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Correlation Between *Sorangium cellulosum* Subgroups and Their Potential for Secondary Metabolite Production

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Phylogenetic analysis of the *groEL1* and *xynB1* gene sequences from *Sorangium cellulosum* strains isolated in Korea previously revealed the existence of at least 5 subgroups (A–E). In the present study, we used sequence analysis of polymerase chain reaction-amplified biosynthetic genes of strains from the 5 subgroups to indicate correlations between *S. cellulosum* subgroups and their secondary metabolic gene categories. We detected putative biosynthetic genes for disorazol, epothilone, ambruticin, and soraphen in group A, group C, group D, and group E strains, respectively. With the exception of KYC3204, culture extracts from group A, group B, and group C strains exhibited no noticeable antimicrobial inhibitory activities. By contrast, culture extracts from group D strains inhibited the growth of *Candida albicans*, whereas culture extracts from group E strains inhibited the growth of *C. albicans* and *Staphylococcus aureus*. High performance liquid chromatography analysis of the culture extracts from the strains of each subgroup revealed unique peak patterns. Our findings indicate the existence of at least 5 subgroups of *S. cellulosum* strains, each of which has the potential to produce a unique set of secondary metabolites.

Key words: *Sorangium cellulosum*, myxobacteria, phylogenetic analysis, strain grouping, secondary metabolite

The myxobacteria are a group of Gram-negative soil bacteria, which produce a diverse range of bioactive secondary metabolites [20, 21]. More than 500 substances, with 100 basic structures, have been isolated from myxobacteria [20]. Approximately half of these substances have been isolated from strains of the cellulolytic species *Sorangium cellulosum* [4]. Owing to the pharmaceutical importance of this species, several thousand strains have been isolated worldwide [4]; approximately 1,000 of these strains have been isolated in Korea [5]. Secondary metabolites produced by *S. cellulosum* were initially isolated from different strains. However, the number of strains known to produce identical metabolites has increased, as the isolated strains have been characterized [7, 14]. The relationships between strains producing the same metabolites remain to be elucidated. We previously reported that *S. cellulosum* strains isolated in Korea could be classified into 5 subgroups based on the DNA sequences of their *groEL1* and *xynB1* genes, which code for heat-shock protein and cellulase, respectively [15]. In the present study, we examined the correlation between the *S. cellulosum* strain subgroups and their potential for secondary metabolite production.

Materials and Methods

Bacterial Strains

S. cellulosum strains were obtained from the collections of the Myxobacteria Bank at Hoseo University, Korea. *Pseudomonas aeruginosa* ATCC 10145, *Staphylococcus aureus* ATCC 25923, and *Candida albicans* ATCC 18804 were purchased from the Biological Resource Center at the Korea Research Institute of Bioscience and Biotechnology.

Media and Culture Conditions

S. cellulosum was cultured on ST21P medium [5], *P. aeruginosa* and *S. aureus* were cultured on NA medium, and *C. albicans* was cultured on YM medium. NA medium contained 0.3% beef extract and 0.5% peptone; YM medium contained 0.3% yeast extract, 0.3% malt extract, 0.5% peptone, and 1% glucose. CYS medium, which was used to prepare culture extracts, contained 0.5% casitone, 0.1% yeast extract, 0.3% soluble starch, 0.1% $MgSO_4 \cdot 7H_2O$, 0.05% $CaCl_2$, 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 0.4% trace element solution, and 0.5 μg/ml cyanocobalamin. The trace element solution contained 100 mg of $MnCl_2 \cdot 4H_2O$, 20 mg of $CoCl_2$, 10 mg of $CuSO_4$, 10 mg of $Na_2MoO_4 \cdot 2H_2O$, 20 mg of $ZnCl_2$, 5 mg of LiCl, 5 mg of $SnCl_2 \cdot 2H_2O$, 10 mg of H_3BO_3, 20 mg of KBr, 20 mg of KI, and 8 g of EDTA Na-Fe^{3+} salt (trihydrate) per liter.

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Table 1. PCR primers used in this study.

Metabolite	Gene	Sequence (5' → 3')		Reference
		Left primer	Right primer	
	xynB1	GGCTTCGGCTACTTCTCGTG	CGTCCACCGACCAGTTCATC	[15]
	groEL1	ACGACGACGGCGACSGTGCTSGC	TCGCCRAAGCCGGGGGCYTTSAC	[9]
Ambruticin	*ambE*	TCTACGGCATCTTCGCAAAC	CAGAGATAAAGGGCCCGAAG	This study
Chivosazol	*chiB*	TGAGCGCTTATGGATTCGAC	GATGGCCTTCCAGGTGATCT	This study
Disorazol	*disB*	CAAGTACACCCGTCTGTGCC	CGATGTTGGATTTCACCGAG	This study
Epothilone	*epoA*	CTGGCTGGTGGGGTATCGCT	TGCTGAAGGGACAAGACGAC	[6]
Etnangien	*sce3190*	GGGAACGGGAAGAGCTACAG	GGTGACTGCAGAGGATCACG	This study
Jerangolid	*jerE*	GACTACGCCTTGCTCCACAA	CACTGGATATGAGGGTTGCG	This study
Leupyrrin	*leuD*	ATCGGACGTCGTGTTTTACG	CGGGAGAGTCCTGGTAGAGC	This study
Myxochelin	*mxcG*	GGATCACCGTCCTGGATCTG	GCTGATCTTGAGCTCGTCGT	This study
Sorangicin	*sorD*	GCGATCTTCTTCAGCCACAA	CCATCCACTTGCTGTTCGTC	This study
Soraphen	*sorB*	GCCCTCTCGCTTTCTCTCGC	CGGCTGATGAGGACGAGGTG	[14]
Spirangien	*spiG*	GGGAAGCTCGTCTTCGTCTT	TAGGTTTCGGTACCAGTGCG	This study
Thuggacin	*tgaC*	GTCACCCTCTGGAACTCGGT	AGGAACTCGATGTCTCCGCT	This study

The DNA sequences of *ambE* from strain So ce10 (accession number, DQ897667), *chiB* from strain So ce56 (DQ065771), *disB* from strain So ce12 (AJ874112), *epoA* from strain So ce90 (AF210843), *sce3190* from strain So ce56 (AM746676), *jerE* from strain So ce307 (DQ897668), *leuD* from strain So ce690 (HM639990), *mxcG* from strain So ce56 (AM746676), *sorD* from strain So ce12 (HM584908), *sorB* from strain So ce26 (U24241), *spiG* from strain So ce90 (AM407731), and *tgaC* from strain So ce895 (GQ981380) were used to design the primers.

Polymerase Chain Reaction and DNA Sequence Analysis

The oligonucleotide primers used to amplify the secondary metabolite genes by polymerase chain reaction (PCR) are listed in Table 1. Purified genomic DNA was used as template DNA. The PCR conditions were as follows: initial denaturation at 95°C for 20 s, annealing at 50–60°C (depending on the gene) for 40 s, and extension at 72°C for 50 s. The PCR products were analyzed by agarose gel electrophoresis. The DNA sequences were determined by Macrogen Inc. (Korea). Sequence analysis was conducted using the BLAST [1], ClustalX2 [13], and TreeView [18] programs.

Preparation of Culture Extracts and Biological Activity Assay

S. cellulosum strains were cultured in 50 ml of CYS medium containing 2 g of Amberlite XAD16 (Sigma, USA). The resin and cells were recovered and extracted twice with 50% acetone, and then twice with 100% acetone. The acetone was evaporated from the extract, and the remaining aqueous phase was extracted with ethyl acetate. The ethyl acetate extract was dried and dissolved in 1 ml of 80% methanol. Paper discs (6 mm in diameter), onto which were absorbed 5 μl of each extract, were placed on agar plates pour-plated with *C. albicans*, *P. aeruginosa*, or *S. aureus*. The plates were incubated at 32°C for 2 days.

High Performance Liquid Chromatography Analysis of Culture Extracts

High performance liquid chromatography (HPLC) separation was performed using an Agilent 1260 VL Infinity Series HPLC System with a Zorbax SB-C18 column (4.6 mm × 150 mm, 5 μm), into which 20 μl of the culture extracts was injected. The mobile phases A and B were water and acetonitrile, respectively, with 0.1% formic acid, and the gradient elution (at a flow rate of 0.2 ml/min) was performed as follows: 0–30 min for 5–100% B (linear gradient), 30–40 min for 100% B (isocratic), followed by 40–50 min of 5% B (isocratic). The eluates were detected with a variable wavelength detector at 254 nm.

RESULTS

Unique Secondary Metabolic Genes of *S. cellulosum* Subgroups

The secondary metabolites for which biosynthetic gene clusters have been cloned from *S. cellulosum* include ambruticin [10], chivosazol [19], disorazol [12], epothilone [17], etnangien [19], jerangolid [10], leupyrrin [11], myxochelin [19], sorangicin [8], soraphen [16], spirangien [3], and thuggacin [2]. If the DNA sequences of these genes are well conserved in different strains, it should be possible to determine by PCR analysis whether certain strains contain specific secondary metabolic genes. To examine the correlations between the 5 *S. cellulosum* subgroups and their secondary metabolic genes, we selected 25 *S. cellulosum* strains (5 from each subgroup) isolated from geographically different locations (Table 2) and isolated their genomic DNAs. When PCR was conducted using the genomic DNA as a DNA template and 12 sets of oligonucleotides that specifically bind to the secondary metabolic genes (Table 1) as primers, the genomic DNA from all 5 group D strains yielded amplified products with the *ambE*-specific primers (Table 3). Only 4 group A strains generated products with the *disB*-specific primers. All 5 group C strains produced products with the *epoA*-specific primers. All 5 group E strains produced products

Table 2. *Sorangium cellulosum* strains used in this study.

Group	Strain	Origin	Accession No.		Source
		City or county	*xynB1*	*groEL1*	
A	KYC3014	Haenam, Jeonnam	GU299049	NA[a]	[15]
	KYC3048	Yeongdeok, Gyeongbuk	GU299053	NA	[15]
	KYC3064	Dangjin, Chungnam	GU299055	NA	[15]
	KYC3074	Danyang, Chungbuk	GU299059	NA	[15]
	KYC3093	Hwacheon, Gangwon	GU299060	NA	[15]
B	KYC3046	Icheon, Gyeonggi	GU299052	GU298980	[15]
	KYC3142	Jeongseon, Gangwon	JN043266	JN043259	This study
	KYC3204	Changnyong, Gyeongnam	GU299065	GU298990	[15]
	KYC3209	Muju, Jeonbuk	GU299070	GU298995	[15]
	KYC3234	Cheongdo, Gyeongbuk	GU299079	GU299001	[15]
C	KYC3013	Jeju, Jeju	GU299048	GU298978	[15]
	KYC3148	Uijeongbu, Gyeonggi	JN043268	JN043261	This study
	KYC3152	Gapyeong, Gyeonggi	JN043269	JN043262	This study
	KYC3205	Changnyong, Gyeongnam	GU299066	GU298991	[15]
	KYC3206	Muju, Jeonbuk	GU299067	GU298992	[15]
D	KYC3080	Danyang, Chungbuk	GU299050	GU298986	[15]
	KYC3147	Yeongwol, Gangwon	JN043267	JN043260	This study
	KYC3153	Paju, Gyeonggi	JN043270	JN043263	This study
	KYC3156	Yongin, Gyeonggi	JN043271	JN043264	This study
	KYC3208	Jinan, Jeonbuk	GU299069	GU298994	[15]
E	KYC3026	Paju, Gyeonggi	NA	JN043257	This study
	KYC3047	Icheon, Gyeonggi	NA	GU298981	[15]
	KYC3076	Anseong, Gyeonggi	NA	JN043258	This study
	KYC3215	Jinan, Jeonbuk	NA	GU298996	[15]
	KYC3241	Yeosu, Jeonnam	NA	JN043265	This study

[a]NA: not available.

with the *sorD*-specific primers, and also the *sorB*-specific primers. With the *spiG*-specific primers, 3 group A strains, 1 group B strain, 5 group C strains, and 5 group E strains produced amplified products. No amplified products were detected from any strains with the *chiB*-, *sce3190*-, *jerE*-, *leuD*-, *mxcG*-, or *tgaC*-specific primers.

The DNA sequence of each amplified product was analyzed to exclude nonspecific amplification. The DNA sequences of the fragments amplified with *disB*-specific primers from 4 group A strains—KYC3014, KYC3064, KYC3074, and KYC3093—shared more than 94.2% identity with the DNA sequence of the *disB* gene encoding a disorazol synthase from *S. cellulosum* So ce12 (accession number, AJ874112). The deduced amino acid sequences of the encoded proteins shared more than 95.2% identity with the DisB protein. These results suggest that all group A strains, except KYC3048, have putative disorazol biosynthetic genes; the DNA sequences of the PCR fragment from these 4 strains shared more than 98.6% identity with one another.

The DNA sequences of the fragments amplified with *spiG*-specific primers in 3 group A strains—KYC3014, KYC3048, and KYC3093—shared more than 98.6% identity with one another. However, they shared less than 75% identity with the DNA sequence of the *spiG* gene encoding a spirangien synthase from *S. cellulosum* So ce90 (accession number, AM407731). Additionally, the amino acid sequences of the deduced proteins shared less than 70% identity with that of the So ce90 strain. Thus, it appears that these amino acid sequences do not encode spirangien synthase, but rather other, unknown, polyketide synthases.

The DNA sequence of the products amplified with *spiG*-specific primers in 1 group B strain–KYC3046–shared more than 97.3% identity with the DNA sequence of the *spiG* gene from the So ce90 strain. This suggests that KYC3046 has a putative spirangien biosynthetic gene unlike those of other B group strains.

The DNA sequences of the fragments amplified with *epoA*-specific primers in all 5 group C strains shared more than 99.2% identity with the DNA sequence of the *epoA* gene encoding an epothilone synthase from *S. cellulosum* So ce90 (accession number, AF210843). In addition, the

Table 3. PCR amplification of metabolite biosynthetic genes in *S. cellulosum* strains from 5 subgroups using gene-specific primers.

Group	Strain	Gene-specific amplification[a] (Sequence similarity; %)[b]						
		ambE	*disB*	*epoA*	*sorD*	*sorB*	*spiG*	*chiB*, *sce3190*, *jerE*, *leuD*, *mxcG*, *tgaC*
A	KYC3014	-	+ (94.7)	-	-	-	+ (NS)[c]	-
	KYC3048	-	-	-	-	-	+ (NS)	-
	KYC3064	-	+ (94.2)	-	-	-	-	-
	KYC3074	-	+ (94.7)	-	-	-	-	-
	KYC3093	-	+ (94.5)	-	-	-	+ (NS)	-
B	KYC3046	-	-	-	-	-	+ (97.3)	-
	KYC3142	-	-	-	-	-	-	-
	KYC3204	-	-	-	-	-	-	-
	KYC3209	-	-	-	-	-	-	-
	KYC3234	-	-	-	-	-	-	-
C	KYC3013	-	-	+ (99.6)	-	-	+ (97.9)	-
	KYC3148	-	-	+ (99.6)	-	-	+ (97.9)	-
	KYC3152	-	-	+ (99.6)	-	-	+ (97.5)	-
	KYC3205	-	-	+ (99.6)	-	-	+ (97.6)	-
	KYC3206	-	-	+ (99.2)	-	-	+ (97.9)	-
D	KYC3080	+ (95.0)	-	-	-	-	-	-
	KYC3147	+ (94.7)	-	-	-	-	-	-
	KYC3153	+ (95.0)	-	-	-	-	-	-
	KYC3156	+ (95.0)	-	-	-	-	-	-
	KYC3208	+ (95.0)	-	-	-	-	-	-
E	KYC3026	-	-	-	+ (NS)	+ (96.1)	+ (NS)	-
	KYC3047	-	-	-	+ (NS)	+ (95.9)	+ (NS)	-
	KYC3076	-	-	-	+ (NS)	+ (96.1)	+ (NS)	-
	KYC3215	-	-	-	+ (NS)	+ (96.1)	+ (NS)	-
	KYC3241	-	-	-	+ (NS)	+ (95.9)	+ (NS)	-

[a]+: amplified; -: no amplification.
[b]DNA sequence similarity of the PCR product with the biosynthetic gene of the metabolite from which the primers were designed.
[c]NS: PCR products were produced, but the DNA sequence identities of the products were <75%.

DNA sequences of the fragments amplified with *spiG*-specific primers from all group C strains shared more than 97.5% identity with the DNA sequence of the *spiG* gene from *S. cellulosum* So ce90 (accession number, AM407731). These results suggest that all group C strains have putative epothilone and spirangien biosynthetic genes.

The DNA sequences of the fragments amplified with *ambE*-specific primers in all group D strains shared more than 94.7% identity with the DNA sequence of the *ambE* gene encoding an ambruticin synthase from *S. cellulosum* So ce10 (accession number, DQ897667). The deduced amino acid sequences of the encoded proteins shared more than 93.8% identity with the AmbE protein. These results suggest that all group D strains have putative ambruticin biosynthetic genes.

The DNA sequences of the fragments amplified with *sorB*-specific primers in the group E strains shared more than 95.9% identity with the DNA sequence of the *sorB* gene encoding a soraphen synthase from *S. cellulosum* So ce26 (accession number, U24241). The deduced amino acid sequences of the encoded proteins shared more than 93.4% identity with the SorB protein. These results suggest that all 5 group E strains have putative soraphen biosynthetic genes.

The DNA sequences of the fragments amplified with *sorD*-specific primers in the group E strains shared more than 99.5% identity with one another. Meanwhile, the fragments amplified with *spiG*-specific primers shared more than 91.7% identity with one another. By contrast, the fragments amplified with *sorD*-specific primers shared less than 53% identity with the *sorD* gene encoding a sorangicin synthase from *S. cellulosum* So ce12 (accession number, HM584908), and the fragments amplified with *spiG*-specific primers shared less than 73% identity with the *spiG* gene from *S. cellulosum* So ce90 (accession number, AM407731). Thus, these amino acid sequences appear not to encode sorangicin or spirangien synthases, but rather other, unknown, polyketide synthases.

In summary, we detected putative disorazol synthetic genes only in the group A strains, putative epothilone synthetic genes only in the group C strains, putative spirangien synthetic genes only in the group C strains and 1 group B strain, putative ambruticin synthetic genes only in the group D strains, and putative soraphen synthetic genes only in the group E strains. Our findings suggest that the strains of each subgroup contain unique secondary metabolic genes.

Antimicrobial Activities of Culture Extracts from the *S. cellulosum* Subgroup Strains

We predicted that, if the strains of each subgroup contain unique secondary metabolic genes, they will also display unique antimicrobial activity profiles. To test this hypothesis, we prepared culture extracts of the 25 strains and assayed their antimicrobial activity against *C. albicans*, *S. aureus*, or *P. aeruginosa*. With the exception of KYC3204, the culture extracts from the group A, group B, and group C strains exhibited no noticeable growth inhibitory activities (Table 4). By contrast, the culture extracts from the group D strains inhibited growth of *C. albicans*, and the culture extracts from the group E strains inhibited growth of *C. albicans* and *S. aureus* (Table 4). Thus, in accordance with our prediction, the distribution of antimicrobial activities among the subgroups was unequal, indicating the presence of correlations between the *S. cellulosum* subgroups and antimicrobial substance production.

Table 4. Antimicrobial activities of the culture extracts from *S. cellulosum* subgroup strains.

Group	Strain	Antimicrobial activities against[a]		
		C. albicans	*S. aureus*	*P. aeruginosa*
A	KYC3014	-	-	-
	KYC3048	-	-	-
	KYC3064	-	-	-
	KYC3074	-	-	-
	KYC3093	-	-	-
B	KYC3046	-	-	-
	KYC3142	-	-	-
	KYC3204	+++	-	-
	KYC3209	-	-	-
	KYC3234	-	-	-
C	KYC3013	-	-	-
	KYC3148	-	-	-
	KYC3152	-	-	-
	KYC3205	-	-	-
	KYC3206	-	-	-
D	KYC3080	+++	-	-
	KYC3147	++	-	-
	KYC3153	+++	-	-
	KYC3156	+	-	-
	KYC3208	++	-	-
E	KYC3026	+	++	-
	KYC3047	+	+	-
	KYC3076	+	++	-
	KYC3215	+	+	-
	KYC3241	+	-	-

[a]+++: diameter of the clear zone >15.0 mm; ++: 10.1–15.0 mm; +: ≤10.0 mm; -: no activity observed.

HPLC Analysis of Culture Extracts from the *S. cellulosum* Subgroup Strains

HPLC analysis of the culture extracts from the 25 strains further revealed correlations between the *S. cellulosum* subgroups and their metabolite production. When the culture extracts from the 25 strains were separated using a Zorbax SB-C18 column, similar peaks were observed with all 25 extracts during the first 25 min of HPLC. However, after 25 min of separation, the strains of each subgroup showed unique peak patterns (Fig. 1). The peak patterns of the culture extracts from the 25 strains were compared (Table 5). Peak A was detected only in the culture extracts of 4 group A strains; peaks B1, B2, and B3 were detected only in the culture extracts of 3 group B strains; peak C was detected only in the culture extracts of 3 group C strains; and peaks E1 and E2 were detected only in the culture extracts of all 5 group E strains.

The culture extracts from group E strains inhibited growth of *C. albicans* and *S. aureus*. Therefore, peaks E1

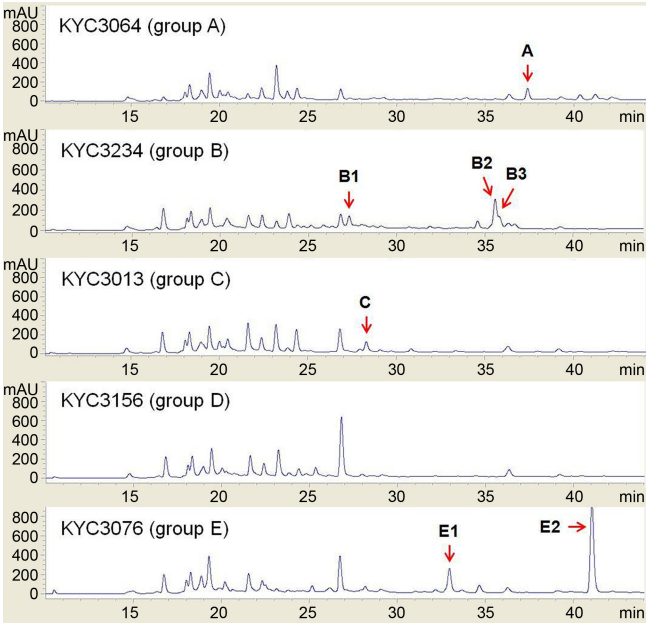

Fig. 1. HPLC chromatograms of the culture extracts from *S. cellulosum* subgroup strains.
Culture extracts from 5 strains belonging to 5 subgroups, respectively, were separated using a Zorbax SB-C18 column. The common peaks detected on the chromatograms of the same subgroup strains are numbered.

Table 5. Common HPLC peaks of the culture extracts from *S. cellulosum* strains.

Group	Strain	Peaks						
		A	B1	B2	B3	C	E1	E2
A	KYC3014	+	-	-	-	-	-	-
	KYC3048	-	-	-	-	-	-	-
	KYC3064	+	-	-	-	-	-	-
	KYC3074	+	-	-	-	-	-	-
	KYC3093	+	-	-	-	-	-	-
B	KYC3046	-	+	+	+	-	-	-
	KYC3142	-	+	-	-	-	-	-
	KYC3204	-	+	+	+	-	-	-
	KYC3209	-	-	-	-	-	-	-
	KYC3234	-	+	+	+	-	-	-
C	KYC3013	-	-	-	-	+	-	--
	KYC3148	-	-	-	-	+	-	-
	KYC3152	-	-	-	-	+	-	-
	KYC3205	-	-	-	-	-	-	-
	KYC3206	-	-	-	-	-	-	-
D	KYC3080	-	-	-	-	-	-	-
	KYC3147	-	-	-	-	-	-	-
	KYC3153	-	-	-	-	-	-	-
	KYC3156	-	-	-	-	-	-	-
	KYC3208	-	-	-	-	-	-	-
E	KYC3026	-	-	-	-	-	+	+
	KYC3047	-	-	-	-	-	+	+
	KYC3076	-	-	-	-	-	+	+
	KYC3215	-	-	-	-	-	+	+
	KYC3241	-	-	-	-	-	+	+

and E2 were fractionated, and their antimicrobial activities were assayed. The peak E1 fraction inhibited growth of *C. albicans* (data not shown), whereas the peak E2 fraction showed no noticeable growth inhibitory activities.

Discussion

S. cellulosum strains can be classified into at least 5 subgroups (A–E) based on the DNA sequences of their *groEL1* and *xynB1* genes [15]. In the present study, PCR detection of the secondary metabolic genes among the subgroup strains indicated correlations between the subgroup classification of *S. cellulosum* strains and their secondary metabolic gene categories. We detected putative disorazol biosynthetic genes only in group A strains, putative epothilone biosynthetic genes only in group C strains, putative spirangien biosynthetic genes only in group C strains and 1 group B strain, putative ambruticin biosynthetic genes only in group D strains, and putative soraphen biosynthetic genes only in group E strains (Table 3). Our antimicrobial activity assay of the culture extracts from the subgroup strains further revealed such correlations. With the exception of KYC3204, the culture extracts from the group A, group B, and group C strains exhibited no noticeable inhibitory activities against *C. albicans* and *S. aureus*. By contrast, the culture extracts from the group D strains inhibited growth of *C. albicans*, whereas the culture extracts from the group E strains inhibited growth of *C. albicans* and *S. aureus* (Table 4). HPLC analysis of the culture extracts from the strains of each subgroup revealed unique peak patterns (Fig. 1 and Table 5). Peak A was detected only in culture extracts of the group A strains, peaks B1, B2, and B3 were detected only in culture extracts of the group B strains, peak C was detected only in culture extracts of the group C strains, and peaks E1 and E2 were detected only in culture extracts of the group E strains. Some of the strains in each subgroup were at variance with these results, indicating that strains within a subgroup do not produce identical substances. Nevertheless, taken together, our results indicate the existence of at least 5 subgroups of *S. cellulosum* strains, each of which has the potential to produce a unique set of secondary metabolites.

Grouping of the strains should facilitate the screening of novel substances from *S. cellulosum*, which is the most proficient secondary metabolite producer among myxobacteria. Instead of cultivating several hundreds of *S. cellulosum* strains under the same conditions, it should be possible to classify the strains into 5 subgroups, and to prepare extracts by culturing a few representative strains from each subgroup under various conditions. Cultivation under various conditions would increase the likelihood of producing and detecting novel compounds. Screening of a rare *S. cellulosum* strain that does not belong to the 5 subgroups would further increase the possibility of finding novel compounds.

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