*J. Microbiol. Biotechnol.* (2008), **18**(8), 1416–1422

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY



# Isolation of *Sorangium cellulosum* Carrying Epothilone Gene Clusters

**Hyun, Hyesook[1], Jinwoo Chung[1], Jihoon Kim[2], Jong Suk Lee[2], Byoung-Mog Kwon[2], Kwang-Hee Son[2], and Kyungyun Cho[1]***

[1]*Myxobacteria Bank, Department of Biotechnology, Hoseo University, Asan 336-795, Korea*
[2]*Molecular Cancer Research Center, Korea Research Institute of Bioscience and Biotechnolgoy (KRIBB), Daejon 305-333, Korea*

Received: November 7, 2007 / Accepted: March 5, 2008

**Epothilone and its analogs are a potent new class of anticancer compounds produced by myxobacteria. Thus, in an effort to identify new myxobacterial strains producing epothilone and its analogs, cellulose-degrading myxobacteria were isolated from Korean soils, and 13 strains carrying epothilone biosynthetic gene homologs were screened using a polymerase chain reaction. A migration assay revealed that *Sorangium cellulosum* KYC3013, 3016, 3017, and 3018 all produced microtubule-stabilizing compounds, and an LC-MS/MS analysis showed that *S. cellulosum* KYC3013 synthesized epothilone A.**

**Keywords:** Myxobacteria, epothilone, *Sorangium cellulosum*

Epothilones are a new class of microtubule-stabilizing agents with a taxol-like mechanism of action from the cellulose-degrading myxobacteria *Sorangium cellulosum* [1, 5]. A semisynthetic epothilone B analog, ixabepilone, which was developed by Bristol-Myers Squibb Co. [9], passed phase III clinical trials and was approved in October 2007 by the United States Food and Drug Administration for use in the treatment of metastatic or locally advanced breast cancer in patients whose tumors are resistant or refractory to currently available chemotherapies. In addition, other analogs are also undergoing various clinical phases [4]. Since epothilones are potent anticancer compounds, it is important to identify the strains and genes biosynthesizing epothilones. Epothilones A and B were first purified from *S. cellulosum* So ce90 and patented by Hoefle *et al.* [6] in 1993. Another research group independently purified them from *S. cellulosum* SMP44 and reported their results in 1995 [1]. The epothilone gene clusters from the So ce90 and SMP44 strains were then cloned and patented in 2000 by two independent research groups at Novartis and Kosan Biosciences, respectively, in the United States [7, 11]. Currently, researchers are trying to increase the yield of epothilone production by expressing the genes in other hosts and optimizing culture conditions, since the wild-type strains produce extremely small amounts of epothilones [3, 12–15, 21]. Researchers are also trying to produce various epothilone analogs by manipulating cloned genes [20].

Myxobacteria are Gram-negative unicellular rod-shaped bacteria that occur in soil everywhere, and synthesize a large number of biologically active secondary metabolites, including epothilones [16, 17, 19]. Myxobacteria are also well known to move by gliding motility and develop multicellular fruiting bodies under starved conditions [2, 8, 23].

Accordingly, in an effort to identify new myxobacterial strains and genes producing epothilones, this study isolated cellulose-degrading myxobacteria and screened for strains carrying epothilone-synthesis genes using a polymerase chain reaction (PCR).

## Materials and Methods

### Media and Culture Conditions

ST21CX and VY/2 agar media were used to isolate cellulose-degrading myxobacteria. The ST21CX agar media consisted of 0.1% $K_2HPO_4$, 0.002% yeast extract, 0.1% $KNO_3$, 0.1% $MgSO_4 \cdot 7H_2O$, 0.1% $CaCl_2 \cdot 2H_2O$, 0.02% $FeCl_3$, 0.01% $MnSO_4 \cdot 7H_2O$, 25 μg/ml cycloheximide, a 0.1% trace element solution, and 1% agar [18], where the trace element solution contained 100 mg $MnCl_2 \cdot 4H_2O$, 20 mg $CoCl_2$, 10 mg $CuSO_4$, 10 mg $Na_2MoO_4 \cdot 2H_2O$, 20 mg $ZnCl_2$, 5 mg LiCl, 5 mg $SnCl_2 \cdot 2H_2O$, 10 mg $H_3BO_3$, 20 mg KBr, 20 mg KI, and 8 g EDTA Na-$Fe^{3+}$ salt (trihydrate) per liter. The VY/2 agar media consisted of 0.5% Baker's yeast, 0.1% $CaCl_2 \cdot 2H_2O$, 0.05% cyanocobalamin, and 1.5% agar [18]. The isolated cellulose-degrading myxobacteria were cultured in CK6 liquid media containing 0.15% $MgSO_4 \cdot 7H_2O$, 0.002% $Fe^{3+}$ citrate, 0.2% $KNO_3$, 0.025% $K_2HPO_4$, 0.5% glucose, and 0.15% $CaCl_2 \cdot 2H_2O$ [18]. All the myxobacterial strains were cultured at 32°C.

### Isolation of Cellulose-Degrading Myxobacteria

The cellulose-degrading myxobacteria were isolated as previously described [18]. Soils collected from all over Korea were placed on a

*Corresponding author
Phone: 82-41-540-5627; Fax: 82-41-548-6231;
E-mail: kycho@ hoseo.edu

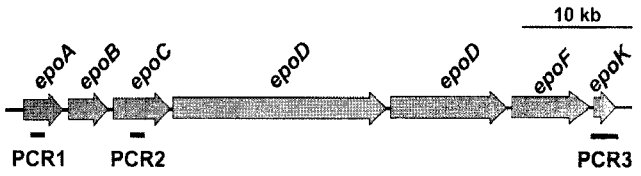


**Fig. 1.** Physical map of the epothilone gene cluster in *S. cellulosum* SMP44.
The map was deduced from reported sequences in the database (AF217189) [7].

filter paper on top of a ST21CX agar plate, and the plates were incubated at 32°C for 1 month to allow the wild myxobacteria in the soil to grow and form fruiting bodies. Mature fruiting bodies were then picked using a sterilized needle and transferred to a new ST21CX agar plate. Swarms of cells that grew on the new ST21CX agar plate were then picked using an inoculation stick and streaked on a VY/2 agar plate to purify the strain as a single colony.

### PCR Amplification of Epothilone Genes

A polymerase chain reaction (PCR) was used to amplify the putative epothilone genes in the myxobacteria. A 515 bp internal DNA fragment of an *epoA* gene homolog (PCR1 in Fig. 1) was amplified using two DNA oligonucleotides, 5'-CTGGCTGGTGGGGTATCGCT-3' (H128) and 5'-TGCTGAAGGGACAAGACGAC-3' (H129), as the PCR primers and the genomic DNA of the isolates as the DNA template. Additionally, a 422 bp internal DNA fragment of an *epoC* gene homolog (PCR2 in Fig. 1) was amplified using two DNA oligonucleotides, 5'-GAACCTCCACGAGCACCCAG-3' (H130) and 5'-TGGCAGACCCAAGGATGACC-3' (H131), as the PCR primers. Furthermore, a 1,625 bp DNA fragment containing a complete *epoK* gene homolog (PCR3 in Fig. 1) was amplified using two DNA oligonucleotides, 5'-ACTCGCATCTCAATCCGCTG-3' (H132) and 5'-CGGCACTTCTTCCGACGTTA-3' (H133), as the PCR primers.

### Analysis of 16S rRNA Sequences

The 16S rRNA genes from the myxobacteria were amplified using two DNA oligonucleotides, 5'-GAGTTTGATCCTGGCTGAG-3' (27f) and 5'-AGAAAGGAGGTGATCCAGCC-3' (1525r), as the PCR primers and the genomic DNA of the isolates as the DNA template. The DNA sequences of the resulting PCR products were then determined by SolGent Co. in Korea using the following DNA oligonucleotides: 5'-CTGCT-GCCTCCCGTA-3' (343r), 5'-TACGGGAGGCAGCAG-3' (357f), 5'-GWATTACCGCGGCKGCTG-3' (519r), 5'-CAGCMG-CCGCGGTAATWC-3' (536f), 5'-ATTAGATACCCTGGTAG-3' (803f), 5'-CCGTCAATTCATTTGAGTTT-3' (907r), 5'-GCAACGAGCGC-AACCC-3' (1114f), and 5'-CGGTGTGTRCAAGGCCC-3' (1385r). A phylogenetic analysis of the isolates was carried out using ClustalW [22]. The 16S rRNA sequences of the isolates were deposited in the GenBank under accession numbers EU242515, EU242516, EU242517, EU242518, and EU242519.

### Morphological Characterization

The vegetative cells of the myxobacteria grown on the VY/2 media were observed using a Nikon E600 phase-contrast microscope, and the fruiting bodies developed on the ST21CX media were observed using a Nikon SMZ1000 stereomicroscope. The microscopic images were then photographed using a Nikon D50 digital camera.

### Preparation of Culture Extract

The bacterial cells and 2% Amberlite XAD-16 resins, cultured in CK6 liquid media for 8 days at 32°C, were treated with acetone to disrupt the cells and extracted with ethyl acetate. After evaporating the ethyl acetate from the extract, the resulting residue was then dissolved in methanol at a concentration of 50 mg/ml.

### Migration Assay

The migration assay was performed in a 6.5-mm Transwell with an 8.0-μm pore polycarbonate membrane insert (Corning, Inc., U.S.A.) according to the manufacturer's protocol. Approximately $5\times10^4$ DLD-1 colon cancer cells expressing PRL-3 were serum-starved overnight and harvested with trypsin/EDTA. The harvested cells were washed once with a serum-free RPMI 1640 medium containing 0.5 mg/ml of a soybean trypsin inhibitor, and then washed twice with a serum-free RPMI 1640 medium. Thereafter, the cells were resuspended in a serum-free medium, and $8\times10^4$ cells in 0.2 ml were added to the upper chamber of the Transwell. Meanwhile, culture extracts in 0.5 ml of an RPMI 1640 medium with 10% fetal bovine serum were placed in the lower chamber of the Transwell. After 24 h of incubation at 37°C in a humidified atmosphere of 5% $CO_2$, the migrant cells that penetrated the membrane and attached to the lower surface of the Transwell were stained with 0.5% crystal violet and counted under a microscope at ×100.

### LC-MS/MS Analysis

Liquid chromatography mass spectrometry (LC-MS) was performed using a Finnigan LCQ Advantage MAX ion trap mass spectrometer (Thermo Electron Co., U.S.A.) equipped with an electrospray ionization (ESI) source. The HPLC separations were performed with a Finnigan Surveyor Modular HPLC System (Thermo Electron Co., U.S.A.), using a Waters XTerra MS C18 (5 μm, 2.1×150 mm; Ireland) with a BetaBasic-18 guard column (2.1×10 mm; Thermo, U.S.A.). The system was operated using Xcalibur software (version 1.3 SP2; Thermo Electron). Mobile phase A was water and mobile phase B was acetonitrile, where both phases contained 0.1% formic acid. The gradient elution at a flow rate of 0.25 ml/min was preformed as follows: 0–20 min 0–80% B (linear gradient) and 20–25 min 100% B (isocratic). The spray needle voltage was 5 kV, the ion transfer capillary temperature 220°C, the nitrogen sheath gas flow rate 55 arbitrary units, and the auxiliary gas flow rate setting 5 arbitrary units. The mass spectra were obtained within a range of *m/z* 200–1,000 in a negative-ion mode. The data-dependent tandem mass spectrometry (MS/MS) experiments were controlled using the menu-driven software provided with the Xcalibur system. All the experiments were performed under automatic gain control conditions.

## RESULTS AND DISCUSSION

### Isolation of Cellulose-Degrading Myxobacteria

Epothilones are known to be produced by the cellulose-degrading myxobacteria *S. cellulosum*. Therefore, as a first step to obtain epothilone-biosynthesizing myxobacteria, cellulose-degrading myxobacteria were isolated. Since myxobacteria occur in soils everywhere [16], soils were collected from all over Korea and placed on a filter paper on top of ST21CX agar plates, and the plates incubated at

32°C for 1 month to allow the wild myxobacteria in the soil to grow and develop fruiting bodies. As ST21CX does not contain any other carbon source, except for cellulose in the form of the filter paper, only cellulose degraders could grow on it. Moreover, the cycloheximide in the medium inhibited the growth of fungi. As a result, only cellulose-degrading bacteria including myxobacteria were able to grow on this medium. *Sorangium* spp. produce a unique form of fruiting body that is distinguishable from those of other myxobacteria [2]. Therefore, after one month of incubation, the fruiting bodies of *Sorangium*-like strains formed on the plate were picked using a sterilized needle and transferred to a new ST21CX agar plate. Swarms of cells that grew on the new plate were then picked using an inoculation stick and streaked on a VY/2 agar plate to isolate the strain as a single colony. The purified isolates were preserved in the form of a fruiting body at -80°C.

## Screening of Myxobacteria Carrying Epothilone Biosynthetic Genes

The epothilone gene clusters from the So ce90 and SMP44 strains have already been cloned and patented by two independent research groups at Novartis and Kosan Biosciences, respectively [7, 11]. Although the genes in the two clusters have been named differently, their DNA sequences are 98% identical. Therefore, since the previously identified strains exhibited very similar epothilone gene clusters, despite being isolated by different research groups, it was hypothesized that any isolates biosynthesizing epothilones in the present collection would also have epothilone gene clusters similar to those of the known strains. Therefore, two sets of PCR primers were designed based on the DNA sequences of the reported epothilone gene clusters. As shown in Fig. 1, the epothilone gene clusters in strains So ce90 and SMP44 consist of 7 genes, named *epoA-F*, plus *epoK* in strain SMP44. Thus, primers H128 and H129 were designed to amplify a 515 bp internal DNA fragment of the *epoA* gene (PCR1 in Fig. 1), whereas primers H130 and H131 were designed to amplify a 422 bp internal DNA fragment of the *epoC* gene (PCR2 in Fig. 1). It was then expected that a PCR using these primer sets would produce the expected sizes of PCR products from the genomic DNA of the isolates carrying the epothilone genes. When testing the genomic DNA of 65 cellulose-degrading isolates, the genomic DNA of 13 isolates produced the expected sizes of PCR products. Fig. 2 shows the PCR products of five representative strains, KYC3013, KYC3016, KYC3017, KYC3018, and KYC3025. Because of the possibility that these PCR products may have resulted from nonspecific PCR amplification, the DNA sequences of the PCR products were analyzed. As a result, the DNA sequences of the PCR products were found to be 99% identical to the *epoC* gene (data not shown), indicating that the 13 isolates carried genes highly homologous to the *epoA* and *epoC* genes of strains So ce90 and SMP44.

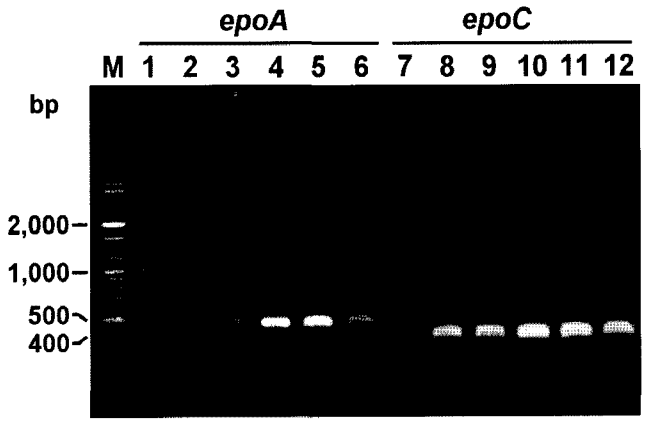


**Fig. 2.** Screening of myxobacteria carrying epothilone gene clusters by PCR amplification.
The genomic DNA of KYC3015 (lanes 1 and 7), KYC3013 (lanes 2 and 8), KYC3016 (lanes 3 and 9), KYC3017 (lanes 4 and 10), KYC3018 (lanes 5 and 11), and KYC3025 (lanes 6 and 12) was used as the DNA templates to amplify a 515 bp internal DNA fragment of the *epoA* gene homologs (lanes 1 to 6) and 422 bp internal DNA fragment of the *epoC* gene homologs (lanes 7 to 12) using a PCR. KYC3015 was the negative control. M, DNA size markers.

## *epoK* Genes of Isolates

The *epoK* gene is the smallest gene in the epothilone gene cluster, as shown in Fig. 3, encoding a P450 enzyme

```
   1 CTGGAAGTTTGCAGCATAGGAGATTTTATGACACAGGAGCAAGCGAATCAGAGTGAGACG
   1                         M  T  Q  E  Q  A  N  Q  S  E  T
  61 AAGCCTGCTTTCGACTTCAAGCCGTTCGCGCCTGGGTACGCGGAGGACCCGTTTCCCGCG
  12 K  P  A  F  D  F  K  P  F  A  P  G  Y  A  E  D  P  F  P  A
 121 ATCGAGCGCCTGAGAGAGGCAACCCCCATCTTCTACTGGGATGAAGGCCGCTCCTGGGTC
  32 I  E  R  L  R  E  A  T  P  I  F  Y  W  D  E  G  R  S  W  V
 181 CTCACCCGATACCACGACGTGTCGGCGGCGTTCCGCGACGAACGCTTCGCGGTCAGTCGA
  52 L  T  R  Y  H  D  V  S  A  A  F  R  D  E  R  F  A  V  S  R
 241 GAAGAATGGGAATCGAGCGCGGAGTACTCGTCGGCCATTCCCGAGCTCAGCGATATGAAG
  72 E  E  W  E  S  S  A  E  Y  S  S  A  I  P  E  L  S  D  M  K
 301 AAGTACGGATTGTTCGGGCTGCCGCCGGAGGATCACGCTCGGGTCCGCAAGCTCGTCAAC
  92 K  Y  G  L  F  G  L  P  P  E  D  H  A  R  V  R  K  L  V  N
 361 CCATCGTTTACGTCACGCGCGATCGACCTGCTGCGCGCCGAAATACAGCGCACCGTCGAC
 112 P  S  F  T  S  R  A  I  D  L  L  R  A  E  I  Q  R  T  V  D
 421 CAGCTGCTCGATGCTCGCTCCGGACAAGAGGAGTTCGACGTTGTGCGGGATTACGCGGAG
 132 Q  L  L  D  A  R  S  G  Q  E  E  F  D  V  V  R  D  Y  A  E
 481 GGAATCCCGATGCGTGCGATCAGCGCTCTGTTGAAGGTTCCGGCCGAGTGCGACGAGAAG
 152 G  I  P  M  R  A  I  S  A  L  L  K  V  P  A  E  C  D  E  K
 541 TTCCGTCGCTTCGGCTCGGCGACTGCGCGCGCGCTCGGCGTGGGTTTGGTGCCCCGGGTC
 172 F  R  R  R  F  G  S  A  T  A  R  A  L  G  V  G  L  V  P  R  V
 601 GATGAGGAGACCAAGACCCTGGTCGCGTCCGTCACCGAGGGGCTCGCGCTGCTCCATGGC
 192 D  E  E  T  K  T  L  V  A  S  V  T  E  G  L  A  L  L  H  G
 661 GTCCTCGATGAGCGGCGCAGGAACCCGCTCGAAAATGACGTCTTGACGATGCTGCTTCAG
 212 V  L  D  E  R  R  R  N  P  L  E  N  D  V  L  T  M  L  L  Q
 721 GCCGAGGCCGACGGCAGCAGGCTGAGCACGAAGGAGCTGGTCGCGCTCGTGGGTGCGATT
 232 A  E  A  D  G  S  R  L  S  T  K  E  L  V  A  L  V  G  A  I
 781 ATCGCTGCTGGCACCGATACCACGATCTACCTTATCGCGTTCGCTGTGCTCAACCTGCTG
 252 I  A  A  G  T  D  T  T  I  Y  L  I  A  F  A  V  L  N  L  L
 841 CGGTCGCCCGAGGCGCTCGAGCTGGTGAAGGCCGAGCCCGGGCTCATGAGGAACGCGCTC
 272 R  S  P  E  A  L  E  L  V  K  A  E  P  G  L  M  R  N  A  L
 901 GATGAGGTGCTCCGCTTCGACAATATCCTCAGAATAGGAACTGTGCGTTTCGCCAGGCAG
 292 D  E  V  L  R  F  D  N  I  L  R  I  G  T  V  R  F  A  R  Q
 961 GACCTGGAGTACTGCGGGGCATCGATCAAGAAAGGGGAGATGGTCTTTCTCCTGATCCCG
 312 D  L  E  Y  C  G  A  S  I  K  K  G  E  M  V  F  L  L  I  P
1021 AGCGCCCTGAGAGATGGGACTGTATTCTCCAGGCCAGACGTGTTTGATGTGCGACGGGAC
 332 S  A  L  R  D  G  T  V  F  S  R  P  D  V  F  D  V  R  R  D
1081 ACGAGCGCGAGCCTCGCGTACGGTAGAGGCCCCCATGTCTGCCCCGGGGTGTCCCTTGCT
 352 T  S  A  S  L  A  Y  G  R  G  P  H  V  C  P  G  V  S  L  A
1141 CGCCTCGAGGCGGAGATCGCCGTGGGCACCATCTTCCGTAGGTTCCCCGAGATGAAGCTG
 372 R  L  E  A  E  I  A  V  G  T  I  F  R  R  F  P  E  M  K  L
1201 AAAGAAACTCCCGTGTTTGGATACCACCCCGCGTTCCGGAACATCGAATCACTCAACGTC
 492 K  E  T  P  V  F  G  Y  H  P  A  F  R  N  I  E  S  L  N  V
1261 ATCTTGAAGCCCTCCAAAGCTGGATAA
 412 I  L  K  P  S  K  A  G  *
```

**Fig. 3.** DNA sequence and deduced amino acid sequence of the putative *epoK* gene from KYC3013.
Predicted Shine-Dalgarno sequence is underlined.

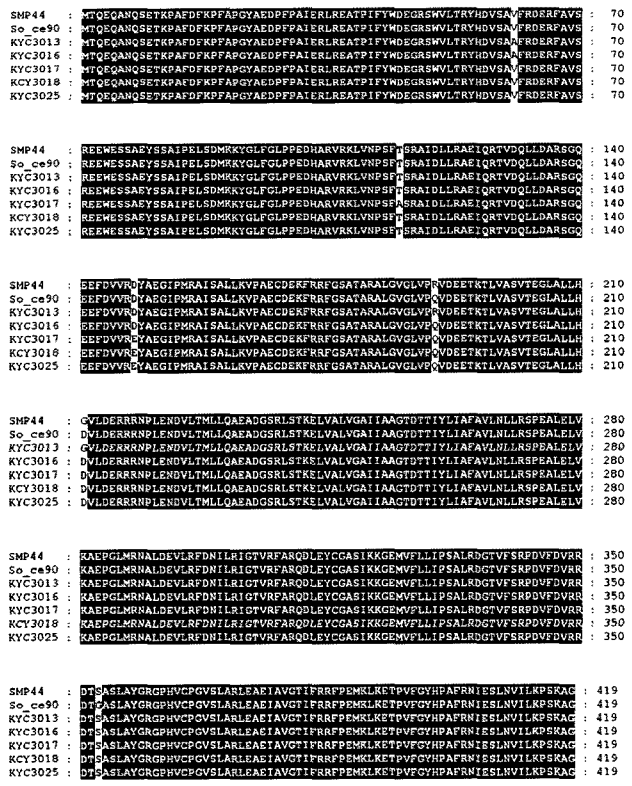


**Fig. 4.** Sequence alignment of putative EpoK proteins from isolates with EpoK proteins from previously reported strains.
The amino acid sequences of the isolates were aligned with those of *S. cellulosum* So ce90 (AAF26924) and SMP44 (AAF62886). The residues conserved in all strains are shaded.

responsible for the epoxidation of epothilones C and D into epothilones A and B, respectively. The amino acid sequences of the EpoK proteins from the two strains So ce90 and SMP44, both of which produce epothilones, are 99% identical. Thus, to analyze one complete gene from the putative epothilone gene clusters of the isolates, the *epoK* gene homologs were PCR amplified from five isolates, KYC3013, 3016, 3017, 3018, and 3025. A new set of PCR primers, H132 and H133, was synthesized and used to PCR amplify the DNA fragment containing the complete *epoK* gene (PCR3 in Fig. 1). When the genomic DNA of the five isolates was used as the respective DNA template, all produced the expected 1,625 bp PCR products. A sequence analysis of the resultant PCR fragments indicated that the DNA sequences of the *epoK* gene homologs from the isolates were 99% identical to those from strains So ce90 and SMP44. The deduced amino acid sequences of the *epoK* gene homologs from the isolates were also 99% identical to those from strains So ce90 and SMP 44. Only 1 to 4 amino acid residues were found to differ in the amino acid sequences of the putative EpoK proteins between the strains. For example, the deduced EpoK homolog from KYC3013 only showed one amino acid difference with the EpoK from SMP44, and four amino acid differences with the EpoK from So ce90 in the amino acid sequence.

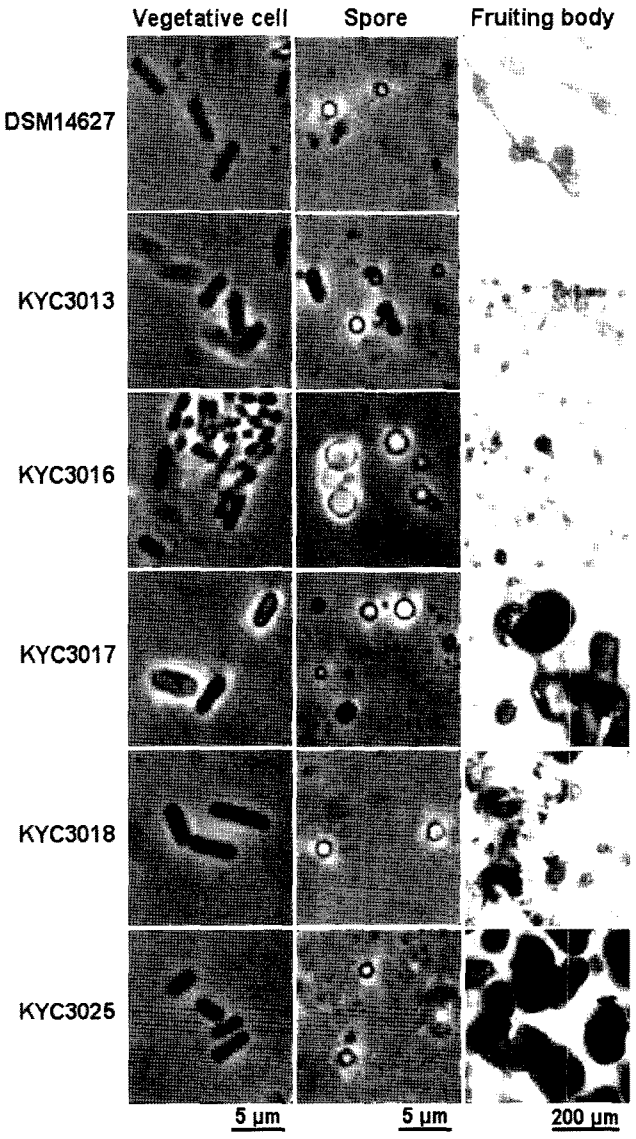


**Fig. 5.** Vegetative cells, spores, and fruiting bodies for the type strain of *S. cellulosum* DSM14627 and isolates carrying putative epothilone genes.

Meanwhile, the deduced amino acid sequences of the *epoK* gene homologs from KYC3018 and KYC3025 were 100% identical, yet their DNA sequences were not identical. In conclusion, this suggested that KYC3013, 3016, 3017, 3018, and 3025 all carried genes exhibiting a high homology to the epothilone biosynthetic genes of strains So ce90 and SMP44.

## Identification of Isolates

The vegetative cells of the five isolates, KYC3013, 3016, 3017, 3018, and 3025, were all rod-shaped, as shown in Fig. 5, and were able to grow using cellulose as the sole carbon source on the ST21CX media. They also formed the characteristic fruiting bodies unique to *S. cellulosum*, suggesting that these isolates are *S. cellulosum*. A phylogenetic analysis using the 16S rRNA sequences of the isolates also

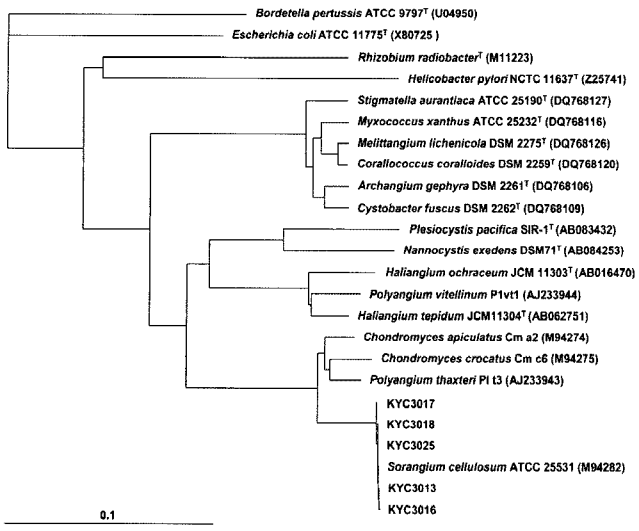


**Fig. 6.** Phylogenetic dendrogram of isolates carrying putative epothilone genes based on 16S rRNA sequences.

supported this. The 1,343 bp partial 16S rRNA sequences from the isolates were found to be 99% identical to that from the *S. cellulosum* type strain, plus they were grouped together in a phylogenetic tree as shown in Fig. 6. Therefore, it was concluded that the isolates were all *S. cellulosum*.

### Microtubule-Stabilizing Activity of Isolates

After screening five strains of *S. cellulosum* carrying epothilone gene-like clusters, the next question was to determine whether these strains produced epothilones. Thus, since epothilones are microtubule-stabilizing agents, the microtubule-stabilizing activity of culture extracts of the five isolates was tested by monitoring the migration of DLD-1 colon cancer cells through the membrane pores of a Transwell (Corning, Inc., U.S.A.). As shown in Fig. 7, the culture extracts of KYC3013, 3016, 3017, and 3018 did exhibit migration inhibition activity (panels C-F), compared with DMSO and KYC1846 as the controls, whereas KYC3025 failed to show any migration inhibition activity, suggesting that KYC3013, 3016, 3017, and 3018 all produced compounds stabilizing microtubules.

Next, the culture extract of KYC3013 was analyzed using LC-MS/MS to determine whether it produced epothilones. When searching for a putative epothilone A using a MS/MS spectral library [10], a major peak was revealed at 13.98 min (arrow) in the mass chromatogram (Fig. 8A). The structure of the compound was also confirmed by comparing the retention time, mass spectrum, and MS/MS spectrum of the compound with those of authentic epothilone A under the same conditions (Fig. 8). As shown in Fig. 8, the authentic epothilone A showed a mass spectrum with a formic acid adductive negative ion ($[M-H+CHOOH]^-$) peak at *m/z* 538. The mass spectrum and tandem mass spectrum for the substance extracted from KYC3013 showed the same patterns as those for the authentic epothilone A. Therefore, it was concluded that the compound was epothilone A and that KYC3013 produced epothilone A.

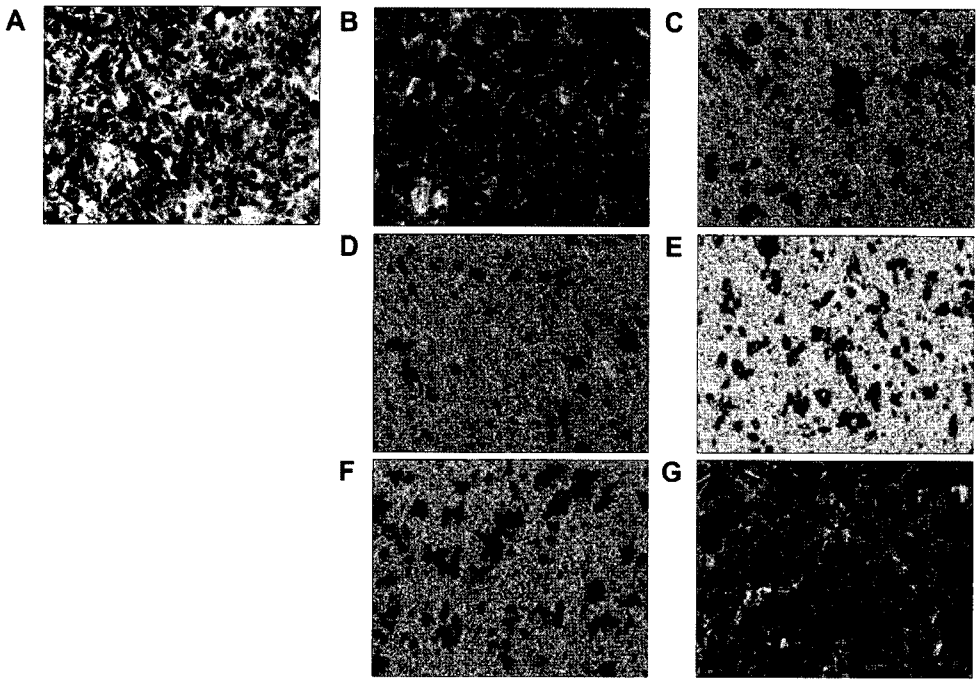


**Fig. 7.** Inhibitory activity of culture extract of isolates towards migration of DLD-1 colon cancer cells.
The migration of DLD-1 colon cancer cells in the presence of a culture extract of KYC1846 (panel B, negative control), KYC3013 (panel C), KYC3016 (panel D), KYC3017 (panel E), KYC3018 (panel F), and KYC3025 (panel G) was observed. Panel A is the control, showing the migrated cells in the presence of DMSO.

In conclusion, cellulose-degrading myxobacteria were isolated and 13 strains of *S. cellulosum* carrying epothilone gene homologs were screened using a PCR, indicating that a PCR can be used as a simple and efficient method for screening strains producing specific bioactive metabolites from collections of wild myxobacteria when the DNA sequences of their genes are known.

In addition, a migration assay revealed that KYC3013, 3016, 3017, and 3018 produced microtubule-stabilizing compounds, and an LC-MS analysis confirmed that KYC3013 synthesized epothilone A. Since epothilones are a potent new class of anticancer compounds, it is important to identify various strains and genes biosynthesizing epothilones. Thus, it is expected that these strains may be used to optimize the culture conditions for the production of epothilones and to produce various epothilone analogs by genetic manipulation.

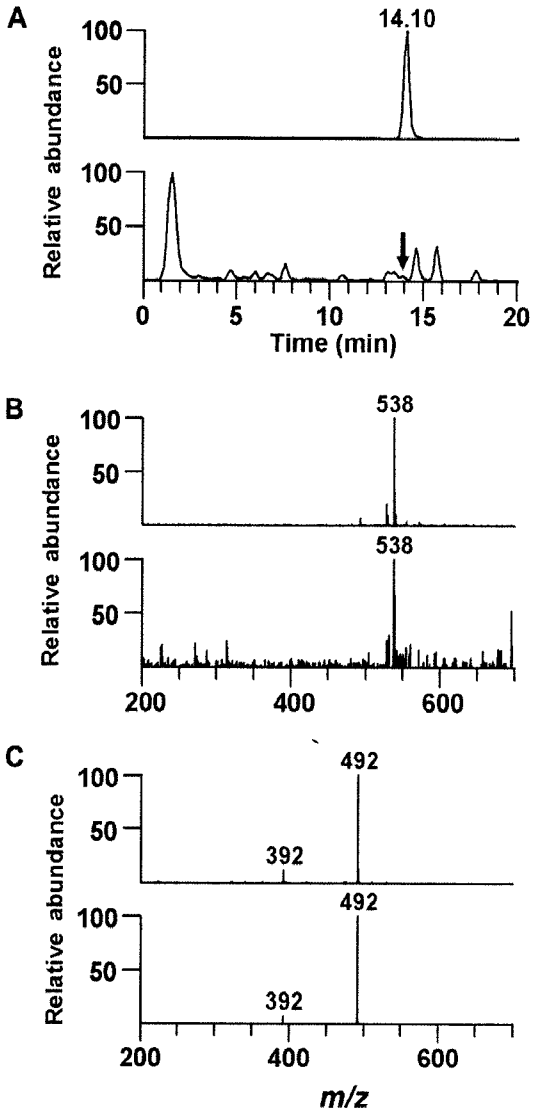


**Fig. 8.** LC-MS/MS analysis of authentic epothilone A and KYC3013 extract.

Selected ion chromatogram (panel A), negative-ion mass spectrum (panel B), and MS/MS spectra (panel C) of epothilone A (upper column, $[M-H+CHOOH]^-$ = *m/z* 538) and KYC3013 extract (lower column).

## Acknowledgment

This research was supported by grants from the Korean Ministry of Science and Technology (21C Frontier Microbial Genomics and Applications Program).

## References

1. Bollag, D. M., P. A. McQueney, J. Zhu, O. Hensens, L. Koupal, J. Liesch, M. Goetz, E. Lazarides, and C. M. Woods. 1995. Epothilones, a new class of microtubule-stabilizing agents with a taxol-like mechanism of action. *Cancer Res.* **55:** 2325–2333.
2. Dawid, W. 2000. Biology and global distribution of myxobacteria in soils. *FEMS Microbiol. Rev.* **24:** 403–427.
3. Frykman, S. A., H. Tsuruta, and P. J. Licari. 2005. Assessment of fed-batch, semicontinuous, and continuous epothilone D production processes. *Biotechnol. Prog.* **21:** 1102–1108.
4. Fumoleau, P., B. Coudert, N. Isambert, and E. Ferrant. 2007. Novel tubulin-targeting agents: Anticancer activity and pharmacologic profile of epothilones and related analogues. *Ann. Oncol.* **5:** v9–v15.
5. Gerth, K., N. Bedorf, G. Höfle, H. Irschik, and H. Reichenbach. 1996. Epothilones A and B: Antifungal and cytotoxic compounds from *Sorangium cellulosum* (Myxobacteria). Production, physico-chemical and biological properties. *J. Antibiot.* **49:** 560–563.
6. Hoefle, G., N. Bedorf, K. Gerth, and H. Reichenback. 1993. Patent DE 4138042.
7. Julien, B., S. Shah, R. Ziermann, R. Goldman, L. Katz, and C. Khosla. 2000. Isolation and characterization of the epothilone biosynthetic gene cluster from *Sorangium cellulosum*. *Gene* **16:** 153–160.
8. Lee, C., J. Chung, J. Kim, and K. Cho. 2006. Identification of a gene required for gliding motility in *Myxococcus xanthus*. *J. Microbiol. Biotechnol.* **16:** 771–777.
9. Lee, F. Y., R. Borzilleri, C. R. Fairchild, S. H. Kim, B. H. Long, C. Reventos-Suarez, G. D. Vite, W. C. Rose, and R. A. Kramer. 2001. BMS-247550: A novel epothilone analog with a mode of action similar to paclitaxel but possessing superior antitumor efficacy. *Clin. Cancer Res.* **7:** 1429–1437.
10. Lee, J. S., D. H. Kim, K. Liu, T. K. Oh, and C. H. Lee. 2005. Identification of flavonoids using liquid chromatography with electrospray ionization and ion trap tandem mass spectrometry with an MS/MS library. *Rapid Commun. Mass Spectrom.* **19:** 3539–3548.
11. Molnar, I., T. Schupp, M. Ono, R. Zirkle, M. Milnamow, B. Nowak-Thompson, *et al.* 2000. The biosynthetic gene cluster for the microtubule-stabilizing agents epothilones A and B from *Sorangium cellulosum* So ce90. *Chem. Biol.* **7:** 97–109.
12. Mutka, S. C., J. R. Carney, Y. Liu, and J. Kennedy. 2006. Heterologous production of epothilone C and D in *Escherichia coli*. *Biochemistry* **45:** 1321–1330.
13. Park, S. W., S. J. Park, S. J. Han, J. W. Lee, D. S. Kim, J. H. Kim, B. W. Kim, J. W. Lee, and S. J. Sim. 2007. Repeated batch

production of epothilone B by immobilized *Sorangium cellulosum*. *J. Microbiol. Biotechnol.* **17:** 1208–1212.
14. Park, S. W., S. H. Choi, Y. J. Yoon, D. H. Lee, D. J. Kim, J.-H. Kim, *et al.* 2006. Enhanced production of epothilones by carbon sources in *Sorangium cellulosum*. *J. Microbiol. Biotechnol.* **16:** 519–523.
15. Regentin, R., S. Frykman, J. Lau, H. Tsuruta, and P. Licari. 2003. Nutrient regulation of epothilone biosynthesis in heterologous and native production strains. *Appl. Microbiol. Biotechnol.* **61:** 451–455.
16. Reichenbach, H. 1999. The ecology of the myxobacteria. *Environ. Microbiol.* **1:** 15–21.
17. Reichenbach, H. and G. Hofle. 1999. Myxobacteria as producers of secondary metabolites, pp. 149–179. *In* S. Grabley and R. Thiericke (eds.), *Drug Discovery from Nature*, Springer Verlag, Berlin.
18. Reichenbach, H. and M. Dworkin. 1992. The myxobacteria, pp. 3416–3487. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (eds.), *The Prokaryotes*, 2nd Ed., Vol. IV. Springer Verlag, New York.
19. Reichengbach, H. 2001. Myxobacteria, producers of novel bioactive substances. *J. Ind. Microbiol. Biotechnol.* **27:** 149–156.
20. Tang, L., L. Chung, J. R. Carney, C. M. Starks, P. Licari, and L. Katz. 2005. Generation of new epothilones by genetic engineering of a polyketide synthase in *Myxococcus xanthus*. *J. Antibiot.* **58:** 178–184.
21. Tang, L., S. Shah, L. Chung, J. Carney, L. Katz, C. Khosla, and B. Julien. 2000. Cloning and heterologous expression of the epothilone gene cluster. *Science* **28:** 640–642.
22. Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22:** 4673–4680.
23. Ward, M. J. and D. R. Zusman. 2000. Developmental aggregation and fruiting body formation in the gliding bacterium *Myxococcus xanthus*, pp. 243–262. *In* Y. V. Brun and L. J. Shimkets (eds.), *Prokaryotic Development*. ASM Press, Washington, D.C.