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EXPLORING THE POTENTIAL OF *Pseudomonas soli* AS A BIOCONTROL AGENT

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The genus *Pseudomonas* includes many bacteria known for their metabolic versatility and biocontrol properties. In a previous study, we found that the cell-free supernatant produced by strain VMAP1 (CFS-AP1) significantly reduced the severity of bacterial spot, caused by *Xanthomonas vesicatoria* (Xv) on tomato plants. After conducting whole-genome sequencing of VMAP1 and subsequent analysis, we identified it as *Pseudomonas soli*, a species about which there is limited information. VMAP1 is the only known *P. soli* strain with demonstrated biocontrol properties. Our previous results showed that although CFS-AP1 did not inhibit growth of Xv in vitro, it disrupted its biofilm-forming ability. However, the molecular mechanisms by which CFS-AP1 interferes with Xv biofilm formation and acts as a biocontrol agent remain unknown. Therefore, we decided to explore the biocontrol potential of VMAP1, both genotypically and phenotypically. First, we searched VMAP1 genome for genes encoding metabolites with biocontrol activity. Using bioinformatic tools, we identified biosynthetic gene clusters that encode metabolites synthesized via both ribosomal and non-ribosomal pathways: hydrogen cyanide (HCN), pyocin R2, pseudopyronines, and xantholysin. We also found genes encoding the two-component GacS/GacA system and the type VI secretion system, both of which are closely associated with biocontrol properties in *Pseudomonas*. In vitro assays confirmed that VMAP1 produces HCN, but volatile compounds produced by this bacterium did not inhibit the growth of Xv. On the other hand, we conducted in vitro assays to determine the effect of CFS-AP1 on factors involved in Xv biofilm formation, such as exopolysaccharide (EPS) production and bacterial motility. CFS-AP1 did not affect EPS production in Xv; however, it impacted swarming, swimming and twitching. We also attempted to identify the metabolites in CFS-

AP1 that might be responsible for the observed biocontrol and anti-biofilm activities. One such metabolite is the cyclic lipopeptide xantholysin (congeners A, B, and C), which synthesis by VMAP1 was confirmed in vitro by electrospray ionisation mass spectrometry. Xantholysin has been shown to have antibacterial activity against various *Xanthomonas* spp., but its effect against Xv has yet to be proven. There is also no evidence that xantholysin exhibiting anti-biofilm activity or triggering defense responses in plants. Thus, we decided to purify xantholysin and test its activity on Xv. Purification of xantholysin is currently in progress. Electron microscopy and nanoparticle tracking analysis showed that VMAP1 produces outer membrane vesicles (OMVs). We are currently analyzing whether these OMVs transport xantholysin or other metabolites with biocontrol activity. Finally, we observed that the CFS-AP1 induce the stomata closure in tomato plants. These results thus far offer valuable insight into the possible biological control mechanisms employed by VMAP1, particularly against Xv.

Palabras clave: *Pseudomonas soli* – Biocontrol – *Xanthomonas vesicatoria* - Anti-biofilm