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Efficacy of *P. oligandrum* affected by its association with bacterial BCAs and rootstock effect in controlling grapevine trunk diseases

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ABSTRACT

The development of biological control agents (BCAs) is a promising and environmentally friendly method to control plant pathogens. In a grapevine nursery greenhouse and for the first time on grafted cuttings (scion cv. Cabernet sauvignon), this two-year study demonstrated the significant efficacy of the oomycete *Pythium oligandrum* as a BCA against two major aggressive fungal pathogens, *Neofusicoccum parvum* and *Phaeomoniella chlamydospora*, which are involved in grapevine trunk diseases (GTDs). By considering the reduction in necrosis lengths within the scion stem, treatments with *P. oligandrum* alone showed the greatest efficacy against the two pathogens (overall average efficacy of 48.3%). This major result was obtained during the two-year bioassay and in cuttings grafted on the two widely used rootstocks, 101-14 and SO4. The biocontrol efficacies of two bacterial strains previously isolated from vineyards, *Pantoea agglomerans* and *Bacillus pumilus*, were also assessed, separately or in combination with *P. oligandrum*. Treatments with each bacterial strain were less effective than treatments with *P. oligandrum*, and the efficacies were not improved when they were applied in combination with the oomycete.

1. Introduction

Grapevine trunk diseases (GTDs), such as Esca, markedly impact the vine and grape industry worldwide. In France, it has been estimated that GTDs are the reason that approximately 11% of vineyards became unproductive in 2008, and approximately 13% in 2012 (Grosman and Doublet, 2012). Because Esca is the most prevalent GTD in Europe, numerous recent studies have focused on this ancient and ubiquitous disease. Esca is currently generally referred to as Esca Complex because the disease is considered to result from the pathogenic activity of several fungal species, including the ascomycete species *Phaeomoniella chlamydospora*, *Phaeoacremonium minimum* and *Neofusicoccum parvum* and basidiomycete species *Fomitiporia mediterranea* (Larignon, 2012). These pathogenic fungi mostly damage wood tissue, causing various types of necrosis and, in most cases, the death of the plant. As the most distinctive symptoms of Esca are central and black punctuate necrosis and discolored xylem (Lecomte et al., 2012; Maher et al., 2012), necrosis length is the main criteria used to assess the attack rates of pathogens associated with GTDs (Laveau et al., 2009; Pouzoulet et al., 2013). Whereas most studies have focused on non-grafted models of grapevine (Fourie and Halleen, 2004; Haidar et al., 2016a, 2016b; Yacoub et al., 2016a), we proposed to study grafted plantlets because they are the most popular system cultivated in French vineyards.

Developing nursery management strategies that protect cuttings are an essential step in reducing the transmission of grapevine trunk diseases during propagation. Although healthy mother plants are used in nurseries, the grafting process itself poses a risk of contamination (Gramaje and Armengol, 2011). Therefore, a method offering protection against GTDs early in the cultivating process could be of great interest.

Biological control with microbial antagonists can offer an environmentally friendly solution and the use of such organisms isolated from vineyards can limit the negative impacts of other products such as chemicals or hot water treatments (Fourie and Halleen, 2004). Moreover, due to their toxicity to the environment and humans, specific pesticides have been withdrawn from the grapevine market. This notably includes the active fungicide ingredient sodium arsenite, banned in Europe in 2001. Since then, no efficient chemical control measure has been developed against GTDs in vineyards (Alabouvette et al., 2006). One alternative strategy is the use of beneficial microbial agents

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as biological control agents (BCAs). Some prior studies have shown the direct or indirect antagonistic activity of several species of bacteria, fungi and/or yeasts towards GTD fungal pathogens (Compant et al., 2012; Bertsch et al., 2013). However, only a few are currently registered as BCAs and commercialized in Europe, according to the EU pesticides database (Ec.europa.eu., n.d.). To select and use a BCA, it is important to note that (i) its efficiency can vary depending on how it is tested and/or screened (laboratory *versus* field conditions); (ii) selected strains must be able to survive and remain within the system (ecosystem, host tissues) they help to protect; and (iii) they need to be compatible with other cultural techniques, notably chemical agricultural inputs (Alabouvette et al., 2006). To maximize the effect of a BCA, an interesting approach is to aim for a synergetic effect between species or strains that display different types of interaction (Compant et al., 2012).

Pythium oligandrum is an oomycete that is naturally present in the rhizosphere of vines (Gerbore et al., 2014b) that has been identified as a helpful organism because of its ability to protect plants from pathogenic attacks. This ability has been demonstrated in several reports since 1986 on a range of crop plants in small-scale field, soil and greenhouse trials (Rey et al., 2008; Benhamou et al., 2012; Gerbore et al., 2014a). The biological control exerted by the chromista species *P. oligandrum* is the result of a complex process, which includes direct effects on pathogenic fungi (mycoparasitism and antibiosis) and plant defense stimulation (Rey et al., 2008; Benhamou et al., 2012). Recently, Yacoub et al. (2017) explored the interaction between grapevine and *P. oligandrum*, showing that significant changes in the grapevine root transcriptome occurred following root colonization by the oomycete. The main changes concerned genes associated with responses to stimuli, and the observed relationship presented similarities with a symbiotic microorganism/root interaction. Moreover, a preliminary greenhouse assay demonstrated that necrosis caused by *P. chlamydospora* in Cabernet Sauvignon grapevine cuttings was reduced by approximately 35% following *P. oligandrum* colonization of the root system (Yacoub et al., 2016a). However, this result was not statistically significant, and the authors did not compare different rootstocks as possibly interacting with *P. oligandrum* BCA efficacy.

Previous recent studies characterized and screened various bacterial strains isolated from Bordeaux vineyards for their antagonistic activity against *N. parvum*, *P. chlamydospora* and *B. cinerea* (Haidar et al., 2016a, 2016b). Among them, *P. agglomerans* (S1) was able to significantly reduce the necrosis length caused by *N. parvum* on ungrafted cutting stems, associated with an intermediate efficiency profile against *B. cinerea*. In similar experimental conditions, *Bacillus pumilus* (S32) and *Paenibacillus* sp (S19) were at the origin of a significant reduction of approximately 30% of the necrosis size caused by *P. chlamydospora* (Haidar et al., 2016b). Therefore, *B. pumilus* (S32) was described as triggering a systemic immune response in grapevine. However, these studies did not investigate potential synergy, interference and/or interaction between two different BCA bacterial strains and they were always carried out in ungrafted plant material.

The major objectives of this study were to quantify and compare the BCA efficacy of *P. oligandrum* under advanced experimental nursery greenhouse conditions: 1) against two aggressive GTD pathogens of prime importance, *N. parvum* (Np) and *P. chlamydospora* (Pch), notably because Po has never been tested against Np; and 2) using two types of grafted plant materials, either the SO4 or the 101-14 rootstock genotype. Similarly, the study aimed at confirming, quantifying and comparing the BCA efficacy of two bacterial strains against these two pathogens, either separately or in combination with *P. oligandrum*. The last complementary objective was to assess the ability of *P. oligandrum* to colonize the root system of the grafted grapevine cuttings following its soil application. This experiment was carried out over two years in a commercial nursery greenhouse.

Table 1
Properties of selected bacterial strains.

Code	Species identification	Origin from grapevine host organ	Targeted pathogen species
S1	*Pantoea agglomerans*	Berries surface	*N. parvum*
S32	*Bacillus pumilus*	Trunk	*P. chlamydospora*

2. Materials and methods

2.1. Microorganisms and media/bacterial and fungal preparations

2.1.1. Pythium oligandrum

P. oligandrum strain “Sto 7” inoculum was prepared through a specific fermentation process and provided by BIOVITIS (Gerbore et al., 2014b). The concentration of the final product was adjusted to 6×10^3 oospores/ml.

2.1.2. Bacterial strains

Two bacterial strains used as BCAs were obtained from the collection of UMR SAVE, INRA, Bordeaux (Table 1). They were previously isolated from French vineyards and screened on various grapevine host organs (Haidar et al., 2016a). In this study, they were used specifically for controlling a GTD pathogen, either *N. parvum* or *P. chlamydospora*. The bacterial strains were grown on TSA (trypticase soy agar) in Petri dishes at 28 °C.

2.1.3. Pathogen strains

The *N. parvum* isolate “Cou 02” was selected from the INRA-UMR 1065 SAVE collection, Bordeaux. This strain was originally obtained in 2008 from a Cabernet Sauvignon grapevine in an experimental INRA vineyard near Bordeaux and was characterized as highly aggressive in previous preliminary studies at INRA (Haidar et al., 2016a, 2016b). *P. chlamydospora* strain “SO44” was selected from the INRA-UMR 1065 SAVE collection, Bordeaux. This strain was originally obtained in 1996 from a Cabernet Franc grapevine in Moncaup, France. It was characterized as highly aggressive in previous studies at INRA (Laveau et al., 2009). The strains were subcultured on Malt Agar (MA) medium and incubated at 22 °C for one month (12 h light/12 h dark). They were retrieved as 4 mm plugs the day before artificial inoculation in bioassays.

2.2. Plant material

For each experiment, grapevine plantlets (*Vitis vinifera* L., cv. Cabernet Sauvignon) were propagated from 2-node woodcuttings in a greenhouse. Plants were grafted on rootstock 101-14 (1st and 2nd year) and SO4 (2nd year), selected because of their potential difference in susceptibility to esca (Liminana et al., 2009). The cuttings were rooted for 30–45 days before infection and grown under controlled conditions (Table 2). The temperature was maintained between 22 and 28 °C. The plants were watered for 2 min per day via a drip system (2 L/h) and fertilized twice a week (nutrient solution N/P/K 20/20/20). Plantlets were drilled a week before the first application. The hole was made on the scion trunk 1 cm below the second node. It was then covered by a layer of protective film (Cellofrais®) to prevent external contamination.

2.3. Experimental design

2.3.1. 1st year bioassay

In the 1st year bioassay, all plants were grafted on rootstock 101-14. Treatments carried out in the 1st year bioassay, as shown in Table 3, included a non-inoculated control: “Y1.Drilled”. Only in the first year assay, another supplementary negative control was “Y1.Non-drilled”, which was not inoculated or drilled. The controls “Y1.Np” and “Y1.Pch”