Table S2. Disulfide-connected peptide fragments of alvinellacin observed after proteolytic cleavage. Peptides with oxidized cysteines were successively digested using the proteases Lys-C and trypsin. The resulting peptides were analyzed by offline nanoESI-Orbitrap MS/MS as shown in Figure S2. The results unambiguously indicated two disulfide linkages between C1-C4 and C2-C3.

Enzyme	Observed disulfide-linked peptides	Observed ions	Disulfide-linked peptide Mw (Da, monoisotopic)	
			theor.	exp. ± SD
Lys-C	$RGC_1YTRC_2WKVGRNGRVC_3MRVC_4T$ (with 2 internal disulfide bonds)	520.849 (5+) 650.810 (4+)	2599.2076	2599.210 ± 0.002
	RGC_1YTRC_2WK | | $VGRNGRVC_3MRVC_4T$ or RGC_1YTRC_2WK |_____|_____ | | $VGRNGRVC_3MRVC_4T$	524.448 (5+) 655.308 (4+)	2617.2182	2617.203 ± 0.001
trypsin	RGC_1YTRC_2WK |_____|_____ | | $NGRVC_3MRVC_4T$	462.014 (5+) 577.265 (4+)	2305.0272	2305.032 ± 0.002
	RGC_1YTRC_2WK |_____|_____ | | VC_3MRVC_4T	495.474 (4+) 660.295 (3+)	1977.8617	1977.865 ± 0.003
	RGC_1YTR | VC_4T	358.833 (3+) 537.746 (2+)	1073.4746	1073.477 ± 0.001
	C_2WK | VC_3MR	314.477 (3+) 471.213 (2+)	940.4082	940.410 ± 0.002