

# Exploring the genome of *Lactobacillaceae* spp. Sy-1 isolated from *Heterotrigona itama* honey

## ABSTRACT

**Background.** Honey produced by *Heterotrigona itama* is highly preferred among consumers due to its high-value as a functional food and beneficial lactic acid bacteria (LAB) reservoir. Fructophilic lactic acid bacteria (FLAB) are a group of LAB with unique growth characteristics and are regarded as promising producers of bioactive compounds. Hence, it is not surprising that LAB, especially FLAB, may be involved with the excellent bioactivity of *H. itama* honey. With the trending consumer preference for *H. itama* honey coupled with increasing awareness for healthy food, the genomic background of FLAB isolated from this honey must, therefore, be clearly understood. In this study, one FLAB strain designated as Sy-1 was isolated from freshly collected *H. itama* honey. Its FLAB behavior and genomic features were investigated to uncover functional genes that could add value to functional food. **Methods.** The fructophilic characteristics of strain Sy-1 were determined, and the genome was sequenced using Illumina iSeq100 and Oxford Nanopore. The average nucleotide identity and phylogenetic analyses based on 16S rRNA, 92 core genes, and whole-genome sequence were performed to unravel the phylogenetic position of strain Sy-1. NCBI Prokaryotic Genome Annotation Pipeline annotated the genome, while the EggNOG-mapper, BLASTKoala, and GHOSTKoala were used to add functional genes and pathways information. **Results.** Strain Sy-1 prefers D-fructose over D-glucose and actively metabolizes D-glucose in the presence of electron acceptors. Genomic annotation of strain Sy-1 revealed few genes involved in carbohydrate transport and metabolism, and partial deletion of *adhE* gene, in line with the characteristic of FLAB. The 16S rRNA gene sequence of strain Sy-1 showed the highest similarity to unknown LAB species isolated from the gut of honeybees. The phylogenetic analyses discovered that strain Sy-1 belonged to the *Lactobacillaceae* family and formed a separate branch closer to type strain from the genera of *Acetilactobacillus* and *Apilactobacillus*. The ANI analysis showed the similarity of the closest relative, *Apilactobacillus micheneri* Hlig3T. The assembled genome of Sy-1 contains 3 contigs with 2.03 Mbp and a 41% GC content. A total of 1,785 genes were identified, including 1,685 protein-coding genes, 68 tRNA, and 15 rRNA. Interestingly, strain Sy-1 encoded complete genes for the biosynthesis of folate and riboflavin. High-performance liquid chromatography analysis further confirmed the high production of folic acid (1.346 mg/L) by Sy-1. **Discussion.** Based on phylogenetic and biochemical characteristics, strain Sy-1 should be classified as a novel genus in the family of *Lactobacillaceae* and a new member of FLAB. The genome information coupled with experimental studies supported the ability of strain Sy-1 to produce high folic acid. Our collective findings support the suitable application of FLAB strain Sy-1 in the functional food and pharmaceutical industries.