



Mikrobiom-Forschung für Qualität und Sicherheit von Milchprodukten



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A bit about FFoQSI



Austrian Competence Center for Feed and Food Quality, Safety and Innovation

Founded in 2017, 66 employees, located in Tulln

One of 21 K1 Centers in Austria across all sciences

Excellent collaborative research between scientific partners and company partners (COMET program managed by FFG)

Funding for eight years (after 4 years interim evaluation)





FFoqQSIs areas of performance





What is microbiomics?





Microbiome: all microorganism existing in an environment (niche)

Very few species (60 in the stomach of a vulture) to ~100.000 (in 1g of soil)

Has brought inspiring new insights in infectious disease research but also in biotechnology





Why groundbreaking: the cultivation bias



Manipulate microbial composition

Which factors influence the Interaction of microbes with food?

Technological factors

vetmedun

Implicit factors

FFoQS

INNOV

Intrinsic factors pH Value A Value Ingredients

Extrinsic factors

. . .

Temperature Humidity



Result is expressed as the number of reads instead of CFU (KBE)



Food microbiome-research at Vetmeduni



RESOURCE - November 11, 1918 20, 195775-5785 E15, Clobins E3, 2024 - Open Access d. Download Full Issue

Unexplored microbial diversity from 2,500 food metagenomes and links with the human microbiome

Necesia Garino 1. Adua Blanco-Migues 1. Martal Pundochi 1. Gauda Mengeni 1. Federica Pinto 1. Alexan Tatt 43.4 Pindo Mangai 1. Federica Amartini 1. Michele Avegrano 1. Core Branceta Ber Filipis 111. Hotargo 2011. Radi Cataren-Fonto 114 Intel Cataren 17. Martal Costello 1. Dominio Childri 1. Narcise M. Guida 107.111. Catalos State 111. Siguras Skilanes 11. Stephen Hotarbon 1. Dominio Childri 1. Narcise M. Guida 107.111. Catalos State 111. Siguras Skilanes 11. Stephen Hotarbon 1. Dominio Childri 1. Narcise M. Guida 107.111. Catalos State 111. Siguras Skilanes 11. Stephen 1. Dominio Childri 1. Narcise M. Guida 107.111. Catalos State 111. Siguras Skilanes 11. Stephen 1. John 1. Analistica 1. Analistica 1. Analistica 1. Analistica 1. Analistica 1. Francesco Aerosa 1. Stephen 1. Analistica 1. Vitor Heidrich 1. Abelando Murgolles 10.111. Vigo Thir Martenason 1418. Omar Rota States 1. 11. Marten Vagne 11.17. Danio Encelini 1.12.17. Paulo Encelini 1.12.17. Paulo Cotter 7.831.94. Nicola Segura 3.12.17.17.19.19. St Educato Paula 1.12.19. Store Marten 1.

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Highlights

- With curatedFoodMetagenomicData, we integrated and analyzed >2,500 food metagenomes

- Over 10.000 prokaryotic and eukaryotic MAGs snoover substantial lood microbial diversity

- Food microbes account for up to an average of 3% of the adult gut microbiome

- Ettain-level analysis highlights potential instances of food-to-gut microbe transmission

nature microbiology

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nature > nature microbiology > articles > article

Article Open access Published: 12 January 2023

Differential carbon utilization enables co-existence of recently speciated Campylobacteraceae in the cow rumen epithelial microbiome

Cameron R. Strachan, Xiaogian A. Yu, Viktoria Neubauer, Anna J. Mueller, Martin Wagner, Oendrim Zebelj, Evelyne Selberherr ⁽²⁾ & Martin F. Polz ⁽²⁾

Nature Microbiology 8, 309-320 (2023) Cite this article

5061 Accesses | 4 Citations | 36 Altmetric | Metrics



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Figures (14)

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Article | Chim access | Fuhlished: 02 August 2024

Microbiome mapping in dairy industry reveals new species and genes for probiotic and bioprotective activities

Francesca De Filippis, Vincanzo Valentino, Min Yap, Baul Caberra-Rubio, Coral Barcentila, Nocoto Carlino, José F. Cobo-Diaz, Narciso Martin Quijada, Inés Caberra-Torre, Patricia Rum-Madiedo, Carlos Sabater, Giurepoine Seguino, Edoardo, Pasoli, Martin Wagner, Abriardo Margolles, Nicola Seguita, Avriino Abares: Ordóbez, Paul D., Center & Danilo Ecolini ⁵⁵

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natureprotocols

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nature > nature protocols > protocols > article

Protocol | Published; 24 January 2024

Improved sampling and DNA extraction procedures for microbiome analysis in food-processing environments

Coral Battenilla, José F. Cobo-Diaz, Francesca De Filippis, Vincenzo Valentino, Baul Cabiera Bubio, Dominic Q'Neit, Lisa Mahler de Sanchez, Federica Amanini, Niccolò Catlino, Altor Blanco-Miguez, Federica Pinto, Inés Calvete-Tome, Carlos Sabater, Susana Delgado, Patricia Buas-Madiedo, Narciso M. Quijada, Monika Doleciol, Sigurfaug Skirnisódite, Stephen Knobloch, Alta Puente, Mercedes López, Miquel Prieto, Vigoó Thór Marteinsson: Martin Wagner, Abelardo Margolles, Nicola Segata, Poul D. Cotter, Danilo Ercolini & Avelino Abunoz. Ordóñez Ello — Steve Isome autors

Nature Protocols 19, 1291–1310 (2024) | Cite this article 2255 Accesses | 4 Citations | 29 Altmetric | Metrics



Some main conclusions



cheese plants; 4 European regions (Italy, Ireland, Austria, Spain; n=1250 samples; five niches (FC, NFC, final product, operator, milk associated samples)

Hard cheeses richer microbiome than soft cheeses

Brine has the highest microbial diversity, least in whey

Cheeses at the end of ripening richer microbiome than at the beginning

FPE higher diversity than the product, and a higher frequency of bacteriocins

 Implified biofilms and microbiomes

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 Published: 02 August 2034

 Microbiome mapping in dairy industry reveals new species and genes for probiotic and bioprotective activities

 Francesca De Filippis, Vincenze Valentino, Min Yap, Baul Cabieta-Bubis, Coral Barcenila, Niccolo Catino, José F Cobo-Diaz, Marciso Martin Dujurda, Irele Calvete-Torre, Patricia Buas-Madieda, Carlos Sabatet, Guisteppina Sequino, Edoardo Pasoli, Martin Wagmer, Abelardo Margolles, Niccola Segata, Avelina Alvarez: Octóber, Baul D, Cotter & Davido Escolini ©

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Who is there in dairy?

239 species (0.01% abundance) found, top three L. lactis, Streotococcus thermophilus and L. cremosis

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Microbiome mapping in dairy industry reveals new species and genes for probiotic and bioprotective activities

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Lactococcus lactis Streptococcus thermophilus Lactococcus cremoris Staphylococcus equorum Brevibacterium aurantiacum Acinetobacter johnsonii Lactobacillus helveticus GGB15717 SGB23955 Lactobacillus delbrueckii Leuconostoc pseudomesenteroides Chromohalobacter japonicus Kocuria salsicia Brachybacterium alimentarium Pseudomonas fluorescens Lactiplantibacillus plantarum Leuconostoc mesenteroides Corynebacterium variabile Kocuria carniphila Pseudomonas fragi Brevibacterium SGB93100 Corvnebacterium casei Moraxella osloensis GGB48227 SGB66234 Acinetobacter guillouiae Lactococcus laudensis Acinetobacter albensis GGB42949 SGB60172 Rothia kristinae Lactococcus raffinolactis Brevibacterium sp 239c

	9.11	4.07	15.07	29.07	15.71	
	11.5	2.87	16.05	26.94	5.61	
-	5.79	3.71	13.58	17.61	15.55	
	6.3	7.37	0.33	1.96	7.01	
_	4.83	4.08	0.15	1.93	2.72	
-	4.45	2.69	1.1	0.04	1.25	
_	0.49	0.17	2.61	1.95	0.6	
-	1.08	3.73	0.75	0.05	4.2	
-	0.6	0.19	1.45	1.57	0.08	
-	0.28	0.17	0.34	1.79	0.79	Deletive
_	0.29	0.23	4.9	0.14	0.01	Relative
-	2.2	1.34	0.29	0	1.68	Abundance
-	1.33	1.39	0	0.52	0.41	
-	0.85	0.62	2.58	0.08	0.32	
-	0.18	0.12	0.04	1.41	0.05	- 1
-	0.38	0.25	0.35	0.92	0.24	20
-	0.83	0.99	0.04	0.43	0.76	
-	0.94	1.08	0.19	0.14	2.01	10
-	0.31	0.25	2.38	0.19	0.08	10
_	1.25	0.91	0	0.2	0.26	
-	0.84	0.46	0	0.55	0.27	0
	0.67	1.2	0.81	0	0.42	
-	0.47	0.26	0.15	0.73	0.12	
-	0.94	0.27	1.09	0.04	1.16	
-	0.28	0.28	0.35	0.68	0.99	
-	0.17	0.42	2.41	0.03	0.14	
-	0.76	0.99	0	0.27	0.17	
-	1.62	0.2	0.13	0	0.01	
	0.08	0.05	1.48	0.47	0.04	
-	0.63	0.5	0	0.42	0.19	
	food contact	non food contact	cheese-related mat	final product	operators	











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Anister | Deep Access | Published 12 August 2004

Microbiome mapping in dairy industry reveals new species and genes for probiotic and bioprotective





heese core microbiome vs. Cheese rind



ND FOOD



Brine as a source







New cellar management







Hard cheeses management



How diverse is the MC?



Autochthonous facility-specific microbiota dominates washed-rind Austrian hard cheese surfaces and its production environment

Colorda, Harchol M.^(1,4), Marris, Dialarra⁽¹⁾, Wagnes, Hartin⁷; Rodrigana-Laboro, David¹, Emergiander, Martin⁴;

Scientiz-Exer. Nuclear 11

From where does it come?



Which part of MC can be cultured?

Which part of the MC is metabolically active?

MC assessment as part of QM (ripening management)

Metatranscriptomic Analyses Unravel Dynamic Changes in the Microbial and Metabolic Transcriptional Profiles in Artisanal Austrian Hard-Cheeses During Ripening

Frantiers in Microbiology - Gaus Access - Witamie 13 - 1 March 3522 - Article number 813400

Quisdo, huncho Martin^{4,0} 🖾 : Quistiol, Moniko⁴: Schnitz-Euser, Stepher⁴: Wagner, Martin^{4,5}:

Setherbert, Extinue* 🖼





How diverse is the MC over the ripening period

vetmeduni

vienna





landwirtschaftskammer vorarlberg

The cheese rind communities are cellar-specific



Relative abundance of 16S rRNA operational taxonomic units

lk

vorarlberg

landwirtschaftskammer



Target gene	OTU no. (number of clones)	*RDP, similarity [%], GenBank accession no. **Best Blast Hits (GenBank nr), similarity [%]	Cheese producing facilities		Relative abundance [%] ee ing Ripening cellars Ripening cellar es I young cheeses old cheeses					ellars ies	Relative abundance among all cellars					
		Селданк ассеззол по.		в	c	0 A	4 0-4 1 A2	0-6 B1	0-6 C2	4-18 A3	6-10 B2	6-14 C1	5	10 1	5 20	L
	OTU 1 - (77)	Halomonas boliviensis strain LC1 (98%), AY245449													_	1
	OTU 2 - (67)	Brevibacterium aurantiacum strain NCDO 739 (98%), X76566														
	OTU 3 - (55)	Staphylococcus equorum strain ATCC 43958 (99%), AB009939														L
	OTU 4 - (28)	Brevibacterium pityocampae strain Tp12 (95%), EU484189				Γ						\square				L
	OTU 5 - (23)	Adveneila kashmirensis strain WT001 (99%), AJ864470														L
	OTU 6 - (19)	Sphingobacterium daejeonense strain TR6-04 (96%), AB249372														L
	OTU 7 - (19)	Brachybacterium conglomeratum strain JCM 11608 (98%), AB537169						\square								L
	OTU 8 - (17)	Alkalibacterium kapii strain T22-1-2 (98%), AB294171														L
	OTU 9 - (14)	Psychrobacter aquaticus strain CMS 56 (94%), AJ584833														
	OTU 10 - (12)	Corynebacterium variabile strain DSM 20132 (99%), AJ222815														
1400	OTU 11 - (9)	Yaniella halofolerans strain YIM 70085 (99%), AY228479				Г										
rRNA	OTU 12 - (8)	Marinobacter maritimus strain CK47 (98%), AJ704395														
	OTU 13 - (6)	Leucobacter chromitresistens strain JG 31 (95%), GU390657														
	OTU 14 - (6)	Citricoccus zhacaiensis strain FS24 (97%), EU305672				Γ										
	OTU 15 - (5)	Leucobacter chromiiresistens strain JG 31 (96%), GU390657														
	OTU 16 - (5)	Pusillimonas ginsengisoil strain DCY25T (92%), EF672088														
	OTU 17 - (5)	Brevibacterium aurantiacum strain NCDO 739 (98%), X76566														
	OTU 18 - (4)	Adveneila kashmirensis strain WT001 (99%), AJ864470														
	OTU 19 - (3)	Brevibacterium permense strain VKM Ac-2280 (98%), AY243343											5			L
	OTU 20 - (2)	Leucobacter selsicius strain M1-8 (96%), GQ352403											1			
	OTU 21 - (2)	Atopostipes suicloacalis strain PPC79 (97%), AF445248											1			
	OTU 22 - (2)	Brachybacterium faecium strain DSM 4810T (99%), X83810											þ			
	OTU 23 - (2)	Nesterenkonia alba strain CAAS 252 (95%), EU566871											þ			
	OTU 24 - (2)	Corynebacterium variabile strain DSM 20132 (98%), AJ222815											35 25	15 10	5 1 0	
	OTU 25 - (2)	Brachybacterium alimentarium strain CNRZ 925 (99%), X91031] 10	20 3	0 40	
	OTU 1 - (127)	Scopulariopsis brevicaulis (99%), JN157617.1														
	OTU 2 - (83)	Pyxidiophora arvemensis AFTOL-ID 2197 (98%), FJ176839.1														
	OTU 3 - (59)	Debaryomyces hansenii (99%), AB628063.1												1		
**18S rRNA	OTU 4 - (40)	Nectria mariannaeae (99%), AB099509.1														
	OTU 5 - (8)	Arachnomyces glareosus isolate CBS 116129 (99%), FJ358341.1														
	OTU 6 - (4)	Acerus immobilis UMMZ BMOC 00-1103-002 AD458 (99%), JQ000104.1														
	OTU 7 - (1)	Starmerel/a sp. 2-1361 (99%), JX515985.1											90 70	50 30 1	050	

Schorhsteiner et al., IJFM 2014



Both bacteria and fungi matter!



Quijada et al., Foods 2018

(b)

vetmeduni vienna





Long-ripenig cellar

Short-ripenig cellar

lk

Species	Short ripening	Long ripening				
Halomonas	Racks, Wall, Air filter, wall	Racks, Wall, Air filter, floor				
Brevibacterium	Shelves, Racks	Racks				
Staphylococcus	phylococcus Shelves					
Psychrobacter	Shelves	Floor, Air filter				
Oceanisphaera	Ceanisphaera Wall					
Pseudo- alteromonas	Air filter	Floor				
Pseudomonas	-	Shelves, Racks,Wall				

Quijada et al., IJFM, 2018









Comparative analysis of culturable versus the non-culturable part of the MC

		Rel. abundance in %; Threshold > <u>5% (molecular)</u>	Rel. abundance in %; Threshold >5% (n=230 isolates)
OTU1	Halomonas boliviensis	19	
OTU2	Brevibacterium spp.	25	30
OTU3	Staphylococcus equorum	14	19
OTU5	Advenella kashmiriensis	7	<5%
OTU6	Sphingobacterium daejeonense	6	<5%
OTU7	Brachybacterium konglomeratum	>5%	10
OTU8	Alkalibacterium kapii	>5%	<5%
OTU10	Corynebacterium spp.	6	8
OTU14	Citricococcus alkalitolerans	<5%	7
	Microbacterium gubensee		6





A modified culture medium for Gram negative rod Halomonas boliviensis (*spp.*)



Modified Halomonas Agar according to Dzieciol

Crystal formation (calcite, magnesian calcite, aragonite, dolomite, monohydrocalcite, hydromagnesite und struvite, was described before, Rivadeneyra et al., 2006)







Diversity

Changes over time

Culture collection

Which species is doing what when?



Which part of the MC is metabolically vermed vier



Quijada et al., Front. Microbiol. 2022



Sequencing of most abundant species: example Brevibacterium

Steady increase of Brevibacterium over ripening time (measured by qPCR)

Three isolates sequenced: one B. auriaticum, two isolates new species in the genus Brevibacterium

Highly detectable by Day_0 , raw milk origin?

Highly proteolytic and lipolytic (gene cluster duplications)

L261, S22 and S111 encode a **putative Linocin-M18 bacteriocin** (active against Listeria)

L261, S22 and S111 habour a putative **histamine** catabolism pathway and

a novel plasmid for adaptation to the cheese environment.









In-House Ripening cultures

No commercial ripening cultures allowed in PDO cheese, but.....

Benefits:

Controlled ripening Treatment of cheese defects Faster ripening

Less food waste Economic benefit Culture mix of main ripening drivers

Smear preparation is pivotal

10 Test wheels: 10⁷ KBE cultures mix plus smear 10 Control wheels: only smear

Smearing 1x/week



Picturecredits: Biorender, Moritz Hartmann





Ripening trials



Sensory with 6 months

Test-Wheels:



- rind more intensive brown colour (10/10)
- riper taste (8/10)
- firmer consistency (9/10)
- no rind or core defects

Control-Wheels:

- impure taste (2/10)
- no rind defects

Final evaluation by sensory panel with 12 months

Sampling of rind and core for microbial comparison along ripening time

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Microbiome gamechanger

Impact on microbial safety: MC decide on +Survival in the processing environment +Transmission patterns (Biofilm) +Growth of pathogens in food

Impact on product stability and quality:

+shelflife

+spoilage and other defects

Impact on technology: supports particular microbial processes by tailored solutions +stabile and efficient ripening microbiome

Impact on nutrional and health aspects : supports choice and physiological properties +breakdown of macromolcules +transfer through gastroenteral barrier +flavour and taste (consumer acceptance)





ΓШΓ



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Credits

ne



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Many more that ...,