Biosynthesis in vitro of bacillamide intermediate-heterocyclic AlaCys\textsubscript{thiazole} by heterologous expression of nonribosomal peptide synthetase (NRPS)

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ABSTRACT

Bacillamide C, a potential natural antialgae active compound, is produced by Bacillus atrophaeus C89 derived from marine sponge Dysidea avara. A nonribosomal peptide synthetase (NRPS) cluster is hypothesized to be involved in the biosynthesis of bacillamide C. The NRPS with a domain string of A1-PCP1-Cy-A2-PCP2-C can be divided into three functional modules. After heterologous expression and purification of module A1-PCP1 and module Cy-A2-PCP2, their catalytic activities were biochemically proven in vitro by the reaction with the apo-PCP domain transformed to the holo-PCP domain through a phosphopantetheinyl transferase, ATP, and substrate amino acids. Five–membered heterocyclic AlaCys\textsubscript{thiazole} with molecular weight of 172.0389 was detected. This proved the formation of the heterocyclic dipeptide AlaCys\textsubscript{thiazole}, which is considered to be a building block for the biosynthesis of bacillamide. This study provides a basis for further biosynthesis of bacillamides.

1. Introduction

Bacillamides, which are nonribosomal peptides, can be categorized as bacillamides A, B, C, D, and E (Fig. S1) (Bloudoff et al., 2017; Ivanova et al., 2007; Jeong et al., 2003; Omura et al., 1975; Socha et al., 2007). In our previous study, neobacillamide A and bacillamide C were isolated from marine Bacillus atrophaeus C89 (Yu et al., 2009). The tryptamide thiazole (bacillamides A–D) or tryptamide thiazoline (bacillamide E) motif as a typical characteristic of bacillamides (Fig. S1), is a building block present in many bioactive cyclic peptides, such as the antibiotic zelkovamycin produced by Streptomyces sp. (Tabata et al., 1999), the protein synthesis inhibitors A-21459A and B from Actinoplanes sp. (Selva et al., 1996), and the immunosuppressive argyrins from the myxobacterium Arcahium gephyra (Sasse et al., 2002). Similarly, bacillamides have multiple biological activities, for example, bacillamide A and its derivatives possess substantial alglidal activities against a range of dinoflagellates and raphidophytes, and exhibit antibiosis against a particular species of cyanobacteria (Jeong et al., 2003; Churro et al., 2009) and bacillamide analogs, thus rendering them novel cytotoxic and anti-inflammatory agents (Kumar et al., 2016).

Bacillamides have been generally synthesized chemically (Fache et al., 2012; Kumar et al., 2016; Li et al., 2009; Wang et al., 2010), only one study reported the biosynthetic pathway of bacillamide E (Bloudoff et al., 2017). Even though the biosynthesis of bacillamide C was optimized, the yield was very low (Jin et al., 2011; Yu et al., 2015). Therefore, investigating the biosynthetic mechanisms of bacillamide C is essential for its mass production and for understanding the biosynthetic pathways of other bacillamides.

Nonribosomal peptides are produced by large multifunctional enzymes (Schwarzer and Marahiel, 2001). A nonribosomal peptide synthetase (NRPS) gene cluster was detected in the genome sequence of B. atrophaeus C89 (Genbank No. NZ_AJRJ01000045.1), which was predicted to synthesize bacillamide C (Liu et al., 2012). Nonribosomal peptide synthesis requires at least three functional domains: the adenylylation (A) domain, which selects and activates the cognate amino acid; the peptidyl carrier protein (PCP) domain, which transports the activated intermediate; and the condensation (C) domain, which catalyzes peptide bond formation, or the cyclization (Cy) domain, which catalyzes heterocycle formation (Marshall et al., 2002). In our previous study, the biosynthetic mechanism of bacillamide C was predicted (Zhang et al., 2018). An NRPS with a domain string of A1-PCP1-Cy-A2-PCP2-C can be divided into three functional modules. The A1-PCP1 module consists of the first A (A1) and the first PCP domain and activates and transports l-alanine. The Cy-A2-PCP2 module consists of a Cy, the second A (A2), and the second PCP (PCP2) domains and activates l-cysteine, catalyzes peptide bond formation, and...
heterocyclizes the product 4-carboxylic acid thiazoline derivative. Moreover, the flavin mononucleotide-containing oxidase (Ox) domain oxidizes the dehydro heterocycle by a two-electron transfer to produce heteroaromatic thiazole. The 3rd module consists of the C domain and catalyzes peptide bond formation between the intermediates synthesized by the A1-PCP1 and Cy-A2-PCP2 modules, and tries to form a dipeptide intermediate (Yuwen et al., 2013). Tryptophan decarboxylation is an extremely rare activity among bacteria (Williams et al., 2018). In our previous study, the AADC enzyme nearest to the NRPS gene cluster in B. atrophaeus C89 was characterized as a highly efficient catalyst for the decarboxylation of L-tryptophan to tryptamine (Yuwen et al., 2013). Tryptophan decarboxylation is an extremely rare activity among bacteria (Williams et al., 2018). In our previous study, the AADC enzyme nearest to the NRPS gene cluster in B. atrophaeus C89 was characterized as a highly efficient catalyst for the decarboxylation of L-tryptophan to tryptamine (Yuwen et al., 2013). Tryptophan decarboxylation is an extremely rare activity among bacteria (Williams et al., 2018).

2. Materials and methods

2.1. Bacterial strains, plasmids, and culture conditions

B. atrophaeus C89 (CCTCC AB 2016282) was isolated from the sponge D. avara in the South China Sea (Li et al., 2007). The plasmid pEASY-E1 (TransGen, Beijing, China) was employed to express the A1-PCP1 and Cy-A2-PCP2 modules. Moreover, E. coli Trans1-T1 (TransGen, Beijing, China) was used to propagate the plasmids, and E. coli BL21 (DE3) (TransGen, Beijing, China) was used as a host for expressing the A1-PCP1 and Cy-A2-PCP2 modules. B. atrophaeus C89 was incubated at 28 °C in a liquid medium containing 0.5% beef extract and 1% peptone. E. coli Trans1-T1 and BL21 were grown in Luria-Bertani (LB) medium at 37 °C.

2.2. Cloning of gene a1-pcp1 and cy-a2-pcp2

The genomic DNA from B. atrophaeus C89 was extracted through lysozyme treatment and phenol-chloroform extraction according to the modified Marmur method (Marmur, 1961; Yuwen et al., 2013). The primers A1-P1F: 5’-ATCGAAATTAGATGTGAGCTGC-3’ and A1-P1R: 5’-GATTACAAGAATCGATGACCT-3’ were employed to amplify the DNA sequence of module A1-PCP1; in addition, the primers C1-A2-P2F: 5’-CCACCCGTTTTAAGATGTCC-3’ and C1-A2-P2R: 5’-GATACGGGTGTTTACATCACTTAC-3’ were used to amplify that of module Cy-A2-PCP2. The sequences of the cloned fragments were examined using T7 promoter and terminator primers. The recombinant plasmid with phosphopantetheinyl transferase gene (sfp) from Bacillus subtilis was provided by Prof. Lin (Huang et al., 2011).

2.3. Gene sequence analysis

Expasy translate tool (http://expasy.org/tools/dna.html) was used for translating nucleotide sequence into protein sequence. Amino acid sequence of NRPS was analyzed using the BLASTP program (https://blast.ncbi.nlm.nih.gov/Blast). Amino acid sequences of the NRPS protein from various species were aligned using MEGA (Molecular Evolutionary Genetics analysis version 6.0) for drawing phylogenetic relationship using UPGMA algorithm.

2.4. Expression and purification of recombinant A1-PCP1 and Cy-A2-PCP2

The DNA sequencing in both orientations and the resulting plasmids were used to transform competent E. coli BL21 (DE3) cells. A single positive colony was inoculated in 5 mL LB medium containing kanamycin (50 μg/mL) or ampicillin (100 μg/mL) at 37 °C for 12 h. The overnight culture was used to inoculate LB medium containing antibiotics and incubated at 37 °C with vigorous shaking until OD600nm reached 0.6; isopropyl β-D-thiogalactopyranoside was added to the medium at a final concentration of 0.5 mM and incubated at 16 °C for 15 h. The cells were centrifuged, resuspended in a binding buffer (300 mM NaCl, 20 mM Tris – HCl, pH 8.0), and lysed through sonication. The debris was removed through centrifugation at 12,000 × g for 20 min. The supernatant was filtered through a 0.45-μm Millipore filter and loaded onto an equilibrated Ni-NTA agarose resin column (Qiagen, Hilden, Germany). The target protein was eluted with an elution buffer (300 mM NaCl, 20 mM Tris – HCl, 200 mM imidazole, pH 8.0) by using gravity flow after the residual proteins were removed with a wash buffer (300 mM NaCl, 20 mM Tris – HCl, 50 mM imidazole, pH 8.0). The purified protein was analyzed using SDS-PAGE in a 10.0% (w/v) polyacrylamide gel. The target protein dissolved in the elution buffer was then transferred to a solution buffer (50 mM NaCl, 20 mM Tris – HCl, pH 8.0) using Millipore 10-kDa-MWCO ultrafilters. The purified protein concentration was determined using the Bradford assay (Sangon, Shanghai, China), and the protein was stored at ~80 °C.

2.5. Alignment analysis of the peptidyl carrier proteins domain, cyclization domain, and condensation domain

Multiple amino acid sequence alignments of domain types (PCP, Cy, and C domains) were performed using the ClustalW2 program (http://www.ebi.ac.uk/Tools/msa/clustalo/).

2.6. Detection of 4-carboxylic acid thiazole derivative

To investigate product formation, according to the method of Duerrfahrt et al. (2004), 200 μM A1-PCP1, 200 μM CyA2PCP2, 20 μM Sfp (purified), 2 mM CoA, 10 mM MgCl2, 2 mM DTT, 5 mM Cys, 5 mM Ala, 5 mM ATP, and 20 mM Tris – HCl were incubated in an assay buffer with a total volume of 1 mL at 37 °C for 2 h and stopped through the addition of 500 μL of 1-butanol/chloroform (4:1; v/v). Reaction in the absence of a substrate amino acid or with the boiled protein was as a control reaction. The lower layer was collected after stratification. After evaporation, the resulting pellet was resuspended in 100 μL of 10% methanol and analyzed through liquid chromatography-high-resolution
mass spectrometry and tandem mass spectrometry using a Waters ACQUITY Ultra Performance Liquid Chromatography system coupled with a Waters Micromass Q-TOF Premier Mass Spectrometer equipped with an electrospray interface (Waters Corporation, Milford, MA). Samples were separated using an Acquity BEH C18 column (100 mm × 2.1 mm i.d., 1.7 μm; Waters, Milford, USA), with gradient solvent A (water with 0.1% (v/v) formic acid) and solvent B (acetonitrile with 0.1% (v/v) formic acid) serving as the mobile phase at a flow rate of 0.40 mL/min. The detailed gradient conditions of the mobile phase are outlined as follows: 0–0.50 min, 2% B; 0.50–6.00 min, 2%–15% B; 6.00–9.00 min, 15%–30% B; 9.00–10.00 min, 30%–50% B; 10.00–11.00 min, 50%–85% B; and 11.00–12.00 min, 85%–100% B. The mass spectrometer was operated in the positive ion electrospray ionization mode (capillary voltage: 3.0 kV; sampling cone: 35 V; collision energy: 4 eV; source temperature: 115 °C; desolvation temperature: 300 °C; desolvation gas: 600 L/h). Two-stage mass spectrometry was also conducted in the positive ion electrospray ionization mode with a collision energy ramp of 12–30 eV instead.

3. Results

3.1. Sequence analysis of NRPS

Recombinant plasmid harboring nprs gene was sequenced and the gene sequence is consistent with the sequence AFJ42298.1 (https://www.ncbi.nlm.nih.gov/nuccore/386833542). Protein sequence of NRPS was compared with other species and the phylogenetic tree was constructed (Fig. 1). The NRPS shared maximum homology (99%) with B. subtilis NRPS.

3.2. Expression of A1-PCP1 and Cy-A2-PCP2 modules

The N-terminally His-tagged A1-PCP1 and Cy-A2-PCP2 domains were expressed in E. coli BL21 (DE3) and purified using affinity chromatography. The molecular weights of A1-PCP1 and Cy-A2-PCP2 were 98 and 116 kDa, respectively (Fig. 2), and the protein purity was determined using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 2). Sfp expressed in E. coli BL21 was purified to convert PCP domains from inactive apo- to cofactor-containing holoforms (Fig. 2). The purified soluble recombinant protein samples were determined by the Bradford assay using Bovine serum albumin (BSA). About 1.5 mg of A1-PCP1 module protein, 1.8 mg of Cy-A2-PCP2 module protein, and 2.1 mg of Sfp protein were obtained from 1-L culture E. coli BL21, respectively.

3.3. Homologous comparison of domains involved in the nonribosomal synthesis of bacillamide

PCP is the site of 4′-PP cofactor binding and substrate acylation. The

3.4. Elucidation of bacillamide intermediate and functional prediction of the cyclization domain

Cy domain in NRPS was predicted to catalyze the heterocyclization of cysteine and alanine to thiazole rings. To confirm this hypothesis, two modules of bacillamide synthetase, namely A1-PCP1 and Cy-A2-PCP2, were expressed and purified (Fig. 2). The NRPS requires post-translational modification of PCP domains with a 4′-phosphopantetheinyl (Ppant) prosthetic group, and this modification for covalent substrate tethering is conducted through a dedicated phosphopantetheinyl transferase. A biosynthetic experiment in vitro was performed, and the product AlaCys(thiazole) was detected by mass spectrometry and two-stage mass spectrometry. One intermediate of bacillamide C—namely 4-carboxylic acid thiazole derivative (C6H5NO2S, mass: 172.03), a cyclic dipeptide thiazole derivative was detected (Figs. 3 and 4). The sample from biosynthetic reaction mixture was confirmed by HR-ESI-MS, which showed an additional peak ([M + H] + 173.0379, identical to the predicted [AlaCyS(thiazole)]) compared with control groups (Fig S2). The biosynthetic process of NRPS domains involved in the synthesis of the intermediate product AlaCyS(thiazole) was predicted. We verified no product was formed in a control reaction in the absence of substrate amino acids or with the boiled protein (Fig 4 and Fig S2). The enzyme-bound amino acids are combined through a nucleophilic attack of either the cysteine amino group or the
heteroatom of the side chain onto the carbonyl C of alanine, and a proton is abstracted through base catalysis, thereby enabling the attack of either the cysteine side chain or the amino group onto the amide bond carbonyl. This results in the formation of a hydroxylated thiazolidine intermediate, which is subsequently dehydrated and dehydrogenated to provide the final thiazole-containing product Ala-Csthiazole (Fig. 5).

4. Discussion

Bacillamides are promising naturally occurring compounds for controlling harmful algal blooms (Churro et al., 2009; Shao et al., 2013). However, the low biosynthetic yield of these compounds has restricted not only their bioactivity studies but also their application in research. Therefore, to improve the potential for mass production of bacillamide C, investigating the biosynthetic mechanisms of bacillamide C is essential. Bleudoff et al. (2017) established a bacillamide synthesis assay by expression and purification of entire bacillamide synthetase with 265-kDa NRPS. Our prior study (Yuwen et al., 2013) speculated that B. atrophaeus C89 can produce tryptamine as a building block of bacillamide C, and the study partially described AADC enzyme in the biosynthesis of bacillamide C. In our previous study, we have found that alanine and cysteine were the optimum substrates for the two adenylation domains (Zhang et al., 2018). The current heterologous expression study further elucidates the biosynthetic mechanism of bacillamide C. The in vitro biosynthesis of the bacillamide C intermediate-AlaCyCsthiazole provides an opportunity for bacillamide C and analog production in the future.

4.1. Synthesis of the bacillamide C intermediate AlaCyCsthiazole

Duerfahrt et al. (2004) suggested that Cy domain in an NRPS catalyzes the heterocyclization of cysteine or serine/threonine to thiazolidine and oxazoline rings through a bimodular model system. However, the productivity of the engineered synthetases is hindered by poor substrate selectivity (Hura et al., 2012). Bleudoff et al. (2017) provided insight into the catalytic mechanisms of the Cy domain and implicated Aspartic acid-Threonine dyad in the catalysis of the cyclodehydration reaction. Mass spectrum of the peak at 9.516 min, [M+H]+ = 173.0386 m/z (a). Reaction in the absence of substrate amino acids was as a control reaction (b). Reaction mixtures with the boiled protein were as a control (c). Each experiment was performed in triplicate.

Table 1
Amino acid alignment of the Peptidyl Carrier Proteins (PCP) domain core region LGGXS (A), the Cy domain core region DXXXXDXXS (B), and C domain core HHXXXDG (C).

<table>
<thead>
<tr>
<th>Domain</th>
<th>Consensus Sequence</th>
<th>Organism</th>
<th>Protein ID</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) peptidyl carrier proteins</td>
<td>LGGXSX</td>
<td>Bacillus atrophaeus</td>
<td>EIM09914</td>
<td>In this study</td>
</tr>
<tr>
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<td>LGGDSI</td>
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<td>NprpCP2</td>
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<td>TycACP</td>
<td>LGGSL</td>
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<td>CAA31623.1</td>
<td>(Turgay et al., 1992)</td>
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<tr>
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<td>LGGHSL</td>
<td>Bacillus subtilis</td>
<td>AUA7653.1</td>
<td>(Kesel et al., 2014)</td>
</tr>
<tr>
<td>B) Cylation domain</td>
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</tr>
<tr>
<td>NprpCy</td>
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<td>Vibrio parahaemolyticus</td>
<td>AAF20720.1</td>
<td>(Schultz et al., 2001)</td>
</tr>
<tr>
<td>VibFCy1</td>
<td>DPLCIDQGS</td>
<td>Bacillus licheniformis</td>
<td>AAP36461.1</td>
<td>(Duerfahrt et al., 2004)</td>
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<tr>
<td>HMWPCy1</td>
<td>DLLMDASS</td>
<td>Yersinia pestis</td>
<td>AAM85057.1</td>
<td>(Deng et al., 2002)</td>
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<tr>
<td>HMWPCy2</td>
<td>DNLLDLGILS</td>
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</tr>
<tr>
<td>C) Condensation domain</td>
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<td>Bacillus atrophaeus</td>
<td>EIM09914</td>
<td>In this study</td>
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<tr>
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<td>Bacillus brevis</td>
<td>CAA31623.1</td>
<td>(Turgay et al., 1992)</td>
</tr>
</tbody>
</table>

Fig. 3. High-performance liquid chromatography–mass spectrometry analysis of product formation catalyzed by in vitro combinatorial biosynthesis. Mass spectrum of the peak at 9.516 min, [M+H]+ = 173.0386 m/z (a). Reaction in the absence of substrate amino acids was as a control reaction (b). Reaction mixtures with the boiled protein were as a control (c). Each experiment was performed in triplicate.

Table 1
Amino acid alignment of the Peptidyl Carrier Proteins (PCP) domain core region LGGXS (A), the Cy domain core region DXXXXDXXS (B), and C domain core HHXXXDG (C).
reaction. In this study, an in vitro assay for dipeptide formation was conducted. After mixing two recombinant protein modules, namely module A1-P1 and module Cy-A2-P2, the Sfp protein, and the corresponding substrate amino acids in an appropriate buffer, we conducted an in vitro assay for dipeptide formation (Figs. 3 and 4). We anticipated that AlaCythiazoline would be produced instead of AlaCysthiazole because of the absence of the Ox domain. Notably, after the in vitro assay, AlaCysthiazole with a molecular weight of 172.03 was detected (Figs. 3, 4 and Fig. S2), and these results are inconsistent with the results of Bloudo et al. (2017), who observed a thiazoline product. The Ox domain can catalyze the oxidation of the heterocyclic rings to produce corresponding thiazole and oxazole derivatives (Duerfahrt et al., 2004; Schneider and Walsh, 2004). The Ox domain from MtaD is involved in myxothiazol biosynthesis (Schneider et al., 2003). In the study of Li et al. (2013), the oxidation of 2-thiazolines and 2-oxazolines was reported to be crucial, and various methods have been reported for this transformation. The introduction of the second double bond into the AlaCythiazoline moiety we observe in our experiments remains unexplained.

Heterocycles such as thiazolidines, thiazoles, and oxazoles exhibit a notable biological activity (Credico et al., 2011), and 4-carboxylic acid derivatives are produced by this synthesis. The use of heterologous hosts for the characterization of biosynthetic enzymes enables the production of natural products and promotes the discovery of new natural products (Watanabe, 2014). In this study, we heterologously expressed and characterized two modules of the complete NRPS enzyme system. We established the reaction system of an artificial bimodular NRPS model system and obtained AlaCythiazoline, which was predicted to be the biosynthetic intermediate of bacillamide C. We present the functional characterization of NRPS clusters in E. coli and the ease of genetic manipulation in E. coli facilitates the development of bacillamide analogs by combinatorial biosynthesis using the functional domains of NRPS clusters.

5. Conclusion

In this research, NRPS module A1-PCP1, module Cy-A2-PCP2 and Sfp protein have been heterologous expressed respectively. Bacillamide biosynthesis intermediate - thiazole derivative was assayed in vitro, after mixing two recombinant proteins module A1-P1 and module Cy-A2-P2, Sfp protein and corresponding substrate amino acids in an appropriate buffer.

Competing interests

The authors declare that they have no competing interests.
The peptidyl carrier protein (PCP) domain are used to shuttle the substrates and the adénylation (A) domains select and activate the cognate amino acid; the thiazoline ring.

The formation between two substrates; and Cy domain performs both inter-

tation (C) domain located at the N-terminus of the module catalyzes amide bond analysis web-site (http://nrps.igs.umaryland.edu). In the domains architecture, manuscript. All authors read and approved the

Acknowledgments

Authors

Fig. 5. Biosynthetic production of the 4-carboxylic acid thiazole derivative (C6H4N2O2S) was predicted by NRPS modules and domains architecture reaction. The domains architecture from NRPS has been analyzed by PKS/NRPS analysis web-site (http://nrps.igs.umaryland.edu). In the domains architecture, the adenylation (A) domains select and activate the cognate amino acid; the peptideyl carrier protein (PCP) domain are used to shuttle the substrates and peptide intermediates between different catalytic domains; and the condensation (C) domain located at the N-termius of the module catalyzes amide bond formation between two substrates; and Cy domain performs both inter-molecular condensation and intramolecular heterocyclization reactions to form thiazoline ring.

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Authors’ contributions

FLZ, NM and YKW conceived the study. FLZ, NM, YKW and YXL analyzed and interpreted the data. FLZ and ZYL drafted and revised the manuscript. All authors read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jbiotec.2018.11.024.

References


Omura, S., Suzuki, Y., Kita, C., Takahashi, Y., Kodaka, Y., 1975. Isolation of a new sulfur-
containing basic substance from a *Thermo actinomyces* species. J. Antibiot. 28, 609–610.


