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Mechanisms of action of aloe polysaccharides and xanthan gum for control of black rot in cauliflower

Caroline Luiz*, Giana Paula Schauffler, Juliane Mendes Lemos-Blainski, Daniel José Rosa, Robson Marcelo Di Piero

Federal University of Santa Catarina, Centro de Ciências Agrárias, Rodovia Admar Gonzaga, 1346, Itacorubi, 88.034-001 Florianópolis, SC, Brazil

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ABSTRACT

Black rot is the main bacterial disease of crucifers. The establishment of this disease in the field can result in significant yield losses. The objective of this study was to evaluate the potential of xanthan gum (GUM) and polysaccharides extracted from *Aloe barbadensis* (aloe polysaccharides—AP) for controlling black rot and eliciting defense mechanisms as well as revealing changes in the physiological behavior of cauliflower. Cauliflower plants were sprayed with distilled water, AP (0.75–6.0 mg mL^{-1}) or GUM (0.25–1.5 mg mL^{-1}), inoculated with *Xanthomonas campestris* pv. *campestris* 4 days later and evaluated for disease severity at 14 days after inoculation. *In vitro* bacterial growth in a culture medium containing AP or GUM (0.0 to 3.0 mg mL^{-1}) was evaluated for checking the antimicrobial activity of the polymers. Defense mechanisms (hypersensitivity reaction—HR, enzyme activities, content of phenolic compounds and flavonoids) and physiological changes (photosynthetic rate, stomatal conductance and transpiration) were quantified from cauliflower plants treated with distilled water, AP (1.5 mg mL^{-1}) or GUM (0.5 mg mL^{-1}), inoculated or not with *X. campestris*. On average, AP reduced bacterial blight symptoms by 68.1% compared to the control. At 0.5 mg mL^{-1}, GUM controlled 74.65% of the disease; however, it caused high levels of phytotoxicity on the leaf surface at 1.5 mg mL^{-1}. There was no direct effect of polysaccharides on the *in vitro* growth of *X. campestris*. Peroxidase activity was increased significantly at 2 and 4 days after GUM application, while AP did not change the activity of this enzyme. There were no cells with HR, and no changes in polyphenol oxidase activity, phenolic compound content, flavonoid content or in the antioxidant activity in plants treated with polysaccharides. The photosynthetic rate in plants sprayed with GUM or AP was 22.55% and 39.10% lower, respectively, than the rate of plants treated with distilled water. On average, the polymers reduced conductance by 54.8%. A similar behavior was observed in the transpiration of the plants. Although GUM decreased black rot in cauliflower, it caused signs of stress and phytotoxicity on leaves. By contrast, the application of *Aloe barbadensis* polysaccharides can be considered as an effective alternative for controlling black rot. This paper also discusses how these polysaccharides reduced the severity of the disease.

1. Introduction

The genus *Brassica* is of great economic importance throughout the world. The principal species is *Brassica oleracea*, which provides a large range of unique cabbage types that include brussel sprouts, broccoli, cauliflower and others (Rakow, 2004). The cauliflower (*B. oleracea* L. var. *botrytis* L.), a botanical variety that stems from the wild cauliflower (*B. oleracea* var. *silvestris*), is extremely important in global horticulture because of the volume produced. According the Food and Agriculture Organization Corporate Statistical Database (FAOSTAT, 2012) commercial plantations produces around 21 million tonnes per year. However, although it is adapted to the soil and climate conditions of the producing regions, the development and yield of cauliflower may be affected by the occurrence of pathogens, such as the *Xanthomonas campestris* pv. *campestris*, the causal agent of black rot.

The black rot was first described by Garman (1894) as a disease of cabbage. The author isolated two types of bacteria from diseased plants in Kentucky, USA. Since then the disease has been identified in all continents where the Brassicaceae family is grown and

* Corresponding author.
E-mail addresses: caroline_luiz@yahoo.com.br (C. Luiz), gischauffler@hotmail.com (G.P. Schauffler), juliane.lemos@yahoo.com.br (J.M. Lemos-Blainski), danielrosa.agro@gmail.com (D.J. Rosa), robson.piero@ufsc.br (R.M. Di Piero).

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is considered to be the most important disease of brassica crops worldwide (Vicente and Holub, 2013).

The use of resistant cultivars, treated seeds and drip irrigation are recommended for the management of the black rot (Heck et al., 2013; Krauthausen et al., 2011; Vicente et al., 2002). Chemical pesticides are often used, however, excessive aplications promote increase in production costs, favor the selection of pesticide-resistant strains, and cause damage to environmental and human health (Sexton et al., 2007). Therefore, there is a growing interest in alternative methods for controlling plant diseases, such as the induced resistance.

Induced resistance is an increase in the level of plant resistance, as a result of activating their apparently inactive genes by using external agents (Stadnik and Maraschin, 2004). Thus, it can be stated that this method is based on plant natural defense reactions against pathogens.

Plant resistance relies on innate immunity of each cell. It envolves anatomical features that act as barriers to the penetration of the pathogen, or the recognition of molecular patterns from microorganisms by the plant, which leads to the activation of defense mechanisms (Jones and Dangl, 2006).

The recognition of pathogen-associated molecular patterns (PAMPs) or microbe-associated molecular patterns (MAMPs) occurs via transmembrane proteins called pattern recognition receptors (PRR). In addition to PAMPs and MAMPs, PRR can recognize damage-associated molecular patterns (DAMPs). These molecules are released by host cells when exposed to attack by pests or microorganisms (Ma et al., 2012).

Molecules from different classes and microorganisms were identified as PAMPs/MAMPs/DAMPs, including bacterial flagellin and Tu elongation factor (EF–Tu) (Shan et al., 2007), fungal ethylene-inducing xylanase (EIX) (Ron and Avni, 2004), various polysaccharides from fungi (Wan et al., 2012) or bacteria (Zeidler et al., 2004) and the peptide AtPep1, which is released from precursor proteins in response to wounding and which triggers an innate immune response (Krol et al., 2010).

After exposure of plants to PAMPs/MAMPs/DAMPs, plants can initiate a defense response that involves the priming of cells. According to Conrath (2011), priming is the phenomenon that enables cells to respond to very low levels of a stimulus in a more rapid and robust manner than non-primed cells. For Conrath et al. (2015), this effect is related to the enhanced accumulation of dormant cellular enzymes functioning in intracellular signal amplification. The authors claim that subsequent challenge by pathogenic microorganisms or abiotic stress could activate these latent signaling enzymes and leading to faster and stronger activation of defense, immunity, and stress tolerance.

Polysaccharides from microorganisms are examples of molecules capable of eliciting mechanisms involved in plant defense and inducing resistance to certain pathogens. Newman et al. (2002), for example, examined the effects of *X. campestris* pv. *campestris* polysaccharides on induced resistance in pepper (*Capsicum annum*) and observed that pretreatment with bacterial polymers (50 mg mL^{-1}) enhanced the expression of genes encoding pathogenesis-related proteins after inoculation with species of *Xanthomonas*.

Xanthan gum, an exopolysaccharide produced by bacteria of the genus *Xanthomonas*, is responsible for the adhesion of bacterial cells to substrates and for favoring the colonization of the surface and internal tissues of plants. Yun et al. (2006a), for instance, demonstrated that xanthan gum is capable of inducing susceptibility in *Nicotiana benthamiana* and *Arabidopsis thaliana* to *X. campestris* pv. *campestris*. According to the authors, the polymer suppresses defense genes and consequently inhibits callose deposition in plants; thus, it is an important factor in bacterial pathogenicity. However, this polysaccharide can elicit defense mechanisms in non-host species of *X. campestris*, such as barley (Antoniazzi et al., 2008; Castro and Bach, 2004).

Microbial polysaccharides are not the only ones responsible for promoting activation or suppression of plant defense mechanisms. The *Aloe barbadensis* parenchyma, rich in secondary metabolites, contains polysaccharides that can increase defense activity of enzymes such as peroxidases, polyphenol oxidases, glucanases and phenylalanine ammonia-lyase, when sprayed on the leaf surface of tomato plants, significantly reducing the severity of bacterial spot (*Xanthomonas gardneri*) (Luiz et al., 2012,2015).

Polysaccharides are precursors in the elicitation of defense mechanisms of various plants species; in addition, they are plentiful, readily available and derived from renewable sources. Therefore, their use in agriculture for reducing plant diseases can be considered as an alternative to conventional pathogen control. Thus, the aim of this study was to evaluate the potential of xanthan gum and polysaccharides extracted from the reserve parenchyma of *A. barbadensis* for control of black rot and elicitation of defense mechanisms; another objective is to show changes in the physiological behavior of cauliflower treated with the polysaccharides.

2. Material and methods

2.1. Plants and pathogen

The experiments were carried out in a greenhouse and in the Phytopathology Laboratory of the Department of Agricultural Sciences, Federal University of Santa Catarina (UFSC), from March 2011 to October 2012.

Cauliflower seeds, from cv. Snowball, susceptible to *X. campestris* pv. *campestris,* were sown in 128-cell styrofoam trays with the substrate Germina Plant and kept in a greenhouse. Two weeks after sowing, the seedlings, around 4 cm high, were transplanted to 2L pots, filled with soil and organic compound at a 4:1 ratio, v/v.

X. campestris pv. *campestris* was isolated from leaves collected in Florianópolis/SC in March 2010. For this purpose, the infected material was washed, and cuts were made in the transition region between the infected and the healthy tissues with a previously flamed scalpel. The fragments were disinfected in alcohol (50%) and sodium hypochlorite (2%). Subsequently, a drop of sterile distilled water was deposited and, through the use of a scalpel, the leaf tissue was macerated. This macerate was plated onto NA medium and kept at 28 °C.

Bacteria grown in culture medium were re-isolated and subjected to Koch's postulates. The bacterial colony that caused the typical symptoms of the disease was preserved at 25 °C in phosphate buffer (8.6 mM K_2HPO_4; 7.4 mM KH_2PO_4).

To obtain the inoculum, the bacteria kept in the buffer were passaged with a platinum loop to Petri dishes containing nutrient agar (NA) [Composition (g/L): meat peptone, 5.0; meat extract 3.0; agar 12.0] (Merck, Darmstadt, Germany). The bacteria grown on this medium were passaged to other Petri dishes, 48 h after the first passage, and incubated on NA medium for a period of 24 h at 28 °C. The suspension of bacterial colonies grown on this medium was prepared with distilled water and adjusted to optical density of 0.3 absorbance units at 600 nm.

2.2. Polysaccharides

To obtain polysaccharides from the reserve parenchyma, *A. barbadensis* leaves, collected on the farm of the company Naturama Sucos Integrais do Brasil Ltda®, were previously washed and their parenchyma was removed, crushed and strained. After that, six volumes of ethanol at 92 GL by one volume of gel were added, and the final mixture was maintained at 4 °C for 24 h for polysaccha-