EXPLORING CIRCULAR BACTERIOCINS: NEW APPROACHES FOR PRODUCTION AND ENGINEERING

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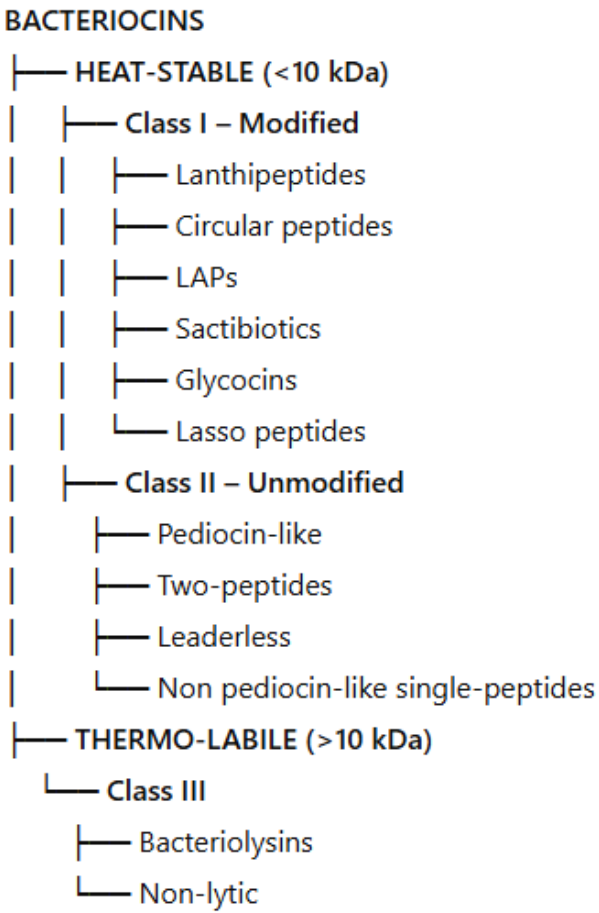
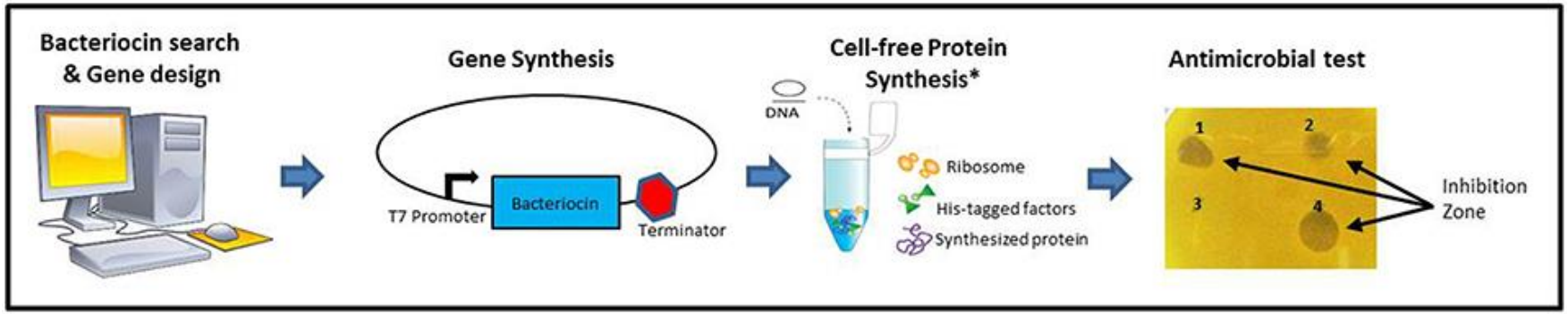
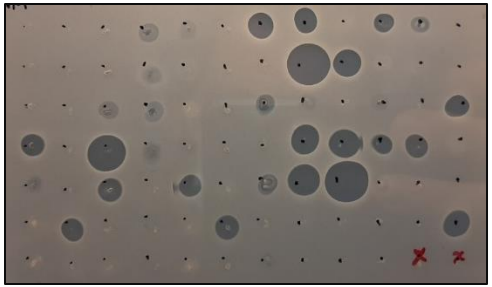

BACTERIOCINS
HEAT-STABLE (<10 kDa)
Class I – Modified
Lanthipeptides
Circular peptides
LAPs
Sactibiotics
Glycocins
Lasso peptides
Class II – Unmodified
Pediocin-like
Two-peptides
Leaderless
Non pediocin-like single-peptides
THERMO-LABILE (>10 kDa)
Class III
Bacteriolysins
Non-lytic

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

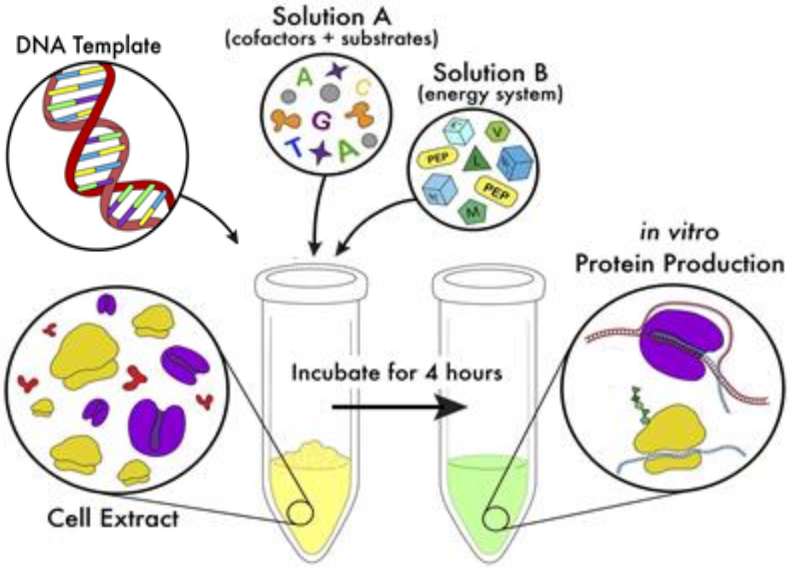
Philippe Gabant* and Juan Borrero†

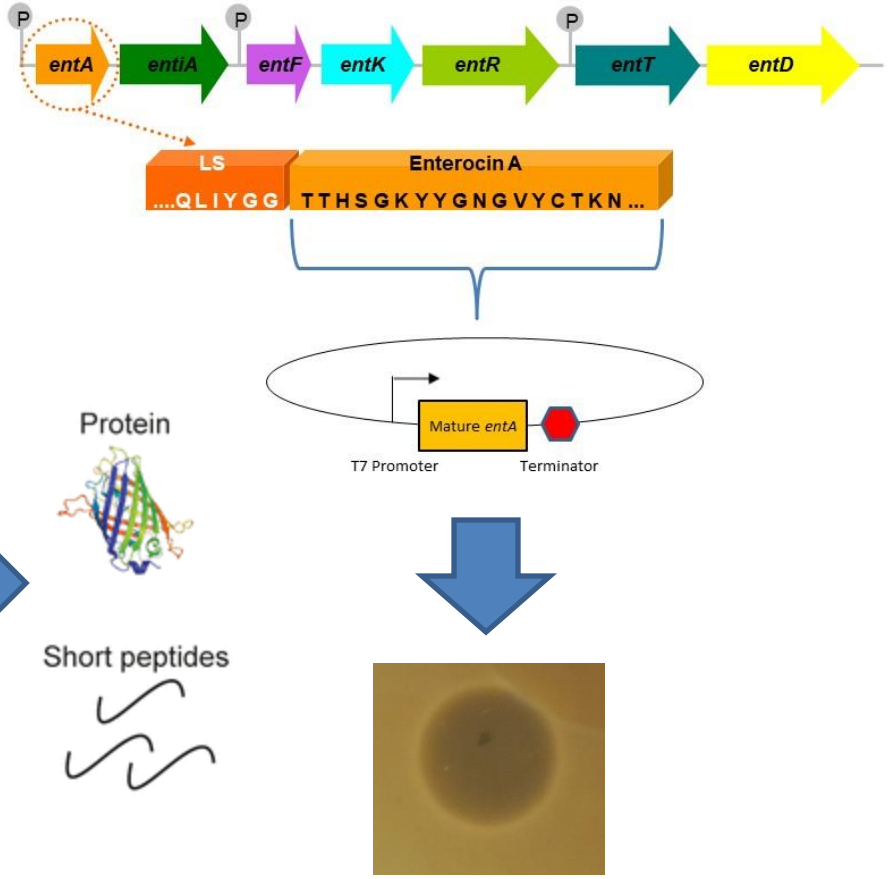




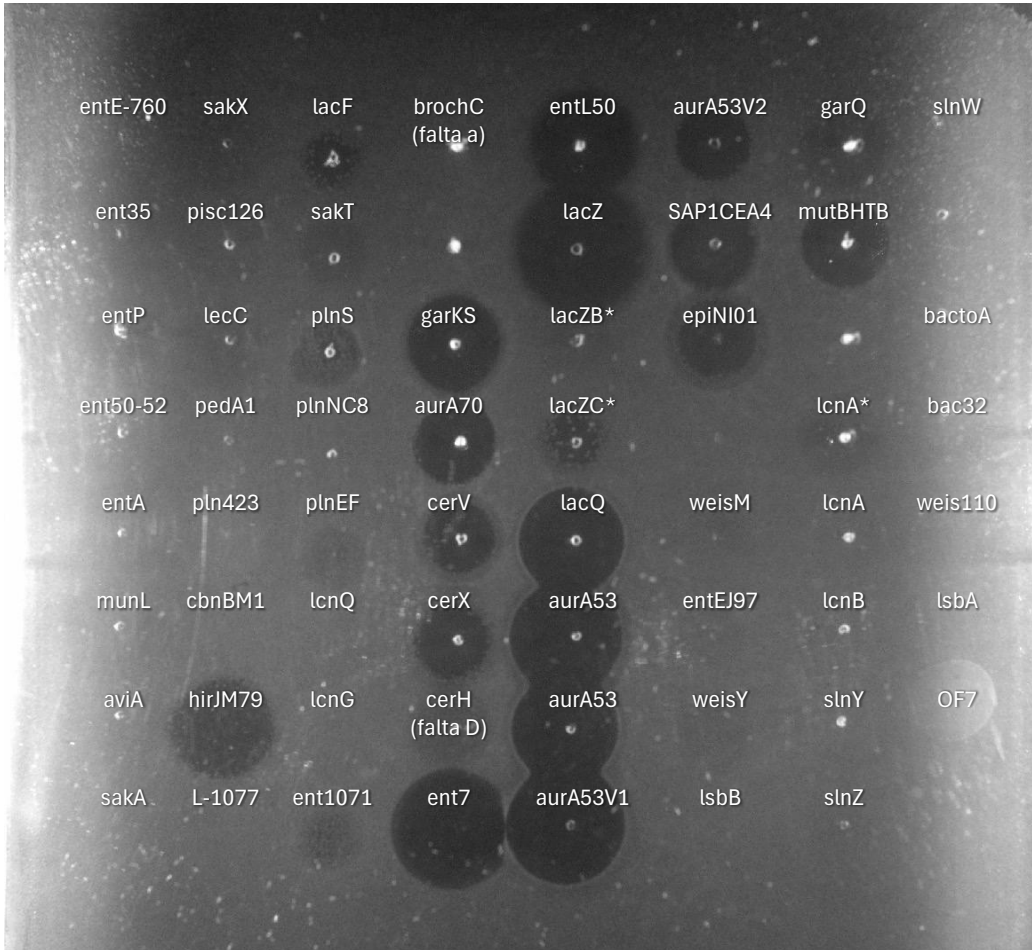
~170 bacteriocins

➢ Cell-free *in vitro* Protein Synthesis





Activity Vs *Streptococcus suis*



BACTERIOCINS

- **HEAT-STABLE (<10 kDa)**
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    - Lanthipeptides
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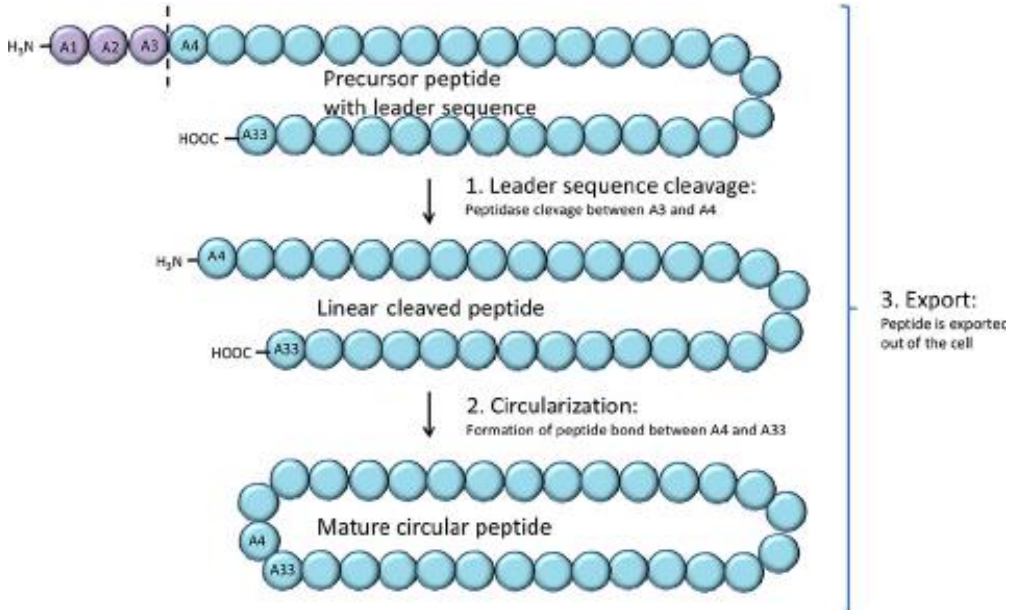
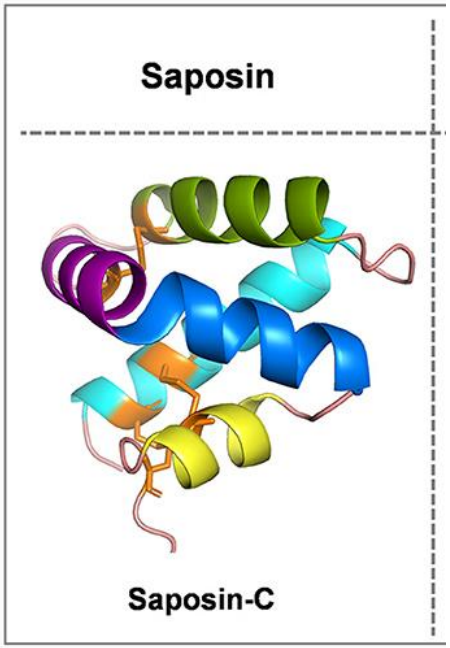
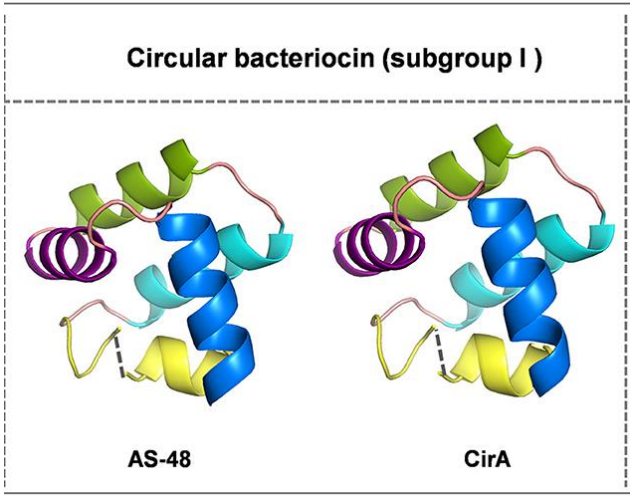
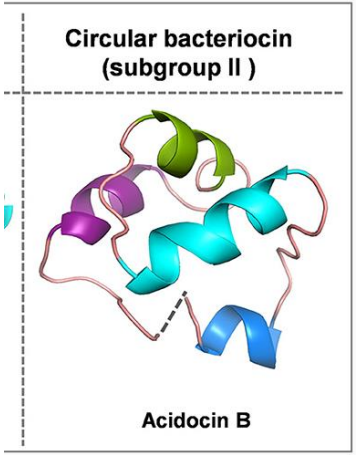


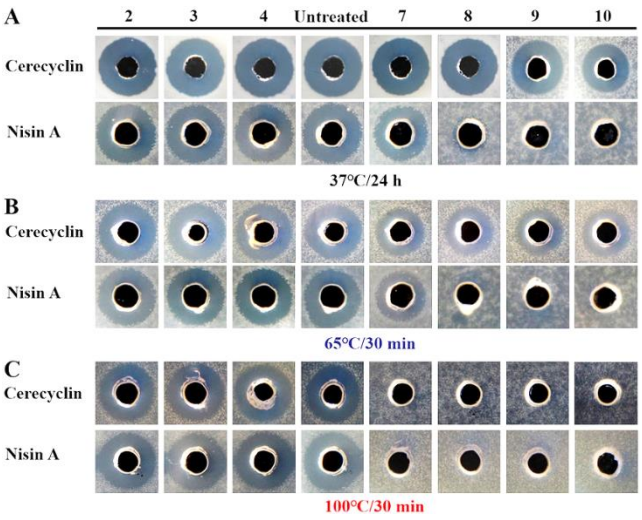


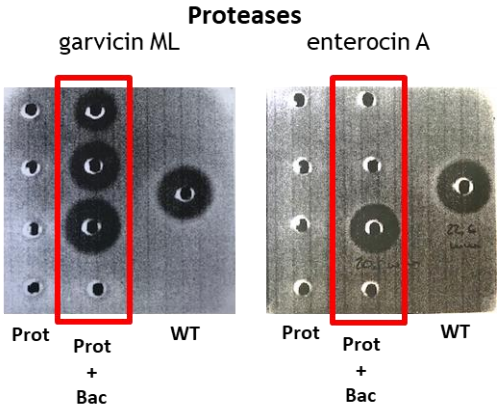




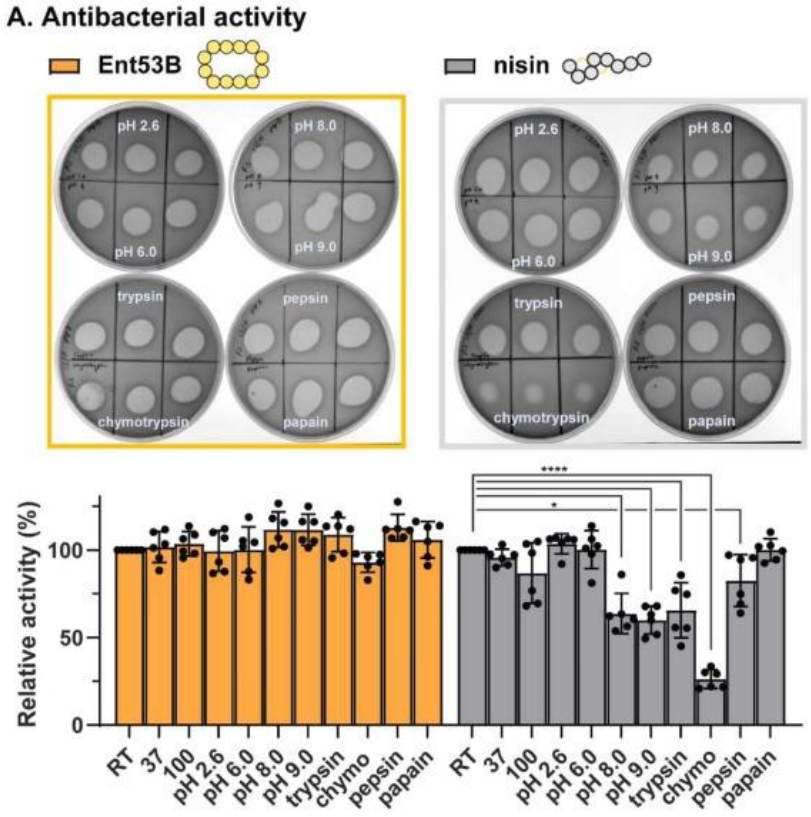
Liu *et al*. Front Microbiol. 2024

Xin et al., 2020, mSystems.





LINEAR Vs CIRCULAR



Wang et al., 2023, Peptides.

Experimentally confirmed circular bacteriocins

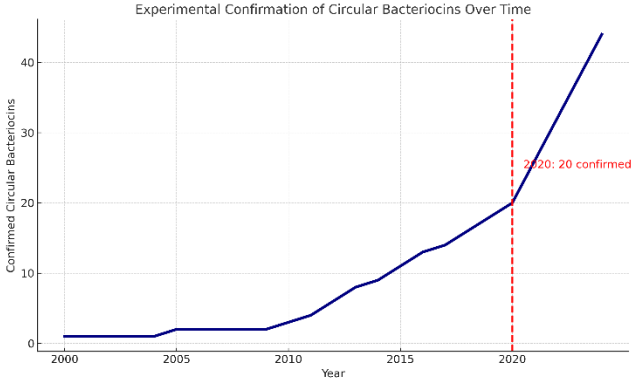
Vezina et al. BMC Microbiology (2020) 20:77
https://doi.org/10.1186/s12866-020-01772-0

BMC Microbiology

RESEARCH ARTICLE Open Access

Bioinformatic prospecting and phylogenetic analysis reveals 94 undescribed circular bacteriocins and key motifs

Ben Vezina, Bernd H. A. Rehm and Andrew T. Smith*



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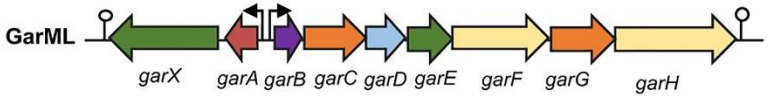
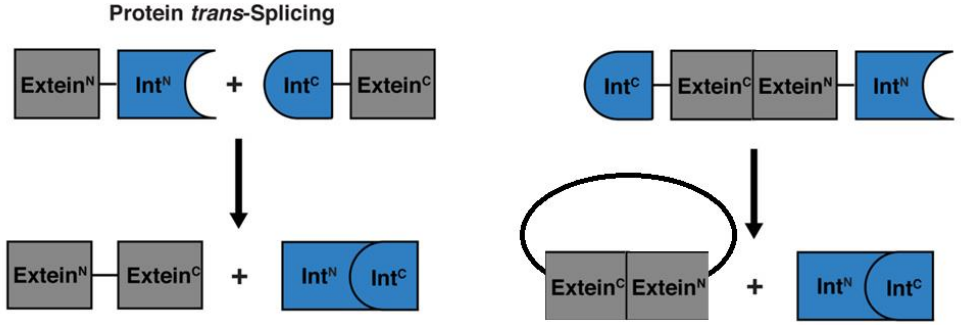
RESEARCH ARTICLE
Applied and Environmental Science

In Silico Analysis Highlights the Diversity and Novelty of Circular Bacteriocins in Sequenced Microbial Genomes

Bingyue Xin,[a,b,c] Hualin Liu,[a] Jinshui Zheng,[a,d] Chuanshuai Xie,[a] Ying Gao,[a] Dadong Dai,[a] Donghai Peng,[a] Lifang Ruan,[a] Huanchun Chen,[c] Ming Sun[a]



Inteins



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

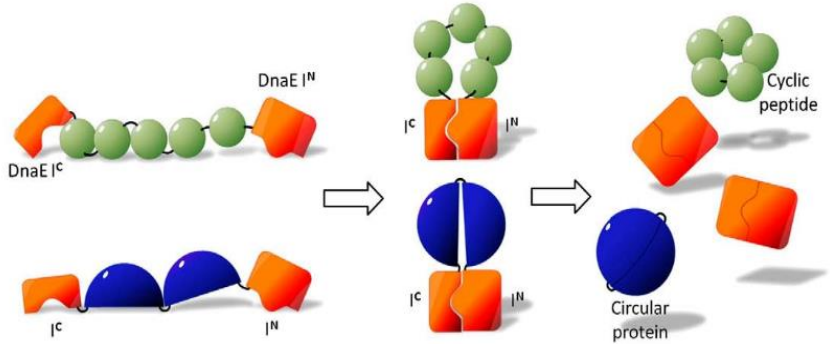
RESEARCH ARTICLE

Production of cyclic peptides and proteins *in vivo*

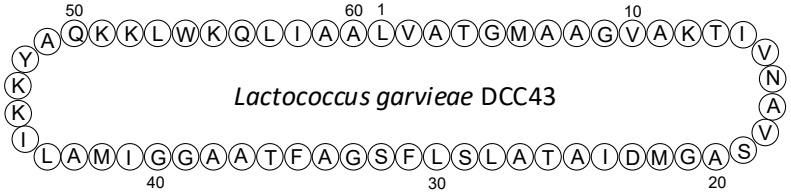
Charles P. Scott, Ernesto Abel-Santos, Mark Wall, Daphne C. Wahnon, and Stephen J. Benkovic

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

Contributed by Stephen J. Benkovic



GARVICIN ML



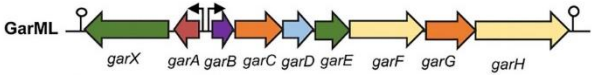
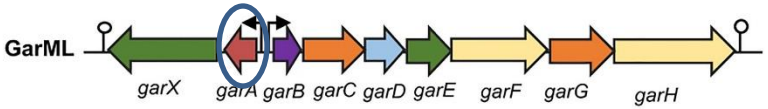


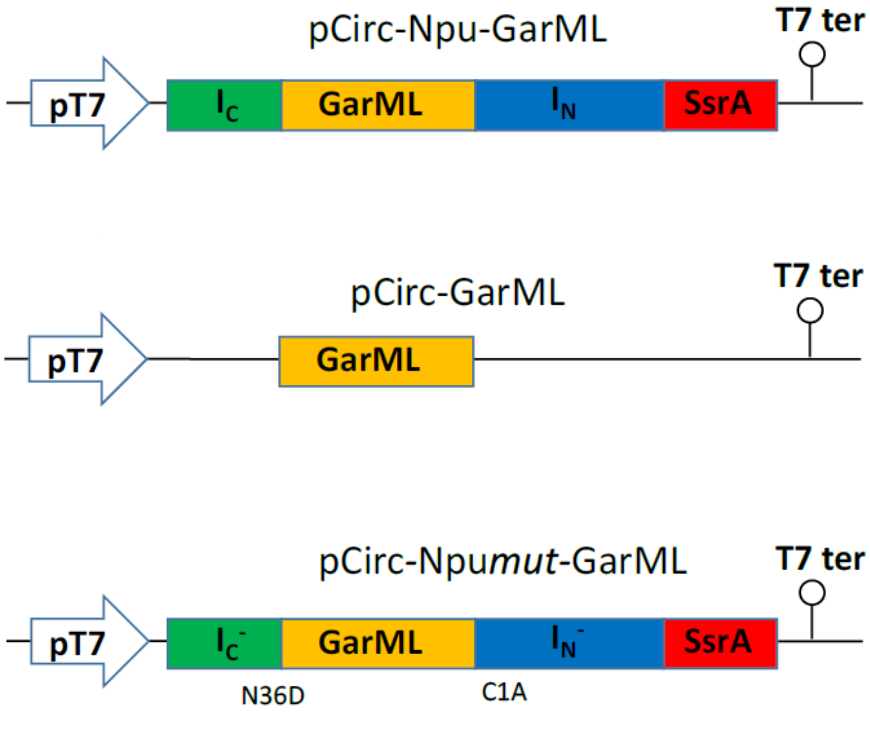
TABLE 1. Antimicrobial activities and inhibitory spectrum of fractions generated from the purification of garvicin ML produced by *L. garvieae* DCC43[a]

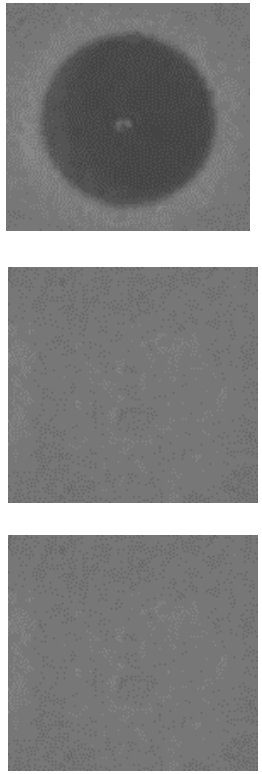
Indicator strain	Source[b]	Halo of inhibition (mm²) using indicated garvicin ML fraction[c]					
		SN	AS	GF	SE	OE	RP
Lactobacillus reuteri 20016	DSM	—[d]	—	—	51	369	378
Lactobacillus helveticus 15009	ATCC	—	—	22	26	129	492
Lactobacillus curvatus 2739	NCFB	—	—	—	—	221	177
Lactobacillus casei 334	ATCC	83	215	153	191	1,048	1,174
Lactobacillus acidophilus 4356	ATCC	—	—	—	25	149	236
Lactobacillus sakei 2714	NCFB	94	204	163	249	1,445	1,445
Lactococcus lactis BB24	FVM	97	186	150	183	941	928
Lactococcus lactis NZ9000	NIZO	—	69	37	77	163	163
Lactococcus lactis DPC5598	DPC	163	98	113	141	1,075	1,541
Lactococcus garvieae 5274	CECT	—	67	71	94	684	617
Lactococcus garvieae 5806	CECT	334	482	413	561	2,807	2,807
Lactococcus garvieae 5807	CECT	291	438	441	529	2,603	2,705
Lactococcus raffinolactis 988	CECT	—	—	—	10	228	228
Pediococcus acidilactici 347	FVM	94	193	153	235	1,398	1,398
Pediococcus pentosaceus FBB61	TNO	21	144	108	193	1,131	1,131
Enterococcus faecium P13	FVM	65	201	160	232	1,217	1,291
Enterococcus faecium L50	FVM	—	63	47	83	574	719
Enterococcus faecalis DBH18	FVM	—	—	—	—	25	25
Enterococcus faecalis P4	IFR	—	—	—	—	86	123
Propionibacterium sp. P6	NCDO	62	119	111	130	684	190
Propionibacterium acidipropionici 563	NCDO	75	153	105	132	662	864
Clostridium tyrobutyricum 3,5 CT	TNO	55	124	83	124	719	651
Clostridium tyrobutyricum 1754	NCDO	58	129	105	126	802	839
Clostridium perfringens 376	CECT	—	45	29	31	351	352
Clostridium botulinum 551	CECT	—	59	174	227	1,232	574
Listeria monocytogenes 4032	CECT	—	22	215	289	1,574	662
Listeria ivanovii 913	CECT	—	139	273	353	1,978	1,020
Listeria seeligeri 917	CECT	34	87	212	292	1,509	684
Listeria grayi 931	CECT	—	127	135	191	1,075	742
Listeria welshimeri 919	CECT	—	—	158	258	1,291	452
Brochothrix thermosphacta 847	CECT	—	—	—	31	255	283
Pseudomonas fluorescens 378	CECT	—	—	—	—	—	—
Escherichia coli JM109	Invitrogen	—	—	—	—	—	—
Escherichia coli MC1000	NIZO	—	—	—	—	—	—
Salmonella paratyphi 554	CECT	—	—	—	—	—	—
Salmonella Typhimurium 443	CECT	—	—	—	—	—	—
Salmonella enteritidis 4396	CECT	—	—	—	—	—	—
Streptococcus pneumoniae FQ26	HRC	69	156	100	110	599	590
Streptococcus pneumoniae 67620	HRC	65	135	94	128	707	596
Streptococcus pneumoniae 15 M-1047	HRC	58	128	96	139	790	790

Borrero *et al*. Appl Environ Microbiol. 2011

Cell-free *in vitro* Protein Synthesis

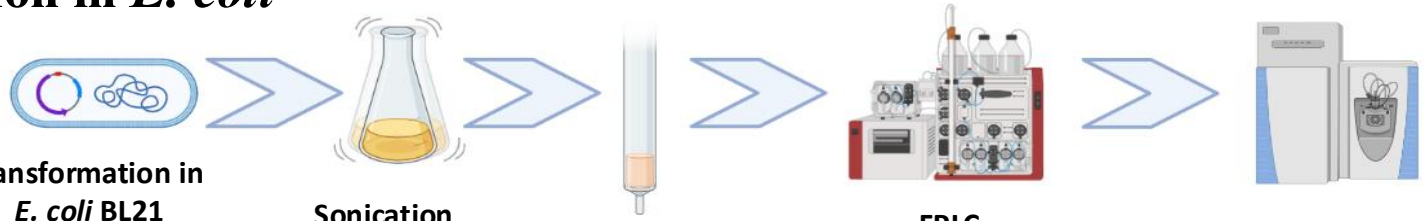


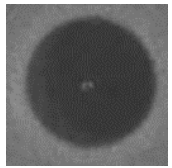


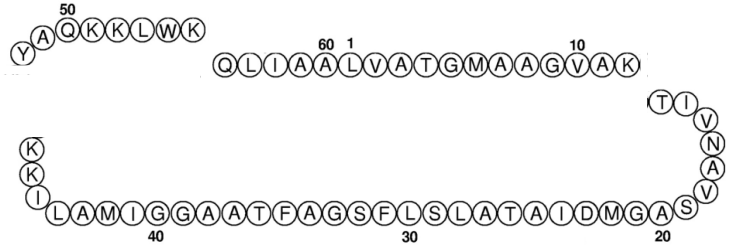


Peña et al. Front Microbiol. 2022

Production in *E. coli*







Skyline		
Sequence	E-1+2	E-3
QLIAALVATG**M**AAGVAK	Si	Si(MS/MS)
QLIAALVATGMAAGVAK	Si (MS/MS)	Si(MS/MS)
IAALVATG**M**AAGVAK	No	Si(MS/MS)
LVATG**M**AAGVAK	No	Si(MS/MS)
LVATGMAAGVAK	Si(MS/MS)	No
YAQKKLWK	Si(MS/MS)	Si(MS/MS)
TIVNAVSAG**M**DIATALSLFSGAFTAAGGIMALIKK	No	Si (MS/MS)
TIVNAVSAGMDIATALSLFSGAFTAAGGIMALIK	No	Si(MS/MS)
TIVNAVSAG**M**DIATALSLFSGAFTAAGGIMALIK	No	Si (MS/MS)
TIVNAVSAG**M**DIATALSLFSGAFTAAGGI**M**ALIK	No	Si (MS/MS)

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Characterized Circular bacteriocins

Non Characterized Circular bacteriocins

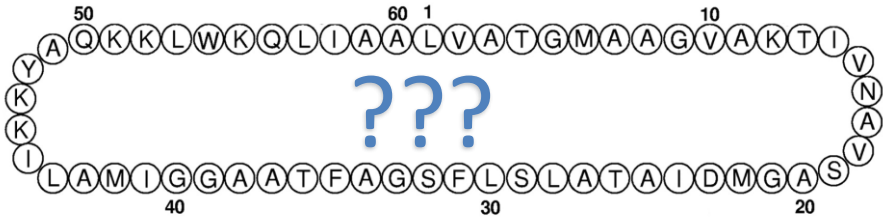
Bacteriocin	Producer	Plasmid	Activity against *L. lactis* IL1403*
Amylocyclicin	*Bacillus amyloliquefaciens* FZB42	pCirc-Npu-Alc	+
Enterocin AS-48	*Enterococcus faecalis* S-48	pCirc-Npu-EntAS48	+
Carnocyclin A	*Carnobacterium maltaromaticum* UAL307	pCirc-Npu-CarA	+
Circularin A	*Clostridium beijerinckii* 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	–

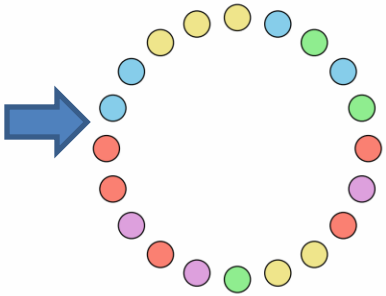
*Samples showing a clear halo of inhibition (+) or no halo of inhibition (–).

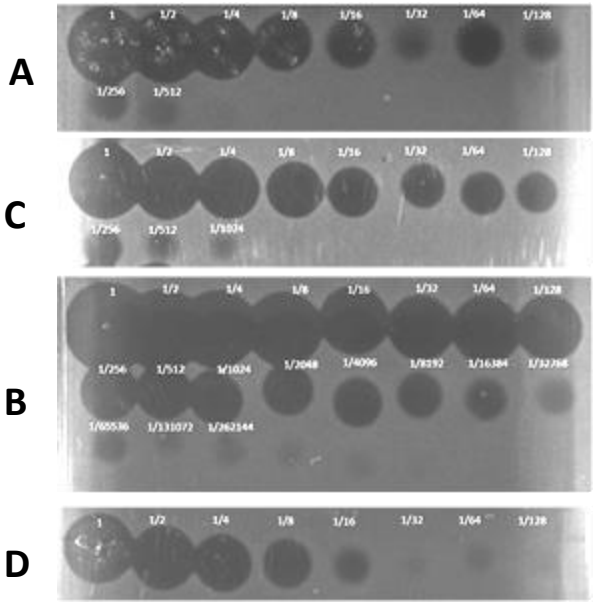
Peña et al. Front Microbiol. 2022

IMPROVEMENTS OF THE SIML SYSTEM

TESTING DIFFERENT CIRCULARIZATION SITES

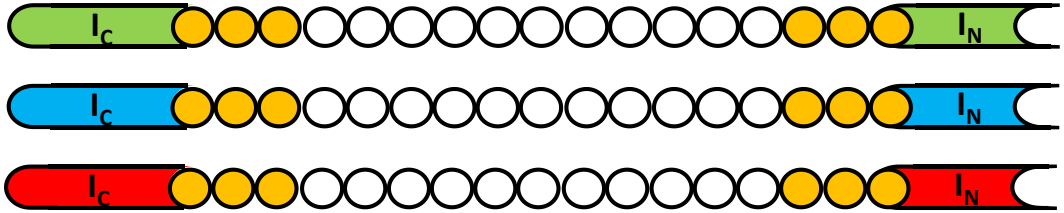




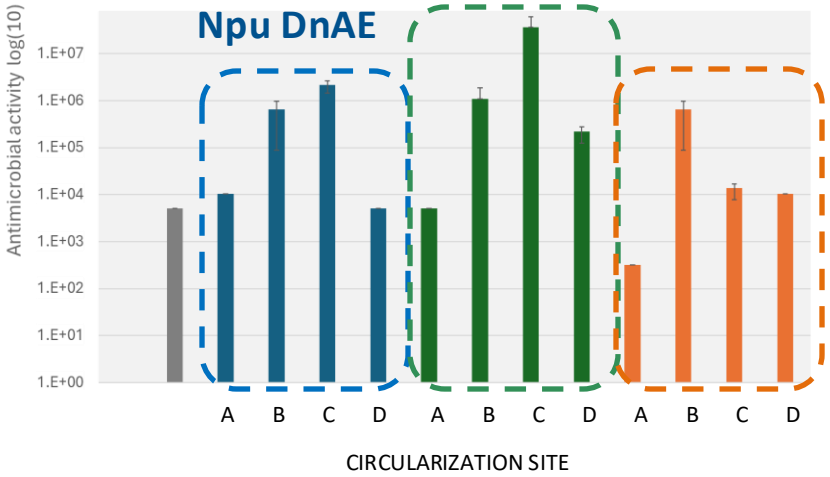


Unpublished results

IMPROVEMENTS OF THE SIML SYSTEM



TESTING DIFFERENT INTEINS



Unpublished results

Table 1. Antimicrobial activity of variants of circular bacteriocins against selected indicator bacteria.

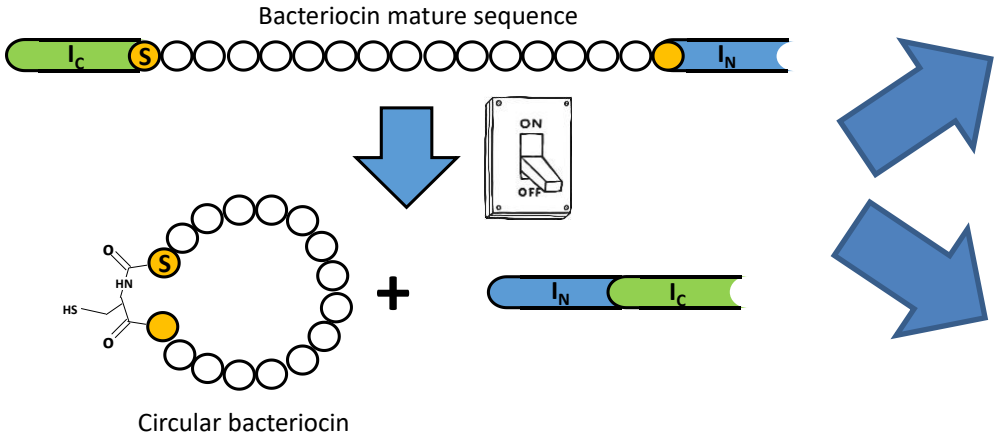
	P. damnosus	*L. garvieae*	*E. faecium*	*L. monocytogenes*	*S. aureus*	*S. suis*	*B. cereus*	*E. rhusiopathiae*	*S. agalactiae*	*P. larvae*	*C. perfringens*	*E. coli*
BAC1	+++	+++	+++	+++	-	+++	+++	+++	+	+++	++	-
BAC2	+++	+++	+++	+++	+	+++	+++	+++	-	+++	++	-
BAC3	++	+	+	+++	-	+	+++	-	-	+++	-	-
BAC4	++	++	-	++	-	-	++	+	-	+	-	-
BAC5	++	+	-	+	-	-	++	-	-	++	-	-
BAC6	++	++	+	+	-	+	++	++	-	+	+	-
BAC7	+				-	-	-	-	-	-	-	-
BAC8	+				-	-	-	-	-	-	-	-
BAC9	++				-	-	+	-	-	-	-	-
BAC10	+++				-	+++	-	+	-	-	+	-
BAC11	+++				-	+	-	++	-	++	-	-
BAC12	++				-	-	-	-	-	-	-	-
BAC13	-				-	-	-	+	-	-	-	-
BAC14	++				-		-	+++	-	++		-
BAC15	+				-	-	-	-	-	-	-	-
BAC16	+++				-	+	++	-	+	+	+	+
BAC17	+++				-	+	++	-	-	+	-	+
BAC18	+++				-	+	++	-	+	++	+	++
BAC19	+++				-	+	++	-	-	+	-	
BAC20	+				-	+	-	-	-	-	-	-
BAC21	++				-	++	-	+	-	-	+	-
BAC22	++	++	+	++	-	+	-	-	-	-	-	-
BAC23	++	++	+	+	-	+	-	++	-	-	+	-
BAC24	++	-	-	+	-		-	-	-	-		-
BAC25	+++	+++	+++	+	-	++	-	-	-	-	++	-
BAC26	+++	+	-	++	-	+	-	-	-	-	-	-
BAC27	-	-	-	-	-	-	-	-	-	-	-	-

MAGADAN MAMMOTH RECOVERED FROM THE PERMAFROST 1977

Antimicrobial activity performed by a spot-on-agar-test (SOAT) and calculated as the diameter of the zone of inhibition, -: no inhibition, +: <5 mm, ++: 5–10 mm, +++: >10 mm.

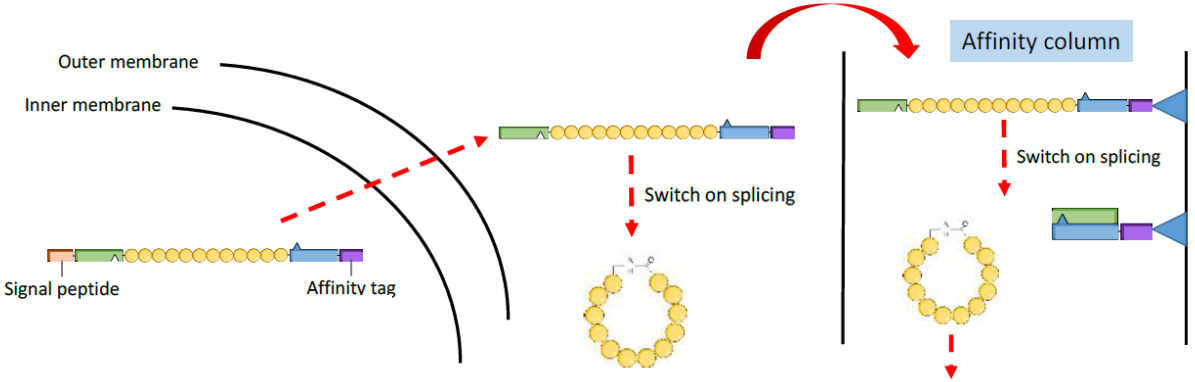
Unpublished results

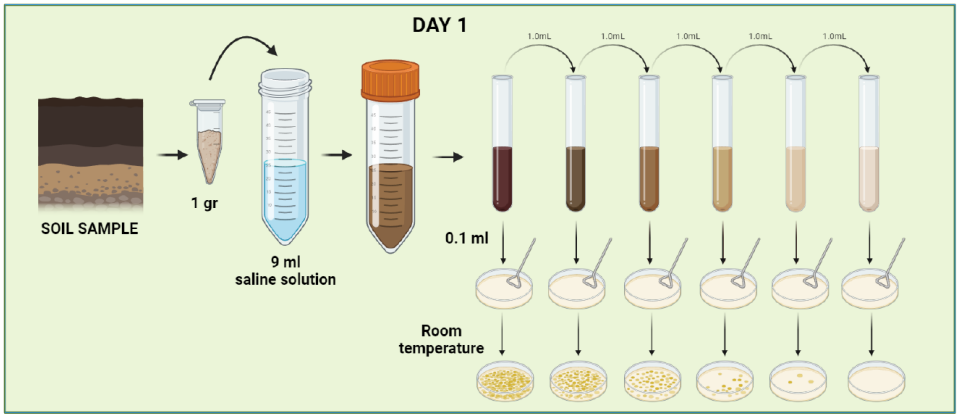
SWITCHABLE INTEINS FOR CONDITIONAL PROTEIN SPLICING

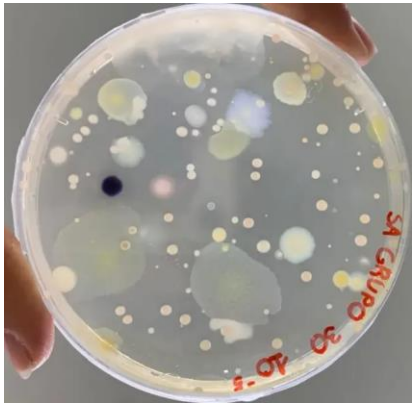


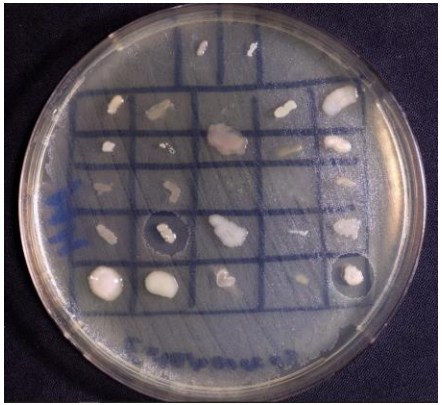
INCREASE PROTEIN YIELDS

IMPROVE PURIFICATION PROCESS









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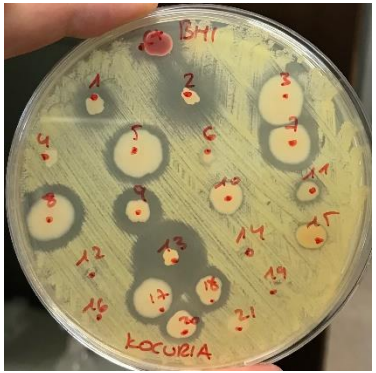
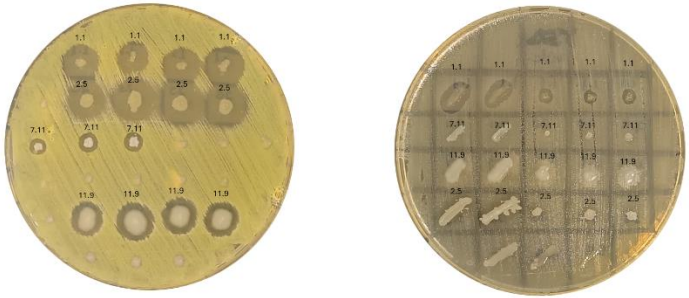
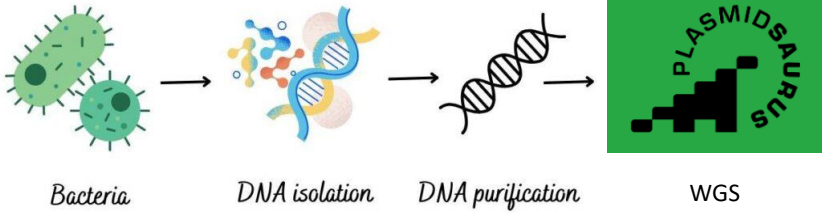


Gram (+)
Kocuria rhizophila

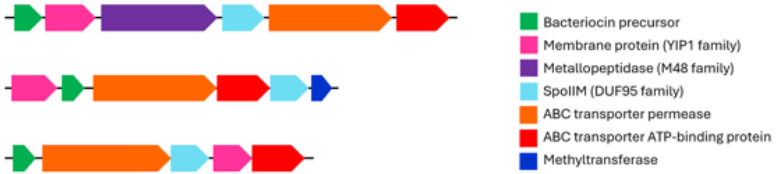
Gram (-)
E. coli Nissle 1917



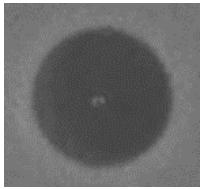
1.1. *Paenibacillus polymyxa*
2.5. *Bacillus pumilus*
7.11. *Staphylococcus epidermidis*
11.9. *Bacillus cereus*



BAGEL4



SIML



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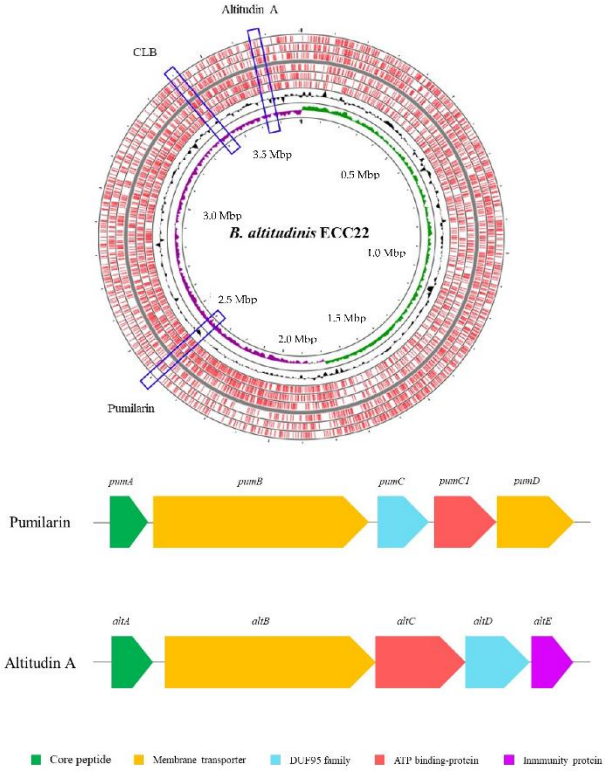
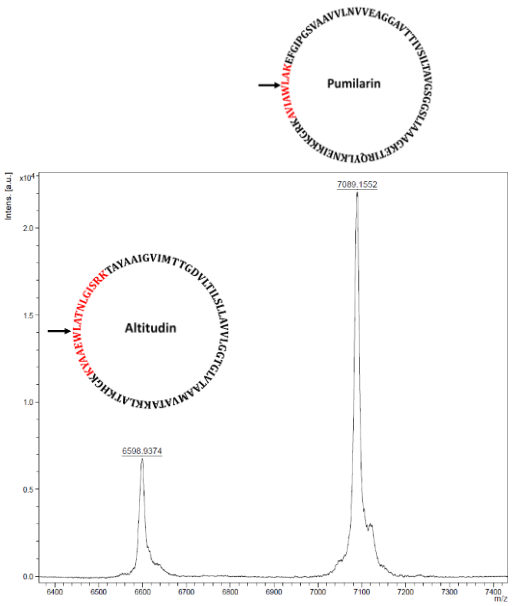
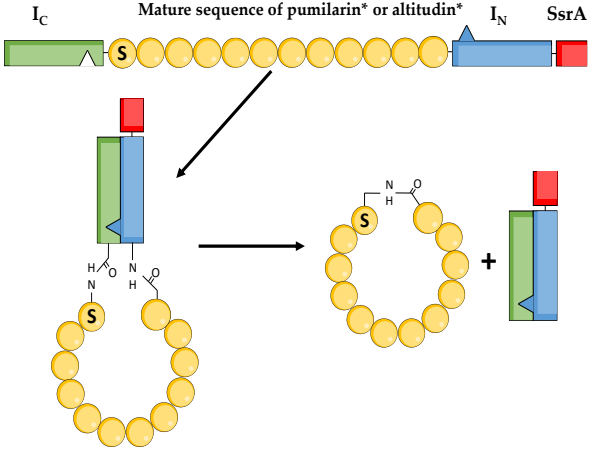
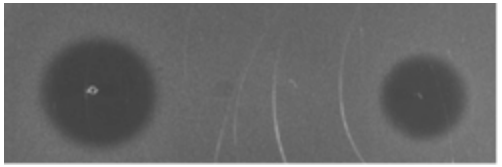
Production of Pumilarin and a Novel Circular Bacteriocin, Altitudin A, by *Bacillus altitudinis* ECC22, a Soil-Derived Bacteriocin Producer

by Irene Lafuente [1], Ester Sevillano [1], Nuria Peña [1], Alicia Cuartero [2], Pablo E. Hernández [1], Luis M. Cintas [1], Estefanía Muñoz-Atienza [1,*] and Juan Borrero [1,*]





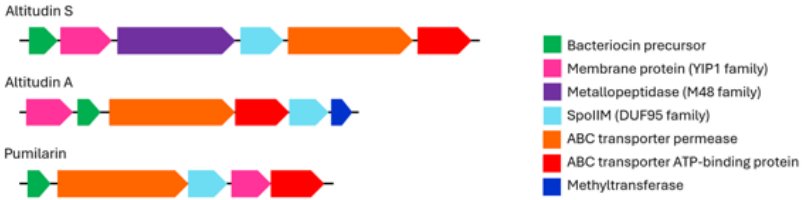


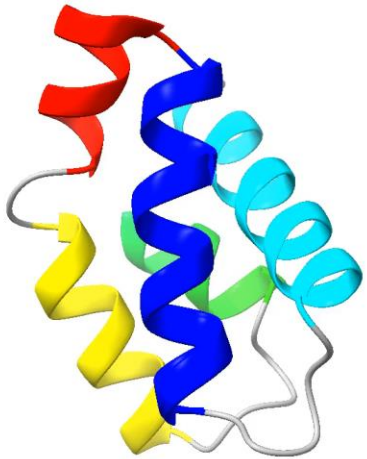


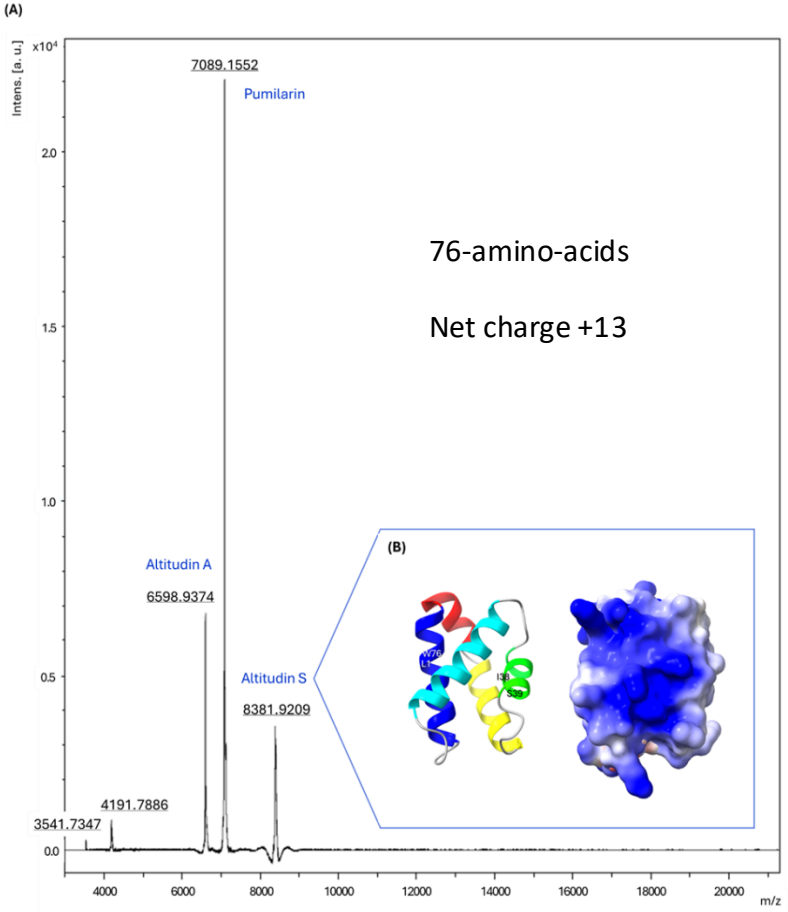
Pumilarin Altitudin A

Lafuente et al., IJMS, 2024

ALTITUDIN S







76-amino-acids

Net charge +13

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PhD students:
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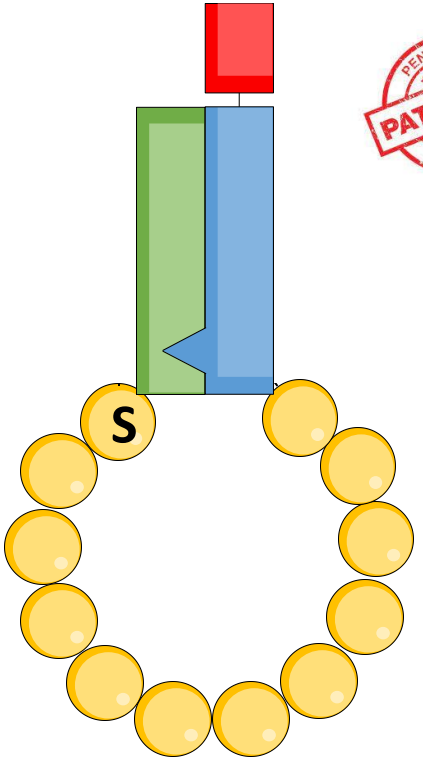
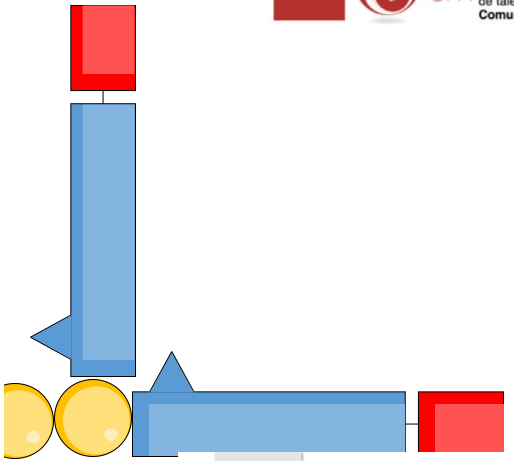
Ester Sevillano
Irene Lafuente





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