

What is a *Lactobacillus*?
The why, who and how of the
re-classification of the genus *Lactobacillus*

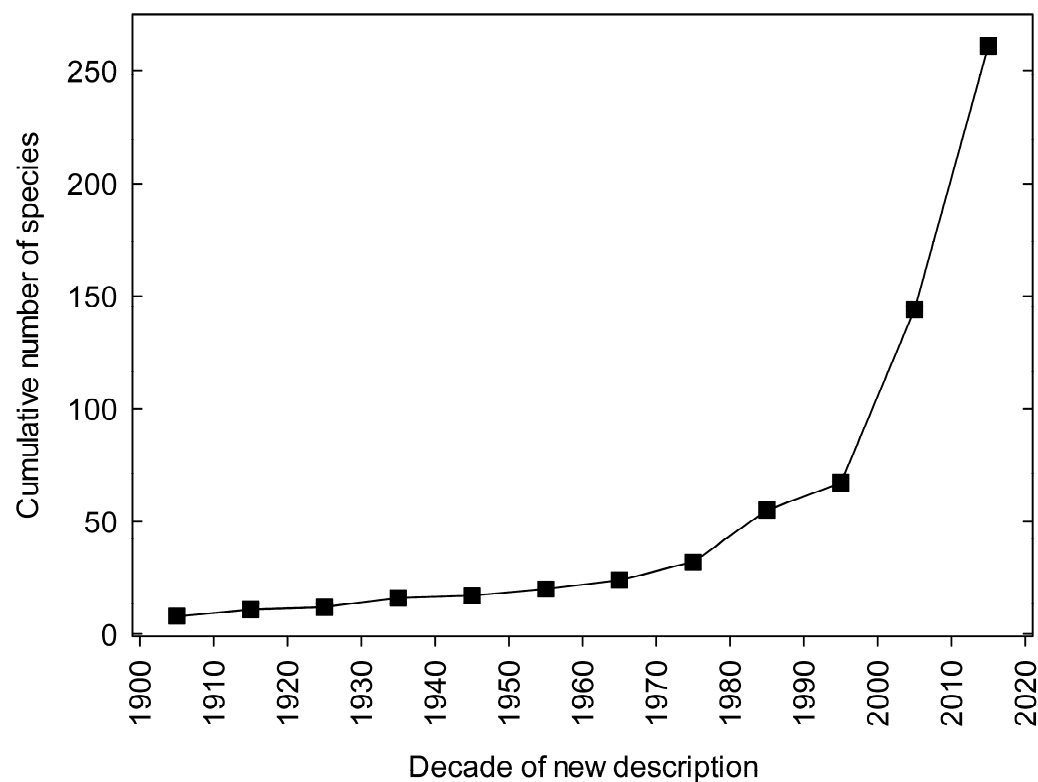
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on behalf of a team of 15 co-autors

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The genus *Lactobacillus* in March 2020



262 species (excluding
pediococci)

84 of these on the International
Dairy Federation list of food
cultures

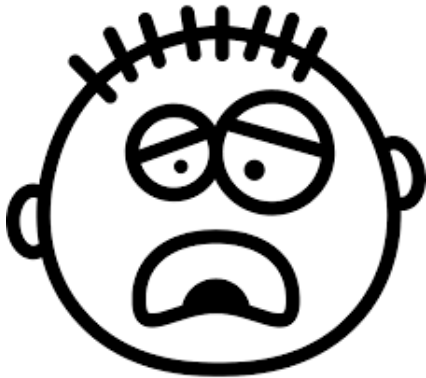
~ 20 produced as starter or
probiotic cultures

37 / 12 species on QPS / GRAS
lists

safe history of use in food

few species related to rare
reports of systemic
infections of critically ill
patients

The new taxonomy of lactobacilli is...



... a pain because no one has time to waste to learn 23 new names.



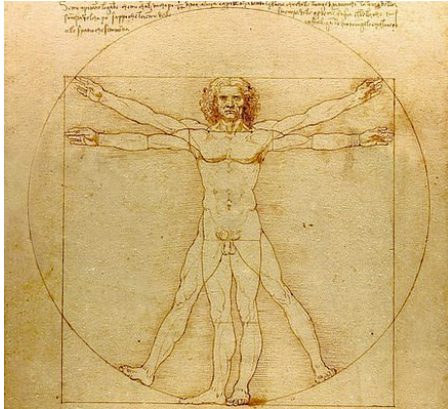
... the best thing since invention of sliced bread because it facilitates communication on all things related to lactobacilli.

“I research human evolution and ecology”



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Genus – Family – Order



Humans
Genus: *Homo*
Family: *Hominidae*
Order: *Primates*



Tapanuli orangutan
Family: *Hominidae*
Order: *Primates*



Neanderthal
Genus: *Homo*
Family: *Hominidae*
Order: *Primates*



Lemur:
Family: *Lemuroidea*
Order: *Primates*

Phylogenetic diversity of lactobacilli

16S rRNA identity (since 1983); ANI and AAI (since 2005);
core genome phylogeny (since 2015)

	16S rRNA homology	AAI range	Eukaryotic example
Species	98.7	>97%	<i>Homo sapiens</i>
Genus	94.5	~65 – 92%	<i>Homo</i>
Family	86.5	~ 60 – 75%	<i>Hominidae</i>
<i>Lactobacillus</i>	86 – 99%	54 %- >90%	
Order	82.0	~ 48 – 62%	Primates
Class	78.5	~ 46 - 60%	
Phylum	75	~ 40 – 50%	

Lactobacilli are...

- ...cocci or rods,
- ...respiring or non-respiring,
- ...homofermentative or heterofermentative,
- ...grow at 4°C or at 45°C or, in few cases, at both temperatures,
- ...fermenting hexoses, pentoses, or both,
- ...producing or consuming lactate,
- ...adapted to vertebrate hosts, insects, or free living,
- ...immune-stimulatory or immune-modulating,
- (...)

Species names allow to quickly determine... ... what?

Latobacilli named after places starting with “H”

Lactobacillus heilongjiangensis

Lactobacillus helsingborgensis

Lactobacillus helveticus

Lactobacillus hokkaidonensis

Lactobacillus huachuanensis

Lactobacillus huananensis

Lactobacillus hulanensis

Lactobacillus hulinensis

EFSA guidance on antimicrobial resistance of microorganisms used in food and feed...

	Ampicillin	Vancomycin	Gentamicin	Kanamycin	Streptomycin	Erythromycin	Clindamycin	Tetracycline
<i>Lactobacillus</i> obligate homofermentative ^(a)	2	2	16	16	16	1	4	4
<i>Lactobacillus acidophilus</i> group	1	2	16	64	16	1	4	4
<i>Lactobacillus</i> obligate heterofermentative ^(b)	2	n.r.	16	64	64	1	4	8 ^(c)
<i>Lactobacillus reuteri</i>	2	n.r.	8	64	64	1	4	32
<i>Lactobacillus</i> facultative heterofermentative ^(d)	4	n.r.	16	64	64	1	4	8
<i>Lactobacillus plantarum/pentosus</i>	2	n.r.	16	64	n.r.	1	4	32
<i>Lactobacillus rhamnosus</i>	4	n.r.	16	64	32	1	4	8
<i>Lactobacillus casei/paracasei</i>	4	n.r.	32	64	64	1	4	4

... is trying to do the right thing but lacks the terminology to do it properly

***Lactobacillus* phylogeny and ecology assessed by comparative genomics**

Salvetti et al., 2012 (16S rRNA)
(doi: 10.1007/s12602-012-9117-8)

14 groups + 10 singletons
+ 4 couples

Core genome phylogeny of lactobacilli:

Zheng et al., 2015 (doi: 10.1128/AEM.02116-15)

Sun et al., 2015 (doi: 10.1038/ncomms9322)

Zheng et al., 2015, Duar et al., 2017:

24 groups

Parks et al., 2018, GBTD (doi: 10.1038/nbt.4229):

16 groups

Salvetti et al., 2018, (doi: 10.1128/AEM.00993-18)

10 groups + 4 singletons

... but: to date, all proposals lack the legitimacy and recognition that is provided by a formal taxonomic re-classification.

Lactobacillus taxonomy: a stack of dirty dishes?

everybody knew that somebody should do it.
anybody could have done it but
nobody did it?





Sander Wuyts



Stijn Wittouck



Jinshui Zheng

Hugh Harris



Paul O'Toole

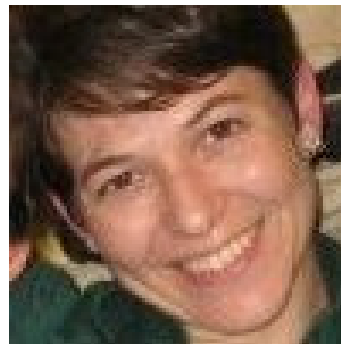
Michael Gänzle



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Charles Franz



Bruno Pot



Paola Mattarelli



Koichi Watanabe



Peter Vandamme

Lactobacillus taxonomy: when did it happen?

INTERNATIONAL
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MICROBIOLOGY**

TAXONOMIC DESCRIPTION

Zheng *et al.*, *Int. J. Syst. Evol. Microbiol.* 2020;70:2782–2858
DOI 10.1099/ijsem.0.004107



A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*

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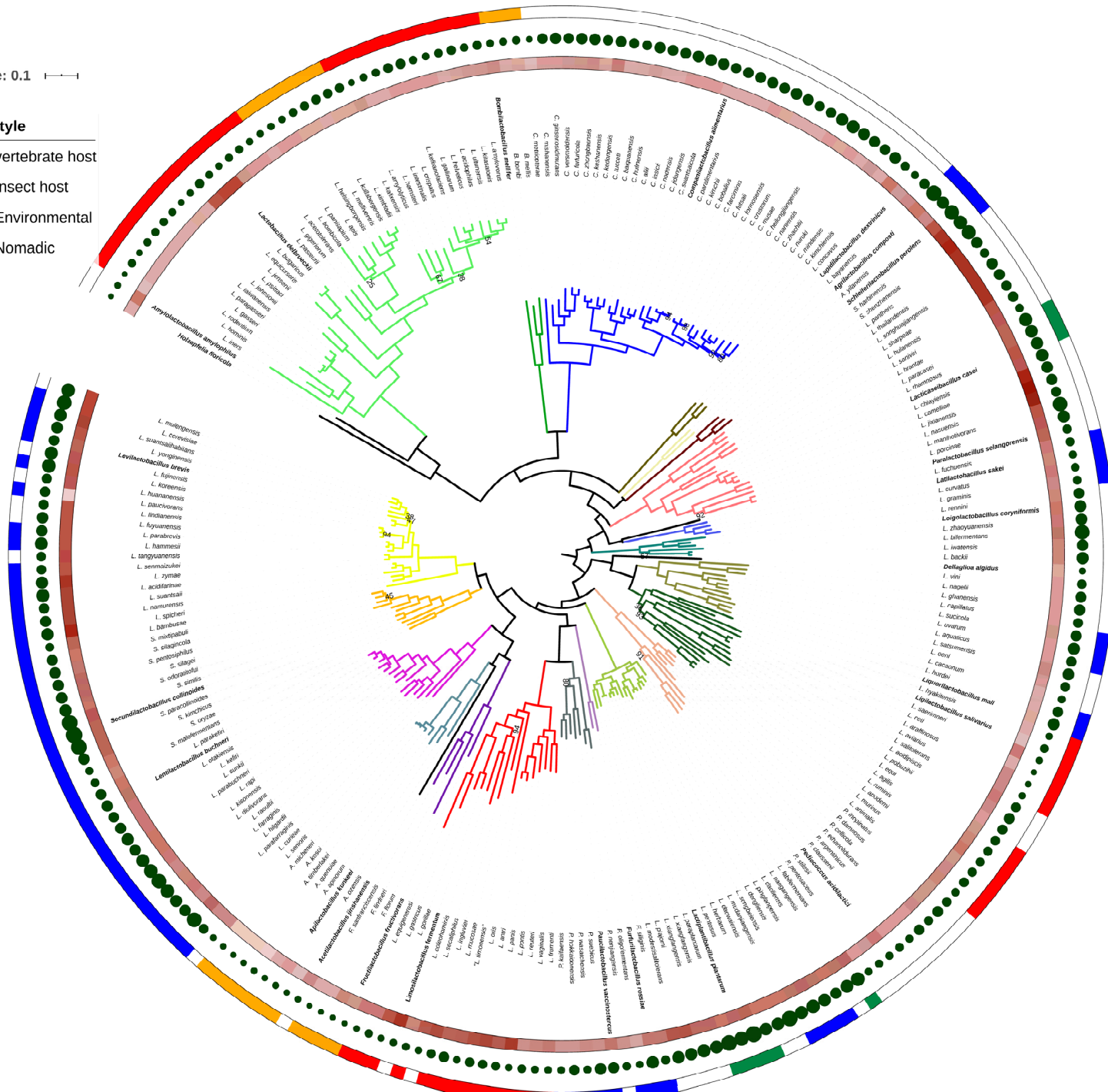
Criteria for delineation of novel genera

- (i) the novel genera are monophyletic
- (ii) intra-genus and inter-genus cAAI values show limited overlap
- (iii) proposed genera are differentiated by ecology, physiology as also supported by signature genes
- (iv) proposed genera are largely consistent with the previous established phylogenetic groups

Tree scale: 0.1

Lifestyle

- vertebrate host
- Insect host
- Environmental
- Nomadic



Current taxonomy *Lactobacillaceae*

- species assigned to *Lactobacillaceae* in March 2020 are assigned to 26 genera:
Lactobacillus, *Pediococcus*, *Paralactobacillus*, and 23 new genera
- *L. delbrueckii* group retains the genus name *Lactobacillus*

The new names translated

Holzapfelia = Wilhelm Holzapfel's lactobacilli

Amylolactobacillus = starch degrading lactobacilli

Lactobacillus = mother lactobacilli (first, ancestral, and representing human vaginal microbiota)

Bombilactobacillus = lactobacilli from bees and bumblebees

Companilactobacillus = companion lactobacilli

Lapidilactobacillus = lactobacilli from stones

Agrilactobacillus = lactobacilli from the field

Schleiferilactobacillus = Karl Heinz Schleifer's lactobacilli

Lacticaseibacillus = relates to cheese

Paralactobacillus = similar to lactobacilli

Latilactobacillus = wide spread lactobacilli

Loigolactobacillus = food spoiling lactobacilli

Dellaglioia = Franco Dellaglio's lactobacilli

Liquorilactobacillus = lactobacilli from liquor or liquids

Ligilactobacillus = uniting (host adapted) lactobacilli

Pediococcus = cocci from spoiled beer

Lactiplantibacillus = related to plants

Furfurilactobacillus = bran-loving lactobacilli

Paucilactobacillus = lactobacilli fermenting few carbohydrates

Limosilactobacillus = slimy lactobacilli

Levilactobacillus = (dough)-leavening lactobacilli

Secundilactobacillus = second lactobacilli

Lentilactobacillus = slow lactobacilli

Apilactobacillus = lactobacilli from bees

Acetilactobacillus = lactobacilli from vinegar

Fructilactobacillus = fructose-loving lactobacilli

Current taxonomy *Lactobacillaceae*

The history of name changes since the mid-20th century is documented in the manuscript.

Rapid search of new and old names on www.lactobacillus.ualberta.ca and <http://www.lactobacillus.uantwerpen.be>

The taxonomy has been included by LMG, NCBI and the Genome Based Taxonomy Database and LMG; EFSA is using old and new names in parallel, DSMZ is working on the transition

ANI or 16S rRNA gene similarity allows robust assignment of new species are assigned to the proposed genera.

A framework for delineation of novel genera is provided.

We can communicate, finally!

- *Lactobacillus* is host adapted, homofermentative, vancomycin sensitive, and lacks pyruvate formate lyase.
- *Lactobacillus* species produce lactate, *Lentilactobacillus* species consume lactate.
- *Apilactobacillus* and *Bombilactobacillus* are adapted to bees and have small genome sizes.
- *Limosilactobacillus* and *Liquorilactobacillus* form biofilms, mediated by glucansucrases or fructansucrases
- Host adapted lactobacilli of the genera *Lactobacillus*, *Ligilactobacillus*, and *Limosilactobacillus* use glutaminase for acid resistance, others do not.

Probiotic lactobacilli

Lactobacillus, *Ligilactobacillus*, and *Limosilactobacillus* species are (mostly) vertebrate host adapted. Most probiotics are derived from these three genera.

Lacticaseibacillus, *Lactiplantibacillus* and *L. fermentum* are nomadic and temporarily persist in the vertebrate intestine. These include probiotics but also organisms that cause – rare – nosocomial infections in severely immunocompromised hosts.

Everything else is everything else.

Lactobacilli in cheese

Lactobacillus and *Streptococcus* species are used as thermophilic starter cultures.

Lacticaseibacillus, *Lactiplantibacillus*, *Ligilactobacillus*, *Limosilactobacillus* species are major representatives of NSLAB (a few more in cold places like Québec).

Everything else is everything else.

Lactobacilli in Chinese grain vinegar

Old nomenclature:

Vinegar mashes (vinegar Pei) are fermented by lactobacilli.

New nomenclature:

Vinegar mashes are fermented by *Lactobacillus* and *Limosilactobacillus* species (and, possibly, *Acetilactobacillus*).

Safety of *Lactobacillaceae*

Old:

Few species of lactobacilli cause rare infections in critically ill patients

Current:

Few species of *Lacticaseibacillus* and *Weissella* cause rare infections in in critically ill patients.

Infections are so rare that most Public Health Agencies nevertheless consider *Lacticaseibacillus rhamnosus* as a safe organism

The case of *Weissella* is not yet settled.

Antibiotic resistance of *Lactobacillaceae*

Lactobacillus, *Amylolactobacillus* and *Holzapfelia* are vancomycin sensitive
All other genera of the *Lactobacillaceae* are intrinsically vancomycin resistant

Some strains of the intestinal lactobacilli (genera *Lactobacillus*, *Ligilactobacillus* and *Limosilacobacillus*) picked up the tetracycline resistance gene *tetW* from their intestinal brethren, other lactobacilli do not carry that gene.

Moving forward:

We can ask questions we could not ask before!

- Does the interaction of nomadic *Lactiplantibacillus* and *Lacticaseibacillus* probiotics with the immune system differ from the interaction of host adapted *Lactobacillus* and *Limosilactobacillus* probiotics?
- Is acid resistance a general property of *Lactobacillus* species?
- Is the ecological role of extracellular fructosidases in *Liquorilactobacillus* and *Lactobacillus* species comparable to oral streptococci?
- Why do lentilactobacilli and limosilactobacilli produce taste active and anti-inflammatory γ -glutamyl peptides?
- What is the role of homofermentative metabolism of pentoses in heterofermentative lactobacilli?
- Can propionic acid formation by *Lentilactobacillus* species be used for food preservation?
- ...

⇒ **onwards and upwards!**

Useful Websites

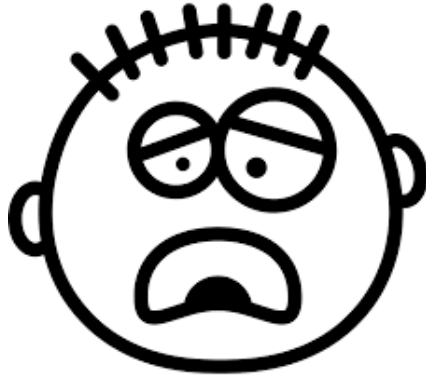
www.lactobacillus.ualberta.ca and www.lactobacillus.uantwerpen.be – “translates” genus names, lists all current and past lactobacilli, and provides links to genome sequences and publications. The websites are regularly updated to include new species that are described

<https://doi.org/10.1099/ijsem.0.004107> ;
open access DOI: <https://doi.org/10.7939/r3-egnz-m294>
the “*Lactobacillus* Monster”

<https://drive.google.com/drive/folders/10gYBZE6wtvhX0FON39yS4w7G75Cni4-K?usp=sharing> – Link to the phylogenetic tree of lactobacilli, which will be occasionally updated.

<https://drive.google.com/drive/folders/10cH7XaNqGQAKdL3VHhd1tzWXIGaTdfkV?usp=sharing> – Link to the Periodic Table of Fermented Foods, which will be occasionally updated.

***Lactobacillus* has changed, lactobacilli have not... do I have conclusions?**



a pain?



... or regulators' work made easier?

**Thanks for your attention - I look
forward to questions.**



Sander Wuyts



Stijn Wittouck



Jinshui Zheng

Hugh Harris



Paul O'Toole

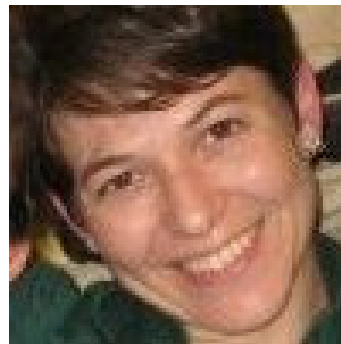
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