SYNGULON
Microbial fermentation technologies for bio-based products

Synthetic biology and formulation of antimicrobial peptides

GREEN WIN

Bio Biotechnology Industry Organization

BIOECONOMY FOR CHANGE

October 16, 2024

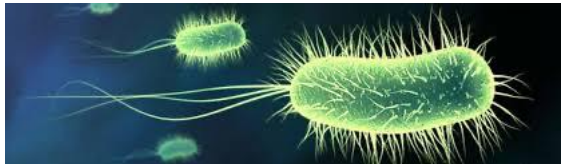
Dr Philippe Gabant, CSO
pgabant@syngulon.com

Structure of the talk

1. What is synthetic biology?
2. Importance of microbes for life on our planet
3. Need of microbial control
4. Antimicrobial peptides (Bacteriocins)
5. PARAGEN Collection
6. Cocktails Formulation
7. Conclusions

What is synthetic biology?

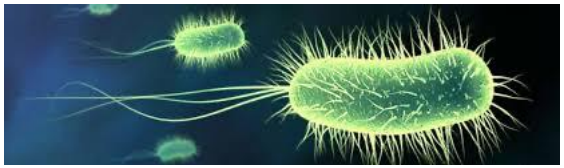
Life-Biology



Industries

What is synthetic biology?

Life-Biology



	Second letter: U	C	A	G	
First letter: U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	GAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

Third letter: U C A G

Industries

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1101010101010100101000010
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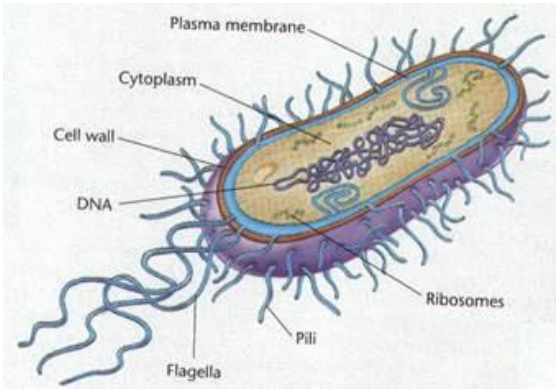
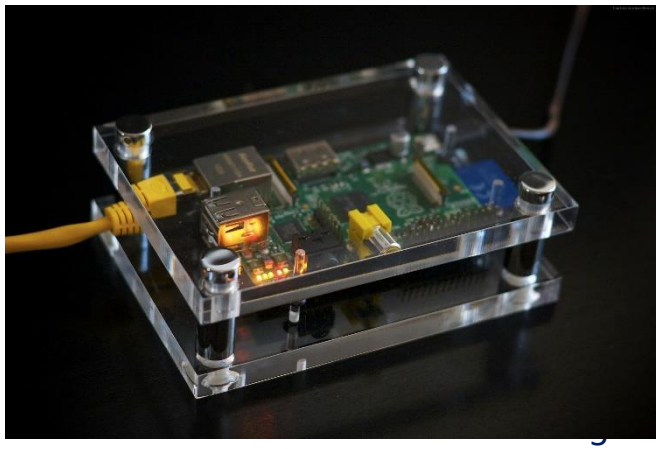
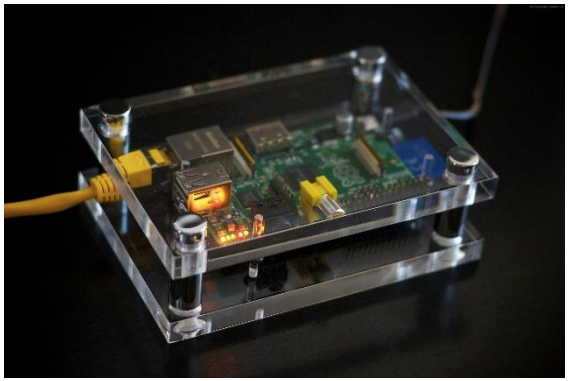
What is synthetic biology?

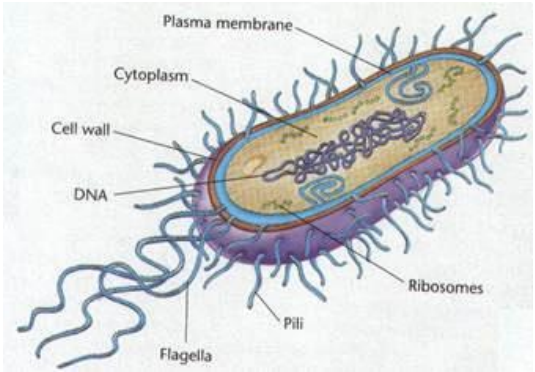




What is synthetic biology?



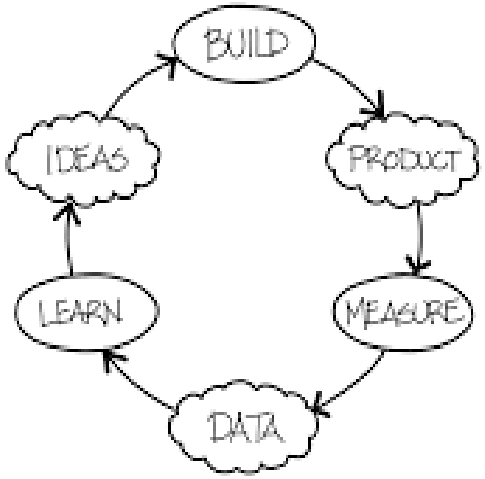
“Blank” chassis
Constructed by modules (parts)
Behavior code based
Non self replicative
Possible contamination by external code



“Evolutionary” based chassis
Constructed by modules (parts)
Behavior code based
Self replicative
Possible contamination by external code

Similarities with IT exists (both code based industries) but fundamental differences exist

Engineering objects

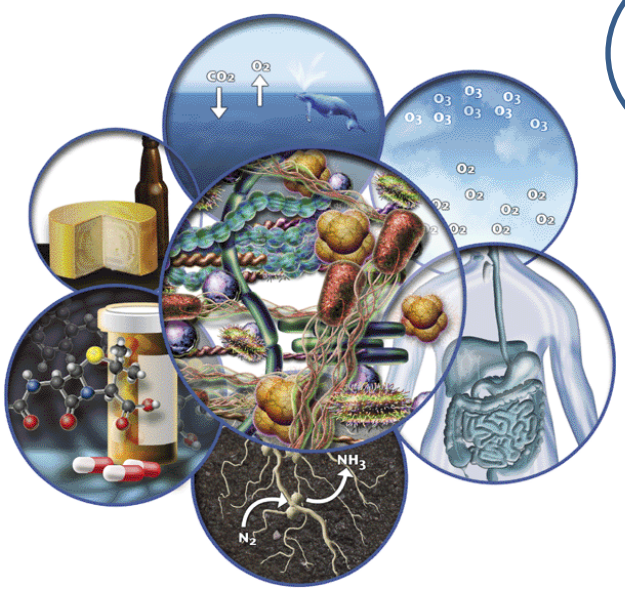


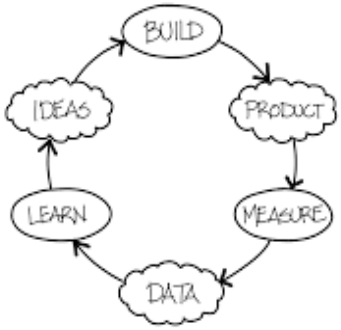
Synthetic biology aims to apply the engineering approaches to living systems

The importance of microbes for life on our planet

- Microbes are the chemical biocatalysers of our ecosystem
- Microbes are collaborating and fighting with each other to reach certain equilibrium to form communities: « microbiota »
- These microbiota have evolved to generate unique chemical reactions via species synergies

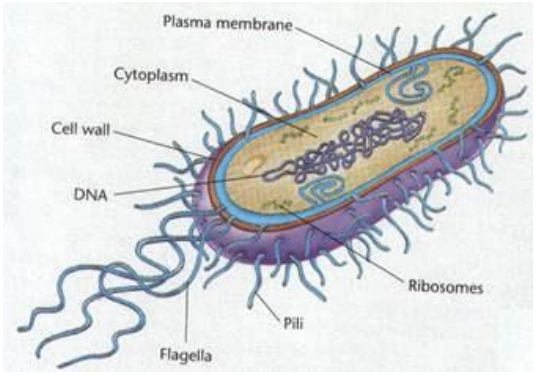
Applications of microorganisms in industries



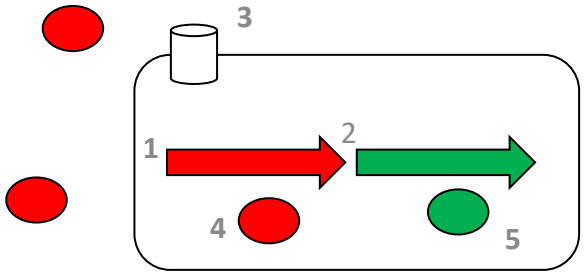


Microbial communities are the biocatalysts of our planet and industries

What is the problem to solve?



The solution would be….



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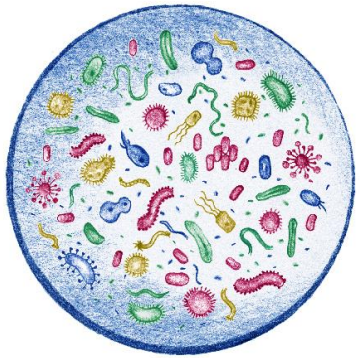

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Design of technologies to improve microbial biocontrol

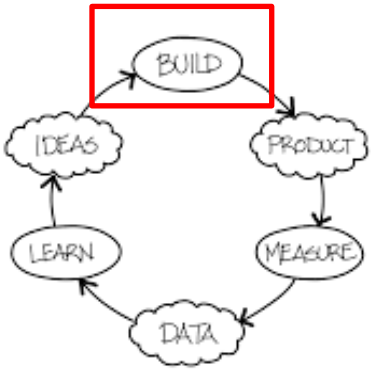
What genes can we use to control microbiota?

(R)Explore the world of bacteriocins

- Discovered in 1925 by Belgian scientist: "**André Gratia** (1893–1950): Forgotten Pioneer of Research into Antimicrobial Agents"
- Heterogenous group of **antimicrobial peptides** produced **ribosomally** by **bacteria**
- Used to **kill related species** to **reduce competition** for resources and space
- **Present a killing specificity ot toxic**

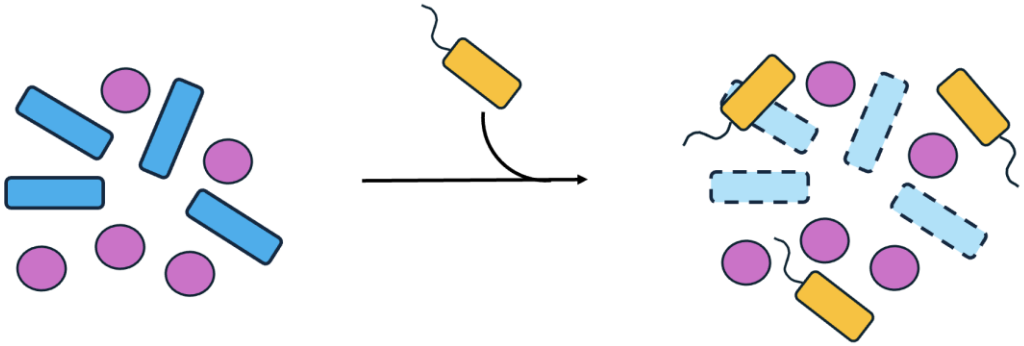
André Gratia

Apply synthetic biology

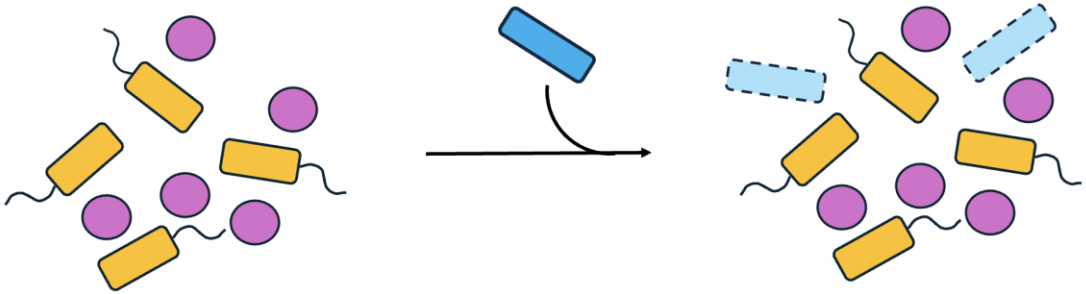


Natural functions of bacteriocins

A) The bacterial insertion within a microbial community is facilitated

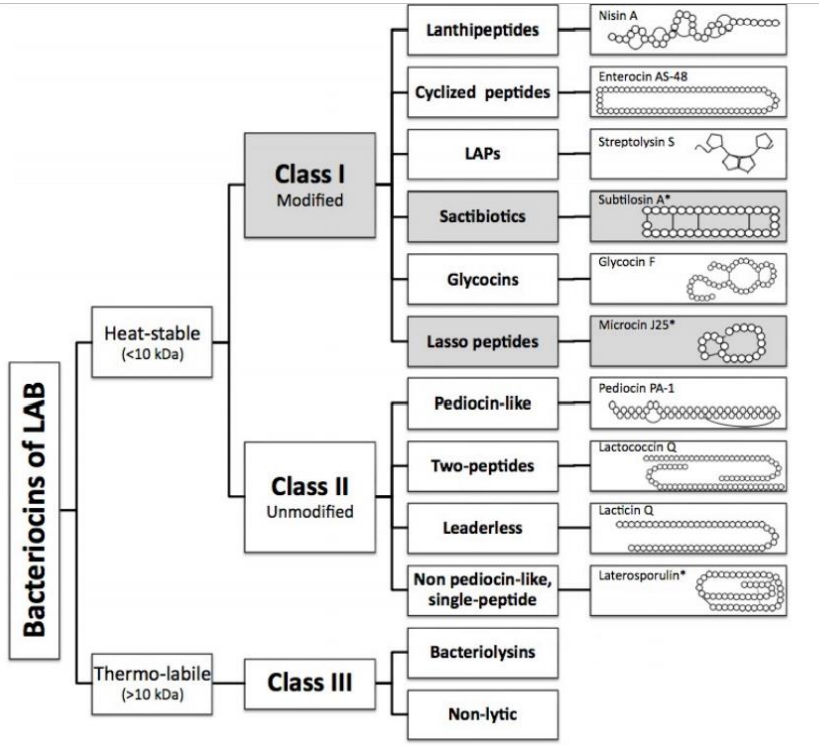


B) The invasion within a microbial community is prevented



Role: protection of ecological niches

Bacteriocins natural diversity



Alvarez-Sieiro et al., 2016, Appl. Microbiol. Biotechnol.

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I	Darobactin	Unusual double ring structure from tryptophan-to-tryptophan, lysine or arginine linkages, rSAM	Darobactin[95]
I	Epipeptide	D-amino acids, rSAM	EpeX[175]
I	Glycocin	S-glycosylation and O-glycosylation of serine/threonine	Pallidocin[121]
I	Lanthipeptide, type I	Methyllanthionine and/or lanthionine residues, LanBC	Kunkecin A[176]
I	Lanthipeptide, type II	Methyllanthionine and/or lanthionine residues, LanM	Roseocin[177]
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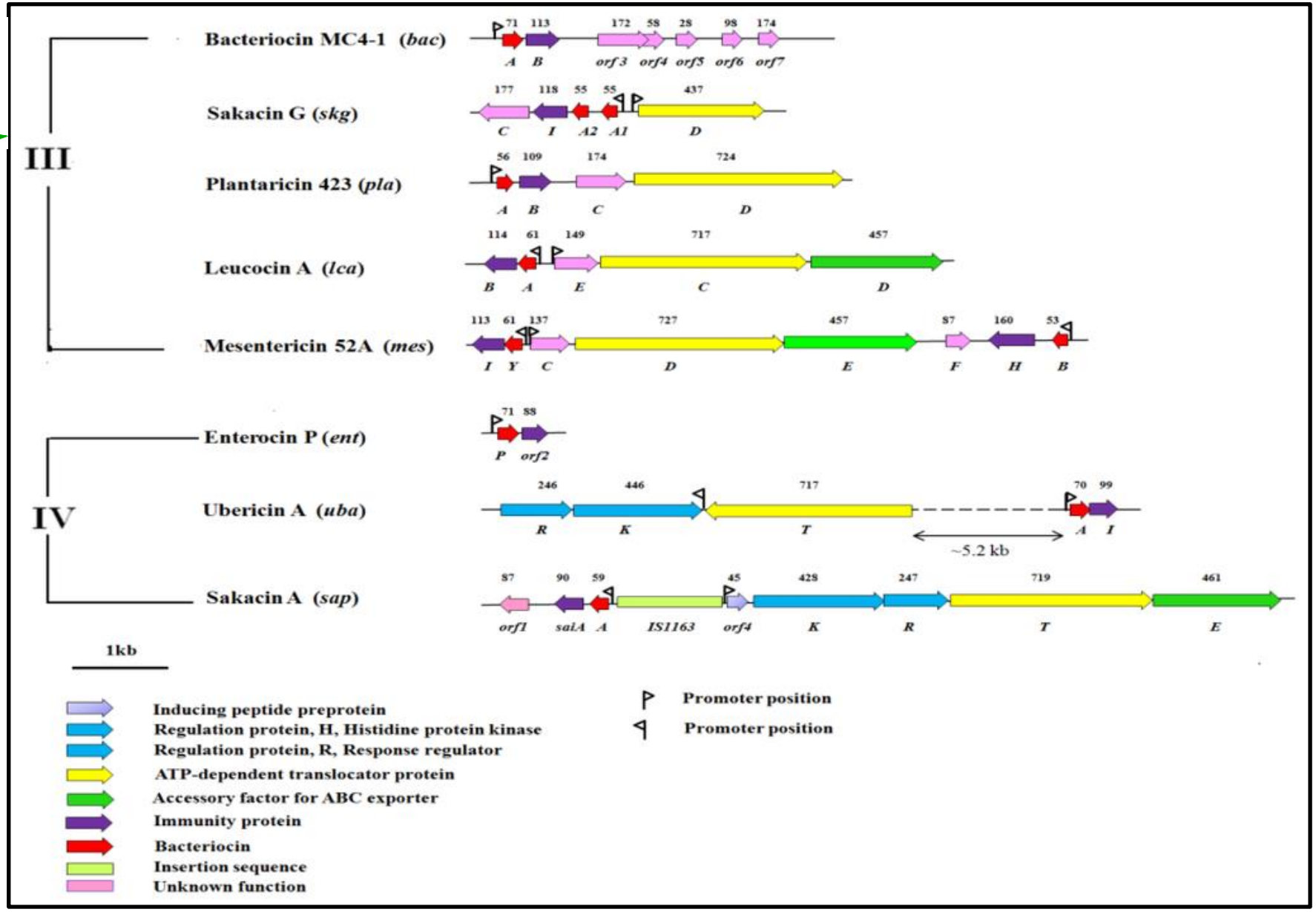
Sugure et al., 2024, Nature reviews microbiology.

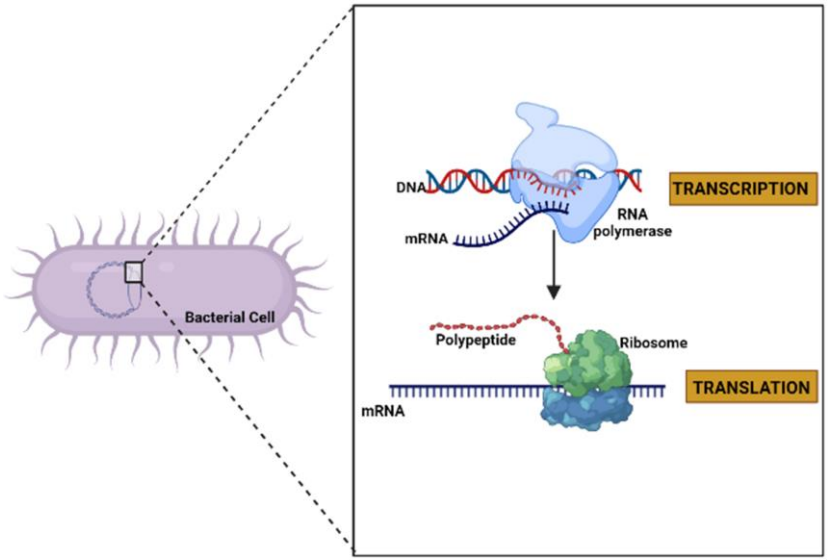


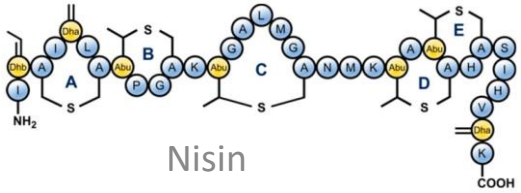
Int. J. Mol. Sci. **2012**, *13*(12), 16668-16707;
Review

Class IIa Bacteriocins: Diversity and New Developments

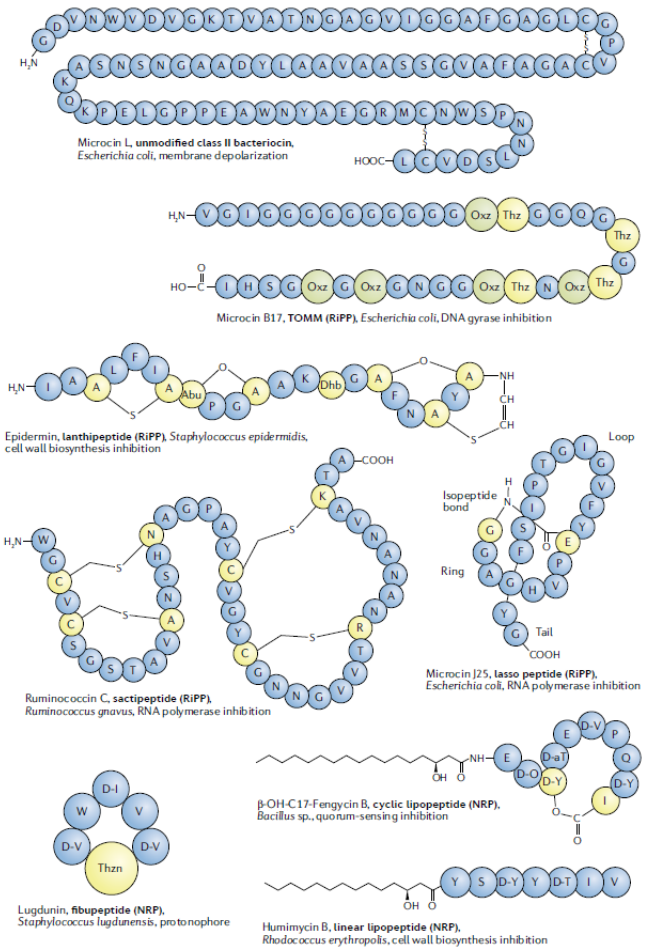
Yanhua Cui 1, Chao Zhang 1, Yunfeng Wang 2,*, John Shi 3, Lanwei Zhang 1,*, Zhongqing Ding 1, Xiaojun Qu 4 and Hongyu Cui 2

Bacteriocins





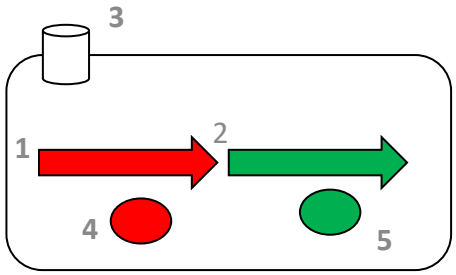
Nisin



Anumudu *et al.,* (2021), Heilbronner *et al.,* (2021), Hurtado-Rios *et al.,* (2022)

Bacteriocins loci fulfill technological requirements

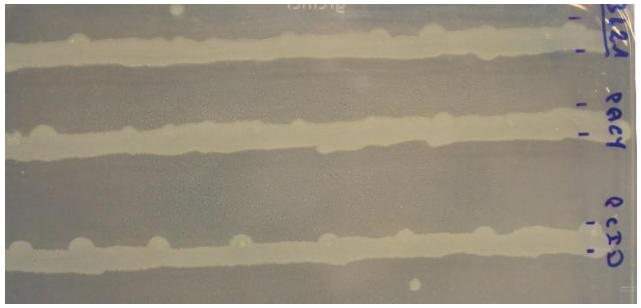
Dr. Mohamed El Bakkoury



BL21(DE3)

BL21(DE3)+pACY

BL21(DE3)+pCID (pACY-MicB17)



BL21(DE3)

BL21(DE3)+pACY

BL21(DE3)+pCID (pACY-MicB17)

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Bacteriocin peer selection for the production of antibiotic selection free biotherapeutic pDNA

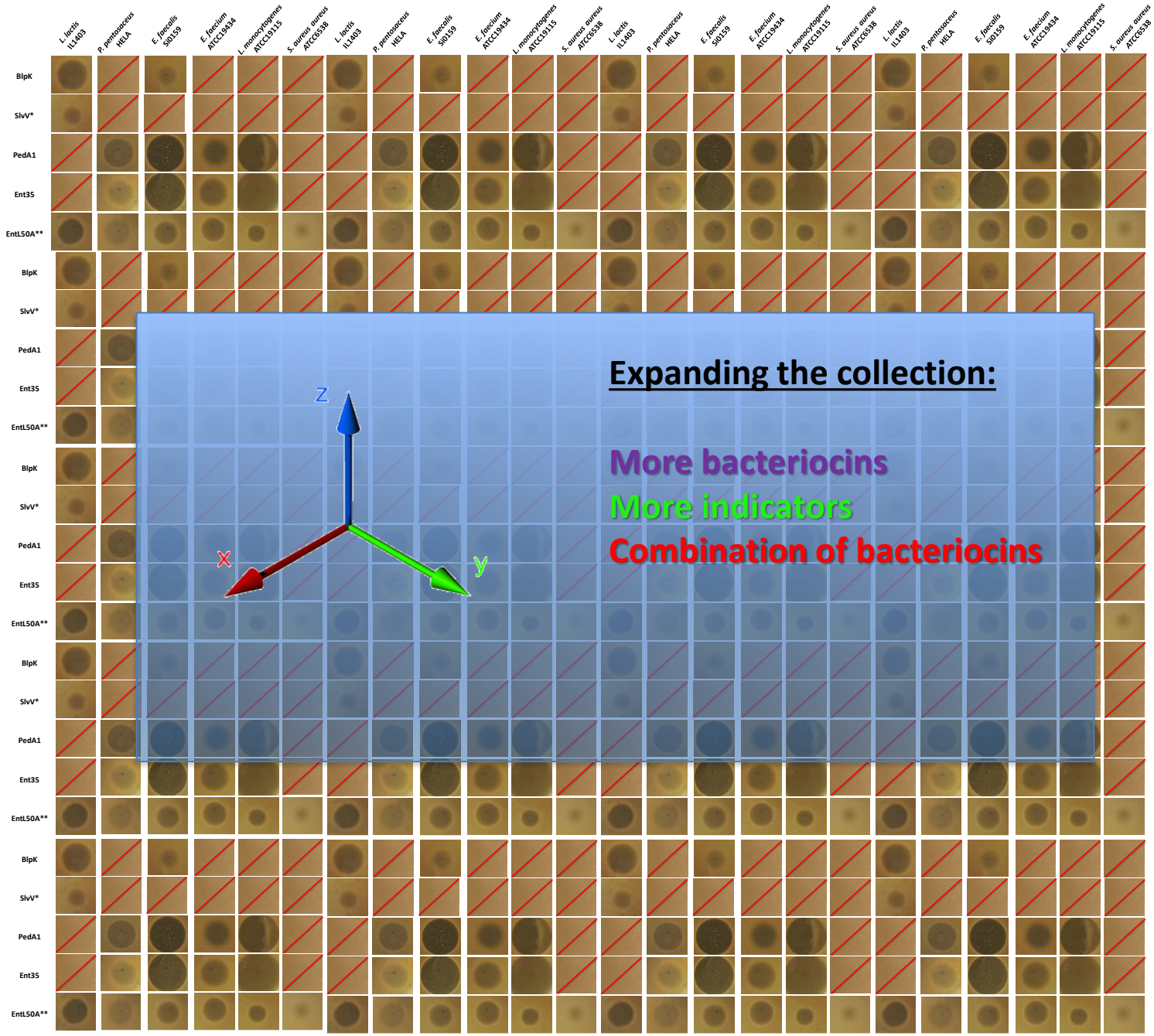
Mohamed El Bakkoury, Luz P. Gómez de Cadiñanos, Philippe Gabant

doi: https://doi.org/10.1101/2023.10.23.563565

This article is a preprint and has not been certified by peer review [what does this mean?].

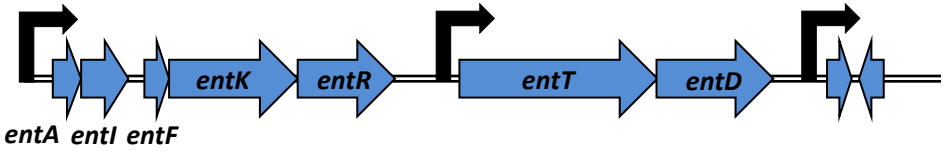
The PARAGEN collection of bacteriocins

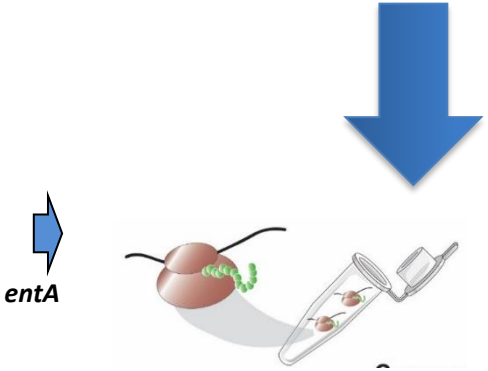
- Have for the first time a collection of active bacteriocins via a collection of biobricks.
- Compare bacteriocins (killing spectrum, stability,..)
- Explore the different technologies to produce bacteriocins (including cocktails)


L. lactis IL1403
P. pentosaceus HELA
E. faecalis S0159
E. faecium ATCC19434
L. monocytogenes ATCC19115
S. aureus aureus ATCC6538
BlpK
SlvV*
PedA1
Ent35
EntL50A**
Expanding the collection:
More bacteriocins
More indicators
Combination of bacteriocins
z
x
y

Cell-free synthesis

MUCH FASTER AND LESS LABOUR INTENSIVE
ALLOWING THE PRODUCTION OF DIFFERENT BACTERIOCINS IN ONE DAY





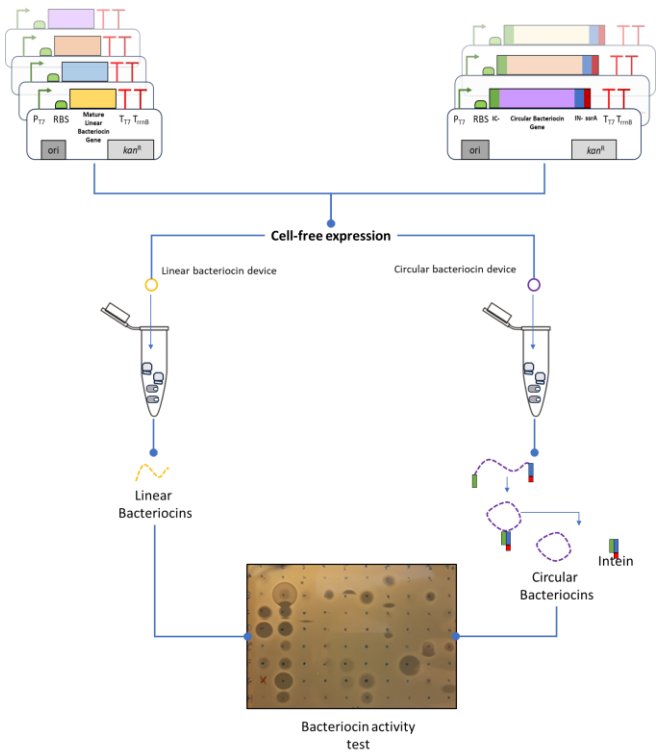
NO ACCESORY, TRANSPORT OR IMMUNITY GENES NEEDED - ALLOWING THE PRODUCTION OF TOXIC PEPTIDES

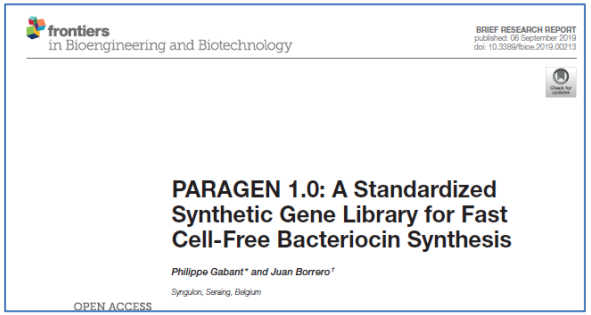
NO LIMITATION IN TERMS OF LENGTH;

COLICIN B (511 aa)

MSDNEGSVPTEGIDYGDTMVVWPSTGRIPGGDVKPGGSSGLAPSMPPGWGDYSPQGIALVQSVLFPGIIRRIILDKELEEGDWSGWSVSV
HSPWGNEKVSAARTVLENGLRGGLPEPSRPAAVSFARLEPASGNEQKIIRLMVTQQLEQVTDIPASQLPAAGNNVPVKYRLTDLMQNGTQY
MAIIGGIPMTVPVVDAVPVPDRSRPGTNIKDVYSAPVSPNLPDLVLSVGQMNTPVRSNPEIQEDGVISETGNYVEAGYTMSSNNHDVIVRFP
EGSGVSPLYISAVEILDSNSLSQRQEAENNAKDDFRVKKEQENDEKTVLTKTSEVIISVGDKVGEYLGDKYKALSREIAENINNFQGKTIRSYDDA
MSSINKLMANPSLKINATDKEAIVNAWKAFNAEDMGNKFAALGKTFKAADYAIKANNIREKSIEGYQTGNWGPLMLEVESWVISGMASAV
ALSLFSLTLGSALIAFGLSATVVGFVGVVIAGAIGAFIDDKFVDELNHKIIK

PARAGEN: standardisation for fast and robust bacteriocin synthesis




frontiers in Bioengineering and Biotechnology

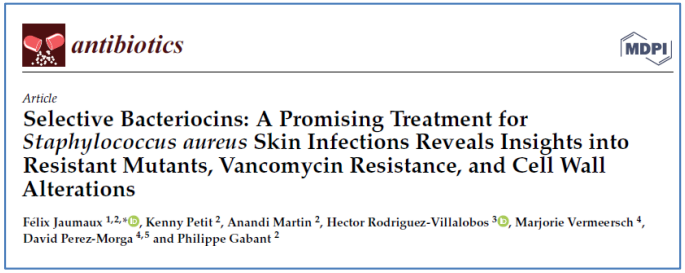
BRIEF RESEARCH REPORT
published: 06 September 2019
doi: 10.3389/fbioe.2019.00213

PARAGEN 1.0: A Standardized Synthetic Gene Library for Fast Cell-Free Bacteriocin Synthesis

Philippe Gabant and Juan Borrero†*

Syngulon, Seraing, Belgium

OPEN ACCESS


antibiotics

MDPI

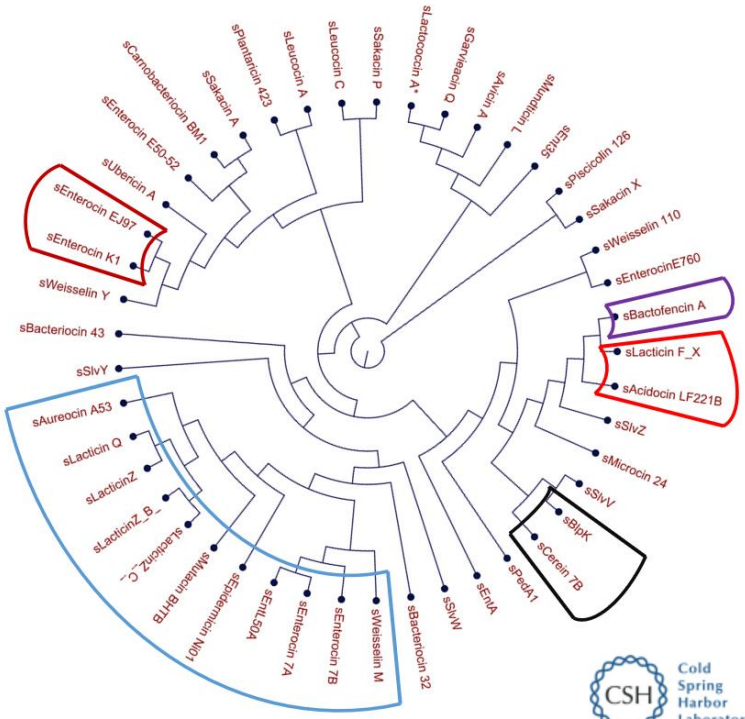
Article

Selective Bacteriocins: A Promising Treatment for *Staphylococcus aureus* Skin Infections Reveals Insights into Resistant Mutants, Vancomycin Resistance, and Cell Wall Alterations

Félix Jaumaux [1,2,*], Kenny Petit [2], Anandi Martin [2], Hector Rodriguez-Villalobos [3], Marjorie Vermeersch [4], David Perez-Morga [4,5] and Philippe Gabant [2]

Gabant & Borrero (2019), Jaumaux *et al.*, (2023)

Expanding PARAGEN by bioinformatic search



UCLouvain

Prof. P. Hols

Julien Damoczi (PhD student)

Cold Spring Harbor Laboratory

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New Results

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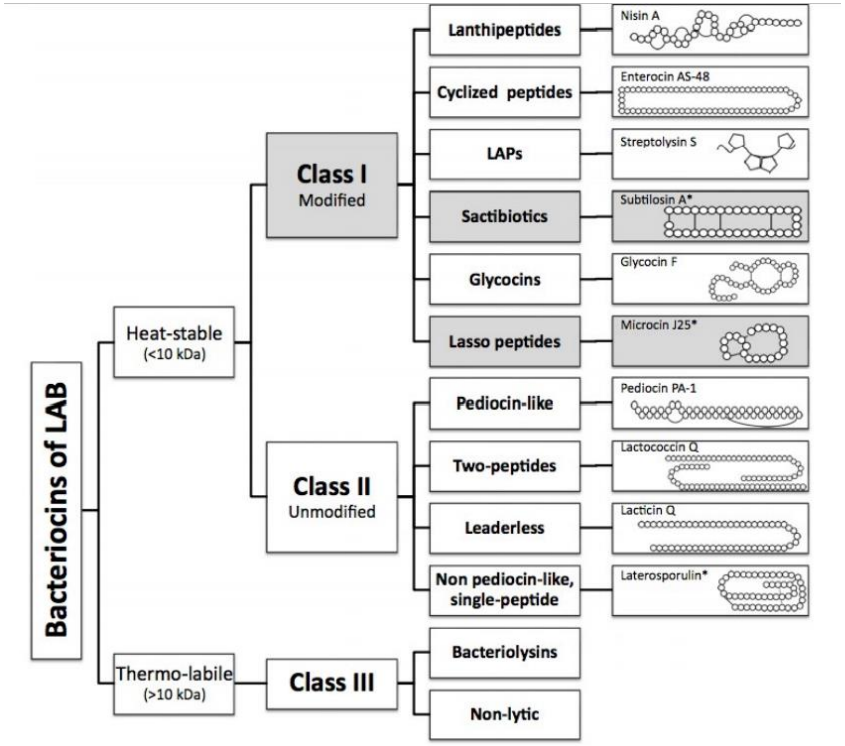
Uncovering the class II-bacteriocin predatiome in salivarius streptococci

Julien Damoczi, Adrien Knoops, Marie-Sophie Martou, Félix Jamaux, Philippe Gabant, Jacques Mahillon, Johann Mignolet, Pascal Hols

doi: https://doi.org/10.1101/2024.03.04.583286

Expanding PARAGEN beyond Class II bacteriocins.



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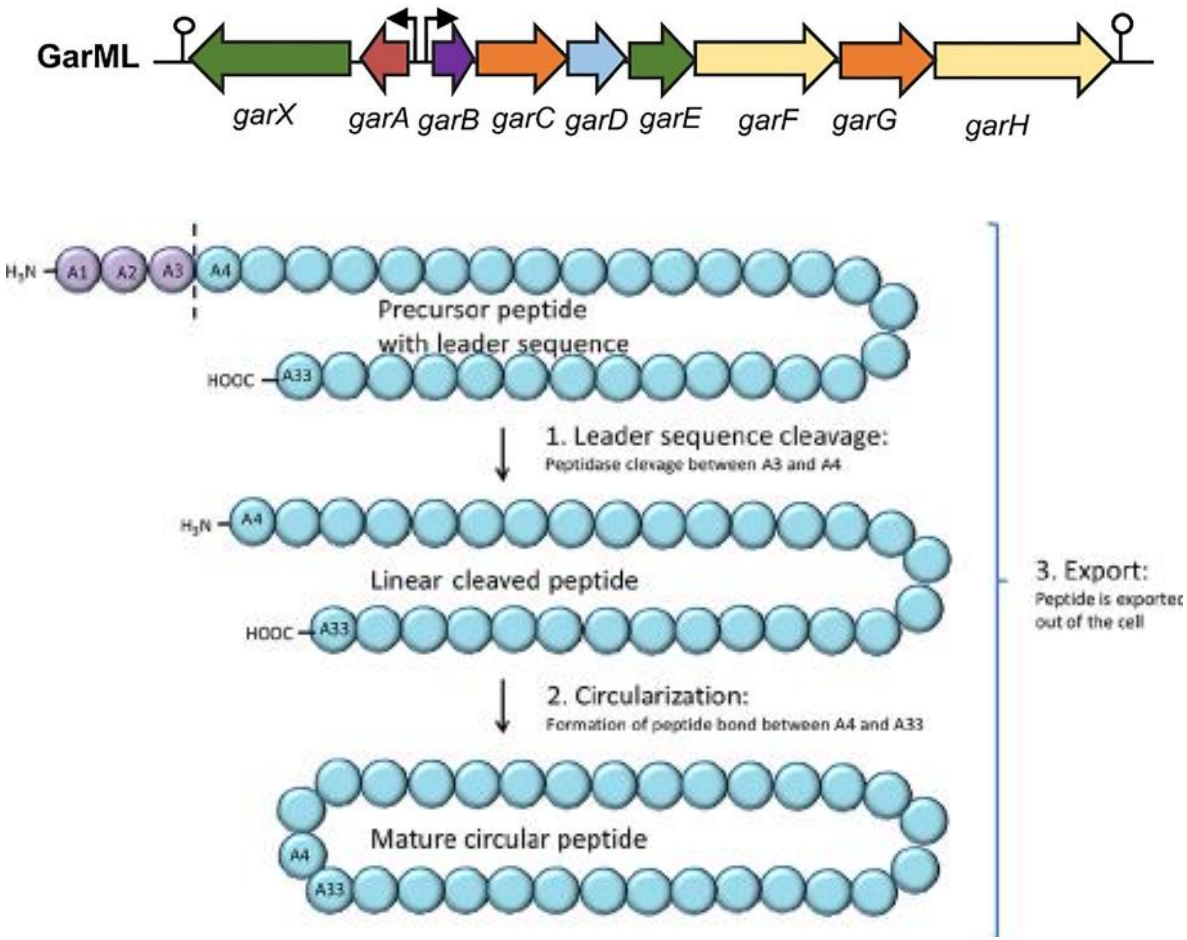
Sugure et al., 2024, Nature reviews microbiology.

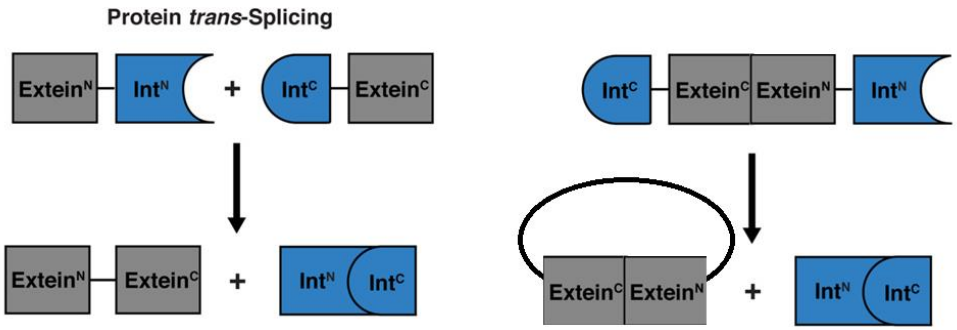
Expanding PARAGEN beyond Class II bacteriocins: the synthetic biology puzzle



Challenge moving from a natural 9 gene circuit to a standard 1 gene PARAGEN Standard: **compacting the code**

Inteins: natural tools

Collaboration with:
Universidad Complutense Madrid (UCM)
Dr. Juan Borrero



PNAS Proceedings of the National Academy of Sciences of the United States of America

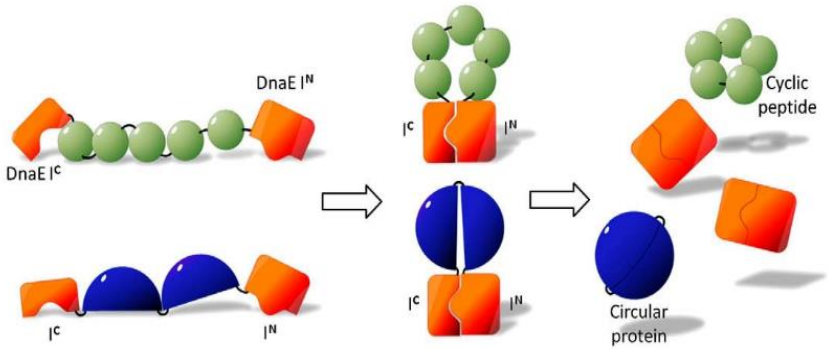
RESEARCH ARTICLE

Production of cyclic peptides and proteins *in vivo*

Charles P. Scott, Ernesto Abel-Santos, Mark Wall, Daphne C. Wahnon, and Stephen J. Benkovic

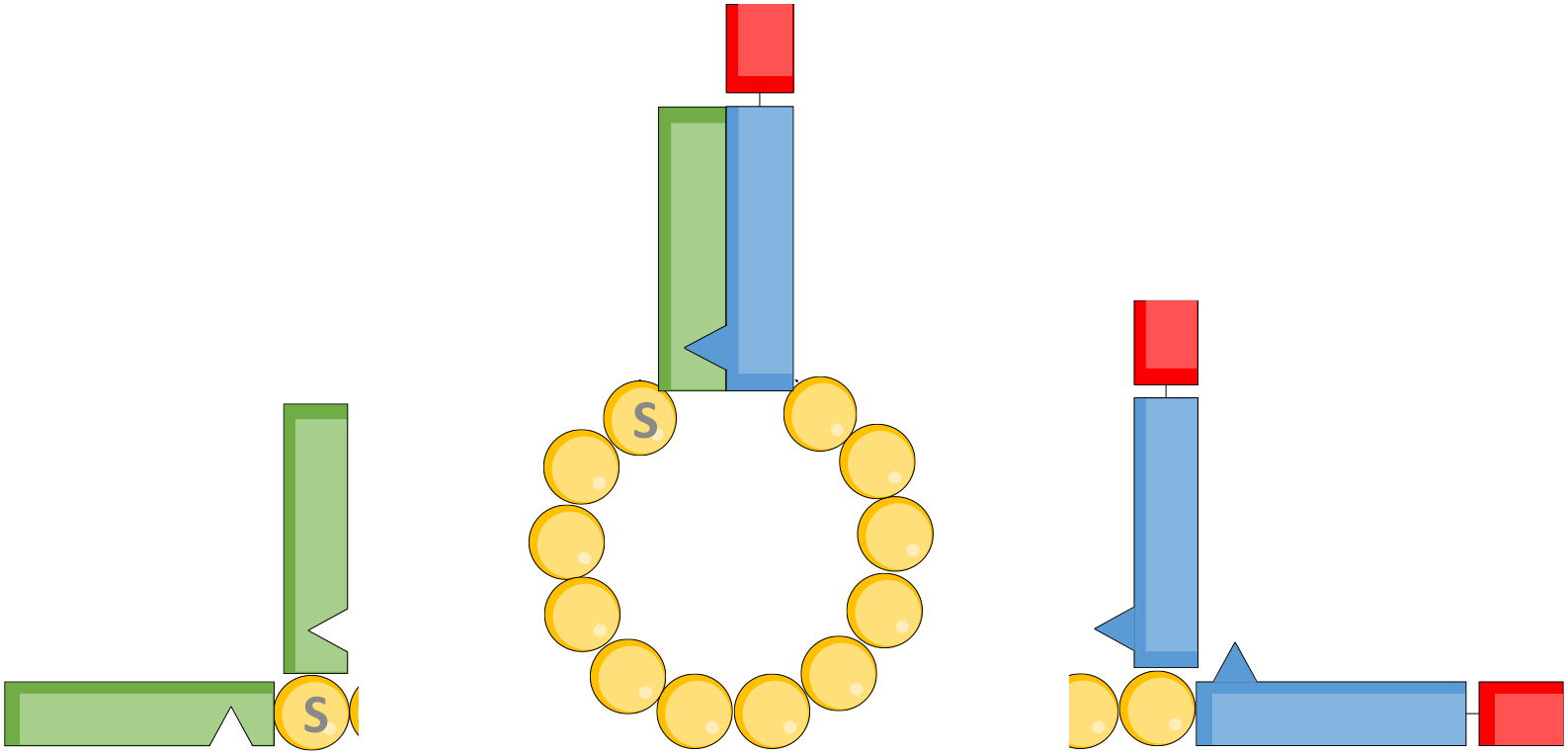
PNAS November 23, 1999 96 (24) 13638-13643; https://doi.org/10.1073/pnas.96.24.13638

Contributed by Stephen J. Benkovic

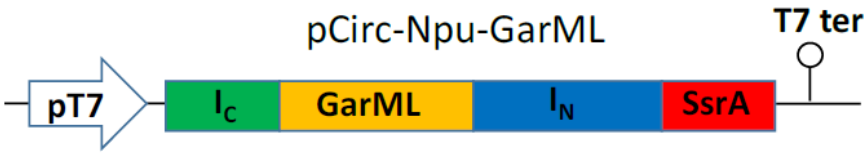


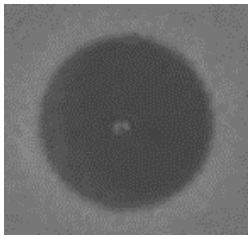
Split Intein Mediated Ligation (SIML) system

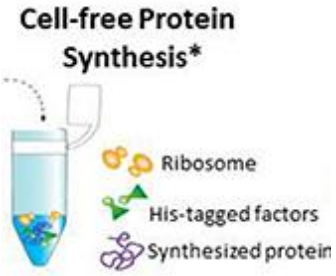
Collaboration with:
Universidad Complutense Madrid (UCM)
Dr. Juan Borrero

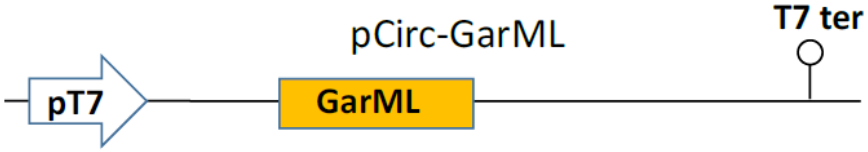


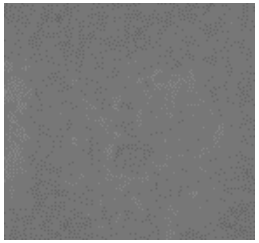
➢ Cell-free *in vitro* Protein Synthesis

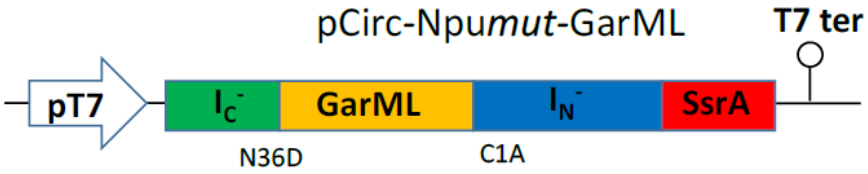


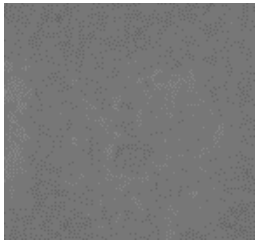


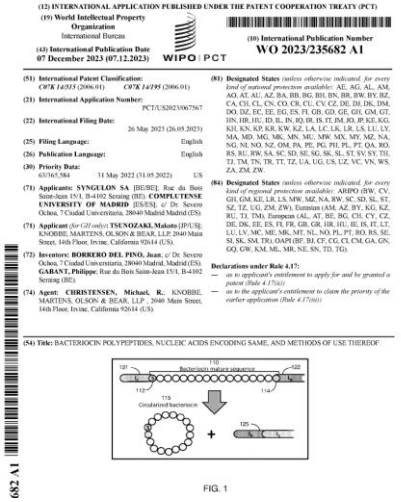












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Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

(54) Title: BACTERIOCIN POLYPEPTIDES, NUCLEIC ACIDS ENCODING SAME, AND METHODS OF USE THEREOF

FIG. 1

682 A1

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In vitro and *in vivo* production and split-intein mediated ligation (SIML) of circular bacteriocins

Nuria Peña[1], Michael J. Bland[2], Ester Sevillano[1], Estefanía Muñoz-Atienza[1], Irene Lafuente[1], Mohamed El Bakkoury[2], Luis M. Cintas[1], Pablo E. Hernández[1], Philippe Gabant[2] and Juan Borrero[1]*

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Expanding PARAGEN to circular bacteriocins: synthetic biology panacea based on inteins

Characterized Circular bacteriocins

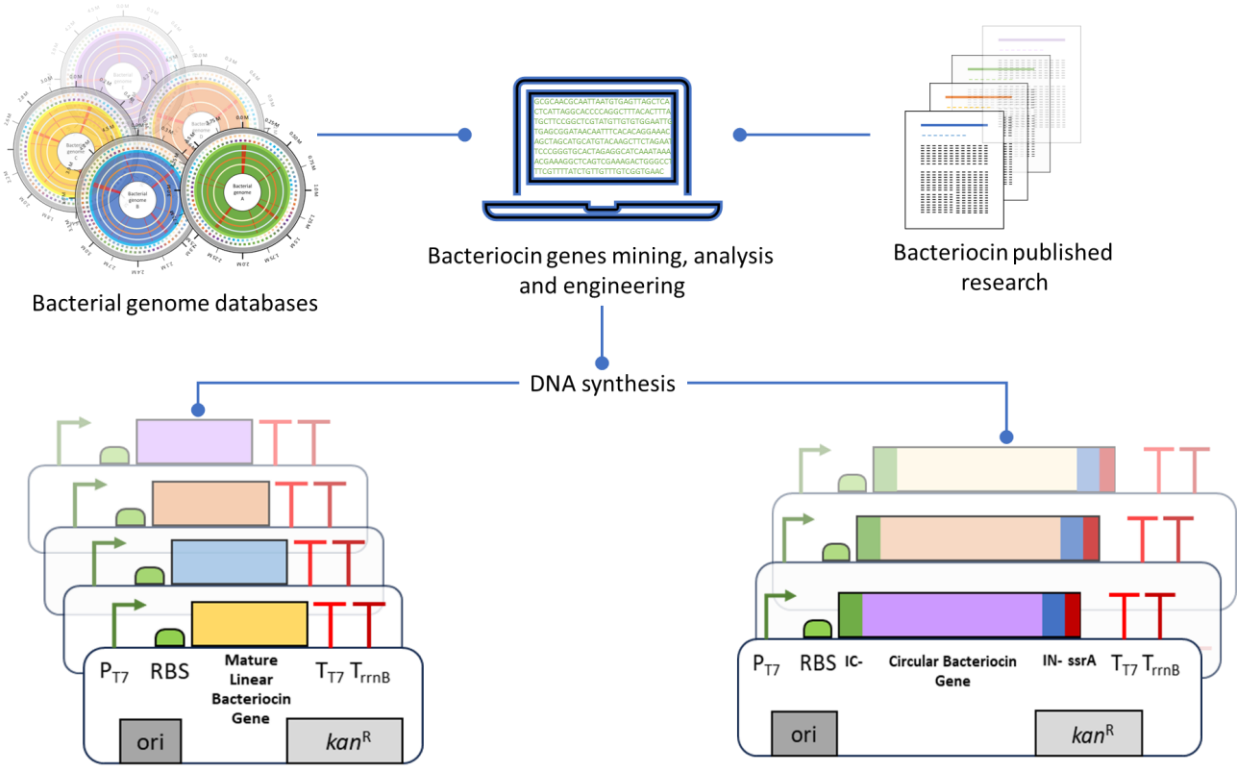
Non Characterized Circular bacteriocins

Bacteriocin	Producer	Plasmid	Activity against *L. lactis* IL1403*
Amylocyclicin	*Bacillus amyloliquefaciens* FZB42	pCirc-Npu-Alc	+
Enterocin AS-48	*Enterococcus faecalis* S-48	pCirc-Npu-EntAS48	+
Carnocyclin A	*Carnobacterium maltaromaticum* UAL307	pCirc-Npu-CarA	+
Circularin A	*Clostridium beijerinckii* ATCC 25752	pCirc-Npu-CirA	+
Enterocin NKR-5-3B	*Enterococcus faecium* NKR-5-3	pCirc-Npu-NKR_5_3B	+
Garvicin ML	*Lactococcus garvieae* DCC43	pCirc-Npu-GarML	+
Leucocyclicin Q	*Leuconostoc mesenteroides* TK41401	pCirc-Npu-LeuQ	+
Uberolysin A	*Streptococcus uberis* 42	pCirc-Npu-UberA	–
Butyrivibriocin AR10	*Butyrivibrio fibrisolvens* AR10	pCirc-Npu-ButAR10	–
Paracyclicin P	*Lactobacillus paracesei* JCM 8130/ DSM 5622	pCirc-Npu-ParP	+
Gassericin A	*Lactobacillus gasseri* LA39	pCirc-Npu-GasA	+
Plantaricyclin A	*Lactobacillus plantarum* NI326	pCirc-Npu-PlcA	+
Cerecyclin	*Bacillus* sp. Xin1	pCirc-Npu-Cer	–
Bacteriocin 3688STDY6124959	*Staphylococcus aureus* 3688STDY6124959	pCirc-Npu-3688STDY	+
Bacteriocin BCW 2997	*Listeria monocytogenes* BCW 2997	pCirc-Npu-BCW_2,997	+
Bacteriocin CF11	*Clavibacter michiganensis* CF11	pCirc-Npu-CF11	–
Bacteriocin NBRC 15376	*Paenibacillus chondroitinus* NBRC 15376	pCirc-Npu-NBRC_15,376	–
Bacteriocin YS111	*Streptococcus suis* YS111	pCirc-Npu-YS111	+
Bacteriocin DSM 15102	*Garciella nitratireducens* DSM 15102	pCirc-Npu-DSM_15,102	+
Bacteriocin AFS089278	*Bacillus toyonensis* AFS089278	pCirc-Npu-AFS089278	–
Bacteriocin TD3	*Bacillus vallismortis* TD3	pCirc-Npu-TD3	–
Bacteriocin NRRL B-24287	*Streptomyces pathocidini* NRRL B-24287	pCirc-Npu-NRLL_B_24,287	–
Bacteriocin AK22	*Alkalibacterium* AK22	pCirc-Npu-AK22	–
Bacteriocin 15,828	*Gemella cuniculi* DSM 15828	pCirc-Npu-15,828	–
Bacteriocin NCTC 12958	*Streptococcus thermophilus* NCTC 12958	pCirc-Npu-NCTC_12,958	+
Bacteriocin UoS2029	*Streptococcus pneumoniae* UoS2029	pCirc-Npu-UoS2029	–

*Samples showing a clear halo of inhibition (+) or no halo of inhibition (–).

Peña et al. Front Microbiol. 2022

Syngulon PARAGEN collection: standardised bacteriocin synthesis

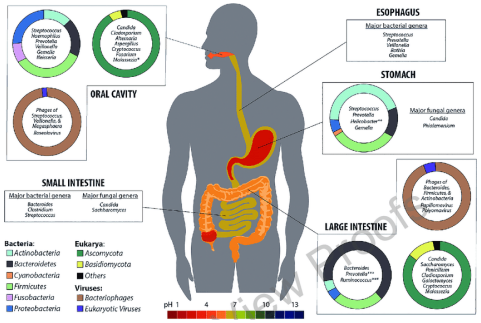


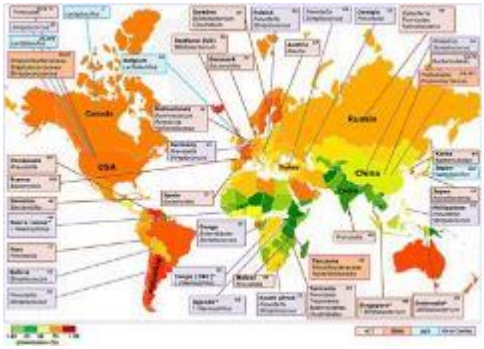
Gabant & Borrero (2019), Peña *et al.,* (2022).

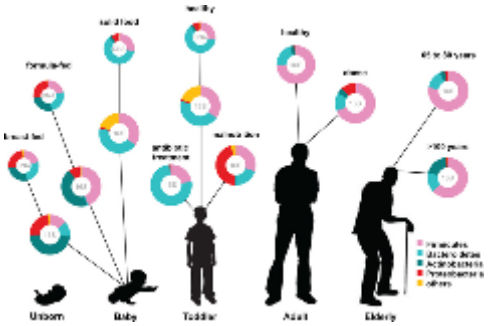
Bacteriocins for precision anti-microbial therapies

Broad spectrum antibiotics
VS
Narrow spectrum bacteriocins

Human microbiota is very variable



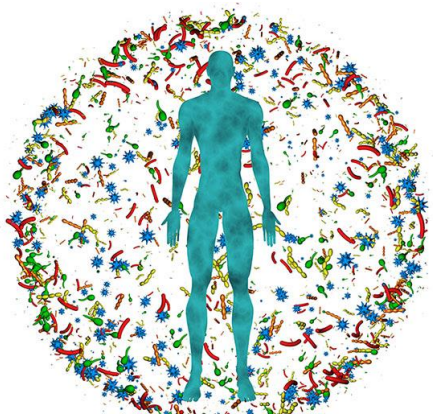


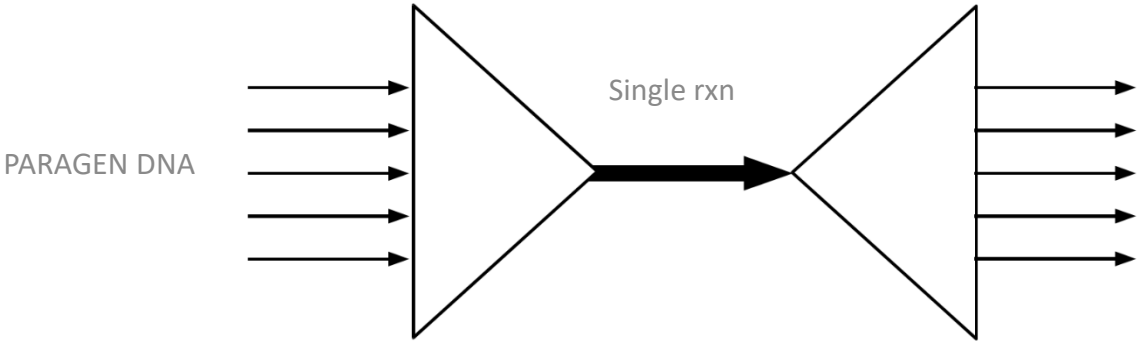


PRECISION MEDICINE

Syngulon PARAGEN collection: how to make a rational and controlled formulation able to shape a microbiota?

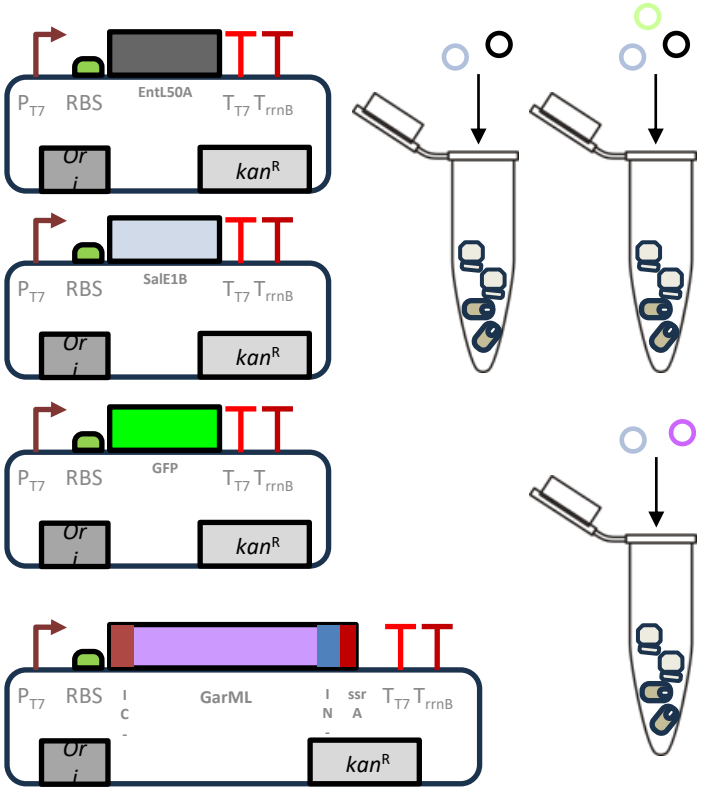
Dr Alex Quinteros Yanes , R&D Project Manager





Use of different genetic code to generate bacteriocins mixtures

A



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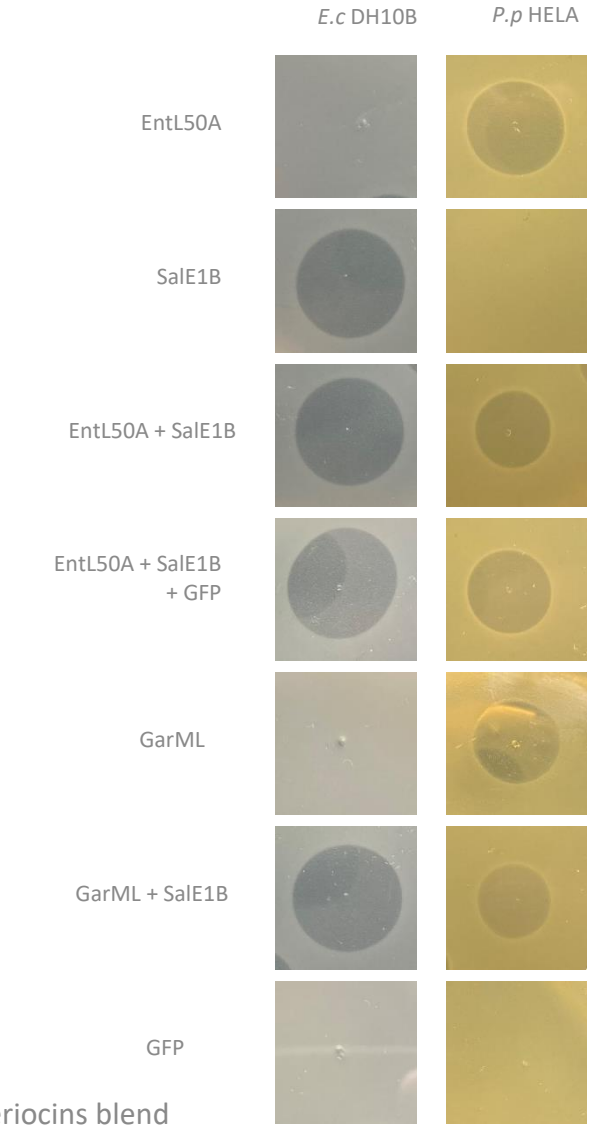
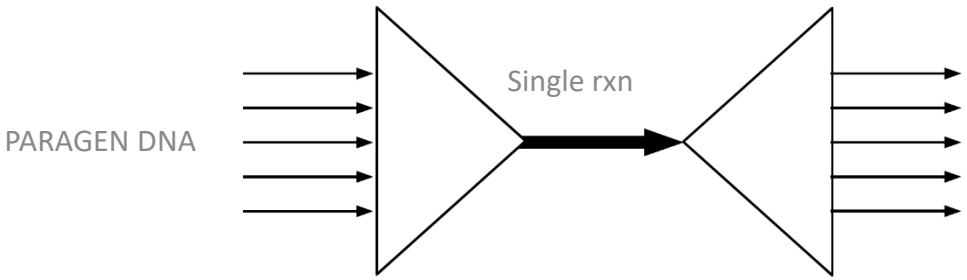
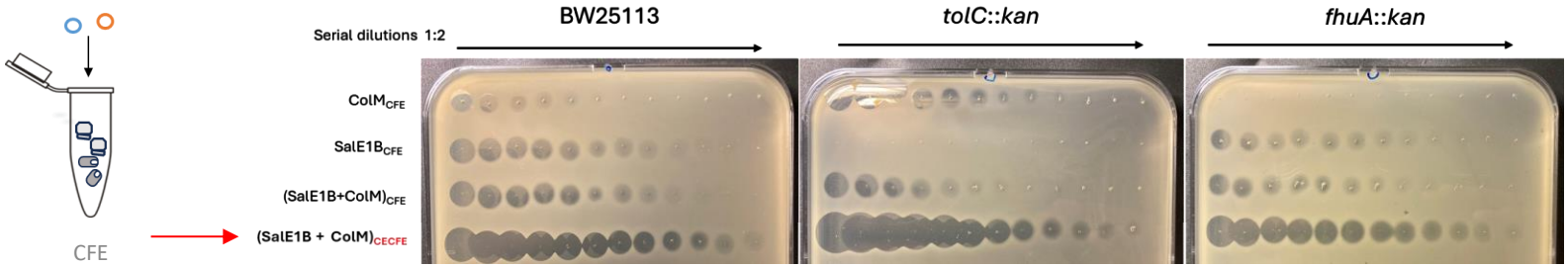
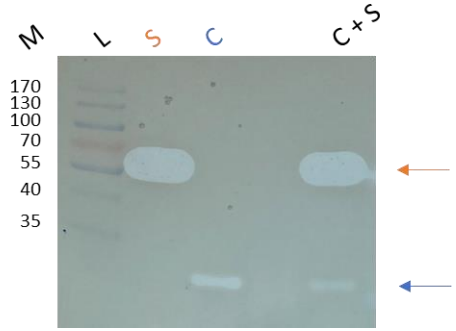
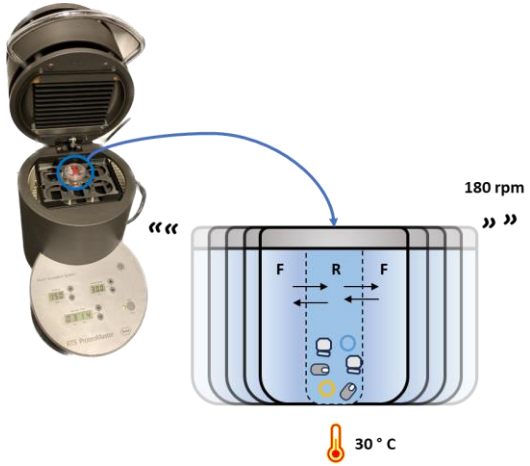




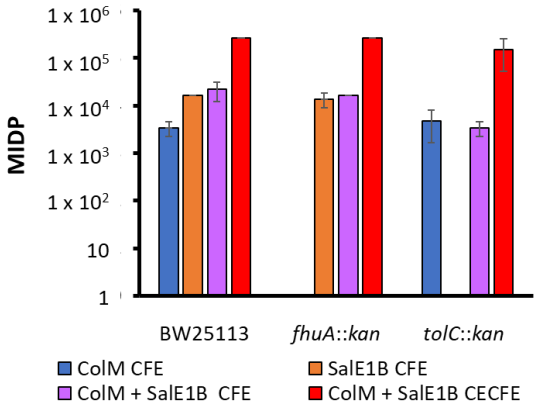
(De)multiplexing and scaling up bacteriocin activity (signals)



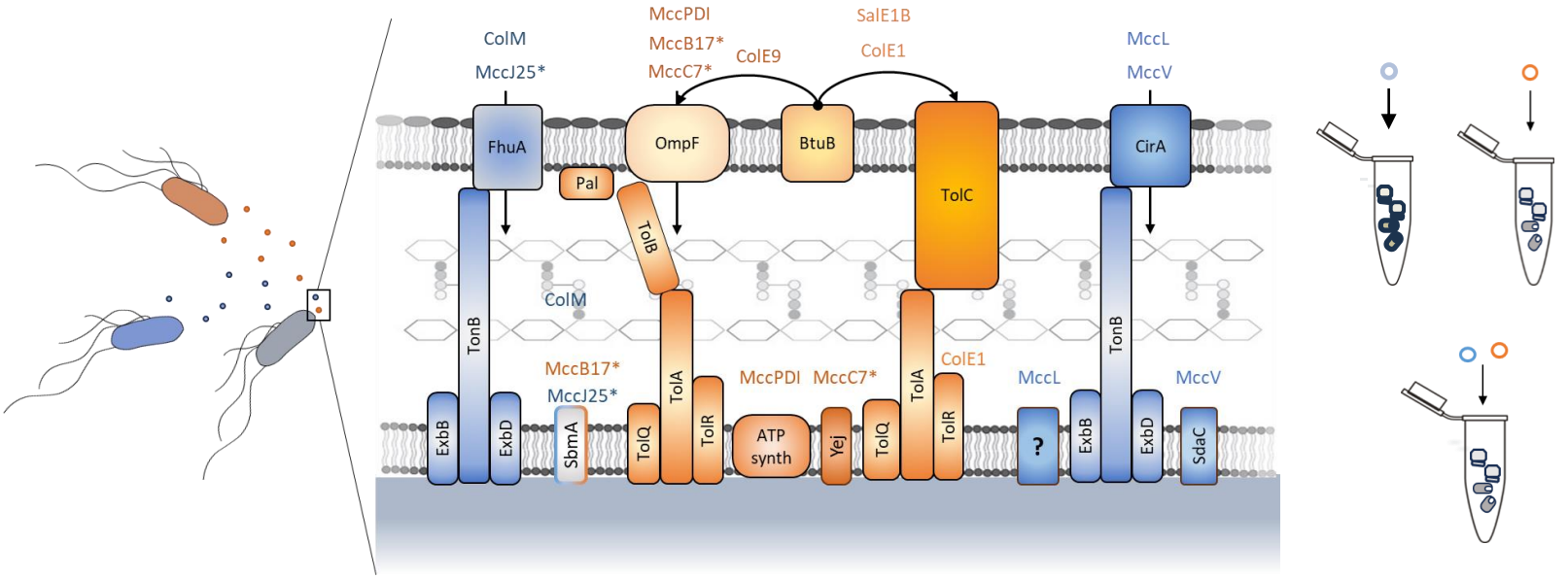


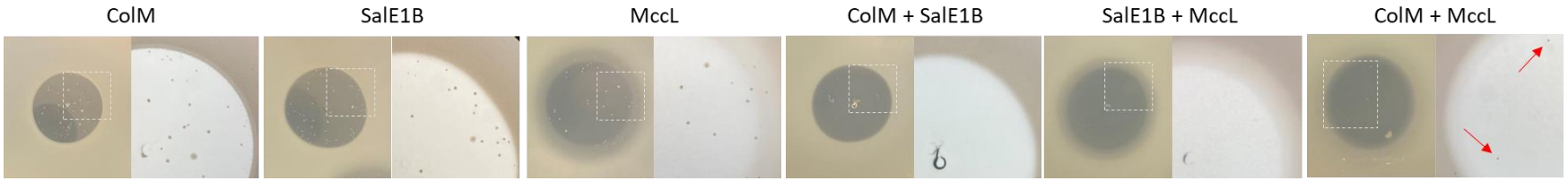


Continuous exchange-CFE

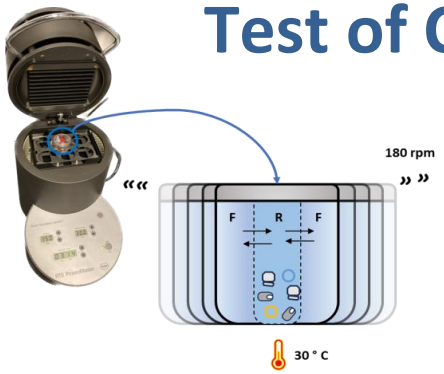


Rational bacteriocin cocktail designs prevent AMR





Test of CFE bacteriocin combinations *in vivo*



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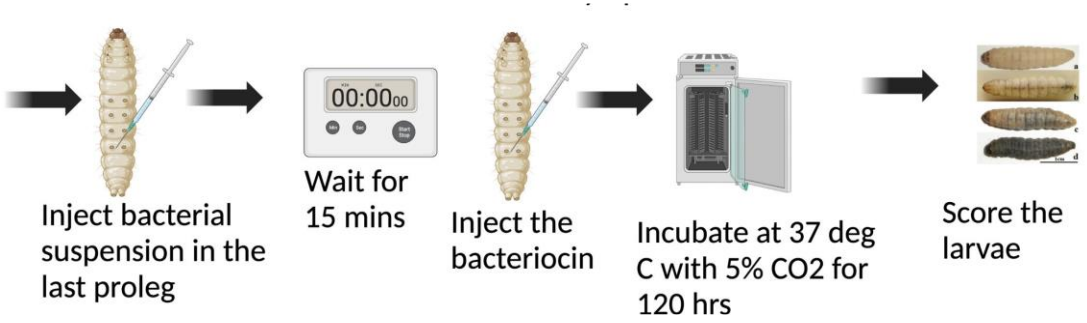

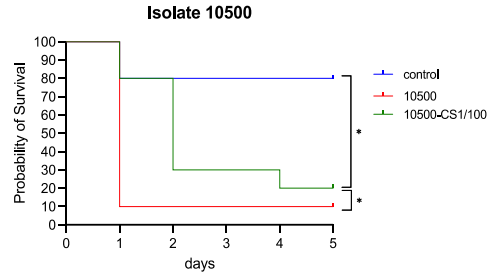

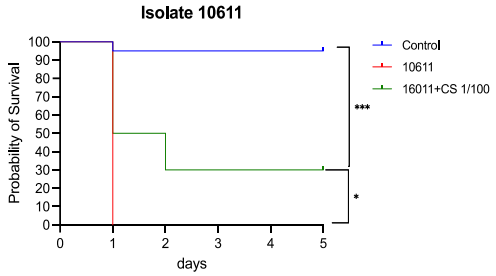

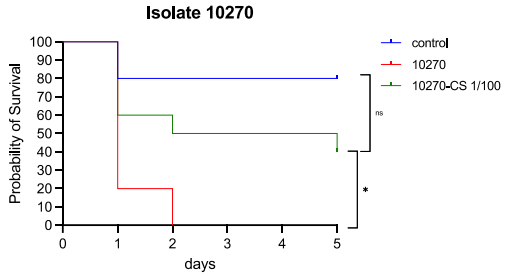

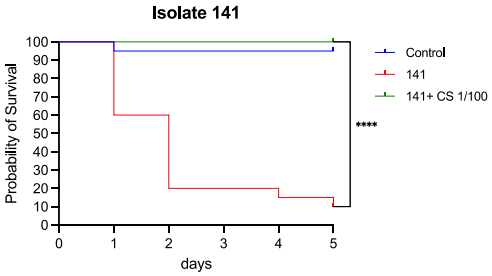

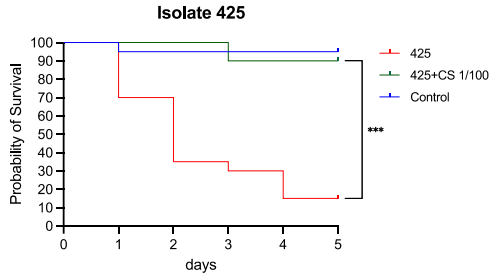

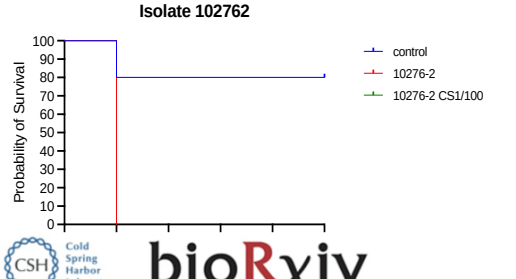

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Bacteriocin cocktail improves survival of *Galleria m* with antibiotic resistant *E. coli* clinical isolates.

New Results

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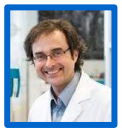
Multiplexing bacteriocin synthesis to kill and prevent antimicrobial resistance

Alex Quintero-Yanes, Kenny Petit, Hector Rodriguez-Villalobos, Hanne Vande Capelle, Joleen Masschelein, Juan Borrero del Pino, Philippe Gabant

doi: https://doi.org/10.1101/2024.09.06.611659

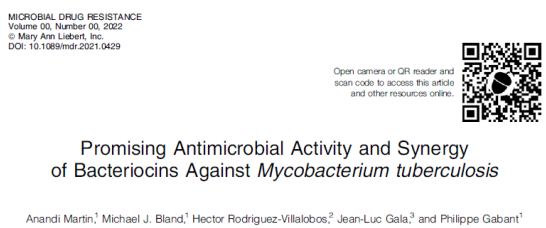


Dr. Joleen Masschelein

Dr. Juan Borrero

Hanne Vande Capelle

Prospective for bacteriocins vectorization and formulation



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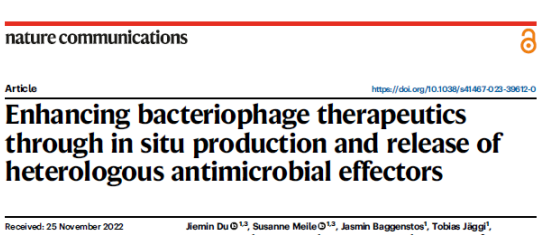
Promising Antimicrobial Activity and Synergy of Bacteriocins Against *Mycobacterium tuberculosis*

Anandi Martin,[1] Michael J. Bland,[1] Hector Rodriguez-Villalobos,[2] Jean-Luc Gala,[3] and Philippe Gabant[1]



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Enhancing bacteriophage therapeutics through in situ production and release of heterologous antimicrobial effectors

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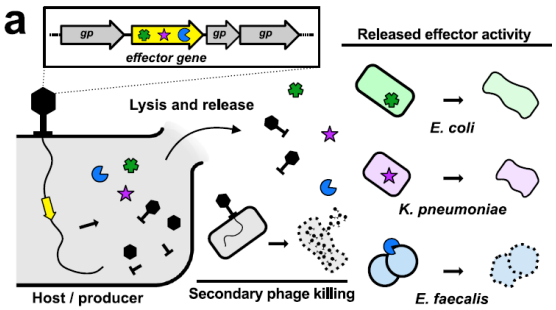

nature communications

Article

https://doi.org/10.1038/s41467-024-50591-8

A bacteriocin expression platform for targeting pathogenic bacterial species

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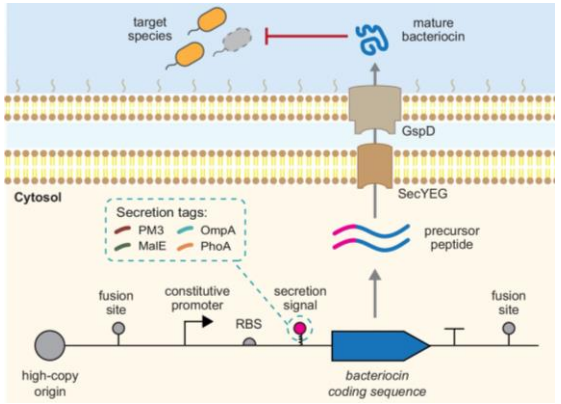
Jack W. Rutter[1,4], Linda Dekker[1,4], Chania Clare[1], Zoe F. Slendebroek[1], Kimberley A. Owen[1], Julie A. K. McDonald[2], Sean P. Nair[3], Alex J. H. Fedorec[1] & Chris P. Barnes[1]



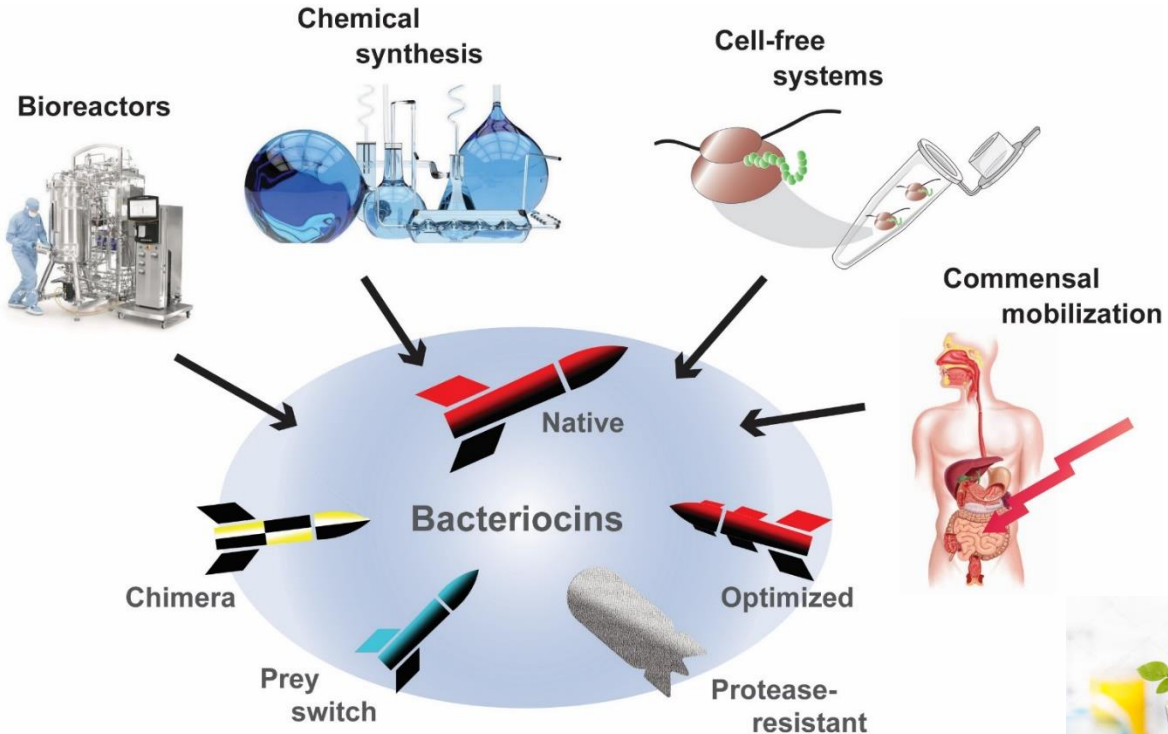
Antibiotic-bacteriocin

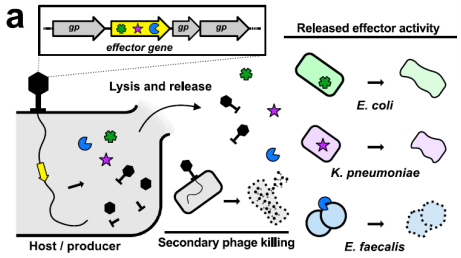
Phage-bacteriocins

Bacteriocins

Tagliaferri, T. *et al.,* (2019), Kranjec, C. *et al.*, (2021), Martin *et al.,* (2021), Du *et* al., (2023), Xiao, G *et al.,* (2023), Rutter *et al.,* (2024).

Bacteriocins production and delivery



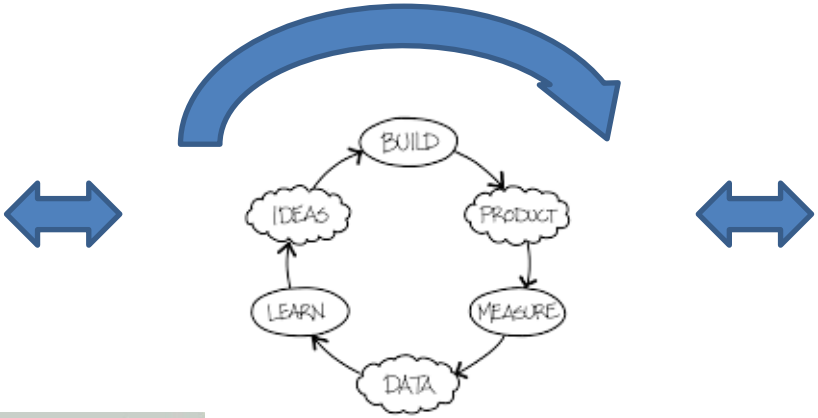


- Production
- Genetic amenability
- Various prey spectrum
- Molecular diversity
- Cyto-friendly
- Stability
- Biological half-life

Adapted from Pascal Hols, Laura Ledesma-García, Philippe Gabant and Johann Mignolet, Trends Microbiology 1685 No. of Pages 13

Take home message

1.Industries are looking for new ways to control microbial flora (microbiota)
2.Synthetic biology allows to apply biological functions at a new level
3.Bacteriocins are natural antimicrobial peptides (AMP) used by bacteria to protect their ecological niche
4. Syngulon has built PARAGEN a unique collection of synthetic bacteriocin genes
5. Via academic collaborations Syngulon is studying the mode of action of bacteriocins
6. Via different industrial partnerships Syngulon is testing applications of bacteriocins



Publications (1)

RESEARCH ARTICLE

Subtle selectivity in a pheromone sensor triumvirate desynchronizes competence and predation in a human gut commensal

Johann Mignolet[1,2‡*], Guillaume Cerckel[1†], Julien Damoczi[1†], Laura Ledesma-Garcia[1], Andrea Sass[3], Tom Coenye[3], Sylvie Nessler[4], Pascal Hols[1]

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Trends in Microbiology

BRIEF RESEARCH REPORT ARTICLE

Front. Bioeng. Biotechnol., 06 September 2019 | https://doi.org/10.3389/fbioe.2019.00213

PARAGEN 1.0: A Standardized Synthetic Gene Library for Fast Cell-Free Bacteriocin Synthesis

Philippe Gabant* and **Juan Borrero†**

Syngulon, Seraing, Belgium

Review

Mobilization of Microbiota Commensals and Their Bacteriocins for Therapeutics

Pascal Hols,[1] Laura Ledesma-García,[1] Philippe Gabant,[2] and Johann Mignolet[1,2,3,*]

Home / Chimica Oggi-Chemistry Today / Vol. 38(4) / Antimicrobial peptides to...

MICHAEL J. BLAND, PHILIPPE GABANT*

*Corresponding author

Syngulon, Seraing, Belgium

BIOTECHNOLOGY

ANTIMICROBIAL PEPTIDES TO SHAPE BIOBASED CHEMICAL PRODUCTION

Keywords: anti-microbial peptides, antibiotics, bacteriocins, biotechnology, industrial fermentation, microbiome, one health

Open Access Perspective

In the Age of Synthetic Biology, Will Antimicrobial Peptides be the Next Generation of Antibiotics?

by Félix Jaumaux, Luz P. Gómez de Cadiñanos and Philippe Gabant *

Syngulon, Rue du Bois Saint-Jean 15/1, 4102 Seraing, Belgium

* Author to whom correspondence should be addressed.

Antibiotics **2020**, *9*(8), 484; https://doi.org/10.3390/antibiotics9080484

ISSN frontiers | Frontiers in Microbiology

TYPE Original Research
PUBLISHED 14 November 2022
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AMERICAN SOCIETY FOR MICROBIOLOGY | Applied and Environmental Microbiology

| Bacteriology | Full-Length Text

Enhancing the antibacterial function of probiotic *Escherichia coli* Nissle: when less is more

Emma Bartram,[1,2] Masanori Asai,[1,3] Philippe Gabant,[4] Sivaramesh Wigneshweraraj[1,2]

OPEN ACCESS

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In vitro and *in vivo* production and split-intein mediated ligation (SIML) of circular bacteriocins

Nuria Peña[1], Michael J. Bland[2], Ester Sevillano[1], Estefanía Muñoz-Atienza[1], Irene Lafuente[1], Mohamed El Bakkoury[2], Luis M. Cintas[1], Pablo E. Hernández[1], Philippe Gabant[2] and Juan Borrero[3]*

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Publications (2)

Microbial Drug Resistance > VOL. 29, NO. 5 | Mechanisms

normal

Promising Antimicrobial Activity and Synergy of Bacteriocins Against *Mycobacterium tuberculosis*

Anandi Martin, Michael J. Bland, Hector Rodriguez-Villalobos, Jean-Luc Gala , and Philippe Gabant

Published Online: 4 May 2023 | https://doi.org/10.1089/mdr.2021.0429

antibiotics

MDPI

Article

Selective Bacteriocins: A Promising Treatment for *Staphylococcus aureus* Skin Infections Reveals Insights into Resistant Mutants, Vancomycin Sensitivity, and Cell Wall Alterations

Félix Jaumaux [1,2], Kenny Petit [2], Anandi Martin [2], Hector Rodriguez-Villalobos [3], Marjorie Vermeersch[4], David Perez-Morga [4,5] and Philippe Gabant [2]

scientific reports

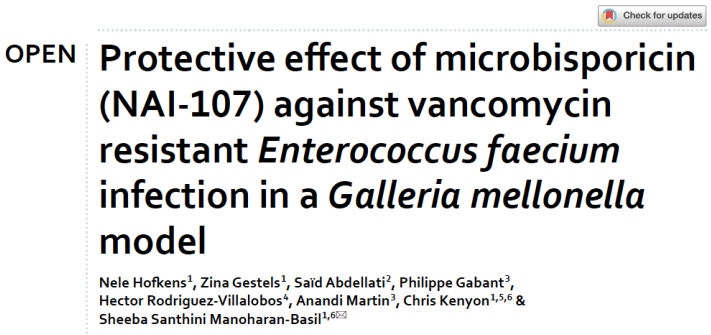
AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Spectrum

Check for updates

| Antimicrobial Chemotherapy | Research Article

Microbisporicin (NAI-107) protects *Galleria mellonella* from infection with *Neisseria gonorrhoeae*

Nele Hofkens,[1] Zina Gestels,[1] Said Abdellati,[2] Irith De Baetselier,[2] Philippe Gabant,[3] Anandi Martin,[3] Christopher Kenyon,[1,4] Sheeba Santhini Manoharan-Basil[1]



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OPEN

Protective effect of microbisporicin (NAI-107) against vancomycin resistant *Enterococcus faecium* infection in a *Galleria mellonella* model

Nele Hofkens[1], Zina Gestels[1], Saïd Abdellati[2], Philippe Gabant[3], Hector Rodriguez-Villalobos[4], Anandi Martin[3], Chris Kenyon[1,5,6] & Sheeba Santhini Manoharan-Basil[1,6]✉

INSTITUTE OF TROPICAL MEDICINE ANTWERP

Other manuscripts in preparation

LinkedIn

Patents (1)

US011932672B2

(12) **United States Patent**
Gabant et al.

(10) **Patent No.: US 11,932,672 B2**
(45) **Date of Patent: Mar. 19, 2024**

(54) **FERMENTATION PROCESS**

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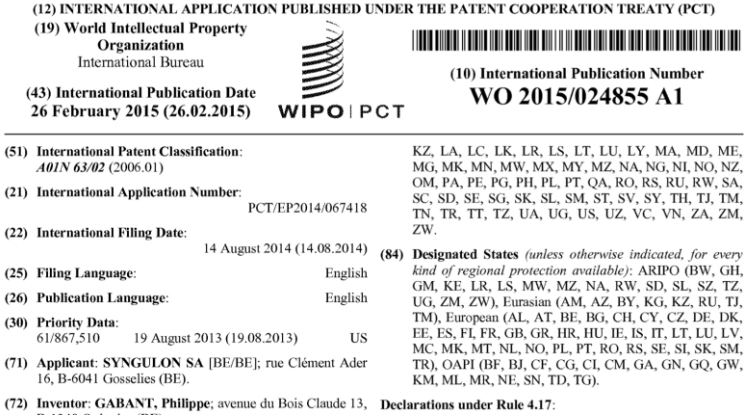
(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 401 days.

(21) Appl. No.: **16/955,720**

(22) PCT Filed: **Dec. 19, 2018**

6,270,969	B1	8/2001	Hartley et al.
6,271,359	B1	8/2001	Norris et al.
6,528,285	B1	3/2003	Biet et al.
7,176,029	B2	2/2007	Bernard et al.
7,183,097	B1	2/2007	Gerdes et al.
7,595,185	B2	9/2009	Gerdes et al.
7,595,186	B2	9/2009	Gerdes et al.
8,318,497	B2	11/2012	Szpirer et al.
8,470,580	B2	6/2013	Gabant et al.
8,476,048	B2	7/2013	Caimi et al.
8,697,426	B2	4/2014	Leana et al.
8,877,504	B2	11/2014	Gabant et al.
9,333,227	B2	5/2016	Gabant
10,188,114	B2	1/2019	Gabant
11,427,800	B2	8/2022	Gabant
11,492,651	B2	11/2022	Gabant
2004/0115811	A1	6/2004	Gabant
2005/0130308	A1	6/2005	Bernard
2005/0260585	A1	11/2005	Szpirer
2010/0330041	A1	12/2010	Bayrock
2013/0115658	A1	5/2013	Szpirer et al.
2013/0280810	A1	10/2013	Gabant et al.
2014/0148379	A1	5/2014	Liu et al.
2014/0178956	A1	6/2014	Leana et al.
2015/0050253	A1	2/2015	Gabant
2021/0238645	A1	8/2021	Gabant
2022/0017573	A1	1/2022	Mignolet et al.



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Declarations under Rule 4.17:

National Committee for Microbiology (NaCoMi)

The Royal Academies for Science and the Arts of Belgium (RASAB)

Precision microbial control: from discoveries to solutions

André Gratia

Together with

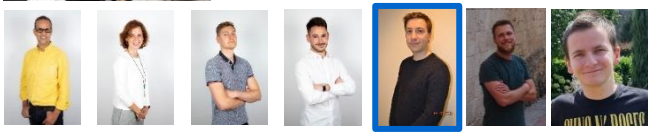
2nd Bacteriocin International Conference

Brussels

May 25 – 28, 2025

Startup in the labs

Guy Hélin, Co-founder, CEO
Dr. Philippe Gabant, Co-Founder, CSO



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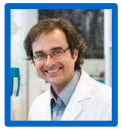

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