

TESTS OF DIFFERENT MEDIA FOR THE COLLECTION AND IDENTIFICATION OF AIR-BORNE SAPROPHYTIC FUNGI*

MARY ANN SWAEBLY, PH.D., CLYDE M. CHRISTENSEN, PH.D., AND
THEODORE A. GRAHEK, B.S., ST. PAUL, MINN.

THE purpose of the extensive work with media favorable for the collection and identification of air-borne fungi recently done at the University of Minnesota, Division of Plant Pathology and Botany, in cooperation with the American Academy of Allergy, was to determine whether the casein hydrolysate medium being used in the present air-borne mold survey is giving the highest possible total mold count or whether other media could be found which would give significantly higher counts.

The test method used in all of the experiments was essentially the same as that recommended by Dr. Leonard Harris in his mimeographed letter to the members of the Academy of Allergy who are cooperating in the mold survey.

In preliminary tests, 2 plates of each of 18 different media were exposed for 12 minutes, the plates being arranged in a randomized block. A new set of plates were exposed on each of 3 successive days. Those media which gave consistently low total mold counts, or which obviously supported the growth of fewer genera than the other media in the series, were eliminated from further tests. Fifteen media were eliminated in the preliminary tests. The 3 remaining media were then used in all of the exposures from June 1 to September 15. The 3 which proved satisfactory in the preliminary tests and which were tested further were: (1) modified Smith-Humfeld medium plus sodium chloride; (2) Waksman's medium plus sodium chloride; and (3) Mehrlich's medium plus sodium chloride.†

The casein hydrolysate medium‡ was used as a standard against which to compare all results throughout the experiment except in the preliminary screening, and was included in all of the exposures.

*Read before the Sixth Annual Meeting of the American Academy of Allergy, Los Angeles, Calif., March 6-8, 1950.

†The formulas for the media used in our comparative tests are as follows:

1. Modified Smith-Humfeld plus sodium chloride:

KH ₂ PO ₄	1Gm.
NaNO ₃	1Gm.
Dextrose	15Gm.
NaCl	75Gm.
Agar	15Gm.
Distilled H ₂ O	1 liter

2. Waksman's medium plus sodium chloride:

K ₂ HPO ₄	1Gm.
MgSO ₄	0.5Gm.
Peptone	5Gm.
Glucose	10Gm.
NaCl	75Gm.
Agar	15Gm.
Distilled H ₂ O	1 liter

3. Mehrlich's medium plus sodium chloride:

KH ₂ PO ₄	1Gm.
MgSO ₄	0.5Gm.
Peptone	5Gm.
Malt extract	5Gm.
Dextrose	15Gm.
NaCl	75Gm.
Agar	15Gm.
Distilled H ₂ O	1 liter

‡Casein hydrolysate plus sodium chloride (prepared Mead Johnson & Company laboratory product):

Casein hydrolysate	50Gm.
NaCl	75Gm.
Agar	15Gm.
Distilled H ₂ O	1 liter

Each medium was judged on the following 4 points considered important in the selection of a substratum for the collection and identification of fungi: (1) The number of identifiable colonies obtained; (2) the number of genera obtained; (3) restricted colony size to eliminate "masking" effect and coalescing of colonies, and (4) facility in identification of the fungus colonies.

Preliminary tests indicated that approximately one mole of NaCl added to any medium greatly increased its usefulness for mold survey work, by practically eliminating bacterial contaminants and by restricting colony size, thus reducing the tendency for the colonies to coalesce. Therefore 75 Gm. of NaCl were added to each liter of medium (Fig. 1).

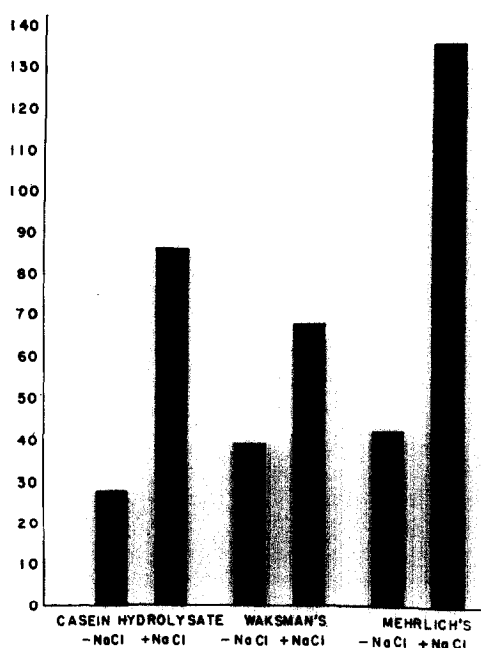


Fig. 1.—Average number of colonies per plate on 3 media with and without sodium chloride.

There were no significant differences in the number of genera obtained on any of the 4 media, except in the case of the modified Smith-Humfeld salt agar which would not support the growth of many of the *Phycomycetes*, especially *Rhizopus* and *Mucor*, but there were considerable differences in number of colonies, and in relative prevalence of some of the common air-borne molds.

The casein hydrolysate medium which is now used in the survey does not compare favorably with several of the other media, even after the addition of the salt. This medium gave the second lowest total mold count of those tested extensively. It permits a profuse growth of many fungi, but this abundant growth was partly responsible for the low total count, since the colonies merged into each other, leaving no sharply defined borders; consequently the colonies failed to stand out as individuals. For example, when one of the *Aspergilli* or *Penicillia* predominated, 2 or 3 colonies would merge into each other, and, unless they were counted with the aid of a microscope before they were clearly

visible to the unaided eye, several colonies could easily be mistaken for one colony. Similarly, if *Rhizopus* or *Mucor* were present, a plate could be completely covered with dense mycelium in 24 to 48 hours and if other fungi were present underneath the dense overgrowth, they could neither be counted nor identified (Fig. 2).

From the standpoint of total mold counts and facility in identification, a modified Smith-Humfeld salt agar was far superior to all others, but because *Rhizopus* and *Mucor* do not grow well on it, its use for survey purposes is perhaps limited. The extremely high average colony count per plate on this medium was due, mainly, to the large number of *Hormodendrum* and *Alternaria* colonies which appeared during the early summer weeks. (Figs. 3 and 4). On this agar, it was possible to get fairly accurate counts of the extraordinarily large number of colonies for two reasons; first, the colonies remained small, and second, the dark reverse side of the sharply defined *Hormodendrum* colonies was clearly visible through the almost transparent agar.

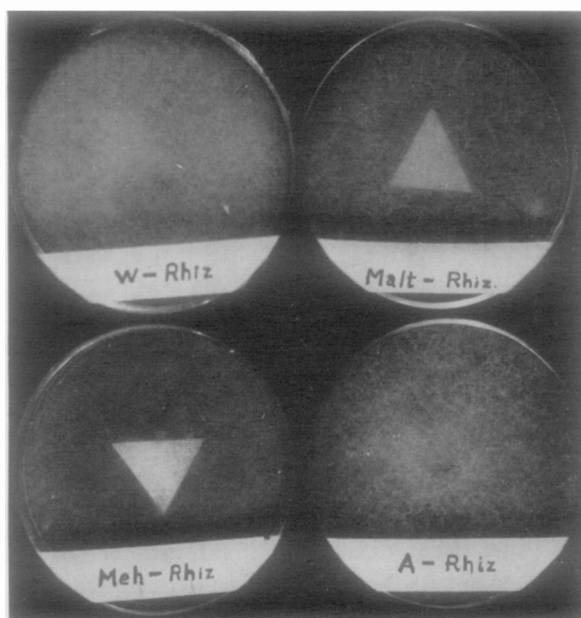


Fig. 2.—Cultures of *Rhizopus* growing on 4 media. W. Waksman's; Meh. Mehrlich; A. casein hydrolysate. White paper triangles beneath the dishes are not visible through the dense mycelium on Waksman's agar and on casein hydrolysate.

Attempts are now being made to modify this agar further so that common air-borne Phycomycetes will grow upon it. If this can be done, this agar should prove to be extremely useful in survey work.

During the first weeks of the experiment Waksman's agar plus salt appeared to be an excellent one for the collection and identification of air-borne fungi because of the adequate but limited growth of the colonies. When *Alternaria* and *Hormodendrum* became prevalent, however, the total count on Waksman's medium failed to rise as sharply as did the total count on the other media. Comparison of the daily figures indicated that this medium consistently

was not permitting the growth of as many colonies as the other media, consequently it was eliminated from the series.

In our tests the most favorable medium was Mehrlich's agar plus sodium chloride. It is light tan in color, fairly transparent, and supports the growth of many common air-borne saprophytic fungi predominant in this region. Its chief advantages are that the colonies remain small and sharply delimited, hence they are clearly visible at maturity as separate entities, eliminating the necessity for observing the plates microscopically before the colonies are mature. After a little practice it is not difficult to identify the fungi by gross cultural observations on this medium, although most fungi appear generally somewhat darker and "dusky" without the bright colors characteristic of many colonies on the casein hydrolysate medium. In our tests Mehrlich's medium gave a high mold count, second only to the modified Smith-Humfeld salt agar, which, as mentioned before, is limited in its usefulness because of its inability to support the *Phycomycetes*.

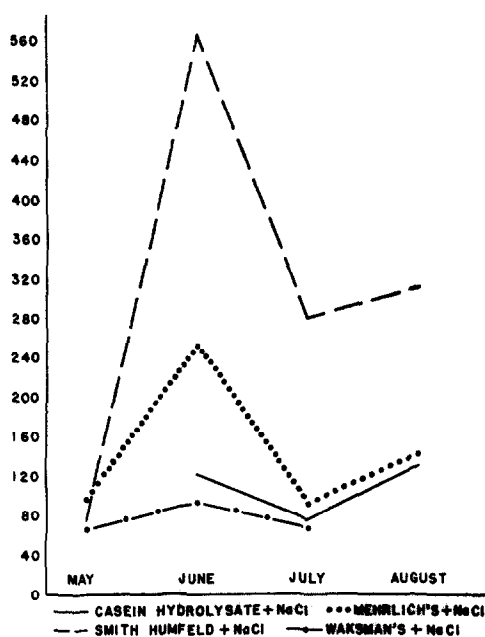


Fig. 3.—Average number of colonies per plate for 4 months on the 4 most favorable media.

Growth of all fungi was adequate for identification, but at the same time the colonies were greatly reduced in size over similar colonies grown on the casein hydrolysate medium, and the border of each colony was sharply defined. *Rhizopus* and *Mucor* grew sufficiently for easy identification, but not densely enough to obscure the other colonies on the plates (Fig. 2).

In the preliminary experiments, a malt salt agar gave very high mold counts, but the fungi were often difficult to identify because of the very dark color of the agar when prepared with a crude grade of malt extract. During the last few weeks of the experiment, however, a highly purified malt extract was obtained and the resulting agar was transparent as well as much lighter in color.

As a result the typical color and texture characteristics of the individual fungi were clearly evident on gross examination, although the same "duskiness" described on the Mehrlich's medium persisted here. Since, however, this form of the malt agar has not been tested extensively under actual survey conditions, we cannot at this time make any definite statement regarding its value for this type of research. It appears to have considerable promise.

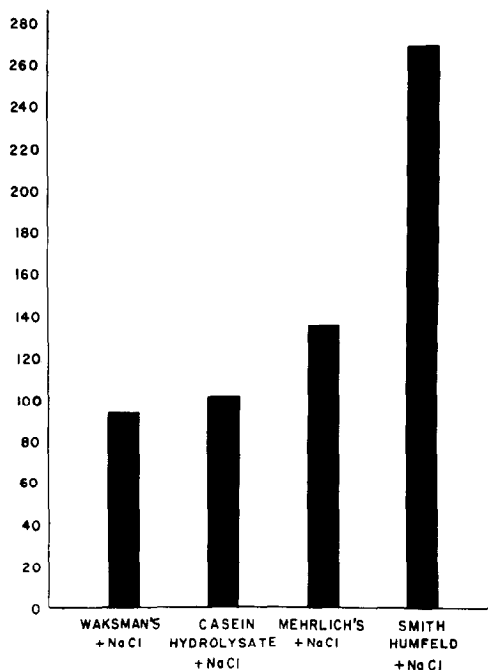


Fig. 4.—Total average number of colonies per plate for 4 months, May, June, July, and August, on the 4 most favorable media.

SUMMARY

1. The experimental results to date indicate that Mehrlich's medium with 75 Gm. of sodium chloride per 1000 c.c. of H_2O will give a significantly higher mold count per plate than the casein hydrolysate medium now employed in the air-borne mold survey. This medium supports the growth of a large number of the air-borne saprophytic fungi common to this region.

2. There is evidence, however, that this medium is still not the best possible, since readings from the modified Smith-Humfeld salt agar show a mold count almost double the mold count obtained on Mehrlich's salt agar and almost triple the mold count from the casein hydrolysate salt medium.

3. As a result, therefore, experiments with a further modified Smith-Humfeld salt medium, a purified malt salt agar, and reduced strengths of the casein hydrolysate medium are being continued.

4. None of these media will disclose the presence of air-borne spores of obligate parasites or spores of some other fungi as mushrooms and wood-rotting fungi which may often be present in considerable numbers, but they may considerably increase the value of the surveys now being conducted.