

Supplementary Material S1

Table S1. Potential cationic antimicrobial peptides from Hb-P and P-Cru identified by RP-HPLC-MS/MS after 3 h of peptic hydrolysis.

Location	Sequence	MM (Da)	ϵ (M ⁻¹ cm ⁻¹)	GRAVY index ^a	Total hydrophobic residues (%) ^a	Net charge at pH 7 ^a	Structure (%) ^b
α (1-28)	VLSAADKANVKA AWGKVGGQAGAHGAEA	2633.4	60234	0.000	50	+1.25	53.57% RC
α (1-29)	VLSAADKANVKA AWGKVGGQAGAHGAEAL	2746.5	61202	0.131	52	+1.25	62.07% RC
α (33-46)	FLGFPTTKTYFPHF	1701.9	48879	0.064	36	+1.25	78.57% RC
α (34-46)	LGFPPTTKTYFPHF	1554.8	42756	-0.146	31	+1.25	84.62% RC
α (37-46)	PTTKTYFPHF	1237.6	34751	-0.810	20	+1.25	90.00% RC
α (66-73)	LTKAVGHL	837.5	11854	0.675	50	+1.25	50.00% RC
α (87-98)	HAHKLRVDPVNF	1431.8	28778	-0.550	42	+1.5	75.00% RC
α (89-98)	HKLRVDPVNF	1223.7	21775	-0.520	40	+1.25	100.00% RC
α (135-141)	VLTSKYR	865.5	11219	-0.457	29	+2	100.00% RC
α (137-141)	TSKYR	653.3	9285	-2.240	0	+2	100.00% RC
β (14-21)	GLWGKVVN	871.5	35861	0.388	50	+1	37.50% RC
β (16-21)	WGKVVN	701.4	33949	-0.050	50	+1	100.00% RC
β (32-42)	LLVVYPWTQRF	1420.8	51991	0.573	55	+1	54.55% RC
β (33-42)	LVVYPWTQRF	1307.7	51023	0.250	50	+1	60.00% RC
β (34-42)	VVYPWTQRF	1194.6	50055	-0.144	44	+1	100.00% RC
β (34-43)	VVYPWTQRF	1341.7	56178	0.150	50	+1	100.00% RC
β (47-86)	GDLSNADAVMGNPKVKAHGKKVLQSFSDGLKHLNLTGTF	4236.2	62028	-0.500	35	+2.5	75.00% AH
β (50-64)	SNADAVMGNPKVKAH	1537.8	22351	-0.573	40	+1.25	80.00% RC
β (50-65)	SNADAVMGNPKVKAHG	1594.8	23295	-0.563	38	+1.25	62.50% RC
β (53-82)	DAVMGNPKVKAHGKKVLQSFSDGLKHLNLT	3245.7	47169	-0.507	37	+2.5	66.67% AH
β (53-86)	DAVMGNPKVKAHGKKVLQSFSDGLKHLNLTGTF	3679.0	56164	-0.512	35	+3.5	67.65% AH
β (54-86)	AVMGNPKVKAHGKKVLQSFSDGLKHLNLTGTF	3563.9	55183	-0.421	36	+4.5	63.64% AH
β (104-108)	FRLLG	604.4	9105	1.100	60	+1	100.00% RC
β (104-109)	FRLLGN	718.4	10164	0.333	50	+1	100.00% RC
β (104-110)	FRLLGNV	817.5	11130	0.886	57	+1	71.43% RC
β (104-111)	FRLLGNVI	930.6	12098	1.338	63	+1	48.39% RC
β (104-112)	FRLLGNVIV	1029.6	13064	1.656	67	+1	77.78% RC
β (104-115)	FRLLGNVIVVL	1340.9	15964	2.258	75	+1	41.67% RC
β (105-109)	RLLGN	571.3	4041	-0.160	40	+1	100.00% RC
β (105-110)	RLLGNV	670.4	5007	0.567	50	+1	100.00% RC
β (105-111)	RLLGNVI	783.5	5975	1.129	57	+1	100.00% RC
β (105-112)	RLLGNVIV	882.6	6941	1.513	63	+1	100.00% RC
β (129-141)	AAFQKVVAGVANA	1244.7	16905	1.008	69	+1	69.23% AH

β(129-147)	AAFQKVVAGVANALAHKYH	1994.1	38186	0.374	58	+2.5	84.21% AH
β(130-141)	AFQKVVAGVANA	1173.7	15950	0.942	67	+1	33.33% RC
β(130-147)	AFQKVVAGVANALAHKYH	1923.0	37231	0.294	56	+2.5	83.33% AH
β(131-136)	FQKVVA	690.4	10116	0.933	67	+1	33.33% RC
β(131-141)	FQKVVAGVANA	1102.6	14995	0.864	64	+1	27.27% RC
β(131-147)	FQKVVAGVANALAHKYH	1852.0	36276	0.206	53	+2.5	82.35% AH
β(132-141)	QKVVAGVANA	955.5	8872	0.670	60	+1	40.00% RC
β(132-147)	QKVVAGVANALAHKYH	1704.9	30153	0.044	50	+2.5	75.00% AH
β(137-147)	GVANALAHKYH	1179.6	25237	-0.191	45	+1.5	45.45% RC
β(140-147)	NALAHKYH	952.5	22372	-0.963	38	+1.5	62.50% RC
β(142-147)	LAHKYH	767.4	20358	-1.000	33	+1.5	100.00% RC

MM: Molecular mass. GRAVY index: grand average of hydropathicity index, which indicates the solubility of the peptides: positive GRAVY (hydrophobic) and negative GRAVY (hydrophilic). ε: molar extinction coefficient. RC: random coil. AH: Alpha helix.

^a Values calculated by using the network antimicrobial peptide database (<http://aps.unmc.edu/AP/>) [1].

^b Values predicted by using the network protein sequence analysis (<https://prabi.ibcp.fr/>) with secondary structure consensus prediction (MLRC, DSC, and PHD) [2].

References

1. Wang, G.; Li, X.; Wang, Z. APD3: the antimicrobial peptide database as a tool for research and education. *Nucleic Acids Research* **2015**, *44*, D1087-D1093, doi:10.1093/nar/gkv1278.
2. Combet, C.; Blanchet, C.; Geourjon, C.; Deléage, G. NPS@: network protein sequence analysis. *Trends in biochemical sciences* **2000**, *25*, 147-150, doi:10.1016/s0968-0004(99)01540-6.

Supplementary Material S2

Table S2. Comparison of potential anionic antimicrobial peptides identified in hydrolysates of Hb-P/P-Cru and Hb-B by basic local alignment search tool (BLAST) [1].

Hb-P/P-Cru hydrolysate			Hb-B hydrolysate			Query Length (AA)	Query Cover (%)	Identity (%)	Reference
Location	Sequence	Net charge at pH 7	Location	Sequence	Net charge at pH 7				
α (71-91)	GHLDLPGALSALSDLHAHKL	-1.25	α (71-91)	GHLDLPGALSALSDLHAHKL	-1,25	21	100	100	Cournoyer <i>et al.</i> [2]
α (110-128)	AAHHPDDFNPSVHASLDKF	-1.25				19	100	74	
α (110-125)	AAHHPDDFNPSVHASL	-1.25	α (110-128)	ASHLPSDFTPAVHASLDKF	-0.5	16	100	69	Adje <i>et al.</i> [3]
α (110-122)	AAHHPDDFNPSVH	-1.25				13	100	62	
α (107-128)	VTLAHHPDDFNPSVHASLDKF	-1.25				22	100	77	
α (107-125)	VTLAHHPDDFNPSVHASL	-1.25	α (107-128)	VTLASHLPSDFTPAVHASLDKF	-0.5	19	100	100	Adje <i>et al.</i> [4]
α (109-125)	LAAHHPDDFNPSVHASL	-1.25				17	100	100	
β (2-13)	VHLSAEEKEAVL	-1.75				12	75	78	
β (2-15)	VHLSAEEKEAVLGL	-1.75	β (1-13)	MLTAEKAAVTAF	-1	14	64	78	
β (14-29)	GLWGKVNVDVGGGAL	-2				16	87	93	Nedjar-Arroume <i>et al.</i> [5]
β (14-32)	GLWGKVNVDVGGGALGRL	-1				19	89	94	
β (16-29)	WGKVNVDVGGGAL	-2	β (1-30)	MLTAEKAAVTAFWGKVKVDVGGGALGRL	-1	14	100	93	
β (16-32)	WGKVNVDVGGGALGRL	-1				17	100	94	
β (98-115)	HVDPENFRLLGNVIVVVL	-0.75	β (98-115)	HVDPENFRLLGNVIVVVL	-0.75	19	100	100	Cournoyer <i>et al.</i> [2]

AA: amino acids. Query length indicates the number of amino acids queried. Query cover indicates the percent of the query length that is included in the aligned segments.

References

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4. Adje, E.Y.; Balti, R.; Kouach, M.; Dhulster, P.; Guillochon, D.; Nedjar-Arroume, N. Obtaining antimicrobial peptides by controlled peptic hydrolysis of bovine hemoglobin. *International Journal of Biological Macromolecules* **2011**, 49, 143-153, doi:<https://doi.org/10.1016/j.ijbiomac.2011.04.004>.
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Supplementary Material S3

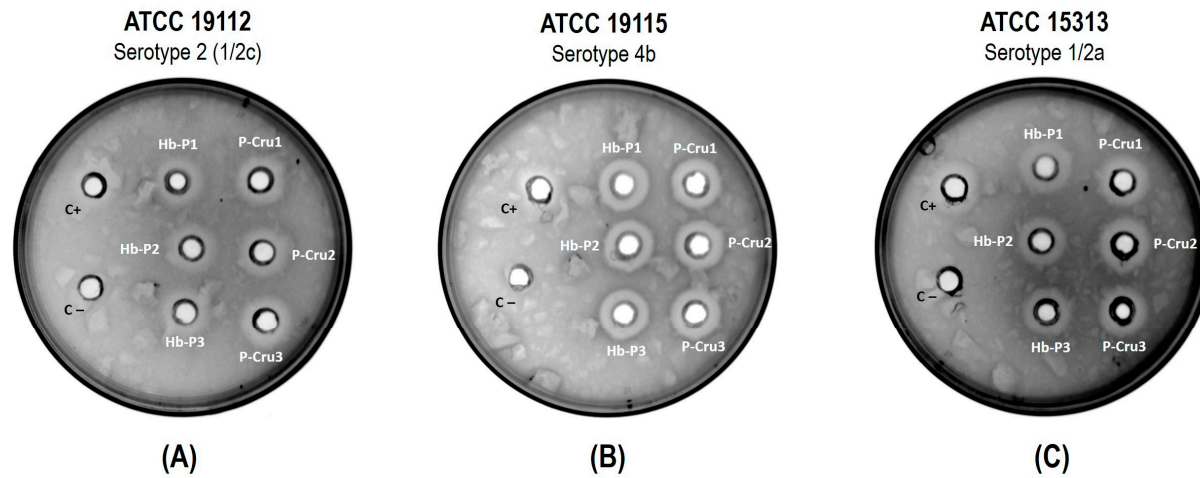


Figure S1. Growth inhibition zones of porcine hemoglobin (Hb-P) and porcine cruor (P-Cru) peptic hydrolysates against *L. monocytogenes* strains ATCC 19112 (A), ATCC 19115 (B), and ATCC 15313 (C). C – is the negative control (sterilized distilled water) and C+ is the positive control (pediocin). Hb-P1, Hb-P2, and Hb-P3 are Hb-P hydrolysate repetitions 1, 2, and 3, respectively. P-Cru1, P-Cru2, and P-Cru3 are P-Cru hydrolysate repetitions 1, 2, and 3, respectively.