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INFLUENCE OF HETEROMETALLIC Ge(IV) - 3d-METALS COMPLEXES ON MICROBIAL RHAMNOSIDASE, GALACTOSIDASE AND PROTEASE ACTIVITY

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In recent years, the attention of researchers has been attracted by coordination compounds of germanium with various bioligands, which may be used both as activator or inhibitor of enzymes. The aim of the work was to investigate the effect of new heterometallic Ge(IV) - 3d-metals complexes with 1-hydroxyethane-1,1-diphosphonic acid and 1,10-phenanthroline on the activity of purified α-L-rhamnosidases produced by Eupenicillium erubescens, Penicillium tardum, Penicillium restrictum, Cryptococcus albidus, α-galactosidase of P. restrictum and proteases with elastolytic and fibrinogenolytic activity of Bacillus sp. The studied compounds (0,1% concentration) activate α-L-rhamnosidase differently depending on its producer. Thus, the activity of α-L-rhamnosidase of E. erubescens was stimulated with $[Co(phen)_3]_4[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 2CH_3COOH\cdot 30H_2O$ by 200%, of Penicillium tardum – with $[Zn(phen)_2(H_2O)_2]_2[Zn(phen)(H_2O)_4]_2[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 18H_2O$ by 200%, of Penicillium restrictum – with $[Ni(phen)_3]_4[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 2CH_3COOH\cdot 26H_2O$ by 67%, of Cryptococcus albidus – wih $[Co(phen)_3]_4[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 2CH_3COOH\cdot 30H_2O$ by 40%. The α-galactosidase of Penicillium restrictum was not affected by the investigated compounds. Bacillus sp. IMV B-7883 elastase was activated with $[Co(phen)_3]_4[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 2CH_3COOH\cdot 30H_2O$ by 70%, but was totally inhibited with $[Fe(phen)_3]_4[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 20H_2O$. The compounds that showed the greatest 200% stimulating effect on the fibrinogenolytic activity of Bacillus sp. IMV B-7883 were $[Fe(phen)_3]_4[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 20H_2O$ and $[Zn(phen)_2(H_2O)_2]_2[Zn(phen)(H_2O)_4]_2[Ge6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 18H_2O$.

K e y w o r d s: heterometallic Ge(IV) - 3d-metals complexes, α-L-rhamnosidases, α-galactosidase, protease, Eupenicillium erubescens, Penicillium tardum, Penicillium restrictum, Cryptococcus albidus, Bacillus sp.

Enzymes, due to their ability to control selective, safe and stable catalysis, are widely used in various branches of industry and medicine. Today, more than 3000 enzymes are known. However, this research is devoted to such enzymes as glycosidases (α-L-rhamnosidase and α-galactosidase) and protease with elastase and fibrinogenolytic activity. α-L-Rhamnosidase (α-L-rhamnoside rhamnohydrolase – EC 3.2.1.40) hydrolytically cleaves the terminal non-reducing α-1,2, α-1,4 and α-1,6 linked L-rhamnose residues in α-L-rhamnosides: glycolipids, glycosides such as plant pigments, gums, resins. Therefore, α-L-rhamnosidase is a technologically important enzyme, in particular for the food industry, including both the food production itself and the improvement of food quality, e.g. to reduce the bitterness of fruit juices, enhance their antioxidant activity, improve the bouquet of wines [1]. α-Galactosidase (EC 3.2.1.22) is a glycosyl hydrolase that is able to cleave, as a rule, with the preservation of their optical configuration, terminal non-reducing residues of α-D-galactose from α-D-galactosides, including galactooligosaccharides, galactomannans, galactolipids. These properties of α-galactosidase can be used to obtain universal donor blood based on the bioconversion of group B(III) erythrocytes

into O-type erythrocytes. α-Galactosidase also has important applications in food biotechnology, particularly in sugar production, where it is used to increase sugar yields and improve sugar quality. Industrial preparations of α-galactosidase allow the widespread use of various soy products as food for humans and animals [2]. In recent years, the special attention of researchers has been attracted to proteolytic enzymes with elastolytic and fibrinogenolytic action, especially those that are able to degrade poorly soluble animal proteins belonging to the group of extracellular matrix proteins, in particular elastin [3], as well as fibrinogen – a blood plasma protein, a precursor to fibrin, whose fibers form the basis of blood clots. Studies of elastase (3.4.21.11) and fibrinogenase (3.4.21.5) are important both in the theoretical aspect for understanding the structure of proteins and peptides, mechanisms of enzymatic catalysis. As for practical significance, elastase is used for the treatment of burn damage and various wounds to remove necrotic tissues and fibrinogenase can be considered as a basis for the creation of drugs aimed at reducing the threat of intravascular thrombosis through limited proteolysis of fibrinogen. Such a wide use of α-L-rhamnosidases, α-galactosidases and proteases with elastase and fibrinogenase activity to solve current chemical-technological and biological-medical problems stimulates researchers to search for compounds that can affect their catalytic activity. Coordination compounds of metals are of particular interest as enzyme activators or inhibitors [4, 5]. The authors [6, 7] investigated a number of supramolecular coordination compounds of germanium, which have a unique structure and properties due to the combination of biometals and biologically active ligands in the molecule. Such ligands include 1-hydroxyethane-1,1-diphosphonic acid (commonly known as etidronic acid), which has significant biological and medical activity primarily due to its ability to interact with ions of other metals and stabilize enzymes that can be inactivated by aggregation caused by metal ions. In recent years, the attention of researchers [8] has been attracted to the coordination compounds of germanium with various bioligands, which are characterized by a variety of activities in combination with unique properties. The authors [9] investigated the effect of coordination compounds of germanium with biologically active hydroxycarboxylic acids on the activity of α-L-rhamnosidases of *Penicillium tardum* and *Penicillium restrictum*. Less information was obtained regarding the effect of complexes with 1-hydroxyethane-1,1-diphosphonic acid. Thus, mixed-ligand complexes of Ge(IV) with one of the metals: Co(II), Ni(II), Cu(II), as well as with 1-hydroxyethane-1,1-diphosphonic acid and 2,2′-bipyridine have a stimulating effect on the activity of fibrinogenase *Bacillus* sp. IMV B-7883, but inhibit the activity of elastase. Therefore, the aim of the work was to investigate the effect of new heterometallic Ge(IV) - 3d-metals complexes with 1-hydroxyethane-1,1-diphosphonic acid with 1,10-phenanthroline on the activity of α-L-rhamnosidases of *Eupenicillium erubescens, Penicillium tardum, Penicillium restrictum, Cryptococcus albidus*, α-galactosidase of *P. restrictum* and proteases with elastolytic and fibrinogenolytic activity of *Bacillus* sp.

Materials and Methods

α-L-Rhamnosidases of *Eupenicillium erubescens, Penicillium tardum, Penicillium restrictum, Cryptococcus albidus*, α-galactosidase of *Penicillium restrictum* and proteases of *Bacillus* sp. IMV B-7883 are investigated in the present study. The processes of culture growth and enzyme production and purification have been described by us earlier [10-13].

α-Galactosidase activity was determined using *p*-nitrophenyl-α-D-galactopyranoside (Sigma, USA) as a substrate [14]. To determine activity, 0.1 ml of the enzyme solution was mixed with 0.2 ml 0.1 M phosphate-citrate buffer (PCB) pH 5.2 and 0.1 ml 0.01 M substrate solution in PCB. The reaction mixture was incubated for 10 min at 37°C. The reaction was stopped by adding 2 ml of 1 M sodium bicarbonate. The amount of released nitrophenol as a result of hydrolysis was determined colorimetrically by the absorption at 400 nm. One unit of enzyme activity was defined as the amount of enzyme that releases 1 μmol of *p*-nitrophenol per min at 37°C in 0.1 M PCB, pH 5.2.

The activity of α-L-rhamnosidase was determined using the Davis method [15] with minor modification. The assay mixture contained 0.2 ml of 0.1% naringin (Sigma, USA) solution in 0.1 M PCB, pH 5.2 and 0.2 ml enzyme solution. After incubation at 37°C for 30 min, the reaction was stopped by adding 5 ml diethylene glycol (90%) and 0.1 ml of 4 N NaOH. The residual naringin was measured at 420 nm. One unit of α-L-rhamnosidase activity was defined as the amount of enzyme that releases 1 μmol of naringin per min in the solution. Elastase

activity was determined colorimetrically by the intensity of the color of the solution upon enzymatic hydrolysis of elastin stained with Congo red [16]. The incubation mixture contains 2.5 ml of 0.01 M Tris-HCl buffer (pH 7.5), 5 mg of elastin stained with 0.002% Congo red solution and 1 ml of the enzyme solution. The reaction mixture is incubated for 5 h at 37°C. The reaction was stopped by keeping the test tubes with the reaction mixture in an ice bath for 30 min. Unhydrolyzed elastin was separated by centrifugation for 10 min at 10 000 g. The color intensity was measured on an SF-26 spectrophotometer by absorption at 515 nm. The amount of enzyme that catalyzes the hydrolysis of 1 mg of substrate per hour under standard conditions was taken as a unit of elastase activity. Specific elastase activity was 4138 U/mg protein.

To determine fibrinogenolytic activity, fibrinogen was used as a substrate [17]. 1 mg of fibrinogen, 1.8 ml of Tris-HCl buffer (pH 7.5) and 0.2 ml of the studied preparation were added to the test sample. Incubate for 30-45 min at 37°C. The reaction was stopped by adding 2 ml of 10% trichloroacetic acid (TCA). TCA was added to the control sample immediately. Samples were kept at room temperature for 20 min and then centrifuged at 10 000 g for 10 min to remove precipitated protein. Absorption was measured on an SF-26 spectrophotometer at a wavelength of 275 nm. The amount of enzyme that, under the conditions of the experiment, increased absorption by 0.01 in 1 min was taken as a unit of activity.

As a result of structure-oriented design, five supramolecular organo-inorganic ensembles, double coordination compounds, were selected as modulators: $[M(phen)_3]_4[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot xCH_3COOH\cdot nH_2O$ (M=Co (**1**), x=2, n=30; Ni (**2**), x=2, n=26; Fe (**5**), x=0, n=20); $[Cu(phen)_2(H_2O)]_2[Cu(phen)(H_2O)_3]_2[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 16H_2O$ (**3**), $[Zn(phen)_2(H_2O)_2]_2[Zn(phen)(H_2O)_4]_2[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 18H_2O$ (**4**), where H_4hedp – 1-hydroxyethane-1,1-diphosphonic acid, phen – 1,10-phenanthroline. They were chemically constructed based on all biologically active components: a hydrophobic cation – a phenanthroline complex of the "metal of life", an anion – a hydrophilic chelate of essential germanium with acid.

The synthesis and properties of these compounds are described in [18]. It was determined that the complexes are of the cation-anion type, comprising a hexanuclear complex anion where Ge atoms are linked by three types of bridging ligands (hydroxy-, oxo-, and 1-hydroxyethane-1,1-diphosphonate) (Fig. 1) and phenanthroline-based cations with varying compositions depending on the 3d-metal (Fig. 2).

The complex qualitative and quantitative composition of these compounds, the features of their structure are complementary to enzymes, and the specificity of whose action is determined by a set of different mechanisms: induced conformity, the effect of convergence of reacting groups, and destabilization of bonds.

When studying the effect of various germanium-containing compounds on the activity of enzymes, we used concentrations of 0.1% (corresponding to the molar concentration range $2.1\text{-}2.8\cdot10^{-4}$ mol/l) and 0.01% (corresponding to the molar concentration range $2.1\text{-}2.8\cdot10^{-5}$ mol/l) (Table) and time of exposure 1 h and 24 h. The studied compounds were dissolved in 0.1% DMSO.

All experiments were performed in 3-5 replicates. Student's *t*-test was used to perform statistical analysis. The data are presented as mean ± standard error ($M \pm m$) and are considered significant at $P < 0.05$. The results presented in the graphs were processed using Microsoft Excel 2007.

Results

The study of the effect of germanium compounds on the activity of α-L-rhamnosidase of *E. erubescens* showed (Fig. 3, *A*, *B*) that they either activated the enzyme under study or their activity was at the control level. The maximum increase in

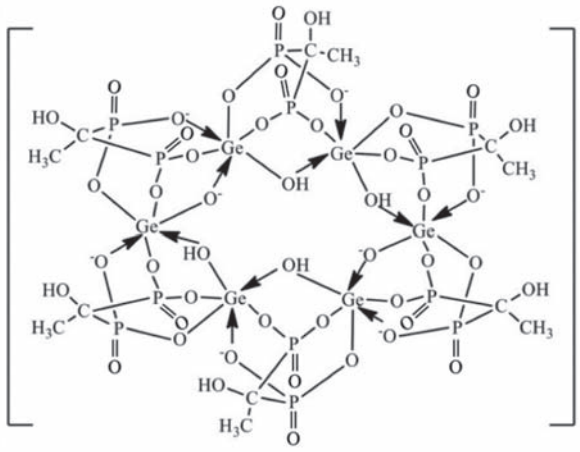

Fig. 1. Scheme of structures of anion $[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]^{8-}$

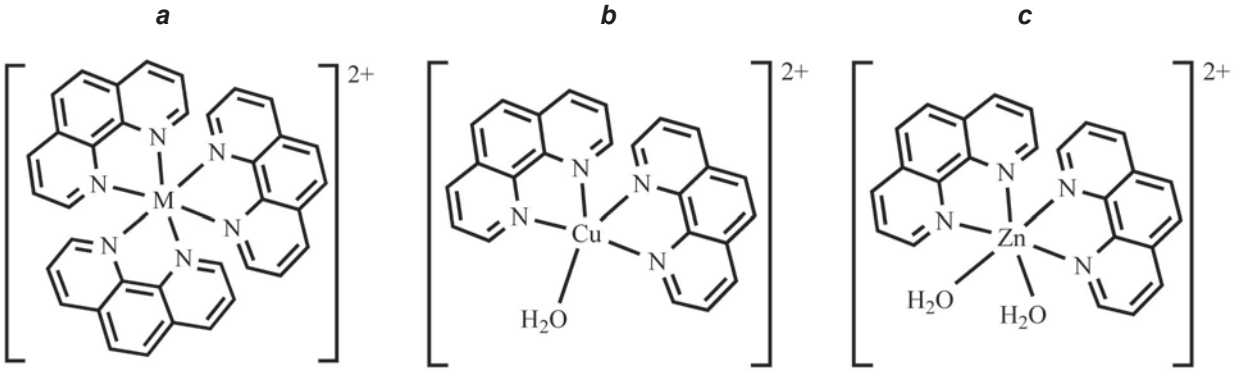

*Fig. 2. Scheme of structures of cations $[M(phen)_3]^{2+}$ (M=Co, Ni, Fe) (**a**), $[Cu(phen)_2(H_2O)]^{2+}$ (**b**), $[Zn(phen)_2(H_2O)_2]^{2+}$ (**c**)*

Table. Mass and molar concentrations of germanium-containing compounds

0.1%	0.01%
$C_M(1)=2.1\cdot10^{-4}$ mol/l	$C_M(1)=2.1\cdot10^{-5}$ mol/l
$C_M(2)=2.1\cdot10^{-4}$ mol/l	$C_M(2)=2.1\cdot10^{-5}$ mol/l
$C_M(3)=2.8\cdot10^{-4}$ mol/l	$C_M(3)=2.8\cdot10^{-5}$ mol/l
$C_M(4)=2.8\cdot10^{-4}$ mol/l	$C_M(4)=2.8\cdot10^{-5}$ mol/l
$C_M(5)=2.2\cdot10^{-4}$ mol/l	$C_M(5)=2.2\cdot10^{-5}$ mol/l

activity was observed with the action of all compounds at a concentration of 0.1% (Fig. 3, *A*, *B*). The greatest activation was observed with the action of compounds 1 (200 and 80%, respectively, at exposure for 1 h and 24 h), 2 (90 and 50%, respectively, at exposure for 1 h and 24 h) and 5 (74 and 60%, respectively, at exposure for 1 h and 24 h). With an exposure time of 1 h, compound 4 increased the activity of the enzyme by 58 and 42%, respectively, for concentrations of 0.1 and 0.01%. A slight activation of 10-29% at 1 h exposure at both concentrations and only 10% at 24 h exposure at a concentration of 0.1% was observed for compound 3. A slightly less activating effect (by 13-16%) was exhibited by compounds 1 and 2 at a concentration of 0.01% at 1 h exposure. It should be noted that when the exposure time with these compounds was increased to 24 h, the activity returned to control values.

The studied compounds exhibited a more diverse effect on the activity of α-L-rhamnosidase *P. tardum* (Fig. 4, *A*, *B*). In this series of experiments, it was established that in most cases, especially at a higher concentration of the substance (0.10%) and a longer exposure time (24 h), an increase in activity was observed. The greatest activation by compounds 4 (200%), 2 (61-67%) and 5 (55-60%) was observed at 0.10% concentration of the substances.

Germanium compounds showed a more diverse effect on the activity of *P. restrictum* α-L-rhamnosidase (Fig. 5, *A*, *B*). In general, it can be noted that at a concentration of 0.1%, compounds 1, 2, 3 and 5 increased the activity of the enzyme by 6-67%. The highest activity (67%) was observed with the action of substance 2 at a concentration of 0.1% and an incubation time of 24 h. However, after 1 h of incubation, the activity increased by 50%. A somewhat smaller stimulation (40%) was provided by compound 1 at a concentration of 0.01% and an incubation time of 1 h. At a concentration of 0.01%, all tested compounds had a slight effect on the P. *restrictum* enzyme. The maximum activation (by 21%) of *P. restrictum* α-L-rhamnosidase was shown by compound 5 when exposed for 24 h and at a concentration of 0.01% (Fig. 5, *A*).

The study of the effect of germanium coordination compounds on *C. albidus* α-L-rhamnosidase showed that a higher concentration of substances (0.10%) and incubation time (24 h) contributed to an increase in enzyme activity (Fig. 6, *A*, *B*). Thus, the activating effect of substance 1 increased from 34 to 40%, and compound 2 from 42 to 86%. The effect of substances 3, 4, 5 at a concentration of 0.10% on the activity of *C. albidus* α-L-rhamnosidase remained at the same level regardless of the incubation time and was 35, 52, and 34%, respectively. While at a concentration of 0.01%, compounds 2, 3 and 5 increased the activity of the enzyme by 42, 14 and 16%, respectively. The effect of other compounds at this concentration was at the control level.

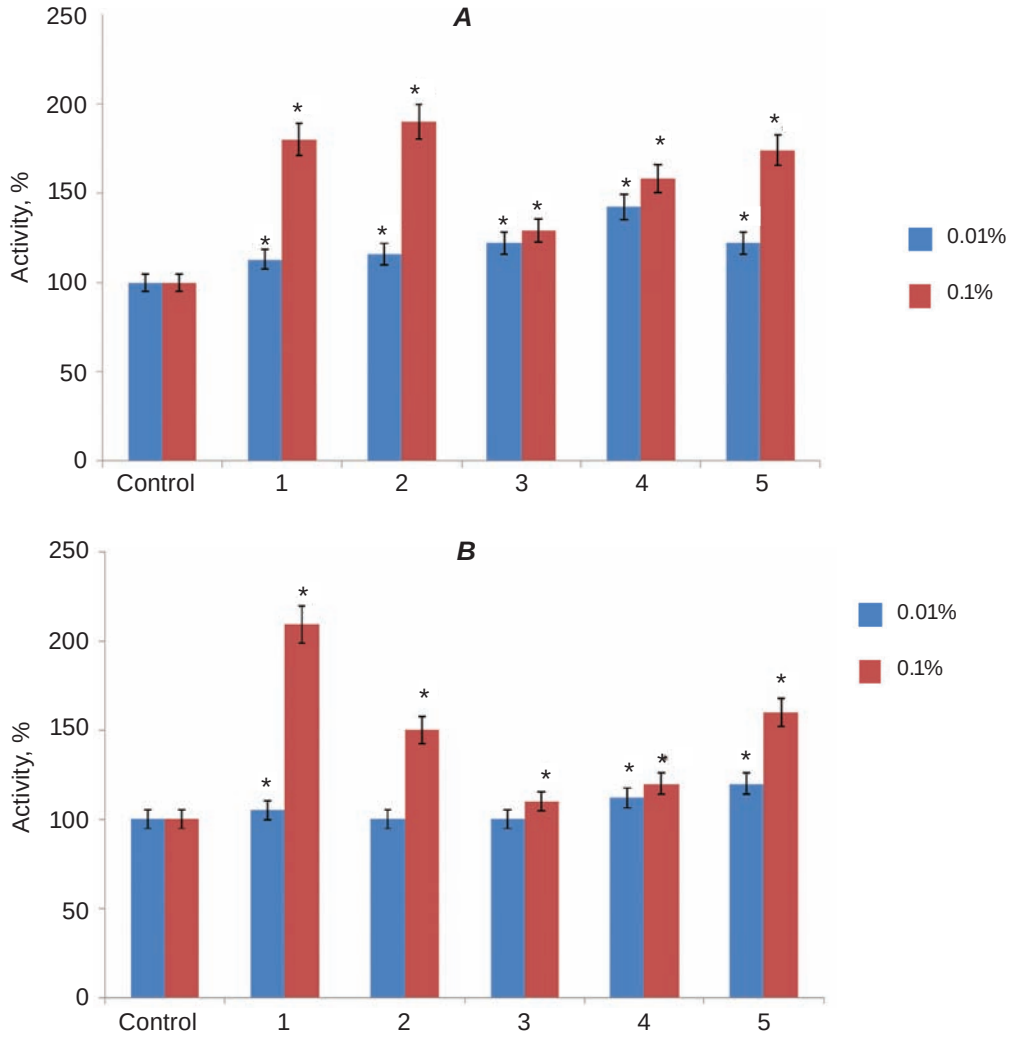

Fig.3. Influence of germanium compounds on the activity of E. erubescens α-L-rhamnosidase. ***A*** *– exposure time 1 h,* ***B*** *– exposure time 24 h, *P < 0.05 compared to control*

None of the studied compounds affected the activity of *P. restrictum* α-galactosidase, which was at the level of control values regardless of the concentration of the compounds or the exposure time.

The study of the effect of germanium coordination compounds on the elastase activity of *Bacillus* sp. IMV B-7883 showed (Fig. 7, *A*, *B*) that only compound 1 displayed an activating effect (70%) in both concentrations and exposure times studied. All other compounds inhibited the activity of the tested enzyme. The greatest inhibitory effect (100%) was exerted by compound 5 at an exposure time of 24 h, in both concentrations.

The study of the influence of the coordination compound 5 at a concentration of 0.01 and 0.1% shows that the inhibitory effect increases with time. Thus, after 20 min of incubation, the activity decreased by 30 and 40%, respectively, at 0.01% and 0.1% concentration of the compound, after 90 min of incubation, complete inhibition was observed (Fig. 8). To determine the nature of the inhibition of elastase *Bacillus* sp. IMV B-7883 by compound 5, dialysis of the reaction mixture against 0.01 M Tris-HCl buffer, pH 7.8 for one day was performed. Complete recovery of activity was observed, which indicates a competitive nature of inhibition.

More interesting was the effect of the studied germanium compounds on the activity of the protease with the fibrinogenolytic activity of *Bacillus* sp. IMV B-7883. None of the studied germanium coordination compounds showed an inhibitory effect on its activity. All compounds increased the activity of the enzyme to a greater or lesser extent. It is shown (Fig. 9, *A*, *B*) that the greatest activation

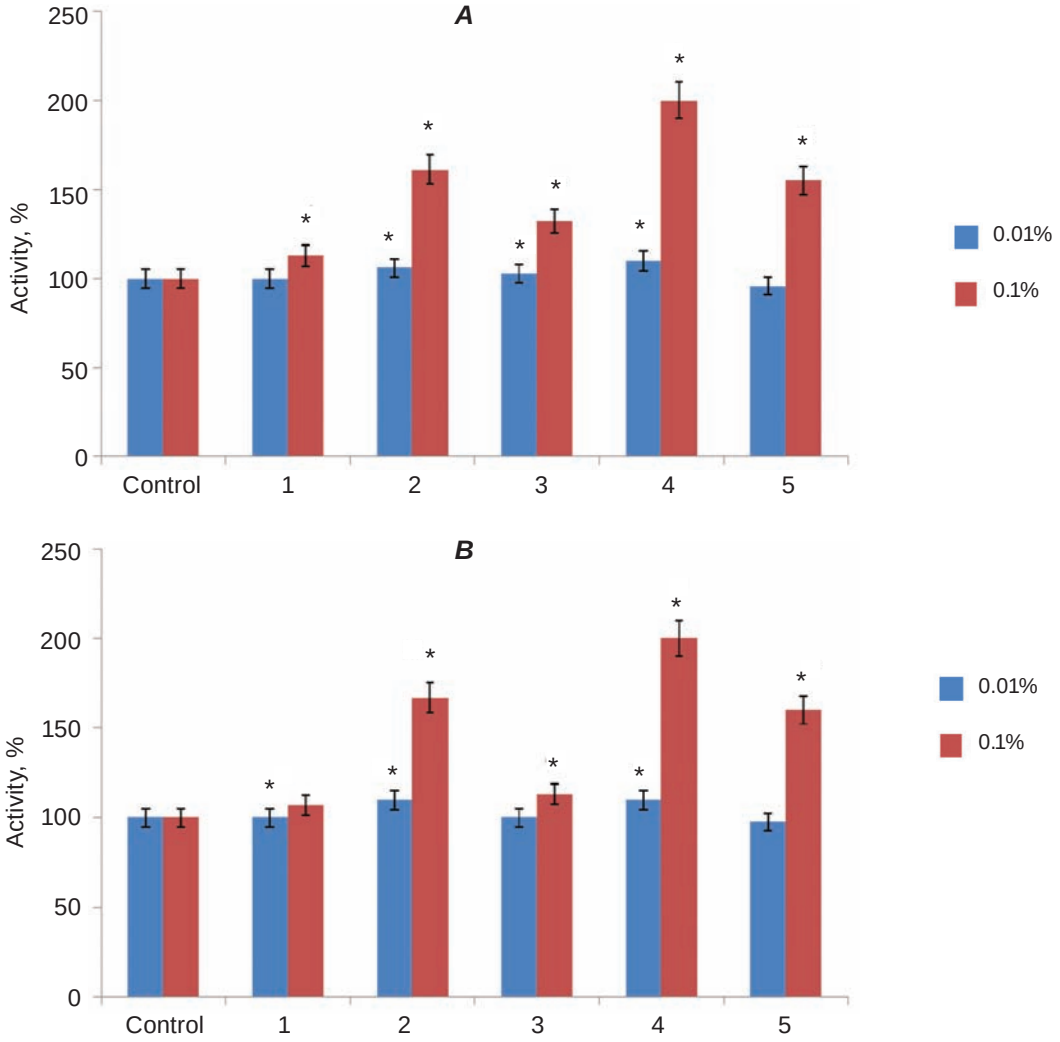

Fig. 4. Influence of germanium compounds on the activity of P. tardum α-L-rhamnosidase. ***A*** *– exposure time 1 h,* ***B*** *– exposure time 24 h, *P < 0.05 compared to control*

was shown by compounds 5 (200%, concentration 0.1%, exposure time 24 h) and 4 (200%, concentration 0.1%, exposure time 1 h). A somewhat lower activation (81%) was observed under the action of compound 5, exposure time 1 h and concentration 0.10 and 62% under the action of substance 4, exposure time 1 h and 24 h, 0.01% concentration.

Compound 1 increased fibrinogenolytic activity by 55% at a concentration of 0.10% and an exposure time of 1 h (Fig. 9, *A*). Compound 3 increased activity by 37%-39%, depending on the concentration and exposure time (Fig. 9, *A*, *B*), while compound 2 showed an activating effect by 35% only at a 0.10% concentration (Fig. 9, *A*, *B*). All compounds, when using a 0.01% concentration, regardless of the exposure time, showed a smaller effect on the activity of *Bacillus* sp. IMV B-7883 fibrinogenase (Fig. 9, *A*, *B*).

Thus, the obtained data on the interaction of germanium complexes with hydroxyethylidene diphosphonic acid (HEDP) and the studied enzymes indicate that they are effective modulators of the activity of rhamnosidases and proteases, but do not affect the activity of α-galactosidase.

Discussion

In modern industrial technological processes, hydrolytic enzymes are of the greatest importance, one of the significant advantages of which, in comparison with other classes of enzymes, is their ability to carry out enzymatic reactions without cofactors and coenzymes. The unique specificity of action and high catalytic activity, as well as the increasing availability of individual enzymes, make them widely used in scientific research in the field of mo-

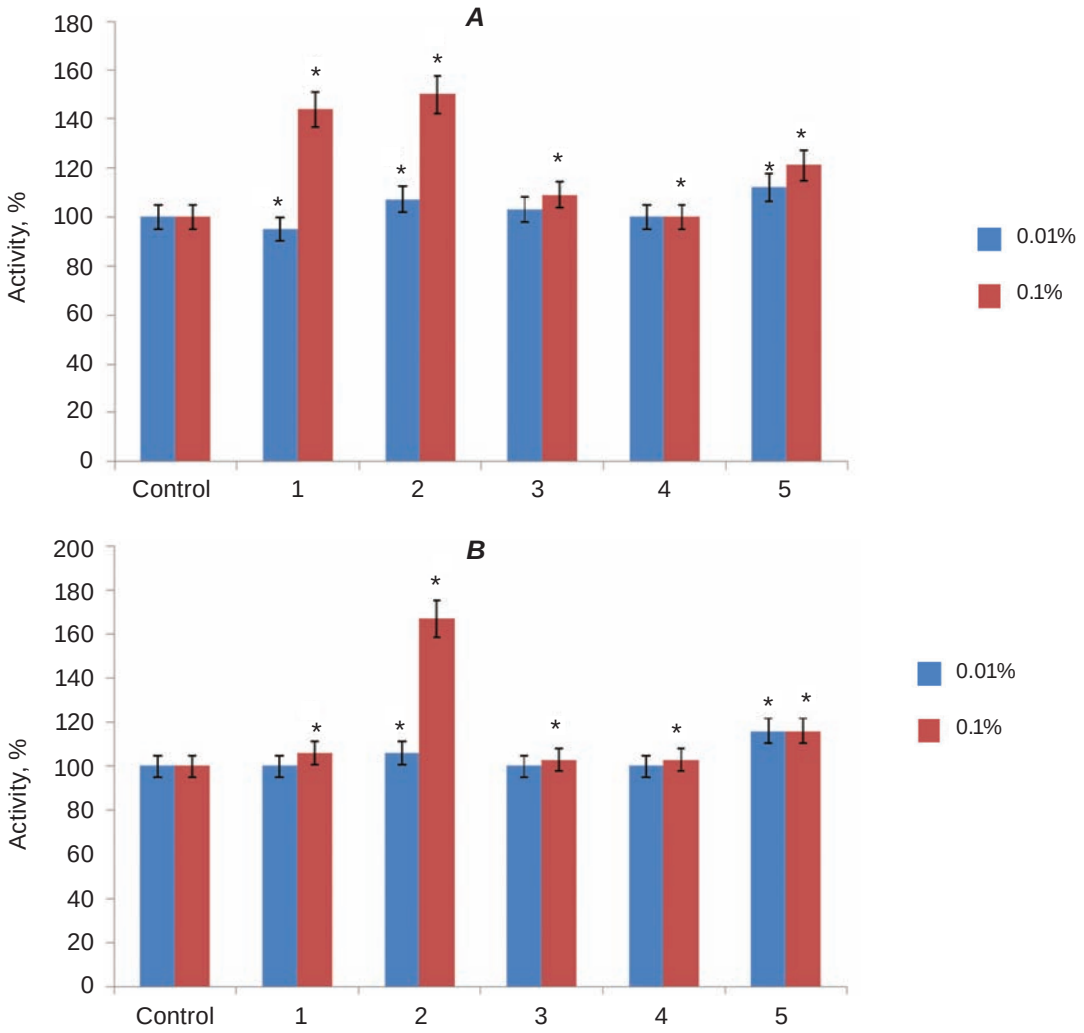

Fig. 5. Influence of germanium compounds on the activity of P. restrictum α-L-rhamnosidase. ***A*** *– exposure time 1 h,* ***B*** *– exposure time 24 h, *P < 0.05 compared to control*

lecular biology and genetics, as well as for solving a number of practical problems in medicine, food, microbiological industry and environmental control. Today, hydrolases have replaced the process of acid hydrolysis in many industries. The most promising for wide application are hydrolytic enzymes, the producers of which are microorganisms due to the unlimited availability of raw materials and great opportunities offered by selection and artificial mutagenesis for directed synthesis. It is known [19, 20] that the efficiency of microbiological production is closely related to the problem of increasing the vital activity of producers, with the development of means of intensification of their growth and increasing productivity. The main factors influencing these processes include physicochemical conditions of cultivation, composition of the nutrient medium and the use of substances that are able to increase not only the growth but also the yield of enzymes and their catalytic activity. In this study, newly synthesized heterometallic Ge(IV) - 3d-metals complexes with 1-hydroxyethane-1,1-diphosphonic acid and 1,10-phenanthroline were used as effectors of the activity of α-L-rhamnosidases *Eupenicillium erubescens, Penicillium tardum, Penicillium restrictum, Cryptococcus albidus*, α-galactosidase *P. restrictum* and proteases with elastolytic and fibrinolytic action *Bacillus* sp.

It has been established that these compounds can either inhibit or activate enzymes, depending on their own composition and the structural characteristics of the enzymes from the studied microbial strains. Thus, different compounds have different activating effects on the same enzyme,

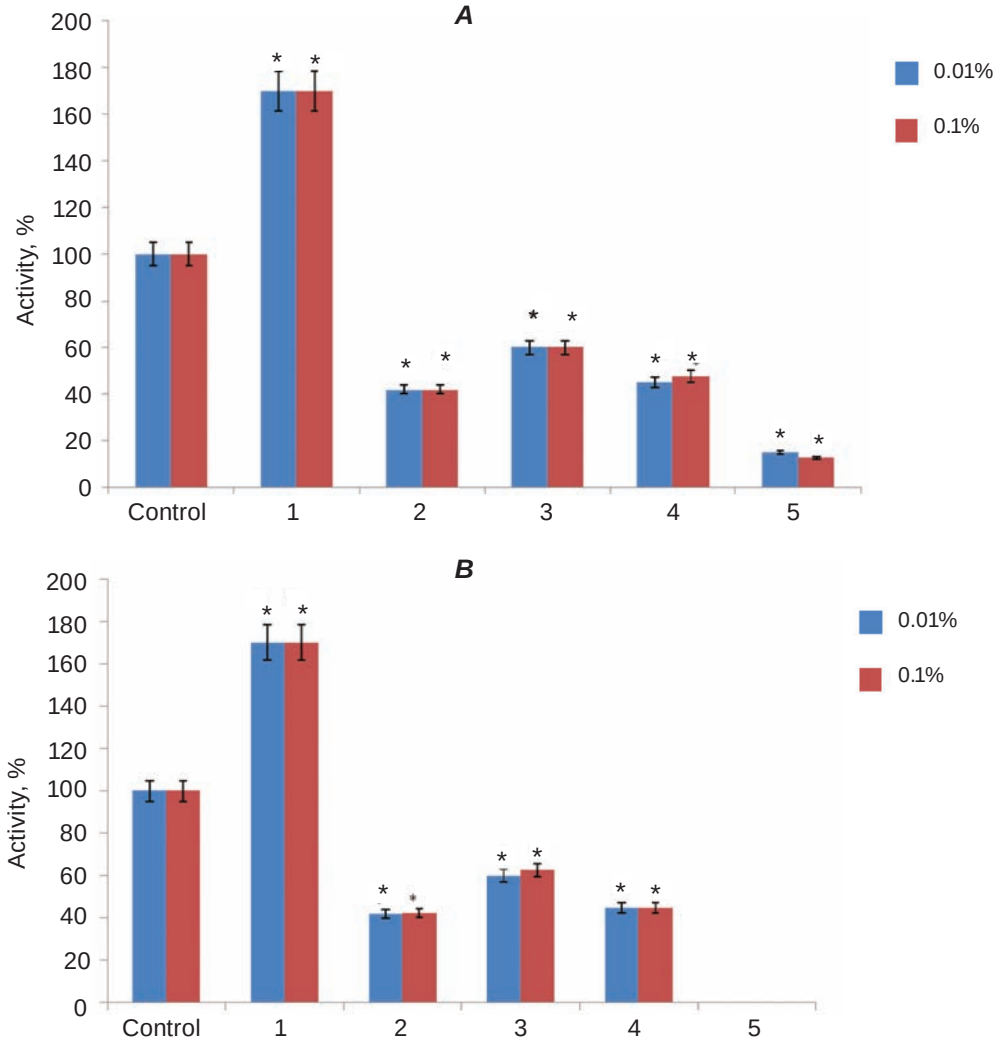

Fig. 6. Influence of germanium compounds on the activity of C. albidus α-L-rhamnosidase. ***A*** *– exposure time 1 h,* ***B*** *– exposure time 24 h, *P < 0.05 compared to control*

α-L-rhamnosidase, when it is derived from different microbial sources. So, the activity of α-L-rhamnosidase *E. erubescens* is stimulated by the compound (1) $[Co(phen)_3]_4[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 2CH_3COOH\cdot 30H_2O$ (200%, 0.1% concentration, exposure time 1 h), *Penicillium tardum* – compound (4) $[Zn(phen)_2(H_2O)_2]_2[Zn(phen)(H_2O)_4]_2[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 18H_2O$ (200%, 0.1% concentration, exposure time 24 h), *Penicillium restrictum* –compound (2) $[Ni(phen)_3]_4[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 2CH_3COOH\cdot 26H_2O$ (67%, 0.01% concentration, exposure time 24 h) and *Cryptococcus albidus* – compound (1) $[Co(phen)_3]_4[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 2CH_3COOH\cdot 30H_2O$ (40%, 0.1% concentration, exposure time 24 h). The activity of *Penicillium restrictum* α-galactosidase was not affected by the coordination compounds studied.

Regarding the elastase activity of *Bacillus* sp IMV B-7883, the compound (1) $[Co(phen)_3]_4[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 2CH_3COOH\cdot 30H_2O$ increased it by 70% (at both concentrations and exposure times tested), while compound (5) $[Fe(phen)_3]_4[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 20H_2O$ inhibited it by 100% (at both concentrations, at an exposure time of 24 h). The greatest stimulating effect on the fibrinogenolytic activity of *Bacillus* sp IMV B-7883 was shown by compounds (5) $[Fe(phen)_3]_4[Ge6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 20H_2O$ (200%, concentration 0.1%, exposure time 24 h) and (4) $[Zn(phen)_2(H_2O)_2]_2[Zn(phen)(H_2O)_4]_2[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 18H_2O$ (200%, concentration 0.1%, exposure time 1 h). Such diverse action of compound 5: inhibitory activity of elastase (100%) and stimulating activity of fibrinogenase (200%) may indicate that the features in the struc-

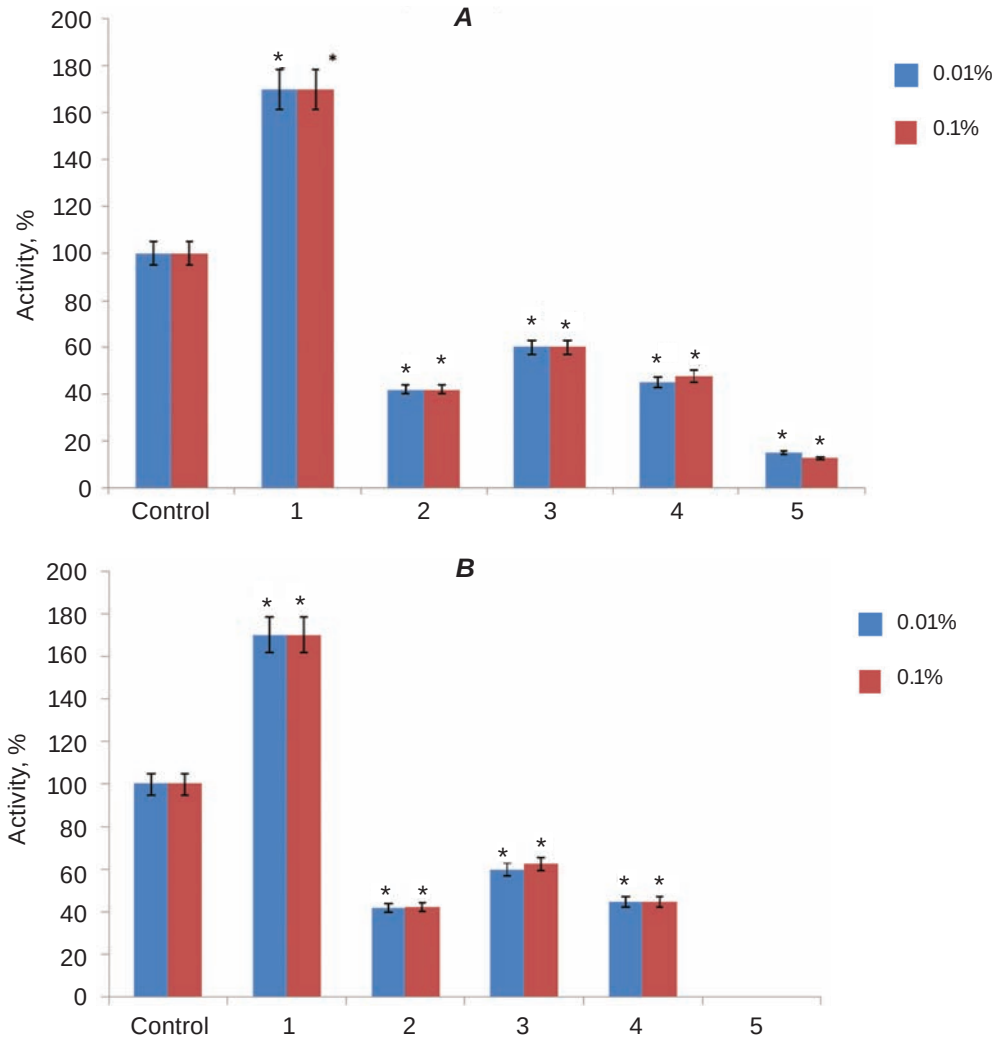

Fig. 7. Influence of germanium compound on the elastase activity of Bacillus sp. IMV B-7883. ***A*** *– exposure time 1 h,* ***B*** *– exposure time 24 h, *P < 0.05 compared to control*

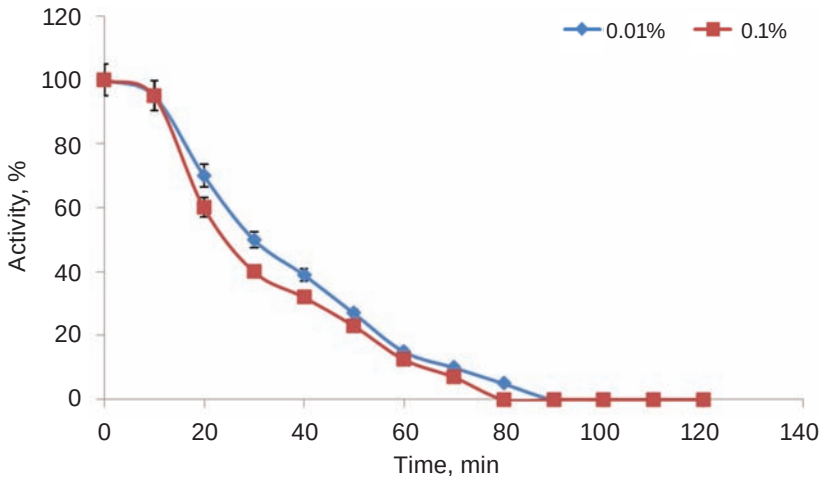

Fig. 8. Influence of compound 5 on the elastase activity of Bacillus sp. IMV B-7883 in dynamic (pH 7.8; t 20°C)

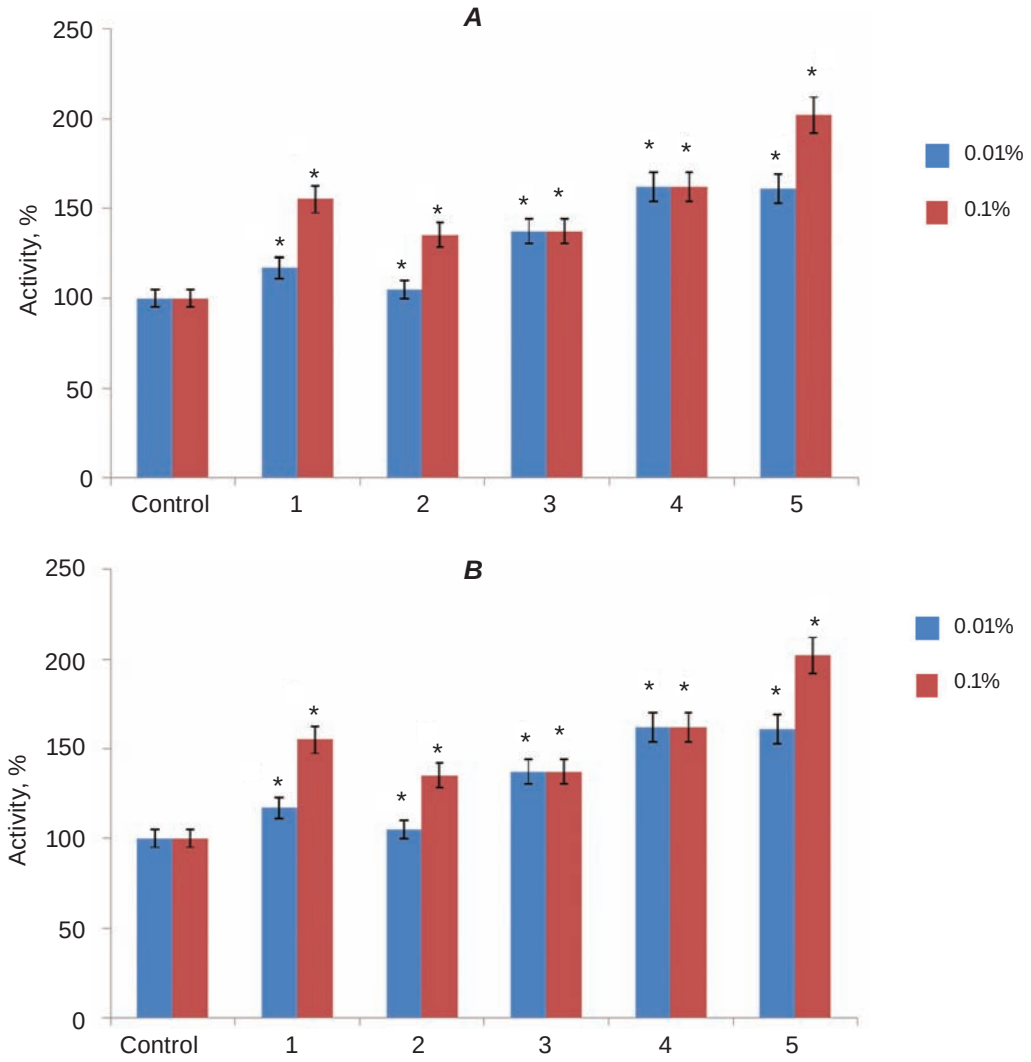

Fig. 9. Influence of germanium compounds on the fibrinogenolytic activity of Bacillus sp. IMV B-7883. ***A*** *– exposure time 1 h,* ***B*** *– exposure time 24 h, *P < 0.05 compared to control*

ture of the catalytic center of the two enzymes play an important role, since all other influencing factors were the same.

The ability of compounds 1-5 to exhibit indifferent action or properties of an activator or inhibitor of protease activity indicates the complexity of the mechanisms of their interaction in the studied systems. In general terms, this can be explained as follows. Potentially, based on the kinetic theory, in a system consisting of an enzyme (E), substrate (S) and modulator (M), the formation of two separate complexes is possible: ES and ESM. It follows that when the modulator exhibits the properties of an activator, the formation of ESM is energetically favorable.

It is in this complex that the effect of the convergence of the reacting groups of the substrate, its conformational rearrangement with a favorable arrangement of these groups for the reaction, is most fully manifested. At the same time, the shape of the hydrophobic filling of the protein globule of the enzyme changes, which is largely determined by the interactions between the structural elements of the amino acids that make up its composition. As a result of these transformations, the activation energy of the catalytic reaction decreases. In the absence of activation, apparently, only the ES complex is realized, the modulator does not have a catalytic effect. In the case of inhibition, competition occurs between the ES and ESM complexes, the degree of which is determined by the energetic advantage of the formation of the complexes, their stability and properties. The competitive nature of inhibition is confirmed by dialysis of the reaction mixture against Tris-HCl

buffer for 24 h, resulted in the enzyme activity being completely restored.

Regardless of the enzyme (except for P. *restrictum* α-galactosidase), in all cases, the dependence of the degree of modulator activity on the composition of the complex cation in its molecule is observed since the anion was the same. Understanding the mechanisms of interaction of these complexes with enzymes opens up broad prospects for their further application. Thus, the coordination compound of germanium (1) $[Co(phen)_3]_4[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 2CH_3COOH\cdot 30H_2O$, which is capable of increasing the activity of α-L-rhamnosidase of *E. erubescens* by 200%, may be useful in the development of therapeutic compositions based on rhamnose-containing substrates. The ability of the compound (5) $[Fe(phen)_3]_4[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 20H_2O$ to inhibit elastase activity by 100% and activate it by 70% by the compound (1) $[Co(phen)_3]_4[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 2CH_3COOH\cdot 30H_2O$ is possibly due to the presence of the Fe ion instead of Co. Indeed, earlier [17] we showed the inhibitory effect of Fe on the elastase activity of *Bacillus* sp. IMV B-7883.

It is noted that the same compounds differently affect elastase and fibrinogenolytic activity, which is associated with a change in the orientation of the substrate bond with the active center of the enzyme, which in turn exhibits specificity: cleaves peptide bonds or activates fibrinogen.

Thus, heterometallic Ge(IV) - 3d-metals complexes with 1-hydroxyethane-1,1-diphosphonic acid and 1,10-phenanthroline are promising compounds for enzyme modification.

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ВПЛИВ ГЕТЕРОМЕТАЛІЧНИХ Ge(IV) - 3d-МЕТАЛІВ НА АКТИВНІСТЬ МІКРОБНИХ РАМНОЗИДАЗ, ГАЛАКТОЗИДАЗИ І ПРОТЕАЗ

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В останні роки увагу дослідників привернули координаційні сполуки германію з різними біолігандами, які можуть бути використані як активатори, так і інгібітори ензимів. Метою роботи було дослідити вплив нових гетерометалічних Ge(IV) - 3d-метал комплексів з 1-гідроксиетан-1,1-дифосфоновою кислотою та 1,10-фенантроліном на активність очищених α-L-рамнозидаз, що продукуються *Eupenicillium erubescens*, *Penicillium tardum*, *Penicillium restrictum*, *Cryptococcus albidus*, α-галактозидази *P. restrictum* та протеаз з еластолітичною та фібриногенолітичною активністю *Bacillus* sp. Досліджувані сполуки (концентрація 0,1%) порізному активували α-L-рамнозидазу залежно від продуцента. Так, активність α-l-рамнозидази *E. erubescens* стимулювалася $[Co(phen)_3]_4[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 2CH_3COOH\cdot 30H_2O$ на 200%, *Penicillium tardum* – $[Zn(phen)_2(H_2O)_2]_2[Zn(phen)(H_2O)_4]_2[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 18H_2O$ на 200%, *Penicillium restrictum* – $[Ni(phen)_3]_4[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 2CH_3COOH\cdot 26H_2O$ на 67%, *Cryptococcus albidus* – $[Co(phen)_3]_4[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 2CH_3COOH\cdot 30H_2O$ на 40%. α-Галактозидаза *Penicillium restrictum* не зазнала впливу досліджуваних сполук. Еластаза *Bacillus* sp. IMB B-7883 активувалася за допомогою $[Co(phen)_3]_4[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 2CH_3COOH\cdot 30H_2O$ на 70%, але повністю інгібувалася $[Fe(phen)_3]_4[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 20H_2O$. Сполуками, що показали найбільший 200% стимулюючий ефект на фібриногенолітичну активність *Bacillus* sp. IMB B-7883 7883, були $[Fe(phen)_3]_4[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 20H_2O$ та $[Zn(phen)_2(H_2O)_2]_2[Zn(phen)(H_2O)_4]_2[Ge_6(\mu\text{-}OH)_4(\mu\text{-}O)_2(\mu\text{-}hedp)_6]\cdot 18H_2O$.

Ключові слова: гетерометалічні Ge(IV) - 3d-метал комплекси, α-L-рамнозидази, α-галактозидаза, протеаза, *Eupenicillium erubescens, Penicillium tardum, Penicillium restrictum, Cryptococcus albidus*, *Bacillus* sp.

References

1. Yadav V, Yadav PK, Yadav S, Yadav KDS. Alpha-L-rhamnosidase: a review. *Proc Biochem*. 2010; 45(8): 1226-1235.
2. Kushwaha A, Kesarwani P, Kushwaha R. Enzymes used for sustainable wet processing in textile industry. *Int J Eng Technol Manag Sci.* 2024; 8(38): 276-283.
3. Varbanets LD, Matseliukh EV. Peptidases of microorganisms and methods of their investigations. K: Naukova Dumka, 2014. 323 p.
4. Çelik E, Özdemir M, Köksoy B, Taskin-Tok T, Taslimi P, Sadeghian N, Yalçın B. New coumarin–thiosemicarbazone based Zn(II), Ni(II) and Co(II) metal complexes: Investigation of cholinesterase, α-amylase, and α-glucosidase enzyme activities, and molecular docking studies. *ChemistrySelect*. 2023; 8 (38): e202301786.
5. Rambabu D, Sharma S, Mayank, Singh N, Shagun, Pooja. Zn-Phenanthroline-dicarboxylate complex: a dual ihibitor of COX-2 and 5-LOX enzymes. *ChemistrySelect*. 2024; 9(23): e202400310.
6. Dunn CJ, Fitton A, Sorkin EM. Etidronic acid. A review of its pharmacological properties and therapeutic efficacy in resorptive bone disease. *Drugs Aging.* 1994; 5(6): 446-474.
7. Povoroznyuk VV, Grygorieva NV, Pekhnyo VI, Resch H, Kozachkova OM, Tsaryk NV. Etidronic acid and its calcium-comprising complexes in the treatment of experimental osteoporosis in estrogen-deficient rats. *J Miner Stoffwechs uskuloskelet Erkrank*. 2018; 25: 7-12.
8. Luo X, Sun J, Kong D, Lei Y, Gong F, Zhang T, Shen Z, Wang K, Luo H, Xu Y. The role of germanium in diseases: exploring its important biological effects. *J Transl Med.* 2023; 21(1): 795.
9. Afanasenko E, Seifullina I, Martsinko E, Konup L, Fizer M, Gudzenko O, Borzova N. Supramolecular salts of Fe(II)/Co(II)/Ni(II)/Cu(II)/Zn(II) 1,10-phenanthroline cations and similar complex tartratostannate(IV) anions: from structural features to antimicrobial activity and enzyme activation. *ChemistrySelect*. 2022; 7(12): e202200280.
10. Gudzenko OV, Varbanets LD. Purification and physico-chemical properties of *Eupenicillium erubescens* alpha-L-rhamnosidase. *Mikrobiol Z.* 2012; 74(2): 14-21. (In Russian).
11. Gudzenko OV, Varbanets LD. Purification and physico-chemical properties of *Penicillium tardum* α-L-rhamnosidase. *Mikrobiol Z.* 2016; 78(1): 13-22. (In Ukrainian).
12. Gudzenko OV, Varbanets LD. Purification and physico-chemical properties of *Cryptococcus albidus* 1001 α-L-rhamnosidase. *Mikrobiol Z.* 2012; 74(6): 16-23. (In Russian).
13. Gudzenko OV., Varbanets LD. Isolation and characterization of *Bacillus* sp. IMV B-7883 proteases. *Ukr Biochem J.* 2023; 95(6): 98-107.
14. Chaplin ME, Kennedy JE. (Eds.) Carbohydrate analysis: a practical approach. Washington, Oxford: IRL Press, 1986.
15. Davis WB. Determination of Flavanones in Citrus Fruits. *Anal Chem.* 1947; 19(7): 476-478.
16. AlShaikh-Mubarak GA, Kotb E, Alabdalall AH, Aldayel MF. A survey of elastase-producing bacteria and characteristics of the most potent producer, *Priestia megaterium* gasm32. *PLoS One*. 2023; 18(3): e0282963.
17. Masada M. Determination of the thrombolytic activity of Natto extract. *Food Style.* 2004; 8(1): 92-95.
18. Tsymbaliuk K, Martsynko O, Dyakonenko V, Finik O, Seifullina I, Shishkina S. Synthesis and structure of heterometallic multiligand Ge(IV) - 3d-metals complexes with 1-hydroxyethane-1,1-diphosphonic acid and 1,10-phenanthroline. *Chem J Moldova.* 2024; 19(2): 28-35.
19. Solanki P, Putatunda C, Kumar A, Bhatia R, Walia A. Microbial proteases: ubiquitous enzymes with innumerable uses. *3 Biotech.* 2021; 11(10): 428.
20. Razzaq A, Shamsi S, Ali A, Ali Q, Sajjad M, Malik A, Ashraf M. Microbial Proteases Applications. *Front Bioeng Biotechnol.* 2019; 7: 110.