

Jean-Daniel Charrière¹, Vincent Dietemann², Benjamin Dainat¹

¹Swiss Bee Research Centre Agroscope Liebefeld Bern Switzerland

²Swiss Bee Research Centre Agroscope Liebefeld Bern Switzerland, Department of Ecology and Evolution University of Lausanne Lausanne Switzerland

Because honey bee colonies contain many individuals in close physical contact, they constitute fertile grounds for numerous pathogens to multiply and spread. Among these, the brood bacteria *Paenibacillus larvae* and *Melissococcus plutonius*, the causative agents of American (AFB) and European foulbrood (EFB) diseases, respectively, are both globally distributed. By affecting larvae, these bacteria can lead to colony weakness and possibly to death. In Switzerland, high numbers of EFB cases were reported each year, leading to increased research efforts into the epidemiology of EFB. During such research, an atypical bacterium was found in addition to *M. plutonius* in an apiary affected by EFB. To identify it, we sequenced De Novo the full genome and described its genome features as well as its physiology and chemotaxonomy. We thus discovered it was a new species, which we named *Paenibacillus melissococcoides*. In this presentation, we will retrace the steps required to identify and name a new bacterium species. Using the genomic information generated, we screened EFB affected colonies in Switzerland and searched genome databases for misidentified matches. We indeed found the possible presence of this bacterium outside Switzerland, suggesting that this species can be found worldwide. The discovery of this new species brings new insights into EFB epidemiology and the suite of bacteria associated with it, as well as a better understanding of the ecology of *Paenibacillus* species when they encounter honey bees. With this information available, it will be possible to study the role of this bacteria in honeybee health and particularly in idiopathic cases, for which brood diseases are suspected, but *M. plutonius* and *P. larvae* are not found.



Paenibacillus melissococcoides: the discovery of a new bacterium associated with a honey bee brood disease

Dainat Benjamin¹, Dietemann Vincent^{1,2}, Charrière Jean-Daniel¹

1 Agroscope Liebefeld, Swiss Bee Research Centre, Bern, Switzerland ; 2 Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland

Apimondia 2025

25th September

Once upon a time in Switzerland...



Florine Ory



apiary with **European foulbrood (EFB)**



symptomatic brood

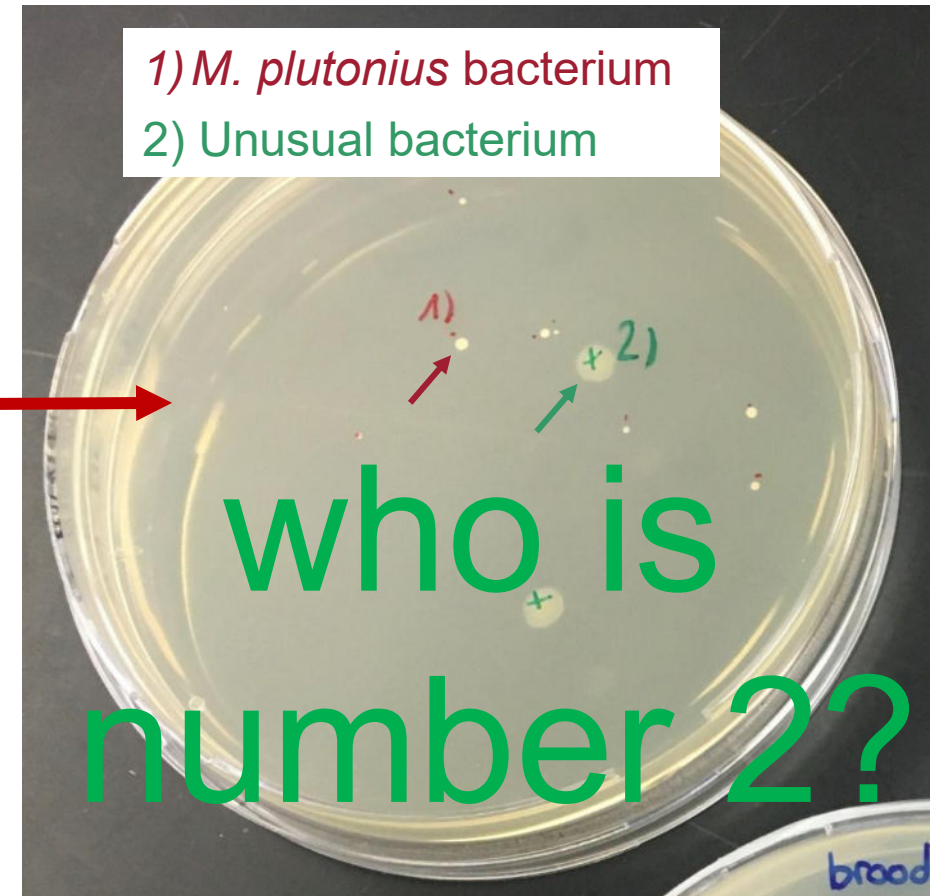
...while studying the epidemiology of EFB...

Where can
Melissococcus plutonius bacteria
be found in brood cells?

And in which quantity?



Worker jelly
in cell with first
instar larva

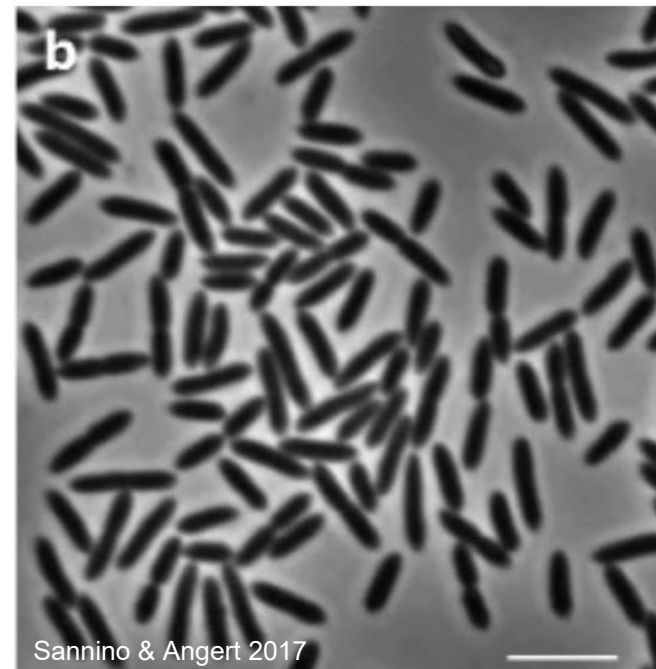


Who is number 2?

Chemical identification of proteins (MALDI)

providing a "fingerprint" of the bacteria based on its unique protein profile

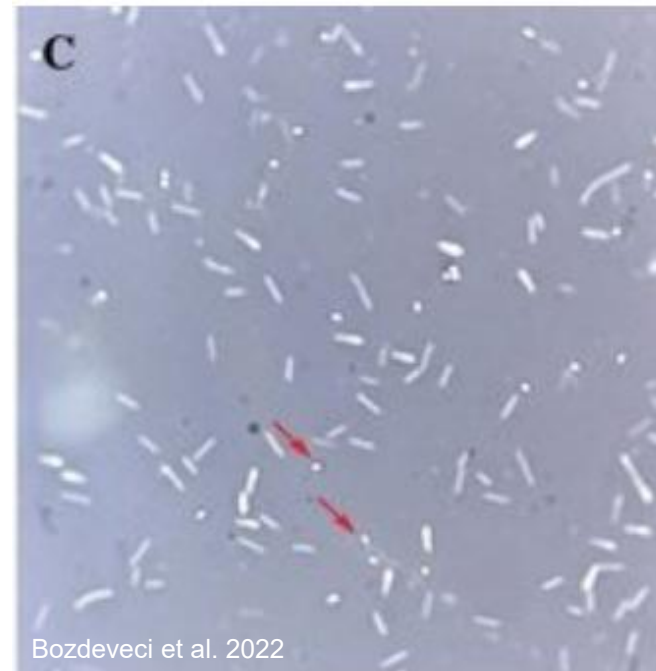
Paenibacillus thiaminolyticus



Who is number 2?

Genetic identification 16S rRNA gene* sequencing

Paenibacillus dendritiformis



Who is number 2?

... belongs to the genus *Paenibacillus*,

as *Paenibacillus larvae*,
the causative agent of american foulbrood

but its classification at the species level is unclear!

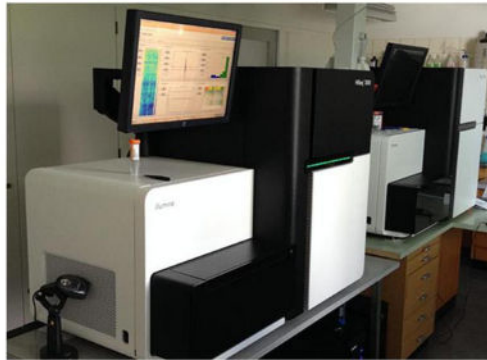


P. thiaminolyticus, *P. dendritiformis*, *P. 'something else'??*

Who is number 2?

Genetic identification full genome sequencing

Next Generation Sequencing (NGS) Platform



The NGS platform was established to facilitate the access to the latest nucleic acid sequencing technologies for researchers from the Universities of [Bern](#) and [Fribourg](#). External customers are also encouraged to inquire for services.

E-Mail: ngs.lab@vetsuisse.unibe.ch

 [PriceList_NGSP_internal_20201023](#) (PDF, 250KB)



Average Nucleotide Identity (ANI)

More than 95% identity = same species

Less than 95% identity = different species

Agroscope

P. dendritiformis / **Number 2** / *P. thiaminolyticus*

91% ANI

It is (very likely) a new species !

***P. melissococcoides* discovery | Apimondia 2025**
B. Dainat, V. Dietemann, JD Charrière

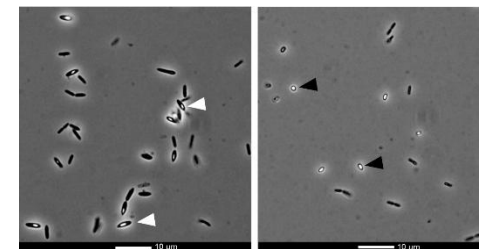
Who is number 2?

Minimal standards to describe a new species

Essential feature	Recommended feature
Microscopic morphology Cell size (width and length), overall shape, shapes of ends of cells, chains, filaments Gram reaction of young culture Motility Presence of storage inclusions Sporangial morphology Spore shape and position Sporangial swelling Presence of parasporal bodies and other inclusions or extracellular deposits Photomicrograph of sporangia	Type of flagellation (by flagellar staining or electron microscopy)
Macroscopic morphology Growth medium used Cultural conditions (temperature, pH, salinity, incubation time) Colony diameter after a given period of incubation Colony shape, elevation, mention of any tendency to spreading or colonial motility, morphology of edges, surface texture, colour, consistency and mention of any tendency to be adherent to the medium	
Physiological characters Recommended growth medium Optimum, maximum and minimum growth temperatures Optimum, maximum and minimum pH for growth Oxygen requirements* NaCl tolerance or requirement (minimum, optimum and maximum concentrations), or stimulation of growth by NaCl Catalase Oxidase Any known special nutritional requirements	Ability to grow on minimal medium (state composition and conditions)
Biochemical characters Acid production from D-glucose Hydrolysis of casein, gelatin and starch Citrate utilization	Gas production from D-glucose Acid production from L-arabinose, D-mannitol, D-xylose and other specified carbohydrates of differential value† Voges-Proskauer reaction Hydrolysis of aesculin and urea Nitrate reduction, and reduction of nitrate or nitrite to nitrous oxide or nitrogen gas Arginine dihydrolase Utilization of propionate and other organic acids as sole carbon sources Utilization of amino acids as sole carbon sources Utilization of carbohydrates as sole carbon sources Egg yolk (lecithinase) reaction‡ Indole production from tryptophan ONPG (o-nitrophenyl β-D-galactopyranoside) hydrolysis
Chemotaxonomic characters Fatty acid profile Cell wall diagnostic diamino acid	Murein structure§ Polar lipid analysis§ Quinone analysis§
Nucleic acid studies 16S rRNA gene sequence (>1400 nt, <0.5% ambiguity) and phylogenetic analysis DNA-DNA relatedness with closely related species	Phylogenetic analysis based on housekeeping protein-coding genes G+C content of DNA§ ΔT _m of hybrid DNA

Microscopic morphology

- Gram staining
- Motility
- Photomicrograph of spores



Macroscopic morphology

- Colony morphology on culture media



Physiology

- Growth media
- Growth conditions (pH, temperature...)

Chemotaxonomic characters

- Fatty acid profiles

fatty acid %	Pm	Pt	Pd
iso-C _{14:0}	0.6		1.6
C _{14:0}	1.3	1.5	2.1
iso-C _{15:0}	8.7		7.4
anteiso-C _{15:0}		43.6	38.4
C _{15:0}	2.3	1.9	
iso-C _{16:0}	5		10.6
C _{16:0}		16.9	16.1
iso-C _{17:0}	7.9	8	
anteiso-C _{17:0}	26.7	15.1	
C _{17:0}	1.2		
iso-C _{18:0}	0.2		0.3
C _{18:0}	0.3		0.4

Genomic studies

- Phylogeny based on 16S rRNA genes
- Phylogeny based on whole genome

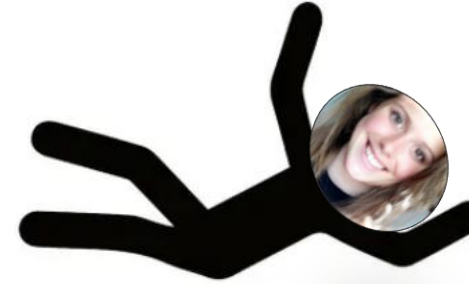
Who is number 2?

...it is new to science!!!

'it' needs a name!!!



P. melissococcoides discovery | Apimondia 2025
B. Dainat, V. Dietemann, JD Charrière



HURRAY!

Who is number 2?

...it is new to science!!!

‘it’ needs a name!!!

<i>P. melissosimile</i>	<i>P. alveus</i>	<i>P. difficile</i>
<i>P. icks</i>	<i>P. alvarium</i>	<i>P. asperum</i>
<i>P. Insidiosa</i>	<i>P. apiumfetus</i>	<i>P. lateo</i>
<i>P. aurantiaco</i>	<i>P. apisalveus</i>	<i>P. casus</i>
<i>P. biformis</i>	<i>P. apium</i>	<i>P. clam</i>
<i>P. aeger</i>	<i>P. reutigensis</i>	<i>P. coloris</i>
<i>P. melissocoram</i>	<i>P. labori(u)s</i>	<i>P. ignotus</i>
<i>P. gelati</i>	<i>P. aegre</i>	<i>P. insidiosa</i>
<i>P. alvaere</i>	<i>P. anfractus</i>	<i>P. basale</i>

INTERNATIONAL
JOURNAL OF **SYSTEMATIC**
AND EVOLUTIONARY
MICROBIOLOGY

2019, volume 69, issue 1A, pages S1–S111



International Code of Nomenclature of Prokaryotes

Prokaryotic Code (2008 Revision)

Charles T. Parker¹, Brian J. Tindall² and George M. Garrity³ (Editors)

¹NamesforLife, LLC (East Lansing, Michigan, United States)

***Paenibacillus melissococcoides* sp. nov.**

Melissococcus, a bacterial genus;
-oides, similar, resembling;
melissococcoides, *Melissococcus*-like

Ory et al., 2023

Paenibacillus melissococcoides

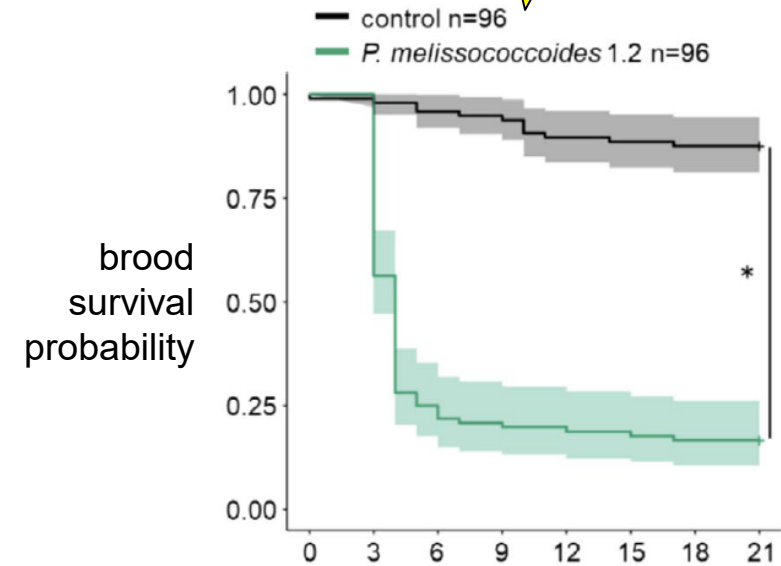
A new pathogen?

In the lab:
Yes!



In the field: detected in 4 idiopathic cases*
Maybe! → secondary invader**

See poster
PP-95
September 24th-25th



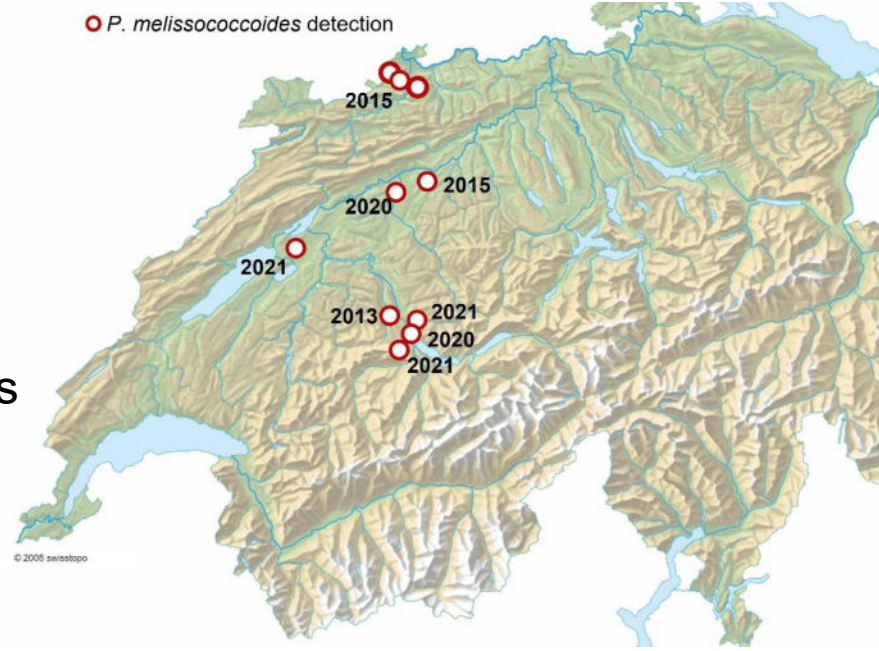
Ory et al., 2025

Paenibacillus melissococcoides

Where to find it?

in Switzerland
in 10 out of 786
EFB-diseased colonies

not detected
in 153 healthy
colonies (2005-2021)



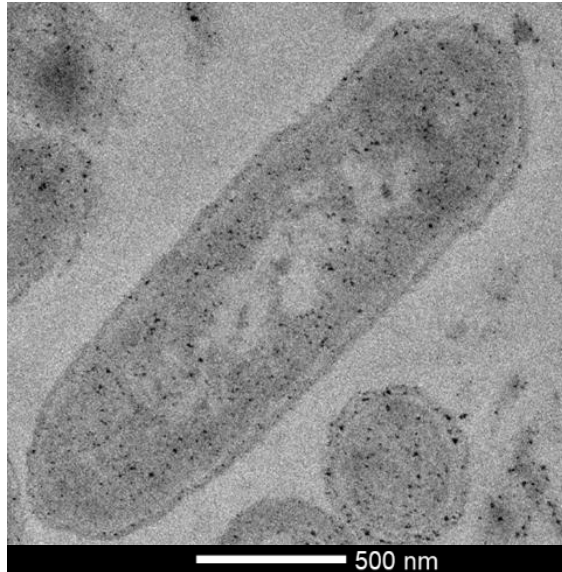
Ory et al., 2025

Italy (database match)
Gaggia et al. 2015

Japan: in 69 of 293 (24%) honey samples from 99 beekeepers
Nakamura et al. 2025



Paenibacillus melissococcoides

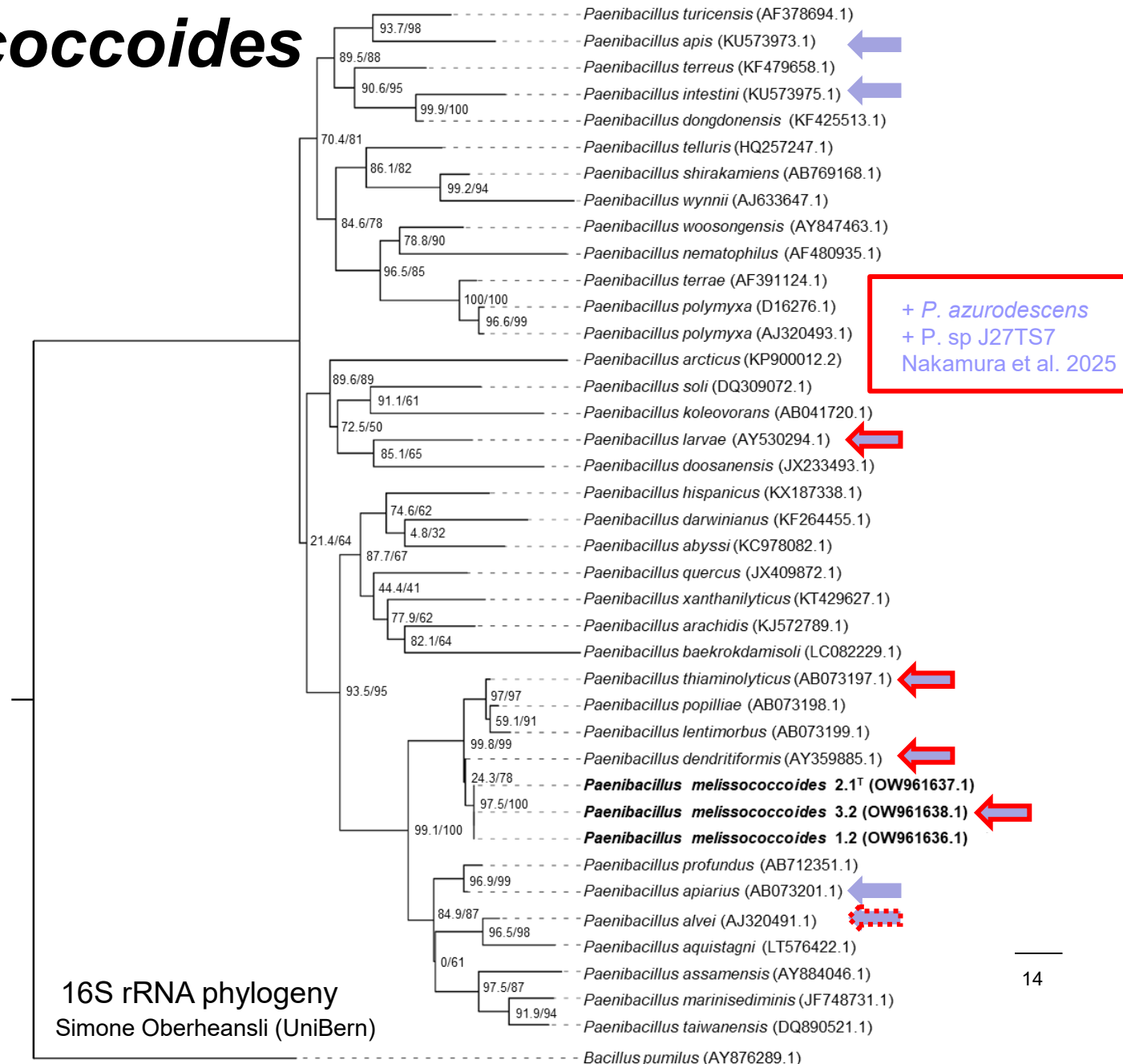


One of 10
Paenibacillus species
associated with
the honey bee

One of the 7 pathogenic species

P. melissococcoides discovery | Apimondia 2025
B. Dainat, V. Dietemann, JD Charrière

Ory et al., 2023



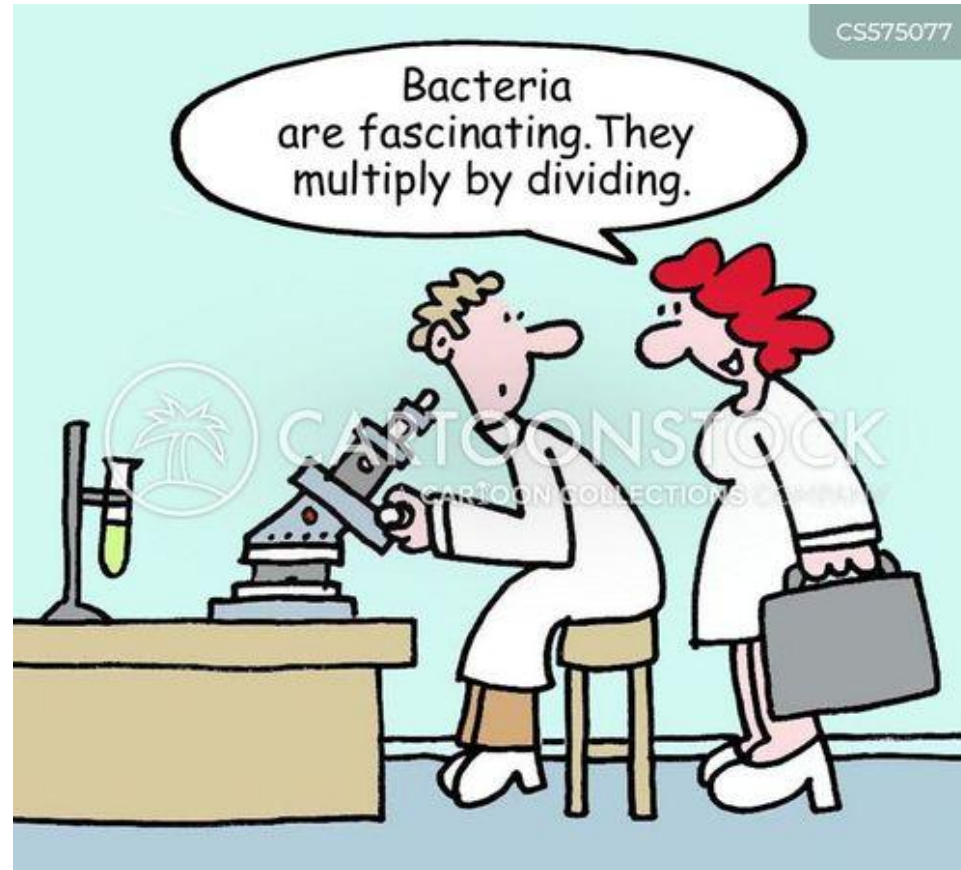
Take home message

Paenibacillus melissococcoides...

- pathogenic for honeybee larvae, maybe for colonies
- is a secondary invader in EFB
- could be common and with a global distribution
- impact on honeybee health to be determined!

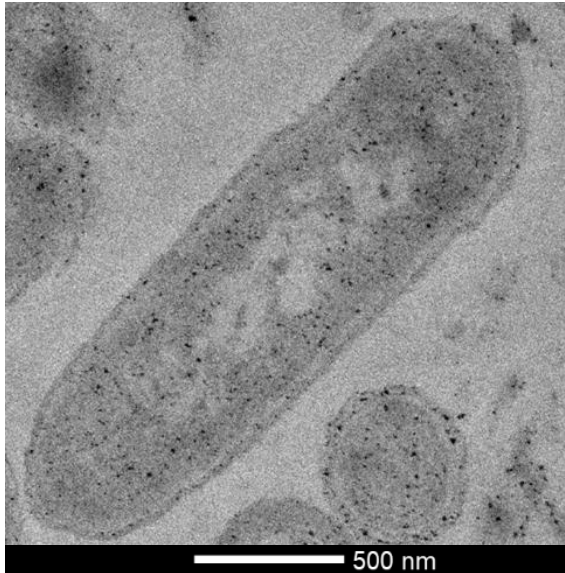


Thank you for your attention !

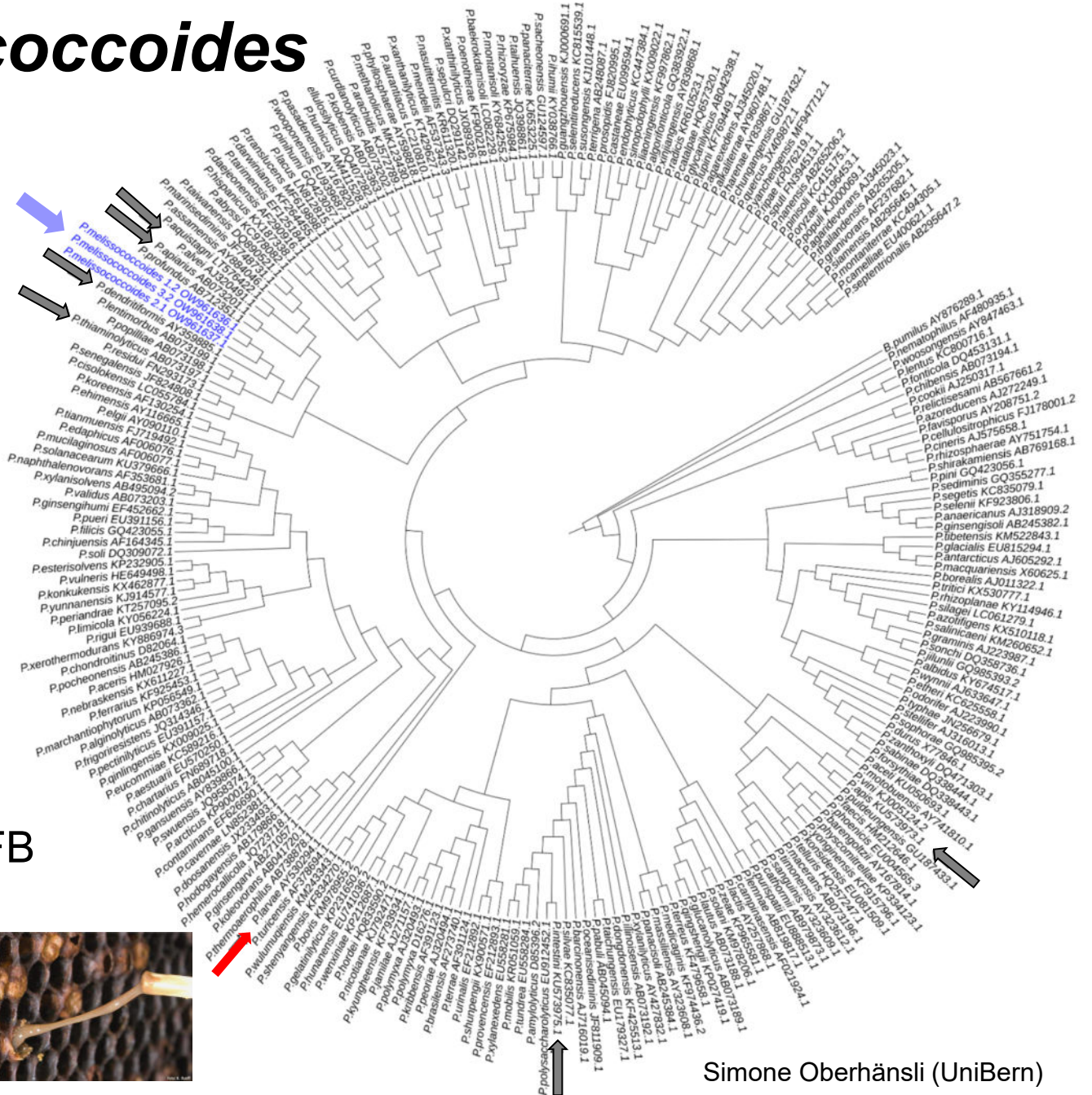




Paenibacillus melissococcoides



→ One of the eight *Paenibacillus* species associated with the honey bee and a secondary agent of EFB

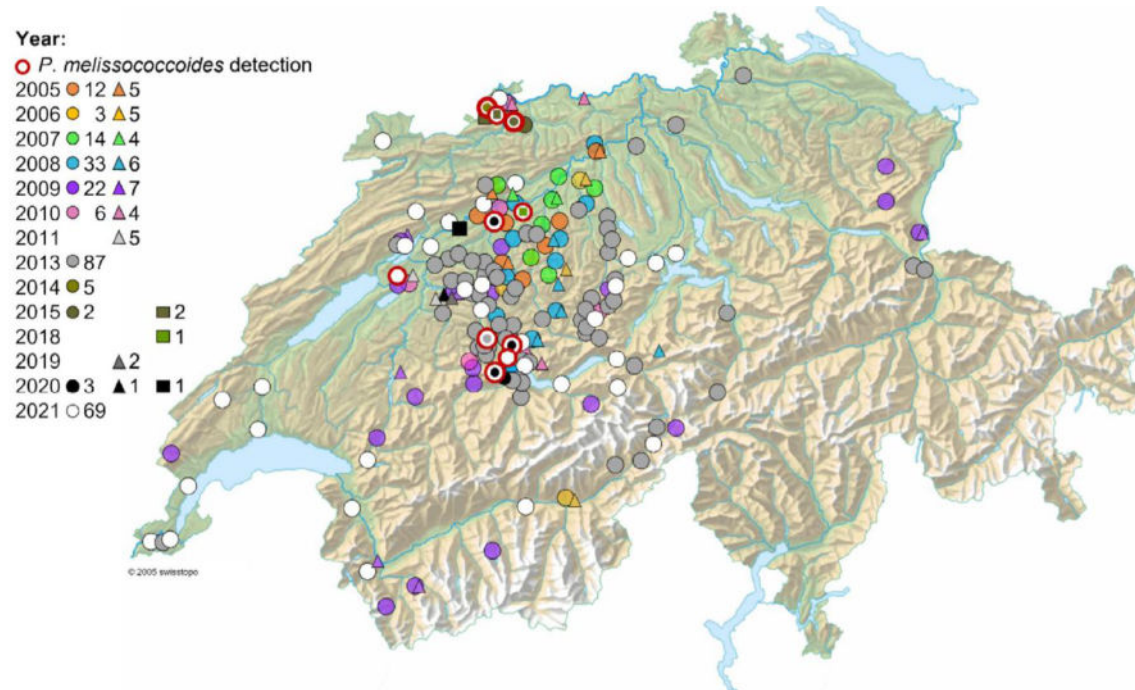


Simone Oberh nsli (UniBern)

Paenibacillus melissococcoides

Where to find it?

in Switzerland



detected in 10 out of 786
diseased colonies
and
not detected in 153 healthy
colonies
sampled between 2005-2021

elsewhere:

Japan: in 24% of 293 honey samples from 99 beekeepers Nakamura et al. 2025

Italy (database match) Gaggia et al. 2015



Comparative analysis

1) Genomic studies

- Phylogeny based on the whole genome **Average Nucleotide Identity (ANI)**

Organisms belonging to the same species typically showed **≥95% ANI** among themselves¹.

Query	DDBJ/EMBL/GenBank accession number	Reference	DDBJ/EMBL/GenBank accession number	ANI (%)
2.1 ^T	GCA_944800085	1.2	GCA_945318265	99.99
1.2	GCA_945318265	3.2	GCA_945318285	99.99
3.2	GCA_945318285	2.1 ^T	GCA_944800085	99.99
2.1 ^T	GCA_944800085	<i>P. dendritiformis</i>	GCA_945605565	92.34
2.1 ^T	GCA_944800085	<i>P. thiaminolyticus</i>	GCA_945318275	91.09
1.2	GCA_945318265	<i>P. dendritiformis</i>	GCA_945605565	92.36
1.2	GCA_945318265	<i>P. thiaminolyticus</i>	GCA_945318275	91.05
3.2	GCA_945318285	<i>P. dendritiformis</i>	GCA_945605565	92.28
3.2	GCA_945318285	<i>P. thiaminolyticus</i>	GCA_945318275	91.03

> 95% ANI, *P. melissococcoides*
2.1^T, 1.2, and 3.2 belong to the same species

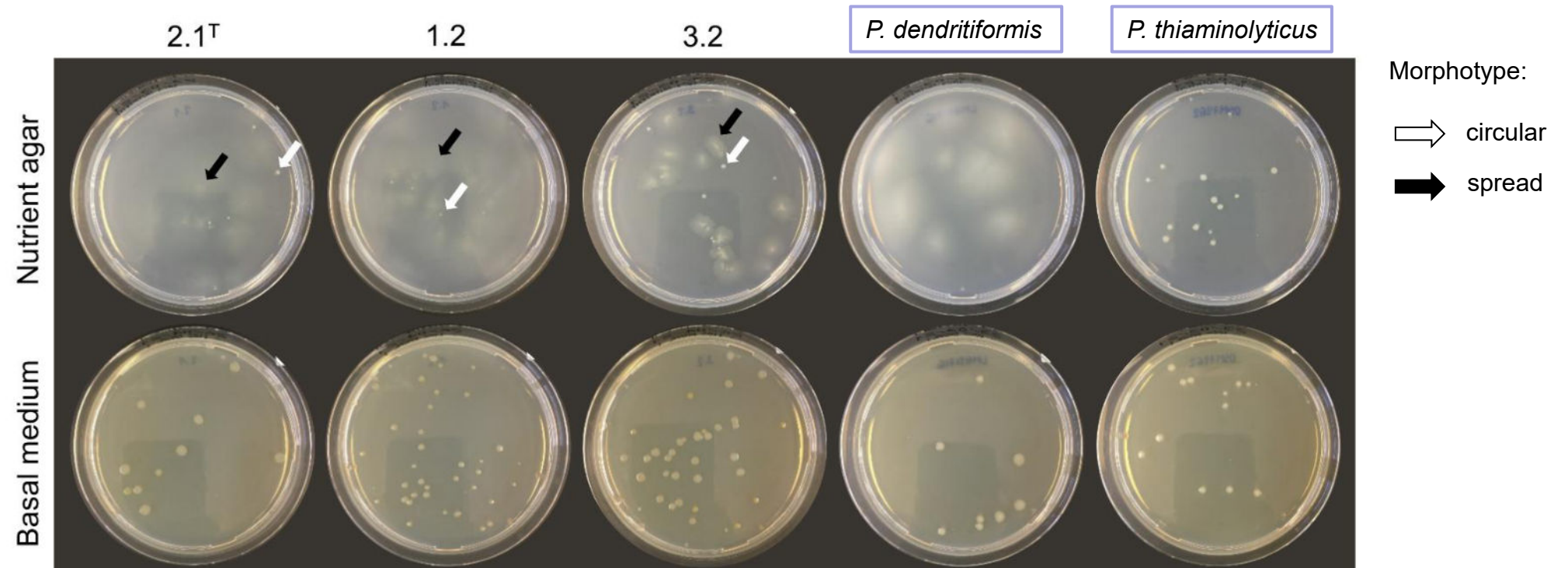
< 95% ANI, *P. melissococcoides*
2.1^T, 1.2, and 3.2 do not belong to *P. dendritiformis*, or *P. thiaminolyticus* species



Comparative analysis

2) Macroscopic morphology

- Colony morphology on media

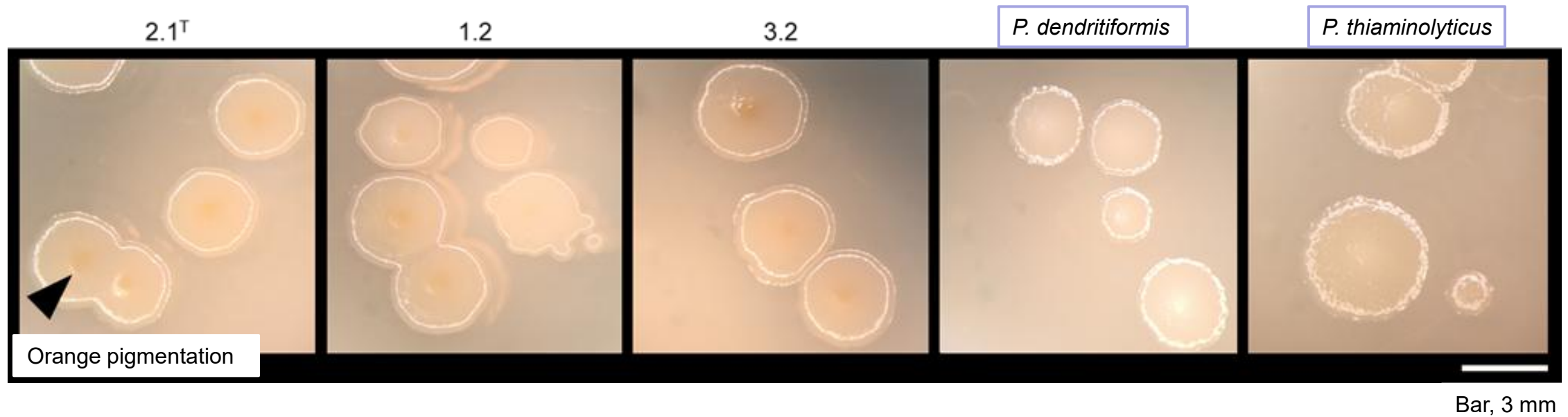




Comparative analysis

2) Macroscopic morphology

- Colony morphology on basal medium





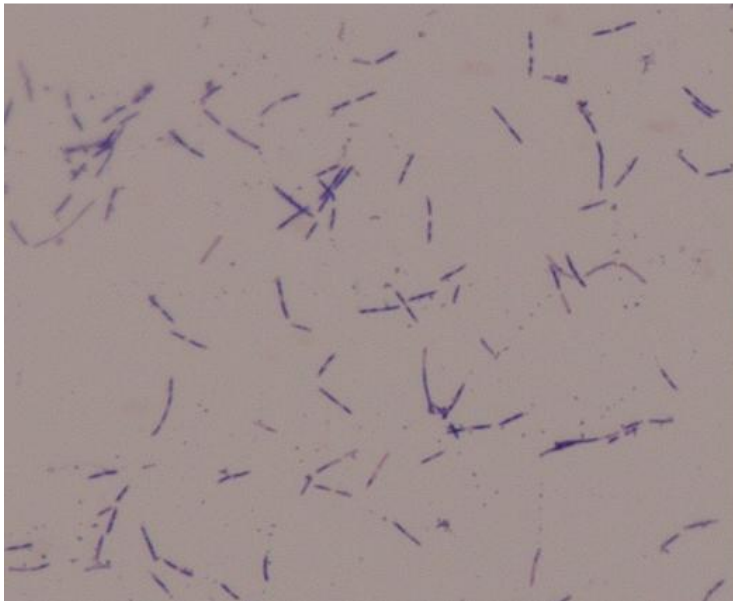
Descriptive analysis

1) Microscopic morphology

- Gram staining

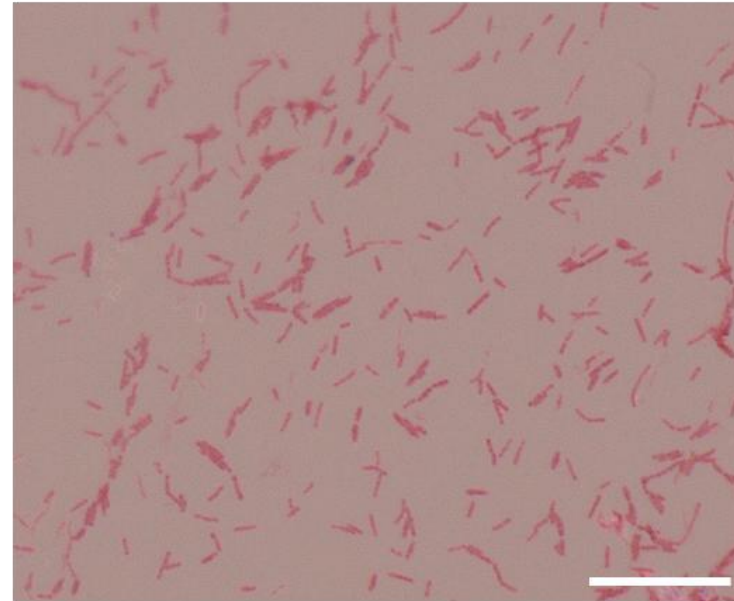
P. melissococcoides sp. nov. type strain 2.1^T

18-h-old culture



Blue/violet = Gram positive

36-h-old culture



Pink = Gram negative

Bar, 20 µm

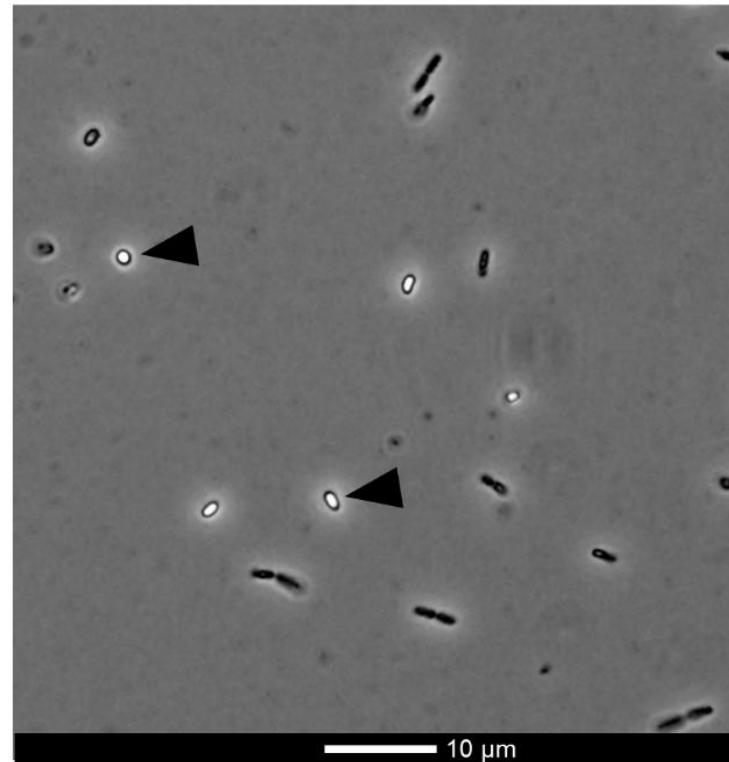
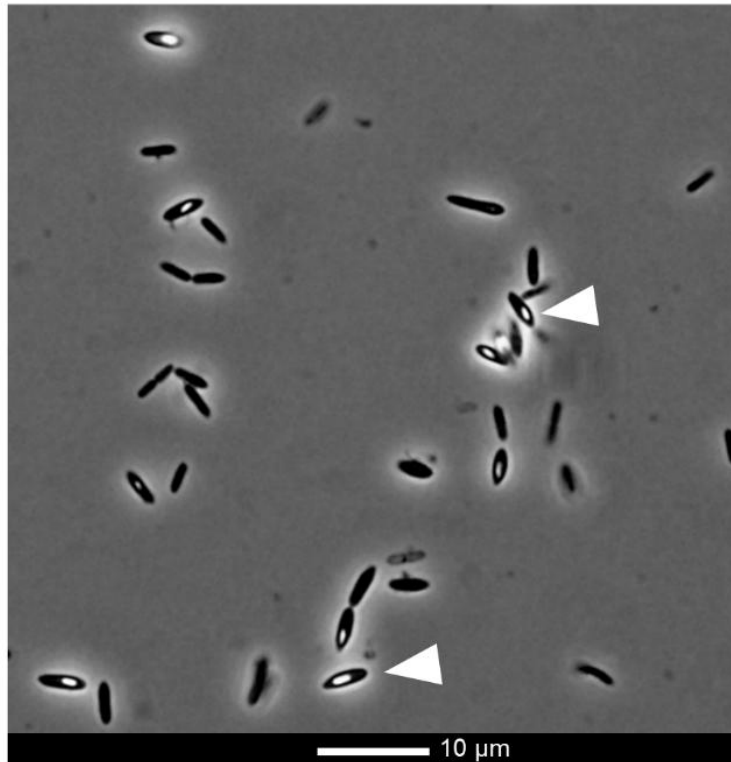
Method: Gram staining
Florine Ory



Descriptive analysis

1) Microscopic morphology

- Endospores formation



▷ endospores
▷ spores

Method: Phase-contrast microscopy
Florine Ory