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CULTURABLE MICROORGANISM FROM FERMENTING HONEY OF STINGLESS BEES

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Sustainable food production depends largely on pollinating insects, with stingless bees (SBs) playing a leading role in this process. Moreover, Bs can produce honey with unique sensory characteristics and biotechnological properties of interest for well-being. Honey from SB naturally ferments due its chemical characteristics and autochthonous microbiota, which is still poorly described. One sample of ca 1.4 kg of honey from the SB *Tetragonisca angustula*, popularly known as Jataí, was collected from a meliponary located at Jardinópolis - São Paulo, Brazil. The initial sample was analysed according the parameters from the São Paulo state legislation: pH, titratable acidity, moisture, water activity - Aw, hydroxymethylfurfural, diastase, water-insoluble solids, ash, pollen, Presence or Absence of *Salmonella* spp., enumeration of coliforms at 45ºC (MPN/g) and Yeast and Molds - YM (CFU/g). Three aliquots of the *T. angustula* honey (ca. 270 g each) were placed in glass bottles with air locks and let spontaneously ferment at 25^{o}C for up four weeks. Samplings were done at times 0, 1, 2, 3 and 4 weeks to determine the microbiota by spread plating decimal dilutions on “Plate Count Agar” (PCA), “de Man Rogosa and Sharpe agar” (MRS), “MRS agar with fructose and cysteine” (MRSfc) and “Glucose Yeast Calcium carbonate agar” (GYC), respectively for the aerobic plate count (APC), lactic acid bacteria (LAB), fructophilic LAB and acetic acid bacteria (AAB), all incubated at 30ºC during 72h. The population of YM was also enumerated along four weeks by spread plating decimal dilutions on Dicloran Rose Bengal Chloramphenicol agar (DRBC) at 25ºC up to 7 days. The fermentation process along four weeks was monitored by measuring pH, titratable acidity and Aw. The LAB and fructophilic LAB were tested for antimicrobial activity against the indicator *Lactobacillus sakei* ATCC 15521 and the isolates selected were presumptively identified by MALDI-TOF.The honey sample collected presented all the parameters very close to the São Paulo legislation: pH 4.36; acidity 46 mEq/kg; 24.4% of moisture, Aw 0.809; HMF <10mg/kg; diastase 60.1 in Goethe scale, water-insoluble solids 0.16g/100g; ash 0.6g/100g; presence of pollen; absence of *Salmonella* spp. in 25g-sample; < 3.0 MPN/g coliforms at 45ºC; and 6.5 x 10^3 CFU/g Yeast and Molds. Initial populations were 1.5 x 10^3 CFU/g for APC, 5.6 x 10^4 CFU/g for LAB, 6.1 x 10^4 CFU/g for fructophilic LAB and 6.5 x 10^3 CFU/g for AAB, but after four weeks of spontaneous fermentation, they decreased respectively to 9 x 10^1 CFU/g, 5.1 x 10^2 CFU/g, 4.8 x 10^2 CFU/g and 7 x 10^1 CFU/g. Along the fermentation the pH dropped from 4.36 to 3.44, while the acidity increased from 46 to 95 mEq/kg, and the Aw did not change (0.809- 0.807). The presumptive identification of the isolates done with MALDI-TOF revealed two species of LAB (*Pediococcus pentosaceus* and *Leuconostoc citreum*) and one species of fructophilic LAB (*Apilactobacillus kunkeei*), which presented antimicrobial activity against the indicator. In the literature, these microorganisms have been associated with probiotic and biopreservative potentials, indicating our results can lead to the discovery of novel antimicrobials and food applications.

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