Fructophilic Lactic Acid Bacteria for Honey Bee Health

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What is fructophilic LAB (FLAB)???

Fructophilic --- fructose + philic = fructose loving

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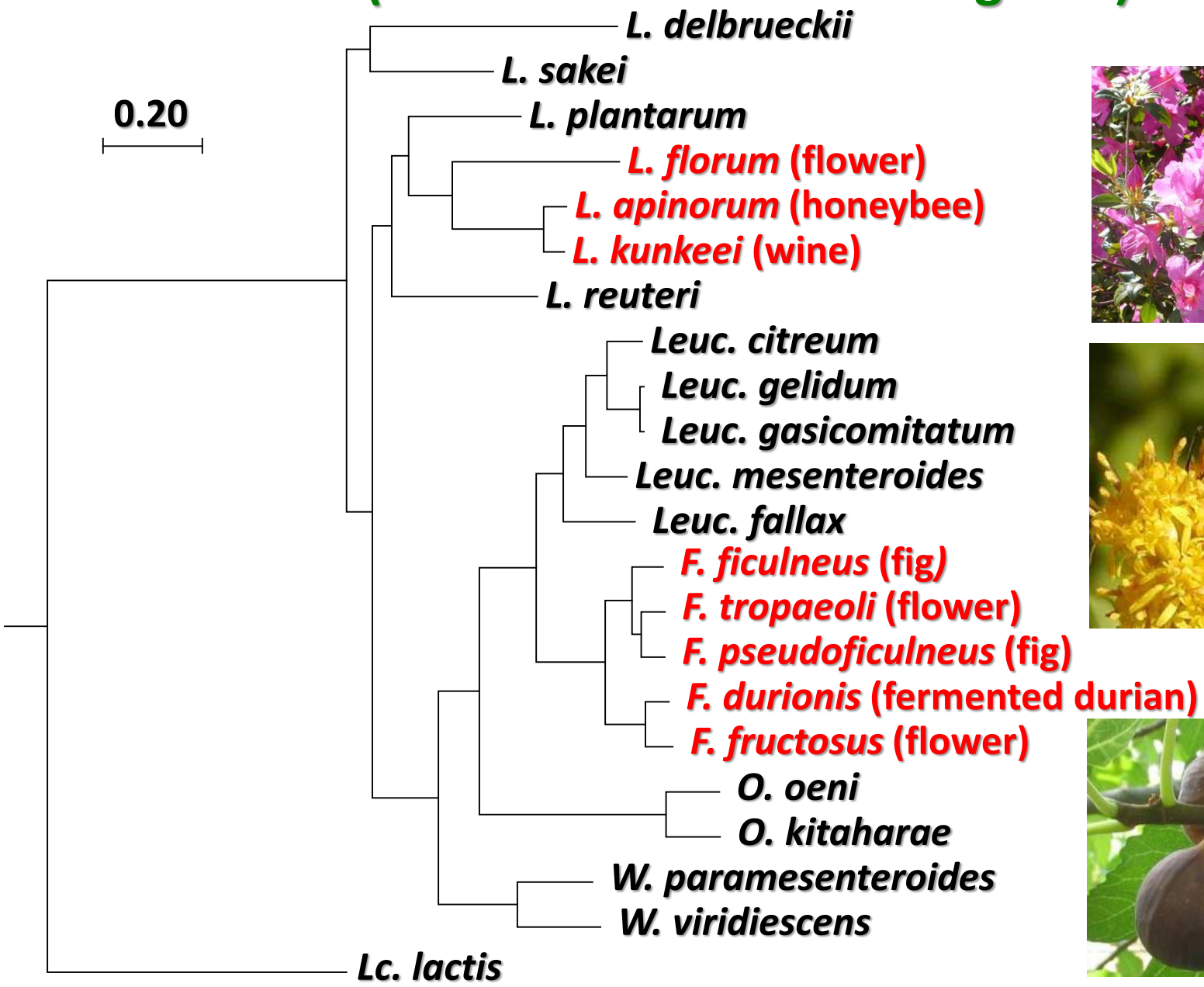
Reclassification of the genus *Leuconostoc* and proposals of *Fructobacillus fructosus* gen. nov., comb. nov., *Fructobacillus durionis* comb. nov., *Fructobacillus ficulneus* comb. nov. and *Fructobacillus pseudoficulneus* comb. nov.

Akihito Endo[1,2,3] and Sanae Okada[1,4]

Description of *Fructobacillus* gen. nov.

Fructobacillus (Fruc.to.ba.cil'lus. N.L. masc. n. *Fructobacillus* arbitrarily derived from fructose and *Lactobacillus*, intended to mean fructose-loving lactic acid-producing bacillus).

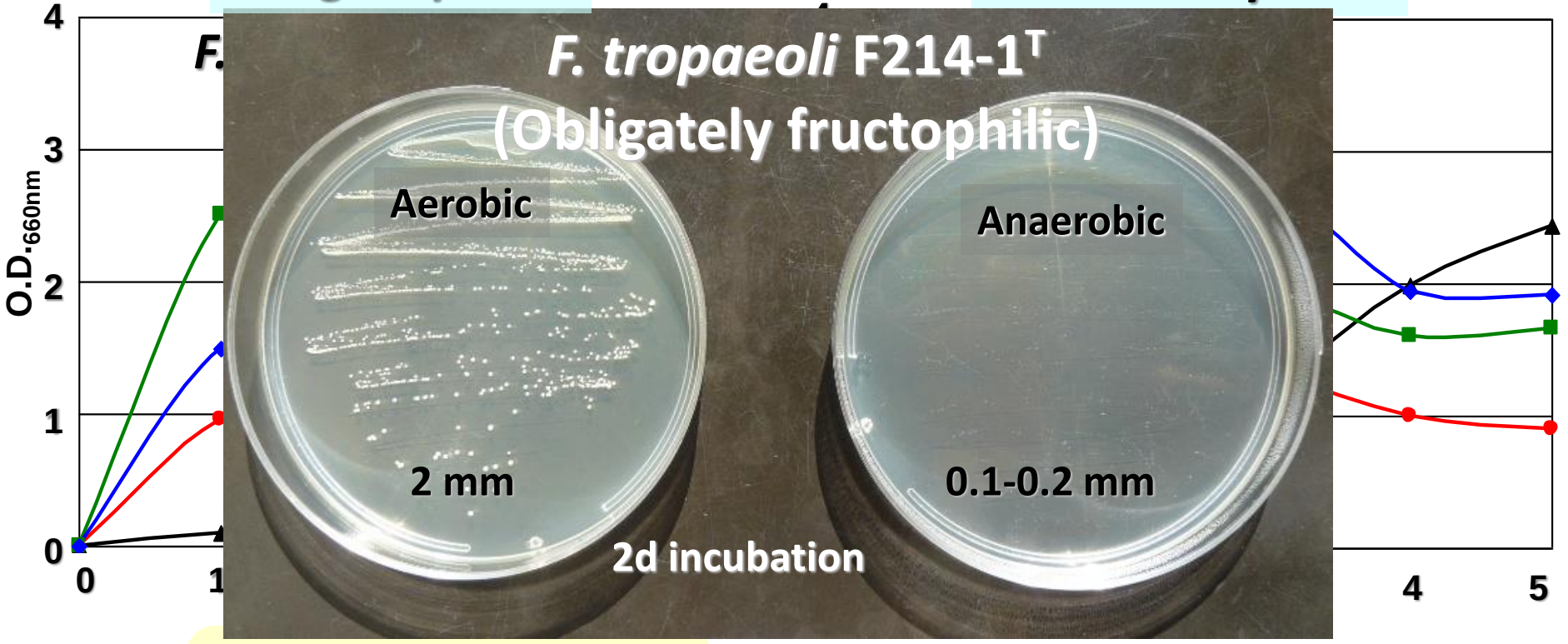
Phylogenetic relationships between FLAB and other LAB (based on 235 conserved genes)



Growth characteristics of FLAB

Obligately FLAB

Facultatively FLAB



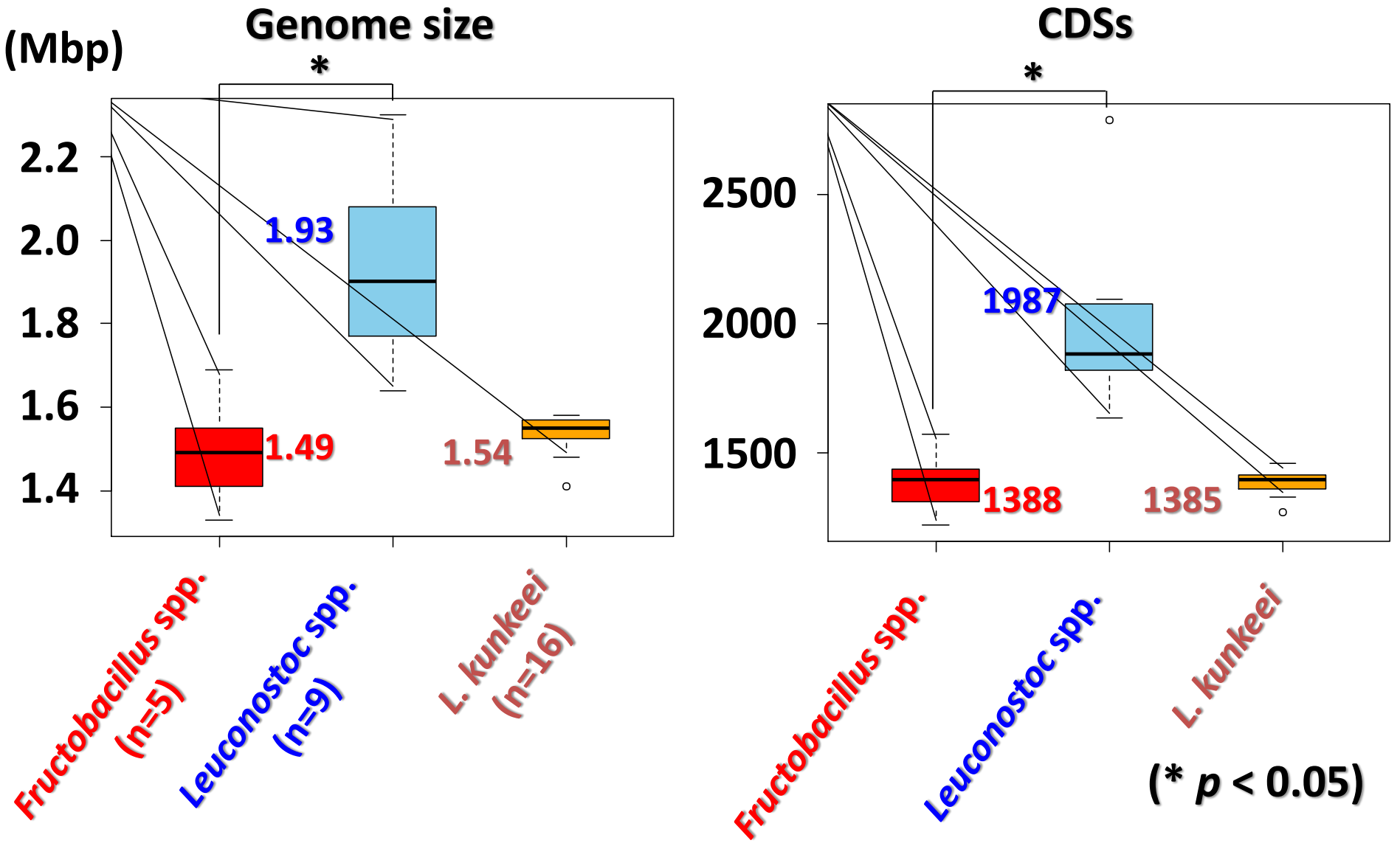
***Fructobacillus* spp.**
Lactobacillus kunkeei
Lactobacillus apinorum

Lactobacillus florum

: glucose
: fructose
: glucose + pyruvate
: glucose (aerobic)

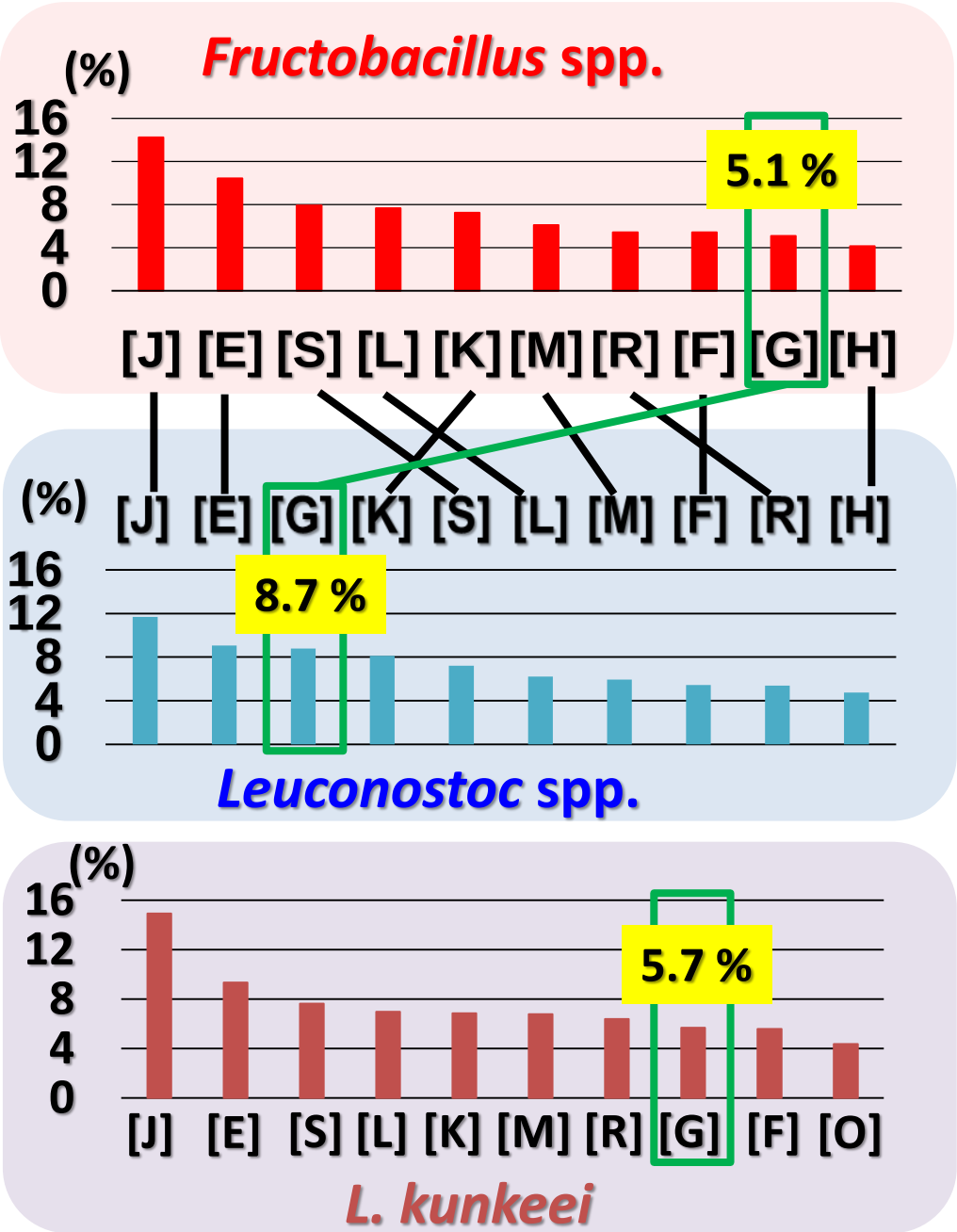
Endo et al. (2009) System. Appl. Microbiol., Endo et al. (2011) Int. J. System. Evol. Microbiol.

Genome size and number of CDSs in *Fructobacillus* spp., *Leuconostoc* spp. and *L. kunkeei*



Endo et al. (2015) BMC Genomics, Maeno et al. (2016) System. Appl. Microbiol.

Top 10 COG classes among the groups



[C] Energy production and conversion
[D] Cell cycle control, cell division etc.
[E] Amino acid transport and metabolism
[F] Nucleotide transport and metabolism
[G] Carbohydrate transport and metabolism
[H] Coenzyme transport and metabolism
[I] Lipid transport and metabolism
[J] Translation and ribosomal structure etc.
[K] Transcription
[L] Replication, recombination and repair
[M] Cell wall membrane etc.
[N] Cell motility
[O] Posttranslational modification etc.
[P] Inorganic ion transport and metabolism
[Q] Secondary metabolites biosynthesis etc.
[R] General function prediction only
[S] Function unknown
[T] Signal transduction mechanisms
[U] Intracellular trafficking, secretion etc.
[V] Defense mechanisms
[X] Mobilome: prophages, transposons

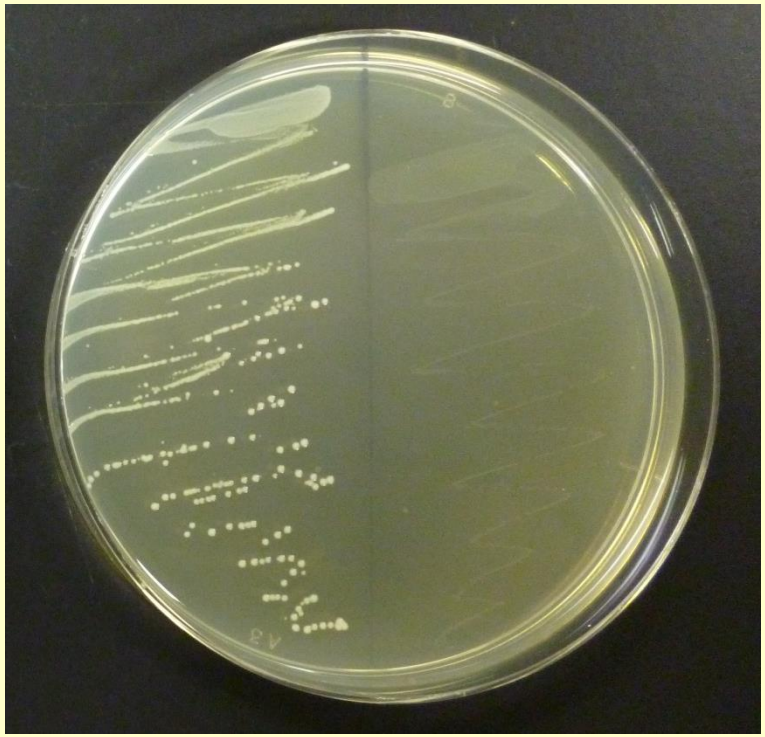
Discriminative pathways among the groups

Aerobic

Leu. mesenteroides *F. fructosus*

Anaerobic

Leu. mesenteroides *F. fructosus*



Fructobacillus-specific- & *Leuconostoc*-specific-genes

4 ≤ : *Fructobacillus* (n=5)
2 ≥ : *Leuconostoc* (n=9)

16 genes

1 ≥ : *Fructobacillus* (n=5)
7 ≤ : *Leuconostoc* (n=9)

114 genes

- **Smaller genome**
- **Less CDS**
- **Simple metabolic systems**

Reductive evolution in *Fructobacillus* spp.

Alcohol dehydrogenase
NAD(P)H dehydrogenase
Chloride peroxidase
Levansucrase
Acylaminoacyl-peptidase
Xanthine
etc.

Pyruvate dehydrogenase E1 component
Pyruvate dehydrogenase E2 component
Transketolase
β-fructofuranosidase
aminopeptidase
dipeptidase
Xaa-Pro dipeptidase
Acetaldehyde/alcohol dehydrogenase (*adhE*)
PTS components
Teichoic acid transport system components
etc.

Endo et al. (2015) BMC genomics

Different habitats among FLAB species

Flowers

Grapes and wine

L. florum

L. kunkeei

F. fructosus

Insects

L. apinorum

F. tropaeoli

F. pseudoficulneus

F. ficulneus *F. durionis*

Fruits and fruits fermentation (other than grapes and wine)

Isolation of FLAB from honeybees and beehives

66 strains of LAB in the initial screening

All were FLAB.

Only 14/354 (4%) strains from flowers and fruits were FLAB.

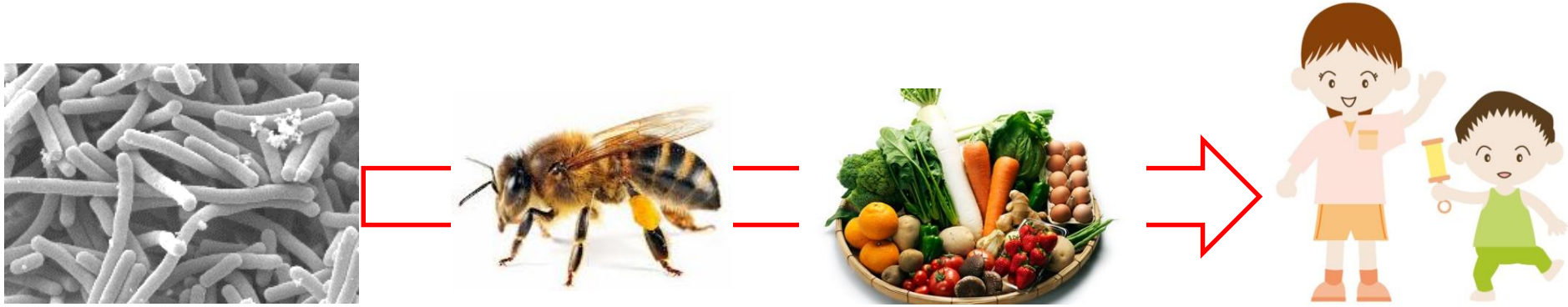
Genetic group by rep-PCR	Origin of grouped strains	
I	flower (10)	*F. fructosus* (99.7%)
II	*A. mellifera*-SW (3), hybridized bee-SW (2), honey (7), larvae (7), bee bread (4), *A. ligustica*-Å (6)	*L. kunkeei* (100%)
III	*A. mellifera*-SW (3)	*F. fructosus* (99.2%)
IV	*A. millifera*-SW (2), *A. ligustica*-SW (4), hybridized bee-SW (2), honey (4), larvae (11), bee bread (8)	*L. kunkeei* (100%)
V	honey (2)	*L. kunkeei* (99.9%)
VI	honey (1)	*L. kunkeei* (99.6%)

Endo & Salminen, System Appl Microbiol (2013)

Summary

FLAB are

- **newly discovered microorganisms.**
- **adapted to their habitats at the genomic level.**
- **members of core microbiota in crop and diet of honeybees.**
- **promising candidates for bee probiotics.**
- **promising symbionts for a vector of paratransgenesis.**



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