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Similarity and dissimilarity of primary structures of some *Streptomyces spp*. genomes and the *Streptomyces globisporus* 1912-2 chromosomal DNA

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Aim. To determine the similarity and dissimilarity of the nucleotide sequences of the *S. globisporus* 1912-2 chromosome and primary structures of genomes of some *Streptomycetes* strains. **Methods.** NCBI tools (BLAST: blastn and bl2seq: megablast) and Internet NCBI databases (Genome, Nucleotide) were used for *in silico* analysis of the primary structure contigs of *S. grlobisporus* 1912-2. **Results.** A few strains with significant identity of their DNA primary structures to the nucleotide sequences of the chromosomal DNA of *S. globisporus* 1912-2 (identity 88–97 %) and a degree of query cover (55–82 %) were identified. Primary structures of genomes of the strains *S. globisporus* C-1027 and *S. griseus* NBRC13350 were chosen as most identical to the nucleotide sequence of the *S. globisporus* 1912-2 chromosomal DNA. No fragments with a homologous primary structure to seven *S. globisporus* 1912-2 contigs were found in *Streptomycetes* spp. from the NCBI databases. **Conclusions.** *S. globisporus* 1912-2 strain is a member of the *S. griseus* clade. We detected a high biosynthetic potential of the strain *S. globisporus* 1912-2 due to many unique nucleotide sequences.

Keywords: *Streptomyces*, primary structure, genome, identity, *in silico* analysis, clade.

Introduction

*Streptomycete*s are characterized by a complex secondary metabolism [1, 2]. They are well known as producers of over two-thirds of clinically useful natural antibiotics (e.g., neomycin, cypemycin, grisemycin, bottromycins, chloramphenicol and many others) [1–5] and some secondary metabolites, which are useful for people (regulators, pigments, proteins, vitamins and some others) [1, 2, 4, 6, 7].

Due to the importance of this species of microorganisms for different areas of human activities great attention is paid to comprehensive study of their genetic, biochemical, physiological characteristics [1, 2]. However, the greatest interest for researchers is the study of genetic information carrier (chromosomes) – their primary structures, construction of the

genetic maps, definition of rules of genome functioning [3–5, 8–10].

With the emergence of data indicating that most *Streptomycetes* contain up to 30 gene clusters involved in the synthesis of secondary metabolites, it has become clear that their biosynthetic potential is underestimated [3, 8, 9, 11, 12]. Nowadays many efforts are made to estimate this potential and to get access to the natural products, the biosynthetic pathways of which are encoded in the genomes of the corresponding *Streptomyces* strains [13–15]. The accumulation of high quality genome sequencing data will strongly contribute to implementation of this task [3, 4, 6–11].

The primary structures are identified for more than 1000 chromosomes of *Actinobacteria*. The genus *Streptomyces* is now one of the most highly sequenced: 50 finished genomic sequences (Complete genome) and some hundreds of draft assemblies are available additionally in the GenBank database of server NCBI in September 2016.

The wild type strain *S. globisporus* 1912 and its variants synthesize some carotenoids, antitumor antibiotics landomycin E and A and a new regulator of the antibiotics biosynthesis and morphogenesis [7, 12, 16]. The nucleotide sequence of *S. globisporus* 1912-2 chromosomal DNA was identified [7].

The aim of this research was *in silico* definition of similarity and dissimilarity of the nucleotide sequences of the *S. globisporus* 1912-2 chromosome and primary structures of some *Streptomycetes* genomes.

Materials and Methods

The sequencing of the genomic DNA of strain *S. globisporus* 1912-2 was carried out in BaseClear B.V., Leiden, Netherlands using described previously procedures [7]. The primary structure of the chromosomal DNA of *S. globisporus* 1912-2 was obtained as a result (in the form of 1438 contigs). The total molecular size of all nucleotide sequences of the chromosomal DNA of *S. globisporus* 1912-2 was 7124511 bp. The nucleotide sequences of a few *Streptomycetes* genomes (*S. globisporus C-1027* (NZ_CP013738.1), *S. pratensis* ATCC 33331 (NC_016114.1), *S. fulvissimus* DSM 40593 (NC_016114.1), *S. griseus subsp. griseus* NBRC 13350 (NC_010572.1), *S. venezuelae* ATCC 10712 (NC_018750.1), S. *venezuelae* ATCC 15439 (NZ_LN881739.1), *S. pristinaespiralis* HCCB 10218 (NZ_CP011340.1), *S. davawensis* JCM 4913 (NC_020504.1), *S. vietnamensis* GIM4.0001 (NZ_CP010407.1), *S. avermitilis* MA-4680 (NC_003155.5), *S. hygroscopicus subsp. limoneus* KCTC 1717 (NZ_CP013219.1), *S. bingchenggensis* BCW-1 (NC_016582.1), *S. violaceusniger* Tu 4113 (NC_015957.1), *S. xiamenensis* 318 (NZ_CP009922.2), *S. sp.* SirexAA-E (NC_015953.1)) from database (Genome) of NCBI server [http://www.ncbi.nlm.nih.gov/genomes/MICROBES)] were tested *in silico*. Comparative analyzes were carried out by BLAST programs (blastn and bl2seq: megablast) [www.ncbi.nlm.nih.gov/blast].

Results and Discussion

Chromosomes of prokaryotes are one double-strand DNAs, which are considered as bacterial genomes. Genetic information coded by the genomes of microorganisms is necessary to provide their metabolism, growth, development and differentiation. Thousands of housekeeping genes (required for the cell vital acti-

vity) – for example tRNA-genes, rRNA-gene clusters and many others – are obligatory presented in the genome. The presence of additional optional genes (such as genes of antibiotic resistances, genes of antibiotic biosynthesis clusters and others) were reported for the *Streptomycetes* genomes. Besides, the coding sequences (structural and regulatory genes) in the genome are identified in many non-coding DNA regions (more than 10 %) [1, 2, 17].

Genetic instability is widespread within the genus *Streptomyces* [8, 17–19]. This important phenomenon includes a high frequency loss (>0.1 % of plated spores) of certain species specific traits, several of which are mapped to the chromosome [20–22]. The insertion and deletions of microbial genomes sequences appeared to be important events in the genome evolution. The balance between these processes is not always kept. As a result, an expansion of some genomes and reduction of others happen [22]. According to the established knowledge of microbial genetics three major natural strategies can be distinguished in the spontaneous generation of genetic variations in bacteria. These strategies are: (1) small local changes in the nucleotide sequence of genome, (2) intra genomic reshuffling of segments of genomic sequences and (3) the acquisition of DNA sequences from another organism [21].

This investigation was carried out by comparative analysis of the primary structures of genomes of some Streptomyces strains. We picked by *in silico* analysis of the GenBank database Genome the genomes of 15 *Streptomyces* strains (Complete genome). The criterion for their selection was a similarity grade of nucleotide sequences of their 16S rRNA-genes and the same genes of *S. globisporus* 1912-2 (AJ132630) [23]. Their range of identity exceeded 97.8 %. The Strains *S. xiamenensis* 318 (95.0 %), *S. bingchenggensis* BCW-1 (97.0 %) and *S. violaceusniger* Tu 4113 (97 %) were used as the out-group in this *in silico* analysis (Fig. 1).

The dendrogramme in Fig. 1, based on the analysis of 16S rRNA fragment sequences,

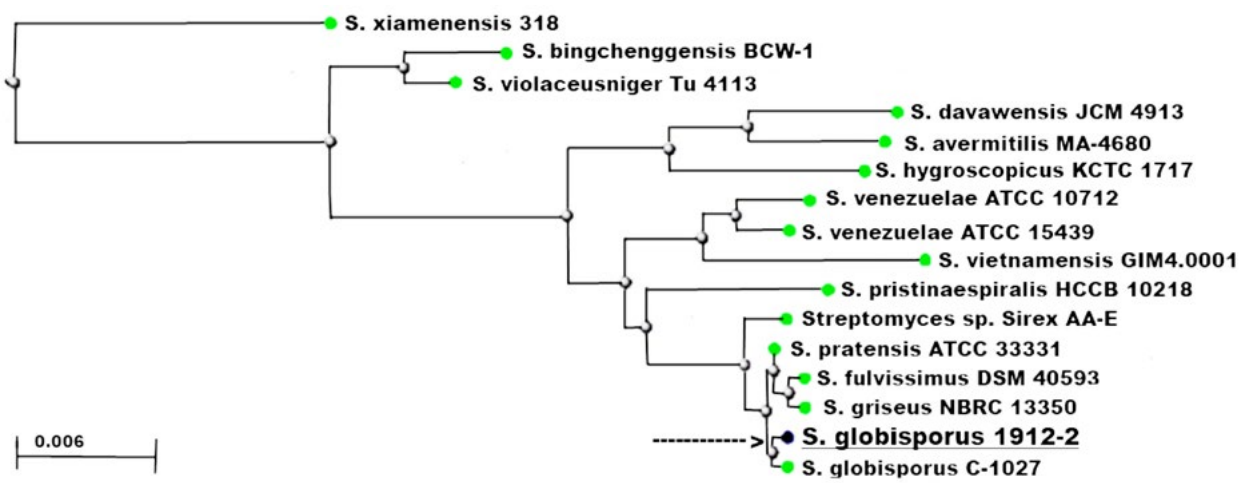

Fig. 1. Phylogenetic tree from alignment analysis of 16S rRNA fragment sequences of 16 *Streptomyces* stains.

Table 1. **Similarity of nucleotide sequences of some *Streptomyces* genomes and the *S. globisporus* 1912-2 contigs library**

Streptomyces species and strains	Genome sizes, Mbp	Query cover, %	E-value	Identity, %
S. globisporus C-1027	7.61	84	0	97
S. griseus NBRC13350	8.55	78	0	96
S. fuvissimus DSM 40593	7.91	73	0	91
S. pratensis ATCC 33331	7.34	55	0	88
S. violaceusniger Tu 4113	11.94	33	0	91
S. xiamenensis 318	5.96	21	0	90

reflects the relationships between some strains Streptomycetes

The similarity of 16S rRNA-genes primary structures of *S. fuvissimus* DSM 40593, *S. globisporus* C-1027, *S. pratensis* ATCC 33331 and *S. griseus* NBRC 13350 to 16S rRNA gene of *S. globisporus* 1912-2 was within the range 99.8 % – 100 %. The trains are members of the *S. griseus* clade [24]. The members of same clade have to share the 16S rRNA gene similarities within the range 97.8 % – 100 % [25]. We assumed the strain *S. globisporus* 1912 to be a member of this *S.griseus* clade (*S. albovinaceus subgroup)*.

We chose 6 genomes of *Streptomyces* strains for further research. A high similarity of four primary structures of *Streptomycetes* genomes and nucleotide sequences of chromosomal DNA of *S. globisporus* 1912-2 was defined *in silico* (Tabl. 1). Strains *S. xiamenensis* 318 and *S. violaceusniger* Tu 4113 were used as the out-group.

An existence of difference in the genomic DNAs maps of different strains of the same species is well known. The presence of different assortments of addition genes (or gene clusters) and different location of homologous genes in chromosomes of such strains were indicated. However, there are certain models in the construction of chromosomes: for example, the same order of localization of homologous genes in similar clusters (synteny), the accumulation of obligatory genes in the central part of the chromosome [26].

Localization of *S. globisporus* 1912-2 DNA fragments (those had homologous primary structures with *S. globisporus* C-1027 ones) was identified using program bl2seq (Fig. 2).

The genus *Streptomyces* members served as a source of new natural products and antibiotics in particular for a long time [1, 2]. Most *Streptomycetes* contain up to 50 gene clusters involved in the synthesis of secondary metabolites [3, 8, 10]. For example, the genomes

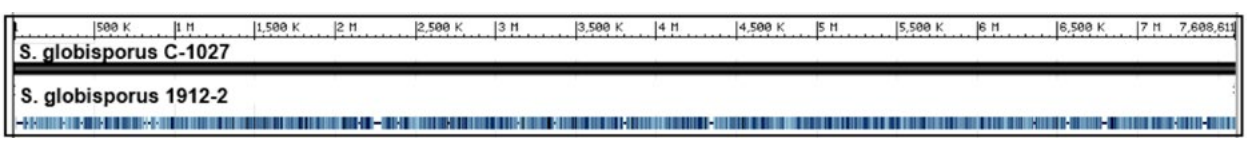

Fig. 2. The localization scheme of chromosomal fragments of the *S. globisporus* 1912-2 (the bottom line) and the *S. globisporus* C-1027 (the upper line) with homologous primary structures.

of *S. griseus* and *S. avermitilis* contain 34 and 30 secondary metabolite clusters, respectively [18]. Some of them are cryptic [9, 11].

We assume that the strain *S. globisporus* 1912 has a high potential to produce secondary metabolites because its genome may also contain several biosynthetic gene clusters. As previously reported, some biosynthetic gene clusters were identified in the chromosome of the *S. globisporus* 1912-2. The clusters of genes for syntheses of antibiotics landomycin E and A, carotenoides and gas vesicle proteins were identified *in silico* in the genome of the *S. globisporus* 1912-2 [7, 12, 27]. The biosynthetic *lnd*-genes cluster was unique and absent in the genomes of all tested *Streptomycetes*. Only three *Streptomycetes* cultures are able to produce the landomycins [7]. The biosynthetic *crt*-genes clusters were identified in genomes of *S. coelicolor A3(2), S. griseus* NBRC13350*, S. setonii* ISP5395, *S. chrysomallus var. carotenoides* and some others [28, 29]. Carotenogenesis of *S. globisporus* 1912-2 does not require induction by illumination or other inducers [12]. The biosynthetic *gvp*-genes clusters were identified in genomes of *S. pratensis* ATCC 33331, *S. xiamenensis* 318 and *S. violaceusniger* Tu 4113 but not in the genome of *S. griseus* NBRC13350 [27].

The strain *S. globisporus* C-1027 is a producer of the antitumor antibiotic C-1027 (lidamycin) [5]. Alignment of the nucleotide sequences of the *S. globisporus* 1912-2 and the lidamycin biosynthetic gene cluster (AY048670.1, 85163 bp) was done by the program bl2seq: megablast. There was not found any genomic DNA fragment of *S. globisporus* 1912-2 homologous by the primary structure to the nucleotide sequence of the lidamycin biosynthetic gene cluster of the strain *S. globisporus* C-1027.

The Strains of the species *S. griseus* are well known as the producers of antibiotic streptomycin [3]. Alignment of the nucleotide sequences of the *S. globisporus* 1912-2 and the streptomycin biosynthetic cluster of *S. griseus* N2-3-11 (Y00459.1, 12203 bp) was done. No genomic DNA fragment of *S. globisporus* 1912-2 homologous by the primary structure to the nucleotide sequence of the *str*-cluster was identified.

A great deal of homologous fragments spread along the whole length of the genomic sequences of two *S. globisporus* strains (1912-2 and C-1027) (Fig. 2). A few of non-homologous fragments (59 contigs) in the sequences of genomes of two strains were found. It might be due to the absence of special *S. globisporus* 1912-2 genes in the *S. globisporus* C-1027 genome (and vice versa) or incomplete definition of the primary structure of the *S. globisporus* 1912-2 chromosomal DNA (which can be done by the next sequencing).

82 contigs of *S. globisporus* 1912-2 had entire non-homologous nucleotide sequences in the genomes of *S. griseus* NBRC13350 but only 37 contigs were not found in any of them. Total molecular size of these 37 *S. globisporus* 1912-2 contigs with non-homologous primary structures to the *S. globisporus* C-1027 and *S. griseus* NBRC13350 amounted to 7.59 % of total nucleotide sequences of tested strain contigs. The identity of nucleotide sequences of the genomes of *S. globisporus* C-1027 and *S. griseus* NBRC13350 was 94 %. In total 70 % of the nucleotide sequence (in fragments) of *S. griseus* NBRC 13350 genome was homologous in their primary structures to the

structure of the *S. globisporus* C-1027 genome (overlap). Many other *S. globisporus* 1912-2 contigs had the fragments with non-homologous nucleotide sequences. For example the homologous fragments in an individual contig (N 2 – 75588 bp) amounted to only 26 % (*S. globisporus* C-1027) and 24 % (*S. griseus* NBRC13350).

Interestingly, there were no homologous nucleotide sequences in the genomes of *Streptomyces spp*. (database Nucletide collection, taxide: 1883 (genus Streptomyces)) to 7 contigs of *S. globisporus* 1912-2 (0.001 %). Further *in silico* studies of the nucleotide sequences of all these non-homologous contigs of the strain *S. globisporus* 1912-2 are necessary to identify new clusters that determine synthesis of unique metabolites. Localization of the unique *lnd*-genes in contigs N 3 and N 220 of *S. globisporus* 1912-2 can be given as an example.

Conclusions

The strain *S. globisporus* 1912-2 was identified as a member of the *S. griseus* clade. Many fragments with unique nucleotide sequences were found in chromosomal DNA of this strain (9.35 % of all primary structure of its chromosome). We assumed a big biosynthetic potential of this strain.

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Подібність і відмінність первинних структур геномів ряду стрептоміцетов і хромосомної ДНК *Streptomyces globisporus* 1912-2

Л. В. Поліщук

Мета – визначити *in silico* подібності та відмінності первинних структур геномів ряду штамів стрептоміцетів і хромосомної ДНК *S. globisporus* 1912-2. **Методи.** Використовували ресурси сервера NCBI (BLAST: blastn

і bl2seq: megablast) для *in silico* аналізу первинної структури контигів *S. grlobisporus* 1912-2 і ряду Інтернет баз даних NCBI (Genome, Nucleotide). **Результати.** Виявлено ряд штамів зі значними показниками гомології їх первинної структури ДНК і нуклеотидної послідовності хромосомної ДНК *S. globisporus* 1912-2 (ступенем ідентичності (88 % – 97 %) і ступенем покриттям (55 % – 82 %)). Максимальна ідентичність нуклеотидній послідовності хромосомної ДНК *S. globisporus* 1912-2 було виявлена у геномів штамів *S. globisporus* C-1027 (97 %) і *S. griseus* NBRC13350 (96 %). Жодного фрагмента з первинною структурою, гомологичною структурам семи контігов *S. globisporus* 1912-2 не було знайдено серед нуклеотидних послідовностей стрептоміцетів з базах даних NCBI. **Висновки.** Встановлено, що штам *S. globisporus* 1912-2 є членом клади *S. griseus*. Ми виявили великий біосинтетичний потенціал штаму *S. globisporus* 1912-2, можливий завдяки його багатьом унікальним нуклеотидним послідовностям.

Ключові слова: стрептоміцет, первинна структура, геном, ідентичність, *in silico* аналіз, клада.

Сходство и отличие первичных структур геномов ряда стрептомицетов и хромосомной ДНК *Streptomyces globisporus* 1912-2

Л. В. Полищук

Цель – определить *in silico* сходство и отличие первичных структур геномов ряда штаммов стрептомицетов и хромосомной ДНК *S. globisporus* 1912-2. **Методы.** Использовали ресурсы сервера NCBI (BLAST: blastn и bl2seq: megablast) для *in silico* анализа первичной структуры контигов *S. globisporus* 1912-2 и ряда Интернет баз данных NCBI (Genome, Nucleotide). **Результаты.** Выявлено ряд штаммов со значительными показателями гомологии их первичной структуры ДНК и нуклеотидной последовательности хромосомной ДНК *S. globisporus* 1912-2 (степенью идентичности (88 % – 97 %) и степенью покрытием (55 % – 82 %)). Максимальная идентичность нуклеотидной последовательности хромосомной ДНК *S. globisporus* 1912-2 было выявлено у геномов штаммов *S. globisporus* C-1027 (97 %) и *S. griseus* NBRC13350 (96 %). Ни одного фрагмента с первичной структурой, гомологичной структурам семи контигов *S. globisporus* 1912-2 не было найдено среди нуклеотидных последовательностей стрептомицетов из базы данных NCBI. **Выводы.** Установлено, что штамм *S. globisporus* 1912-2 является членом клады *S. griseus*. Мы выявили большой биосинтетический потенциал штамма *S. globisporus* 1912-2, возможный благодаря его многим уникальным нуклеотидным последовательностям.

Ключевые слова: стрептомицет, первичная структура, геном, идентичность, *in silico* анализ, клада.

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