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GENOMIC ANALYSIS OF AN ANTARCTIC ACTINOMYCETE *MICROMONOSPORA ENDOLITHICA* STRAIN AA-459

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***Aim**. To investigate the genome annotation of M. endolithica AA-459 for the presence of unique genes and their combinations, as well as to assess the production potential of the strain by analysing secondary metabolism clusters. **Methods**. Genome annotation was performed using the RAST tool, and the search for secondary metabolism clusters was performed using the AntiSMASH tool. **Results**. Genome annotation identified 6,593 coding regions, including 9 rRNA and 67 tRNA genes. Functional characterization revealed genes spanning 24 subsystems. Protein metabolism was represented by 235 genes, carbohydrate metabolism involved 266 genes, 281 genes involved in amino acid metabolism, 99 genes associated with lipid and fatty acid metabolism. AntiSMASH analysis identified 17 potential secondary metabolite clusters in the genome of M. endolithica AA-459. Notably, two clusters showed high homology to those of known compounds. **Conclusions**. The genome analysis of M. endolithica AA-459 demonstrated the high production potential of the strain, which indicates the importance of the Antarctic region in the context of new compound discovery. However, the number of described compounds from this region remains low, which may be due to the lack of optimal conditions for the expression of the relevant genes. Further research in this area will reveal the biotechnological potential of Antarctic actinomycetes.*

***Keywords**: bacteria, actinomycetes, Micromonospora, Antarctic, genomic analysis.*

Introduction. Infectious diseases are the second leading cause of death globally, surpassed only by coronary heart disease. They account for 15 % of all global deaths, with just five pathogens (*Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*) responsible for half of all cases. *S. aureus* alone causes over one million deaths annually. Infectious diseases are prevalent in both developing and high-income countries; for instance, *S. aureus* accounts for 25 % of sepsis cases in Europe (Brüssow, 2024). The rapid development of antibiotic resistance exacerbates this global health crisis. Methicillin-resistant *S. aureus* (MRSA) alone has caused more than 100,000 deaths worldwide, with numbers continuing to rise. Other pathogens, such as *Mycobacterium tuberculosis*, are also becoming increasingly resistant to antibiotics, complicating tuberculosis treatment. Additionally, fungal infections are emerging as a significant threat, particularly during the COVID-19 pandemic. Human fungal diseases differ fundamentally from bacterial infections. As eukaryotic pathogens, fungi share many similarities with human host cells, making the development of effective antifungal compounds challenging. Furthermore, fungi are capable of infecting diverse hosts and causing systemic diseases. Some fungal infections have severe consequences for human health. For example, approximately 220,000 new cases of cryptococcal meningitis are reported globally each year, resulting in 181,000 deaths. In Latin America, histoplasmosis is among the most common opportunistic infections in people living with HIV/AIDS, with an alarming mortality rate of approximately 30 % (Rodrigues & Nosanchuk, 2021). Global warming further accelerates the evolution of mold fungi, potentially enabling them to overcome the human body's thermal barrier, increasing the risk of new fungal infections previously unseen in humans. Addressing these challenges will require the development of effective vaccines, novel antimicrobial agents, and fungicides to curb the spread and impact of infectious diseases.

The classical approach to discovering new antibiotics involves testing the ability of microorganisms to inhibit the growth of pathogenic strains.

This method has led to the discovery of most clinical antibiotics, including β-lactams, aminoglycosides, polymyxins, and glycopeptides. These antibiotics, or their semi-synthetic derivatives, remain the cornerstone of infection treatment, with some compounds in use for over 70 years (Stokes et al., 2020). The primary producers of antibiotics are molds and bacteria belonging to the *Bacilli, Deltaproteobacteria,* and *Actinomycetes* classes. Among these, actinomycetes are particularly valuable, accounting for approximately 70% of modern antibiotics. Actinomycetes are unicellular (cocci or bacilli) or mycelial bacteria characterized by a high G + C content in their genomes. These microorganisms are widespread, inhabiting soil and aquatic ecosystems and often forming symbiotic relationships with eukaryotic organisms (Selim et al., 2021). Actinomycetes isolated from extreme environments are of special interest, as stressful conditions can drive the evolution of unique bioactive compounds and enzymes (Hui et al., 2021). Antarctica, with its extreme conditions — low temperatures, limited water availability, high UV radiation, and nutrient scarcity — is a promising region for studying extremophilic bacteria. Furthermore, its relative isolation promotes unique evolutionary processes, potentially leading to novel biochemical pathways. For our bioinformatics study, we selected *Micromonospora endolithica* strain AA-459, which was isolated from the McMurdo Dry Valleys in Antarctica (Hirsch et al., 2004). Investigating the biotechnological potential of this strain may enhance our understanding of the role of Antarctic actinomycetes in the discovery and development of new therapeutic agents.

Materials and methods

The genome of *M. endolithica* AA-459 was obtained from The National Centre for Biotechnology Information. Genome annotation was performed using the RAST tool (Aziz et al.,2008), and the search for secondary metabolism clusters was performed using the AntiSMASH (v.7.0) tool (Blin et al., 2023).

Results and discussion

The genome of *Micromonospora endolithica* AA-459 was assembled into two contigs with a total length of 7,026,184 base pairs and a G + C content of 72.5 %. Genome annotation identified 6,593 coding regions, including 9 rRNA and 67 tRNA genes. Functional characterization revealed genes spanning 24 subsystems. Specifically, 103 genes were associated with DNA metabolism, including DNA topoisomerases type I and II and DNA repair genes of the *RecFOR* pathway. Nineteen genes were involved in the metabolism of aromatic compounds, identifying pathways for the degradation of quinate, benzoate, biphenyl, p-hydroxybenzoate, and salicylates. Among transporters, sodium-dependent transporters for phosphorus, copper, and magnesium were identified. Protein metabolism was represented by 235 genes, including selenoprotein genes, which are known for their antioxidant functions (Byun & Kang, 2011) — particularly relevant in Antarctica, where UV exposure is elevated (Bernhard & Stierle, 2020). Carbohydrate metabolism involved 266 genes, including those for butanol synthesis and the degradation of chitin, xylose, mannose, arabinose, ribose, glycerol, trehalose, lactose, maltose, and sucrose. Among 34 genes linked to cell wall and exopolysaccharide synthesis, genes for rhamnose-containing glucans were identified. Genes conferring resistance to heavy metals, including mercury, cobalt, zinc, cadmium, and copper, were also present. The genome contained 99 genes associated with lipid and fatty acid metabolism and 281 genes involved in amino acid metabolism. Genes for phosphate and polyphosphate metabolism were also detected. Additionally, genes encoding for the biosynthesis of biotin, folate, riboflavin, thiamine, pyridoxine, cobalamin, and coenzymes A and 420 were identified. While the role of vitamins in microbial metabolism remains underexplored, it is known that adding pantothenic acid to culture media can increase actinomycete populations (Carrothers et al., 2015). Symbiotic microorganisms that produce vitamins can also stimulate plant growth and development (Palacios et al., 2014). These findings suggest that Antarctic actinomycete metabolism could serve as a source of novel genes and metabolic products, including potentially cold-resistant compounds.

AntiSMASH analysis identified 17 potential secondary metabolite clusters in the genome of *M. endolithica* AA-459 (Fig. 2). Notably, two clusters showed high homology to those of known compounds. Cluster 7 was 100% identical to the SapB protein cluster, a lanthipeptide responsible for aerial mycelium and spore formation in certain actinomycetes (Kodani et al., 2004). Cluster 17 displayed 88% similarity to the loseolamycin A1 cluster, which produces a compound active against Gram-positive bacteria and certain weeds (Lasch et al., 2020). Additionally, a cluster showing 66 % similarity to the legonoxamine A-B cluster was identified (Maglangit et al., 2019).

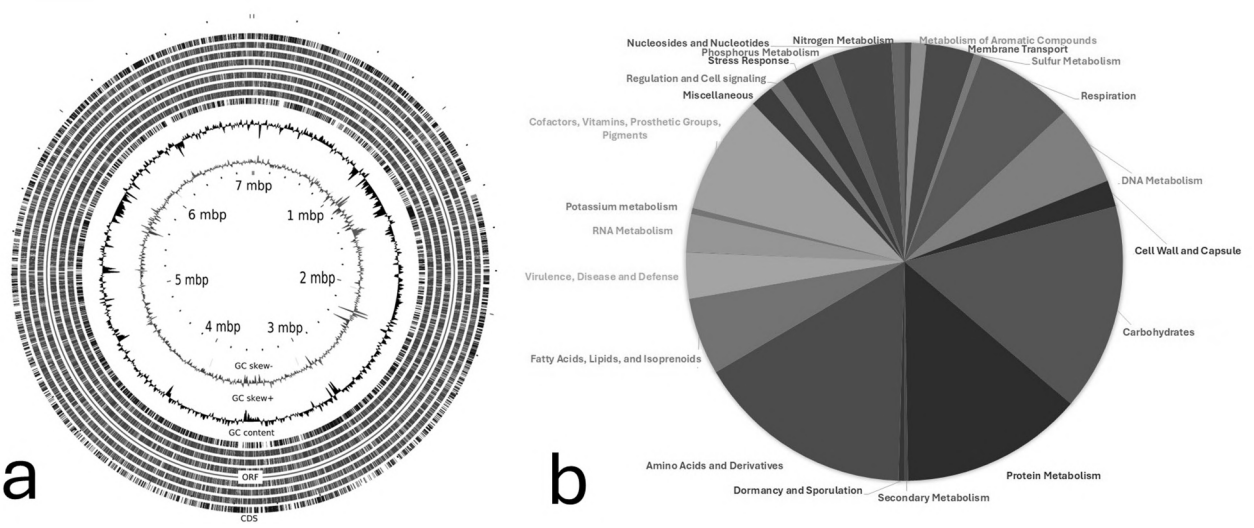

Figure 1. Genome map of Micromonospora endolithica AA-459 **(a)** and classification of annotated genes into subsystems using the RAST service **(b)**. From the outside to center ring: annotated genes (coding sequences, CDS) on the forward and reverse strands and the GC content.

Legonoxamines belong to the group of hydroxamate siderophores, which function in metal chelation. Siderophores commonly inhibit pathogen growth, enhance mineral nutrition, and assist in heavy metal detoxification (Makar et al., 2023). The remaining clusters showed lower homology but included various peptide compound clusters, such as non-ribosomal peptides, thiopeptides, and linear azol(in)e-containing peptides.

The latter are often antimicrobial and can inhibit protein synthesis by binding to 23S rRNA (Travin et al., 2019). A cluster for the synthesis of N-acetylglutaminylglutamine amide, a dipeptide that provides osmotic stress protection in bacteria, was also identified (Sagot et al., 2010). These findings highlight the high biosynthetic potential of the Antarctic strain *M. endolithica* AA-459.

Region	Type	From	To	Most similar known cluster		Similarity
Region 1.1	terpene	604,801	625,742	tetrachlorizine	Polyketide	13%
Region 1.2	RiPP-like	776,591	787,409	lymphostin/neolymphostinol B/lymphostinol/neolymphostin b	Polyketide+NRP	30%
Region 1.3	PKS-like , T1PKS , NRPS	1,277,400	1,411,869	chlorothricin/deschlorothricin	Polyketide:Modular type I polyketide+Polyketide:Iterative type I polyketide+Saccharide:Oligosaccharide	48%
Region 1.4	thiopeptide , thioamitides , LAP	1,508,002	1,560,867	meilingmycin	Polyketide	2%
Region 1.5	RiPP-like	1,598,094	1,609,578			
Region 1.6	NI-siderophore	1,727,123	1,763,019	legonoxamine A/desferrioxamine B/legonoxamine B	Other	66%
Region 1.7	LAP	1,800,809	1,824,412			
Region 1.8	T2PKS , arylpolyene	1,961,182	2,033,727	paramagnetoquinone 1/paramagnetoquinone 2	Polyketide	25%
Region 1.9	RRE-containing	2,070,416	2,091,528			
Region 1.10	lanthipeptide-class-iii	2,277,607	2,300,210	SapB	RiPP:Lanthipeptide	100%
Region 1.11	terpene	2,521,877	2,543,133	oryzanaphthopyran A/oryzanaphthopyran B/oryzanaphthopyran C/oryzanthrone A/oryzanthrone B/chlororyzanthrone A/chlororyzanthrone B	Polyketide	6%
Region 1.12	NRPS-like , NRPS , T1PKS , linaridin	3,363,322	3,444,009	caerulomycin A	NRP+Polyketide	8%
Region 1.13	RRE-containing	3,522,728	3,543,900			
Region 1.14	NAGGN	4,449,317	4,464,054			
Region 1.15	terpene	5,126,046	5,147,053	phosphonoglycans	Saccharide	3%
Region 1.16	terpene	5,207,401	5,228,312	isorenieratene	Terpene	25%
Region 1.17	T3PKS	6,074,143	6,115,192	loseolamycin A1/loseolamycin A2	Polyketide	88%

Figure 2. Clusters of secondary metabolites detected in the genome of Micromonospora endolithica AA-459 using AntiSMASH service.

Conclusions

Analysis of the *Micromonospora endolithica* AA-459 genome revealed the presence of a large number of genes for the synthesis of various compounds. And the lack of similarity in the predicted clusters may indicate that their products have not been previously described. However, Antarctica remains a poorly explored region, and the number of secondary metabolites isolated from there is critically low. The main reason for the low biotechnological potential of this region is the lack of a standardised approach to the cultivation of native microorganisms. In addition, such strains usually have a high level of silent clusters, which can rarely be activated by traditional approaches. That is why such work will allow us to create an overview of the potential of Antarctic strains, and a comprehensive gene analysis will allow us to identify regulatory systems, the manipulation of which will provide knowledge about the processes of evolution in the extreme conditions of Antarctica.

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ГЕНОМНИЙ АНАЛІЗ АНТАРКТИЧНОГО АКТИНОМІЦЕТА *MICROMONOSPORA ENDOLITHICA* AA-459

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Мета. Провести анотацію геному *M. endolithica* AA-459 на наявність унікальних генів та їхніх комбінацій, а також оцінити продукційний потенціал штаму за допомогою аналізу кластерів вторинного метаболізму. **Методи**. Анотування геному проводили за допомогою програми RAST, а пошук кластерів вторинного метаболізму — з використанням програми AntiSMASH. **Результати**. За даними анотації геному ідентифіковано 6 593 кодуючі ділянки, серед яких 9 генів рРНК та 67 генів тРНК. Функціональна характеристика виявила гени, що охоплюють 24 підсистеми. Білковий метаболізм представлений 235 генами, вуглеводний метаболізм — 266 генами, амінокислотний метаболізм — 281 геном, метаболізм ліпідів та жирних кислот — 99 генами. Аналіз AntiSMASH виявив 17 потенційних кластерів вторинних метаболітів у геномі *M. endolithica* AA-459. Показово, що два кластери показали високу гомологію до кластерів відомих сполук. **Висновки**. Аналіз геному *M. endolithica* AA-459 продемонстрував високий продукційний потенціал штаму, що свідчить про важливість антарктичного регіону в контексті відкриття нових сполук. Однак, кількість описаних сполук з цього регіону залишається низькою, що може бути пов'язано з відсутністю оптимальних умов для експресії відповідних генів. Подальші дослідження в цьому напрямі дозволять розкрити біотехнологічний потенціал антарктичних актиноміцетів.

Ключові слова: бактерії, актиноміцети, *Micromonospora*, Антарктика, геномний аналіз.