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BURKHOLDERIA CEPACIA A BIOLOGICAL MODEL FOR PLANT PATHOGEN CONTROL AND HEAVY METAL REMEDIATION

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 objective of this study was to demonstrate through in vitro assays the importance of the bacterium belonging to the Burkholderia cepacia complex as a biological resource for the biological control of phytopathogens through the release of secondary metabolite-like compounds and the ability to promote plant growth and assist plants in adaptation and abiotic stress produced by the presence of heavy metal pollutants. The results and scientific evidence carried out by the Agricultural Bioprospecting research group show that B. cepacia produces secondary metabolites with activity against Colletotrichum gloeosporioides and the ability to tolerate high concentrations of lead, cadmium, mercury and nickel.

Keywords. Bacterial complex, biological control, remediation, endophyte**.**

1. INTRODUCTION
Endophytic bacteria colonize plant tissues, providing a protective mechanism against fluctuating environmental conditions and showing a non-significant effect for the bacteria (Latz et al., 2018). Phytoremediation of pollutants and contaminants in the environment is another eco-friendly technology and sustainability application that endophytic bacteria can provide (Deng and Cao, 2017; Feng et al., 2017). There is also known evidence of applications of endophytic bacteria in protecting plants against biotic and abiotic stress conditions, promoting growth and directly and indirectly contributing to plants tolerating high concentrations of pollutants, contributing to the process of endophyte bacteria-assisted phyco-remediation (He et al., 2020). At the present time it is considered that endophyte-mediated bioremediation was undertaken as a prospective method to clean soil contaminated with heavy metals. The metals present in soil pose environmental heavy metals and may be toxic to human health (Domka et al., 2019; Franco-Franklin et al., 2021). In addition, bacterial

endophytes promote the bioaccumulation of heavy metals (thereby reducing metal phytotoxicity (Ma et al., 2016).

Bacterial endophytes inhibit or kill pathogens, and thereby protect the plant against disease onset. Similarly, if a pollutant inhibits the germination of seeds or plant growth, endophytes eliminate the pollutant via biodegradation (Mercado-Blanco and Lugtenberg, 2014, Mercado-Blanco and Lugtenberg, 2014).

The Burkholderia cepacia bacterial complex (BCC), or simply Burkholderia cepacia, or Pseudomonas cepacia, is a group of Gram-negative, non-fermenting, aerobic, catalase-producing bacteria composed of at least nine different species (Mahenthiralingam et al., 2005).

Burkholderia cepacia has been demonstrated in vitro to tolerate different concentrations of metals, promote plant growth and exert biocontrol against phytopathogens. Based on the scientific evidence of the contributions of Burkholderia cepacia in productivity, sustainability, crop protection and in the recovery of environments contaminated with toxic compounds, it was proposed to isolate and evaluate the benefits of *B. cepacia* as a biocontrol agent and biological resource to help plant species to adapt to the presence of heavy metals.

MATERIALS AND METHODS

For the isolation of endophytic bacteria the following process is followed as described in figure 1.

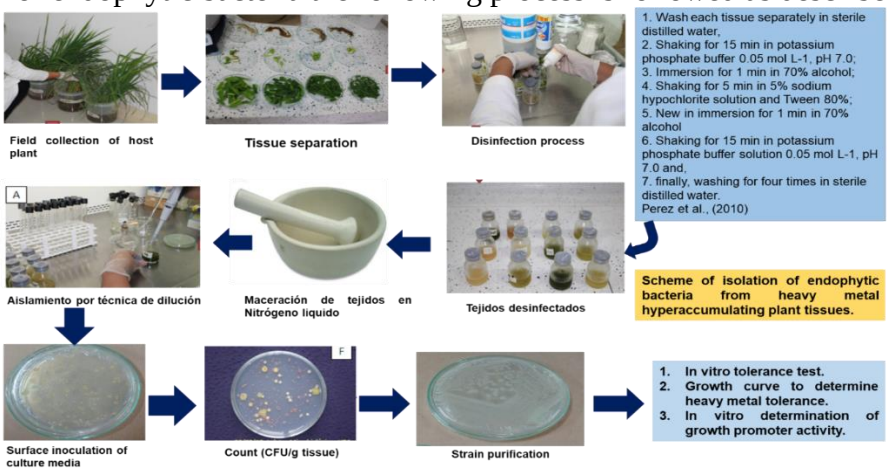

Figure 1. Scheme for isolation of endophytic bacteria from tissues of plants. Source: Pérez et al., 2023.

For the determination of antimicrobial activity of Burkholderia cepacia the following methodology is used as described in figure 2.

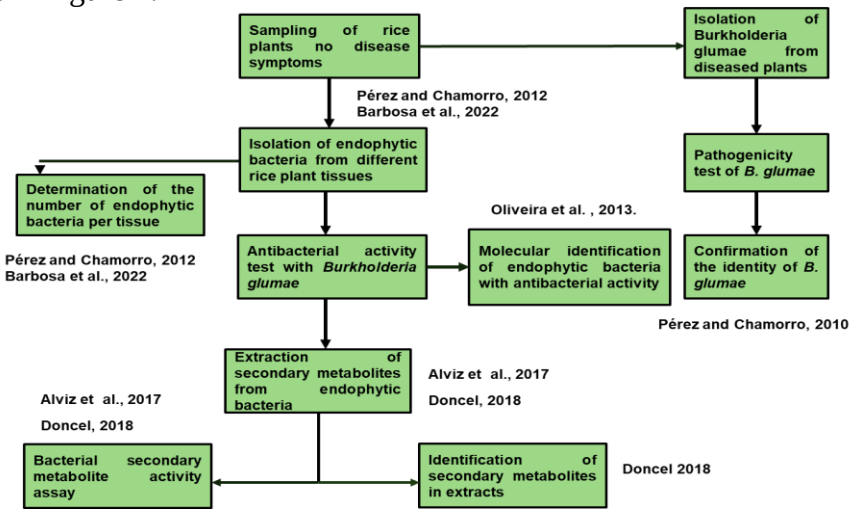

Figure 2. Stages in the process of evaluation of secondary metabolite-like compounds produced by endophytic bacteria isolated from rice plants against *Burkholderia glumae*.

To evaluate the in vitro tolerance activity to different concentrations of heavy metals, the following steps are carried out as described in figure 3.

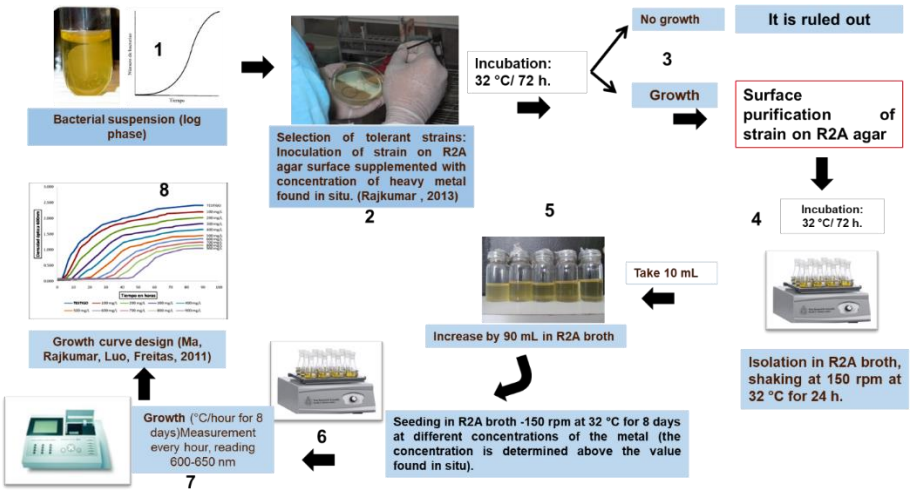

Figure 3. Schematic of tolerance test of endophytic bacterial isolates to different concentrations of the heavy metal, which is to be used in the form of salts. Source: Pérez et al., 2023.

RESULTS AND DISCUSSION

As a result of heavy metal tolerance activity by Burkholderia cepacia in the different works the research team reports the following results as described in table 1.

Burkholderia cepacia, is present in soil and water (Glowicz et al., 2018), also as an endophytic bacterium of Lupinus luteus with resistance to Co, Cu, Cd, Hg, Ni, Pb and Zn, highlighting the ability to help its host to adapt to unfavorable conditions (Ma et al., 2016). In addition, it possesses growth-promoting activity through nitrogen fixation, siderophores, phosphate solubilization, biocontrol of plant pathogens and bioremediation *(*Mathew et al., 2014; Ghosh et al., 2016; Rojas et al., 2018*)*.

Table 1. *Bukholderia cepacia* isolated from different plant species with the capacity to remediate heavy metals and promote plant growth in the Colombian Caribbean

Host plant	Endophytic bacterium	Origin	Function	Reference
Rice	*Burkholderia cepacia*	Department of Córdoba, Colombia	Lead tolerance, nitrogen fixation, phosphate solubilization and siderophore production	Pérez et al., 2015
Paspalum arundinaceum	*Burkholderia cepacia*	Department of Bolívar, Colombia	Mercury tolerance, nitrogen fixation, phosphate solubilization and siderophore production	Pérez et al., 2016
Rice	*Burkholderia cepaceae*	Department of Córdoba, Colombia	Cadmium tolerance, nitrogen fixation, phosphate solubilization and siderophore production	Ayubb et al., 2017
Rice	*Burkholderia cepaceae*	Department of Córdoba	Cadmium tolerance, nitrogen fixation, phosphate solubilization and siderophore production	Ayubb et al., 2017
Aquatic macrophytes	*Burkholderia cepacia*	Department of Sucre, Colombia	Mercury tolerance, nitrogen fixation, phosphate solubilization and siderophore production	Torres et al., 2019
Pasture	*Burkholderia cepacia*	Department of Sucre, Colombia	Cadmium tolerance, siderophore production	Pérez et al., 2022(a)
Pasture	Burkholderia cepacea	Department of Sucre, Colombia	Led tolerance, siderophore production	Pérez et al., 2022(b)
Yam	*Burkholderia Cepacea*	Department of Sucre, Colombia	Cadmium tolerance, siderophore production	Pérez et al. 2023(a)
Yam	*Burkholderia cepacia*	Department of Sucre, Colombia	Cadmium tolerance, 1-aminocyclopropane-1-carboxylic acid deaminase (ACC) production	Pérez et al. 2023(b)

Likewise, studies carried out by Pérez et al., *(2016),* identified *Burkholderia cepacia* species KJ935925 as an endophytic bacterium associated with *Paspalum arundinaceum* plant tissue, with the ability to resist in vitro to high mercury concentration.

Torres et al., 2019, isolated Burkholderia cepacia as endophytic bacteria from Neptunia oleracea Lour, Eichhornia crassipes (Mart.) Solms and Paspalum repens Bergius. and evaluated in vitro the ability to tolerate different concentrations of mercury and nickel.

Doncel and Pérez, (2017) isolated Burkholderia cepacia from yam plants in the department of Sucre and demonstrated in vitro the ability to produce metabolites with antifungal activity against the phytopathogenic fungus Colletotrichum gloeosporioides. As a result of this study, the research team found four strains of endophytic bacteria that show high homology with sequences of bacterial species related to the Burkholderia cepacia complex associated with yam plants (Dioscorea rotundata), producing secondary metabolite-like compounds with inhibitory activity against C. gloeosporioides, as shown in figure 4.

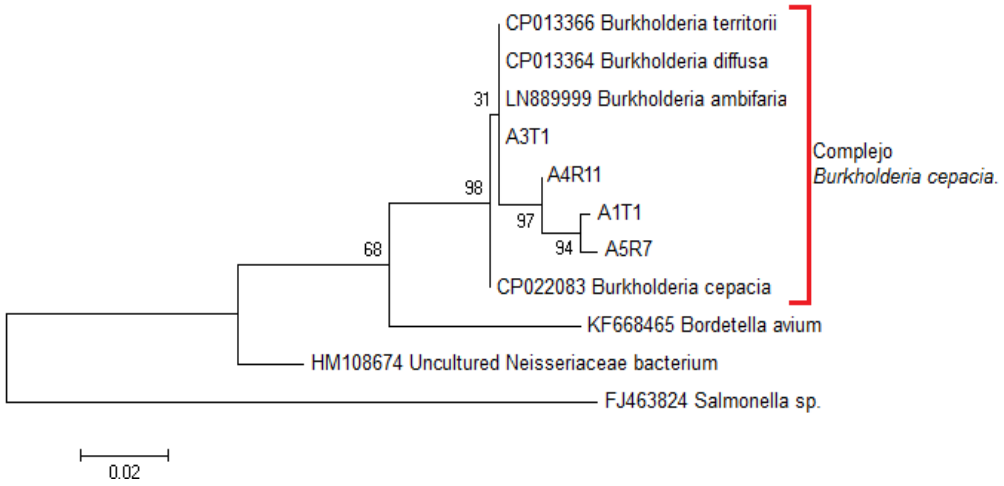

Figure 4. Phylogenetic tree derived from 16S rDNA gene sequencing analysis using primers F948β and R1492 of endophytic bacterial strains A1T1, A3T1, A4R11 and A5R7 isolated from yam culture and showing similarity with homologous sequences obtained from NCBI. Source: Doncel, 2018.

On the other hand, the Burkholderia cepacia complex is considered as important bacteria in the integrated management of plant diseases (Hernández et al., 1997), because they possess the ability to colonize plant organs and tissues and produce a wide variety of secondary metabolites such as siderophores, antibiotics and quinolysidine alkaloids with inhibitory activity against fungi and phytopathogenic bacteria. Evidence from studies concludes that Burkholderia cepacia is a plant endophyte of great importance for agricultural sustainability, mainly due to the production of a wide range of active metabolites, which positively affect healthy plant growth and development (Bevivino et al., 2000).

CONCLUSION

The complex of endophytic bacteria belonging to *Burkholderia cepacia* is part of the genomic bank of the research group in Agricultural Bioprospecting of the University of Sucre, who evaluated in vitro the antimicrobial capacity against phytopathogens of commercial interest and tested the tolerance of this species of endophytic bacteria to different concentrations of heavy metals. The results of the different studies show that *B. cepacia* is an integrative biological resource for promoting plant growth, assisting in heavy metal phycoremediation processes and as a biocontrol agent with plant pathogens in the Colombian Caribbean.

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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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