SCIENTIFIC HORIZONS

Journal homepage: https://sciencehorizon.com.ua
Scientific Horizons, 25(11), 20-30

UDC 619:576.895:619:616.98:579.873.21T
DOI: 10.48077/scihor.25(11).2022.20-30

Influence of *Sitophilus Oryzae* on Biological Properties of *Mycobacterium Bovis*

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Article's History:
Received: 10/05/2022
Revised: 11/23/2022
Accepted: 12/16/2022

Suggested Citation:
Zazharskyi, V., Alifonova, K., Bilan, M., Kozak, N., & Kasianenko, O. (2022). Influence of *Sitophilus Oryzae* on biological properties of *Mycobacterium Bovis*. *Scientific Horizons*, 25(11), 20-30.

Abstract. The active spread of tuberculosis around the world has been an urgent issue for many decades, but even now, there is no data on all available methods of introducing the pathogen into a previously healthy territory and the mechanisms of mycobacteria activity, and this makes it impossible to eradicate the disease completely. The purpose of this study was to determine the effect of the rice weevil (lat. *Sitophilus oryzae*) on the dissociative forms of mycobacterium tuberculosis, their viability, biochemical properties and virulence through passage of the pathogen through the insect's body. To fulfil this purpose, the following methods were used: biological (experimental infection of laboratory animals), anatomical pathology and determining the viability of mycobacteria by counting colony-forming units. The effect on pathogenic properties of dissociative forms of *Mycobacterium bovis* (passage 118) after passage through the body of the rice weevil in an experiment on guinea pigs was determined and summarized. The change in the viability of mycobacteria isolated in the dynamics of the experiment in comparison with the original culture was described. The plasticity of the biochemical activity of the pathogen after passing through the body of the beetle was proven, and the influence of the macroorganism on the enzymatic properties of mycobacterium tuberculosis was analysed. The phenomena of the activation of enzymatic systems, which are associated with the adaptation and survival of mycobacteria in the conditions of a changed habitat, were substantiated. The practical value of this study lies in the improved understanding of the processes of the spread of mycobacterium tuberculosis and the established role of insects in the transmission of infection, with the prospect of developing measures to prevent and combat the disease

Keywords: mycobacteria, rice weevil, dissociative strain, biochemical properties, colony-forming units, variability

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INTRODUCTION

The issue of the rapid spread of tuberculosis among animals and humans has been relevant for many years throughout the world, as well as in Ukraine. This disease poses a great epizootological threat and is accompanied by considerable economic and social losses (Pérez-Morote *et al*., 2020). Two important problems are still the most understudied. The first is the development of anti-tuberculosis preparations to fight infection (Bihdan *et al*., 2018; Gotsulya *et al*., 2020; Hotsulia *et al*., 2021). Considerable attention is paid to elucidating the antimicrobial effect of plant extracts and derivatives of 1,2,4-triazole-3-thiols (Palchykov *et al*., 2019; Zazharskyi *et al*., 2019; Zazharskyi *et al*., 2020). The second is the question of determining all possible ways of the tuberculosis pathogen entering a previously safe territory, to interrupt the epizootological chain and prevent new foci of infection. The spread of mycobacterium tuberculosis is ensured by the exceptional resistance of mycobacteria to the action of physical and chemical factors due to the presence of lipids in the composition of the cell wall, their ability to change to adapt the microorganism in an unfavourable environment and to resist the protective forces of the macroorganism (Queiroz *et al*., 2017). Changes in the morphology, biological and biochemical properties of *M. bovis* are not accidental, they are related to the maintenance of the viability of the microbe in the surrounding environment. It is proved that mycobacteria can change both genotypic and phenotypic traits. To survive, mycobacteria can change their morphological features, tincture, cultural properties, as well as virulence and enzymatic activity. A typical example of the variability of the causative agent of tuberculosis is the dissociation of mycobacteria. Dissociative forms of *M. bovis* are characterized by a change in the metabolic processes of the bacterial cell, which contributes to a wide range of survival of the microbe in the external environment (Tkachenko *et al*., 2020).

As a result of adaptation to the conditions of existence, the pathogen has an expansive range of biological and mechanical vectors. *M. bovis* actively circulates in nature and maintains its viability due to the large number of potential reservoirs in nature. Crispell *et al.* (2019) described the possibility of both intraspecific and interspecific transmission of the pathogen. Some authors demonstrate that insects of various species can be a source of tuberculosis infection. Thus, Cano *et al.* (2018) indicate that Hemiptera insects are potential carriers of Mycobacterium ulcerans. Silva *et al.* (2022) found that Triatomine bugs are carriers of mycobacteria, namely species of *Mycobacterium tuberculosis complex*.

During the experimental infection of beetles of the rice weevil species (Latin *Sitophilus oryza*e) with *M. bovis* by the method of contamination of wheat with the pathogen's suspension, it was determined that the bacteria stay viable in the body of weevils until the 50th day, and are released by the beetles into the environment until the 30th day after infection (Tkachenko *et al*., 2021). However, the issue of changes in the biological properties of mycobacteria after passage through the body of beetles is understudied and requires further research. The expansion of knowledge in this area, namely on the change in the properties of the pathogen due to the replacement of the habitat, is important and explains the relevance of the subject under study.

The purpose of this study was to figure out the influence of the rice weevil *Sitophilus oryzae* on the biological and biochemical properties of dissociative forms of *M. bovis*, to investigate the mechanism of adaptation of mycobacteria in an unfamiliar environment. The tasks of the study: to establish changes in virulence, viability (by the method of counting the number of colony-forming units) and biochemical activity of mycobacterium after passage through the body of beetles in the dynamics of the experiment.

MATERIALS AND METHODS

The research was conducted in the conditions of the educational laboratory of the Department of Infectious Diseases of Animals of the Dnipro State Agrarian and Economic University during 2020-2021. The subject of the research is guinea pigs and cultures of the dissociative form of *Mycobacterium bovis*: the initial culture of 118 passages (240th generation), which was stored at 3.0 ± 0.5°C in the museum of the department, and cultures obtained after passage through the organism of the rice weevil (with beetle homogenate 30 days after infection; from the grain on which the insects were kept during the experiment after the second to fifth transplantation (on Days 8, 12, 20, and 30 of the experiment) to pre-sterilized grain). This paper is the second stage of the study. At the first stage, it was established that beetles can keep mycobacteria in their bodies for 50 days and release them, contaminating objects of the external environment for 30 days (the methodology and detailed results of the experiment can be found in the previous paper). To fulfil the purpose of this study, it was conducted in three stages:

Stage I (biological research). To determine the influence of beetles on the change in virulence of the dissociative form of *M. bovis*, the original culture of passage 118, cultures isolated from grain suspensions on Days 8, 12, 20, and 30, on which infected beetles were kept, were used, as well as the culture obtained from the homogenate of beetles on Day 30 of the experiment.

Guinea pigs were divided into 7 experimental groups and one control group (3 animals in each group). Animals of the Group I were infected with the original strain of mycobacteria, which was not exposed to the body of beetles; Group II – culture obtained from grain on Day 4 of the experiment, Group III – on Day 8, Group IV – on Day 12, Group V – on Day 20, Group VI – on Day 30, Group VII – culture obtained from the beetle

homogenate on Day 30 of the experiment. The control group stayed intact throughout the entire study period. Before infection, the animals were quarantined for 14 days, during which time they were subjected to an allergy test (tuberculinization) to rule out natural tuberculosis. Tuberculinization was carried out by injecting a process solution (25 IU in a volume of 0.1 cm^3) of PPD-tuberculin for mammals intradermally, the reaction was recorded after 24 and 48 hours.

Guinea pigs were infected subcutaneously in the inner part of the thigh with a suspension of experimental mycobacteria in the amount of 1.0 mg of microbial mass per 1.0 cm^3 of isotonic 0.9% sodium chloride solution. During the experiment (90 days), the animals were clinically examined and weighed weekly, and on Days 30, 60, and 90, the sensitizing properties of dissociative forms of mycobacteria were determined before and after passage through beetles by tuberculinization. The result of the biological sample was evaluated by conducting a anatomical pathology, cultural, and microscopic examination. After the autopsy, pathological changes in the lungs, liver, spleen, and lymph nodes were determined. Virulence was assessed by the degree of damage to internal organs, which was expressed in points (M.V. Trius scheme). According to this scheme, specific lesions were expressed by pluses ("+" – single; "++" – several; "+++" – numerous nodules) followed by conversion into digital indicators (each "+" in lymph nodes equals 1; in the spleen – 2; in the liver – 3; in the lungs – 4), respectively, the maximum index of damage to the body is 30 points. In addition, a suspension was prepared from internal organs according to the method of A.P. Alikaeva. The resulting suspension was sown on Mordovsky ("Nove") nutrient medium with a pH of 6.5 (an Adwa AD1030 pH meter was used to determine the pH value).

Stage II (viability of mycobacteria). At the second stage of the study, the viability of the microbial cells of the original culture of mycobacteria and cultures obtained after passage through the body of beetles was determined by evaluating colony-forming units (CFU) by conducting serial dilutions. To conduct the research, *Eppendorf*-type microtubes were used, in which sterile isotonic 0.9% sodium chloride solution was introduced in the amount of 0.5 cm^3 in the first microtube and 0.4 cm^3 in all subsequent microtubes. Test cultures were collected from the test tubes with a bacteriological loop, squeezed between two sheets of filter paper and weighed 50.0 mg (0.05 g) on a torsion balance. The weighted mass of mycobacteria was introduced into microtube No. 1 and suspended by thorough mixing with a bacterial loop. With an insulin syringe, 0.1 cm^3 of the obtained suspension was taken, after which it was introduced into microtube No. 2, mixed thoroughly, 0.1 cm^3 was taken again and transferred to the next test tube up to and including the tenth. Subsequently, 0.1 cm^3 of suspension was taken from each microtube with separate insulin syringes, which was placed in two tubes with Mordovsky nutrient medium and distributed on the surface. In the future, the test tubes were placed in a thermostat at +37 ± 0.5°C.

When determining the viability of microbial cells of the initial culture of dissociants and the culture obtained from grain on Day 8 of the experiment, the calculation was made in the eighth dilution, upon determining the number of CFU of the culture that grew from the sowing of homogenized beetles on Day 30 of the experiment and the culture obtained from grain on Day 12 of the experiment, counting was carried out in the seventh dilution. When determining the viability of cultures obtained from grain on Days 20 and 30 of the experiment, the calculation was made in the sixth dilution. These dilutions were chosen because at lower dilutions continuous growth was observed, and at higher dilutions colony growth was absent. After the occurrence of colony growth, the number of viable microorganisms in 1 gram of culture was calculated mathematically.

Stage III (biochemical activity). The initial culture of dissociants and cultures isolated after passage through beetles were examined for changes in biochemical activity. Catalase, peroxidase, dehydrogenase activity, the presence of nitrate reduction and the ability to hydrolyse Tween 80 were determined.

Catalase and peroxidase activity were determined simultaneously according to the modified method of Bogen (1981). A 2.0% solution of hydrogen peroxide and a 0.5% solution of pyrogallol A were added to the experimental cultures. Catalase activity was measured 15 and 30 minutes after the reaction was started. Reactions were assessed by visual inspection based on the turbulent flow and release of the number of oxygen bubbles: (+++) – considerable release of bubbles; (++) – moderate; (+) – single; (-) – no bubbles are released. Peroxidase activity of dissociants was determined after 1.5-2 hours by evaluating the above reaction. The calculation was carried out visually, determining the formation of brown pigment in the experimental colonies as a result of the transformation of pyrogallol due to the action of the peroxidase enzyme into purpurogallin in the presence of hydrogen peroxide: (+++) – dark brown colouration of the colonies; (++) – brown; (+) – pale brown; (-) – the colour does not change.

Dehydrogenase activity was established in agglutinating tubes. For this, 4.0 cm^3 of a suspension of microbial cells with a concentration of 10.0 mg/cm^3 in a phosphate buffer of pH 7.4-7.6 was mixed with 1.0 cm^3 of a 1.0% glucose solution and 0.1 cm^3 of 0.02% solution of methylene blue. Sterile vaseline oil was layered on the contents obtained in the test tube. The test tubes were incubated in a thermostat at +38 ± 0.5°C and the time of dye discolouration was monitored. The

result of the reaction was evaluated after 15-30 minutes and 24 hours. Test tubes with mycobacterial suspension and methylene blue without glucose served as controls.

At the next stage, the nitrate reductase activity of mycobacteria was evaluated. The presence of nitrate reduction was determined as follows: 10.0 mg of the wet biological mass of the experimental culture was weighed on a torsion balance and placed in a test tube containing 1.0 cm^3 of 0.067 M phosphate buffer in (pH .1) with 0.1% – with sodium nitrate solution. After suspension, the cultures were seasoned for 20-22 hours at +37.0 ± 0.5°C. Nitrate formation was checked by adding two drops of a 2.0% solution of paradimethylaminobenzaldehyde (P-dimethylaminobenzaldehyde) to a 1.0% solution of hydrochloric acid in a test tube.

The hydrolysis reaction of Tween 80 was determined by the modified method of Wein. The following reagents were used for the reaction: 1/15 M phosphate buffer (pH 7.0) – 100.0 cm^3, Tween 80 – 0.5 cm^3, basic red 0.1% – 2.0 cm^3. All reagents were mixed and poured into 4.0 cm^3 tubes and autoclaved for 15 minutes at +120.0±0.5°C. Subsequently, the test tubes were placed in a thermostat for 24 hours to check for sterility. Three bacteriological loops of each experimental culture were emulsified in test tubes with a substrate and incubated in a thermostat for 10 days. The reaction was recorded after 4 hours, on Days 5 and 10. The result was considered positive if a pink or red colour appeared within 10 days, with a negative result, the colour did not appear. Data in the tables are presented as x ± SD (standard deviation).

When working with animals, the provisions of Article 26 of the Law of Ukraine No. 5456-VI "On the Protection of Animals from Cruelty" dated 10/16/2012 (Law of Ukraine No. 3447-IV), "General Ethical Principles of Animal Experiments", approved on The First National Congress on Bioethics (Reznikov, 2003), the requirements of the European Convention "On the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes" (European convention, 1986), the Declaration "On the Humane Treatment of Animals" (Universal Declaration, 2007).

RESULTS AND DISCUSSION

The introduction of a suspension of experimental mycobacteria to guinea pigs did not cause clinical and pathological signs inherent in the course of tuberculosis in any of the experimental groups, an ulcer was not formed at the place of introduction of the suspension. During the experiment, the animals were active and gained weight (Fig. 1). When studying the sensitization of experimental cultures, it was established that guinea pigs do not respond to the administration of PPD-tuberculin in any of the experimental groups.

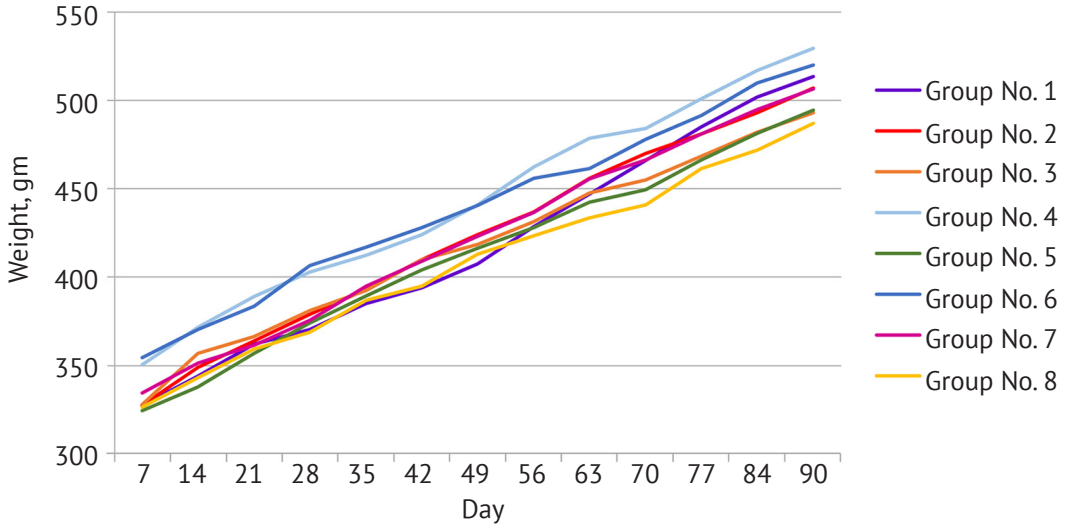

***Figure 1**. Dynamics of changes in body weight of experimental guinea pigs during the experiment*
***Source:** compiled by the authors*

3 months after infection, the animals were euthanized, and an anatomical pathology study was performed. In the internal organs (lungs, liver, spleen) and lymph nodes, no changes inherent in tuberculosis were detected. The index of damage to the body of experimental guinea pigs of all groups is 0 points. When inoculating the suspension from organs and lymph nodes on Mordovsky ("Nove") nutrient medium, the growth of smooth, rounded, even-edged orange colonies was observed, which were morphologically identical to the dissociative forms of *M. bovis* (passage 118) for 8-10 days from the organs of guinea pigs of I-IV and VII experimental groups. On Day 12, no growth was detected from the organs of guinea pigs of groups V-VI in test tubes from the control group of animals.

At the next stage of the study, the viability of microbial cells was established by calculating CFU. It was found that the number of colonies that grew from the original culture during the eighth dilution was

338.0 ± 29.7, and from the culture obtained on Day 4 of the experiment from the grain suspension – 321.0 ± 9.9; on Day 8 – 293.0 ± 15.6 colonies in 6.4×10^{-5} mg of microbial mass, i.e., in 1 gram of culture 528125000.0≈5.3×10^{8}, 501562500.0≈5.0×10^{8} and 457812500.0≈4.6×10^{8} viable microorganisms, respectively.

Viable mycobacteria in the culture obtained from the grain suspension on Day 12 of the experiment and the culture from the homogenate of beetles on Day 30 was calculated during the seventh dilution. It was found that the number of colonies was equal to 350.0 ± 43.8 and 442.0 ± 32.5 in 3.2×10^{-4} mg of bacterial mass, respectively. That is, 109375000.0≈1.1×10^{8} and 138125000.0≈1.4×10^{8} bacteria in 1 g of culture.

The CFU of the cultures obtained from the grain suspension Days 20 and 30 was calculated in the sixth dilution. It was found that 1.6×10^{-3} mg of microbial mass contains 237.0 ± 39.6 and 209.0 ± 24.0 microbial units, i.e., 14812500.0≈1.5×10^{7} and 13062500.0≈1.3×10^{7} microbial units in 1 g of culture (Table 1).

***Table 1**. Evaluation of colony-forming units of the dissociative form of Mycobacterium bovis (118 passages) in the dynamics of the experiment*

	Number of grown colonies on the nutrient medium											M ± m	The amount of CFU in 1 g of bacterial mass
	Dilution multiplicity												
		I	II	III	IV	V	VI	VII	VIII	IX	X		
Source culture (118 passage)	Sample No. 1	c.g.	c.g.	c.g.	c.g.	c.g.	c.g.	c.g.	317	n.g.	n.g.	338.0 ± 29.7	$5.3*10^{8}$
	Sample No. 2	c.g.	c.g.	c.g.	c.g.	c.g.	c.g.	c.g.	359	n.g.	n.g.		
Culture obtained from grain on Day 4 of the experiment	Sample No. 1	c.g.	c.g.	c.g.	c.g.	c.g.	c.g.	c.g.	314	n.g.	n.g.	321.0 ± 9.9	5.0*108
	Sample No. 2	c.g.	c.g.	c.g.	c.g.	c.g.	c.g.	c.g.	328	n.g.	n.g.		
Culture obtained from grain on Day 8 of the experiment	Sample No. 1	c.g.	c.g.	c.g.	c.g.	c.g.	c.g.	c.g.	282	n.g.	n.g.	293.0 ± 15.6	$4.6*10^{8}$
	Sample No. 2	c.g.	c.g.	c.g.	c.g.	c.g.	c.g.	c.g.	304	n.g.	n.g.		
Culture obtained from grain on Day 12 of the experiment	Sample No. 1	c.g.	c.g.	c.g.	c.g.	c.g.	c.g.	319	n.g.	n.g.	n.g.	350.0 ± 43.8	$1.1*10^{8}$
	Sample No. 2	c.g.	c.g.	c.g.	c.g.	c.g.	c.g.	381	n.g.	n.g.	n.g.		
Culture obtained from grain on Day 20 of the experiment	Sample No. 1	c.g.	c.g.	c.g.	c.g.	c.g.	209	n.g.	n.g.	n.g.	n.g.	237.0 ± 39.6	$1.5*10^{7}$
	Sample No. 2	c.g.	c.g.	c.g.	c.g.	c.g.	265	n.g.	n.g.	n.g.	n.g.		
Culture obtained from grain on Day 30 of the experiment	Sample No. 1	c.g.	c.g.	c.g.	c.g.	c.g.	192	n.g.	n.g.	n.g.	n.g.	209.0 ± 24.0	$1.3*10^{7}$
	Sample No. 2	c.g.	c.g.	c.g.	c.g.	c.g.	226	n.g.	n.g.	n.g.	n.g.		
Culture obtained from homogenized beetles on Day 30 of the experiment	Sample No. 1	c.g.	c.g.	c.g.	c.g.	c.g.	c.g.	419	n.g.	n.g.	n.g.	442.0 ± 32.5	$1.4*10^{8}$
	Sample No. 2	c.g.	c.g.	c.g.	c.g.	c.g.	c.g.	465	n.g.	n.g.	n.g.		

***Source:** c.g. – continuous growth of colonies on nutrient medium; n.g. – no growth*

It was found that beetles can reserve and secrete viable mycobacteria and contaminate objects in the environment. At the same time, the authors observed a tendency to decrease the viability of the pathogen isolated by weevils over time. The number of colony-forming units in cultures isolated from grain on Day 4 of the experiment decreased by 5.03% compared to the initial strain, on Day 8 – by 13.32%, on Day 12 – by 79.29%, on Day 20 – by 97.20%, on Day 30 – by 97.53%, respectively. The viability of *M. bovis* in the body of beetles for 30 days (in the homogenate) decreased by 73.85% from the initial culture. This may indicate that the body of the beetle is a favourable environment for the reservation of mycobacteria, and therefore rice weevils may contribute to the activation of the epizootic process of tuberculosis and mycobacterial infections of animals. As a result

of conducting a biochemical study, it was established that indicators of the enzymatic activity of mycobacteria are variable. Passage of dissociative forms of *M. bovis* through the body of the rice weevil led to an increase in the activity of the following enzymes: dehydrogenase, nitrate reductase, and the ability to hydrolyse Tween 80 (Table 2).

***Table 2**. Biochemical activity of the dissociative form of M. bovis (118 passages) in the dynamics of the experiment (n = 3)*

	Catalase activity		Peroxidase activity 2 h	Dehydrogenase activity		Nitrate reductase activity	Hydrolysis of Tween 80		
	15 min	30 min		15-30 min	24 h		4 h	5 days	10 days
Source culture (118 passage)	++	++	–	–	–	±	–	–	+
Culture obtained from grain on Day 4 of the experiment	++	++	–	–	–	±	–	–	+
Culture obtained from grain on Day 8 of the experiment	++	++	–	–	±	+	–	–	+
Culture obtained from grain on Day 12 of the experiment	++	++	–	–	±	+	–	–	+
Culture obtained from grain on Day 20 of the experiment	++	++	–	–	±	+	–	–	+
Culture obtained from grain on Day 30 of the experiment	++	++	–	–	±	+	–	–	+
Culture obtained from homogenized beetles on Day 30 of the experiment	++	++	–	–	±	+	–	–	+

***Note:** + – positive reaction; - – negative reaction; ± – doubtful reaction*
***Source:** compiled by the authors*

The principle of the catalase reaction lies in the splitting of hydrogen peroxide under the action of the catalase enzyme into water and atomic oxygen, which is accompanied by the release of oxygen bubbles and the transition of pyrogallol to purpurogallin in the presence of hydrogen peroxide under peroxidase. All cultures under study had moderate catalase activity (Fig. 2). Peroxidase activity was negative in all experimental samples.

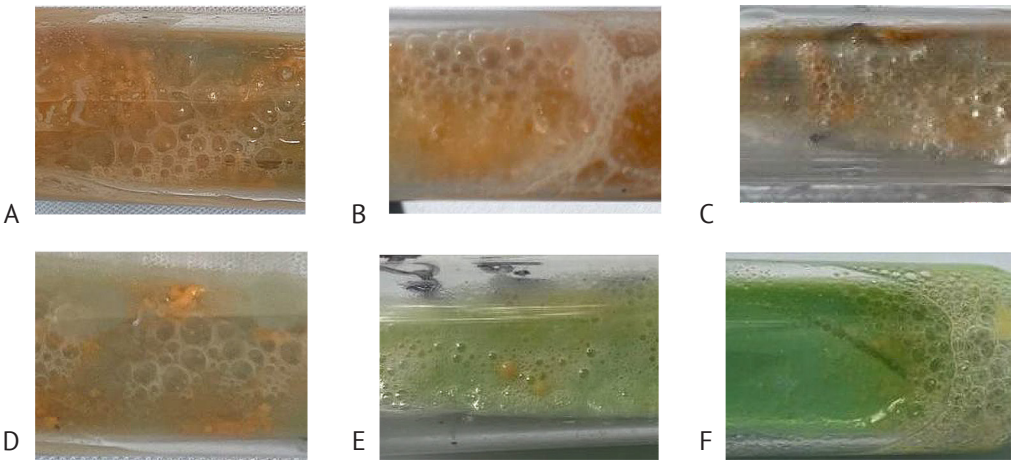

***Figure 2**. Calculation of catalase activity of experimental cultures: A – culture obtained from grain on Day 4, B – on Day 8, C – on Day 12, D – on Day 20, E – on Day 30, F – from homogenate of beetles on Day 30*
***Source:** photographed by the authors*

When studying the activity of dehydrogenase, no discolouration of the methylene blue solution was found in any of the cultures after 15-30 minutes. The dehydrogenase activity of the original culture and the culture obtained from grain on Day 4 after 24 hours was also negative. However, cultures obtained from the homogenate of weevils on Day 30 of the experiment and from the beetle-contaminated grain on Days 8, 12, 20, and 30 of the experiment partially decolorized the solution in 24 hours (Fig. 3).

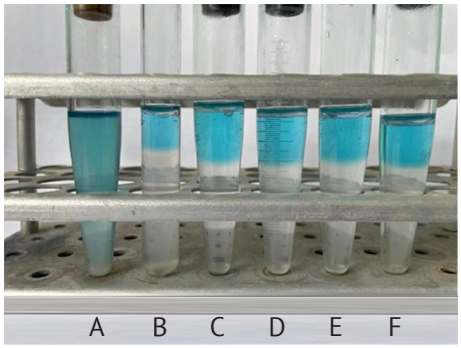

Figure 3. *Calculation of dehydrogenase activity of experimental cultures: A – culture obtained from grain on Day 4, B – on Day 8, C – on Day 12, D – on Day 20, G – on Day 30, D – from homogenate of beetles on Day 30*
Source: *photographed by the authors*

Nitrate reductase activity of mycobacteria lies in accounting for the reduction of nitrite from nitrate by visual observation of the colour reaction with P-dimethylaminobenzaldehyde, with a positive reaction, a yellow colour is formed, with a negative reaction, the colour of the solution does not change. Investigating the activity of nitrate reduction, it was established that the result was questionable in the original culture and the culture obtained from grain on Day 4, while all other experimental cultures acquired the ability to reduce nitrates (Fig. 4).

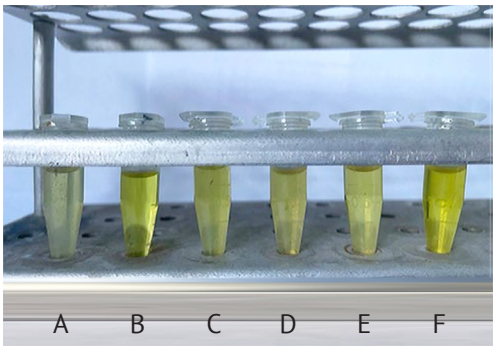

Figure 4. *Calculation of nitrate reductase activity of experimental cultures: A – culture obtained from grain on Day 4, B – on Day 8, C – on Day 12, D – on Day 20, G – on Day 30, D – from homogenate of beetles on Day 30*
Source: *photographed by the authors*

The result of Tween 80 hydrolysis in all experimental cultures was negative after 4 hours and after 5 days. However, on Day 10 of observation, a positive result (the formation of a pink-red colour) was found in all the samples under study (Fig. 5).

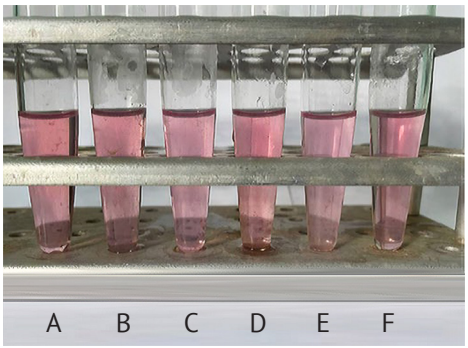

Figure 5. *Calculation of hydrolysis reaction of Tween 80 in experimental cultures: A – culture obtained from grain on Day 4, B – on Day 8, C – on Day 12, D – on Day 20, E – on Day 30, F – from homogenate of beetles on Day 30*
Source: *photographed by the authors*

Thus, the research results demonstrate that the passage of mycobacteria through the body of the rice weevil leads to certain changes in the biochemical properties of the bacterial cell. However, passage through insects does not lead to restoration of virulent properties of dissociative forms of the pathogen. Therewith, the number of viable cells in the mycobacteria isolated from the homogenate of beetles and grain contaminated by them decreases but stays sufficient to infect laboratory animals.

This study determined the effect of the rice weevil on the viability, pathogenicity, and biochemical properties of a dissociative strain of *M. bovis*. The obtained results demonstrate that the dissociative forms of *M. bovis* are non-pathogenic for guinea pigs. The animals did not develop an ulcer at the place of introduction of the suspension of the original strain of the pathogen and the cultures obtained as a result of passage through the body of the rice weevil. Allergic reaction to the introduction of PPD-tuberculin for mammals and anatomical pathology changes were not detected. The results of the experiment show that dissociants do not restore virulent properties after passing through a living organism, which indicates a persistent loss of pathogenicity of the original strain. However, despite the absence of visible clinical and anatomical pathology changes in guinea pigs, it was possible to isolate mycobacteria from their internal organs. The obtained data do not contradict the study of Tkachenko *et al.* (2020), which describes the absence of an infectious process in laboratory animals infected with dissociative forms of *M. bovis* after long-term storage. As is known, mycobacteria that have lost their virulent properties can survive and replicate in the macroorganism without causing immunopathological processes.

Scientific works of researchers have proven that mycobacteria can change to adapt to their surrounding environment and, accordingly, have a large number of reservoirs and factors of disease transmission (Bañuls *et al*., 2015).Mycobacteria have high adaptive properties and can survive in adverse conditions (for instance, in conditions of starvation) without showing growth for up to two years with the ability to fully recover. When studying the viability of microbial cells after passing through the weevil, it was determined that mycobacteria stay viable in the beetle's body for a long time, and therefore it is a favourable environment for the pathogen to be reserved and can potentially contribute to the activation of the epizootic process of tuberculosis and mycobacterial infections of animals. Mycobacteria, due to the structure of the cell wall, are resistant to the action of gastric enzymes of insects and can be released by them with saliva and excrement. In addition, existing data suggest that infected insects can be captured by favourable animals. In their gastrointestinal tract, due to the action of gastric juice, insects are digested, but the acid-fast pathogen stays viable and can persist in the macroorganism or be released into the environment and contaminate it. Fischer *et al.* (2003) conducted an experimental oral infection of *Blatta orientalis* cockroach nymphs with mycobacterial suspension and found that the pathogen accumulated in the insect's body and was released into the environment. The researchers isolated viable mycobacteria in cockroach excrement 3 days after infection, and in cockroach homogenate after 10 days, which also indicates the ability to reserve the pathogen for a long time.

Indicators of the enzymatic activity of mycobacteria are extremely important. Enzymes take part in ensuring the energy metabolism of the microbial cell and provide the protective function of the microbe and contribute to its adaptation to environmental factors (Arora *et al*., 2018). As a result of conducting a biochemical study, it was established that indicators of the enzymatic activity of mycobacteria are variable. Li *et al.* (1998) and Manca *et al.* (1998) claim that catalase activity contributes to the resistance and survival of microorganisms and suggest that this enzyme is a factor in the virulence of mycobacteria. However, this study did not confirm this assumption.

Dehydrogenase activity is based on the detection of the redox enzyme dehydrogenase and metabolic products. This enzyme is responsible for the protection of the bacterial cell and adaptation in the conditions of the macroorganism, takes part in ensuring the antioxidant function of the microbe. The results confirm the existing data, the activity of dehydrogenase increased after the contact of mycobacteria with beetles. Comparable studies are demonstrated by other scientific papers indicating an increase in the activity of this enzyme after passing through a living organism (Tkachenko *et al*., 2021).

Fritz (2002) states on the example of *M. bovis BCG* that nitrates are important for the metabolism of the microbial cell and take part in providing it with energy; moreover, they can maintain metabolism even without oxygen. The results of research by a number of scientists claim that the activity of nitrate reductase is partially responsible for the survival of mycobacteria in host cells (Hayashi *et al*., 2010). The obtained results, according to which non-pathogenic dissociative forms of mycobacteria can stably reduce nitrates, do not contradict the studies of Sohaskey & Modesti. (2009), who claim that only the virulent strain of *M. bovis* has weak nitrate reductase activity.

Considering the opposite of the existing data, it can be concluded that relying solely on the ability of mycobacteria to reduce nitrates, it is not possible to unambiguously characterize the pathogenicity of the strain. It can be assumed that the activity of this enzyme is not directly related to the virulence of the pathogen. The ability of mycobacteria to hydrolyse Tween 80 is provided by mycobacterial phospholipase A, which catalyses lipid hydrolysis. The essence of the reaction is that Tween 80 binds neutral red and before the reaction the experimental solution has a straw-yellow colour, but

due to the release of neutral red, the colour is restored from pink to red (with a positive reaction). According to the assumption of Deb *et al.* (2009), this activity can contribute to the survival of the bacterial cell in conditions of starvation and be used by the pathogen as a carbon source.

The obtained data suggest that changes in the biochemical parameters of mycobacteria occur as a result of increased survival and adaptation of the microbe to new environmental conditions. Such assumptions do not contradict other studies on the high degree of variability of biological properties of *M. bovis*, including biochemical activity. It is believed that mycobacteria in unfavourable conditions of existence can maintain their viability for more than 650 days. At the same time, a decrease in the viability of the pathogen (decrease in CFU) is observed. In this study, against the background of a decrease in the number of CFUs in the microbial cell, the mechanisms of adaptation to the change in habitat are activated, the activity of redox enzymes increases, and the microbial cell is supplied with energy by increasing the reduction of nitrates.

Therefore, analysing the results of this study and the research of other scientists, it can be stated that the change in the properties of mycobacteria is related to the adaptation and survival of the microbial cell in a new habitat.

CONCLUSIONS

Summarizing the obtained results, it becomes clear that mycobacteria can change the properties of the bacterial cell to adapt to existence in changed conditions. The biochemical activity of the pathogen undergoes primary changes, and this, in turn, may indicate that synthetase systems, namely enzyme systems, are mainly responsible for the survival of the microbe. Enzymatic activity of dissociative forms of mycobacteria is variable. Cultures obtained after passage through the body of beetles acquire the ability to decolorize methylene blue solution in 24 hours (dehydrogenase activity) and have higher nitrate reductase activity compared to the original strain. Changes in the enzymatic activity of cultures after passing through the body of the rice weevil are associated with ensuring the adaptation of the microbial cell in a new habitat.

Analyzing the pathogenic properties of the pathogen, it can be stated that mycobacteria have a stable genome. Dissociative forms of *M. bovis* (118 passage, 240 generation) do not restore virulent properties after passage through the body of beetles, which indicates persistent changes in mycobacteria towards reduced pathogenicity. Rice weevils are incapable of increasing virulence. At the same time, it can be noted that dissociative forms of *M. bovis* have a stable genotype with loss of pathogenicity.

Estimating the number of CFU dissociants, we observe a tendentious loss of viability in the dynamics of the experiment. As a result of passage through the organism of the rice weevil, the number of viable mycobacteria decreased by 5.03-97.53% in grain contaminated with beetles during Days 8-30 after infection compared to the original strain, and by 73.85% in the homogenate from beetles after 30 days from the moment of infection. Prospects for further research are to improve disinsection measures and develop ways to interrupt the epizootic chain and prevent infected insects from entering the safe territory.

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Вплив *Sitophilus oryzae* на біологічні властивості *Mycobacterium bovis*

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Анотація. Активне поширення туберкульозу по всьому світу є актуальною проблемою протягом багатьох десятиліть, але й дотепер немає даних щодо всіх наявних методів занесення збудника на раніше благополучну територію та механізмів діяльності мікобактерій, а це, в свою чергу, унеможливлює повне викорінення хвороби. Метою даної роботи було визначення впливу рисового довгоносика (лат. *Sitophilus oryzae*) на дисоціативні форми мікобактерій туберкульозу, їх життєздатність, біохімічні властивості та вірулентність шляхом пасажування збудника через організм комахи. Для досягнення мети були застосовані наступні методи дослідження: біологічні (експериментальне інфікування лабораторних тварин), патологоанатомічні та визначення життєздатності мікобактерій методом підрахунку колонієутворюючих одиниць. Було визначено та узагальнено вплив на патогенні властивості дисоціативних форм *Mycobacterium bovis* (118-й пасаж) після пасажування через організм рисового довгоносика в експерименті на морських свинках. Описано тенденційну зміну життєздатності мікобактерій виділених у динаміці досліду в порівнянні з вихідною культурою.Доведено пластичність біохімічної активності збудника після проходження через організм жука та проаналізовано вплив макроорганізму на ферментативні властивості мікобактерій туберкульозу. Обґрунтовано явища активізації ферментативних систем, які пов'язані з адаптацією та виживанням мікобактерій в умовах зміненого середовища існування. Практична цінність роботи полягає в удосконалені розуміння процесів поширення мікобактерій туберкульозу та встановлені ролі комах у передачі інфекції з перспективою розробки заходів профілактики та боротьби з захворюванням

Ключові слова: мікобактерії, рисовий довгоносик, дисоціативний штам, біохімічні властивості, колонієутворюючі одиниці, мінливість