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DETECTION OF SULFATE-REDUCING BACTERIA FROM VARIOUS ECOTOPES BY REAL-TIME PCR

D. R. Abdulina[1]
G. O. Iutynska[1]
A. I. Aniskina[2]
M. M. Nikitin[2]

[1] Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine, Kyiv

[2] GenBit LLC, Moscow, Russia

E-mail: adara@ukr.net

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The study of detection effectiveness of sulfate-reducing bacteria (SRB) in the samples from various ecotopes by microbiological (cultural by serial dilutions) and molecular biological (by real-time PCR) methods with designed test-systems lyophilized on the silicon microchip was performed. The developed DSRM and SRB2 test-systems for detection of the functional gene *dsrA* presence, encoding one of the key enzyme of dissimilatory sulphate-reduction pathway — dissimilatory sulfite reductase were used. It was found that the minimal determined SRB titres in water samples were 10^4 cells/ml and in soil samples they were 10^2–10^5 cells/g of absolutely dry soil. In natural and man-caused samples, the amount of SRB detected by the microbiological method was correlated with calculated values determined by the molecular biological method, Pearson's indexes were $r = 0.41$–0.69 ($k = 11$, $P \leq 0.01$–0.05). Thus, real-time PCR assay with designed test systems lyophilized on the silicon microchips is a high-quality and rapid method for the detection of SRB in various natural and man-caused ecotopes.

Key words: *dsrA* gene, test-systems, sulfate-reducing bacteria, biocorrosion, real-time PCR.

Losses from corrosion of the metal underground structures and closed technical systems were estimated approximately 1–6% of the gross domestic product (GDP) of developed countries [1, 2]. However, all existing methods for ensuring the operational reliability of underground structures are based on the principles of corrosion assessment as an exclusively electrochemical process. However, according to expert estimates, 80–90% of corrosion destruction of metal structures in the underground and marine environment is directly or indirectly caused by the activity of sulfate-reducing bacteria (SRB) which are dangerous agents of microbial corrosion of metals (biocorrosion). More than 20% of losses in the oil and gas industry, which cost more than $100 million annually, are the result of biocorrosion [3–5]. Among the main factors of biocorrosion, a special role belongs to corrosive SRBs, capable to sulphidogenesis [6–9].

Presently the soils corrosion aggressiveness factor as a result of activity of corrosive-relevant bacteria is not taken into account both in the design of underground structures and in the development of protective methods against corrosion. Therefore, there is need for identification and continuous monitoring of SRB. The most common method of soil biocorrosion activity monitoring, which is noticed in current regulations, is the quantitative measuring of sulfate-reducing and thionic bacteria [4, 10, 11]. However, the detection of bacteria by the classical microbiological method of cultivation on nutrient media is expensive, time-consuming, and requires a specially equipped microbiological laboratory. The total time of determination of the SRB's availability and quantity takes up to 10–14 days.

New approaches in the detection of SRB are molecular biological methods, which are based on the determination of functional genes encoding key enzymes of dissimilatory sulphate-reduction pathways by real-time PCR. Last decade, a rapid reduction in cost and simplification of these methods has been observed due to the development of large-scale production of microchip systems and reducing the reagents consumption. Nowadays,

microchip systems are widely used in medicine, crop production and animal husbandry to detect the pathogens of bacterial and viral diseases of humans, animals and plants [12, 13]. However, there is no available test-systems for the detection of SRB in various natural and man-made ecotopes using specific primers for the α-subunit of the functional gene encoding the dissimilatory sulfite reductase (*dsrA,* EC 1.8.99.3). The analysis of this gene is performed only as a part of the metagenomic complex product Geochip 5.0 (Glomics) [14].

Previously, we have designed the test systems for the detection of SRB by real-time PCR assay. It was found that 3 of 6 test systems had showed the specificity. The most specific test system was SRB2 designed on the basis of the nucleotide sequence of the α-subunit of *dsrA* gene of the *Desulfovibrio* bacteria and DSRM test-system specific to all the SRB genera [15].

The aim of the study was to determine the effectiveness of the developed molecular biological method for detecting SRB in natural and man-caused samples by real-time PCR in microchip format in comparison with the classical microbiological method.

Materials and Methods

Objects of study. Collection SRB strains *Desulfovibrio desulfuricans* UCM B-11501, *Desulfovibrio vulgaris* UCM B-11502, *Desulfovibrio* sp. UCM B-11503 (Ukrainian Collection of Microorganisms of the Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine) were used. The presence of SRB in soils, biofoulings, sludges, sewage and sea waters samples using microbiological and molecular biological methods was detected.

Sampling for analysis. For microbiological studies, soil samples (10–50 g) were taken with sterile spatula in sterile plastic or paper bags. The samples of waters, sludges, foulings were taken in volume of 50–100 ml in sterile containers. Before the analysis, the samples were stored at +4 °C, no more than 2–3 days. Determination of soil moisture was carried out by drying at 105 °C to a constant mass of absolutely dry soil (ADS).

The cultivation of the collection SRB strains were performed in the liquid Postgate "B" media under microaerophilic conditions, at the temperature of 28 °C, during 7–10 days up to the stationary phase of bacterial growth [16]. The cell titres were determined by the method of serial dilutions and the number of bacterial cells in 1 ml of an aqueous sample (or in 1 gram of absolutely dry soil) using MacCradey's tables were calculated [17].

Preparation of bacterial biomass. To isolate DNA wet SRB biomass (up to 1 g) was put to a 5–10 ml test tubes, 1.0 ml of saturated citric acid solution was added (133 g of citric acid in 100 ml of distilled water), and mixed intensively by vortex until the biomass was completely suspended and metal sulphides were dissolved. Further, the bacterial biomass was kept in a thermostat at 28–30 °C for 1–2 h, centrifuged at 13.000 rpm for 10–15 min (5415R, Eppendorf) and then re-suspended in 0.5 ml of distilled water.

DNA isolation. Isolation of total genomic DNA from the SRB biomass was performed using reagent kits DNA Sorb-B (AmpliSens, Russia) or DNA PROBA-CTAB reagent kit (DNA Technology, Russia) according to the manufacturers' instructions. Total DNA from water samples was isolated using the reagent kit DNA Probe-CTAB (DNA Technology, Russia). Before isolation of total DNA from the soil samples, it was prepared soil suspension as mentioned in the laboratory manual. To isolate total DNA from soil samples two methods were used: 1) commercial reagent kit DNeasy PowerSoil Kit (Qiagen, USA); 2) guanidine and isothiocyanate method followed by DNA purification in agarose gel according to methodical recommendations [18]. DNA concentration was measured using the SmartSpec Plus (Biorad, USA) spectrophotometer.

Test-systems. Test-systems consisting of the primer pairs and fluorescent probes were developed on the basis of available in the GenBank database nucleotide sequences of the α-subunit of the *dsrA* gene of SRB and designed and characterized earlier [15]. For the development of test systems, the Oligo 6.0 Primer Analysis Software program (Molecular Biology Insights, Inc., Colorado, CO, USA) was used. Sequences of primers and fluorescent probes are listed in the Table 1.

Microchips used in the study. In this study two types of silicon (Si) microchips (30 and 48-well) were developed and tested (Fig. 1).

Preparation of microchips for analysis. PCR mixture consisted of hot-start Taq DNA polymerase (5.0 u/μl) ("Sibenzyme", Russia), dNTP mix (0.5 mmol/l each), primers (5.0 mmol/l each), probe (2.5 mmol/l), deionized water, stabilizing solution (containing tregalose (100mM), polyvinyl pyrrolidone (2%), Tween 20 (1%) with deionized water). The mixtures were added

to each microchip wells (including internal, positive and negative controls) and lyophilized with freeze-drying FreeZone6 (Labconco, USA) using developed technology (Genbit LLC, Russia).

DNA samples mixed with a PCR buffer ("Sibenzyme", Russia) at the ratio of 1:8.5 were added to the previously prepared microchip. The microchip was installed into holder cartridge and the whole reaction zone was covered with a sealing layer of mineral oil (620 µl). The test samples were added to wells under the sealing oil layer according to the microchip topology (Fig. 1). As a positive control the total DNA (C+) isolated from the SRB were used. The negative control (C-) was deionized water.

Real-time PCR in microchips. Real-time amplification was performed on two-beam AriaDNA Microchip Amplifier (Lumex-Marketing, Russia). Thermal cycling conditions for amplification was as follows and included initial denaturation (94 °C, 180 s), followed by 45 cycles of denaturation (94 °C, 5 s) and primer annealing (56 °C, 30 s). Signal recording (FAM detection channel) was performed at the end of annealing step. The ROX detection channel was used for IC. Processing of the results of PCR analysis recording and counting of threshold cycles (C_t) was performed automatically using the AriaDNA ver.1.4. software (Lumex-Marketing, Russia).

The detection limit determination for designed test-system was determined in a series of model experiments. To detect the

Table 1. **Specific test-systems used for real-time PCR on silicon microchips**

Specificity to SRB	The name of the test system	Primers and probes	Sequences primers and probes (5/-3/)	Length of PCR product (bp)
Desulfovibrio	SRB2	SRBF2 SRBR2 SRB2Probe	ACC CAC TGG AAG CAC G ACG GTG TGG AAG TGC G FAM-CGGGCTGGTCACAGTAACGG-BHQ1	113
All SRB genera	DSRM	DSR1FM DSR1RM DSR1Probe	AAGGAACCCCGCACCAAC TTATCTCAGGTGTCTCTTGCGG FAM-TCGCGATCCCAGCCACCAGG-BHQ1	102

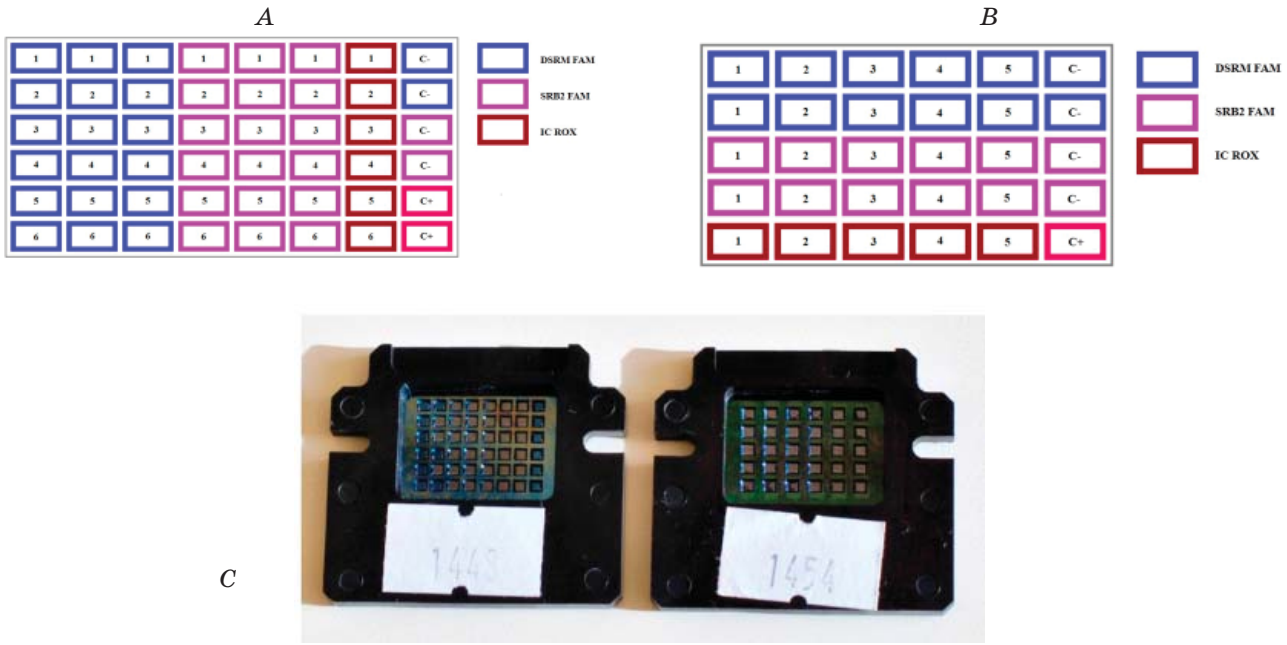

Fig 1. **Topology of PCR silicon-based microchips with lyophilized test-systems for 48-cells (*A*) and 30-cells (*B*), their surface (*C*):**
1–6 — the number of samples loaded into the microchip, in 3 repeats (FAM detection channel), C+ — positive control; C– — negative control; IC — internal control sample (ROX detection channel)

minimum determined number of bacteria in the samples the curves of representation of the dependence between bacterial titre and threshold cycles from real-time PCR were fitted. Data for constructing dependencies were obtained from a series of experiments with known titres of test cultures *D. desulfuricans* UCM B-11501, *D. vulgaris* UCM B-11502, *Desulfovibrio* sp. UCM B-11503. The initial titres of the SRB strains were 10^9 cells/ml (g). A series of dilutions of bacterial cells were prepared in sterile water, as well as in the soil, excavated near the water pipe-line. The soil was not sterilized, but beforehand the amount of SRB and the presence of *dsrA* genes were checked. From water and soil series of bacterial dilutions, total DNA was isolated and real-time PCR was performed on 3-6 microchips repeats. The total number of repeats was $N = 9–12$.

Statistical analysis. Averaging and approximation of the obtained data ($M \pm m$, $P < 0.05$), construction of regression curves of the threshold cycles (C_t) from lg SRB (cells/ml (g)) were performed using the MS Excel 2010 software package. The calculation of paired Pearson's correlation indexes (r) was performed using the *Statistica* program software ver. 10 (StatSoft Inc., USA http://www.statsoft.com/).

Results and Discussion

The molecular biological method for detecting of corrosive-relevant SRB is based on the determination of functional gene encoding key enzymes of the dissimilatory sulphate-reduction pathway, in particular the α-subunit of the dissimilatory sulfite reductase gene (*dsrA*). Early, we had designed the test-systems for the detection of SRB by real-time PCR assay [15], which allow detecting effectively the presence of the *dsrA* gene. However, there are no data about comparative studies of the effectiveness of detection SRB presence in various natural and man-caused samples using newly developed molecular approach and microbiological method.

To estimate detection limit of the developed test-system, serial dilutions of SRB in soil and water samples were performed. This value is one of the quantitative characteristics of test-systems. Dilutes of SRB cells in sterile tap water and in soil, excavated near the water pipeline, were used for model experiments. The initial titres of SRB cultures were next for *Desulfovibrio* sp. UCM B-11503 ($10^8–10^9$ cells/ml), *D. desulfuricans* UCM B-11501 ($10^8–10^9$ cells/ml) and *D. vulgaris* UCM B-11502 (10^8 cells/ml).

Total DNA was isolated from the prepared series of diluted SRB cultures and the presence of the *dsrA* gene was determined by real-time PCR using the reaction threshold cycle (C_t) values. The PCR curves for estimation of the detection limit i.e. the minimum SRB titres are shown in Fig. 2 (the series of limit dilutions of *D. vulgaris* UCM B-11502 in tap water). The Ch1 line on the graph is the threshold line for the first beam of the fluorescent probe (FAM), drawn automatically by the microchip amplifier's program software above the background. The intersection point of the fluorescence curve with the threshold line Ch1 gives the threshold cycle value (C_t). The earlier the reaction curve crosses the Ch1 line, the lower threshold cycle value is and PCR runs more effective [15].

As appeared on the graphs, the real-time PCR curves corresponded to a series of limit dilutions both in terms of fluorescence intensity, which went down with decreasing bacterial titre (from 900–1 000 to 100–200). Threshold cycles for the DSRM test-system detected C_t were in range 21–31 (Fig. 2, *A*) and for the SRB2 test-system from 27 to 37 (Fig. 2, *B*).

From the obtained data of 9–12 repeats linear dependences of the number of SRB cells (lg cells/ml or cells/g) on the values of threshold cycles (C_t) of real-time PCR were derived to build standard regression curves (Fig. 3). The standard deviation of the average threshold cycles did not exceed 5%. The points of intersection of red lines indicate the values of reliable threshold cycles (C_t) do not exceed 35 cycles and the limit determined amount (titre) of SRB in the sample.

For test-systems tested on the SRB cultures diluted in water samples had shown that the detection limit of the *dsrA* gene corresponded to the follows bacterial titres — 10^4 cells/ml of the water sample (line 1, Fig. 3, *A*). In soil samples, the detection limit of SRB titres were differed for two used methods and amounted in $10^4–10^5$ cells/g of absolutely dry soil (line 3, Fig. 3, *B*). The method of DNA isolation according to the recommendations [18] (line 2, Fig. 3, *B*) was more sensitive, since the determined amount of SRB was 3 orders of magnitude less compared with data obtained for PCR with the DNA samples extracted with the commercial reagent kit. The minimum determined SRB titre was 10^2 cells/g of ADS. Thus, the minimum determined amount (lg of SRB cells) in soil samples was 2 orders of magnitude more than in water samples.

To compare different methods, we determined the amount of SRB in samples from natural and man-caused ecotopes (Table 2). For analysis, 13 samples were taken from the following ecotopes: the Black sea, Odessa region (sludge samples OL4, OL5; sea water OL6, OL7); wastewater treatment plant Chernihiv city (sewage waters samples Ch2, Ch4; biofoulings Ch1, Ch3, Ch5); main gas-pipeline, Poltava region (soil from the pipe surfaces P5, P7 and control soil P9, P10). In all the studied samples SRB were detected by both methods, i.e. microbiological and molecular biological using real-time PCR. For the studied samples, the dynamics of real-time PCR are shown in Fig. 4.

It was revealed that in the samples from the Odessa region, i.e. OL4, OL5 samples from the bottom of the estuary, the SRB titres determined by microbiological method were 10^6–10^8 cells/ml, and in OL6, OL7 sea water samples the amount of SRB were lower by 2–4 orders of magnitude (10^4–10^6 cells/ml). Detection of the presence of the *dsrA* gene in the total DNA isolated from these samples showed that in the OL4 sample with a high SRB titre (10^8 cells/ml), the C_t values obtained from PCR curves for two test-systems DSRM and SRB2 were 31.15 and 32.46, respectively. For OL5 and OL7 samples with SRB titre (10^6 cells/ml), the C_t values for the DSRM test-system were 31.65-32.13, which is less effective because the threshold cycles are higher. For the OL6 sample with the lowest SRB titre, it was noted that the gene was detected by only one SRB2 test system (C_t = 34.50). It was noticed that the obtained C_t values are reaching to the reliable threshold determination (35 cycles), i.e. the values above which are considered unreliable (Table 2; Fig. 4, *A*). Thus, the smaller number of bacteria in the sludge and seawaters samples corresponds to the highest C_t values, which is considered with the data of regression curves (Fig. 3). The SRB titre obtained by the microbiological method in most samples was higher than the values calculated from the regression curves.

The data obtained for samples from the man-caused ecotope (Chernihiv wastewater treatment plant) showed a similar relationship between the number of bacteria and the threshold cycles C_t values. For Ch1, Ch3 biofouling samples, the determined SRB titres were high (10^8 cells/ml) with obtained subsequent threshold cycles for the DSRM test-system were C_t = 28.50–32.07 and for the SRB2 test system C_t = 29.49–30.84. In Ch2, Ch5 sewage waters samples, the SRB titres 10^7 cells/ml were in one order less than in below mentioned samples. The threshold cycles for the DSRM test-system C_t = 29.58–31.15 values were

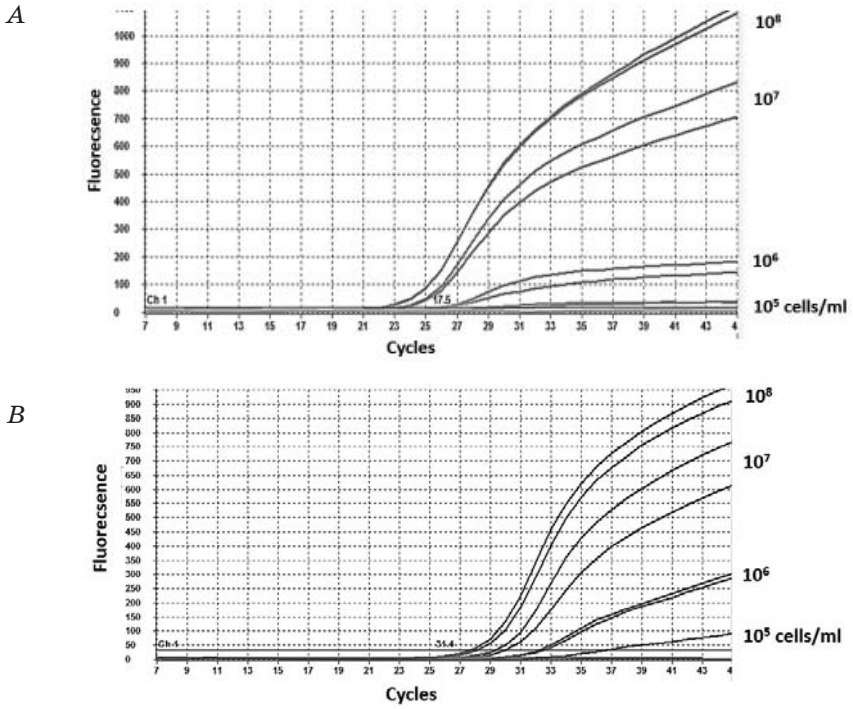

Fig. 2. **Real-time PCR curves of determining the presence of dsrA genes in a series of limit dilutions of *D. vulgaris* UCM B-11502 in water samples:**
A — DSRM test-system, *B* — SRB2 test-system. The graphs show the titre values for *D. vulgaris* UCM B-11502 test culture corresponding to the samples from which the DNA was extracted

Table 2. **Comparison of the microbiological method for determining the amount of SRB with the real-time PCR method in microchip format**

No.	The source of sample isolation	Sample name	Microbiological method	Molecular biological real-time PCR method			
			lg SRB, cells/g ADS (or 1 ml of water)	C_t, cycles		lg SRB (by regression curves)	
				DSRM	SRB2	DSRM	SRB2
Water, sludge, biofouling							
1	Odessa estuary and water from Black sea (Kryzhanovka, Odessa region.)	OL4 (sludge, estuary)	8.0	31.15	32.46	5.28	5.04
2		OL5 (sludge, estuary)	6.0	31.65	–	5.13	0
3		OL6 (sea water)	4.0	–	34.50	0	4.29
4		OL7 (sea water)	6.0	32.13	28.87	4.98	6.36
5	Wastewater treatment plant (KP "Chernihiv Vodokanal", Chernihiv)	Ch1 (biofouling)	8.0	28.50	29.49	6.08	6.13
6		Ch3 (biofouling)	8.0	32.07	30.84	5.0	5.64
7		Ch5 (biofouling)	7.0	31.15	27.75	5.28	6.77
8		Ch2 (waste water)	7.0	29.58	29.32	5.75	6.20
9		Ch4 (waste water)	5.0	34.56	33.62	4.25	4.62
Soils							
10	Soils, near the main gas-pipeline (Poltava region)	P5 (lower tangent)	4.0	34.96	–	3.45	0
11		P7 (side tangent)	5.0	34.34	–	3.82	0
12		P9 (control)	4.0	–	30.69	0	6.30
13		P10 (control)	6.0	27.12	26.97	8.20	7.63
Pearson's correlation indexes (r)						0.69	0.41

Note: "– " — threshold cycles are not defined (no reaction occurred).

for one unit higher than for Ch1, Ch3 samples. For the SRB2 test-system, the C_t values (27.75–29.32 cycles) were similar to the above-described samples.

In the Ch4 sample with the lowest SRB titre (10^5 cells/ml) the threshold cycles values were high (34.56 and 33.62), for two test-systems DSRM and SRB2, respectively (Table 2; Fig. 4, *B*). So, the amount of the SRB detected by the molecular biological method was less than for microbiological one.

For soil samples taken near the main gas-pipeline (Poltava region), the DSRM test-system detected the target gene in 3 total DNA samples, in contrast to the SRB2 test-system, which detect the gene in 2 samples (Table 2; Fig. 4, *B*). It was also noted that in the P10 sample with the largest among this ecotope SRB titre (10^6 cells/g of absolutely dry soil) the *dsrA* gene was detected with the C_t values 27.12 and 26.97, for the DSRM and SRB2 test-systems, respectively. For P5, P9 samples with 2 orders less SRB titres (10^4 cells/g of absolutely dry soil), the detection of the *dsrA* gene was less effective. Threshold cycles for DSRM and SRB2 test-systems were 34.96 and 30.69, respectively. It was noted that in water samples the *dsrA* gene was detected more effectively than in soil samples. A lot of factors can influence on the effectiveness of determination of the *dsrA* gene both in water and soil samples. As mentioned in the work [19] the soil environment is heterogeneous and multicomponent, that can effect on the quality of the isolated DNA and, accordingly, on the effectiveness of PCR analysis.

Thus, in the total DNA isolated from natural and man-caused samples the presence of the α-subunit of dissimilatory sulphite reductase (*dsrA* gene) and, accordingly, the presence of SRB by real-time PCR with using of the lyophilized silicon microchips was determined in 11 of the 13 samples using DSRM test-system and 10 of the 13 samples using SRB2 test-system.

To compare the effectiveness of SRB detection by microbiological and molecular biological methods, Pearson's correlation

indexes were calculated for the results obtained from different methods. To gain this, the derived regression equations based on data obtained from real-time PCR with designed test-systems for SRB titres (presented in lg) were calculated (Fig. 3). Calculated Pearson's pair correlation indexes (Table 2), were positive, which indicates the coincidence of the data obtained by two methods. Thus, according to data from 13 samples, the Pearson's correlation indexes for the DSRM test system was $r = 0.69$ (k = 11, for $P \leq 0.01$), which indicates a positive average tightness of the linear dependence, and for SRB2 test-system $r = 0.41$ (k = 11, for $P \leq 0.05$), which indicates a weak tightness of the linear dependence. Based on the number of triggers and correlation analysis data, we could suppose that the DSRM test-system is reliable and effective.

Thus, microbiological measurements are obviously the most reliable and sensitive quantitative method for detecting bacteria, but this method is more expensive and, importantly, time-consuming without the possibility of full automatization the detection process. The use of real-time PCR with the developed test-systems on the lyophilized silicon microchips are cheaper, high-quality and rapid method for the detection of SRB in various natural and man-caused ecotopes.

When conducting large-scale construction the molecular biological studies of soils with the goal to detect the presence of the corrosive-relevant bacteria are essentially needed. Such expertise will help to avoid the severe consequences that arise during operating underground structures.

Potential areas of industry and national economy where the use of developed test-systems is recommended are: oil and gas industry (to assess the corrosion activity of soils in the design and laying of oil and gas pipelines); microbiological control of oil and oil products pollution; monitoring of reservoir waters; monitoring of corrosion destruction of tanks, tankers and closed communications, etc.; development and testing of new inhibitors of biocorrosion caused by SRB [20–22]. In addition, the detection of SRB is necessary during the searching and checking the quality

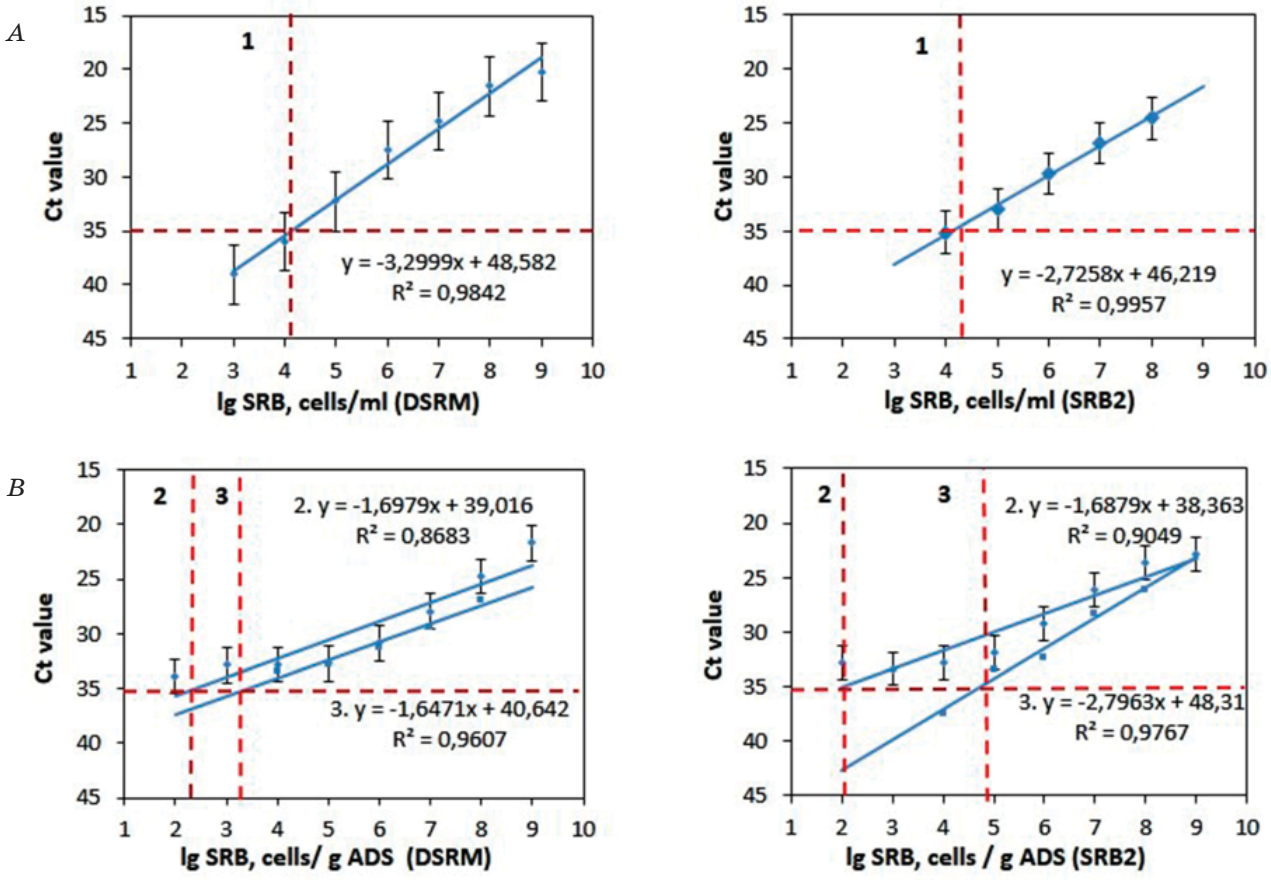

Fig. 3. **Standard regression curves of a series of limit dilutions of SRB in water (*A*) and soil (*B*) samples ($N = 9–12$, $P \leq 0.05$):**

DSRM, SRB2 microchip lyophilized test systems. Line 1: sample preparation using DNA PROBA-CTAB reagent kit (DNA Technology, Russia, cat. P-004/2); line 2: sample preparation as indicated in the guidelines [18]; line 3: sample preparation using the DNeasy PowerSoil Kit (Qiagen, USA)

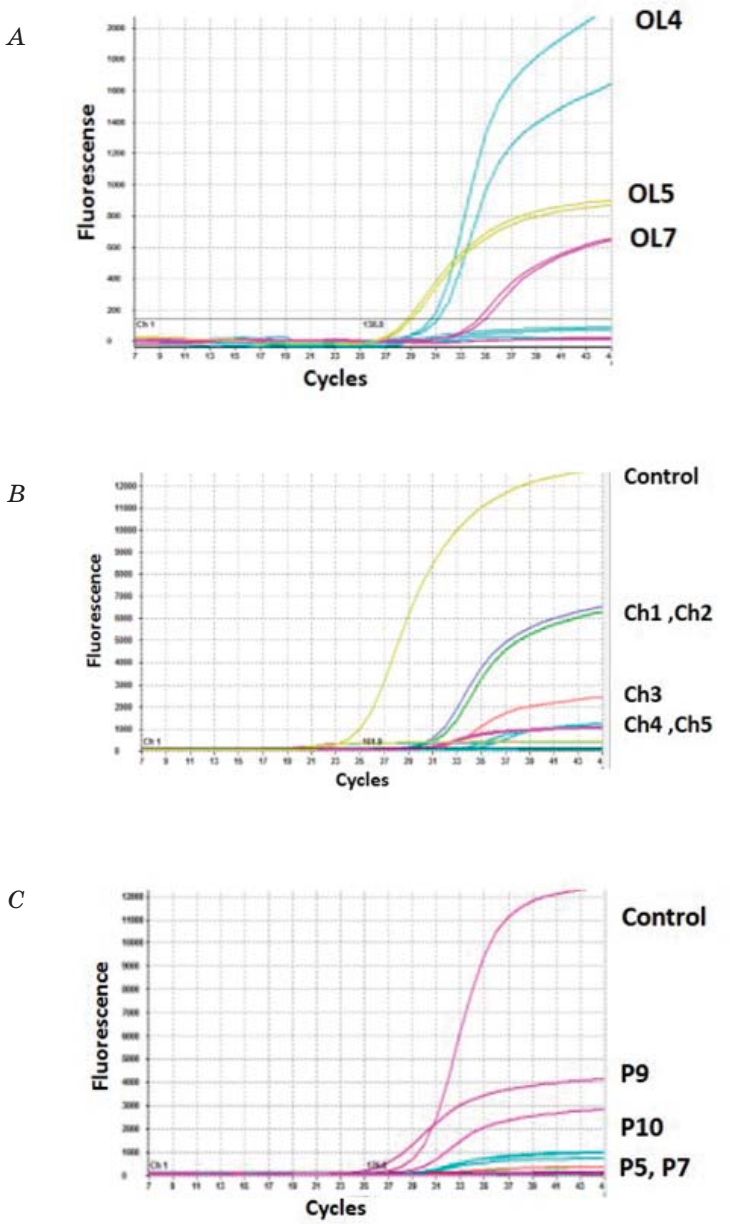

Fig. 4. **Real-time PCR curves obtained from DSRM test-system for DNA extracted from natural and man-caused samples:**

A — sludge samples (OL4, OL5), sea water (OL7) (Black sea, Odessa region); *B* — sewage waters (Ch2, Ch4), biofoulings (Ch1, Ch3, Ch5) (wastewater treatment plant, Chernihiv); *C* — soils near the main gas-pipeline (P5, P7, P9, P10) (gas-pipeline, Poltava region)

Control — positive control of DNA isolated from SRB cultures of *D. vulgaris* UCM B-11502 or *D. desulfuricans* UCM B-11501.

of deposits of curative muds for the creation of hospitals and recreational resorts. In monitoring of the sanitary state of soils and assessing their potential corrosion activity for municipal and industrial construction, it is also necessary to study the presence of SRB [4, 20, 21].

Advantages of bioprospecting: the use of test-systems in microchip format will be cost-effective for the following reasons such as speed and accuracy of determination, efficiency, and comparative reduction of the analysis cost, the possibility of the process automation and the introduction of serial production of microchips. Also, it is possible to use test-systems for monitoring and detection of SRB in such samples as soils, waste waters and biofoulings with a view to identify corrosion spots on the objects with increased man-caused loading to predict the future possible occurrence of extreme situations.

After comparative analysis of the two methods, we can conclude that microbiological

measures are highly reliable and sensitive, and can be used as a quantitative method, but they are expensive, time-consuming, needed for highly qualified personnel and there is impossibility of full automation. The proposed molecular-biological PCR assay using lyophilized test-systems on a silicon microchips is a cheap, effective, high-quality express method for the detecting the presence of SRB in a various samples, such as water, soil, biofouling, etc. This method has the ability to automate the determination process, as well as the introduction of serial production of microchip systems.

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ДЕТЕКЦІЯ СУЛЬФАТВІДНОВЛЮВАЛЬНИХ БАКТЕРІЙ У РІЗНИХ ЕКОТОПАХ МЕТОДОМ ПЛР У РЕЖИМІ РЕАЛЬНОГО ЧАСУ

Д. Р. Абдуліна[1], *Г. О. Іутинська*[1], *А. І. Аніскіна*[2], *М. М. Нікітін*[2]

[1]Інститут мікробіології і вірусології ім. Д. К. Заболотного НАН України, Київ
[2]ТОВ ГенБіт, Москва, Російська Федерація

E-mail: adara@ukr.net

Проведено дослідження ефективності виявлення сульфатвідновлювальних бактерій у різних екотопах мікробіологічним (культуральним методом граничних розведень) та молекулярно-біологічним (ПЛР у режимі реального часу) методом за допомогою розроблених тест-систем на ліофілізованих кремнієвих мікрочипах. Було використано тест-системи DSRM та SRB2 для виявлення функціонального гена *dsrA*, що кодує один із ключових ензимів процесу дисиміляційної сульфатредукції — дисиміляційну сульфітредуктазу. Встановлено, що гранично визначені титри сульфатвідновлювальних бактерій у водних зразках становили 10^4 клітин/мл води, у ґрунтових — від 10^2 до 10^5 кл/г абсолютно сухого ґрунту. В природних та техногенних зразках кількість сульфатвідновлювальних бактерій, що було визначено мікробіологічним методом, корелювала з розрахованими значеннями титрів, визначеними за допомогою молекулярно-біологічного методу, індекси Пірсона становили $r = 0{,}41–0{,}69$ ($k = 11$, $P \le 0{,}01–0{,}05$). Таким чином, ПЛР у реальному часі з розробленими тест-системами на кремнієвих мікрочипах є надійним та якісним експрес-методом для детекції сульфатвідновлювальних бактерій у різних природних та техногенних екотопах.

Ключові слова: *dsrA* ген, тест-системи, сульфатвідновлювальні бактерії, біокорозія, ПЛР у режимі реального часу.

ДЕТЕКЦИЯ СУЛЬФАТРЕДУЦИРУЮЩИХ БАКТЕРИЙ ИЗ РАЗЛИЧНЫХ ЭКОТОПОВ МЕТОДОМ ПЦР В РЕЖИМЕ РЕАЛЬНОГО ВРЕМЕНИ

Д. Р. Абдулина[1], *Г. А Иутинская*[1], *А. И. Анискина*[2], *М. М. Никитин*[2]

[1]Институт микробиологии и вирусологии им. Д. К. Заболотного НАН Украины, Киев
[2]ООО ГенБит, Москва, РФ

E-mail: adara@ukr.net

Проведено исследование эффективности выявления сульфатредуцирующих бактерий в образцах из разных экотопов микробиологическим (культуральным методом предельных разведений) и молекулярно-биологическим методом (ПЦР в режиме реального времени) с помощью разработанных тест-систем на лиофилизированных кремниевых микрочипах. Были использованы тест-системы DSRM и SRB2 для выявления наличия функционального гена *dsrA*, кодирующего один из ключевых энзимов диссимиляционной сульфатредукции — диссимиляционную сульфитредуктазу. Установлено, что минимально определяемые титры сульфатредуцирующих бактерий в водных образцах составляют 10^4 кл/мл, в грунтовых — от 10^2 до 10^5 кл/г абсолютно сухого грунта. В природных и техногенных образцах количество сульфатредуцирующих бактерий, выявленное микробиологическим методом, коррелировало с рассчитанными значениями титров, определенными с помощью молекулярно-биологического метода, индексы Пирсона составили $r = 0.41–0.69$ ($k = 11$, $P \le 0.01–0.05$). ПЦР в режиме реального времени с разработанными тест-системами на кремниевых микрочипах является надёжным качественным экспресс-методом для детекции сульфатредуцирующих бактерий в различных природных и техногенных экотопах.

Ключевые слова: *dsrA* ген, тест-системы, сульфатредуцирующие бактерии, биокоррозия, ПЦР в режиме реального времени.