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THE STUDYING OF THE GALLERIA MELLONELLA LARVAE GUT MICROBIOME AND DETERMINING ITS SPECIES COMPOSITION

D.D. Kairat[1], V.S. Kiyan[1],
T.S. Todosiichuk[2], A.M. Smagulova[1], A.K. Zhumalin[1],
[1]S. Seifullin Kazakh Agrotechnical University,
[2]National Technical University of Ukraine

Abstract

Galleria mellonella is common pest of honeybee hives and can be found everywhere where beekeeping is practicing with the exception of regions with a harsh climate and high attitude. *Galleria mellonella* is one of the common testing organism in investigation of several pesticides, insect pathogens and biological active substances. However, most properties of this organism remain unstudied. Immunity and high surviving abilities of organisms closely related to their microbiome and symbiosis. Digestive tract (gut) of *Galleria mellonella* had been studied to obtain knowledge about its microbiological composition via microbiology and molecular genetic analysis techniques. Gut microbiome was habitat to various bacterial species, mostly to *Bacillus* genus. Intestine microbiome of larvae populated with enormous amount and types of bacteria gives its host several abilities to digest range of different material and playing defensive role from foreign gut intruders by secreting enzymes and antibacterial substances. Investigation of mentioned microorganisms from gut were studied and characterized.

Key words: *Galleria mellonella, pest, larvae, gut microbiome, molecular genetic characteristics, cultivation, 16S rRNA sequencing.*

Introduction

Galleria mellonella, often called as “greater wax moth” is moth from the family *Pyralidae* of *Lepidopteran* order. Greater wax moth is wide spread, and can be found where honeybees are cultivated. *G. mellonella* was first reported as a pest insect in Asia, but then spread to northern Africa, Great Britain, some parts of Europe, northern America, and New Zealand [1]. It has been reported in twenty-seven African countries [2], nine Asian countries [3], in North American countries [4], three South American countries [5], Australia [6], ten European countries [7], and five island countries [8]. This pest may spread further, to invade more areas as the climate and their natural habitats are changing from human activities.

Galleria mellonella mainly well characterized for its parasitization on honeybee combs and hives. Female adult insects shortly after emergence lay their eggs inside a beehive, where small cracks or holes are present. Wax moth often can be found in weak honeybee colonies, whenever strong colonies of honeybees can easily deal with foreign intruders, but wax moth females prefer to lay their eggs in strong and healthy colonies over weaker ones [9].

There are also several usages of *Galleria mellonella* in biology and medicine [10]. For the past two decades, microbiologists have searched alternatives to mammals for studying the molecular basis of virulence and for testing antimicrobial drugs. Tsai et al. made a literature review which reported the value of *Galleria mellonella* larvae as a model for investigating bacterial pathogens. The authors highlight many of the attractive features of this model: when compared with mammals, *G. mellonella* larvae are cheaper and easier to maintain, they do not require specialized laboratories or equipment and work with *G. mellonella* does not require ethical approval. Unlike many alternative models *G. mellonella* can be maintained at 37^{o}C. It can be an important feature of this model is the ease with which the larvae can be injected with precise doses of pathogen, allowing the relative virulence of strains and mutants to be compared [11].

In a limited number of studies done by Péchy-Tarr M. et al. showed that preparations from either bacteria or fungi that have been injected into *G. mellonella* to study their toxicity were less virulent to the larvae. In many cases, the toxins studied are known to be insecticidal and *G. mellonella* larvae provide a good model to further investigate toxicity [12].

Wojda et al. made researches about *G. mellonella* immunity, describing anatomical and physiological barriers of insects, protecting them against invasion by microorganisms [13]. While *Drosophila melanogaster* is used to study the genetic aspect of insect immunity, *Galleria mellonella* can serve as a good model for biochemical research [14]. Given the size of the insect, it is possible to easily obtain hemolymph and other tissues as a source of many immune-relevant polypeptides. Therefore, a larva serves as a model to study the virulence mechanisms of human pathogens. In addition, Wojda et al. affirm that antibacterial and antifungal peptides derived from insects and proteins are considered for their potential to be applied as alternatives to antibiotics [15].

According to the research done by Paolo Bombelli et al. biodegradation of polyethylene is possible by larvae of the wax moth *Galleria mellonella*, producing ethylene glycol [16]. However, question that whether the hydrocarbon-digesting activity of *G. mellonella* derives from the organism itself, or from enzymatic activity of larvae gut microbiome remains unsolved.

The scientific research is based on studying of the *Galleria mellonella* larvae gut microbiome to determine microbiome species composition and the possibility of its use in biotechnology.

Materials and Methods

Larvae collection and gut cultivation. *Galleria mellonella* larvae or greater wax moth caterpillars were obtained from Aqmola Region apiaries, in the city of Nur-Sultan, Kazakhstan. Before gut isolation, larvae were pretreated with 70% ethanol for 2-3 minutes, in order to avoid contamination from the caterpillar surface.

After 70% ethanol treatment caterpillars were moved to glass slide and prepared, inner digestive system of caterpillars moved to Petri dish under sterile conditions with MPA (meat peptone agar) culture medium. 12.8 g of dry MPA culture medium was dissolved in 400 ml of distilled water and mixed, then autoclaved at 121^{o}C for 20 min. Culture medium then spilled into four sterile Petri dishes and cultivated at 37^{o}C in incubator. 3 dishes were with caterpillar digestive systems and one for a control in order to analyze if contamination occur from the culture medium.

During the incubation, colonies of microorganisms were formed. In order to get an axenic culture we used titration to spread out different microorganism types to form smaller, independent colonies. For further identification we used DNA extraction to make molecular genetic analysis via 16S rRNA sequencing.

DNA extraction. For DNA extraction “Biosilica bacterial DNA extraction kit” (“Biosilica”) was used. Isolation was performed according to the instruction for use of this kit.

Determining the quality and quantity of isolated DNA. The quality of genomic DNA was monitored by electrophoresis on a 1.5% agarose gel. Quantitative DNA analysis was performed based on the ultraviolet light absorption ratio of DNA/RNA molecules and their concentration in the sample. Quantitative analysis was performed using a BioPhotometer plus (Eppendorf) spectrophotometer. The DNA/RNA absorption maximum is approximately 260 nm. In addition, determining the presence of impurities was carried out at 280 and 320 nm, which made it possible to determine pure nucleic acids ratio, A_{320} = 0, A_{260}/A_{280} ratio 1.8 and higher, indicating pure DNA. All measurements were carried out in a buffer solution (TE) at neutral pH. TE was used to prepare the working concentration (10 ng/μl).

Electrophoretic analysis of samples and qualitative determination of isolated DNA. Analysis of the amplified target DNA fragments, as well as qualitative analysis of the isolated DNA was performed by separating DNA fragments in an agarose gel (agarose concentration from 1-2%, depending on the length of the analyzed fragment), in the presence of an intercalating agent, ethidium bromide, which was used to further DNA visualization. Electrophoresis was carried out in a Max Fill HU10 horizontal electrophoresis chamber and a Consort EV 243 current source. 1x TAE buffer was used as electrode buffer.

Documentation of the obtained results was performed using the documentation system of Bio-Print gels. The size of the molecules of the analyzed DNA samples was

determined by comparing their electrophoretic mobility in the gel with the mobility of the markers – a DNA fragment of known molecular weight. DNA Ladder 1kb (Fermentas) was used as a molecular weight marker.

16S ribosomal RNA is the component of prokaryotic ribosome that undergoes slow changes during the evolution of the encoding gene, using these properties 16S rRNA is commonly used in sequencing and in reconstructing phylogenies. The 16S rRNA was amplified using the primer pair: forward 16S rRNA-8F (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse 16S rRNA-806R (5'-GGACTACCAGGGTATCTAAT-3').

Amplification of DNA samples. For with all primers in a total volume of 20 μl, a mixture was prepared containing 25 ng of DNA, 1 unit. Taq DNA Polymerase (Fermentas), 0.2 mM of each dNTP, 1^{x} PCR buffer, 2.5 mM $MgCl_2$, 10 pmol of each primer. The PCR program was run on a Master cycler Gradient, (Eppendorf) amplificator.

PCR samples purification. PCR samples were purified from oligonucleotide residues by dephosphorylation using alkaline phosphatase (SAP – shrimp alkaline phosphatase) and endonuclease. A mixture was prepared in a total volume of 10 μl for each sample – dH_2O – 7.25 μL, 10^{x}PCR Buffer – 1.0 μl, $MgCl_2$ – 1.0 μl, SAP (5 mM) – 2.5 μl, Exonuclease I (5 units/μL) – 0.125 μl. The resulting mixture was added to each PCR product, placed in a thermal cycler under the following conditions: 37°C – 30 min, 85°C – 15 min, 4°C – ∞. Sample preparation for sequencing carried out by precipitation with an alcohol-acetate mixture.

DNA sequencing. The components of a standard set of reagents for the sequencing reaction were prepared in a 0.2-ml thin-walled thermocyclic tube, in some cases it can becarried out in a microplate well. A standard set of reagents for cyclic sequencing using CEQ WellRED terminator dyes (partially mixed) eliminates the numerous steps of pipetting components and possible errors. Since the volume of the added "basic mixture" is only 8 μl, there remains more free volume for the introduction of the DNA matrix and primer.

Thermal cycling program: 96 °C for 20 seconds, 50 °C for 20 seconds and 60 ° C for 4 min. For 30 cycles and followed by aging at 4 °C.

Sample preparation for loading to CEQ 8000 analyser. If the deposition was carried out in test tubes, then resuspended samples were transferred to the corresponding wells of the microplate for CEQ samples. One drop of light mineral oil put on the surface of each of the resuspended samples. The sample plate loaded to CEQ 8000 and run the selected method.

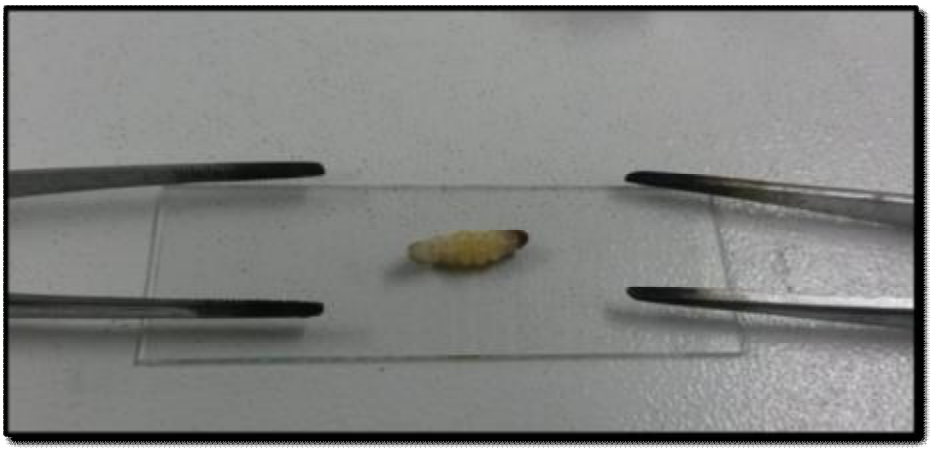
DNA matrix preparation. A matrix was prepared in a volume sufficient for accurate quantification and verification of purity. Then, separation was started using capillary electrophoresis. The detection of laser-induced signals occurs through four spectral channels. After completion of the analysis, a nucleotide sequence is provided in text form.

The analysis of the obtained nucleotide sequences was carried out using the software package (CEQuence

Investigator, etc.), and using international nucleotide sequence databases (Blast, ENSEMBL, GeneBank, etc.).

Results

Isolation of larvae digestive system and obtaining an anxietic culture. All larvae samples preparation and cultivation were carried out in sterile conditions under 2nd class laminar flow cabinet (picture 1).



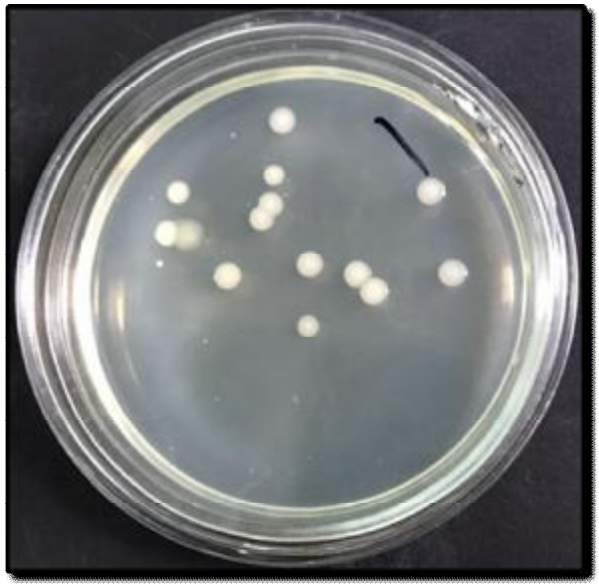
Picture 1 – *Galleria mellonella* larvae preparation

Larvae were treated with 70% ethanol and prepared, inner digestive system of caterpillars moved to Petri dish under sterile conditions. MPA (meat-peptone agar) was choosen as culture medium, due to its multipurpose properties in cultivation of microorganisms. Culture medium then spilled into sterile Petri dish and cultivated at 37°C in incubator. One dish was for a control purposes in order to analyse contamination and sterility. Primary colonies were formed after 24 hours of cultivation (picture 2).

Picture 2 – Primary colony of gut microorganisms

As seen in picture 2, primary colonies of microorganisms, isolated straightly from gut spread around all culture media surface, which made their species identification difficult. To isolate single colonies, we carried out work on isolation by dilution method. After formation of primary colonies MPB (meat-peptonebroth) liquid culture medium was prepared for further facilitation of dilution methods to spread out different microorganism species.

400 ml of MPB with gut microorganisms were cultivated at 37^{o}C in incubator for 24 hours. After cultivation, 100 µl of culture media were diluted in 1 ml sterile, autoclaved water. After 6th dilution 100 µl of sample was removed to Petri dish with MPA that was used as control in previous steps for economy reasons. Petri dish was then moved to incubator for further cultivation at 37^{o}C. After 24 hours colonies of microorganisms were formed (picture 3).



Picture 3 – Separate colonies of gut microorganisms after dilution

According to picture 3, 8 different and 15 total colonies were formed. After independent colonies formed, microorganisms separately were removed to new culture medium. Colonies were round shaped and varying from size of 0.1 mm to 0.7 mm. Round colonies were opaque and do not showed transparency properties. Surface of colonies were mostly smooth and glistering, raised from agar but varied to totally dull ones. By consistency colonies were mostly viscid only dull colony showed mucus-like property.

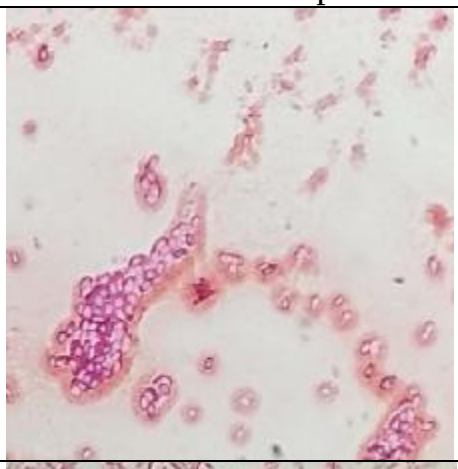
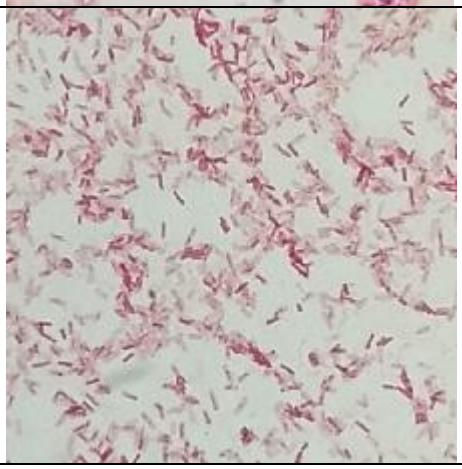
Morphology of microorganisms. We used Gram staining methods to dye and to visualise bacterial cells through microscopy for further morphological description. Samples of microorganisms numbered 1, 6 and 8 isolated from the intestine of *G.*

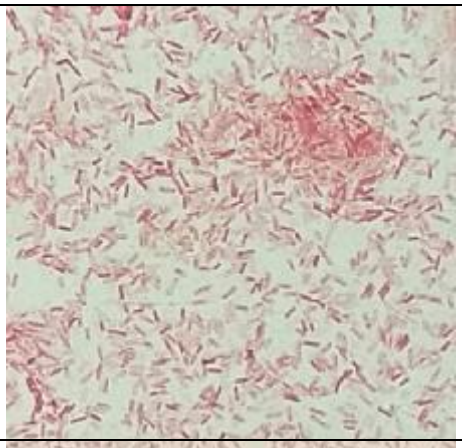
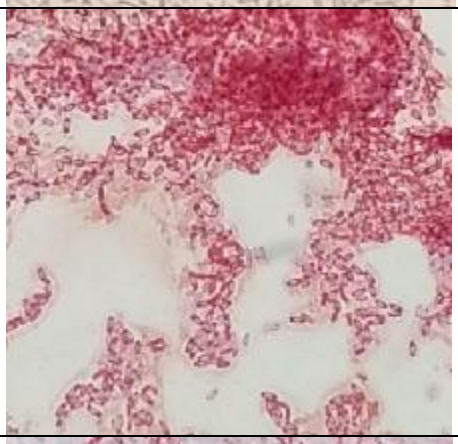
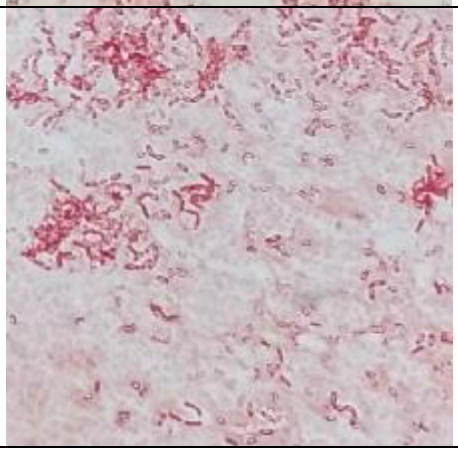
mellonella larvae werecoloured with crystal violet dye after treatment with safranin red dye and were characterized as Gram-positive, rod shaped and spore forming bacteria. This properties can be described as *Bacillus* genus properties.

Sample of microorganisms numbered 3 isolated from gut was well coloured with safranin red dye showing that bacteria were gram negative, rod shaped and non spore forming.

Under the microscopy the sample of microorganisms numbered as 5 showed Gram negative properties. No spores were observed. Morphological structure of microorganisms was rod shaped. Unfortunately, cell structure and flagellum or cilia was not seen. More detailed information can be seen in table 1.

Table 1 - Morphological description of microorganisms.

Sample	Picture of sample	Description
1		Bacterial cells in sample #1 were rod shaped and Gram positive. Membranes of bacterial cells were coloured with gentian violet and safranin dye. Spores were formed inside bacterial cells and were not colored with dyes. Size of cells varied from 1,0 to 2,0 μm long. Properties of *Bacillus* genus supposed.
2		Microorganisms in sample #2 were rod shaped and showed Gram negative properties due to cell membrane colouring with safranin dye. Forming of spores was not observed. Size of cells varied from 1,5 to 3,0 μm. *Rhizobium* spp. Characteristics can be presumed.

3		In sample #3 microorganisms were rod shaped and Gram negative due to cell membrane colouring with safranin dye. Forming of spores was not observed. Size of cells varied from 1,5 to 3,0 μm. *Rhizobium* spp. characteristics can be presumed.
5		Sample #5 had microorganisms with rod shaped and Gram negative properties. Membrane of bacterial cells were coloured with safranin dye. Forming of spores was not observed. Size of cells varied from 1 to 5 μm long. Properties of current bacterial strain show *Pseudomonas spp.* characteristics.
6		Bacterial cells in sample #6 were rod shaped and Gram positive. Membranes of bacterial cells were coloured with gentian violet and safranin dye. Spores were formed inside bacterial cells and were not coloured with dyes. Size of cells varied from 1,0 to 2,0 μm long. Properties of *Bacillus* genus supposed.
8		In sample #8 microorganisms were rod shaped and Gram positive due to cell membrane colouring with gentian violet and safranin dye. Forming of spores was observed in bacterial cells. Size of cells varied from 1,0 to 2,0 μm long. Properties of *Bacillus* genus supposed.

Several microorganisms including strains 1, 6 and 8 were selected to further study, since they showed differences in morphology and microscopic analysis.

Biochemical properties of

microorganisms. After independent colonies of microorganisms formed, we separately placed them into new fresh medium for *Enterobacter* identification with bromothymol blue. Main principle of identification was based on changing of pH values and culture medium colour. Graphical representation of bromothyhol pH values can bee seen at picture 4.

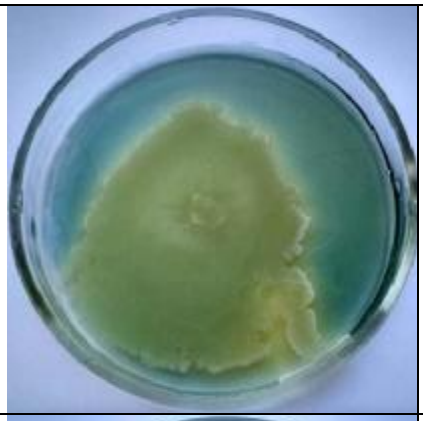
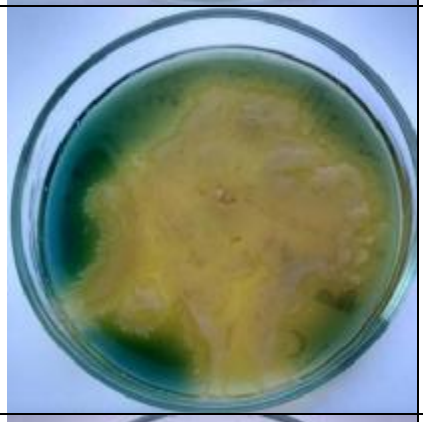
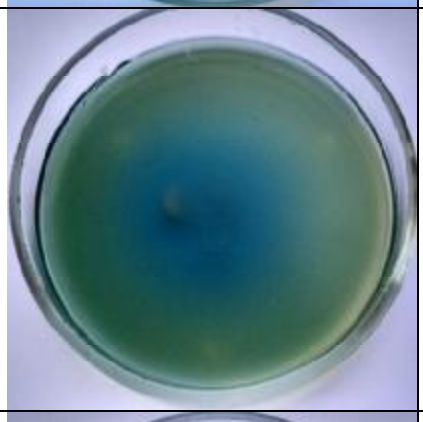
Picture 4. pH scale of bromothymol blue

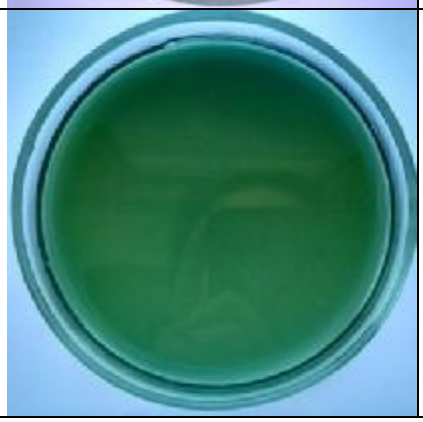
Bromothymol working pH values range from 5,8 to 7,6. Dye if often used for measuring the presence of carbonic acids in liquids. Acid dissociation constant (pK_a) of bromothymol is equal to 7,10 at neutral pH (pH=7,0).

In 6 Petri dishes culture medium colour changed from greenish-blue to yellow, meaning that pH in medium decreased to acidic degrees. Maltose as carbon source and fish pancreas hydrolyzate as amino acids source were main nutrition sources in culture medium. Decomposition of maltose by microorganisms leads to forming glucose in cytoplasm that leads to glycolysis pathway and forming of acetate as product. One Petri dish numbered as 5, changed culture medium colour to blue from greenish-blue, leading to pH increase to basic values. Visual representation of biochemical analysis can be seen at table 2.

Table 2 – Biochemical analysis of strains

Sample	Picture	Description
1		Bacterial strain #1 changed culture media into dark greenish-yellow colour on all the surface, therefore changing pH values according to scale is slightly acidic, pH is equal to 6,0 - 6,2. *Bacillus* genus microorganisms were represented in dish.

2		Bacterial strain in second Petri dish changed colour of culture medium to dark yellow at some points and greenish mostly on the colony surface. pH values was not same, visually can be estimated between range of 5,8 to 6,1. *Rhizobium* genus microorganisms were represented in this dish.
3		Microorganisms in Petri dish number 3 changed pH of culture media into more acidic degrees according to light yellow colour. pH of culture media was ranged from 5.3 to 5.5. *Rhizobium* genus microorganisms were represented in this dish.
5		Petri dish number 5 after 12 hour of cultivation changed its colour not to light green or yellow, but to blue, meaning that pH of this culture media was changed to basic values. pH of culture media was 7,5 - 7,8 depending on area. *Pseudomonas* genus microorganisms were represented in this dish.
6		Bacterial strain #6 changed culture media into greenish yellow colour at corners and slightly blue at the centre of Petri dish. pH values of culture ranged from 6,7 to 7,1. *Bacillus* genus microorganisms were represented in this dish.

8		Culture medium with bacterial strain #8 changed colour to dark yellow. pH values were ranging from 5,5 at the centre to 6,2 on corner sides. *Bacillus* genus microorganisms were represented in this dish.
Control		Control, sterile Petri dish without microorganisms at neutral pH of 7,0.

According to table 2, *Bacillus* and *Rhizobium spp.* supposed bacterial strains changed culture medium colour to yellow. Bromothymol blue in current Petri dishes indicated acidic conditions, meaning that metabolic pathways in those strains degrade maltose into acidic compounds.

Pseudomonas spp. changed color of culture medium to blue, meaning more basic compounds were present. Metabolic pathways difference between strains *Bacillus* and *Rhizobium* could be explained. *Pseudomonas spp.* strains often take part in nitrogen cycle in soil and can use different energy sources like nitrogenous compounds that other microorganisms do not usually use.

Molecular genetic characteristics of microorganisms. After DNA sequencing nucleotide sequence of samples analysed using CEQunce software and searched for matches via GeneBank international nucleotide database. 16S rRNA identification results of larvae gut microbiome microorganisms can be seen at table 3.

Table 3 – 16S rRNA identification results

Isolate	Suggestions of GenBank	GenBank accession number	Identity value, %
1	*Bacillus amyloliquefaciens* strain XQ51	KU291367.1	96
	Bacillus amyloliquefaciens strain 10626	KX268481.1	96
	Bacillus amyloliquefaciens strain BCRh10	KT153606.1	96
2	*Rhizobium* sp. P5-19-30-1	LC368035.1	99

	Agrobacterium tumefaciens strain AL4	MG819299.1	99
	Agrobacterium tumefaciens strain AL5	MG819298.1	99
3	*Rhizobium* sp. P5-19-30-1	LC368035.1	97
	Agrobacterium tumefaciens strain AL4	MG819299.1	97
	Agrobacterium tumefaciens strain AL5	MG819298.1	97
5	*Pseudomonas* sp. NF-2	EU180220.1	98
	Pseudomonas sp. Strain ZX01	MG760359.1	98
	Pseudomonas chengduensis strain W105	MF554604.1	98
6	*Bacillus velezensis* strain FD-UOS1	MG952530.1	98
	Bacillus velezensis strain DKU NT 04	CP026533.1	98
	Bacillus velezensis strain SB-12	MF321851.1	98
8	*Bacillus subtilis* strain VSS2	KR296931.1	92
	Bacillus velezensis strain FD-UOS1	MG952530.1	92
	Bacillus velezensis strain DKU NT 04	CP026533.1	92
Control E. coli	*Escherichia coli* strain L2	KT824795.1	87
	Escherichia coli strain UTI05	MG913260.1	87
	Escherichia coli strain KBN10P04869	CP026473.1	86

According to table 3, molecular genetic analysis confirmed the assumptions based on morphological analysis, microscopy and biochemical analysis. *E. coli* was used as control strain. Molecular genetic analysis allowed and narrowed the detection of more *Bacillus* strain species: *B. velezensis*, *B. subtilis* and *B. amyloliquefaciens. Rhizobium radiobacter* or in other words, *Agrobacterium tumefaciens* had been detected. Several *Pseudomonas* strain DNA had been observed. DNA purity and identity values were at high degrees.

Conclusion

Scientific studies of *Galleria mellonella* gut microbiome strains and their biochemical, morphological, molecular genetics properties. Gut microbiome of wax moth larvae showed great multitude. Different metabolic pathways of gut microorganisms and their enzymatic differences give an ability to degrade several molecular complex substances like honeycomb wax. Microbial species multiplicity also helps host organisms to counter internal invasions by extracting antimicrobial metabolites and maintaining conditions in an interior of organism which are harmful to others. Such properties of wax moth larvae microbiome can act as valuable tools for the study of host and pathogen interactions. Using insect larvae can facilitate the identification of bacterial pathogens and give possibilities to discover new components that are involved in host innate immune responses and bacterial interactions.

According to the scientific research carried out by Yang et al. gut microorganisms consisted of two main bacterial strains involved in degradation of polyethylene (PE) that were isolated from this worm's gut, *Enterobacter asburiae* YT1 and *Bacillus sp.* YP1 [17]. Using 16S rRNA sequencing we obtained similar results, proving that *Galleria mellonella* microbiome consists of the following microorganisms: *Bacillus amyloliquefaciens, Pseudomonas spp., Bacillus velezensis, Rhizobium radiobacter, Bacillus subtilis.* Additionally, scientific research done by Kyaw et al. Reports that *Pseudomonas* strain which presents in wax moth larvae gut microbiome causes weight losses of up to 20% in the tested PE within 120 days [18]. Bacterial strain presented in current study unfolds opportunities for solution of several ecological issues of landfill and aquatic environment pollution with PE.

Bacterial strains isolated from larvae gut separately can be used in biotechnology, agriculture and ecology. Further investigations of bacterial properties must be performed. Our results show that wax moth larvae gut composition characteristics included variety of microorganisms.

More detailed studies of *Galleria mellonella* gut microbiome and possible useful properties of microorganisms and whole organism itself need further investigations.

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ИЗУЧЕНИЕ МИКРОБИОМА КИШЕЧНИКА ГУСЕНИЦЫ *GALLERIA MELLONELLA* И ОПРЕДЕЛЕНИЕ ЕЁ ВИДОВОГО СОСТАВА

Д.Д. Кайрат[1], В.С. Киян[1],
Т.С. Тодосийчук [2], А.М. Смагулова[1], А.Х. Жумалин[1].
[1]Казахский агротехнический университет им. С. Сейфуллина,
[2]Национальный технический университет Украины «Киевский политехнический институт имени Игоря Сикорского»

В научной статье проводилось исследование с использованием личинки восковой моли – *Galleria mellonella,* являющимся распространенным вредителем диких пчел и пчеловодства. Также в статье приводятся примеры использования восковой моли как организма, на которых проводят исследования воздействия некоторых пестицидов, возбудителей болезней насекомых и влияния биологически активных веществ. Утверждается, что большинство свойств этого организма остается неизученным. Иммунитет и высокая выживаемость личинок тесно связаны с их микробиомом и симбиозом. С этой целью авторы исследовали пищеварительный тракт организма *Galleria mellonella* для получения информации о его микробиологическом составе с помощью микробиологических и молекулярно-генетических методов. Кишечный микробиом был средой обитания для различных видов бактерий, в основном для рода *Bacillus*. Было предположено, что микробиом кишечника личинок, населенных огромным количеством и типом бактерий, дает хозяину ряд некоторых возможностей для перевариваривания различных веществ и играет защитную роль от чужеродных кишечных патогенных организмов путем выделения ферментов и антибактериальных веществ в кишечную среду. Исследование показало видовой состав микроорганизмов состоящий из следующих микроорганизмов: *Bacillus amyloliquefaciens, Pseudomonas spp., Bacillus velezensis, Rhizobium radiobacter, Bacillus subtilis.*

Ключевые слова: *Galleria mellonella,* вредители, личинки, кишечный микробиом, молекулярно-генетическая характеристика, культивирование, секвенирование 16S рРНК.

*GALLERIAMELLONELLA*БАЛАҢҚҰРТЫНЫҢІШКІ МИКРОБИОМЫН ЖӘНЕ ОНЫҢ ЕРЕКШЕЛІКТЕРІН АНЫҚТАУ

Д.Д. Қайрат[1], В.С. Киян[1],
Т.С. Тодосийчук[2], А.М. Смагулова[1], А.К. Жумалин[1],
[1]С.Сейфуллин атындағы Қазақ агротехникалық университеті,
[2]Украина ұлттық техникалық университеті «Игорь Сикорский атындағы Киев политехникалық институты»

Ғылыми мақалада балауызды көбелек – *Galleria mellonella* личинкасын зерттеу жүргізілді, ол жабайы аралар мен бал араларындағы кең таралған зиянкесі болып табылады. Мақалада сонымен қатар балауыз көбелекті модельдік организм ретінде қолдану мысалдары келтірілген, онда кейбір пестицидтердің, жәндіктер ауруының қоздырғыштары мен биологиялық белсенді заттардың әсері зерттеледі. Бұл организмнің көптеген қасиеттері зерттелмеген күйінде қалады деп айтылады. Личинкалардың иммунитеті және жоғары төзу деңгейі олардың микробиом симбиозымен тығыз байланысты. Осы мақсатта авторлар микробиологиялық және молекулярлы-генетикалық әдістерді қолдана отырып, оның микробиологиялық құрамы туралы ақпарат алу үшін *Galleria mellonella* денесінің ас қорыту жолдарын зерттеді. Ішек микробиомы бактериялардың әр түрлі түрлеріне, негізінен *Bacillus* тұқымдасына, мекен етті. Бактериялардың көп саны мен түрі бар личинкалардың ішек микробиомасы иесіне әртүрлі заттарды сіңіруге белгілі мүмкіндіктер береді және ферменттер мен бактерияға қарсы заттарды ішек ортасына бөліп шығару арқылы бөгде ішек патогенді организмдерге қарсы қорғаныс рөлін атқарады. Зерттеу келесі микроорганизмдерден тұратын микроорганизмдердің түрлік құрамын көрсетті: *Bacillus amyloliquefaciens, Pseudomonas spp., Bacillus velezensis, Rhizobium radiobacter, Bacillus subtilis.*

Кілттік сөздер: *Galleria mellonella*, зиянкестер, личинкалар, ішек микробиомы, молекулалық-генетикалық сипаттама, микроағзаларды культивирлеу, 16S рРНҚ секвенирлеу.