

Observations on OMV Release and Cyanobacterial-Bacterial Biofilm Formation within *Azolla microphylla* Using AFM

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Introduction

Azolla is a small floating water fern and the only plant known to associate symbiotically with cyanobacteria and bacteria within leaf cavities and the megasporocarps of the fern. Our previous work (see in ref) showed that outer membrane vesicles (OMVs) were released from the cyanobacteria and were proposed to be involved in the formation of both the envelope surrounding the cyanobacteria when in leaf cavities, and the cyanobacteria-endobacteria biofilm in the sporocarps.

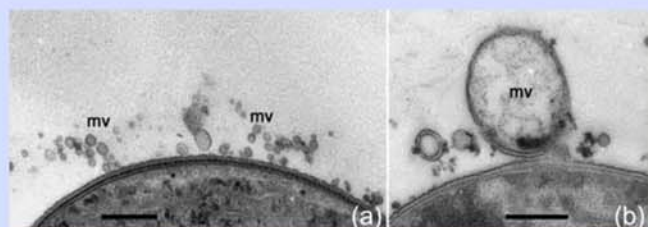


Fig.1 Transmission electron microscopy micrographs illustrating the release of cyanobacterial membrane vesicles. (a) Numerous membrane vesicles (mv) are seen being released from a cyanobacterial cell; (b) Close-up of several small membrane vesicles. Bars, 10 nm (a); 0.5 μm (b). Reprinted from ref.

Results

In present study, atomic force microscopy (AFM) was for the first time used to demonstrate that not only the cyanobacteria but also the bacteria occupying the *Azolla* leaf cavities produced abundant OMVs. The OMVs were discharged from cyanobacterial and bacterial cells and ranged from 50 to 500 nm in diameter. However the OMVs released by the bacteria were generally much smaller. OMVs from the cyanobacteria may have merged with each other, grown in size and subsequently formed the envelope surrounding the akinets (cyanobacterial spores) within the sporocarps and cyanobacterial and bacterial OMVs may have fused to form a film-like structure which enwrapped the cyanobacterial-bacterial community within mature leaf cavities and the inducium chamber of the megasporocarps.

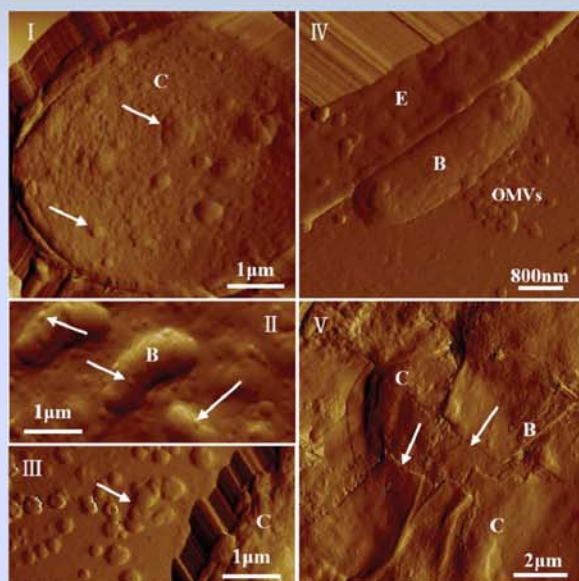


Fig.2 OMVs release and biofilms formation within *Azolla*-cyanobacterial association (C:cyanobacterial cell; B:bacteria; E: the envelop of the cyanobacterial cell; OMVs: outer membrane vesicles; Arrows: OMV or biofilm-like structure).

More Observation on Cyano and bacterium by AFM

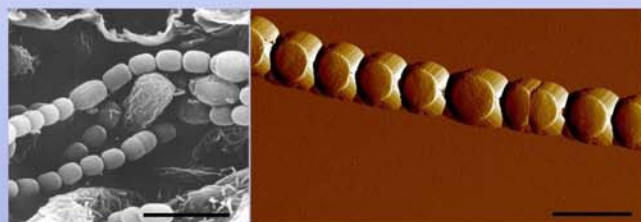


Fig.3 Cyanobacteria observed by SEM and AFM. Bars, 10 μm .

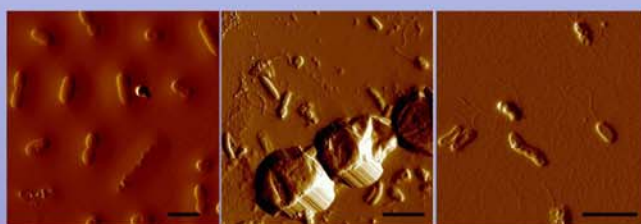


Fig.4 Cyanobacteria Co-exist bacterium observed by AFM. Bars, 3 μm .

Reference Zheng et al. , 2009, *New Phytologist*, 181: 53-61

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