Screening and Isolation of a Novel Polyene-Producing Streptomyces Strain Inhibiting Phytopathogenic Fungi in the Soil Environment

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Microbial-based eco-friendly biological substances are needed to protect crops from phytopathogenic fungi and replace toxic chemical fungicides that cause serious environmental issues. This study screened for soil antifungal Streptomyces strains, which produce rich, diverse, and valuable bioactive metabolites in the soil environment. Bioassay-based antifungal screening of approximately 2,400 Streptomyces strains led to the isolation of 149 strains as tentative antifungal producers. One Streptomyces strain showing the most potent antifungal activities against Candida albicans and Fusarium oxysporum was identified as a putative anti-phytopathogenic soil isolate that is highly homologous to Streptomyces rubrisoli (named S. rubrisoli Inha 501). An in vitro antifungal assay, pot-test, and field-test against various phytopathogenic fungi confirmed that S. rubrisoli Inha 501 is a potential novel phytopathogenic fungicide producer to protect various crops in the soil environment. Whole-genome sequencing of S. rubrisoli Inha 501 and an anti-SMASH genome mining approach revealed an approximately 150-kb polyene biosynthetic gene cluster (BGC) in the chromosome. The target compound isolation and its BGC analysis confirmed that the giant linear polyene compound exhibiting the anti-phytopathogenic activity in S. rubrisoli Inha 501 was highly homologous to the previously reported compound, neotetrafibricin A. These results suggest that a bioassay-based screening of a novel antifungal Streptomyces strain followed by its genome mining for target compound BGC characterization would be an efficient approach to isolating a novel candidate phytopathogenic fungicide that can protect crops in the soil environment.

Keywords: Streptomyces, phytopathogenic fungicide, polyene, BGC, genome mining, biosynthetic gene cluster

INTRODUCTION

For a long time in traditional agriculture, chemical fungicides have been used to control phytopathogenic fungi, such as Fusarium, Botrytis, and Colletotrichum, which cause severe damage in crop production (Dean et al., 2012; Parnell et al., 2016). On the other hand, the use of chemical fungicides has been restricted because of human toxicity and ecosystem...
destruction (Ab Rahman et al., 2018). Alternative methods are being pursued to protect crops from phytopathogenic fungi using soil-rich microorganisms, such as *Pseudomonas*, *Bacillus*, and *Streptomyces* species, to minimize these toxic compounds in the soil environment. Several commercial products containing these microbial strains are potential biocontrol agents against various phytopathogenic fungi (Parnell et al., 2016; Ab Rahman et al., 2018; Abbasi et al., 2019). Among them is a genus called *Streptomyces*, a high G + C Gram-positive bacteria, which are ubiquitous in the soil environment. *Streptomyces* produce various useful bioactive secondary metabolites, such as antibiotics, antiviral, anticancer, anti-inflammatory, antiparasitic, and antioxidant compounds (Manivasagan et al., 2014; Jakubiec-Krzesniak et al., 2018; Bu et al., 2019). In recent years, many *Streptomyces* species present in plant roots have also shown beneficial effects on crops by controlling phytopathogenic fungi or the secretion of plant growth hormones and increasing the possibility of agricultural applications (Shi et al., 2018; Kim et al., 2019).

Polycyclic compounds typically comprise a polylactone ring with 20–40 carbon atoms, including 3–8 conjugated double bonds. The most characterized antifungal polycyclics used primarily to treat severe fungal infections are polycyclic macrolides, such as the tetraene-containing nystatin A1 and heptaene-containing amphotericin B (Caffrey et al., 2016; Zhang et al., 2017). The primary antifungal mechanism of polycyclic antimicrobials is dependent on the interactions between the antibiotic molecules and ergosterol that appear to occur through the polycyclic region of the macrolactone core. In addition to these typical macrocyclic polycyclic compounds, there are also linear aminopolycyclic polycyclic compounds containing an aminoo- or guanidino-moiety, such as linearycyclic, ECO-02301, mediomycin, and neotetrafibrin (Figure 1, Caffrey et al., 2016; Zhang et al., 2017). The polycyclic core is biosynthesized by a multi-modular giant enzyme complex called polyketide synthase (PKS), and the following processes are programmed by each domain included in PKS. In general, the acyltransferase (AT) domain selects and loads an extender unit (acyl-CoA) to the acyl-career protein (ACP) domain and the keto-synthese (KS) domain then catalyzes a decarboxylative condensation. Optionally, the keto-reductase (KR), dehydratase (DH), and enoylreductace (ER) domains reduce the β-keto group to a β-hydroxy group, an α,β-double bond, and a saturated bond, respectively. Finally, the thioesterase (TE) domain cleaves the polyketide chain from PKS. The chain is modified by post-PKS modification enzymes, including P450 hydroxylases and glycosyltransferases (Caffrey et al., 2016; Zhang et al., 2017). The major antifungal mechanism of these polycycles is considered the formation of ion channels via fungal ergosterol binding that mediates the leakage of cellular K+ and Mg2+, which leads to the death of fungal cells (Neumann et al., 2016).

The traditional activity-based screening strategy is still the most widely practiced approach for selecting useful and diverse bioactive metabolites produced by soil *Streptomyces* species. Recently, alternative state-of-the-art technologies, including microbial genome mining, BGC cloning, heterologous expression, and pathway refactoring, have been pursued to complement the weaknesses of the traditional approaches, such as re-isolation and low expression issues under typical laboratory culture conditions (Lee et al., 2020). In particular, the genome mining strategy is an approach that analyzes and utilizes functional microbial genomes through bioinformatic analyses, enabling the establishment of effective strategies for predicting the pathways of various bioactive metabolite biosynthetic gene clusters (BGCs) present in the bacteria (Zerikly and Challis, 2009; Lee et al., 2020). Although a bioinformatics-based prediction using a genome mining approach still needs to be supplemented through extensive laboratory work, novel bioactive compounds can be synthesized from bacteria to various derivatives in the desired location, or natural compounds can be synthesized using sophisticated and rational reprogramming (Ziemert et al., 2016; Awakawa et al., 2018).

In this study, the bioassay-based antifungal screening of approximately 2,400 *Streptomyces* strains led to the isolation of 149 strains as tentative antifungal producers. One of these *Streptomyces* strains, showing the most potent antifungal activities against *Candida albicans* and *Fusarium oxysporum*, was identified as a putative polycyclic-producing soil isolate, highly homologous to *Streptomyces rubrisoli* (named *S. rubrisoli* Inha 501). An in vitro antifungal assay, pot-test, and field-test against various phytopathogenic fungi confirmed that *S. rubrisoli* Inha 501 could be a good candidate for a novel phytopathogenic fungicide to protect various crops in the soil environment.

**MATERIALS AND METHODS**

**Strains and Growth Conditions**

*S. rubrisoli* Inha501 was distributed from Industrial Biomaterial Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), South Korea. The strain was grown routinely in ISP2 agar (malt extract 10 g, yeast extract 4 g, glucose 2 g, agar 20 g per liter) at 30°C for the sporulation and seed culture. The R5 medium (sucrose 51.5 g yeast extract 2.5 g, peptone 5 g, malt extract 3 g, glucose 10 g, sucrose 340 g, and 10N NaOH 0.7 ml per liter) was used to produce the I-NTF (Inha-neotetrafibrin A). All *Escherichia coli* strains were incubated at 37°C in Luria–Bertani medium supplemented with the appropriate antibiotics where needed. *Candida albicans* ATCC 14053, *Aspergillus niger* ATCC 9642, *Fusarium oxysporum* f. sp. lacticac KACC (Korean Agricultural Culture Collection) 42795, *Fusarium oxysporum* f. sp. gladioli KACC 40051, *Fusarium solani* KACC 44891, *Fusarium graminearum* KACC 47495, *Fusarium verticillioides* KCTC (Korean Collection for Type Cultures) 6065, *Fusarium semitectum* KCTC 16672, Botrytis cinerea KACC 40574, *Colletotrichum gloeosporioides* KACC 40003, *Curvularia lunata* KACC 40861, and *Alternaria alternata* KACC 40019 were grown on PDA medium (potato starch 4 g, glucose 2 g, agar 15 g per liter) at 28°C for 2–7 days.

**Antifungal Pot-Test and Field-Test**

The fermentation TSB broth of *S. rubrisoli* Inha 501 was tested for *in vivo* antifungal activity against Fusarium wilt. In the case
of the pot-test, eight of each red pepper, strawberry, and tomato seedlings were prepared, respectively. The prepared seedlings were grown for 2 weeks in vinyl pots (diameter 10 cm) in a growth room at 22 ± 1°C. Four seedlings of tree crops were treated with 100 ml of *S. rubrisoli* Inha501 culture medium (10⁶ CFU/ml) along with a negative control treated only with 100 ml of TSB. After 24 h, the potted seedlings were wounded on the stem and then treated with *F. oxysporum* KACC 40051. After 2 weeks, the appearance of the seedlings was examined to measure the degree of Fusarium wilt infection (0: disease-free ~ 100: crop failure). The pot-to-pot significance difference test was performed at the 95% level by the DMRT method (Kramer, 1957). In the case of field tests, tests were conducted in accordance with “the pesticide registration test standards and methods” in Korea (Rural Development Administration, 2008). In the evaluation, the minimum incidence rate in the untreated seedlings was more than 10%, and the effectiveness was assessed by comparing the morbidity rate with the untreated seedlings 10 days after the final *S. rubrisoli* Inha501 treatment. As for the placement of the test seedlings, the test was carried out in three repetitions of the randomized complete block design and the complete randomized design (Zerbe, 1979; Scott and Milliken, 1993). Two red pepper and tomato greenhouses each infected with Fusarium wilt were prepared. The prepared seedlings were grown in soil (10 m²) in a growth room at 29 ± 3°C for 4 weeks. The test seedlings were treated with 10 L of *S. rubrisoli* Inha501 culture medium.
(2 × 10^3 CFU/ml) and the untreated seedlings were treated with 10 L of TSB. The pot-to-pot significance difference test was performed at the 95% level by the DMRT method (Kramer, 1957). The more detailed results of antifungal pot-test and field-test are provided as Supplementary Figure 2.

**Genome Sequencing and Assembly of S. rubrisoli Inha 501**

The S. rubrisoli Inha501 genome was sequenced at Macrogen (Korea) using both the PacBio RSII (Pacific Biosciences, United States) and Illumina HiSeq (Illumina, United States) platforms. The library preparation for Illumina and PacBio sequencing was performed using the TruSeq DNA sample prep kit for Illumina (NE, United States) and the PacBio DNA Template Prep Kit 1.0 (Pacific Biosciences, United States), respectively. The library insert sizes were 350 bp for Illumina sequencing and 20 kb for PacBio RS SMRT sequencing. The de novo assembly of sequenced fragments was performed using Canu (v1.7) software. A high-quality sequence was obtained by performing an error correction of the assembled contig using Pilon (v1.21) software. The annotation was performed using Prokka (v1.12b) software.

**Production and Purification of Polyene Compound**

The S. rubrisoli Inha501 was inoculated in 200 ml of TSB medium at 30°C and 220 rpm for 48 h. The pre-cultures were added to 2 L of R5 medium in a 5 L bioreactor for batch fermentation. After 120 h of cultivation, the culture broth was extracted in 2 L of n-butanol. The extract was concentrated using a vacuum evaporator. The concentrated extract was dissolved in methanol and loaded onto a column packed with a C18 reversed-phase silica gel (Daiso, Japan) and eluted with methanol-water (30:70, v/v) to remove any residual sugar from the production media. The extracts with the sugar removed were purified using a silica gel (Daiso, Japan) and eluted with methanol-water (30:70, v/v) to remove any residual sugar from the production media. The culture extracts provided from the S. rubrisoli Inha501 by homologous recombination. The desired mutant was selected on apramycin-included ISP2 agar medium, and its genotypes were verified by PCR.

**In vitro Assays for Biological Activities**

The Clinical and Laboratory Standards Institute document M27-A3 was adapted to the in vitro antifungal assay (Clinical and Laboratory Standards Institute, 2008). After fungus was cultured in PDB medium at 30°C for 1~7 days, the cultured solution was diluted with PDB medium until the OD value reached 0.3 at 530 nm. A working suspension was prepared by a 1,200 dilution with RPMI-1640 broth media (with glucose and phenol red, without bicarbonate, Sigma-Aldrich, United States), which resulted in 5.0 × 10^2 to 2.5 × 10^3 cells per μl. Ten microliter of the DMSO containing polyene antibiotics at various concentrations (25~1,600 μg/ml) were added to the working suspension of 990 Μl and the mixtures were then incubated at 30°C without shaking for 2~7 days. The colorimetric change in the mixture from red to yellow indicated the growth of the fungus. The minimum inhibitory concentration (MIC) was determined by measuring the minimum concentration that changed the color to yellow. The experiment was performed in duplicate.

**RESULTS**

**Screening and Isolation of an Antifungal Soli Streptomyces Species**

A total of 2,419 Streptomyces culture extracts provided from the actinomycetes cell collection center in the Industrial Biomaterial Research Center, Korea Research Institute of Bioscience and Biotechnology (KIRIBB), Korea was screened to isolate the strains
showing antifungal activities against C. albicans (a fungal strain for the confirmation of antifungal activity) and F. oxysporum (a phytopathogenic fungal strain) (Figure 2 and Supplementary Figure 1). 149 antifungal culture extracts showing antifungal activity were first selected, followed by the additional selection of 51 antifungal strains showing a typical polyene spectrum assayed by 2-dimensional HPLC analysis (Figure 2, Guo et al., 2000). Among the 51 strains tested, one Streptomyces sp. isolated from organic green tea fields in Jeolla Province in Korea (34°51′31.7″N 127°08′48.1″E) was selected as a final candidate to control phytopathogenic fungi, showing the strongest antifungal activities against C. albicans and F. oxysporum. 16s rRNA sequence-based phylogenetic analysis showed that the strain isolated above exhibited 98.9% similarity of S. rubrisoli FXJ1.725 (KC137300) and was called S. rubrisoli Inha501 (Figure 3A).

Laboratory-scale antifungal bioassays were performed to assess the potential applications for the development of phytopathogenic fungicide using the isolated S. rubrisoli Inha501 against various phytopathogenic fungi, F. oxysporum, F. solani, F. graminearum, F. verticilliodes, F. semitectum, Aspergillus niger, Botrytis cinerea, Colletotrichum gloeosporioides, Curvularia lunata, and Alternaria alternata. As shown in Figure 3B, S. rubrisoli Inha501 showed a strong and broad spectrum of phytopathogenic antifungal activities against most phytopathogenic fungi tested.

The controllability of phytopathogenic fungi in several crops was tested by performing pot-scale tests with the S. rubrisoli Inha501 culture in the plants infected with F. oxysporum (KACC 40051). As shown in Table 1A, the fungal pathogen control rate for each red pepper, strawberry, and tomato was...
TABLE 1 | In vivo antifungal activity of S. rubrisoli Inha501 against Fusarium wilt.

(A) Pot-test against F. oxysporum.

| Pot test | Tomato* | Strawberry* | Red pepper* |
|----------|---------|-------------|-------------|
|          | Disease incidence (%) | DMRT | Control rate (%) | Disease incidence (%) | DMRT | Control rate (%) | Disease Incidence (%) | DMRT | Control rate (%) |
| Inha501  | 35.0 bc | 63.2 | – | 15.0 b | 48.8 | a | 23.1 bc | 70.6 |
| No-treatment | 95.0 a | – | – | 63.2 | – | – | 78.8 a | – |

Coefficient of variation – a 13.0%, b 21.2%, c 19.3%.

(B) Field-test against Fusarium wilt

| Field test I* | Tomato | Field test II* | Tomato |
|---------------|--------|---------------|--------|
| Disease incidence (%) | DMRT | Control rate (%) | Disease incidence (%) | DMRT | Control rate (%) |
| Inha501       | 5.3 b | 65.0           | Inha501 | 6.3 b | 51.5 |
| No-treatment  | 15.4 a | –              | No-treatment | 13.1 a | – |

| Field test III* | Red pepper | Field test IV* | Red pepper |
|-----------------|------------|---------------|------------|
| Disease incidence (%) | DMRT | Control rate (%) | Disease incidence (%) | DMRT | Control rate (%) |
| Inha501       | 5.1 b | 57.5           | Inha501 | 10.5 b | 55.1 |
| No-treatment  | 12.0 a | –              | No-treatment | 23.14 a | – |

Coefficient of variation – a 15.5%, b 10.7%, c 17.9%, d 29.4%.

observed at 70.6, 69.2, and 63.2%, respectively. Moreover, the fungal pathogen control rate in the field-scale test was 56.3 and 58.2% for the naturally infected red pepper and tomato, respectively, implying a positive outlook for its registration as a biocontrol microbial agent (Table 1B and Supplementary Figure 2).

Whole-Genome Sequencing and Characterization of Biosynthetic Gene Clusters

Whole-genome sequencing was performed to identify the biosynthetic gene cluster (BGC) present in the S. rubrisoli Inha501 chromosome, which is responsible for producing the target antifungal compound. The complete genome size was 8,249,972 bp, and the G + C content was 70.19% (Figure 4A and Supplementary Table 1). The whole genome sequence was deposited in Strategic Initiative for Microbiomes in Agriculture and Food with an accession number igem-0000408. The S. rubrisoli Inha501 genome contained 2 plasmids (85,489, 32,253 bp), 7,318 genes, 75 tRNAs, and 18 rRNAs (Supplementary Table 1). The encoding gene sequences were aligned with the eggnog databases to predict the putative gene functions (Huerta-Cepas et al., 2019). Among them, 6,881 genes were successfully annotated with EggnoG, accounting for 94% of all genes (Figure 4B).

The antiSMASH 5.0 program revealed 35 tentative BGCs of the secondary metabolites present in the S. rubrisoli Inha501 chromosome (Blin et al., 2019). BGC # 22 was predicted to be the most likely BGC to synthesize the target linear polyene compound (Figure 4C). Bioinformatic analysis of BGC #22 predicted the typical biosynthetic pathway of the target polyene compound and revealed a significant similarity to the BGC of the previously reported neotetrafibrincin A (NTF A) (Table 2 and Supplementary Figure 3). LC-MS analysis of the purified target polyene compound (>90% purity) in the S. rubrisoli Inha501 culture showed a signal at m/z 1226.73 (calculated mass, 1227.73), which is the same as the signal at m/z 1226.72 for [C₆₇H₁₀₄NO₁₉]⁻ of NTF A (Zhang et al., 2017; Supplementary Figure 4).

The PKS gene (Inha501-4694) of BGC #22 was disrupted in the S. rubrisoli Inha501 chromosome to confirm that BGC #22 is responsible for biosynthesis of the NTF A-like compound (Figure 5A and Supplementary Figure 5). As expected, the BGC #22 PKS gene-disrupted mutant strain failed to produce NTF A-like compound, suggesting that the BGC #22 is indeed responsible for the biosynthesis of NTF A-like compound in S. rubrisoli Inha501 (tentatively called Inha-neotetrafibrincin A, I-NTF) (Yoo et al., 2011; Hong et al., 2013; Zhang et al., 2017; Figure 5B and Supplementary Figure 6).

Biological Activities of I-NTF

The purified I-NTF was evaluated for its antifungal activity by in vitro assays using a paper disc. The antifungal activity of I-NTF was confirmed in C. albicans and 11 phytopathogenic fungi. The size of the inhibition zone was larger than the controls.
FIGURE 4  (A) Circular whole-genome map drawn by applying the annotation result of the S. rubrisoli Inha501 chromosome. Marked characteristics are shown from the outside to the center; CDS on the forward strand, CDS on the reverse strand, tRNA, rRNA, GC content, and GC skew  (B) Classification of CDS by eggNOG annotation (C) Analysis of secondary metabolite biosynthetic gene clusters by antiSMASH 5.0.

Amphotericin B and Nystatin A1 (Supplementary Figure 7). The antifungal activity was measured by examining the MIC (minimum inhibitory concentration) evaluation assays of the purified I-NTF using the colorimetric change in the RPMI-1640 media containing the fungus (Clinical and Laboratory Standards Institute, 2008; Supplementary Figure 8). The MIC of I-NTF against C. albicans, A. niger, and F. oxysporum was lower than nystatin A1 but higher than that of amphotericin B (Table 3). Interestingly, the MIC value of I-NTF against F. verticillioides, F. semitectum, C. lunata, and A. alternata was lower than nystatin A1 and amphotericin B (Table 3). In summary, the antifungal activity of I-NTF produced by S. rubrisoli Inha501 against 12 fungi (containing 11 phytopathogenic fungi) was confirmed.

DISCUSSION

In place of synthetic fungicides containing various environmental and health issues, considerable research for the isolation and application of eco-friendly microorganisms has been conducted. In this study, activity-based screening allowed the isolation of 149 strains of antifungal Streptomyces that exhibited antifungal activity out of the 2,419 strains tested. Existing activity-based screening strategies had limitations that the type and chemical structure of target compounds produced from selected strains were unknown until accurate structural analysis was completed. PCR screening approaches using the primers specific to polypeptide specific P450 genes were also implemented to select the novel strain producing a specific structural family,
TABLE 2 | Annotation of the ORFs in the I-NTF gene cluster.

| ORF    | Sizea | Predicted function         | S. aizunensis and S. neyagawaensis homologb | Accession number |
|--------|-------|----------------------------|---------------------------------------------|------------------|
| I-NTF A | 234   | Thioesterase               | ORF1 (65/75%)                               | AAX98176.1       |
| I-NTF B | 920   | Transcriptional regulator  | ORF2 (55/65%)                               | AAX98177.1       |
| I-NTF C | 210   | Response regulator         | ORF3 (75/84%)                               | AAX98178.1       |
| I-NTF D | 417   | Sensor kinase              | ORF4 (65/76%)                               | AAX98179.1       |
| I-NTF E | 182   | Putative membrane protein  | ORF5 (64/75%)                               | AAX98180.1       |
| I-NTF F | 163   | Putative membrane protein  | ORF6 (82/89%)                               | AAX98181.1       |
| I-NTF G | 520   | Putative membrane protein  | ORF7 (63/75%)                               | AAX98182.1       |
| I-NTF H | 373   | Glycosyltransferase        | ORF8 (74/82%)                               | AAX98183.1       |
| I-NTF PKS I | 9792 | Type I PKS                 | Tfb1 (72%-80%)                              | BAW35651.1       |
| I-NTF PKS II | 9298 | Type I PKS                 | Tfb2 (68/76%)                               | BAW35652.1       |
| I-NTF PKS III | 6493 | Type I PKS                 | Tfb3 (72/79%)                               | BAW35653.1       |
| I-NTF PKS IV | 1633 | Type I PKS                 | Tfb4 (74/83%)                               | BAW35654.1       |
| I-NTF PKS V | 5121 | Type I PKS                 | Tfb5 (74/81%)                               | BAW35655.1       |
| I-NTF PKS VI | 5309 | Type I PKS                 | Tfb6 (71/79%)                               | BAW35656.1       |
| I-NTF PKS VII | 3169 | Type I PKS                 | Tfb7 (78/85%)                               | BAW35657.1       |
| I-NTF PKS VIII | 7352 | Type I PKS                 | Tfb8 (75/82%)                               | BAW35658.1       |
| I-NTF PKS IX | 3755 | Type I PKS                 | Tfb9 (74/81%)                               | BAW35659.1       |
| I-NTF I   | 317   | Acyltransferase            | ORF18 (66/75%)                              | AAX98193.1       |
| I-NTF J   | 298   | Putative membrane protein  | ORF19 (65/76%)                              | AAX98194.1       |
| I-NTF K   | 349   | ABC transporter            | ORF20 (68/79%)                              | AAX98195.1       |
| I-NTF L   | 335   | Sugar dehydratase/epimerase| ORF21 (58/65%)                              | AAX98196.1       |
| I-NTFM    | 210   | Sugar epimerase            | ORF22 (69/82%)                              | AAX98197.1       |
| I-NTF N   | 355   | Sugar nucleotidytransferase| ORF23 (74/81%)                              | AAX98198.1       |
| I-NTF O   | 328   | Sugar dehydratase/epimerase| ORF24 (77/82%)                              | AAX98199.1       |
| I-NTF P   | 227   | Thioesterase               | ORF25 (55/66%)                              | AAX98200.1       |
| I-NTF Q   | 467   | Acyl CoA ligase            | ORF26 (79/87%)                              | AAX98201.1       |
| I-NTF R   | 544   | Amine oxidase              | ORF27 (76/83%)                              | AAX98202.1       |
| I-NTF S   | 226   | Phosphopantetheinyl transferase| ORF28 (56/67%)                        | AAX98203.1       |
| I-NTF T   | 303   | Metallophosphoesterase     | ORF29 (80/84%)                              | AAX98204.1       |
| I-NTF U   | 952   | Transcriptional regulator  | ORF30 (51/64%)                              | AAX98205.1       |
| I-NTF V   | 533   | Carboxylase/carboxyltransferase| ORF31 (86/91%)                    | AAX98206.1       |
| I-NTF W   | 332   | Aminohydrolase             | ORF32 (79/90%)                              | AAX98207.1       |

aSize in the number of amino acids.
bS. aizunensis NRRL B-11277 is the production strain of ECO-02301 (ORF 1∼32) (McAlpine et al., 2005). S. neyagawaensis NR0557 is the production strain of neotetrafibricin A (Tfb 1∼9) (Zhang et al., 2017). The homolog shows amino acid sequence identity%/similarity%.

such as polypeptide compounds (Hwang et al., 2007). Complete genome sequencing of the selected strain complemented the weakness of the activity-based strategy, and the BGC analysis made it feasible to predict the tentative structure of the target compound.

Through the whole genome sequence analysis of S. rubrisoli Inha501, 35 putative BGCs were predicted, of which seven BGCs contained the PKS genes as the core biosynthetic genes. Thanks to the co-linearity nature of the sequence, the biosynthetic pathway of the type I PKS system and its structure are relatively easy to predict in accordance with the usual rules of the PKS multi-domain modular system. Through anti-SMASH analysis of all BGCs present in the S. rubrisoli Inha501 strains, it was predicted that the target antifungal compound encoded by the BGC #22 was the previously reported giant linear polypeptide compound called NTF A (Zhang et al., 2017). The compound produced by S. rubrisoli Inha501 was tentatively named I-NTF (Inha neotetrafibricin) until a more definite structure analysis was completed. The gene-knockout experiments confirmed that BGC #22 is responsible for the biosynthesis of an I-NTF. Interestingly, I-NTF biosynthetic gene knockout showed a decrease in overall antifungal activity in S. rubrisoli Inha501, but the activity did not disappear completely, suggesting that there might be additional functional antifungal BGCs in S. rubrisoli Inha501, such as BGC #5 (80% similarity to tautomycetin) and BGC #25 (81% similarity to tubercidin) (Choi et al., 2017; Liu et al., 2018).

The newly isolated S. rubrisoli Inha501 confirmed its antifungal activity against various phytopathogenic fungal strains and showed its potential applications as a bio-control agent through pot-test and field-test for actual crops, such as strawberries, tomatoes, and red peppers. Optimization of the culture condition and its formulation study is currently being pursued for the development of microbial fungicides of S. rubrisoli Inha501. The synergistic approach between...
activity-based screening and genome analysis highlights the efficient isolation of novel strains, such as *S. rubrisoli* Inha501, which contains a giant linear polyene BGC and a strong anti-phytopathogenic compound to protect various crops growing in the soil environments.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the government-funded whole genome sequence is deposited in the local members-only genome database. Requests to access the datasets should be directed to eungsoo@inha.ac.kr.

AUTHOR CONTRIBUTIONS

H-SP, S-SC, and E-SK designed the experiments. H-SP, H-JN, and S-HK performed the experiments. H-SP and E-SK wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fbioe.2021.692340/full#supplementary-material

Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874. doi: 10.1093/molbev/msw054

Lee, N., Hwang, S., Kim, J., Cho, S., Palsson, B., and Cho, B. K. (2020). Mini review: genome mining approaches for the identification of secondary metabolite biosynthetic gene clusters in Streptomyces. Comput. Struct. Biotechnol. J. 18, 1548–1556. doi: 10.1016/j.csbj.2020.06.024

Liu, Y., Gong, R., Liu, X., Zhang, P., Zhang, Q., Cai, Y. S., et al. (2018). Discovery and characterization of the tubercidin biosynthetic pathway from Streptomyces tubercidicus NBRC 13090. Microb. Cell Fact. 17:131. doi: 10.1186/s12934-018-0978-8

Manivisagan, P., Venkatesan, J., Sivakumar, K., and Kim, S. K. (2014). Pharmacologically active secondary metabolites of marine actinobacteria. Microbiol. Res. 169, 262–278. doi: 10.1016/j.micres.2013.07.014

McAlpine, J. B., Bachmann, B. O., Piraee, M., Tremblay, S., Alarco, A. M., Zazopoulos, E., et al. (2005). Microbial genomics as a guide to drug discovery and structural elucidation: ECO-02301, a novel antifungal agent, as an example. J. Nat. Prod. 68, 493–496. doi: 10.1021/n1040166

Neumann, A., Wieczor, Z., Zielinska, J., Baginski, M., and Czub, J. (2016). Membrane sterols modulate the binding mode of amphotericin B without affecting its affinity for a lipid bilayer. Langmuir 32, 3452–3461. doi: 10.1021/acs.langmuir.5b04433

Parnell, J. J., Berkla, R., Young, H. A., Sturino, J. M., Kang, Y., Barnhart, D. M., et al. (2016). From the lab to the farm: an industrial perspective of plant beneficial microorganisms. Front. Plant Sci. 7:1110. doi: 10.3389/fpls.2016.01110

Rural Development Administration (2008). Pesticide Registration Test Standards and Methods. Available online at: https://www.law.go.kr/LSW/admRulInfoP.do?admRulSeq=2000000060683 (accessed February 11, 2008).

Scott, R. A., and Milliken, G. A. (1993). A SAS program for analyzing augmented block designs. Crop Sci. 33, 865–867.

Shi, L., Niew, T. T., Ge, B., Zhao, W., Liu, B., Cui, H., et al. (2018). Antifungal and plant growth-promoting activities of Streptomyces roseofuscus strain NKZ-259. Biol. Control 125, 57–64. doi: 10.1016/j.biocontrol.2018.06.012

Yoo, H. G., Kwon, S. Y., Sarki, S., and Kwon, H. J. (2011). A new route to DTPD-6-deoxy-L-talose and DTPD-L-rhamnose: DTPD-L-rhamnose 4-epimerase in Burkholderia thailandensis. Bioorg. Med. Chem. Lett. 21, 3914–3917. doi: 10.1016/j.bmcl.2011.05.030

Zerbe, G. O. (1979). Randomization of the analysis completely randomized design extended to growth and response curves. J. Am. Stat. Assoc. 74, 215–221.

Zerikley, M., and Challis, G. L. (2009). Strategies for the discovery of new natural products by genome mining. ChemBioChem 10, 625–633. doi: 10.1002/cbic.200800389

Zhang, L., Hashimoto, T., Qin, B., Hashimoto, J., Kozono, I., Kawahara, T., et al. (2017). Characterization of giant modular PKSs provides insight into genetic mechanism for structural diversification of aminopolyl polyketides. Angew. Chem. Int. Ed. Engl. 56, 1740–1745. doi: 10.1002/anie.201611371

Ziemert, N., Alanjary, M., and Weber, T. (2016). The evolution of genome mining in microbes—a review. Nat. Prod. Rep. 33, 988–1005. doi: 10.1039/cn00052h

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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