



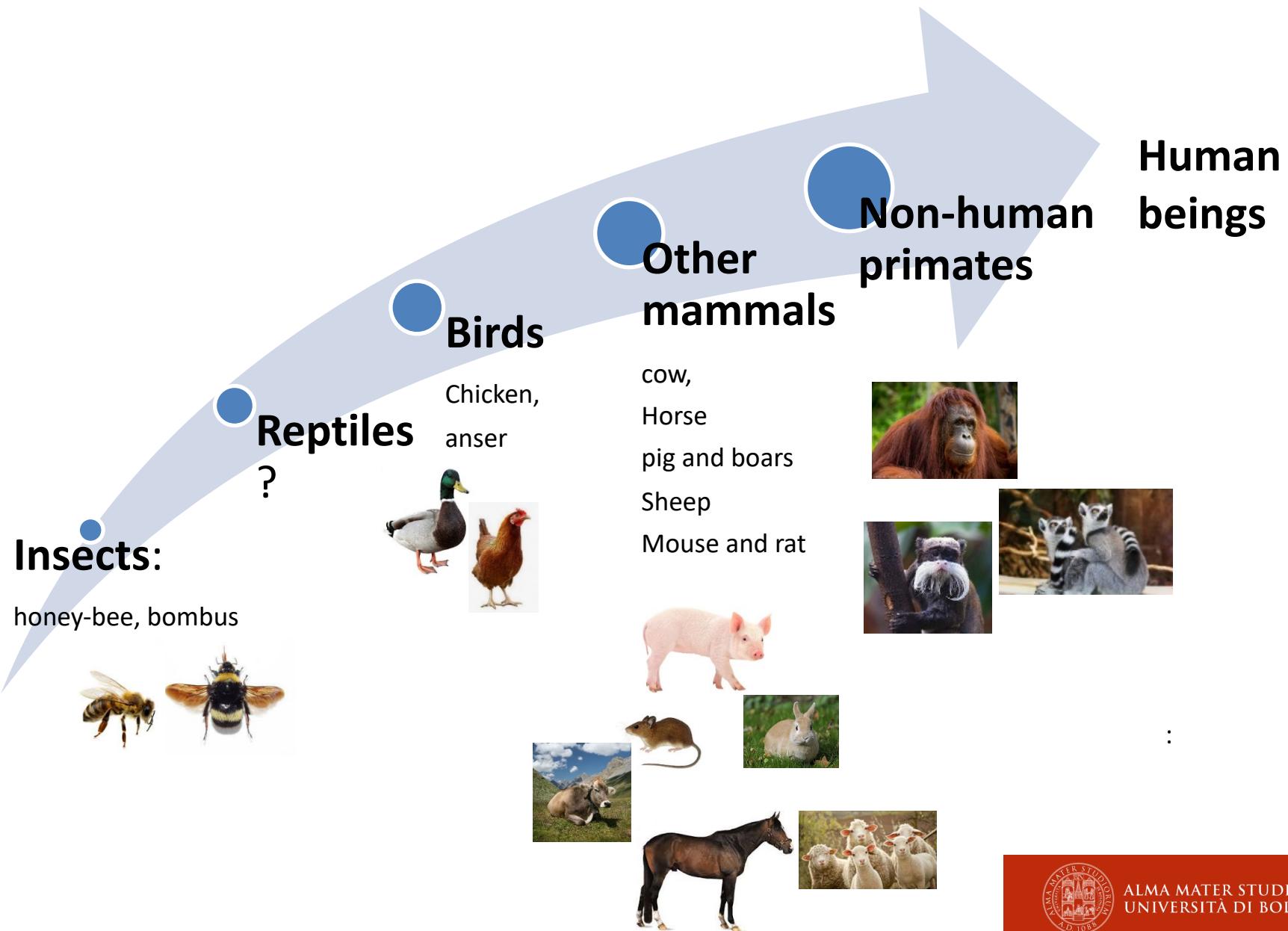
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UNIVERSITÀ DI BOLOGNA

NON-HUMAN PRIMATES BIFIDOBACTERIACEAE: A RESERVOIR FOR APPLICATION FOR ANIMAL WELL-BEING

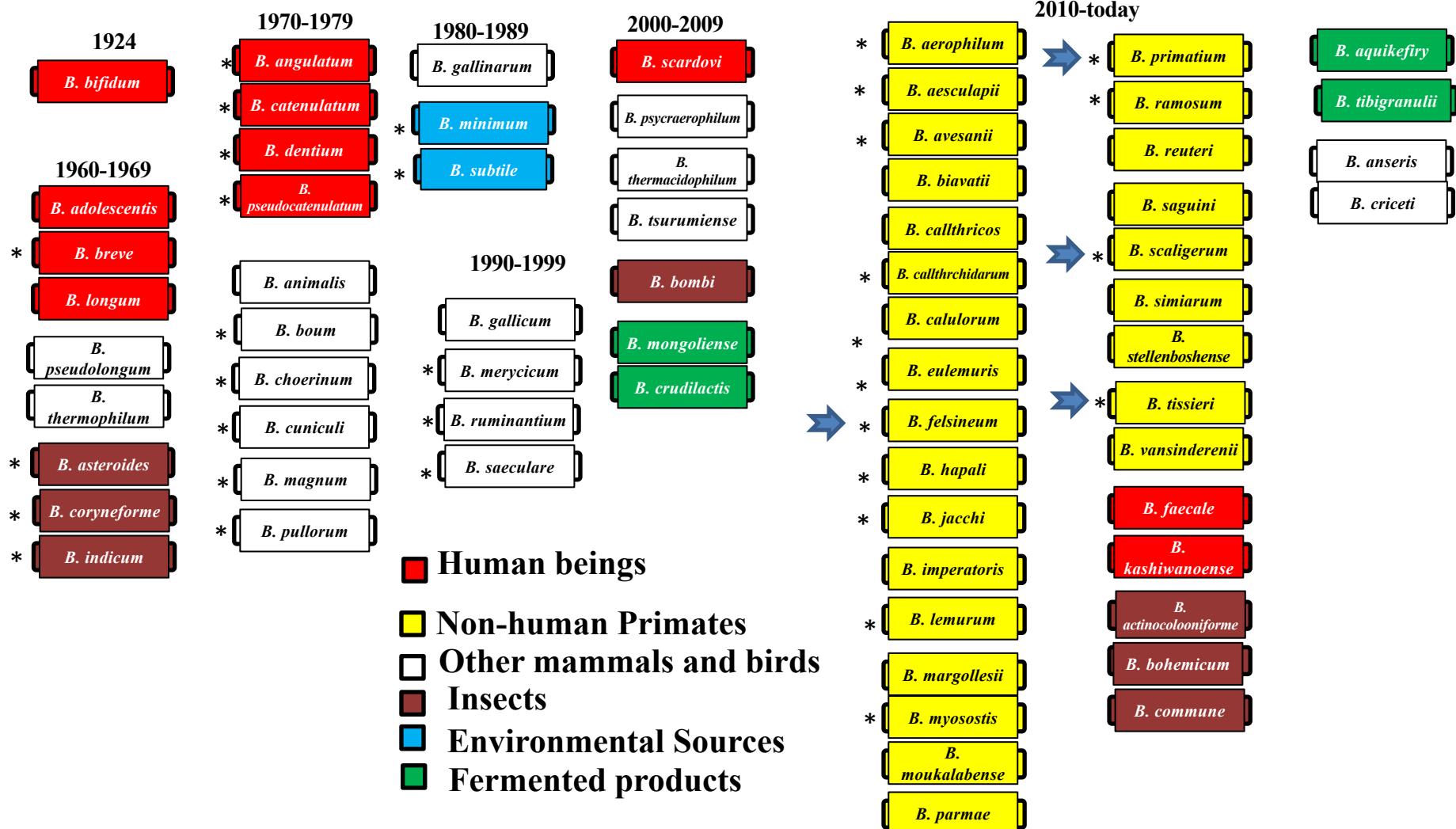
**Modesto M., Sciavilla P., Scarafìle
D., Evangelisti B., Spiezio C.,
Sandri C., Mattarelli P.**

Department of Agricultural and Food
Sciences, University of Bologna, Italy

Bifidobacteria occurrence in different body animals



76 bifidobacterial species updated at June 2019





Systematic and Applied Microbiology

Available online 23 August 2018

In Press, Corrected Proof



Bifidobacterium primatum sp. nov., *Bifidobacterium scaligerum* sp. nov., *Bifidobacterium felsineum* sp. nov. and *Bifidobacterium simiarum* sp. nov.: Four novel taxa isolated from the faeces of the cotton top tamarin (*Saguinus oedipus*) and the emperor tamarin (*Saguinus imperator*)



Monica Modesto ^a, Edoardo Puglisi ^b, Andrea Bonetti ^a, Samanta Michelini ^{a, c}, Caterina Spiezio ^d, Camillo Sandri ^d, Barbara Sgorbati ^{a, e}, Lorenzo Morelli ^b, Paola Mattarelli ^a

Bifidobacteria occurrence in different non-human primates



Lemur catta: *B. lemurum*



Eulemur macaco:

B. eulemuris



Cotton top tamarin:

Saguinus oedipus

B. felsineum

B. primatum

B. scaligerum

B. simiae

B. vansinderenii

B. aerophilum

B. ramosum

B. avesanii



Red-handed tamarin

Saguinus midas

B. biavatii *

*B. saguini**

*B. stellenboschense**



Common marmoset

Callithrix jacchus

*B. callitrichicos**

: *B. reuteri**

B. myosotis; *B. tissieri*

B. hapali; *B. aesculapii*

B. catulorum; *B. callitrichicos*



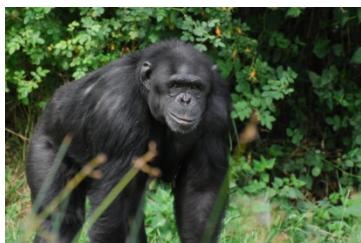
Gelada

Alloscardovia theropiteci



Gorilla

B. moukalabense



Chimpanzee

B. dentium

B. angulatum

B. catenulatum

B. pseudocatenulatum



Orangutang

B. adolescentis

NO successfully isolation from

- Barbary macaque
- Saki
- *Hapalemur alaotrensis*
- *Chlorocebo aethiops*



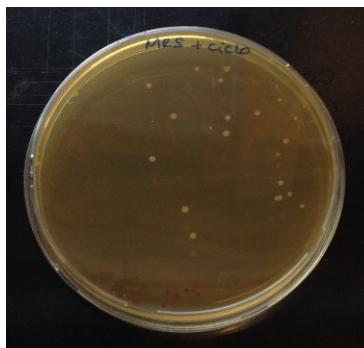
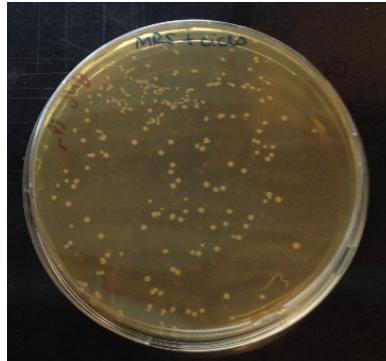
*ENDO et al. SAM, 2012, 35: 92-97



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Bifidobacterial isolation

MRS medium



Selective agents

mupirocin (100 mg/l)

mupirocin (50 mg/l)
acetic acid (1 ml/l)

mupirocin (50 mg/l)
acetic acid (1 ml/l)
8HQ (90 mg/l)

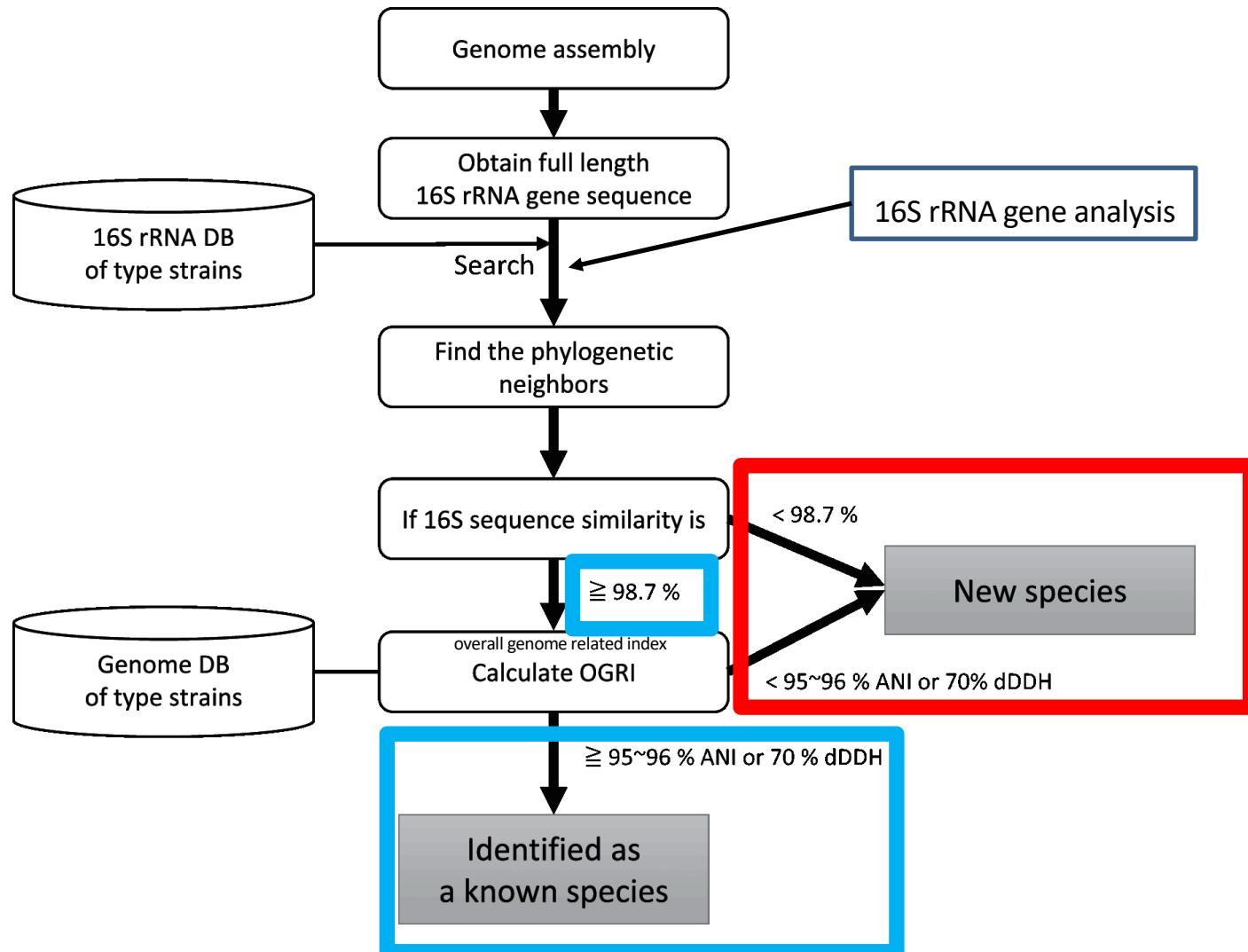
mupirocin (50 mg/l)
8HQ (70 mg/l)
amfotericin B (1 mg/l)

mupirocin (50 mg/l)
acetic acid (1 ml/l)
norfloxacin (200 mg/l)



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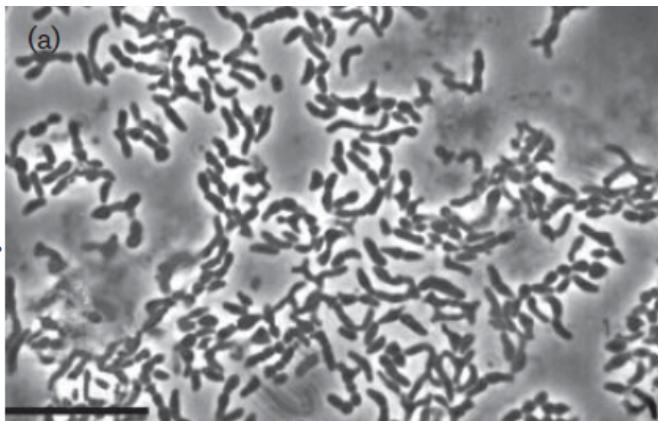
16S rRNA sequence of representatives of groups



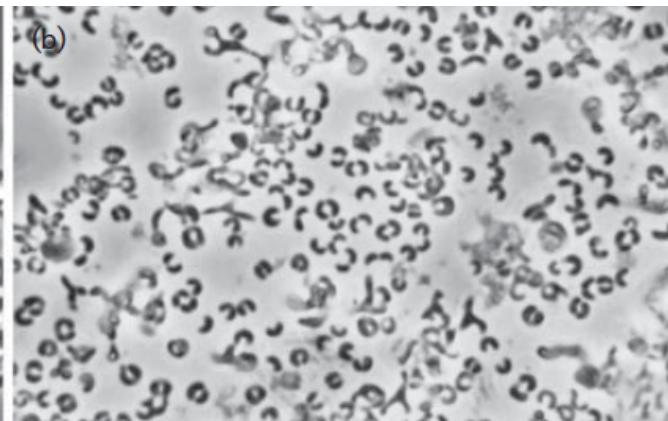
Chun J, Oren A, Ventosa A, Christensen H, Arahal DR, da Costa MS, Rooney AP, Yi H, Xu XW, De Meyer S, Trujillo ME..Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol. 2018 Jan;68(1):461-466

16S rRNA > 98.7%

Species type strain	16S r RNA	ANI	isDDH	type strain closest neighbour
<i>B. eulemuris</i>	99,80%	92,24%	51,60%	<i>B. lemurum</i>
<i>B. catenulatum</i>	99,1%	91,72%	47,30%	<i>B. pseudocatenulatum</i>
<i>B. thermophilum</i>	98,8%	85,48%	33%	<i>B. thermacidophilum</i>
<i>B. saeculare</i>	99,3%	96,76%	37,50%	<i>B. pullorum</i>
<i>B. pullorum</i>	99,7%	88,65%	38,10%	<i>B. gallinarum</i>



B. eulemuris



B. lemurum

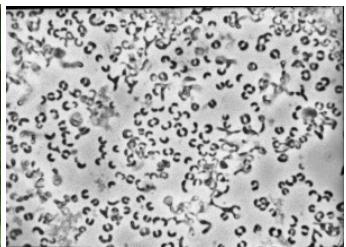


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New bifidobacterial species in primates described by University of Bologna (2014-2019)

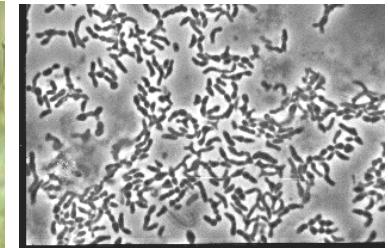
Old World Monkey

Lemur catta



B. lemurum
LMM 3T

Eulemur macaco



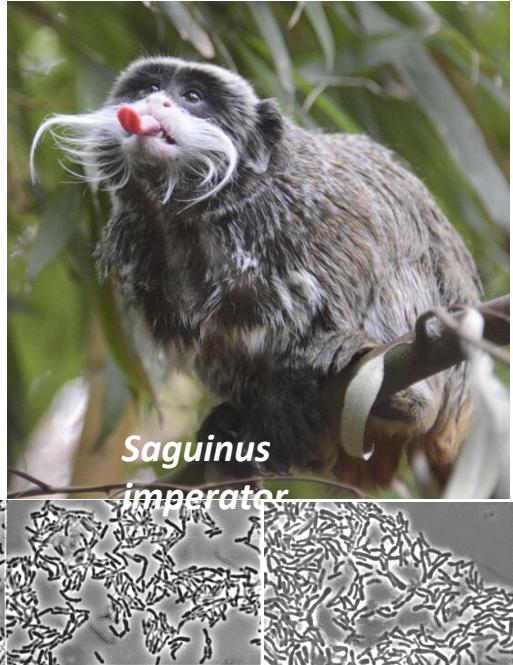
B. eulemuris
LMM E3



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New bifidobacterial species in primates described by University of Bologna (2014-2019)

New World Monkey



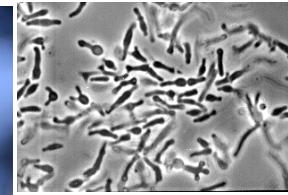
B. callitrichidarum
TRI 5

B. simiarum
TRI 7

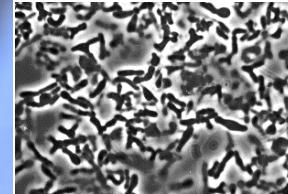


B. ramosum
TRE 1

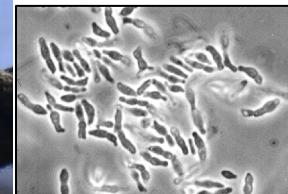
B. avesanii
TRE C



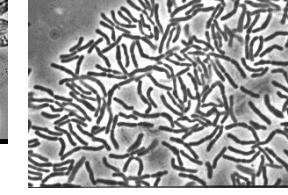
B. primatum TRE 1



B. scaligerum TRE D



B. aerophilum TRE



B. felsineum TRE H

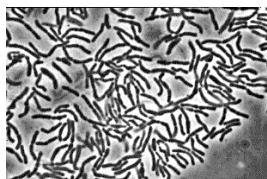
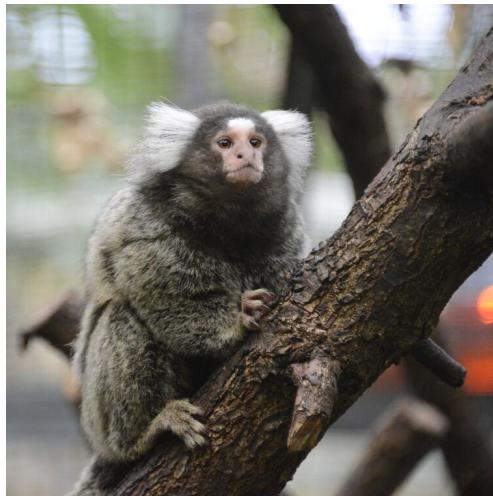


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New bifidobacterial species in primates described by University of Bologna (2014-2019)

New World Monkey

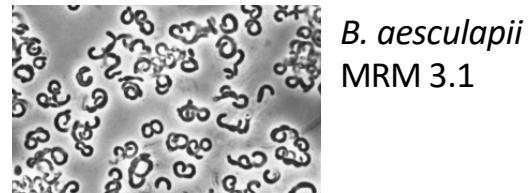
Callithrix jacchus (adult)



*B.
catulorum*
MRM 8.19

MARMOSET

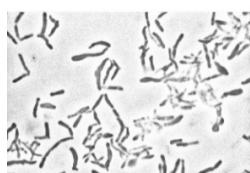
Callithrix jacchus (baby)



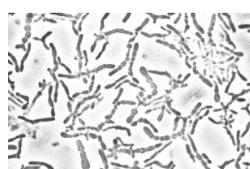
*B.
aesculapii*
MRM 3.1



*B.
myosotis*
MRM 5.9



*B.
tissieri*
MRM 5.18



*B.
hapali*
MRM 8.14

Diet: 70% guar gum

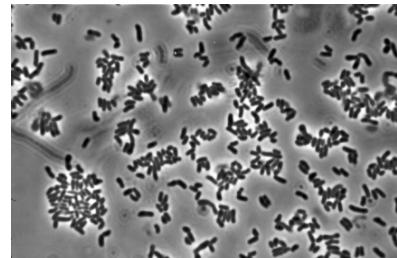


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New bifidobacterial species in primates described by University of Bologna (2014-2019)

New World Monkey

Theropithecus gelada



Alloscardovia theropiteci GLDI 4/2

Diet: only grass

Bifidobacterial species in human beings: ADULTS

1 species

Species	N° samples with a single species	N° of samples with more than one speies	N° of isolates
<i>B. pseudocatenulatum</i>	12		134
<i>B. longum</i>	8		59
<i>B. adolescentis</i>	9		68
<i>B. catenulatum</i>		4	53
<i>B. adolescentis</i>		4	20
<i>B. bifidum</i>			12
<i>B. adolescentis</i>		7	35
<i>B. longum</i>			33
<i>B. pseudocatenulatum</i>		2	15
<i>B. adolescentis</i>			5
<i>B. pseudocatenulatum</i>			9
<i>B. longum</i>			6
<i>B. adolescentis</i>			7
<i>B. catenulatum</i>		1	8
<i>B. pseudocatenulatum</i>			4
<i>B. adolescentis</i>			7
<i>B. catenulatum</i>		1	8
<i>B. longum</i>			1
<i>B. bifidum</i>			1
<i>B. longum</i>		2	11
<i>B. adolescentis</i>			6
<i>B. catenulatum</i>			3
Total	Samples 52		Isolates 516

2 species

3 species

Bifidobacterial species in humans: INFANTS

1 species

Species	Nº of samples	Nº of isolates
1 species	80	
<i>B. longum</i> subsp. <i>infantis</i>	26	123
<i>B. breve</i>	17	137
<i>B. Longum</i> subsp. <i>longum</i>	17	87
<i>B. bifidum</i>	7	38
<i>B. pseuocatenulatum</i>	7	41
<i>B. dentium</i>	3	11
<i>B. catenulatum</i>	3	14
Combiantion of :		
2 species	51	308
3 species	12	12
4 species	4	4
Absence of bifidobacteria	46	
Total	Samples 193	Isolates 775

2 species

3 species

4 species



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Bifidobacterial species in non-human primates

Species	Lemurs		New World Monkeys		
	<i>Lemur catta</i>	<i>Eulemur macaco</i>	<i>Callithrix jacchus</i>	<i>Saguinus oedipus</i>	<i>Saguinus imperator</i>
	ring-tailed lemur	black lemur	8 species common marmoset	top-cotton tamarin	emperor tamarin
<i>B. callithricos</i> (Endo et al, 2012)			26 (30.4%)		1 (5.3%)
<i>B. reuteri</i> (Endo et al, 2012)			12 (28.2%)		
<i>B. aesculapii</i>			28 (30.4%)		
<i>B. myosotis</i>			11 (11.7%)		
<i>B. tissieri</i>			8 (8.6%)		
<i>B. hapali</i>			5 (5.2%)		2 (10.5%)
<i>B. catulorum</i>			1 (1%)		
MRM_9.3 (sp. nov. to be described)			1 (1%)		
<i>B. aerophilum</i> (Cluster I)				4 (9.1%)	1 (5.3%)
<i>B. avesani</i> (Cluster II)				1 (2.3%)	
<i>B. ramosum</i> (Cluster III)				1 (2.3%)	3 (15.8%)
<i>B. primatium</i> (Cluster IV)				19 (43.2%)	
<i>B. callithricos</i> (Cluster V) (Endo et al., 2012)				6 (13.6%)	
<i>B. felsineum</i> (Cluster VI)				1 (2.3%)	
<i>B. simiarum</i> (Cluster VII)				6 (13.6%)	
<i>B. vansinderenii</i> (cluster VIII) (Duranti et al, 2017)				4 (9.1%)	2 (10.5%)
<i>B. scalarigerum</i> (Cluster IX)				1 (2.3%)	
<i>B. myosotis</i> (Cluster X)				1 (2.3%)	
<i>B. callithrichidarum</i> (Cluster XI)					5 (26.3%)
Cluster XII					1 (5.3%)
Cluster XIII					1 (5.3%)
Cluster XV					1 (5.3%)
Cluster XVI					1 (5.3%)
Cluster XVII					1 (5.3%)
<i>B. lemurum</i>	4 (100%)	25 (62.6%)			
<i>B. eulemuris</i>		15 (37.5%)			
TOTAL OF ISOLATES (N° 192)	N° 4	N° 40	N° 92	N° 44	19



“Academics taxonomists should recognise that their product
is the **working technical language** of microbiology”

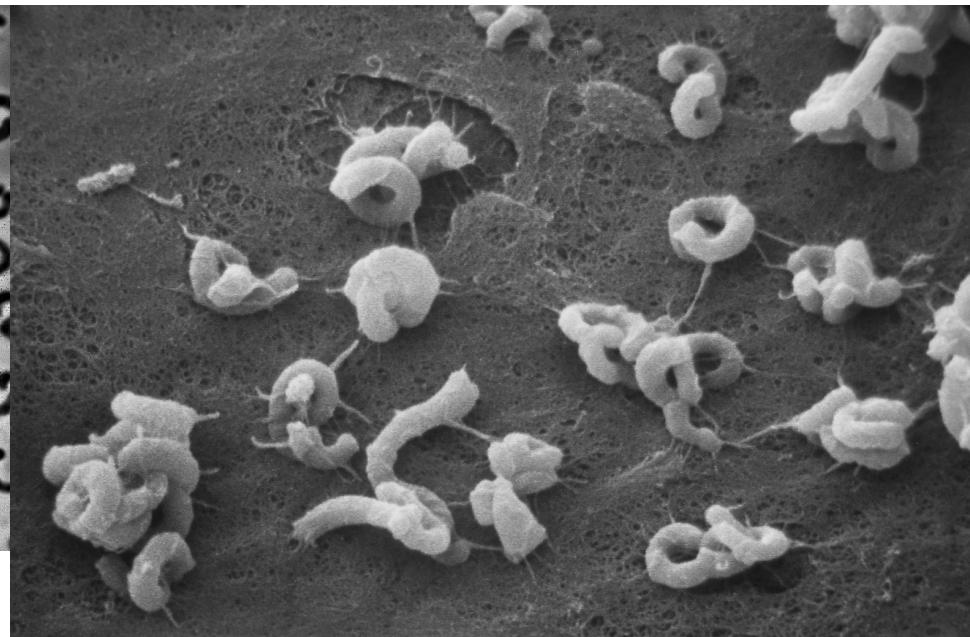
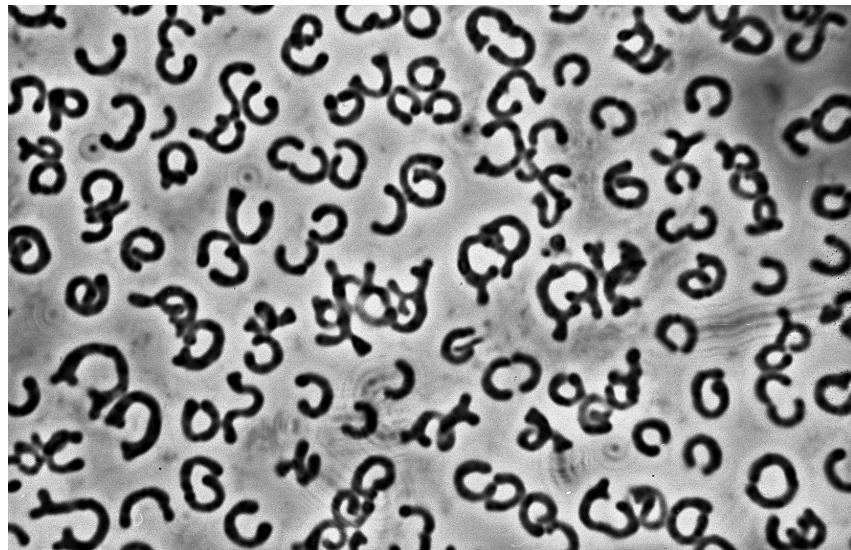
Magee JT (1993) J Med Micro

**Study of the potentiality of utilization of new
bifidobacteria not isolates from humans for application**



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Bifidobacterium aesculapi from baby marmoset



Cell loads and EPS detection in TPY medium

Strain	Cell load (log cfu/mL)	EPS (µg/mL)	Cell load (log cfu/mL)	EPS (µg/mL)	
	Glucose 1.5%	Glucose 1.5%	Glucose 2%	Glucose 2%	
Marmoset strains	MRM_3.1	8.94 ± 0.27 ^A	→ 231.61 ± 5.25 ^A	8.67 ± 0.05 ^A	→ 123.92 ± 10.20 ^A
	MRM_4.2	8.82 ± 0.30 ^A	83.46 ± 2.20 ^B	7.92 ± 0.10 ^B	92.51 ± 8.45 ^B
	MRM_4.6	8.15 ± 0.14 ^B	→ 196.48 ± 8.50 ^C	7.85 ± 0.09 ^B	→ 127.67 ± 6.34 ^A
	MRM_4.7	8.55 ± 0.42	→ 162.88 ± 7.35 ^D	7.68 ± 0.13 ^B	→ 135.89 ± 3.45 ^A
	MRM_4.8	8.34 ± 0.15 ^B	114.01 ± 7.28 ^E	8.47 ± 0.16 ^A	218.99 ± 8.12 ^C
	MRM_5.13	8.72 ± 0.21 ^A	44.52 ± 6.32 ^F	8.18 ± 0.86 ^A	41.82 ± 2.10 ^D
	MRM 8.7	8.32 ± 1.46 ^B	→ 106.75 ± 8.34 ^E	7.52 ± 0.86 ^{B,C}	→ 102.10 ± 5.23 ^B
	RE06	9.88 ± 1.46 ^C	7.53 ± 2.50 ^G	7.52 ± 0.15 ^{B,C}	15.45 ± 3.10 ^E
	DSM 23967	8.92 ± 0.42 ^A	12.88 ± 3.00 ^G	7.90 ± 0.23 ^B	–

For each column considered, values with the same superscript letter are not statistically different ($P > 0.05$).

^a Under the detection limit.





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Technological potential of *Bifidobacterium aesculapii* strains for fermented soymilk production



F. Patrignani^{a,*}, M. Modesto^b, Samanta Michelini^b, Maria Cristina Sansosti^b, Diana I. Serrazanetti^a, Linnea Qvirist^c, Lorenzo Siroli^a, Lucia Camprini^a, Paola Mattarelli^b, Rosalba Lanciotti^a

^a Department of Agricultural and Food Sciences, Alma Mater Studiorum, University of Bologna, Campus of Food Science, Piazza Goidanich 60, 47521 Cesena, Italy

^b Department of Agricultural Sciences, Alma Mater Studiorum, University of Bologna, viale Fanin 42, 40127, Bologna Italy

^c Department of Biology and Biological Engineering, Chalmers University of Technology, S-41296 Gothenburg, Sweden



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***B. aesculapii* strains**

- grew very well in soymilk, producing considerable amounts of EPS, and resulting in high product viscosity and firmness values.
- according to the data of the panel test, the fermented milk obtained from MRM 4.6 obtained also the highest scores for general acceptance.
- the performances were comparable with those reported by the literature for the industrial *Bifidobacterium* strains
- these results are very promising and useful for the further scaling-up of the process to obtain function fermented soymilk.

New bifidobacteria as probiotics

Should we follow the classic criteria of strain origin?

Conclusion

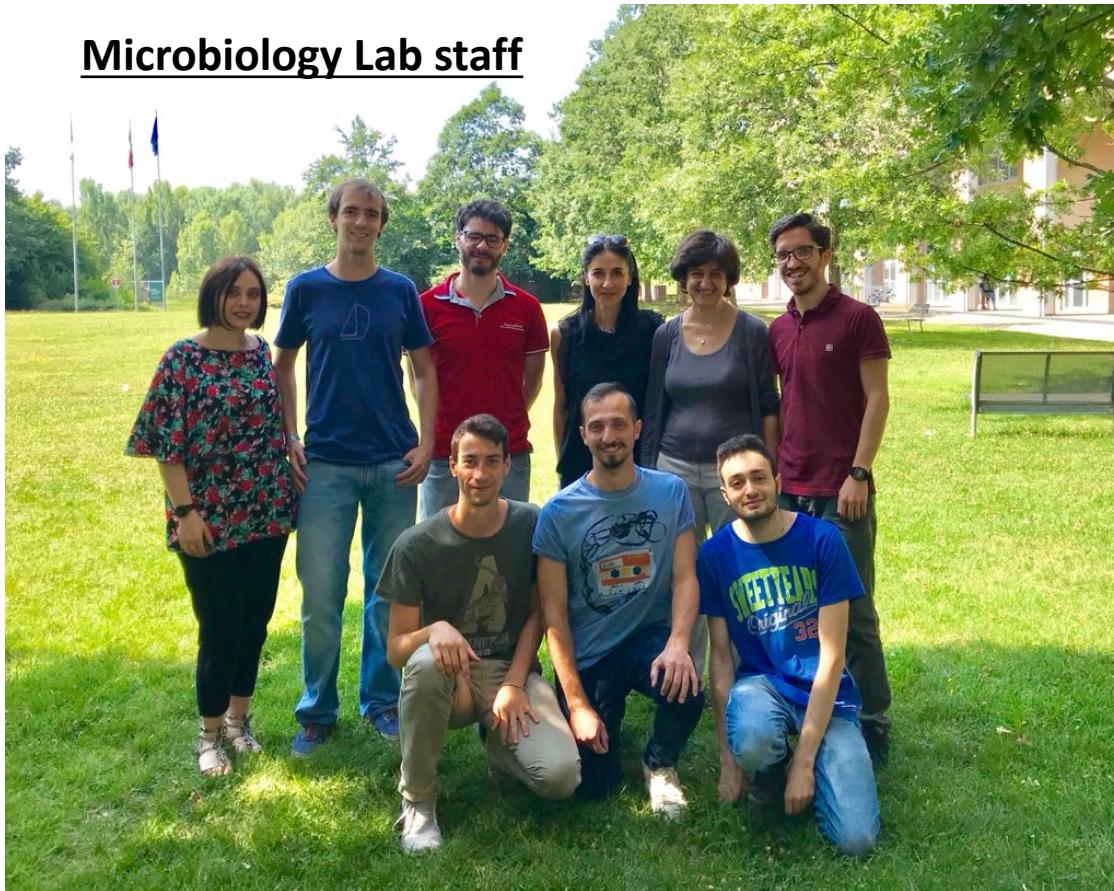
Non-human primates *Bifidobacteriaceae* could be a reservoir for application for animal and human well-being

The exploitation of probiotic properties of strains isolated from non-human primates requires the existence of isolates

The storage in culture collections, which assure the maintenance with appropriate methodologies of bifidobacterial cultures for intended health and biotechnological uses, are FUNDAMENTAL

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Microbiology Lab staff



**Barbara Sgorbati
Rosalba Lanciotti
Francesca Patrignani**



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Thank you