Original Research Article

Translocation of subunit PPSE in plipastatin synthase and synthesis of novel lipopeptides

Ling Gao a,b,1, Wenjie Ma a,1, Zhaoxin Lu a, Jinzhong Han a, Zhi Ma a, Hongxia Liu a, Xiaomei Bie a,∗

a Nanjing Agricultural University, College of Food Science and Technology, Nanjing, 210095, China
b Jiangnan University, The Key Laboratory of Industrial Biotechnology, Wuxi, 214122, China

A R T I C L E   I N F O

Keywords: Nonribosomal peptide synthase (NRPS) is a unique molecular assembly mechanism with high hybridity. Its recombination is conducive to the development of novel lipopeptides. However, there are few reports on NRPS subunit recombination of plipastatin at present. In this paper, plipastatin synthase was modified by the forward movement of subunit PPSE and the replacement of the communication-mediating (COM) domain. The results showed that ppsABE, a new assembly line, could synthesize novel lipopeptides such as cycle pentapeptide (C16–14β-OHFA-E-O-cyclo(Y-T-I)), and its antimicrobial activity against Rhizopus stolonifer and Staphylococcus aureus was better than that of plipastatin. However, the reactivity of ppsABCE disappeared, but the substitution of COM mutant/COM mutant or COM mutant/COM mutant for COM mutant/COM mutant could restore its activity and conduct the biosynthesis of linear hexapeptide (C16–12β-OHFA-E-O-Y-T-E-A/V) and heptapeptide (C17–13β-OHFA-E-O-Y-T-E-A-I). Collectively, these findings indicated that the COM donor domain at the C-terminus of PPSE and the compatible COM domain is an important tool for communication between nonpartner subunits. Moreover, the integrity and selective compatibility of the COM acceptor domain of subunit PPSE are essential to promote the interaction between PPSE and other subunits. This work further complemented the rules of NRPS subunit recombination and provided a theoretical basis for the development of novel high-efficiency lipopeptides.

1. Introduction

Bacillus species are often applied as biocontrol strains owing to their ability to produce a variety of biocontrol agents [1–4]. Cyclic lipopeptides of Bacillus, such as surfactin, iturin, and plipastatin (fengycin), have well-known potential in biotechnology and biopharmaceutical applications due to their biosurfactant properties, antibacterial, antifungal, and antiviral activities [5–7]. Structurally, these lipopeptides share a typical cyclic structure comprised of β-amino or β-hydroxy fatty acid integrated into a peptide moiety. This particular structure also makes their chemical synthesis time-consuming and laborious. In contrast, some microorganisms could synthesize cyclic lipopeptides by the multicarrier thiotemplate mechanism of nonribosomal peptide synthase (NRPS). The NRPS has an interesting feature, its modular design, where the individual modules act as building blocks for incorporating single amino acid components into the final lipopeptide product [8]. Each module has a core C-A-T domain responsible for recognizing, activating, and loading the corresponding amino acid [9]. Some modules also have an Epimerization (E) domain, which can transform L-type amino acid to D-type amino acid to maintain the correct three-dimensional structure of the lipopeptides [10,11]. In addition, there is a thioesterase (Te) domain at the end of the termination module, which is responsible for cyclizing and releasing the mature peptide products [12].

The modular structure endows NRPS systems with natural hybridity. In recent years, combinatorial biosynthesis mediated by the recombination of NRPS has become a potential way to synthesize novel lipopeptides. Jiang et al. [13] obtained high-efficient and low toxicity surfactin lipopeptide derivatives without Leu or Asp by knocking out a single module in surfactin synthase. In previous studies in our

Peer review under responsibility of KeAi Communications Co., Ltd.
Corresponding author. Nanjing Agr Univ, Coll Food Sci & Technol, Nanjing, 210095, China.
E-mail addresses: gasaoling@jiangnan.edu.cn (L. Gao), 2019108002@njau.edu.cn (W. Ma), fmb@njau.edu.cn (Z. Lu), 2013208003@njau.edu.cn (J. Han), 2015208002@njau.edu.cn (Z. Ma), 2013208002@njau.edu.cn (H. Liu), bxm43@njau.edu.cn (X. Bie).
* Mean joint first authors.

https://doi.org/10.1016/j.synbio.2022.09.001
Received 5 May 2022; Received in revised form 1 August 2022; Accepted 7 September 2022
Available online 20 September 2022

© 2022 The Authors. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
The main reason for this is that the docking between modules and the forward movement of the Te domain, and a series of linear lipopeptide products were synthesized [14].

Table 1

| Strains | Mass ions (m/z) | Peptide sequence | Products |
|---------|----------------|-----------------|----------|
| pB2-L   | 1435.8, 1449.8, 1463.8, 1477.8, 1491.8, 1505.8, 1519.8, 1533.8 | C14-21 \(-\text{OHFA}-\text{E}-\text{O}-\text{Y}-\text{T}-\text{E}-\) \(\beta\)-pentapeptide | Plipastatin |
| LP7     | 875.55, 889.57, 903.58 | C15-18 \(-\text{OHFA}-\text{E}-\text{O}-\text{Y}-\text{T}-\text{E}-\) \(\beta\)-pentapeptide | Cycle |
| LP8     | 980.5435, 994.5923, 1006.5950, 1022.6102 | C16-17 \(-\text{OHFA}-\text{E}-\text{O}-\text{Y}-\text{T}-\text{E}-\) \(\beta\)-hexapeptide | Linear |
| LP10    | 980.5435, 994.5923, 1006.5950, 1022.6102, 1107.6545, 1121.6705 | C17-18 \(-\text{OHFA}-\text{E}-\text{O}-\text{Y}-\text{T}-\text{E}-\) \(\beta\)-heptapeptide | Cycle |

Fig. 1. Schematic representation of subunit PPSE translocation in plipastatin synthetases. (A) Plipastatin synthase operon; (B) COM domain between synthase subunits; (C) Strategy of subunit PPSE translocation.

2. Materials and methods

2.1. Strains and culture media

The bacterial strains were grown in Luria Broth (LB) medium, supplemented when needed with erythromycin (10 g/mL for B. subtilis) and kanamycin (10 g/mL for B. subtilis, 50 g/mL for E. coli). Landy fermentation medium buffered with 0.1 M 3-(N-morpholino) propane sulfonic acid (MOPS) was used to produce lipopeptides [23].

2.2. Plasmid construction

All DNA fragments were PCR-amplified from the genomic DNA of B. subtilis 168 with pfu DNA polymerase (Fermentas, MA, USA). The primers (Table S1) were purchased from Sangon Biotech (Shanghai, China) and contain the desired restriction endonuclease sites for the subsequent cloning of PCR products. Standard procedures were applied for all DNA manipulations [24]. DNA sequencing confirmed the identity of all plasmids constructed.

A schematic representation of plasmid construction for deletion and substitution is shown in Fig. S1. The 1422 bp upstream fragment ppsB’ and the 945 bp downstream fragment ppsE’ were amplified with the primers ppsB-F/BEsoe-R and BEsoe-F/ppsE-R, respectively. Based on the 24 bp overlapping region in BEsoe-F and BEsoe-R, both fragments were used simultaneously as the template for the subsequent fusion PCR with the primers ppsB-F and ppsE-R. The resulting fusion fragment ppsB’-ppsE’ (containing COMpsps/COMppse) was cloned into the pMD19-T vector via TATA cloning. After digestion with Sall and KpnI, the fusion fragment was ligated into pKS2 to obtain the final deletion vector pKS-DE. Similarly, the fusion fragment ppsC’-ppsE’ (containing COMppsc/COMppse) was constructed by a splice overlap extension polymerase chain reaction (SOE-PCR) using the primers ppsC-F and CEsoe-R as well as CEsoe-F and ppsE-R to yield (after cloning) the deletion vector pKS-CE. The fusion fragment ppsC’-ppsE’ (containing COMppsc/COMppse) was amplified by SOE-PCR using the primers ppsC/F and ppsC/R as well as ppsC/F and ppsC/R to give pKS-CD. The fusion fragment ppsC’-ppsE’ (containing COMppsc/COMppse) was amplified by SOE-PCR using the primers ppsC/F and ppsC/R as well as ppsC/F and ppsC/R to obtain pKS-DE.

2.3. B. subtilis strain construction

The translocation of subunit PPSE in plipastatin NRPS was achieved by a homologous recombination approach mediated with a two-step
replacement recombination procedure [25]. Plasmids were transferred into Bacillus subtilis pB2-L by natural competence as previously described. After the transformation of the plasmid into the host, the entire plasmid was inserted into the chromosome via a single crossover between the target gene and a homologous sequence on the plasmid when grown in LB medium at 37 °C (a nonpermissive temperature for plasmid replication). Subsequent growth of the cointegrates in LB medium at the permissive temperature (30 °C) leads to a second recombination event, resulting in kanamycin- and erythromycin-sensitive clones with either the parental or mutant sequence. PCR analysis and sequencing were performed to confirm the mutant strain (the kanamycin and erythromycin resistance genes could not be amplified, but the upstream and downstream regions of the homologous sequence could be amplified). To this end, the deletion plasmid pKS-BE or pKS-CE was transformed into the wild-type strain B. subtilis pB2-L to give B. subtilis mutants LP7 (ppsB∷COMDppsB∷ppsE) and LP8 (ppsC∷COMDppsC∷ppsE). The mutant strain LP8 was transformed with plasmids pKS-CD or pKS-DE, generating B. subtilis mutants LP9 (ppsC∷COMDppsD∷ppsE) and LP10 (ppsC∷COMDppsE∷ppsE).

2.4. Product analysis

Production and purification of lipopeptides were performed as described previously. Extracts were analyzed by high-resolution liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) using a G2-XS Q-TOF mass spectrometer (Waters, USA). Samples were loaded onto a UPLC column (2.1 × 100 mm ACQUITY UPLC BEH C18 column containing 1.7 μm particles) and eluted with a solvent gradient of 5–95% buffer B for 22 min (buffer A, 0.1% [v/v] formic acid in H2O; buffer B, 0.1% [v/v] formic acid in acetonitrile) at a flow rate of 0.4 mL min\(^{-1}\) and monitored at 205 nm.

Mass spectrometry was performed using an electrospray source in positive ion mode within a mass range of 50–1500 m/z. Ionization was performed with a capillary voltage of 2.5 kV, collision energy of 40 eV, source temperature of 120 °C, and desolvation gas temperature of 400 °C. Data acquisition and processing were performed using MassLynx 4.1 (Waters, USA).

2.5. Analysis of biological activity

Using Rhizopus stolonifer, Fusarium oxysporum, Aspergillus ochraceus, and Staphylococcus aureus as indicator microorganisms, the antibacterial
activity of the samples was detected by the Oxford cup plate diffusion method (double-layer nutrient agar, 10 mL in the upper layer and 5 mL in the lower layer). The methanol extract of the fermentation product of mutant strains LP7, LP8, and LP9 was filtered and sterilized with a 0.45 μm PVDF membrane. Then, 100 μL of filtrate was added to the Oxford cup and cultured at 37°C for 24 h, and the bacteriostatic effect was observed and recorded.

3. Results

3.1. Translocation of the PPSE subunit in plipastatin synthetases

To obtain plipastatin derivatives with a shorter peptide sequence, the PPSE subunit, comprising a module for incorporating Ile and the terminal Te domain, was moved forward into the end of the PPBS or PPSC subunit in plipastatin synthase. The knockout plasmids (pKS-BS and pKS-CE) containing the appropriate constructs were transferred into B. subtilis pB2-L, and then translocations of the subunit PPSE were generated by completely deleting PPSC and PPSD to give the corresponding mutant strains LP7, LP8, and LP9, as shown in Fig. 1C. The native donor COMppsA domain at the C-terminus of subunit PPBS and native acceptor COMppsB domain at the N-terminus of subunit PPSE were maintained to facilitate the interaction between the nonpartner subunit PPBS and PPSE in mutant strain LP7. Similarly, the constructed pair COMppsA/COMppsB was used to facilitate contact between the nonpartner subunits PPSE and PPSE in mutant strain LP8.

After verifying the correct genotype, the fermentation products of the recombinant strains B. subtilis LP7 and LP8 were analyzed by high-resolution ESI-MS. A series of molecular ions with m/z values at 875.5488, 889.5644, and 903.5801 (Table 1 and Fig. 2A) were detected from the crude extract of LP7, which were consistent with the mass ions of the truncated hexapeptide Cβ-OH-fatty acid containing 17 carbons linked to the N-terminus of the plipastatin. Further support for this result was obtained from an analysis of the ESI-MS/MS spectrum of [M+H]+ ions at 903.5801 m/z, as shown in Fig. 2B. The cyclic molecule fragments yielded characteristic products -y3 (378.20) and -b2 broken at the Orn-Tyr bonds, corresponding to the macrocyclic moiety and fatty acid chain along with Glu-Orn residues. Other fragment ions at m/z 115.09 (Orn) and 136.3 (Tyr), and internal fragmentation ions at m/z 215.14 (Tyr + Ile), m/z 689.44 (M + H-Tyr-Ile+), m/z 671.44 (M + H-Tyr-Ile-H2O)+, m/z 653.43 ((M + H-Tyr-Ile-2H2O)+, and m/z 885.57 (M + H2O)+ were also detected, which confirmed the sequence of the cyclic pentapeptide C18-OHFA-Glu-Orn-cyclo(Tyr-Thr-Ile). The precursor ions m/z 875.5488 and 889.5644 differed from m/z 903.5801 with multiples of 14 Da in mass, indicating that the cyclic pentapeptides with identical peptide sequences had variable chain lengths of the β-OH fatty acid (C16 and C17).

In contrast, we failed to detect any lipopeptide related to plipastatin in crude extracts of B. subtilis LP8, which suggested that the hybrid enzyme ppA/BCE was inactivated. Previous studies have shown that the interaction between subunits of the NRPS complex relies on the interplay of compatible sets of donor and acceptor COM domains [26]. Thus, we speculated that the COM pair COMppsA/COMppsB was incompatible, preventing the crosstalk between enzymes PPSC and PPSE, resulting in the abrogation of this hybrid biosynthetic system in LP8.

3.2. Swapping of the COMppsA/COMppsB domain

To validate our speculation and restore the productivity of the hybrid synthase ppA/BCE, we substituted the incompatible COMppsA/COMppsB domain with the native compatible COM pair COMppsC/COMppsD and COMppsE/COMppsF (Fig. 3A). This cloning step was performed in a way that ensured the maintenance of the primary sequences of the conserved core motifs “TLSD” and “MQEGMFLH”, which were used as fusion sites (Fig. 3B).

The corresponding COM domain swaps were generated, and the integrity of the resulting B. subtilis strains LP9 (COMppsC/COMppsD and LP10 (COMppsD/COMppsE) was verified as described above. Subsequently, lipopeptide production was analyzed. As shown in Fig. 4A, the high-resolution ESI-MS revealed a series of molecular ions with m/z values of 980.5435, 994.5923, 1008.5950, and 1022.6102, which were consistent with the mass ions of the truncated hexapeptide C16-17β-OHFA-Glu-Orn-Tyr-Thr-Glu-Ala-Val. The structures of the derivatives were further confirmed using ESI-MS/MS analysis. For example, in the ESI-MS/MS spectrum of the [M+H]+ ions at 1022.6102 m/z (Fig. 4B), the observed b- and y-fragment ions permitted coverage of the entire sequence. This confirmed that the amino acid composition of the putative linear hexapeptide was Glu-Orn-Tyr-Thr-Glu-Val, where the β-OH fatty acid containing 17 carbons was linked to the N-terminus of the peptide. Other molecular ions were derived from this peptide moiety, and the 14 Da differences again indicated variations of the sixth amino acid.
acid (Ala or Val) and the length of the β-OH FA chain. Remarkably, no expected shortened heptapeptide could be observed in the crude lipopeptide extracts of *B. subtilis* LP9. According to these results, the COM<sup>D</sup>/COM<sup>A</sup> domain swap restored the productivity of ppsABCE, leading to a truncated assembly line with PPSC, PPSE, and Te domains that produces hexapeptides with the sequence C<sub>16-17</sub>β-OHFA-Glu-Orn-Tyr-Thr-Glu-Ala/Val but does not allow for a productive interaction between the natural nonpartner subunits PPSC and PPSE.

In contrast, ESI-MS and ESI-MS/MS detection results for *B. subtilis* LP10 (COM<sup>D</sup>/COM<sup>A</sup>) revealed the formation of the expected shortened heptapeptide product. As shown in Fig. 5, the predicted linear heptapeptides C<sub>17</sub>β-OHFA-Glu-Orn-Tyr-Thr-Glu-Ala-Ile (m/z calcd for C<sub>54</sub>H<sub>90</sub>N<sub>8</sub>O<sub>16</sub>[M+H]<sup>+</sup>, found 1107.6545, RT: 11.59 min) and C<sub>18</sub>β-OHFA-Glu-Orn-Tyr-Thr-Glu-Ala-Ile (m/z calcd for C<sub>55</sub>H<sub>92</sub>N<sub>8</sub>O<sub>16</sub>[M+H]<sup>+</sup>, found 1121.6705, RT: 12.22 min) were detected. Using MS/MS spectra of the precursor ions [M+H]<sup>+</sup> at m/z 1121.6705, b- and y-fragment ions were assigned, which confirmed the sequence of the heptapeptide as C<sub>18</sub>β-OHFA-Glu-Orn-Tyr-Thr-Glu-Ala-Ile (Fig. 6). These results indicated that the compatible COM<sup>D</sup>/COM<sup>A</sup> domain swap restored the productivity of the hybrid NRPS system ppsABCE, enforced communication between the natural nonpartner enzymes PPSC and PPSE, and eventually catalyzed the synthesis of shortened heptapeptides.

### 3.3. Biological activity

Based on the above results, the antimicrobial activity of lipopeptide analogues from LP7, LP9, and LP10 was analyzed. Previous studies have shown that the biological activity of plipastatin is mainly reflected in its antifungal activity [27,28]. As shown in Fig. 7, the cyclic pentapeptide produced by LP7 maintained good antifungal activity, especially against *Rhizopus stolonifer*, but the antifungal activity of linear hexapeptide and linear heptapeptide were decreased significantly.

In addition, plipastatin also has antimicrobial activity against *Staphylococcus aureus* [29]. It can reduce the intestinal colonization ability of *Staphylococcus aureus* by inhibiting its quorum sensing [30] and thus has good application prospects in the treatment of infections caused by *Staphylococcus aureus*. As shown in Fig. 7, the methanol extracts of pB2-L and LP7 had a clear inhibition zone on *Staphylococcus aureus*, and the diameter of LP7 was significantly larger than that of pB2-L. In contrast, LP9 and LP10 had no antibacterial activity against *Staphylococcus aureus*. This result showed that the change in structure...
affected the biological activity of plipastatin. The new cyclic penta-peptide exerted an excellent inhibitory effect on *Staphylococcus aureus* and fungi, which provided a new and efficient potential drug for the treatment of *Staphylococcus aureus*.

4. Discussion

In NRPS, in addition to the selective specificity of the amino acid fusion module, the synthesis of specific lipopeptides also depends on proper communication between subunits [31–33]. The COM domain plays a vital role in promoting proper communication between cooperative enzyme subunits, preventing nonselective interactions between noncooperative enzyme subunits, and finally ensuring the synthesis of peptides according to specific assembly lines. With molecular dynamics simulation, Fage et al. [20] proposed that through T7D mutation, the selection specificity of COM$_{ppsB}$ could be converted to COM$_{ppsE}$, and its recognition activity to the homologous receptor COM$_{ppsB}$ could be weakened. This result showed that the COM domain is easy to transpose. In this study, a new biosynthetic assembly line, ppsABE, was successfully constructed by moving the subunit PPSE forward to the C-terminus of PPSB. It could catalyze the synthesis of a new cyclic pentapeptide (shown in Table 1). These results indicated that COM$_{ppsB}$ and COM$_{ppsE}$ have strong compatibility and could promote the protein–protein interaction between subunits PPSB and PPSE and condense the peptidyl substrate Glu-Orn-Tyr-Thr-S-ppan with the substrate Ile. However, it should be noted that this compatibility is not universal. For
example, knockout of PPSD in this study caused a loss of the ability to synthesize lipopeptides in the hybrid synthetase ppsABCE in the mutant strain LP8, indicating that COM\textsubscript{ppsD} and COM\textsubscript{ppsc} are mismatched and incompatible with each other, just as COM\textsubscript{TycA} cannot interact with COM\textsubscript{TycC} [17].

The results of COM domain substitution showed that after replacing the receptor domain COM\textsubscript{ppsc} of subunit PPSE in the hybrid synthetase ppsABCE with COM\textsubscript{ppsd} to form a matched and compatible COM\textsubscript{ppsc}/COM\textsubscript{ppsd}, the mutant strain LP9 could produce linear hexapeptide. This result revealed that COM\textsubscript{ppsc}/COM\textsubscript{ppsd} restored the ability of ppsABCE to synthesize lipopeptides to a certain extent. However, it cannot guide the interaction between subunit PPSC and PPSE and promote the condensation of the peptidyl substrate Glu-Orn-Tyr-Thr-Glu-Ala/Val-S-ppan with substrate Ile. In contrast, after COM\textsubscript{ppsc} in ppsABCE was replaced with COM\textsubscript{ppsd}, the matched and compatible COM\textsubscript{ppsc}/COM\textsubscript{ppsd} might promote the communication and interactions between subunit PPSC and PPSE and encourage the condensation of the peptidyl substrate Glu-Orn-Tyr-Thr-Glu-Ala-S-ppan with substrate Ile. In contrast, after COM\textsubscript{ppsc} in ppsABCE was replaced with COM\textsubscript{ppsd}, the matched and compatible COM\textsubscript{ppsc}/COM\textsubscript{ppsd} might promote the communication and interactions between subunit PPSC and PPSE and encourage the condensation of the peptidyl substrate Glu-Orn-Tyr-Thr-Glu-Ala-S-ppan with substrate Ile.

In our previous research, we found that the 10IleT-Te linker plays an indispensable role in protein–protein interactions and directing peptide product synthesis. However, this study demonstrated that COM\textsubscript{ppsd} and COM\textsubscript{ppsc} were also compatible in the hybrid synthetase ppsABE. Hahn et al. [35] proposed that the replacement of COM\textsubscript{ppsc} with COM\textsubscript{ppsd} restored the ability of ppsABCE to synthesize lipopeptides to a certain extent. However, it cannot guide the interaction between subunit PPSC and PPSE and promote the condensation of the peptidyl substrate Glu-Orn-Tyr-Thr-Glu-Ala/Val-S-ppan with substrate Ile. In contrast, after COM\textsubscript{ppsc} in ppsABCE was replaced with COM\textsubscript{ppsd}, the matched and compatible COM\textsubscript{ppsc}/COM\textsubscript{ppsd} might promote the communication and interactions between subunit PPSC and PPSE and encourage the condensation of the peptidyl substrate Glu-Orn-Tyr-Thr-Glu-Ala-S-ppan with substrate Ile.

In the plipastatin synthase system, COM\textsubscript{ppsc}/COM\textsubscript{ppsd} are a pair of compatible and matched COM domains. However, this study demonstrated that COM\textsubscript{ppsd} and COM\textsubscript{ppsc} were also compatible in the hybrid synthetase ppsABE. Hahn et al. [35] proposed that the replacement of COM\textsubscript{ppsc} with COM\textsubscript{ppsd} restored the ability of ppsABCE to synthesize lipopeptides to a certain extent. However, it cannot guide the interaction between subunit PPSC and PPSE and promote the condensation of the peptidyl substrate Glu-Orn-Tyr-Thr-Glu-Ala-S-ppan with substrate Ile. In contrast, after COM\textsubscript{ppsc} in ppsABCE was replaced with COM\textsubscript{ppsd}, the matched and compatible COM\textsubscript{ppsc}/COM\textsubscript{ppsd} might promote the communication and interactions between subunit PPSC and PPSE and encourage the condensation of the peptidyl substrate Glu-Orn-Tyr-Thr-Glu-Ala-S-ppan with substrate Ile.

The results of COM domain substitution showed that after replacing the receptor domain COM\textsubscript{ppsc} of subunit PPSE in the hybrid synthetase ppsABCE with COM\textsubscript{ppsd} to form a matched and compatible COM\textsubscript{ppsc}/COM\textsubscript{ppsd}, the mutant strain LP9 could produce linear hexapeptide. This result revealed that COM\textsubscript{ppsc}/COM\textsubscript{ppsd} restored the ability of ppsABCE to synthesize lipopeptides to a certain extent. However, it cannot guide the interaction between subunit PPSC and PPSE and promote the condensation of the peptidyl substrate Glu-Orn-Tyr-Thr-Glu-Ala/Val-S-ppan with substrate Ile. In contrast, after COM\textsubscript{ppsc} in ppsABCE was replaced with COM\textsubscript{ppsd}, the matched and compatible COM\textsubscript{ppsc}/COM\textsubscript{ppsd} might promote the communication and interactions between subunit PPSC and PPSE and encourage the condensation of the peptidyl substrate Glu-Orn-Tyr-Thr-Glu-Ala-S-ppan with substrate Ile.

In our previous research, we found that the 10IleT-Te linker plays an important role in the forward transformation of the Te domain [14]. In contrast, this study demonstrated that the interaction and compatibility between COM domains are of great significance for maintaining the activity of NRPS synthase and found that COM\textsubscript{ppsb} could selectively promote the communication and interactions between subunit PPSC and PPSE and encourage the condensation of the peptidyl substrate Glu-Orn-Tyr-Thr-Glu-Ala-S-ppan with substrate Ile.
interact with COM\textsuperscript{pspE} in addition to COM\textsuperscript{pspC}. With the interaction between COM domains, a new assembly line ppsABE and ppsABCE was constructed, and ppsABE could synthesize a cyclic pentapeptide, which has better antibacterial activity against \textit{L. Gao et al.}

CRediT authorship contribution statement

Yuliani H, Perdani MS, Savitri I, et al. Translocation of the thiostreucin domain for the redesign of plipastatin synthetase. Sci Rep 2016;6:38407. http://10.7164/antibiotics.39.888.

[35] Hahn M, Stachelhaus T. Harnessing the potential of communication-mediating domains for non-ribosomal peptide production in \textit{Rhizopus stolonifer}. Syst Biotechnol 2022;7:677–88. http://10.1371/journal.pone.0249211.

[41] Kim WE, Patel A, Hur GH, et al. Mechanistic probes for the epimerization domain of nonribosomal peptide synthetases. ChemBioChem 2019;20:147–52. http://10.1002/cbic.201900459.

[24] Fage CD, Kosol S, Jenner M, et al. Communication breakdown: dissecting the COM interfaces between the subunits of nonribosomal peptide synthetase. ACS Catal 2021;11:10802–13. http://10.1021/acscatal.1c02113.

[40] Zhi Ma: Formal analysis, Investigation, Data analysis, Writing – original draft. Xiaomei Bie: Conceptualization, Methodology, Resources, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was supported by grants from the National Natural Science Foundation of China (Grant No. 31972174) and the National Key R&D Program of China (Grant No. 2018YFC1602500).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.synbio.2020.09.001.

References

[1] Pandin C, Darsonval M, Mayeur C, et al. Biofilm formation and synthesis of antimicrobial compounds by the biocontrol agent \textit{Bacillus velezensis} Q5713 in an agasurus biopeas compost microcosm. Appl Environ Microbiol 2019;85. http://10.1128/AEM.00327-19.

[9] Marahiel MA. A structural model for multimodular NRPS assembly lines. Nat Prod Rep 2017;34:618–37. http://10.1039/c6nr00409k.

[1] Pandin C, Darsonval M, Mayeur C, et al. Biofilm formation and synthesis of antimicrobial compounds by the biocontrol agent \textit{Bacillus velezensis} Q5713 in an agasurus biopeas compost microcosm. Appl Environ Microbiol 2019;85. http://10.1128/AEM.00327-19.

[7] Yuliani H, Perdani MS, Savitri I, et al. Antimicrobial activity of biosurfactant derived from \textit{Bacillus subtilis} C19. In: 5th international conference on energy and environment research (ICEER, (2018), vol. 153; 2018. p. 274–80. http://10.1016/j.jmb.2016.09.007.

[3] Zakataeva NP, Nikitina OV, Gronskiy SV, et al. A simple method to introduce new and modified biosurfactants. Biosurfactants; 2010. p. 158–69.

[11] Hahn M, Stachelhaus T. Selective interaction between nonribosomal peptide synthetases is facilitated by short communication-mediating domains. P Natl Acad Sci USA 2004;101:15585–90. http://10.1073/pnas.0404921101.

[37] Sur S, Romo TD, Gronsfeld A. Selectivity and mechanism of fengycin, an antimicrobial lipopeptide, from molecular dynamics. J Phys Chem B 2018;122:7616–26. http://10.1021/acs.jpcb.7b09488.

[20] Fage CD, Kosol S, Jenner M, et al. Communication breakdown: dissecting the COM interfaces between the subunits of nonribosomal peptide synthetase. ACS Catal 2021;11:10802–13. http://10.1021/acscatal.1c02113.

[27] Tuseg K, Aas T, Hirai M, et al. The genes degp, pps, and lpa-8 (slp) are responsible for conversion of Bacillus subtilis 168 to plipastatin production. Antimicrob Agents Chemother 1999;43:2183–92. http://10.1128/AAC.43.9.2183.

[10] Kim WE, Patel A, Hur GH, et al. Mechanistic probes for the epimerization domain of nonribosomal peptide synthetases. ChemBioChem 2019;20:147–52. http://10.1002/cbic.201900459.

[2] Pylro VS, Franco Dias AC, Andreote FD, et al. Closed genome sequence of \textit{Bacillus sacchari} strain CBMAI 1303, a bacterium applied for phytopathogen biocontrol. Microbiol Resour Ann 2019;8. http://10.1128/MRA.01507-18.

[5] Yadav M, Eswari JS. Modern paradigm towards potential target identification for antiviral (SARS-ncov-2) and anticancer lipopeptides: a pharmacophore-based approach. Avicenna J Med Biotechnol (AJMB) 2022;14:70–7. http://10.3390/ajmb.v14i1.8172.

[34] Siewers V, San-Bento R, Nielsen J. Implementation of communication-mediating domains for non-ribosomal peptide production in \textit{Rhizopus stolonifer}. Syst Biotechnol 2022;7:677–88. http://10.1371/journal.pone.0249211.

[34] Siewers V, San-Bento R, Nielsen J. Implementation of communication-mediating domains for non-ribosomal peptide production in \textit{Rhizopus stolonifer}. Syst Biotechnol 2022;7:677–88. http://10.1371/journal.pone.0249211.

[29] Vanittanakom N, Loeffler W, Koch U, et al. Fengycin—a novel antifungal lipopeptide antibiotic produced by \textit{Bacillus subtilis} F-293. J Antibiot 1986;39:888–901. http://10.1002/ajmb.20210.

[18] Chiocchini C, Linne U, Stachelhaus T. In vivo biocombinatorial synthesis of lipopeptides by COM domain-mediated reprogramming of the surfactin biosynthetic complex. Chem Biol 2006;13:899–908. http://10.1016/j.chembiol.2006.06.015.

[16] Corpuz JC, Sanlley JO, Burkart MD. Protein-protein interface analysis of the non-ribosomal peptide synthetase peptidyl carrier protein and enzymatic domains. Synthetize in Microbial Natural Products. Biotechnol Bioeng 2010;106:841–56. http://10.1002/bit.22739.

[25] Zakataeva NP, Nikitina OV, Gronskiy SV, et al. A simple method to introduce new and modified biosurfactants. Biosurfactants; 2010. p. 158–69.