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Effects of a lectin-like protein isolated from *Acacia farnesiana* seeds on phytopathogenic bacterial strains and root-knot nematode

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ABSTRACT

Acacia farnesiana lectin-like protein (AFAL) showed bacterioestatic effects against *Xanthomonas axonopodis* pv. *passiflorae* (Gram-negative) and *Clavibacter michiganensis michiganensis* (Gram-positive), with the latter being more sensitive. This effect is probably due to the ability of AFAL to interact with the bacterial cell wall where we observed that AFAL induced macroscopic change. The maximum bacterial growth inhibition was approximately 78% when incubated with Gram-negative strains, and as high as 92% percent for the Gram-positive one. The antibacterial effect of flavonoids (rutin, quercetin and morin) was also observed using low concentrations against both bacterial strains. Prior incubation of both with AFAL at high concentrations increases the inhibitory effect of flavonoids on bacterial growth. The potential use of AFAL as a control agent against the root-knot nematode *Meloidogyne incognita* was investigated as well, showing anti-nematode properties involving both egg hatching and motility. In the juvenile second-stage, AFAL showed reduction in larval mobility when measured against a control group. The results suggest that AFAL is effective against *M. incognita* and could be used as a component of integrated pest management programs. These data also suggest that lectins probably play a role in plant defense not only against invertebrate phytopathogens, herbivores and fungi but also against bacteria.

1. Introduction

Pathogenic microorganisms bind, invade and colonize the tissues of the host to mount a successful infection. Surface proteins, carbohydrates and pili are used by all bacterial pathogens for this type of infection. The interaction with the host tissue is made through specific receptor ligands that determine the range and the site of infection [1,2].

Almost all microorganisms express surface-exposed carbohydrates that are potential lectin-reactive sites. The ability of lectins to complex with microbial glycoconjugates makes it possible to employ them as probes and sorbents for whole cells, mutants, and other cellular constituents (e.g. metabolites). Microbial receptors for lectins consist of several unique chemical structures [3].

Most phytopathogenic bacteria, are Gram-negative (e.g. *Xanthomonas axonopodis* pv. *passiflorae*). However, Gram-positive phytopathogens are important, since they cause great losses in crop cultivation. Bacterial canker caused by *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) is a recurrent and serious disease of field- and greenhouse tomatoes in many countries [4–6]. The disease leads to vascular infections, wilting, chlorosis, and eventual death of the plant. Disease control is difficult because of the lack of commercially acceptable, resistant tomato cultivars. Chemical control of the disease relies on the use of antibiotics (such as streptomycin) and copper compounds, which prevent bacterial multiplication and further infection. Unfortunately, antibiotics have led to the resistant bacterial populations, and the use of antibiotics is banned or severely limited in many countries [7].

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Some soil nematodes also cause diseases in plants, root-knot nematode (*Meloidogyne incognita)* eggs hatch in the soil as second-stage juveniles (J2), which penetrate the host root in the elongation zone (Ripoll et al.) [8], establishes permanent feeding sites forming giant cells adopting a sedentary lifestyle. The eggs are then released in masses on the surface of the root gall [9]. The symptoms of these infections are poor development due to root damages, defoilage, mineral deficiency, clorosis, and low water absorption [10].

Many strategies have been recommended to treat *Meloidogyne* spp., *Xanthomonas* spp. and, *Clavibacter* spp. infections but, at times they are inefficient and difficult to implement. Plant molecules with phytopathogenic properties controlling plant phytopathogens could be an alternative for small farmers, (low cost and non-toxic effects).

Strategies to combat some phytopathogens use molecules from plant metabolism such as alkaloids, flavonoids and terpens [11]. Flavonoids are found in fruits, vegetables, nuts, seeds, stems, flowers, tea, wine, propolis and honey and certain beverages, and have diverse beneficial biochemical and antioxidant effects. Natural products are increasingly the subject of anti-infective research, and many have flavonoids which possess antifungal, antiviral and antibacterial activity [12]. The ability of flavonoids to protect against attack by microbes and insects has been explored in many studies. The widespread distribution of flavonoids, their variety and low toxicity compared to other active plant compounds (for instance, alkaloids) are well-established [13]. The synergistic effect of flavonoids and lectins against plant pathogens, such as bacteria and nematodes has been noted as well [12].

Plant lectins are proteins or glycoproteins that display at least one non-catalytic domain that binds reversibly to specific mono- or oligosaccharide residues on the cell membrane [14]. Lectins are constitutively expressed in seeds, leaves, bark, roots, tubers, and fruits [15]. It has been suggested that they play a role, not only in plant defense against predators and pathogens [16–18], but also in nodulation [19]. Differently from antibiotics commonly used lectins are wildly distributed on nature and can be found in all kinds of organisms, being biodegradable and incapable to accumulate on soil or other organism who feed the organisms treated by them. Antibiotics are commonly known to eradicate bacteria by inhibit some metabolic procedures with lectins which can act by the carbohydrate recognition, making microorganisms' growth and development difficult.

A. farnesiana get special attention and was investigated in this work due to reports of Arias and co-workers [20], and El Abbouyi and co-workers [21] on various biological activities of different species of Acacia. Some of these biological activities are related to the lectin fraction of albumin seeds of *A. farnesiana*, which justify using this organism as biological source of protein. The Acacia species is an important producer of secondary metabolites with significant biological activity such as antitumor, anti-inflammatory, antibacterial and anti-parasitic [22]. With the aim of finding a compound less aggressive to the environment that combats phytobacteria AFAL was used, testing its ability to inhibit the growth of *C. michiganensis*, *X. axonopodis* and the nematode *M. incognita*.

Phaseolus vulgaris seeds contain a mixture of isolectins (PHA) which usually bind to 1,6-branched *N*-acetyl-D-glucosamine containing N-glycans. A PHA-like lectin that binds to chitin can represent a more potent defense protein against insects, fungi, nematodes, and bacteria than the similar ones [23]. AFAL is a chitin-binding protein purified from the albumin fraction of soluble extracts from *A. farnesiana* seeds. The complete amino acid sequencing obtained by tandem mass spectrometry confirms its similarity with PHA-like lectins. AFAL exhibits time-dependent oligomerization which can explain the variation in the affinity for chitin. This is due to the time dependence of contact between the polysaccharide and AFAL. Once AFAL presents the recurrent variations in the quaternary structure assembly, this may enhance or minimize the chitin-binding properties leading to differential actions of AFAL in different cellular environments [24]. The purpose of the present study was to investigate this protein's antimicrobial activity against Gram-positive (*C. michiganensis*) and Gram-negative (*X. axonopodis*) bacteria and its potential to control the nematode *M. incognita*.

2. Materials and methods

2.1. Protein isolation

The seeds of *Acacia farnesiana*, commonly known as "coronha" in Brazil, were collected in the state of Ceara, Brazil. The species are deposited at Herbarium Prisco Bezerra (EAC) from Biology Department of Federal University from Ceara under the accession number EAC 20273.

Seeds were separated from fruits, dried and finely ground in a coffee mill, and the flour was used for experimental purposes. The protein extraction was performed as previously described by Santi-Gadelha and co-workers [24]. The protein extract was dialyzed against water with several changes for 48 h and centrifuged at 20,000*g* for 20 min at 4 °C. The supernatant (albumin protein fraction) was submitted to hemagglutinating activity and ion-exchange chromatography on DEAE–Sephacel (2.0 × 20 cm), equilibrated with 0.025 M Tris–HCl buffer, pH 7.4. The adsorbed proteins were eluted with a linear gradient of 0–1 M NaCl in 0.025 M Tris–HCl buffer . The eluate was monitored at 280 nm, and fractions (1.5-mL) were collected manually and tested for hemagglutinating activity toward rabbit erythrocytes [25]. The purity of all protein preparations was monitored by polyacrylamide gel electrophoresis (12% gels) as described by Laemmli [26].

2.2. Antibacterial activity of AFAL

The bacterial strains used were donated by L.O.S. Beriam (Centro Experimental Central do Instituto Biológico – CEIB Laboratório de Bacteriologia Vegetal Caixa Postal 70 CEP 13001–970 – Campinas – SP – Brasil). The strains are registered under the numbers below: *C. michiganensis* subsp. *michiganensis*; ICMP International Collection of Microorganisms from Plants, Auckland, New Zealand) 13696; *X. axonopodis* pv. *passiflorae*; ICMP (International Collection of Microorganisms from Plants, Auckland, New Zealand) 3151.

In this article we used two different parameters to evaluate the effect of AFAL concentrations on the growth rates of *X. axonopodis*, and *C. michiganensis*. One method was the direct count of viable organisms expressed as CFU in semisolid agar. Another approach was estimating bacterial cell viability based on detection of cellular ATP using the Bac Titer-GloTm assay. The antibacterial assay performed here was conducted as described by Oliveira and co-workers [27] and Oliveira co-workers [28]. Bacterial strains of *X. axonopodis* pv. passiflorae (Gram-negative) and *C. michiganensis* subsp. michiganensis (Gram-positive) were collected from fresh agar plates and suspended in sterilized distilled water ($A_{650} = 3 \times 10^8$ CFU). Aliquots of the bacterial suspension were diluted to 10^3 CFU/mL and incubated with *A. farnesiana* lectin-like protein (150 μg/mL) for 1 h at 37 °C; after incubation, survival was determined on nutrient (Difco) plates ($n = 5$). In addition, we tested for bacterial cell viability using the Bac Titer-GloTm assay (Promega) which was conducted according to manufacturer's instructions. The antimicrobial effect of the protein was tested against *X. axonopodis* pv. passiflora and *C. michiganensis* subsp. michiganensis, also using scanning electron microscopy (SEM). The samples were taken for examination after the incubation time