

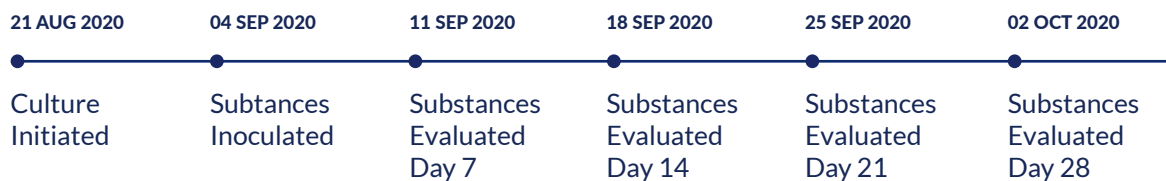
NYCOTE® COATINGS ABILITY TO RESIST FUNGAL ATTACK CASE STUDY #5

TEST METHOD BACKGROUND AND STUDY TIMELINE

ASTM G21: General Information

ASTM International, formerly the American Society for Testing and Materials (ASTM), is an internationally recognized organization that develops and publishes product and testing standards. ASTM G21 is a qualitative test method designed to assess the ability of synthetic materials to resist fungal attack. The method is typically conducted over a 28 day period, where treated materials are inoculated with a pooled suspension of fungal spores, incubated, then compared to untreated controls at intervals. The untreated controls serve as references for fungal resistance. A diverse array of fungal species are used in this method, so it is considered to be a good indicator of fungal resistance in a variety of environments.

Study Timeline

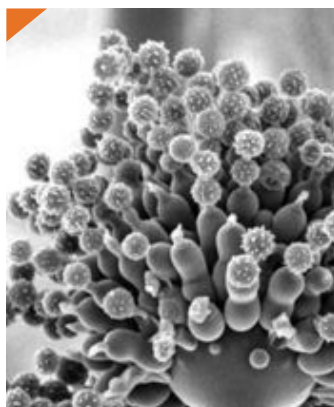


Products Included In This Study:

Nycote 7-11® and Nycote 99®

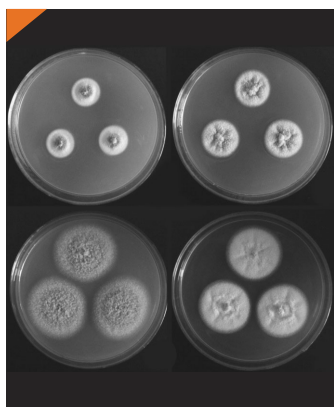
Test Microorganism Information

The test microorganism selected for this test:



***Aspergillus brasiliensis* 9642**

This fungi is a conidiophore, or a sexual spore generating aerobic fungus. *A. brasiliensis*, formerly listed as a strain of *A. niger*, is related to other *Aspergillus* species in that they produce spores which are highly resistant to chemical and environmental conditions. *A. brasiliensis* is commonly used as a benchmark fungus for antimicrobial fungicides and preservatives used in pharmaceutical and personal care products.



***Talaromyces pinophilus* 11797**

This fungi is a facultative tonophile meaning it can survive and grow in extreme conditions such as arid or high pH environments which is uncharacteristic of other mold fungi. This species is known to utilize molecular components from several materials as a food source, namely cotton and paper products. *T. pinophilus*, formerly listed as a strain of *Penicillium funiculosum*, is associated with rotting fruit and seeds and is commonly used in fungus resistance of articles.

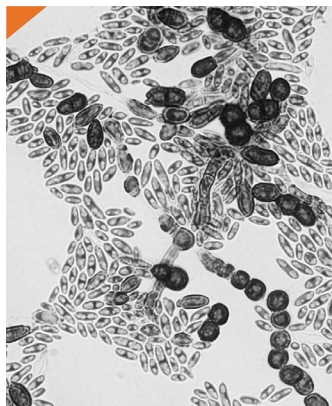


***Chaetomium globosum* 6205**

This fungi is a saprotrophic species that is normally found in soil, air, and plant debris. *C. globosum* thrives in cellulose rich areas such as seeds, textiles, straw, and sacking. Typically prevalent in homes with water damaged areas, *C. globosum* is one of the more prevalent fungi encountered in household environments. This prevalence makes *C. globosum* a commonly used model for fungus resistance testing.

Test Microorganism Information

The test microorganism selected for this test:



Aureobasidium pullulans 15233

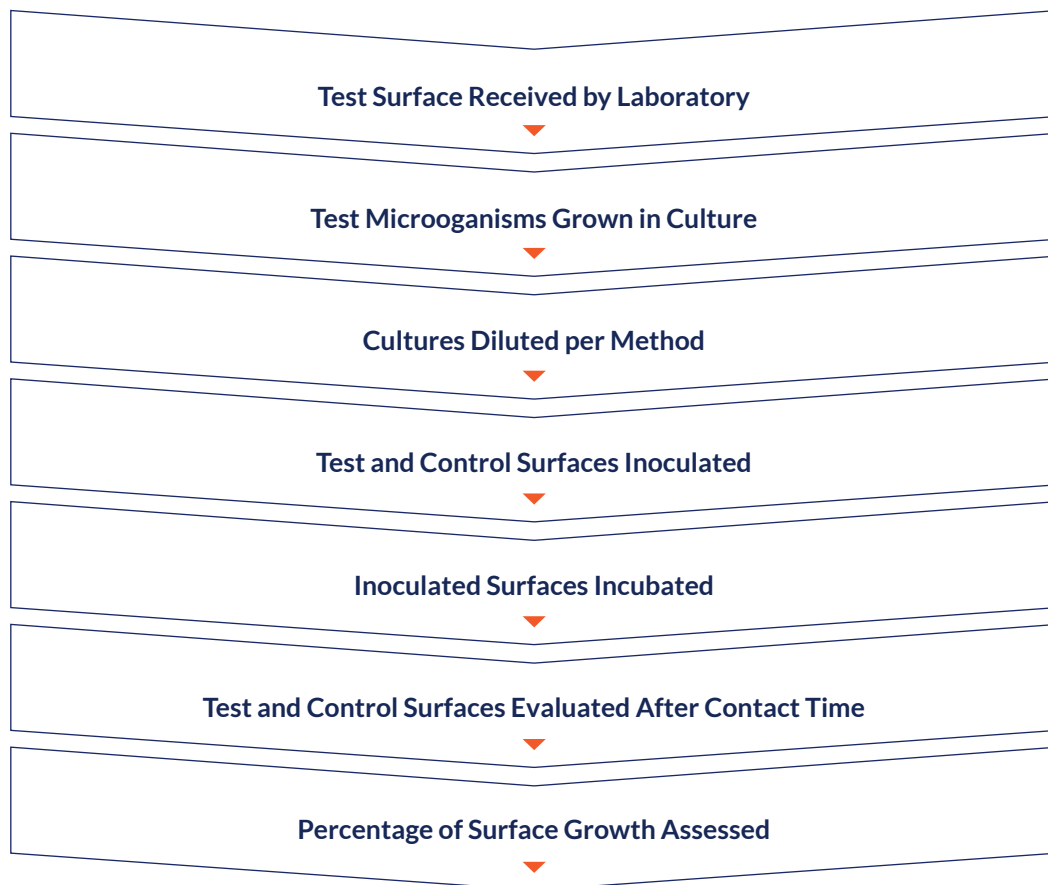
This fungi is a ubiquitous saprotroph meaning it can be found in a multitude of environments and process nutrients by extracellular digestion of dead or decayed organic matter. *A. pullulans* has been known to cause pneumonitis (humidifier lung) over extended periods of exposure. This fungi is often employed in fungal resistance testing due to its ability to prevail in numerous environments and its ability to metabolize organic matter.



Trichoderma virens 9645

This fungi typically prevalent in soil and decayed wood. *T. virens* is often used in the agriculture industry as it is beneficial to crop production and plant metabolism because of its ability to produce antibiotics and parasitize other fungi. Because of its widely accepted use, it is a recommended microorganism for fungi resistance testing of adhesives and lumber.

Diagram of the Procedure



Summary of the Procedure

- The test fungi are prepared individually, prior to the test, by growth on agar.
- Suspensions of fungi are standardized by dilution in a buffered saline solution, and then pooled into a single suspension.
- Test and control substances are aseptically placed on agar plates.
- The pooled suspension of fungi is then applied to the surface of test and control substances using a sprayer. Sufficient spray is applied to wet the surface of the test substance.
- Inoculated test and control substances are placed in a sealed, humid environment and incubated for the predetermined contact times.
- At the conclusion of each contact time, visual assessments of each sample are made, noting the percentage of fungal growth on the inoculated surfaces of both test and control substances.
- Based on the percentage of growth observed, a numerical score is assigned to each substance.

PARAMETERS

Criteria for Scientific Defensibility of an ASTM G21 Study

1. The average number of viable spores of each fungal species shall be approximately 1×10^6 spores.
2. Greater than 85% growth observed on inoculated untreated control substances after 14 days of incubation.

Testing Parameters

	Surface	Size	Replicates
Test Substance	Treated Glass Surfaces	2" x 2"	Triple
Control Substance	Sterile Filter Paper	2" x 2"	Triple

Culture Growth Media	PDA and Rabbit Food Agar
Culture Growth Time	7–14 days
Culture Suspension Media	Mineral Salts Broth
Inoculum Application	Spray
Inoculum Concentration	1.0×10^6 spores/mL
Test Plating Media	Mineral Salts Agar

OBSERVATIONS

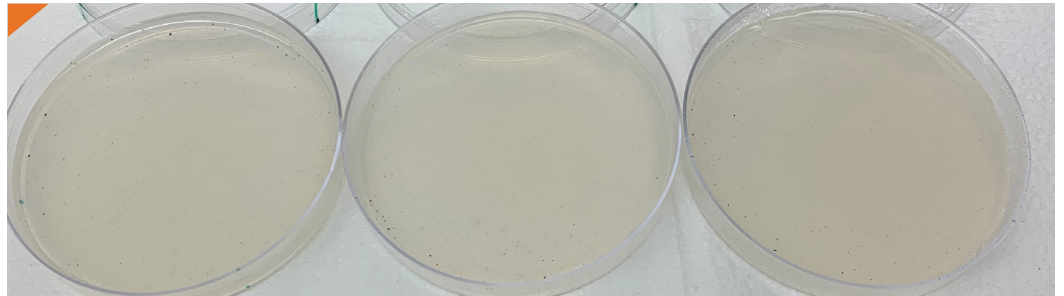
Study Modifications

No further modifications were made to the method for this study.

Study Notes

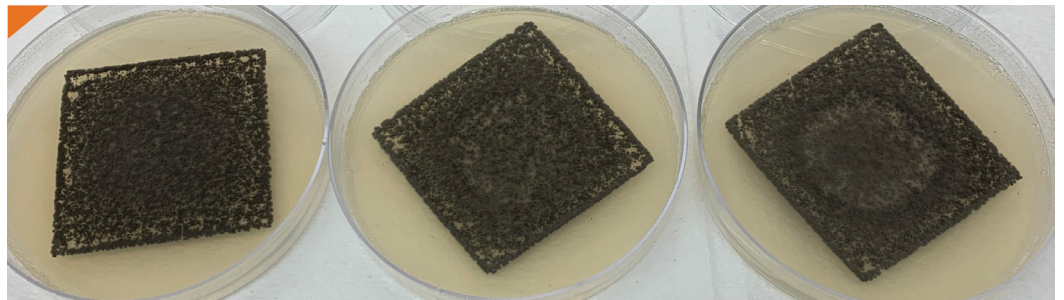
No additional notes were made for this study.

Study Photographs – Day 28



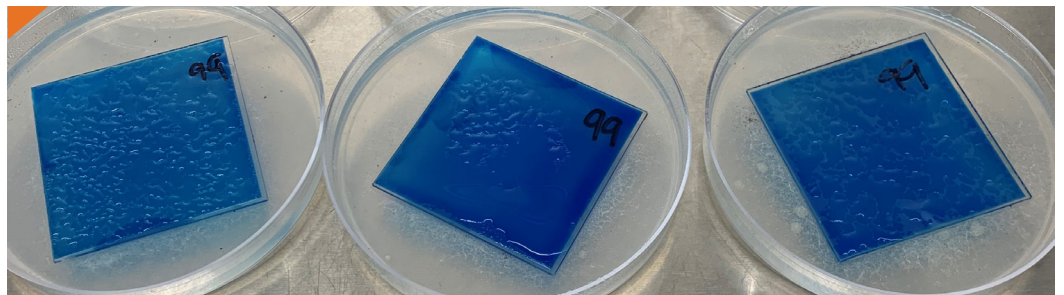
Negative Controls

No fungal growth was observed on the inoculated mineral salt agar replicates used in this study.



Positive Controls

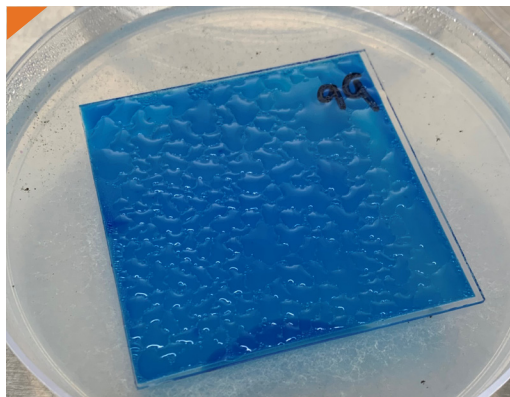
Heavy fungal growth was observed on both inoculated filter paper replicates.



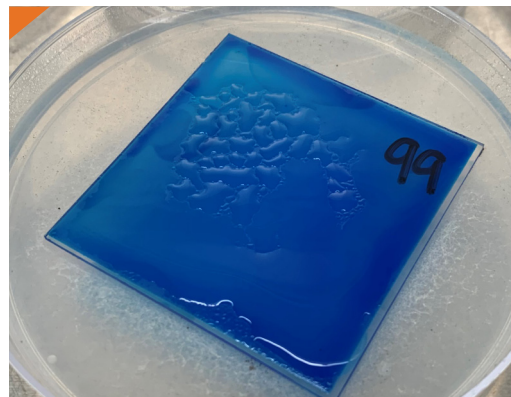
The photograph above shows the three replicates (1-3, Left to Right) of the Nycote 99 Ecoshield, sample on day 28 of incubation after inoculation with pooled fungal spores. No fungal growth was observed on the test surfaces of the three replicates.

OBSERVATIONS

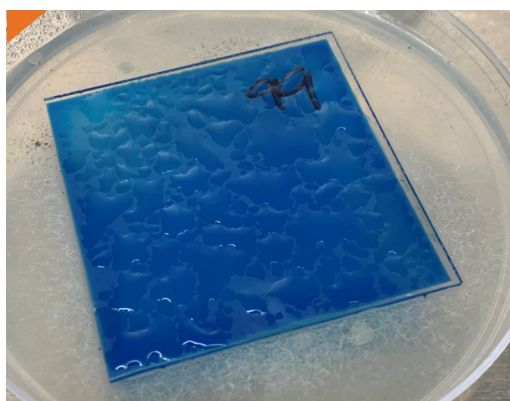
Study Photographs – Day 28



The photograph above shows a close-up of replicate 1 of the Nycote 99 Ecoshield sample on day 28 of incubation after inoculation with pooled fungal spores.



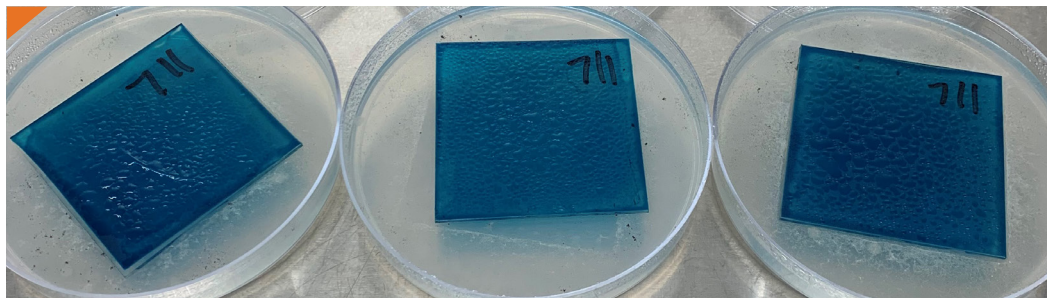
The photograph above shows a close-up of replicate 2 of the Nycote 99 Ecoshield sample on day 28 of incubation after inoculation with pooled fungal spores.



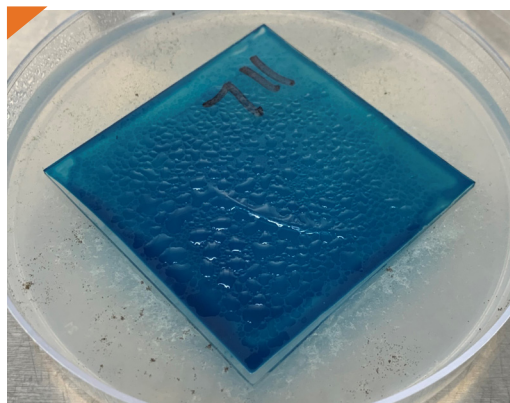
The photograph above shows a close-up of replicate 3 of the Nycote 99 Ecoshield sample on day 28 of incubation after inoculation with pooled fungal spores.

OBSERVATIONS

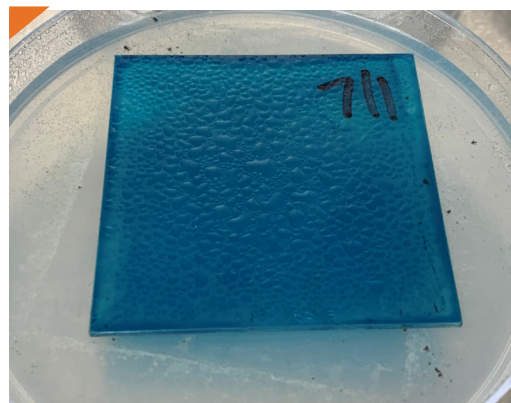
Study Photographs – Day 28



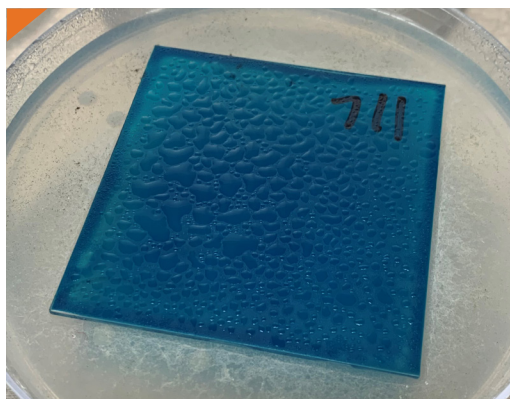
The photograph above shows the three replicates (1-3, Left to Right) of the Nycote 7-11 sample on day 28 of incubation after inoculation with pooled fungal spores. No fungal growth was observed on the test surfaces of the three replicates.



The photograph above shows a close-up of replicate 1 of the Nycote 7-11 sample on day 28 of incubation after inoculation with pooled fungal spores.



The photograph above shows a close-up of replicate 2 of the Nycote 7-11 sample on day 28 of incubation after inoculation with pooled fungal spores.



The photograph above shows a close-up of replicate 3 of the Nycote 7-11 sample on day 28 of incubation after inoculation with pooled fungal spores.

RESULTS

Control Results

Neutralizer: N/A
Growth Confirmation: Pure
Media Sterility: Sterile

Calculations

No calculations are made for this study.

Observations of growth on test substances is rated by the method according to the following table:

Score	Description
0	No Growth Detected on Surface of Sample
1	Traces of Growth Detected on Sample (< 10%)
2	Light Growth Detected on Sample (10%-30%)
3	Medium Growth Detected on Sample (30%-60%)
4	Heavy Growth Detected on Sample (60%-Complete)

Sample	Incubation Time and Growth Score per Replicate			
	Day 7	Day 14	Day 21	Day 28
Negative Control	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
Positive Control	4, 4, 4	4, 4, 4	4, 4, 4	4, 4, 4
Nycote 99	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
Nycote 7-11	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0