Supporting Information

Synthesis and Characterization of Heterodimers and Fluorescent Nisin Species by Incorporation of Methionine Analogs and Subsequent Click Chemistry

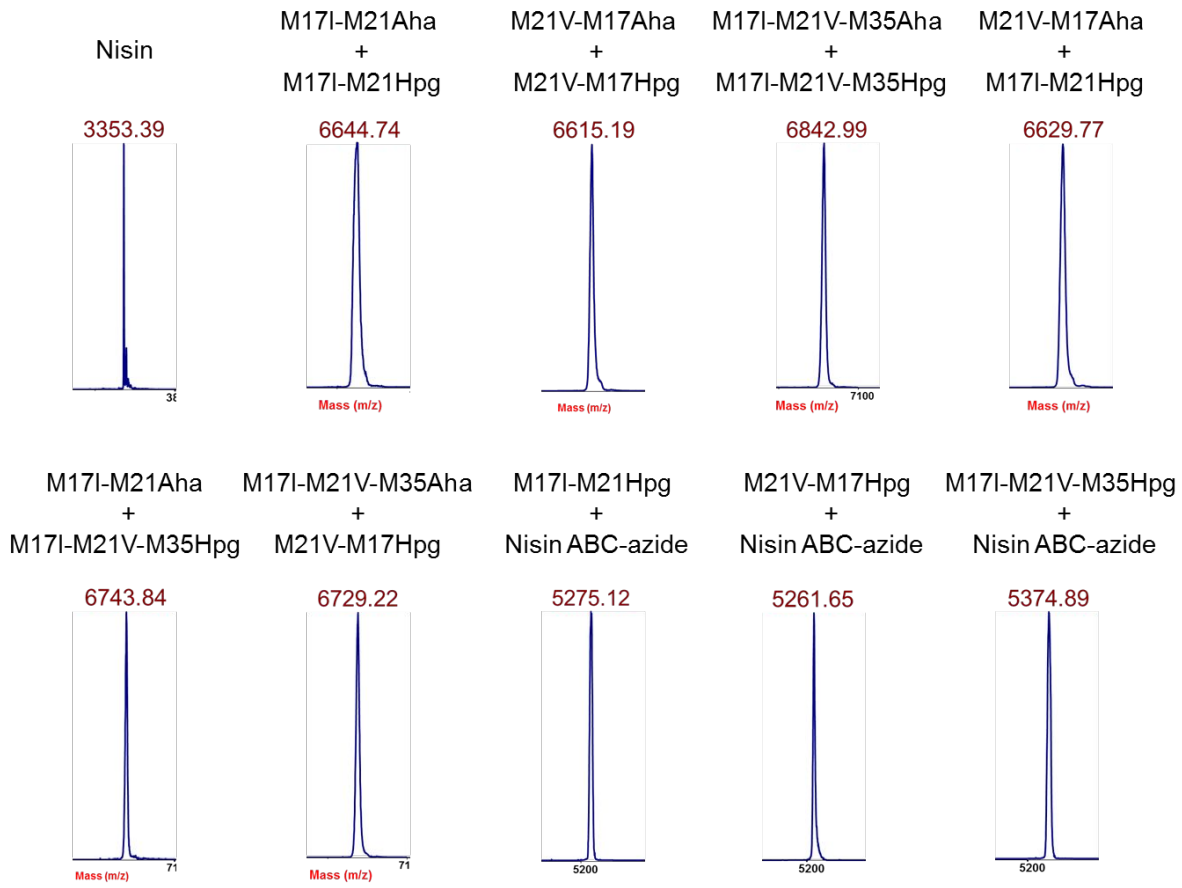
Jingjing Deng, Jakob H. Viel, Jingqi Chen and Oscar P. Kuipers*

Department of Molecular Genetics, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands.

* Corresponding author: Email: o.p.kuipers@rug.nl

Supplementary Figures

A

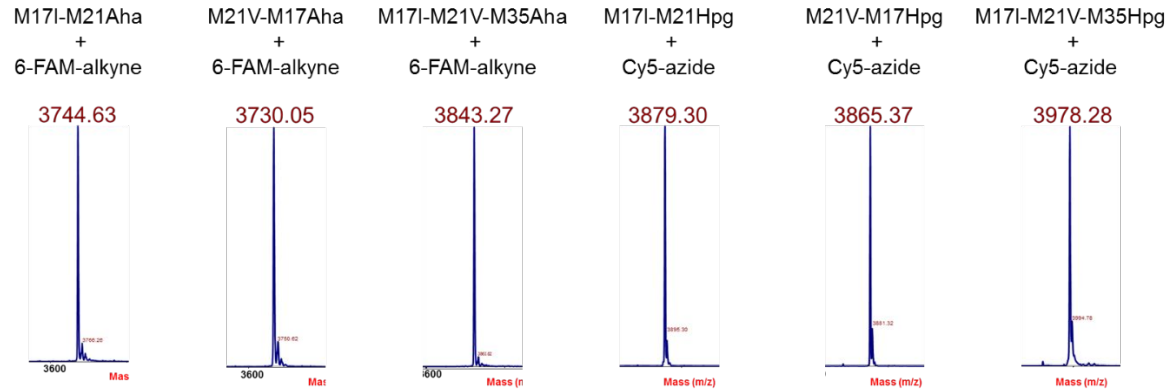


B

Peptides	Predicted mass (Da)	Measured Mass (Da)
Nisin	3353.03	3353.39
M17I-M21Aha + M17I-M21Hpg	6644.97	6644.74
M21V-M17Aha + M21V-M17Hpg	6616.93	6615.19
M17I-M21V-M35Aha + M17I-M21V-M35Hpg	6843.23	6842.99
M21V-M17Aha + M17I-M21Hpg	6630.95	6629.77
M17I-M21Aha + M17I-M21V-M35Hpg	6744.10	6743.84
M17I-M21V-M35Aha + M21V-M17Hpg	6730.08	6729.22
M17I-M21Hpg + Nisin ABC-azide	5276.39	5275.12
M21V-M17Hpg + Nisin ABC-azide	5262.37	5261.65
M17I-M21V-M35Hpg + Nisin ABC-azide	5375.52	5374.89

Supplementary Figure 1. A). MALDI-TOF analysis of nisin, dimeric nisin cosntructs and nisin hybrids. B). Predicted and measured mass of nisin, dimeric nisin constructs and nisin hybrids.

A



B

Peptides	**Predicted mass (Da)**	**Measured Mass (Da)**
M17I-M21Aha + 6-FAM-alkyne	3744.36	3744.63
M21V-M17Aha + 6-FAM-alkyne	3730.34	3730.05
M17I-M21V-M35Aha + 6-FAM-alkyne	3843.49	3843.27
M17I-M21Hpg + Cy5-azide	3879.36	3879.30
M21V-M17Hpg + Cy5-azide	3865.34	3865.37
M17I-M21V-M35Hpg + Cy5-azide	3978.49	3978.28

Supplementary Figure 2. A). MALDI-TOF analysis of fluorescently labeled nisin variants. B). Predicted and measured mass of fluorescently-labeled nisin variants.

Supplementary Tables

Supplementary Table 1. Strains and plasmids used in this study.

Strains or Plasmids	Characteristics	References
Strains		
Lactococcus lactis NZ9000	*pepN::nisRK*; Expression host strain	[1]
Indicator strains		
Micrococcus flavus		Lab collection
Straphylococcus aureus CAL	Methicillin resistant (MRSA)	The University Medical Center Groningen, The Netherlands
Straphylococcus aureus MW2	Methicillin resistant (MRSA)	The University Medical Center Groningen, The Netherlands
Entercoccus faecium LMG 16003	Avoparcin and vancomycin resistant (VRE)	Laboratory of Microbiology, Gent, Belgium
Entercoccus faecalis LMG 16216	Vancomycin resistant (VRE)	Laboratory of Microbiology, Gent, Belgium
Bacillus cereus ATCC 14579		[2]
Listeria monocytogenes LMG 10470		[3]
Plasmids		
pIL3EryBTC	EryR, *nisBTC*, modification and transport of lantibiotics	[4]
pCZ-nisA	CmR, *nisA*, encoding NisA, under the control of P*czc*D promoter	[5]
pCZ-nisA-M17I	Point mutant of pCZ-nisA, with the Met 17 of nisin changed to Ile	This work
pCZ-nisA-M21V	Point mutant of pCZ-nisA, with the Met 21 of nisin changed to Val	This work
pCZ-nisA-M17I-M21V-M35	Point mutant of pCZ-nisA, with the Met 17 and 21 of nisin changed to Ile and Val, respecitviely, with Met 35	This work
pCZ-nisA-I4M-M17I-M21V	Point mutant of pCZ-nisA, with the Ile 4, Met 17 and Met 21 of nisin changed to Met, Ile and Val, respecitviely	This work
pNZnisP8H	CmR, *nisP*, encoding NisP mutant, with 8 histines	[6]

Supplementary Table 2. Primers used in this study

Mutants	Primer	Sequence
M17I	pCZ-F	aacagtagtggcctcgtagc
	M17I-Rev	gctgttttcatgttacaaccaatcagagctcctgttttac
	M17I-Fwd	gtaaaacaggagctctgattggttgtaacatgaaaacagc
	pCZ-R	tagtctcggacattctgctc
M21V	pCZ-F	aacagtagtggcctcgtagc
	M21V-Rev	tacaatgacaagttgctgtttttacgttacaacccatcagagctc
	M21V-Fwd	agctctgatgggttgtaacgtaaaaacagcaacttgtcattgtag
	pCZ-R	tagtctcggacattctgctc
M17I-M21V-M35	NheI-For	atcagctagcacggaatagacatggtgttc
	M35-Rev1	ctacaatgacaagttgctgtttttacgttacaaccaatcagagctcctgttttac
	M35-Rev2	taccgcatgcctgcaggcttacattttgcttacgtgaatactacaatgacaagttg
I4M-M17I-M21V	NheI-For	atcagctagcacggaatagacatggtgttc
	I4-Rev1	cagagctcctgttttacaaccgggtgtacatagcgacatacttgtaatgcgtggtg
	I4-Rev2	acaatgacaagttgctgtttttacgttacaaccaatcagagctcctgttttac
	I4-Rev3	taccgcatgcctgcaggcttatttgcttacgtgaatactacaatgacaagttgctg

References

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