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Fascinating fructophilic lactic acid bacteria associated with various fructose-rich niches

SUMMARY

Fructophilic lactic acid bacteria (FLAB) are recently described group of lactic acid bacteria (LAB) that prefer fructose instead of glucose as a carbon source. FLAB have been isolated from fructose-rich niches such as flowers, fruits, fermented fruits, and gastrointestinal tracts of insects whose diet is based on fructose. These bacteria are divided into obligate and facultative fructophilc lactobacilli based on biochemical features. All FLAB are heterofermentative microorganisms, which during fermentation of carbohydrates, in addition to lactic acid, produce also acetic acid, and alcohol as end-products. The fructophilic bacteria, inhabiting the honeybee guts positively impact the health of their hosts, improve their longevity, and are promising probiotic candidates. These symbionts of honeybees play a key role in the production of honey by bees and are present in a large number in fresh honey. The combination of osmolarity with antibacterial, and therapeutic properties of these bacteria make fresh honey optimal alternative for future wound healing.

Keywords: fructophilic lactic acid bacteria, *Apis mellifera*, probiotics, honey dressing, [*GAR*$^+$] prions

INTRODUCTION

Fructophilic lactic acid bacteria are recently discovered sub-group of lactic acid bacteria (LAB) that under anaerobic conditions prefer fructose over glucose as a carbon and energy source. They grow well on glucose in the presence of oxygen or when pyruvate or fructose are external electron acceptors (18, 23, 36, 41). These properties distinguish FLAB from other lactic acid bacteria. Fructophilic lactic acid bacteria, as other LAB, are Gram-positive, non-motile rods occurring singly, in pairs, or as chains (Fig. 1). Pentoses are not fermented by FLAB, which suggests the lack of pentose isomerases or epimerases for pentose fermentation (19). All fructophilic lactic acid bacteria catabolize only a limited number of carbohydrates (2–5 out of 49 carbohydrates tested) as a result of a lower number of the genes for carbohydrate metabolism than in other LAB (19, 20, 22, 23, 25, 41). Furthermore, it is necessary to underline that in *Fructobacillus* sp. only one gene for phosphoenolpyruvate: sugar phosphotransferase system (PTS) was identified (23, 26). Such genes were not found in *L. kunkeei L. apinorum,* and *L. florum* genomes*,* whereas in the genomes of other LAB from 13 to 36 PTS genes were identified (23, 36, 37). Fructophilic lactic acid bacteria have been isolated from different fructose-rich niches such as: flowers, fruits, fermented foods made from fruits, and from the gastrointestinal tracts of different insects, e.g. honeybees, bumblebees, tropical fruit flies, *Camponotus* ants, whose diet is based on fructose (1, 5, 20, 24, 36). Up to now, only three fructophilic *Lactobacillus* sp. have been identified, i.e. *Lactobacillus kunkeei* originally isolated from grape wine as a spoilage organism (15) and next, from flowers, fresh honey, and honeybee guts (39, 41), *Lactobacillus florum* isolated from flowers (20, 22), and *Lactobacillus apinorum* origi-

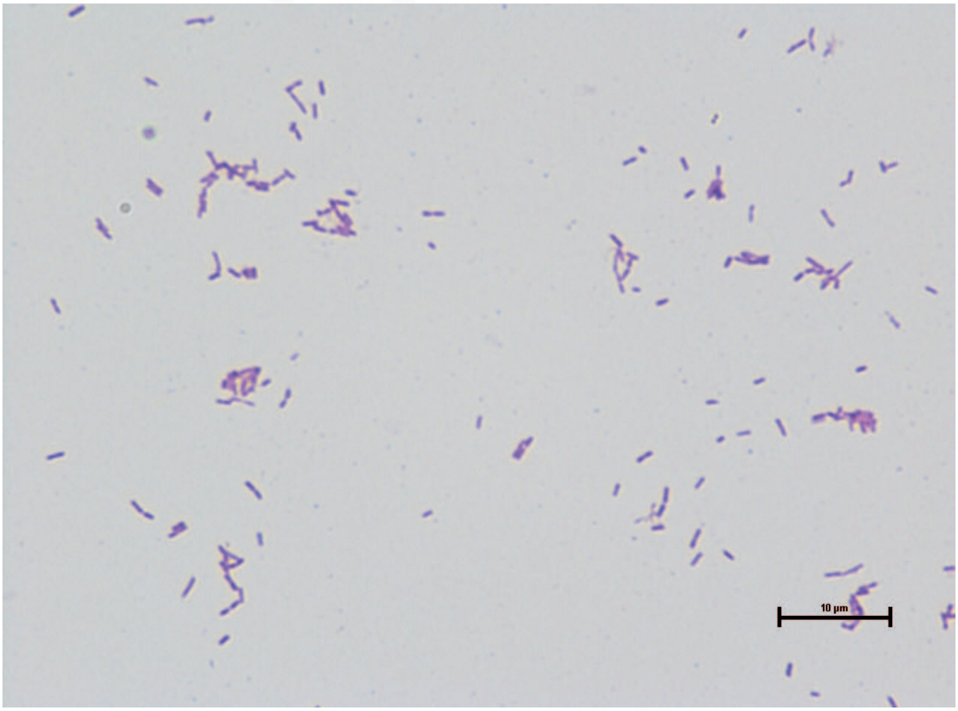

Fig. 1. Gram-stained cells of *Lactobacillus kunkeei* CH1 strain (Pachla et al. (2018) grown in MRS broth. Bar, 10 µm.

nally isolated from the guts of honeybees (36). As fructophilic lactic acid bacterium, strain 123–20 of *L. brevis*, isolated from wild flower (Durban, South Africa) was also classified (39). Representatives of FLAB are also *Fructobacillus* sp. such as: *Fructobacillus fructosus*, originally described as *Lactobacillus fructosus*, whose growth was stimulated by tomato juice containing fructose, *Fructobacillus durionis* isolated from durian fermented fruits, *Fructobacillus tropaeoli* isolated from a nasturtium flower, *Fructobacillus ficulneus* isolated from ripe figs, and *Fructobacillus pseudoficulneus* isolated from bananas, figs, flowers, and cane juice (5, 19, 21, 22, 34). All these fructobacilli are phylogenetically closely related to the genera *Leuconostoc, Oenococcus,* and *Weissella* (22, 24, 41). Earlier, they were classified to the genus *Leuconostoc* (24). The type species of the genus *Fructobacillus* is *F. fructosus*, which has been originally isolated from flowers, and next, from wine and the beverage taberna produced in Mexico by the fermentation of the *Acrocomia* aculeate palm sap (1, 33).

Recent analysis of *L. kunkeei* and *Fructobacillus* sp. genomes revealed their niche-specific evolution (8, 23, 26, 36, 37). The genomes of these bacteria are smaller (respectively 1.41–1.58 Mbp and 1.33–1.68 Mbp) than the genomes of most of other lactobacilli (1.28-3.62 Mpb) and contain significantly less genes encoding proteins (CDS) (coding sequences). *L. kunkeei* and *Fructobacillus* species lost some genes determining carbohydrate transport and metabolism, as well the genes involved in respiration (genes for the tricarboxylic acid cycle [ATC] and biosynthesis of ubiquinone [coenzyme Q] and other terpenoid quinones). These data clearly support hypothesis of reductive evolution of fructophilic lactic acid bacterium genomes as a result of the adaptation of bacteria to specific fructose-rich niches (23, 26, 36, 37).

BIOCHEMICAL CHARACTERISTICS

Based on biochemical features, fructophilic lactic acid bacteria are separated into two sub-groups, i.e. obligatory and facultative FLAB (22, 23). The first sub-group comprises *L. kunkeei*, *L. apinorum*, and *Fructobacillus* sp., which under anaerobic conditions exhibit poor growth on glucose, but accelerated growth on glucose in the presence of external electron acceptors such as pyruvate, oxygen or fructose. Obligate FLAB convert glucose into almost equimolar amounts of lactic acid and acetic acid, and trace amounts of ethanol, i.e. 1:0.9–1.2:0.005-0.01 (19, 22, 24, 41). The low production of ethanol in *L. kunkeei* results from the absence of acetaldehyde dehydrogenase (ALDH) and a low activity of alcohol dehydrogenase (ADH) (23). The second sub-group of FLAB includes *L. florum* bacteria and a few biotypes of the species *L. brevis* (20, 39). Facultative FLAB grow well on fructose and glucose in the presence of the external electron acceptors, which enhance glucose metabolism. However, these bacteria grow on glucose without the electron acceptors, but at a delayed rate. Facultative FLAB convert glucose to lactic acid, ethanol, and acetic acid at a ratio of 1:1:0.2, respectively (20, 22). It was found that obligate FLAB do not contain *adhE* genes (*Fructobacillus* sp. *L. apinorum*) or have incomplete *adhE* gene (*L. kunkeei*), whereas facultative FLAB (*L. florum*) harbor the complete *adhE* gene and exhibit ADH and ALDH activities (23, 26, 36, 37).

It should also be emphasized that fructophilic lactic acid bacteria tolerate and grow even in the presence of 30% fructose in the culture medium (5, 19, 41). This property of FLAB enables them to survive and colonize the intestinal tract of honeybees containing large amounts of sugars including fructose, which is the main component of nectar.

Lactic acid bacteria are classified into two groups according to their hexose sugar fermentation pathway, i.e. homo- heterofermentative LAB (32). The homofermentative bacteria dissimilate hexoses *via* Embden-Meyerhof-Parna's (EMP) pathway generating from 1 mol of hexose 2 mol of lactic acid and 2 mol of ATP, whereas the heterofermentative bacteria convert hexoses to equimolar amounts of lactic acid, acetic acid or ethanol, and carbon dioxide, by using pentose-phosphate pathway and yielding 1 mol of ATP per mol of hexose fermented (11, 13) (Fig. 2). All fructophilic

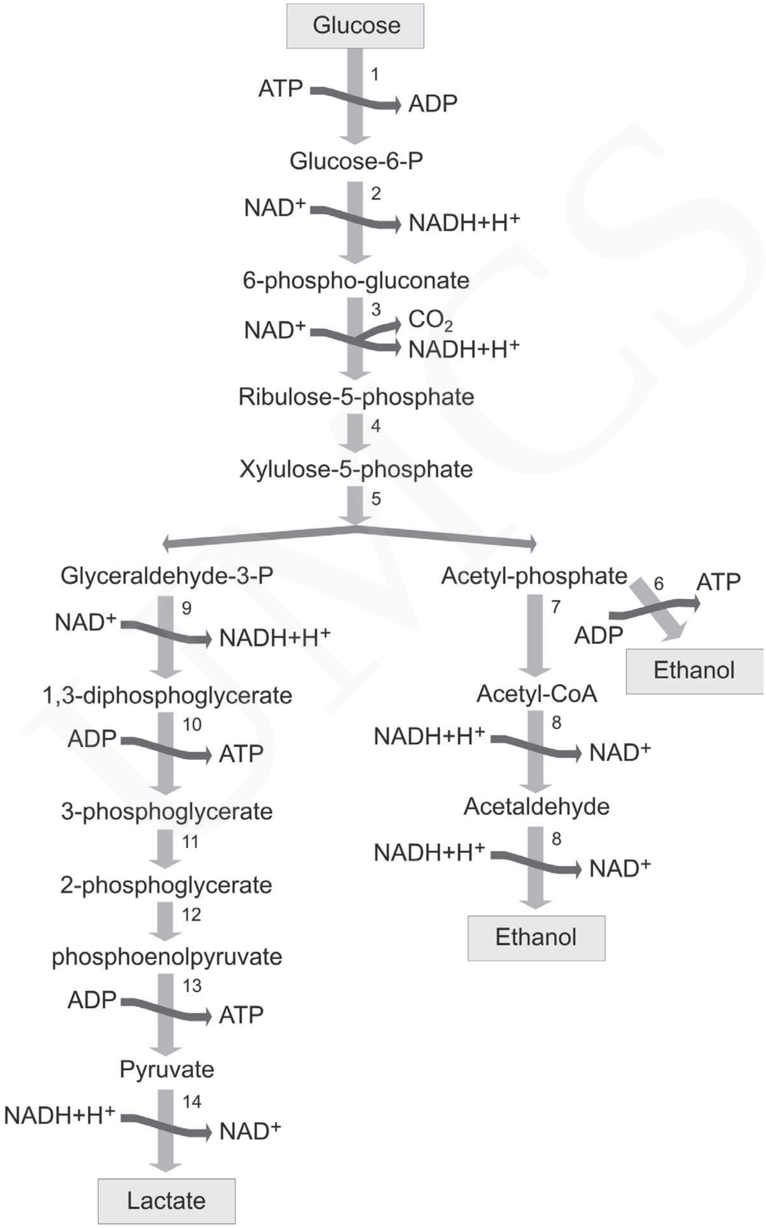

Fig. 2. Heterofermentative pathway of glucose fermentation by lactic acid bacteria (LAB). 1. Hexokinase; 2. Glucose-6-phosphate dehydrogenase; 3. 6-phosphogluconate dehydrogenase; 4. Ribulose-5-phosphate-3-epimerase; 5. Phosphoketolase; 6. Acetate kinase; 7. Phosphotransacetylase; 8. Acetaldehyde/alcohol dehydrogenase; 9. Glyceraldehyde phosphate dehydrogenase; 10. Phosphoglycerate kinase; 11. Phosphoglycerate mutase; 12. Enolase; 13. Pyruvate kinase; 14. Lactate dehydrogenase (adopted from Endo et al. 2018).

lactic acid bacteria are heterofermentative bacteria. Obligate FLAB, besides lactic acid, produce almost equimolar amounts of acetate, and residual amounts of ethanol (19, 22, 41). The conversion of acetyl-CoA to acetate (not to ethanol) yields 1 additional ATP molecule and NAD^+ have to be regenerated from NADH by reduction of pyruvate, fumarate or oxygen (11)

ADVANTAGES OF FLAB FOR PEOPLE AND ANIMALS

Fructophilic lactic acid bacteria and other LAB are important symbionts of the intestinal tract of honeybees. These microorganisms have attracted attention of many researchers and beekeepers due to their positive impact on health and lifespan of honeybees. The special interest of researchers, in this regard, have received fructophilic acid bacteria of the species *L. kunkeei*, which are dominant residents of the intestinal tract of honeybees and the first bacteria that settle in the stomach of emerging honeybees (46). These microorganisms promoted health and increased survival of honeybees when they were added as probiotic into sugar syrup used to feed honeybees (9, 10, 35, 42–44). It was found that they prevent pathogen infections by: production of lactic acid, acetic acid, bacteriocins, competition with pathogens for the same receptors in the epithelial cells of bee guts, maintaining the balance of the intestinal microflora, stimulation of the host immune system, and promotion of host defense due to the fact that intestinal cells produce many immune molecules in response to pathogens (4, 7, 27, 30, 40). Fundamental properties in the selection of probiotic strains used as honeybee food supplements are their resistance to acidity occurring in the gastrointestinal tract of bees and survival in this acidic environment.

Probiotics are defined as “live microorganisms which, administrated in adequate amounts, confer health benefits on the host” (16, 47). Probiotic strains should have the beneficial effects on a host’s health, produce antimicrobial substances against enteric pathogens, adhere to the intestinal epithelium cells of the host, must be nonpathogenic, withstand transit through a gastrointestinal tract, should not carry transferable antibiotic resistance, and must survive during processing and storage (16, 47). The use of LAB as probiotics for honeybees is of particular importance in the preventing and combating diseases. Due to the social organization of the honeybee colonies, the intra-colony transmission of diseases is rapid, mainly through the nurse worker bees that feed the bee brood (28).

In recent years, the marked deterioration in the health status of honeybees and significant increase of their mortality have become very serious concern in many countries. The major reason for declining of honeybee populations are highly contagious diseases of honeybee brood such as: American foulbrood (AFB) and European foulbrood (EFB) caused by *Paenibacillus larvae* and *Melisococcus plutonius*, respectively (17). Recent studies reported that LAB, including fructophilic lactic acid bacteria isolated from honeybees, exhibit antagonistic effect towards *P. larvae* and *M. plutonius* and significantly reduce mortality of honeybee larvae (2, 6, 29, 30, 46, 48). It was also found that *L. kunkeei* strains diminish the number of *Nosema ceranae* spores (microsporidian parasites) in adult honeybees (6). These data clearly indicate that lactic acid bacteria including FLAB are of particular importance in preventing and combating diseases in honeybees and aspire to be used as probiotics.

Over the years, beekeepers have used the antibiotic for prevention and treatment of bacterial infections. The common use of antibiotics to fight bacterial infections resulted in the emergence of antibiotic-resistant microorganisms in the honeybee guts and transmission of the antibiotic resistance genes between individual bacteria. (29, 38). Furthermore, the administration of antibiotics to honeybees may contribute to dangerous contamination of honey with antibiotic residues. (3, 14).

These harmful consequences of antibiotic overuse have mobilized researchers to look for alternative tools to combat infectious diseases. For centuries, honey has been used in folk medicine for healing sore throat and wound infections. Therapeutic potential of honey may be explained by its high osmolarity, low water activity (below 0.60), acidic pH (usually between 3.2–4.5), presence of organic acids, hydrogen peroxide, beedefensin-1 (known also as royalisin), methyglyoxal (MGO), and phenolic compounds with antioxidant properties such as flavonoids and phenolic acids (40). Fructophilic and other LAB symbionts of honeybees play a key role in the honey production and participate in the antibacterial activity of honey by forming many different antimicrobial metabolites. Viable LAB have been found in large number in fresh honey (10^8/g fresh honey) (40, 46). These bacteria die after a couple of weeks because of low water content in honey but metabolites of LAB are preserved in ripe honey. The antimicrobial properties of honey, to a large extent, are associated with lactobacilli (including *L. kunkeei*, the dominant species not only of honeybee guts but also honey), that participate in healing of chronic wounds infected by pathogens such as e.g.: *Pseudomonas aeruginosa, Streptococcus pyogenes, Staphylococcus aureus* by producing organic acids (lactic, acetic, formic acids) and forming environment hostile for pathogens (40, 46).

The osmotolerance and antibacterial activity of FLAB make honey dressing with viable fructophilic lactobacilli good alternative to antibiotics for future treatment of ulcers, bed sores, and other surface infections associated with burns and wounds.

FRUCTOPHILIC *L. KUNKEEI* BACTERIA INDUCE [*GAR*$^+$] PRIONS IN *SACCHAROMYCES CEREVISIAE*

Describing fructophilic lactic acid bacteria, it is necessary to emphasize the ability of *L. kunkeei* to induce the formation of [*GAR*$^+$] prions in *S. cerevisiae* cells during grape juice fermentation (12). The prions are proteins which can take many conformations even without changes in nucleic acid sequences (45). The name [*GAR*$^+$] of yeast prions refers to ability of these proteins to bypass glucose-associated repression. The bracket means that [*GAR*$^+$] is cytoplasmically inherited, italic indicates that this protein functions as a genetic element, and the capital letters point to [*GAR*$^+$] element dominance in the genetic crosses (31). The induction of [*GAR*$^+$] prions in *S. cerevisiae* by *L. kunkeei* leads to metabolic changes in the yeast cells growing on glucose (12). The yeasts reduce the consumption of glucose and simultaneously start to use other carbon and energy sources. The [*GAR*$^+$] proteins alter the properties of regulatory protein that in the presence of glucose suppresses the yeast growth on other carbon sources and allows yeasts to use a wide variety of carbon sources in the presence of glucose. The genetic network that controls the chemical induction of [*GAR*$^+$] proteins in yeasts includes several genes whose functions remain unknown. [*GAR*$^+$] prions are advantageous to both organisms, i.e. to *L. kunkeei* because yeasts make less ethanol and therefore, bacteria have more favorable conditions for growth, as well as to yeasts because their growth and longevity are improved due to possibility to utilize many different carbon sources (12).

CONCLUSION

Fructophilic lactic acid bacteria, which inhabit the gastrointestinal tract of honeybees and protect their hosts from harmful pathogens and contribute to maintaining a good health of honeybee colonies have received considerable interest for their use as probiotics promoting honeybee health. FLAB play also a key role in the honey production by bees. They contribute to antibacterial and therapeutic properties of honey. Honey, with bioactive, antibacterial substances produced by FLAB and other lactic acid bacteria, is a promising candidate to be alternative tool in wound management against different human and animal infections.

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