Exploring the use of endophytes to improve grafting of *T. cacao* regional material with fine flavor potential

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Introduction

The plant microbiome has been considered one of the determining factors in plant health and productivity. Multiple studies have shown that inoculation of plants with growth-promoting bacteria produces a change in root architecture, increasing its surface area, which accelerates plant growth. The use of these microorganisms has been mostly focused on short-cycle crops where the growth effect has direct economic repercussions on the grower. However, this beneficial effect could also have applications in perennial crops such as cacao (*Theobroma cacao* L.). Colombian cacao has the potential to produce fine flavor cocoa beans, especially regional materials of the municipality of San Andrés de Tumaco (Nariño, Colombia). However, the production of these regional materials faces several challenges due to their low grafting success (IMC-67 rootstock). One possible alternative to improve the propagation of these materials is to promote plant cell growth at the junction of a graft union using Plant Growth Promoting Bacteria (PGPB). Here, we explore the potential of PGPB to improve grafting in commercial and regional materials.

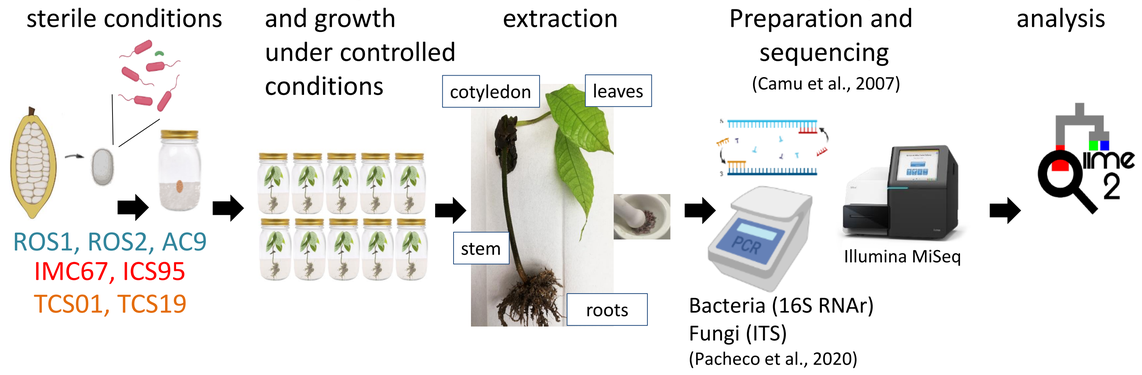
Objectives

This research seeks to explore the differences and similarities of the endophytic communities of regional and universal cacao scion materials to determine if there is a relationship between these endophytic communities and grafting success, as well as to isolate endophytic microorganisms with growth-promoting capacity and evaluate their effect on the grafting success of regional cocoa materials.

Materials & Methods

METATAXOMY : BACTERIAL DIVERSITY

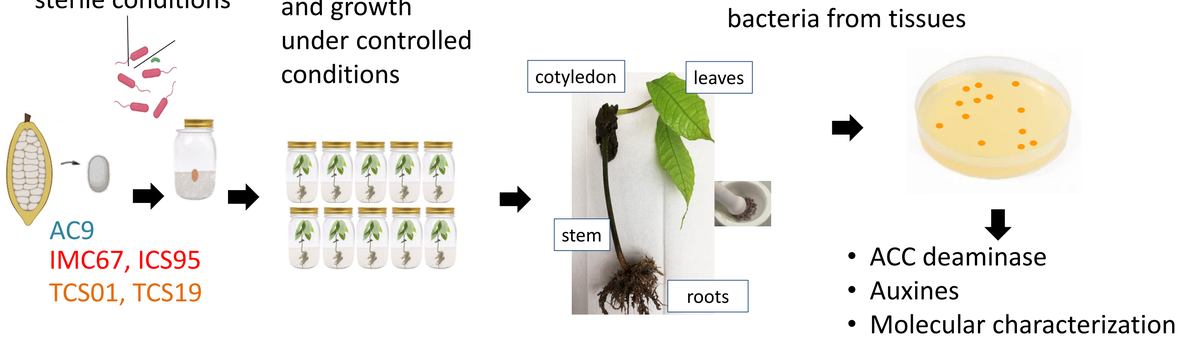
1. Germination under sterile conditions
2. Emergence and growth under controlled conditions
3. Tissue DNA extraction
4. Library Preparation and sequencing (Camu et al., 2007)
5. Bioinformatic analysis



Bacteria (16S RNAr)
Fungi (ITS)
(Pacheco et al., 2020)

SEED ENDOPHYTES ISOLATION AND CHARACTERIZATION

1. Germination under sterile conditions
2. Emergence and growth under controlled conditions
3. Isolation of bacteria from tissues



- ACC deaminase
- Auxines
- Molecular characterization (16S RNAr)

GRAFTING AND GROWTH PROMOTING EVALUATION

1. Inoculation of promising bacteria during grafting
2. Evaluation of the success of grafting

P2102, P2501 *Bacillus*

P2508, P2510 *Pseudomonas*

Results & Discussion

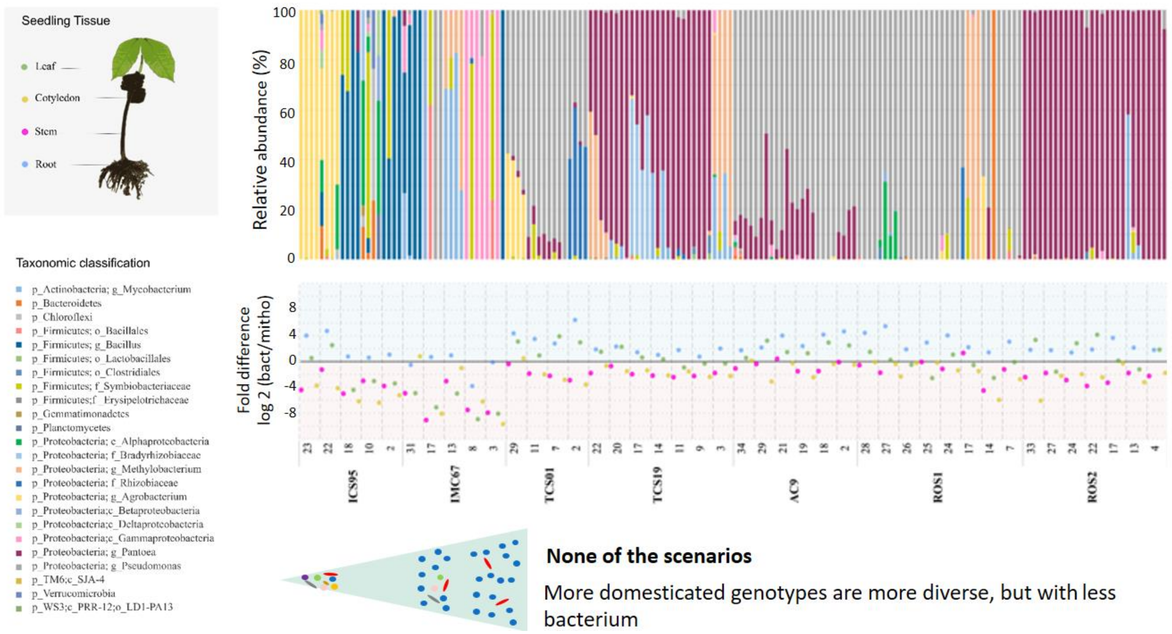


Figure 1. Taxonomic classification of metagenomic libraries of the bacterial communities from tissues from seeds of four commercial cacao genotypes, ICS95, IMC67, TCS01, TCS19 and three regional genotypes AC9, ROS1 and ROS2. Percent of classified reads at the family based on GreenGenes database. Fold difference between the abundance of bacteria reads versus mitochondria reads.

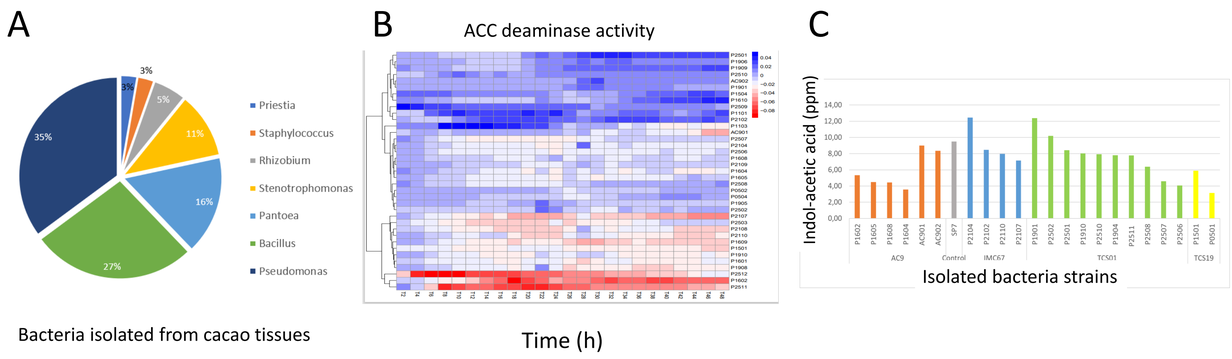


Figure 2. Seed endophytes isolated from tissues of cacao seedlings. A. Molecular identification to genus level of bacterial isolates isolated from plant tissues of cocoa plants multiplied in vitro. B. ACC deaminase activity of isolated bacteria during 48h. C. Production of indol-acetic acid (auxin) of isolated bacteria strains.

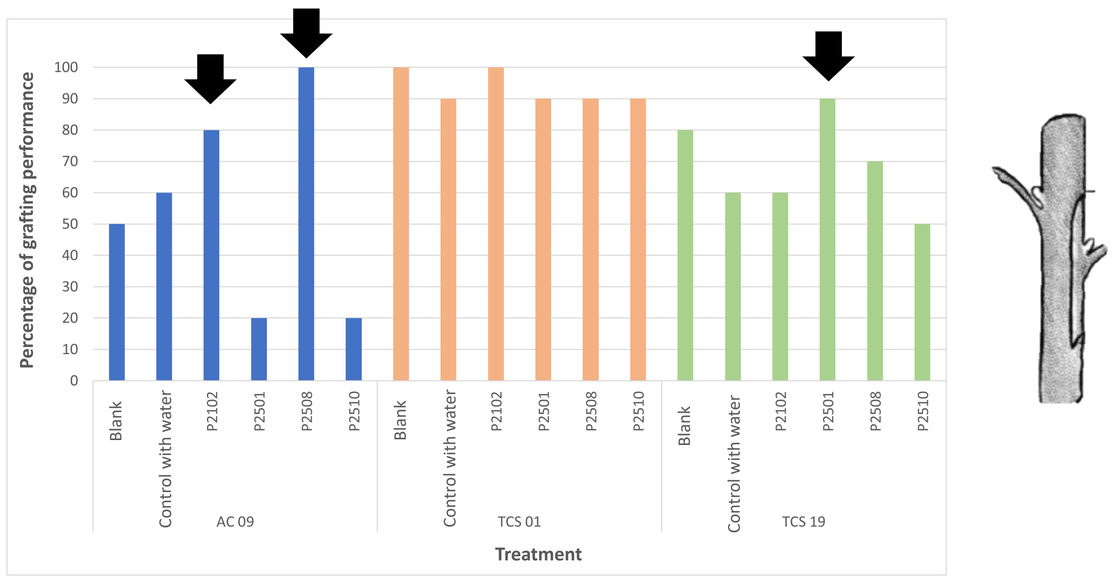


Figure 2. Evaluation of grafting performance of three cacao genotypes (AC09, TCS01 and TCS19 in function of the inoculated strain

Conclusions

This study allowed the identification of the endophytic microbiome associated with plant tissues of cacao plants of commercial and regional materials from Tumaco, Nariño.

The two most abundant bacteria genera were Pseudomonas and Pantoea. The fungal community was very similar in which the genus *Penicillium* was dominant.

Forty-four bacterial endophytic morphotypes were isolated from different tissues obtained from sterile seed. Fifty percent of the isolated morphotypes are potential producers of 1-indole-3-acetic acid. ACC deaminase-producing morphotypes were found, which vary in the time of onset of enzymatic activity and the maximum absorbance reached. Four isolates showed both growth-promoting activities (P2501, P2510, P2102, P2508), with P2102 and P2510 as the major ACC producers.

An increase in the grafting success of regional cocoa material AC9 using the cocoa endophytic strains of Bacillus and Pseudomonas genera was observed, especially using the isolate P2508.

References

Pacheco-Montealegre, M. E., Dávila-Mora, L. L., Botero-Rute, L. M., Reyes, A., Caro-Quintero, A. 2020. Fine Resolution Analysis of Microbial Communities Provides Insights into the Variability of Cocoa Bean Fermentation. Frontiers in Microbiology, 11, 650.

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