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SHORT COMMUNICATION

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Carotenoid synthesis in *Streptomyces setonii* ISP5395 is induced by the gene *crtS*, whose product is similar to a sigma factor

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Abstract In many species of actinomycetes, carotenogenesis can be photoinduced. The capacity to respond to photoinduction is, however unstable and, in various strains of Streptomyces, is lost at a relatively high frequency. In *Streptomyces setonii* ISP5395, which normally produces no carotenoids, carotenoid-producing mutants can be obtained following protoplast regeneration. We report here the characterization of a gene, *crtS*, which was isolated from one such mutant and can confer on wild-type *S. setonii* ISP5395 cells the capacity to synthesize carotenoids. Sequence analysis of *crtS* reveals an open reading frame, which shows homology to genes that encode alternative sigma factors in *Bacillus subtilis*. We propose that *crtS* encodes a sigma factor which is necessary for the expression of a cryptic gene(s) for carotenoid biosynthesis in *S. setonii* ISP5395.

Key words *Streptomyces setonii* ISP5395 · Cryptic gene · Carotenogenesis · Sigma factor

Introduction

Our earlier studies (Koyama et al. 1976; Kato et al. 1981; 1986) have revealed that photoinduced carotenogenesis is found in Actinomycetes and related microorganisms. We found that 70 out of the 375 species of belonging to the genus *Streptomyces* that we examined synthesized carotenoids after photoinduction. The remainder synthesized carotenoid constitutively or not at all. This characteristic photoinduced carotenogenesis in various strains of *Streptomyces* is lost at relatively higher frequencies, as are other known unstable phenotypes of streptomycetes.

Carotenoid-producing mutants of *Streptomyces setonii* ISP5395, which normally produces no carotenoids, were obtained at a frequency of 10^{-4} to 10^{-5} after protoplast regeneration. Expression of cryptic genes can be induced through protoplast regeneration, as has been shown in *S. griseus*, in which a cryptic kanamycin resis-

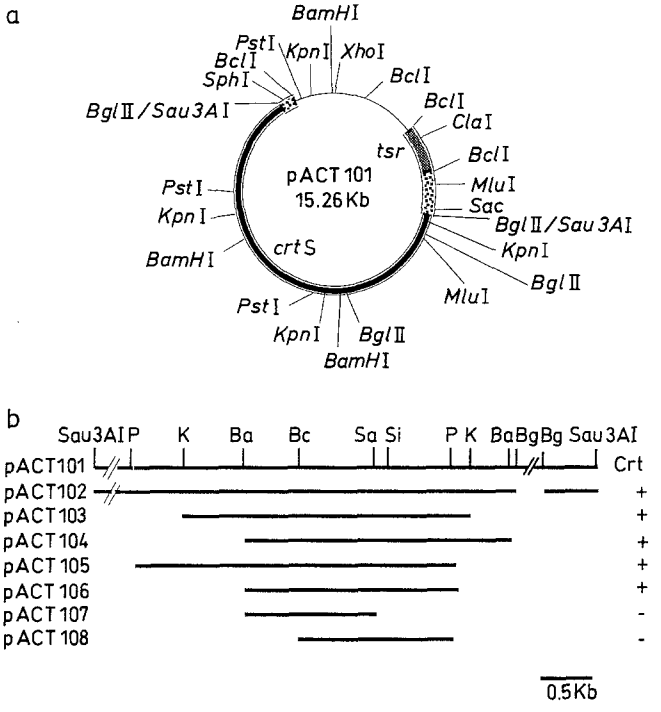

Fig. 1A,B Restriction map of pACT101 and subcloning of *crtS*. **A** pACT1 which confers carotenoid synthesis capacity of *S. setonii* ISP5395 carries a 9.5 kb insert at the *Bgl* II site of pIJ702. **B** Each of the restriction fragments indicated with **thick lines** was cloned into the appropriate site of pIJ702. These constructs were used to transform *S. setonii* ISP5395. The column on the right indicates presence (+) or absence (-) of carotenoid synthesis by the transformants. The 1.75 kb *BamH* I-*Pst* I fragment of pACT106 was cloned into pUC18/19. Each plasmid was sequentially deleted with *Exo*III nuclease. Those deleted plasmids were used as templates for nucleotide sequencing of both strands. Abbreviations; P, *Pst* I; K, *Kpn* I; Ba, *BamH*I; Bc, *Bcl* I; Sa, *Sac* I; Sl, *Sal* I; Bg, *Bgl* II

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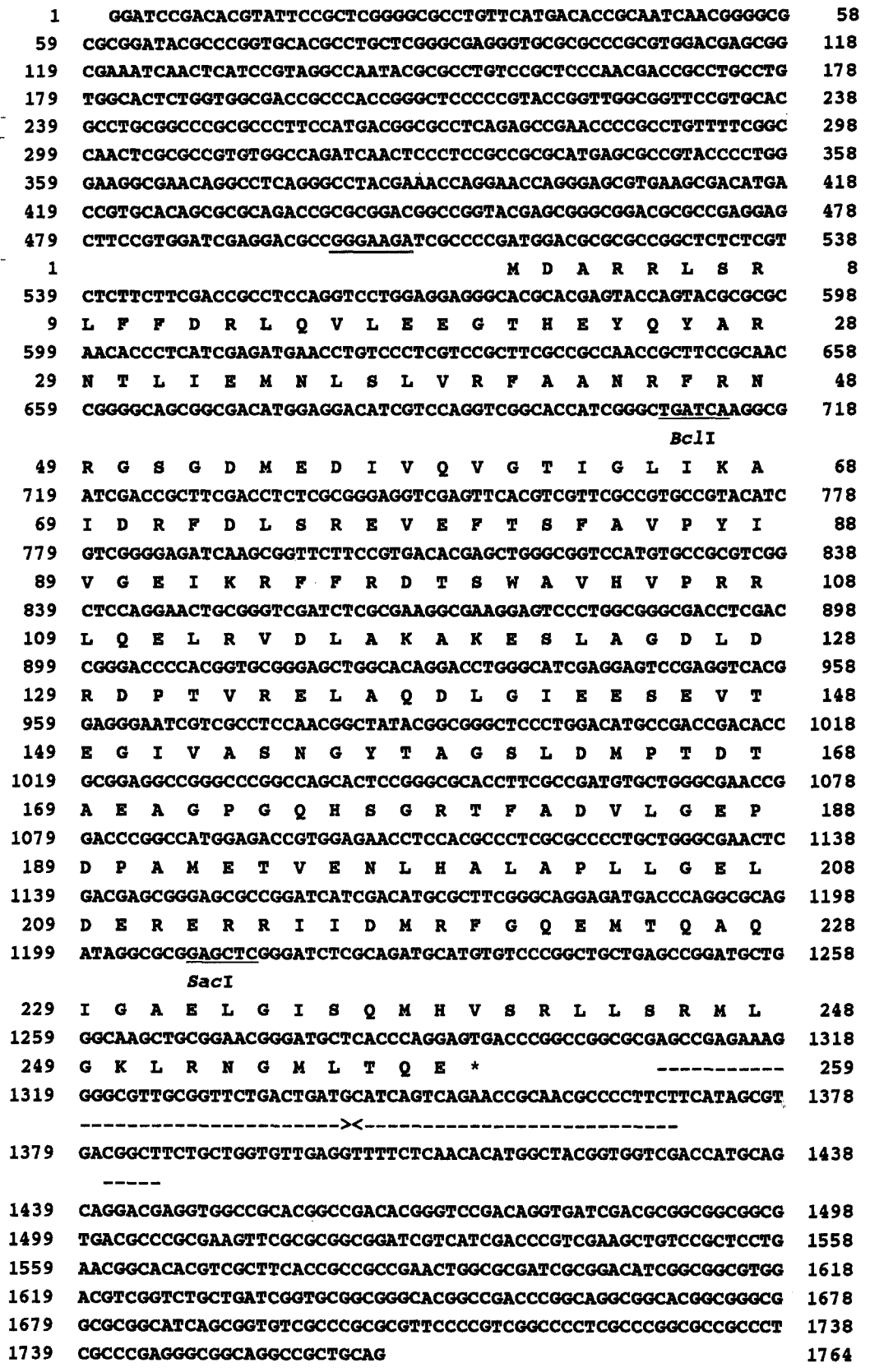

Fig. 2 Nucleotide sequence of *crtS* and flanking regions and deduced amino acid sequence of the *crtS* gene product. Restriction sites shown in the sequence are referred to in the text. A potential ribosome binding site is *underlined*, and the inverted repeat sequence giving a putative stem-loop structure is shown as *horizontal arrows*. The sequence accession number in the EMBL Data Library is D17466

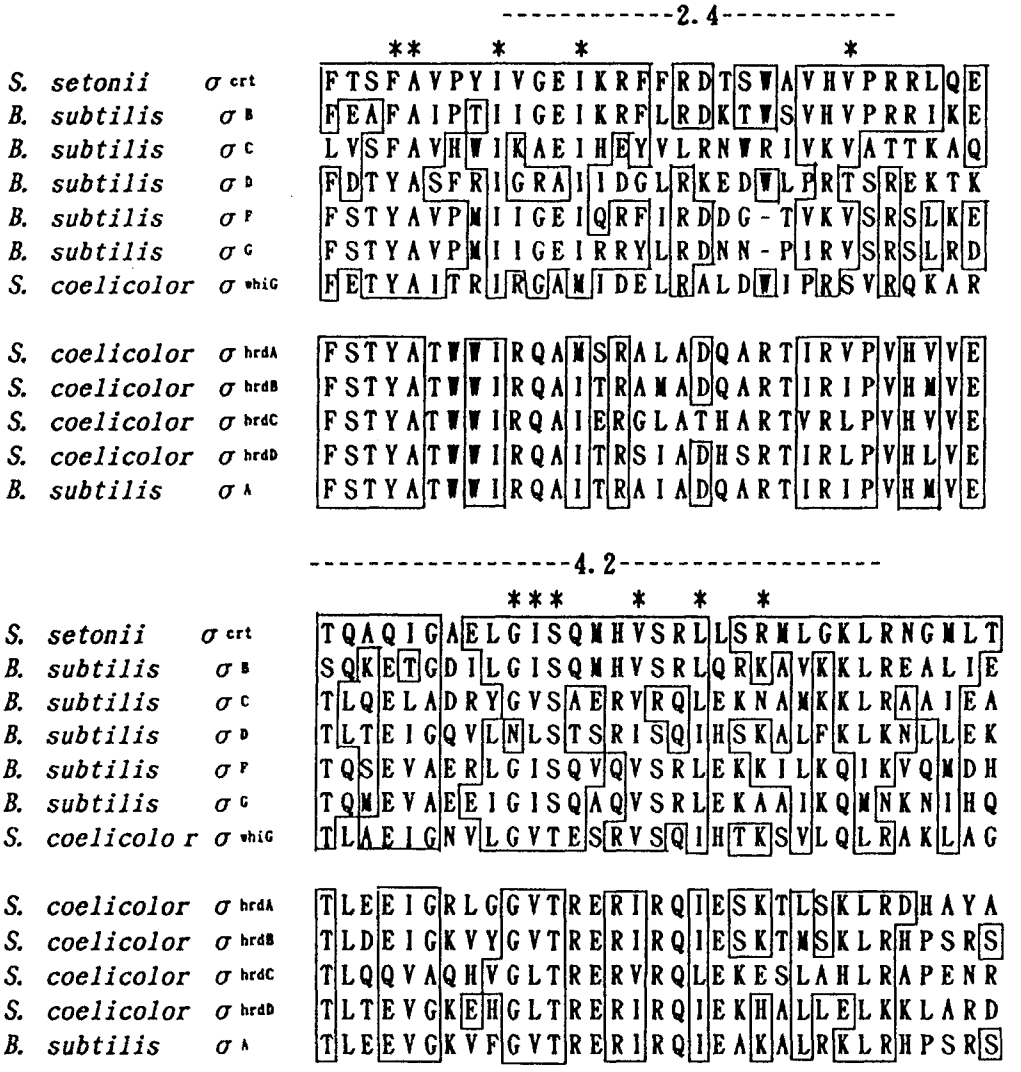

Fig. 3 Alignment of the amino acid sequences of the regions essential for promoter recognition by sigma factors(2.4 and 4.2) in the *crtS* gene product and other sigma factors. Amino acids identical or equivalent to that at the corresponding position in σ^{crt} are *boxed*. Equivalent amino acids are defined as follows: (I, L, M, V); (K, H, R); (D, E, N, Q); (S, T); (A, G) and (F, W, Y). *Asterisks* indicate amino acid residues highly conserved among sigma factors

tance gene was activated by a single base substitution in the putative promoter region (Hotta et al. 1988; Ishikawa et al. 1988; Ishikawa and Hotta 1991).

To identify the gene(s) responsible for the derepression of carotenoid production following protoplast regeneration of the carotenoid non-producer *S. setonii* ISP5395, we cloned and sequenced the inducing gene from a carotenoid-producing mutant PRY2 of *S. setonii* ISP5395, which had acquired the ability to synthesize carotenoids.

Chromosomal DNA of the carotenoid-producing mutant PRY2 was partially digested with *Sau*3AI, and ligated with *Bgl*II-digested pIJ702. *S. setonii* ISP5395 was transformed with the ligated DNA. Among the thiostrepton-resistant transformants, some yellow colored transformants were obtained. That the pigmentation of the transformants was not solely a result of protoplast regeneration, was confirmed by the fact that only yellow Thio^r transformants were obtained when strain ISP5395 was re-transformed with plasmid DNA isolated from one of the original yellow transformants. The yellow pigments produced by such transformants were extracted as described before (Kato et al. 1989) and shown to be a mixture of carotenoids, based their absorption spectra (420, 445 and 470 nm). Hence these transformants carry a sequence, designated *crtS*, that enables *S. setanii* ISP5395 to synthesize carotenoids. The corresponding plasmid pACT 101 (Fig. 1A), was characterized by restriction mapping and each of the restriction fragments indicated by thick lines in Fig. 1B was cloned into the appropriate site of pIJ702 (Bibb and Cohen 1982). *S. setonii* ISP5395 was transformed with each of the constructed plasmids and the transformants were tested for carotenoid synthesis. The results (Fig. 1B)revealed that the region essential for carotenoid synthesis was the 1.75 kb *Bam*H I- *Pst* I fragment of pACT101. That neither pACT107 nor 108 could induce carotenoid synthesis suggested that both *Bcl* I and *Sac* I sites are present in the *crtS* sequence.

The 1.75 kb *BamH* I-*Pst* I fragment containing *crtS* was subcloned into pUC118 and 119. Overlapping deletions of the recombinant plasmids were generated using the Kilo-Sequence Deletion kit (Takara Shuzo Co.). The nucleotide sequences of both strands were determined by

the dideoxy termination method (Sanger 1977). Computer analysis of the nucleotide sequence of the 1.75 kb *BamHI-Pst I* fragment (Fig. 2) revealed a possible ORF (positions 516 to 1295) with a high G+C content. That both *Bcl* I (790 nt) and *Sac* I (1209 nt) sites were present within the ORF agrees with the subcloning results, which showed that both restriction sites lie in the essential region of *crtS*. Furthermore, usage of codons rare in *Streptomyces* genes is also infrequent in the ORF. Therefore we postulate that this ORF, located in the 1.75 kb *BamH* I-*Pst* I fragment, represents *crtS*.

In the nucleotide sequence of *crtS*, preceding the putative initiation codon(ATG), a possible ribosome binding site (underlined in Fig. 3) was detected, which is complementary to the 3′ end of the 16S RNA (Bibb and Cohen 1982). Downstream of the ORF, there was an inverted repeat located at positions 1309–1386, which could form a stem-loop structure, assumed to be a Rho-independent terminator (Platt 1986).

We concluded that the *crtS* ORF which activates the cryptic carotenoid synthesis capacity of *S. setonii* ISP 5395 starts with an ATG initiation codon at position 516 and extends to position 1295, encoding a 29.12 kDa protein consisting of 259 amino acids (Fig. 2).

Sequences similar to that deduced for *crtS* were sought in available databases. Computer comparisons of the deduced amino acid sequence of CtrS with that of other proteins showed that the putative gene product of *crtS* is similar to the alternative RNA polymerase sigma factor B (Binnie et al. 1986), which participates in spore formation in *Bacillus subtilis*. The deduced amino acid sequence of *CrtS* also had similarity to the alternative sigma factor F (Yudkin 1987) of *B. subtilis* but had relatively lower similarity with sigma G (Masuda et al.1988) and sigma WhiG (Chater et al. 1989).

As reported by Helmann and Chamberlin (1988), there are two highly conserved regions (2.4 and 4.2) in the various sigma factors, both required for the recognition of gene promoters. A comparison of the amino acid sequence of the promoter recognition regions 2.4 and 4.2 of the known sigma factors with the *crtS* product is shown in Fig. 3. Both the amino acid sequences of *CrtS* corresponding to the regions 2.4 and 4.2 were similar to those of sigma B and other alternative sigma factors of *B. subtilis* as well as to sigma WhiG of *Streptomyces coelicolor*.Although the overall similarity between the *crtS* product and the four sigma factors *HrdA,B, C* and *D*, isolated from *S. coelicolor* (Takahashi et al. 1991),was lower than the similarity between the *CrtS* product and the alternative sigma factors of *B. subtilis*, the amino acid sequences in regions 2.4 and 4.2 were almost identical.

These results indicate that *crtS* is not a structural gene of carotenoid biosynthesis, but a gene encoding a sigma factor which is necessary for the expression of the gene(s) for carotenoid biosynthesis in *S. setonii*.

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