Exploring of Nano-biomolecules for the Detection and Control of Bacterial Plant Diseases

Toufic Elbeaino
Miloud Sabri, Kaoutar El Handi, Ben Slimen Amani., Orges Cara
CIHEAM- IAM Bari, Via Ceglie 9, 70010 Valenzano, Bari, Italy
elbeaino@iamb.it

To date, the main challenge for controlling plant bacterial diseases is to find effective ecofriendly and practical antibacterial molecules able to contrast their spread in nature. Although banned in EU countries, the use of antibiotics against bacterial infections is dramatically ongoing in non-EU countries, causing serious antibiotic resistance challenges. In the last decade, the use of nano-biomolecules, such as the bacteriophages (bacteria killer), the antimicrobial peptides (AMPs), and Aptamers (Apta), has attracted interest as promising approaches to cope with plant diseases infections and control, due to their high antipathogenic activity, low toxicity to the host plant and environment and the slower emergence of resistance. In this context, the efficiencies of these three types of nano-biomolecules were explored through the identification, characterization, and application on challenging phytopathogenic bacteria, *i.e*., *Erwinia amylovora* (*Ea*), *Xylella fastidiosa* (*Xf*), and *Xanthomonas campestris* pv. campestris (*Xcc*); notorious for their high threats to global agriculture, causing severe diseases in hundreds of plants and fruit tree species.

In our study, two new bacteriophages species, named "*Erwinia amylovora* bacteriophage EP-IT22" and "*Xylella fastidiosa* bacteriophage MATE 2", belonging to the family *Myoviridae* and the genus *Carpasinavirus* were identified, respectively [1, 2]. EP-IT22 showed a circular genome of 174,346 bp containing 310 open reading frames (ORFs), whereas MATE 2 showed to have a linear genome with 63,695 bp (95 ORFs), and lacked lysogenic, virulence, antibiotic resistance, and toxin genes. *In-vivo* assays, EP-IT22 prevented fire blight symptoms in pear plants 40 days post-inoculation [2]; whereas MATE 2 showed *In-vitro* antibacterial activity on *Xf* cells.

At the AMPs level, the *Lactococcus lactis* subsp. *lactis* strain ATCC 11454 (*L. lactis*), known for its production of nisin A, and a cocktail of AMPs were used as control strategies for *Ea* and *Xf*. The results showed that 4 AMPs, out of 8 tested, and nisin A were involved in a strong antagonistic activity against both bacteria [3, 4]. The minimal lethal concentration of the AMPs and nisin A were *ca*. 0.6 mg/mL. *In-planta* tests, the AMPs and nisin A demonstrated the ability to tackle *Ea* and *Xf* infections within *Nicotiana benthamiana* plants that remained asymptomatic 74 days post inoculation.

The attempt to develop Xf-specific aptamers was conducted through the Cell-SELEX method, comprising 10 rounds of exposure to *Xf* and 4 rounds of counter-selection to a similar bacterium (*Xcc*), to increase the ligand specificity of the aptamers for *Xf*. Analyses of sequence libraries obtained by Cell-SELEX identified 2 highly competent aptamers on which to develop innovative laboratory diagnostic assays for *Xf*. The efficiency of nano-biomolecules in the diagnostics and control of phytopathogens is steadily increasing, making them perfect for smarter, innovative, and environmentally friendly use in agriculture.

References

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