PROKARYOTIC DIVERSITY OF THE AQUATIC ECOSYSTEM LA IZVOR (CHISINAU MUNICIPALITY) AND ITS INDUSTRIAL POTENTIAL

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Abstract: Microbial diversity and enzymatic activity of bacterial strains isolated from water samples, bottom sediments and biofilms of lake were investigated and identified by PCR and sequencing of the 16S rRNA gene. Screening procedures included decimal dilutions performing with incubation at 37°C, isolation of pure non-pathogenic bacterial cultures, isolate cultivation on agar in Petri plates at 30°C and tests on enzyme production. Enzymatic activity of 65 selected strains (*Bacillus*, *Planococcus*, *Micrococcus*, *Paenibacillus*, *Arthrobacter*, *Lysinibacillus*, *Bhargavaea*, *Peribacillus*, *Kocuria*) was determined used tests on plates and by spectrophotometric approach. The isolates found to be a promising candidate for agriculture, textile, food and pharmaceutical application by showed enzyme-producing of amylase, lipase, cellulase, especially catalase.

Keywords: lake; bacteria; identification; enzymes; production; spectrophotometric assay

INTRODUCTION

Microorganisms have been used for a long time as an endless and invaluable source of useful compounds. Screening of new strains of microorganisms with high production of biologically active substances is drawing attention and represents important research tendency in the world. Evaluation the impact of environmental factors on microbial composition of water ecosystem depends of inorganic (salts, mineral acids, metal compounds, sulfates, cyanides, chemical waste) and organic water pollutants include bacteria and pollutants from wastewater, fertilizers, pesticides, agricultural substances, forestry, food processing industry. Microorganisms that have adapted to water quality and environment conditions such as: temperature, pH, biological and dissolved oxygen, carbon dioxide, content of organic and mineral substances are able to produce unique secondary metabolites [12, 31].

For example, in marine sediments were investigated the diversity of industrial enzyme-producing marine bacteria, isolates possessed abilities to produce amylase, lipase and protease. Identified strains belong to phyla *Proteobacteria*, *Firmicutes Bacteroidetes* and genera *Bacillus, Cobetia, Halomonas, Pseudomonas, Psychrobacter, Myroides, Planococcus, Sporosarcina, Wangia* [8, 9, 23, 46, 48].

Also it well known that hot springs isolates also like marine bacteria are able to produce a wide range of industrial enzymes and are considered as one of the main producers of α-amylase, α-glucosidase, α-galactosidase, cellulases, chitinase, lipase, protease. Some of these strains have been identified as *Aeromonas* sp., *Alteromonas* sp., *Arthrobacter* sp., *Clostridium* sp., *Enterobacter* sp., *Flavobacterium* sp., *Pseudomonas* sp., *Psychrobacter* sp., *Streptomyces* sp., *Vibrio* sp., *Marinobacter* sp. and *Bacillus* sp. [2, 12, 15, 45].

But not only bacteria of marine or hot springs origin synthesized valuable metabolites. Kingdom Bacteria are dominant in the microbial diversity of freshwater and hydrologically closed lakes - ecosystems rich in the resources and conditions for microbial growth, identified as *Proteobacteria*, *Firmicutes*, *Actinobacteriota*, *Cyanobacteria*, *Acidobacteriota*, *Bacteroidota*, *Deinococcota* etc. [20].

Bacterial strains, special *Bacillus* sp. isolated from lake have ability to synthesis various extracellular enzymes like α-amylase and proteases, particular alkaline proteases which are wide applied in modern food biotechnology bakery, egg yolk industry and refinement of vegetable oils [22]. From lake Khubsugul and thermal springs of the Baikal region researcher Suslov identified strains *Bacillus* sp. with high biochemical activity of the protease, amylase, lipase, phosphatase and phospholipase enzymes [35].

According to the modern scientists bacterial enzymes can be potential used in bioremediation, in degradation of organic and non-organic pollutants, natural and synthetic polymers, and also in processes of sterilization in medicine and bleaching in textile industry. Bacterial catalases, important for protecting the cell from oxidative damage [11, 33], due to purification and biochemical properties are able to be applied in process of biodegradation as a substitute for toxic chlorate compounds [5, 44]. Sediment soil microorganisms in lakes able to carry out biodegradation of contaminant compound, such as polycyclic aromatic hydrocarbons [25]. Obtaining the active and stable microbial enzymes for purification would be safe, effective and practical, because food (as an indicator of the milk quality for mastitis) and plastic waste remain a problem [4, 31, 36].

It is important to note that in addition to industrial implementation of bacterial enzymes, researchers identified potential of aquatic bacteria with high antagonistic activity due to developed enzymatic complex, and their potential is quite broad and promising from the food industry (used in dairy products, baking, beverages) to therapeutic drugs [34,

41, 47]. Babich et al. identified *Pseudomonas* sp., *Bacillus* sp., *Micrococcus* sp. and *Acinetobacter* sp. representatives from Baikal lake, which metabolites being bacteriocins of broad spectrum and are able to antibacterial effect on microorganisms pathogenic for humans such as: *Salmonella, Escherichia coli, Bacillus cereus, Enterococcus faecalis, Candida albicans, Staphylococcus aureus* [3].

Our research was carried out to isolate from aquatic environment of lakes from La Izvor park (Chisinau) new strains of enzyme-producing bacteria, potential for antifungal and antibacterial activity promising for agriculture and other application.

MATERIAL AND METHODS

To investigate the prokaryotic diversity the water, bottom sediments and biofilms samples were collected in sterile bottles, delivered to the laboratory for microbiological analyses. Samples were collected from three united wide water basins. The geographical coordinates of collected samples were: 1) 47°02'49.9"N, 28°47'10.4"E; 2) 47°02'44.2"N, 28°47'18.9"E; 3) 47°03'00.2"N, 28°47'26.0"E; 4) 47°02'53.7"N, 28°47'42.5"E; 5) 47°02'59.6"N, 28°47'59.3"E (Fig. 1).

In our studies we used method of inoculation on medium as nutrient, selective, indicator, with different compositions [32, 43]. A total of 213 bacterial strains were isolated from water, bottom sediments and biofilms samples at the lakes of La Izvor park, of which 148 being pathogenic were eliminated from research. Isolates were identified based on the morphological, cultural and biochemical features using different tests. Morphological and tinctorial features were investigated at microscopy OPTIKA B-510PH.

Bacterial isolates were screened using agar plates for testing amylase, catalase, celullase and lipase production, respectively. After incubation at 30°C for 24-48 hours, starch hydrolysis was determined by flooding the plates with iodine solution. For cellulase-producing strains was used solid media containing carboxymethylcellulose. Lipid hydrolysis of the isolates was screened using agar medium with twin-80. The promising isolates showing zones of clearance around the colonies indicating enzyme producing were selected for further study. For measuring catalase activity were used qualitative analysis by glass-drop test and quantitative analysis by spectrophotometric and colorimetric approach. Quantitative assay at wavelength 240 nm is based on the ability of hydrogen peroxide to interact with ammonium molybdate forming a stable colored complex [17]. Catalase activity was expressed both in µKat/L and mmoles of decomposed H_2O_2 /min/mg protein. The protein content was determined according to Lowry's method [24].

The bacterial identification was determined by amplification and sequencing of the 16S rRNA. The Polymerase Chain Reaction (PCR) conditions included denaturation for 5 min at 96°C, 40 cycles of 30 min at 96°C, 30 s at 50°C, 2 min at 72°C and the final extension for 5 min at 72°C using (AGAGTTTGATCMTGGCTCAG) and 1100R (GGGTTGCGCTCGTT) primers. Thereafter PCR products were cleaned with alcohol following the manufacturer instructions and were sequenced in forward directions using Applied Biosystems model 3500 Dx Genetic Analyzer and Advanced SEQ SupreDye Cycle Sequencing Kit.

The calculation of the statistical indices was performed using the MS Excel 2010 software. All experiments were carried out in triplicate and data was expressed as mean ± standard deviation (SD). A p-value <0.05 was considered statistically significant.

RESULTS

From two hundred thirteen bacterial strains isolated from water, bottom sediments and biofilms samples 65 strains were selected for further investigations, 148 were pathogenic microorganisms. The primary identification of bacterial strains was based on colony and cell morphology, appearance and Gram staining.

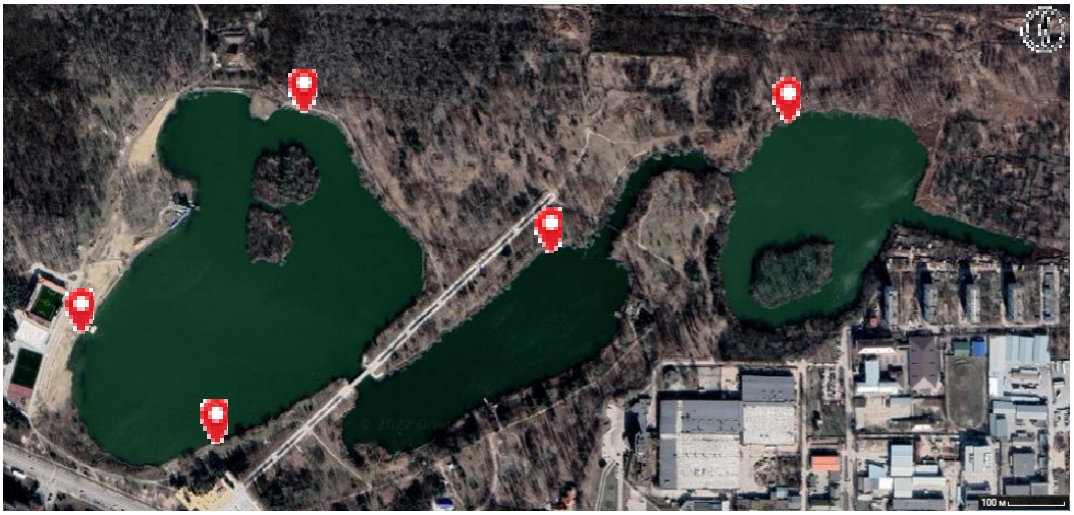

Figure 1. Location of the La Izvor park and lakes on the map Chisinau city, Republic of Moldova (source: Google Maps)

The microscopic examination showed spherical and rod-shape cells, Gram positive and most of Gram positive are Gram-variable strains. By Gram-staining were identified Gram-positive strains *Bacillus* sp., *Kocuria* sp., *Micrococcus* sp., *Planococcus* sp., *Bhargavaea* sp., where some strains of *Micrococcus* sp. and *Planococcus* sp. were Gram-variable, *Paenibacillus* sp., *Peribacillus* sp. are also Gram-variable, Gram-negative *Lysinibacillus* sp., *Arthrobacter* sp. It has been shown that all selected strains grow well in the nutrient agar medium, showed colonies of different forms and colors after incubation at 37°C for 24-48 h (Fig. 2).

The colony morphology characteristics included forms, irregular for *Bacillus* spp., *Peribacillus* spp., *Planococcus* spp. strains, circular to the rest, as well as differences in their margins such as wavy of *Lysinibacillus* spp. or undulate of *Bacillus* spp. The color of the isolates ranged from cream-white (*Arthrobacter* spp., *Bacillus* spp., *Paenibacillus* spp.), cream-yellow (*Planococcus* spp., *Bhargavaea* spp., *Kocuria* spp., *Peribacillus* spp., *Lysinibacillus* spp.,) and yellow (*Micrococcus* spp.). Their sizes ranged from small (*Arthrobacter* spp., *Bhargavaea* spp., *Micrococcus* spp., *Paenibacillus* spp.) to medium and large (*Bacillus* spp., *Peribacillus* spp., *Lysinibacillus* spp.).

The results of identification based on cultural and morphological properties were supplemented after study of biochemical characteristics as well as genetic analysis.

Isolates were screened to evaluate their ability to produce enzymes amylase, catalase, lipase and cellulase. Determination of enzymatic activity in the studied strains of bacteria isolated from lakes showed that they are able to synthesis extracellular enzymes. There was only one strain which did not produce no one of the targeted enzymes. Based on the clear zone production on agar plate, out of 65 bacteria isolates, 37 (56.9%) bacterial strains were able to produce amylase, 30 (46.1%) strains – cellulase, 23 (35.3%) bacteria were lipase producers and 59 (90.7%) strains produced catalase using qualitative analysis, bubbles clearly indicated a catalase positive result. Thirty-four isolates (52.3%) were able to produce three or four enzymes, (most from water and bottom sediments samples), while 14 (21.5%) strains were able to produce only one enzyme (mainly it were bacteria isolated from biofilms).

Bacillus spp., *Paenibacillus* spp., *Peribacillus* spp. and *Planococcus* spp. being isolated from water, were identified as more active strains for amylase, catalase and celullase production, for catalase production – strains from bottom sediments and biofilms. *Kocuria* spp., *Bacillus* spp. and *Micrococcus* spp. isolated from biofilms were characterized active lipase-producers. It is important to note that identified strain of *Bhargavaea* sp. was characterized with active enzymatic features at complex to four enzymes amylase, catalase, lipase and cellulase. Furthermore, hydrolytic bacterial enzymes of selected strains demonstrated antagonistic properties against *E. coli*

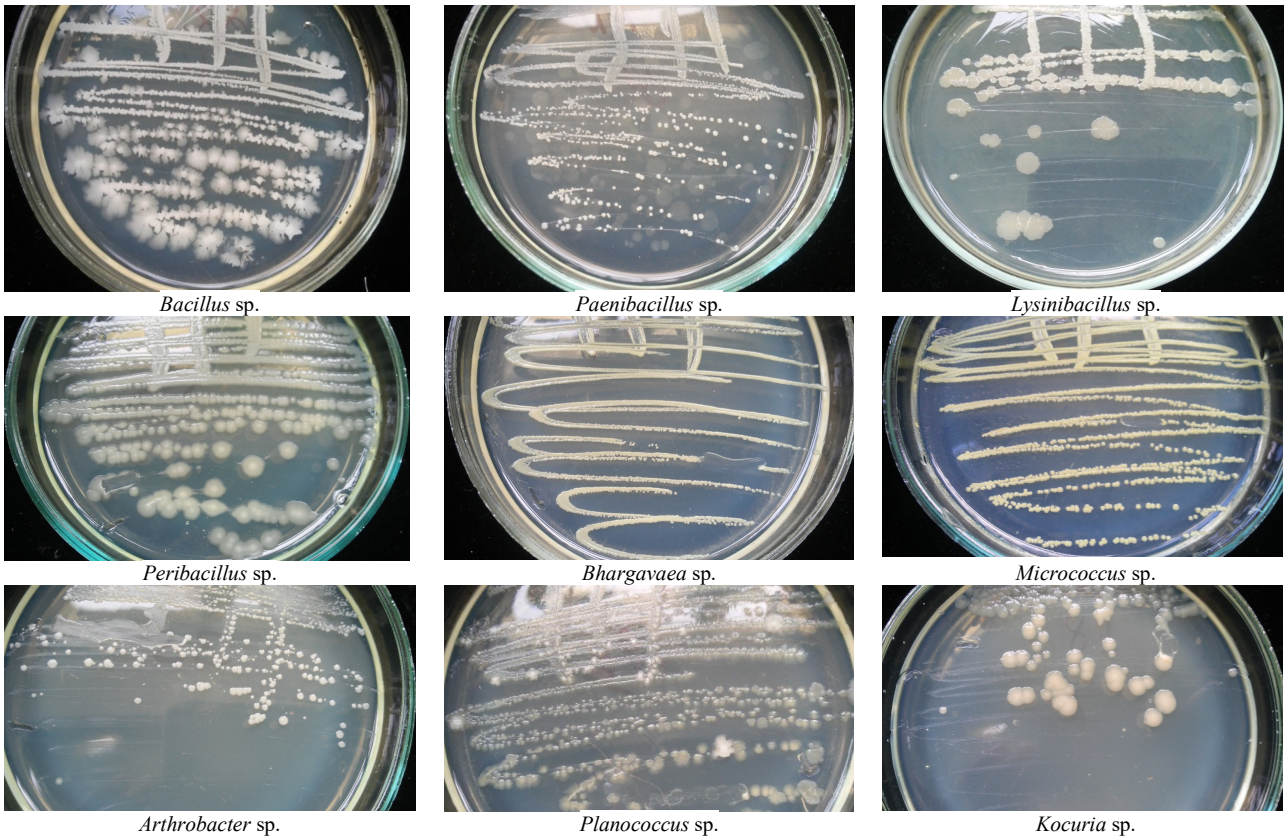

Figure 2. Morphological and cultural properties of isolated strains on agar plate

and phytopathogens *Fusarium* sp., *Alternaria* sp., *Botrytis* sp., *Aspergillus* sp. what is important for various biotechnological applications.

Thus, selected species isolated in pure cultures were molecularly identified by amplification and Sanger sequencing of the 16S rRNA. According to the analysis of sequencing results, some of isolated strains had the same 16S rRNA sequence. In this way, identified strains belong to nine different bacterial species (Table 1).

The most prevalent of the isolated bacterial species were *Bacillus* spp. strains predominant in samples collected from water, bottom sediments and biofilms. *Peribacillus* spp. and *Paenibacillus* spp. strains were widespread from water and bottom sediments, and *Kocuria* spp. from water and biofilms. Worth noting that lake samples demonstrated habitat specific bacterial communities, the number and diversity of bacteria were different - strains belonging to the genera *Planococcus* and *Lysinibacillus* were successfully identified only from water samples, genera *Arthrobacter* and *Bhargavaea* only from bottom sediments and *Micrococcus* spp. only from biofilms.

Bacterial diversity in Lake of La Izvor park based on the 16S rRNA gene sequences suggested twenty the most active and perspective bacteria to replenish the National Collection of Non-pathogenic Microorganisms (NCNM) of the Institute of Microbiology and Biotechnology of Technical University of Moldova.

The determination of catalase activity was carried out in the log phase of growth, after 48 hours of cultivation on the nutrient agar medium, this is the phase in which the increase cell mass can be determined quantitatively. Catalase is an important antioxidant enzyme that prevent cell oxidative damage, industrial demand for this enzyme is growing due to ability to degrading hydrogen peroxide to water and oxygen [17, 18]. Thus, quantitative determination of catalase activity in cell biomass of 20 active microorganisms showed that 3 strains have higher catalase activity, *Bacillus safensis* CNMN-BB-26 228,94 mmol/min/mg protein, *Pseudomonas poae* CNMN-Ps-09 270,16 mmol/min/mg protein and 380,68 mmol/min/mg protein to *Bacillus velezensis* CNMN-BB-14 (Table 2).

According to the data, *Peribacillus* strains showed high catalase activity per liter of biomass varied between 38.52-41.54 µKat/L, but because the protein content is low this index relatived to mmol/min/mg protein was lower, respectively.

DISCUSSION

The results obtained in this study are consistent with the literature, which also noted that natural microbial community is the most important reservoir of potential microbial resources and its valuable components. For example, the study reported by Yadav A.N. and colleagues demonstrated that prokaryotic

Table 1. Prokaryotic diversity of the aquatic ecosystem La Izvor

Water	Bottom sediments	Biofilms
Bacillus spp.	*Bacillus* spp.	*Bacillus* spp.
Peribacillus spp.	*Peribacillus* spp.	*Kocuria* spp.
Paenibacillus spp.	*Paenibacillus* spp.	*Micrococcus* spp.
Kocuria spp.	*Arthrobacter* spp.	
Planococcus spp.	*Bhargavaea* sp.	
Lysinibacillus spp.	*Pseudomonas* sp.	

Table 2. Catalase activity and protein content of bacterial strains isolated from the aquatic ecosystem La Izvor and deposited in NCNM

Strains	Sampling site	Catalase activity, µKat/L	Protein content, % ADB	Catalase activity, mmol/min/mg protein
Pseudomonas poae CNMN-Ps-09	bottom sediments	34.52±1.52	43.48±0.40	271.42±2.46
Bacillus velezensis CNMN-BB-12	water	37.76±1.67	10.52±0.20	70.79±1.35
Bacillus velezensis CNMN-BB-13	water	33.17±1.46	20.02±0.79	117.11±4.73
Bacillus velezensis CNMN-BB-14	bottom sediments	47.97±2.12	44.08±1.19	375.45±4.27
Bacillus velezensis CNMN-BB-15	bottom sediments	32.57±1.44	17.85±0.69	103.13±4.07
Bacillus velezensis CNMN-BB-16	bottom sediments	31.35±1.38	20.58±0.30	116.95±1.68
Bacillus velezensis CNMN-BB-17	biofilms	36.74±1.62	21.84±0.20	145.77±1.31
Bacillus velezensis CNMN-BB-18	biofilms	28.97±1.28	14.86±0.40	76.45±2.07
Micrococcus yunnanensis CNMN-BM-19	biofilms	32.59±1.44	27.10±1.19	162.52±3.98
Micrococcus yunnanensis CNMN-BM-20	biofilms	0.36±0.02	33.57±0.20	2.14±0.01
Paenibacillus pabuli CNMN-BPp-21	water	11.66±0.51	24.87±0.79	51.33±1.66
Planococcus ruber CNMN-BP-22	water	28.62±1.26	30.94±1.39	155.72±3.15
Peribacillus simplex CNMN-BP-23	water	41.54±1.83	10.06±0.30	76.35±2.22
Planococcus chinensis CNMN-BP-24	water	35.01±1.55	19.97±0.69	123.61±4.37
Bacillus safensis CNMN-BB-25	water	23.64±1.04	27.40±0.59	115.32±2.53
Bacillus safensis CNMN-BB-26	water	48.20±2.13	26.39±0.20	228.06±1.72
Bacillus safensis CNMN-BB-27	bottom sediments	0.09±0.02	30.74±0.59	0.49±0.02
Peribacillus simplex CNMN-BP-28	bottom sediments	38.52±1.70	13.70±0.30	96.04±2.06
Bacillus rugosus CNMN-BB-29	bottom sediments	28.17±1.24	26.69±0.79	133.31±4.02
Micrococcus aloeverae CNMN-BM-30	biofilms	0.09±0.02	27.05±0.89	0.43±0.02

organisms isolated from lake ecosystems have been identified as *Actinobacteria*, *Cyanobacteria*, *Acidobacteria*, *Firmicutes*, *Proteobacteria* phylum, and freshwater bacterial representatives belonging to predominant genera such as *Arthrobacter*, *Bacillus*, *Lysinibacillus*, *Methylobacter*, *Paenibacillus*, *Planococcus*, *Proteus*, *Pseudomonas*, *Rhodococcus*, *Staphylococcus*, *Streptomyces*, etc. [42]. Authors highlight industrial potential of these strains as a source of active enzyme substances, for example, *Planococcus* sp., isolated from the deep-sea mud, have the ability to synthesize an extracellular protease essential for applications in detergent industry [7]. *Arthrobacter* species which have the ability to nitrate reduction [6, 14] and *Lysinibacillus* is well-known for its insecticidal activity against various insects, also as biostimulant and an agent in effective bioremediation [1, 19].

Aquatic bacterial isolates with antifungal activity through the production of metabolites such as extracellular lytic enzymes, siderophores, salicylic acid, antibiotics were studied and proved by many scientists. It is known that such industrial enzymes are important for various biotechnological applications, also extracellular hydrolytic bacterial enzymes effect on fungal growth inhibition, for example, genus *Bacillus* are showing high level of cellulolytic activity with antifungal activity against phytopathogens [21, 27, 28, 30, 38].

According to the literature, secondary metabolites of *Bacillus velezensis* isolated from Lake Bogoria can be used as potential biocontrol agents against mycelial inhibition of *Fusarium solani* strains [40]. Molecular weight oligopeptides isolated from metabolites produced by *Pseudomonas*, *Bacillus*, *Micrococcus* and *Acinetobacter* representatives, have a broad spectrum of antimicrobial activity and were identified as bacteriocins [3]. Moreover, from Qurugol Lake a new strain of *Tabrizicola aquatica* have been isolated and identified with considerable antagonistic effect against *Escherichia coli*, *Rhizobium radiobacter*, *Pseudomonas syringae*, *Erwinia amylovora*, *Botrytis cinerea* and *Fusarium oxysporum* [37].

Ecology and agriculture needs in the development of new eco-friendly methodologies based on biological sources. There are a lot of scientific results proved of different microorganisms (and theirs metabolites) application as control agents and as a solution for inhibition of the phytopathogens growth for the reason that often using of pesticides follow to pollution and chemical compounds accumulation in plants, lethal impact to beneficial soil organisms and negative to consumer health.

Bacillus strains (reclassification of *Bacillus beijingensis* strain as *Bhargavaea beijingensis*) [39] have a lot of benefits for new biotechnologies because are unlimited source of proteins, substances for plant growth and enzymes [10, 13]. So, for example, catalase is an intracellular enzyme discovered in all aerobic bacteria and in most facultative anaerobes and it prevents not only cell oxidative damage, but also plays an important role in medicine (cancer treatment, diabetes, atherosclerosis, as a protein therapeutic agent for the treatment of diabetes, Alzheimer's disease, Parkinson's disease, vitiligo), food industry (products with a long shelf life, immobilized enzyme, etc.) [26, 29], but also in bioremediation. Hydrogen peroxide (H_2O_2) widely used as a bleaching agent for natural and synthetic textiles, as cleaning solution for semiconductor industry. Such industrial wastewater characterized by the presence of toxic impurities and chemicals affected to the health of plants, animals, humans and must be treated to eliminate. Treatment with chemicals has a number of disadvantages, due to toxicity it also damages the activated sludge. The approach involved the enzymatic (catalase) degradation of H_2O_2 is a safer alternative, it has the capacity to decompose more than one million molecules of hydrogen peroxide, per molecule of enzyme. Bacterial catalase and rest microbial enzymes are preferably source than enzymes of plant or animal origin due to their cost-effective, easy, catalytic activity, stability [4, 16].

Experiments have shown that bacterial strains isolated from the aquatic ecosystem of lake from the genera *Pseudomonas*, *Bacillus, Planococcus* and *Peribacillus* were identified as active catalase-producing bacteria, also showed ability to inhibit the growth of test microorganisms *E. coli, Fusarium*, *Alternaria*, *Botrytis*, *Aspergillus*. Our data are in accordance with the literature, according to which it results that the representatives of *Bacillus* sp., *Peribacillus* sp., *Planococcus* sp., *Paenibacillus* sp., *Micrococcus* sp., *Lysinibacillus* sp., *Arthrobacter* sp., *Bhargavaea* sp., *Pseudomonas* sp. are perspective for biotechnological applications due to synthesis of valuable metabolites with utility in different industries: food, textil, pharmaceutical industry, agriculture, ecology, etc.

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REFERENCES

[1] Ahsan, N., Shimizu, M., (2021): *Lysinibacillus* species: their potential as effective bioremediation, biostimulant, and biocontrol agents. Reviews in Agricultural Science, 9: 103-116.

[2] Alrumman, S., Mostafa, Y.S.M., Al-Qahtani, S., Taha, T.H.T., (2018): Hydrolytic enzyme production by thermophilic bacteria isolated from Saudi hot springs. Open Life Sciences, 13: 470-480.

[3] Babich, O., Shevchenko, M., Ivanova, S., Pavsky, V., Zimina, M., Noskova, S., Anohova, V., Chupakhin, E., Sukhikh, S., (2021): Antimicrobial potential of microorganisms isolated from the bottom sediments of lake Baikal. Antibiotics (Basel), 10(8): 927-948.

[4] Bhandari, S., Poudel, D.K., Marahatha, R., Dawadi, S., Khadayat, K., Phuyal, S., Shrestha, S., Gaire, S., Basnet, K., Khadka, U., Parajuli, N., (2021): Microbial enzymes used in bioremediation. Journal of Chemistry, 2021: 1-17.

[5] Brown-Peterson, N.J., Salin, M.L., (1993): Purification of a catalase-peroxidase from *Halobacterium halobium*: characterization of some unique properties of the halophilic enzyme. Journal of Bacteriology, 175(13): 4197-4202.

[6] Bukin, S.V., Pavlova, O.N., Manakov, A.Y., Kostyreva, E.A., Chernitsyna, S.M., Mamaeva, E.V., Pogodaeva, T.V., Zemskaya, T.I., (2016): The ability of microbial community of lake Baikal bottom sediments associated with gas discharge to carry out the transformation of organic matter under thermobaric conditions. Frontiers in Microbiology, 10(7): 690.

[7] Chen, K., Mo, Q., Liu, H., Yuan, F., Chai, H., Lu, F., Zhang, H., (2018): Identification and characterization of a novel cold-tolerant extracellular protease from *Planococcus* sp. CGMCC 8088. Extremophiles, 22(3): 473-484.

[8] Cheng, T.H., Ismail, N., Kamaruding, N., Saidin, J., Danish-Daniel, M., (2020): Industrial enzymes-producing marine bacteria from marine resources. Biotechnology reports (Amsterdam, Netherlands), 27: e00482.

[9] Dang, H., Zhu, H., Wang, J., Li T., (2009): Extracellular hydrolytic enzyme screening of culturable heterotrophic bacteria from deep-sea sediments of the southern Okinawa Trough. World Journal of Microbiology and Biotechnology, 25(1): 71-79.

[10] Danilova, I., Sharipova, M., (2020): The practical potential of *Bacilli* and their enzymes for industrial production. Frontiers in Microbiology, 11: 1782.

[11] Dantas, A.S., Andrade, R.V. de Carvalho, M.J., Felipe, M.S.S., Campos, É.G., (2008): Oxidative stress response in *Paracoccidioides brasiliensis*: assessing catalase and cytochrome c peroxidase. Mycological Research, 112(6): 747-756.

[12] El-Hassayeb, H.E.A., Abdel-Aziz, S.M.Z., (2016): Screening, production and industrial application of protease enzyme from marine bacteria. International Journal of Current Microbiology and Applied Sciences, 5(7): 863-874.

[13] Errington, J., Aart, L.T.V., (2020): Microbe profile: *Bacillus subtilis*: model organism for cellular development, and industrial workhorse. Microbiology (Reading), 166(5): 425-427.

[14] Eschbach, M., Möbitz, H., Rompf, A., Jahn, D., (2003): Members of the genus *Arthrobacter* grow anaerobically using nitrate ammonification and fermentative processes: anaerobic adaptation of aerobic bacteria abundant in soil. FEMS Microbiology Letters, 223(2): 227-230.

[15] Fulzele, R., Desa, E., Yadav, A., Shouche, Y., Bhadekar, R., (2011): Characterization of novel extracellular protease produced by marine bacterial isolate from the Indian Ocean. Brazilian Journal of Microbiology, 42(4): 1364-1373.

[16] Gan, J.S., Ashraf, S.S., Bilal, M., Iqbal, H.M.N., (2022): Biodegradation of environmental pollutants using catalase-based biocatalytic systems. Environmental Research, 214(2): 113914.

[17] Hadwan, M.H., (2018): Simple spectrophotometric assay for measuring catalase activity in biological tissues. BMC Biochemistry, 19(1): 7.

[18] Kauldhar, B.S., Sooch, B.S., (2016): Tailoring nutritional and process variables for hyperproduction of catalase from a novel isolated bacterium *Geobacillus* sp. BSS-7. Microbial Cell Factories 15(1): 7.

[19] Kämpfer, P., Martin, K., Glaeser, S.P., (2013): *Lysinibacillus contaminans* sp. nov., isolated from surface water. International Journal of Systematic and Evolutionary Microbiology, 63(9): 3148-3153.

[20] Kiama, C.W., Njire, M.M., Kambura, A.K., Mugweru, J.N., Matiru, V.N., Wafula, E.N., (2021): Isolation and characterization of bacteria from lakes Olbolosat and Oloiden, Kenya. African Journal of Microbiology Research, 15: 1-19.

[21] Khan, N., Martínez-Hidalgo, P., Ice, T.A., Maymon, M., Humm, E.A., Nejat, N., Sanders, E.R., Kaplan, D., Hirsch, A.M., (2018): Antifungal activity of *Bacillus* species against *Fusarium* and analysis of the potential mechanisms used in biocontrol. Frontiers in Microbiology, 9: 2363.

[22] Kiersztyn, B., Siuda, W., Chróst, R.J., (2012): Persistence of bacterial proteolytic enzymes in lake ecosystems. FEMS Microbiology Ecology, 80(1): 124-134.

[23] Li, Y., Wu, C., Zhou, M., Wang, E.T., Zhang, Z., Liu, W., Ning, J., Xie, Z., (2017): Diversity of cultivable protease-producing bacteria in Laizhou Bay sediments, Bohai Sea, China. Frontiers in Microbiology, 8: 405.

[24] Lowry, O., Roseboug, N., Lewis Farr, A. Randall, R. (1951): Protein measurement with the folin phenol reagent. Journal of Biological Chemistry, 193(1): 256-275.

[25] McNally, D.L., Mihelcic, J.R., Lueking, D.R., (1998): Polycyclic aromatic hydrocarbon degrading microorganisms in Great Lakes sediments. Journal of Great Lake Research, 24(2): 392-403.

[26] Motta, J.F.G, Freitas, B.C.B., Almeida, A.F., Martins, G.A.S., Borges, S.V., (2023): Use of enzymes in the food industry: a review. Food Science and Technology, Campinas, 43: e106222.

[27] Muhamad, A.K., Shakhatreh, J.H.J., Emad, I.H., Majed, M.M., Safwan, M.O., Abdul-salam, F.J., Mutaz, A.A., (2017): Microbiological analysis, antimicrobial activity, and heavy-metals content of Jordanian Ma'in hot-springs water. Journal of Infection and Public Health, 10(6): 789-793.

[28] Mulamattathil, S.G., Bezuidenhout, C., Mbewe, M., Ateba, C.N., (2014): Isolation of environmental bacteria from surface and drinking water in Mafikeng, South Africa, and characterization using their antibiotic resistance profiles. Journal of Pathogens, 2014: 371208.

[29] Nandi, A., Yan, L.G, Jana, C.K., Das, N., (2019): Role of catalase in oxidative stress-and age-associated degenerative diseases. Oxidative Medicine and Cellular Longevity, 2019: 1-19.

[30] Petatán-Sagahón, I., Anducho-Reyes, M.A., Silva-Rojas, H.V., Arana-Cuenca, A., Tellez-Jurado, A., Cárdenas-Álvarez, I.O., Mercado-Flores, Y., (2011): Isolation of bacteria with antifungal activity against the phytopathogenic fungi *Stenocarpella maydis* and *Stenocarpella macrospora*. International Journal of Molecular Sciences, 12(9): 5522-5537.

[31] Raveendran, S., Parameswaran, B., Ummalyma, S.B., Abraham, A., Mathew, A.K., Madhavan, A., Rebello, S., Pandey, A., (2018): Applications of microbial enzymes in food industry. Food Technology and Biotechnology, 56(1): 16-30.

[32] Salcher, M.M., Šimek, K., (2016): Isolation and cultivation of planktonic freshwater microbes is essential for a comprehensive understanding of their ecology. Aquatic Microbial Ecology, 77(3): 183-196.
[33] Schrader, M., Fahimi, H.D., (2006): Peroxisomes and oxidative stress. Biochimica et Biophysica Acta - Molecular Cell Research, 1763(12): 1755-1766.
[34] Slanina, V., Bogdan-Golubi, N., (2021): Studierea bacteriilor izolate din namolul lacurilor parcului „La Izvor". pp. 112-114. In Slanina, V., Bogdan-Golubi, N., (eds.): Materialele ale Conferinței "Integrare prin cercetare și inovare". Ştiinţe ale naturii și exacte. Chișinău.
[35] Suslova, M., (2007): Distribution and diversity of sporeforming bacteria of the genus *Bacillus* in aquatic ecosystems. (in Russian). Manuscript of PhD thesis, Universitatea de Stat din Orientul Îndepărtat, Ulan-Ude, Republic of Buryatia, Russian Federation.
[36] Tamoor, M., Samak, N.A., Jia, Y., Mushtaq, M.U., Sher, H., Bibi, M., Xing, J., (2021): Potential use of microbial enzymes for the conversion of plastic waste into value-added products: a viable solution. Frontiers in Microbiology, 12: 777727.
[37] Tarhriz, V., Eyvazi, S., Shakeri, E., Hejazi, M.S., Dilmaghan, A., (2020): Antibacterial and antifungal activity of novel freshwater bacterium *Tabrizicola aquatica* as a prominent natural antibiotic available in Qurugol Lake. Pharmaceutical Sciences, 26(1): 88-92.
[38] Thawabteh, A.M., Swaileh, Z., Ammar, M., Jaghama, W., Yousef, M., Karaman, R., Bufo, A.S., Scrano, L., (2023): Antifungal and antibacterial activities of isolated marine compounds. Toxins (Basel), 15(2): 93.
[39] Verma, P., Pandey, P.K., Gupta, A.K., Seong, C.N., Park, S.C., Choe, H.N., Baik, K.S., Patole, M.S., Shouche, Y.S., (2012): Reclassification of *Bacillus beijingensis* Qiu et al. 2009 and *Bacillus ginsengi* Qiu et al. 2009 as *Bhargavaea beijingensis* comb. nov. and *Bhargavaea ginsengi* comb. nov. and emended description of the genus *Bhargavaea*. International Journal of Systematic and Evolutionary Microbiology, 62(10): 2495-2504.
[40] Wekesa, T.B., Wekesa, V.W., Onguso, J.M., Wafula, E.N., Kavesu, N., (2022): Isolation and characterization of *Bacillus velezensis* from Lake Bogoria as a potential biocontrol of *Fusarium solani* in *Phaseolus vulgaris* L. Bacteria, 1(4): 279-293.
[41] Yadav, N., Kour, D., Yadav, A.N., (2018): Microbiomes of freshwater lake ecosystems. Journal of Microbiology and Experimentation, 6: 245-248.
[42] Yadav, A.N., Yadav, N., Kour, D., Kumar, A., Yadav, K., Kumar, A., Rastegari, A.A., Sachan, S.G., Singh, B., Chauhan, V.S., Saxena, A.K., (2019): Bacterial community composition in lakes. pp. 1–71. In Bandh, S.A., Shafi, S., Shameem, N., (eds.): Freshwater Microbiology. Academic Press. Cambridge.
[43] Zarnea, G., Mihăilescu, G., Velehorschi, V., (1992): Principii şi tehnici de microbiologie generală. Editura Universitara, Bucureşti, 330 p.
[44] Zeng, H.W., Cai, Y.J., Liao, X.R., Zhang, F., Zhang, D., (2011): Production, characterization, cloning and sequence analysis of a monofunctional catalase from *Serratia marcescens* SYBC08. Journal of Basic Microbiology, 51(2): 205-214.
[45] Zhang, C., Kim, S.K., (2010): Research and application of marine microbial enzymes: status and prospects. Marine Drugs, 8(6): 1920-1934.
[46] Zhang, X.Y., Han, X.X., Chen, X.L., Dang, H.Y., Xie, B.B., Qin, Q.L., Shi, M., Zhou, B.C., Zhang, Y.Z., (2015): Diversity of cultivable protease producing bacteria in sediments of Jiaozhou Bay, China. Frontiers in Microbiology, 6: 1021.
[47] Zhang, Z., Wu, C., Shao, S., Liu, W., Wang, E.-T., Li, Y., (2020): Diversity of protease-producing bacteria in the Bohai Bay sediment and their extracellular enzymatic properties. Acta Oceanologica Sinica, 39(8): 1-11.
[48] Zhou, M.Y., Wang, G.L., Li, D., Zhao, D.L., Qin, Q.L., Chen, X.L., Chen, B., Zhou, B.C., Zhang, X.Y., Zhang, Y.Z., (2013): Diversity of both the cultivable protease-producing bacteria and bacterial extracellular proteases in the coastal sediments of King George Island, Antarctica. PLoS One, 8(11): e79668.

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