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Inhibition Index of Secondary Metabolite-Like Extracts of Bacillus Cereus Against Phytopathogenic Bacteria in Rice Cultivation

Yelitza Aguas Mendoza[1], Alexander Pérez Cordero[2], Donicer E. Montes Vergara[3]

Abstract

The presence of diseases in rice cultivation has become a limiting factor in rice yield and production. The bacterial panicle blast disease has generated large economic losses in the agricultural sector. One of the strategies commonly used by farmers to control the disease is the application of agrochemicals, but these can contaminate the environment and many microorganisms have acquired resistance. Endophytic bacteria are considered a tool to control pathogens through the production of secondary metabolites. The aim of this work was to evaluate in vitro the concentrated fraction of secondary metabolites produced by Bacillus cereus against Burkholderia glumae and Burkholderia plantarii which were characterized by gas chromatography coupled to mass spectrometry (GC-MS). The inhibitory effect against B. glumae and B. plantarii was observed by metabolite impregnation test on filter paper discs. The metabolites geraniol, geranate and sulcatone were identified and showed in vitro antagonism against the plant pathogens. The production of microbial secondary metabolites may be an alternative to replace agrochemicals in the future.

Keywords: *Extracts, Metabolites, Inhibition, Bacteria, Pathogenic, Rice Crop.*

Introduction

According to (Perfetti et al., 2013), agriculture is considered an economic activity in developed countries, providing a source of employment and income for the rural sector. In Colombia, coffee, maize and rice have been the most important productive crops in the last three decades due to the area cultivated (Agronet, 2017). Rice is the most important crop in the world; approximately 50% of the world's population depends on this grass as a staple food. Because this species is considered a staple food crop and because of its high consumption, rice is cultivated under different agro-ecological conditions, especially in the Colombian coast where the environment has an adequate influence on the growth and development of cultivars (Sripongpangkul et al., 2000; Tatis et al., 2011).

The presence of diseases caused by phytopathogens has become a limiting factor in rice production and yield, and these are mainly caused by bacteria, including Burkholderia glumae and Burkholderia plantarii (Mitchell & Teh, 2005). These phytopathogens cause the disease known as bacterial blast of the rice panicle, whose incidence has increased in recent years causing a decrease in production, as well as gaining tolerance to commercial agrochemicals, which is why the responsible entities have established agronomic zones throughout the country, seeking to control the disease through integrated crop management (Beltrán et al., 2013; Quesada

[1] Universidad de Sucre, Facultad de Ingeniería, Colombia, https://orcid.org/0000-0003-4880-4510.
[2] Universidad de Sucre, Facultad de Ciencias Agropecuarias, Colombia https://orcid.org/0000-0003-3989-1747, Email: alexander.perez@unisucre.edu.co, (Corresponding Author)
[3] Universidad de Sucre, Facultad de Ciencias Agropecuarias, Colombia, https://orcid.org/0000-0002-2860-0505.

& García, 2014; Zhou, 2014).

Currently, the search continues for innovative biological alternatives through active ingredients that allow disease control and that are environmentally friendly, which is why emphasis has been placed on the use of beneficial organisms, mainly those called endophytic microorganisms. Endophytic bacteria are defined as an important group of symbionts that live in plant tissues without causing disease symptoms (Pelaez & Londoño, 2017; Pérez et al., 2018; Porras & Bayman, 2011). Several studies have shown that endophytic bacteria, especially the genus Bacillus promote plant growth through the production of siderophores, production of enzymes such as phosphatases and organic acids that have the ability to dispose phosphorus to forms assimilable by the plant (Ramírez & Urbano, 2014; Rojas et al., 2017; Villareal et al., 2018).

The production of secondary metabolites from endophytic bacteria has become an alternative to replace agrochemicals because they can cause environmental damage and serious consequences on human health (Montoya et al., 2014; Rojas & Bedoya, 2013; Segura, 2015). The aim of the study was to evaluate in vitro the fraction of secondary metabolites produced by B. cereus against the phytopathogenic bacteria *B. glumae* and *B. plantarii*.

Materials and Methods

Bacterial. The species used in this study are part of the strain bank collection of the Bioprospección Agropecuaria group, microbiological research laboratory of the University of Sucre and identified as LIM- Bacillus cereus, which was activated and purified in culture medium.

Liquid fermentation. A pure colony of B. cereus was taken and inoculated in Luria Bertani (LB) liquid medium and shaken for 48 hours at 30°C. After this time, 1ml of bacterial growth was taken and deposited in 400ml of 3s medium. After this time, 1 ml of bacterial growth was taken and placed in 400 ml of 3s medium. The medium was left in constant agitation for 72 hours (Ariza & Sanchez, 2012).

Extraction and identification of secondary metabolites from *B. cereus*. 100 mL of the fermented medium was taken to be centrifuged at 7000 rpm for 45 minutes. To each filtrate 80 ml of ethyl acetate was added, then the organic fraction was collected and concentrated using a rotary evaporator. The concentrate was analyzed by gas chromatography coupled to mass spectrometry (GC-MS).

Inhibitory activity of secondary metabolites of *B. cereus* against *B. glumae* and *B. plantarii*. To evaluate the antimicrobial activity, the agar disc diffusion technique was used, which consisted of impregnating 20µL sterile filter paper discs with the concentrated microbial extract and inoculating them onto the surface of King B medium previously inoculated with *B. glumae* and B. *plantarii*. The boxes were incubated for 3 days at a temperature of 34°C (Cuellar & Hussein, 2009). The percentage inhibition was determined using the following formula (Barraza et al., 2017):

%IB= 1-(Aai/Acc)*100

Where: Aai: average growth of treatment, Acc: average growth of control test.

Statistical análisis. A completely randomized design was applied to determine the percentage inhibition of secondary metabolites. Also, the Duncan multiple range test was applied to establish the difference in the percentage of inhibition against B. glumae and B. plantarii. Four

replicates per treatment were carried out. Data were analyzed in the free version of InfoStat software.

Results and Discussion

Figure 1 shows the results of the 100% extract inhibition test of *Bacillus cereus* against *Burkholderia glumae* and *Burkholderia plantarii* on King B agar medium.

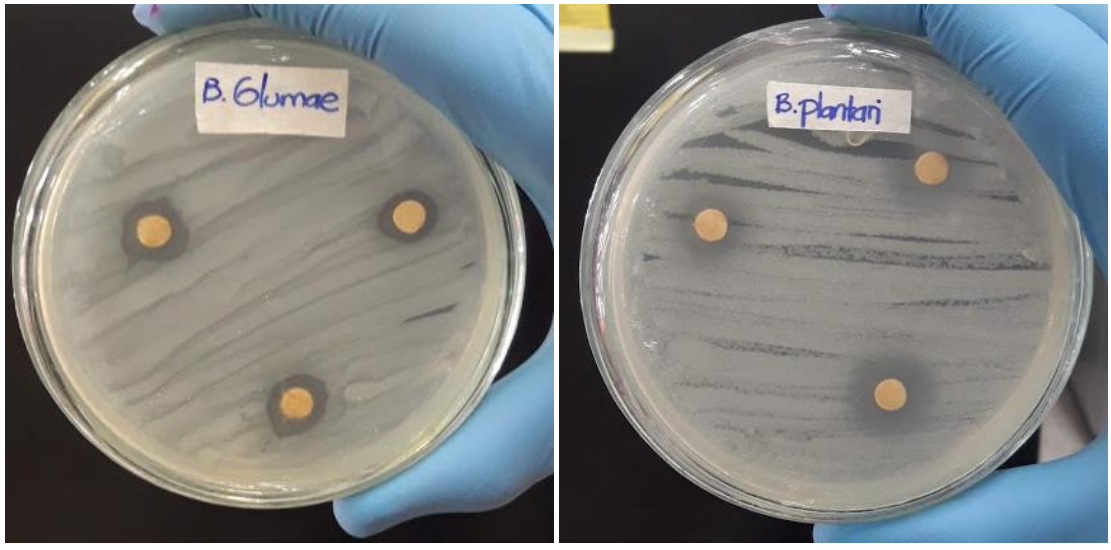

Figure 1.

Inhibition activity assay of the concentrated metabolite fraction of B. cereus against B. glumae and B. plantarii on King B. agar medium. Source: Microbiological Research Laboratory.

Figure 2 shows the results of the percentage inhibition of *Bacillus cereus* against *Burkholderia glumae* and *Burkholderia plantarii* using 4 concentrations of Bacillus cereus bacterial extract. The inhibition index was evaluated in terms of inhibition in mm presented by the disc impregnated with *B. cereus* extract suspension at different concentrations. The results show that at 75% concentration of B. cereus bacterial extract showed inhibition of 12 mm for *Burkholderia glumae* and 8 mm for *Burkholderia plantarii*. No significant difference was found between 75% and 100% extract concentrations.

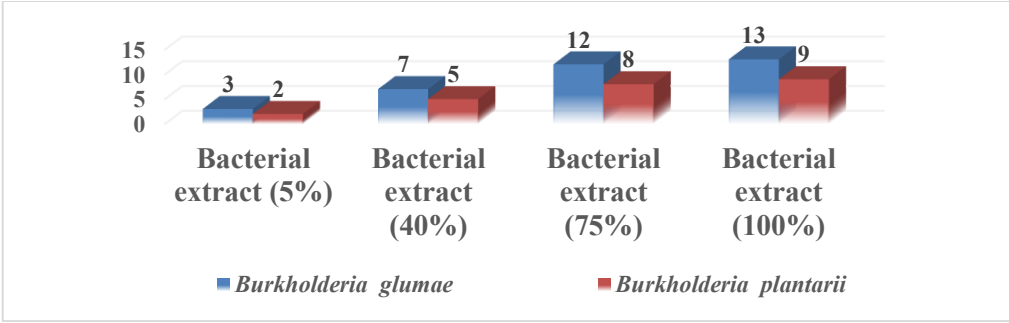

Figure 2.

Inhibition index of bacterial extract of *Bacillus cereus* at different concentrations on the plant pathogenic bacteria *Burkholderia glumae* and *Burkholderia plantarii*.

Figure 3 shows the inhibitory activity of *Bacillus cereus* extract at 100% concentration against *Burkholderia glumae*, the maximum growth of the pathogenic bacterium was 0.31 nm at 16 hours after the start of the experiment, corresponding to a bacterial inhibition rate of *Bacillus cereus* of 56.33%, compared to the growth of *B. glumae* (control). With regard to the inhibitory activity of the B. cereus extract against *Burkholderia plantarii*, at 18 hours with a growth of 0.27 nm corresponding to a bacterial inhibition rate of the Bacillus cereus extract of 52.62%, compared to the growth of *B. plantarii* (control) (figure 4).

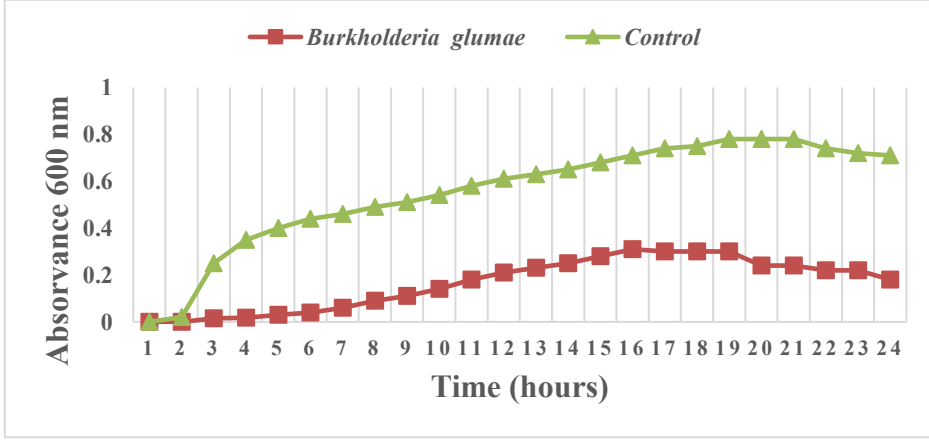

Figure 3. Growth Behaviour of *Burkholderia Glumae* in the Presence Of 100% Suspension Extract of *Bacillus Cereus.*

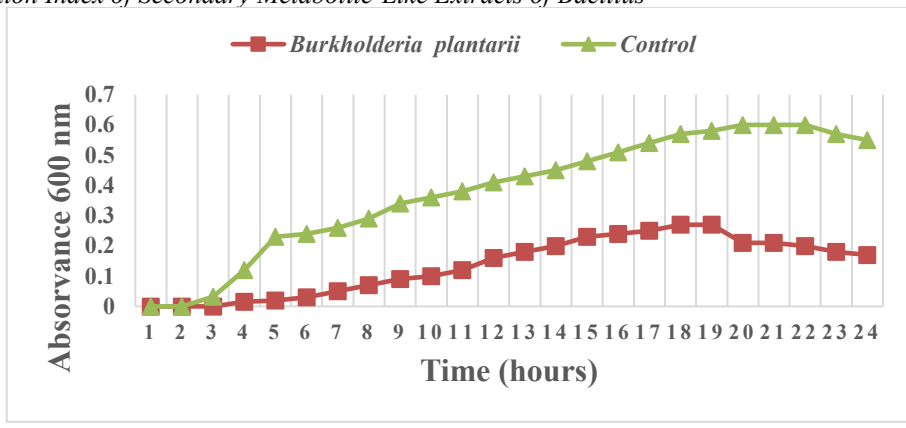

Figure 4. Growth Behaviour of *Burkholderia Plantarii* in the Presence of 100% Suspension Extract of *Bacillus Cereus*.

From the chromatographic analysis, 42 compounds were identified of which geraniol had an area percentage (30%), sulcatone (20%) and geranate (26%), which are possibly producing inhibitory activity against pathogens affecting the rice crop (figure 5).

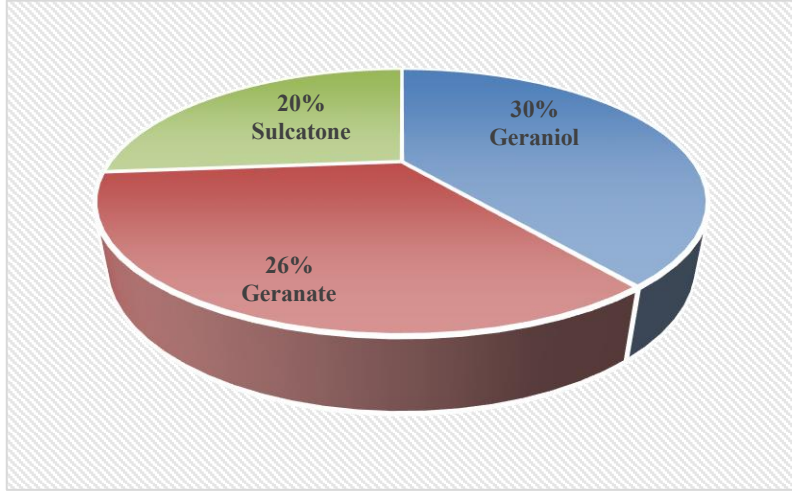

Figure 5. Secondary Metabolites Present in *Bacillus Cereus*

Source: Microbiological Research Laboratory. University Of Sucre, 2022).

In this study, *B. cereus* was isolated from Lippia spp. It has been shown that many species of endophytic bacteria produce secondary metabolite-like substances that are structurally similar to the molecules produced by host plants, which is possibly a co-evolutionary process between host plants and resident microorganisms (Wang & Dai, 2011), These monoterpenes act on the cell membrane of pathogens generating an imbalance in the lipid membrane which causes an increase in the fluidity of the membrane causing a loss of potassium ions (Bard et al., 1988; Linde et al., 2016; Stashenko et al., 2014)

As reported by Tapia (2018), he showed that geraniol was present in the extracted oil Clinopodium pulchellum (Kunth) (Lamiaceae) which showed inhibitory activity with Candida albicans. Lorenzi et al. (2009), reported that many of the bacterial species of clinical interest have developed some resistance to the antibiotics commonly used to inhibit them. Evidence from studies shows that combinations of antibiotics with geraniol to counteract growth at the in vitro level. The results obtained were that combining the essential oil with phenylalanine arginine β-naphthylamide produced synergistic activity against Enterobacter aerogenes, Escherichia coli, Pseudomonas aeruginosa and Acinetobacter baumannii.

Evidence from studies on the production of microbial metabolites has emphasised the genus Bacillus. Many of the species belonging to this genus have become biotechnological tools in order to control pathogens that affect the yield of crops of agricultural interest and thus reduce the application of chemical pesticides (Daraz et al., 2020; Chen et al., 2020). In a study conducted by Ariza and Sánchez (2012), which aimed to characterise the metabolites produced by B. subtilis against Fusarium sp. the results obtained were a controlling effect against the fungus through the production of Iturin A with an inhibition percentage of 70%.

Zhang et al. (2020) demonstrated that B. velezensis can inhibit Alternaria solani in vitro by producing metabolites such as iturins and acetophenone which significantly affected the hyphae by perforation and swelling where the microbial metabolite acted. In addition, Sidorova et al. (2020) demonstrated that the optimal production of metabolites produced by B. subtilis is found at a pH ranging between 6.0-8.0 and at a temperature between 25°C and 30°C. They also reported by chromatographic analysis the metabolites synthesized as surfactin and Iturin A, which showed inhibition against Fusarium sp. These data can give us an indication for the manufacture of effective fungicides to protect crops from any pathogen.

Conclusions

In this study, secondary metabolites produced by B. cereus inhibited in vitro the plant pathogens B. glumae and B. plantarii which affect rice yield. Large-scale production of metabolites from the genus Bacillus can be considered as an alternative for the management of different diseases in the field and as a substitute for agrochemicals.

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Author Contribution

Alexander Perez Cordero: experiment execution, data analysis. Donicer Montes V and Yelitza Aguas M, conceptualization, writing - revision and editing. All authors have read and approved the manuscript.

Conflict of Interest

All the authors of the manuscript declare that they have no conflict of interest.

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