Discovery and **Characterization** of Novel Microviridin-like RiPPs

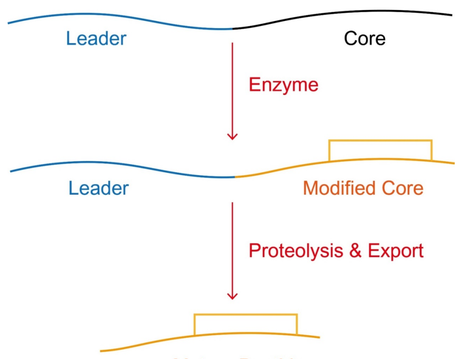
Mingyu Choi, Hyunbin Lee, Seokhee Kim*

Department of Chemistry, Seoul National University, Gwanak-Gu, Seoul 151-747, Korea

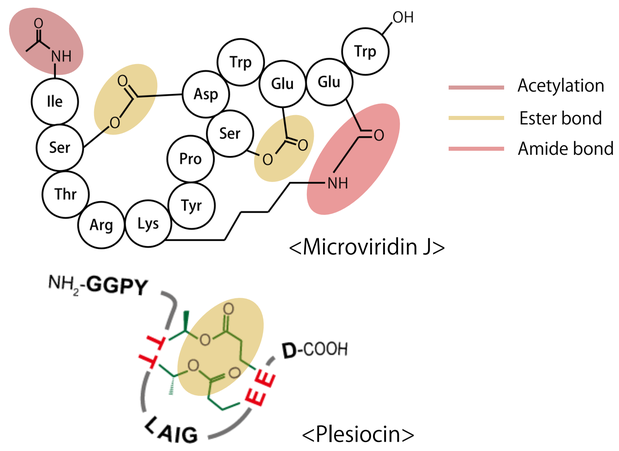
Introduction

Biosynthetic logic of RiPP is relatively simple and amenable to bioengineering

RiPP = **Ri**bosomally synthesized and **P**ost-translationally modified **P**eptides



<Biosynthesis of RiPP>



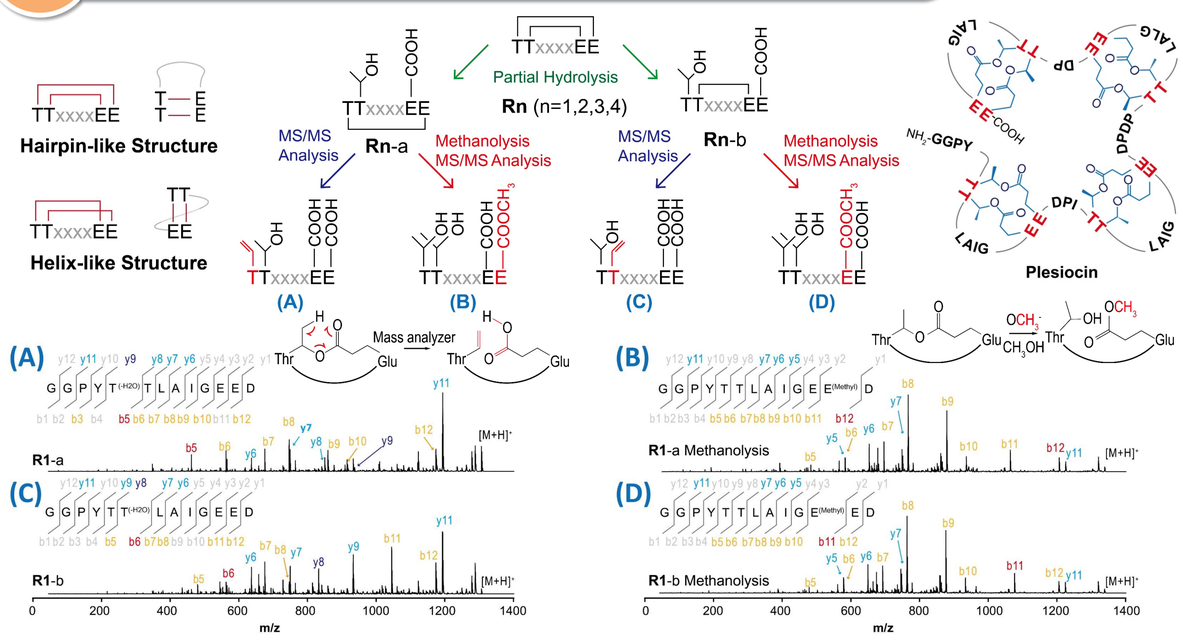
<Microviridin J>

<Plesiocin>

Microviridin, a subfamily in RiPP, can be a good model system to study by its simple PTM

Recent Studies

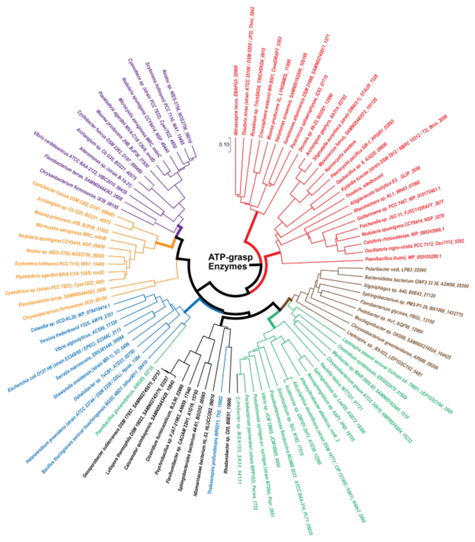
Plesiocin has 4 hairpin-like bicyclic repeats



Connectivity of microviridin-like RiPPs can be determined by MS^2 and hydrolysis / methanolysis

Bio Informatic Study

Various sequences, but distinct consensus are found on Microviridin-like RiPPs

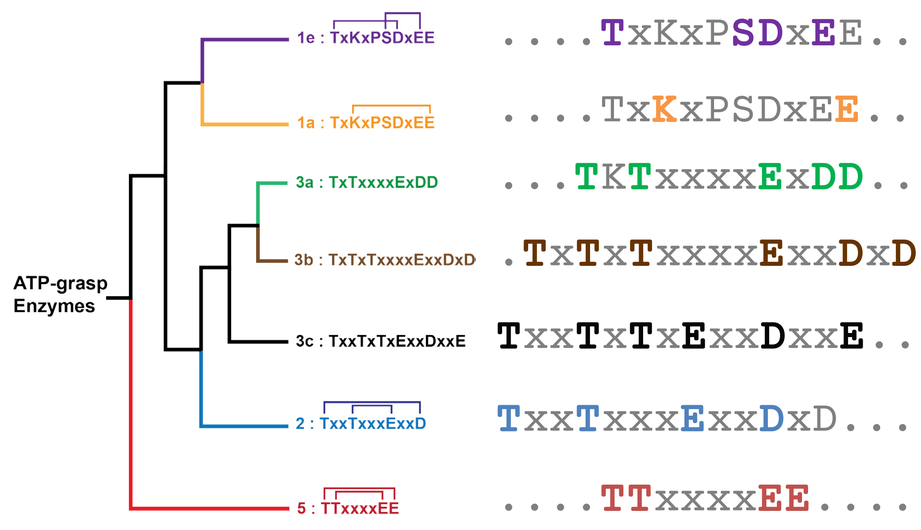


<Phylogenetic analysis of ATP-grasp enzymes>

Group 3a	*Vibrio sp.* JCM 18905	..PFILNYATER..
	C. gleum ATCC 35910	..PLILDYKIVR..
	N. italica	..PILLRYGTPR..
	Acidovorax sp. GW101-3H11	..PLLTAASIER..
	Consensus	..PLILxYxxxR..
Group 3b	*C. greenlandense*	..NLVLNYFE..
	Algoriphagus sp. A40	..PLILKFAE..
	Pedobacter sp. Hv1	..PFIFQFKE..
	Leptospira sp. B5-022	..PFILRYKE..
	Consensus	..PhILxrxE..
Group 3c	*G. subterraneus* DSM 17957	..PLLKYR..
	I. bacterium HL-53	..PYLISK..
	S. bacterium 44-61 (1)	..PILNFR..
	S. bacterium 44-61 (1)	..PILHFR..
	Consensus	..PILxrR..
Group 5	*P. pacifica* SIR-1	..LFIEDL..
	S. aurantiaca (strain DW4/3-1)	..LFVQDL..
	Geobacillus sp. 8	..LFIEEL..
	K. tusciae	..LFIEQL..
	Consensus	..LFIEaL..

a : acidic
b : basic
h : hydrophobic
r : aromatic
x : all

<Leader Sequence Alignment>



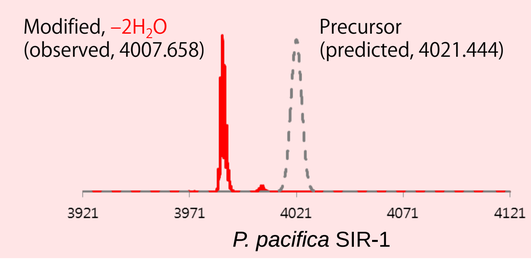
< Summary of Phylogenetic Tree > < Key residues in core sequence > < Core Sequence Logo >

Bioinformatic analysis shows that there are various subfamilies in microviridin-like RiPPs

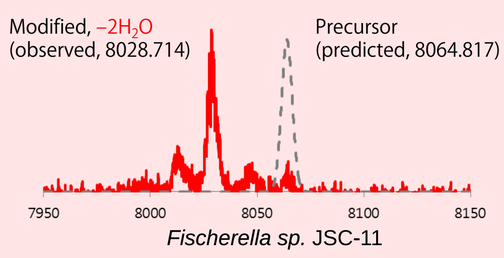
Cross-linking Pattern Analysis

Members of RiPPs of same subfamily show equal level of dehydration

Group 5 (TTxxxxEE) : -2 H_2O from each repeat

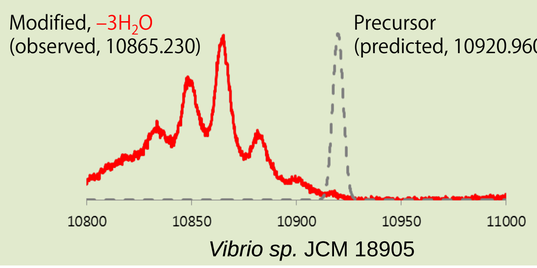


P. pacifica SIR-1

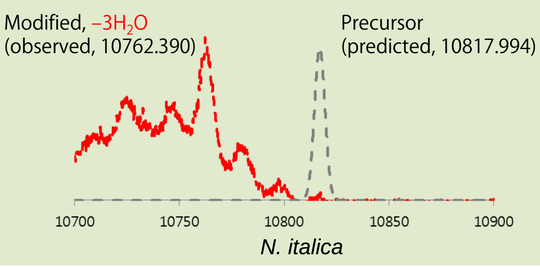


Fischerella sp. JSC-11

Group 3a (TKTxxxxExDD) : -3 H_2O from each repeat

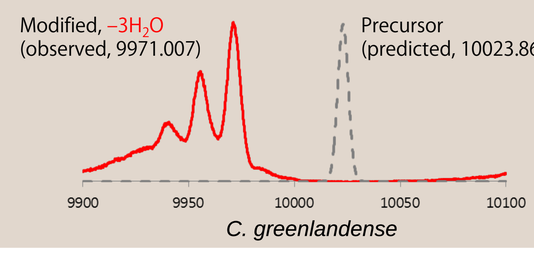


Vibrio sp. JCM 18905

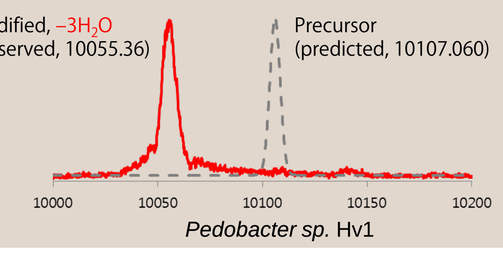


N. italica

Group 3b (TxTxTxxxxExSDSD) : -3 H_2O from each repeat

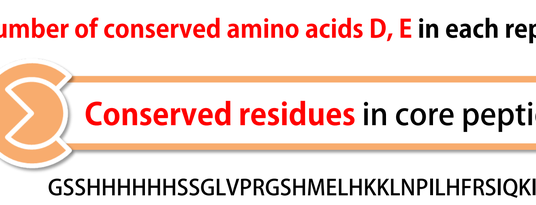


C. greenlandense



Pedobacter sp. Hv1

Group 3c (TxxTxTxExxDxxE) : -3 H_2O from each repeat



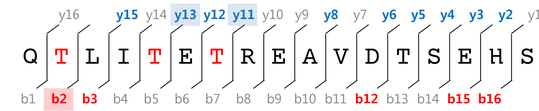
I. bacterium HL-53

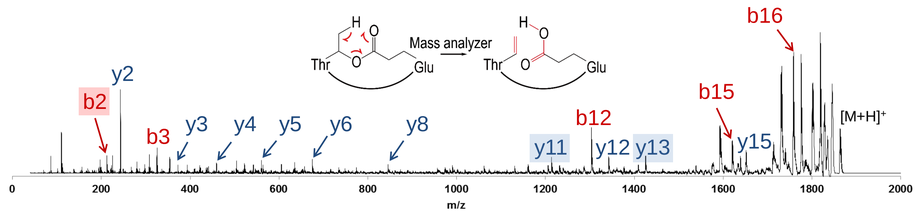
S. bacterium 44-61 (1)

Number of conserved amino acids D, E in each repeat correlates to the dehydration level in each subfamily

Conserved residues in core peptides participate in ω-esterification

GSSHHHHHHSSGLVPRGSHMELHKKLNPILHFRSIQKIQKPDRKDGDALTLANKDEDFKLRHKF // QTLITETREAVDTSEHS

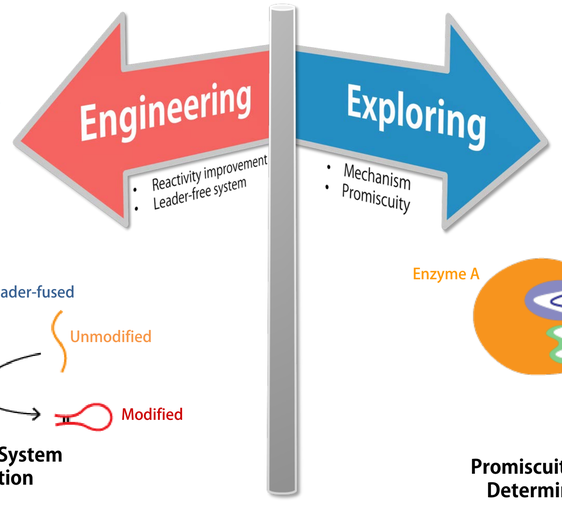




MS^2 analysis showed that -OH group on well-conserved threonines participates in ω-esterification

Future Plan

[Reactivity Improvement]



Further study will focus on understanding the biosynthesis of these RiPPs and engineering

Reference

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Laboratory of Proteolytic Systems