MICROCHEM LABORATORY

STUDY REPORT

Study Title

Ability of PURETi Group, LLC's Test Substance to Resist Fungal Attack

Test Method

ASTM International Method G21
Standard Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi

Study Identification Number

NG9871

Study Sponsor

Bikash Rajkarnikar
PURETi Group, LLC
R & D Laboratory
249 Research Drive, #2
Milford, CT 06460
(203) 451-4163
bikash@pureti.com

Test Facility

Microchem Laboratory
1304 W. Industrial Blvd
Round Rock, TX 78681
(512) 310-8378
Testing Conducted by: C. Craney

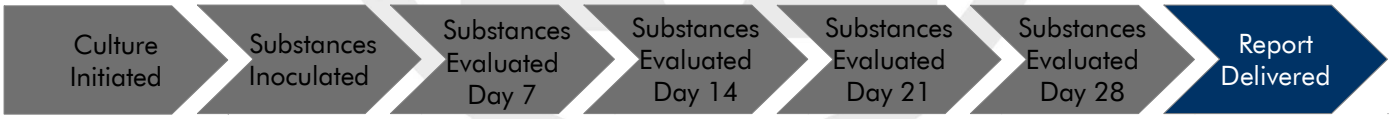
ASTM G21: General Information

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.

Laboratory Qualifications Specific to the ASTM G21

Microchem Laboratory began conducting the ASTM G21 test method in 2010. Since then, the laboratory has performed numerous ASTM G21 tests on a broad array of test substances, against method specific and non-method specific fungal species. The laboratory also has experience with regard to modifications of the ASTM G21 test method in order to accommodate specific customer requirements or test substance needs. Every ASTM G21 test at Microchem Laboratory is performed in a manner appropriate for the test substances submitted by the Study Sponsor, while maintaining the integrity of the study.

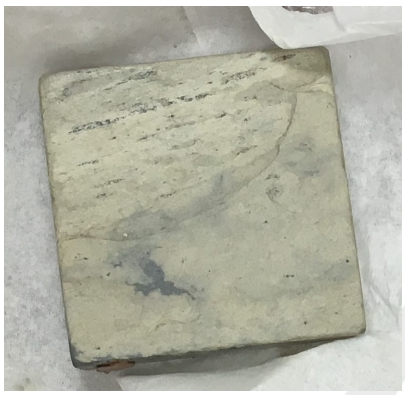
Study Timeline



02 JAN 2018 | 12 JAN 2018 | 19 JAN 2018 | 26 JAN 2018 | 02 FEB 2018 | 09 FEB 2018 | 14 FEB 2018

Test Substance Information

The test substance was received on 12 DEC 2018 and the following pictures were taken.



To the left: Photograph shows tested side of a Test Sample.

To the left: Photograph shows x marked untested side of a Test Sample.

Test Substances Received: PureTi treated cement samples

Test Substances arrived in dimensions that were optimal for the conduct of the study. Test substances were not cut down or altered for the conduct of this study.

Test Microorganism Information

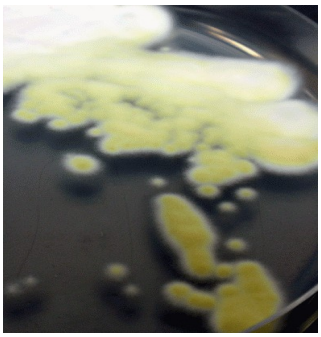
The test microorganisms selected for this test:

***Aspergillus brasiliensis* 9642**

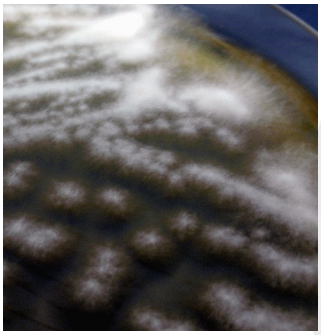
This fungi is a conidiophore, or a sexual spore generating aerobic fungus. *A. brasiliensis*, formerly listed as a strain of *A. niger*, is related to other *Aspergillus* species in that they produce spores which are highly resistant to chemical and environmental conditions. *A. brasiliensis* is commonly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

Test Microorganism Information

The test microorganisms selected for this test:



Penicillium funiculosum 11797
This fungi is a faculative 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. *P. funiculosum* is associated with rotting fruit and seeds and is commonly used in fungus resistance of articles.

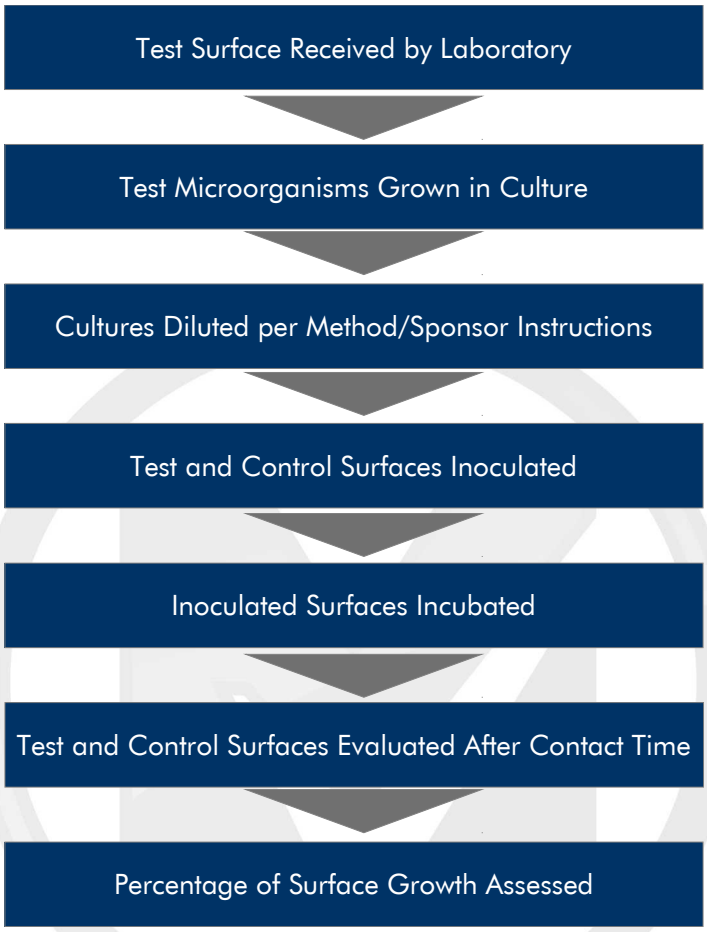


Chaetomium globosum 6205
This fungi is a saprotrophic species that is normally found in soil, air, and plant debris. *C. globosum* thrives is cellulose rich areas such as seeds, textiles, straw, and sacking. Typically prevalent in homes with water damaged areas, *C. globosum* is one of the more prevalent fungi encountered in household environments. This prevalence makes *C. globosum* a commonly used model for fungus resistance testing.

Trichoderma virens 9645
This fungi typically prevalent in soil and decayed wood. *T. virens* is often used in the agriculture industry as it beneficial to crop production and plant metabolism because of it's ability to produce antibiotics and parasitize other fungi. Because of it's widely accepted use, it is a recommended microorganism for fungi resistance testing of adhesives and lumber.

Aurobasidium 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 it's ability to prevail in numerous environments and it's ability to metabolize organic matter.

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.

Criteria for Scientific Defensibility of an ASTM G21 Study

For Microchem Laboratory to consider an ASTM G21 study to be scientifically defensible, the following criteria must be met:

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

Passing Criteria

ASTM does not specify a performance criteria, therefore it may be established by the Study Sponsor.

Testing Parameters used in this Study

Test Substance:	Cement	Test Substance Size:	2″x 2″
Control Substance:	Sterile Filter Paper	Control Substance Size:	2″x 2″
Replicates:	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 x 10^6 spores/ml	Test Plating Media:	Mineral Salts Agar
Observation Times:	7, 14, 21, 28 days	Contact Temp.:	30°C ± 2°C

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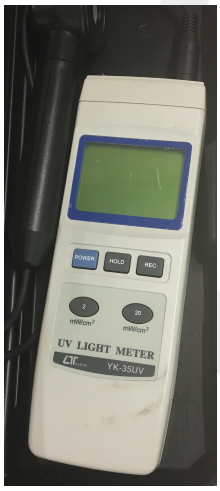
Study Modifications

The test samples were exposed to UV-A light for duration of 28 day incubation. A 15 watt Sylvania 18 inch T8 Black Light Fluorescent Tube was used. The study sponsor provided a light meter which was used to set-up the distance of the light source to the test and control samples that provided a UV-A intensity of 1mW per cm^2. The light meter was used at each 7 day observation point to confirm the light intensity was at 1mW per cm^2.

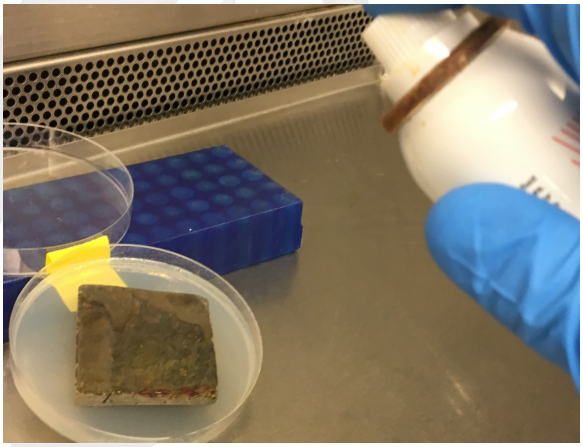
Study Notes

Positive Controls were run in parallel to the duration of testing without exposure to the UV-A light.

Study Photographs



Pictured above is the light meter used in this study to measure the light intensity of the UV-A light.



The photograph above shows inoculation of a test sample with the pooled fungal suspension via atomizer application.

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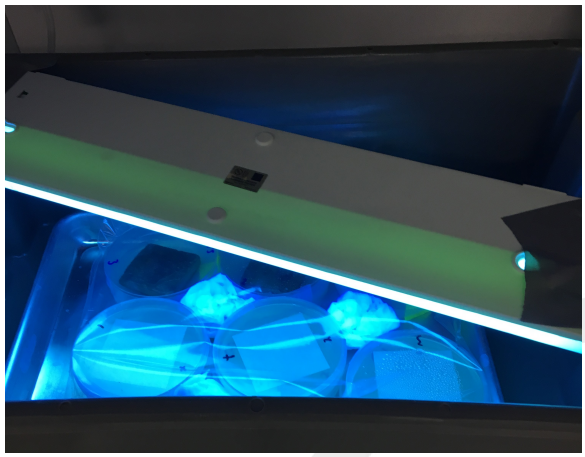
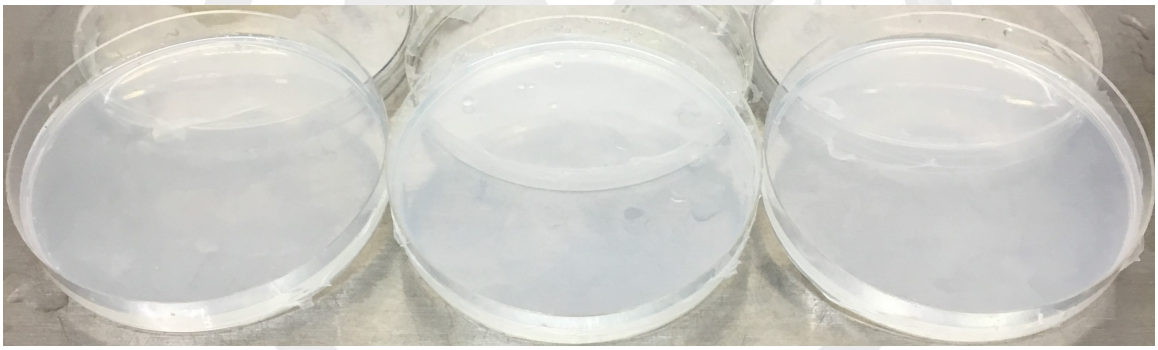
Study Photographs

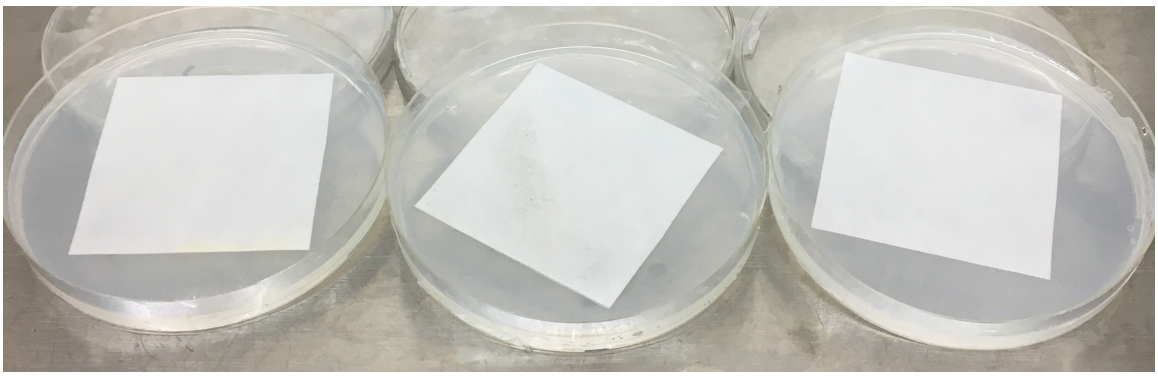


Pictured on the left is the study setup. Specifically, positice control and test substance exposed to UV-A light at an intensity of 1mW/cm^2.

Study Photographs – Day 28



Negative Controls: Mineral Salts Agar inoculated with pooled fungal spore suspension without filter paper.



Positive Controls: Mineral Salts Agar with filter paper inoculated with pooled fungal spore suspension and incubated with exposure to UV-A light.

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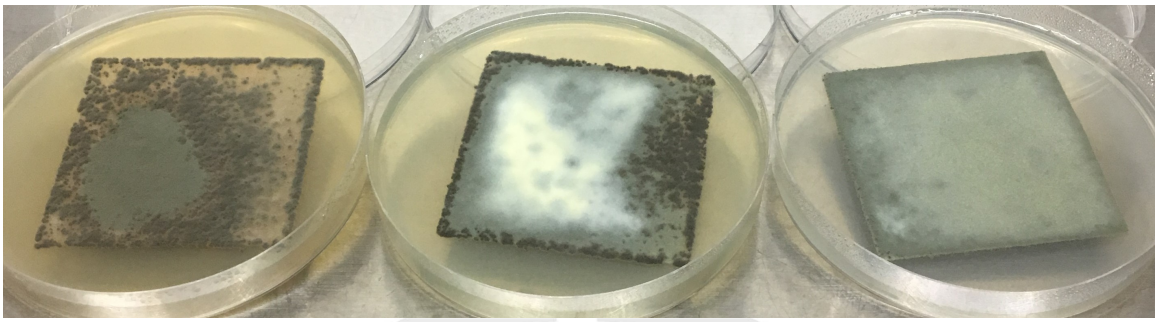
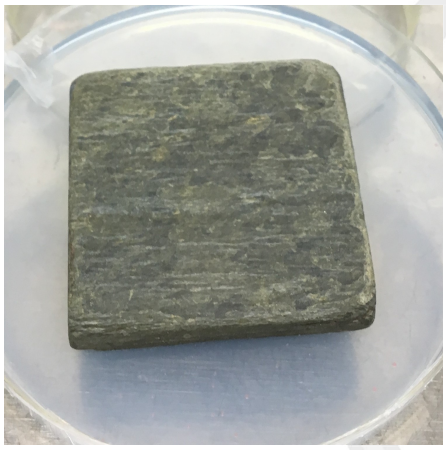
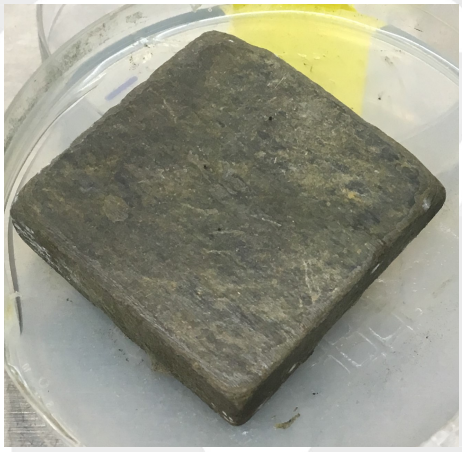
Study Photographs – Day 28



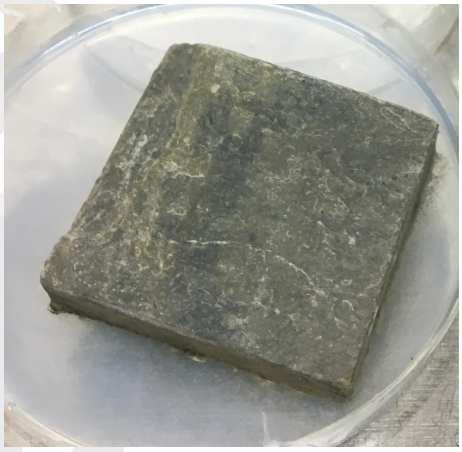
Positive Controls incubated without exposure to UV-A light.



Test Sample Replicate1



Test Sample Replicate 2



Test Sample Replicate 3

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Control Results

Neutralizer: Not Applicable
Media Sterility: Confirmed Sterile
Growth Confirmation: Growth Confirmed

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)

Results of the Study

Sample	Incubation Time and Growth Score			
	Day 7	Day 14	Day 21	Day 28
Microchem Positive Controls	1 / 1 / 1	1 / 2 / 1	2 / 3 / 2	1 / 2 / 1
Microchem Positive Controls (without UV exposure)	4 / 4 / 4	4 / 4 / 4	4 / 4 / 4	4 / 4 / 4
Microchem Negative Controls	0 / 0 / 0	0 / 0 / 0	0 / 0 / 0	0 / 0 / 0
Test Sample	0 / 0 / 0	0 / 0 / 0	0 / 0 / 0	0 / 0 / 0

The three numbers listed per sample per day represent the growth score for each sample replicate.

The results of this study apply to the tested substances(s) only. Extrapolation of findings to related materials is the responsibility of the Sponsor.