МИКРОБИОЛОГИЧЕСКИЙ АНАЛИЗ БИОМАССЫ *TENEBRIO MOLITOR*

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MICROBIOLOGICAL ANALYSIS OF TENEBRIO MOLITOR BIOMASS

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АННОТАЦИЯ

В статье рассмотрен микробиологический анализ биомассы *Tenebrio molitor*, в будущем проведен микробиологический анализ высушенных образцов биомассы Tenebrio molitor в связи с тем, что она имеет большое научное и практическое значение для предотвращения возникновения повреждений у организмов, потребляющих эту биомассу (человека, крупного рогатого скота, птиц, рыб и др.) На основании проведенных работ проведены исследования на основе природной высушенной биомассы личинок *Tenebrio molitor.* Учитывая весьма сжатые сроки, отведенные на наши исследования, микробиологический анализ съедобных насекомых, представляющих собой огромный экономический ресурс и требующих длительного времени, направление наших исследований направлено на определение бактериальной флоры личинок *Tenbrio molitor.*

Ключевые слова: Tenebrio molitor, микробиологический анализ, Bacillus thuringiensis, Bacillus firmus.

ABSTRACT

The article discusses the microbiological analysis of *Tenebrio molitor* biomass; in the future, a microbiological analysis of dried samples of *Tenebrio molitor* biomass will be carried out due to the fact that it is of great scientific and practical importance for preventing damage to organisms that consume this biomass (humans, cattle, birds, fish, etc.) Based on the work carried out, studies were carried out on the basis of natural dried biomass of *Tenebrio molitor* larvae. Taking into account the very short time allocated for our research, microbiological analysis of edible insects, which represent a huge economic resource and require a long time, the direction of our research is aimed at determining the bacterial flora of *Tenebrio molitor* larvae.

Key words: Tenebrio molitor, microbiological analysis, Bacillus thuringiensis, Bacillus firmus.

There are very few scientific resources on the microbiological flora of edible insects in the world, and scientific research work on further clarification of the microbiological flora is one of the urgent issues.

Bacteria belonging to the genus Staphylococcus, Streptococcus, Bacillus, Proteus, Pseudomonas, Escherichia, Micrococcus, Lactobacillus and Acinetobacter are recorded as the main microbiological flora of insects [Agabou and Alloui, 2010; Amadi et al., 2005; Braide et al., 2011; Giaccone, 2005].

According to information provided by FAO, pathogenic (entomopathogenic) bacteria found in insects are harmful to humans and animals [FAO, 2013]. Therefore, there is a theory that, in addition to bacteria found in insects, microflora from outside during their cultivation (naturally or accidentally falling into buildings and structures, natural or accidental microbes in food products, etc.) and during processing or storage processes can be dangerous. [ANSES, 2015].

Also, a large number of pathogenic microbes found in insects are widely used in pest control, and are usually included in the fourth group of dangerous microbes and are considered safe for humans and warm-blooded animals. For example, in South America, insect pathogens used in the GRAS system to control pests are generally considered harmless, but in the EU countries, they are considered harmful to humans and animals if they have the status of QPS (classification of presumption of safety), that is, if they are specially added to food or feed against pests [Sundh et al. ., 2012; Leuschner et al., 2010)]. If there are microbes or their metabolic products that are not included in these safety groups, it is appropriate to conduct special tests on them and draw a conclusion on their further consumption.

Therefore, ensuring the safety of invertebrate pathogens is an integral part of listing them as biological control agents prior to commercialization [Eilenberg et al., 2015].

Klunder and his colleagues performed a microbiological analysis of the cultures of Tenebrio molitor and Brachytrupes sp.) in newly bred insects and noted that mainly spore-forming bacteria and enterobacteria were found [Klunder et al., 2012]. In these studies, it was found that the composition and condition of the substrate used for feeding insects directly affects the number of microbes found in the intestines of insects. However, it has been noted that the substrate and taxonomy used in feeding do not depend on the bacterial profile in the insect's gut [Colman et al., 2012]. It can be concluded that it is advisable to approach the insect biomass in each prepared large batch individually. Peña-Pascagaza from the University of Javeriana, Colombia, and his colleagues, using the most modern methods of microbiological analysis of Tenebrio molitor larvae, that is, sequencing based on 16S rRNA, noted that the microflora can be found in the following table [Peña-Pascagaza PM, López-Ramírez NA, Ballen-Segura MA. Tenebrio molitor and its gut bacteria growth in polystyrene (PS) presence as the sole source carbon, Universitas Scientiarum, 25 (1): 37-53, 2020. doi: 10.11144/Javeriana.SC25-1.tmai]. The results of the microbiological analysis presented by them in this table specifically emphasized that the yellow flour beetle appeared when polystyrene was used as the only source of carbon.

From the scientific sources listed in Table 3.1.1, it can be seen that based on the results of microbiological analysis of the Tenebrio molitor food-eating insect, the presence of microbiological objects belonging to the genera Bacillus anthracis, Stenotrophomonas, Pantoea and Erwinia was recorded in their composition. In addition, several genera of microorganisms, such as enterococci, spore-forming aerobic bacteria, fermenting yeasts, Escherichia coli, are widely covered in scientific sources [Yan X. et al., 2023].

Therefore, in the course of our research, we tried to carry out their microbiological analysis using the samples of Tenebrio molitor nutritive insect grown in the scientific laboratory of the Institute of Microbiology of the Academy of

Sciences of the Republic of Uzbekistan of the Tashkent Institute of Chemical Technology, Department of "Biotechnology".

On the basis of the conducted research, studies were conducted on the basis of natural dried and dried biomass of Tenebrio molitor larvae. In particular, based on the results of the preliminary research, a microbiological analysis of the larvae of the feeding insect Tenebrio molitor before drying was carried out.

Taking into account that the time allocated for our research is very short, microbiological analysis of nutritious insects is a huge economic resource and requires a long time, we focused our research on determining the bacterial flora of Tenbrio molitor larvae.

According to the results, the following microbiological isolates were isolated: Staphylococcus warneri, Staphylococcus succinus, Bacillus thuringiensis, Bacillus firmus, Pseudomonas aeruginosa, Bacillus cereus, Serratia marcescens, Pseudomonas mosselii, Enterobacter cloacae, Enterobacter asburiae and Cronobacter sp.

The obtained results, morphological and biochemical characteristics of bacterial isolates isolated from the biomass of Tenebrio molitor larvae before drying are recorded in Table 3.1.2. In particular, when we analyzed bacterial isolates isolated from the biomass of Tenebrio molitor larvae before drying, it was found that isolates belonging to the genus Staphylococcus ranged from 1.22×105 to 2.04×105 cells in terms of the number of colonies (KOE/ml). It was also noted that all of these isolates showed a positive reaction according to the G reaction characteristic. In addition, when studying the mobility of the isolates, the mobility of bacteria belonging to the genus Staphylococcus was not recorded.

Table 1

Microbiological flora of Tenebrio molitor larvae when polystyrene is used as a carbon source

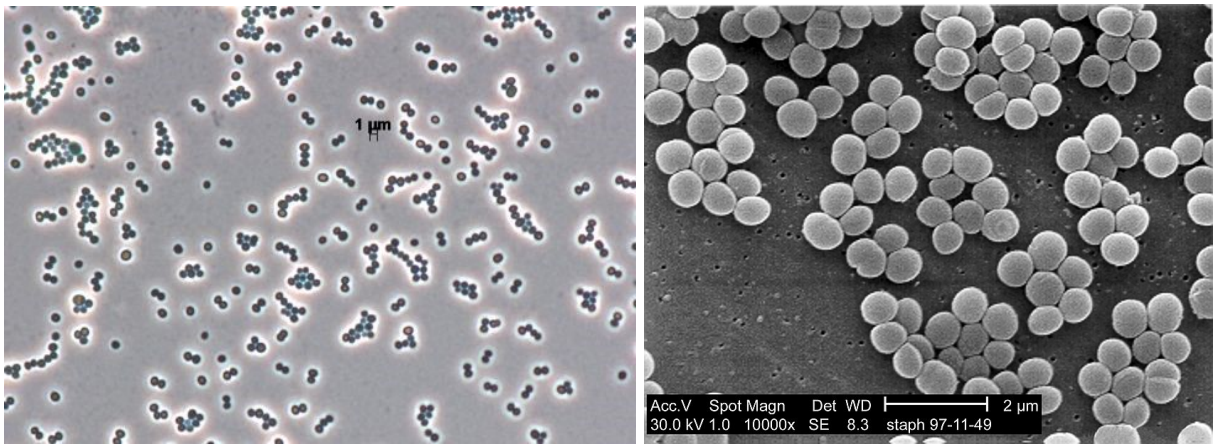
[Peña-Pascagaza et al., 2020]

№	Isolation number	Morphological description	Biochemical description	Molecular identification
1	1G	It consists of cells that form colonies of white round shape with wavy edges and bacillary morphology.	Gram positive bacteria produce gas by fermenting lactose or sucrose. Indole or catalase positive, motile.	Bacillus anthracis
2	3G	It consists of cells that form colonies of white round shape, having a bacillary morphological form, with smooth edges.	Gram negative bacteria ferments sugar to form gas, positive for citrate and catalase, motile.	Stenotrophomonas sp.
3	5G	It consists of cells that form colonies of yellow color with smooth edges, having a bacillary morphological form.	Gram-negative bacterium, ferments sugar to form gas, positive for VP and oxidase, motile.	Stenotrophomonas sp.
4	8G	It consists of cells forming white round colonies with a bacillary morphological form, complete and smooth edges.	Gram-positive bacteria ferment glucose and sucrose without producing gas. Catalase and oxidase positive, mobile.	Bacillus sp.
5	10G	It consists of cells forming white colonies with a bacillary morphological form, with full edges.	Gram negative bacteria, does not ferment glucose, positive for citrate and catalase, motile.	Stenotrophomonas sp.

6	11E	It consists of cells forming white colonies with a bacillary morphological form, with full edges.	Gram negative bacteria ferment lactose and sucrose without producing gas. VP, positive for citrate and catalase, motile.	Pantoea agglomerans
7	12E	It consists of cells forming white colonies with a bacillary morphological form and wavy edges.	Gram negative bacteria, ferments lactose and sucrose without producing gas, is mobile.	Erwinia persicina
8	13E	It consists of cells forming white colonies with a bacillary morphological form and wavy edges.	Gram positive bacteria ferment glucose and sucrose without producing gas. Catalase positive, mobile.	Bacillus sp.
9	14E	It consists of cells forming white colonies with a bacillary morphological form and wavy edges.	Gram positive bacteria ferment glucose and sucrose to form gas. Catalase positive, mobile.	Bacilus anthracis

When analyzing the isolates, the detection of Warneri and Gallinarum species of the genus Staphylococcus was noted (Fig. 3.1.1, A).

In addition, isolates belonging to the thuringiensis and Firmus species of bacteria of the spore-forming genus Bacillus were isolated. During the research, it was noted that species belonging to this genus are very aggressive, and the shape of the cells is rod-shaped (Table 3.1.2).



А. Б.

Figure 3.1.1. Microscopic view of an isolate of Staphylococcus warneri [Yann He´chard et al., 2005] (A-cells confluent cells during growth, B-cells coccoid appearance)

It was observed that colonies of Bacillus thuringiensis isolate showed white color, whereas Bacillus firmus isolates showed milky cream color unlike thuringiensis.

Also, it was noted that bacteria belonging to the genus Staphylococcus are resistant to standard heat exposure, on the contrary, isolates of Bacillus thuringiensis, Bacillus firmus and Pseudomonas aeruginosa are resistant.

During the research, it was observed that all isolates mentioned in table 3.1.1 are resistant to the effect of 6.5% NaCl solution, but only Staphylococcus warneri and gallinarum species are resistant to 10% NaCl solution, Bacillus thuringiensis, Bacillus firmus and Pseudomonas aeruginosa isolates it was noted that it was unbearable. Among the isolates shown in Table 3.1.1, the oxidase activity of the isolates belonging to the genera warneri and gallinarum of the genus Staphylococcus and the species thuringiensis and firmus of the genus Bacillus was negative, but it was noted that the isolate of Pseudomonas aeruginosa showed positive activity.

Also, according to catalase activity, it was found that all isolates, that is, warneri and gallinarum species of Staphylococcus genera, as well as isolates of Bacillus thuringiensis, Bacillus firmus and Pseudomonas aeruginosa, showed a positive result.

In the studies on the reaction to Candelabrum, the data recorded on catalase activity were repeated, that is, the negative activity of isolates belonging to the genera Staphylococcus warner and gallinarum species and Bacillus thuringiensis and firmus species from the isolates shown in Table 3.1.1 on the reaction to Candelyabr, Pseudomonas aeruginosa isolate on the reaction to Candelyabr showed positive activity.

Based on biochemical analysis, it was found that the species of Staphylococcus warneri and gallinarum showed a negative reaction in terms of nitrate recovery, while

the isolates of the genus Bacillus thuringiensis and firmus and the isolate of Pseudomonas aeruginosa showed positive activity.

Table 2

Morphological and biochemical characteristics of bacterial isolates isolated from the biomass of Tenebrio molitor larvae before drying (larvae grown on the basis of wheat bran, 35 days old)

№	Sample tests	*Staphylococcus warneri*	*Staphylococcus gallinarum*	*Bacillus thuringiensis*	*Bacillus firmus*	*Pseudomonas aeruginosa*
1	Cell count (CFU/ml)	$2,04\times10^5$	$1,22\times10^5$	$3,21\times10^5$	$1,12\times10^5$	$3,17\times10^5$
2	Reaction description of G	+	+	+	+	+
3	Mobility	-	-	+	+	+
4	Cell shape	coccoid	coccoid	rod-shaped	rod-shaped	rod-shaped
5	Color of colonies	white	yellow	white	milky	milky
6	Spore formation	-	-	+	+	+
7	Heat test	-	-	+	+	+
8	Fluorescence to KB*	-	-	-	-	+
9	NaCl resistance (6.5%)	+	+	+	+	+
10	NaCl resistance (10%)	+	+	-	-	-
11	Oxidase	-	-	-	-	+
12	Catalase	+	+	+	+	+
13	Staining in methyl red	-	-	-	-	-
14	Nitrate recovery	-	-	+	+	+
15	Acid formation of	+	+	-	+	+

	arabinose					
16	O/F test	F	F	F	F	O
17	Starch hydrolysis	-	-	+	+	+
18	Cellulose hydrolysis	-	-	-	-	-
19	Lignin hydrolysis	-	-	+	+	-
20	Allergic (HR) reaction	-	-	-	++	++

Изох: *+ - positive; – - negative; *Resistant to 16% NaCl; *Reaction to chandelier: + - weak reaction; ++ -strong reaction; +++- very strong reaction.*

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