Metagenomics and expression analysis reveals that members of the Erwinaceae and Enterobacteriaceae are active players in the initial steps of cocoa fermentation.

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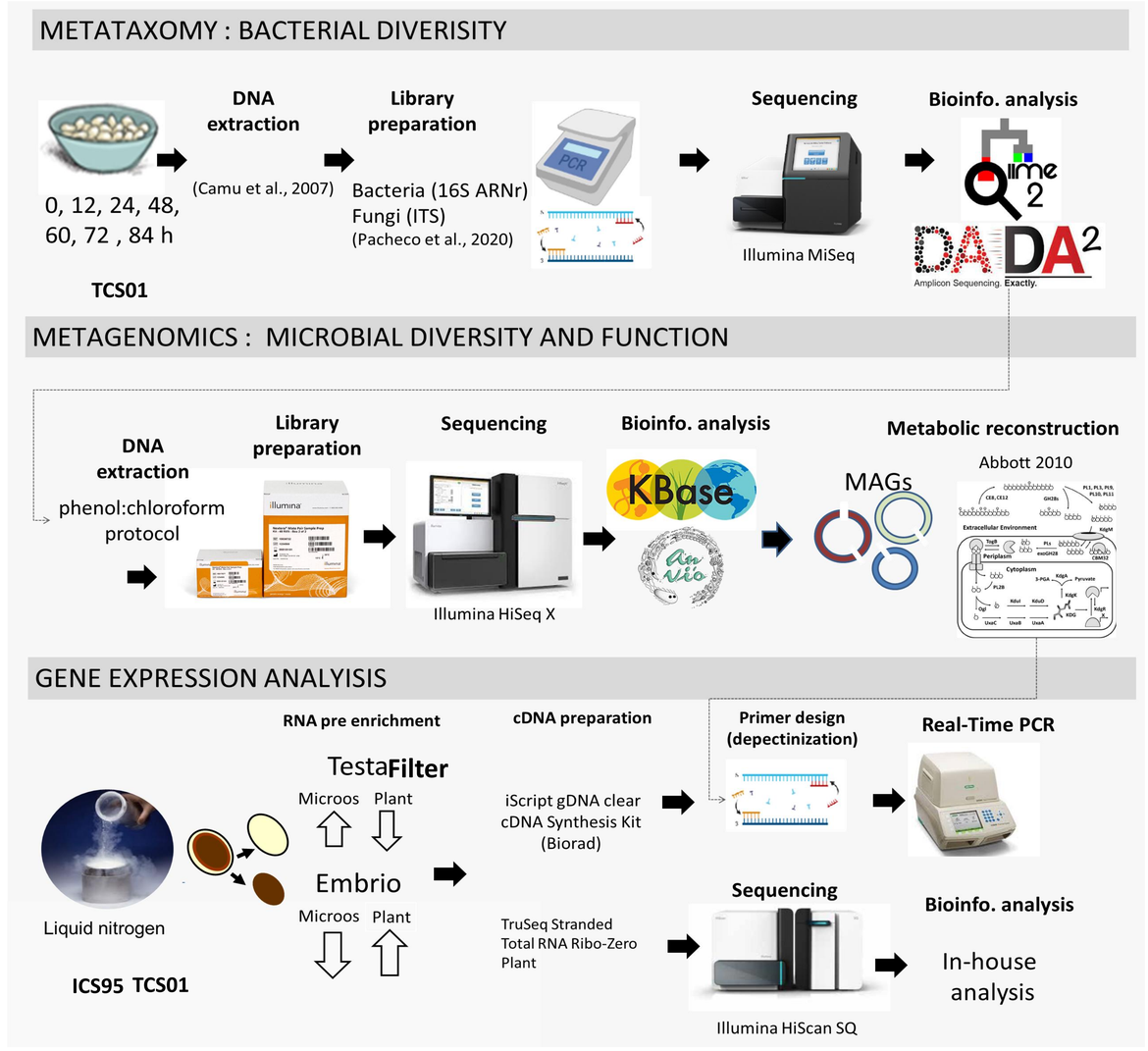
Introduction

Cocoa fermentation is one of the fundamental processes for generating chocolate flavor and aroma, followed by drying and roasting. The fermentation process is driven by environmental microbes that colonize and degrade the cocoa pulp producing a wide range of metabolic end-products. Currently, there is a large amount of information related to the role of yeast, lactic acid bacteria, and acetic acid bacteria in cocoa fermentation which contrasts with the limited information available for the bacteria belonging to the Erwinaceae and Enterobacteriaceae family. These microbes might be capable of metabolizing the cocoa pulp-bean mass. Therefore, they might have a more critical role in the metabolic processes of cocoa bean fermentation than previously thought. Despite the growing evidence of enterobacteria ubiquity in the fermentation process, it is still unclear if they play an active role during fermentation, as most of the degradation activity of cocoa pulp is attributed to yeast.

Objective

to determine the relevance (presence, distribution, and gene expression rates) of the Enterobacteriaceae and Erwinaceaea families during the initial times of the fermentation process.

Materials & Methods



Results & Discussion

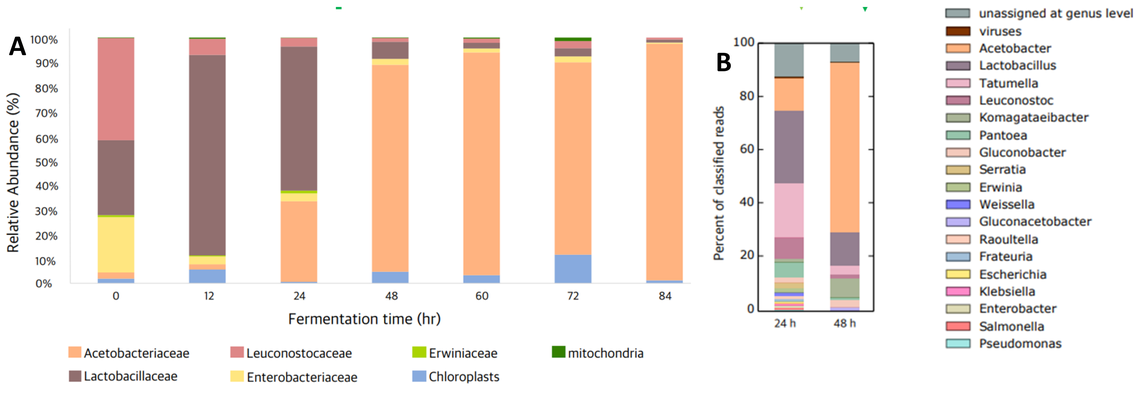


Figure 1. Taxonomic composition of the bacterial communities involved in the fermentation of cocoa beans of the material TCS01 from Santander (Colombia). Assessment of the diversity using 16S ARNr (A) and shot-gun metagenomics (B).

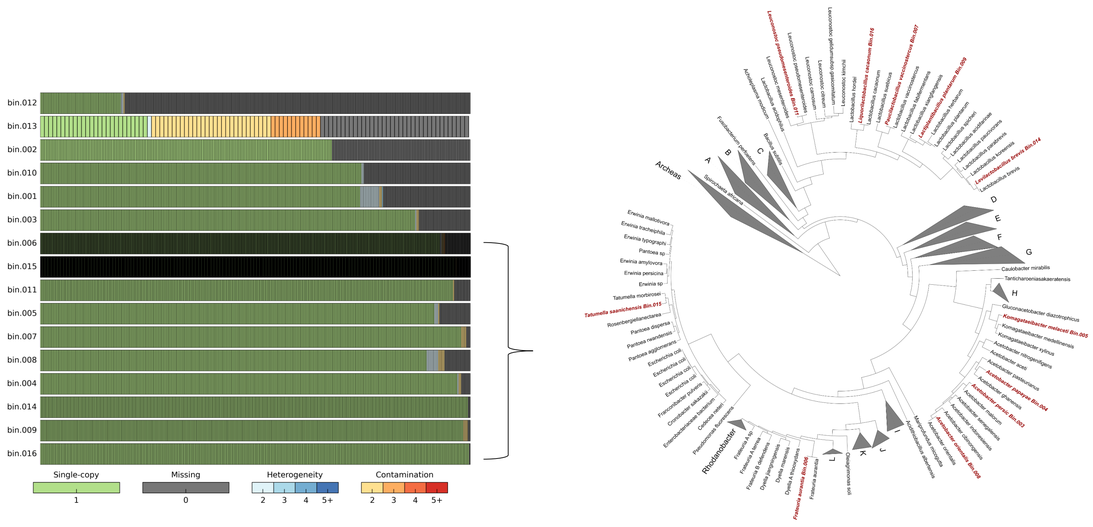


Figure 2 . Metagenome-assembled genomes (MAGs) were obtained from the TCS01 fermentation. The quality assessment of MAGs (Left) and their phylogenetic relationship (Right) are shown.

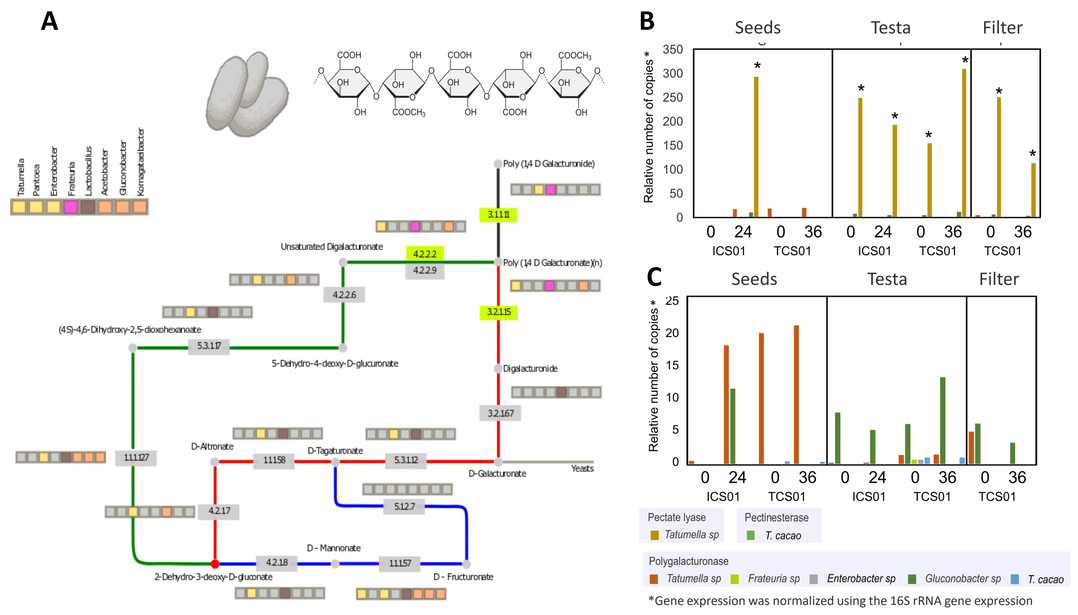


Figure 3. Function potential and expression of genes involved in pectin depolymerization. The metabolic reconstruction of the depectinization pathway and the identification of genes in the metagenomes and the taxa carried them are shown (**A**). The evaluation of expression of genes of some of the initial steps was evaluated by qPCR at 0, 24, and 36 h in two cacao genotypes (ICS1 and TCS01) of fermentation (**B** and **C**)

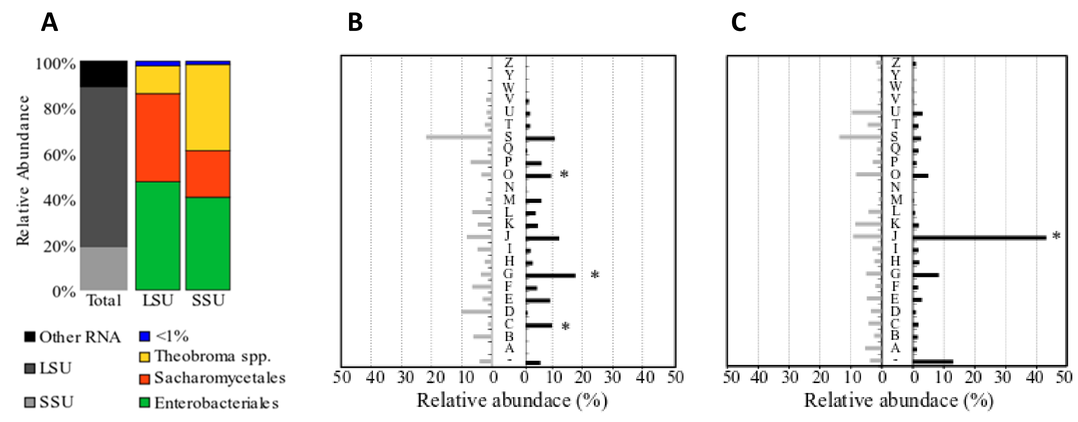


Figure 4. Meta transcriptomics Analysis. The relative abundance rRNA reads were classified in LSU (Large Subunit and Small subunit (SSU) using the Silva database (**A**). Functional characterization of the expressed protein-coding gene was done by mapping the reads against the genomes of *Tatumella* (**B**) and *Hanseniospora* (**C**). The average relative abundance of functional categories (COGs) was quantified for the available genomes (gray left bars) and the relative abundance of gene expression profiles (black bars).

Conclusions

Our results show that bacteria from *Tatumella* genus play essential roles in the initial steps of the depectinization pathway, as corroborated by the metagenomic assembly and the evaluation of gene expression by qPCR. The metatranscriptomic analysis shows that in terms of ribosomal rRNA genes, the Enterobacteriales have a higher expression than yeast (probably being more active) and that the protein-coding genes overexpressed in *Tatumella* belong to the Carbohydrate transport and metabolism (G category), while in Hansenospora, most genes are related to genes translation (J category). Overall, Our results show that *Tatumella* is an active player in the initial steps of cocoa fermentation, and their contribution and technological application deserve further research.

References

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Acknowledgments

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