APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 2008, p. 5571–5574 Vol. 74, No. 17
0099-2240/08/$08.00+0 doi:10.1128/AEM.00224-08

PCR Detection of Type I Polyketide Synthase Genes in Myxobacteria▽†

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Received 25 January 2008/Accepted 30 June 2008

The diversity of type I modular polyketide synthase (PKS) was explored by PCR amplification of DNA encoding ketosynthase and acyltransferase domains in myxobacteria. The sequencing of the amplicons revealed that many PKS genes were distantly related to the published sequences. Thus, myxobacteria may be excellent resources for novel and diverse polyketides.

Myxobacteria are gram-negative, rod-shaped, gliding bacteria with a high G+C content whose unique characteristic is the process of multicellular development that leads to fruiting body formation. Furthermore, myxobacteria are considered to be a rich source of antibiotics that are rarely produced by other microorganisms. Interestingly, the majority of bioactive compounds isolated from myxobacteria contain polyketide structures (1, 2, 22). Therefore, myxobacterial strains seem to have many novel polyketide synthase (PKS) genes and to produce hitherto unknown polyketides. While the presence of novel PKS genes in an organism is a good indicator of the production of novel polyketide molecules in this organism, reports on analyses of PKS genes in taxonomically diverse myxobacterial strains have been limited (17). Here, we characterized PKS genes in various myxobacterial strains of nine different genera, which were derived not only from terrestrial but also from marine environments.

PCR amplification of PKS genes in myxobacteria. Two primer sets, 5′-GCSATGGAYCCSCARCARCGSVT-3′/5′-GTSCCSGTSCCRTGSSCYTCSAC-3′, reported by Schirmer et al. (23), and 5′-TTCSTSTTYMCSGGVCAGG-3′/5′-GSGGGCYSABYTCSABGAA-3′, designed in this study, with conserved regions in *mta* (25), *cta* (4), *mel* (28), *epo* (27), *sti* (8), *tub* (GenBank accession no. EAU69663), *mch* (GenBank accession no. AJ698723), and *chi* (21) were used for the amplification of DNA encoding the ketosynthase (KS) and acyltransferase (AT) domains, respectively. The reaction mixture contained a 0.2 mM concentration of each deoxynucleoside triphosphate, 0.5% (vol/vol) dimethyl sulfoxide, a 1 μM concentration of degenerate primers, 12.5 ng/μl of genomic DNA, and 0.025 units/μl of EX-*Taq* Hot Start version polymerase (TaKaRa Bio, Shiga, Japan) in 1× EX-*Taq* PCR buffer. Amplification of KS sequences was performed with an initial denaturation step (94°C, 5 min), followed by 25 cycles of denaturation (94°C, 30 s), annealing (66°C, 30 s), and extension (72°C, 1 min). A final extension was performed at 72°C for 5 min. For the amplification of AT sequences, 35 cycles of denaturation (94°C, 30 s), annealing (64°C, 30 s), and extension (72°C, 1 min) were employed instead of the 25 cycles used in the PCR amplification of KS. KS and AT DNAs (about 680 bp and 810 bp, respectively) were amplified from all 20 strains listed in Table 1. The PCR products were cloned, and the sequences were compared to the published sequences by performing a BLAST search against sequences in the GenBank/EMBL/DDBJ databases. For convenience, they were classified into two categories: "known genes," exhibiting significant similarity (>70% identity of amino acid sequences) to published sequences, and "novel genes," exhibiting less significant similarity (<70%) to the published sequences. The cutoff value of 70% to separate "known" and "novel" PKS genes was chosen because the amino acid sequences of KS domains involved in the synthesis of structurally related polyketide molecules were, in almost all cases, more than 70% identical to each other in *Streptomyces* (our unpublished observations).

Among the strains used in this study, nine have been observed to produce 10 different polyketides (Table 1). Among the 10 polyketides, biosynthesis genes have been identified for 7 polyketides, namely, myxothiazol (*mta*), cystothiazole (*cta*), myxalamid (*mxa*/*mmx*) (5, 24), disorazol (*dis*/*dsz*) (2, 15), ambruticin (*amb*) (13), epothilone (*epo*), and spirangien (*spi*) (6). As expected, sequences similar to these genes were found in the corresponding producers (data not shown).

Novel PKS genes observed in myxobacteria. In addition to the known PKS genes described so far, many PKS gene sequences whose sequence identities to known genes are less than 70% were also found in this analysis. All PKS genes detected in the *Myxococcus xanthus* type strain were almost identical (>98% identity) to those in *M. xanthus* DK 1622, whose genome project has been completed (9), and therefore no novel PKS gene was found. On the other hand, all 19 of the other strains possessed novel PKS genes (Table 1).

The percentages of novel genes in terrestrial *Cystobacterineae* and terrestrial *Sorangiineae* strains were 24% and 40%, respectively, while that in marine *Sorangiineae* was 100% (Fig. 1). Furthermore, the sequence similarities of the PKS genes found in the marine strains were quite low compared to those

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† Supplemental material for this article may be found at http://aem.asm.org/.

▽ Published ahead of print on 7 July 2008.

TABLE 1. Tested myxobacterial strains and numbers of PKS sequences obtained in this study

Taxonomic group	Strain/product(s) (reference[s])[a]	Isolation origin	No. of PKS sequences[b]	
			Novel	Known
Terrestrial *Cystobacterineae*	*Myxococcus xanthus* ATCC 25232^T (3)	Soil (from ATCC)		9
	Myxococcus sp. strain M1017/myxothiazol	Soil, Kanagawa, Japan	6	10
	Myxococcus flavescens AJ 12298/myxovirescin (19, 26)	Soil, Kanagawa, Japan	2	7
	Myxococcus stipitatus AJ 12587/phenalamid (14)	Soil, Kanagawa, Japan	3	7
	Cystobacter fuscus AJ 13278/cystothiazole (20), myxalamid (16)	Soil, Kanagawa, Japan	1	10
	Melittangium lichenicola ATCC 25946	Soil (from ATCC)	3	7
	Stigmatella aurantiaca ATCC 25190^T	Soil (from ATCC)	2	6
Terrestrial *Sorangiineae*	*Chondromyces apiculatus* HT-1	Goat feces, Okinawa, Japan	6	1
	Sorangium cellulosum KU-4/disorazol	Soil, Tokyo, Japan	6	13
	S. cellulosum IS-3/ambruticin	Soil, Yokohama, Japan	1	6
	S. cellulosum IS-4	Soil, Yokohama, Japan	3	
	S. cellulosum YA-2/epothilone, spirangien	Soil, Yokohama, Japan	6	9
	S. cellulosum EW4/epothilone	Dead angleworm, Tokyo, Japan	3	11
	Sorangineae myxobacteria HB-1	Bacterial mat, Hakuba Spring, Japan	1	1
Marine *Sorangiineae*	*Plesiocystis pacifica* SIR-1^T (10)	Seagrass, Iriomote Island, Japan	3	
	Plesiocystis sp. strain SIS-2	Sea sand, Ishigaki Island, Japan	7	
	Enhygromyxa salina SHK-1^T (11)	Sludge, Saroma Lake, Japan	5	
	Enhygromyxa sp. strain SMH-02-3/nannochelin	Sea sand, Miura Peninsula, Japan	7	
	Enhygromyxa sp. strain SYM-1	Sea sand, Izu Peninsula, Japan	5	
	Haliangium tepidum SMP-10^T (7)	Seagrass, Miura Peninsula, Japan	10	

[a] Strains: from the culture collection of Ajinomoto Co., Inc., Japan. Product: identified in the joint research by Ajinomoto Co., Inc. and Nagoya University.
[b] Numbers of non-redundant sequences in each strain. Novel: identity to the best match of BLAST search was <70%. Known: >70% identity.

found in terrestrial strains: almost half of the marine PKS genes exhibited less than 55% identity to the reported sequences.

Phylogenetic diversity of PKS sequences. The phylogenetic relationship of myxobacterial PKS sequences was reconstructed by the NJ method (Fig. 2; see Fig. S1 in the supplemental material). Most of the sequences detected in *Sorangium cellulosum* EW-4 were similar to those in *S. cellulosum* YA-2. Often, but not always, PKS in one strain of *Cystobacterineae* was similar to that in another strain of *Cystobacterineae*; however, most other sequences were nonredundant, indicating that different strains have different PKS genes.

PKS sequences were organized into many clades. In addition to the clades of reported PKS genes, many novel clades were constructed. These clades were often composed of only *Cystobacterineae* sequences or only *Sorangiineae* sequences, suggesting the relationship between the taxonomy and the distribution of PKS genes, although some PKS clades included sequences derived from different suborder strains. In general, the correlation between the phylogenetic positions based on 16S rRNA gene sequences and those based on PKS genes is low, and the distribution of PKS genes in different bacterial strains is often explained by horizontal gene transfer (12, 18). However, in myxobacteria, horizontal gene transfer between different suborders might not be so frequent, and different taxa of myxobacteria seem to possess different PKS genes.

Interestingly, some marine *Sorangiineae* sequences formed marine-specific clades which were well separated from the sequences of terrestrial strains (Fig. 2). Such phylogenetically novel clades found in this study would be especially interesting for exploring PKS genes involved in the synthesis of novel metabolites. The remarkable novelty and diversity of PKS genes in marine strains indicate the importance of marine myxobacteria as sources for exploring novel polyketide compounds.

Nucleotide sequence accession numbers. DNA sequences obtained in this study were deposited in the DDBJ under accession numbers AB376371 to AB376541.

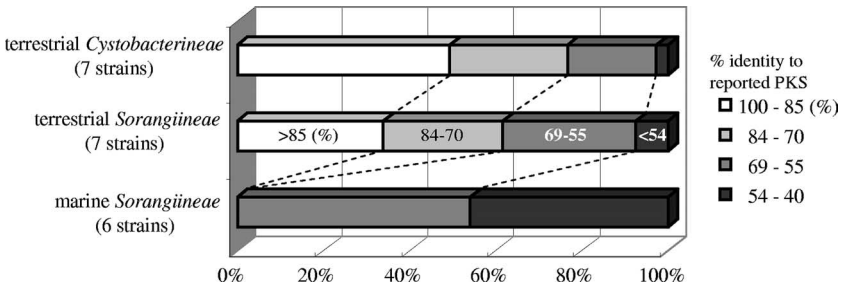

FIG. 1. Percentages of novel PKS sequences in myxobacteria of different categories.

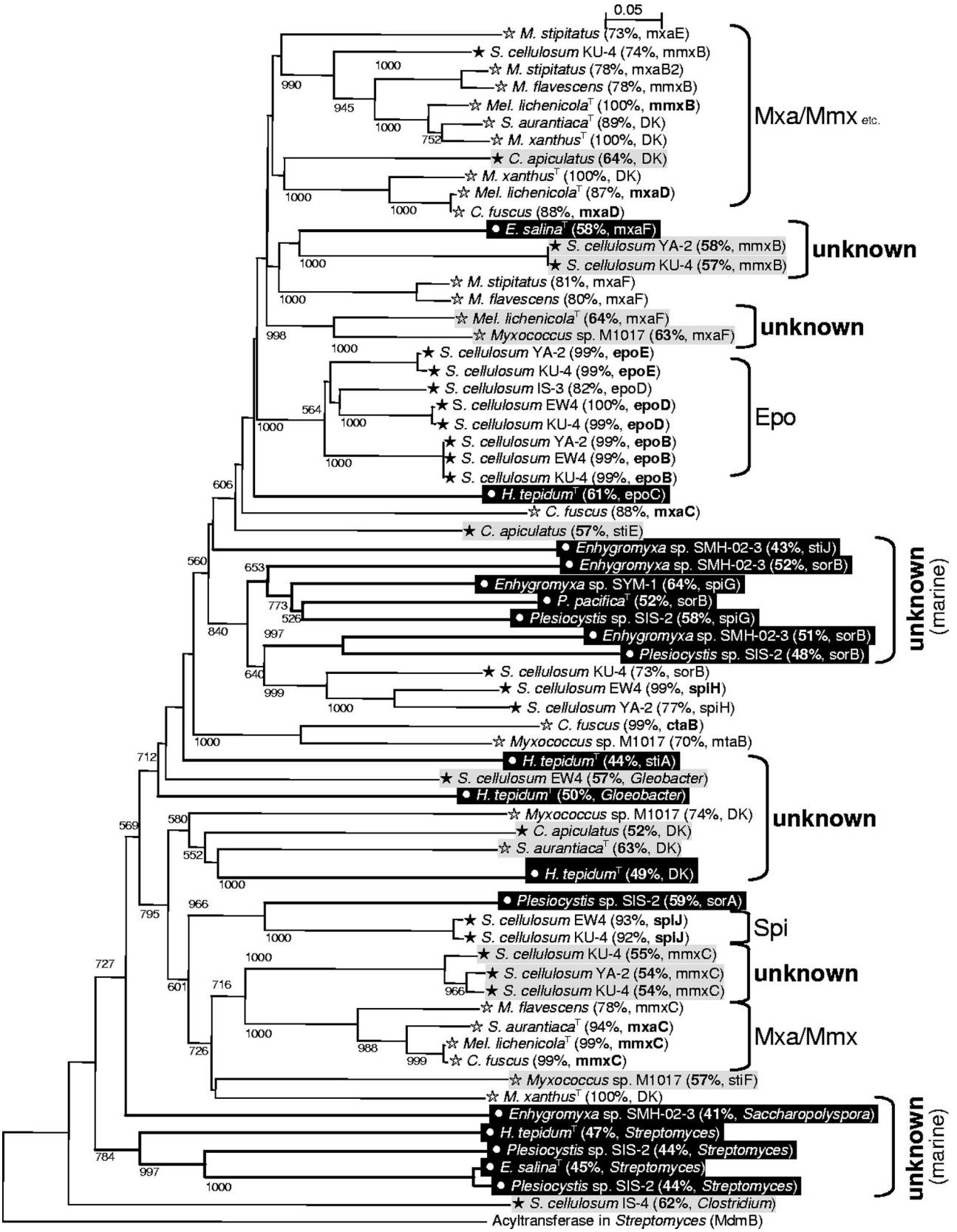

FIG. 2. Phylogenetic trees of of AT domains in PKSs from myxobacteria. Bootstrap values of >500 calculated from 1,000 bootstrap trees (neighborhood-joining algorithm) are indicated at the nodes. Bars indicate 5% amino acid sequence divergence. Identity to the best-matched PKS genes (or their genomes) found in the BLAST search are indicated in brackets. DK, PKS genes in the genome of *M. xanthus* DK 1622 (9). Sequences with <70% identity are shaded in gray or black. Symbols: open star, terrestrial *Cystobacterineae*; filled star, terrestrial *Sorangiineae*; open circle (and black background), marine *Sorangiineae*.

This work was supported by a grant from the New Energy and Industrial Technology Development Organization, Japan (P02038).

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