How to harness biosynthetic gene clusters of lasso peptides

Mini Review

How to harness biosynthetic gene clusters of lasso peptides

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Abstract

Lasso peptides produced by bacteria have a very unique cyclic structure ("lasso" structure) and are resistant to protease. To date, a number of lasso peptides have been isolated from proteobacteria and actinobacteria. Many lasso peptides exhibit various biological activities, such as antibacterial activity, and are expected to have various applications. Based on study of genome mining, large numbers of biosynthetic gene cluster of lasso peptides are revealed to distribute over genomes of proteobacteria and actinobacteria. However the biosynthetic gene clusters are cryptic in most cases. Therefore, the combination of genome mining and heterologous production is efficient method for the production of lasso peptides. To utilize lasso peptide as fine chemical, there have been several attempts to add new function to lasso peptide by genetic engineering. Currently, a more efficient lasso peptide production system is being developed to harness cryptic biosynthetic gene clusters of lasso peptide. In this review, the overview of lasso peptide study is discussed.

Introduction

In recent years, medium-sized molecules have attracted attention as candidate molecules for drug development. Small molecule drugs can be administered orally and have low immunotoxicity, but these drugs may cause side effects due to their low specificity. On the other hand, high molecular weight protein drugs have high specificity and few side effects, but their application range is narrow, and there is a problem of immunotoxicity. Peptides with medium sizes (0.5-5 kDa) are expected to have the advantages of both molecules. Naturally occurring peptides derived from bacteria are attractive sources for medium-sized molecules. Among bacterial peptides, “lasso peptides” are very promising compounds due to its structural varieties with biological activities [36, 11, 68, 69]. Lasso peptides are a group of peptides named after “lasso” (a rope with a noose at one end). This class of peptides has been reported from bacteria, including proteobacteria and actinobacteria. Lasso peptide is comprised of 14-33 amino acids in length and possesses a slipknot structure as a motif at the *N*-terminus. In the lasso peptide molecule, an isopeptide bond is present between the amino group of the *N*-terminal amino acid and the β- or γ-carboxyl group of Asp or Glu at the 7th - 9th position from the *N*-terminus, forming a macrolactam ring. The lasso peptide microcin J25 was isolated from *Escherichia coli*, and it is regarded as the archetype of lasso peptides [90, 94, 5, 104, 1].

In the case of microcin J25, the 1st Gly and 8th Glu residues form isopeptide bonds, resulting in a macrolactam ring (Fig. 1A). Normally, the *C*-terminal linear peptide part (tail) normally passes through the macrolactam ring (orange part, FYG in microcin J25, Fig. 1A). The loop part is between the tail and ring parts, bending and supporting the linear part to get though the ring (green part, YFVGIGTPIS in microcin J25, Fig. 1A). This unique three-dimensional structure is a motif structure of lasso peptides that provides resistance to protease digestion. Bulky amino acids such as Tyr at the tail sequence sometimes prevent unthreading of the tail from the ring (F and Y in microcin J25, Fig. 1A).

Based on the biosynthetic system, lasso peptides are classified as ribosomally synthesized and post-translationally modified peptides (RiPPs) [40]. As shown in Fig. 2A, the lasso peptide biosynthetic gene cluster (BGC) of proteobacteria consists of the following four genes: a precursor peptide coding gene (gene A), two maturation enzyme coding genes (gene B, peptidase; gene C, cyclase), and an ATP-binding cassette transporter gene (gene D). The precursor peptide is modified by protein C to form an isopeptide bond between the amino group of the *N*-terminal amino acid and the β- or γ-carboxyl group of Asp or Glu at the 7th - 9th position (Fig. 2B). The leader peptide (orange part in Fig. 2B) is cleaved off by the function of protein B. This set of four genes

is common in the lasso peptide BGC of proteobacteria, although the transporter gene (gene D) is not essential. The BGC of the lasso peptide of actinobacteria also has a similar gene set. The B proteins of the BGC of actinobacteria are split (B1 and B2) and correspond to protein B of proteobacteria (Fig. 2A). The gene cluster is a very simple and comparatively small gene cluster (~5000 bp), and it can be targeted for heterologous expression to produce new lasso peptides using *E. coli* as a host cell. According to advancements in efficient genome sequencing methods, massive amounts of bacterial genome data have been deposited in databases. Now, it is not difficult to find new BGCs of lasso peptides from bacterial genome data. In this review, we discuss the history of lasso peptide discovery and the current status of heterologous production of lasso peptide using cryptic biosynthetic gene clusters. In addition, we introduce intriguing attempts to add new functions to lasso peptide by genetic engineering.

Genome mining method for BGCs of lasso peptide

The genome mining method has become a powerful tool to find new lasso peptides due to the accumulation of bacterial genome data. There are several genome mining tools, such as AntiSmash [6], RiPPER [92], and RippMiner [2], for secondary metabolites, including lasso peptides. The prediction system for RiPPs, named RODEO (Rapid ORF

Description and Evaluation Online) [98], was developed, and six new lasso peptides have been experimentally verified with this system. Without these tools, it is easy to find lasso peptide gene clusters just by using a Blastp search, which is a common method for protein similarity searches. In the case of lasso peptides in proteobacteria, genes B and C are essential for biosynthesis (Fig. 2A). For instance, the process to find BGCs is as follows: 1) search analogous genes using known B genes, such as *mcjB*, of microcin J25 biosynthesis; 2) check the area near gene B to find genes A and C and find bacterial genomes that have both genes. Gene A is sometimes not annotated by automated annotation systems that find ORFs from databases, since it is a very short sequence (40-60 amino acids) without any functional motif, such as the active site of the enzyme. In that case, it is necessary to manually find gene A from the raw DNA sequence of the area near gene B or gene C with genome data. By genome mining, Maksimov et al. investigated more than 3000 prokaryotic genome data in search for BGCs of lasso peptides and identified 76 bacteria that had BGCs of lasso peptides [70].

Bioactivities of lasso peptides

A large number of lasso peptides that exhibit various physiological activities have been reported from proteobacteria and actinomycetes (Table 1). Microcin J25 shows

specific antibacterial activity against *Escherichia* and *Salmonella*. Microcin J25 is taken up into cells via siderophore receptors [72]. Microcin J25 inhibits RNA polymerase in cells and exhibits antibacterial activity [19]. Since microcin J25 has potent antibacterial activity, attempts were made to modify the constituent amino acids of microcin J25 to produce improved peptides with stronger and more specific antibacterial activity. Since lasso peptides are generated by enzymatic reactions, there are restrictions on the amino acids that can be substituted, and a method for creating variants of microcin J25 using computer calculations was developed [79]. In the gene cluster of microcin J25, a gene library in which amino acids of the precursor peptide have been substituted has been prepared, and a variant having a stronger activity against *Salmonella enterica* was obtained [81]. In addition, it was shown that the size of the macrolactam ring is also important for the formation of the lasso peptide structure in the modification of microcin J25 [22]. Recently, the microcin J25 analogous peptide citrocin (Fig. 1B) was reported to have weaker antibacterial activity than microcin J25 [12]. Interestingly, citrocin showed higher RNA polymerase inhibitory activity than microcin J25, which indicated that uptake into bacterial cells may limit the antibacterial activity of citrocin.

Many lasso peptides have been isolated from actinomycetes. Anantin is the first microbial antagonist of atrial natriuretic peptide [103, 105]. RES-701 type lasso

peptides are antagonists of endothelin type B receptor, which are expected to be promising for treating diseases caused by elevated levels of endothelins [76, 78, 107]. BI-32169 is a glucagon receptor antagonist that can potentially be applied as a new antidiabetic therapy [46, 55, 87]. Lariatins isolated from the actinobacterium *Rhodococcus jostii* K01-B0171 showed potent and specific antimicrobial activity against mycobacteria [44, 45]. Lariatin A inhibited the growth of *Mycobacterium tuberculosis* at quite a low concentration: MIC value of 0.39 μg/mL. The mechanism of specific antimycobacterial activity is of great interest; however, the antibacterial mechanism is still unknown. Lassomycin (Fig. 2B) was discovered as an "unthreaded form" lasso peptide from the rare actinobacterium *Lentzea kentuckyensis* [30]. Interestingly, the *C*-terminal carboxyl group of lassomycin is methylated, which is unusual for lasso peptides. Lassomycin exerted specific antimicrobial activity towards a variety of mycobacterial species, including multidrug-resistant and extremely drug-resistant *M. tuberculosis*, with an MIC value of 0.8-3 μg/mL. The mechanism of potent antimycobacterial activity of lassomycin is dysregulation of the ClpP1P2 protease by interaction with the ClpC1 ATPase. Propeptins as inhibitors of prolyl endopeptidase have been isolated from the rare actinobacterium *Microbispora* sp. [25, 52, 53]. Siamycin-type lasso peptides, including RP 71955 [29, 39] (aborycin [86]), siamycin I

[65] (NP-06 [16] or MS-271 [48, 109]), siamycin II [17, 20, 99] and specialicin [49], were isolated from actinobacteria belonging to the *Streptomyces* genus. Interestingly, MS-271 (siamycin I) has D-Trp at the *C*-terminus residue, and this D-Trp may be converted from L-Trp by specific epimerase [27]. Siamycin-type lasso peptides were reported to have anti-HIV and antibacterial activities. In addition, MS-271 (siamycin I) had inhibitory activity against smooth muscle myosin light chain kinase [48, 109]. Chaxapeptin [24, 71], sungsanpin [100] and ulleungdin [95], were reported to suppress cell invasion (and migration) by the human lung cancer cell line A549.

Heterologous production of lasso peptides

Many lasso peptides of actinomycetes are produced by natural producers, whereas few lasso peptides of proteobacteria are produced by natural producers (Table 1). Even if lasso peptides are produced, they are produced only in trace amounts; for example, the cases of caulosegnin [35] and astexin [115]. Therefore, heterologous production using *Escherichia coli* as a host seems to be necessary for the production of lasso peptides of proteobacteria. For instance, the general expression vector pET-41a was used to clone the capistruin gene cluster [54]. The resulting construct was transformed into *E. coli* BL21(DE3), which led to the production of capistruin. By culturing in an

153 oligotrophic M20 medium, capistruin was successfully produced with a yield of 0.2
154 mg/L. Heterologous production by *E. coli* was also performed for the lasso peptide
155 caulosegnin [35]. The production of caulosegnins was optimized by the strategy that
156 integrating a new terminator and *mcjBCD* promoter upstream of the modifying enzymes
157 to regulate expression levels [80]. As a result, the production of caulosegnins was
158 achieved with a yield of 0.1-0.3 mg/L. In heterologous expression using *E. coli*, the *C*-
159 terminus tail part of the lasso peptide is normally susceptible to endogenous proteases,
160 resulting in a *C*-terminus truncated lasso peptide. Recently, pandonodin (Fig. 1B), a
161 lasso peptide with a long *C*-terminal tail (18 amino acid residues), was reported to be
162 produced by heterologous production [10]. Interestingly, pandonodin possesses 6 amino
163 acids disulfide-bonded macrocycle as a steric lock in addition to macrolactam ring. For
164 heterologous production of lasso peptides using *E. coli* as a host, an oligotrophic
165 medium such as M9 or M20 medium is often used to simplify isolation procedure of the
166 lasso peptide from culture medium. The growth of *E. coli* in oligotrophic medium is
167 very slow and weak, even though lasso peptides are produced. This is thought to be one
168 reason for low production. Under such circumstances, a new production system for
169 lasso peptides needs to be developed. Lasso peptides are classified as secondary
170 metabolites. Lasso peptides are hardly produced when proteobacteria are cultured in the

laboratory. On the other hand, there have been no reports on screening for natural producers of lasso peptides in proteobacteria. Many bacteria belonging to the genus *Sphingomonas* have lasso peptide biosynthesis gene clusters. We thought that natural producers could be discovered by screening for lasso peptide production using various media. As a result of screening, three strains of *Sphingomonas* (*S. macrogoltabidus*, *S. herbicidovorans*, and *S. subterranea*) were found to produce lasso peptides when they were cultured in M9 medium (Kodani, unpublished data). Among these three strains, a novel lasso peptide, subterisin (Fig. 2B), was isolated from *S. subterranea*, and it is noteworthy that subterisin was obtained with a high yield of 15.0 mg/L [62] when modified basal medium was used for culture. However, production was abolished when rich nutrient medium such as LB medium was used for cultivation of *S. subterranea*. This result indicates that the production of lasso peptide may be strictly controlled by the genetic system that responds to the culture condition. The gene cluster for subterisin production is shown in Fig. 2C [62]. This alignment of gene clusters is also frequently found in gene clusters of other proteobacterial lasso peptides. GntR-like transcription regulators exist upstream of *sbrA*, *B*, and *C*, which biosynthesize subterisin. Therefore, this gene cluster may be regulated by GntR. Other genes encoding FecI-like protein, FecR-like protein, TonB-dependent receptor, and isopeptidase are arranged in the

189 opposite direction (Fig. 2C). Regarding isopeptidase, it has been shown that the lasso
190 peptides sphingopyxin and astexin specifically cleave the isopeptide bond in the
191 macrolactam ring of the corresponding lasso peptide [26, 67]. The FecI-like protein,
192 FecR-like protein and TonB-dependent receptor are involved in the regulation of
193 siderophore uptake and gene expression. On the basis of this gene cluster arrangement,
194 it was suggested that the function of the lasso peptide was involved in iron uptake,
195 similar to siderophores [11]. In the future, the functions of lasso peptides will be
196 clarified by analyzing the production control system of lasso peptides using a natural
197 producer such as *S. subterrane*a. We performed heterologous production of subterisin
198 with an expression system using the pET-41a vector and *E. coli* BL21(DE3). However,
199 trace amounts of subterisin (<0.1 mg/L) were produced in the *E. coli* expression system
200 (unpublished data), although the natural host *S. subterranea* produced subterisin with a
201 high production yield of 15.0 mg/L.
202 In general, heterologous production of lasso peptides in *E. coli* results in low yields.
203 To solve this problem, we proposed that the natural lasso peptide producer *S.*
204 *subterranea* may be suitable as a host for heterologous production. A shuttle vector for
205 *Sphingomonas* was already reported [77], so a shuttle vector between *E. coli* and
206 *Sphingomonas* was constructed according to the report. There was also a report on a

207 consecutive promoter that works for sphingomonads [47]. The promoter was
208 incorporated into a shuttle vector and further downstream of the lasso peptide gene
209 cluster present in the genome of the α-proteobacterium *Brevundimonas diminuta*. As we
210 expected, *S. subterranea* harboring the expression shuttle vector produced a novel lasso
211 peptide, brevunsin, with a yield of 10.2 mg/L [58]. This yield was obtained without
212 optimizing the culture conditions, and it is considered that the yield can be improved by
213 further optimization.

214 Recently, the fungal pathogen *Burkholderia gladioli* was used as a host to
215 heterologously produce the lasso peptide burhizin, and the yield was 1 mg/L [8]. In the
216 case of burhizin, it was reported that six amino acids at the *C*-terminus were cleaved off
217 from the peptide when *E. coli* was used as a heterologous production host. In addition,
218 heterologous production of the lasso peptide capistruin was performed using a
219 *Burkholderia* bacterium as a host [61]. It was reported that deletion of spliceostatin
220 biosynthetic genes from the host genome increased the production of capistruin by 4.3
221 times. It was also reported that one of the five transformants produced a very large
222 amount of capistruin at 116 mg/L.

223 Among the lasso peptides of actinomycetes, homologous expression of the lasso
224 peptide lariatin was accomplished using the *larA* (lariatin precursor encoding gene)

225 mutant of actinomycete *Rhodococcus jostii* [41]. High production of the lasso peptide
226 lariatin (105 μg/mL) was reported in this system. To clarify the structure-activity
227 relationship, variants of lartiatin substituted with one Ala were produced by genetic
228 engineering. As a result, isopeptide bond-forming amino acids (Gly1 and Glu8) and
229 amino acids that constitute branches (Arg7 and Trp9) were determined to be essential.
230 Loop amino acids (Val10 and Gly11) were also indicated to be important.
231 An orthogonal two plasmid expression system was established using one plasmid
232 possessing the gene "*Streptomyces* Antibiotic Regulatory Protein (SARP)" and the other
233 plasmid with a specific promoter of SARP for the expression of the lasso peptide BGC
234 [75]. Several lasso peptides have been produced using a system with *S. albus* or *S.*
235 *lividans* as hosts. In the cloning process, the general promoter for actinomycetes
236 functions in *E. coli*, causing a problem in molecular cloning. With this orthogonal
237 system, the SARP-specific promoter does not function in *E. coli* but does function in *S.*
238 *albus* or *S. lividans*.
239 Acetylated lasso peptide albusnodin was produced in *S. coelicolor* and *S. lividans* by
240 heterologous expression [117]. Surprisingly, homologous expression did not result in
241 the production of albusnodin by *S. albus*. In addition, a new lasso peptide, fuscanodin,
242 was discovered by performing heterologous production of the lasso peptide gene cluster

243 of the thermophilic actinomycete *Thermobifida fusca* using *E. coli* as a host [60]. At the
244 same time, in vitro synthesis of the lasso peptide fuscanodin was accomplished, and it
245 clarified the kinetics of the initial reaction. Lasso peptide biosynthetic enzymes are
246 normally unstable, and using a stable thermophilic actinomycete enzyme is thought to
247 be important for in vitro reactions. Almost at the same time, the same compound was
248 reported to be enzymatically synthesized in vitro with a different name, fusilassin
249 (fuscanodin) [21]. Although in vitro synthesis of lasso peptides was reported
250 (Fuscanodin/Fusilassin), heterologous production is still the main method to explore
251 new lasso peptides, and a more efficient production system is needed.
252

253 **Giving new biological functions to lasso peptides by genetic engineering**

254 Attempts have also been made to utilize lasso peptides as fine chemical materials.
255 Allen & Link introduced two cysteines into microcin J25 and cleaved it with enzymes,
256 and self-association occurred, resulting in catenane-like molecules [4]. The lasso
257 peptide benenodin-1 was reported to form two conformers that change with heat energy
258 [119]. This is considered to be applicable to molecular devices with thermal switching
259 functions. In addition, a variant in which Arg was introduced into the loop region of
260 benenodin-1 was prepared and digested with trypsin to prepare a rotaxane-like molecule

261 [119]. The challenge to fuse a lasso peptide with a functional protein was accomplished
262 [118]. The lasso peptide astexin-1 was fused with green fluorescent protein via a
263 flexible linker. This result allowed for phage and bacterial display systems for the high-
264 throughput screening of lasso peptide libraries for new functions. Hegemann et al. used
265 genetic recombination to introduce the RGDF sequence into the loop region of microcin
266 J25 to produce an integrin inhibitory lasso peptide [32]. The original function of
267 microcin J25 was antibacterial, and it is a very challenging attempt to add a new
268 different function to lasso peptides.
269
270 **Perspective of lasso peptide research**
271 At present, the existence of a large number of lasso peptide gene clusters has been
272 revealed. The precursor peptides have large diversity in amino acid sequences. There are
273 two directions to utilize lasso peptides as functional medium-sized molecules for
274 pharmaceuticals in the future.
275 1. Modification of the lasso peptide amino acid sequence: There are still few examples of
276 drastic modification of the amino acid sequence, as in the case of microcin J25. The
277 substrate recognition site of the modified enzyme of the lasso peptide for the core peptide
278 is not yet clear. Therefore, it is reported that production is reduced by modifying the amino

acid of the core peptide in the precursor peptide. It is expected that the understanding of the substrate recognition of the core peptide by the modifying enzyme and the engineering of the modifying enzyme will enable the production of various lasso peptide variants.

2. Discovery of new bioactive lasso peptides by genome mining: At present, genomic information of a large number of bacteria has accumulated due to the development of DNA sequencing technology. The amino acid sequence of the precursor peptide of lasso peptides is very diverse. Therefore, lasso peptides containing a bioactive amino acid motif are searched. Furthermore, heterologous production is carried out using the biosynthetic gene cluster of the lasso peptide.

From the viewpoint of application, a yield on the order of mg is required for lasso peptide production for structure determination and bioactivity screening. In vitro production of lasso peptides is currently difficult due to enzyme instability, and lasso peptides are mainly produced in vivo. Several researchers have indicated new heterologous production systems for various host bacteria. Further improvement of expression systems (genetic engineering of hosts, optimization of promoters, culture conditions, etc.) is required to increase the productivity of lasso peptides.

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300

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743

744 Table 1. The list of lasso peptides

Actinobacteria			
[Natural production] *Trace amount in natural production.			
Peptide	**Producing strain**	**Biological activities**	**References**
Aborycin/ RP 71955	*Streptomyces griseoflavus* TÜ 4072 *Streptomyces* sp. SCSIO ZS0098 *Streptomyces* sp. SP9440	Anti-HIV Antibacterial	[86, 93] [39, 29]
Achromosin	*Streptomyces achromogenes* subsp. *Achromogenes* NBRC 12735^T	Antibacterial	[50]
Actinokineosin	*Actinokineospora spheciospongiae* DSM 45935^T	Antibacterial	[97]
Anantin **Anantins B$_1$&B$_2$**	*Streptomyces coerulescens* DSM 4777/4788 *Streptomyces* sp. NRRL S-146	Atrial natriuretic peptide (ANP) antagonist Antibacterial	[103, 105] [98]
BI-32169	*Streptomyces* sp. DSM 14996	Glucagon receptor antagonist	[87, 55, 46]
Canucins A&B	*Streptomyces canus* NRRL B-3980	-	[111]
Cattlecin/ Moomysin	*Streptomyces cattleya* NBRC 14057 *Streptomyces cattleya* NRRL 8057	-	[96] [98]
Chaxapeptin	*Streptomyces leeuwenhoekii* strain C58	Antibacterial Inhibition in cell invasion of lung cancer cell	[24]
Citrulassin A	*Streptomyces albulus* NRRL B-3066	-	[98]
Huascopeptin	*Streptomyces huasconensis HST28^T*	-	[18]
Humidimycin	*Streptomyces humidus* F-100,629	Caspofungin activity potentiator	[101]
Lariatin A **Lariatin B**	*Rhodococcus jostii* K01-B0171	Antimycobacterial	[44, 45, 43, 42, 14, 41, 106]
Lagmysin	*Streptomyes* sp. NRRL S-118	-	[98]
Lassomycin	*Lentzea kentuckyensis* sp.	Antimycobacterial	[30]
LP2006	*Nocardiopsis alba* NRRL B-24146	Antibacterial	[98]
Propeptin **Propeptin-2**	*Microbispora* sp. SNA-115	Antibacterial Prolyl endopeptidase inhibitor	[52, 25] [53]
RES-701-1&-2 **RES-701-3&-4**	*Streptomyces* sp. RE-701 *Streptomyces* sp. RE-896	Endothelin type B receptor antagonist	[76, 107] [78]
Siamycin I/ MS-271/ NP-06 **Siamycin II**	*Streptomyces* sp. AA6532 *Streptomyces* sp. M-271 *Streptomyces* sp. SKH-2344 *Streptomyces* sp. AA3891	Anti-HIV Antibacterial Inhibitor of myosin light chain kinase	[20, 99, 65] [48, 109, 27] [16] [17, 99]
Specialicin	*Streptomyces specialis* JCM 16611^T	Anti-HIV Antibacterial	[49]
Sphaericin	*Planomonospora sphaerica* JCM 9374^T	Antibacterial	[59]
SRO15-2005*	*Streptomyces roseosporus* NRRL 15998	-	[51]
Streptomonomicin	*Streptomonospora alba* YIM 90003	Antibacterial	[74]

Sungsanpin	*Streptomyces* sp. SNJ013		Inhibition in cell invasion of lung cancer cell	[100]
Ulleungdin	*Streptomyces* sp. KCB13F003		Inhibition in cell invasion of lung cancer cell	[95]
[Heterologous production]				
Peptide	**Native host**	**Heterologous host**	**Biological activities**	**References**
9401-LP1	*Streptomyces* sp. ADI94-01	*Streptomyces albus*	-	[75]
9810-LP	*Streptomyces* sp. ADI98-10	*Streptomyces albus*	-	[75]
Aborycin	*Streptomyces* sp. SCSIO ZS0098	*Streptomyces coeliecolor*	Anti-HIV Antibacterial	[93]
Alubusnodin	*Streptomyces albus* DSM 41398	*Streptomyces lividans*	-	[117]
Chaxapeptin	*Streptomyces leeuwenhoekii* strain C58	*E. coli* BL21(DE3) *Streptomyces albus* *Streptomyces coelicolor*	Inhibition in cell invasion of lung cancer cell	[71] [31]
***Des*-citrulassin A**	*Streptomyces albulus* NRRL B-3066	*Streptomyces lividans*	-	[98]
Fuscanodin/ Fusilassin	*Thermobifida fusca* DSM 43792	*E. coli* BL21 *E. coli* BL21(DE3)	-	[60] [21]
Humidimycin	*Streptomyces humidus* CA-100629	*Streptomyces albus* *Streptomyces coelicolor*	Caspofungin activity potentiator	[91]
Leepeptin	*Streptomyces leeuwenhoekii* C34^{T}	*Streptomyces coelicolor*	-	[31]
MS-271	*Streptomyces* sp. M-271	*Streptomyces lividans*	Anti-HIV Antibacterial Inhibitor of myosin light chain kinase	[27]
Snou-LP	*Streptomyces noursei* ATCC 11455	*Streptomyces lividans*	-	[75]
Sviceucin	*Streptomyces sviceus* DSM 924	*Streptomyces coelicolor*	Antibacterial	[64]
Proteobacteria				
[Natural production] *Trace amount in natural production.				
Peptide	**Producing strain**		**Biological activities**	**References**
Acinetodin	*Acinetobacter gyllenbergii* CIP 110306^{T}		RNA polymerase inhibition	[73]
Astexins-1&-2*	*Asticcacaulis excentricus* CB48 DSM 4724		-	[115]
Burhizin-23	*Paraburkholderia rhizoxinica* HKI454		-	[8]
Capistruin	*Burkholderia thailandensis* E264 DSM 13276		RNA polymerase inhibition	[56, 63]
Caulosegnins I&II*	*Caulobacter segnis* DSM 7131		-	[35]

Citrocin	*Citrobacter braakii* ATCC 51113^T		RNA polymerase inhibition	[12]
Microcin J25	*Escherichia coli* AY25		RNA polymerase inhibition	[90]
Mycetohabins - 15&16	*Mycetohabitans rhizoxinica* HKI454		-	[8]
Subterisin	*Sphingomonas subterranea* NBRC 16086^T		-	[62]
[Heterologous production]				
Peptide	**Native host**	**Heterologous host**	**Biological activities**	**References**
Acinetodin	*Acinetobacter gyllenbergii* CIP 110306^T	*E. coli* BL21(DE3)	RNA polymerase inhibition	[73]
Astexins-1 **Astexins-2&-3**	*Asticcacaulis excentricus* CB48 DSM 4724	*E. coli* BL21(DE3) *E. coli* BL21	Antibacterial -	[115] [70, 118] [67, 3, 66]
Benenodin-1	*Asticcacaulis benevestitus* DSM 16100	*E. coli* BL21	-	[9, 119, 23]
Brevunsin	*Brevundimonas diminuta* NBRC 12697^T	*Sphingomonas subterranea*	-	[58]
Burhizin **Burhizin-23**	*Burkholderia rhizoxinica* HKI454 DSM 19002 *Mycetohabitans rhizoxinica* HKI454	*E. coli* BL21(DE3) *Burkholderia gladioli* pv *agaricicola* HKI0676	-	[37] [8]
Capistruin	*Burkholderia thailandensis* E264 DSM 13276	*E. coli* BL21(DE3) *E. coli* BL21 *Burkholderia* sp. FERM BP-3421	RNA polymerase inhibition	[56, 54, 63] [83, 7] [61]
Caulonodins I-VII	*Caulobacter* sp. K31	*E. coli* BL21(DE3)	-	[37, 116]
Caulosegnins I-III	*Caulobacter segnis* DSM 7131	*E. coli* BL21(DE3)	-	[35, 33]
Citrocin	*Citrobacter pasteurii* CIP 55.13^T	*E. coli* BL21	RNA polymerase inhibition	[12]
Klebsidin	*Klebsiella pneumonia* 4541-2	*E. coli* BW25113	RNA polymerase inhibition	[73]
Microcin J25	*Escherichia coli* AY25	*E.coli* several strains	RNA polymerase inhibition	[90, 94, 110, 5, 104, 1, 89, 102, 84, 15, 28, 80, 57, 79, 81, 22, 82, 83, 108, 32, 85, 4, 88, 7, 12]
Mycetohabins	*Mycetohabitans rhizoxinica* HKI454	*E. coli* BL21(DE3)	-	[8]

Pandonodin	*Pandoraea norimbergensis* DSM 11628	*E. coli* BL21	-	[10]
Rhodanodin	*Rhodanobacter thiooxydans* LCS2 DSM 18863	*E. coli* BL21(DE3)	-	[37]
Rubrivinodin	*Rubrivivax gelatinosus* IL44	*E. coli* BL21(DE3)	-	[37]
Sphingonodins I&II	*Sphingobium japonicum* UT26	*E. coli* BL21(DE3)	-	[37]
Sphingopyxins I&II	*Sphingopyxis alaskensis* RB2256 DSM 13593	*E. coli* BL21(DE3)	-	[37, 26]
Syanodin I	*Sphingobium yanoikuyae* XLDN2-5	*E. coli* BL21(DE3)	-	[37]
Ubonodin	*Burkholderia ubonensis* MSMB2207	*E. coli* BL21	RNA polymerase inhibition	[13]
Xanthomonins I-III	*Xanthomonas gardneri* DSM 19127 *Xanthomonas citri pv. mangiferaeindicae* LMG 241	*E. coli* BL21(DE3)	-	[38]
Zucinodin	*Phenylobacterium zucineum* HLK1	*E. coli* BL21(DE3)	-	[37]
Firmicutes				
[Heterologous production]				
Peptide	**Native host**	**Heterologous host**	**Biological activities**	**References**
Paeninodin	*Paenibacillus dendritiformis* C454	*E. coli* BL21(DE3)	-	[114, 34, 113, 112]
Pseudomycoidin	*Bacillus pseudomycoides*	*E. coli* BL21(DE3)	-	[120]

745

746 Figure legends

747 Fig. 1. A) Lasso structure model of microcin J25 with amino acids. Blue: macrolactam

748 ring forming amino acids. Green: Loop part forming amino acids. Orange: Tail part

749 forming amino acids. B) Three dimensional structures of lasso peptides.

750 Fig. 2. A) Typical lasso peptide biosynthetic gene clusters in proteobacteria and

751 actinobacteria. B) Functions of proteins B and C to biosynthesize mature lasso peptide

752 from precursor. C) Biosynthetic gene cluster of subterisin.

753

754 Figure 1

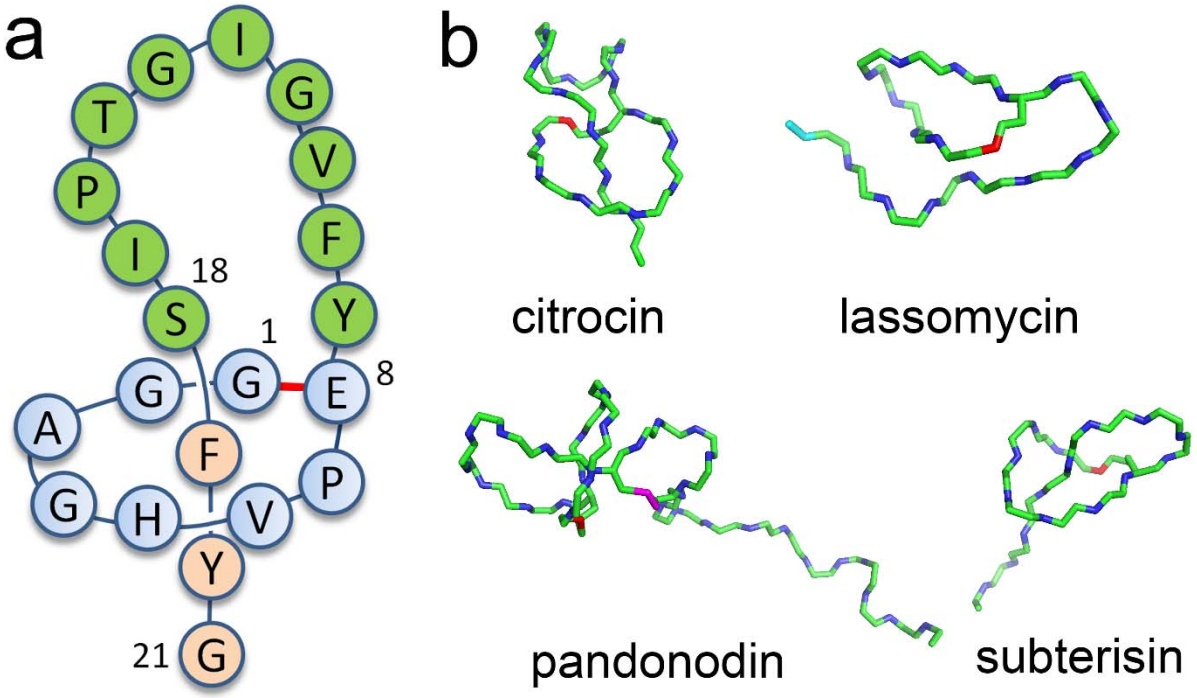
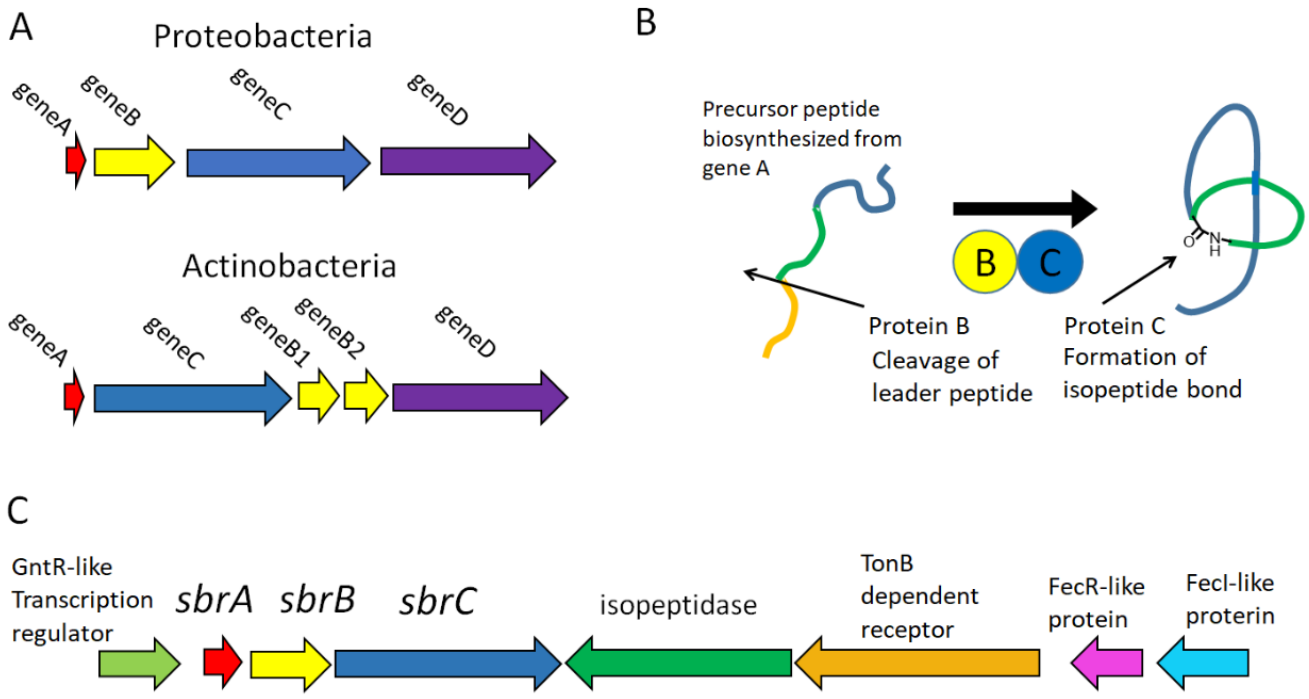


Figure 1. Kodani&Unno

755 Figure 2


A
Proteobacteria
geneA
geneB
geneC
geneD
Actinobacteria
geneA
geneC
geneB1
geneB2
geneD
B
Precursor peptide
biosynthesized from
gene A
B
C
Protein B
Cleavage of
leader peptide
Protein C
Formation of
isopeptide bond
C
GntR-like
Transcription
regulator
sbrA
sbrB
sbrC
isopeptidase
TonB
dependent
receptor
FecR-like
protein
FecI-like
proterin