

Title: Genomic analysis of biotechnological properties of *Acetobacter senegalensis* from Brazilian cocoa fermentation

Authors: Almeida, OGG; Pereira, MG; De Martinis, ECP.

Institution: University of São Paulo, Faculty of Pharmaceutical Sciences of Ribeirão Preto. São Paulo state, Brazil.

Abstract:

Acetic acid bacteria (AAB) are important in spontaneous cocoa fermentation, production of vinegar and beverages, among other biotechnological applications. These are Gram-negative bacteria usually thermo and acid tolerant, which are traits of great interest for food processing. In this research, distinct *Acetobacter senegalensis* strains (GYC10, GYC12, GYC19, GYC27 and MRS7) were selected from a Brazilian spontaneous cocoa fermentation, and they were characterized by whole genome sequencing, using the Illumina® NovaSeq6000 (paired-end 2x150bp). For genome assembly, the raw-reads obtained were quality-filtered to remove the adapter sequences and the low-quality base calls ($Q30 \leq 30$). Next, all the public genomes of *A. senegalensis* were downloaded from Genbank for comparison with the newly sequenced isolates. Genome completeness was evaluated with BUSCO (database rhodospirillales_odb10) and the taxonomy was confirmed by average nucleotide identity (ANI). Pangenome analysis was performed with the selected strains plus two Metagenome-Assembled Genomes (MAGs) of *A. senegalensis* previously obtained. Functional annotation was done with the MicrobeAnnotator pipeline and the Rastk subsystems database. Overall, the results revealed high-quality genomes for the novel strains (average of 99.9% of completeness), and unequivocal identification at species level (ANI > 97%). Moreover, according to functional annotation, all *A. senegalensis* presented potential to utilize lactate, maltose, trehalose, mannose and glycogen, but the metabolism of sucrose was restrict to strains GYC10, GYC12 and LMG23690. Besides, all *A. senegalensis* strains presented genes related with osmotic, periplasmic and oxidative stress response. Pangenome analysis revealed genes related to thermotolerance (chaperonins and ABC transporters) and acid resistance (PQQ-complex enzymes and alcohol dehydrogenase) were conserved in all strains, with multiple copies. These results highlight the adaptive potential of AAB in harsh environments and contribute to expand genomic public databases for supporting a more precise determination of AAB ecology.

Key-words: acetic acid bacteria, whole-genome sequencing, pangenome analysis, cocoa fermentation.

Funding: The São Paulo Research Foundation – FAPESP (Proc. #17/13759-6 and #18/13564-3).