Elastic Properties of *Aspergillus nidulans* Studied with Atomic Force Microscopy

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ABSTRACT

In this study an atomic force microscope (AFM) was used as a nanoindentor to measure cell wall mechanical properties of the model fungus *Aspergillus nidulans* in both hyphal[5] and spore[6] forms. For fungal hyphae the nanoindentation data were compared to finite element analysis (FEMLAB v3.0, Burlington MA) to simulate AFM indentation because no suitable analytical models were available. The elastic modulus of wild type hyphae grown in complete medium was determined to be 110 ± 10 MPa. This decreased to 64 ± 4 MPa when grown in 0.6 M KCl, implying growth medium osmotic conditions have significant effects on cell wall elasticity. These values are comparable with other microbial systems (e.g., yeast and bacteria). Comparisons with a mutant strain (Δ*csmA*) further indicate that the differences in mechanical properties may be dependent on varying molecular structure of hyphal cell walls as opposed to wall composition. AFM images showed characteristic "rodlet" protein structures covering spore surface. This feature can be removed by sonication. Nanoindentation measurements on the spores showed that rodlet-covered spores had surface stiffness of 110 ± 10 N/m and cell wall elastic modulus of 6.6 ± 0.4 GPa, both lower than those of rodlet-free spores. These results imply the rodlet layer is significantly softer than the underlying portion of the cell wall.

Keywords: afm, fungi, elastic modulus, indent, spores

Filamentous fungi are used to produce an exceptionally wide range of products [1-3], comprising approximately half of the world's pharmaceutical and biotechnology market [4]. Most of these products are produced in fermentations where productivity is strongly influenced by hyphal breakage or fragmentation. Fragmentation in turn, depends on the mechanical properties of the fungal hyphae, in particular the elastic modulus (measure of stiffness). Unfortunately, relatively little information is available on the mechanical properties of any filamentous microbe, as the equipment used is typically difficult to operate and not commercially available [5, 6]. In contrast, atomic force microscopy (AFM) is relatively straight forward and commercially available instruments can be used to measure mechanical properties of biological material [7, 8]. This is generally accomplished by monitoring the deflection of an AFM cantilever, as its tip deforms a sample. "Force curves" are collected, and the resulting data is fit to models describing the mechanics of contact, allowing determination of mechanical properties.

The goal in this study was to develop methods to determine the elastic moduli (*E*; measure of "stiffness") of filamentous fungus *A. nidulans*, in forms of both hyphae and spores, using an AFM approach. To accomplish this, both wild type *A. nidulans* and a mutant strain lacking an important cell wall chitin synthase gene (Δ*csmA*) were grown in shake flasks. During growth, hyphae were harvested and immobilized on polylysine-coated cover slips. Subsequent AFM testing was carried out in aqueous buffer. As shown in Fig. 1, hyphae were considered to be a cylindrical shells with an internal pressure, deformed by an externalnormal force. A representative AFM force-displacement curve is shown in Fig. 2. The contact region shows a linear response, and the slope *m* can be used to calculate cell wall spring constant k_w according to the following equation:

$$k_w = \frac{k_c \cdot m}{1 - m} \qquad (1)$$

where k_c is cantilever spring constant. A shell model was defined and a finite element modeling was performed (FEMLAB v3.0, Burlington, MA) and validated (using analytical solutions). This allowed us to use k_w, along with cell dimensions *R* and *h* (determined via electron microscopy) to calculate elastic modulus *E*. We note wild type hyphae have significantly lower elastic modulus (from 100 to 59 MPa) when grown in the presence of 0.6 M potassium chloride, implying high molarity stress has a strong effect on cell wall elasticity.

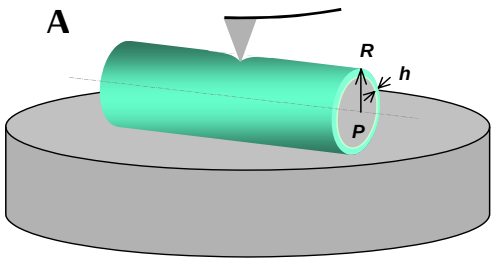

Figure 1 Schematic illustrations of indentation experiment. The hypha is submerged in PBS buffer and immobilized on a polylysine-coated cover glass. The cantilever tip exerts a normal force and deforms the hyphal wall. The hypha is idealized as an infinitely long, circular cylindrical shell with outer radius *R* and wall thickness *h*, inflated with internal pressure *P*.

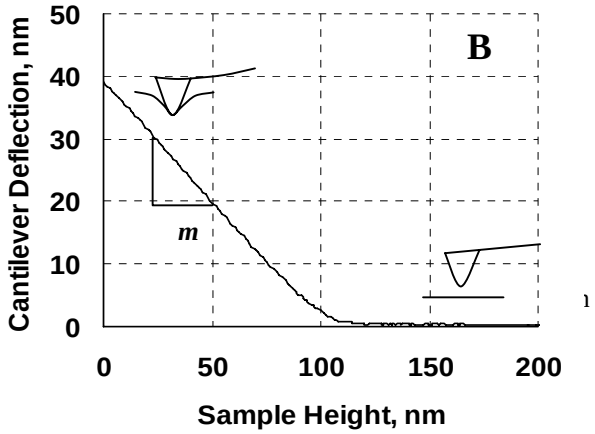

Figure 2. A force curve taken on a wild type *A. nidulans* hypha. The cantilever deflection is zero in the noncontact region. In the sloped contact region the cantilever tip deforms the hyphal wall. The curve is linear with a slope *m* in the contact region. The spring constant of the cantilever is 0.47 N/m

surface topography and micromechanical properties of *A. nidulans* spores grown on potato dextrose agar plates. "Untreated" spores were scratched from sporulated mycelial mats and tapped over a poly-L-lysine coated coverslip which was then used for atomic force microscopy. Alternatively, "sonicated" spores were obtained from agar plates, suspended in sterile deionized water and subjected to sonication before being subjected to AFM testing. To assess the influence of proteins covering the spore surface, wild type spores were compared with spores from isogenic *rodA*$^+$ and *rodA*$^-$ strains. Tapping mode AFM images of wild type and *rodA*$^+$ spores in air showed characteristic "rodlet" protein structures covering a granular shaped spore surface (Fig. 3). In comparison, *rodA*$^-$ spores were rodlet free but showed a similar granular surface structure as the wild type and *rodA*$^+$. Rodlets were removed

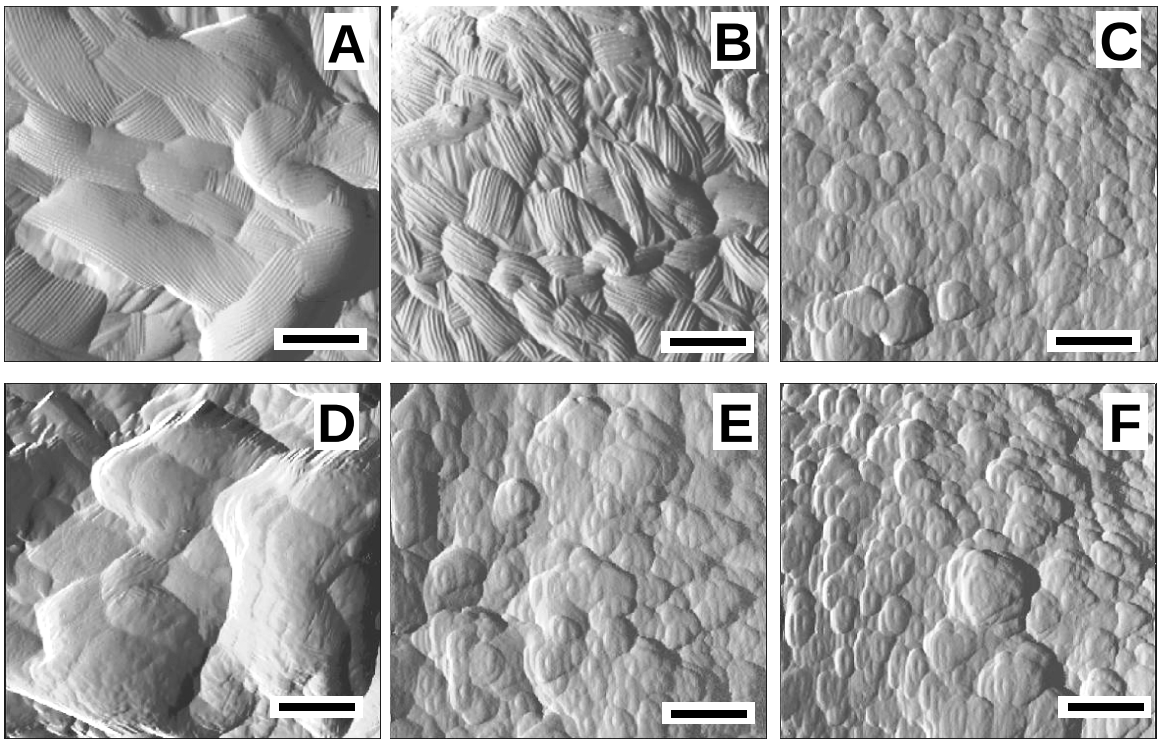

Figure 3. Images generated using AFM in tapping mode of the surface morphology of (A-C) "untreated" and (D-F) "sonicated" *A. nidulans* spores; (A and D) wild type, (B and E) *rodA*$^+$, and (C and F) *rodA*$^-$. Untreated wild type (A) and *rodA*$^+$ (B) spores have rough surfaces with a rodlet layer on each, while *rodA*$^-$ spores (C) exhibited granular materials on a rodlet free surface. All spores were observed to lose nearly all rodlets after sonication (D-F), uncovering the underlying region of granular materials. Bar length: 0.2 m.

from *rodA*$^+$ spores by sonication, uncovering the underlying granular layer. Both rodlet-covered and rodlet-free spores were subjected to nanoindentation measurements, conducted in air, which showed the stiffness to be 110 ± 10, 120 ± 10 and 300 ± 20 N/m, and elastic modulus to be 6.6 ± 0.4, 7.0 ± 0.7 and 22 ± 2 GPa, for wild type, *rodA*$^+$ and *rodA*$^-$, respectively. These results imply the rodlet layer is significantly softer than the underlying portion of the cell wall.

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