Atomic resolution etching of the external proteinaceous protective membrane of *Ulocladium* and *Aspergillus 1-4* spores in vivo.

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Abstract

High resolution AFM images of immobilized *Ulocladium* sp and Aspergillus 4-1 sp cultures on silicon wafers reveal cease of biological activity after laser illumination at 157 nm.

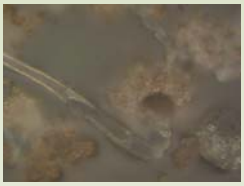
Laser light dissociates the external multilayered proteinacious membrane of the spores reducing their thickness to a critical value prior to cell explosion due to the high internal pressure of the nucleus.

The use of 157 nm laser is an effective and controllable method for stopping biological activity of *Ulocladium sp and Aspergillus 4-1 spores* in artifacts.

Experimental

❖ *Ulocladium sp* spores were collected from mycelia cultures grown in agar, the aggregation containing 1,2x105 spores/ml with 20 % /hour rate.
❖ *Aspergillus 4-1* filamentous fungus, was isolated in pure culture from salted lake Baia Baciului (Romania) environmental conditions which are too extreme for survival of other organisms except halophylic bacteria.
❖ *Aspergillus sp 4-1* strain was grown at 28^{0}C on Petri dishes with glucose-yeast extract-agar in salt water from the lake as nutrient media.

Monolayer aggregation of ulocladium sp spores grown on Si substrate. The average length of the spores was 10 μm.



Aspergillus sp.4-1 fungi . Conidiophore with vesicle and chains of spores.

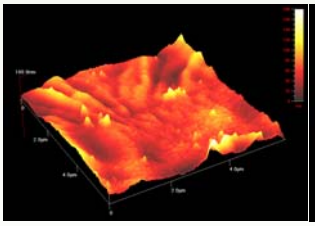
❖The spores were de-hydrated and then they were illuminated with a number of laser pulses of known fluence at 157 nm.

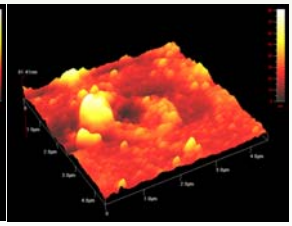
❖The exposed surface was investigated by Optical and AFM microscope

The experimental set up consists of the laser apparatus at 157nm which is the VUV exposure tool, the focusing optics and the high precision translation stage where the samples were placed. The optical paths, were flushed with high purity nitrogen gas to provide VUV transparency, as oxygen absorbs strongly below 185 nm. The distance between the last CaF2 lens of the focusing optics and the samples was 1 cm and samples were irradiated either in vacuum or in N2 ambient temperature.

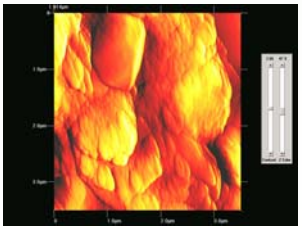
Results

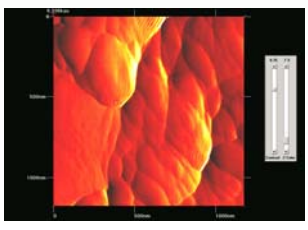
Prior to irradiation



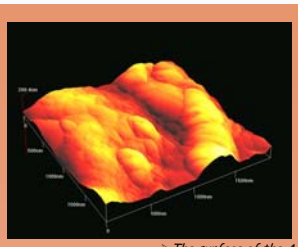


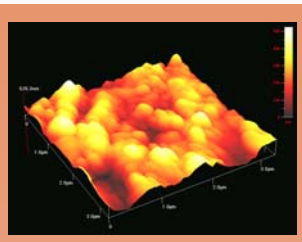
AFM images of Ulocladium sp spores.
➢ *Large conic holes 200-500 nm wide on the top of the surface which become narrower towards the centre.*
➢ *Nubs 100 nm long.*
➢ *Regularly spaced nods or rod let patterns 10-20 nm long.*
➢ *Around the hole there are spaced nods and rodlets.*
➢ *The spore wall consisted of two zones and the holes are discontinuities which connect the two layers*





AFM images of Aspergillus 4-1 sp spores.
➢ *The surface consists of granular domains with dimensions 100-200 nm.*
➢ *Higher resolution images (phase mode) reveals the presence of rodlet-like structures in the surface of the granular domains. The rodlets are approximately 20nm wide and a few hundred nm long, (left image)*





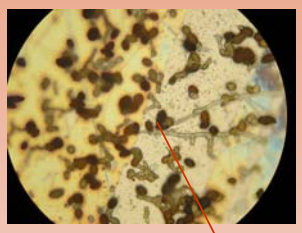
➢ *The surface of the Aspergillus 4-1 sp*
➢ *Part of the conidiophore can be seen on the righ (left image)*

After irradiation

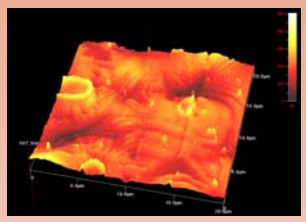
Ulocladium sp spores

❖The population of a monolayer culture was successfully destroyed following illumination with 150 laser pulses at the fluence of 1mJ/cm2 per pulse.

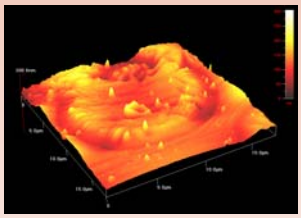
❖A thin layer of 0.3 nm was removed on the average from the external membrane per pulse, and a thin layer of 45 nm had to be removed from the external membrane before cell explosion.



Exposed at 157nm 1mJ/cm^{2} per pulse



➢ *AFM image of one spore consisted of two cells following illumination at 157 nm.*
➢ *The spore was exploded after illumination indicating that the nucleus material is under high pressure.*



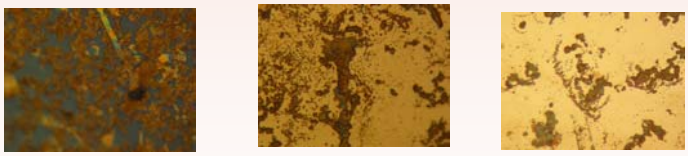
➢ *AFM image of two connected spores.*
➢ *They seem to be empty from the nucleus material.*

Aspergillus sp spores

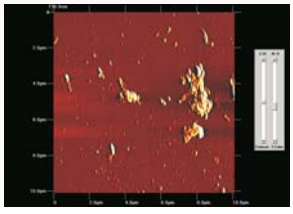
❖Experiments were done with laser fluence of 0.1, 0.5 and 1 mJ/cm2 per pulse. For all the three cases changes were observed for a total dose of ~ 200 mJ/cm2 a fact, which indicated that the ablative rate of the external proteinacious membrane of fungus is proportional with the number of photons falling on its surface.

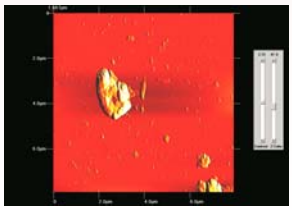
❖The ablative rate was found to be ~ 0.3 nm for a laser fluence of ~1 mJ/cm2.The ablative rate was increased to ~ 1 nm for a laser fluence of ~1 mJ/cm2 when the experiment was done at high vacuum ~ 10-6 mbar.

❖After the laser treatment no rodlet-like structure has been observed in the surface of the destroyed spores.



Monolayer aggregation of Aspergillus sp spores grown on Si substrate . The total dose of the laser is increased from the left to the right.





➢*AFM images of the exposed Aspergillus 4-1 spores at 157nm.*
➢ *Parts of spores with dimensions approximately 100-200nm, spread in the area of destroyed spores can be seen.*
➢*A destroyed spore can be seen and a part of about 250nm thick has been removed from the center of the spore.*