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Fluorescent pseudomonads as biocontrol agents for sustainable agricultural systems

Monica Höfte [a,*], Nora Altier [b]

[a] Laboratory of Phytopathology, Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, Gent, Belgium
[b] Plant Protection, National Institute for Agricultural Research, Instituto Nacional de Investigación Agropecuaria, INIA Las Brujas, Canelones, Uruguay

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Abstract

The highly diverse genus *Pseudomonas* contains very effective biocontrol agents that can increase plant growth and improve plant health. Biocontrol characteristics, however, are strain-dependent and cannot be clearly linked to phylogenetic variation. Isolate screening remains essential to find suitable strains, which can be done by testing large local collections for disease suppression and plant-growth promotion exemplified in a case study on forage legumes in Uruguay or by targeted screening for *Pseudomonas* spp. which produce desirable secondary metabolites, as demonstrated in a case study on cocoyam in Cameroon. In both case studies, access to reference strains from public and private collections was essential for identification, phylogenetic studies and metabolite characterization.
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1. Introduction

1.1. Biological control

Biological control of plant diseases, in its widest sense, is any means of controlling disease or reducing the amount or effect of pathogens that relies on biological mechanisms or organisms other than man (Campbell, 1989). Within this definition, it also includes cultural practices such as crop rotation and soil amendments that affect pathogenic microorganisms. A more narrow approach is to restrict biological control to the artificial introduction of living microorganisms into the environment to control the pathogen. As such, these biopesticides are an alternative or supplementary way of reducing the use of chemical pesticides in agriculture. There is a genuine commercial interest in biopesticides, since they can be used in rotation with chemical pesticides to reduce the development of pathogen resistance. Biocontrol can also be used in situations where no control is currently available, where conventional pesticides cannot be used due to re-entry or residue concerns or where the product must be certified organic (Fravel, 2005). The paper of Fravel (2005) lists 14 bacteria and 12 fungi registered with the United States Environmental Protection Agency for control of plant diseases. Among the 14 registered bacterial biocontrol agents, six are based on *Bacillus*, five on *Pseudomonas*, two on *Agrobacterium* and one on *Streptomyces*. *Pseudomonas* spp. are particularly suited as biocontrol agent because they can use many exudates as nutrient source, they are abundantly present in natural soils, especially in the rhizosphere, they have a high growth rate, they can be directly plant-growth-promoting and they have the ability to control diseases by a variety of mechanisms. They are also the most extensively studied group of bacterial biocontrol agents, since *Pseudomonas* bacteria are amenable to mutation and modification using genetic tools (Chin-A-Woeng et al., 2003).

* Corresponding author. Tel.: +32 9 2646017; fax: +32 9 2646238.
E-mail addresses: monica.hofte@ugent.be (M. Höfte), naltier@lb.inia.org.uy (N. Altier).

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1.2. Role of culture collections in biocontrol research

Culture collections play a very important role in research as a source of authenticated biological material. They accept deposits subject to publication to enable confirmation of results and further studies, and offer safe, confidential patent deposit services (Smith, 2003). In relation to agriculture, specific collections are available mainly dealing with plant pathogenic microorganisms (Kang et al., 2006) and nitrogen-fixing bacteria such as *Rhizobium* (World Federation for Culture Collections, 2010), but to our knowledge, there are no culture collections dedicated to bacterial biocontrol agents.

In biocontrol research, screening is a critical step in the development of biocontrol agents, and the ultimate success of biocontrol depends on how well the searching and screening process is done (Fravel, 2005). The places to look for potential control agents must be selected carefully and the control agent eventually selected must be able to survive and grow in the environment in which it is expected to operate (Campbell, 1989). As stated by Campbell (1989), isolates from culture collections rarely produce useful organisms for the field because they are usually adapted to the high nutrient levels in common media. However, culture collections are important in biocontrol research because reference strains are needed in taxonomic and phylogenetic studies to identify the newly isolated biocontrol agents and to study their genotypic and phenotypic diversity.

In this review, we focus on Pseudomonas as a case study of biological control agents in relation to culture collections because they are widely used and studied and highly diverse, which poses challenges to taxonomy and phylogeny. After a brief general overview about *Pseudomonas* taxonomy, plant-associated pseudomonads and the diversity of *Pseudomonas* biocontrol agents, two case studies will focus on the isolation and screening of effective *Pseudomonas* biocontrol agents in Uruguay and Cameroon. Microorganisms from Africa and South America are typically underrepresented in culture collections. Of the 574 culture collections registered with the World Data Center for Microorganisms (World Federation for Culture Collections, 2010), 11 collections are located in Africa and they contain only 0.81% of the microorganisms in these culture collections. A case study on the holdings of the environmental prokaryotes available at the American Type Culture Collection (Floyd et al., 2005) has revealed that only 2.8% of the accessions come from Africa and only 1.8% from South America. One question concerns whether isolation and screening of *Pseudomonas* biocontrol agents in Uruguay and Cameroon will deliver new *Pseudomonas* spp. with undescribed mode of actions.

2. Fluorescent pseudomonads as biocontrol agents

2.1. Pseudomonas *taxonomy*

Pseudomonas is one of the most ubiquitous bacterial genera in the world and different species have been isolated from very diverse ecological niches. Since its discovery, the genus *Pseudomonas* has undergone numerous taxonomic changes and a detailed history of *Pseudomonas* taxonomy can be found in a recent paper by Peix et al. (2009). Currently, only the representatives of rRNA group I Palleroni et al. (1973) are included in the genus *Pseudomonas* and up to now, 128 species have been validly described for this genus, including the fluorescent pseudomonads that have the capacity to produce fluorescent pyoverdine-type siderophores under low-iron conditions. Most species are saprophytes that are commonly found in water and soil; 23 species are pathogenic to plants, including *Pseudomonas syringae* with 36 pathovars affecting different plants. In addition, 16 species are associated with diseases in humans and animals (Peix et al., 2009). Some species, such as *Pseudomonas aeruginosa*, are ubiquitous and can be associated with both plants and animals. Some of the saprophytic species have interesting characteristics and are used in biotechnological applications to improve plant growth and plant health, but also in water and soil bioremediation.

2.2. Plant-associated Pseudomonas *spp*

Plant-associated *Pseudomonas* include both beneficial and pathogenic isolates (Höfte and De Vos, 2006), which colonize the same ecological niches and possess similar mechanisms for plant colonization (Preston, 2004). Pathogenic, saprophytic and plant-growth-promoting strains are often found within the same species. Strains that improve plant growth and plant health, also called plant-growth-promoting bacteria (Kloepper et al., 1980) or plant-probiotic fluorescent pseudomonads in analogy with probiotic bacteria and yeasts in the gastrointestinal tract (Haas and Keel, 2003; Picard and Bosco, 2008), are commonly found within *Pseudomonas fluorescens*, *Pseudomonas putida* and *Pseudomonas chlororaphis* (=*Pseudomonas aureofaciens*), but also isolates of *P. aeruginosa* and *Pseudomonas syringae* have been identified as efficient biocontrol agents (Anjaiah et al., 1998; Audenaert et al., 2002; Janisiewicz and Marchi, 1992). The most commonly reported mechanisms of biocontrol by fluorescent *Pseudomonas* spp. include production of antibiotics, hydrogen cyanide, lytic exoenzymes (Thomashow and Weller, 1996), cyclic lipopeptides (Raaijmakers et al., 2006), competition for nutrients and niches (Kamilova et al., 2005), competition for iron mediated by siderophores, competition for carbon (Thomashow and Weller, 1996) and induced systemic resistance (De Vleesschauwer and Höfte, 2009). The antibiotics 2,4-diacetylphloroglucinol (DAPG), pyoluteorin, pyrrolnitrin and different phenazine derivatives have been described in biocontrol *Pseudomonas* spp. as the main cause of their antagonistic activity (De La Fuente et al., 2004; Thomashow and Weller, 1996; Weller et al., 2007). Additionally, many rhizospheric fluorescent *Pseudomonas* spp. have the capacity to stimulate plant growth, by increasing the availability and uptake of mineral nutrients via phosphate-solubilizing enzymes or by enhancing root growth and morphology via the production of phytohormones such as auxin (Vessey, 2003).

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