Original Article

Antibacterial Activity Of Bacillus Subtilis Ah013002.3 Against Phytopathogenic Bacteria In Rice Crops

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Abstract

Isolate R1A2LIM from the genomic bank of the Agricultural Bioprospecting research group of the University of Sucre and identified as Bacillus subtilis AH013002.3 was isolated from roots of rice varieties (F473, F2000 and FMocarí) without symptoms of bacterial blast disease in the Colombian Caribbean. The isolate was purified and used to evaluate in vitro antibacterial activity against Burkholderia glumae. The inhibitory activity test shows 100% inhibition of the R1A2LIM isolate against Burkholderia glumae. The isolate was molecularly identified by sequencing technique as Bacillus subtilis AH013002.3.

Keywords: Endophytic bacteria, tissues, species, locality, avocado seedlings.

1. INTRODUCTION

Rice is the third most important crop after wheat and maize (FAO, 2018). Is considered one of the main foods in the family food basket and is characterized by its high nutritional content (Martínez et al., 2005). In Colombia, rice cultivation ranks first in terms of economic value among short-cycle crops (Maqueira et al., 2009). Rice is ranked as the second rice-producing country in Latin America and the Caribbean after Brazil and Peru; and on a global scale it ranks 22nd with a 0.4% share (FAO, 2013; Mazuera, 2010).

The bacterial Burkholderia glumae, the causal agent of the bacterial panicle blight of rice and seedling rot (Nandakumar et al., 2009), is a typical example of plant pathogens that are favored by biotic challenges due to changes in environmental conditions. The presence of B. glumae can be devastating, severely reducing yield due to decrease in grain weight, sterility of florets, inhibition of seed germination, and the formation of empty grains at the end of the crop cycle (Fory et al., 2014).

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Burkholderia glumae Kurita and Tabei, 1967 (Burkholderiaceae) is the etiological agent of the disease called bacterial panicle blast of rice (Pedraza et al., 2018). The symptomatology of this disease is evidenced in the panicle of the plant, causing grain abortion which generates large economic losses in rice production (Ham et al., 2011).

The genus of bacteria Bacillus represents a large group of biocontrol bacteria. Bacillus strains can inhibit the growth of plant pathogens through releasing a wide variety of secondary metabolites with good antimicrobial properties, such as bacilysin, surfactin, fengycin, subtilosin A, and so on (Leclere et al., 2005; Nam et al., 2016).

The aim of this study was to evaluate in vitro the inhibitory activity of the isolate R1A2LIM identified as Bacillus susbtilis against the phytopathogenic bacterium Burkholderia glumae causing bacterial blast in rice cultivation in the Colombian Caribbean.

2. MATERIAL AND METHODS

2.1 Collection of plant material. A zig-zag sampling was carried out collecting 3 complete rice plants of the varieties F473, F2000 and FMocarí. The samples were labelled with their respective variety and date of collection. The samples were stored and preserved in plastic boxes at 4 °C for transport to the Microbiological Research Laboratory of the University of Sucre and processed within 24 hours after collection.

2.2 Isolation of R1A2LIM. For the disinfection process, the methodology proposed by Pérez et al. (2010) was followed, which consists of washing the root, stem, leaf and panicle tissues previously cut to 1 cm. After the disinfection process, each tissue was placed in a porcelain dish and macerated until a homogeneous mixture was obtained, which was then inoculated in 9 ml of peptone and left in agitation for 24 hours at a temperature of 34 °C. From the homogenate, serial dilutions were made and seeded by diffusion on R2A agar surface and incubated at 28 °C for 72 hours. In addition, B. glumae was activated on King B agar medium. The selected morphotypes were purified and maintained on R2A agar for further in vitro evaluation against B. glumae. The isolate was coded as R1A2LI and is stored in the genome bank of the microbiological research laboratory of the University of Sucre.

2.3 In vitro antimicrobial activity of R1A2LIM against B. glumae. Filter paper discs were impregnated with 20µl of suspension of the isolate identified with R1A2LIM and placed on King B agar medium pre-inoculated with B. glumae. The inoculated Petri dishes were incubated at 32°C for 72 hours. The positive control used was oxolinic acid and sterile water as a negative control (Rojas et al., 2012). The percentage inhibition was calculated using the formula (Da/C)*100. Where Da corresponds to the inhibition of the bacteria in each treatment and C corresponds to the inhibition of the control. The assay was performed in triplicate. R1A2LIM isolates for which inhibitory activity was observed were selected for molecular identification by sequencing technique (Pérez et al., 2015).

2.4 Molecular identification of R1A2LIM. The extraction of genomic DNA of R1A2LIM that showed antibacterial activity was performed and the respective amplification of the 16S rRNA gene was carried out by PCR technique, following the methodology proposed by Oliveira et al. (2013). The amplified and purified product was sent for sequencing to Macrogen. The sequences obtained were compared with sequences from the Genbank database. Base alignment was performed in the Clustal W program; phylogenetic inferences were obtained by Neighbor Joining method based on the kimura-2-parameter model with 1,000 bootstrap test in the MEGA X program.

3. RESULTS AND DISCUSSION

Figure 1 shows the percentage inhibition shown by isolate R1A2LIM against Burkholderia gluma. It can also be seen that the percentage inhibition of this isolate was similar to that shown by oxolinic acid.

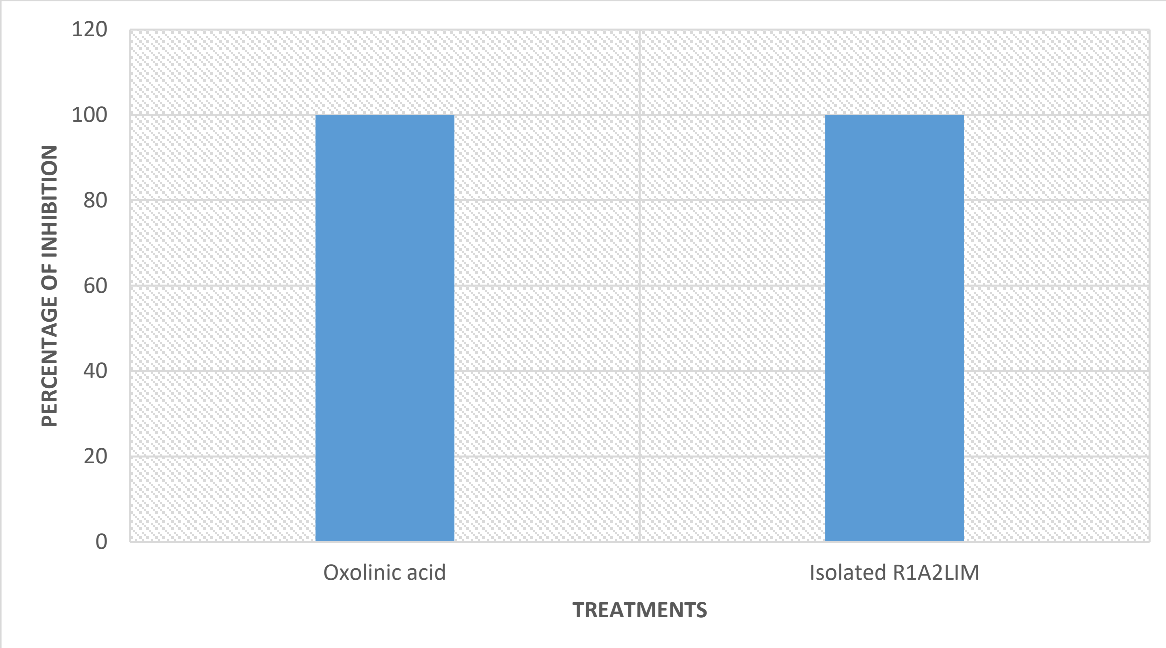

Figure 1. In vitro percentage inhibition of R1A2LIM isolate and oxolinic acid against B. glumae.

Figure 2 shows the presence of the R1A2LIM isolate by tissue type and rice variety. The analysis indicates that the highest presence of the isolate was found in roots of the rice variety F2000 in relation to the other plant tissues (stem, leaf and inflorescence) and rice variety analyzed (F747 and FMocari).

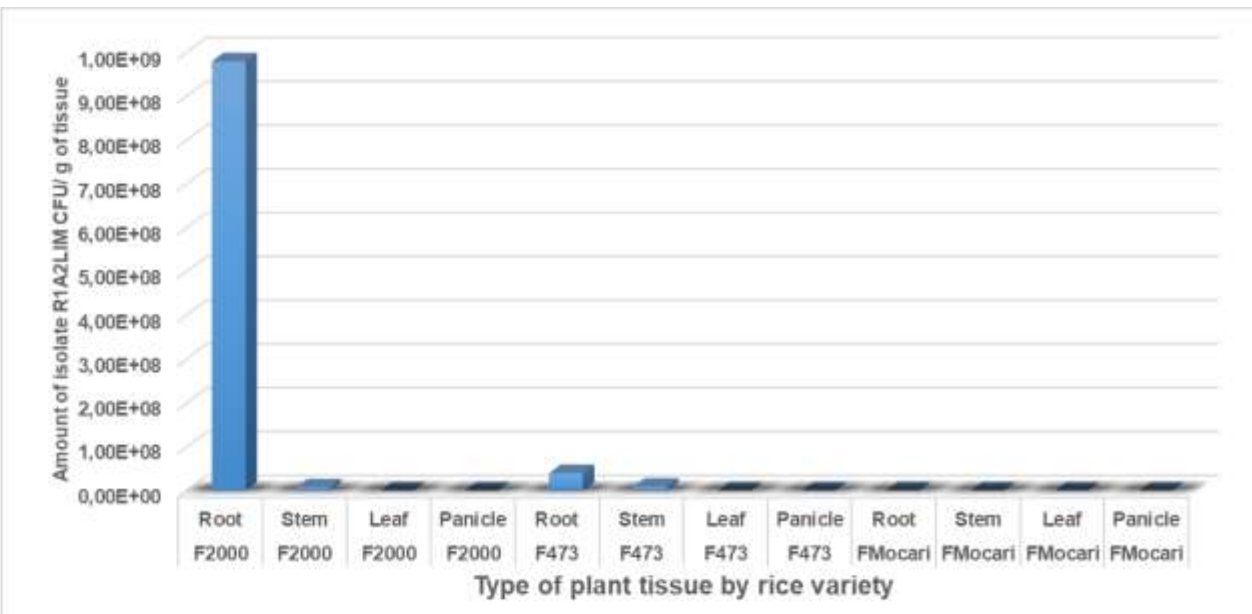

Figure 2. Amount of R1A2LIM isolate in relation to plant tissue type and rice variety.

There was also a difference in the amount of R1A2LIM isolate with respect to tissue and rice variety. The tissues of the rice varieties showed significant differences (p-value < 0.05), with higher colonization observed in the root (9.8x108, 4.0 x 107 and 6.0 x 105 CFU/g tissue), respectively in relation to stem (7.0x107, 3.0 x 106 and 4.0 x 104 CFU/g tissue), leaf (2.0x105, 7.0 x 104 and 3.0 x 104 CFU/g tissue) and panicle (3.0x104, 1.3 x 104 and 6.3 x 103 CFU/g tissue) (figure 2).

The amount of endophytic bacteria varies according to the rice plant tissue. The main reason why there is a higher colonization in the root than in other plant tissues may be due to the fact that the roots are in intimate contact with the environment which has a high diversity of microorganisms (Senthilkumar et al., 2011). Bacilio et al. (2001), indicated that the density is higher in the intercellular junction zone of the root epidermis, this is probably due to the space and the possibility of mobility provided by these regions, because the mucilaginous layer that is found covering the epidermis has less tension in this area. Molecular studies on bacterial diversity indicate that the root has a higher population density compared to the stem and leaves. This is due to the great diversity of bacteria in the soil and the site of entry of bacteria into the plant through this organ (Mano & Morisaki, 2008).

The results of the sequence analysis show that the isolate identified as R1M2LIM shows a high homology (100%) with sequences stored in the GenBank genome sequence bank with Bacillus subtilis strain AH013002.3 (figure 3).

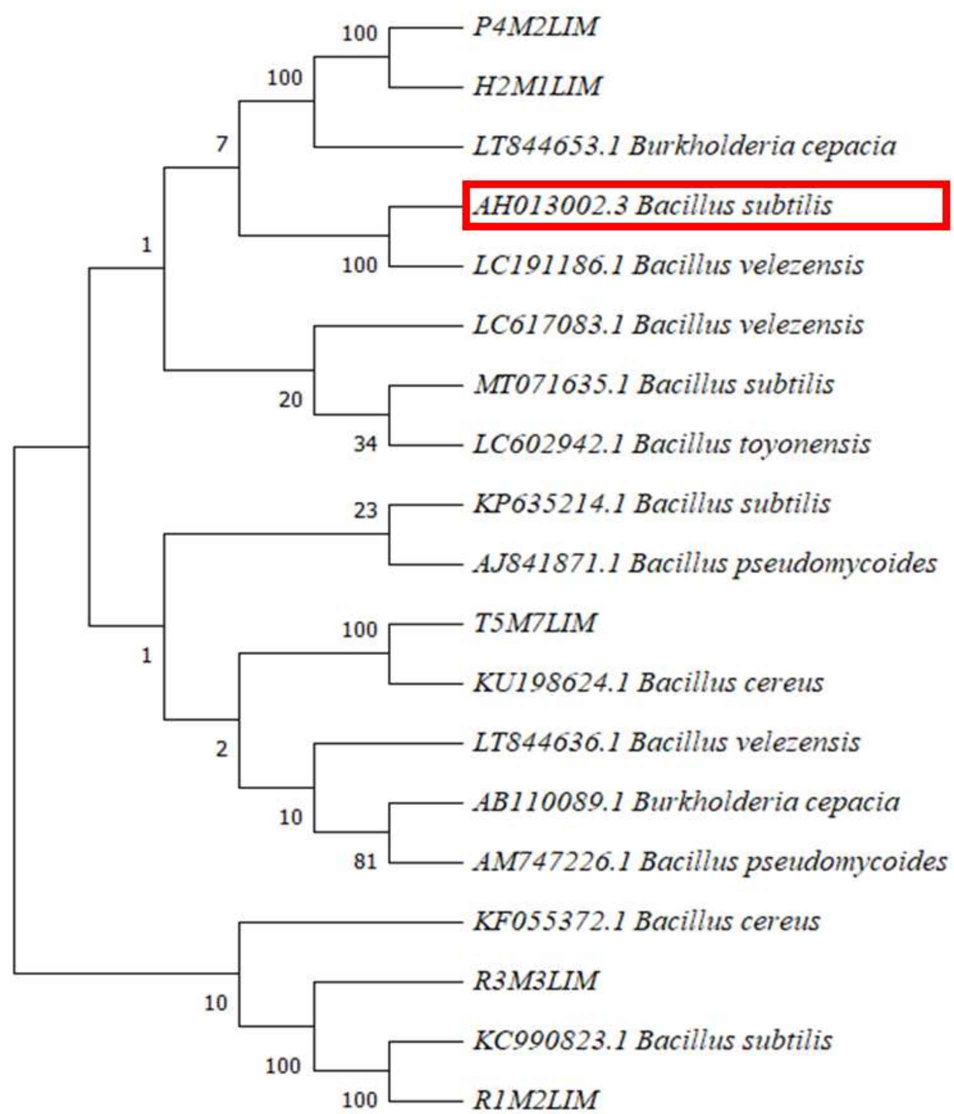

Figure 3. Dendogram of phylogenetic relationships of endophytic bacteria isolated from plant tissues of rice plants using the Neighbor-joining method from 16S rRNA gene sequences. H: leaf; P: panicle; T: stem; R: root; M: morphotypes; LIM: Microbiological Research Laboratory.

According to Yanes (2012), the strain of bacteria identified as B. subtilis provides effective control of fungal and bacterial diseases. The potential of this bacterium is based on its ability to produce a wide range of bioactive molecules, which show antifungal properties, together with low toxicity and high biodegradability, in addition, the ability to form endospores, provide a high level of resistance to extreme environmental conditions.

According to reports by Tejera et al., 2013; Layton et al., 2011; Yesid and Sánchez, (2012), the antagonistic capacity of B. subtilis lies mainly in its ability to produce different metabolites with antifungal and antibacterial capacity, within the group of antifungal metabolites are surfactins, phengicins and iturins; in addition to a variety of lytic enzymes among which lipases, proteases and β-glucanases stand out.

4. CONCLUSION

The isolate R1A2LIM, obtained from roots of commercial rice varieties, which was identified by sequencing technique as Bacillus subtilis AH013002.3, showed in vitro 100% inhibition on the growth of the phytopathogenic bacterial strain known as Burkholderia glumae causing the disease known as panicle blast. Further studies will determine the type of secondary metabolite directly related to the antibacterial activity of this isolate of endophytic bacteria.

5. ACKNOWLEDGMENTS

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6. COMPETING INTERESTS.

The authors declare no competing interests.

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