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STREPTOMYCETE PLASMIDS CONTAINING BIOSYNTHESIS GENE CLUSTERS OF ANTITUMOR ANTIBIOTIC LIDAMICIN

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Anticancer antibiotic lidamycin is produced by *Streptomyces globisporus* C-1027 strain. The lidamycin biosynthesis cluster (LDM-cluster) is localized on its plasmid SGLP1.

Aim. To identify and characterize plasmid-localized gene clusters potentially involved in lidamycin biosynthesis in streptomycetes.

Methods. Nucleotide sequences of streptomycetes from the Internet database Nucleotide Collection on the National Center for Biotechnology Information were objects of this study. Search for probable LDM clusters was performed using the Basic Local Alignment Search Tool. The LDM-cluster sequence of *S. globisporus* C-1027 was used as a query in BLASTN analysis.

Results. The database contains information on the primary structures of thousands of chromosomes and dozens of plasmids of streptomycetes, which are fully defined (Compete genome). BLASTN-analysis of primary structures of DNAs revealed the presence of probable LDM clusters in 6 streptomycete plasmids. Nucleotide sequences of 7 plasmids were only partially identical — they all contained sequences that were similar to the fragment 7,747 bp — 112,237 bp of SGLP1.

Conclusions. The findings have demonstrated that LDM clusters are predominantly localized on plasmids in Streptomyces species. Although the identified plasmids share substantial sequence similarity — spanning approximately 104.5 kb — with the reference SGLP1 plasmid, they are not genetically identical.

Key words: *Streptomyces*, cluster, plasmid, nucleotide sequence, computerized analysis, lidamycin biosynthesis.

Lidamycin has been shown to exert significant antitumor effects across a wide range of cancers, including liver, breast, pancreatic, colon, lung, gastric, and brain cancers, as well as lymphoma and myeloma [1]. In addition to its anticancer properties, lidamycin also exhibits antimicrobial activity, primarily against Gram-positive bacteria. However, it is not effective against Mycobacterium species or Gram-negative bacteria [2].

Lidamycin (LDM) is an enediyne antibiotic produced by *Streptomyces globisporus* C-1027 [1–3]. The biosynthetic gene cluster for lidamycin (LDM-cluster, 72,676 bp) of *S. globisporus* C-1027 was located in the linear plasmid SGLP1 (167,754 bp) [4–6]. The LDM cluster has been identified, sequenced, and heterologously expressed [6].

We hypothesize that LDM clusters are much more common in the genomes of streptomycetes. However, some of them are

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silent clusters. The development of scientific technologies in molecular biology and bioinformatics makes it possible to identify the presence of such clusters in streptomycete genomes [7].

Aim — To identify and characterize plasmid-localized gene clusters potentially involved in lidamycin biosynthesis in streptomycetes.

Materials and Methods

Objects of this search were nucleotide sequences of chromosomes and plasmids of streptomycetes deposited in databases at the National Center for Biotechnology Information (NCBI). Primary DNA structures were analyzed using the Basic Local Alignment Search Tool (BLASTN: megablast). The LDM-cluster sequence of *S. globisporus* C-1027 was used as a query for computerized analysis of nucleotide sequences.

Results and Discussion

As established, the LDM-cluster (72672 bp) is localized on a plasmid SGLP1 (CP013739). The lidamycin biosynthesis cluster (LDM-cluster) includes 58 genes (WQO_33745 — WQO_34030).

To accomplish the task, a computerized analysis of nucleotide sequences of chromosomes and plasmids of streptomycetes was carried out. Only the results of BLASTN-analysis of heredity factors were considered, sequences of which were determined in full (complete genomes). Such sequences are deposited in the public database Nucleotide Collection at the server NCBI. The database contains information on fully defined primary structures of thousands of chromosomes and dozens of plasmids of streptomycetes.

BLASTN-analysis of DNA primary structures revealed query-like sequences in hundreds of streptomycetes. In most cases, these were small fragments of both plasmid and chromosomal DNAs. Only six factors of heredity have been identified in streptomycetes, structures of which contained sequences similar to the complete sequence of the LDM cluster. These were sequences of 6 plasmids (Table).

The plasmids had molecular sizes ranging from 104,367 bp to 294,500 bp. Five plasmids are maintained in cells of 5 of the species *S. globisporus*, and one plasmid was found in cells of the *S. parvusus* F-G-2 strain.

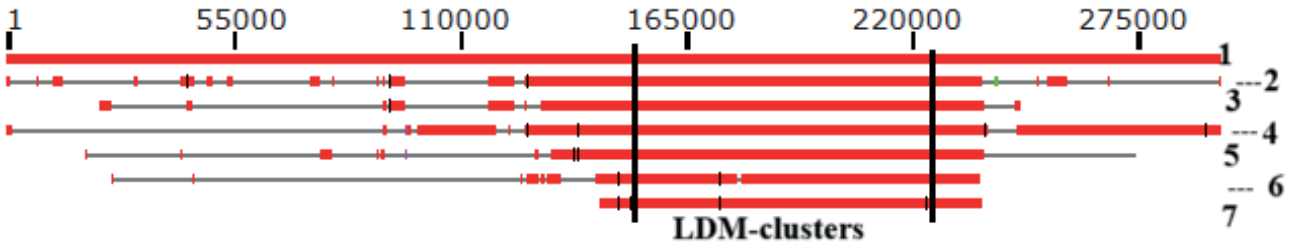
The nucleotide sequences of seven plasmids were partially identical, all containing sequences similar to the fragment 7,747–112,237 bp of SGLP1 (*S. globisporus* C-1027). The conditional localization of plasmid sequences identical to the plasmid CP109060 sequence is visualized in Figure.

The gene clusters for the biosynthesis of the vast majority of antibiotics are usually located on streptomycete chromosomes [8]. However, in a few cases such cluster is situated on a large plasmid. For example, the molecular size of a pKSL plasmid is 520 kb [9]. Some of them have been studied in detail: SCP1 and pSV1 for methylenomycin, pSLA2-L for lankacidin, lankamycin, pPZG103 for oxytetracycline, SGLP1 and pTFH56 for lidamycin, pKSL for salinomycin [4, 5, 9–11]. Most of these plasmids are linear, with the exception of the pSV1 plasmid [8].

S. globisporus C-1027 and *S. globisporus* TFH56 strains are known to produce the antitumor agent lidamycin [5, 11]. LDM-clusters both strains localize on plasmids [5, 11]. Gene sequences for lidamycin biosynthesis were not found in other *Streptomyces spp*, as reported in 2019 [11].

Information about identified plasmids

Plasmids	Molecular length, bp	Annotation number, GenBank	Streptomycete host strains
unnamed1	216726	CP108547	*S. globisporus* NBC_01211
unnamed1	294500	CP109060	*S. globisporus* NBC_01004
pTFH56	127820	CP029362	*S. globisporus* TFH56
unnamed1	231877	CP109147	*S. globisporus* NBC_01745
unnamed1	104367	CP108593	*S. globisporus* NBC_01178
pG-2-2	214348	CP135078	*S. parvulus* F-G-2



Distribution of sequences similar to the plasmid CP109060 sequence of S. globisporus NBC_01004 (Query) in streptomycete plasmid seguences:
Lines: *1* — CP109060, *2* — CP109147, *3* — CP029362 (pTFH56), *4* — CP108547, *5* — CP013739 (SGLP1), *6* — CP135078, *7* — CP108593

Among the plasmids selected in our studies was a pTFH56 plasmid (Table), which is known to contain an LDM-cluster [11]. This confirms the validity of the method we have chosen. As noted above, all 6 identified plasmids (including pTFH56) containing LDM-clusters and SGLP1 are large (Table).

The synthesis of the antibiotic methylenomycin is determined by *mmy/mmf*-clusters localized on 2 plasmids. It has been established that the SCP1 plasmid is linear, and the pSV1 plasmid has a cyclic form. Among the established plasmids, there are plasmids of both forms. Five linear plasmids (SGLP1) and one cyclic plasmid (pG-2-2) were identified.

Conclusions

These studies have shown that LDM clusters, as a rule, are localized on plasmids. Plasmids that have LDM clusters can be both linear and cyclic in form. The identified plasmids and SGLP1 are not identical — they only contain similar 104.5 kb fragments of their sequences. Streptomycetes, which host plasmids with clusters, can belong to different species.

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Conflicts of Interest
The author declare no conflicts of interest.

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ПЛАЗМІДИ СТРЕПТОМІЦЕТІВ, ЩО МІСТЯТЬ КЛАСТЕРИ ГЕНІВ БІОСИНТЕЗУ ПРОТИПУХЛИННОГО АНТИБІОТИКУ ЛІДАМІЦИНУ

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Протипухлинний антибіотик лідаміцин продукується штамом *Streptomyces globisporus* C-1027. Кластер біосинтезу лідаміцину (LDM-кластер) локалізований на його плазміді SGLP1.

Мета. Знайти стрептоміцети, ДНК яких містить ймовірні LDM-кластери.

Методи. Об'єктами дослідження були нуклеотидні послідовності стрептоміцетів з Інтернет-бази *Nucleotide Collection* на сервері *the National Center for Biotechnology Information*. Пошук ймовірних LDM-кластерів проводився за допомогою програм *Basic Local Alignment Search Tool*. Послідовність LDM-кластера *S. globisporus* C-1027 використовували як запит у BLASTN-аналізі.

Результати. База даних містить інформацію про первинні структури тисяч хромосом і десятків плазмід стрептоміцетів, які повністю визначені (*Compete genome*). BLASTN-аналіз первинних структур ДНК виявив наявність ймовірних LDM-кластерів у 6 плазмідах стрептоміцетів. Нуклеотидні послідовності 7 плазмід були ідентичні лише частково — усі вони містили послідовності, подібні до фрагмента 7747 п.н.–112237 п.н. SGLP1.

Висновки. LDM-кластери, як правило, локалізовані лише на плазмідах. Ідентифіковані плазміди та SGLP1 не є ідентичними — вони містять лише подібні 104,5 п.н.-фрагменти своїх послідовностей.

Ключові слова: *Streptomyces*, кластер, плазміда, нуклеотидна послідовність, комп'ютерний аналіз, біосинтез лідаміцину.