Supplementary material:

S. pneumoniae 801
MNNSIMASEFGLNKIEKAFLFS--LLVSVVTIVAVG-N=IYWVCGKFGIELAPGWYQDIVD FVSSGGTIVEAFAAVAGVTLPAWVGPVLAAFGLASA

Streptococcus sp. 2_136FA
MLIAVG-N=IYWICGKFGIRLAPGWYQDIVD FVSAGGSIVDAFAIIAGITLPAWIAPVLAGFGVVSA

B. fibrisolvens AR10 (Butyrivibriocin AR10)
MSKKQIMS--NCISIALLIALIPN=IYFIADKMGIQLAPAWYQDIVN WVSAGGTLTTGFAIIVGVTVPAWIAEAAAAFGIASA

L. (para)casei
MTKMEKTVLTIGSFVIAALMVTILFTN=IYFIANKLGIHLAPGWYQDMVN YVSAGGSLAGAFSVVAGVTLPAWIVPIATAFGAVSA

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Figure S1. Alignment of peptides identified with BAGEL3 that show similarity to Butyrivibriocin AR10 (1). The end of the (predicted) leader is indicated with an = sign. The butyrivibriocin AR10 variants we identified are highly homologous except for the leader sequences.

C. saccharolyticus DSM 8903	3MLF	=VAA	TLG	IPET	LATT	IVN	IIL	TGS	FIV S	IIL	ALT	SILT	AGVS	AVILA	AGGN	ITAFI	NLV	KSI	<va< th=""><th>QLG</th><th>LRGA</th><th>ILW</th></va<>	QLG	LRGA	ILW
C. bescii DSM 6725	MLY	=VAA	TLG	IPET	LAVT	IVN	IIL	TGS	FIV S	III	ALT	SILT	AGVS	AIIL	EGGW	ITAFI	NLV	KSF	(VA	QLG	LRGT	ILW
G. kaustophilus HTA426	MSLLAL	=VAG	TLG	VSQS	IATT	VVS	IVL	TGS	FLIS	IIL	GIT.	AILS	GGVD	AILE	IG-W	ISAFV	ATV	KKI	IVA	ERG	KAAA	IAW
Geobacillus sp C56 T3	MSLLAL	=VAG	TLG	VSQA	VATT	VVS	IVL	TGS	FLIS	IIL	GIT.	AILS	GGVD	AILE	IG-W	ISAFV	ATV	KKI	IVA	ERG	KAAA	IAW
B. cereus H308197	-MGLFH	=VAS	KFK	VSQG	IAGG	VVA	AVL	NAG	FLIS	AIG	AVT	VVMS	GGID	AILE	/G-W	ITAFV	ATV	KEζ)AA	KRG	TAGA	VAW
B. cereus IS075	-MGLFH	=VAS	KFK.	ISQG	IAGG	VVA	AVL	NAG	FLI S	AIG	AVT	VVMS	GGID	AILE	/G-W	TAFV	ATV	KEς	DAA	KRG	TAGA	VAW
B. cereus H308197C	-MDLFH	=VAS	KLS	ISKG	VAAG	VVS	TVL	NAG	FLI T	AIG	AVT	VALS	GGID	AILEV	/G-W	IATEV	TV	KEÇ	DAA	KRG	TAGA	VAW
B. coagulans XZL9	-MGLFH	=VAS	KFH	VSAG	IASG	VVT	AVL	HAG	FIAS	IIG	AVT	VVMS	GGVD	AILD	1G-W	TAFI	AEV	KHI	AK	EYG	KKRA	IAW
C. beijerinckii (Circularin A)	MFL	=VAG	ALG	VQTA	AATT	IVN	VIL	NAG	LVI	VLG	IIA	SIAS	GGAG	TLMT	IG-W	ATEK	ATV	QKI	AK	QS-	MARA	IAY
C. perfringens E str JGS1987	7MFD	= LAG	MLG	VDFY	AAKK	VID	IVA	TAG	FVAS	VIG	IVA	AVAG	TGG	IG	/G	II	AAA	KAI	AK	KYG	TKAA	ATW
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Figure S	2. Alignment	of peptide	s identified	with	BAGEL3	that	show	similarity	to	Circularin-A	(2).	The e	nd c	of the
(predicted) leader is ind	dicated with	an = sign.											

B. thuringiensis IBL 4222	MFE =	IMGYFG	VDRGWATKI	VNAI DAVGW	GVAAASAIA	AIVTAGGL	VTSAMID	VAIFTVKD	FLRRNLKAQAV	VAW
B. thuringiensis HD 789	MFE =	IMGYFG	VDRGWATKI	VNAIDAVGW	GVAAASAIA	AIVTAGGLA	VTSAMID	VAIFTVKD	FLRRNLKAQAV	VAW
B. cereus VD184	MFE =	• VMGYFG	VDRGWATKI	VNAIDAVGW	GVAAAGTIA	AIISAGGLA	VTSAMIDA	AAIITVKS	YLKRNLKAQA	IAW
Bacillus sp. 1NLA3E	MLE=	AMGFFG	VGKTLATQI	VNVVDAVGY	AAIAVSTIM	AILSAGGL	APTAAAID	FAIIYIKK	KIANNLKAQA:	IVW
P. larvae B3650	MKGGILLFQ=	IAGYLG	ISTGMAKKI	VDVIDAAGW	AFVAVSTIM	AILSAGGL	VTSAMVD	YAIIYVKD	LLKRNLKAQA:	IVW
S. uberis 0140J (Uberolysin)	MDILLE=	LAGYTG	IASGTAKKV	VDAIDKGAA	AFVIISIIS	TVISAGALO	GAVSASAD	FIILTVKN	YISRNLKAQAV	VIW
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Figure S3. Alignment of peptides identified with BAGEL3 that show similarity to Uberolysin (3). The end of the (predicted) leader is indicated with an = sign.

Strain	Relevant properties	Reference
B. pumilus B4107	Pumilarin producer	Genome announcement
B. pumilus KMM62	Indicator strain	Laboratory collection
B. subtilis 168	Indicator strain	Laboratory collection
<i>E. faecalis</i> VE14089	Indicator strain	Laboratory collection
<i>E. faecalis</i> JH2-2	Indicator strain	Laboratory collection
<i>E. faecalis</i> V583	Indicator strain	Laboratory collection
S. pneumoniae D39	Indicator strain	Laboratory collection
<i>M. flavus</i> B423	Indicator strain	NIZO food research

Supplementary Table 1. Strains used in this work.

L. monocytogenes	Indicator strain	Laboratory collection				
B. cereus ATCC14579	Indicator strain	Laboratory collection				
S. aureus RN6390B	Indicator strain	Laboratory collection				
<i>E. coli</i> DH5a	Indicator strain	Laboratory collection				
Lactococcus lactis NZ9000	Indicator strain	Laboratory collection				
Mycobacterium smegmatis	Indicator strain	Laboratory collection				
S. aureus MRSA CAL	MRSA Indicator strain	Girbe Buist UMCG				
S. aureus MRSA MW2	MRSA Indicator strain	Girbe Buist UMCG				



Figure S4. Distribution of the identified circular bacteriocins over the different taxa. In total 59 unique (at least one AA difference in the leader or core peptide) sequences were identified.



Figure S5. HPLC profile at 280 nm of pumilarin (control) and pumilarin cleaved with different proteases. Light green corresponds to the uncleaved peptide, dark green to the proteolysis with trypsin, red with a-chymotrypsin, brown with proteinase K, blue with endoprotease Glu-C, pink with pepsin and purple with protease XIV. The separation was performed according to (4).

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- 3. Wirawan RE, Swanson KM, Kleffmann T, Jack RW, Tagg JR. Uberolysin: a novel cyclic bacteriocin produced by *Streptococcus uberis*. Microbiology. 2007 May;153(Pt 5):1619–30.
- 4. Montalbán-López M, Spolaore B, Pinato O, Martínez-Bueno M, Valdivia E, Maqueda M, et al. Characterization of linear forms of the circular enterocin AS-48 obtained by limited proteolysis. FEBS Lett. 2008 Sep 22;582(21-22):3237–42.