# Fungal Surface Measurements: Water Contact Angles

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## Abstract

Fungal surface properties have been implicated as one of the main factors affecting fungal colonization and adhesion to plant surfaces. Characterization of fungal surfaces through hydrophobic measurements is important for understanding its function. Water contact angles are a direct and simple approach for characterization of fungal surface hydrophobicity. The objective of this study was to evaluate if utilization of undisturbed fungal cultures coupled with versatile image analysis allow for more accurate contact angle measurements. Fungal cultures were grown on agar slide media and contact angles were measured utilizing a modified microscope and digital camera setup, with Low Bond Axisymmetric Drop Shape Analysis Model (LB_ADSA) for contact angle determination. Fungal strains were categorized into hydrophobic, hydrophilic and a newly defined hydroamphiphilic class containing fungi taxa with changing hydrophobicity.

## Introduction

Fungal surface hydrophobicity is one of the surface properties that influence microbial interactions at the fungal interface (Van Loosdrecht *et al.* 1987; Gannon *et al*. 1991). Key functions include supporting internal turgor pressure, appressorium formation in the cells and also providing structure, shape, adhesion and aggregation (Dague *et al.* 2007). Determination of structural and physical properties of microbial surfaces provides further understanding of their functions. Current methods employed to characterize/assess microbial cell surface hydrophobicity include binding to aliphatic acids, hydrocarbons, microsphere assay, colony imprints, dielectric permittivity, hydrophobic interaction chromatography, imprint assay, rolling drop assay, salt aggregation test and two phase partitioning (Doyle 2000). These methods are subjected to criticism as they are indirect methods to quantify hydrophobicity of the microbial cell surfaces. Time, temperature, pH, ionic strength, and interaction species concentration are factors that affect these methods especially when dealing with adhesion techniques (Ofek and Doyle 1994). Some fungi exhibit persistent surface hydrophobicity which makes these techniques applicable; however others exhibit changing hydrophobicity that results in degradation of surface hydrophobicity with manipulations.

The objective of this study was to develop a simple and rapid method for assessing water contact angles on fungal cultures grown on slide media using a modified microscope. With this

method, we can measure degree of hydrophobicity through contact angles in an effort to determine fungal hydrophobic properties. To assess if this method is able to quantify fungal surface property successfully, we compared our results to fungal strains analyzed in previous studies.

## Materials and methods

Cultures of *Alternaria* sp. (Kunze) Wiltshire SMCD 2122, *Penicillium aurantiogriseum* Dierckx SMCD 2151, *Cladosporium cladosporioides* (Fresen.) G.A. de Vries SMCD 2128, *Cladosporium minourae* Iwatsu SMCD 2130, *Suillus tomentosus* (Kauffman) Singer UAMH 9089/SMCD 2263, *Cenococcum genophilium* Fr. (Strain UAMH 5512) SMCD 2264, *Trichoderma harzianum* Rifai SMCD 2166, *Mortierella hyalina* (Harz), W. Gams SMCD 2145, *Laccaria laccata* Scop & Cooke) UAMH 10033 /SMCD 2265 and *Laccaria trichodermophora* G.M. Muell SMCD 2267 were obtained from the Saskatchewan Microbial Collection and Database (SMCD) and the University of Alberta Microfungus Collection and Herbarium (UAMH). Fungal mycelial plugs from an active growing culture were inoculated on the center of the slide media and incubated in sterile Petri plates. Inoculated slides were then placed on sample stage. Images of 5 to 6 water droplets of 10µL were taken from one edge of the slide to the other edge in two replicates. Additional measurements on the PDA slide were preformed to assess reproducibility of contact angles of the strains. Contact angle images were obtained by using a modified stereo microscope with a horizontal view path coupled with Nikon Cool Pix 8400 camera, high resolutions images were analyzed using the Low Bond Axisymmetric Drop Shape Analysis Model of Drop Shape Analysis (LB_ADSA) plug in which is available online for free at http://bigwww.epfl.ch/demo/dropanalysis/ (Stalder et al., 2006).

## Results and Discussion

**Table 1.** Comparison of Contact Angles Obtained from the Modified Microscope Approach on Fungal Slide Cultures with Similar Fungal Species from Literature.

| Fungus Culture | Time | PDA† | MMN‡ | Fungal Surface Classification | Literature Fungal surface classification |
|---|---|---|---|---|---|
| | (Days) | $\theta_w$ | $\theta_w$ | | |
| *Cladosporium cladosporioides* | 10 | 142 ° ± 1 (106±2) § | 135 ° ± 1 (100±3) § | Hydrophobic | *Cladiosporium sp.* Hydrophobic (Smits et al., 2003) |
| *Cladosporium minourae* | 10 | 142 ° ± 5 (106±2) § | 141 ° ± 1 (100±3) § | Hydrophobic | *Cladiosporium sp.* Hydrophobic (Smits et al., 2003) |
| *Penicillium aurantiogriseum* | 10 | 128 ° ± 1 | 124 ° ± 1 | Hydrophobic | n/a |
| *Alternaria sp.* | 5 | 122 ° ± 1 | 124 ° ± 2 | Hydrophobic | n/a |
| *Suillus tomentosus* | 30 | 89 ° - 134 ° | 96 ° - 118 ° | Hydroamphiphilic | *Suillus tomentosus* Hydrophobic (Unestam, 1991) ¶ |
| *Trichoderma harzianum* | 3 | 61 ° - 117 ° (27±3) § | 43 ° - 108 ° (25±3) § | Hydroamphiphilic | *Trichoderma harzianum* Hydrophilic (Smits et al., 2003) |
| *Cenococcum geophilum* | 30 | 68 ° -133 ° | 74 ° - 81 ° | Hydroamphiphilic | *Cenococcum geophilum* Hydrophilic (Unestam and Sun, 1995 ) ¶ |
| *Laccaria laccata* | 30 | 0 ° | 0 ° | Hydrophilic | *Laccaria laccata* Hydrophilic (Unestam and Sun, 1995 ) ¶ |
| *Laccaria trichodermophora* | 21 | 0 ° | 53 ° - 82 ° | Hydrophilic | *Laccaria sp*. Hydrophilic (Unestam and Sun, 1995) ¶ |
| *Mortierella hyalina* | 7 | 59 ° ± 1 | 31 ° - 51 ° | Hydrophilic | n/a |

† Potato Dextrose Agar Media
‡Melin Norkrans Media
§ Referenced Values
¶ No measured contact angle

*Alternaria* sp.*, C. cladosporioides, C. minourae and P. aurantiogriseum* illustrated hydrophobic surface properties due to contact angles measurement > $90^o$ (Table 1). Water contact angles were evaluated from the point of inoculation to the end of the slide to assess how hydrophobicity changes with time. We observed only a slight difference in the contact angles assessed with the four hydrophobic strains as the standard deviation was low (Table 1). *Cladosporium* strains showed typically higher contact angle values, but were still within the same hydrophobicity classes (Table 1).

*M. hyalina, L. laccata* and *L. trichodermophora* had water contact angles readings < $90^o$ and thus were classified as hydrophilic (Table 1). *L. trichodermophora* and *M. hyalina* did however show an increase in the contact angle as the placed droplets approached the area of inoculation. This resulted in a large range of contact angle values (Table 1.). The range of the values may be attributed to the growth of a second layer of hyphae that caused the increased contact angles (Smits *et al.* 2003). Therefore, defining these fungi as hydrophilic might not be correct because of the change in fungal surface hydrophobicity at different growth stages.

*S. tomentosus* and *T. harzianum* had a contact angle > $90^o$ at the point of inoculation, but contact angles became smaller as growth further away from the point of inoculation. At the end of growth, contact angles were < $90^o$ on the slide. Therefore, these two strains showed hydrophobic characteristics at the point of inoculation, but growth further away from the point of inoculation showed hydrophilic characteristics. However, *S. tomentosus* was classified as hydrophobic by Unestam (1991) and *T. harzianum* was described as hydrophilic by Smits et al. (2003). Unestam (1991) speculated that hydrophobic fungus must also have hydrophilic structures to aid in uptake of water and result in its hydrophilic characteristics. As *T. harzianum* aged in culture it started to produce hydrophobic spores that further affected the water contact angle (Smits *et al*. 2003). This behaviour is important when working with fungi surface hydrophobicity and because of its implications we proposed a new *Hydro (Greek: water) – amphiphilic (Greek: loving both)* class containing fungal taxa with changing hydrophobicity. With the additional of a new class, emerging hydrophobicity expression patterns with in the same species can be quantified more accurately. This provides more insights into the functional significance of fungal surface properties.

Although previous methods had standardized the conditions of measurements, they seem to manipulate the hydrophobic property of the fungus. Growth conditions have a major impact on contact angle measurements and as fungi age or reach maturity, it starts to secrete hydrophobic moieties in order to obtain adaptable structures. Our proposed method allowed for actively growing fungal cultures to be assessed for their hydrophobic property. With this method, we may be able to evaluate the relationship between nutrient conditions and hydrophobic surface property. A major benefit of this direct method is free of fungal manipulation. Fungal surface hydrophobicity is a dynamic property, and has shown to be caused by a variety of factors (Wösten and de Vocht, 2000). Most of research proposed the production of hydrophobic proteins called hydrophobins that contributed to the hydrophobic cell surface property (Wessel *et al.* 1991; Wessel 1992). An inhibition of hydrophobin productions through gene deletions and knockout mutations causes a shift in hydrophobic surface property (Teertstra *et al*. 2006). Further research should examine these implications and factors that may affect the expression of these genes.

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