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INTRODUCTION

- Panama is the largest consumer of rice in Central America (68 kg / person) (FAO, 2013), being one of the essential foods in the diet of the population.

- The bacterial blight of the rice panicle, caused by *B.glumae* is one of them, acquiring great importance in recent years. Another pathogen of high incidence in rice cultivars is the fungus *P. oryzae* considered one of the most important phytopathogens in this crop.

- In recent decades the biological control of pests and diseases in agriculture has acquired great importance in the face of the phytosanitary problems caused by the indiscriminate use of chemical pesticides, which has resulted in severe problems of contamination to the environment and has increased the resistance of phytopathogens, increasing their virulence (Quesada and García, 2014).

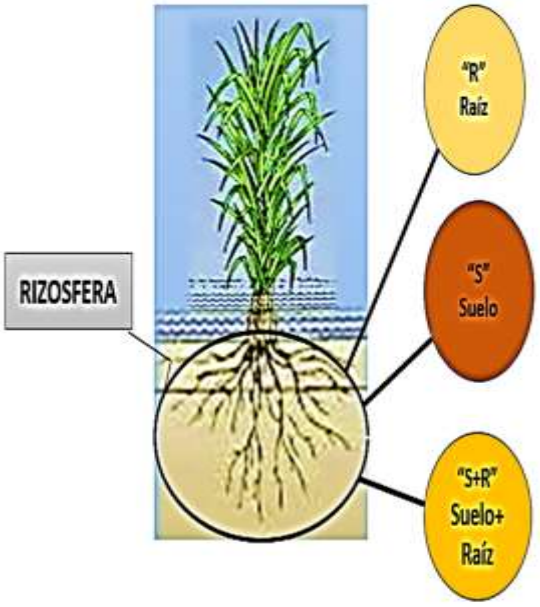
- The rhizosphere, due to its environment rich in energy and nutrients, hosts large populations of most of the groups of microorganisms in the soil. The microbial flora found in this can play an antagonistic action against phytopathogens (Jaramillo, 2002).

- The phyllosphere is considered as adverse for the colonization of organisms, as there is no source of nutrients there. The microbial communities of this space are diverse and include microorganisms that can be found as epiphytes on the surface of the plant or endophytes within the tissues of the plant.

- The espermosphere is a zone of influence of the seed in germination that produces stimulating exudates or inhibitors of the activity and / or growth of the autochthonous populations of the soil.

METHODOLOGY

- The samples were collected in the province of Coclé, Republic of Panama, near the UTM coordinates (Datum WGS84) 591361.06 m E; 943492.23 m N.
- The sampling was carried out in a 10 m2 plot with rice variety Gab 8 during three phenological stages (vegetative, reproductive and maturative).



METHODOLOGY

- **Rhizosphere. Isolation of rhizospheric bacteria.**

- From each of the collected plants the rhizospheric zone was separated, dividing it into three sections, the exorhizosphere or rhizospheric soil (S), the root that involves both the endorhizosphere and rhizoplane (R) and a mixture of rhizospheric soil with roots (S + R). 30 g of the sample were weighed from each section, and homogenized in 100 ml of peptonated water. These were placed in a seward® homogenizer at 200 RPM for 1 min. For the bacteria, serial dilutions of 10^{-1} to 10^{-7} were made for each of the sections of the rhizosphere (homogenized in peptonated water). 0.7 ml of each dilution was added in trypticase soy agar (TSA) (Alpha Biosciences®) and incubated at 28 ° C for 24 h. After the incubation, the bacterial colonies were isolated in each dilution **(Benitez *et al*., 2007).**

- **Rhizosphere. Isolation of rhizospheric fungi**.

- Similar to the rhizospheric bacteria, serial dilutions were made and 0.7 ml was added from the 30 g sample taken from each of the sections of the rhizosphere of each dilution in potato dextrose agar (PDA) (Alpha Biosciences ®) **(Benitez *et al*., 2007).**

METHODOLOGY

- **Phyllosphere. Isolation of epiphytic bacteria**

- The methodology developed for the isolation of epiphytic bacteria, was performed in the sections of the apex, middle and basal areas of the collected leaves. Surface disinfection was carried out with water for 5 min. to eliminate all soil residues. After each section, 10 ml of peptone water was added and homogenized for 1 min at 200 rpm (Stomacher Seward®). From the solution in peptone water, serial dilutions (10-1 to 10-7) of each leaf section (apical, middle and basal) were prepared, from which aliquots were taken and they inoculated on trypticase soy agar (TSA) for the isolation of total bacteria, and were incubated at 28 ° C for 72 h **(Benitez *et al*., 2007).** Subsequently, the selection of colonies was made.

- **Phyllosphere.Isolation of endophytic fungi**

- For the isolation of endophytic fungi, healthy leaves were selected and each section of the leaf was cut into 2x2 mm squares, sections of plant tissue were placed on potato dextrose agar (PDA), incubated at 28 ° C (Schulz *et al*., 2002), exposed to 16 h light and 8 h of darkness. As fungal growth was observed, hyphae points were taken to perform microcultures, for later taxonomic identification.

METHODOLOGY

- **Espermosphere**

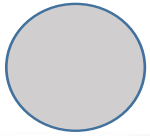
For the isolation of bacteria and fungi, 100 grams of rice seeds were weighed to which 200 mL of sterile distilled water was added. Then they were placed in a Stomacher (400 circulator) and shaken for 10 minutes. With the decanted water serial dilutions of $1x10^{-1}$ to $1x10^{-7}$ were made and spread on trypticase soy agar and potato dextrose agar, subsequently incubated at 30 ° C for 24 h.

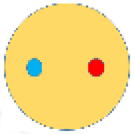
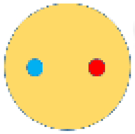
- **Antagonism tests**

The antagonism tests consisted of dual confrontations between the autochthonous microorganisms isolated from the rhizosphere, phyllosphere and espermosphere against the phytopathogens *B. glumae* and *P. oryzae*, in order to quantify the possible inhibition of the growth of the pathogenic microorganisms. The in vitro antagonism test was based on the methodology of **Alvis *et al* (2017)** with minor modifications and consisted of dual confrontations between the B.glumae strain and the isolated microorganisms.

- **Identification of Microorganisms**

For the identification of bacteria with antagonistic capacity against *B.glumae*, we used the API biochemical identification system (API 20 E and one of API 50 CH) Biomereux®. The strains with inhibition halo ≥ 4mm in diameter were considered as a significant antagonist. The monosporic cultures were identified with the help of the taxonomic keys were identified (Watanabe, 2010).

Enfrentamientos				
Replica 1	**Replica 2**	**Replica 3**	**Controles**	**Observación**
 ***B.glumae*** difusa en medio TSA Bacterias aisladas de la rizósfera	 ***B.glumae*** difusa en medio TSA Bacterias aisladas de la rizósfera	 ***B.glumae*** difusa en medio TSA Bacterias aisladas de la rizósfera	***Control 1*** Se coloca 1 ml de ***B.glumae*** en el plato Petri y luego se agrega agar TSA homogeneizando y dejando solidificar.  ***B.glumae*** *difusa en medio TSA*	Los tratamientos se incubaron a temperatura ambiente. La evaluación se hizo por medición del diámetro de las zonas de inhibición de crecimiento alrededor de la gota radial (mm), el día 2, 5 y 8 luego de la inoculación. La sola presencia de halos de inhibición alrededor de la gota será considerada como una respuesta cualitativa antagónica entre cepas.
Método: difusión en agar -Se coloca 1 mililitro de ***B.glumae*** en el plato Petri -Se agrega agar TSA homogeneizando y dejando solidificar. -Luego de solidificado se divide en 4 secciones, colocando en cada sección una gota (tres micro litros) de la bacteria aislada y previamente revivificada (una bacteria distinta en cada sección).			***Control 2*** En un plato Petri con medio TSA, se colocan 3 µl de las soluciones bacteriana con las que se trabajan (una bacteria en cada sección).  *Plato Petri con medio TSA* *Bacterias aisladas de la rizósfera*	Para el cálculo del potencial antagonista, se mide el diámetro del halo de inhibición y se resta del diámetro de la colonia de la bacteria.

<table>
<tr><th colspan="3">Enfrentamientos</th><th></th><th></th></tr>
<tr><th>Replica 1</th><th>Replica 2</th><th>Replica 3</th><th>Controles</th><th>Observación</th></tr>
<tr>
<td> 
Plato Petri con medio PDA
Disco de la cepa fúngica aislada de la rizósfera
Disco de la cepa de P. oryzae</td>
<td> 
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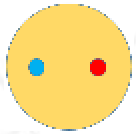
<td> 
Plato Petri con medio PDA
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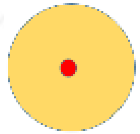
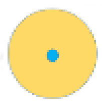
 
Se colocó un disco de 5mm del fitopatógeno P.oryzae en un plato Petri con medio PDA.
Plato Petri con medio PDA
Disco de la cepa de P. oryzae</td>
<td rowspan="2">Se marca una línea divisoria (centro del plato Petri), para observar la invasión del espacio de cada microorganismo.

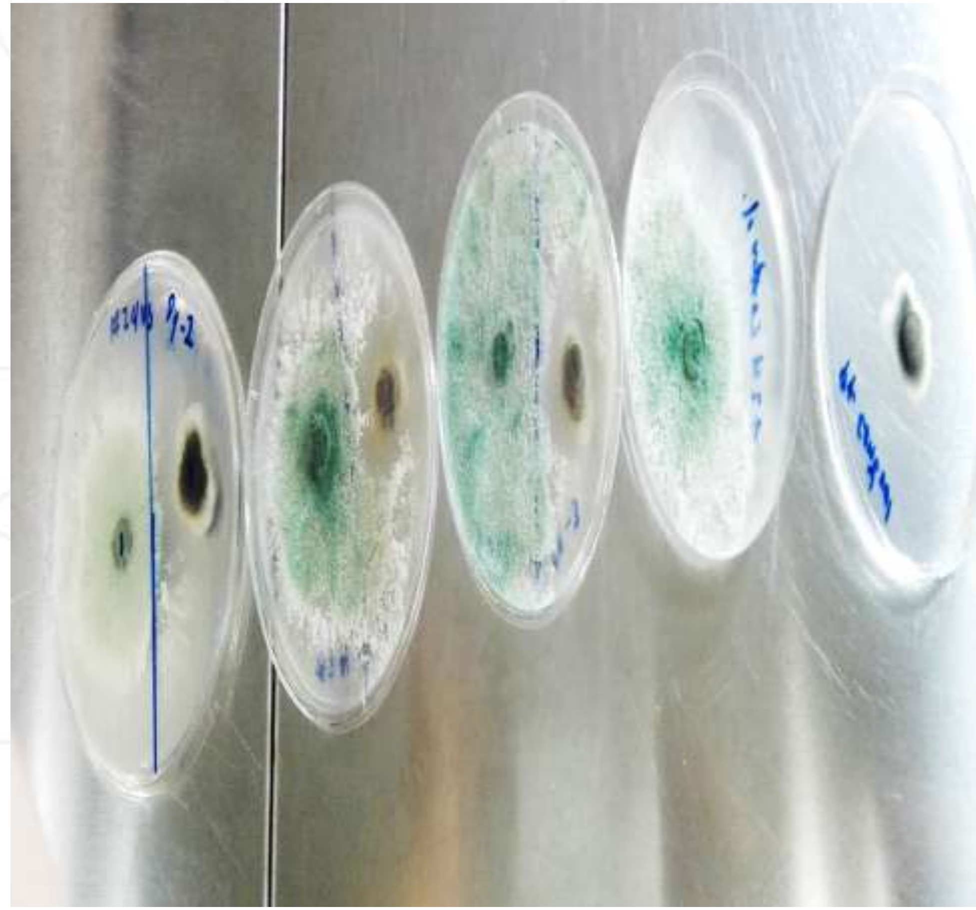
Se tomará la medida del crecimiento micelial del patógeno Pyricularia y se calcula el porcentaje de inhibición con la formula

$PICR = \frac{R1 - R2}{R1} \cdot 100$

Donde R1 es el radio mayor (radio patógeno-testigo) y R2 es el radio menor (radio del patógeno en cul-tivo dual).

Los tratamientos se incuban a temperatura ambiente. Las mediciones se realizan los días 3, 5 y 8 posterior</td>
</tr>
<tr>
<td colspan="3">Método: Cultivo dual

-En PDA, se sembrará un disco (5 mm) del hongo aislado a 15 mm del centro del plato,
-En el sentido opuesto también a 15 mm del centro, se colocará un disco de 5 mm de diámetro del patógeno Pyricularia.</td>
<td>Control 2
 
Se colocó un disco de 5mm de la cepa fúngica aislada de la Rizósfera en un plato Petri con medio PDA.
Plato Petri con medio PDA</td>
</tr>
</table>



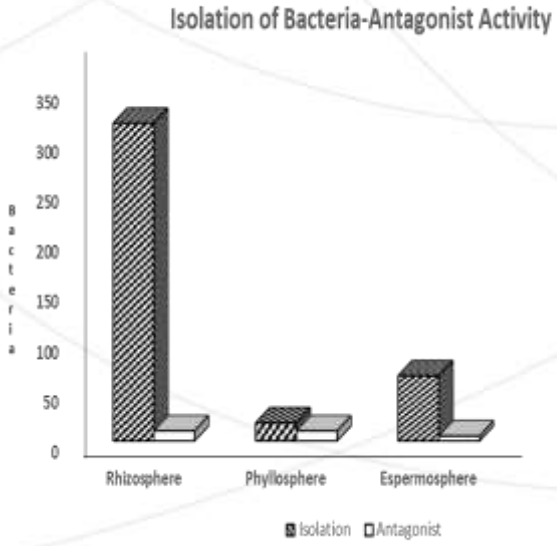
RESULTS AND DISCUSSION

- Soil samples taken for physicochemical analysis revealed a percentage of organic matter 1.04, a pH of 5.80 and a sand-silt-clay composition of 70-8-12%.

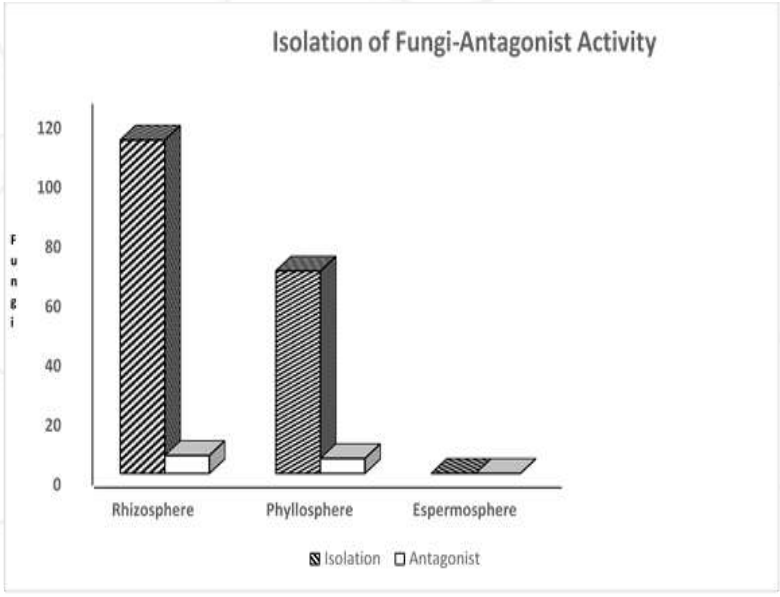
ELEMENTS					
Parameter	*P*	*K*	*Ca*	*Mg*	
	mg/l	*mg/l*	*Cmol/Kg*	*Cmol/Kg*	
Results	17.00	79.00	6.80	1.80	
Interpretation	low	medium	high	high	
Parameter	*Al*	*Mn*	*Fe*	*Zn*	*Cu*
	Cmol/Kg	*mg/l*	*mg/l*	*mg/l*	*mg/l*
Results	0.40	105.30	128.00	2.00	5.00
Interpretation	low	high	high	low	medium

RELATIONS						
Parameter	*Ca/Mg*	*(Ca+Mg)/K*	*K/Mg*		*Mg/K*	*Ca/K*
Results	3.78	42.57	0.11		8.91	33.66
Interpretation	normal	out of range	out of range		Normal	out of range
Parameter	*CICE*	*Saturation Al*	*K/CICE*	*Ca/CICE*	*Mg/CICE*	*Sat. of bases*
Results	9.20	4.35	2.20	73.90	19.56	95.65
Interpretation	low	low	medium	high		

- In the three sections (rhizosphere, phyllosphere and espermosphere), a total of 400 bacteria and 180 fungi were isolated, of which 25 bacteria had antagonist activity against *B. glumae* and 11 fungi against *P. oryzae*.

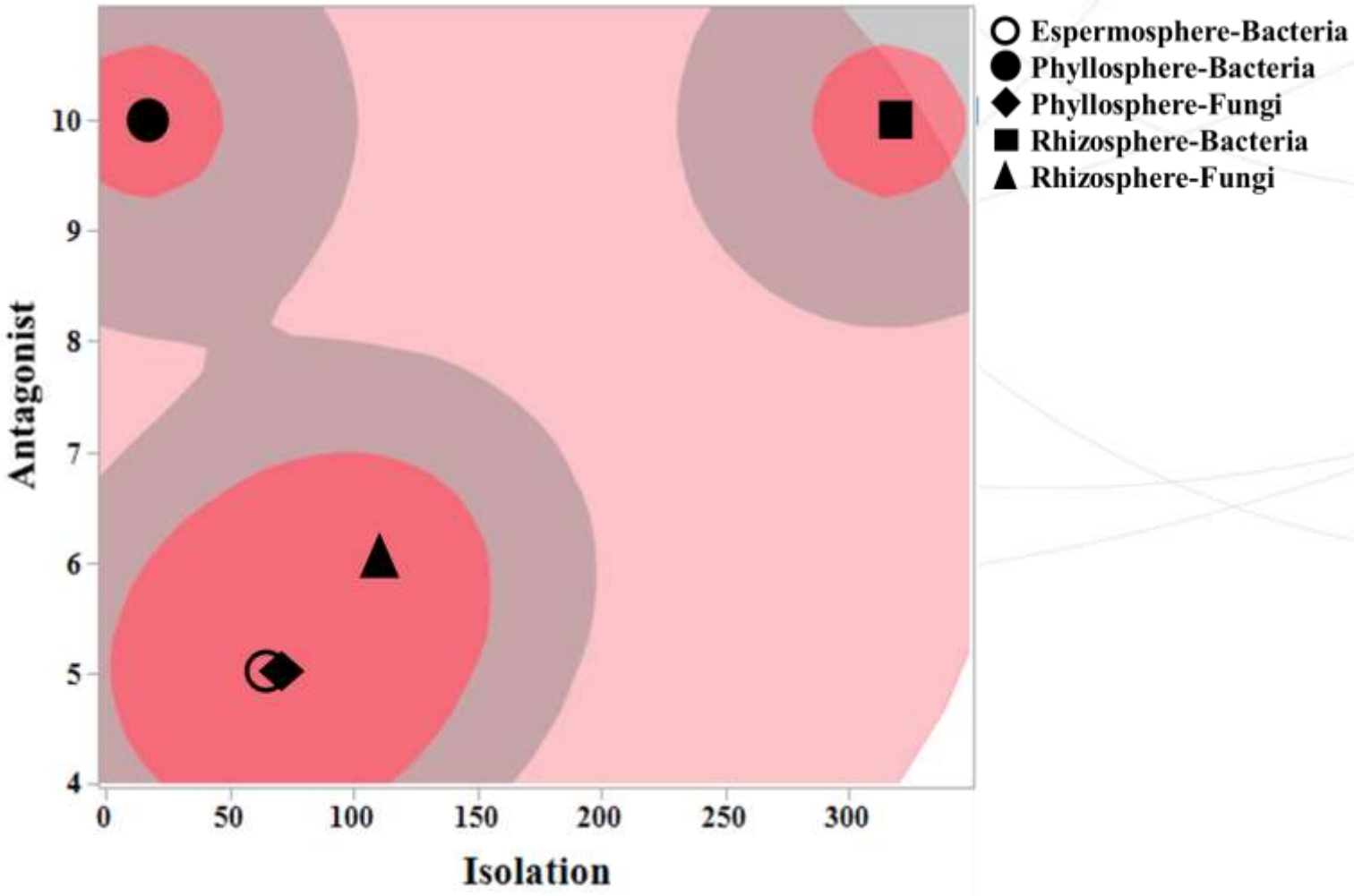


Isolation of bacteria and antagonistic activity against *B. glumae* in rhizosphere, phyllosphere and espermosphere in rice plants.

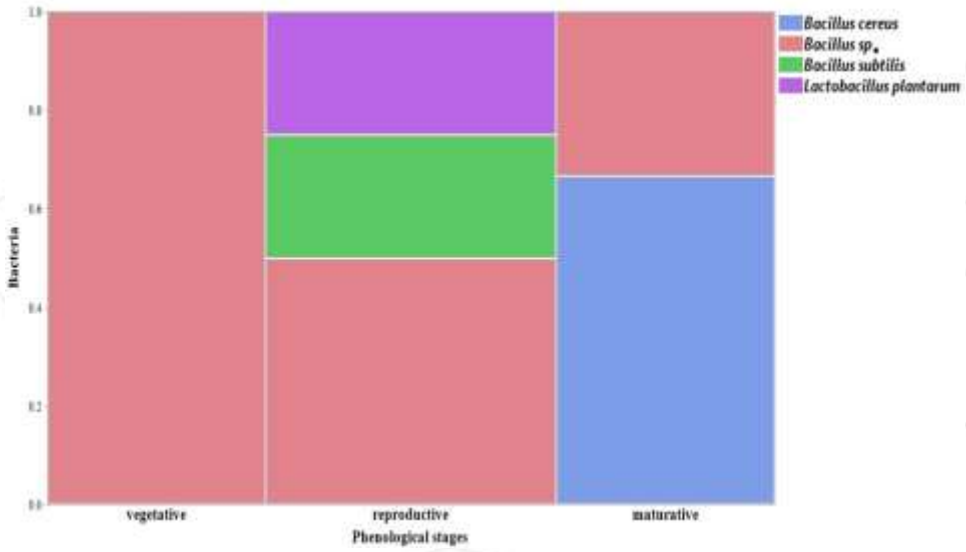


Isolation of fungi and antagonistic activity against *P. oryzae* in rhizosphere, phyllosphere and espermosphere in rice plants.

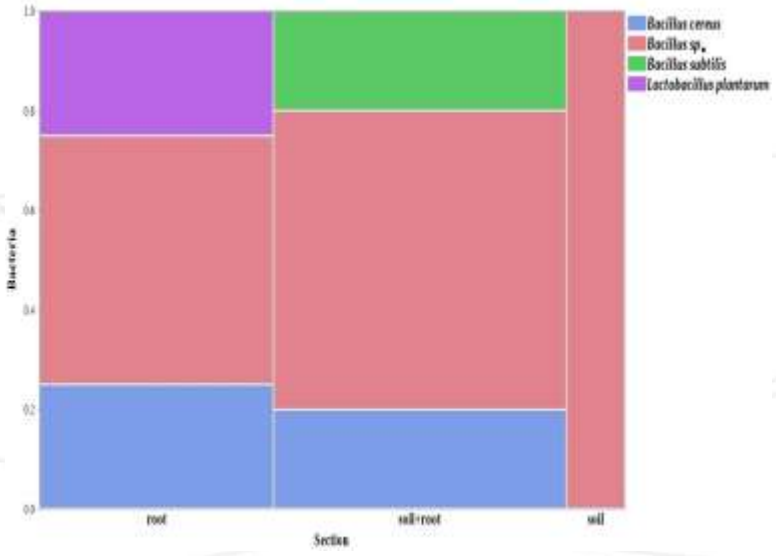
Conglomerate analysis of the number of isolates and antagonistic activity of the rhizosphere, phyllosphere and espermosphere.



- The predominance and distribution of the genus *Bacillus* in the phenological stages and the parts of the rhizosphere, suggesting the ubiquity of this bacterium for any of these sites.

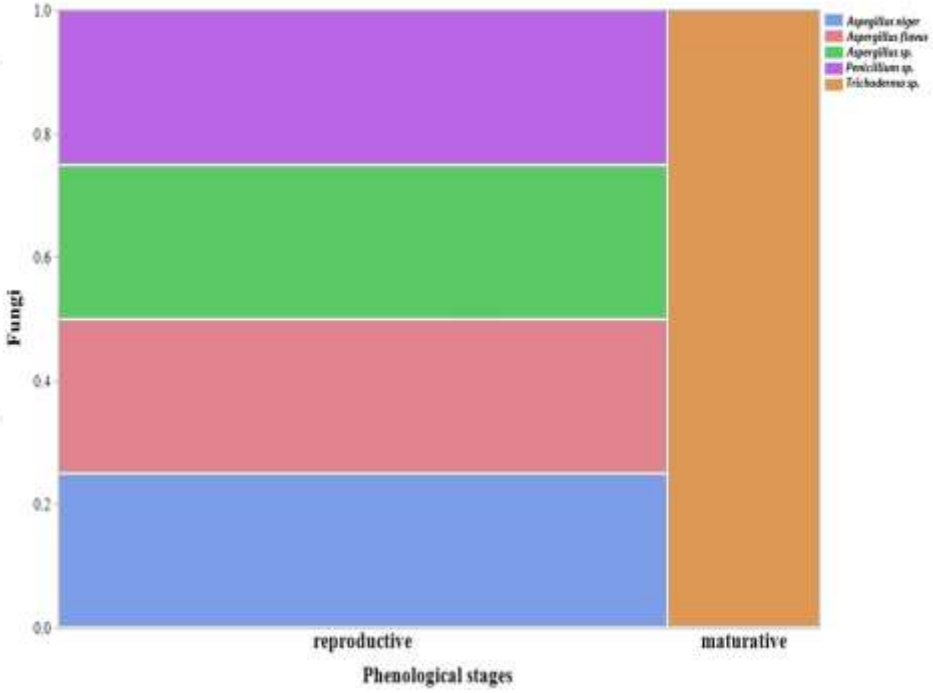


Distribution of the genus Bacillus in the phenological stages of the rice plant. The distribution of *Bacillus* subtilis, *B. cereus*, and *L. plantarum* is also indicated.

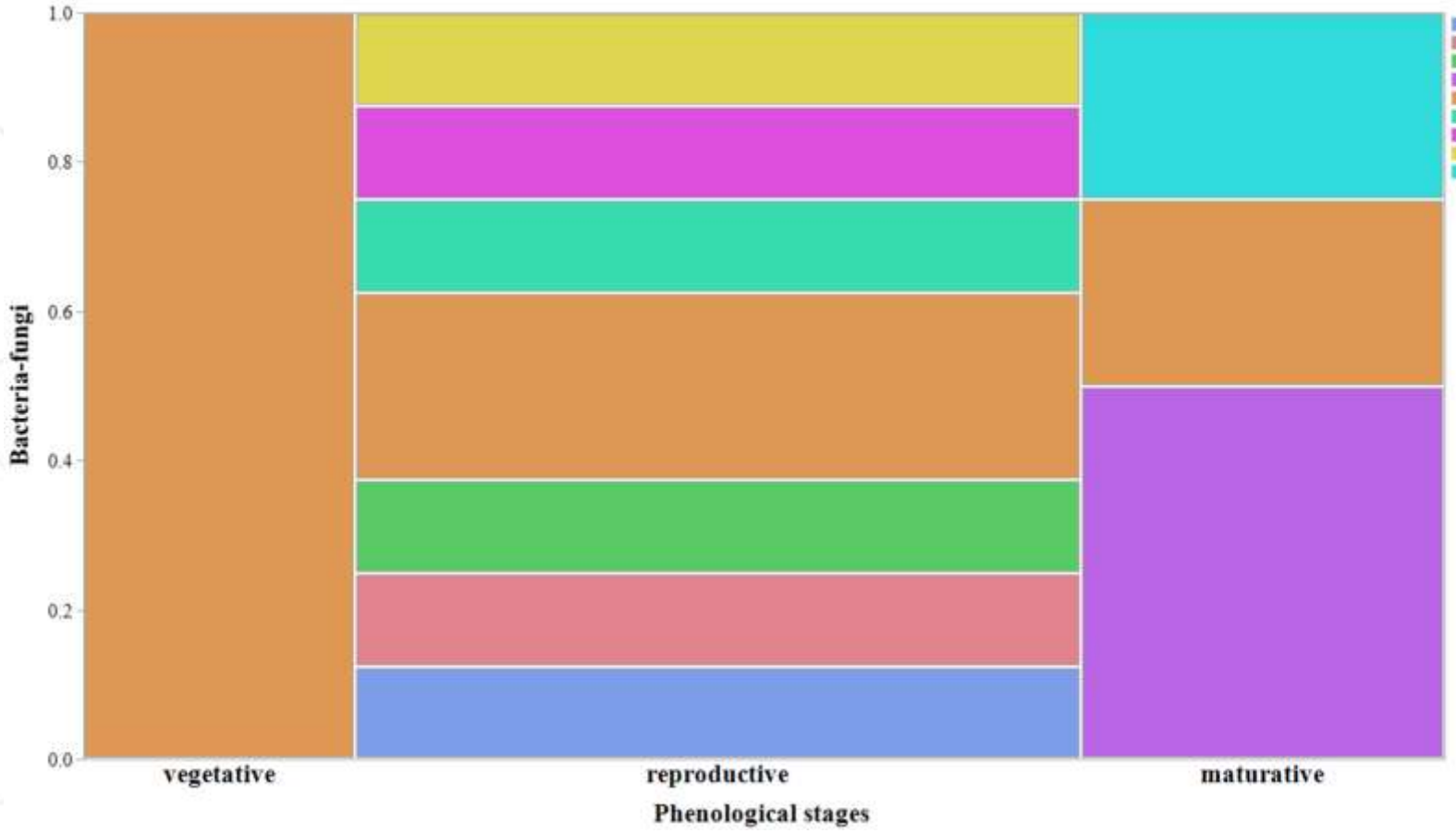


Distribution of the genus Bacillus in the three sections of the rhizosphere of the rice plant. Also included are *B. cereus*, *B. subtilis* and *L. plantarum.*

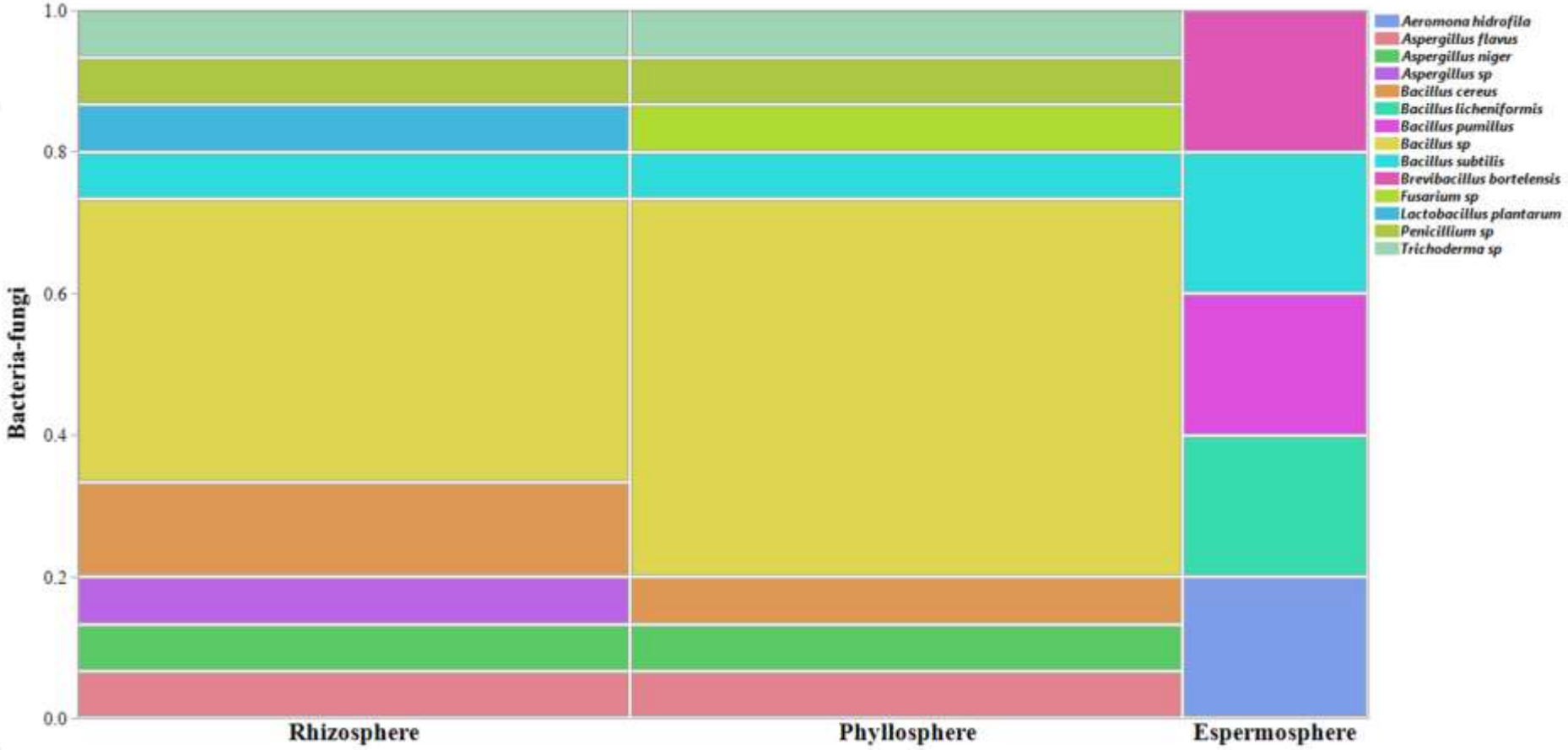
- In relation to the rhizosphere fungi, it was determined that there were no significant differences between the phenological stages and the sections of the rhizosphere, in terms of the number of total fungal isolations.



Distribution of antagonistic fungi against *P. oryzae* in the phenological stages of rice. The predominance of *Trichoderma* sp in the maturation phase can be observed.



Distribution by phenological stages of antagonistic bacteria and fungi against pathogens isolated in rice culture.



Distribution of antagonists count *B. glumae* and *P.oryzae* in rice culture. The description includes the presence of fungi and bacteria in the rhizosphere, phyllosphere and espermosphere.

Thank you for your attention