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Detection of Hydroxyapatite Associated with Xylella Fastidiosa Biofilms: Evidence of Mineralization and Potential Role in Xylem Vessel Occlusion

Ayron Andrey Da Silva Lima [1], Duber Marcel Murillo Munar [1], Mariana De Souza E Silva [2], Antonio Augusto G. Von Zuben [1], Alessandra Alves De Souza [2], Breno Leite [1], Mônica Alonso Cotta [1]

[1] University of Campinas, Campinas, Brazil

[2] Citrus Research Center "Sylvio Moreira", Cordeirópolis, Brazil

Xylella fastidiosa (Xf) is a Gram-negative bacterium that poses a significant threat to global agriculture. In Europe, it causes Olive Quick Decline Syndrome, leading to olive tree death, while in Brazil, it negatively impacts citrus farming, causing citrus variegated chlorosis. The disease mechanism involves several factors, including interactions between Xf cells and surfaces, the influence of divalent ions on adhesion and biofilm formation, the composition of xylem components, and xylem pH. To understand how these parameters affect Xf biofilm development, we developed a new formulation of XFM, a medium for Xf growth. We modified XFM by adding phosphate buffer, CaCl2, and reduced glutathione, using it to culture Xf on silicon and gold surfaces at three different pH levels (6, 7, and 8) for 24 hours. The same protocol was applied to control samples, replacing XFM with Periwinkle wilt (PW) medium. Results showed large biofilm coverage on both surfaces with modified XFM, while PW cultures showed mostly single cells. The largest biofilm was observed at pH 6, linked to higher Ca2+ availability. For the first time, hydroxyapatite was detected in Xf biofilm by X-ray diffraction, revealing the beginning of a calcification. In conclusion, the results indicate that plant vessels become occluded not just by cells and extracellular polymeric substances, but also by mineral deposits.