

微生物に見出した新規で多様なアミド結合形成酵素の網羅的解析と利用

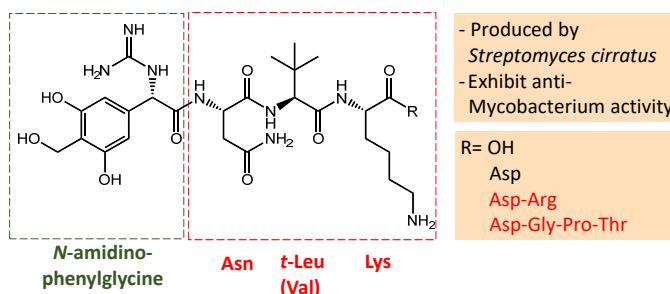
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Abstract

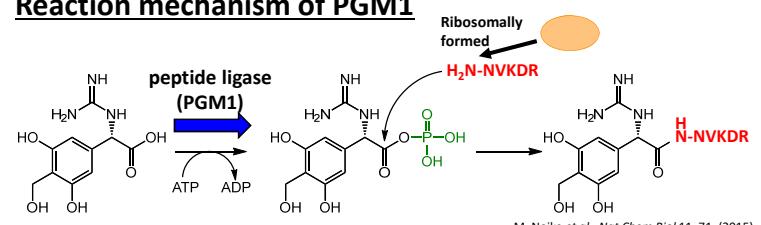
We identified a novel peptide ligase (PGM1) involved in pheganomycins biosynthesis. The enzyme activates a carboxyl group of an N-amidino-phenylglycine derivative by phosphorylation in the presence of ATP and catalyzes the amide bond formation between the N-amidino-phenylglycine derivative and ribosomally synthesized peptides. This is the first example of an ATP-grasp enzyme catalyzing the amide bond formation with peptides as nucleophiles. We identified gene clusters containing a peptide ligase ortholog in actinobacteria by BLAST analysis. These gene clusters were heterologously expressed in *Streptomyces lividans* and *Streptomyces albus* to probe the function of the orthologs. We detected a specific metabolite in culture broth of the transformant. We also identified a novel ATP-grasp enzyme, which is involved in peptidoglycan biosynthesis and catalyzed ligation between L-Glu and UDP-MurNAc-L-Ala. Besides, a novel epimerase catalyzing the epimerization of the terminal L-Glu of the product was also identified.

Background

Structure of pheganomycins (PGMs)



Reaction mechanism of PGM1

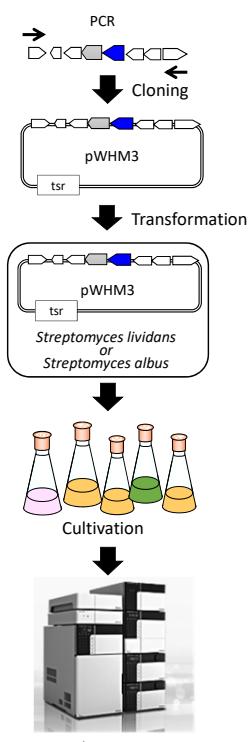


Biosynthetic gene cluster of PGMs

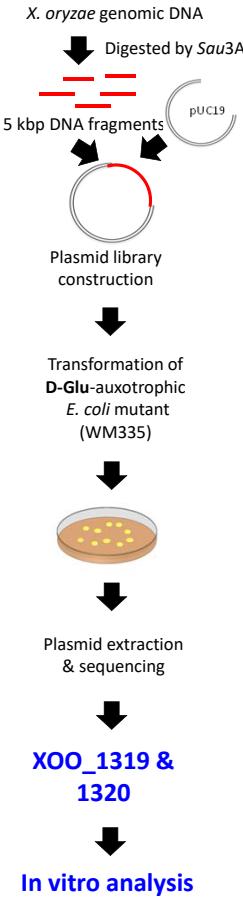
MEREIVWTEIEESDLAAVVSASNVKDGPVTSSNVKDR (38 amino acids)

Method

ATP-grasp ligase



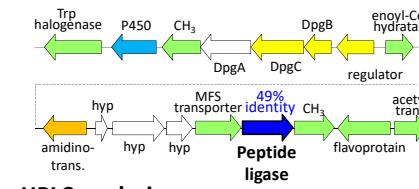
Peptidoglycan



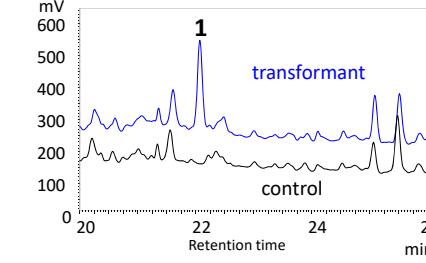
Results

ATP-grasp ligase

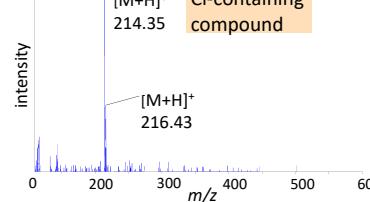
Nocardiopsis baichengensis



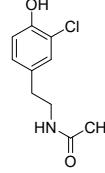
HPLC analysis



MS spectrum of peak 1



NMR analysis



Peptidoglycan

XOO_1320 Glu ligase assay

Assay mixture

UDP-MurNAc-L-Ala	1 mM
ATP	10 mM
β-Mercaptoethanol	2.5 mM
MgCl ₂	20 mM
(NH ₄) ₂ SO ₄	25 mM
Tris-HCl (pH 8.0)	100 mM
D-Glu or L-Glu (control)	
XOO_1320	5 µg/ml

XOO_1319 epimerase assay

Assay mixture

UDP-MurNAc-L-Ala-L-Glu	1 mM
ATP	10 mM
β-Mercaptoethanol	2.5 mM
MgCl ₂	20 mM
(NH ₄) ₂ SO ₄	25 mM
Tris-HCl (pH 8.0)	100 mM
XOO_1319 or XOO_1320	5 µg/ml

With D-Glu

With L-Glu

Low yield

High yield

UDP-MurNAc-L-Ala-D-Glu

MW = 879.1

Retention time [min]

UV 260 nm

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