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Discovery of Argyrin-Producing *Archangium gephyra* MEHO_001 and Identification of Its Argyrin Biosynthetic Genes

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Argyrins are a group of anticancer and antibacterial octapeptide bioactive substances isolated from myxobacteria. In this study, we showed that the myxobacterium *Archangium gephyra* MEHO_001, isolated in Korea, produces argyrins A and B. MEHO_001 cells tend to aggregate when cultured in liquid media. Hence, a dispersion mutant, MEHO_002, was isolated from MEHO_001. The MEHO_002 strain produced approximately 3.5 times more argyrins than that produced by the wild-type strain MEHO_001. We determined the whole-genome sequence of *A. gephyra* MEHO_002 and identified a putative argyrin biosynthetic gene cluster comprising five genes, *arg1-arg5*, encoding non-ribosomal peptide synthases and tailoring enzymes. Inactivation of *arg2* by plasmid insertion disrupted argyrin production. The amino acid sequences of the proteins encoded by *arg2-arg5* of *A. gephyra* MEHO_002 were 90-98% similar to those encoded by the argyrin biosynthetic genes of *Cystobacter* sp. SBCb004, an argyrin-producing myxobacterium with identical domain organization.

Keywords: ***Archangium gephyra*, argyrin, myxobacteria, secondary metabolite**

Introduction

Argyrins are a group of octapeptide bioactive substances isolated from the myxobacteria *Archangium gephyra* and *Cystobacter* sp. [1, 2]. They have also been isolated from the actinobacterium *Actinoplanes* sp. under the names A21459 A and B [3, 4]. Argyrins A–H have been isolated from wild-type myxobacteria [1], while argyrin I–L, A2, F3, and G3 are produced by the heterologous expression of the argyrin biosynthetic genes of *Cystobacter* sp. SBCb004 in *Myxococcus xanthus* [5].

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Argyrins display antibacterial activity against the intrinsically drug-resistant pathogen *Pseudomonas aeruginosa* [1]. Argyrin B exhibits antibacterial activity by inhibiting protein synthesis by acting on elongation factor G (EF-G) and cytotoxicity by acting on mitochondrial elongation factor G1 (EF-G1) [6]. Argyrins also have immunosuppressive [1, 2, 7] and anti-tumorigenic [8–10] activities. Argyrin A shows anticancer activity by inhibiting the cyclin kinase inhibitor $p27^{kip1}$-degrading proteasome [8]. Cyclin kinase inhibitor $p27^{kip1}$ is a protein that prevents cell division by blocking G1–S transition [11]. Argyrin B is a non-competitive immunoproteasome inhibitor [12]. Recent studies have shown that argyrin F treatment may be useful as an additional therapy for pancreatic adenocarcinoma [13].

An argyrin biosynthetic gene cluster has been identified and reported in the genome of *Cystobacter* sp. SBCb004

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[5]. The *Cystobacter* sp. SBCb004 argyrin biosynthetic gene cluster comprises five genes, *arg1–arg5*, which encode non-ribosomal peptide synthases (NRPS) and tailoring enzymes. However, argyrin biosynthetic genes have not been reported in *A. gephyra* and *Actinoplanes* sp.

Argyrins may potentially be used as attractive lead structures for the development of antibacterial and anticancer agents. However, very few argyrin-producing strains are known worldwide; none have previously been isolated in Korea. In this study, we report the discovery of the argyrin-producing strain *A. gephyra* MEHO_001 isolated in Korea and the identification of its argyrin biosynthetic gene cluster by determining and analyzing its whole-genome sequence.

Materials and Methods

Strains and culture conditions

A. gephyra MEHO_001 (KYC2615) is a myxobacterial strain isolated from Korean soil [14]. *A. gephyra* MEHO_002 and *A. gephyra* MEHO_006 were constructed in this study. *Bacillus subtilis* ATCC 6051, *Escherichia coli* ATCC 11775, *P. aeruginosa* ATCC 10145, and *Staphylococcus aureus* ATCC 25923 were obtained from the Korean Collection for Type Culture (KCTC). *P. aeruginosa* CCARM 0024 was obtained from the Culture Collection of Antimicrobial Resistant Microbes (CCARM, Korea). *A. gephyra* was cultured in CYS medium [15] at 32℃. Bacteria other than *A. gephyra* were cultured in tryptic soy broth and trypticase soy agar [16].

Plasmid and strain construction

A. gephyra MEHO_002 was isolated by subculturing the wild-type MEHO_001 strain in the CYS liquid medium. The cells dispersed in the liquid medium were then spread on CYS agar plates, incubated for 5 days, and the S-motility-defective colonies were isolated. *A. gephyra* MEHO_006 was constructed by inserting the pJO119 plasmid into the *arg2* gene of the MEHO_002 strain. The pJO119 plasmid was constructed by inserting an internal fragment of the *arg2* gene (510 bp), which was PCR-amplified using 5′-CTGGTCCTGATCGAGTCAA-3′ and 5′-CCTGGACGTATCCAACTCAA-3′ primers and *A. gephyra* MEHO_002 genomic DNA as template, into the pCR2.1 plasmid (Promega, USA). The pJO119 plasmid was introduced into *A. gephyra* MEHO_002 by electroporation and kanamycin-resistant transformants were selected as previously reported [17]. Since pCR2.1 cannot replicate in *A. gephyra*, only cells carrying a plasmid inserted on the chromosome by homologous recombination grew in the presence of kanamycin. Plasmid insertion into the *arg2* gene was confirmed using PCR, using a primer that binds to the pCR2.1 vector DNA (5′-TGTAAAACGACGGCCAGT-3′) and another that binds to the chromosomal DNA near the insertion site (5′-CTGGAGACGCACGATGAAG-3′).

Preparation of culture extracts

Myxobacterial strains were cultured in CYS liquid media with 1% Amberlite XAD-16 resin (Sigma, USA) for 5 days. The resin was then harvested and extracted using methanol. The methanol was evaporated from the extract, and the residue was dissolved in 1:1 (v/v) ethyl acetate and water. After the ethyl acetate and water layers were separated, the ethyl acetate layer was recovered and dried. The dried residue was dissolved in methanol.

High performance liquid chromatography (HPLC)

Argyrins were analyzed using an HPLC system (1260 VL Infinity Series; Agilent, USA) equipped with a Zorbax SB-C18 column (4.6 × 150 mm, 5 μm; Agilent). Water with 0.1% formic acid and acetonitrile with 0.1% formic acid were used as mobile phase A and mobile phase B, respectively. Gradient elution at 0.5 ml/min flow rate was performed as follows: 0–5 min 30% B (isocratic), 5–25 min 30–60% B (linear gradient), 25–30 min 60–100% B (linear gradient), and 30–35 min 100% B (isocratic).

Argyrins were purified using a Zorbax SB-C18 PrepHT column (21.2 × 250 mm, 7 μm; Agilent). Water with 0.1% formic acid and acetonitrile with 0.1% formic acid were used as mobile phase A and mobile phase B, respectively. Gradient elution at 6 ml/min flow rate was performed as follows: 0–50 min 40–50% B (linear gradient) and 50–60 min 100% B (isocratic).

Antimicrobial assay

Paper discs (6 mm diameter; Advantec MFS, Japan) containing 20 μg of the antimicrobial compounds were

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dried, placed on trypticase soy agar plates inoculated with test microorganisms, and incubated at 32℃ for 18 h.

Liquid chromatography-mass spectrometry (LC-MS) analysis

LC-MS was performed using an Accelar UHPLC (Thermo Scientific, USA) equipped with an Acquity UPLC® BEH C18 column (2.1 × 150 mm, 1.7 μm) and an LTQ-Orbitrap XL high-resolution mass analyzer located at Gyeonggi Bio-Center (Korea). Water with 0.1% formic acid and acetonitrile with 0.1% formic acid were used as mobile phase A mobile phase B, respectively. The gradient elution at 0.4 ml/min flow rate was performed as follows: 0–1 min 30% B (isocratic), 1–15 min 30–60% B (linear gradient), 15–25 min 60–100% B (linear gradient), and 25–27 min 100% B (isocratic).

Nuclear magnetic resonance (NMR) analysis

NMR analysis was performed using an Ascend™ 700 MHz NMR spectrometer (Bruker, Germany) equipped with a dual $^{1}H/^{13}C$ cryogenic probe system located at Gyeonggi Bio-Center. ^{1}H-^{1}H correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC) were used to elucidate the constituents of compounds.

Sequence analysis

Genomic DNA was isolated using a previously described method [18]. Whole genome sequencing was performed by Macrogen (Korea). Secondary metabolic genes were analyzed using the Antibiotics & Secondary Metabolite Analysis SHell (AntiSMASH) program [19]. DNA and amino acid sequences were analyzed using BLAST [20]. The nucleotide sequence of the argyrin biosynthetic gene cluster was deposited in the GenBank DNA sequence database (Accession Number, MW448249).

Results

Production of argyrins by *A. gephyra* MEHO_001

A. gephyra MEHO_001 (KYC2615) was originally identified as a tubulysin biosynthetic gene-carrying strain among the 81 myxobacteria strains of the family *Cystobacteraceae* [14]. HPLC analysis of *A. gephyra* MEHO_001 culture extract indicated that the peaks at 20.8 min and 22.2 min (MT001 and MT002, respectively) were significantly higher than others (Fig. 1). To determine their identity, the peak fractions were separated and analyzed using LC-MS. The m/z values of MT001 and MT002 were 825.3048 ($[M+H]^{+}$) and 839.3214 ($[M+H]^{+}$), respectively (Fig. 2). The prediction of chemical compositions based on the m/z value suggested that MT001 might be argyrin A and MT002 might be argyrin B, since the theoretical molecular weights of argyrin A and B ($[M+H]^{+}$) are 825.3137 and 839.3294, respectively. Moreover, the theoretical molecular weights of tubulysin A and B ($[M+H]^{+}$) are 844.4525 and 830.4368, respectively.

To test whether MT001 and MT002 are argyrins, two peak substances were purified and analyzed using an NMR spectrometer. ^{1}H-^{1}H correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC) were used to elucidate the constituents of MT001 and MT002. MT001 appeared to consist of 2-(1-aminoethyl)-thiazole-4-carboxylic acid, tryptophan, 4′-methoxytryptophan, glycine, alanine, dehydro-alanine, and sarcosine (Fig. 3A, Table S1), indicating that MT001

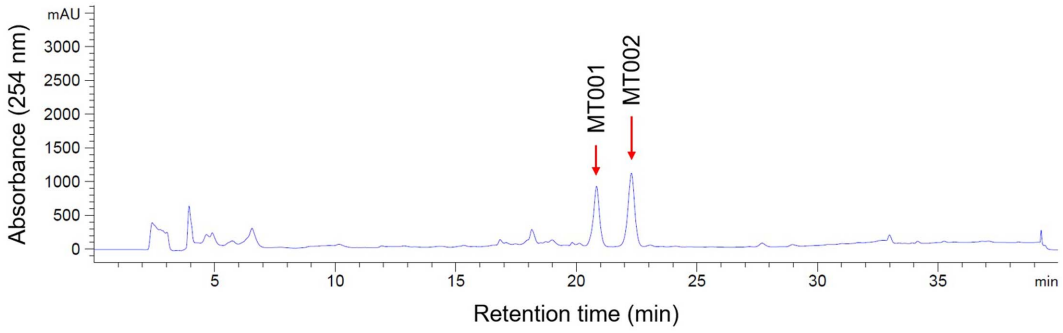

Fig. 1. High-performance liquid chromatogram of *Archangium gephyra* MEHO_001 culture extract.

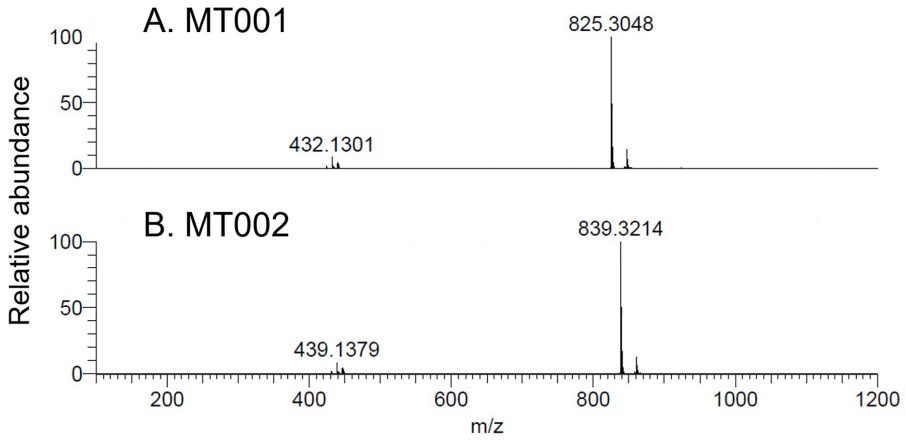

Fig. 2. The mass spectra of MT001 and MT002 produced by *Archangium gephyra* MEHO_001.

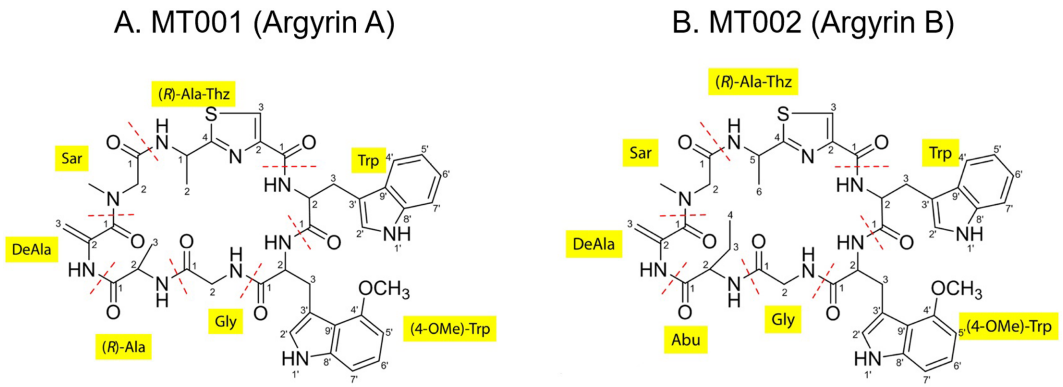

Fig. 3. The chemical structure of MT001 and MT002 determined by nuclear magnetic resonance (NMR) analysis. Ala-Thz, 2-(1-aminoethyl)-thiazole-4-carboxylic acid; Trp, tryptophan; (4-OMe)-Trp, 4'-methoxytryptophan; Gly, glycine; Ala, alanine; DeAla, dehydro-alanine; Sar, sarcosine; Abu, α-aminobutyric acid.

is identical to argyrin A. MT002 was analyzed to be identical to MT001 except that alanine is replaced by α-aminobutyric acid (Fig. 3B, Table S2), indicating that MT002 is identical to argyrin B. Therefore, it was concluded that *A. gephyra* MEHO_001 produces argyrin A and B.

Antibacterial activity of argyrins

Argyrins inhibit the growth of some bacteria, such as *P. aeruginosa* [1]. Therefore, we tested whether the *A. gephyra* MEHO_001-produced argyrin A and B have the same antibacterial activity spectrum. When a paper disc containing 20 μg argyrin A or B was placed on an agar plate inoculated with *P. aeruginosa* ATCC 10145 or CCARM 0024, the argyrins inhibited their growth (Table 1). However, under the same conditions, the growth of *B. subtilis*, *E. coli*, and *S. aureus* was not affected. This indicated that the argyrins produced by *A. gephyra* MEHO_001 have the same antibacterial activity spectrum.

Table 1. Antibacterial activity of argyrin A and argyrin B.

Microorganism	Diameter of inhibition zone (mm)[a]	
	Argyrin A	Argyrin B
Bacillus subtilis ATCC 6051	-	-
Escherichia coli ATCC 11775	-	-
Pseudomonas aeruginosa ATCC 10145	8	8
Pseudomonas aeruginosa CCARM 0024	9	9
Staphylococcus aureus ATCC 25923	-	-

[a]Determined by disc diffusion method using 6 mm diameter paper discs containing 20 μg compound.

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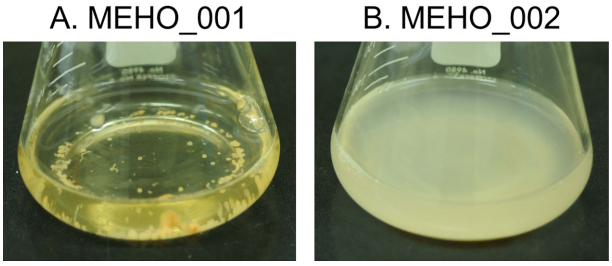

Fig. 4. The growth of *Archangium gephyra* MEHO_001 and MEHO_002 in liquid medium. The wild-type strain MEHO_001 and the dispersion mutant MEHO_002 were cultured in CYS medium for 5 days.

Isolation of the dispersion mutant of *A. gephyra* MEHO_001

Myxobacterial cells tend to clump and stick to the wall of culture vessels when cultured in liquid media. Thus, measuring cell density and manipulating genes for mutant construction is difficult. We therefore attempted to isolate mutants that disperse in a liquid medium. It is known that impairing S-motility in myxobacteria causes cell dispersion [21]. S-motility-defective mutants form colonies that are distinct from wild-type colonies on agar plates. Using these characteristics, we subcultured *A. gephyra* MEHO_001 in CYS liquid medium, spread the dispersed cells on a CYS agar plate, incubated the plates, and then isolated the colonies that appeared to have lost S-motility (Fig. S1). Four colonies were screened from eight cultures that were passaged independently. Among them, a strain that had completely dispersed in CYS liquid medium was selected (MEHO_002 strain; Fig. 4). LC-MS analysis of the culture extracts indicated that the mutant MEHO_002 and wild-type MEHO_001 strains produced the same argyrin A and B (Fig. S2). When the wild-type strain MEHO_001 was cultured in CYS medium for 5 days, it produced 1.4 mg/l argyrin A and 1.9 mg/l argyrin B. Under the same conditions, the dispersion mutant MEHO_002 produced 5.1 mg/l argyrin A and 6.7 mg/l argyrin B, resulting in a 3.5-fold increase in yield.

Identification of the argyrin biosynthetic genes

An argyrin biosynthetic gene cluster was first identified and reported in *Cystobacter* sp. SBCb004 [5]. However, the argyrin biosynthetic genes of *A. gephyra* have not been reported to date. We determined the genome sequence of *A. gephyra* MEHO_002 and analyzed the existence of argyrin biosynthetic genes. Whole-genome *de novo* sequencing generated 13,208,011 bp nucleotide sequences composed of 15 contigs. Analysis of secondary metabolic genes using AntiSMASH revealed the presence of 42 putative secondary metabolic independent genes or gene clusters in the longest contig. One of them was predicted to be a putative argyrin biosynthetic gene cluster. The 31 kb cluster comprises five genes. The amino acid sequences of the proteins encoded by the genes were more than 90% similar to those encoded by the argyrin biosynthetic genes of *Cystobacter* sp. SBCb004, and the domain compositions were the same. Therefore, these genes were named

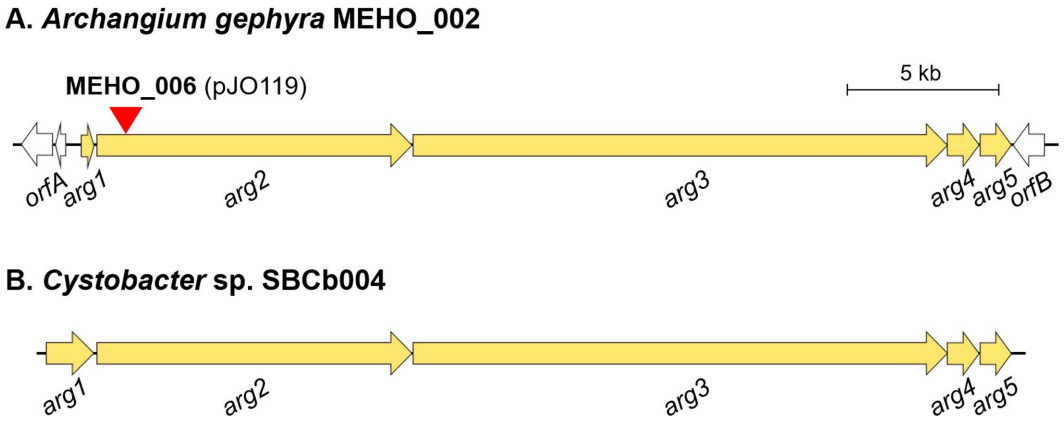

Fig. 5. The argyrin biosynthetic gene clusters. The argyrin biosynthetic gene cluster of *Archangium gephyra* MEHO_002 (accession number, MW448249) and *Cystobacter* sp. SBCb004 (accession number, MK047651) [5] are shown. The inverted triangle indicates pJO119 plasmid insertion site.

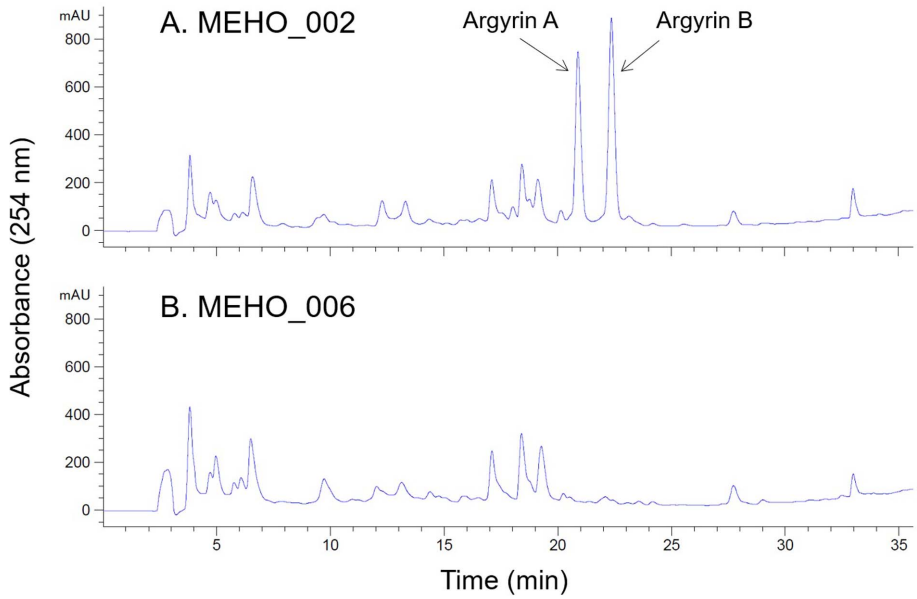

Fig. 6. High performance liquid chromatograms of *Archangium gephyra* MEHO_002 and MEHO_006 culture extracts.

arg1–arg5 (Fig. 5).

To prove that the *arg1–arg5* genes of *A. gephyra* MEHO_002 encode the argyrin biosynthetic enzymes, the pJO119 plasmid with an internal DNA fragment (510 bp) of the *arg2* gene was constructed and inserted into the *arg2* gene of the chromosome to knockout the gene. The resultant MEHO_006 strain was cultured in CYS medium to prepare a culture extract, which was then analyzed by HPLC. It was found that the MEHO_006 strain did not produce argyrin A and B (Fig. 6). These results directly showed that *arg1–arg5* genes are argyrin biosynthetic genes.

Table 2. Comparison between the argyrin biosynthetic genes of *Archangium gephyra* MEHO_002 and *Cystobacter* sp. SBCb004.

A. gephyra MEHO_002					*Cystobacter* sp. SBCb004	
Gene	Product size (aa)	Predicted function	NRPS motif predicted by antiSMASH	Identity/ Similarity (%)	Gene	Product size (aa)
orfA	89	Fragment of rRNA methyltransferase		(92/93)		
arg1	111	Fragment of Arg1		(96/98)	*arg1*	670
arg2	3,546	NRPS	A(N/A)-PCP-E-C-A(ser)-PCP-C-A(gly)-nMT	92/95	*arg2*	3,538
arg3	5,958	NRPS	C-A(N/A)-PCP-C-A(cys)-PCP-C-A(N/A)-PCP-C-A(N/A)-PCP-C-A(gly)-PCP-TE	93/95	*arg3*	5,946
arg4	336	O-methyltransferase		95/98	*arg4*	336
arg5	384	Tryptophan 2,3-dioxygenase		86/90	*arg5*	383
orfB	156	Hypothetical protein				

A, adenylation domain; aa, amino acid; ACP, acyl-carrier protein domain; C, condensation domain; cys, cysteine; E, epimerization domain; gly, glycine; N/A, not available; nMT, nitrogen methyltransferase; NRPS, non-ribosomal peptide synthetase; PCP, peptidyl-carrier protein; ser, serine, TE, thioesterase domain.
Identity/Similarity: Identity and similarity to the corresponding proteins encoded by *Cystobacter* sp. SBCb004.

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Analysis of the argyrin biosynthetic genes

Two NRPS subunits encoded by *arg2* and *arg3* genes form the argyrin core structure [5]. The amino acid sequences of the proteins encoded by the *arg2* and *arg3* genes of *A. gephyra* MEHO_002 were 92% and 93% identical to the two NRPS subunits Arg2 and Arg3 of *Cystobacter* sp. SBCb004, respectively, and the domain organization of each protein was also identical (Table 2). The amino acid sequences of the proteins encoded by the *arg4* and *arg5* genes of *A. gephyra* MEHO_002 were 98% and 90% identical to the Arg4 (O-methyltransferase) and Arg5 (tryptophan 2,3-dioxygenase) proteins of *Cystobacter* sp. SBCb004, respectively (Table 2). However, the *arg1* gene of *A. gephyra* MEHO_002 (336 bp) is only about one-sixth of the size of the *arg1* gene of *Cystobacter* sp. SBCb004 (2,013 bp) (Table 2). In addition, only the first 77 amino acids of the 111 amino acid protein were similar to the 670 amino acid protein encoded by the *arg1* gene of *Cystobacter* sp. SBCb004 (Fig. 3S). Therefore, the *arg1* gene in *A. gephyra* MEHO_002 appeared to be a gene that does not function properly due to fragmentation.

Discussion

Myxobacteria are gram-negative bacteria that produce diverse types of secondary metabolites. More than 600 substances, including derivatives, have been isolated to date [22–24]. Secondary metabolites isolated from myxobacteria exhibit various biological activities. *A. gephyra* MEHO_001 was originally screened as one of the eight strains carrying tubulysin biosynthetic genes in a previous study [14]. Thus, it also produces tubulysins in addition to argyrins, similar to *Cystobacter* sp. SBCb004, which produces both argyrins and tubulysins. However, among the eight strains with tubulysin biosynthetic genes, only the MEHO_001 strain produces argyrins, suggesting that the production of both argyrins and tubulysins is not a common characteristic. Although *A. gephyra* MEHO_001 produces both argyrins and tubulysins, their production does not appear to be correlated. The argyrin biosynthetic gene cluster is located at a completely different locus from the tubulysin biosynthetic gene cluster on the chromosome, and the production time is also different. Tubulysin production started after 5 days of incubation and reached a maximum on day 7, whereas argyrin production started on day 3 and reached a maximum on day 5. In addition, tubulysin biosynthetic gene inactivation did not affect argyrin production [25], and argyrin biosynthetic gene inactivation did not affect tubulysin production.

The *A. gephyra* MEHO_001 strain exhibited cellular aggregation when cultured in liquid. These characteristics hinder its mass cultivation using fermenters and make it difficult to manipulate genes for mutant construction; thus, a dispersion mutant, MEHO_002, was isolated from the culture of the MEHO_001 strain. HPLC analysis of the culture extract indicated that MEHO_002 produced approximately 3.5 times more argyrins than that produced by the MEHO_001 strain. The growth of the MEHO_002 strain was completely dispersed in the liquid medium. The increase in yield could be due to an increase in gene expression or simply due to an increase in the number of cells of the dispersion mutant following its acclimatization to the liquid medium. HPLC analysis of the culture extract showed that the content of other substances also increased (Figs. 1 and 6). Thus, it is plausible that the increase in production yield was simply due to an increase in the number of cells.

We identified an argyrin biosynthetic gene cluster comprising five genes, *arg1–arg5*, in *A. gephyra* MEHO_002, which is similar to that in *Cystobacter* sp. SBCb004. The *arg2* and *arg3* genes were predicted to encode two NRPS subunits required for argyrin core structure biosynthesis. The *arg4* and *arg5* genes were predicted to encode two putative modifying enzymes, o-methyltransferase and tryptophan 2,3-dioxygenase, respectively. However, the *arg1* gene of *A. gephyra* MEHO_002 is only approximately one-sixth the size of the *arg1* gene of *Cystobacter* sp. SBCb004. The *arg1* gene of *Cystobacter* sp. SBCb004 encodes a radical SAM-dependent methyltransferase, which is assumed to C-methylate the tryptophan residue incorporated by the module 7 of argyrin synthase complex [5]. No intact isoform of this gene is found in the genome of *A. gephyra* MEHO_002. Therefore, it is possible that *A. gephyra* MEHO_002 is unable to produce argyrin C and D, which carry a methyl group on the tryptophan residue incorporated by the module 7 in *A. gephyra* MEHO_002.

Argyrins have antibacterial, immunosuppressive [1, 7], and anti-tumorigenic activities [8, 9, 13]. They are

attractive lead compounds for the development of antibacterial and anticancer agents. In this study, we showed that *A. gephyra* MEHO_001 isolated in Korea produced argyrins A and B. We also isolated a dispersion mutant, strain MEHO_002, and identified the argyrin biosynthetic gene cluster from this strain. The isolated strains and genes may be used as important materials for improving argyrin production and the production of derivatives by genetic manipulation in the future.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

References

1. Sasse F, Steinmetz H, Schupp T, Petersen F, Memmert K, Hofmann H, *et al*. 2002. Argyrins, immunosuppressive cyclic peptides from myxobacteria. I. Production, isolation, physico-chemical and biological properties. *J. Antibiot*. **55**: 543-551.
2. Vollbrecht L, Steinmetz H, Hofle G, Oberer L, Rihs G, Bovermann G, *et al*. 2002. Argyrins, immunosuppressive cyclic peptides from myxobacteria. II. Structure elucidation and stereochemistry. *J. Antibiot*. **55**: 715-721.
3. Ferrari P, Vekey K, Galimberti M, Gallo GG, Selva E, Zerilli LF. 1996. Antibiotics A21459 A and B, new inhibitors of bacterial protein synthesis. II. Structure elucidation. *J. Antibiot*. **49**: 150-154.
4. Selva E, Gastaldo L, Saddler GS, Toppo G, Ferrari P, Carniti G, *et al*. 1996. Antibiotics A21459 A and B, new inhibitors of bacterial protein synthesis. I. Taxonomy, isolation and characterization. *J. Antibiot* **49**: 145-149.
5. Pogorevc D, Tang Y, Hoffmann M, Zipf G, Bernauer HS, Popoff A, *et al*. 2019. Biosynthesis and heterologous production of argyrins. *ACS Synth. Biol*. **8**: 1121-1133.
6. Nyfeler B, Hoepfner D, Palestrant D, Kirby CA, Whitehead L, Yu R, *et al*. 2012. Identification of elongation factor G as the conserved cellular target of argyrin B. *PLoS One* **7**: e42657.
7. Ley SV, Priour A, Heusser C. 2002. Total synthesis of the cyclic heptapeptide Argyrin B: a new potent inhibitor of T-cell independent antibody formation. *Org. Lett*. **4**: 711-714.
8. Nickeleit I, Zender S, Sasse F, Geffers R, Brandes G, Sörensen I, *et al*. 2008. Argyrin A reveals a critical role for the tumor suppressor protein $p27^{kip1}$ in mediating antitumor activities in response to proteasome inhibition. *Cancer Cell*. **14**: 23-35.
9. Bulow L, Nickeleit I, Girbig AK, Brodmann T, Rentsch A, Eggert U, *et al*. 2010. Synthesis and biological characterization of argyrin F. *ChemMedChem* **5**: 832-836.
10. Stauch B, Simon B, Basile T, Schneider G, Malek NP, Kalesse M, *et al*. 2010. Elucidation of the structure and intermolecular interactions of a reversible cyclic-peptide inhibitor of the proteasome by NMR spectroscopy and molecular modeling. *Angew. Chem. Int. Ed. Engl*. **49**: 3934-3938.
11. Polyak K, Lee MH, Erdjument-Bromage H, Koff A, Roberts JM, Tempst P, *et al*. 1994. Cloning of p27Kip1, a cyclin-dependent kinase inhibitor and a potential mediator of extracellular antimitogenic signals. *Cell* **78**: 59-66.
12. Allardyce DJ, Bell CM, Loizidou EZ. 2019. Argyrin B, a non-competitive inhibitor of the human immunoproteasome exhibiting preference for β1i. *Chem. Biol. Drug Des*. **94**: 1556-1567.
13. Chen X, Bui KC, Barat S, Nguyen MLT, Bozko P, Sipos B, *et al*. 2017. Therapeutic effects of Argyrin F in pancreatic adenocarcinoma. *Cancer Lett*. **399**: 20-28.
14. Hyun H, Choi J, Kang D, Kim Y, Lee P, Chung GJY, *et al*. 2021. Screening of myxobacteria carrying tubulysin biosynthetic genes. *Microbiol. Biotechnol. Lett*. **49**: 32-38.
15. Shin H, Youn J, An D, Cho K. 2013. Production of antimicrobial substances by strains of myxobacteria *Corallococcus* and *Myxococcus*. *Korean J. Microbiol. Biotechnol*. **41**: 44-51.
16. Zimbro MJ, Power DA, Miller SM, Wilson GE, Johnson JA (eds.). 2009. Difco and BBL manual: Manual of microbiological culture media. 2nd ed. Becton Dickinson and Co., Sparks, MD.
17. Cho K, Zusman DR. 1999. AsgD, a new two-component regulator required for A-signalling and nutrient sensing during early development of *Myxococcus xanthus*. *Mol. Microbiol*. **34**: 268-281.
18. Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, N.Y., USA.
19. Blin K, Shaw S, Steinke K, Villebro R, Ziemert N, *et al*. 2019. antiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. *Nucleic Acids Res*. **47**: W81-W87.
20. Johnson M, Zaretskaya I, Raytselis Y, Mereshuk Y, McGinnis S, Madden TL. 2008. NCBI BLAST: a better web interface. *Nucleic Acids Res*. **36**: W5-W9.
21. Shimkets LJ. 1986. Correlation of energy-dependent cell cohesion with social motility in *Myxococcus xanthus*. *J. Bacteriol*. **166**: 837-841.
22. Gerth K, Pradella S, Perlova O, Beyer S, Müller R. 2003. Myxobacteria: proficient producers of novel natural products with various biological activities - past and future biotechnological aspects with the focus on the genus *Sorangium*. *J. Biotechnol*. **106**: 233-253.
23. Schaberle TF, Lohr F, Schmitz A, Konig GM. 2014. Antibiotics from myxobacteria. *Nat. Prod. Rep*. **31**: 953-972.
24. Hyun H, Cho K. 2018. Secondary metabolites of myxobacteria. *Korean J. Microbiol*. **54**: 175-187.
25. Choi J, Park T, Kang D, Lee J, Kim Y, Lee P, *et al*. 2021. Analysis of tubulysin biosynthetic genes in *Archangium gephyra*. *Microbiol. Biotechnol. Lett*. **49**: 458-465.

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