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ANTIBIOSIS OF ENDOPHYTIC BACTERIA AGAINST PHYTOPATHOGENIC BACTERIA IN RICE CULTIVATION

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

[1]*Universidad de Sucre, Facultad de Ciencias Agropecuarias, Colombia, https://orcid.org/0000-0003-3989-1747
[2]Universidad de Sucre, Facultad de Ciencias Agropecuarias, Colombia, donicer.montes@unisucre.edu.co, https://orcid.org/0000-0002-2860-0505
[3]Universidad de Sucre, Facultad de Ingeniería, Colombia, yelitza.aguas@unisucre.edu.co https://orcid.org/0000-0003-4880-4510

***Corresponding Author:** Alexander Pérez Cordero
*Universidad de Sucre, Facultad de Ciencias Agropecuarias, Sincelejo, Sucre, Colombia
alexander.perez@unisucre.edu.co

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ABSTRACT
The aim of this study was to isolate endophytic bacteria from different tissues of commercial rice varieties and to test in vitro their antibacterial activity against *Burkholderia glumae* causing bacterial blast disease of rice. The results of this study report a high diversity of culturable endophytic bacteria with the ability to produce secondary metabolite type extracts with the capacity to inhibit in vitro the growth of B. glumae. The results obtained from the extracts at different extraction times have similar effects to that shown by the chemical control with oxolinic acid. Further studies will identify the groups of metabolites produced by these bacteria and their possible use for field management of *B. glumae*.

Keywords: bacterial extracts, inhibition, phytopathogen, rice.

INTRODUCTION

Rice (*Oryza sativa L.*) is one of the most consumed major cereals of economic interest worldwide. The production system is affected by various plant pathogens capable of causing significant economic losses in crop production and productivity (Fahad et al., 2014; Ruzmi et al., 2017; Stuart et al., 2020). Bacterial species identified as Burkholderia glumae and Burkholderia gladioli are considered the pathogenic agents of the disease known as rice panicle blast (Ham et al., 2011; Ham et al., 2018). This disease is most prevalent at the flowering stage and causes infertility, with discoloration and staining of the developing glume due to toxoflavin secretion (Lee et al., 2016).
Rice plant-associated endophytic bacteria in their process of co-evolved with the rice plant to establish themselves to the plant and reduce diseases in the shared ecological niche by employing different strategies (Ham et al., 2011). In that perspective, biological control, using native endophytic bacteria, has shown promise (Barra et al., 2014; Choi et al., 2018). Strategies based on biological mechanisms such as anti quorum it sensing, quorum quenching (Fetzner, 2015), induced systemic resistance

(Chung et al., 2015) and production of antimicrobial metabolites (Ramos et al., 2019) are the most promising for disease management in the field (Shrestha et al., 2016).

Endophytic bacteria are colonizers of healthy plant tissues without symptoms of disease or intoxication, which live on the surface of clean tissues or in plant extracts (Hallmann et al., 199; Sessitsch et al., 2004). They have been isolated from a variety of plant species including rice, yams, pastures, medicinal and aromatic plants among others.

Biological control of crop diseases and pests using antagonistic microorganisms has been an environmentally friendly alternative to the use of chemical pesticides (Moenne-Loccoz et al., 2001) and is being widely studied in many plant diseases, using a diverse group of antagonistic microorganisms as part of integrated disease management programmes.

Based on the above, the strategy was to evaluate in vitro the inhibitory capacity of extracts of endophytic bacteria isolated from rice plants against *Burkholderia glumae*.

MATERIALS AND METHODS

Isolation, counting, strain separation, antibacterial activity testing of cells and metabolite compounds, strain identification by molecular sequencing and identification of the composition of microbial metabolites were performed following the scheme as described in the process in figure 1.

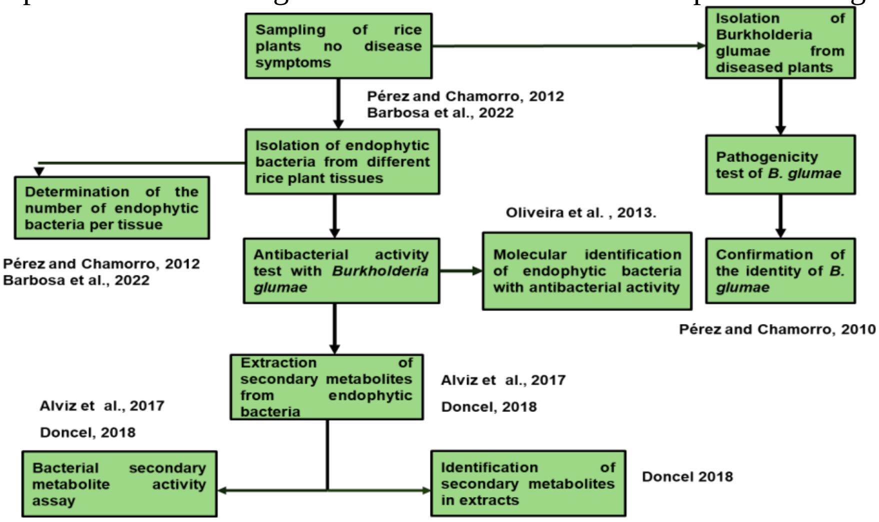

Figure 1. Stages in the process of evaluation of secondary metabolite-like compounds produced by endophytic bacteria isolated from rice plants against Burkholderia glumae.

RESULTS AND DISCUSSION

The sampling of rice plants was carried out at the facilities of the experimental farm La Victoria, in the locality of Mocarí, municipality of Montería, department of Córdoba, Colombia, as shown in figure 2.

Figure 2. Sampling site of commercial rice varieties for isolation of endophytic bacteria and secondary metabolite-type compounds against *Burkholderia glumae.*

A high diversity of endophytic bacterial strains was isolated from different rice plant tissues on R2A agar medium surfaces, as shown in figure 3.

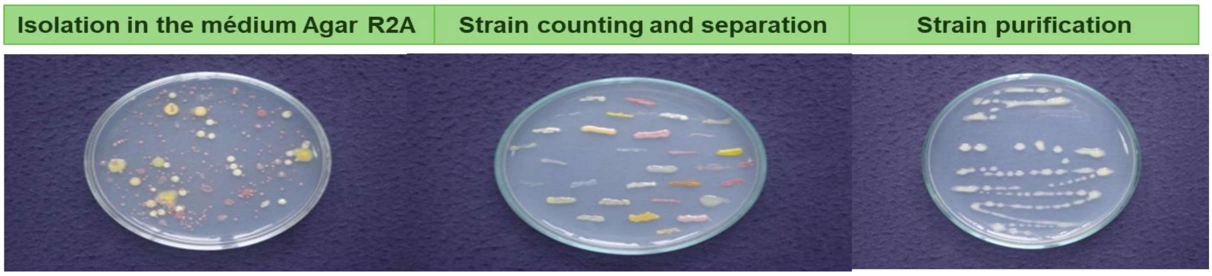

Figure 3. Process of isolation, separation and purification of endophytic bacterial strains from different rice tissues.

Figure 4 shows the antibacterial activity test of microbial metabolites of endophytic bacteria against B. glumae. The figure shows the inhibition halo activity of the bacterial extracts compared to the positive control with oxolinic acid.

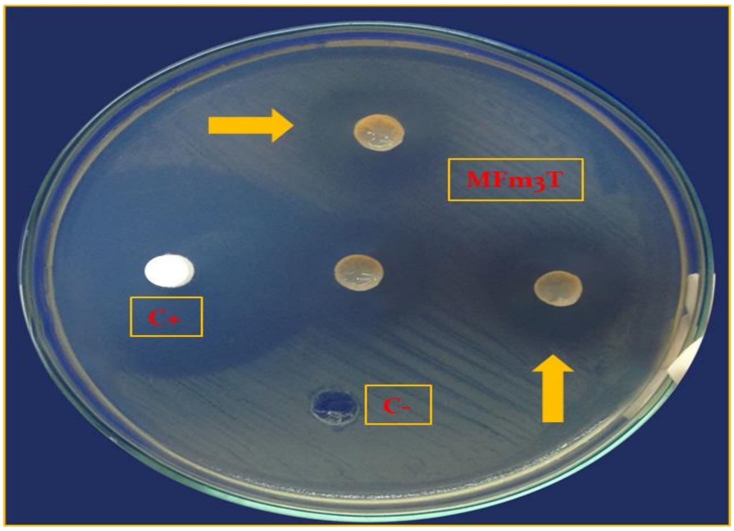

Figure 4. Antibacterial activity by production of antimicrobial compounds from endophytes bacterial isolated from rice varieties against *B. glumae*.

Figure 5 shows the antibacterial activity of endophytic bacterial cell extracts at different extraction times and the activity compared to the effect of the positive control using the chemical control with oxolinic acid.

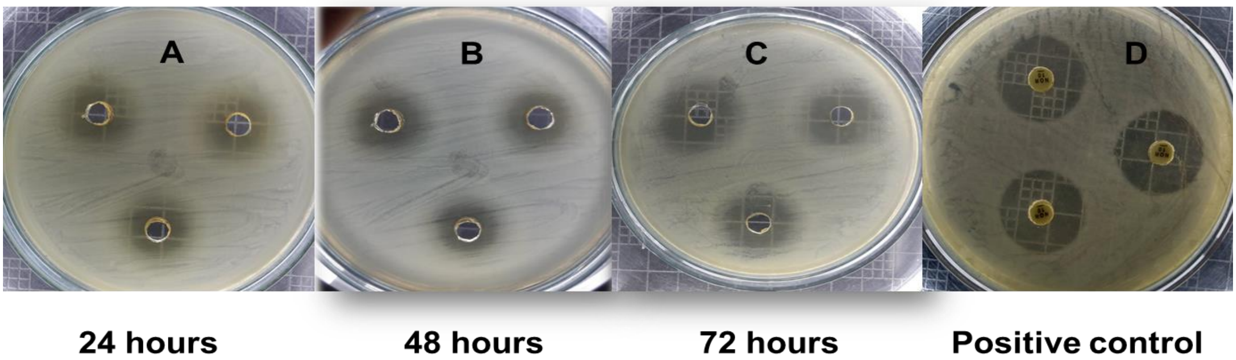

Figure 5. Antibacterial activity of crude extracts of endophytic bacteria against B. glumae at different exposure times.

Figure 6 shows the PCR amplification products of 16S rDNA fragment, with primers F984GC/R1378, of the total of endophytic bacteria associated with the Fmocari strain. Figure 6 shows the PCR amplification products of 16S rDNA fragment, with primers F984GC/R1378, of the total of endophytic bacteria associated with the strain F733.

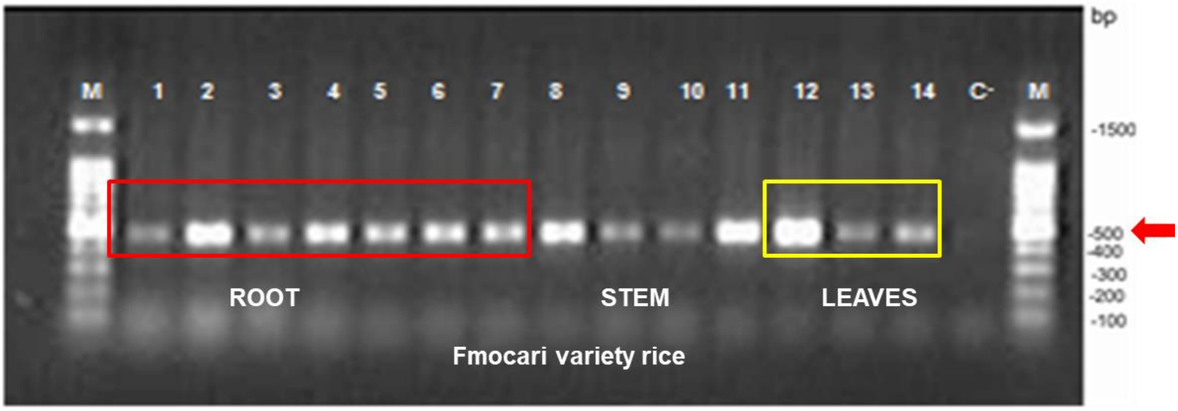

Figure 6. Agarose gel electrophoretic profile at 1.2 % of the PCR amplification product of 16S rDNA fragment, with primers F984GC/R1378, of the total of endophytic bacteria associated with the Fmocari variety. 1-7: roots, 8-11: stem, 12-14: leaf, M-100 bp DNA Ladder, C-: negative control.

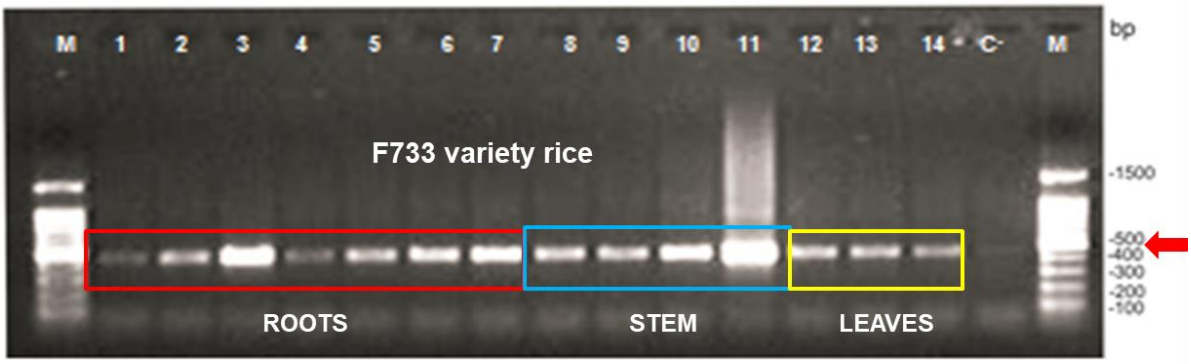

Figure 7. Electrophoretic profile in agarose gel at 1.2 % of the PCR amplification product of 16S rDNA fragment, with primers F984GC/R1378, of the total of endophytic bacteria associated with variety F733. 1-7: roots, 8-11: stem, 12-14: leaf, M-100 bp DNA Ladder, C-: negative control.

Various endophytic bacteria have shown antimicrobial activity against different plant pathogens. Verma et al., 2009, conducted studies on the antimicrobial activity of endophytes associated with the neem tree, Azadirachta indica, in India. The endophytes were identified as Streptomyces sp., Streptosporangium sp. and Nocardia sp. Assays with extracts of these endophyte strains showed activity against Pseudomonas fluorescens, B. subtilis, S. aureus, E. coli, C. albicans, Trichophyton sp., Microsporum sp., Aspergillus sp., Pythium sp. and Phytophthora sp.

Similarly, Machavariani et al., (2014), isolated endophytic bacteria from Aloe arborescens, Mentha arvensis, Lysimachia nummularia, Fragaria vesca and Arctium lappa; native to Russia. These isolates were identified as Nocardiopsis, Streptomyces and Micromonospora. The results of the well diffusion assays showed activity against S. aureus, Micrococcus luteus, B. subtilis, E. coli, P. aeruginosa and the fungus S. cerevisiae. The strain extract showed activity against E. coli, Salmonella sp, B. subtilis, E. faecium, S. aureus and C. albicans. Significant disease suppression was also observed for wheat plants colonised with B. subtilis (Liu et al., 2009) and for banana plants pre-inoculated with endophytic Pseudomonas and Burkholderia (Fishal et al., 2010).

CONCLUSION

The results of this study show a high diversity of culturable endophytic bacteria with the ability to inhibit in vitro the growth of the phytobacterium Burkholderia glumae causing bacterial blast in rice.

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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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