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QUARTERLY

Sequences homological with the β-ketoacyl synthase gene — their distribution within *Streptomyces* DNA

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It is well known that *Streptomyces* strains produce a multitude of so-called secondary metabolites that include a vast array of polyketide compounds, a diverse class of products that encompass antibiotics, pigments and immunosuppressants that have applications in agriculture, industry and medicine. Polyketide compounds show considerable structural heterogeneity but have a common mode of biosynthesis. The synthases that catalyze these reactions vary in their structural organization in different microorganisms. In the so-called type I system, exemplified by the deoxyerythronolide B synthase of *Saccharopolyspora erythraea* and by the 6-methylsalicylic acid synthase of *Penicillium patulum*, the biochemical functions are carried out by distinct domains on a large multifunctional polypeptide [1–2]. The respective genes constitute large open reading frames that comprise repeated modules coding for the entire set of biochemical functions. The type II system is a multienzyme complex that consists of separate polypeptides coded by individual genes for each step in carbon chain elongation [3–4].

The striking structural homology between polyketide synthase genes of different microbial species has facilitated detailed analyses on their structure and organization. Homologous genes show a significant level of nucleotide sequence similarity and this allows the assignment of putative functions to identified open reading frames [5]. This suggests that cloned DNA coding for one synthase might work as a hybridization probe for the detection of other synthases.

The primary aim of the present study was to design a DNA probe that was a part of the polyketide biosynthesis genes and which could be used to recognize these genes irrespective of their type of organization.

DNA sequencing and sequence analysis

Our previous hybridization studies (Kuczek & Mordarski, unpublished) indicated that repeated DNA sequences of *Streptomyces* strain ISP 5485, different from rDNA[1], show extensive homology with genomic DNA from other *Streptomyces* strains. One of such restriction fragments (*Sal*I fragment, 1.6 kb) was cloned in a pUC18 vector. This fragment was used as a hybridization probe against Southern blots of various enzymes digested DNA from several other *Streptomyces* strains. Hybridization results, concerning both heterologous DNA and that from *Streptomyces* strain ISP 5485, showed multiple homology bands thereby indicating a repeated character of the sequence. The fragment was sequenced by using the dideoxy chain termination method of Sanger *et al.* [6]. The sequence (GeneBank Database accession number L20249), which was established as a 1582 bp fragment, had a G+C content of 74.7% which is slightly more than a typical value for

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[1]Abbreviations: DEBS, deoxyerythronolide B synthase; nt, nucleotide; rDNA, ribosomal DNA.

genomic DNA from streptomycetes [7]. Neither stop codons nor transcription start signals were found within the sequence which was found to be unique when compared with corresponding information held in the EMBL data base. The highest homology value, 60% nucleotide identity over the entire sequenced fragment, was with the sequenced DNA operon of deoxyerythronolide B synthase (DEBS) of *Sacch. erythraea* [8]. The 5'-end region of the sequenced fragment (approx. 500 bp) showed greater than the entire fragment similarity to the putative β-ketoacyl synthase gene of *Sacch. erythraea*, that is 71% nucleotide identity (Fig. 1).

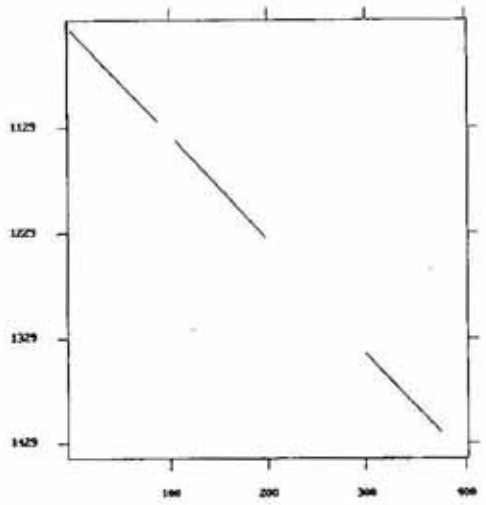

Fig. 1. DOTPLOT comparison of the sequenced DNA (horizontal axis) with the keto synthase domains of deoxyerythronolide B synthase within the first open reading frame [2].
Each dot represents a comparison of two 20-nt-long sequences where at least 15 nt are identical between the two sequences.

Comparison with information held in available data bases also showed that there was significant nucleotide sequence homology (55%–56% nucleotide identity over the entire sequenced fragment) with other polyketide synthase genes (not shown).

Probe synthesis and hybridization

It was anticipated that the conserved fragment of the putative β-ketoacyl synthase gene might provide a useful hybridization probe for the detection of analogous genes involved in the biosynthesis of polyketides in streptomycetes. Two possibilities were considered, one that the probe might be specific for type I synthases given the cloned DNA similarity to deoxyerythronolide B synthase or, secondly, that the probe might be capable of recognizing a conserved fragment of the condensing enzyme sequence independent of the type of synthase operon organization.

Primers complementary to the best matching region of the deoxyerythronolide B synthase gene were chosen. The conditions of the PCR reaction [9] were optimized to amplify a 330 bp fragment which was nonradioactively labelled during the amplification reaction; the labelled PCR product was detected by using the enhanced chemiluminescence method [10]. The product of the PCR was used to probe DNA from numerous streptomycetes in order to determine how common the sequence was within their genomic DNA sequences. Thirty-two *Streptomyces* strains and the one of *Saccharopolyspora* (Table 1) were selected either on the basis of their known ability to synthesize different classes of polyketides or because their capacity to synthesize polyketides, as opposed to other antibiotics, was not known. The probe was hybridized to DNA isolated directly onto membranes [11]. DNA from all the tested polyketide producers hybridized with the probe as did DNA from some of the non-polyketide producers.

The test strains were assigned to four groups (Table 1). Group one contained *Streptomyces* strain ISP 5485 and several macrolide producers. The corresponding polyketide synthases of these strains seem to have a similar type I organization [8, 12–14]. Group II contained the tetracycline producers. All of these organisms gave a positive hybridization signal and were considered to have type II synthases [15]. The third group of strains which synthesized polyketides included organisms that produced different classes of compounds. DNA from all these strains hybridized with the probe. Test strains producing antibiotic compounds that are not synthesized through the polyketide pathway (group IV) responded in various ways to the probe.

It can be anticipated from the alignment data that the probe should only give a signal upon hybridization with DNA of type I polyketide synthases (macrolide producing strains comprising group I). However, DNA of all of the

Table 1

Name, source and antibiotics produced by the test strains. Hybridization results indicated as + or –

	Strain		Antibiotic synthesized	
	Saccharopolyspora erythraea	NCIB 8233	erythromycin	+
	Streptomyces coriofaciens	ISP 5485	unknown	+
	S. umbofaciens	ATCC 15154	spiramycin	+
I	*S. avermitilis*	K 139	avermectin	+
	S. chartreusis	ISP 5085	chartreusin	+
	S. fradiae	PCM 2330	tylosin	+
	S. rochei	ISP 5231	borrelidin	+
	S. rimosus	ATCC 10970	chlorotetracycline	+
II	*S. varsoviensis*	ISP 5346	oxytetracycline	+
	S. viridifaciens	IAW 127	tetracycline	+
	S. albus	JII 1160	polyether antibiotic	+
	S. bobili	ISP 5956	cinerubin-like antibiotic	+
	S. canescens	DSM 40001	ascosin A & B	+
	S. coelicolor	A3(2) M110	prodigiosine, whiE	+
	S. coelicolor	ISP 5233	heptaene antibiotic	+
III	*S. griseus*	ATCC 23345	griseusin	+
	S. murinus	PCM 2369	actinomycin X	+
	S. parvulus	ATCC 12434	actinomycin D	+
	S. spitsbergensis	PCM 2404	prodigiosine-like	+
	S. albus	G	paulomycin	-
	S. azureus	ATCC 1421	thiostrepton	-
	S. cavourensis	DSM 40300	flavensomycin	-
	S. clavuligerus	NRRL 3585	cephamycin	+
	S. griseocarneus	ISP 5004	hydroxystreptomycin	+
	S. griseus	ATCC 3478	streptomycin	-
IV	*S. hygroscopicus*	ISP N736	bialaphos	-
	S. hygroscopicus	ISP 5578	hygromycin	-
	S. jumonjinenesis	ISP G4	istamycin, fortimycin	-
	S. kanamyceticus	ISP 5500	kanamycin	+
	S. lavendulae	ISP 5069	streptothricin	+
	S. purpurascens	PCM 2299	rhodomycin	-
	S. purpurascens	PCM 2344	chloramphenicol	-
	S. toyocaensis	PCM 2168	toyocamycin	-

strains potentially producing polyketides gave a positive signal. It also seems that the probe is sufficiently conservative to hybridize with DNA coding for different classes of polyketide synthases. Some of the positive results can be explained by the presence of multiple biosynthetical pathways as both types of polyketide synthases have been reported from a single strain [16–17]. It can also be deduced that positive hybridization signals might be given by polyketide synthase genes in strains which have yet to be examined for polyketide products.

Distribution of similar sequences within DNA of other *Streptomyces* strains

The primers referred to earlier were used to amplify, through PCR, an approximately similar sized fragment to that from *Streptomyces* strain ISP 5485 from *S. coelicolor* A3(2) M110, *S. coelicolor* ISP 5233, *S. fradiae* PCM 2330, *S. rimosus* ATCC 10970, *S. griseocarneus* ISP 5004 and *Sacch. erythaea* NCIB 8233. The amplified fragments of the DNA of *S. coelicolor* A3(2), *S. coelicolor* ISP 5233 and *S. rimosus* ISP 5260 were also partially sequenced, and the sequences and their putative translation products compared with one another and with DEBS of *Sacch. erythraea*. A similar nucleotide, as well as a protein sequence, was found between the sequences amplified from *S. rimosus* ATCC 10970 and *S. coelicolor* ISP 5233, but there was no significant similarity of these sequences with DEBS. The putative translation product of the DNA fragment amplified from *S. coelicolor* A3(2) was to a significant degree similar to DEBS (Fig. 2). The

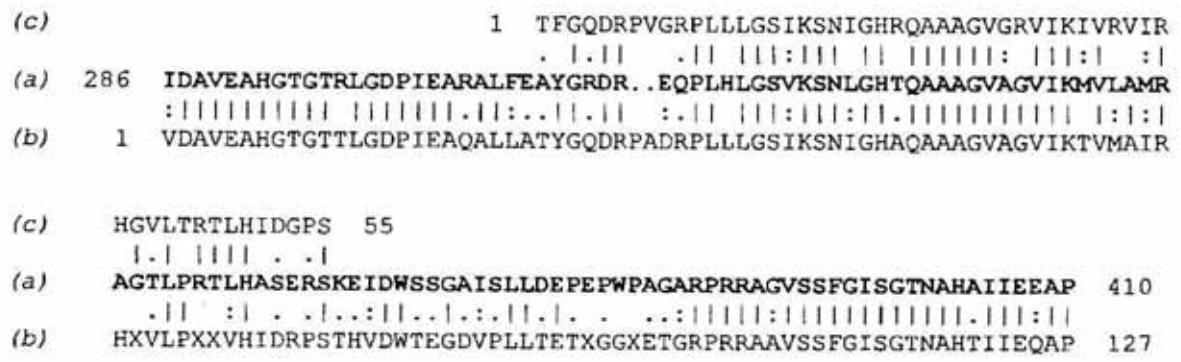

Fig. 2. Comparison of the putative translation products with that of the β-ketoacyl synthase gene of DEBS from Sacch. erythraea: (a) the fragment of putative ketoacyl synthase (amino acids 286 - 410), (b) translated 5'-end of the analysed sequence (127 amino acids); 73% similarity with DEBS and 63% identity (c) translated PCR product of S. coelicolor A3(2) M110; 74% similarity with DEBS and 56% identity.

results suggest a considerable amino-acid sequence heterogeneity of the putative translation products.

Despite the heterogeneity of the putative translation products of the DNA sequences recognized by the probe there seems to exist some DNA regions within putative genes which have a comparatively conservative nucleotide sequence. Designing primers complementary to such regions might enable the detection of these genes by PCR. Such studies would take advantage of the repeated character of the gene and of the widespread occurrence of the basic condensing enzyme from a polyketide biosynthetic pathway. The distribution of sequences similar to the gene proved to be more widespread among streptomycetes than predicted. The coding capability of a genome for a non-polyketide antibiotic does not exclude the possibility of the existence of a polyketide synthase operon. Moreover, a β-ketoacyl synthase as a basic condensing enzyme of the complex can be involved in more than one biosynthetic pathway.

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