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SIMILARITY OF GENOMIC SEQUENCES OF FIVE *STREPTOMYCES GLOBISPORUS* STRAINS

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The relevance of the research is that its results, firstly, will be useful in classifying streptomycetes to lower-order taxa, and secondly, can be used in studies on the evolution of organisms at the molecular level. Similar researches are widely conducted on housekeeping genes (for example, 16S rRNA). However, it is interesting to study successful applying in such researches of genes that determine not essential for survival proteins. ***The purposes of this research were*** *to determine similarity of genomic sequences of 5 Streptomyces globisporus strains and to study whether it is possible to use the analysis of nucleotide sequences of genes encoding non-essential proteins or clusters of such genes (for example crt-genes) in determining the kinship of streptomycetes.* ***Materials and methods.*** *Genomic sequences of 5 Streptomyces globisporus strains (C-1027, TFH56, NRRL B-2709, NRRL B-2293, and 1912-4Crt) were in NCBI databases. Computerized analysis of chromosomal DNAs sequences of streptomycetes were carried out by means of BLAST programs.* ***Results.*** *Genomic sequences of 5 S. globisporus strains were analyzed by BLAST program and similarity of lot of their characteristics were found. But many differences in sequences of 5 genomes were determined. It was found that the nucleotide sequences (of both the entire genome and its individual fragments) of S. globisporus NRRL B-2293 strain are the most different from the sequences of the other 4 chromosomes. The specific organization of its crt cluster can serve as a good example of such a distinction. We assume that it is necessary to revise the affiliation of NRRL B-2293 strain to the S. globisporus species.* ***Conclusions.*** *When performing classification (in addition to the traditionally used characteristics of genomes), we propose to analyze both non-essential genes and gene clusters – their presence in the genomes of streptomycetes, the level of similarity of nucleotide sequences of genes, and the organization of gene clusters.*

Keywords: Streptomyces globisporus, crt-cluster, similarity of genomic sequences.

Since 1977 DNA sequencing of organisms takes place with impressive progression. Sequencing of genomes of various taxa organisms (viruses, microorganisms, protozoa, animals, and human) is conducted. Different databases contain information about millions of sequences of separate genes and complete genomes. For example the amount of sequenced complete genomes of streptomycetes will attain 500 soon. Presently there is no necessity to prove the value of determination and study of DNA sequences. Everyone knows the usefulness of such research for the development of science and practice.

Current study results will be useful in classification of streptomycetes to lower order taxons and in the study of molecular evolution of organisms on the whole. Similar researches are widely conducted on housekeeping genes (for example, 16S rRNA) [1–3, 5–7, 8, 13–17]. However, it is interesting to study the homology of sequences of genes determining non-essential proteins.

The use of special techniques for studying the evolution of microorganisms at the molecular level showed its fundamental advantages compared with phenotypic approaches: it opened the possibility to determine the affinity of individual organisms that may not have common phenotypic traits [13].

In addition, the determination of similarity level of genomic structures of streptomycetes will allow to select promising strains of microorganisms.

The purposes of this study were to determine similarity of genomic sequences of 5 *Streptomyces globisporus* strains and to measure usefulness for definition the degree of affinity of nucleotide sequences of gene clusters.

Materials and methods. Genome sequences of 5 *S. globisporus* strains C-1027 (NZ_CP-013738.1), TFH56 (NZ_CP029361.1), NRRL B-2709 (NZ_JNZK00000000.1), NRRL B-2293 (NZ_JODW00000000.1), and 1912-4Crt (NZ_QWFA00000000.1) were taken from NCBI databases [www.ncbi.nlm.nih.gov/nucleotide/]. These strains are representatives of *Streptomyces albovinaceus* subgroup of *Streptomyces griseus* group.

Computerized analysis of sequences of streptomycetes genomes were carried out by means of BLAST programs [www.ncbi.nlm.nih.gov/blast].

Results. Current taxonomic classification of prokaryotes is based on polyphasic taxonomy. This approach combines genomic and phenotypic characteristics of a strain. Minimum amount of genomic information required for description of novel bacterial species must include its phylogenetic classification, DNA–DNA relatedness and the mol% G+C content of DNA [1–8, 13–17].

Some characteristics of 5 *S. globisporus* strains genome sequences are in the Table 1. Information was taken from annotations of genomes in NCBI databases (GenBank).

Only part of *S. globisporus* 1912-Crt4 genome was sequenced, the total size of this part is 7.37 Mbp. It fragment is smaller than sizes of *S. globisporus* C-1027, *S. globisporus* TFH56, *S. globisporus* NRRL B-2709 or *S. globisporus* NRRL B-2293 genomes. We suppose that the sequenced part of *S. globisporus* 1912-Crt4 genome represents about 95% of the initial *S. globisporus* 1912 strain chromosome [10]. These 5 strains are members of the same taxon (*S. albovinaceus* subgroup of *S. griseus* group) [10].

It is interesting that the size of *S. globisporus* NRRL B-2293 strain genomic DNA is bigger than chromosome sizes of other four strains from *S. albovinaceus* subgroup (Table 1). For example, this strain genome size (8.63 Mbp) is 11.86% bigger than molecular size of *S. globisporus* C-1027 strain genome (7.61 Mbp).

It was found by BLAST analysis of 5 streptomycetes strains genome sequences, that genomic structure of *S. globisporus* NRRL B-2293 strain is the least similar to the other 4 strains (Table 1, 2).

The problem of species identification in prokaryotic taxonomy has led to the emergence of various bacteria classification systems. The International Committee on Systematics of Prokaryotes recommended to use the G+C ratio in hierarchical classification [16, 18]. It should be noted that the G+C content of *S. globisporus* NRRL B-2293 genomic DNA is higher (72.40 mol%) than in other 4 *S. globisporus* strains (72 mol%) (Table 1).

Streptomycetes usually have only 1 crt-cluster in their genomes [10]. Members of *S. griseus* group are an exception – 2 or more crt-clusters are found in the genomes of several strains from this clade.

Two crt-clusters were identified in some strains from *S. albovinaceus* subgroup: *S. globisporus* 1912-4Crt, *S. globisporus* C-1027, and *S. globisporus* TFH56. One of them has typical organization: two operons with seven genes (**crtEIBV-><-crtUTY**), but the second one has 6 genes (**crtEIBV-><-crtUY**). At the same time only 1 cluster with typical organization was found in *S. globisporus* NRRL B-2709 genome. *S. globisporus* NRRL B-2293 strain contains only 1 atypically organized crt-cluster in genome (**<-crtT-crtEIBV-><-crtUY**). The least homology with crt-cluster sequence of *S. globisporus* 1912-4Crt strain was observed for NRRL B-2293 strain (Fig. 1).

Two cryptic gvp-clusters were found in the genome of *S. globisporus* 1912-2 [10]. It is interesting to define the presence of sequences homologous to the gvp-cluster genes in the genomes of other *S. globisporus* strains. Such gvp-clusters were not found in *S. globisporus* C-1027 strain genome, but in the NRRL B-2709 strain genome both gvp-clusters were detected. TFH56 and NRRL

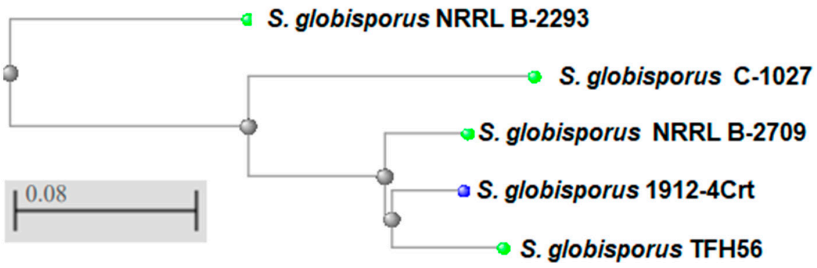

Fig. 1. Phylogenetic tree of *S. globisporus* strains based on similarity of complete crt-cluster sequences. Query – the sequences of crt-cluster of 1912-4Crt strain.

B-2293 strains have only one gvp-cluster (Table 3). Genomic sequences of 3streptomycetes strains gvp-clusters have enough percentage of homology; genomic sequence of NRRL B-2293 strain gvp-cluster is the least similar to other strains (Table 3).

It is interesting that genomes of 3 strains (from 5 analyzed) contained 2 plasmids. Presence of pSG1912-1 and pSG1912-2 plasmids in the genome of *S. globisporus* 1912 and their molecular sizes (10.3 kp and 22.4 kb respectively) was determined by analytical method of DNA research [9]. Sequences of pTFSG1 (127.8 kb) and pTFSG2 (50.1 kb) plasmids of TFH56 strain and SGLP1 (167.8 kb) and pSGL1 (7.2 kb) plasmids of C-1027 strain were determined and added to GenBank database.

Table 1

Some characteristics of genomic sequences of *S. globisporus* strains

Attributes of genomic sequences	*S. globisporus* strains				
	C-1027	TFH56	B-2709	B-2293	1912-4Crt
Genome size, bp	7608611	7488586	7454528	8632737	7365300
Number of contigs	–	–	84	271	466
G+C content, mol%	71.55	71.54	71.70	72.40	71.50
Number of replicons	1	1	1	1	1
Number of plasmids	2	2	0	0	2*
Total number of genes	7015	6916	6784	7932	6862
Number of rRNA genes	18	18	12	10	13
Number of rRNA operons	6	6	1	1	0
Total number of RNA genes	88	88	82	84	75
Number of tRNA genes	67	67	67	71	62
Protein coding genes	6640	6369	6293	7316	6345
Value of DNA sequences	Whole genome sequencing	Whole genome sequencing	Whole genome shotgun sequencing	Whole genome shotgun sequencing	Whole genome shotgun sequencing
The strains sources	China, soil	South Korea, tomato flowers	USA, soil	USA, soil	Armenia, soil

Note: * – determined by analytical method of DNA research [9].

Table 2

Similarity of *S. globisporus* strains genomic sequences

Strains (subject)	Similarity of sequences (query)				
	C-1027	TFH56	NRRL B-2709	NRRL B-2293	1912-4Crt
C-1027	–	Qc = 89% Ev = 0.0 I = 97.1	Qc = 81% Ev = 0.0 I = 95.92%	Qc = 38% Ev = 0.0 I = 91.01%	Qc = 82% Ev = 0.0 I = 96.08%
TFH56	Qc = 90% Ev = 0.0 I = 96.88%	–	Qc = 83% Ev = 0.0 I = 94.56%	Qc = 37%, Ev = 0.0 I = 90.98%	Qc = 84% Ev = 0.0 I = 95.88%
NRRL B-2709	Qc = 80% Ev = 0.0 I = 95.9%	Qc = 83% Ev = 0.0 I = 94.56%	–	Qc = 37% Ev = 0.0 I = 90.92%	Qc = 81% Ev = 0.0 I = 95.89%
NRRL B-2293	Qc = 43% Ev = 0.0 I = 91.01%	Qc = 37% Ev = 0.0 I = 90.98%	Qc = 42% Ev = 0.0 I = 90.89%	–	Qc = 43% Ev = 0.0 I = 90.88%
1912-4Crt	Qc = 82% Ev = 0.0 I = 96.08%	Qc = 84% Ev = 0.0 I = 95.9%	Qc = 80% Ev = 0.0 I = 95.89%	Qc = 37% Ev = 0.0 I = 90.89	–

Legend: Qc – query cover, Ev – E value, I – identity.

Table 3

Similarity of gvp-clasters sequences of 4 *S. globisporus* strains (subject) to the sequences of 2 gvp-clasters of *S. globisporus* 1912-2 (query)

S. globisporus strains (subject)	Value of sequence identity of gvp-clasters	
	gvp-claster #1 Contig_264 (query)	gvp-claster #2 Contigs_756 and 781 (query)
TFH56 NZ_CP029361.1	N	7175843 bp - 7181314 bp Qc = 100%; Ev = 0.0; I = 93.1%
C-1027 NZ_CP013738.1	N	N
NRRL B-2709 JNZK00000000.1	JNZK01000016.1 57833 bp - 63500 bp Qc = 100%; Ev = 0.0; I = 92.2%	JNZK01000016.1 65387 bp - 71389 bp Qc = 99%; Ev = 0.0; I = 91.2%
NRRL B-2293 JODW00000000.1	JODW01000051.1 53909 bp - 59362 bp Qc = 96%, Ev = 0.0, I = 74.2%	N

Legend: N – gvp-clusters did not found; Qc – query cover, Ev – E value, I – identity.

Several differences in the characteristics of *S. globisporus* strains genome sequences from *S. albovinaceus* subgroup were found (Table 1-3). Characteristics of the NRRL B-2293 strain sequence had more differences. It is interesting to determine the level of 16S rRNA-genes similarity of *S. albovinaceus* subgroup strains. The sequence of 16S rRNA-gene of *S. globisporus* C-1027 strain was used as a query sequence.

The sequences of 16S rRNA genes of 3 strains (C-1027 – WQO_RS04730, TFH56 – DIJ69_RS07015, and 1912-4Crt – D3105_15675) were completely identical (Qc = 100%; I = 100%; Ev = 0.00), similarity level of 16S rRNA gene of B-2709 (IF53_RS0133755) strain was slightly less (Qc = 100%; I = 99.8%; Ev = 0.00), but the least level of gene similarity (IH67_RS41710) was in B-2293 (Qc = 96%, I = 98.0%, Ev = 0.00) strain. Similarity of 16S rRNA-genes sequences of 5 *S. globisporus* strains were within the 98.1-100% range (Fig. 2). In accordance with accepted agreements, members of the same clade should have 97.8-100% similarity of 16S rRNA gene sequences [8].

Discussion. The presented research is a part of study of evolution of organisms at molecular level that are conducted in many laboratories. Basic criteria of the obtained data analysis are identity of sequences of genes/proteins, similarity of their functions, synteny of genes in chromosomes [10].

Such researches are widely conducted on housekeeping genes (as rule it is 6 obligatory genes – 16S rRNA, recombinase A, ATP-dependent helicase HrpA, subunit B of DNA gyrase, sigma RNA polymerase factor, sigma RNA polymerase E factor). But it is necessarily to study the

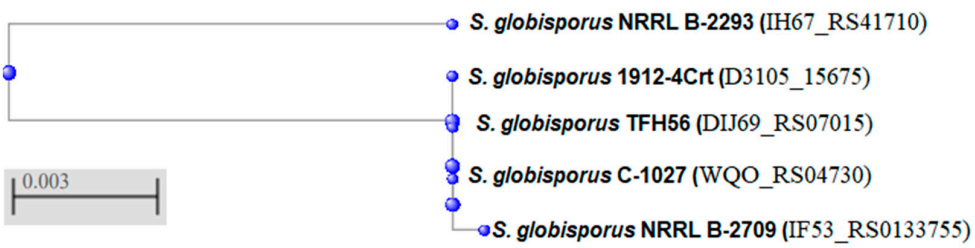

Fig. 2. Phylogenetic tree of *S. globisporus* strains based on similarity of 16S rRNA gene sequences. Query – 16S rRNA sequences of *S. globisporus* C-1027 strain.

similarity of gene sequences that are responsible for synthesis of "non-essential proteins". It is important to determine the possibility of their use (both separate gene and all genome) in taxonomy of microorganisms and in the study of evolution on molecular level. We have previously shown that the similarity of facultative genes structures (on the example of beta galactosidase genes) correlates with the similarity of housekeeping genes (16S rRNA) [12].

Some characteristics of 5 *S. globisporus* strains genomic sequences were analyzed and level of their similarity was determined. Sequences of both entire genomes and a number of individual components (genes, clusters) were analyzed.

Although *S. globisporus* NRRL B-2293 strain has high similarity level of 16S rRNA gene (Qc = 96%, I = 98.0%) with the same genes of other 4 strains from *S. globisporus* subgroup, it differs significantly both in the degree of similarity of its complete genome sequence (Qc = 43%, I = 90.9%) and in the organization of its crt-cluster (Table 2).

We also determined the level of similarity of nucleotide sequences of some other housekeeping genes (recA, gyrB, rpoB, atpB, trpB) to confirm our assumption about degree of interrelation of these strains. The smallest similarity of nucleotide sequences of these 5 selected essential genes of *S. globisporus* NRRL B-2293 strain with the sequences of the same genes of other 4 *S. globisporus* strains was revealed by computerized (BLAST) analysis.

The partial results of the gyrB gene sequences alignment are shown in the Table 6 as an example. The sequence of the *S. globisporus* C-1027 strain recA gene (WQO_RS26770) was used as a query subrange (Table 4).

Modern classification of prokaryotes combines genomic and phenotypic characteristics of strains, such as structure of genes, DNA G+C content.

It should be noted that the genomic sequence (both the entire genome and its individual fragments) of *S. globisporus* NRRL B-2293 strain had the greatest amount of differences from the sequences of other 4 strains. The specific organization of its crt-cluster can be an example of such difference.

Earlier, we declared (using *S. albus/albidoflavus, S. hygroscopycus* strains and some others as examples) that the similarity of cluster organization scheme correlates with similarity of 16S rRNA structures [11]. So our assumption that *S. albus* J1027, *S. albus* SM254, and *S. sampsonii* KJ40 strains belong to the same clade was confirmed by other authors. *S. albus* J1027 and *S. albus* SM254 strains were later classified by Luzhetskyy A (Lviv, Ukraine) as S. *albidoflavus* J1027 (accession NC_020990.1, GenBank) and SM254 (NZ_CP014485.1). These 2 strains were placed to the *S. albidoflavus* group as *S. sampsonii* KJ40.

The crt-cluster from *S. globisporus* NRRL B-2293 strain genome is organized according to the cluster scheme, which was reported by us earlier for *S. hygroscopicus* strains. It may be necessary to consider whether the strain NRRL B-2293 belongs to the *S. globisporus* species.

Analysis of the same species streptomycetes genomes showed that both the degree of differences in some characteristics of DNA molecules (G+C content of DNA, molecular sizes) and the structural features of their components (genes, clusters) determine the remoteness of their relationships and their simultaneous comparison may be useful for classification of prokaryotes.

Table 1

Similarity of 4 *S. globisporus* strains recA-genes sequences to *S. globisporus* C-1027 gene (Query)

Streptomycetes strains	Genes of strains, bp	Indexes of similarity of gyrB-genes
S. globisporus TFH56	DIJ69_RS06880	I = 98.2%, Qc = 100.0%, M/G = 20/0, Ev = 0.0
S. globisporus NRRL B-2709	IF53_RS0130790 NZ_JNZK01000027	I = 97.87%, Qc = 100.0%, M/G = 24/0, Ev = 0.0
S. globisporus 12-4Crt	D3105_RS24135 NZ_QWFA01000145	I = 97.4%, Qc = 100.0%, M/G =29/0, Ev = 0.0
S. globisporus NRRL B-2293	IH67_RS0110965 NZ_JODW01000040	I = 83.5%, Qc = 90.2%, M/G = 167/0, Ev = 0.0

Legend: Qc – query cover, Ev – E value, I – identity, M – Mismatches, G – Gaps.

We can assume that for conducting taxonomic classification (in addition to the traditionally used genome characteristics) it will be useful to analyze gene clusters – their presence in the streptomycetes genome, sequence of genes, and architecture of clusters.

СХОЖІСТЬ ПОСЛІДОВНОСТЕЙ ГЕНОМІВ П'ЯТИ ШТАМІВ ВИДУ *STREPTOMYCES GLOBISPORUS*

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Резюме

Актуальність проведеного дослідження полягає в тому, що його результати, по-перше, будуть корисні при класифікації стрептоміцетів до таксонів нижчого порядку, по-друге – використовуватимуться в дослідженнях по еволюції організмів на молекулярному рівні. Аналогічні дослідження широко проводяться на генах домашнього господарства (наприклад, 16S рРНК). Однак цікаво вивчити успішність застосування в таких дослідженнях генів, що визначають необов'язкові білки. **Метою дослідження** було встановити схожість нуклеотидних послідовностей геномних ДНК п'яти штамів виду *Streptomyces globisporus* і визначити можливість використання при визначенні спорідненості стрептоміцетів аналізу нуклеотидних послідовностей генів, що кодують неважливі для виживання білки або кластери таких генів (на прикладі crt-генів). **Матеріали і методи**. Первинні структури геномів 5-ти штамів *Streptomyces globisporus* C-1027, TFH56, NRRL B-2709, NRRL B-2293 і 1912-4Crt були об'єктами наших досліджень. Інформація була отримана з баз даних сервера NCBI. Комп'ютерний аналіз послідовності геномів стрептоміцетів проводився за допомогою програм BLAST. **Результати.** Послідовності геномів 5-ти штамів *S. globisporus* були проаналізовані за допомогою програми BLAST. Була виявлена схожість багатьох їх характеристик, але було визначено також існування багатьох відмінностей в послідовностях цих геномів. Виявлено, що нуклеотидна послідовність геному (як всього геному, так і його окремих фрагментів) штаму *S. globisporus* NRRL B-2293 найбільш відрізняється значеннями аналізованих характеристик від послідовностей інших 4 штамів. Ми передбачаємо, що необхідно переглянути належність штаму NRRL B-2293 до виду *S. globisporus.* Ексклюзивна організація його crt-кластера може слугувати вдалим прикладом такого роду. **Висновок.** При проведенні класифікації буде корисно (як додаток до характеристик геному, що традиційно використовуються) аналізувати як факультативні гени, так і кластери генів – їх присутність в геномах стрептоміцетів, рівень схожості нуклеотидних послідовностей генів і організацію кластерів генів.

*Ключові слова***:** *Streptomyces globisporus*, crt-кластер, схожість нуклеотидних послідовностей геномних ДНК.

СХОДСТВО ПОСЛЕДОВАТЕЛЬНОСТЕЙ ГЕНОМОВ ПЯТИ ШТАММОВ ВИДА *STREPTOMYCES GLOBISPORUS*

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Резюме

Актуальность проведенного исследования заключается в том, что его результаты, во-первых, будут полезны при классификации стрептомицетов до таксонов низкого порядка, а во-вторых – могут быть использованы в исследованиях по эволюции организмов на молекулярном уровне. Аналогичные исследования широко проводятся на housekeeping генах (например, 16S рРНК). Однако интересно изучить успешность применения в таких исследованиях генов, определяющих несущественные белки. **Цель данного исследования** – установить сходство нуклеотидных последовательностей геномных ДНК пяти штаммов вида *Streptomyces globisporus* и определить возможность использования при определении родства стрептомицетов анализа первичных структур генов, кодирующих несущественные для выживания белки или кластеры таких генов (на примере crt-генов). **Материалы и методы.** Последовательности геномных ДНК пяти штаммов (C-1027, TFH56, NRRL B-2709, NRRL B-2293 и 1912-4Crt) вида *S. globisporus* представлены в базах данных NCBI. Компьютерный анализ после-

довательностей хромосомных ДНК стрептомицетов проводился с помощью программы BLAST. **Результаты**. Последовательности геномов 5-ти штаммов *S. globisporus* проанализированы с помощью программы BLAST и обнаружено сходство многих их характеристик. Было определено также наличие множества различий в последовательностях этих геномов. Было обнаружено, что нуклеотидные последовательности (как всего генома, так и его отдельных фрагментов) штамма *S. globisporus* NRRL B-2293 наиболее отличаются от последовательностей хромосом других 4 штаммов. Специфическая организация его crt-кластера может служить хорошим примером такого различия. Мы предполагаем, что необходимо пересмотреть принадлежность штамма NRRL B-2293 к виду *S. globisporus*. **Заключение.** При проведении классификации будет полезно (в дополнение к традиционно используемым характеристикам геномов) анализировать как факультативные гены, так и кластеры генов – их присутствие в геномах стрептомицетов, уровень сходства нуклеотидных последовательностей генов и организацию кластеров генов.

Ключевые слова: Streptomyces globisporus, crt-кластер, подобие нуклеотидных последовательностей геномных ДНК.

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