DOI: http://dx.doi.org/10.18524/2307-4663.2023.2(58).287369

UDC 579.222.3

V.V. Subota, A.M. Ostapchuk, M.D. Shtenikov
Odesa I. I. Mechnikov National University,
2, Dvorianska str., Odesa, 65082, Ukraine;
e-mail: viktoriia27subota98@gmail.com

DEEPENED GENOMICS-METABOLOMICS CHARACTERISTICS OF THE BACTERIA OF THE BACILLUS GENUS ISOLATED FROM DEEP-SEA

Marine bacteria from the genus Bacillus are attracting increasing attention as a source of large amounts of bioactive metabolites. The biosynthetic potential of marine bacteria from the genus Bacillus is not sufficiently studied. The previous study of authors could not give a complete result, so it was decided to reanalyze the metabolites not identified at that time, taking into account the development of existing databases. Therefore, the work ***aimed*** *to reanalyze the metabolome already performed on Bacillus velezensis ONU 553, Bacillus pumilus ONU 554, and Bacillus subtilis ONU 559 strains isolated from the Black Sea sediments.* ***Methods.*** *The general genome identification was performed using the online version of the Dictionary of Natural Products database, and search for biosynthetic clusters in genome of Bacillus velezensis ONU 553 - with antiSMASH 7.0.* ***Results and Conclusion.*** *Genomic-metabolic profiling of strains led to the identification of several new biological compounds – 7 and 1 metabolites in Bacillus velezensis ONU 553 and Bacillus pumilus ONU 554 strains, respectively, and to identify biosynthetic clusters of iturin- and helipeptin-like peptides in Bacillus velezensis ONU 553 strain for the first time. A new biosynthetic cluster was found in the Bacillus velezensis ONU 553 strain*

Key words: Bacillus, Spergualin, Bacillomycin, Helipeptin, deepwater sediments.

Until recently, soil microorganisms were the most productive sources of new antibiotics and anticancer compounds. However, the gradual decrease in the frequency of discovery of new biologically active compounds from representatives of this biotope pushes researchers to less studied ones. An example of such a biotope can be the sea, as an environment inhabited by life from the earliest times [8]. This study is aimed at deepening our knowledge of the biosynthetic potential of marine bacteria from the genus *Bacillus*.

It is known that the oceans occupy 70% of the world's surface, 95% of which have depths of more than 1000 m [2]. In the course of adaptation to conditions of high pressure, high salinity, lack of light and low oxygen concentration, metabolism, we can assume the formation of a special genetic pool of determinants of secondary metabolites, which distinguishes marine prokaryotes from their terrestrial relatives. This increases the probability of discovering structurally unique natural products in them.

Marine environments are already known as sources of strains with specific biosynthetic potential. For 50 years from 1948 to 1998 the number of new marine natural compounds exceeded 20 000, 2% of which were isolated from deepwater organisms [15]. However, due to the limitations of sample collection technologies, fewer microorganisms have been isolated from deep-sea environments than from coast and land.

Representatives of the genus *Bacillus* are promising for research to identify new metabolites. Examples of antibiotics produced by *Bacillus* spp include the lipopeptide bacitracin of *B. licheniformis* or *B. subtilis*, cyclic peptides polymyxin of *Paenibacillus polymyxa* and gramicidin of *Brevibacillus brevis*, as well as antibiotics of such groups as amicoumacins, polyketides and their derivatives [16]. In recent years, several lipopeptide antibiotics have received FDA approval for the treatment of infections caused by multidrug-resistant bacteria, (e.g., daptomycin), and others are at various stages of clinical and preclinical trials [13]. *Bacillus* produces several of well-known examples of these compounds, including surfactins and fengycins. In addition to these better-known examples, these organisms generate a number of other classes of lipopeptides with potent antimicrobial activity.

Lipopeptides from *Bacillus* and *Paenibacillus* spp. are secondary metabolites produced by non-ribosomal peptide synthetases (NRPS) [3]. They usually contain amino acids of both L and D configurations, which leads to increased stability in the presence of proteolytic enzymes of target organisms, including human plasma proteases. This can potentially be used for patient treatment by oral administration and intravenous injection. The N-termini of lipopeptides of these species are usually acylated with branched-chain fatty acids, which often contain a β-hydroxyl functionality (usually in the R-configuration) [10]. In most, but not all cases, lipopeptides are cyclized by an ester or amide bond between a heteroatom on the side chain of an amino acid or lipid tail and the C-terminus, which limits the product's configurational flexibility. Nonproteinogenic amino acids are regularly incorporated into the peptide chain and provide additional diversity to this class of antibiotics. The mechanism of action of many lipopeptides involves membrane disruption, and due to the reorganization hurdle of membrane reorganization by target cells, relatively few resistant strains develop [3, 7].

This work aims to re-annotate the metabolite spectrum, of the genus *Bacillus* strains isolated from the Black Sea sediments. The metabolome obtained by author in 2018 [11] contains compounds that were not identified or had questionable identification due to the limitations of the database, and therefore it is of interest to reanalyze them, given the additions to the database in last years. Therefore, it was decided to re-annotate the metabolomes to supplement the knowledge about the biosynthetic potential of Black Sea bacteria from the genus *Bacillus*.

Materials and methods

Previously studied strains of *Bacillus velezensis* ONU 553, *Bacillus pumilus* ONU 554 and *Bacillus subtilis* ONU 559 were used, which were at one time selected for their antagonistic activity from more than 240 strains of endospore-forming facultative anaerobic bacteria isolated from deep-sea sediments of the Black Sea [11].

Exometabolite spectra of the strains obtained in the course of work [11] were used as the main material for this study.

Reannotation was performed using the Dictionary of Natural Products database. The 2019 version of the program was used for identification of exometabolome components in work [11]. Currently, all unidentified or questionably identified compounds have been re-identified using the current online version.

The identification and analysis of biosynthetic clusters were performed using the antiSMASH 7.0 server. Comparison of the predicted product for biosynthetic clusters of NRPS was performed using the NORINE database [5].

Results and discussion

In the exometabolomic data of *Bacillus velezensis* ONU 553, 7 metabolites were additionally identified, *Bacillus pumilus* ONU 554 – 1, and *Bacillus subtilis* ONU 559 – 0, respectively. The integrated data obtained during the analysis of the samples are presented in Table 1.

Previously, variants of the most studied class of non-ribosomal peptides, surfactins, were identified among the secondary metabolites of the studied strains [3, 11]. The newly found metabolites are presented in Table 1. From this result, we were interested in iturin-like peptides: Bacillopeptin and Bacillomycin.

Bacillomycin Lc, a new antifungal antibiotic of the iturin class, was isolated from the *Bacillus subtilis* strain in a set of two structural analogs - Bacillomycin L_{c0} and Bacillomycin L_{c4}. The biosynthetic cluster of bacillomycin contains a set of A-domains with the following specificities: Asn, D-Tyr, D-Asn, Ser, Glu, D-Ser, Thr. It is known from a previous study [13] that a number of clusters were found in the genome of *Bacillus velezensis* ONU 553, but no such cluster was found to correspond to Bacillomycin at that time. Using antiSMASH, genome of ONU 553 was checked out and we found that the bacillomycin cluster was not separately represented in its output, and it was a cluster in the same region as fungycin (Table 2). The region of fungycin is 1.884.833 – 1,.940.000, and the bacillomycin cluster with the region 1.965.000 – 2.021.950.

In the case of bacillomycin L_c, the carboxylic acid is at position 1 (L-aspartic acid) and the amide is at position 5 (L-glutamine). Alternatively, the structure can be interpreted as a substitution of L-serine for L-proline in bacillomycin D [4].

Some *Bacillus* species can produce cyclic lipopeptides, and these lipopeptides are promising agents that contribute to the biocontrol of plant diseases. The cyclic lipopeptide plipastatin variant Bacillopeptin was isolated from the fermentation broth of *Bacillus amyloliquefaciens*, which originates from marine sediments.

In 1981, the water-soluble peptide Spergualin was isolated from the culture filtrate of *Brevibacillus latersporus* and studied as a new anticancer or antibiotic substance [1].

Spergualin (1-amino-19-guanitido-11,15-dihydroxy-4,9,12-triazathioprinon-adecane-10,13-dione) has a synthetic derivative 15-deoxyspergualine. It's analog, 15-deoxyspergualine, later became widely known as a promising new immunosuppressant.

The exact mechanism of action of 15-deoxyspergualine is precisely unknown. It specifically binds to the heat shock protein Hsp 70 [1] and is believed to

Table 1

Compounds identified in *Bacillus velezensis* ONU 553 and *Bacillus pumilus* ONU 554 extracts

Compounds identified in *Bacillus velezensis* ONU 553 extracts					
RT[1]	**M[2], Da**	**Name[3]**	**Accurate Mass[4], Da**	**Biological Source[5]**	**Use / Importance[5]**
5.08	1020.5174	Bacillomycin L_{C0}	1020.516802	*Bacillus subtilis* FR-2	Displaysgoo dantifungal activityandcy totoxicity
5.39	1034.5333	Bacillopeptin B	1034.528426	*Bacillus amyloliquefaciens* SH-B74	Inhibitsevera lfungalpatho gensinvitro
5.52	403.2969	Spergualin	403.2907027	*Bacillus latersporus*	Unknown
5.71	1048.5489	Bacillomycin L_{C4}	1048.54543	*Bacillus subtilis*	Displaysgoo dantifungal activityandcy totoxicity
6.33	1490.83	Agrastatin B	1490.831944	*Bacillus subtilis*	Inhibitsevera lfungalpatho gensinvitro
Compounds identified in *Bacillus pumilus* ONU 554 extracts					
7.71	241.2408	13-Methyltetradecanamide. Bacillamidin F	241.240564	*Bacillus pumilus*	Displaysgoo dantifungal activityandcy totoxicity

Note: 1 – release time of the compound in min; 2 – Molecular mass of compound, identified in this study; 3 – Name of identified compound; 4 – Molecular mass of identified compound according to the database (Dictionary of Natural Products, CRC Press); 5 – source and characteristics of identified compound according to the database.

Table 2

Characteristics and localization of clusters, found in the genome of *B. velezensis* ONU 553

No	Cluster	Localisation, bp		The most similar known cluster	Similarity, %
		From	To		
1	Nonribosomalpept idesynthetase	311.938	376.796	Surfactin	**91**
2	Polyketidesynthase	956.092	997.336	Butyrosin	**7**
3	Terpenoidsynthesi senzymes	1.084.807	1.101.975	–	–
4	Trans AT-polyketidesynthase	1.406.685	1.494.504	Macrolactine	**100**
5	Combined nonribosomal peptide synthetase /Trans AT-polyketidesynthase	1.717.970	1.827.173	Bacillaene	**100**
6	**NRPS/Trans AT-polyketidesynthase**	**1.884.833**	**1.940.000**	**Bacillomycin**	**100**
7	NRPS	1.965.000	2.021.950	Fengycin	**100**
8	Terpenoidsynthesi senzymes	2.044.982	2.066.865	–	–
9	Polyketidesynthase III type	2.117.302	2.158.402	–	–
10	Trans AT-tranferase	2.274.263	2.380.436	Difficidin	**100**
11	Bacteriocin	299924	300217	LCI	–
12	Bacteriocin	3052374	3049942	Amylocyclicin	–
13	Nonribosomalpept idesynthetase	3.003.787	3.054.939	Bacillibactin	**100**
14	Nonribosomalpept idesynthetase	3.337.057	3.397.053	–	–
15	Other	3.599.120	3.640.538	Bacilysin	**100**

have its main effect by inhibiting the activation of the transcription factor NFκB in antigen-presenting cells and monocytes [6].

The antibiotic Agrastatin was found in *B. subtilis* AQ713 and has a broad fungicidal spectrum *in vitro*. Agrastatin is a powerful tool for controlling the gray leaf rot of beans and geraniums, as well as the early blight of tomato seedlings caused by *Alternaria solani*. Downy mildew of grapes caused by *Plasmopara viticola* is also effectively controlled by Agrastatin, and its effectiveness in this regard is comparable to that of the synthetic fungicide metalaxyl [6].

Using the antiSMASH 7.0 program, an unidentified NRPS cluster was detected in the genome (Fig. 1). When analyzing this cluster using NORINE nonribosomal peptides database, the sequence of the A-domains was found to be similar to a metabolite from marine sponges, namely the antimicrobial peptide helipeptine.

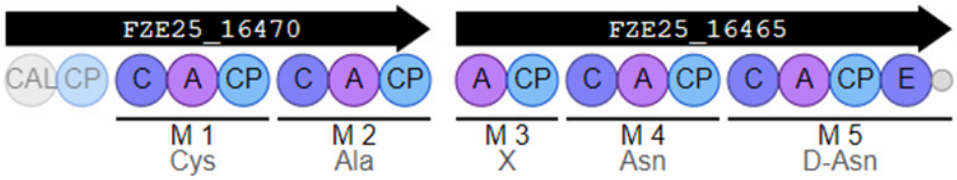

Fig.1. The domain structure of the products of the cluster NRPS from the genome of *Bacillus velezensis* ONU 553 identified by antiSMASH

Bacillamidin F found in *Bacillus pumilus* ONU 554 is a hydrolysis product of an undetected amicoumacin. In a previous study [11], a cluster that may be responsible for the synthesis of amicoumacin AI-77A, as well as this amicoumacin itself, was already found in the strain under investigation.

As a result of the repeated metabolomic analysis, it was found that all studied strains of Black Sea bacteria of the genus *Bacillus* synthesize compounds whose structure fits into a small number of known structural categories. It should be noted that even though 4 years have passed since the last identification, there are still a number of compounds in the metabolomes that cannot be surely identified.

All the identified clusters of additional metabolites of the studied bacillus strains – Agrastatin, Bacillopeptin, Bacillomycin, and Spergualin, isolated from the Black Sea sediments, are interesting from a biological point of view and may be useful for future biotechnological research. A new biosynthetic cluster was found in the *Bacillus velezensis* ONU 553 strain, which was not known in this strain before – Bacillomycin. In the genome of *Bacillus velezensis* ONU 553 was detected a biosynthetic cluster of NRPS, whose product is similar to the metabolite of marine sponges, the antimicrobial peptide helipeptine. That together with the detection of unidentified biosynthetic clusters in the genome of ONU 553 indicates the prospects for the detection of previously unknown compounds.

В.В. Субота, А.М. Остапчук, М.Д. Штеніков
Одеський національний університет імені І. І. Мечникова,
65058 вул. Дворянська, 2, Одеса, Україна,
e-mail: viktoriia27subota98@gmail.com

ПОГЛИБЛЕНА ГЕНОМНО-МЕТАБОЛОМНА ХАРАКТЕРИСТИКА БАКТЕРІЙ РОДУ BACILLUS, ВИДІЛЕНИХ З ГЛИБОКОВОДНИХ ВІДКЛАДЕНЬ ЧОРНОГО МОРЯ

Реферат

Морські бактерії приваблюють все більше уваги як джерело великої кількісті біоактивних метаболітів. Біосинтетичний потенціал морських бацил не є достатньо вивченим. Попередне дослідження авторів не могло дати повного результату, тому було прийнято рішення провести переаналіз не ідентифікованих тоді метаболітів, з урахуванням доповнення існуючих баз даних. ***Метою*** *роботи було провести переаналіз уже наявних метаболомних даних для штамів Bacillus velezensis ONU 553, Bacillus pumilus ONU 554, та Bacillus subtilis ONU 559, виділених із донних відкладень Чорного моря.* ***Методи****. Ідентифікацію сполук проводили за допомогою використання онлайн версії програми Dictionary of Natural Products, а пошук біосинтетичних кластерів у геномі Bacillus velezensis ONU 553 – з використанням antiSMASH 7.0.* ***Результати та висновки.*** *Геномно-метаболомне профілювання штамів призвело до ідентифікації декількох раніше не виявлених сполук – 7 та 1 метаболітів у штамів Bacillus velezensis ONU 553 та Bacillus pumilus ONU 554, відповідно. Виявлено раніше не виявлені біосинтетичні кластери – ітурин-подібних пептидів та геліпептид-подібних пептидів у Bacillus velezensis ONU 553.*

Ключові слова: Bacillus, спергуалін, бациломіцин, геліпептин, глибоководні донні відкладення.

СПИСОК ВИКОРИСТАНОЇ ЛІТЕРАТУРИ

1. *Banerjee T., Singh R.R., Gupta S., Surolia A., Surolia N.* 15-deoxyspergualin hinders physical interaction between basic residues of transit peptide in PfENR and Hsp70-1 // IUBMB. life. – 2012. – V. 64 (1). – P. 99–107.
2. *Childress J.J., Fisher C.R.,* The biology of hydrothermal vent animals: Physiology, biochemistry, and autotrophic symbioses // Oceanograph. Mar. Biol. – 1992. – V. 30. – P. 337–441
3. *Cochrane S.A., Vederas J.C.* Lipopeptides from *Bacillus* and *Paenibacillus* spp.: a gold mine of antibiotic candidates. Med Res Rev. 2016; 36 (1); 4–31.
4. *Eshita S.M, Roberto N.H., Beale J.M., Mamiya B.M., Workman R.F.* Bacillomycin Lc, a new antibiotic of the iturin group: isolations, structures, and antifungal activities of the congeners // J Antibiot. – 1995. – V. 48 (11) . – P. 1240–1247.
5. *Flissi A., Ricart E., Campart C., Chevalier M., Dufresne Y., Michalik J., Jacques P., Flahaut C., Lisacek F., Leclère V., Pupin M.* Norine: Update of the nonribosomal peptide resource // Nucleic acids research. – 2020. – V. 48 (1). – P. 465–469.

6. *Halloran P.F.* Molecular mechanisms of new immunosuppressants // Clin Transplant. – 1996. – V. 10 (1 Pt 2) . – P. 118–123.
7. *Heisey R.M, Huang J., Mishra S.K., Keller J.E., Miller J.R., Putnam A.R., D'Silva T.D.* Production of valinomycin, an insecticidal antibiotic, by *Streptomyces griseus* var. flexipertum var. Nov // J Agric Food Chem. – 1988. – V. 36. – P. 1283–1286.
8. *Hughes C.C., Fenical W.* Antibacterials from the sea // Chemistry–A European Journal. – 2010. – V. 16 (42) . – P. 12512–12525.
9. *Morioka M., Kato K., Umezawa K.* Design and synthesis of 15-deoxyspergualin–biotin conjugates as novel binding probes for target protein screening // The Journal of Antibiotics. – 2016. – V. 69 (7). – P. 574–578.
10. *Murai A., Amino Y., Ando T.* Absolute configuration of the beta-hydroxyl fatty acid constituent of permetin A // J Antibiot. – 1985. – V. 38 (11) . – P. 1610–1613.
11. *Ostapchuk A.M., Shtenikov M.D., Ivanytsia V. O.* Exometabolites of endospore-forming bacteria of *Bacillus* genus identified by genomic-metabolomic profiling // Ukrainian Biochemical Journal. – 2020. – V. 92 (6) . – P. 154–164.
12. *Pirri G., Giuliani A., Nicoletto S.F., Pizzuto L., Rinaldi A.C.* Lipopeptides as anti-infectives: A practical perspective // Cent. Eur. J. Biol. – 2009. – V. 4 (3). – P. 258–273.
13. *Shtenikov M.D., Ostapchuk A.M., Vasylieva N.Y., Luzhetskyy A.M., Rückert C., Kalinowski J., Ivanytsia, V.O.* Characteristics of genome of *Bacillus velezensis* ONU 553 strain isolated from the bottom sediments of the Black Sea // MicrobiolBiotechnol. – 2020. – V. 82 (3) . – P. 14–21.
14. *Schorlemmer H.U., Dickneite G., Seiler F.R.* Treatment of acute rejection episodes and induction of tolerance in rat skin allotransplantation by 15-deoxyspergualin // Transplant Proc. – 1990. – V. 22. – P. 1626–1630.
15. *Skropeta D.* Deep-sea natural products // Natural Product Reports. – 2008. – V. 25 (6). – P. 1131–1166.
16. *Tyurin A.P., Efimenko T.A., Prokhorenko I.A., Rogozhin E.A., Malanicheva I.A., Zenkova V.A., Efremenkova O.V., Korshun V.A.* Chapter 12 - Amicoumacins and related compounds: chemistry and biology // Stud Nat Prod Chem. – 2018. – V. 55. – P. 385–441.
17. *Valentin B.B., Vinod V., Beulah M.C.* Biopotential of secondary metabolites isolated from marine sponge *Dendrilla nigra* // Asian Pac. J. Trop. Dis. – 2011. – V. 1. – P. 299–303.

REFERENCES

1. Banerjee T, Singh RR, Gupta S, Surolia A, Surolia N. 15-deoxyspergualin hinders physical interaction between basic residues of transit peptide in PfENR and Hsp70-1. *IUBMB life.* 2012; 64(1): 99–107.
2. Childress JJ, Fisher CR. The biology of hydrothermal vent animals: Physiology, biochemistry, and autotrophic symbioses. *Oceanograph. Mar. Biol.* 1992; 30: 337–441
3. Cochrane SA, Vederas JC. Lipopeptides from *Bacillus* and *Paenibacillus* spp.: a gold mine of antibiotic candidates. *Med Res Rev.* 2016; 36(1): 4–31.

4. Eshita SM, Roberto NH, Beale JM, Mamiya BM, Workman RF. Bacillomycin Lc, a new antibiotic of the iturin group: isolations, structures, and antifungal activities of the congeners. *J Antibiot.* 1995; 48(11): 1240–1247.
5. Flissi A, Ricart E, Campart C, Chevalier M, Dufresne Y, Michalik J, Jacques P, Flahaut C, Lisacek F, Leclère V, Pupin M. Norine: Update of the nonribosomal peptide resource. *Nucleic acids research*. 2020; 48(1): 465–469.
6. Halloran PF. Molecular mechanisms of new immunosuppressants. *Clin Transplant.* 1996; 10(1 Pt 2): 118–123.
7. Heisey RM, Huang J, Mishra SK, Keller JE, Miller JR, Putnam AR, D'Silva TD. Production of valinomycin, an insecticidal antibiotic, by *Streptomyces griseus* var. flexipertum var. nov. *J Agric Food Chem.* 1988; 36: 1283–1286.
8. Hughes CC, Fenical W. Antibacterials from the sea. *Chemistry–A European Journal.* 2010; 16(42): 12512–12525.
9. Morioka M, Kato K, Umezawa K. Design and synthesis of 15-deoxyspergualin–biotin conjugates as novel binding probes for target protein screening. *The Journal of Antibiotics.* 2016; 69(7): 574–578.
10. Murai A, Amino Y, Ando T. Absolute configuration of the beta-hydroxyl fatty acid constituent of permetin A. *J Antibiot.* 1985; 38(11): 1610–1613.
11. Ostapchuk, AM, Shtenikov MD, Ivanytsia VO. Exometabolites of endospore-forming bacteria of *Bacillus* genus identified by genomic-metabolomic profiling. *Ukrainian Biochemical Journal.* 2020; 92(6): 154–164.
12. Pirri G, Giuliani A, Nicoletto SF, Pizzuto L, Rinaldi AC. Lipopeptides as anti-infectives: A practical perspective. *Cent Eur J Biol.* 2009; 4(3): 258–273.
13. Shtenikov MD, Ostapchuk AM, Vasylieva NY, Luzhetskyy AM, Rückert C, Kalinowski J, Ivanytsia, VO. Characteristics of genome of *Bacillus velezensis* ONU 553 strain isolated from the bottom sediments of the Black Sea. *MicrobiolBiotechnol.* 2020; 82(3): 14–21.
14. Schorlemmer HU, Dickneite G, Seiler FR. Treatment of acute rejection episodes and induction of tolerance in rat skin allotransplantation by 15-deoxyspergualin. Transplant Proc. 1990; 22: 1626–1630.
15. Skropeta D. Deep-sea natural products. Natural Product Reports. 2008; 25(6): 1131–1166.
16. Tyurin AP, Efimenko TA, Prokhorenko IA, Rogozhin EA, Malanicheva IA, Zenkova VA, Efremenkova OV, Korshun VA. Chapter 12 - Amicoumacins and related compounds: chemistry and biology. *Stud Nat Prod Chem.* 2018; 55: 385–441.
17. Valentin BB, Vinod V, Beulah MC. Biopotential of secondary metabolites isolated from marine sponge *Dendrilla nigra*. *Asian Pac. J. Trop. Dis*. 2011; 1: 299–303.

Стаття надійшла до редакції 07.09.2023 р.