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# Microbial Diversity of Aquatic Environment as Source of Enzymes

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RESEARCH ARTICLE

## Abstract

This research consisted of studying the ability of new strains of microorganisms isolated from water of the "La izvor" lake system to synthesize enzymes: amylase, catalase, cellulase, lipase and selecting those with significant enzymatic potential. Express tests were performed to determine the enzymatic capacity: amylase, catalase, cellulase and lipase of 25 strains of actinobacteria, 25 strains of bacteria, 8 strains of microalgae and cyanobacteria, 35 strains of fungi. When determining the enzymatic capacity, specific indicators were used for each enzyme: amylase - Lugol's solution, catalase - $H_2O_2$ (3%), cellulase – carboxymethyl cellulose and Congo red, lipase – Tween 80. As a result of this study, 3 strains of actinobacteria, 9 strains of bacteria, 4 strains of fungi, and 5 strains of microalgae and cyanobacteria with significant enzymatic potential as prospects for biotechnology were selected.

**Keywords:** bacteria, cyanobacteria, fungi, amylase, catalase, cellulase, lipase.

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## INTRODUCTION

Enzymes are biological catalysts that facilitate the conversion of substrates into products by providing favorable conditions that lower the activation energy of the reaction. An inexhaustible source of enzymes are various microorganisms (Vingiani et al., 2019). Enzymes are considered the most efficient catalysts for industrial processes. Nowadays, bacterial and fungal enzymes are widely used in the food industry (i.e., amylase, protease, lipase, glucose oxidase, pectinase, and tannase) due to their high specificity and the smallest number of by-products formed during their production (Mir Khan et al., 2020). However, algae have interesting metabolic pathways related to the biogeochemical cycles of nitrogen, phosphorus and carbon, which could serve as sources of enzymes with industrial, biomonitoring and bioremediation applications. Algae are a polyphyletic group of organisms that live in different and, in some cases, extreme environments. These characteristics and their metabolic adaptations make them ideal sources for new enzymes, which have potential uses in various fields, from human health applications, carbon sequestration, to mine effluent treatment. In addition, seven algal enzyme complexes are described: carbonic anhydrase, hydrogenase, lipoxygenase, nitrilase, nitrogenase, phosphatase, and thiolase (Karigar et al., 2011). Microalgae consist of a diverse group that includes prokaryotic cyanobacteria and eukaryotic photosynthetic microorganisms that inhabit freshwater and marine habitats. They are important biomass factories that can grow in various environments and have enzyme-mediated metabolisms, which makes them good candidates for enzyme production with industrial applications and in environmental bioremediation. Enzymes derived from photosynthetic

microorganisms include cellulases, galactosidases, proteases, lipases, phytases, laccases, amylases, antioxidant enzymes, and enzymes associated with carbohydrate accumulation and carbon concentration. In addition, recent reports on microalgae genomics reveal a variety of novel genes that should be investigated for biotechnological applications. Exploring the genetic diversity of algae will also enable the efficient use of photosynthetic microorganisms as biofactories of recombinant enzymes that will be useful to industry (Brasil et al., 2017).

Most of the above-mentioned are of interest, because the listed classes of enzymes are used in various industries, including medicine and agriculture which present the leading areas of biotechnology. The total volume of the microbial enzyme market sector, including enzymes, their activity results, substrates, as well as devices based on them, is estimated at billions of US dollars and is constantly growing (Adrio et al., 2014; Li et al., 2012). Today, the coupling enzymes of cellulase, glycosidase and pectinase represent a significant part of the world production of enzymes, and the scope of their use is constantly supplemented with new promising directions. Cellulolytic enzymes are synthesized by microorganisms from different taxonomic groups. The most promising of all microorganisms are fungi of the genera *Aspergillus*, *Cladosporium*, *Fusarium*, *Neurospora*, *Penicillium*, *Trichoderma*, etc. Enzymes of cellulase, hemicellulase, pectinase are used into animal and poultry feed. The main benefit is their capacity by break down grain components to improve the nutritional value of the feed or to replace the digestive enzymes of animals or birds, and also contribute to silage production (Velázquez-De Lucio et al., 2021).

Actinobacteria represent one of the most diverse groups of filamentous bacteria able to survive in a range of ecological niches due to their bioactive potential. They are commonly isolated from soils. Actinobacteria have gained particular importance as the most potent source of antibiotics and other bioactive secondary metabolites (Kandasamy et al., 2012; Solecka et al., 2012).

Actinobacteria are considered a promising source of a wide range of enzymes. Some of them are produced on an industrial scale, but many others remain to be exploited. Actinobacteria have the ability to degrade a wide range of hydrocarbons, pesticides, aliphatic and aromatic compounds (Sambasiva Rao et al., 2012). They carry out microbial transformations of organic compounds, an area of great commercial value. Members of many genera of actinobacteria have potential for use in the bioconversion of underutilized agricultural and urban wastes into high-value chemicals (Crawford, 1988). Some actinobacteria secrete enzymes responsible for degrading lignocellulose from lignin, cellulose and hemicellulose, others may secrete enzymes that can only partially accomplish this breakdown (Mason et al., 2001).

Among actinobacteria, streptomycetes are an important link in the production of half of the metabolites known as antibiotics, antitumor agents, as well as enzymes and their inhibitors (El-Naggar, 2016; Hosseini et al., 2016). For example, out of 35 strains of actinobacteria isolated from Jerusalem soil samples, 3 showed the ability to produce protease, 4 - lipase, 5 - tyrosinase and 2 - phosphatase. In addition, all isolates were positive for amylase and catalase and many for cellulase (Mansour et al., 2015).

A study has shown that a strain of the genus *Streptomyces* is an active source of amylase, used in medical research, food, textile, paper and other industries and occupies up to 25% of the world (Chakraborty et al., 2012).

Streptomycetes, like micromycetes, are active producers of lipases (which are of great industrial use, especially in the food industry and in the production of detergents) as well as in the food, leather, perfumery, textile, bakery, and other industries (Aly et al., 2012).

Cellulases play an important role in the bioregulation of plant residues, which have been considered as a source of infection of cultivated plants (Phitsuwan et al., 2013). Data showing that actinobacteria are one of the most efficient prokaryotes for cellulase production (Rajagopal et al., 2017). The ability of representatives of the genus *Micromonospora* isolated from freshwater lakes to decompose cellulose is described in article of de Menezes et al. (2008). According to investigations of Veiga et al. (1983) it was shown that out of 36 strains of actinobacteria isolated from marine sediments, only 19 strains showed the ability to degrade cellulose. Further studies by de Menezes et al. (2008) showed that a number of strains of the genus *Micromonospora* isolated from freshwater lakes are capable of destroying cellulose.

According to the literature, out of 65 streptomycete isolates from marine bottom samples, only 8 strains showed positive amylolytic activity (4-20 mm) and only 1 strain – *Streptomyces* sp. S6 is considered the most efficient source of amylase synthesis (Rengasamy and Thangaprakasam, 2018). Among the 24 strains of streptomycetes, 7 strains had the ability to synthesize amylase and protease, the best indicators were observed in *S. albus*, *S. scabies*, and *S. gougerotii* (Mansour et al., 1994).

Lipase-producing strains have been identified from actinobacteria, mainly representatives of the *Streptomyces* genus. Strains were isolated from soda lakes in southeastern Siberia, soils in Africa, and other regions (Sorokin et al., 2014; Aly et al., 2012).

Most aquatic bacteria are a rich source of hydrolytic enzymes, such as amylases, lipases, proteases, phospholipases, catalases and other important industrial enzymes, and have drawn attention to the ability of lake bacteria to synthesize various extracellular enzymes (Mudryk and Podgorskar, 2006). Similarly, the enzyme known as protease produced by microorganisms has industrial potential due to its wide biochemical applications in food industries, medicinal formulations, detergents, and waste treatment (Saurabh, 2007). Currently, most of the enzyme

market is occupied by alkaline proteases due to its varied applications and major proportions of these are derived from *Bacillus* species. Lipolytic lake bacteria were also found to produce the enzyme known as phospholipase. This enzyme plays a key role in baking and is used in bread making, egg yolk industry and vegetable oil refining. The article by Syed et al., (2009) reported that an enzyme called α-amylase is important in many industrial processes and constitutes 25% of the world's enzyme market. The amylase enzyme used in the hydrolysis of starch molecules, in its simple form releases glucose and other metabolizable sugars (Sindhu et al., 1997). All these industrial enzymes are selected based on the capacity of the potential of microbes and their gene expression in microbial hosts or the cloned version, with commercially attractive quantities (De Maria et al., 1997).

Thus, in several industrial processes different types of enzymes are needed, because enzymes are the most efficient catalysts, offering much more competitive processes compared to chemical catalysts (Vingiani et al., 2019). The purpose of the research was to determine ability of the new strains of microorganisms isolated from the water of the "La izvor" lake system to synthesize enzymes: amylase, catalase, cellulase, lipase and to select those with significant enzymatic potential.

## MATERIALS AND METHODS

The following research was conducted within the National Collection of Non-pathogenic Microorganisms (NCNM) of the Institute of Microbiology and Biotechnology of the Technical University of Moldova. Research area was located in the "La izvor" lake system (Republic of Moldova, Chisinau Municipality). Random samples were collected in sterile bottles from water column. The geographical coordinates of collected samples were: 1) 47°02'44.2"N 28°47'18.9"E; 2) 47°02'53.7"N 28°47'42.5"E; 3) 47°02'59.9"N 28°48'01.0"E (Figure 1). The samples were not pre-treated. After that, serial dilutions were carried out using distilled water to dilute the samples to $10^{-1}$ - $10^{-8}$ (Hussein et al., 2018; Yu et al., 2015).

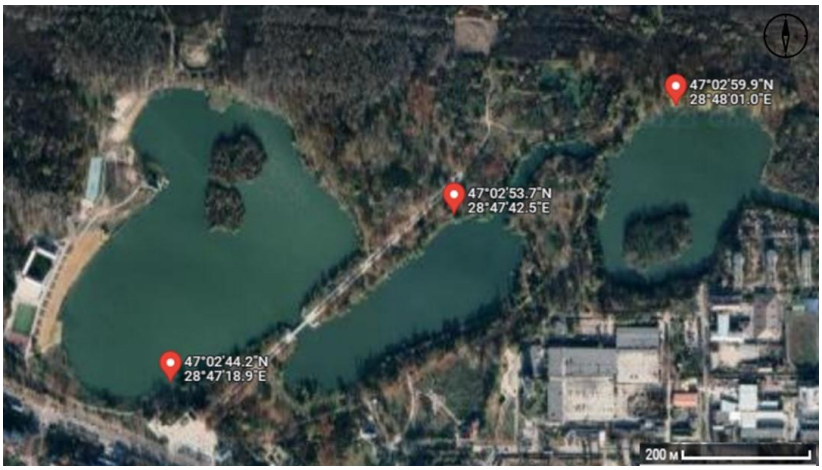


**Figure 1.** Location of the "La izvor" lake system on the map Chisinau Municipality, Republic of Moldova (source: Google Maps)

For study actinobacteria, were isolated 8 genera on special selective nutrient media (containing carbon, phosphorus, mineral salts, and antibiotic sources) in Petri dishes by inoculation of diluted samples: *Actinomadura*; *Actinoplanes*; *Frankia*; *Geodermatophilus*; *Micromonospora*; *Nocardia*; *Rhodococcus*; *Streptomyces*.
The bacteria were isolated by serial dilution technique on nutrient agar medium Liofilchem (Italy). Spread plate technique was carried out to isolate the organism form the diluted sample and incubated for 24 hours at 37°C.
Fungi are typically isolated by plating a sample on a Petri dish containing wort agar (5.0°B, pH=5.8-6.0), supporting the growth of a variety of fungi incubated for 7-10 days at 28-30°C.

Isolation of cyanobacteria and microalgae was carried out by successive dilutions and inoculation on liquid mineral cultivation media and agar media on Petri dishes (especially on Gromov 6 medium) for 14 days at 18-25°C. The identification of microorganisms was carried out with optical microscopes (Lomo Mikmed - 2; Optika - B-292) using determinants for actinobacteria (Parte et al., 2012), bacteria (Zarnea, 1994), fungi (Dugan, 2017), microalgae and cyanobacteria (Bellinger and Sigee, 2015; Nienaber and Steinitz-Kannan, 2018).

Amylase screening: actinobacteria strains were grown on ISP-4 medium at 28°C for 72 hours. Bacteria strains were cultivated on nutrient agar medium for 48 hours at 37°C, and micromycete strains on malt-agar medium at 28°C for 96 hours. Microalgae and cyanobacteria were cultivated on Gromov 6 medium for 14 days at 18-25°C. After incubation, 3 mL of 1% iodine was poured into each Petri dish, and the appearance of the transparent zone around the colonies indicates the synthesis of amylase (Rengasamy and Thangaprakasam, 2018).

Catalase screening: bacterial strains were grown on nutrient agar medium for 48 hours at 37°C, micromycete strains on malt-agar medium at 28°C for 96 hours, microalgae and cyanobacteria were on Gromov 6 medium for 14 days at 18-25°C, and actinobacteria strains on Gause medium at 28°C for 14 days. All the isolates obtained were verified, following the method of Mahon et al., (2011) – glass-drop. Positive reactions were evident by immediate effervescence (bubble formation).

Cellulase screening: bacterial strains were cultivated on agar-nutritive medium, micromycete strains on malt-agar medium, supplemented with 10 g/L carboxymethyl cellulose for 48 hours (37°C) and 96 hours (28°C), respectively; microalgae and cyanobacteria on Gromov 6 medium with 10 g/L carboxymethyl cellulose for 14 days at 18-25°C, and the actinobacteria strains were cultivated on CMC (carboxymethyl cellulose medium), for 72 hours at 28°C. After incubation, 3 mL of 1% Congo red was poured into each Petri dish. The surface of the agar medium was washed of excess dye with distilled water, and the appearance of red or yellow area around the colonies indicates the level of cellulase synthesis (Rajagopal et al., 2017).

Lipase screening: bacterial strains were cultivated on nutrient agar medium, micromycete strains on malt-agar medium, microalgae and cyanobacteria on Gromov 6 supplemented with 10 g/L Tween 80, for 48 hours (37°C), 96 hours (28°C), and 14 days (18-25°C), respectively; actinobacteria strains were grown on the medium: $H_2O$ dist. – 900 mL, peptone – 10.0 g/L, NaCl – 5.0 g/L, $CaCl_2*H_2O$ – 0.1 g/L, agar – 20.0 g/L, Tween 80 – 10.0 g/L, pH=7.0, for 72 hours, at 28°C. The Tween 80 solution (10 g/L) is autoclaved separately, cooled to 50°C and mixed together with the agar medium. After cultivation, the appearance of the radiant zone around the colony serves as an indicator (Aly, 2012).

## RESULTS AND DISCUSSIONS

Studies were carried out in which the enzymatic activity of actinobacteria isolated from the "La izvor" lake system was determined. Thus, the new strains isolated from water showed their enzymatic activity in different ways. Out of 25 strains of actinobacteria, isolated from water, 16 strains were characterized by a low activity of amylase (+) (Table 1).

**Table 1.** Enzymatic activity of actinobacteria strains isolated from the water of the "La izvor" lake system

| Actinobacteria genus | Strain № | Amylase | Catalase | Cellulase | Lipase |
|---|---|---|---|---|---|
| ***Actinomadura*** | **A 1.1** | - | - | - | - |
| | **A 1.2** | + | + | + | + |
| ***Actinoplanes*** | **A 2.1** | + | ++ | - | ++ |
| | **A 2.2** | + | + | - | - |
| | **A 2.3** | + | ++ | - | - |
| ***Frankia*** | **A 3.1** | + | + | - | - |
| ***Geodermatophilus*** | **A 4.1** | + | + | - | - |
| | **A 4.2** | - | ++ | - | - |
| | **A 4.3** | - | ++ | - | - |
| ***Micromonospora*** | **A 5.1** | + | ++ | + | + |
| | **A 5.2** | + | + | + | - |
| | **A 5.3** | - | - | - | - |
| ***Nocardia*** | **A 6.1** | + | + | + | - |
| | **A 6.2** | + | + | - | ++ |
| ***Rhodococcus*** | **A 7.1** | - | +++ | - | - |
| | **A 7.2** | - | - | - | - |
| ***Streptomyces*** | **A 8.1** | + | + | - | - |
| | **A 8.2** | + | ++ | + | + |
| | **A 8.3** | + | ++ | + | + |
| | **A 8.4** | + | + | - | - |
| | **A 8.5** | - | + | - | - |
| | **A 8.6** | - | + | - | ++ |
| | **A 8.7** | - | - | - | - |
| | **A 8.8** | + | - | + | - |
| | **A 8.9** | + | ++ | - | - |

Note: - lack of enzymatic activity; + - activity at a weak level; ++ - medium level activity; +++ - high level activity.

The highest catalase synthesis activity was established in the strain *Rhodococcus* A 7.1, and some representatives of the genera *Actinoplanes*, *Geodermatophilus*, *Micromonospora*, showed medium level activity. The catalase weak activity level was registered by *Streptomyces* strains. Only 7 strains of actinobacteria showed weak level of cellulolytic activity. One strain each from *Actinoplanes*, *Nocardia*, and *Streptomyces* possesses medium lipase activity.

Also of particular interest are 3 strains in which the enzymatic activity of 3 enzymes was detected (*Actinoplanes* A 2.1, *Micromonospora* A 5.1, *Nocardia* A 6.2).

According to Riesco et al. (2022), *Micromonospora* spp. possess amylase, cellulase, and catalase activity. The data obtained for 6 strains showed that all of them have a positive reaction to catalase, when in the presented studies only 2 out of 3 have it. *Actinoplanes* strains isolated by Ara et al. (2010), showed lack or low lipase activity, whereas in present work, a strain with medium level activity was found. *Nocardia* is to be demonstrated a good producer of lipase enzyme, but only one water isolate showed a medium level activity (Nesbit and Gunasekaran, 1993).

To determine the enzymatic properties, bacteria were cultivated on specific nutrient media. Thus, from 25 strains of bacteria, the presence of amylase was established in 19 strains, catalase in 22 strains, cellulase in 16 strains and lipase in 2 strains. The mentioned strains showed medium enzymatic activity (Table 2).

**Table 2.** Enzymatic activity of bacteria strains isolated from the water of the "La izvor" lake system

| Bacteria genus | Strain № | Amylase | Catalase | Cellulase | Lipase |
|---|---|---|---|---|---|
| ***Bacillus*** | **1** | ++ | - | ++ | - |
| | **2** | - | ++ | ++ | ++ |
| ***Lysinibacillus*** | **3** | - | ++ | - | - |
| | **4** | - | - | - | - |
| ***Peribacillus*** | **5** | ++ | - | - | - |
| ***Bacillus*** | **6** | - | ++ | - | ++ |
| ***Planococcus*** | **7** | ++ | ++ | ++ | - |
| ***Bacillus*** | **8** | ++ | ++ | ++ | - |
| | **9** | - | ++ | ++ | - |
| | **10** | ++ | ++ | - | - |
| | **11** | ++ | ++ | ++ | - |
| ***Paenibacillus*** | **12** | ++ | ++ | ++ | - |
| ***Bacillus*** | **13** | ++ | ++ | - | - |
| | **14** | + | + | ++ | - |
| | **15** | ++ | ++ | ++ | - |
| | **16** | ++ | ++ | ++ | - |
| ***Planococcus*** | **17** | - | - | ++ | - |
| ***Kocuria*** | **18** | + | + | ++ | - |
| ***Peribacillus*** | **19** | + | + | - | - |
| ***Bacillus*** | **20** | ++ | ++ | ++ | - |
| | **21** | ++ | + | ++ | - |
| | **22** | ++ | ++ | ++ | - |
| | **23** | ++ | ++ | - | - |
| | **24** | ++ | ++ | + | - |
| | **25** | ++ | ++ | - | - |

Note: - lack of enzymatic activity; + - activity at a weak level; ++ - medium level activity; +++ - high level activity.

According to the enzyme activity study of the total number of 25 strains isolated from water, the most active were 9 strains (№): *Bacillus* (2, 8, 11, 15, 16, 20, 22); *Planococcus* 7; *Paenibacillus* 12. Several research confirmed that *Bacillus*, *Planococcus*, and *Paenibacillus* ssp. can serve as potential source of amylase (Figure 2), catalase, and cellulase (Labuzek et al., 2003; Malika and Javed, 2021; Pason and Kyu, 2006; Hu and Liu, 2021; Saeed et al., 2023). However, the isolated bacteria had almost no lipolytic activity.

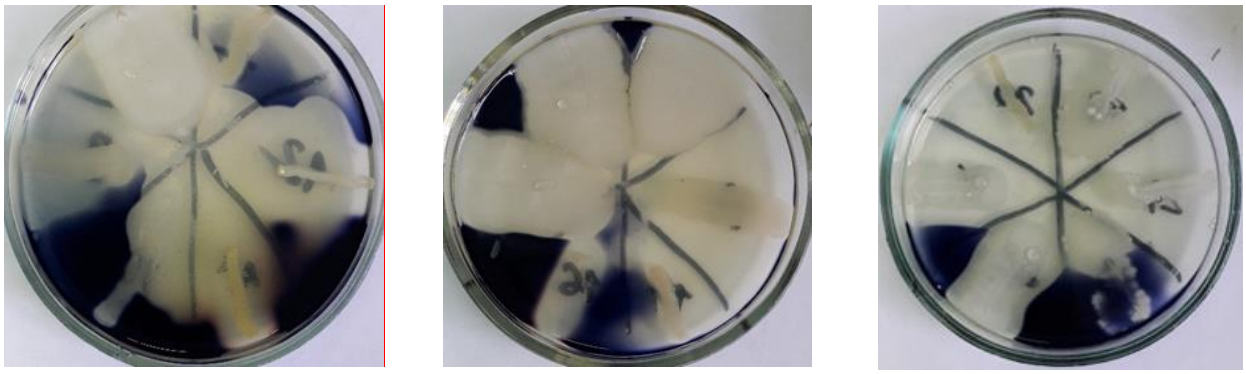

**Figure 2.** Amylase activity of *Planococcus* sp. 7; *Paenibacillus* sp. 12; *Kocuria* sp. 18; *Bacillus* sp. 20, 21, 22, 23, 24, 25

To determine the enzymatic activity of microalgae and cyanobacteria from the water of the "La izvor" lake system, 8 strains were selected that are of interest for biotechnology according to their biochemical properties and characteristics such as: good growth, antagonistic activity against phytopathogenic bacteria and fungi, stress resistance on growth conditions change, enzymatic abilities according to literature data (Valluri, 2022). The results of the enzyme activity study using express methods, namely amylolytic, catalase, cellulase, and lipolytic activity, are presented in Table 3.

**Table 3.** Enzymatic activity of microalgae and cyanobacteria strains isolated from the water of the "La izvor" lake system

| Strain sp. | Amylase | Catalase | Cellulase | Lipase |
|---|---|---|---|---|
| ***Oscillatoria planctonica*** | +++ | - | +++ | ++ |
| ***Chlorella vulgaris*** | + | ++ | ++ | ++ |
| ***Nostoc verrucosum*** | +++ | +++ | +++ | ++ |
| ***Oscillatoria brevis*** | - | ++ | ++ | ++ |
| ***Oscillatoria amphibia*** | - | - | ++ | ++ |
| ***Spirulina major*** | + | + | +++ | + |
| ***Anabaena variabilis*** | - | +++ | +++ | - |
| ***Aphanizomenon flos-aquae*** | - | + | + | - |

Note: - lack of enzymatic activity; + - activity at a weak level; ++ - medium level activity; +++ - high level activity.

As a result of the experiments on the enzymatic properties, it can be mentioned that *Nostoc verrucosum* had a high enzymatic activity (+++). The *Spirulina major* strain presented a high cellulase activity (+++), and the amylolytic, catalase, lipase activities were more diminished (+). In the case of *Chlorella vulgaris*, medium activities (++) were expressed for catalase, cellulase, and lipase; and amylase showed a lower (+) activity. *Oscillatoria brevis* showed medium catalase, cellulase, and lipase activity (++), and *O. planctonica* - high cellulase activity (+++), and medium lipase activity (++). It should also be mentioned that *Anabaena variabilis* expressed a high catalase and cellulase activity (+++), and *Aphanizomenon flos-aquae* showed lower enzymatic activities in comparison with other strains of microalgae studied. As a result of this study, it was demonstrated that the most active strain is *Nostoc verrucosum*. According to literature data, *Nostoc* sp. are strong producers of amylase and cellulase (Reyes-Sosa et al., 2010; Galetović et al., 2023).

The next stage of the investigations consisted in the testing of 35 isolates, which belong to the most representative genera of fungi isolated from the water basin "La izvor". These are representatives of the genera *Aspergillus*, *Penicillium*, *Trichoderma*, *Fusarium*, *Mucor*, *Rhizopus*, which are considered to possess significant enzymatic properties.

Table 4 shows the results obtained on the fungi isolated from water. The presented data demonstrate that fungi isolated from water possess different enzymatic activity. Thus, in 12 isolates, catalase activity was at a medium level (++), in 11 strains - weak (+), and in 12 strains this activity was absent (-). Amylase, lipase, and cellulase activities were also weak in most strains tested. Only 4 strains recorded a medium enzymatic activity (++) of the 3 enzymes. In the rest of the strains, the activity of these enzymes is weak (+) or absent (-). The activity of amylase was not manifested in 10 strains, of lipase in 13 strains, and of cellulase in 12 strains. None of the tested strains showed the

activity of the studied enzymes at an average level, and 3 enzymes showed an average enzyme activity - strains A 12 and A 14.

**Table 4.** Enzymatic activity of fungi strains isolated from the water of the "La izvor" lake system

| Gungi genus | Strain № | Amylase | Catalase | Cellulase | Lipase |
|---|---|---|---|---|---|
| *Talaromyces* | A 1 | + | + | + | + |
| | A 2 | - | + | + | + |
| | A 3 | - | + | ++ | + |
| *Penicillium* | A 4 | + | + | + | ++ |
| | A 5 | ++ | + | + | ++ |
| | A 6 | - | + | ++ | + |
| | A 7 | ++ | + | - | + |
| | A 8 | ++ | - | + | + |
| | A 9 | ++ | + | - | - |
| *Trichoderma* | A 10 | - | + | + | + |
| | A 11 | ++ | + | - | - |
| | A 12 | ++ | - | ++ | ++ |
| | A 13 | ++ | ++ | + | + |
| | A 14 | ++ | ++ | + | ++ |
| | A 15 | + | ++ | + | + |
| | A 16 | ++ | + | + | + |
| *Mucor* | A 17 | ++ | ++ | - | - |
| | A 18 | + | + | - | - |
| *Fusarium* | A 19 | - | - | - | - |
| | A 20 | ++ | - | - | - |
| *Alternaria* | A 21 | - | + | - | + |
| | A 22 | - | + | - | + |
| | A 23 | + | + | + | + |
| | A 24 | + | - | + | - |
| | A 25 | + | - | + | - |
| | A 26 | - | + | + | + |
| *Fusarium* | A 27 | + | + | + | + |
| | A 28 | ++ | + | - | + |
| | A 29 | - | - | - | - |
| *Aspergillus* | A 30 | - | - | - | - |
| | A 31 | + | - | - | - |
| | A 32 | + | + | + | + |
| | A 33 | + | + | + | + |
| | A 34 | + | + | + | + |
| | A 35 | + | - | + | - |

Note: - lack of enzymatic activity; + - activity at a weak level; ++ - medium level activity; +++ - high level activity.

For further research, the strains that showed significant enzymatic activity and may present biotechnological interest were selected. These are the strains: *Penicillium* A 5, *Trichoderma* (A 12, A 13, A 14), which will complete

the NCNM. As demonstrated by other researchers, these strains are potential sources of amylase and catalase (Figure 3) (Balkan and Ertan, 2005; Zhou et al., 2020).

**Figure 3.** Catalase activity of *Trichoderma* sp. 13, 14, 15

## CONCLUSIONS

As a result of the research, it was established that the strains of microorganisms isolated from water possess different enzymatic activity. Actinobacteria and bacteria are more active in catalase among all enzymes, thereby ensuring their survival against oxidative stress. Microalgae and cyanobacteria are most often sources of cellulase, being photoheterotrophic they required cellulose for growth in the absence of other carbon sources. Fungi, on the other hand, have a wider enzymatic activity; many of them are capable of synthesizing all the enzymes being studied. The strains of microorganisms possessing significant enzymatic activity have been selected and will supplement the NCNM, namely: 3 strains of actinobacteria (*Actinoplanes* A 2.1, *Micromonospora* A 5.1, *Nocardia* A 6.2), 9 strains of bacteria (№ 2, 7, 8, 11, 12, 15, 16, 20, 22), 4 strains of fungi (A 5, A 12, A 13, A 14), 5 strains of microalgae and cyanobacteria (*Nostoc verrucosum*, *Oscillatoria planctonica*, *Chlorella vulgaris*, *Spirulina major*, *Anabaena variabilis*). These cultures will be further studied to identify potential producers of bioactive substances of biotechnological interest.

**Author Contributions:** T.S. Conceived and designed the analysis; S.B. Collected the data; M.B., N.B.-G., V.S., O.Ț., C.M. Contributed data or analysis tools; T.S. Performed the analysis; T.S., M.B. Wrote the paper.

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**Conflicts of Interest**

The authors declare that they do not have any conflict of interest.

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