were dried to a constant weight in a 100 °C oven and reweighed. The difference between the 2 weights was taken as the dry weight of the sample.

**Chemicals**

All chemicals used were of reagent grade or of the highest purity available commercially. Amino acids and the anthrone reagent were purchased from Calbiochemical Co., Los Angeles, Calif., and deoxyribose, ribose and crystalline bovine serum albumin fraction V from Nutritional Biochemical Corp., Cleveland, Ohio.

**Results and discussion**

**Chemical composition of 48 h cultures**

The yeast cells of *O. kalrai* were grown in both a tryptone broth and a nitrogen salts medium for 48 h. The mean values from 4 chemical analyses are presented in table 1. No significant difference in the amount of RNA was found when the cells were grown in the different media, while DNA and protein did vary to some degree. Carbohydrate and lipid levels, however, varied significantly. Higher levels of carbohydrate and lower levels of lipid were found in cells grown on the nitrogen salts medium. Conversely, cells grown in the tryptone enriched medium contained lower levels of carbohydrate and higher levels of lipid.

The results of chemical analysis on 48 h cultures indicate that variations in composition of the growth medium causes an alteration on the chemical composition of the cells.

**Table 1. Chemical Composition of 48 h Yeast Cells of *Oidiodendron kalrai***

<table>
<thead>
<tr>
<th>Components</th>
<th>Tryptone broth medium</th>
<th>Nitrogen salts medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>1.05 ± 0.2</td>
<td>1.72 ± 0.2</td>
</tr>
<tr>
<td>RNA</td>
<td>5.60 ± 0.4</td>
<td>4.90 ± 0.5</td>
</tr>
<tr>
<td>Protein</td>
<td>30.10 ± 1.0</td>
<td>34.80 ± 1.0</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>29.70 ± 1.0</td>
<td>38.10 ± 1.0</td>
</tr>
<tr>
<td>Lipid</td>
<td>30.30 ± 1.3</td>
<td>20.00 ± 1.0</td>
</tr>
<tr>
<td>Recovery</td>
<td>96.75</td>
<td>99.52</td>
</tr>
</tbody>
</table>

* Chemical composition is expressed as percent of dry weight.