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MODIFICATION OF *BACILLUS SP.* IMV B-7883 ELASTASE ACTIVITY BY THE HETERO-METALLIC CARBOXYLATOGERMANATES/STANNATES

In recent years, some researchers have found that although many Gram-negative and Gram-positive bacteria secrete elastase, the bacterial forms of elastase have either a low activity or harmful effects. Therefore, further research is needed in isolating and screening microorganisms that produce a high level of elastase activity. Previously we selected strain Bacillus sp. IMV B-7883, which exhibits fairly high elastase activity. To increase its activity, we chose one of the well-known approaches, in particular, the use of a number of coordination compounds capable to influence elastase activity. In this regard, the ***purpose*** *of this work was to study the effect of such coordination compounds as hetero-metallic carboxylatogermanates/stannates on the elastase activity of Bacillus sp. IMV B-7883.* ***Methods.*** *The object of the investigation was the strain of Bacillus sp., deposited in the Ukrainian Collection of Microorganisms under the number IMV B-7883, isolated from soil. The culture was grown under conditions of submerged cultivation at 28 °C, with a mixing speed of the nutrient medium of 244 rpm for three to six days (72—144 hours). We used an enzyme purified from the supernatant of the culture liquid by precipitation with 90% ammonium sulfate, with further fractionation on neutral and charged carriers. Elastase activity was determined colorimetrically by the intensity of the color of the solution upon enzymatic hydrolysis of elastin stained with Congo red. As modifiers of enzyme activity, hetero-metallic carboxylatogermanates/stannates were used.* ***Results.*** *Of the 15 studied in this work coordination compounds presented by hetero-metallic carboxylatogermanates/stannates, only 1* $[Ba(H_2O)_6][Ge_2(OH)_2(C_6H_8O_7)_2]\cdot nH_2O$, *n=2 and 3* $[Ni\ (H_2O)_6][Ge_2(OH)_2(C_6H_8O_7)_2]\cdot nH_2O$, *n=4, depending on the concentration used and incubation time, increase the elastase activity by only 3—5%. All other compounds*

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have an inhibitory effect. ***Conclusions.*** *Obtained data on the inhibitory effect of hetero-metallic carboxylatogermanates/stannates on the elastase activity of Bacillus sp. IMV B-7883 provide new information which may help in solving the issue of the mechanism of interaction between enzymes and complex chemical molecules.*

Keywords: *Bacillus sp. IMV B-7883, elastase activity, hetero-metallic carboxylatogermanates/stannates complexes.*

Research on proteases is important both in a theoretical aspect — for understanding the structure of proteins and peptides, the mechanism of enzymatic catalysis, and in a practical sense. So, they are characterized by high necrotic activity but do not affect healthy tissue, lyse viscous purulent exudates, and exhibit thrombolytic and anti-inflammatory effects. Today, a number of protease producers of various origins are known. But the most promising for widespread use are microorganisms that are capable of rapidly multiplying and synthesizing biologically active substances under controlled conditions (Mótyán et al., 2013; Yang et al., 2023; López-Otín & Bond, 2008). The ability of microorganisms to release significant amounts of proteases into the environment facilitates the task of their isolation and purification. Since the microbial cell, depending on the living conditions, is surrounded by different protein substrates, proteases of both broad specificity, hydrolyzing several substrates, and highly specific ones, acting exclusively on a specific substrate, are synthesized. Researchers' attention is drawn to elastases (EC 3.4.21.36), serine proteases catalyzing cleavage of carboxyl groups present on small hydrophobic amino acids, such as glycine, alanine, and valine. Its primary role is the breakdown of elastin, a protein that imparts elasticity to connective tissue. Elastase is the only enzyme that targets, solubilizes, and degrades elastin. Clinically, elastase is used as a vascular dilator drug. In industries of food processing, elastase is used to tenderize meat and improve meat quality. In recent years, some researchers have found that although many Gram-negative and Gram-positive bacteria secrete elastase, the bacterial forms of elastase have either a low activity or harmful effects. Therefore, further research is needed in isolating and screening microorganisms that produce a high level of elastase (Chen et al., 2007). An analysis of the works of a number of researchers (Kotb et al., 2023; Mechri et al., 2019; Su et al., 2020) gave us the opportunity to assume that promising microorganisms are representatives of the genus *Bacillus*, which synthesize peptidase with elastase activity. And, indeed, we previously (Gudzenko et al., 2023a) selected strain *Bacillus* sp. IMV B-7883, which exhibits fairly high elastase activity. We (Gudzenko et al., 2020) have previously shown that a number of metal coordination compounds can exhibit both stimulating and inhibitory effects on the activity of a number of enzymes. In this regard, the purpose of this work was to study the effect of such coordination compounds as hetero-metallic carboxylatogermanates/stannates on the elastase activity of *Bacillus* sp. IMV B-7883.

Materials and Methods. The object of the investigation was the strain of *Bacillus* sp., deposited in the Ukrainian Collection of Microorganisms under the number IMV B-7883, isolated from soil.

Bacillus sp. IMV B-7883 was submerged and cultivated in Erlenmeyer flasks on a liquid medium of the following composition, (g/L): KH_2PO_4 — 1.6; $MgSO_4 \times 7H_2O$ — 0.75; $ZnSO_4 \times 7H_2O$ — 0.25; $(NH_4)_2SO_4$ — 0.5; maltose — 1.0; gelatin — 10.0; yeast autolysate — 0.15, pH — 7.0. The strain was cultivated for three to six days (72—144 hours) in flasks on a shaker (100 mL of medium, 28 °C, 244 rpm). The inoculum was taken on the same medium for 24 hours and inoculated in flasks at a quantity of 10^5—10^6 colony-forming units (CFU). Cells were separated from the culture liquid medium by centrifugation at 5000 g for 30 min. The enzyme was purified from the supernatant of the culture liquid by precipitation with 90% ammonium sulfate, with further fractionation on neutral and charged carriers, as we described previ-

ously (Gudzenko & Varbanets, 2023). Elastase activity was determined colorimetrically by the intensity of the color of the solution upon enzymatic hydrolysis of elastin stained with Congo red (Trowbridge & Moon, 1972). The incubation mixture contained 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 culture liquid supernatant. The reaction mixture was incubated for 5 hours at a temperature of 37 °C. The reaction was stopped by keeping the test tubes with the reaction mixture in an ice bath for 30 minutes. Unhydrolyzed elastin was separated by centrifugation for 10 min at 10000 g. The color intensity was measured on a 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.

As modifiers of enzyme activity, three groups of compounds were used.

Group 1 of compounds (1—5) includes coordination compounds of Ge(IV) with (Ba(II), (Co(II), Ni(II), Cu(II), Zn(II)), and gluconic acid. The synthesized complexes correspond to the formulas $[M(H_2O)_6][Ge_2(OH)_2(C_6H_8O_7)_2]\cdot nH_2O$ (M = Ba(1), n=2; Co(2), n=4; Ni(3), n=4; Cu(4), n=4; Zn(5), n=3). They was described previously in (Gudzenko et al., 2023b).

Group 2 of compounds (9—14) includes mixed-ligand germanium-3d-metal complexes: $[Ni(bipy)_3][Ge(HCit)_2]\cdot 3H_2O$ (9); $[Ni(phen)_3][Ge(HCit)_2]\cdot 2H_2O$ (10); $[\{Cu(bipy)_2\}_2Ge(m\text{-}Cit)_2]\cdot 12H_2O$ (11); $[\{Cu(phen)_2\}_2Ge(m\text{-}Cit)_2]\times \times 13H_2O$ (12); $[Zn(bipy)_3][Ge(HCit)_2]\cdot 2H_2O$ (13), and $[Zn(phen)_3][Ge(HCit)_2]\cdot 3H_2O$ (14) including 1,10-phenanthroline, 2,2'-bipyridine, and citric acid H_4Cit. The synthesis of these compounds was described previously (Seifullina et al., 2016, 2017a, 2017b; Gudzenko et al., 2023a).

Group 3 of compounds (15—18) of novel supramolecular salts with 1,10-phenanthroline 3d-metals cations and malatostannate(IV) anion $[Fe(phen)_3]_2[\{Sn(HMal)_2(Mal)\}Cl]\cdot 14H_2O$ (15), $[Co(phen)_3]_2[\{Sn(HMal)_2(Mal)\}Cl]\cdot 14H_2O$ (16), $[Ni(phen)_3]_2[\{Sn(HMal)_2(Mal)\}Cl]\cdot 14H_2O$ (17), $[Cu(phen)_3]_2[\{Sn(HMal)_2(Mal)\}Cl]\cdot 10H_2O$ (18).

It was established that malate anion $[Sn(HMal)_2(Mal)]^{3-}$ contains bidentate $HMal^{2-}$ and Mal^{3-} forms. The Sn atoms are six-coordinated and their polyhedrons are distorted octahedrons. The specific feature of compounds 15—18 is the presence of the additional Cl^- anion, which compensates the charge of the two $[M(phen)_3]^{2-}$ cations and additionally connects them to each other.

When studying the effect of various germanium-containing compounds on the activity of enzymes, we used concentrations of 0.1 and 0.01% and time of exposure 0.5 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 ± m) and are considered significant at $p < 0.05$. The results presented in graphs were processed using Microsoft Excel 2007.

Results. Study of the influence of coordination compounds of group 1 (1—5) on the elastase activity of *Bacillus* sp. IMV B-7883 showed (Fig. 1 *a*, *b*) that compounds 1 and 3 at a concentration of 0.1% and an exposure time of 1 hour increased activity only by 3—5%, which is not reliable enough compared to control. Increasing the action time to 24 hours (Fig. 1, *b*) returned the activity to the initial values under the action of substance 3 but did not reduce activation by compound 1. In general, when studying the effect of the compounds of group 1, it was shown that the difference in the action time appeared only for substance 2 in concentration 0.1%. The other compounds 1, 3, 4, 5 showed similar effects both at 1 hour and at 24 hours of incubation. It was shown that the highest degree of inhibition (62.5%) of elastase activity in this group was exhibited by compound 2 at a concentration of 0.1%. At a lower concentration, the inhibitory effect decreased to 37.5%. The inhibition by compound 5 at a concentration of

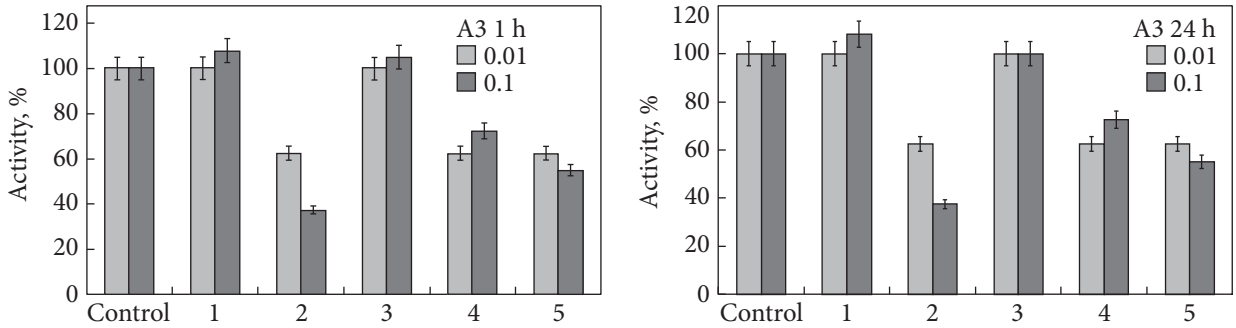

Fig. 1. Influence of germanium compounds of group 1 on the elastase activity of *Bacillus* sp. IMV B-7883: A — exposure time 1 hour, B — exposure time 24 hours

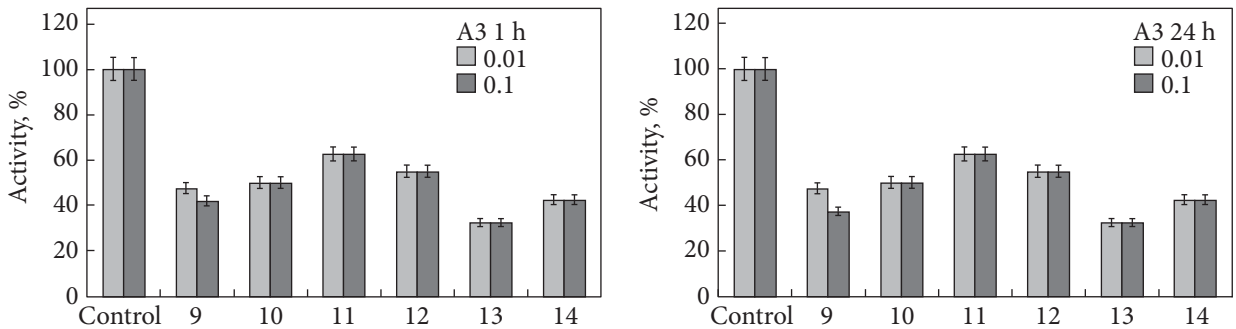

Fig. 2. Influence of germanium compounds of group 2 on the elastase activity of *Bacillus* sp. IMV B-7883: A — exposure time 1 hour, B — exposure time 24 hours

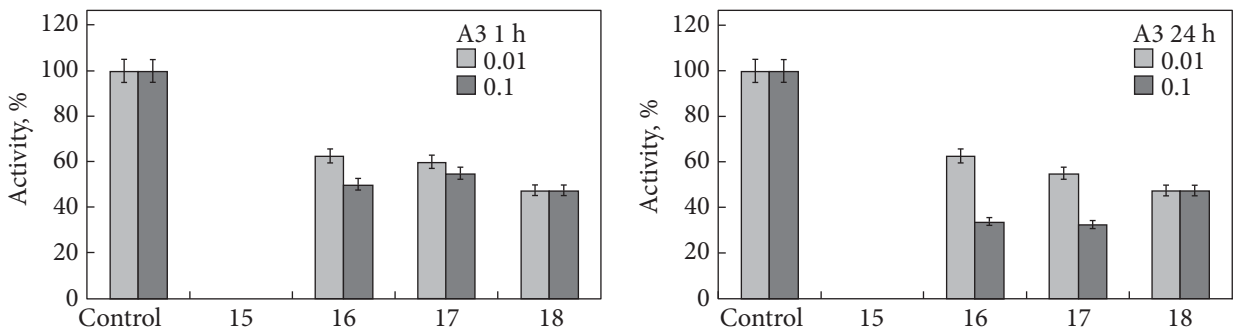

Fig. 3. Influence of germanium compounds of group 3 on the elastase activity of *Bacillus* sp. IMV B-7883: A — exposure time 1 hour, B — exposure time 24 hours

0.1% was slightly lower (45%). For compound 4 at a concentration of 0.1%, the inhibitory effect was at the level of 26.5%. When studying the effect of the compounds of group 1 at a concentration of 0.01%, the same degree of inhibition of elastase activity (37.5%) by substances 2, 4, 5 was shown, while the effect of compounds 1 and 3 remained at the control level.

A study of the effect of compounds of group 2 (9—14) showed (Fig. 2 A, B) that all of them inhibited the elastase activity of *Bacillus* sp. IMV B-7883 to a greater or lesser extent. The degree of inhibition did not depend on time and the concentration of substances. The greatest inactivation (67.5%) was observed with compound 13. Compounds 14 (57.5%), 10 (50%), 12 (45%),

and 11 (37.5%) had a fairly strong inhibitory effect. The degree of inhibition by compound 9 depended on the concentration of the substance and the exposure time. The greatest inactivation (62.5%) was observed with an action time of 24 hours and a compound concentration of 0.1%. With an action time of 1 hour, the degree of inhibition was 10% lower compared to 24 hours.

Group 3 compounds (15—18) also showed (Fig. 3 A, B) an inhibitory effect on the elastase activity of *Bacillus* sp. IMV B-7883. Compound 15 resulted in complete inhibition of activity at both concentrations and at different times of action. The effect of compound 18 was also independent of time and concentration: inhibition of enzyme activity by 52.5% was observed under all experimental conditions. Compounds 16 and 17 inhibited the enzyme to a greater extent at higher concentrations of the substance (0.1%). It was shown that at a concentration of 0.01%, compound 16 reduced enzyme activity by 37.5, regardless of the incubation time, whereas at a 0.1% concentration of this substance, elastase activity decreased by 50%, and at an incubation time of 24 hours — by 66.25%. At a 0.01% concentration of substance 17, elastase activity decreased by 40%, and at an incubation time of 24 hours — by 45%. An increase in concentration did not contribute to higher inhibition with an exposure of 1 hour and amounted to 45%. With an action time of 24 hours, the highest degree of inhibition (67.5%) by substance 17 was noted.

Discussion. To increase the activity of enzymes, a number of approaches can be used, in particular, the optimization of cultivation conditions, the use of inducers of both synthetic and natural origin. But in recent years, special attention of researchers has been directed to the search for substances that can directly affect the activity of enzymes, in particular, coordination compounds of transition metals. Coordination compounds of germanium, cobalt, and nickel with organic ligands have already proven themselves as promising enzyme activators (Wiltschi, et al., 2020; Kaul & Asano, 2012; Atanasov et al., 2021), and low toxicity and a wide spectrum of biological activity encourage researchers to synthesize new complex compounds (Insuasty et al., 2020). Earlier (Gudzenko et al., 2023a, 2023b, 2023c), we showed that various germanium complexes with bioligands can be recommended for targeted search for α-L-rhamnosidase effectors. It was shown that the most effective compound was tris(bipyridine) nickel(II) with μ-dihydroxysalarogermanate (IV), which increased the activity of α-L-rhamnosidase of *E. erubescens* by 9.7 times; and *P. tardum* 8 times (exposure time 24 h, effector concentration 0.1%), as well as tris(bipyridine)nickel(II) bis(citrate) germanate hydrate (IV), the maximum activating effect of which (when using 0.1% concentration on α-L-rhamnosidase of *E. erubescens)* was by 2.5 times, and *P. tardum* — by 5 times.

Unfortunately, of the 15 coordination compounds studied in this work, the effect of the two compounds was almost at the control level (3—5%): 1 $[Ba(H_2O)_6][Ge_2(OH)_2(C_6H_8O_7)_2]\cdot nH_2O$, n = 2 and 3 $[Ni\ (H_2O)_6][Ge_2(OH)_2(C_6H_8O_7)_2]\cdot nH_2O$, n = 4. All the other compounds had an inhibitory effect. The obtained results regarding the inhibitory effect of the studied coordination compounds are of great practical and theoretical interest. They can be used for practical purposes to develop new medicines: for the treatment of cardiovascular diseases (Korkmaz et al., 2010) (to reduce the level of elastase in the blood and prevent atherosclerosis) (Wen et al., 2018); treatment of diabetes (to reduce the level of elastase in the blood and prevent diabetic retinopathy (Kunder et al., 2022), and treatment of chronic obstructive pulmonary diseases (to reduce the level of elastase in them). In a theoretical sense, enzyme inhibitors can be used to elucidate some of their mechanisms of action.

The complexity of the mechanisms of interaction between metal coordination compounds and enzymes is evidenced by our previously obtained data (Afanasenko et al., 2023) that novel supramolecular salts with 1,10-phenanthroline 3d-metals cations and malatostannate(IV) an-

ion —15, 16 and 17 in the concentration 0.1% at exposure time of 1 hour increased the activity of *Cryptococcus albidus* α-L-rhamnosidase by 2.44—2.55 times. Enlarging the exposure time up to 24 h leaded to the activation by 21—90%, while at the concentration 0.01%, all compounds slightly inhibited the studied enzyme. Analysis of the data obtained from studying the effect of coordination compounds on the activity of *Cryptococcus albidus* α-L-rhamnosidase and elastase of *Bacillus* sp. IMV B-7883 makes it possible to assume that in addition to the structure of the effector, the structure of the enzyme is important for being active.

Of course, further studies are needed to confirm the potential of the investigated coordination compounds for the treatment of diseases associated with the elastin dysfunction.

Although the compounds described in this work are inferior to previously studied activators and inhibitors of a similar nature in terms of their effectiveness toward elastase activity, they provide new information that may help in solving the issue of the mechanism of interaction between enzymes and complex chemical molecules.

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МОДИФІКАЦІЯ ЕЛАСТАЗНОЇ АКТИВНОСТІ *BACILLUS* SP. IMV B-7883 ГЕТЕРОМЕТАЛІЧНИМИ КАРБОКСИЛАТОГЕРМАНАТАМИ/СТАНАТАМИ

В останні роки деякі дослідники виявили, що хоча багато грамнегативних і грампозитивних бактерій продукують еластазу, її бактеріальні форми або мають низьку активність, або шкідливі. Тому необхідні подальші дослідження щодо виділення та скринінгу мікроорганізмів, які продукують високий рівень еластази. Раніше ми відібрали штам *Bacillus* sp. IMV B-7883, який проявляє досить високу еластазну активність. Для підвищення його активності ми обрали один із відомих підходів, зокрема використання ряду координаційних сполук, здатних впливати на активність еластази. У зв'язку з цим **метою** даної роботи було вивчити вплив таких координаційних сполук як гетерометалічні карбоксилатогерманати/станати на еластазну активність *Bacillus* sp. IMB B-7883. **Методи**. Об'єктом дослідження був виділений із ґрунту штам *Bacillus* sp., депонований в Українській колекції мікроорганізмів під номером IMB B-7883. Культуру вирощували в умовах глибинного культивування при 28 °C зі швидкістю перемішування живильного середовища 244 об/хв впродовж 3—6 діб (72—144 години). Використовували фермент, очищений з супернатанту культуральної рідини осадженням 90% сульфатом амонію з подальшим фракціонуванням на нейтральних і заряджених носіях. Активність еластази визначали колориметрично за інтенсивністю забарвлення розчину при ферментативному гідролізі еластину, забарвленого конго червоним. Як модифікатори ферментативної активності використовували гетерометалічні карбоксилатогерманати/станати. **Результати.** З 15 досліджених у цій роботі координаційних сполук, представлених гетерометалічними карбоксилатогерманатами/станатами, лише 1 $[Ba(H_2O)_6][Ge_2(OH)_2(C_6H_8O_7)_2] \times nH_2O$, n = 2 та 3 $[Ni(H_2O)_6][Ge_2(OH)_2(C_6H_8O_7)_2] \times$ nH2O, n = 4, залежно від використовуваної концентрації та часу інкубації, підвищували активність еластази лише на 3—5 %. Всі інші сполуки мають гальмівну дію. **Висновки.** Отримані дані щодо інгібуючої дії гетерометалічних карбоксилатогерманатів/станатів на еластазну активність *Bacillus* sp. IMV B-7883 надають нову інформацію, яка може допомогти у вирішенні питання щодо механізму взаємодії між ферментами та складними хімічними молекулами.

Ключові слова: *Bacillus sp. IMV B-7883, активність еластази, гетерометалічні карбоксилатогерманатні/станатні комплекси.*