

Variation in Colonial Morphology of Fungal Isolates Exhibited by Different Malt Extract Products in Agar Media

S. Abbott¹, D. Spero¹, W. Phillips², A. Y. Hsiung², J. Hardy²;

¹Natural Link Mold Lab, Inc., Sparks, NV, ²Hardy Diagnostics, Santa Maria, CA.

ABSTRACT

Substantial variation in the performance of malt extract agars (MEA) was noted among suppliers of prepared media in the environmental mycology industry. Using the same media recipe, but varying the source of malt extract products was found to produce significant variation in the colonial morphology of fungal isolates. Variation included growth rate, degree of sporulation, colony color, colony texture, and degree of colony sulcation on both obverse and reverse sides. Consistent growth patterns and well developed morphological features are critical for accurate species level identification. Species of *Penicillium* exhibited some of the greatest variability, and were often poorly developed on commercial media. Identification using the Pitt ID protocols is greatly aided by use of a high quality media formulation. Other fungi such as *Aspergillus* and *Stachybotrys* species, performed noticeably better on some media than others. Phylloplane fungi such as *Alternaria*, *Ulocladium*, *Epicoccum* and *Cladosporium* sporulate readily on optimal quality malt extract media but may produce an abundance of sterile mycelium without spores on some formulations. Extensive growth trials utilizing 10 different malt extract agar formulations and using 18 species of fungi resulted in an optimal MEA media recipe to provide excellent morphological growth characters over a broad range of features for a wide selection of organisms.

INTRODUCTION

Malt Extract Agar (MEA) is a commonly used non-selective agar for isolation and identification of fungi. Indoor air quality (IAQ)/environmental laboratories and field investigators rely on this media to recover fungi from a variety of indoor and outdoor sources. Many commonly recovered fungi grow and sporulate well on this media, but we observed substantial variation in performance among commercially available products from a wide range of sources. The formulation of the media is the key to getting good results. Often MEA formulations stimulate vegetative growth with little or no production of reproductive structures, which are critical to the identification process. This can lead to a situation where primary isolation plates are covered with vigorously growing fungal colonies but few can be identified. Unchecked vegetative growth can overgrow adjacent colonies on the plate making enumeration problematic. Other morphological anomalies associated with certain formulations of MEA are the tendencies of certain genera to become sulcate or umbonate. This is a situation where the mycelium grows densely and the colonies buckle under the stress making them wrinkle in the case of sulcation or grow tall in the case of umbonation. This is often accompanied with a reduction of sporulation and in extreme cases can cause the colony to split apart.

The ideal MEA would have semi-restricted colonies, good sporulation, suppression of aerial mycelium as well as the suppression of sulcation / umbonation. Early trials indicated substantial variation associated with using different malt extract products, while little variation was observed by altering the other ingredients in the standard malt extract recipe. Ten formulations using different malt extract sources were compared in order to find one that exemplified the criterion presented above. By process of elimination a formulation was selected for detailed comparison with the commercially available formulation. The findings of that comparison are presented below.

MATERIALS AND METHODS

Eighteen isolates of fungi commonly recovered indoors were inoculated on two MEA formulations. The comparisons were made between the original commercially available formulation and a new formulation, which was selected as the best performer of the experimental lots initially compared. Both formulations used John Pitt's MEA recipe which was developed for *Penicillium* identification and is provided in Table A (Pitt 1988). The difference between the two formulations differed only in the source or 'brand' of the malt extract ingredient, the formula itself remained constant. Three point inoculations were made on the different formulations on 85 mm petri dishes from spores liberated from mature colonies. The fungi were allowed to grow for seven days in the dark at 25°C. At seven days measurements and observations were made. Colony diameter, degree of umbonation / sulcation, degree of aerial / sterile mycelium, degree of sporulation, and presence and color of diffusing pigment and exudate were recorded. Colony measurements were made from the reverse and the average of the three colonies was recorded. The degree of umbonation/sulcation was measured qualitatively and in relation to their counterpart on the other formulation. Aerial / sterile mycelium and sporulation measurements were in relation their counterpart on the other formulation.

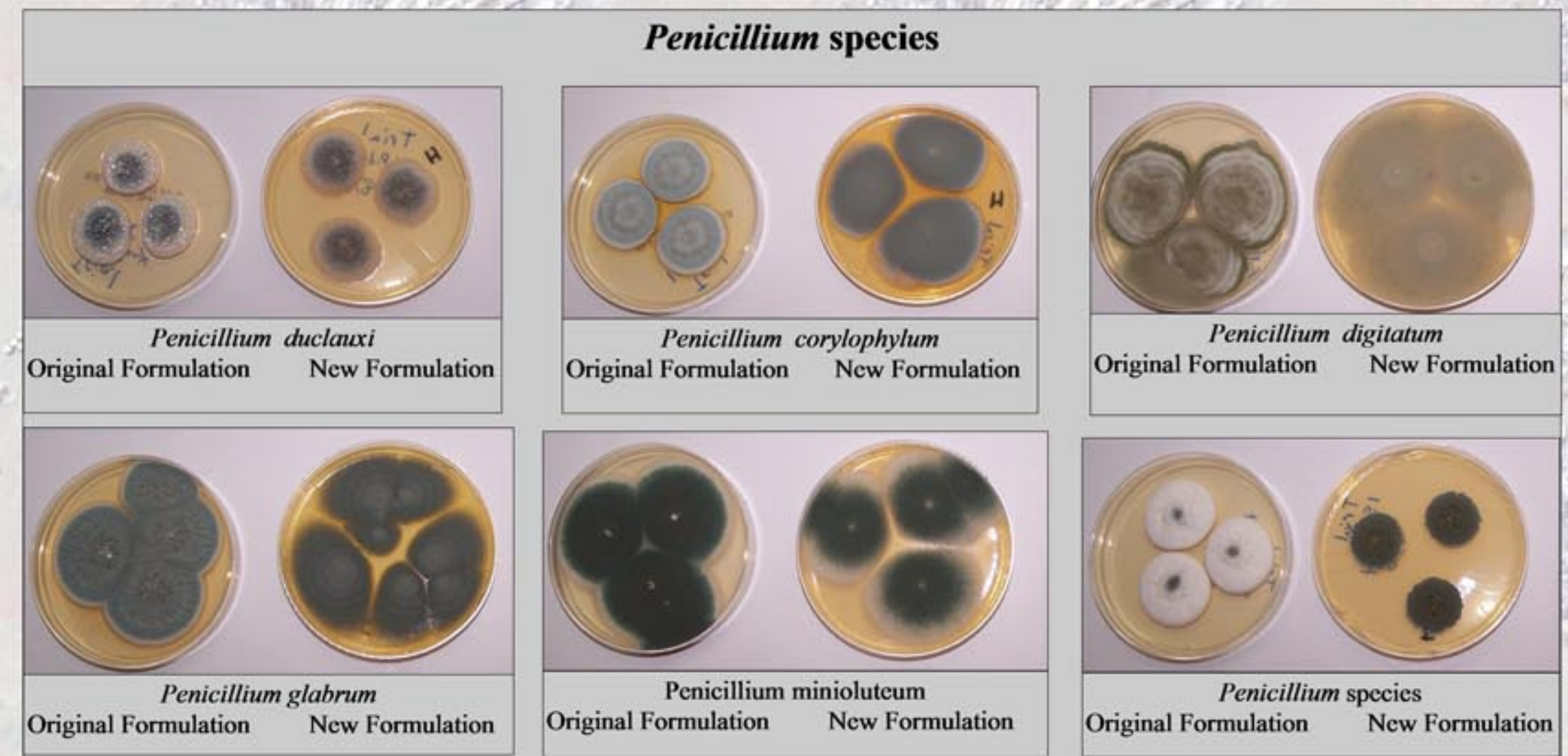
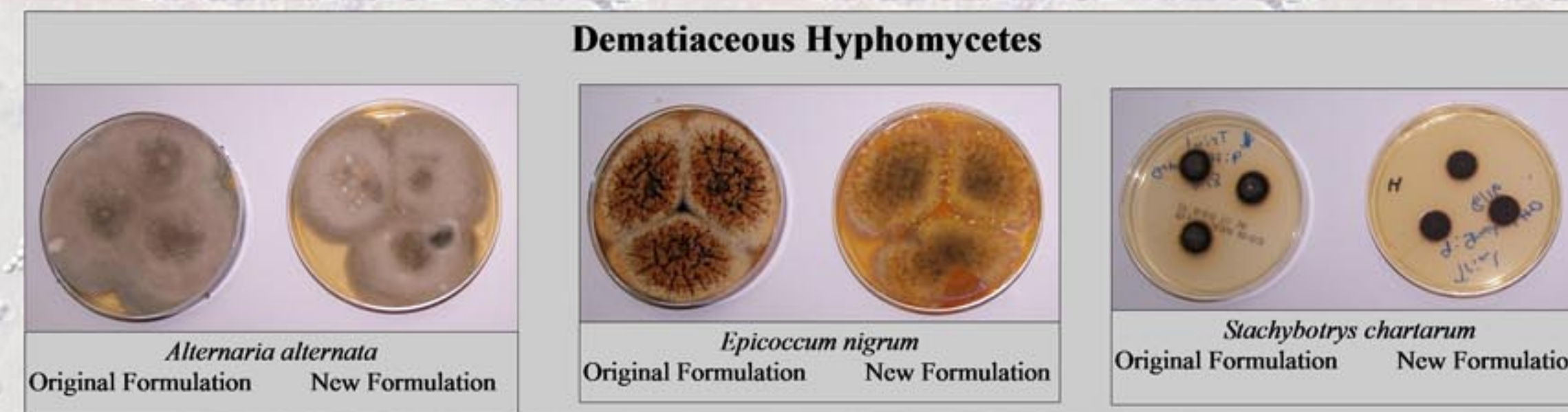
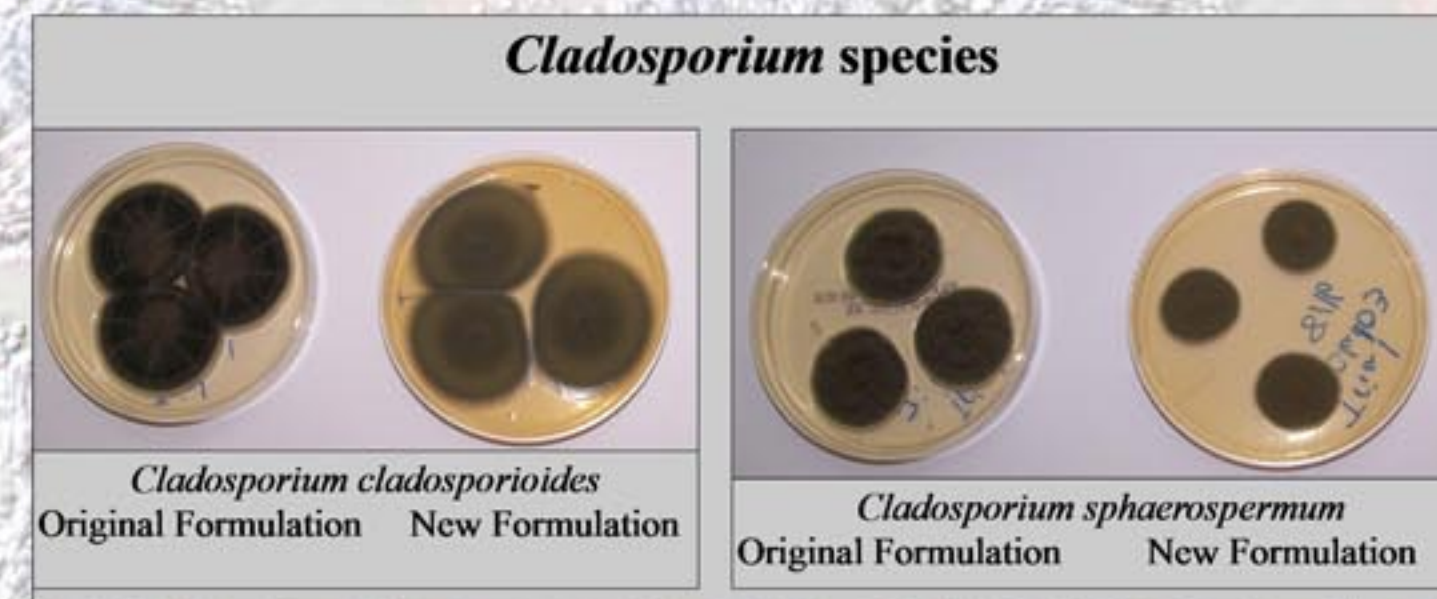
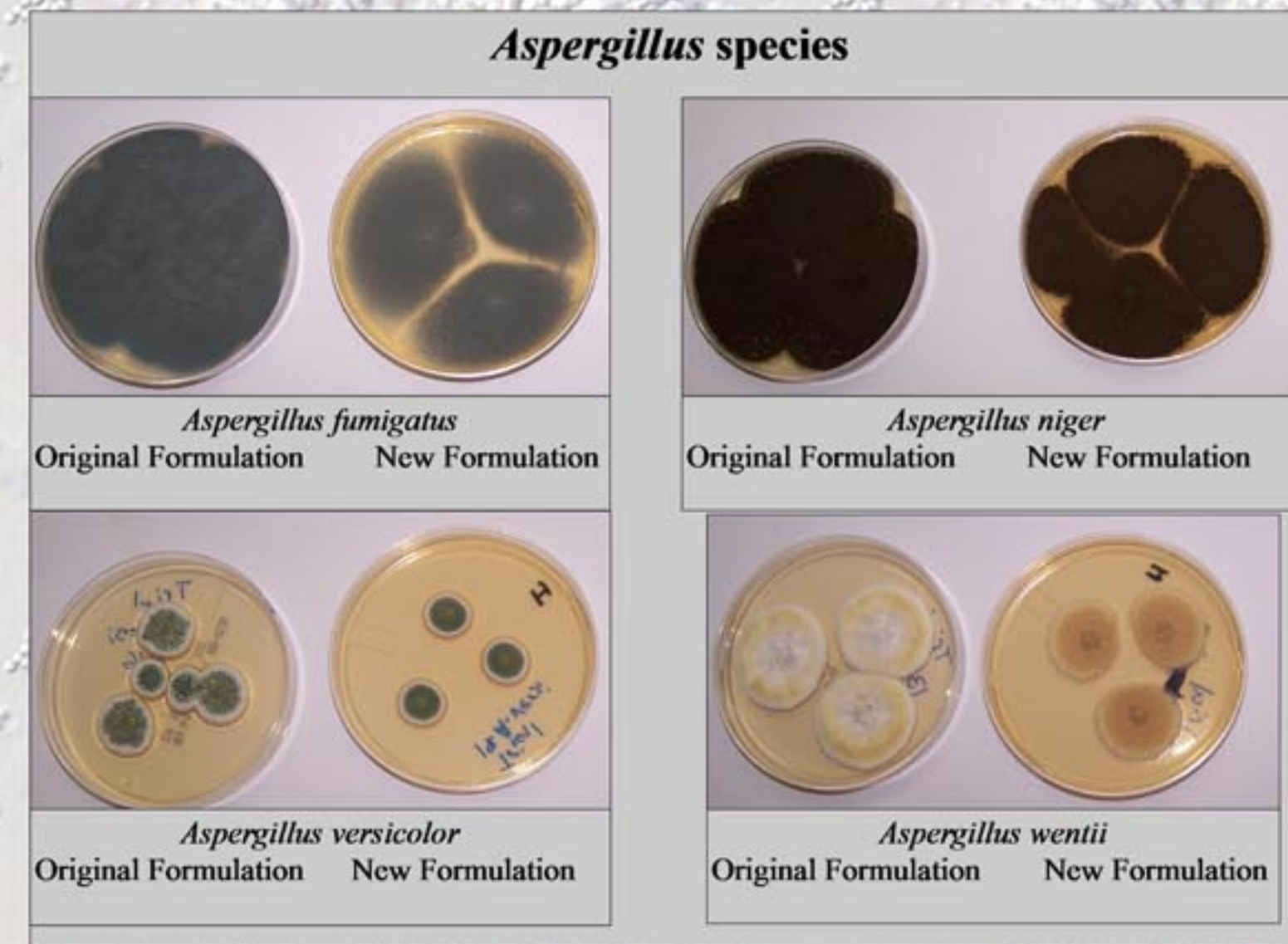
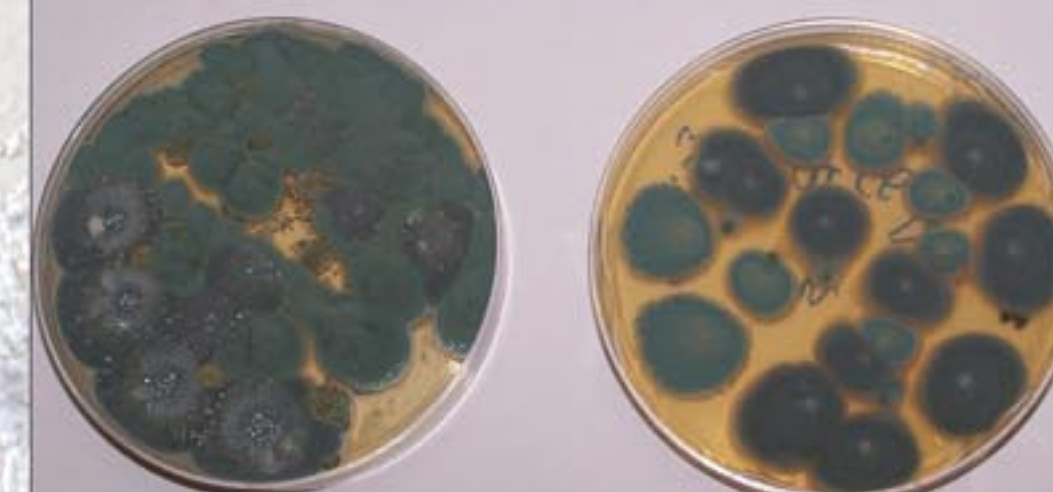


Table A: MEA recipe

Malt Extract Agar (MEA)

Malt extract, powdered	20 g
Peptone	1.0 g
Glucose	20 g
Agar	20 g
Water, distilled	1 L

Primary Isolation Demonstration



Recovery of a wide variety of fungi from primary isolation plates was improved by:

- reducing spread of aerial mycelium and confluent overgrowth of dominant organisms
- restricting colonics which could be easily isolated and enumerated
- improving expression of morphological features allowing for different species to be quickly recognized and differentiated

RESULTS

Table B: Comparison of morphological features on two MEA formulations for various species.

Species	Colony diameter	Umbonation / Sulcation	Aerial / Sterile mycelium	Sporulation
<i>Aspergillus fumigatus</i>	33	heavy radial sulcation	heavy with lots of minute exudate droplets	moderate
<i>Aspergillus niger</i>	34	slight umbonation	sparsely	heavy
<i>Aspergillus versicolor</i>	1			
<i>Aspergillus wentii</i>	58	plane	heavy w/ copious exudate	poor
<i>Cladosporium cladosporioides</i>	49	plane	moderate	moderate
<i>Cladosporium sphaerospermum</i>	25	heavy umbonation	moderate	heavy
<i>Dematiaceous Hyphomycetes</i>	60	moderate radial sulcation	heavy	heavy
<i>Alternaria alternata</i>	51	plane	sparsely	heavy
<i>Stachybotrys chartarum</i>	60	moderate radial sulcation	heavy	heavy
<i>Epicoccum nigrum</i>	54	plane	sparsely	heavy
<i>Penicillium duclauxi</i>	22	umbonate	heavy around edges	moderate
<i>Penicillium corylophyllum</i>	24	plane	sparsely	heavy
<i>Penicillium digitatum</i>	2			
<i>Penicillium glabrum</i>	37	heavy radial sulcation	heavy around edges	moderate
<i>Penicillium minioluteum</i>	45	plane	sparsely	heavy
<i>Penicillium species</i>	28	moderate umbonation	heavy very floccose w/ sterile mycelium	very poor
<i>Stachybotrys chartarum</i>	30	slight umbonation	moderate	moderate/ heavy

DISCUSSION AND SUMMARY

The new formulation performed better than the original commercially available formulation using the criteria discussed in the introduction. Colonies tended to be more restricted. Sulcation/ umbonation was reduced or in most cases eliminated. Production of aerial sterile mycelium was suppressed while sporulation increased. This formulation also reduced the production of exudate.

MEA agar is the most commonly used agar to recover fungi from indoor environments. Having a good formulation supports accurate identification and enumeration of fungi recovered. The recipe of the MEA formula did not change. The malt extract source itself, not there concentrations was the factor effecting colony morphology.

REFERENCES

Pitt, J. 2000. A Laboratory Guide to Common *Penicillium* species. Food Science Australia, NSW, Australia.

ACKNOWLEDGEMENTS

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