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Review Article

Biological Control Potential of *Aneurinibacillus migulanus* against *Phytophthora* Species

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ABSTRACT

Aneurinibacillus migulanus strains show potential in biocontrol against a wide range of plant pathogens, including Ascomycota, Basidiomycota and Oomycetes. However, suggested that production of a single antibiotic cyclic peptide, gramicidin S (GS), by the two strains, is not the sole mechanism of inhibition but the combination between the antibiotic and biosurfactants activity together. The sequenced genomes of *Aneurinibacillus* prompted us to apply genome mining techniques to identify the bioactive potential of A. *migulanus* to provide insights into the secondary metabolite arsenal of the genus *Aneurinibacillus*. Eleven secondary metabolite biosynthetic gene clusters were detected in the *Aneurinibacillus* species. These secondary metabolites play an important role in inhibition the growth for a wide range of plant pathogens and their ability to enhance the activity of antibiotics that has been improved in many studies.

Other biosynthetic gene clusters have been detected that may have a significant effective against different pathogens such as bacteriocins, microcins, non-ribosomal peptides, polyketides, terpenes, phosphonates, lasso peptides and linaridins. Chitinolytic potential and iron metabolism regulation also needed more investigation. With increasing numbers of biocontrol bacterial genomes being sequenced and mined, the use of approaches similar to those described in this paper will lead to an increase in the numbers of environmentally friendly natural products available to use against plant disease.

Keywords: Biological control; *Phytophthora spp*; *Aneurinibacillus migulanus*; Plant pathogens; Secondary metabolites

INTRODUCTION

Oomycete pathogens are present in a broad range of environments and are responsible for some of the most economically damaging diseases of both animals and plants [1,2]. This class in the Stramenopila is characterized by an ability to reproduce rapidly under suitable conditions [2]. Motile zoospores released from sporangia, disperse in water and infect suitable host tissues in the surrounding environment [3].

The genus *Phytophthora* includes many species that cause serious plant diseases [4]. Within the genus, 10 clades have been identified using molecular techniques and phylogenetic analyses [5]. Many *Phytophthora* species can, under conditions conducive to pathogen growth, have devastating impacts on agricultural and horticultural crops, and on natural ecosystems [3]. Therefore, treatments are urgently required for the long-term management of the damage caused by these organisms. Although some agrochemicals are active against *Phytophthora* spp., it is desirable to develop sustainable treatments that are more environmentally friendly than xenobiotic chemicals. For these reasons, research on the potential use of naturally occurring bacteria and fungi for use as Biological Control Agents (BCAs) against plant diseases is common [6]. Certain BCAs are known to reduce the impacts of diseases caused by *Phytophthora* species [5]. For example, *Bacillus altitudinis* JSCX-1 effectively inhibited mycelial growth and zoospore germination of *Phytophthora sojae* through increasing the production of reactive oxygen species and callose deposition in soybean leaves [7,8].

Although some apparently effective fungal antagonists of plant diseases are known, bacterial antagonists have, arguably, shown more promising results to date. Bacteria in the genus *Bacillus* are active against *P. infestans* either directly, through antibiosis, or indirectly, through the induction of plant defence systems [9,10]. Several studies also highlighted the potential of *Bacillus* spp. for the suppression of *Phytophthora capsici* in various crops [11-13].

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LITERATURE REVIEW

The mechanisms of action of BCAs are poorly understood. Biological-control treatments provide disease protection in three main ways: (a) production of antibiotics or other molecules that are deleterious to pathogen development, (b) competition with pathogen for nutrients and space, and (c) induction of plant resistance [10]. In addition, BCAs may produce a variety of polysaccharide degrading enzymes active against the pathogen cell wall and can also form large multicellular assemblages known as biofilms, which may present a physical barrier to ingress of pathogens [14,15].

Several *Bacillus* species are thought particularly suitable for BCAs, as different isolates within the genus have shown marked antagonistic activity against a large range of plant pathogens, including fungi, oomycetes and bacteria [6,16-18]. Many *Bacillus* spp. are known to produce antibiotics and lipopeptide biosurfactants active against plant pathogens, including oomycetes [19-21]. For example, *B. amyloliquefaciens* PG12 produced lipopeptides that showed antagonist activity against *Phytophthora infestans* and *Phytophthora capsica in vitro* assay [22].

Species of *Bacillus* are well-known for inhibitory activity against plant pathogens [6]. For example, many strains of *Bacillus subtilis* are known to inhibit mycelial growth of fungal pathogens *in vitro* [23-25] through the production of antibiotics, including biosurfactants such as surfactin. *Bacillus subtilis* strain GA1 inhibited mycelial growth of *Botrytis cinerea* by up to 70% [24]. Also, B. *subtilis* ME488 showed an inhibition in *in vitro* inhibition assays for *Phytophthora* cactorum, *Phytophthora cambivora*, *Phytophthora capsici*, *Phytophthora cinnamomi* and *Phytophthora* cryptogea [26]. Similar results were obtained, where two strains of *A. migulanus* inhibited growth of a range of *Phytophthora* spp.; the strain Nagano, however, was more effective than NCTC 7096 in inhibiting growth. The Nagano strain is known to produce three different gramicidin cyclopeptides differing in the lengths of the lipid side-chains, and also produces exopolysaccharides with potential surfactant activities; in contrast, NCTC 7096 produces only GS in any quantity and does not produce exopolysaccharides [18,27].

Aneurinibacillus migulanus (syn. Brevi*bacillus brevis*) has been shown to be an effective biocontrol agent (BCA), inhibiting plant pathogens *via* production of cyclic peptides, particularly gramicidin S [18,27,28-30]. In addition, A. *migulanus* strain Nagano produces a biosurfactant which decreases surface tension on plant surfaces, and consequently inhibits spore germination, adding to the action of gramicidin S [18,31]. For example, A. *migulanus* Nagano effectively reduced grey mould caused by *Botrytis cinerea* on leaves of Chinese cabbage and reduces the disease severity symptoms on tomato leaves [28].

There were clear differences between the three strains of *Aneurinibacillus migulanus* in terms of their abilities to affect the growth of a wide range of *Phytophthora* species in dual culture assays and in apples, further supporting the results described elsewhere [18,27]. A. *migulanus* Nagano was more effective than NCTC 7096 or E1 at inhibiting pathogen growth. The relative abilities of these strains to affect rot in apples caused by two *Phytophthora* species, *P. cryptogea* and *P. rosacearum*, both known to cause diseases on apples [32], was also demonstrated by the apple inoculation technique developed in this work. *Phytophthora species* in clades 2 and 7 grew more rapidly than those in clades 3 and 4 in the dual culture assays. Moreover, P. *psychrophila* was incubated at 20°C, rather than 25°C, due to its lower temperature optimum [33]. Strains of *Bacillus subtilis* also differed in their abilities to reduce damage caused by *Phytophthora capsici* to red pepper plants [34]. Two isolates were particularly effective in reducing disease and improved growth of plants raised from seed treated with these isolates [34]; a similar plant growth-promoting effect has previously been reported with A. *migulanus* Nagano [29].

Species of *Bacillus* are well-known for inhibitory activity against plant pathogens [6]. For example, many strains of *Bacillus subtilis* are known to inhibit mycelial growth of fungal pathogens *in vitro* [23-25] through the production of antibiotics, including biosurfactants such as surfactin. *Bacillus subtilis* strain GA1 inhibited mycelial growth of *Botrytis cinerea* by up to 70% [24]. Also, *B. subtilis* ME488 showed an inhibition in *in vitro* inhibition assays for *Phytophthora cactorum*, *Phytophthora cambivora*, *Phytophthora capsici*, *Phytophthora cinnamomi* and *Phytophthora cryptogea* [26]. The Nagano strain is known to produce three different gramicidin cyclopeptides differing in the lengths of the lipid side-chains, and also produces exopolysaccharides with potential surfactant activities; in contrast, NCTC 7096 produces only GS in any quantity and does not produce exopolysaccharides [18,27].

The present report is the first to report differential sensitivities of *Phytophthora* clades to inhibition by A. *migulanus* Nagano, where there were a variety of the *Phytophthora* species sensitivity respond to the effects of Nagano [18]. The reasons for this differential activity require further investigation, although it was probably due to varying sensitivity to gramicidins, and/or the biosurfactants produced by Nagano [31]. In addition, the mode of action of GS, which targets respiration and metabolism as functional processes in the cell [35], is likely to be involved in the differential effects on *Phytophthora* spp. The vulnerability to antibiotics or other inhibitors of *Phytophthora species* could relate to other factors that are known in the *A. migulanus* Nagano genome [27]. It was noted that the slower growing *Phytophthora species* were more sensitive to A. *migulanus* Nagano than the faster growing species such as *P. plurivora* MKDF-179 [27]. These differences may be due to changes in the production of antibiotics and other secondary metabolites by A. *migulanus* Nagano with time [18,27]; further work is required to determine the relationships between pathogen growth rates and sensitivity to these naturally produced antibiotics.

In addition, the mode of action of GS, which targets respiration and metabolism as functional processes in the cell [35], is likely to be involved in the differential effects on *Phytophthora* spp. The vulnerability to antibiotics or other inhibitors of *Phytophthora* species could relate to other factors that are known in the *A. migulanus* Nagano genome [27]. These differences may be due to changes in the production of antibiotics and other secondary metabolites by *A. migulanus* Nagano with time [8,18]; further work is required to determine the relationships between pathogen growth rates and sensitivity to these naturally produced antibiotics.

Edwards and Seddon [28] reported that *A. migulanus* Nagano was highly effective against the fungus *Botrytis cinerea*, reducing spore germination and infection development, both *in vitro* and in *planta*. Comparable results were reported previously where *Pinus contorta* foliage treated with *A. migulanus* Nagano and subsequently inoculated with the needle blight pathogen *Dothistroma septosporum* developed significantly fewer lesions than those treated with NCTC 7096 [27]; in contrast, NCTC 7096 showed no significant ability to control *D. septosporum* infection. Further comparable results were reported by Chandel et al. [29] where tomato plants treated with *A. migulanus* Nagano were protected against wilt caused by *Fusarium oxysporum f.sp. lycopersici*. Alenezi et al. [18] reported that tomato leaves treated with *A. migulanus* Nagano provided greater reduction in development of *B. cinerea* infections on tomato foliage than the treatment with *A. migulanus* NCTC 7096.

As suggested above, it is likely that both the additional gramicidins and the biosurfactant produced by *A. migulanus* Nagano were responsible for the differences in biological control activities between Nagano, NCTC 7096 and E1. There are numerous other reports of the use of *Bacillus* isolates against *Phytophthora* diseases of plants, emphasizing the potential for these bacteria to be used in agriculture, horticulture and landscape situations [26,36-38] probably due to the considerable number of additional variables in plant tissues affecting the growth of both the bacteria and the pathogens [28].

DISCUSSIONS AND CONCLUSION

The abilities of strains of *Aneurinibacillus migulanus*, Nagano, NCTC 7096, to inhibit the growth of *Phytophthora* species was compared using *in vitro* dual culture assays in many previous studies by Alenezi. All *Phytophthora* species tested was more strongly inhibited by *A. migulanus* Nagano compared to NCTC 7096 and E1 did not inhibit growth of any *Phytophthora* species.

In addition, an in *planta* method, should be tested by using the inoculation of *Phytophthora* species inoculated into fruit pre-treated with the strains of *A. migulanus*, to determine the effects of the bacteria on the development of rot in an in *planta* system. *Phytophthora rosacearum* MKDF-148 and *P. cryptogea* E2 and other thirteen species of *Phytophthora*, where tested against both NCTC 7096, Nagano or the E1 mutant, *A. migulanus* Nagano was more active against the development of *Phytophthora* on dual culture assay test causing greater growth reductions The reductions in pathogen growth *in vitro* can be supported by further in *planta* test. The *A. migulanus* Nagano acts as a candidate for biological control against species of *Phytophthora*. Moreover, the use of apple inoculation technique proved a useful first in *planta* screen for biological control activity.

This report provided strong evidence that *A. migulanus* Nagano is consistently more effective in inhibiting growth of a wide range of *Phytophthora species* than *A. migulanus* NCTC 7096, suggesting that the Nagano strain could be useful in preventing such as apple storage rot caused by *Phytophthora* species. The fruit test may also prove to be a useful screening tool in determining the relative abilities of different bacterial isolates to inhibit the growth of fungal and fungus-like pathogens in plant tissues.

Further work is required to investigate the secondary metabolism of different strains of *A. migulanus* and the use of these organisms in the management and control of plant diseases and other bioremediation study; the genomes of *A. migulanus* encode for many potential metabolites and other factors which could be active against plant pathogens. Further testing of the ability of *A. migulanus* Nagano to reduce losses to post-harvest diseases on fruits is required, using standard storage facilities. Pathogens other than *Phytophthora* species should be included, such as *Sclerotinia laxa* and *Penicillium expansum*. Moreover, it will be useful to consider combinations of *A. migulanus* Nagano with other bacterial BCAs, possibly with different precise modes of action, to determine if the range of diseases controlled could be expanded.

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