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THERMOPHILIC SULFUR-REDUCING BACTERIA *MOORELLA THERMOACETICA* NADIA-3, ISOLATED FROM "NADIIA" PIT SPOIL HEAP OF CHERVONOHRAD MINING REGION

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Introduction. Thermophilic sulfate-reducing bacteria attract attention of scientists as the potential agents of purification of wastewater polluted by sulfur and its compounds, heavy metal ions and organic compounds. These bacteria oxidize different organic substrates using metals with variable valency as electron acceptors and transform them into non-toxic or less toxic forms for living organisms. However, wastewater contains high concentrations of different toxic xenobiotics, particularly, metal ions that have negative influence on living organisms. For this reason, it is important to use resistant strains of microorganisms for the purification of wastewater.

The aim of this work was to identify the thermophilic sulfur-reducing bacteria, isolated from “Nadiia” pit spoil heap of Chervonohrad mining region, and to study their properties.

Materials and Methods. Thermophilic sulfur-reducing bacteria were isolated from the samples of rock of “Nadiia” pit heap at 50 cm depth. Bacteria were cultivated in TF medium under the anaerobic conditions in anaerostates. Cell biomass was measured turbidimetrically using the photoelectric colorimeter KFK-3 (λ = 340 nm, 3 mm cuvette). Hydrogen sulfide content was measured photoelectrocolorymetrically by the production of methylene blue. Organic acids content was measured by high performance liquid chromatography. Cr(VI), Fe(III), Mn(IV) and NO_3^- content was measured turbidimetrically.

Results. Thermophilic sulfur-reducing bacteria were isolated from the rock of “Nadiia” pit heap of Chervonohrad mining region. They were identified as *Moorela thermoacetica* based on the morpho-physiological and biochemical properties and on the results of phylogenetic analysis. *M. thermoacetica* Nadia-3 grow in the synthetic TF medium, have the shape of elongated rods, are gram-positive, endospore-forming.

They form light brown colonies. Optimal growth was observed at 50–55 °C, pH 6.5–7. The bacteria utilize glucose, starch, fructose, maltose, lactose, sodium lactate, arabinose, cellulose, maltose, glycerol, fumarate, and ethanol as carbon sources. The highest sulfidogenic activity of *M. thermoacetica* Nadia-3 was found in media with glycerol, lactose, and glucose. *M. thermoacetica* Nadia-3 reduce SO_4^{2-}, $S_2O_3^{2-}$, Fe(III), NO_3^-, Cr(VI) compounds besides elemental sulfur. They accumulate biomass at $K_2Cr_2O_7$ concentrations of 0.1–1 mM. Sulfur reduction is not the main way of energy accumulation.

Conclusions. Thermophilic chromium-resistant sulfur-reducing bacteria *M. thermoacetica* Nadia-3, that produce hydrogen sulfide during the oxidation of different organic compounds, were isolated from the rock of "Nadiia" pit heap. They reduce Fe(III), Cr(VI), NO_3^-, SO_4^{2-}, $S_2O_3^{2-}$, besides elemental sulfur.

Keywords: thermophilic sulfur-reducing bacteria, elemental sulfur, glucose, starch

INTRODUCTION

Moorella thermoacetica bacteria (formerly known as *Clostridium thermoaceticum*) are thermophilic bacteria, obligate anaerobes that belong to phylum *Firmicutes*, class *Clostridia*, order *Thermoanaerobacterales*, family *Thermoanaerobacteriaceae*, genus *Moorella* [3, 9, 12]. They are capable of obtaining energy by autotrophic (acetogenesis) and heterotrophic (homoacetogenesis) ways of metabolism [8]. Acetogens is a joint group of obligate anaerobic bacteria that fix CO_2 through the metabolic pathway known as the reductive acetyl-CoA pathway or Wood–Ljungdahl pathway [4, 8]. Bacteria use the Wood–Ljungdahl pathway for the simultaneous assimilation of CO_2 and ATP generation to conserve energy by converting acetyl-CoA to acetate. The ability of *M. thermoacetica* to utilize a wide spectrum of organic and inorganic compounds and universalism of energy accumulation enables them to play a significant role in many different ecosystems such as soil, marshes and gastrointestinal tract of animals [3, 8].

Interest to acetogenic bacteria has increased within recent years due to their potential ability to produce acetate from syngas. Thermophilic acetogens are of significance, since their use would reduce gas cooling requirements, allow for cost-efficient recovery of products with a relatively low boiling point, and decrease the risk of contamination [13].

The aim of this work was to identify the thermophilic sulfur-reducing bacteria isolated from "Nadiia" pit spoil heap of Chervonohrad mining region and to study their properties.

MATERIALS AND METHODS

Thermophilic sulfur-reducing bacteria were isolated from the rock samples collected at the depth 30–50 cm from "Nadiia" pit (peak and base) and Central Enrichment Factory (CEF) (central terrace) heaps. Multiple re-inoculation of separate colonies was performed to isolate pure cultures. Bacteria were cultivated for 10–14 days at 30 °C in the anaerostates on the agarized TF medium [10]. Generators GENboxanaer (France) were used to absorb oxygen. The conditions of anaerobiosis were confirmed with the use of anaerobic conditions' indicator AnaerIndicator (bioMeriux, France). Purity of isolated cultures was controlled microscopically.

Bacteria were grown in liquid TF medium to study their ability to utilize different carbon sources and electron donors [10]. The medium was sterilized at 0.75 atm for

30 min, poured into test tubes (25 mL), and closed with sterile rubber stoppers. The medium was inoculated with a suspension of cells at the concentration of 0.2 g/L and cultivated at 30 °C for 5–14 days.

Cell biomass was determined by turbidimetric method on the photoelectric colorimeter KFK-3 (λ = 340 nm, cuvette length – 3 mm). The content of hydrogen sulfide was found by photoelectric colorimetry method on the formation of methylene blue [14].

Organic acids (lactate, acetate) content was measured by high performance liquid chromatography (HPLC). The chromatographic system consisted of two Varian ProStar 210 pumps (Agilent Technologies, Singapore), Polaris 5 C18-A column (Agilent Technologies, Netherlands), 250×4.6 mm in Varian ProStar 500 column module (Agilent Technologies, Australia), Varian ProStar 335 UV-visible photodiode array detector (Agilent Technologies, Australia). 0.2% solution of trifluoroacetic acid in 1st class water (obtained using water purification system Adrona Crystal E Bio with ultrafilter Milipore (Adrona, Latvia)) was used as a mobile phase. Chromatographic separation was performed in 0.2% trifluoroacetic acid for 8 min. Mobile phase flow was 1.5 mL/min, sample volume – 20 μl. Chromatograms were recorded at 210 nm [11]. Column temperature was 35 °C.

Identification of thermophilic sulfur-reducing bacteria was performed on the basis of morpho-physiological properties and the results of nucleotide sequence of 16S rRNA gene analysis. Total DNA was isolated from 1 mL of culture, grown in TF medium at 55 °C for 14 days. Cells were sedimented by centrifugation, isolation of total DNA was performed by soft lysis method [6]. Cells were lysed by NaCl (final concentration 0.5 M) and sodium dodecyl sulfate (final concentration 2%) at 65 °C for 15–20 min. Proteins were sedimented by salting-out with 5 M potassium acetate (final concentration 2 M) with further centrifugation. DNA was sedimented by isopropanol, washed by 70% ethanol and dissolved in the deionized water. The isolated DNA was visualized by electrophoresis and stored at 20 °C. 16S rDNA was amplified from the total DNA of strain using universal primers 27F AGAGTTTGATCCTGGCTCAG and 1492R GGTTACCTTGTTACGACTT [15]. Amplicone of expected size (approx. 1,5 kbps) was purified using “QiaQuick” kit (“Qiagen”, США) and sequenced by Sanger method using the abovementioned primers. Sequences were collected using Geneious application (Biomatters, Ltd, New Zelland) and analysed using BLASTn with 16S rRNA database (Bacteria and Archaea: 16S ribosomal RNA project, NCBI, USA).

The content of Cr(VI), Fe(III), Mn(IV) and NO_3^- in the culture liquid was determined using qualitative measurement [7]. The initial concentration of cells in all experiments was equal to 0.2 g/L, and elemental sulfur – to 1 mM. Incubation lasted for 14 days.

Statistical processing of the results was performed by a computer program Microsoft Excel XP using Student’s *t*-test. The obtained results were presented as mean ± standard deviation (x ± SD). To assess the significance of the difference between the statistical characteristics of two alternative sets of data, we conducted one-way analysis of variance. The differences between samples were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Rock samples were collected from the depth of 30–50 cm of “Nadiia” pit (peak and base) and CEF (central terrace) heaps to isolate thermophilic sulfur-reducing bacteria. “Nadiia” pit rock temperature was 40–55 °C; humidity – 12–16 %, and of CEF rock – 75–80 °C and 9–10 %, respectively. pH of “Nadiia” pit rock was 4.0–5.6, of CEF rock – 3.3 (**Table 1**).

Table 1. **Sample collection sites and their general characteristics**

Таблиця 1. **Місця відбору проб і їхня загальна характеристика**

Sample	Sample collection site	Depth of collection, cm	pH of soil extract	Humidity of soil, %	Temperature of soil, °C
Viseiska 1	base	30	5.18	16.9	19
Viseiska 2	base	50	4.60	12.2	18
CEF 1	peak	30	3.31	9.4	19
CEF 2	peak	50	3.34	11.4	18
Nadiia 1	peak	50	5.60	16	55
Nadiia 2	slope	50	4.0	12	40

Thermophilic sulfur-reducing bacteria were detected in the rock, by inoculating collected samples in 4 different media with elemental sulfur: 480G [10], TYE [2], TF [5], MSH [10] at 55 and 65 °C. Growth of the bacteria in TF i MSH media was found. Growth was more intensive in TF medium, than in MSH.

Rock samples were dilluted for 100–200 times and inoculated into Petri dishes to isolate thermophilic sulfur-reducing bacteria. The dilution and volume were adjusted to obtain 100–150 colonies per plate. Cultures were grown for 14–16 days at 55 °C in anaerostates on the agarized TF medium. Glucose was an electron donor in it and elemental sulfur – electron acceptor. Multiple re-inoculation of separate colonies was performed to isolate pure cultures. Isolation was followed by microscopic control.

From 60 isolated strains one (Nadia-3), which had good growth in selective medium and produced the highest amount of hydrogen sulfide, was used for further research.

Colonies of sulfur-reducing bacteria Nadia-3 have light-brown color. Optimal growth temperature – 50–55 °C, pH – 6.5–7.0 (**Table 2**). It was found as a result of electron microscopic studies that Nadia-3 bacteria have the shape of elongated rods, cell size – 4–10 × 1–2 μm (**Fig. 1**). Bacteria are gram-positive, they form endospores.

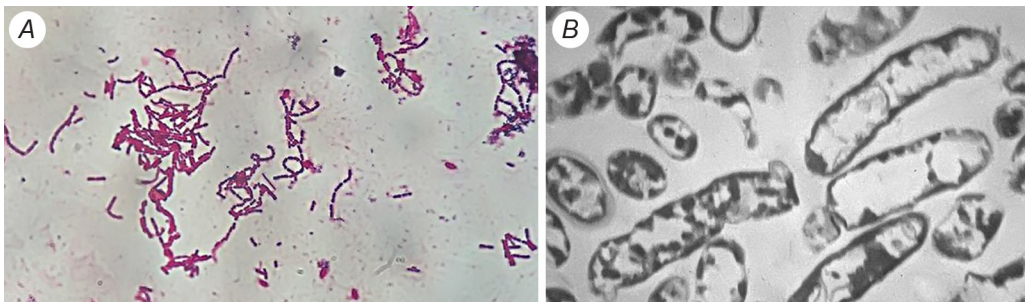

Fig. 1. Cells of thermophilic sulfur-reducing bacteria Nadia-3: ***A*** – light microscpy (×1600); ***B*** – electron microscopy (x15 000)

Рис. 1. Клітини термофільних сірковідновлювальних бактерій штаму Nadia-3: ***A*** – світлова мікроскопія (×1600), ***B*** – електронна мікроскопія (x 15 000)

Nadia-3 bacteria grow in media with sodium lactate, sodium acetate, ethanol, glycerol, fumarate, fructose, arabinose, maltose, lactose, glucose, and starch as carbon sources. They do not grow in media with butanol, raffinose, ramnose, succinate, or malate (**Table 2**).

Nadia-3 bacteria are capable of utilizing Fe(III), Cr(VI), NO_3^-, SO_4^{2-} and $S_2O_3^{2-}$ compounds besides elemental sulfur as electron acceptors (**Table 2**).

Table 2. **Characteristics of Nadia-3 strain and related starins**

Таблиця 2. **Властивості штаму Nadia-3 і споріднених штамів**

Property	*M. thermoacetica* Nadia-3	*M. perchloratireducens* An10 [1, 9]	*M. thermoacetica* JCA-5801 [1, 3, 9]
Gram stain	gram-positive	gram-positive	gram-positive
Cell shape	rod-shaped	rod-shaped	rod-shaped
Cell size, μm	1–2×4–10	0.5×2–9	0.4–1.2×5–14
Optimal			
Temperature, °C	50–55	55–60	55–60
pH	6.5–7	6.5–7.0	6.9
Electron donor and carbon source			
Sodium acetate	+	–	NS
Sodium lactate	+	–	±
Ethanol	+	–	+
Buthanol	–	–	+
Glycerol	+	–	–
Raffinose	–	–	NS
Rhamnose	–	–	–
Succinate	–	–	NS
Malate	–	NS	NS
Fumarate	+	–	NS
Fructose	+	+	+
Arabinose	+	NS	NS
Maltose	+	NS	NS
Lactose	+	NS	NS
Glucose	+	+	+
Starch	+	NS	NS
Electron acceptor			
S^0	+	NS	NS
SO_4^{2-}	+	+	+
$S_2O_3^{2-}$	+	+	NS
Fe(III) compounds	+	NS	NS
NO_3^-	+	+	+
Cr(VI)	+	NS	NS
Perchlorate	NS	+	–

Comments: "–" – growth absent; "+" – growth present; "NS" – not studied

Примітки: "–" росту немає; "+" – наявний ріст; "NS" – не досліджено

The ability of Nadia-3 strain to hydrolyse different organic compounds was studied using RapID^{TM}ONE system. Nadia-3 can hydrolyse alyphatic thiol, fatty acid ester, glucose, sorbitol, adothynol. They do not hydrolyse urea, arginine, ornitine, lysine, glucuronides, galactosides, glucosides, xyllosides, glucoseaminides, mallonate, proline, γ-glutamyl, pyrrolidine, They do not produce indole (**Table 3**).

Table 3. **Physiological and biochemical characteristics of thermophilic sulfur-reducing bacteria Nadia-3, determined using RapID ONE test**

Таблиця 3. **Фізіолого-біохімічні властивості термофільних сірковідновлювальних бактерій Nadia-3, визначені з використанням RapID ONE тесту**

N	Test code	Substarte	Reaction
1	URE	Urea	–
2	ADH	Arginine	–
3	ODC	Ornitine	–
4	LDC	Lysine	–
5	TET	Alyphatic thiol	+
6	LIP	Fatty acid ester	+
7	KSF	Glucose	+
8	SBL	Sorbitol	+
9	GUR	*p*-Nitrophenol-β,D-glucuronide	–
10	ONPG	*p*-Nitrophenol-β,D-galactoside	–
11	βGLU	*p*-Nitrophenol-β,D-glucoside	–
12	βXYL	*p*-Nitrophenol-β,D-xylloside	–
13	NAG	*p*-Nitrophenol-*p*-acetyl-β,D-glucosaminide	–
14	MAL	Mallonate	–
15	PRO	Proline-β-naphtylamide	–
16	GGT	γ-Glutamyl-β-naphtylamine	–
17	PYR	Pyrrolydine-β-naphtylamine	–
18	ADON	Adothynol	+
18	IND	Triptophane	–

Comments: "+" – positive reaction; "–" – negative reaction

Примітки: "+" позитивна реакція; "–" – негативна реакція

Sequencing of 16S rRNA gene fragment was performed to identify Nadia-3 bacteria. Search of homologous nucleotide sequences to 16S rRNA gene of Nadia-3 strain (GenBan MW940839) among the known strains of SILVA 138.1 2020.08. database was done using BLASTN application. This allowed finding the most closely related species to this strain – *Moorella thermoacetica,* whose 16S rRNA gene exhibited 98 % of identity to our sample.

In order to perform the phylogenetic analysis, a phylogenetic tree was built using the algorithm of Neighbor Joining at phylogeny.fr (PhyML v3.0) server. 11 sequences of the representative strains were selected among the sequences with the highest identity

values to build the phylogenetic tree. The studied sequence was marked as *M. thermoacetica* Nadia-3. *M. thermoacetica* Nadia-3 16S rRNA gene sequence shows a high level of identity to the sequences of 16S rRNA genes of *Moorella* sp. (99.5 %), *M. thermoacetica* JCA-5801 (99.2 %), *M. glycerini* JW (99 %), *M. mulderi* DSM (91.25 %) and *M. perchloratireducens* An10 (99.5 %) strains (**Fig. 2**).

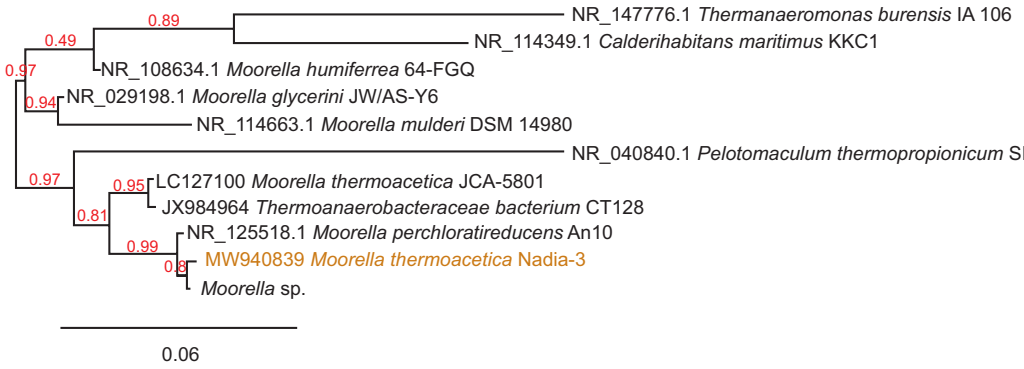

Fig. 2. Unrooted phylogenetic tree (NJ algorithm) of 16S rRNA genes from *Moorella thermoacetica* Nadia-3 and its closest counterparts found via BLASTN search. Bootstrap values are shown on the nodes

Рис. 2. Філогенетичне дерево (алгоритм NJ) генів 16S рРНК *Moorella thermoacetica* Nadia-3 та їх найближчих родичів, отримане через пошуки BLASTN. Значення Bootstrap показано у вузлах

Main differences between *M. thermoacetica* Nadia-3 strain and the most related strains of *M. perchloratireducens* species – *M. perchloratireducens* An10 and *M. thermoacetica* JCA-5801 are presented in **Table 2**. In contrast to *M. thermoacetica* Nadia-3, *M. perchloratireducens* An10 and *M. thermoacetica* JCA-5801 have higher optimal growth temperature – 55–60 °C, do not utilize sodium acetate, ethanol, glycerol and fumarate as carbon sources. *M. perchloratireducens* An10 and *M. thermoacetica* JCA-5801 do not reduce elemental sulfur, Fe(III) or Cr(VI) compounds.

The studied physiological and biochemical properties of *M. thermoacetica* Nadia-3 strain (see **Table 2**) and the results of phylogenetic analysis (see **Fig. 2**) indicate that they belong to *M. thermoacetica* – thermophilic acetogenic bacteria, strict anaerobes, which belong to *Moorella* genus, *Thermoanaerobacteriaceae* family, *Thermoanaerobacterales* order, *Clostridia* class, *Firmicutes* phylum [9, 11].

The study of the effects of different organic and inorganic compounds on the growth and biomass accumulation by thermophilic bacteria *M. thermoacetica* Nadia-3 showed that bacteria accumulated the highest biomass (1.5–3.5 g/L) in media with glucose, starch, mannose, lactose, sodium lactate at the presence of elemental sulfur. Lower growth was found in media with arabinose, cellulose, maltose, fructose, glycerol, fumarate, ethanol. Bacteria did not accumulate biomass in media with raffinose, rhamnose, malate, succinate, buthanol (**Table 4**).

The highest sulfidogenic activity was found in the media with lactose, glucose and starch. The highest hydrogen sulfide accumulation (approx. 1 mM) was found in the medium with glucose.

We studied the peculiarities of *M. thermoacetica* Nadia-3 growth in media with different organic compounds in the absence of elemental sulfur. Bacteria grew utlizing

glucose, starch, fructose, maltose, mannose, lactose, sodium lactate, arabinose, cellulose, glycerol, fumarate and ethanol as carbon sources (**Table 4**). Hence, sulfur reduction is not the main way of energy conservation by the studied bacteria.

Table 4. **Accumulation of biomass and hydrogen sulfide by *M. thermoacetica* Nadia-3 bacteria in media with different organic compounds**

Таблиця 4. **Нагромадження біомаси і гідроген сульфіду бактеріями *M. thermoacetica* Nadia-3 у середовищах із різними органічними сполуками**

Carbon source	Biomass, g/L (M±m, n = 3)		Hydrogen sulfide, mM (M±m, n = 3)
	at the presence of sulfur	at the absence of sulfur	
Glucose	3.50±0.15	3.40±0.14	0.98±0.05
Starch	3.40±0.12	3.20±0.11	0.80±0.04
Fructose	1.10±0.05	0.90±0.04	–
Maltose	0.55±0.01	0.52±0.01	0.05±0.00
Mannose	3.40±0.13	3.30±0.10	0.04±0.00
Lactose	2.70±0.12	2.50±0.10	0.94±0.04
Sodium lactate	1.50±0.07	1.20±0.05	0.39±0.01
Arabinose	0.64±0.02	0.50±0.02	–
Cellulose	0.45±0.02	0.43±0.02	0.04±0.00
Glycerol	0.43±0.02	0.40±0.02	0.24±0.01
Fumarate	0.55±0.03	0.50±0.02	0.05±0.00
Ethanol	0.40±0.02	0.40±0.01	0.30±0.01
Raffinose	–	–	–
Rhamnose	–	–	–
Malate	–	–	–
Succinate	–	–	–
Buthanol	–	–	–

Comment: "–" – growth absent

Примітка: "–" – немає росту

The ability of *M. thermoacetica* Nadia-3 strain to utilize NO_3^-, Fe(III) and Cr(VI) as electron acceptors was studied. KNO_3 at concentrations of 5, 20 and 30 mM, Fe(III) citrate at concentrations of 1, 5, 10, 20 and 30 mM and potassium dichromate at concentrations of 0.1, 0.5, 1 mM were added to the culture medium instead of elemental sulfur for this purpose. Medium with elemental sulfur was the control. Bacteria accumulated the highest biomass at KNO_3 concentration of 20 mM. Biomass level was 2.7 g/L (**Fig. 3**), which is 1.2 times lower than in the control.

Bacteria grew the best at Fe(III) citrate concentration of 1 mM, accumulating 1.8 g/L of biomass, which is 2 times lower than in the medium with elemental sulfur. Biomass accumulation decreased by 4 times at higher Fe(III) citrate concentrations. *M. Thermoacetica* Nadia-3 accumulated approximately 3.4 g/L of biomass at $K_2Cr_2O_7$ concentration of 0.1–1 mM, which is the same as in the control (**Fig. 3**).

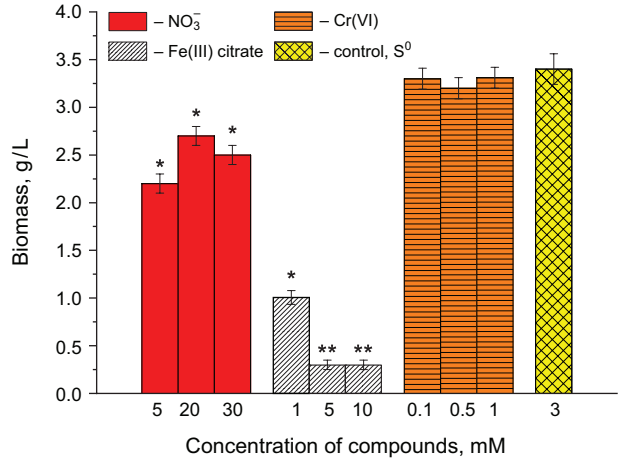

Fig. 3. Accumulation of biomass by *M. thermoacetica* Nadia-3 bacteria under the influence of different electron acceptros (*−P<0.05; **−P<0.01 – reliable changes of biomass, compared to control) (M±m, n = 3)

Рис. 3. Нагромадження біомаси бактеріями *M. thermoacetica* Nadia-3 за впливу різних акцепторів електронів (*−P<0,01; **−P<0,01 – вірогідні зміни біомаси порівняно з контролем) (M±m, n = 3)

Chromatographic analysis of the culture liquid after the cultivation of thermophilic *M. thermoacetica* Nadia-3 bacteria in the medium with glucose showed that lactate is accumulated (1 g/L). the initial glucose concentration was 3 g/L.

The isolated *Desulfuromonas* sp. YSDS-3 and *M. thermoacetica* Nadia-3 strains are different form strains described in literature by biochemical properties and may be of high practical interest, particularly, for the purification of environment from a number of organic compounds as well as elemental sulfur, nitrates, hexavalent chromium, and iron compounds.

CONCLUSIONS

Thermophilic sulfur-reducing bacteria of Nadia-3 strain were isolated from the rock of “Nadiia” pit heap of Chervonohrad mining region. They are identified as *Moorela thermoacetica* on the basis of morphological, physiological and biochemical properties, and phylogenetic analysis. Optimal temperature of *M. thermoacetica* Nadia-3 growth is 50–55 °C, pH – 6.5–7. *M. thermoacetica* Nadia-3 reduce elemental sulfur in media with glucose, starch, fructose, maltose, lactose, sodium lactate, arabinose, maltose, glycerol, ethanol, and fumarate, accumulating up to 1 mM of hydrogen sulfide. The bacteria reduce S^0, Fe(III), Cr(VI), NO_3^-, SO_4^{2-} and $S_2O_3^{2-}$.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Animal Rights: This article does not contain any studies with animal subjects performed by any of the authors.

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ТЕРМОФІЛЬНІ СІРКОВІДНОВЛЮВАЛЬНІ БАКТЕРІЇ *MOORELLA THERMOACETICA* NADIA-3, ВИДІЛЕНІ З ВІДВАЛУ ШАХТИ “НАДІЯ” ЧЕРВОНОГРАДСЬКОГО ГІРНИЧОПРОМИСЛОВОГО РАЙОНУ

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Вступ. Термофільні сірковідновлювальні бактерії привертають увагу дослідників як потенційні агенти очищення стічних вод, забруднених сіркою та сполуками сульфуру, йонами важких металів і органічними сполуками. Ці бактерії окиснюють різні органічні субстрати з використанням металів зі змінною валентністю як акцепторів електронів і перетворюють їх на нетоксичні або менш токсичні для живих організмів форми. Проте стічні води містять високі концентрації різноманітних токсичних ксенобіотиків, зокрема, йонів металів, що згубно впливають на живі організми. Тому важливо для очищення стічних вод використовувати резистентні штами мікроорганізмів.

Мета цього дослідження полягала в ідентифікації термофільних сірковідновлювальних бактерій, виділених з відвалу шахти “Надія” Червоноградського гірничопромислового району, та в дослідженні їхніх властивостей.

Матеріали та методи. Термофільні сірковідновлювальні бактерії виділяли з проб породи відвалу шахти “Надія” на глибині 50 см. Бактерій культивували у середовищі TF за анаеробних умов в анаеростатах. Біомасу клітин визначали турбідиметрично на фотоелектроколориметрі КФК – 3 (λ = 340 нм, довжина кювети 3 мм). Вміст гідроген сульфіду визначали фотоелектроколометрично за утворенням метиленової сині. Концентрацію органічних кислот визначали методом високоефективної рідинної хроматографії. Вміст Cr(VI), Fe(III), Mn(IV) і NO_3^- визначали турбідиметрично.

Результати досліджень. Із породи відвалу шахти “Надія” Червоноградського гірничопромислового району виділено термофільні сірковідновлювальні бактерії, які на основі морфофізіологічних і біохімічних властивостей та за результатами філогенетичного аналізу ідентифіковано як *Moorela thermoacetica.* Встановлено, що бактерії *M. thermoacetica* Nadia-3 ростуть у синтетичному середовищі TF, мають форму витягнутих паличок, грампозитивні, утворюють ендоспори. Колонії світло-коричневого кольору. Оптимальний ріст спостерігають за температури 50–55 °C,

pH 6,5–7. Як джерело карбону використовують глюкозу, крохмаль, фруктозу, мальтозу, лактозу, натрій лактат, арабінозу, целюлозу, мальтозу, гліцерин, фумарову кислоту, етанол. Найвищу сульфідогенну активність бактерій *M. thermoacetica* Nadia-3 виявлено у середовищі з гліцерином, лактозою, глюкозою. Окрім елементної сірки, бактерії *M. thermoacetica* Nadia-3 відновлюють SO_4^{2-}, $S_2O_3^{2-}$, сполуки Fe(III), NO_3^-, Cr(VI). Нагромаджують біомасу за концентації $K_2Cr_2O_7$ 0,1–1 мМ. Сіркоредукція не є основним способом одержання енергії.

Висновки. З породи відвалу шахти "Надія" виділено термофільні хромрезистентні сірковідновлювальні бактерії штаму *M. thermoacetica* Nadia-3, які під час окиснення різних органічних сполук продукують гідроген сульфід. Окрім елементної сірки, відновлюють Fe(III), Cr(VI), NO_3^-, SO_4^{2-}, $S_2O_3^{2-}$.

Ключові слова: термофільні сірковідновлювальні бактерії, елементна сірка, глюкоза, крохмаль

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