ASCOSPORE AGAR

INTENDED USE
Remel Ascospore Agar is a solid medium recommended for use in qualitative procedures for the cultivation of ascomycetous yeasts, such as Saccharomyces cerevisiae.

SUMMARY AND EXPLANATION
McClary, Nulty, and Miller studied the effect of potassium acetate versus sodium acetate in the sporulation of Saccharomyces.\(^1\) Ascospore Agar is the potassium acetate-yeast extract-glucose medium of McClary et al. modified from Adams' previous sodium acetate medium.\(^2,3\) Potassium acetate was found to be superior to sodium acetate in its ability to enhance the production of ascospores.

PRINCIPLE
The features by which most fungi are identified are related to the mode of sporulation and the morphology and arrangement of the spores produced.\(^1\) In the case of some fungi (e.g., Saccharomyces), sexual reproduction results in the production of sexual spores in a structure called an ascocarp. This contains smaller sacs called ascii, each of which contains ascospores. Ascospores are produced on this medium and are visible microscopically after staining or in a wet-mount preparation.\(^4\)

REAGENTS (CLASSICAL FORMULA)*

\[
\begin{align*}
\text{Potassium Acetate} & : 10.0 \text{ g} \\
\text{Yeast Extract} & : 2.5 \text{ g} \\
\text{Dextrose} & : 1.0 \text{ g} \\
\text{Agar} & : 30.0 \text{ g} \\
\text{Demineralized Water} & : 1000.0 \text{ ml}
\end{align*}
\]

pH 6.4 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. Implement appropriate procedures to verify that the test isolate is a yeast.
2. Inoculate Ascospore Agar with a portion of a colony and streak for isolation.
3. Incubate at 25-30°C for 3-10 days. Examine daily for growth.
4. Make a thin smear of the growth on a microscope slide and heat-fix.
5. Stain with 5% aqueous malachite green for 3 minutes. Wash with tap water.
6. Decolorize with 95% ethyl alcohol for 30 seconds. Wash with tap water.
7. Counterstain with 5% aqueous safranin for 30 seconds. Wash with tap water and allow to dry.
8. Examine under oil immersion at 400 x to 1000 x magnification. Ascospores (1 to 4 per ascus) appear in tetrahedral or linear arrangement and stain green, while the vegetative portion of the fungus stains red.

Note: The ascospore stain is the preferred method; alternatives include the acid-fast stain and the wet mount preparation.\(^4\)

Pour Tube: Melt the pour tube in a boiling water bath and cool to 45-50°C. Mix and dispense into a sterile petri dish and proceed with the instructions above.

QUALITY CONTROL
All lot numbers of Ascospore Agar have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL

\begin{itemize}
  \item \textit{Saccharomyces cerevisiae} ATCC® 24903
  \item \textit{Candida albicans} ATCC® 10231
\end{itemize}

INCUBATION

\begin{itemize}
  \item Aerobic, 3-6 days @ 25-30°C
\end{itemize}

RESULTS

\begin{itemize}
  \item \textit{Saccharomyces cerevisiae}: Ascospores formed
  \item \textit{Candida albicans}: No ascospores
\end{itemize}

BIBLIOGRAPHY


Refer to the front of Remel Technical Manual of Microbiological Media for General Information regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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