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TECHNICAL MANUSCRIPT 381

# YEAST SPORULATION ON TWO COMMONLY AVAILABLE MEDIA

Warren P. Iverson

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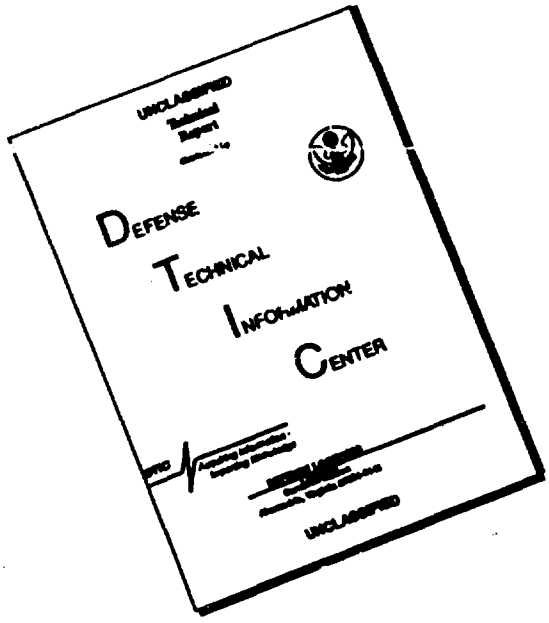


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TECHNICAL MANUSCRIPT 381

# YEAST SPORULATION ON TWO COMMONLY AVAILABLE MEDIA

Warren P. Iverson

Special Operations Division
COMMODITY DEVELOPMENT AND ENGINEERING LABORATORY

Project 1C522301A061

March 1967

# YEAST SPORULATION ON TWO COMMONLY AVAILABLE MEDIA


## ABSTRACT

Two commercially available media (nutrient agar and trypticase soy broth plus agar) induced sporulation of several yeast strains that sporulate with difficulty. In a comparison with the highly successful potassium acetate medium, the majority of 16 strains of Saccharomyces also sporulated. The two media appear useful because of their ease of preparation.


A yeast strain must be able to produce ascospores to allow taxonomic and genetic studies. No single medium appears to suffice for the sporulation of all yeast strains, so several media must be used. Preparation of most of these media is somewhat involved. Attempts to produce sporulation in some yeast strains showed that trypticase soy broth (TSB)* plus agar (1.5%) as well as nutrient agar (NA)* induced fair to good sporulation of commercial bakers' yeast in 3 days, after two or three necessary transfers on a presporulation medium (malt extract agar).** To determine the usefulness of these two media for sporulation, 12 yeasts that sporulated with difficulty,*** plus a commercial bakers' yeast and a Saccharomyces strain (102) isolated in 1923 from the soil were compared for sporulation on the two media indicated above, and on carrot slices, McKelvey's agar,[1] and vegetable (V-8) juice agar.[2]

The TSB plus agar and the NA were prepared in plates; the other media were prepared in screw-capped test tubes. Three transfers at 2- to 3-day intervals were made on malt extract agar before inoculation of the sporulation media. The temperature of incubation was 20 to 25 C for 1½ months. The results are presented in Table 1. As indicated in the table, no one medium was successful in producing sporulation of all the strains. The greatest number (six) of yeasts sporulated on vegetable juice agar, followed by McKelvey's agar, TSB, and NA (five each).

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* Baltimore Biological Laboratory, Inc., 2201 Aisquith St., Baltimore, Maryland.

** Difco Laboratories, Detroit, Michigan.

*** Obtained from Dr. L.J. Wickerham, Northern Utilization Research and Development Division, Peoria, Illinois.

TABLE 1. SPORULATION[a] OF FOURTEEN YEAST STRAINS (SEVEN GENERA) ON FIVE SPORULATION MEDIA

| Strain | Culture Number[b] | Medium | | | | |
|---|---|---|---|---|---|---|
| | | TSB | NA | Vegetable Juice | McKelvey's | Carrot |
| Bakers' yeast | | 3 | 3 | - | - | - |
| *Saccharomyces* sp. (102) | | 1 | 1 | 2 | - | - |
| *Lipomyces* sp. | Y-138 | - | 3 | - | 2 | rare |
| *Hansenula wingei* | Y-2340 | - | - | - | 1 | - |
| *Saccharomyces elongosporus* | Y-2176 | - | - | 1 | - | - |
| *Saccharomyces elongosporus* | YB-4240 | - | rare | - | - | - |
| *Saccharomyces elongosporus* | YB-4239 | - | - | 1 | - | - |
| *Metschnikowia zobellii* | Y-5387 | - | - | 3 | - | - |
| *Metschnikowia krissi* | Y-5389 | - | - | 1 | - | 1 |
| *Debaromyces phaffii* | YB-5161 | rare | - | - | - | - |
| *Endomycopsis fibuliger* | Y-25 | 2 | - | 2 | 1 | 3 |
| *Endomycopsis fibuliger* | Y-1062 | - | - | - | - | - |
| *Kloeckera apiculata* | Y-2336 | rare | 1 | - | 2 | - |
| *Hansenula wickerhamii* | YB-4943 | - | - | - | 1 | - |

a. rare = one ascus per several fields.
1 = <5%
2 = 5 to 20%
3 = 20 to 50%

b. Northern Utilization Research and Development Division Culture Collection numbers.

Because TSB and NA appeared to be favorable media for the sporulation of Saccharomyces, it was of interest to compare sporulation on these two media with that on a potassium acetate (0.1 M), yeast extract (0.25%), glucose (0.1%) medium, highly successful for inducing sporulation in this genus.[3] Sixteen strains of alcohol and wine yeasts, including a strain of Saccharomyces anamensis,* were selected. These strains with one exception had been transferred at 6-month intervals on malt agar for more than 20 years and for the past 4 years on Sabouraud dextrose agar.** Malt extract agar** was used as the presporulation medium for NA and TSB plus agar. The presporulation medium for the potassium acetate medium consisted of 20 g glucose, 2 g $(NH_4)_2SO_4$, 2 g $KH_2PO_4$, 5 g yeast extract (Difco), 15 g agar, and distilled water to 1,000 ml.[3] Two transfers were made at 2-day intervals on plates of the presporulation medium with a 3-day interval between transfers from presporulation to sporulation media. The incubation temperature was 20 to 25 C. The results are indicated in Table 2. The greatest number of four-spored asci was produced on the potassium acetate medium. Although this medium appears to be best for the sporulation of Saccharomyces, the other two media appear to be useful as general sporulation media because of their ease in preparation.

TABLE 2. SPORULATION OF SIXTEEN STRAINS OF SACCHAROMYCES ON THREE SPORULATION MEDIA

| Sporulation Medium | Strains with Asci[a] after | | | Per Cent Cells with Asci after | Strains with 50% or More Asci[b] after |
|---|---|---|---|---|---|
| | 2 days | 1 wk | 2 wk | 1 wk | 1 wk |
| Potassium acetate, yeast extract, glucose agar | 11/16 | 14/16 | 15/16 | 7 - 92 | 11/14 |
| Nutrient agar | 8/16 | 13/16 | 13/16 | rare - 45 | 0/13 |
| Trypticase soy broth + agar | 1/16 | 11/16 | 12/16 | rare - 35 | 0/11 |

a. Strains producing asci/Total no. of strains.
b. Strains producing ≥50% asci/Total no. of strains producing asci.

* Obtained from Dr. Allgeier, Fort Detrick.
** Difco Laboratories, Detroit, Michigan.

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14. Key Words

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