Identity of carotenoid biosynthetic genes of *Streptomyces* and their activation in *S. globisporus* 1912-4Crt

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Abstract

High identity of the carotenoid biosynthetic gene (crt) sequences (92–95%) was shown in *S. globisporus* 1912, *S. globisporus* TFH56 and *S. griseus* NBRC 13350. The homology of the crt genes of the other 53 *Streptomyces* strains was lower, ranging from 75% to 88%. Two direct and non-punctual repeats (NPRs) of 21 bp and spontaneous deletion of 119 bp, including the sequence of 96 bp between two non-punctual repeats, one NPR from 3’-side and 2 neighboring bp, have been identified in the sequence of the lycopene cyclase gene crtY of the strains 1912-2 and 1912-4Crt of *S. globisporus*, correspondingly. Both NPRs begin from the same specific site (5’-GGGGGCG-3’) that may play a role in the site-specific recombination, appearance of deletion and activation of the carotenoid biosynthesis in *S. globisporus* 1912-4Crt. The crtY gene of the different species of *Streptomyces* genus and bacteria contain the not identical 5’-GGGGCG-3’ site in the both NPRs. The mutant strain 1912-4Crt presents biotechnological interest for genetic selection of the active producer of beta-carotene and lycopene.

Keywords: *Streptomyces globisporus* 1912. Crt gene cluster; Crt gene identity; Non-punctual repeats; Spontaneous deletion

Introduction

Carotenoids belong to the natural pigments, synthesizing by plants, some bacteria, Streptomycetes and fungi, and playing an important role in the life of animals and humans as bio stimulants, vitamin A substitutes, antioxidants, coloring and tumor inhibiting compounds [1]. Representatives of the genus Streptomyces are known to be the main industrial producers of the different antibiotics widely used in medicine, veterinary and agriculture [2]. Genetic study of the different strains of *Streptomyces* showed the presence of the carotenoid biosynthetic gene clusters in their genomes in the functionally inactive state. Activation of the transcription of these cryptic cr genes in *S. coelicolor* A3(2) and *S. griseus* IPO 13350 requires the induction of a stress-responsible sigma factor through illumination of the culture with blue or increasing the copy number of the cr genes [3-4]. In *S. globisporus* 1912 and *S. albus* J1074 the carotenoid producing mutants can appear in a spontaneous manner [5-6]. At present the genomes of many Streptomyces strains of the different species were sequenced and submitted to GenBank. Complete sequence of the carotenoid biosynthetic gene cluster of the strain *S. globisporus* 1912 and whole genome shotgun sequencing project of the strain *S. globisporus* 1912-4Crt were submitted to Genbank (Click: GenBank Overview; Nucleotide: Streptomyces globisporus strain 1912; Streptomyces globisporus strain 1912-4Crt (Contigs 307 and 141 FASTA).

The aim of this study was the comparative analysis of the identity of the carotenoid biosynthetic genes of the sequenced genomes of the different *Streptomyces* species and search of a possible cause of a spontaneous activation of the crt gene cluster in the red-orange mutant *S. globisporus* 1912-4Crt. The Biogenesis and Maturation of Exosomes

Exosomes are formed during earlier steps of endosomal pathway and released upon fusion of multiple vesicle bodies (MVBs) with the plasma membrane. As part of cellular system for membrane traffic, formation of exosomes shares a lot of key components with endocytic process. For instance, it was shown that the formation of exosomes requires Endosomal Sorting Complex Required for Transport (ESCRT), although ESCRT-independent mechanisms exist in certain circumstances. ESCRT consists of four complexes, ESCRT-0, -I, -II and -III, plus several accessory components, such as VPS4, TSG101. ESCRT-0, together with flat clathrin coats, forms a protein network on endosomal membranes, capturing ubiquitinated cargo proteins and initiating their sorting into the MVB pathway. ESCRT-I and -II complexes was shown to be responsible for membrane deformation into buds containing sequestered cargo, while ESCRT-III provides the core membrane-remodeling activity driving MVB formation.

ALG-2-interacting protein X (ALIX) is a protein that interacts with several ESCRT proteins and is thought to be involved in the germination and shedding process. *In vitro* binding assays shown that syndecans, syntenin and ALIX form tripartite complexes (syndecan–syntenin–ALIX), which plays a specific role in the biogenesis of a major class of exosomes, the loading of exosomes with specific cargo, or both. In addition, as regulators of syntenin exosomes, the small GTase ADP ribosylation factor 6 (ARF6) and its effector phospholipase D2 (PLD2) were found to be able to affect exosomes by controlling the budding of intraluminal vesicles (ILVs) into multivesicular bodies (MVBs). Apart from this, autophagy-related gene 5(ATG5) has been shown to mediate acidification of the MVB lumen and allows MVB–PM (plasma membrane) fusion, knocking-out of ATG5 significantly reduces exosome release. Interestingly, the ATG12–ATG3 complex has also been found to regulate exosome biogenesis through their interaction with ALIX, indicating potential reciprocal regulation between autophagosome formation and exosome biogenesis.
Materials and Methods

Isolation of strains

The initial wild-type strain S. globisporus 1912 was isolated from a soil sample of Armenia and is keeping in the Ukrainian Collection of Microorganisms at the D.K. Zabolotny Institute of Microbiology and Virology of NASU as the strain S. globisporus Ac-2098. The strain 1912-2, the producer of 200 mg L\(^{-1}\) of the antitumor antibiotic landomycin E, was received by action of nitrosoguanidine on the spores of 1912 strain (Figure 1a) [7]. The strain 1912-4Crt, the spontaneous mutant of 1912, produced beta-carotene (7.0 mg L\(^{-1}\)) and lycopene (3.24 mg L\(^{-1}\)) in flask culture (Figure 1b) [7].

![Figure 1: Pigmentation of the colonies of S. globisporus strains: 1912-2 (a) and 1912-4Crt (b).](image)

Isolation of chromosomal DNA

The young mycelium of the strains 1912-2 and 1912-4Crt after sedimentation by centrifugation was washed by TE buffer, pH 8.0 and resuspended in the same buffer containing 300 mg lysozyme in 1.0 mL. The procedure of mycelium lysis, DNA deproteinization and purification followed the standard protocol of the Kirby method [8]. The precipitated DNA was dissolved in TE buffer, pH 8.0 and stored at 4°C.

2.1-2.3 and electrophoresis showed one compact and high-situated strip of DNA in agarose gel. These data confirm the high molecular weight of the DNA preparations.

DNA sequencing

The sequencing of the genome DNA of the strains 1912-2 and 1912-4Crt was carried out in BaseClear B.V., Leiden, Netherlands using the following procedures. The FASTQ sequence reads were generated according to Illumina Casava pipeline version 1.8.3. Initial quality assessment was based on data passing the Illumina Chastity filtering. Subsequently, reads containing adapters and/or PhiX control signal were removed using an in-house filtering protocol. The second quality assessment was based on the remaining reads using the FASTQ quality control tool version 0.10.0. The quality of the FASTQ sequences was enhanced by trimming off low-quality bases using the “Trim sequences” option of the CLC Genomics Workbench version 6.0.4. The quality-filtered sequence reads were puzzled into a number of contig-sequences. The analysis has been performed using the “De novo assembly” option of the CLC Genomics Workbench version 6.0.4. The optimal k-mer size was automatically determined using KmerGenie [9]. The contigs were linked and placed into scaffolds or supercontigs. The orientation, order and distance between the contigs were estimated using the insert size between the paired-end and/or matepair reads. The analysis has been performed using the SSPACE Premium scaffold version 2.3 [10]. The gapped regions within the scaffolds are (partially) closed in an automated manner using GapFiller version 1.10 [11].

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Results

The comparative analysis of the crt genes identity of the representatives of the different Streptomyces species was carried out by means of the BLAST program [www.ncbi.nlm.nih.gov/blast] (Table 1). High identity (92-95%) of the crt genes of S. globisporus 1912, S. globisporus TFH56 and S. griseus NBRC 13350 was shown. The crt genes of these three strains have also the same size. The homology of crt genes of the other 53 strains of Streptomyces was lower (71-88%). The crtE gene among all 7 genes of the crt cluster has the lowest identity (72-85%).

<table>
<thead>
<tr>
<th>NN</th>
<th>Strain (GenBank accession N)</th>
<th>Crt genes, direction of transcription (arrows), bp N and identity with 1912 Crt genes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>S. globisporus 1912 (KM349312)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>crtE</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1206</td>
</tr>
<tr>
<td>2</td>
<td>S. globisporus TFH56 (CP029361.1)</td>
<td>1209</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(85)</td>
</tr>
<tr>
<td>3</td>
<td>S. griseus NBRC 13350 (AP009493.1)</td>
<td>1209</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(85)</td>
</tr>
<tr>
<td>4</td>
<td>S. violaceoruber S21 (CP020570)</td>
<td>1158</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(80)</td>
</tr>
<tr>
<td>5</td>
<td>S. fulvissimus DSM 40593 (CP005080.1)</td>
<td>1064</td>
</tr>
</tbody>
</table>
Table 1: Identity of Crt genes of the different Streptomyces strains.

Phylogenetic tree of the eight crtY genes of Streptomyces was constructed by means of the Blastn (Distance tree) program of GenBank (Figure 2), using the data of Table 2. One can see good coincidence of the identity results obtained for all seven Crt genes of the different strains and only one crtY gene.

Table 2: Identity of crtY genes of Streptomyces with 1912 crtY gene.

The crtY gene encoding enzyme lycopene cyclase in 1912-2 strain contains two direct and non-punctual repeats (NPRs, underlined) of 21 bp, flanking the sequence of 96 bp. These two NPRs differ one from another by the four underlined bases. The specific sites of the first 6 bp (5′-GGGGCG-3′) are the same in both NPRs of the strain 1912-2 and contain substitutions of some base pair in the crtY gene sequence of the other Streptomyces strains (Table 3).
Discussion

Identity of the carotenoid genes of the different Streptomyces strains was established ranging from 71% to 95%. The most identity (92% - 95%) of crt genes was shown for S. globisporus 1912, S. globisporus TFH56 and related S. griseus NBRC 13350. Phylogenetic tree of the eight crtY genes of the different strains of Streptomyces agrees with the data presented in the Tables 1 and 2.

The cluster of crt genes is localized in the end of a linear chromosome of the streptomycetes in the neighborhood of the terminal inverted repeat (TIR) region where frequent DNA rearrangement is observed [13]. Direct repeats stimulated excision and generation of single point mutations, small insertions or replacements and deletions of different size in bacterial genes [14].

The frequency of the spontaneous appearance of the direct crt+ mutations in 1912-2 strain, activating beta-carotene production, was relatively high [5]. Loss of the above-mentioned property by 1912-4Crt strain in its turn was also increased [15]. Two direct and non-punctual repeats of 21 bp were found in the crtY gene of 1912-2 strain beginning from the same specific sites 5′-GGGGCGG-3′. We suppose that these sites play role in a site-specific recombination and genetic instability. This assumption was confirmed by the spontaneous deletion of 21 bp observed in the red-orange strain 1912-4Crt.

The representatives of the different Streptomyces species contain only one above mentioned specific site in the NPR of crtY gene meanwhile the second one has some substitutions of the base pairs preventing a site-specific recombination and deletion appearance (Table 3) [16-18].

Conclusion

One can suppose that NPRs were introduced into crtY genes of different bacteria in the distant past and their function is not clear today. One hypothesis suggests the increase of complexity of the carotenoid gene clusters of Streptomyces throughout evolution.

Scientists use metabolic engineering in order to increase the biotechnological production of carotenoids in non-carotenogenic microorganisms. The strain 1912-4Crt presents biotechnological interest as the perspective one for genetic selection of a high producer of beta-carotene and lycopene. On the first stage of selection the mutant strain 1912-4Lcp Hp7, a derivative of 1912-4Crt, was received producing 63.0 mg L⁻¹ of lycopene in flask culture.

References


