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Bacterial selection for biological control of plant disease: criterion determination and validation

Monalize Salete Mota[a,*], Cesar Bauer Gomes[b], Ismail Teodoro Souza Júnior[a], Andréa Bittencourt Moura[a]

[a] Universidade Federal de Pelotas, Departamento de Fitopatologia, Pelotas, RS, Brazil
[b] Embrapa Clima Temperado, Pelotas, RS, Brazil

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ABSTRACT

This study aimed to evaluate the biocontrol potential of bacteria isolated from different plant species and soils. The production of compounds related to phytopathogen biocontrol and/or promotion of plant growth (CRBPGs) in bacterial isolates was evaluated by measuring the production of antimicrobial compounds (ammonia and antibiosis) and hydrolytic enzymes (amylases, lipases, proteases, and chitinases) and phosphate solubilization. Of the 1219 bacterial isolates, 92% produced one or more of the eight compounds evaluated, but only 1% of the isolates produced all the compounds. Proteolytic activity was most frequently observed among the bacterial isolates. Among the compounds which often determine the success of biocontrol, 43% produced compounds which inhibit mycelial growth of *Monilinia fructicola*, but only 11% hydrolyzed chitin. Bacteria from different plant species (rhizosphere or phylloplane) exhibited differences in the ability to produce the compounds evaluated. Most bacterial isolates with biocontrol potential were isolated from rhizospheric soil. The most efficient bacteria (producing at least five CRBPGs), 86 in total, were evaluated for their biocontrol potential by observing their ability to kill juvenile *Mesocriconema xenoplax*. Thus, we clearly observed that bacteria that produced more CRBPGs had a higher efficacy for nematode biocontrol, which validated the selection strategy used.

Introduction

Concerns regarding food safety and the environment have led to reduced use of agrochemicals and the development of sustainable agriculture. In this context, the focus of biological control studies reflects the desire of several sectors to develop sustainable methods for plant disease control.[1] However, efficient antagonists must be obtained for biological control to become a reality.

Soil microorganisms coexist in association with plant roots and interfere with plant performance and microbial community structure. Bacteria are estimated to occupy between 7%

* Corresponding author.
E-mail: monalizem@gmail.com (M.S. Mota).
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and 15% of the total root surface area.[2] Of these, some bacteria positively affect plants and have been designated as plant growth-promoting rhizobacteria (PGPR).[3]

Rhizobacteria can indirectly or directly promote positive effects on plants. Indirectly, they suppress pathogens mediated by competition and the production of antimicrobial compounds and lytic enzymes. Directly, they solubilize minerals and cause a wide range of changes in the rhizosphere, which promotes higher efficiency in the absorption of water and macro- and micronutrients by plants and changes in phytohormone concentrations, nitrogen fixation, and siderophore production.[4–6]

In addition to the rhizosphere, the phylloplane is a source of antagonists; however, bacteria from this area are still underused as biological control agents, especially compared to rhizobacteria.[7] Kishore et al.,[8] found that both rhizoplane and phylloplane bacteria promote peanut seedling growth. In addition, bacteria that colonize the shoots have a better chance of surviving and multiplying in a nutrition-rich environment such as the soil, whereas in the phylloplane, they are exposed to high temperatures, moisture content fluctuations, and limited nutrient availability.

In vivo biocontrol agent selection is not a simple task due to the diversity of agents and interactions with the host plant, and therefore, efficient search methods are required. Thus, it is necessary to develop efficient selection strategies to reduce costs and increase the possibility of selecting organisms that can be produced in a large scale at low cost and that maintain their viability and efficiency for long periods. In 1997, Schisler and Slininger[9] divided the selection process into three categories: (i) choosing the appropriate pathosystem, (ii) choosing the adequate method, and (iii) characterizing the isolates and evaluating efficiency. In recent years, this concept has evolved, and other groups of researchers have proposed initial selection criteria based on evaluations in the absence of the host.[10,11]

In this sense, in vitro tests are appropriate during the initial selection steps due to the large number of microorganisms that can be evaluated and, especially, their low cost. Thus, due to the need for alternative management strategies for difficult-to-control pathogens, and considering that the initial steps for biocontrol agent selection should be performed in the absence of the host, the present study aimed to (i) characterize bacteria to determine their in vitro potential for the production of compounds related to phytopathogen biocontrol and/or promotion of plant growth (CRBPGs); (ii) select bacterial isolates with the highest number of CRBPGs; and (iii) validate the selection process by studying the effect of the bacteria selected on the ringed peach nematode.

Material and methods

Origin of the bacteria

A total of 1219 bacteria that belonged to the collection of the Plant Bacteriology Laboratory (Laboratório de Bacteriologia Vegetal – LBV) at the Universidade Federal de Pelotas, Brazil, were used. The bacteria were obtained from different niches (phylloplane, rhizosphere, and soil) and grouped according to their isolation source: fig tree (*Ficus carica* L. – 55), Gramineae (72), Leguminoseae (151), Liliaceae (219), peach tree (*Prunus persica* L. – 297), *Tagetes* sp. (51), non-rhizospheric soil (309), and others (65 – tomato plant (*Solanum lycopersicum* L.), Brassicae, and culture medium contaminants).

Evaluation of CRBPG production

The abilities to produce antimicrobial compounds (antibiosis and ammonia production) and hydrolytic enzymes (amylases, lipases, proteases, and chitinases) and to solubilize phosphates were evaluated. Bacteria that previously exhibited or did not exhibit the ability to produce each compound studied were used as positive and negative controls, respectively.

The ability of bacteria to produce antibiotic compounds against *Monilinia fructicola* (Winter) Honey, the causal agent of peach brown rot was evaluated. Four bacteria arranged equidistant at the edges were streaked on each Petri dish containing 523 medium[12] and in the center, a mycelial disk containing the fungus previously grown in a potato dextrose agar (PDA). After seven days at $22 \pm 2\,^{\circ}C$, scores were assigned according to the inhibition zone of the fungal growth as follows: 0 – no inhibition; 1 – $\leq$10 mm; 2 – $\geq$11 and $\leq$20 mm; and 3 – $\geq$21 mm).

Ammonia production was observed by the presence of a yellow-orange precipitate after five days of incubation at $28\,^{\circ}C$.[13]

Starch hydrolysis was evaluated according to the method of Schaad (1988). After four days of incubation at $28\,^{\circ}C$, the addition of Lugol's Iodine allowed the visualization of clear zones around the bacterial colony, indicative of starch hydrolysis, and the bacteria were classified using the scale described for antibiotic compounds.

Lipid hydrolysis was evaluated in medium containing 1% Tween 80, according to the method used by Fahy and Persley,[14] after four and seven days of incubation at $28\,^{\circ}C$ and verified by the presence of a milky white precipitate surrounding the colonies.

Two substrates were used to evaluate the ability of the bacteria to hydrolyze proteins, Litmus® milk (Difco) and 5% gelatin medium, as described by Schaad.[15] After four and ten days of incubation at $28\,^{\circ}C$, the medium changed from milky to translucent (Litmus) or became liquefied after being refrigerated at $4\,^{\circ}C$ for 1 h (gelatin). Chitin hydrolysis was evaluated using 0.5% chitin medium as the sole carbon source[16] and calcium phosphate solubilization was evaluated in NBRIP culture medium,[17] both activities were observed at seven, 14, and 21 days of incubation at $28\,^{\circ}C$ to verify the degradation zone of each compound.

The hydrolysis of chitin, lipid and proteins (Litmus® milk and gelatin) was determined in a semiquantitative way, using different incubation times for evaluating. The intensity of production was low for positive reaction after 10 (lipids and proteins) or 21 days (chitin) of incubation; middle if the positive reaction happened after 14 days of incubation (chitin); and high when the positive reaction occurred after four (lipids and proteins) or seven days of incubation (chitin).

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