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DRAFT GENOME SEQUENCE OF *NONOMURAEA* SP., A BACTERIA INHABITING BLACK TRUFFLE TUBER  
*AESTIVUM* FRUITBODY

Research article

Potapova N.A.<sup>1</sup>, Dmitrieva M.E.<sup>2</sup>, Malygina E.V.<sup>3</sup>, Morgunova M.M.<sup>4</sup>, Listopad A.S.<sup>5</sup>, Lipatova O.E.<sup>6</sup>, Emshanova V.A.<sup>7</sup>,  
Martynova E.I.<sup>8</sup>, Batalova A.A.<sup>9</sup>, Axenov-Gribanov D.V.<sup>10,\*</sup>

<sup>1</sup>ORCID : 0000-0002-3818-9270;

<sup>2</sup>ORCID : 0000-0002-9229-1954;

<sup>3</sup>ORCID : 0000-0002-2673-0226;

<sup>4</sup>ORCID : 0000-0002-7939-1432;

<sup>6</sup>ORCID : 0009-0005-7286-5673;

<sup>10</sup>ORCID : 0000-0003-2020-6084;

<sup>1, 2, 3, 4, 5, 6, 7, 8, 9, 10</sup> Irkutsk state university, Irkutsk, Russian Federation

\* Corresponding author (denis.axengri[at]gmail.com)

**Abstract**

*Nonomurea* is a rare genus of Gram-positive aerobic bacteria belonging to actinobacteria. The genome sequence of novel *Nonomurea* sp. isolated from the truffle *Tuber aestivum* sample. The bacterial strain is genetically similar to *N. glycinis* and *N. quangzhouensis*. We report this genome to accelerate investigation of the *Nonomurea* genus for medical and biotechnological purposes, as well as exploration of species inhabiting *T. aestivum* fruitbodies. Assembly completeness of genome was 98.60%. GC content was 70.22% in consistency with *Nonomurea* genus. The genome was annotated and predicted 9805 genes. The size of the genome is 10 Mb. We found clusters associated with polyketides, terpenes and NRP. Thus, the symbiotic organisms of truffle *T. aestivum* can be interesting and perspective object for biomedical and genetic engineering research.

**Keywords:** actinobacteria, *Nonomurea* sp., *Tuber aestivum*, genome.

ГЕНОМ БАКТЕРИАЛЬНОГО ШТАММА *NONOMURAEA* SP., ВЫДЕЛЕННОГО ИЗ ПЛОДОВОГО ТЕЛА  
ЧЕРНОГО ТРЮФЕЛЯ *TUBER AESTIVUM*

Научная статья

Потапова Н.А.<sup>1</sup>, Дмитриева М.Е.<sup>2</sup>, Малыгина Е.В.<sup>3</sup>, Моргунова М.М.<sup>4</sup>, Листопад А.С.<sup>5</sup>, Липатова О.Е.<sup>6</sup>,  
Емшанова В.А.<sup>7</sup>, Мартынова Е.И.<sup>8</sup>, Баталова А.А.<sup>9</sup>, Аксёнов-Грибанов Д.В.<sup>10,\*</sup>

<sup>1</sup>ORCID : 0000-0002-3818-9270;

<sup>2</sup>ORCID : 0000-0002-9229-1954;

<sup>3</sup>ORCID : 0000-0002-2673-0226;

<sup>4</sup>ORCID : 0000-0002-7939-1432;

<sup>6</sup>ORCID : 0009-0005-7286-5673;

<sup>10</sup>ORCID : 0000-0003-2020-6084;

<sup>1, 2, 3, 4, 5, 6, 7, 8, 9, 10</sup> Иркутский государственный университет, Иркутск, Российская Федерация

\* Корреспондирующий автор (denis.axengri[at]gmail.com)

**Аннотация**

*Nonomurea* представляет собой редкий род грамположительных аэробных бактерий, относящихся к актинобактериям. Геномная последовательность нового вида *Nonomurea* sp., выделенного из симбиотического сообщества трюфеля *Tuber aestivum*, была секвенирована и аннотирована в ходе настоящего исследования. Показано, что выделенный штамм бактерий генетически сходен с *N. glycinis* и *N. quangzhouensis*. Полнота сборки генома составила 98,60%, а размер – 10 Мб. Содержание GC-оснований составило 70,22%, что в целом соответствует роду *Nonomurea*. Геном был аннотирован, в ходе чего выявлено 9 805 генов. Обнаружены кластеры, связанные с поликетидами, терпенами и нерибосомальными пептидами. Таким образом, симбиотические организмы трюфеля *T. aestivum* могут стать интересным и перспективным объектом для биомедицинских и биотехнологических исследований.

**Ключевые слова:** актинобактерии, *Nonomurea* sp., *Tuber aestivum*, геном.

**Introduction**

Genus *Nonomurea* includes Gram-positive aerobic bacteria inhabiting diverse environments [1], [2], [3], [4]. They produce natural products important in medicine, biotechnology and other industries. To date, different antibiotics, anticancer and antipsychotic compounds, as well as many others directed to various diseases, have been extracted from *Nonomurea* species [1]. Capability of *Nonomurea* species to produce secondary metabolites and their precursors is not well studied: not more than 50 natural products have been described and characterized for bacteria of this genus. However, one of the giant sources of new potential medical, biotechnological and other compounds is living organisms, including bacteria and fungi. The potential and ease of obtaining such compounds cannot be overshadowed by computer design of compounds and drugs. Therefore, studies of novel organisms especially known as carriers of possible useful compounds are of a high priority.

## Research methods and principles

Summer or black truffle *Tuber aestivum* is a fungus of the Tuber genus. This species can be found in Europe, including Russia, and is widely known for its black fruit bodies located underground. Fruit bodies are of a size 2-8 cm and are covered by black plaques which gave name to this truffle. *T. aestivum* grows in symbiosis with the roots of trees and also has many bacteria, fungi and protists inhabiting its fruitbody. All these living organisms, in relation to their presence and amount, can influence the condition of truffle, leading it to prosperity and oppression. As the truffles are promising organisms for different industries including biotechnology, information about organisms inhabiting them is necessary, for instance, for the comprehensive analysis and interpretation and prognosis of metabolite production. Therefore, detailed analysis of each of such organisms can improve the knowledge of *T. aestivum*.

We sequenced, assembled and analyzed the genome of the novel *Nonomuraea* species isolated from the truffle *Tuber aestivum*. 16S rRNA sequence retrieved from the assembled contigs using ContEst16S tool [5] showed similarity to 16S rRNA of *N. glycinis* [6] (GenBank accession number KY328642, 99.47% identity) and *N. guangzhouensis* [7], [8] (GenBank accession number KC417349, 99.79% identity). Difficulty in species determination is often observed for *Nonomureae*, probably, due to genetic similarity between strains and incomplete genomics information for strains in GenBank.

Strain of *Nonomuraea* sp. LPB2021202275-12-8 was isolated from wild ascocarp of *Tuber aestivum* collected in coniferous forest in Krasnodar, Russia (GPS 43.61824381815124, 39.84066203618042). The ascocarp surface was cleaned with a toothbrush. Then the ascocarp was split and the central part of the tuber gleba was dissected, homogenized in Tryptic Soy Broth (TSB, Kat. T8907, Merck, Germany) and plated on Potato Dextrose Agar nutrient medium (PDA, Kat. 1.10130, Merck, Germany). The spread plate method followed by the streak plate method was used to isolate a single colony of the strain. Ten passages were performed to ensure the purity of clones. Then confirmation of culture purity was checked during PCR identification of strain with universal eubacterial primers (27F 5'-AGAGTTTGATYMTGGCTCAG-3' and 1510R 5'-TACGGYTACCTTGTACGACTT-3') used for 16S rRNA gene amplification. Then analysis of sequence chromatogram quality was performed as in [9].

For DNA isolation, the strain was cultured for 36 hours in liquid TSB nutrient medium in Erlenmeyer flasks with deflectors at 28°C and shaking rate 180 rpm. DNA was extracted using MP Biomedicals™ Gnome™ DNA Isolation Kit (USA), for library preparation we used KAPA HyperPrep kit (Roche Sequencing Solutions, USA). The genome was sequenced on Illumina MiSeq using 250 bp paired-end reads.

As the next step, we performed genome assembly. The Uncicycler (v.0.5.0) tool performs raw reads quality control during assembly, so we performed genome *de novo* assembly using this tool with default parameters [10]. Then we kept only those contigs which were longer than 1000 bp and checked them for contamination. This procedure was performed in two steps: inspecting GC content using written scripts to observe which contig deviates from the mean GC value as well as from known GC value for *Nonomuraea* genus. As the second step we performed blastn and blastx (online program v. 2.15.0 on the NCBI website, accessed on 15.07.2024) procedures for each contig with the default parameters to check whether there are hits related not to *Nonomuraea* genus [11], [12]. In total, 129 contigs longer than 1000 bp of a total length 10075022 bp with the average coverage of 1.53x survived after the filtering procedure. The length of the genome is similar to a few known genome sequences of *Nonomuraea* species [8]. Still, there were described longer assembled genomes [2], [14]. It shows that, according to genome length, our species does not differ dramatically from the other sequences of species of this genus. Among assembled contigs of the studied strain, we found two short length assemblies marked by Uncicycler as circular, i.e. meaning they are plasmids, sized 102852 bp (4.11x) and 5386 bp (2.83x). The following analysis revealed that they carried genes usually found in plasmids, supporting the fact of their correct identification. Assembly completeness was 98.60% (BUSCO v.5.4.7 and streptosporangiales\_odb10 dataset) and showed a high [15], [16]. Assembly GC content was 70.22% in consistency with *Nonomuraea* genus [1], [3], [13], assembly N50 was 206573 bp, L50 was 13 (QUAST v.5.2.0) [16], [17].

## Main results

Then we annotated the genome with PGAP (online program v.6.7 on the NCBI website, accessed on 08.03.2024) and predicted 9876 genes located in this genome. Annotation of secondary metabolite biosynthesis gene clusters was retrieved from antiSMASH (v.7.0, 19). In total, 30 genetic clusters were detected. Twenty-seven clusters were presented as novel and unknown. Among detected informative clusters we found clusters which were predicted to be associated with polyketides (alkylresorcinol, 100% similarity), terpenes (geosmin, 100% similarity) and NRP (coelichelin, 72% similarity). In theoretical and experimental studies it was shown that alkylresorcinols being natural chemical compounds that have anticancer activity, can be potential regulators of different processes including immune, antimicrobial and metabolic. For geosmin there are no observations about antimicrobial activity, but it may have warning chemical activity. It gives a smell similar to the soil smell and can attract or repel organisms. Coelichelin is less studied than two previous compounds and is involved in interactions with metals in cells. Taken together, found clusters have a potential for different medical and biotechnological purposes and can be considered in detail in further studies.

Overall, our study presents a novel genome sequence of *Nonomuraea* sp. isolated from *T. aestivum* and describes it for possible valuable secondary metabolites. This information can be used in further studies in a search for different drugs, including antibiotics, and extends the knowledge of genomes and their functions for this rare genus.

### 3.1. Data availability

Genome assembly project is available in NCBI BioProject database with accession number PRJNA1075312, assembly is available in NCBI Genome with accession number GCF\_037044575.1, raw reads are available in NCBI SRA database with accession number SRX24330061, genome annotation is available in NCBI Gene database with accession number GCF\_037044575.1. antiSMASH report is available on FigShare 10.6084/m9.figshare.26303158.

## Conclusion

The truffle fungi is a well known source of microorganisms. They includes both bacteria, and truffle associated fungi. Our previous study revealed the symbiotic microorganisms related to such genera as *Rahnella*, *Enterobacter*, *Stenotrophomonas*, *Acinetobacter*, *Citrobacter*, *Comamonas*, *Enterohabdus*, *Klebsiella*, *Lactobacillus*, *Massilia*, *Nitrobacter*, *Nocardioides*, *Pedobacter*, *Ralstonia*, *Raoultella*, *Rahnella*, *Rhizobium*, *Sphingobacterium*, and *Staphylococcus*. *Nonomurea* sp., isolated in these study, was first time detected from the truffles.

Thus, the symbiotic organisms of truffle *T. aestivum* can be interesting and perspective object for biomedical and genetic engineering research.

## Финансирование

The research received support from the Russian Science Foundation through projects 20-76-00001 (strain isolation) and 22-76-10036 (genome sequencing, analysis, and annotation).

## Конфликт интересов

Не указан.

## Рецензия

Все статьи проходят рецензирование. Но рецензент или автор статьи предпочли не публиковать рецензию к этой статье в открытом доступе. Рецензия может быть предоставлена компетентным органам по запросу.

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## Conflict of Interest

None declared.

## Review

All articles are peer-reviewed. But the reviewer or the author of the article chose not to publish a review of this article in the public domain. The review can be provided to the competent authorities upon request.

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