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REVIEW

Use of beneficial bacteria and their secondary metabolites to control grapevine pathogen diseases

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Abstract Grapevine is one of the most important economic crops yielding berries, wine products as well as derivates. However, due to the large array of pathogens inducing diseases on this plant, considerable amounts of pesticides—with possible negative impact on the environment and health—have been used and are currently used in viticulture. To avoid negative impacts of such products and to ensure product quality, a substantial fraction of pesticides needs to be replaced in the near future. One solution can be related to the use of beneficial bacteria inhabiting the rhizo- and/or the endosphere of plants. These biocontrol bacteria and their secondary metabolites can reduce directly or indirectly pathogen diseases by affecting pathogen performance by antibiosis, competition for niches and nutrients, interference with pathogen signaling or by stimulation of host plant defenses. Due to the large demand for biocontrol of grapevine diseases, such biopesticides, their modes of actions and putative consequences of their uses need to be described. Moreover, the current knowledge on new strains from the rhizo- and endosphere and their metabolites that can be used on grapevine plants to counteract pathogen attack needs to be discussed. This is in particular with regard to the control of root rot, grey mould, trunk diseases, powdery and downy mildews, pierce's disease, grapevine yellows as well as crown gall. Future prospects on specific beneficial microbes and their secondary metabolites that can be used as elicitors of plant defenses and/or as biocontrol agents with potential use in a more sustainable viticulture will be further discussed.

Keywords *Vitis vinifera* L. · Diseases · Biocontrol · Beneficial bacteria · Secondary metabolites

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Introduction

Grapevine is one of the most important economic crops, mainly because of the use of their berries for red, white, and rosé wine. This represents more than 7.5 million ha of cultivated surfaces in the world with 27 million t of wine produced by year as described for 2009 (FAOSTAT 2011). However, grapevine plants

can be infected and colonized by a large variety of pathogenic microorganisms such as deleterious fungi, oomycetes and bacteria (Gouadec et al. 2007). These vine diseases can have drastic effects on the host plants, on berries, but also on wine qualities and their sensorial and organoleptic properties (Gouadec et al. 2007), resulting in economic losses for the wine growers and producers (van Helden 2008).

Pesticides have been or are currently applied in the vineyard to avoid the outbreak of vine pests or diseases, to manage the surrounding flora, to increase grape yield and to ensure wine quality (Leroux 2003; Pezet et al. 2004). As for instance in France more than 30,000 t $year^{-1}$ of fungicides and bactericides have been used for grapevine production (FAOSTAT 2011). For Europe, the International Organization of Vines and Wine estimates that 70,000 t of fungicides are used annually on around 3.8 million hectares of land dedicated to viticulture (http://www.endure-network.eu/). Worldwide, on average 35 % of all pesticides are used for viticulture. The continuous use of phytosanitary products during the last decades has been, however, accompanied by an increasing awareness of the problems arising from intensive pesticide use. Consequences of intensive pesticide use include their persistence in soils, contamination of the environment, as well as appearance of resistant pathogenic strains (Leroux 2004). Additionally, specific pesticides have been withdrawn from the market due to their negative impact on human health and the environment (Amaro and Mexia 2003). Development of new active molecules targeting vine pests without undesired impact is possible. However, due to increasing costs to develop these new molecules, other alternative solutions have also been proposed.

To reduce the use of phytosanitary products, genetically modified (GM) plants have been propagated to control vine pests and diseases (see for examples the studies of Ferreira et al. 2004; Agüero et al. 2005; Vidal et al. 2006; The Local Monitoring Committee et al. 2010). However, this alternative strategy has not been and is still not widely accepted. So far, no GM grapevine has been commercialized (The Local Monitoring Committee et al. 2010). Many regions, especially in Europe, are generally not in favour of cultivation of GM crops (Marshall 2009), so there is a need for other solutions.

One of the alternative strategies to reduce the use of pesticides in grapevine production corresponds to the use of beneficial bacteria as biocontrol agents (Bent 2006). Since the rhizosphere concept of Lorenz Hiltner describing that the soil surrounding roots is influenced by plants and by microorganisms (Hiltner 1904; Hartmann et al. 2008), a large number of studies have demonstrated that part of the rhizobacteria inhabiting the rhizosphere can stimulate plant growth (plant growth-promoting rhizobacteria; PGPR) as well as protect plants against pathogen infections (biocontrol strains) (Berg 2009; Lugtenberg and Kamilova 2009). Plant growth promotion (e.g. achieved by hormone stimulation or changed nutrient availability) and biocontrol activities of particular rhizobacteria strains are distinct issues. However, in practice this is often hard to dissect as bacteria can show both activities. Also, particularly in field or in greenhouse trials, biocontrol bacteria might promote plant growth by reducing pathogenic pressures. Biocontrol by beneficial bacteria might be achieved by direct antibiosis, competition for niches and nutrients, interference with pathogen signalling or by inducing plant resistance (Fig. 1, Berg 2009; Lugtenberg and Kamilova 2009). Moreover biocontrol might be achieved by degradation of virulence factors or phytotoxins of pathogens, thereby leading to reduction of disease symptoms (Compant et al. 2005a). Considerable literature information has shown that rhizobacteria can secrete various secondary metabolites (SMs). Both rhizobacteria and SMs produced by them can act on pathogens by depriving the pathogens of nutrients (competition), lysing cells and/or blocking specific functions related to pathogen growth (antibiosis) and act therefore as biocontrol agents (Berg 2009; Compant et al. 2005a; Lugtenberg and Kamilova 2009). Rhizobacteria and their SMs are also known to induce plant defense reactions leading to a systemic resistance or priming of above-ground parts to be more resistant to subsequent pathogen infections (Berg 2009; van Loon 2008; van Loon and Bakker 2005), and this can be used for grapevine protection against phytopathogenic diseases.

Already since the nineteenth century with the description of bacteria-like structures by Woronin (the so-called *Frankia* sp.) and the work of Galippe and di Vestea (see Compant et al. 2010a, 2012) with bacteria other than root nodulating strains, it has been widely accepted that specific microsymbionts can also colonize different host plants and plant parts. Although sources of colonization of these endophytic

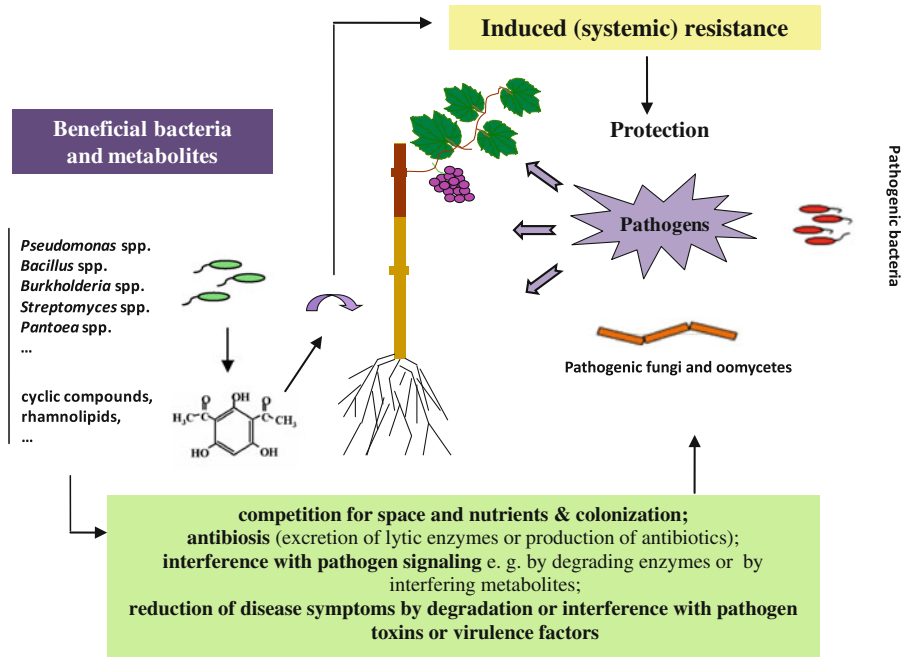

Fig. 1 Drawing summarizing the potential mechanisms involved in the control of grapevine pathogen diseases by beneficial bacteria and their secondary metabolites

bacteria could be the anthosphere, the caulosphere, the phyllosphere or the spermosphere, the prevailing opinion suggests colonization of a large fraction of the endophytic population from the rhizosphere as described by microscopic, genetic as well as metagenomic evidences (Hallmann 2001; Hallmann and Berg 2007; Compant et al. 2010a).

As rhizobacteria, also endophytes are known to stimulate host plant growth and can act as biocontrol agents to alleviate infection by pathogenic strains, in particular cases even to higher levels than root-restricted bacteria (Welbaum et al. 2004; Hallmann and Berg 2007). Bacterial endophytes inhabiting plant internal tissues are also a source of SMs that may act as elicitors of plant defenses or as antimicrobial agents with potential use to control disease (Qin et al. 2011).

Different elicitors of plant defenses are known from beneficial bacteria, both from the rhizo- and the endosphere of plants. This includes a variety of primary bacterial constituents such as flagella (flagellin) or lipopolysaccharides (LPS) but also SMs with high structural diversity specific for certain strains (Qin et al. 2011; van Loon and Bakker 2005). In addition, continuous research and discovery of novel elicitors and strains from different environments, particularly from harsh ecosystems, will likely yield novel strains and elicitors capable of triggering plant defenses and enabling resistance. This is especially interesting for the reduction of the use of pesticides in viticulture, where—in France—up to 50 % of the total pesticide entry is used for only 3.3 % of cultivated surfaces and in EU 3.5 % of the cultivated land receives 15 % of the total pesticide entry representing 20–22 kg of pesticide per ha used for grapevine (Compant 2011; Compant and Mathieu, 2011).

The role of both rhizobacteria and endophytes in biocontrol of plant diseases or for a sustainable management of agriculture has been highlighted (van Loon and Bakker 2005; Lugtenberg and Kamilova 2009) and information on the usage of beneficial microbes in viticulture is currently emerging. Research performed on specific strains have moreover allowed the description of SMs secreted by specific strains (both rhizo- and endosphere colonizing bacteria), which may be responsible for their effects on pathogen targets and/or on resistance mechanisms of grapevine plants (Compant and Mathieu 2011). Additionally, new beneficial bacterial strains and SMs to control plant diseases with potential use in viticulture are continuously described (Compant 2011). Nevertheless, a better understanding of how and which microorganisms or bacterial metabolites can

be used to reduce disease pressure in grapevine plants is needed. In this review, the use of beneficial bacteria and their metabolites used to control various grapevine diseases caused by fungi, oomycetes or bacteria is described. This also includes the description of mechanisms involved in plants, on phytopathogen diseases reduction, but also on the origin of strains and metabolites used to control grapevine diseases. Future prospects for a better delivery of inoculants or elicitors are also provided. Understanding the mechanisms through which beneficial bacteria and their metabolites act on phytopathogens and plant responses is a pre-requisite for a better delivery of bacterial microsymbionts in the field, but also for fundamental research or bioprocesses development.

Beneficial bacteria and biocontrol of grapevine fungal and oomycetes diseases

The research performed so far has demonstrated that specific strains of both rhizo- and endophytic bacteria as well as some of their secreted secondary metabolites can inhibit pathogens affecting grapevine (Fig. 1). In the following paragraphs the focus will be on fungal trunk diseases, Fusarium root rot, grey mould, powdery and downy mildew as serious diseases affecting viticulture and on beneficial bacteria strains reducing these diseases (Table 1). Their effects under controlled and field conditions are discussed.

Biocontrol of wilt and root rot caused by *Fusarium* spp.

Wilts and root rots of grapevine caused by fungal pathogens such as *Armillaria* spp. *Fusarium* spp. and *Verticillium* dahlia Kleb. have been occasionally reported (Garrido et al. 2004; Gubler et al. 2004; Zhang et al. 2009; Ziedan et al. 2011). In the following part we will exemplify the biocontrol of wilt and root rot caused by *Fusarium* spp., which are of regional importance, particularly in warm vine regions such as Australia, Brazil, Egypt (Garrido et al. 2004; Highet and Nair 1995; Ziedan et al. 2011) and may also cause problems in combination with phylloxera feeding (Granett et al. 1998). Depending on the rootstock (Omer et al. 1999), *Fusarium oxysporum* E.F. Sm. & Swingle (Nectriaceae) can cause reduced plant growth, affects the survival of young plants and the yield and productivity of grapevine (Highet and Nair 1995). Incidences on vineplants suffering from this fungus have been described recently in Egypt, where *F. oxysporum* isolates on grapevine plants (Cv. crimson) caused vascular wilt (on 66.7 % of the cases) and root-rot syndrome (33.3 %) (Ziedan et al. 2011). Another species of *Fusarium*, *F. solani* Sny. & Hans, can also lead to rootstock deficiency (Andrade 1993; Grasso 1984; Gugino et al. 2001). To tackle the problem of *Fusarium* infections in grapevine, Ziedan et al. (2010) studied biocontrol bacteria to alleviate vine plant infections by *Fusarium* spp. Seven strains of *Streptomyces* spp. isolated from grapevine rhizospheric soil, were screened for antagonistic activities towards *F. oxysporum*. All isolates showed antifungal activities. One isolate identified as *Streptomyces alni* exhibited the highest activity, which was correlated to an inhibition of fungal growth, malformation, lysis of hyphae as well as inhibition of normal branches and conidia of conidiophores on dual culture plates. This indicates a direct antibiosis effect of this biocontrol strain, potentially mediated by the effect of a hitherto uncharacterized antibiotic (Ziedan et al. 2010). Under greenhouse and field conditions, the use of *S. alni* was associated with a reduction of root rot infection. An increase of grape yield of cv. Superior was also noted. In combination with the biofertiliser "Rhizobacterin" containing the *Klebsiella planticola* strain BIM B-161 the *S. alni* strain was even more effective (Ziedan et al. 2010). The obtained results suggest that the *S. alni* strain could be successfully used in combination with biofertilisers for controlling root-rot of grapevine, especially in organic farming systems.

In addition to *S. alni*, the *Pseudomonas fluorescens* isolate NRC10, a rhizobacterial strain isolated from the grapevine root environment, might have the potential to control *Fusarium* rot in grapevine plants (Ziedan and El-Mohamedy 2008). A number of fluorescent Gammaproteobacteria such as *P. fluorescens* are well-known to act as biocontrol or PGPR agents as well as inhibiting the rhizosphere of grape plants (Svercel et al. 2009, 2010). For strain NRC10 it was demonstrated that it can attach or adhere fungal hyphae of *Fusarium* spp. It can also penetrate fungal cell walls and can be responsible for morphological changes of fungal hyphae, and conidiospores as well as of partial degradation of fungal cell walls and sclerotia (Ziedan and El-Mohamedy 2008). Mechanistically, both production of lytic enzymes by the

Table 1 List of examples of biocontrol beneficial strains having biocontrol properties on phytopathogens of grapevine diseases

Biocontrol strain	Mechanisms described	Phytopathogen	Disease	Evidence reference	References
Streptomyces spp.	Antibiosis	*Fusarium oxysporum* E.F. Sm. & Swingle	Fusarium wilt and root rot	Field	Ziedan et al. (2010)
Pseudomonas fluorescens isolate NRC10	Antibiosis + ISR	*Fusarium oxysporum* E.F. Sm. & Swingle	Fusarium wilt and root rot	Field	Ziedan and El-Mohamedy (2008)
Bacillus subtilis strain	Antibiosis	*Eutypa lata* (Pers.) Tul. & C. Tul.	Eutypa dieback	*In planta*	Ferreira et al. (1991)
B. subtilis strain Blα	Antibosis + interference with virulence factors	*Eutypa lata* (Pers.) Tul. & C. Tul.	Eutypa dieback	On wood disks	Schmidt et al. (2001)
Erwinia herbicola strains JII/E2 and JII/E4	Antibosis + interference with virulence factors	*Eutypa lata* (Pers.) Tul. & C. Tul.	Eutypa dieback	On wood disks	Schmidt et al. (2001)
Actinomycete strain A123	Antibosis + interference with virulence factors	*Eutypa lata* (Pers.) Tul. & C. Tul.	Eutypa dieback	On wood disks	Schmidt et al. (2001)
Bacillus subtilis AG1	Antibiosis	*Lasiodiplodia theobromae* (Pat.) Griffon & Maubl.	Grapevine canker	In vitro	Alfonzo et al. (2009)
Bacillus subtilis AG1	Antibiosis	*Phaeomoniella chlamydospora* (W. Gams, Crous, M.J. Wingf. & Mugnai) Crous & W.Gams	Esca disease	In vitro	Alfonzo et al. (2009)
Bacillus subtilis AG1	Antibiosis	*Phaeoacremonium aleophilum*W. Gams, Crous, M.J. Wingf. & Mugnai	Esca disease	In vitro	Alfonzo et al. (2009)
Bacillus circulans GI 070	Antibiosis	*Botrytis cinerea* (Pers.)	Grey mould	In vitro	Paul et al. (1997)
Bacillus sp.	Antibiosis + ISR	*Botrytis cinerea* (Pers.)	Grey mould	*In planta*	Krol (1998)
P. fluorescens strain	Antibiosis + ISR	*Botrytis cinerea* (Pers.)	Grey mould	*In planta*	Krol (1998)
Pseudomonas fluorescens PTA-268, PTA-CT2	Antibiosis + ISR	*Botrytis cinerea* (Pers.)	Grey mould	Field	Magnin-Robert et al. (2007),Trotel-Aziz et al. (2006, 2008), and Verhagen et al. (2011)
Bacillus subtilis PTA-271	Antibiosis + ISR	*Botrytis cinerea* (Pers.)	Grey mould	*In planta*	Trotel-Aziz et al. (2006, 2008) and Verhagen et al. (2011)
Pantoea agglomerans PTA-AF1 and PTA-AF2	Antibiosis + ISR	*Botrytis cinerea* (Pers.)	Grey mould	Field	Magnin-Robert et al. (2007), Trotel-Aziz et al. (2006, 2008), and Verhagen et al. (2011)
Acinetobacter Iwoffii PTA-113 and PTA-152	Antibiosis + ISR	*Botrytis cinerea* (Pers.)	Grey mould	Field	Magnin-Robert et al. (2007) Trotel-Aziz et al. (2006, 2008), and Verhagen et al. (2011)
Pseudomonas aeruginosa (7NSK2)	ISR	*Botrytis cinerea* (Pers.)	Grey mould	*In planta*	Verhagen et al. (2010)

Table 1 continued

Biocontrol strain	Mechanisms described	Phytopathogen	Disease	Evidence reference	References
P. fluorescens (strains CHA0, Q2-87 and WCS417)	ISR	*Botrytis cinerea* (Pers.)	Grey mould	*In planta*	Verhagen et al. (2010)
P. putida (WCS358)	ISR	*Botrytis cinerea* (Pers.)	Grey mould	*In planta*	Verhagen et al. (2010)
Burkholderia phytofirmans strain PsJN	ISR	*Botrytis cinerea* (Pers.)	Grey mould	*In planta*	Ait Barka et al. (2000, (2002)
Streptomyces spp.	Antibiosis	*Botrytis cinerea* (Pers.)	Grey mould	In vitro	Loqman et al. (2009)
Micromonospora spp.	Antibiosis	*Botrytis cinerea* (Pers.)	Grey mould	In vitro	Loqman et al. (2009)
Streptomyces sp.	Antibiosis + ISR	*Botrytis cinerea* (Pers.)	Grey mould	*In planta*	Lebrihi et al. (2009a, b)
OxB related to *Cupriavidus* sp.	Degradation of virulence factors	*Botrytis cinerea* (Pers.)	Grey mould	On leaves	Schoonbeek et al. (2007)
B. pumilus B-30087	Antibiosis + ISR	*Erysiphe necator* Schw.	Powdery mildew	*In planta*	Lehman et al. (2000)
Bacillus strains ATCC 55608 and 55609	Antibiosis	*Erysiphe necator* Schw.	Powdery mildew	Field	Sawant et al. (2011)
Serratia marcescens MSU-97	Antibiosis	*Plasmopara viticola* (Berk. and Curt.) Berl. and de Toni	Downy mildew	In vitro	Strobel et al. (2005)
Streptomyces sp. ANK313	Antibiosis	*Plasmopara viticola* (Berk, and Curt.) Berl. and de Toni	Downy mildew	In vitro	Abdalla et al. (2011)
A non-tumorigenic strain (F2/5) of *Agrobacterium vitis*	Competition, signal interference + ISR	*Agrobacterium vitis*	Crown gall	*In planta*	Burr and Reid (1994) and Burr et al. (1997)
Agrobacterium vitis strain E26 or VAR03-1	Competition, signal interference + ISR	*Agrobacterium vitis*	Crown gall	*In planta*	Kawaguchi et al. (2007, 2008)
Pseudomonas aureofaciens B-4117	Antibiosis	*Agrobacterium vitis*	Crown gall	*In planta*	Khmel et al. (1998)
P. fluorescens CR330D *and 1100-6*	Antibiosis, competition	*Agrobacterium vitis*	Crown gall	*In planta*	Khmel et al. (1998)
Bacillus subtilis EN63-1	Antibiosis	*Agrobacterium vitis*	Crown gall	*In planta*	Khmel et al. (1998)
Bacillus sp. EN71-1	Antibiosis	*Agrobacterium vitis*	Crown gall	*In planta*	Khmel et al. (1998)
Rahnella aquatilis HX2	Antibiosis	*Agrobacterium vitis*	Crown gall	Field	Khmel et al. (1998)
Strains of *Enterobacter agglomerans, Rahnella aquatilis*, and *Pseudomonas* spp.	Antibiosis	*Agrobacterium vitis*	Crown gall	*In planta*	Bell et al. (1995)
X. fastidiosa Syc86-1, EB92-1, PD95-6, PD91-2, EB92-1	Competition + ISR	*Xylella fastidiosa*	Pierce's disease	Field	Hopkins (2005)

biocontrol bacteria or production of antifungal metabolites have been discussed, as such mechanisms and modes of actions have been described for closely related *P. fluorescens* strains (Ziedan and El-Mohamedy 2008). Soil treatment of cv. Thompson Seedless with *P. fluorescens* NRC10 can significantly reduce additionally root rot percentage and disease severity in the field. It has been further shown that inoculation of *P. fluorescens* NRC10 on soil of grape plants induced an increase of fruit yield in an Egyptian vineyard (Ziedan and El-Mohamedy 2008). This demonstrates the potential of this isolate for application directly in the field.

Both examples cited before show that there are alternatives to pesticide use to control *Fusarium* sp. contamination on vine plants. However, considerable information is still required on how these strains can protect grape plants against root rot disease. In particular it is not clear at the moment if and which SMs are involved in the root rot inhibition. Additionally, activation of plant defense reactions leading to resistance may play a role in the reduction of the infection. It may be speculated that jasmonate and ethylene dependent induced resistance is important in enhanced grapevine resistance to Fusarium rot—at least after *P. fluorescens* treatment—since the contribution of these signal pathways in enhanced resistance in *Arabidopsis* after treatment with different *P. fluorescens* strains is well established (van der Ent et al. 2009; van Wees et al. 2008).

Biocontrol of fungal trunk diseases

Trunk diseases can be caused by various fungal taxa and have been widely reported as severe diseases infecting grapevine plants. The diatrypaceous fungus *Eutypa lata* (Pers.) Tul. & C. Tul. is known to cause one of the major symptoms, the Eutypa dieback. Other fungi of this family have been also shown to be associated with the disease, and have been isolated from necrotic tissues in shoots, at margin of canker in cordons, trunks, spurs or from surface of decorticated bark or wood of grapevines (Trouillas et al. 2010). Associated species are *Cryptovalsa ampelina* (Incertae sedis) (Nitschke) Fuckel, *Diatrype stigma* (Hoffm.) Fr. and *Eutypa leptoplaca* (Mont.) Rappaz causing vascular necrosis (Trouillas and Gubler 2010) as well as *Cryptosphaeria pullmanensis* Glawe, *Cryptovalsa ampelina*, *D. stigma*, *D. whitmanensis* J.D. Rogers & Glawe, and *E. leptoplaca* infecting and causing lesions in green shoots (Trouillas and Gubler 2004, 2010; Trouillas et al. 2010). Reassessment of concept of *Eutypa lata* has allowed to support that another associated fungus, *E. armeniaceae* Hansf. & M.V. Carter, is synonymous of *E. lata* (Rolshausen et al. 2006). Eutypa dieback results in significant economic damage on grapevine plants. Infected grapevines show a wedge-shaped staining of dead wood, gradually decline in productivity and eventually die. Dieback can also lead to stunted grapevine shoot, cupped and chlorotic leaves with necrotic margins, as well as to reduced qualitative yield productivities (Carter 1991; Kotze 2008).

Historically, management of Eutypa dieback relied on sanitary practices as well as the protection of the surface area of pruning woods by phytosanitary products (Carter and Price 1974; Rolshausen and Gubler 2005; Bester et al. 2007). At the moment, apart from fungicide use, various *Trichoderma* strains are in discussion as potential biocontrol agents for dieback (John et al. 2004; Halleen et al. 2010; Kotze 2008). However, also an endophytic strain of *Bacillus subtilis*, which was isolated from grape wood arm of cv. Chenin Blanc infected with *E. lata*, was under discussion as it can reduce the pathogen infection, colonization as well as the disease (Ferreira et al. 1991). This strain can inhibit mycelial growth, induce malformation of hyphae as well as reduce ascospore germination in in vitro tests indicating a direct antibiosis effect of the strain. Interestingly, it has been further demonstrated that spraying a suspension of this strain on grape wood reduces infection with the pathogenic agent (with a 100 % reduction; Ferreira et al. 1991). This demonstrates the potential of a beneficial endophytic bacterium to control *E. lata* infection. Other potential biocontrol bacteria also exist. Following the study of Ferreira et al. (1991), Munkvold and Marois (1993) tried to identify effective bacterial strains to control *E. lata* in the field. However, only a small fraction of three strains of more than 150 active strains in the laboratory on wood has been tested in the field in these experiments and tests failed to find a biocontrol agent (Munkvold and Marois 1993). In 2001, it has been demonstrated that 121 isolates (from different origins, belonging to Actinomycetes, *Bacillus* spp., *Erwinia herbicola* and *Pseudomonas* spp.) of 188 tested could exhibit antagonistic activity towards *E. lata* in vitro (Schmidt et al.

2001). One *B. subtilis* strain (B1α), two *E. herbicola* strains (JII/E2 and JII/E4) and one actinomycete (strain A123) have shown the highest degree of antagonism on grape wood discs. The use of such strains could allow a reduction of 70 to 100 % of the pathogen infection and its colonization over a four week period as demonstrated by the experiments. *Erwinia herbicola* JII/E2 and *B. subtilis* B1α inhibited growth of six different *E. lata* isolates on wood. Moreover, inhibition of the fungus by these strains correlated with a reduction in fungal hydrolase activity, which is highly correlated with mycelial growth in wood, demonstrating the strong ability of these strains to reduce *E. lata* growth and their potential for application (Schmidt et al. 2001). What could be verified is if bacterial biocontrol strains are also effective against *E. lata* in the field. Nevertheless, an effective biocontrol strain against Eutypa dieback has high potential in application, especially if this strain could also control a number of other fungi causing similar symptoms/other trunk diseases. These include for instance members of Botryosphaeriaceae.

Botryosphaeria dothidea (Moug.) Ces. & De Not., *Diplodia seriata* De Not., and *B. stevensii* Shoemaker are the cause of "Black Dead Arm" (BDA) in France (Larignon and Dubos 2001). The disease is characterized by wood streaking and red patches at the margin of the leaves, and large areas of chlorosis and deterioration between the veins (Larignon and Dubos 2001). However the occurrence of the Botryosphaeriaceae is not always linked to BDA disease. Virulence and symptoms of Botryosphaeriaceae have been reported as different according to cultivars and countries. For example, no symptoms of BDA were found associated with the same species on grapevines in Portugal (Phillips 2002). Nevertheless Botryosphaeriaceae members have been frequently isolated from grapevines showing decline or dieback symptoms in different regions/countries as in Egypt (El-Goorani and El Meleigi 1972), California, USA (Gubler et al. 2005), Arizona, USA, Mexico (Leavitt 1990), Europe (Hungary, France, Italy, Portugal, Spain; Rovesti and Montermini 1987; Lehoczky 1974; Phillips 1998; Larignon and Dubos 2001; Luque et al. 2005), South Africa (van Niekerk et al. 2004), Chile (Auger et al. 2004), and Australia (Castillo-Pando et al. 2001).

Although it is often difficult to distinguish symptoms of Botryosphaeriaceae from the ones caused by other fungal pathogens such as *E. lata*, *E. leptoplaca* and *Phomopsis viticola* (Sacc.), a number of different members have been associated with the disease such as *Diplodia seriata, Neofusicoccum australe* Slippers, Crous & M.J. Wingf., *B. dothidea, N. luteum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, *N. parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, *B. stevensii, Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. (Úrbez-Torres et al. 2006) and the anamorphs *Diplodia sarmentorum* (Fr.:Fr.) Fr., *D. porosum* Niekerk & Crous, *Fusicoccum viticlavatum* Niekerk & Crous, and *F. vitifusiforme* Niekerk & Crous (van Niekerk et al. 2004). Recent advances in control of Botryosphaeriaceae infection have shown that beneficial microbes could control some of the species mentioned above. In particular, in vitro assays have shown that the heat stable metabolites of *Bacillus subtilis* AG1 can inhibit the growth of *Lasiodiplodia theobromae* (Alfonzo et al. 2009). Recent screening also shows that a considerable number of bacterial isolates from the rhizosphere and/or endosphere from grapevine, as well as from harsh environments, can reduce in vitro growth of *D. seriatia* and *N. parvum* (unpublished information). However there is still as yet no work related to determine the potential of beneficial bacteria to control Botryosphaeriaceae infection in the field. This is partly due to the fact that beneficial bacteria acting as a biocontrol agent should not only reduce Botryosphaeriaceae infection but also other fungi responsible for trunk diseases.

Esca (also known as black measles in the USA) is attracting more consideration in viticulture and has long been considered a single disease, which normally affects adult or old vines. Although different fungi have been correlated with the disease, three main fungi, *Phaeomoniella chlamydospora* (W. Gams, Crous, M.J. Wingf. & Mugnai) Crous & W. Gams and *Phaeoacremonium aleophilum* W. Gams, Crous, M.J. Wingf. & Mugnai (corresponding to causal agents of petri disease) together with *Fomitiporia mediterranea* M. Fisch., have been mainly associated with esca (Surico et al. 2008). However these fungi can lead to five related syndromes. This forms the esca disease complex with potentially dramatic consequences up to death of the grapevine plant (Graniti et al. 2000). The syndromes are brown wood streaking of rooted cuttings, Petri disease with brown wood streaking in young vines, young esca (also recently called phaeotracheomicosis), white rot, and esca

proper (addition of young esca with white rot; Gramaje and Armengol 2011; Graniti et al. 2000; Mostert et al. 2006; Surico et al. 2008). The three main fungi *Pa. chlamydospora*, *Pm. aleophilum* and *F. mediterranea* are generally spread by spores released from infected vines or other host plants during wet conditions and are dispersed by wind currents. Infection on fresh pruning wounds is believed to be the main cause of entrance for fungi causing trunk disease symptoms (Graniti et al. 2000). Although some pesticides have been employed to reduce infection of these diseases, commercial use has been restricted and has been disputed in the case of the use of sodium arsenate (Chiarappa 2000). Researches on biocontrol agents have started to find alternative strategies to reduce petri disease, young esca, white rot and esca proper. This has been concentrated on beneficial fungi such as *Trichoderma* spp. strains (Fourie and Halleen 2006; Halleen et al. 2010; Kotze 2008), but beneficial bacteria have been studied as well. In particular, in vitro assays have shown that metabolites of *Bacillus subtilis* AG1 described above can—in addition to *Lasiodiplodia theobromae*—inhibit the growth of fungi involved in trunk diseases such as *Pm. aleophilum*, and *Pa. chlamydospora* (Alfonzo et al. 2009). Other bacteria are currently being tested as biocontrol agents to reduce diseases caused by the fungi (unpublished results). Although the first results in lab showed promising findings to protect the decline of vine resulting from trunk diseases, more work is required for the use of such strains or metabolites. Especially, additional testing in plants and long term management in the field is needed to ensure the required level of protection.

Searching the mechanism involved is needed for a better delivery of bacterial inoculants and for the application of bacterial metabolites in the field. Some of those so far tested biocontrol strains and their metabolites seem to have a direct effect on the growth of fungi in grapevine wood, either by growth inhibition or by inhibition of fungal enzymatic activities. What should be studied further is how far activation of plant defenses is also playing a role in bacterial biocontrol of trunk diseases. Search for strains with the potential to degrade phytotoxic disease factors of Eutypa dieback and esca disease pathogens (Christen et al. 2005) might provide an additional strategy to control trunk diseases. Since only limited means for the control of trunk disease exist, development of bioncontrol strains will be an important factor in the future for controlling trunk disease in viticulture.

Biocontrol of grey mould caused by *Botrytis cinerea*

Grapevine is not only infected by fungi affecting trunks and roots, but also by fungi deteriorating fruit setting and quality such as *Botrytis cinerea* Pers. (Sclerotiniaceae). *B. cinerea* is known to be responsible for grey mould and Botrytis bunch rot affecting young fruit, during the ripening process and making the grapes inappropriate for wine making. The potential of specific strains to control grey mould has been demonstrated by a number of beneficial bacteria. Strains belonging to Pseudomonadaceae, Bacillaceae, Enterobacteriaceae, Actinobacteria as well as Burkholderiaceae have been shown to have a positive effect on grey mould control (Compant et al. 2011).

An endospore forming bacterial strain (GI 070) belonging to the species *Bacillus circulans*, was described as antagonistic to *B. cinerea* (Paul et al. 1997). The bacterial culture and its filtrate can completely suppress the fungus in Petri-dishes and reduce grey mould symptoms on grapevine in vitro plantlets (Paul et al. 1997). In another study, Krol (1998) studied 17 isolates on 282 leaf-derived endophytic strains exhibited antagonistic activities to *B. cinerea*. However, only two isolates (one *Bacillus* sp. and one *P. fluorescens* strain) limited the disease development on grapes (Krol 1998). Both studies demonstrate that different bacteria have the potential to control grey mould symptoms on grapes, but also show that in vitro antagonistic activities have limited prediction in activities *in planta* and that induced plant resistance might play a major role in the observed effects.

In another study the potential of different bacteria isolated from the rhizosphere or the endosphere of different plant parts of healthy field-grown grapevine plants cv. Chardonnay was evaluated for biocontrol of grey mould symptoms (Trotel-Aziz et al. 2006, 2008). Twenty-six out of 282 bacterial strains, all of them isolated from vineyards and belonging to *Pseudomonas, Bacillus, Acinetobacter* and *Pantoea* demonstrated protective activity (85–100 %) against *Botrytis cinerea* on dual culture plates. The biocontrol activity of the bacteria *Pseudomonas fluorescens* PTA-268, PTA-CT2, *Bacillus subtilis* PTA-271,

Pantoea agglomerans PTA-AF1 and PTA-AF2, as well as *Acinetobacter lwoffii* PTA-113 and PTA-152 was moreover demonstrated on in vitro plantlets cv. Chardonnay. Differential induction of defense-related responses such as lipoxygenases, phenylalanine ammonia-lyases and chitinases in grapevine leaves was correlated with the protection (Trotel-Aziz et al. 2006, 2008). Moreover treatment with the strains *P. agglomerans* AF2, *B. subtilis* 271, *A. lwoffii* 113 and *P. fluorescens* CT2 enhanced oxidative burst and production of the phytoalexin resveratrol in grapevine leaves, which was well correlated with the enhanced resistance to *B. cinerea* (Verhagen et al. 2011). Verhagen et al. (2010) also showed that *Pseudomonas aeruginosa* (7NSK2), *P. fluorescens* (strains CHA0, Q2-87 and WCS417) and *P. putida* (WCS358) could induce resistance to *B. cinerea* in grapevine, which was correlated to a different extent with phytoalexins and oxidative burst production. The authors showed that inducing resistance in the plant is a major mechanism observed in protection against *B. cinerea* and also demonstrated that the bacterial metabolites salicylic acid (SA), 2,4-diacetylphloroglucinol (DAPG), pyochelin and pyoverdin contributed to this resistance, but are not the only chemical factors involved.

In field experiments during four consecutive years, the potential of the beneficial strains described before were also demonstrated, and the severity of grey mould disease on grapevine leaves and berries was reduced (Magnin-Robert et al. 2007). This was correlated to different levels of protection, depending on the bacterial strain used (in total seven) and of the inoculation method (Magnin-Robert et al. 2007). The state of plant resistance was associated with a stimulation of plant defense responses such as chitinase and β-1,3-glucanase activities (with known botryticidal activities) in both leaves and berries (Magnin-Robert et al. 2007), again indicating a major contribution of enhanced plant resistance in response to the biocontrol strains. Highest activities were, however, dependent on plant organs. *Acinetobacter lwoffii* PTA-113 and *Pseudomonas fluorescens* PTA-CT2 showed highest protection in leaves, and *A. lwoffii* PTA-113 or *Pantoea agglomerans* PTA-AF1 in berries, suggesting that different strains can be more appropriate for treatment of specific organs (Magnin-Robert et al. 2007).

Use of the endophytic plant growth-promoting bacterium, *Burkholderia phytofirmans* strain PsJN (Sessitsch et al. 2005), isolated from onion root infected with *Glomus vesiculiferum* in Germany (Nowak et al. 1995) has been demonstrated as enabling the reduction of infection of *B. cinerea* on grapevine plants (Ait Barka et al. 2000, 2002). It has been additionally shown that this β-proteobacterium could improve host plant growth as well as establishes rhizospheric and endophytic subpopulations in various organs and systemically spreads inside grapevine plants (Compant et al. 2005b, 2008a, b). Although no experiment was done in the field to evaluate the potential of such strains under natural conditions as well as its persistence inside soil and internal tissues during a long period, a recent study has demonstrated that the species *B. phytofirmans* could be naturally present in the vineyard (Lo Picollo et al. 2010). It can establish subpopulations in leaves of grapevine plants as demonstrated in Italy (Lo Picollo et al. 2010) and could therefore be used for application on grape although this needs to be tested under field conditions.

Attempts to use members of the Actinomycetales such as *Streptomyces* spp. or *Micromonospora* spp. to control *B. cinerea* have also been studied (Loqman et al. 2009; Lebrihi et al. 2009a, b). Some soil strains of these bacteria can allow grapevine in vitro plantlets to withstand grey rot (Loqman et al. 2009). Experiments corresponding to the use of other *Streptomyces* sp. strains have also shown that a protection can occur under greenhouse conditions (Lebrihi et al. 2009a, b). Moreover, cyclic bacterial metabolites (tetracyclopeptides) secreted by these latter strains can induce protection directly by antibiosis or indirectly by inducing various plant defense responses leading to protective effects (Lebrihi et al. 2009a, b). However, due to large arrays of various Actinomycetes secreting bio-active compounds, further experiments need to be conducted with attempts to find new bioactive compounds as well as new strains for *B. cinerea* control.

Research on new elicitors secreted by bacteria has recently also demonstrated that not only microbes can reduce infection *B. cinerea* but also their SMs alone. Glycolipids biosurfactants such as rhamnolipids secreted by *Pseudomonas aeruginosa* used in food protection, in cosmetology and for industrial applications can reduce grapevine disease such as the Botrytis rot. The effect of rhamnolipids was recently assessed on *B. cinerea* as well as on grapevine using cell

suspension cultures and in vitro-plantlets of cv. Chardonnay (Varnier et al. 2009). Rhamnolipids can have direct antifungal properties by inhibiting spore germination and mycelium growth of the fungus. They can also efficiently protect grapevine against the disease. Defenses were associated to a Ca^{2+} influx, mitogen-activated protein kinase activation and reactive oxygen species production as early events (Varnier et al. 2009). Induction of plant defenses including expression of a wide range of defense genes, hypersensitive response (HR)-like response explained parts of the mechanisms involved in plant resistance. Additionally, rhamnolipids potentiated defense responses induced by chitosan elicitor and by the culture filtrate of *B. cinerea* (Varnier et al. 2009), suggesting that the combination of rhamnolipids with other effectors could participate in grapevine protection against the grey mould disease.

A recent study demonstrated another possibility to control *B. cinerea* caused diseases. An important virulence factor of *B. cinerea* with broad activity is oxalic acid. Schoonbeek et al. (2007) therefore investigated an interesting approach to reduce *B. cinerea* caused symptoms by looking for bacteria capable of degrading oxalic acid. The authors found an active oxalic acid degrading strain named oxB, which is closely related to *Cupriavidus campinensis.* Strain oxB could limit grey mould symptoms on leaves and strongly reduce disease symptoms in inflorescences under laboratory conditions.

In summary, biocontrol of *B. cinerea* by beneficial bacteria seems to be achieved mainly by activation of induced resistance in the plants. A number of strategies using beneficial bacteria to fight *B. cinerea* are in discussion and application potential seems to be higher than for the other diseases discussed. However, this is partly owed to the fact that the *B. cinerea* phytopathosystem is easy to study under laboratory conditions. Widening the search for new active strains and bacterial metabolites should allow developing an even broader portfolio of biocontrol strains, which would allow a more stable usage under different conditions, with different cultivars as well as allowing a better rotation system to overcome reduction of efficiency.

Biocontrol of powdery mildew (*Erysiphe necator*)

Powdery mildew of grapevines (*Erysiphe necator* Schw., syn. *Uncinula necator*, anamorph *Oidium tuckeri*) spread from America to Europe in the mid of the nineteenth century has ever been since a serious issue for the European wine industry causing loss and diminished quality of grapevine fruits. *E. necator* is known as infesting all green tissues and typically grows in round areas on young leaves, which become chlorotic and can become senescent and fall off prematurely. Inflorescences and young berries may become completely covered by the mildew (Gadoury et al. 2012; Pearson and Goheen 1988). Elder berries become more resistant to *E. necator*, but even low number of *E. necator* might have an effect on subsequent grey mould infestations (Ficke et al. 2002). Control of *E. necator* is mainly achieved by the use of an array of fungicides, but also by a number of inorganic substances, above all sulphur. Attempts to use biological control include various fungi, parasitic fungi such as *Ampelomyces quisqualis* and the mycophagous mite *Orthotydeus lambi* (Gadoury et al. 2012; Kiss 2003). However, bacteria such as some *Bacillus* strains have been tested for their capability to restrict the growth of *E. necator*. Seedlings of cv. Chardonnay were protected by *B. pumilus* B-30087 almost as effectively as the chemical fungicide myclobutanil at 25 ppm, althought in vitro growth of a number of different fungi was not affected by this bacterium. This indicates either a specific direct inhibition mechanism or a defense activation effect allowing the plant to successfully combat *E. necator* infections. It has been suggested therefore that a water soluble antifungal metabolite smaller than 10000 Daltons and different from zwittermicin A may play a role in the effects of *B. pumilus* B-30087 (Lehman et al. 2000).

Other *Bacillus* strains have also been patented to fight against *E. necator*. The *Bacillus* strains ATCC 55608 and 55609 were almost as effective against *E. necator* as metalaxyl in assays in grapevine plants. These strains produce antifungal substances including zwittermicin-A, which might play a vital role in the interaction (Marrone et al. 1999). More recently, Sawant et al. (2011) conducted field studies with Milastin K, a formulation of *B. subtilis*, over three years with cv. Thompson seedless. They observed that under low and medium *E. necator* pressure the pathogen could be controlled effectively, while under high pathogen pressure the effect was not as effective as sulphur.

While putatively effective and good candidates are known for bacterial biocontrol of *E. necator,* what remains to be studied is whether this can compete with

cheap and effective sulphur treatments. However, *Bacillus* strains and bacterial SMs acting as bioeffectors may also have the advantage to be used in combination with synthetic or inorganic antifungal compounds. These combinatory applications are however more difficult with sensitive mycophagous mites and parasitic fungi.

Biocontrol of downy mildew (*Plasmopara viticola*)

Plasmopara viticola (Berk. and Curt.) Berl. and de Toni is another problematic grapevine pathogen introduced to Europe from America in the second half of the nineteenth century. It is the causative agent of downy mildew resulting in severe losses in grapevine production especially in more humid areas of Europe and North America. Pathogen infection results at first as yellow spots on leaf surfaces and growth of sporophores on the opposite lower leaf sides can be observed. Later on, it can cause losses through defoliation and killing of shoots and deteriorating fruit quality. In favorable weather conditions and without protective measurements losses may rise up to 75 % (Gessler et al. 2011; Pearson and Goheen 1988). *P. viticola* is an oomycete and relies as such on a zoospore stage, at which grapevine plants are invaded via stomata (Riethmueller et al. 2002). This entry mechanism may play a role in the effectiveness of biological control of the disease with oligosaccharides such as oligogalacturonides (OGA), which affects stomata regulation. Nevertheless other defense mechanism must be induced by certain oligosaccharides since PS3 (sulfated laminarin) induces protection to *P. viticola* but does not affect stomatal closure (Allègre et al. 2009). Also bacteria and their SMs have been patented as potential inhibitors of oomycetes including *P. viticola*. The effect of *Serratia marcescens* MSU-97 specifically on oomycetes have been shown in vitro. The active SM is a small cyclic peptide named serratamolide with membrane activity inhibiting oomycetes (Strobel et al. 2005). More recently, a terrestrial actinomycete, *Streptomyces* sp. ANK313 was shown to produce the chinone khatmiamycin, which shows motility inhibition and causes lysis of zoospores of *P. viticola* (Abdalla et al. 2011). It remains to be seen if these and other beneficial bacteria also have a positive effect on downy mildew control *in planta* and in vineyards and if biocontrol strains can also boost grapevine defenses against *P. viticola.* Future applications of any of the biocontrol measurements can help to reduce the intensive use of copper and pesticides required for downy mildew control.

The majority of information on bacterial biocontrol of diseases caused by fungi and oomycetes can be found for grey disease caused by *Botrytis cinerea*. This does not necessarily reflect a limitation of the use of bacterial biocontrol for severe grapevine diseases such as powdery mildew, downy mildew and trunk diseases, but might also simply reflect the easiness of screenings for activity against *B. cinerea* and the widespread use of *B. cinerea* as test fungus in a number of laboratories. Future research for the use of bacteria for biocontrol should also focus on downy mildew and trunk diseases. Of course, different types of strains might be effective against these pathogens, also due to their different life conditions and location *in planta*, but for a broader practical application of biocontrol strains a wider portfolio and/or combinatory use of strains with the ability to control major grapevine diseases are necessary.

Beneficial bacteria and biocontrol of grapevine bacterial diseases

In addition to phytopathogenic fungi, bacteria infecting grape plants are the causal agents of severe diseases: *Agrobacterium vitis* causes crown gall (Sule and Burr 1998; Stafford 2000; Escobar and Dandekar 2003), *Candidatus* Phytoplasma vitis and *Ca.* Phytoplasma solani cause flavescence dorée (FD) and bois noir (BN) (Constable 2010), *Xylophilus ampelinus* arms bunches (Ridé 1996) and *Xylella fastidiosa* causes Pierce's disease (Hopkins 1989). Although different strategies have been used to control them, research of biocontrol agents to control these vine diseases has shown the potential of different bacterial strains to reduce bacterial infections (Table 1, Fig. 1). This is especially important for bacterial diseases that are difficult to treat with conventional pesticides and localized in the phloem or xylem vessels.

Biocontrol of *Agrobacterium vitis*

Crown gall disease of grapevines occurs especially in climates where cold winter temperatures can cause

wounds, which are the main entry points for the pathogen. The disease incidence can be very high in affected vineyards and nurseries resulting in reduced growth and potentially the death of the plants (Burr and Otten 1999; Creasap et al. 2005). Currently few strategies for disease management of *A. vitis* exist. As an example for biocontrol of bacterial disease, a non-tumorigenic strain (F2/5) of *Agrobacterium vitis* has been shown to inhibit the in vitro growth of 21 of 25 *A. vitis* and two of ten *A. tumefaciens* biovar 1 pathogenic strains (Burr and Reid 1994). When applied to wounds on potted woody grape trunks (*Vitis vinifera* L. cvs. Chardonnay and Riesling) in the greenhouse, the gall sizes were moreover significantly reduced for seven of ten *A. vitis*, one of two *A. tumefaciens* biovar 1 and one of one biovar 2 strains, demonstrating the potential of a non-tumorigenic strain for field application. Co-inoculation of F2/5 with the pathogen was moreover at least as effective as pre-inoculation with F2/5. When the pathogen was inoculated prior to F2/5, the level of control was however greatly reduced (Burr and Reid 1994). However, caution should be taken in the application of strains belonging to species containing pathogenic strains. Burr and Reid (1994) demonstrated that the biocontrol strain was non-tumorigenic and that none of the three plasmids of strain F2/5 can hybridize with a probe consisting of the T-DNA from *A. tumefaciens* strain C58. However, the use of close relatives of pathogenic strains for biocontrol presents the risk that non-pathogenic biocontrol strains might mutate or acquire virulence plasmids, especially if the exact mechanisms of protection are not well understood (Seemüller and Harries 2010).

To investigate the mechanisms involved in biocontrol by the strain F2/5, agrocin-minus mutants were constructed. The mutants of strain F2/5 controlled grape crown gall as well as the wild-type strain (Burr et al. 1997), indicating that agrocin is not a major factor in the mechanism of biological control. Tumorigenic *Agrobacterium* strains attach to grapevine cells before infection. Therefore a competition of biocontrol strains for attachment sites may reduce infection pressure of pathogenic strains (Shim et al. 1987). Attachment of tumorigenic strains (CG49 and K306) and biological control strains (F2/5 and the agrocin-minus mutant 1077) was also often reduced when mixtures of the strains were applied. However, high concentrations of all strains attached suggest that competition for attachment sites is not a factor involved in the mechanism of biological control (Burr et al. 1997). Transfer of T-DNA to grape by CG49 was prevented or greatly inhibited in the presence of F2/5 or 1077, although the Ti plasmid virulence genes of the phytopathogens were induced by exudates from grape shoots that had been previously inoculated with F2/5 (Burr et al. 1997). Alternative mechanism of plant protection by non-tumorigenic strains might include induced resistance of the plants or bacterial signal interference. Although the mechanism of how F2/5 could control crown gall clearly needs further investigation, non-pathogenic *Agrobacterium* strains promise interesting strategies to control the disease.

Other non-tumorigenic strains have also been used on grapevine plants such as *Agrobacterium vitis* strain E26 or VAR03-1 (Kawaguchi et al. 2007, 2008; Wei et al. 2009). In biological control tests strain VAR03-1 was especially effective in reducing the incidence of gall formation on grapevine and reduced gall size by 84–100% in comparison to the positive control (Kawaguchi et al. 2005, 2007, 2008). To minimize the potential risks of using biocontrol *Agrobacterium* strains, polymerase chain reaction and Southern blot analyses were used to determine that five essential virulence genes (*virA*, *virG*, *iaaH*, *iaaM* and *ipt*) were not present in strain E26 controlling crown gall disease (Wei et al. 2009). This suggests that this strain is unlikely to elicit crown gall symptoms in either host or non-host plants.

Not only non-tumorigenic strains of *Agrobacterium* spp. could control crown gall disease, but also strains from other taxa. *Pseudomonas aureofaciens* B-4117, *P. fluorescens* CR330D and 1100-6, *Bacillus subtilis* EN63-1, *Bacillus* sp. EN71-1, as well as *Rahnella aquatilis* HX2, can inhibit for instance the growth of a wide range of plant pathogens, including *A. tumefaciens*, when tested on agar media or on grapevine plants. The *P. aureofaciens* strain B-4117 persisted moreover on the root surfaces of inoculated vine cuttings and in non-sterile soil (Khmel et al. 1998). In growth chamber studies, *P. fluorescens* '1100-6' that reduce crown gall disease was also found to survive on the rhizoplane of grapevines for six months and predominantly occupied xylem and pith tissues (Eastwell et al. 2006), demonstrating a rhizo- and endosphere competence of this beneficial strain. With *Rahnella aquatilis* HX2, it has been shown in field trials that immersion of the basal ends of grape cuttings with HX2 cell suspension inhibited or even

completely prevented crown gall formation caused by *A. vitis* K308 (30.8 % compared to 93.5 % in plants without HX2). Strain HX2 was found in the grape rhizosphere, grown under field conditions, for up to 90 days after inoculation and did not influence the mean population sizes of selected members of the microflora (Chen et al. 2007).

The production of an antibacterial substance ("ABS") was suggested to be an important factor in the biocontrol process by strain HX2 used to control crown gall as described by Chen et al. (2009) and Guo et al. (2009). ABS is a thermostable and alkali-sensitive substance containing sugar(s) and an unknown moiety with an absorption maximum at 285-nm. ABS displays a broad activity spectrum against 13 test isolates of phytopathogenic bacteria including *Agrobacterium*. *Agrobacterium* spp. strains were additionally more sensitive to ABS than other tested strains, with larger inhibition zones and lower minimal inhibitory concentration. The metabolite did not cause bacterial cell lysis, no leakage of cytoplasmic materials from cells of *A. vitis* but it rather inhibits RNA and protein synthesis in tumorigenic *A. vitis* (Chen et al. 2009).

Although the extent of disease control depends on the grape variety tested, the results suggest that there is potentially beneficial effect in using the antagonists to diminish the influence of latent rootstock infection of crown gall. Other bacteria preventing crown gall of grapevine are endophytes of xylem sap of vine plants grown in Nova Scotia, Canada. Despite variation was noted in performing in vitro antibiosis, 24 strains were catalogued to have a strong inhibitory effect on *A. vitis* (Bell et al. 1995). This includes strains of *Enterobacter agglomerans*, *Rahnella aquatilis*, and *Pseudomonas* spp. Soil microcosm studies with a *xylE*-marked *A. vitis* strain showed in particular that one of these endophytes (an isolate of *P. corrugata*) is able to control population numbers of agrobacteria *in situ*. *In planta* trials with *V. vinifera* cv. Chardonnay showed that less than 47 % in comparison to the positive control treatment produced galled vines, demonstrating significant biocontrol of the disease by three of the endophytes (Bell et al. 1995).

Biocontrol of grapevine yellows caused by phytoplasmas

In grapevine, infections with phytoplasmas 16S rDNA group I, II, III, V and XII-A and XII-B corresponding to different *Candidatus Phytoplasma* species have been described and economically most important are *Ca. Phytoplasma australiense* (16S rDNA group XII-B) causing Australian grapevine yellows, *Ca.* Phytoplasma solani (XII-A, Stolbur) causing bois noir (BN) and *Ca.* Phytoplasma vitis (V) causing flavescence dorée (FD) (Constable 2010). In Europe, BN and FD frequently occur in wine producing countries. Infection of plants results in reddening (red varieties) or yellowing of leaves, backward curling of leaf edges, shoots failing to harden off, shoots may die back and berries may shrivel and dry early. BN and FD are transmitted by phloem sucking insects, but with distinct epidemiology. FD is transmitted by the leafhopper *Scaphoideus titanus*, which is monophagous on grapevine in Europe and can transmit FD from grapevine to grapevine. BN on the other hand is transmitted by the planthopper *Hyalesthes obsoletus*, not able to fulfill a lifecycle on grapevine. The insects feed on herbs including nettle and bindweed, which are believed to be the main reservoir hosts of BN. Transmission to grapevine from these hosts is believed to be rather an accident (Constable 2010; Maixner 2011). Alternative vectors of BN have however also been discussed (Constable 2010; Riedle-Bauer et al. 2008). The different epidemiology has an impact on disease management, which relies on viticultural practices and insecticide treatments to reduce vector pressure, since no practical methods except the largely banned and expensive antibiotic treatments are available to treat Phytoplasma infected plants at the moment.

A potential mechanism of how bacterial diseases can be controlled is by cross protection with mild or avirulent strains of the disease causing agents (Seemüller and Harries 2010). Such cross protection with avirulent strains has been observed with phytoplasma (*Ca. Phytoplasma prunorum*) infected apricots, where infections with avirulent or mild strains seem to have a pre-immunizing effect (Seemüller and Harries 2010), either competing with disease causing phytoplasmas or enhancing the resistance of colonized plants. Given the risks of such cross protection and the limited knowledge how cross protection is achieved, application of this strategy is limited. Nevertheless there is an interest for such biocontrol applications in bacterial diseases difficult to control, especially in areas where disease pressure is very high.

Established beneficial bacteria like *Bacillus* spp. or *Pseudomonas* spp. cannot directly compete with phytoplasmas due to their different *in planta* location.

However, since beneficial bacteria can prime plants and may induce resistance to a wide array of pathogens (Kloepper et al. 2004, van Loon 2007), an effect on phloem colonizing phytoplasmas can also be expected. In this respect it is interesting to note that in all grapevine yellows, spontaneous remission and recovery has been described (Constable, 2010). Bulgari et al. (2001) recently demonstrated that lower diversity of endophytic bacteria exists in Phytoplasma infected leaves of grapevine plants. This can be the results of a direct interaction between phytoplasmas and endophytic bacteria or a phytoplasma mediated plant response that restructured endophytic bacterial community. Isolation of endophytic bacteria in healthy, or especially in plants showing remission and their uses on grapevine could be therefore interesting for biocontrol of the disease.

Repeated biocontrol treatment with various inducers of plant resistance such as benzothiadiazole and glutathione/oligosaccharines mixtures lead to enhanced remission in BN affected grapevines (Romanazzi et al. 2009). Very recently, the concept of inducing enhanced resistance to phytoplasma with beneficial bacteria has been evaluated using *Chrysanthemum* as model organism. Results showed that pretreatment with *Pseudomonas putida* S1Pf1Rif decreases the negative effects on plant growth infected with chrysanthemum yellows phytoplasma (CYP), but had no effect on CYP viability and proliferation (Gamalero et al. 2010). A combination treatment of *P. putida* S1Pf1Rif and the fungus *Glomus mossae* BEG12 resulted in slightly increased resistance and a delay of symptoms in CYP infected and non-resistant plants (D'Amelio et al. 2011). *G. mossae* could also reduce symptoms of the stolbur phytoplasma causing BN in grapevine in tomato (Lingua et al. 2002). It would be interesting to see if beneficial microorganisms also have an effect on symptom reduction of phytoplasma disease in grapevine plants under greenhouse and field conditions.

Biocontrol of *Xylella fastidiosa*

Pierce's disease has been well described in South-Eastern US and occurs in several regions in North and Central America (Hopkins 2005). The causal agent of this disease is *X. fastidiosa*, which colonizes intensively xylem vessels after being transmitted by a sharpshooter (Cicadellidae). Symptoms on affected grapevines include yellow and brown color on leaves and eventually a sudden collapse of the foliage or a gradual death over a period of one to five years after plantation, with strong impact on the ability to produce wine in the affected regions (Almeida et al. 2005; Baumgartner and Warren 2005; Chatterjee et al. 2008; Hopkins 2005). This has lead to study potential solutions for control.

Several strains of avirulent endophytic *X. fastidiosa* can provide reduction in symptom development as described with cv. Carignane in greenhouse and field experiments (Hopkins 2005). In a two-year assay on cv. 'Himrod' in the vineyard, strain Syc86-1 (isolated from sycamore), but not strain PD-1 (derived from grapevine), was effective in limiting the development of Pierce's disease. In tests on new vineyard plantings of cv. Flame Seedless and cv. Cabernet Sauvignon, six non pathogenic strains of *X. fastidiosa* were evaluated for biological control of the natural progression of Pierce's disease (Hopkins 2005). However, only one strain (EB92-1) provides good control of the disease. Genome sequencing of strain EB92-1 revealed its very close resemblance to pathogenic *X. fastidiosa* strains, but lacks ten putative virulence genes (Zhang et al. 2011). Grape strain PD95-6 showed lower disease severity in Flame Seedless when compared with non-treated vines. Strain PD91-2 delayed symptoms in Cabernet Sauvignon for 12 to 18 months, and strain EB92-1 (isolated from elderberry) but not strain Syc86-1 indeed allowed reduction of the disease in both cultivars. Biological control by inoculation of susceptible grapevines with benign strains of *X. fastidiosa*, especially strain EB92-1, appears therefore to possibly control Pierce's disease in commercial vineyards in Florida, USA as well as in other areas (Hopkins 2005) where the disease occurs or could appear in the future. However the use of avirulent strains closely related to pathogenic *X. fastidiosa* strains cross protecting grapevine against Pierce's disease might bear risks as avirulent strains may mutate or acquire virulence genes. In areas such as the southeastern United States, where Pierce's disease strongly limits grapevine production (Hopkins 2005), these risks might be acceptable.

Several biocontrol agents have been tested or are under consideration for biocontrol of the discussed bacterial diseases. The effect of avirulent strains of these pathogens might be the result of niche competition and/or interference of signals with aggressive

pathogens strains. Alternatively and additionally, effects of these biocontrol strains on enhanced plant resistance and plant immunity must be taken into consideration. This type of mechanism is also more likely involved in the biocontrol ability of bacteria inhabiting distinct habitats in the plant than the respective plant disease causing bacteria. Little evidence exists so far for direct antibiotic effects of biocontrol SM on bacterial pathogens inside plants. However SMs might also change plant defense mechanisms leading to altered resistance to bacterial pathogens. Future research will show which of the discussed mechanism is of major importance for application of biocontrol strains in the control of bacterial grapevine diseases.

Conclusions and future prospects

Considerable information on the possibility to use biocontrol agents of bacterial origin to fight a variety of grapevine diseases affecting yield and productivity has become available. In this review we focused on fungi responsible for trunk diseases, root rot by *F. oxysporum*, grey mould induced by *B. cinerea,* powdery mildew caused by *Erysiphe necator,* downy mildew caused by the oomycete *Plasmopara viticola* as well as on the bacterial pathogens *X. fastidiosa*, *Ca. Phytoplasma* spp. and *A. vitis*. Continuous research for effective beneficial bacteria, associated SMs and study of their mechanism is very important to allow the development of effective biocontrol agents and to allow sufficient disease management for these and other grapevine diseases in viticulture. There are not enough examples of biocontrol agents and SMs used for grapevine in our opinion. A current need for practical use of beneficial bacteria or their metabolites corresponding to a portfolio of different products would allow a more efficient disease treatment. The research for mechanisms involved can be of high importance for a better understanding of the processes involved and should subsequently also lead to better applications in disease management. Only few mechanisms enabling vine plant resistance have yet been demonstrated. For a number of bacterial metabolites their antifungal or antibacterial properties to vine pathogens have not even been tested yet. Additionally studying effect of new biocontrol bacteria as well as new metabolites having the abilities to control crop disease or to stimulate plant defense reactions is of special importance for fundamental knowledge and development. In case of a climate change scenario (Compant et al. 2010b), some strains isolated from desert soil can be promising agents as they are adapted to more extreme conditions (unpublished results). However, the colonization process, the persistence in soil, as well as the mechanisms allowing host plant protection should be obligatorily studied before field delivery and marketing.

A natural microflora can inhabit the vine host plants, both in the rhizosphere and the endosphere of various plant organs. Any application of specific microbe(s) should lead to study its behaviour inside grape plants and also the interaction with the natural microflora. The intensive use of pesticides in viticulture may also have a strong impact on endophyte composition. Nevertheless the aspect of potential alteration of microflora by biocontrol agents shall not be neglected. All these aspects should be considered for both fundamental knowledge in beneficial bacteria-plant interactions as well as for further improvement of bacterial biocontrol in the vineyard, i.e. for a sustainable management of viticulture.

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