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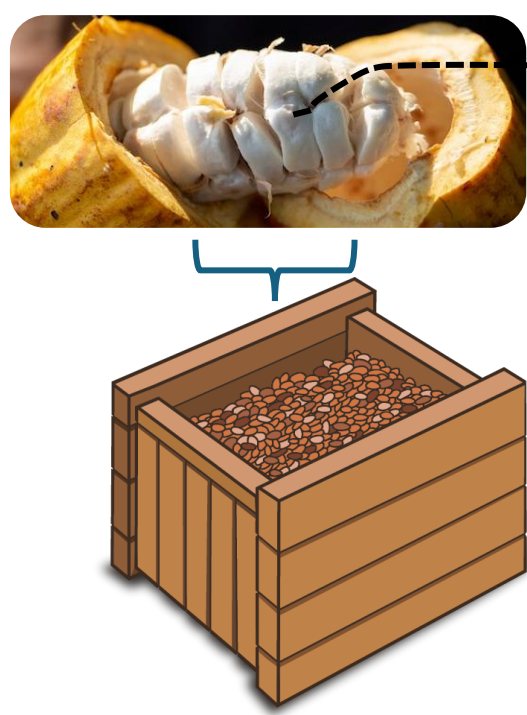
ABSTRACT

Colombian cocoa, classified as fine and aromatic by the ICCO, owes its quality to genetics, agroecological factors, and post-harvest processes like fermentation and roasting. These processes form flavor precursors—proteins, carbohydrates, polyphenols, and sugars—contributing to chocolate’s floral, nutty, and fruity notes. The microbial community, especially bacteria and yeast, significantly influences cocoa’s sensory qualities. While metataxonomic studies exist, none have investigated gene expression in the microbiome and cocoa seeds during fermentation. This metatranscriptomic study focuses on gene expression in cocoa seeds and the microbiome to understand their roles in flavor and aroma development. Cacao pods were collected from 20 farms in Arauca, and fermentation was conducted in triplicate. Samples from pulp and cotyledons were taken at 0, 24, and 48 hours. RNA extraction and sequencing were performed, followed by bioinformatics analysis. Gene expression in *Theobroma cacao* revealed the breakdown of vicilin (a flavor-related protein) and genes linked to senescence and germination, highlighting the seeds’ role in producing flavor precursors. Furthermore, key metabolic pathways were found in the pulp microbiome, including the Ehrlich pathway, phenylalanine metabolism, sugar fermentation, amino acid and protein biosynthesis and butanoate metabolism. These pathways are involved in producing metabolites associated with fine flavor and aroma notes.

INTRODUCTION

Biochemical transformation of cocoa beans during fermentation

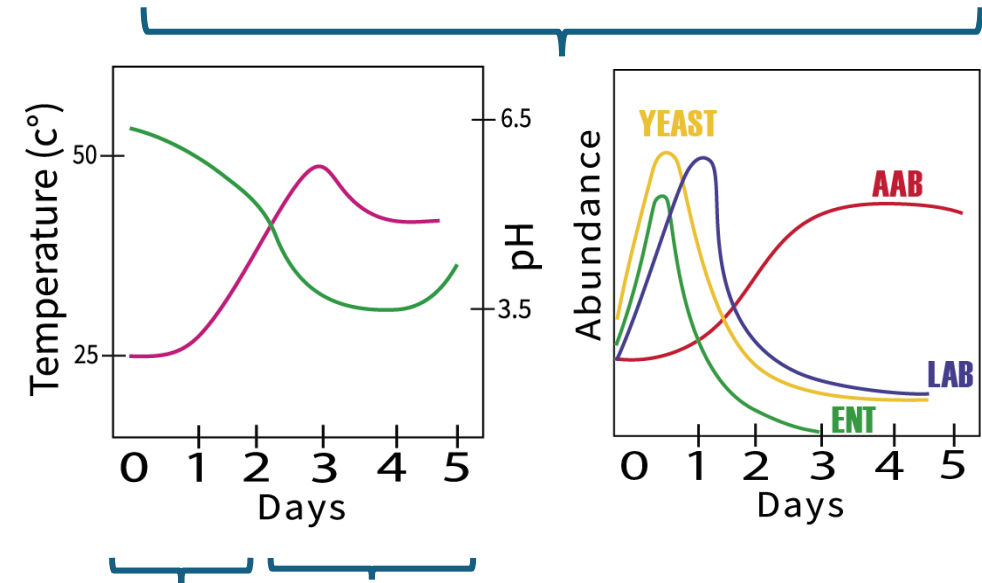
Physical Changes



Water = 84,4%
Dry weight = 15,6%
Sugar = 11% (fructose, glucose, sucrose)
Fat = 1,6%
Protein = 0,2%
Citric acid = 0,5% – 3%
Pectin = 1% -2%
Pentosans = 2% - 3%
Amino acids, peptides, minerals, vitamins.

Biochemical Changes

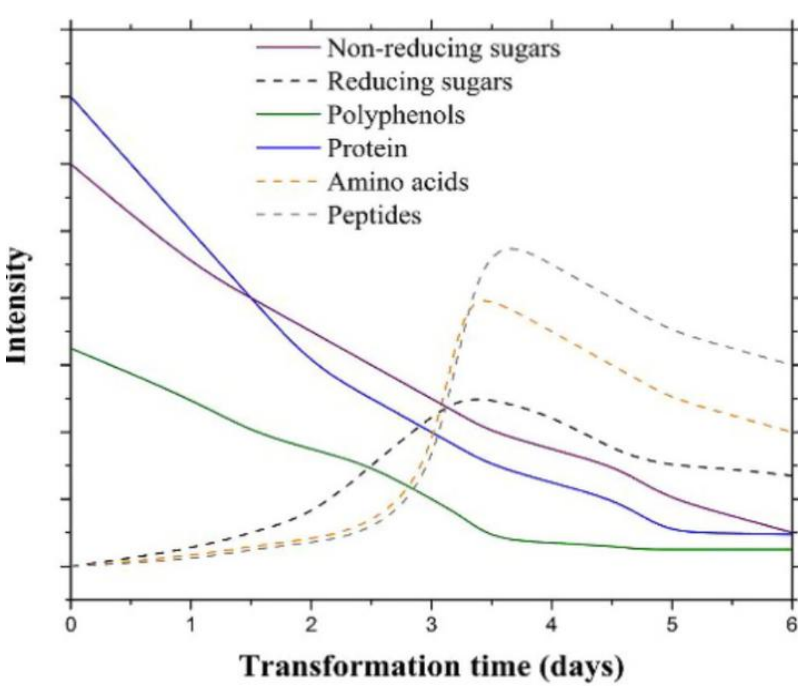
Water = 31-39%
Fat = 30% - 32%
Protein = 8% – 10% (vicilin-albumin)
Pentosans = 4% - 6%
Starch = 4% - 6%
Glucose = 2% -3%
Sucrose = 2% - 3%
Polyphenols = 5% - 6%
Theobromine 1% - 6%
Citric acid = 1% (oxalic – malic)
Caffeine = 0.2% - 1%



Anaerobic phase
Aerobic phase

Santander et al., 2019; Mayorga-Gross et al., 2016

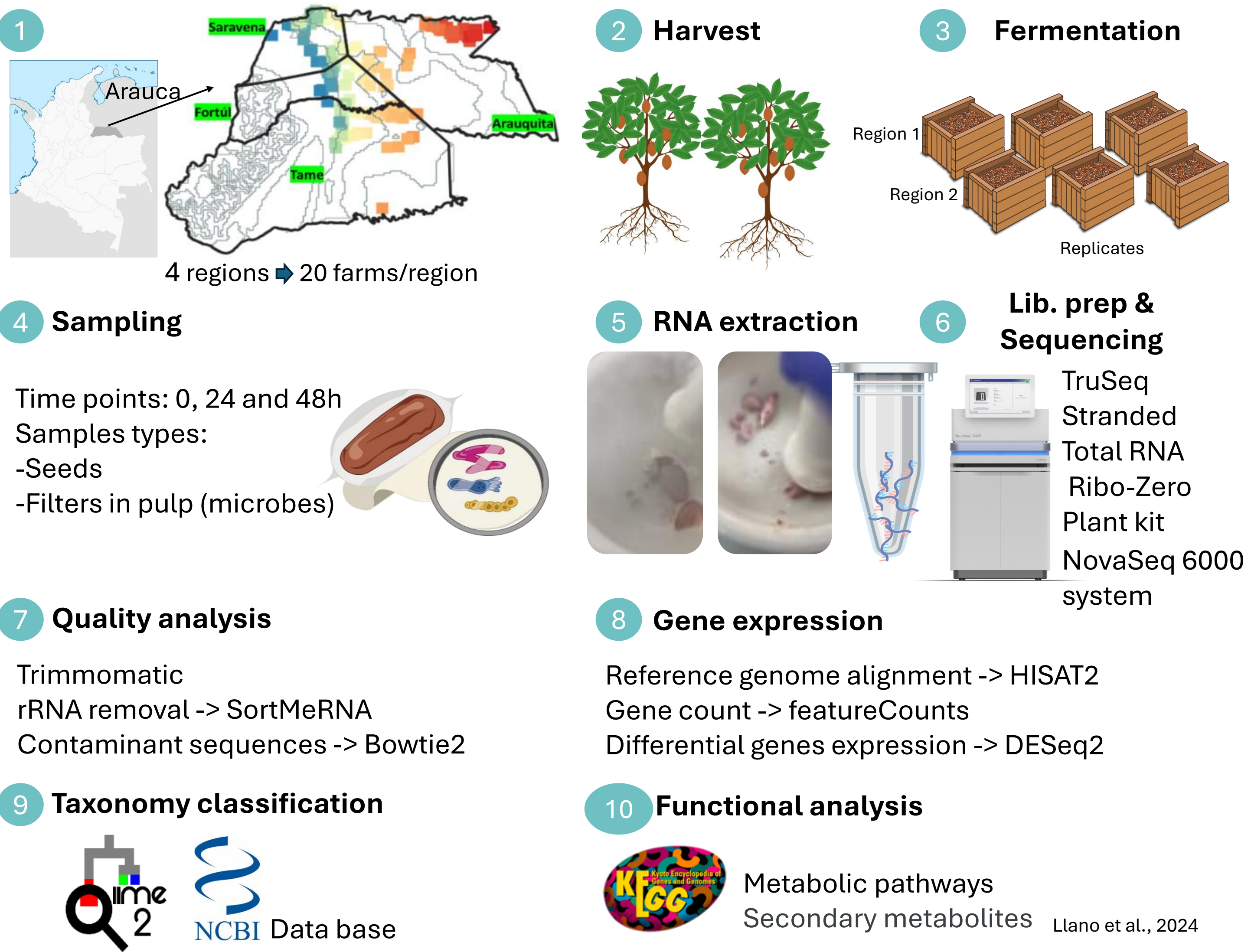
Proteolysis, hydrolysis and oxidation



Metabolic pathways
Flavor – related?

Flavor precursors

METHODOLOGY



RESULTS

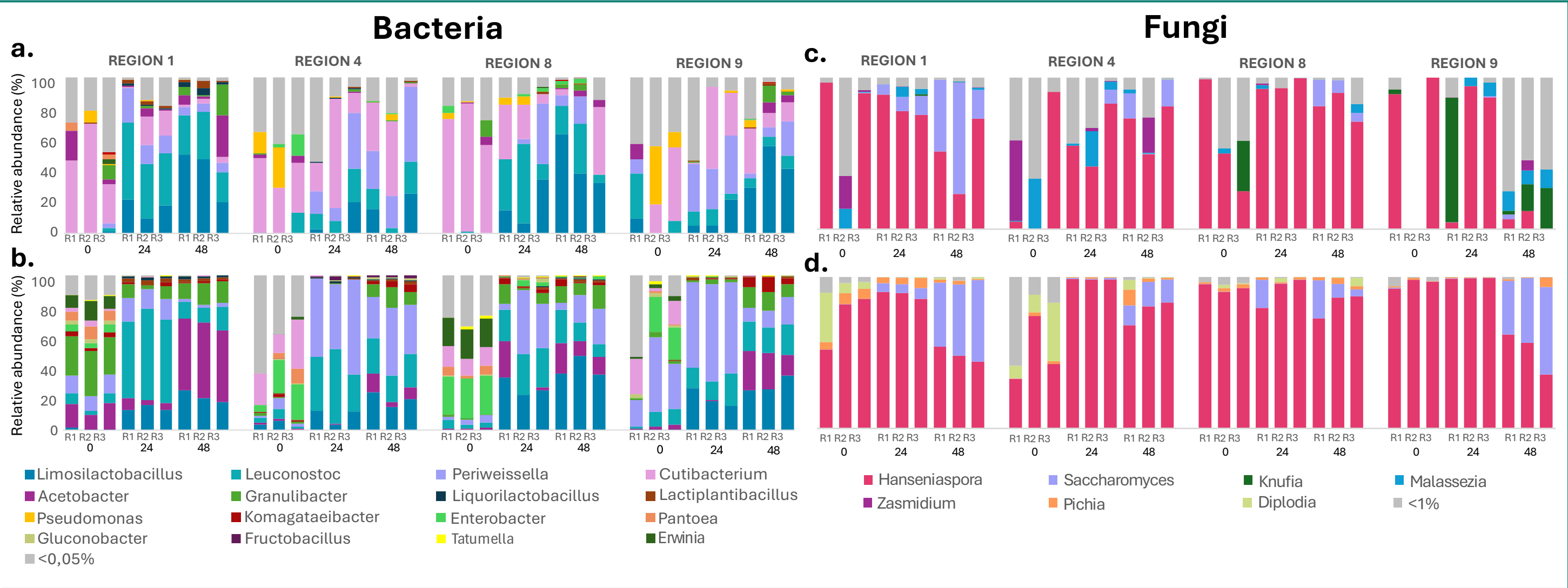


Fig. 1. Relative abundance of microorganisms. a. Bacteria in the seed. b. Bacteria in the pulp. c. Fungi in the seed. d. Fungi in the pulp. R1,R2,R3: replications.

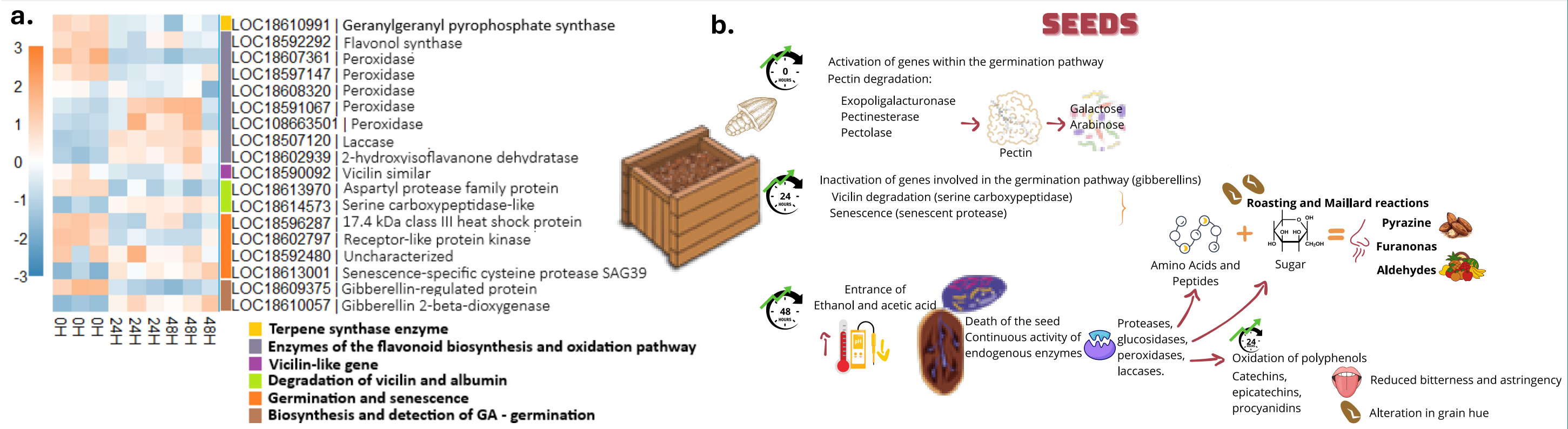


Fig. 2. Differential expression of genes in seeds during fermentation. a. Gene expression of some important genes. b. Involvement of gene expression in seed in the generation of flavor and aroma of chocolate.

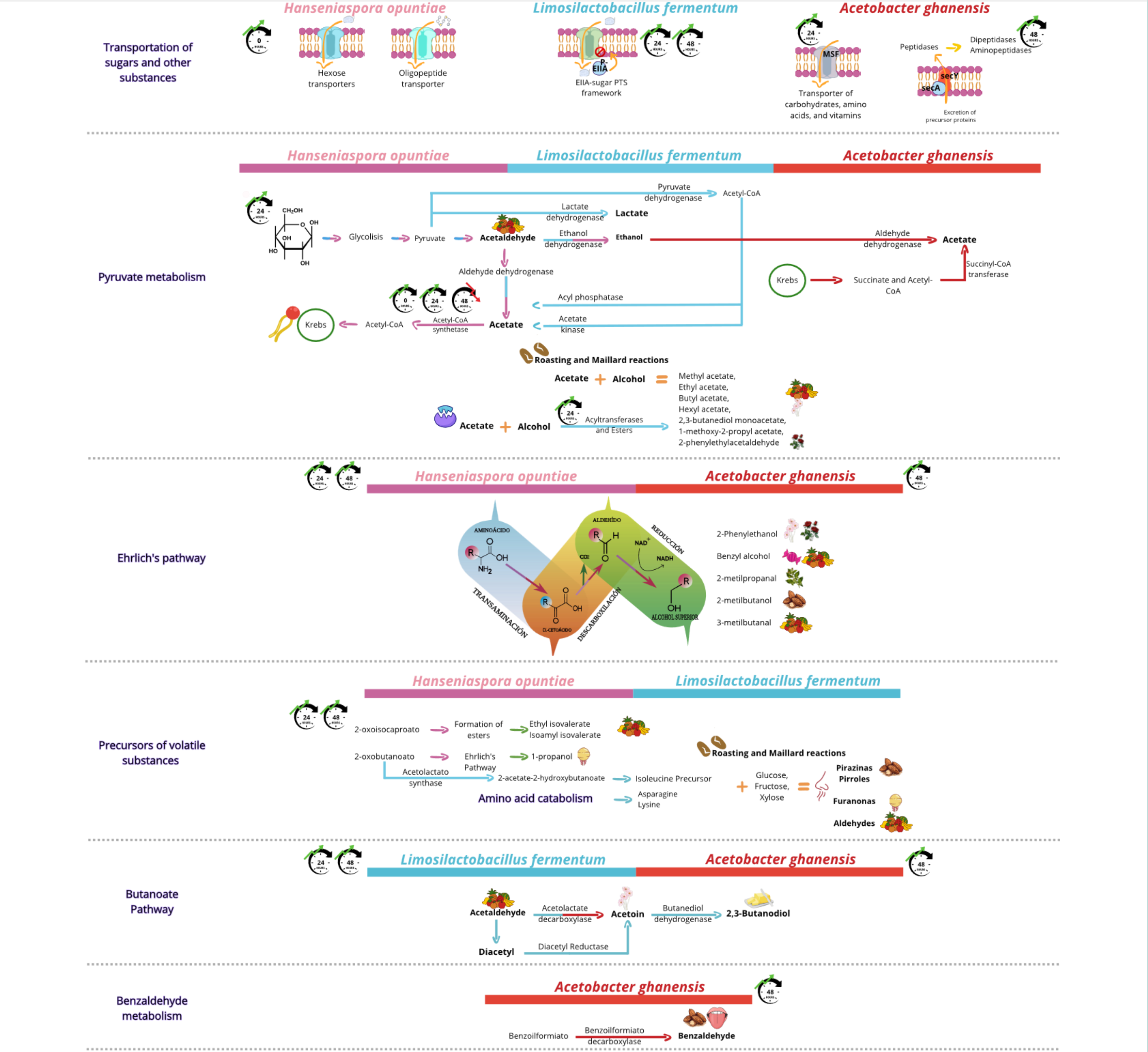


Fig.3. Metabolic pathways and differentially expressed genes in microorganisms related to flavor and aroma.

CONCLUSIONS

1. Metatranscriptomics enabled the identification of metabolically active microorganisms during cocoa fermentation, revealing their functional role in flavor development.
2. Gene activation and metabolic pathways directly involved in the chocolate sensory profile occur in both the seed and the pulp microbiome during fermentation.
3. This biotechnological approach, based on metatranscriptomics, represents a significant advance in understanding cocoa fermentation. It also provides a scientific framework that producers, researchers, and technicians can leverage to enhance the competitiveness of Colombian cocoa in international markets, promoting the production of beans with desirable and reproducible sensory profiles.

ACKNOWLEDGMENTS

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