

The spectrum and suppression of antiviral resistance in bacterial isolates from the *Ralstonia solanacearum* complex (CRS).

Guilherme Lage Guimarães^{1*}, Vanessa Sessa Dian², Alicia Dutra de Oliveira Moreira³, André da Silva Xavier⁴.

¹ Laboratório de Biotecnologia Agrícola e Ambiental, Universidade Federal do Espírito Santo. Alto Universitário, s/nº, Guararema, 29500-000, Alegre-ES, Brasil.

² Departamento de Agronomia, Universidade Federal do Espírito Santo. Alto Universitário, s/nº, Guararema, 29500-000, Alegre-ES, Brasil.

* Corresponding author. E-mail: guilherme.15.lage@gmail.com

The *Ralstonia solanacearum* complex (RSC) comprises a group of Gram-negative soil-dwelling bacteria with a wide global distribution and extensive genetic diversity. Species within the RSC are among the major phytopathogens affecting economically important crops such as tomatoes, tobacco, bananas, and potatoes, causing bacterial wilt by obstructing vascular tissues. With the aim of proposing innovative management strategies that generate low environmental impact, given the lack of effective methods to ensure disease-free or low-incidence cultivation, the present research aimed to develop a viral cocktail to elucidate the suppressive potential in the emergence of *R. solanacearum* lines insensitive to bacteriophages (BIMs), with the intention of supporting antiviral resistance management and strengthening future phage therapy proposals. Through an overlay assay, BIMs were obtained after bacterial isolates of *R. solanacearum* and *R. pseudosolanacearum* were challenged with different viral isolates, both of which were prospectively collected in the state of Espírito Santo. The bacterial species were inoculated with each virus individually and in combination, forming a cocktail. The BIMs were also subjected to a Spot-test assay to investigate whether there would be an overlap in the resistance profile of these bacterial isolates when inoculated with different viral isolates. In this assay, each isolated BIM was challenged again with each of the viral isolates individually and with the viral cocktail to assess the resistance spectrum. To understand the multiple resistance profile on BIMs, genetic material from each of the bacteriophage was extracted for molecular and bioinformatic analyses. The genomes of the bacteriophage isolates were sequenced and analyzed on bioinformatics platforms. The results obtained show that bacterial isolates of the same species exhibited biological and genomic differences and that each of the BIMs exhibits multiple resistance when challenged with other *Ralstonia* phages. It was also found that the bacteriophage isolates share extensive genomic similarity, with an intergenomic similarity rate exceeding 95% among the phages, corroborating the multiple antiviral resistance of the BIMs. Knowledge of antiviral mechanisms in bacterial populations is a key component in the biocontrol of phytopathogenic microorganisms through phage therapy, contributing to the development of new strategies to control bacterial resistance phenotypes with a view to the success of phage therapy.

Key words: *Ralstonia solanacearum*; Phage therapy; BIMs; Bacteriophages; Similarity; Genome.

Espectro e supressão de resistência antiviral em isolados de mutantes insensíveis à bacteriófagos (BIMs) do Complexo *Ralstonia solanacearum*.

O complexo *Ralstonia solanacearum* é um grupo de bactérias Gram-negativas que habitam o solo e afetam cultivos como tomate, tabaco, banana e batata, causando murcha bacteriana. Esta pesquisa visa desenvolver um coquetel viral para suprimir mutantes insensíveis a bacteriófagos (BIMs) do *R. solanacearum*. Os BIMs exibiram resistência múltipla quando desafiados contra outros fagos de *Ralstonia* quando avaliou-se o espectro de resistência. Esses estudos podem informar estratégias futuras de controle da resistência bacteriana em fagoterapia.

Palavras-chave: *Ralstonia solanacearum*; Fagoterapia; BIMs; Bacteriófagos.