PRODUCTION OF CHLAMYDOSPORES BY 
CANDIDA ALBICANS CULTIVATED ON DILUTE 
OXGALL AGAR 

by 

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When Candida albicans is grown under suitable conditions of 
temperature, nutrition and oxygen tension, it produces characteristic 
chlamydospores. The production of these spores, usually in great 
numbers is a property commonly used in routine work for the rapid 
identification of this fungus. Two other species of Candida of medi-
cal importance, Candida stellatoidea (MARTIN et al., 1937) and 
Candida tropicalis (POLLACK & BENHAM, 1957) also form chlamy-
dospores but neither produces these as abundantly as C. albicans. 

Many culture media (WINNER & HURLEY, 1964) have been re-
commended for the production of chlamydospores since the intro-
duction by BENHAM (1931) of corn meal agar. This medium is still 
the best known and probably the most widely used medium for this 
purpose. The addition of 1.0 % tween 80 by ROSENTHAL & FUNARI 
(1958) made the medium more reliable. This chemical had already 
been used by TASCHDJIAN (1957) in another medium for the same 
purpose. BENHAM's petri dish method of identifying C. albicans 
on corn meal agar was to cut slits in the medium with a needle in-
fected with the culture. KLIGMAN (1950) showed that covering the 
inoculated area with a coverslip increased the production of chlamy-
dospores. 

None of the media recommended for the rapid identification of 
C. albicans has been universally accepted. Two important reasons 
for this may be the lack of uniformity in the constituents of the me-
dia and failure to follow carefully the recommended methods of 
preparation. We have tried many of these modifications but have 

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always returned to corn meal agar or, in recent years, to corn meal agar with tween 80 and neomycin. Our method of preparing this medium usually gives good results but it is time consuming and requires a great deal of patience and care. Oxgall, like tween 80, creates a low surface tension but, unlike the latter substance, it has a slight nutritional value. We therefore explored the use of dilute oxgall in agar and found that it did in fact encourage the production of chlamydospores. Oxgall was used by Seeliger (1955) to induce filament formation by C. albicans; and by Pavlatou & Marselou (1956) to produce chlamydospores. The latter authors used it in three ways: in undiluted form, in a coagulated egg base and in a potato-carrot base. Our studies have shown that dilute bile in a plain agar gel is an excellent medium for the production of chlamydospores by C. albicans.

**Materials and Methods**

Cultures used in this investigation included strains of C. albicans, C. tropicalis, C. pseudotropicalis, C. krusei and C. parapsilosis from our stock collection as well as some strains of C. stellatoidea supplied by Dr. Norman Conant of Duke University, Durham, North Carolina. Also studied were 199 consecutive vaginal and cervical swabs submitted by physicians in a transport medium. These were positive for C. albicans on either or both media. Strains isolated from sputum, throat swabs, urine and skin were also examined. All species from our stock collection had been identified by fermentation (Martin et al., 1937) and assimilation (Lodder & Kreger-van Rij, 1952) tests.

The concentration of bile most suitable for the production of chlamydospores was determined by preparing varying concentrations ranging from 0.6 to 10% in 1.8% agar. These were inoculated with 58 strains of C. albicans isolated from routine specimens. The methods of preparing the bile agar and the control medium, corn meal-tween 80 agar, are given below.

**Oxgall agar medium**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
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<tbody>
<tr>
<td>Oxgall (Difco)</td>
<td>20 g</td>
</tr>
<tr>
<td>Agar (Difco)</td>
<td>18 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

Dissolve the oxgall in the water, add the agar and heat to boiling to dissolve. Sterilize at 15 pounds for 15 minutes. Cool to about 50°C and distribute in 12 to 15 ml amounts into plastic petri plates.

**Corn Meal-Tween 80 agar**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
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<tbody>
<tr>
<td>Corn meal (yellow)</td>
<td>80 g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>2000 ml</td>
</tr>
<tr>
<td>Agar (Difco)</td>
<td>20 g</td>
</tr>
<tr>
<td>Tween 80</td>
<td>10 ml</td>
</tr>
<tr>
<td>Neomycin</td>
<td>0.1 g</td>
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