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Short Communication

Biological activities and genome of marine *Streptomyces verrucosisporus* CPB1-1^{T}

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Abstract

The CPB1-1^{T} strain of *Streptomyces verrucosisporus* has been isolated from marine sediment in Thailand. The objective of this study was to determine for the first time the biological activity and to obtain the genome sequence of this type strain. The crude ethyl acetate extract of the type strain had potent antibacterial activity against Gram-positive bacteria (*Bacillus cereus* and *Enterococcus faecium*) with a minimum inhibitory concentration (MIC) of 1.56 µg/mL. In addition, it showed anti-*Mycobacterium tuberculosis* activity with a MIC value of 6.25 µg/mL. The crude extract had no activity against the Gram-negative bacteria *Plasmodium falciparum*, nor cancer cell lines. The genome analysis revealed that the strain CPB1-1^{T} had several biosynthetic gene clusters of secondary metabolites including terpene, polyketide, lanthipeptide, lasso peptide and thiopeptide.

Introduction

Microbial natural products are important resources for drug discovery as microbial secondary metabolites have long been beneficial as pharmaceutical agents including antibiotics, anticancer agents, immunosuppressants, fungicides, herbicides, insecticides and nematicides (Ziemert et al., 2014). The Actinobacteria is the phylum of Gram-positive bacteria with a high guanine-cytosine content (G+C) content in the genome (Barka et al., 2015). Among these bacteria, the genus *Streptomyces*, which belongs to the family *Streptomycetaceae*, has long been recognized as the most important bioactive compound producer (Bérdy, 2005). During the past several decades, large numbers of *Streptomyces* have been isolated from terrestrial habitat, especially soil and consequently, the opportunity to isolate novel *Streptomyces* species has decreased (Solanki et al., 2008).

The ocean is the largest ecosystem on Earth and marine habitats are believed to be a rich source of novel actinobacteria which might be able to produce unique bioactive compounds (Dhakal et al., 2017). A good example is the *Salinispora*, consisting of obligate marine actinobacteria, which produce at least 23 different secondary metabolites, antiprotealide, cyanosporide A, desferioxamine B, enterocin, lomiaviticin A, salinosporamide A, sporolide A, salinilactam, sioxanthi, pacificanone A, salinipyrone A, saliniketal A, arenicolide A, saliniquinone, cyclomarin A, cyclomarazine, arenimycin, arenamide A, staurosporines, isopimara-8, 15-dien-19-ol, rifamycin B, mevinolin and lymphostin (Jensen et al., 2015). Phongsopitanun et al. (2016) proposed a novel marine *Streptomyces* species, isolated from marine sediment collected in Thailand, which was named *S. verrucosisporus*. The type strain of this new species is CPB1-1^{T} (=JCM 18519^{T} = PCU 343^{T} = TISTR 2344^{T}). However, the biological activities and the genome of the type strain have not been determined. Consequently, this study screened the biological

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activities of the type strain of *S. verruosisporus* CPB1-1^T. In addition, the genome of the type strain was sequenced and analyzed.

Materials and Methods

Microorganism

Streptomyces verrucosisporus CPB1-1^T (= JCM 18519^T = PCU 343^T = TISTR 2344^T) was obtained from the culture collection of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand. The strain was activated on International Streptomyces Project (ISP) medium no. 2 agar (yeast extract 4 g, glucose 4 g, malt extract 10 g, agar 18 g, distilled water 1 L, pH 7.8; Shirling and Gottlieb, 1966) supplemented with seawater before use. The spore morphology was observed on the culture grown on ISP2 agar at 30°C for 14 d using a light microscope (CH2; Olympus; Japan) and a scanning electron microscope (JSM-5410LV, JEOL; Japan) according to Itoh et al. (1989).

Fermentation and extraction

The medium was prepared using artificial seawater. The inoculum was cultured in 150 mL of ISP2 broth in 500 mL Erlenmeyer flasks using shaking conditions at 180 revolutions per minute (rpm) at 30°C for 4 d (Inova 4230; New Brunswick Scientific; Germany). Then, 1 mL of inoculum was transferred into the Erlenmeyer flasks, containing 250 mL of ISP2 medium. The production medium (10 L) was incubated at 30°C under shaking conditions at 180 rpm for 10 d. At the end of the fermentation period, the culture broth, including mycelia and spores, was partitioned with ethyl acetate three times. The ethyl acetate layer was collected and evaporated to dryness. Finally, 2.54 g of a deep-brown-colored gum was obtained.

Biological activities

The antimicrobial activities and anticancer activities of the crude extract of the strain CPB1-1^T were determined. Antimalarial activity against *Plasmodium falciparum* K1, a multi-drug resistant strain, was determined using the microculture radioisotope technique (Desjardins et al., 1979) with dihydroartemisinin and mefloquine as positive controls. Antimicrobial activity was tested against *Bacillus cereus* ATCC 11778 and *Candida albicans* ATCC 90028 and cytotoxicity against KB (oral human epidermoid carcinoma, ATCC CCL-17), MCF-7 (human breast cancer, ATCC HTC-22) and NCI-H187 (human small cell lung cancer, ATCC CRL-5804) cell lines and evaluated using resazurin microplate assay (O'Brien et al., 2000; Sarker et al., 2007). Amphotericin B and vancomycin were used as positive controls for anti-*C. albicans* and anti-*B. cereus*, respectively. Ellipticine and doxorubicin were used as positive controls for anti-KB and anti-NCI-H187, while doxorubicin and tamoxifen were used as positive controls for cytotoxicity against MCF-7 cells. Anti-*Mycobacterium tuberculosis* H37Ra (ATCC 25177) and anti-phytopathogenic fungi (*Collectotrichum capsici* BMGC 106, *Collectotrichum gloeosporioides* BMGC107, *Curvularia lunata* and *Magnaporthe grisea* BCC10261) were determined using green fluorescent protein microplate assay (Changsen et al., 2003; Chutrakul et al., 2013). Isoniazid, ofloxacin, rifampicin, streptomycin and ethambutol were used as positive controls for anti-*M. tuberculosis* activity. Amphotericin B was used as a positive control for anti-phytopathogenic fungi. Antibacterial activity was performed against *Enterococcus faecium* ATCC 51559, *Escherichia coli* ATCC 25922, *Acinetobacter baumannii* ATCC 19606, *Klebsiella pneumoniae* ATCC 700603 and *Pseudomonas aeruginosa* ATCC 15692 using the standard protocols obtained from the Clinical and Laboratory Standard Institute (Wayne, 2006a, b). Rifampicin and erythromycin were used as positive controls for anti-*A. baumannii*, anti-*E. coli* and anti-*K. pneumoniae*. Erythromycin and chloramphenicol were used as positive controls for anti-*P. aeruginosa*. Tetracycline HCl was used as a positive control for anti-*E. faecium.*

Genome sequencing and bioinformatics

Genomic DNA was extracted using a PureLink™ Genomic DNA mini kit (Invitrogen; USA). The genomic DNA was sequenced using the MiSeq sequencing system (Illumina; USA). The bioinformatics data of the genome were analyzed using the bacterial bioinformatics database and analysis resource (PATRIC; Wattam et al., 2014). The biosynthetic gene cluster in the genome was determined using the antiSMASH database (Blin et al., 2019).

Results and Discussion

Biological activities

The strain CPB1-1^T grew well on ISP2 medium supplemented with the artificial seawater. Grayish-green aerial masses were observed at day 14 of culture. An open-looped spiral spore chain was observed on the aerial masses and a warty surface was observed on the mature spore (Fig. 1). These results confirmed the previous study of the morphology of the type strain (Phongsopitanun et al., 2016). The crude ethyl acetate extract of strain CPB1-1^T showed potent antibacterial activity against Gram-positive bacteria (*B. cereus* and *E. faecium*), with a minimum inhibitory concentration (MIC) of 1.56 µg/mL. The activity of the crude extract against *B. cereus* was close to that for vancomycin, the positive control (MIC = 2 µg/mL). However, the extract had no activity against tested Gram-negative bacteria (*E. coli*, *K. pneumoniae*, *A. baumannii* and *P. aeruginosa*) nor against the tested yeast *C. albicans*. The anti-*Mycobacterium tuberculosis* had an MIC of 6.25 µg/mL. However, this value was much higher than for the positive controls used for the treatment of tuberculosis, such as rifampicin, streptomycin, isoniazid, ofloxacin and ethambutol which had MIC values of 0.025 µg/mL, 0.625 µg/mL, 0.047 µg/mL, 0.781 µg/mL and 1.88 µg/mL, respectively. Based on this study, the antimalarial activity of the crude ethyl acetate of strain CPB1-1^T was negative against *Plasmodium falciparum* and against the anti-phytopathogenic fungi *Colletotrichum capsici*, *Colletotrichum gloeosporioides*, *Curvularia lunata* and *Magnaporthe grisea*. The anticancer activity against KB, MCF7 and NCI-H187 was negative.

Genome analysis

The draft genome of strain CPB1-1^T was submitted to GenBank (accession number JADEYH000000000) and is publicly available. The assembled genome of strain CPB1-1^T had 105 contigs, with a total length of 6,063,600 bp with an average G+C content of 70.03%. The genome had 5,820 protein coding sequences (CDS), 60 transfer RNA (tRNA) and 3 ribosomal RNA (rRNA) genes. Based on the analysis of the biosynthesis gene clusters of secondary metabolites using antiSMASH, strain CPB1-1^T had several biosynthetic gene clusters of secondary metabolites including terpene, polyketide, lanthipeptide, lasso peptide and thiopeptide, as shown in Table 1. Among these biosynthetic gene clusters, six clusters (SapB, ectoine, desferrioxamin B, isorenieratene, alkylresorcinol and pristinol) had a similarity value of 100% (Table 1). These compounds are commonly found in various *Streptomyces* species. SapB, a lanthipeptide-like, is a morphogenetic peptide which is used for the formation of aerial mycelia of filamentous bacteria (Kodani et al., 2019). Ectoine, 1,4,5,6-tetrahydro-2-methyl-4-pyrimidine carboxylic acid, is a compatible solute that is commonly produced by *Streptomyces* species to prevent osmotic stresses (Sadeghi et al., 2014). The presence of the ectoine biosynthetic gene cluster in the genome of strain CPB1-1^T was not unexpected because the strain was isolated from marine sediment. Desferrioxamines are a siderophore, a high-affinity, iron-chelating compound, which is commonly found in *Streptomyces*. Desferrioxamine B, Desferal, is used for the treatment of iron overload in humans (Barona-Gómez et al., 2004). Isorenieratene, a terpenoid, is an aryl carotenoid mostly found in green photosynthetic bacteria and some *Streptomyces* species such as *S. argillaceus, S. mediolani, S. odorifer, S. lividans, S. coelicolor* and *S. griseus* (Krügel et al., 1999; Becerril et al., 2018). Alkylresorcinol, synthesized by typeIII polyketide synthase, is a relatively rare phenolic lipid found in wheat, rye, barley and *Streptomyces* strains (Funabashi et al., 2008). However, the biological functions of alkylresorcinol in *Streptomyces* are not completely understood (Núñez-Montero et al., 2019). Pristinol is sesquiterpene alcohol with an unusual skeleton from *Streptomyces pristinaespiralis* (Klapschinski et al., 2016). These compounds have no antimicrobial activity. Therefore, the antibacterial activity of the crude extract observed in this study must arise from the other unidentified compounds.

This study was the first to report on the biological activities and the genome sequence of the type strain of *Streptomyces verrucosisporus*. Although most of the tests produced negative results, these data should help other researchers by saving time and cost in further investigation of this strain.

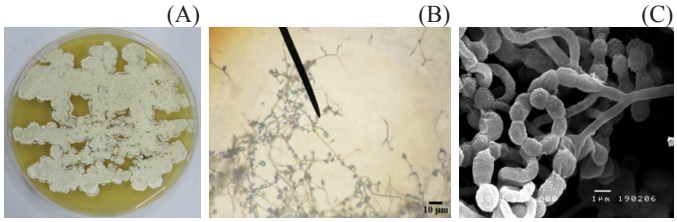

Fig. 1 Culturing characteristics and morphology of *Streptomyces verrucosisporus* CPB1-1^T (A); microscopic image showing arrangement of spore chain (B); scanning electron micrograph showing spore surface (C), where all images taken after the strain was grown on ISP2 medium at 30°C for 14 d

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Table 1 Distribution of biosynthetic gene cluster in *Streptomyces verrucosisporus* CPB1-1^T, showing only biosynthetic gene clusters with similarity values greater than 20%

Cluster	Type	Most similar known cluster (class)	Similarity (%)
1	Lipolanthine	SapB (Ripp: Lanthipeptide)	100
2	Ectoine	Ectoine (other)	100
3	Siderophore	Macrotetrolide (polyketide)	33
4	Terpene	Hopene (terpene)	30
5	Siderophore	Desferrioxamin B (other)	100
6	Terpene	Isorenieratene (terpene)	100
7	Melanin	Grizazone (terpene)	60
8	T2PKS, PKS- like	LL-D49194α1 (polyketide)	39
9	T3PKS	Alkylresorcinol (polyketide)	100
10	Terpene	Pristinol (Terpene)	100
11	Lassopeptide	SRO15-2005 (RiPP:Lassopeptide)	75
12	T1PKS	Lavendiol (polyketide)	22
13	Thiopeptide	Nosiheptide (RiPP:Thiopeptide)	61
	NRPS,T1PS, butyrolactone	Neocarzinostatin (polyketide: Iterative type 1 + polyketide: Enediyne type 1)	50
14	T2PKS, terpene, NRPS-like	Spore pigment	66

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