SOME CORROSIVE BACTERIA ISOLATED FROM THE TECHNOGENIC SOIL ECOSYSTEM IN CHERNIHIV CITY (UKRAINE)

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Abstract:

The soil microbiome is exposed to technogenic influence during the operation of metal structures. There are quantitative and qualitative changes in the microbiota of the technogenic ecosystem. During the study of the technogenic soil ecosystem (ferrosphere), samples of which were taken in the field (Chernihiv, Ukraine: 51°29'58"N, 31°16'09"E), the presence of corrosively active microbial cenosis was established: sulfate-reducing, denitrifying, iron-reducing (using acetate as the only electron donor, and Fe (III) as the only electron acceptor) and ammonifying bacteria. The predominant representatives of corrosively active groups of bacteria were isolated. They were identified as *Bacillus simplex*, *Streptomyces gardneri*, *Streptomyces canus* (ammonifying bacteria), *Fictibacillus* sp. (ammonifying bacteria with iron-reducing ability), *Anaerotignum (Clostridium) propionicum* (organic acid-producing bacteria), *Desulfovibrio oryzae* (sulfate-reducing bacteria) based on some microbiological, physiological and biochemical, genetic features. Strains of heterotrophic and hemolitotrophic bacteria (individual representatives and their associations) isolated from the technogenic ecosystem can be used in both industrial and technological spheres. The interaction of isolated bacteria in the process of microbial induced corrosion is a prospect for further research.

Key words: 16S rRNA gene, microbial induced corrosion, phenotypic characteristics.

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INTRODUCTION

Microbial induced corrosion (MIC) is the result of the activity of microorganisms in the form of a biofilm on a metal or another corroding surface. The development of corrosive groups occurs in the technogenic ecosystem the ferrosphere, which is the area of soil that comes into contact with metal surfaces (Antonovskaya et al., 1986; Costerton et al., 1995; James et al., 1995; Lewandowski, 2000; Pilyashenko-Novokhatny, 2000; Andreyuk et al., 2002, 2005; Purish and Asaulenko, 2007; Purish et al., 2009). The taxonomic composition is represented by bacteria of the genera Pseudomonas, Arthrobacter, Bacillus, Desulfovibrio, Streptomyces, Thiobacillus (Ait-Langomazino et al., 1991). It has been experimentally proved that changes occur in the microbial group during the formation of a steel biofilm on the surface (Purish and Asaulenko, 2007; Purish et al., 2009). In the first stages of biofilm formation, the dominant position is occupied by heterotrophic denitrifying (DNB) and ammonifying bacteria (AMB), which contribute to the creation of anaerobic conditions on the steel surface. Next sulfate-reducing (SRB) and iron-reducing bacteria (IRB) develop in the biofilm (Purish and Asaulenko, 2007; Purish *et al.*, 2009). The dominance of the previous physiological group creates optimal conditions for the functioning of the next. It is shown that in the case of metal corrosion, gram-negative bacteria are 80% more dominant than gram-positive among the isolated heterotrophic bacteria. The overall distribution was *Pseudomonas* sp. (20%), *Bacillus* sp. (10%), *Gallionella* sp. (10%) and *Vibrio* sp. (10%) (Nuňez, 2007).

It has become possible recently to define a wider range of microbial variety in natural environments using molecular-genetic approaches (Stahl *et al.*, 1984; Giovannoni *et al.*, 1990; Amann *et al.*, 2002). Today, the number of reports of molecular genetic research in areas with corrosion is growing. In particular, corrosion-active microbial groups of nuclear waste storage (Horn *et al.*, 2003; Engel *et al.*,

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2019), sewage systems (Vincke *et al.*, 2001; Okabe *et al.*, 2007; Satoh *et al.*, 2009), copper pipes (Pavissich *et al.*, 2010), pipelines, seal rings, steel plates, stainless steel coupons in seawater (Lopez *et al.*, 2006; Bermont-Bouis *et al.*, 2007; Li *et al.*, 2017; Capão *et al.*, 2020; Salgar-Chaparro *et al.*, 2020), gas pipelines (Zhu *et al.*, 2003; Jan-Roblero *et al.*, 2004), oil pipelines (Magot *et al.*, 1997; Neria-Gonzalez *et al.*, 2010) have been investigated. A higher complexity of microbial groups than observed with bacterial culture approaches was found out. However, no common microbial groups or general trends were observed in these studies, as the areas of corrosion studied were diverse and complex.

The aim of this study was to isolate the predominant representatives of ammonifying, iron-reducing, organic acid-producing and sulfate-reducing bacteria from the technogenic soil ecosystem (ferrosphere), and to identify them using microbiological and molecular-genetic methods.

MATERIAL AND METHODS

Sampling

A soil selection of the technogenic ecosystem (ferrosphere) was carried out in the field in November from the surface of a metal structure (fence support, 15 years immersed in sod-podzolic soil) from generally accepted sampling depth 0.7 m in studies of the sulfidogenic microbial community (Abdulina et al., 2011). Sampling site is located in Chernihiv (Ukraine), 51°29'58"N 31°16'09"E (Fig. 1). The sample was collected using a sterile glass flask and was immediately transported to the laboratory of T.H. Shevchenko National University "Chernihiv Colehium" and stored at 4°C in the refrigerator. Further definition of the determination of number of bacteria of corrosive dangerous groups, the isolation of bacteria and the studies of cultural, morphological, physiological and biochemical properties of isolates were carried out in T.H. Shevchenko National University "Chernihiv Colehium".

Determination of the number of bacteria of corrosive dangerous groups

Quantitative content of bacteria of corrosive dangerous groups in soil ferrosphere was determined by the classical method of serial ten-fold dilutions that relies on executing of dilution series of soil suspension. The soil suspension was carried out by shaking 1 g soil in 99 ml sterile water during 10 min for desorption the bacteria from soil particles into solution. Elective media (liquid and solid) were inoculated with 1 ml and 0.1 ml of the appropriate dilution, respectively. We used the solid medium meat-peptone agar (MPA) to calculate the number of AMB and the liquid medium: the Giltaya's medium to calculate the number of DNB; the medium of Postgate's "B" to calculate the number of SRB (Romanenko and Kuznetsov, 1974; Aseeva *et al.*, 1991);



Fig. 1. Location of sampling site.

the medium FWA-Fe (III) citrate (with acetate as the only donor of electrons and Fe (III) as the only electron acceptor) to calculate the number of IRB (Lovley and Phillips, 1988). A general number of bacteria was determined in 1 g of absolutely dry (abs. dry) soil, the humidity of which was determined by the weight method (Vaschenko *et al.*, 1982).

Isolation of the strains of bacteria and identification

The isolation of bacteria was carried out from dilution 10⁻¹–10⁻⁶ (depending on the number of microorganisms of the corresponding corrosive group) on MPA (ammonifying bacteria – ChNPU F1, ChNPU F3, NUChC F2 isolates), FWA-Fe (III) citrate medium (iron-reducing bacteria -ChNPU ZVB1 isolate), Postgate's "B" medium (organic acid-producing bacteria (NUChC Sat1 isolate) and sulfate-reducing bacteria (NUChC SRB1, NUChC SRB2 isolates). The purity of the culture was verified by microscopy and cultivation on various nutrient media (potato agar, oat agar, peptone-yeast agar, Chapek's medium, starch-ammonia agar). An optical microscope Delta Optical Genetic Pro with increase ×400 and ×1000 and electron microscope BS-540 (Tesla, Czechoslovakia) with magnification x16000 were used to study the morphology of bacteria. Determination of the size of bacterial cells was carried out using the Aigo ScopeImage 9.0 computer program.

The studies of cultural, morphological, physiological and biochemical properties of isolated bacteria were carried out by the conventional methods (Methods of general bacteriology, 1984). Methods of molecular genetic study of bacteria (sequence of 16S rRNA gene fragments and phylogenetic analysis) were also used by the procedures which were described by Tkachuk et al. (2017) and were carried out in Danylo Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine. Briefly, to perform 16S rRNA gene sequencing genomic DNA was isolated from pure bacterial cultures and amplification with 27F and 1492r primers (Lane, 1991) was carried out. PCR-products were purified and sequenced in both directions using ABI 310 (Applied Biosystems). Obtained sequences were compared with those deposited in GenBank database with BLASTn and the highest revealed percent of similarity between sequences (>98,7%) was taken to

identify species. In case of multiple similarity sequences with >98.7% were searched and analyzed for variable and parsimony-informative sites with MEGA6 (Tamura *et al.*, 2003) to specify identification.

Statistical analysis of experimental data

Methods of mathematical statistics were used in processing the data on the number of microorganisms (Plohinskij, 1970). The number of microorganisms on liquid media was determined using McCready tables and on solid media – taking into account the mean square deviation.

RESULTS

Microbiological analysis of the soil from ferrosphere allowed us to determine a number of microorganisms of corrosion-active groups. It has been found out that the number of sulfate-reducing bacteria in the investigated soil is quite high $- 6.6 \times 10^4$ cells/1 g of abs. dry soil. The number of denitrifying bacteria in the soil of the ferrosphere appeared to be similar with SRB (6.6×10^4 cells/1 g of abs. dry soil). However, the number of iron-reducing bacteria in the studied soil was insignificant including 10^1 cells/1 g of abs. dry soil. The number of heterotrophic ammonifying bacteria in the soil of the ferrosphere is $1.19\pm0.03 \times 10^6$ of colony-forming units/1 g of abs. dry soil.

Thus, the number of microorganisms of corrosive dangerous groups in the studied technogenic soil indicates presence of corrosively active microbial cenosis.

Next, we isolated the predominant representatives of corrosive dangerous groups of bacteria from the soil technogenic ecosystem. The results of the study of cultural-morphological and physiological-biochemical properties of the isolated bacteria are presented (Tables 1–7).

Microbiological characteristics according to Bergey's Manual of Systematic Bacteriology (Bergey's Manual of Systematic Bacteriology, 2005, 2009, 2012) prove that the isolated bacteria can belong to the *Bacillaceae* family (ChNPU F1, ChNPU ZVB1 isolates), *Streptomyces gardneri* (ChNPU F3 isolate), *Streptomyces canus* (NUChC F2 isolate), the *Clostridiaceae* family (NUChC Sat1 isolate), the *Desulfovibrionaceae* family (NUChC SRB1, NUChC SRB2 isolates).

To define species of isolates 16S rRNA, the gene sequencing was performed. As a result nucleotide sequences of gene fragments were obtained and compared to those deposited in GenBank database (Table 8). Generally, the length of 16S rRNA gene fragment varied from 483 bp to 1368 bp and was informative for genus or species, in most cases, identification. The phylogenetic analysis including detailed nucleotide composition analysis and construction of phylogenetic dendrogram was carried out where the defining of species was controversially.

Based on microbiological, physiological and biochemical, genetic features, the investigated bacteria are identified as Bacillus simplex, Streptomyces gardneri, Streptomyces canus, Fictibacillus sp., Anaerotignum (Clostridium) propionicum and Desulfovibrio oryzae (Table 8).

DISCUSSION

Bacteria of the Bacillus genus deserve attention as one of the representative microorganisms for MIC research (Wang et al., 2020). There are no reports of involvement of B. simplex and Fictibacillus sp. in microbial damage to materials. However, according to the analyzed publications, microbial damage to metals depends on the studied species and strain of bacteria of the Bacillus genus (Rajasekar and Ting, 2010; Bano and Qazi, 2011; Bragadeeswaran et al., 2011; Du et al., 2014). The metabolites of Bacillus sp. contain cyclic peptides, antibiotics, polyglutamic acid, polyaspartic acid and polypolysaccharide, and can form biofilms with spores. Research showed that extracellular polymeric substances produced by Bacillus was the main component of the biofilm formed on the metal surface and provided the biologically active compound which affected the corrosion process. More and more attention has been paid to the corrosion effect of Bacillus on metals, but the mechanism is still unclear, and there is still much controversy (Wang et al., 2020).

Streptomycetes of isolated species (*S. gardneri, S. canus*) are also not known to participate in the biodamage of materials. However, the ability to produce biologically active substances known to streptomycetes determines the interest in microbial corrosion studies involving these bacteria (Jayaraman *et al.*, 1997; Nnabuk Eddy Okon, 2010; Pacheco da Rosa *et al.*, 2013, 2016; Bleich *et al.*, 2015).

The *D. oryzae* species is currently poorly studied. In the available literature there is a single brief description of the species only (Salgar-Chaparro and Silva-Plata, 2008). Some *D. oryzae* strains have been isolated from a community of oil-related water associated with the corrosion process (Salgar-Chaparro and Silva-Plata, 2008; Duque *et al.*, 2011).

Among bacteria, secreting organic acids and exopolymers or slime clostridia deserve attention. They have been isolated from the sites of corrosion (Pope et al., 1984; Beech and Gaylarde, 1999; Zhu et al., 2003; Oliveira et al., 2011), sulfidogenic bacterial consortia (Herro and Port, 1993; Agrawal et al., 2010; Monroy et al., 2011; Ilhan-Sungur et al., 2017; Qiu et al., 2017). Herewith, the mechanisms of influence on the process of MIC and the consequences depend on the species of clostridia. In general, the corrosion hazard for metal coatings reveals the products of clostridium metabolism (organic acids, slim, H₂, H₂S), followed by synergism between Clostridium sp. and SRB, which helps to increase the corrosive aggressiveness of the environment (Beech and Gaylarde, 1999; Zhu et al., 2003; AlAbbas et al., 2013; Bala and Chidambaram, 2014). Co-cultivation of D. oryzae with A. propionicum (without electron donors, the presence of sulfates and yeast extract) showed the formation of hydrogen sulfide by sulfate-reducing bacteria, which was not observed in the case of cultivation of monocultures (Tkachuk et al., 2020). In this case, a phenomenon

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Table 1. Cultural and morphological properties of ChNPU F1 isolate.

| Sign | Characteristic | |
|---------------------------------|---|--|
| Colonies on the MPA | Colonies are superficial, grayish-white, 1–2 mm in diameter (1 st day) and 3–6 mm (2 nd days). The shape of the colonies is rounded, the edges are uneven, wavy. The profile is convex, the structure is homogeneous the surface is smooth, shiny. The texture is soft. Colonies are translucent, opalescent in transmitted light after 18 hours of cultivation. On the 2 nd day the colonies are opaque. On the 7 th day the colonies become concentric, the profile acquires a conical shape. | |
| Growth in the MPB | The solution is moderately uniformly turbid, the film is thin, smooth; no smell; no pigmentation; sediment is significant, soft | |
| Colonies on potato agar | The isolate grows on the 1 st day. The shape of the colonies is rounded, grayish-white with opalescent wavy edges. The diameter of the colonies is 2–3 mm on the 1 st day and 15–18 mm on the 3 rd day. The profile of the colonies is flattened, crater-like on the 3 rd day, the structure is homogeneous, the surface is smooth and shiny. The texture is soft. | |
| Colonies on peptone-yeast agar | The isolate grows on the 1 st day. Colonies are with a diameter of 0.1 mm (1 st day) and 3–5 mm (3 rd day), whitish-gray, round with jagged edges. The structure of the colonies is fine-grained, the profile is flattened, the surface is uneven, folded, matte. The texture is soft. | |
| Colonies on Chapek's medium | The isolate grows on the 3 rd day. Colonies are small (diameter 0.1 mm), grayish-white, rounded with jagged, wavy edges. The profile is convex, the surface is smooth, shiny. The texture is soft. | |
| Colonies on starch-ammonia agar | The isolate grows on the 2 nd day. Growth is weak. Colonies are of irregular shape, membranous, 4–5 mm in diameter, white-gray, with uneven, wavy edges. The structure of the colonies is fine-grained, the profile is flattened, the consistency is soft. | |
| The shape and location of cells | Cells in young culture (18 hours) are rod-shaped, with rounded ends, 1–2 cells are located. In the old culture (2 months) the cells are rod-shaped with rounded ends, 1–2 cells are located, in chains of 4 or more cells. | |
| Cell length | 5.80±0.25 μm | |
| Cell motility | Weak motility | |
| Endospores, their location | Available, central location | |
| Gram stain | Gram-negative | |

Table 2. Cultural and morphological properties of ChNPU F3 isolate.

| Sign | Characteristic | | |
|---------------------------------|---|--|--|
| Colonies on the MPA | Colonies are superficial, round, 0.5–3 mm in diameter, yellowish-gray with smooth edges. The structure is homogeneous, the profile is convex, the surface is smooth, matte. The consistency is oily. On the 14 th –30 th days of cultivation, colonies up to 10 mm in diameter have a weak white air mycelium. | | |
| Colonies on oat agar | Colonies are gray, rounded, 0.5–1.0 mm in diameter. The edges are fringed, the surface is matte, the profile is convex, the consistency is firm. Air mycelium is not expressed. The reverse side of the colonies without different pigments (yellowish-gray color). Over time, the diameter of the colonies on oat agar increases (2.5–3.0 mm), the shape becomes rounded with a roller, the profile is crater-like, air mycelium is not expressed. On the 10 th day of cultivation, the colonies have white air mycelium, weakly expressed. | | |
| Growth in the MPB | The solution is moderately uniformly turbid, the film is thin, smooth; no smell; no pigmentation; sediment is significant, soft | | |
| Colonies on potato agar | The isolate grows on the 3 rd day. Colonies are rounded, folded, with wavy edges, yellowish-white, 2–3 mm in diameter. The profile is convex, the structure is fine-grained, the surface is matte, the texture is soft. When the colony is removed by the bacteriological loop, the substrate mycelium remains. Air mycelium is not expressed. Pigments are not formed. | | |
| Colonies on peptone-yeast agar | The isolate grows on the 3 rd day. Colonies with a diameter of 2–3 mm are rounded with wavy edges, white. The surface is folded, matte, flat profile, fine-grained structure, soft texture. There is a substrate mycelium. Air mycelium is not expressed. Pigments are not formed. | | |
| Colonies on Chapek's medium | The isolate grows on the 3 rd day. Colonies with a diameter of 1 mm round shape with fringed edges, white The surface is matte. The profile is flattened, the structure is homogeneous, the consistency is firm. There i a substrate mycelium. Air mycelium is not expressed. Pigments are not formed. | | |
| Colonies on starch-ammonia agar | The isolate grows on the 2 nd day. Colonies are with a diameter of 0.1 mm, rounded, grayish-white, convex r profile, fringed edges, fine-grained structure, firm consistency. There is a substrate mycelium. Air mycelium is not expressed. | | |
| The shape and location of cells | Form a branched mycelium with spores of round shape, arranged in a chain of 5-10 spores or more | | |
| Endospores, their location | None | | |
| Gram stain | Gram-positive | | |

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Sign Characteristic Colonies on the MPA The isolate grows on the 4th day, the colonies are rounded, 2–4 mm in diameter, gray-brown, the surface is folded, leathery, matte, coarse-grained structure, firm consistency. Releases a dark brown pigment. Air mycelium is not formed. Colonies on oat agar Colonies are superficial, gray-white, 2–4 mm in diameter. The shape is rounded with a roller, the profile is crater-shaped, the surface is folded, the consistency is fibrous. On the 14th–20th day, a well-defined white air mycelium develops. On the underside of the colony is brown. The isolate forms a brown pigment, which

| Table 3. Cultural and morphological p | properties of NUChC F2 isolate. |
|---------------------------------------|---------------------------------|
|---------------------------------------|---------------------------------|

| Colonies on oat agar | Colonies are superficial, gray-white, 2–4 mm in diameter. The shape is rounded with a roller, the profile is crater-shaped, the surface is folded, the consistency is fibrous. On the 14 th –20 th day, a well-defined white air mycelium develops. On the underside of the colony is brown. The isolate forms a brown pigment, which over time (1–2 months) becomes reddish | |
|---------------------------------|--|--|
| The shape and location of cells | Bacteria form a branched mycelium with spores arranged in a chain of 10–50 spores or more. Spore chains are spiral, but there are some spore chains straight and loop-like. | |
| Endospores, their location | None | |
| Gram stain | Gram-positive | |

Table 4. Cultural and morphological properties of ChNPU ZVB1 isolate.

| Sign | Characteristic | | |
|---|--|--|--|
| Colonies on FWA-Fe (III) citrate medium | Colonies grow on the 7 th day under both anaerobic and aerobic cultivation conditions. Colonies are superficial, amber in color, 4 mm in diameter, irregularly shaped, flattened, wavy edges, matte surface, coarse-grained structure, soft texture. | | |
| Colonies on the MPA | Colonies of the isolate grow at 29°C on the 1 st day under aerobic conditions. Colonies are superficial, round, yellowish-gray, 1–2 mm in diameter, convex profile, wavy edges, shiny surface, smooth, homogeneous structure, soft texture | | |
| Growth in the MPB | The solution is moderately uniformly turbid; the film is thin, well defined, smooth; sediment is significant, loose; there is a specific smell; no pigmentation | | |
| Colonies on potato agar | Colonies grow on the 3 rd day, rounded, jagged edges, hairy. The profile is flattened. The surface is shiny. The structure is homogeneous. The texture is soft. | | |
| Colonies on peptone-yeast agar | Colonies grow on the 1 st day, small, 2–3 mm in diameter, yellowish-gray color. The shape is rounded. The edges are uneven. The surface is smooth, shiny. The profile is flattened with the acting center. The structur is fine-grained. The texture is soft. | | |
| Colonies on Chapek's medium | It does not grow | | |
| Colonies on starch-ammonia agar | Weak, membranous growth | | |
| The shape and location of cells | Slightly curved sticks with rounded ends, combine into long curved threads surrounded by a cover, some cells are stretched | | |
| Cell length | 4.355±0.204 μm | | |
| Cell motility | Motility | | |
| Endospores, their location | Available, central location | | |
| Gram stain | Gram-positive | | |

Table 5. Cultural and morphological properties of NUChC Sat1 isolate.

| Sign | Characteristic | |
|--|---|--|
| Colonies on the Postgate's "B" medium (anaerobic conditions) | Colonies are deep, grayish-beige, round, soft, 2–3 mm in size | |
| Shape and location of cells | Stick-shaped, slightly curved, with rounded ends, single | |
| Cell length | 4.671±0.387 μm | |
| Cell motility | Motility, peritrihous | |
| Endospores, their location | Spores are sub-terminal and inflate cells | |
| Gram stain | Gram-positive | |

Table 6. Cultural and morphological properties of NUChC SRB1 and NUChC SRB2 isolates.

| Sign | Characteristic | |
|--|---|--|
| Colonies on the Postgate's "B" medium (anaerobic conditions) | Clear black SRB colonies with a diameter of 1 mm (NUChC SRB1) and 2 mm (NUChC SRB2) | |
| The shape and location of cells | Vibrios with rounded ends, respectively, monotrichs | |
| Cell length | 4.6±0.3 μm (NUChC SRB1), 1.8±0.1 μm (NUChC SRB2) | |
| Cell motility | Motile | |
| Endospores, their location | No sign | |
| Gram stain | Gram-negative | |

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Table 7. Some physiological and biochemical properties of isolated bacteria.

| Isolate | Physiological and biochemical properties |
|---------------------------|--|
| ChNPU F1 | Catalase-positive; oxidase-negative; aerobic; citrate utilize; do not form indole; formic acid fermentation is not carried out; casein is not hydrolyzed; do not form hydrogen sulfide and cellulases; ammonia is formed |
| ChNPU F3 | Catalase-positive; oxidase-negative; aerobic; do not utilize citrate; utilize urea; do not form indole; formic acid fermentation is not carried out; levan is not synthesized; starch, fats, casein, gelatin are hydrolyzed; do not form hydrogen sulfide and cellulases; ammonia is formed |
| NUChC F2 | Catalase-positive, oxidase-negative; aerobe, do not utilize glucose, but grow due to the amino acids peptone, releasing ammonia; hydrolyze starch and casein; do not hydrolyze lipids and urea; citrate is utilized; do not break down aromatic amino acids; do not form hydrogen sulfide; ammonia is produced; levansaccharase is absent; formic acid fermentation is not carried out, Fe (III) is reduced |
| ChNPU ZVB1 | Catalase and oxidase-positive, facultative anaerobes, glucose is utilized both under aerobic conditions (with the formation of acid but not gas) and under anaerobic conditions (with the formation of acid but not gas); citrate urea is not utilized; do not form indole; formic acid fermentation is not carried out; levan is synthesized; fats and gelatin are hydrolyzed; starch, casein are not hydrolyzed; have the phospholipase; do not grow on mannitol salt agar, which may be due to sensitivity to high concentrations of NaCl; form ammonia; do not form hydrogen sulfide and cellulase |
| NUChC Sat1 | Catalase- and oxidase-negative; the isolate assimilates lactate, but does not assimilate glycerol, dulcite, ethanol, mannitol, sorbitol, arabinose, lactose, ramnose, raffinose, starch, sucrose as a source of Carbon. |
| NUChC SRB1, NUChC SRB2 | Catalase-negative, oxidase-positive; able to use lactate and formate as electron donors. Acetate, fumarate, propionate, malate, fructose and glucose are not used as electron donors; NUChC SRB1 isolate uses sulfate and thiosulfate as electron acceptors, but does not use sulfite, fumarate and nitrate. NUChC SRB2 isolate uses sulfate, thiosulfate and fumarate as electron acceptors, but does not use sulfite, fumarate, but does not use sulfite and fumarate as electron acceptors, but does not use sulfite and fumarate as electron acceptors, but does not use sulfite and nitrate. |

 Table 8. Ecological and trophic group, the number of the registration of nucleotide sequences of isolated and identified bacteria.

| Species or genus | Ecological and trophic group | Strain | Number of registration in the GenBank |
|--|--|------------|--|
| Bacillus simplex | ammonifying bacteria | ChNPU F1 | KX349220 |
| Streptomyces gardneri | ammonifying bacteria | ChNPU F3 | KX349221 |
| Srteptomyces canus | ammonifying bacteria | NUChC F2 | MG924748 and MG924855 |
| Fictibacillus sp. | ammonifying and iron-reducing bacteria | ChNPU ZVB1 | KX349222 |
| Anaerotignum (Clostridium) propionicum | organic acid-producing bacteria | NUChC Sat1 | MG924854.1 |
| Derulferikuis errores | milfate no ducin a heatenia | NUChC SRB1 | MT102713.1 |
| Desulfovibrio oryzae | sulfate-reducing bacteria | NUChC SRB2 | MT102714.1 |

of syntrophy seems to take place and the electron donor appears due to the use of the yeast extract compounds by the NUChC Sat1 strain.

CONCLUSIONS

Thus, the soil microbiome is exposed to technogenic influence during the operation of metal structures in it, there are quantitative and qualitative changes in the microbiota. At the same time, strains of heterotrophic and hemolitotrophic bacteria (individual representatives and their associations) isolated from the technogenic ecosystem can be used in both industrial and technological spheres. The interaction of isolated bacteria in the process of microbial induced corrosion is a prospect for further research.

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REFERENCES

- Abdulina, D.R., Asaulenko, L.G., Purish, L.M., 2011. Dissemination of corrosive aggressive bacteria in soils of different biotopes (Rozpovsiudzhennia koroziino-ahresyvnykh bakterii u gruntakh riznykh biotopiv). Studia Biologica 5 (1), 11–16. (in Ukrainian).
- Agrawal, A., Vanbroekhoven, K., Lal, B., 2010. Diversity of culturable sulfidogenic bacteria in two oil-water separation tanks in the north-eastern oil fields of India. Anaerobe 16 (1), 12–18.
- Ait-Langomazino, N., Sellier, R., Jonquet, G., Trescinski, M., 1991. Microbial degradation of bitumen. Experientia 6, 533–539.
- AlAbbas, F.M., Williamson, Ch., Bhola, Sh.M., Spear, J.R., Olson, D.L., Mishra, B., Kakpovbia, A.E., 2013. Microbial Corrosion in Linepipe Steel Under the Influence of a Sulfate-Reducing Consortium Isolated from an Oil Field. Journal of Materials Engineering and Performance 22 (11), 3517–3529.
- Amann, R.J., Stromley, J., Devereux, R., Key, R., Stahl, D.A., 1992. Molecular and microscopic identification of sulfate-reducing bacteria in multispecies biofilms. Applied and Environmental Microbiology 58, 614–623.
- Andreyuk, E.I., Kozlova, I.A., Kopteva, Zh.P., Pilyashenko-Novokhatny, A.I., Zanina, V.V., Purish, L.M., 2002. Ferrosphere – formation zone corrosive community of microorganisms (Ferrosfera – zona formirovaniya korrozionno-aktivnogo soobschestva mikroorganizmov). Reports of the NAS of Ukraine 3, 157–161. (in Russian)

Andreyuk, K., Kozlova, I., Koptieva, Zh., Pilyashenko-Novokhatny,

A., Zanina, V., Purish, L., 2005. Microbial Corrosion of Underground Structures, Naukova Dumka, Kyiv. (in Ukrainian with English summary).

- Antonovskaya, N.S., Kozlova, I.A., Andreyuk, E.I., 1986. *Thiobacillus thioparus* – active agent in steel corrosion. Mikrobiologii Zhurnal 1, 36–41. (in Russian with English summary)
- Aseeva, I.V., Babieva, I.P., Byzov, B.A., Goosev, V.S., Dobrovolskaya, T.G., Zvyagintsev, D.G., Zenova, G.M., Kozhevin, P.A., Kurakov, A.V., Lysak, L.V., Marfenina, O.E., Mirchink, T.G., Polyanskaya, L.M., Panikov, N.S., Skvortsova, I.N., Stepanov, A.L., Umarov, M.M., 1991. Methods of soil microbiology and biochemistry (Metodyi pochvennoy mikrobiologii i biohimii). In: Zvyagintsev, D.G. (Ed.), Moscow University Press, Moscow. (in Russian).
- Bala, D.D., Chidambaram, D., 2014. Effect of anaerobic microbial corrosion on the surface film formed on steel. ECS Transactions 58 (41), 137–149.
- Bano, A.Sh., Qazi, J.I., 2011. Soil Buried Mild Steel Corrosion by Bacillus cereus-SNB4 and its Inhibition by Bacillus thuringiensis-SN8. Pakistan Journal of Zoology 43 (3), 555–562.
- Bergey's Manual of Systematic Bacteriology, 2005. Second Edition, Volume 2, The Proteobacteria, Part C. The Alpha-, Beta-, Delta-, and Epsilonproteobacteria. Brenner, D.J., Krieg, N.R., Staley, J.T. et al., Springer, New York.
- Bergey's Manual of Systematic Bacteriology, 2009. Second edition, Volume 3, The Firmicutes. De Vos, P., Garrity, G.M., Jones, D., Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer, K.-H., Whitman, W.B. Springer, New York.
- Bergey's Manual of Systematic Bacteriology, 2012. Second edition, Volume 5, The Actinobacteria, Part A. Goodfellow, M., Kämpfer, P., Busse, H.-J., Trujillo, M.E., Suzuki, K.-I., Ludwig, W., Whitman, W.B. Springer, New York.
- Beech, I.B., Gaylarde, Ch.C., 1999. Recent advances in the study of biocorrosion: an overview. Revista de Microbiologia 30 (3), 117– 190.
- Bermont-Bouis, D., Janvier, M., Grimont, P.A., Dupont, I., Vallaeys, T., 2007. Both sulfate-reducing bacteria and *Enterobacteriaceae* take part in marine biocorrosion of carbon steel. Journal of Applied Microbiology 102, 161–168.
- Bleich, R., Watrous, J.D., Dorrestein, P.C., Bowers, A.A., Shank, E.A., 2015. Thiopeptide antibiotics stimulate biofilm formation in *Bacillus subtilis*. Proceedings of the National Academy of Sciences (PNAS) 112 (10), 3086–3091.
- Bolton, N., Critchley, M., Fabien, R., Cromar, N., Fallowfield, H., 2010. Microbially influenced corrosion of galvanized steel pipes in aerobic water systems. Journal of Applied Microbiology 109, 239–247.
- Bragadeeswaran, S., Jeevapriya, R., Prabhu, K., Sophia Rani, S., Priyadharsini, S., Balasubramanian, T., 2011. Exopolysaccharide production by *Bacillus cereus* GU812900, a fouling marine bacterium. African Journal of Microbiology Research 5 (24), 4124–4132.
- Capão, A., Moreira-Filho, P., Garcia, M., Bitati, S., Luciano Procópio, L., 2020. Marine bacterial community analysis on 316L stainless steel coupons by Illumina MiSeq sequencing. Biotechnology Letters 42, 1431–1448.
- Costerton, J.W., Lewandowski, Z., Caldwell, D.E., Korber, D.R., Lappin-Scott, H.M. Microbial Biofilms, 1995. Annual Review of Microbiology 49, 711–745.
- Du, J., Li, S., Liu, J., Yu, M., 2014. Corrosion behavior of steel Q235 co-influenced by *Thiobacillus thiooxidans* and *Bacillus*. Beijing Hangkong Hangtian Daxue Xuebao. Journal of Beijing University of Aeronautics and Astronautics 40 (1), 31–38.
- Duque, Z., Ibars, J.R., Sarró, M.I., Moreno, D.A., 2011. Comparison of sulphide corrosivity of sulphate- and non-sulphate-reducing prokaryotes isolated from oilfield injection water. Materials and Corrosion 62 (9999), 1–7.
- Engel, K., Ford, S.E., Coyotzi, S., McKelvie, J., Diomidis, N., Slater, G., Neufeld, J.D., 2019. Stability of Microbial Community Pro-

files Associated with Compacted Bentonite from the Grimsel Underground Research Laboratory. mSphere 4 (6) e00601-19. https:// doi.org/10.1128/mSphere.00601-19

- Giovannoni, S.J., Britschgi, T.B., Moyer, C.L., Field, K.G., 1990. Genetic diversity in Sargasso Sea bacterioplankton. Nature, 345, 60–63.
- Herro, H.M., Port, R.D., 1993. The Nalco guide to cooling water system failure analysis, 1st ed., McGraw-Hill, New York, pp. 420.
- Horn, J., Carrrillo, C., Dias, V., 2003. Comparison of the Microbial Community Composition at Yucca Mountain and Laboratory Test Nuclear Repository Environments. CORROSION /2003 (San Diego, CA, March 16–20, 2003), Paper No. 03556. NACE International, Houston.
- Ilhan-Sungur, E., Ozuolmez, D., Çotuk, A., Cansever, N., Muyzer, G., 2017. Isolation of a sulfide-producing bacterial consortium from coolingtower water: Evaluation of corrosive effects on galvanized steel. Anaerobe 43, 27–34.
- James, G.A., Beaudette, L., Costerton, J.W., 1995. Interspecies bacterial interactions in biofilm. The Journal of Industrial Microbiology and Biotechnology 15 (4), 237–262.
- Jan-Roblero, J., Romero, J.M., Amaya, M., Le Borgne, S., 2004. Phylogenetic characterization of a corrosive consortium isolated from a sour gas pipeline. Applied Microbiology and Biotechnology 64, 862–867.
- Jayaraman, A., Earthman, J.C., Wood, T.K., 1997. Corrosion inhibition by aerobic biofilms on SAE 1018 steel. Applied Microbiology and Biotechnology 47, 62–68.
- Lane, D.G., 1991. Nucleic acids techniques in bacterial systematic. In: Stackebrandt, E., Goodfellow, M. (Eds), Nucleic Acid Techniques in Bacterial Systematic, John Wiley and Sons, New York, 115–175.
- Lewandowski, Z., 2000. Structure and Function of Biofilms. In: Evans, L.V. (Ed.) Biofilms: Recent Advances in Their Study and Control, 1–17, Harwood Academic Publishers.
- Li, X., Duan, J., Xiao, H., Li, Y., Liu, H., Guan, F., Zhai, X., 2017. Analysis of Bacterial Community Composition of Corroded Steel Immersed in Sanya and Xiamen Seawaters in China via Method of Illumina MiSeq Sequencing. Frontiers in microbiology 8, 1737.
- Lopez, M.A., Serna, F.J.Z., Jan-Roblero, J., Romero, J.M., Hernandez-Rodriguez, C., 2006. Phylogenetic analysis of a biofilm bacterial population in a water pipeline in the Gulf of Mexico. FEMS Microbiology Ecology 58, 145–154.
- Lovley, D.R., Phillips, E.J.P., 1988. Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganes, Applied and Environmental Microbiology 54 (6), 1472–1480.
- Magot, M., Ravot, G., Campaignolle, X., Ollivier, B., Patel, B.K., Fardeau, M.L., Thomas, P., Crolet, J.L., Garcia, J.L., 1997. *Dethiosulfovibrio peptidovorans* gen. Nov., sp. Nov., a new anaerobic, slightly halophilic, thiosulfate-reducing bacterium from corroding offshore oil wells. International Journal of Systematic and Evolutionary Microbiology 47, 818–824.
- Methods of general bacteriology: in three volumes (Metodyi obschey mikrobiologii), 1984. Gerhardt, F. et al. (Ed.), 3, Mir, Moscow. (in Russian).
- Monroy, O.A.R., Gayosso, M.J.H., Ordaz, N.R., Olivares, G.Z., Ramírez, C.J., 2011. Corrosion of API XL 52 steel in presence of *Clostridium celerecrescens*. Materials and Corrosion 62 (9), 878–883.
- Neria-Gonzalez, I., Wang, E.T., Ramirez, F., Romero, J.M., Hernandez-Rodriguez, C., 2006. Characterization of bacterial community associated to biofilms of corroded oil pipelines from the southeast of Mexico. Anaerobe 12, 122–133.
- Nnabuk Eddy Okon, 2010. Fermentation product of *Streptomyces griseus* (albomycin) as a green inhibitor for the corrosion of zinc in H₂SO₄. Green Chemistry: Letters and Reviews 3 (4), 307–314.

- Nuňez, M., 2007. Prevention of metal corrosion: new research. Nova Science Publishers, Inc., New York, pp. 310.
- Okabe ,S., Odagiri, M., Ito, T., Satoh, H., 2007. Succession of sulfur-oxidizing bacteria in the microbial community on corroding concrete in sewer systems. Applied and Environmental Microbiology 73, 971–980.
- Oliveira, V.M., Lopes-Oliveira, P.F., Passarini, M.R.Z., Menezes, C.B.A., Oliveira, W.R.C., Rocha, A.J., Sette, L.D., 2011. Molecular analysis of microbial diversity in corrosion samples from energy transmission towers. Biofouling 27 (4), 435–447.
- Pacheco da Rosa, J., Korenblum, E., Franco-Cirigliano, M.N., Abreu, F., Lins, U., Soares, R.M.A., Macrae, A., Seldin, L., Coelho, R.R.R., 2013. *Streptomyces lunalinharesii* Strain 235 Shows the Potential to Inhibit Bacteria Involved in Biocorrosion Processes. Hindawi Publishing Corporation BioMed Research International, Article ID 309769.
- Pacheco da Rosa, J., Tiburcio, S.R.G., Marques, J.M., Seldin, L., Coelho, R.R.R., 2016. *Streptomyces lunalinharesii* 235 prevents the formation of a sulfate-reducing bacterial biofilm. Brazilian journal of microbiology 47, 603–609.
- Pavissich, J.P., Vargas, I.T., Gonzalez, B., Pasten, P.A., Pizarro, G.E., 2010. Culture dependent and independent analyses of bacterial communities involved in copper plumbing corrosion. Journal of Applied Microbiology 109, 771–782.
- Pope, D.H., Duquette, D.J., Johannes, A.H., Wayner, P.C., 1984. Microbially influenced corrosion of industrial alloys. Materials Performance 23 (4), 14–15.
- Pilyashenko-Novokhatny, A.I., 2000. Possible distribution of functions between the components of corrosion-hazardous aggregates of microorganisms in the general process of microbially induced corrosion (Mozhlyvyi rozpodil funktsii mizh skladovymy koroziinonebezpechnymy sukupnostiamy mikroorhanizmiv v zahalnomu protsesi mikrobno indukovanoi koroziii). Materials IV International. Conference-exhibitions "Problems of corrosion and anticorrosion. Protection of materials" (Corrosion-200). G.V. Karpenko Physical-Mechanical Institute of the National Academy of Sciences of Ukraine, Lviv. 564–567. (in Ukrainian)
- Plohinskij, N.A., 1970. Biometrics (Biometriya). Izdatel'stvo Moskovskogo universiteta, Moskva. (in Russian)
- Purish, L.M., Asaulenko, L.G., 2007. Dynamics of succession changes in sulfidogenic microbial association under conditions of biofilm formation on the surface of steel. Mikrobiologii Zhurnal 69 (6), 19-25. (in Ukrainian with English summary)
- Purish, L.M., Asaulenko, L.G., Ostapchuk, A.M., 2009. Features of development of mono- and associative cultures of sulfate-reducing bacteria and formation of exopolymer complex. Mikrobiologii Zhurnal 71 (2), 20–26. (in Ukrainian with English summary)
- Qiu, Y.-Y., Guo, J.-H., Zhang, L., Chen, G.-H., Jiang, F., 2017. A highrate sulfidogenic process based on elemental sulfur reduction: cost-effectiveness evaluation and microbial community analysis. Biochemical Engineering Journa 128, 26–32.

- Rajasekar, A., Ting, Y.-P., 2010. Microbial Corrosion of Aluminum 2024 Aeronautical Alloy by Hydrocarbon Degrading Bacteria Bacillus cereus ACE4 and Serratia marcescens ACE2. Industrial & Engineering Chemistry Research 49, 6054–6061.
- Romanenko, V.I., Kuznetsov, S.I., 1974. Ecology of microorganisms of fresh reservoirs (Ekologiya mikroorganizmov presnyih vodoemov), Nauka, Leningrad. (in Russian).
- Salgar-Chaparro, S.J., Darwin, A., Kaksonen, A.H., Machuca, L.L., 2020. Carbon steel corrosion by bacteria from failed seal rings at an offshore facility. Scientific reports 10 (1), 12287. https://doi. org/10.1038/s41598-020-69292-5
- Salgar-Chaparro, S.J., Silva-Plata, B.A., 2008. Caracterizacion de la comunidad microbiana residente en aguas de produccion de tres campos de explotacion petrolera, con especial enfasis en grupos asociados a procesos corrosivos. Proyecto. Universidad Industrial de Santander. (in Spanish with English summary)
- Satoh, H., Odagiri, M., Ito, T., Okabe, S., 2009. Microbial community structures and in situ sulfate-reducing and sulfur-oxidizing activities in biofilms developed on mortor specimens in a corroded sewer system. Water Research 43, 4729–4739.
- Stahl, D.A., Lane, D.I., Olsen, G.L., Pace, N.R., 1984. Analysis of hydrothermal vent-associated symbionts by ribosomal RNA sequences. Science, 224, 409–411.
- Su, H., Mi, Sh., Peng, X., Han, Y., 2019. The mutual influence between corrosion and the surrounding soil microbial communities of buried petroleum pipelines. RSC Advances 9, 18930–18940.
- Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30 (12), 2725–2729.
- Tkachuk, N., Zelena, L., Mazur, P., Lukash, O., 2020. Genotypic, physiological and biochemical features of *Desulfovibrio* strains in a sulfidogenic microbial community isolated from the soil of ferrosphere. Ecological questions 31 (2), 79–88.
- Tkachuk, N.V., Zelena, L.B., Parmynska, V.S., Yanchenko, V.O., Demchenko, A.M., 2017. Identification of heterotrophic soil ferrosphere bacteria and their sensitivity to the pesticide linuron, Mikrobiologii Zhurnal 9 (4), 75–87. (in Ukrainian with English summary).
- Vaschenko, I.M., Lange, K.P., Merkulov, M.P., 1982. Workshop on the basics of rural farming (Praktikum po osnovam selskogo hazyaystva), Prosveschenie, Moskva. (in Russian).
- Vincke, E., Boon, N., Verstraete, W., 2001. Analysis of the microbial communities on corroded concrete sewer pipes – a case. Applied Microbiology and Biotechnology 57, 776–785.
- Wang, Y.S., Liu, L., Fu, Q., Sun, J., An, Z.Y., Ding, R., Li, Y., Zhao, X.D., 2020. Effect of *Bacillus subtilis* on corrosion behavior of 10MnNiCrCu steel in marine environment. Scientific Reports 10, 5744. http://dx.doi.org/10.1038/s41598-020-62809-y
- Zhu, X., Lubeck, J., Kilbane II J.J., 2003. Characterization of microbial communities in gas industry pipelines. Applied and Environmental Microbiology 69, 5354–5363.