1 **Supplemental Material**

2 TABLE S1. Chemical shift assignments for acidocin B.

	HN	Hα	Hβ	Others
Ile-1	7.95	3.81	1.95	γCH_3 0.81, γCH_2 1.25, 1.54, δCH_3 0.83
Tyr-2	7.75	4.27	3.26, 3.09	δCH 7.20, εCH 6.83
Trp-3	8.24	4.31	3.63, 3.44	δ_1CH 7.36, εNH 10.04, ζ_2CH 7.43, η_2CH 7.10, ζ_3CH 6.94, ε_3CH 7.50
Ile-4	8.50	3.56	2.12	γCH_3 1.01, γCH_2 1.33, 2.21, δCH_3 0.98
Ala-5	8.06	4.21	1.53	
Asp-6	8.08	4.37	2.75, 2.63	
Gln-7	8.06	3.72	1.36	γCH_2 1.00, 1.57, εNH_2 6.39, 6.33
Phe-8	7.83	4.55	3.34, 2.83	δCH 7.33, εCH 7.23, ζCH 7.19
Gly-9	7.86	3.98, 3.85		
Ile-10	7.62	4.09	1.68	γCH_3 0.76, γCH_2 1.10, 1.42, δCH_3 0.79
His-11	8.20		3.25, 3.07	
Leu-12	8.12	4.45	1.67	γCH 1.55, δCH_3 0.88
Ala-13	8.26	4.43	1.45	
Thr-14	8.54	3.99	4.25	γCH_3 1.27
Gly-15	8.30	4.25		
Thr-16	7.98	3.90	4.17	γCH_3 1.47
Ala-17	8.54	3.99	1.47	
Arg-18	7.91	3.93	1.91	γCH_2 1.78, 1.68, δCH_2 3.22, 3.30, εNH 7.20
Lys-19	7.75	4.13	1.98	γCH_2 1.45, δCH_2 1.61, 1.73, εCH_2 2.99
Leu-20	7.93	4.13	1.86, 1.82	γCH 1.64, δCH_3 0.92, 0.89
Leu-21	8.17	3.99	1.76, 1.69	γCH 1.56, δCH_3 0.90, 0.88
Asp-22	8.36	4.41	2.85, 2.71	
Ala-23	7.77	4.23	1.60	
Val-24	7.99	3.96	2.24	γCH_3 0.98, 1.07
Ala-25	8.31	4.28	1.49	
Ser-26	7.92	4.39	4.00, 4.04	
Gly-27	8.23	4.03, 3.96		
Ala-28	8.04	4.37	1.43	
Ser-29	8.21	4.52	3.95	
Leu-30	8.23	4.24	1.76, 1.70	γCH 1.56, δCH_3 0.91, 0.86
Gly-31	8.40	4.01, 3.90		
Thr-32	7.95	4.11	4.28	γCH_3 1.26
Ala-33	8.06	4.20	1.42	
Phe-34	8.24	4.34	3.17	δCH 7.21, εCH 7.23, ζCH 7.16
Ala-35	7.95	3.99	1.49	
Ala-36	7.80	4.17	1.52	
Ile-37	7.98	3.92	1.96	γCH_3 0.93, γCH_2 1.69, 1.25, δCH_3 0.86
Leu-38	7.64	4.14	1.68, 1.63	γCH 1.52, δCH_3 0.80, 0.78

Gly-39	7.84	4.06, 3.85		
Val-40	7.35	4.24	2.18	γCH_3 0.93, 0.97
Thr-41	7.98	4.39	4.04	γCH_3 1.16
Leu-42	8.32	4.31	1.61	δCH_3 0.82, 0.74
Pro-43		4.38	2.25, 1.91	γCH_2 1.23, 1.01, δCH_2 3.77, 3.01
Ala-44	8.65	3.99	1.53	
Trp-45	7.59	4.47	3.48, 3.34	$\delta_1 CH$ 7.55, εNH 10.31, $\zeta_2 CH$ 7.44, $\eta_2 CH$ 7.04, $\zeta_3 CH$ 6.88, $\varepsilon_3 CH$ 7.27
Ala-46	6.72	3.89	0.79	
Leu-47	7.31	3.98	1.71	γCH 1.58, δCH_3 0.84, 0.88
Ala-48	7.95	4.18	1.54	
Ala-49	8.05	4.18	1.60	
Ala-50	8.11	4.01	1.48	
Gly-51	8.13	3.94		
Ala-52	7.92	4.01	1.49	
Gly-54	8.23	4.05, 3.95		
Ala-55	8.04	4.22	1.86	
Thr-56	7.89	4.12	4.32	γCH_3 1.34
Ala-57	8.16	4.38	1.57	
Ala-58	8.23	4.31	1.50	

TABLE S2. Structure calculation statistics for acidocin B.

NOE restraints	909
short-range, |i-j|≤1	558
medium-range, 1<|i-j|<5	296
long-range, |i-j|≥5	55
average target function value	0.37 ± 0.06
RMSD for residues 1-58 (full peptide)	
backbone atoms (Å)	1.48 ± 0.47
heavy atoms (Å)	1.82 ± 0.50
RMSD for the α-helices	
backbone atoms (Å)	1.26 ± 0.44
heavy atoms (Å)	1.47 ± 0.50
Ramachandran Plot	
Φ/Ψ in most favored regions	74.6%
Φ/Ψ in additionally allowed regions	25.4%
Φ/Ψ in generously allowed regions	0.0%
Φ/Ψ in disallowed regions	0.0%

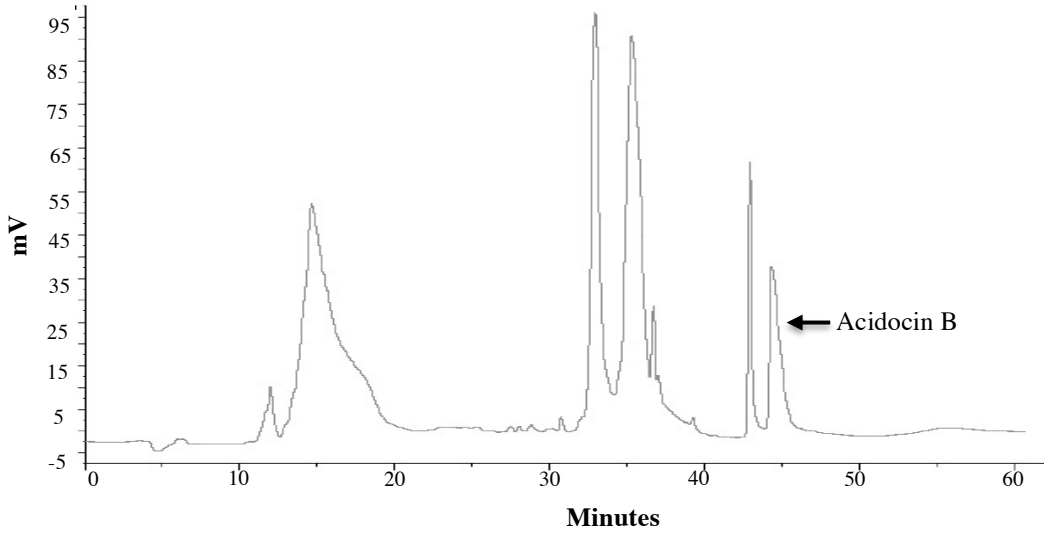

FIG. S1. RP-HPLC trace of acidocin B from *L. acidophilus* M46. Acidocin B eluted at 44 min.

FIG. S2. Superimposition of the 20 lowest energy conformers calculated for acidocin B. The arrow indicates the linkage of the N- and C-termini.

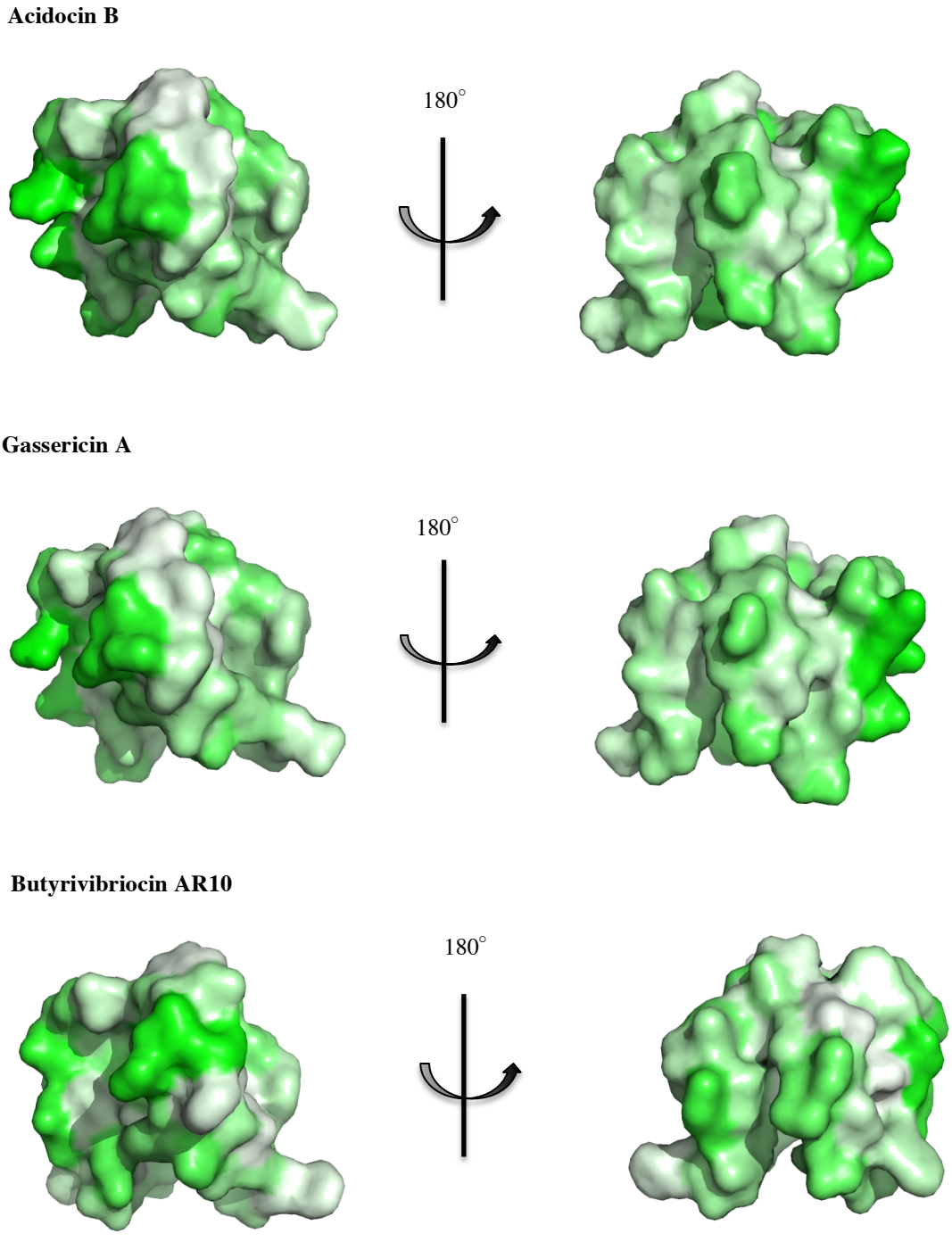

FIG. S3. Hydrophobic surface maps of the predicted structures of gassericin A and butyrivibriocin AR10 derived from homology modeling (SWISS-MODEL) (1) using the structure of acidocin B as template. Structures were generated using PyMOL (2).

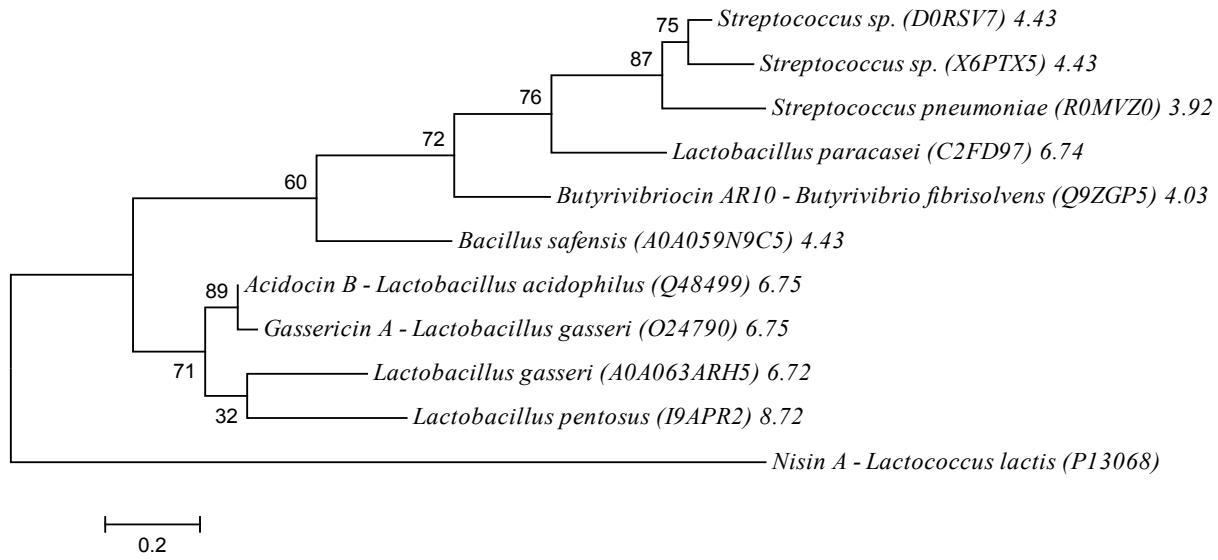

FIG. S4. Molecular phylogenetic analysis of known and putative members of subgroup ii circular bacteriocins with nisin A as outgroup. Acidocin B, gassericin A, and butyrivibriocin AR10 are the known members of this subgroup. Putative members (source organism indicated) were identified through BLAST (3) analysis using acidocin B protein sequence and 40% identity as cut-off. The isoelectric point (pI) of each peptide, as predicted using ExPASy Prot Param tool (4), is indicated at the end of each name. The cleavage site during maturation was predicted through alignment with known bacteriocins and only the mature peptide was considered for calculation of pI. The evolutionary history was inferred by using the maximum likelihood method based on the JTT matrix-based model (5). The tree with the highest log likelihood (-827.3975) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 11 amino acid sequences. All positions containing gaps and missing data were

eliminated. There were a total of 49 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (6).

References

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