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Characterization and evaluation of the endophyte *Bacillus* B014 as a potential biocontrol agent for the control of *Xanthomonas axonopodis* pv. *dieffenbachiae* – Induced blight of Anthurium

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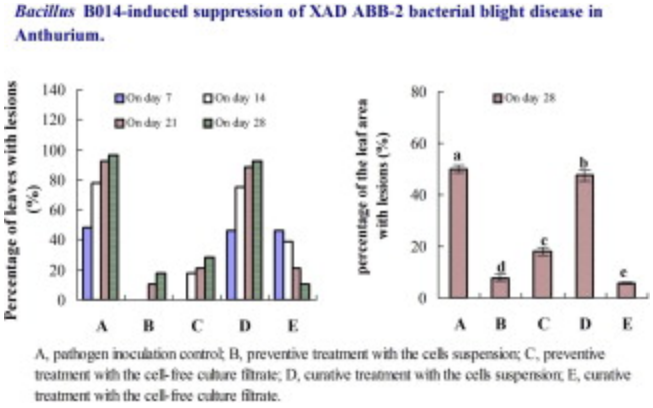
Abstract

A *Bacillus* isolate (B014) demonstrating strong inhibition against the pathogen *Xanthomonas axonopodis* pv. *dieffenbachiae* (XAD) was obtained from healthy tissue of an Anthurium plant and was selected for further characterization. Based on the sequence analysis of the 16S rDNA gene and the *gyr*A gene, the *Bacillus* B014 isolate was identified as *Bacillus amyloliquefaciens*. *Bacillus* B014 was shown to produce anti-XAD metabolites that have a high resistance to a wide range of pH values, high temperatures and digestion by proteinase K. PCR-based detection showed that *Bacillus* B014 can potentially coproduce iturins and surfactins. Applying the *Bacillus* B014 cell suspension or its cell-free culture filtrate to the leaves of an Anthurium

pot plant before challenge with the pathogen XAD decreased the percentage of leaves with lesions to 17.86% and 28.57%, the percentage of the leaf area with lesions to 7.86±1.25% and 18.14±1.44%, respectively, when compared to 96.30% and 49.85±1.58% in control Anthurium plants challenged only with the bacterial pathogen. The *Bacillus* B014 cell suspension or its cell-free culture filtrate also induced an increase in the activities of the defense-related enzymes phenylalanine ammonia lyase, peroxidase and polyphenol oxidase, when compared to control Anthurium plants challenged only with the pathogen. The *Bacillus* B014 cell-free culture filtrate also inhibited further disease development of Anthurium plants already demonstrating disease symptoms caused by XAD. In conclusion, the newly isolated *B. amyloliquefaciens* B014 is a promising candidate as a biological agent to control bacterial blight caused by XAD, particularly in the Anthurium plant.

Graphical abstract



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Highlights

► *Bacillus* B014 showed strong antagonism against blight pathogen XAD ABB-2. ► *Bacillus* B014 produced anti-XAD ABB-2 metabolites with high heat-stability. ► *Bacillus* B014 dramatically reduced the disease development caused by XAD ABB-2. ► *Bacillus* B014 induced increase of enzymes activities in Anthurium plant.

Introduction

Anthurium (*Anthurium andreanum* Linden ex André), the second largest tropical flower, is cultivated throughout the tropics as well as in temperate areas. However, bacterial blight caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* (XAD), previously known as *Xanthomonas campestris* pv. *dieffenbachiae*, has significantly affected Anthurium production throughout the world. This disease, first reported in Brazil in 1960 and subsequently in Hawaii in 1971, was responsible for the decline of the Hawaiian Anthurium industry in the 1980s (Elibox and Umaharan, 2008) and has now been reported in most countries producing Anthurium (Ji et al., 2004, Alvarez et al., 2006, Laurent et al., 2009).

At present, the chemical control of bacterial blight in Anthurium is still not successful. In fact, some chemicals used in the past have been shown to increase the severity of the disease (Alvarez et al., 2006, Elibox and Umaharan, 2008). Because bacterial blight pathogen was serious resistance to antibiotics, most of antibiotics are incapable of killing the bacterium under normal use rates (Elibox and Umaharan, 2008). Several resistant (tolerant) cultivars have been developed and grown in recent years. However, susceptible cultivars remain in high demand because of their desirable flower shapes and colors. Alternative methods of disease control are needed to ensure that the crop is protected from disease outbreaks.

Biological control through the use of beneficial microorganisms colonizing on the rhizosphere, surface and inner tissues of healthy plants has emerged as a promising alternative to chemical pesticides as a more rational and safer crop management. There is a large body of literature reporting the potential use of beneficial microorganisms in controlling plant diseases on numerous types of plants (Sharma et al., 2009, Maksimov et al., 2011). However, there are few publications reporting biocontrol studies on Anthurium plant diseases.

The genus Bacillus is the best characterized and most utilized biocontrol bacteria due to its effective root colonization and sporulation ability (Hassan et al., 2010, Hu et al., 2010). Many of these Bacilli have been shown to be capable of producing many antibiotics with a wide variety of structures and activities (Stein, 2005, Perez-Garcia et al., 2011). *Bacillus* species are also well known as producers of lipopeptides (LPs). LPs impart successful biocontrol effects not only by inhibiting pathogen growth, but also by facilitating root colonization, enhancing the spread of biocontrol agents and reinforcing the host resistance potential (Ongena and Jacques, 2008, Arguelles-Arias et al., 2009, Jourdan et al., 2009). Several genetic studies have

revealed LPs as key biocontrol agent molecules for controlling plant diseases (Chen et al., 2009, Chaisit et al., 2010, Arrebola et al., 2010).

The present study was conducted to obtain an endophytic *Bacillus* that was inhibitory to the pathogenic XAD and to characterize and evaluate the selected *Bacillus* isolate B014 for its biocontrol potential against bacterial blight of Anthurium caused by XAD.

Section snippets

Preparation of bacterial pathogen XAD

XAD ABB-2, a highly virulent pathogen causing bacterial blight in Anthurium, was originally isolated by our laboratory from the leaf of an Anthurium plant showing the typical symptoms of bacterial blight. XAD ABB-2 was grown in medium containing 300 $\mu g mL^{-1}$ of penicillin, streptomycin, or kanamycin. For preparation of the inoculum, the frozen stock of XAD ABB-2 was streaked onto a nutrient agar (NA) plate (gL^{-1}: yeast extract, 3; tryptone, 10; NaCl, 5; agar, 20) containing 200 $\mu g mL^{-1}$ of kanamycin…

Isolation and selection of the endophytic *Bacillus* B014 that is inhibitory to XAD ABB-2

A total of seven *Bacillus* isolates was obtained from the inner tissue of healthy Anthurium plants. All of the isolates were screened for their inhibitory activities against XAD ABB-2 by performing a dual culture assay. Among them, four isolates showed inhibitory activity against the indicator microorganism, with inhibition zones from 12.6 to 35.64 mm in diameter, with the *Bacillus* isolate designated B014 displaying the strongest inhibitory activity against XAD ABB-2 growth with an inhibition…

Discussion

Xanthomonas axonopodis pv. *dieffenbachiae* (XAD) causes Anthurium blight, which is regarded as the most threatening disease for the Anthurium industry worldwide. However, no reliable control method so far has been developed for this disease. In this study, we have shown for the first time the isolation and characterization of a *Bacillus* strain showing potential biocontrol against this plant disease.

In the present study, *Bacillus* B014 was obtained from the inner tissue of healthy Anthurium plants …

Acknowledgments

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An overview of plant defense-related enzymes responses to biotic stresses
2021, Plant Gene

Citation Excerpt :

…Activation of downstream protective enzyme β-1,3-glucanase was involved in wheat resistance to Diuraphis noxia aphid (Moloi and van derWesthuizen, 2006). The Bacillus spp. can minimize the disease intensity and dramatically induce the expression of β-1,3-glucanase and PAL in inhibited or threatened

plants (Prathuangwong and Buensanteai, 2007; Li et al., 2012, 2015). Overexpression of β-1,3-glucanase in the association alongside some pathogen-related enzymes was known to stimulate defensive responses and decrease the disease incidence against rhizome rot in turmeric caused by Pythium aphanidermatum (Anusuya and Sathiyabama, 2014, 2015a, 2015b, 2015c) and Pyricularia grisea in finger millet plants (Sathiyabama and Manikandan, 2018, 2021).…

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Pseudomonas taiwanensis (MTCC11631) mediated induction of systemic resistance in Anthurium andreanum L against blight disease and visualisation of defence related secondary metabolites using confocal laser scanning microscopy

2020, Biocatalysis and Agricultural Biotechnology

Citation Excerpt :

…PAL, PPO and PO enzymes along with phenolic compounds were produced after treatment of anthurium plants with PGPR. PPO and PO enzymes were also observed in anthurium plants treated with B.amyloliquifaciens to suppress blight disease in anthurium plants by (Li et al., 2012). Similar involvement of enzymes, proteins and phenolic compounds were reported by Ramamoorthy et al. (2002) and Potiatti et al. (2009) in tomato roots and potato (Raghavendra et al., 2013).…

Show abstract

Control of postharvest fungal pathogens in pome fruits by lipopeptides from a Bacillus sp. isolate SL-6

2020, Scientia Horticulturae

Citation Excerpt :

…Numerous studies have reported different actions of members of the Bacillus genus as biocontrol agents with their protective capability against phytopathogenic agents (Kumar et al., 2011; Radhakrishnan et al., 2017). It is well known that cell-based biocontrol agents are more suitable for preventing plant diseases, whereas the metabolites extracted from their cultures are effective in their curative potential (Li et al., 2012). Besides, in a broader perspective, the demand of Bacillus spp. lipopeptides for applications in animal and human welfare is growing, due to their low toxicity, high biodegradability, low irritancy and good compatibility with human skin (Meena et al., 2018).…

Show abstract

Biohardening of micropropagated banana using endophytic bacteria to induce plant growth promotion and restrain rhizome rot disease caused by Pectobacterium carotovorum subsp. carotovorum

2018, Scientia Horticulturae

> *Citation Excerpt :*
>
> …The cell suspension and cell-free culture filtrate of endophytic B. amyloliquefaciens (B014) have been reported to inhibit X. axonopodis pv. dieffenbachiae causing anthurium leaf blight disease (Li et al., 2012). In our study, tissue culture plantlets biohardened with B. subtilis during both, primary and secondary hardening stages, showed enhanced disease resistance against rhizome rot pathogen.…

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