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Microflora of *Galleria mellonella* L.

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In natural conditions the larvae of the wax-moth, *Galleria mellonella* L., feed only on beeswax, whose main component is wax. The ability to use wax as food is a rare phenomenon among animals. Besides *Galleria mellonella* this ability also occurs in *Achroea grisella* which feeds on beeswax too and in *Aphomia* and *Melissoblates* which feed on bee and bumblebee honeycombs. *Epirops* digests wax excreted on the body surface of cicades that serve as their food (30). An African bird, *Indicator*, is enabled to digest wax by the bacteria occurring in its digestive tract (41).

Among microorganisms the ability to digest wax is also a rare phenomenon. So far, few species able to decompose this substance have been described. They include *Micrococcus ureae, Aspergillus flavus, Pseudomonas* sp. and *Micrococcus* sp. (21, 22), and some other un identified bacteria (12, 18, 52).

Metalnikow (40, 54) was the first scientist to carry out systematic studies on the morphology, anatomy and physiology of the wax-moth. His works and, later on, the studies of others (12, 16, 18, 39, 44) showed that wax constitutes about 50 per cent of the total mass of beeswax, and that it is a substance which provides the larvae of *Galleria mellonella* with both energy and substrates for the formation of their own bodies. According to some scientists (12, 16, 44, 62) about one half of the wax consumed by the larvae does not appear in the excrements but is metabolized in the bodies of the larvae.

Beeswax is a compound substance containing high molecular fatty acids or their esters with alcohols (cerotic, melissic and palmitic acids; mylissyl, ceryl and cetyl alcohols) as well as high molecular carbohydrides.

The biochemical transformation of wax into the fats of wax-moth larvae, which do not principally differ from the fats of other animals (39, 43) was studied by Niemierko and Włodawer and Przełęcka (42, 44, 45, 48, 69).

Assimilation by the larvae of the fatty acid component of wax has not been questioned (12, 16, 18, 40, 44, 62, 69). However, in the diet of the larvae, one cannot substitute the particular fatty acids, occurring in the composition of wax, for wax itself (10, 11, 40).

The use of carbohydrides and alcohols is still a controversial problem. Some

workers (44,69) report that both substances actively participate in the metabolism, others (12,16, 18, 40, 62) argue the larvae ability to assimilate carbohydrides.

Apart from lipid-like substances, the larvae of the wax-moth take some unidentified nitrogen compounds from beeswax. The quantities of nitrogen in beeswax are small and changeable. They constitute 2—3 per cent in several years old honeycomb (40, 70). They come from pollen and substances produced by bees and maggots (70).

Beeswax also completely covers the vitamin requirements of wax-moth larvae. Nicotinic acid was found to be an important growth factor for *Galleria mellonella* (51). In cultures grown on artificial media it was also necessary to add some vitamins of group B.

Undoubtedly, digesting of wax takes place in the larvae digestive tracts. However, we have no data which would make it possible to find out whether beeswax is decomposed by the enzymes of the larvae or by the bacteria in their digestive tracts. Dickman (12), Florkin (18) and Rybicki (52) are of the opinion that digesting takes place with the active participation of the bacteria. On the other hand, Metalnikow (40) and Mankiewicz (38) attribute it to the enzymes. The latter hypothesis seems to be supported by the reports of Niemierko and Włodawer (44) and Włodawer (69), as well as by the most convincing study by Waterhouse (62) carried out on axenic cultures. He showed that bacteria-free larvae can assimilate alcohols, fatty acids and wax esters but that they make use of paraffin C_{30} neither directly nor after decomposition by bacterial flora.

It is also interesting to observe the fact that wax-moth larvae are capable of digesting waxes occurring in the cell walls of acid-fast bacilli. Similarly to beeswax, extracted waxes of *Mycobacterium* serve as a good nutriment for the larvae (38, 40).

Wax-moth larvae are resistant to infection by tubercule bacilli. Two days after the administration of even relatively high doses to the organisms, one cannot isolate any live bacteria in the organisms of the larvae. The resistance of the larvae is thought by Metalnikow (40) to be connected with the ability of metabolizing wax which leads to the damage of the cell walls of the bacilli. In resistance an important role is played by phagocytosis (8). Extracts from wax-moth larvae were used in an experimental treatment of guinea pig tuberculosis (40). In vitro the extracts from wax-moth larvae sensitized the bacilli to antibiotics and chemotherapeutics, to which they are normally resistant (13, 14, 25, 26, 31, 46).

It is not clear yet whether the microflora which occur in wax-moth larvae participate in their anti-bacillary resistance.

So far the microflora of *Galleria mellonella* have been studied only fragmentarily (56, 47).

Numerous studies have been devoted to those microorganisms which are pathogenic to insectes. In the case of economically important insectes, such as silk-moths or bees, the recognition of a pathogenic microorganism makes it possible to prevent diseases destroying whole populations. Thus, the microflora of *Bombyx mori* and *Apis mellifera* have been objects of studies from the second half of the 19th century till today (1, 33, 34, 55, 61). Equally numerous studies have concerned the bacteria pathogenic for insects (2, 19, 24, 27, 29, 32, 35, 36, 37, 50, 53, 56, 64, 65, 66, 67, 68).

Unlike chemical preparations, most entomopathogenic microorganisms can be characterized by specificity towards certain species of insects. This allows one to use them as a biological weapon. In the fight against the wax-moth, *Bacillus thuringensis*, attacking *Lepidoptera*, is widely used. Some of its numerous strains are adapted to a given kind of insects (19, 24, 28, 53, 64, 65).

Carrying out studies on the virulence of microorganisms for the larvae of *Galleria mellonella*, various scientists have also obtained some data on the microflora composition of these insects. Poltiew (47) determined its composition on the surface of eggs, in the larvae, pupae and mature individuals. The studies were conducted on insects growing in natural conditions — in beehives. They did not deal with quantitative changes in the number of microorganisms, or particular species in relation to the age of the larvae, or the section of the alimentary tract in which they were localized, or, finally, the role played by them in the processes of digesting and regular growth.

Bucher and Williams (6) examined bacterial floras of mature larvae and pupae of *Galleria mellonella* in laboratory cultures grown on an artificial diet. The aim of the paper was the determination of the bacterial species which exert some influence on the state of health of wax-moth larvae used for generating parasite nematodes and insects. They showed that the presence of *Streptococcus faecalis* — constantly occurring in wax-moth larvae — prevets the development of pathogenic bacteria, and thus it plays an important role in the resistance of the insect.

It seemed justified, therefore, to undertake a study on the microflora in the ontogenesis of *Galleria mellonella* developing on a normal diet of beeswax in laboratory cultures, taking into account age, stage of development and localization in particular sections of the intestine.

MATERIALS AND METHODS

Insects were taken from a culture growing on beeswax in a thermostat at 30°C. The studies were carried out on eggs, larvae in various ages, pupae and imagines. The presence of microorganisms within eggs was checked on stained microtome sections prepared in the Zoology Department, Maria Curie-Sklodowska University. The interiors were sterile.

The larvae were examined in the first, third and fourth weeks of life, and directly before pupation. Their lengths in respective weeks were 10, 15 and 25 mm. Microorganisms were isolated from the whole alimentary tract and from its particular sections — anterior, medial and posterior intestines.

In experiments with pupae, large larvae (25—30 mm) were transferred into separate jars with beswax. The jars were examined twice a day and selected pupae were put on Petri dishes. This allowed an exact determination of the pupae age. Microgorganism isolation was performed on 1-, 3-, 5-, 7-, and 9-day-old pupae. On the tenth day there appeared mature individuals used for further experiments.

Larvae, pupae and imagines were submerged for 2 minutes in 70 per cent ethanol in order to disinfect their exterior surfaces and to kill the insects. Sterility was controlled by rolling a given form of the insect over a Petri dish covered with nutrient agar.

Inoculation of intestinal contents was carried out by three methods:

1. The insect was mounted on a sterile cork, sectioned, and the alimentary tract was then ground on a Petri dish with an appropriate medium.

2. The isolated alimentary tract was submerged in 1 ml of physiological solution, homogenized and inoculated on plates.

3. In the case of isolation from particular sections of the intestine, larvae were cut into three pieces:

a) the first two body segments — in which the anterior intestine is located

b) the section from the third to the ninth body segments containing the medial intestine

c) the section from the tenth to the fourteenth body segments in which the posterior intestine is located. Prepared intestine sections were inoculated directly, or after suspending the contents in the physiological solution on plates with an appropriate medium.

In the studies on the number of microorganisms the intestine contents were diluted.

For microorganism isolation the following media were used: nutrient agar *pH* 6.5, 7.6, agar with glucose (2 per cent), blood agar (10 per cent of equine blood), agar and serum (10 per cent of bovine serum), agar with starch (2 per cent), agar with larvae extract after Rybicki, medium after Chapman, medium after Garibaldi, medium after Paszkiewicz, medium after Sabourand, medium with wheat bran, Endo medium, medium after Wrzosek.

After 24-hour incubation at 37°C the morphology of the culture was described. From representative colonies preparations were made, stained by Gram's method, and spread on slope nutrient agar.

In the case of the Wrzosek medium, which was the only liquid medium used in this study, after incubation and examination of the preparations stained by Gram s method, microorganisms were inoculated on plates with nutrient agar. They were incubated in aerobic conditions. The colonies developed in this way served as preparations stained by Gram's method, and they were compared with those obtained directly from the anaerobic medium.

Pure strains were identified by commonly used microbiological tests. Bergey's classification was used.

Experiments with microorganisms occurring in besswax were carried out in a similar way. Isolation was performed from homogenates of wax in physiological solution.

RESULTS

Over five hundred strains were obtained from larvae, pupae and imagines of *Galleria mellonella* isolated on fifteen media. On the basis of morphological, physiological and growth features they were divided into six systematic groups:

1. Gram-positive cocci, producing no catalase, corresponding in their physiological and growth features to *Streptococcus* group D, mainly *Streptococcus faecalis;*

2. Gram-positive cocci, catalase-positive, belonging to genera *Micrococcus, Staphylococcus* and *Sarcina;*

3) aerobic, spore-forming bacilli from genus *Bacillus;*

4) Gram-negative bacteria;

5) actinomycetes;

6) fungi.

Strains belonging to *Streptococcus*, which formed a group of bacteria occurring in all stages of insect development, with the exception of eggs, were included in the genus. The taxonomic features of this group are unusually uniform and they correspond to the features of *Streptococcus faecalis*. Only one of the strains seems most similar to *Streptococcus equinus*.

Physiological and growth properties of Gram-positive cocci producing catalase make it possible to include them in the genera *Staphylococcus*, *Micrococcus* and *Sarcina*.

Aerobic spore-forming bacilli from the genus *Bacillus* were encountered in all developmental stages of the insect but in a small percentage of individuals. The physiological properties of this group are not uniform. Only strains antagonistic to acid-fast bacilli were included in the genus. *Bacillus subtilis* was represented most frequently; *Bacillus cereus*, *Bacillus lentus* and *Bacillus licheniformis* were isolated in single cases.

Gram-negative bacteria, actinomycetes and fungi were isolated very rarely. Six strains of bacteria were isolated from larvae, one from pupa, and one from imago. Similarly, actinomycetes were isolated; two strains were isolated from larvae, one from a pupa, three strains of fungi were isolated from larvae.

Because of sporadic occurrence, these microorganisms were not included in the genus, with the exception of one of the actinomycetes. It is the strain *Streptomyces griseus*, which is strongly antibiotic to *Candida albicans*.

No strict anaerobic bacteria were found in the microflora of *Galleria mellonella*.

Internal microflora occur in all the developmental stages of the wax-moth, except in the eggs. In stained preparations of microtome sections of the eggs no microorganisms were discovered.

The percentage distribution of particular groups of microorganisms isolated from individuals in various stages of development is not the same (Table 1).

Table 1. Percentage contents of particular groups of microorganisms isolated from various developmental stages of *Galleria mellonella* L.

Stage of development	Catalase--negative cocci	Catalase--positive cocci	Aerobic spore--forming bacilli	Bacilli	Actinomycetes	Fungi
Larvae	54.1	21.6	21.1	1.7	0.6	0.9
Pupae	83.3	1.4	12.8	1.4	0.7	0
Imagines	87.9	0	9.1	3.0	0	0

Calculations for 490 strains isolated.

The larval period can be characterized by the greatest variety of flora. The number of genera becomes smaller in pupal stage and is the smallest in imagines (Table 2).

During the whole development of *Galleria mellonella*, cocci of group D, *Streptococcus faecalis*, are the dominating microorganisms. *Streptococcus faecalis* is the only microorganism found in 40 per cent of larvae and in up to 77.8

Table 2. Percentage of individuals of *Galleria mellonella* containing microorganisms from various groups

Time in weeks \ Microorganisms	Catalase-negative cocci	Catalase-positive cocci	Aerobic spore-forming bacilli	Bacilli	Actinomycetes	Fungi
Larvae						
1st week	52.5	26.4	11.5	1.8	0	1.8
3rd week	55.3	26.3	10.5	6.6	0	1.3
4th week	57.6	21.6	18.0	0	1.4	1.4
Pupae						
1st day	88.9	2.2	8.9	0	0	0
3rd day	87.5	0	12.5	0	0	0
5th day	91.9	0	5.4	2.7	0	0
7th day	89.5	2.6	7.9	0	0	0
9th day	90.4	0	4.8	0	4.8	0
Imagines						
1st day	93.8	0	3.1	3.1	0	0

The values given are based on the analysis of several series of 50 individuals of every age each.

Table 3. Percentage of *Galleria mellonella* L. individuals containing microorganisms from one group only

Time in weeks \ Microorganisms	Catalase-negative cocci	Catalase-positive cocci	Aerobic spore-forming bacilli	Bacilli	Acetinomycetes	Fungi
Larvae						
1st week	40	14	0	0	0	0
3rd week	42	6	6	0	0	0
4th week	41	6	9	0	0	0
Pupae						
1st day	77.8	0	0	0	0	0
3rd day	67.7	0	0	0	0	0
5th day	69.0	0	0	0	0	0
7th day	66.8	0	0	0	0	0
9th day	76.2	0	0	0	0	0
Imagines						
1st day	62.3	0	0	0	0	0

per cent of pupae. In imagines it occurs in all individuals containing bacteria and most frequently it is the only microorganism (Table 3).

However, this microorganism does not always occur in beeswax given as food. In ten portions of wax, coming from various sources, only half of them contained cocci and in quantities of several thousand per 1 g. In the wax, however, on which the wax-moth larvae fed, the dominating microorganism

is *Streptococcus faecalis*. The number of fungi undergoes reduction while other microorganisms in the wax, coming from the original source and the wax-moth culture, do not undergo any significant changes (Table 4).

Table 4. Number of microorganisms in thousand/1 g of bees-wax

Portion of bees-wax	Before administration to culture				Within culture (after 1 week of larvae feeding)			
	Catalase--negative cocci	Catalase--positive cocci	Aerobic spore--forming bacilli	Fungi	Catalase--negative cocci	Catalase--positive cocci	Aerobic spore--forming bacilli	Fungi
1	6	36	5	1	1	1	1	0
2	2	7	14	1	330	12	0	1
3	0	2	0	12	300	36	1	1
4	0	1	1	8	120	1	2	1
5	2	16	1	10	82	52	0	1
6	2	2	6	2	66	13	4	1
7	2	8	20	12	10	4	2	1
8	0	16	4	1	290	30	1	1
9	0	4	0	18	36	12	12	0
10	2	2	2	6	14	14	1	0

The mean values of three repetitions given.

Studies on the numerousness of bacterial populations in insects of various ages revealed great differences between individuals. Within the same age group, in individuals from the same culture, one may find sterile insects, insects in which the number of bacteria does not exceed 1000 cells, and insects containing bacterial populations of the order 10^6 or even 10^7.

The mean size of bacterial populations in larvae is the smallest in the first week of life. In that period one observes the highest percentage of individuals containing less than 10^3 microorganisms. At the same time, a small percentage of larvae contains large bacterial populations. The percentage of larvae with small populations decreases in the third week of life, while the number of larvae with a large number of microorganisms, reaching sometimes 10^7 cells, increases. Hence, the mean number of bacteria in one larva is the highest in this age group. In the fourth week of life, the percentage of larvae containing a small number of bacteria does not change, but the number of individuals with populations over one million cells decreases, which is reflected in the lower mean of the number of microorganisms in one insect (Tables 5 and 6).

In one-day-old pupae the mean number of bacteria is the same as in larvae in the fourth week of life, i.e. in the period directly preceding cocooning. On the third and fifth days one observes a slight decrease in the number of microorganisms, and again a small increase on the seventh and ninth days (Table 5). In pupae, no individuals with bacterial populations exceeding 5×10^6 cells have been observed (Table 6).

Among imagines there is the highest percentage of individuals with the smallest number of the bacteria. The number of microorganisms in one imago does not exceed 10^5 cells (Tables 5 and 6).

Table 5. The mean number of bacteria in various developmental stages of the wax-moth

Developmental stage		No. of bacteria/individual
Larvae		
	1st week	$\frac{1.7\times10^5}{0—6.4\times10^6}$
	3rd week	$\frac{1.3\times10^6}{0—1.6\times10^7}$
	4th week	$\frac{8.7\times10^5}{0—9.9\times10^6}$
Pupae		
	1st day	$\frac{8.9\times10^5}{0—4.1\times10^6}$
	3rd day	$\frac{3.6\times10^5}{0—2.2\times10^6}$
	5th day	$\frac{3.9\times10^5}{0—2.5\times10^6}$
	7th day	$\frac{5.9\times10^5}{0—3.3\times10^6}$
	9th day	$\frac{5.6\times10^5}{0—3.6\times10^6}$
Imago		
	1st day	$\frac{1.1\times10^4}{0—5.6\times10^4}$

Table 6. Percentage of *Galleria mellonella* individuals in subsequent developmental stages containing various numbers of bacterial populations

	Numerousness of bacterial population/% of individuals					
	$0—10^3$	$10^3—10^5$	$10^5—5\times10^5$	$5\times10^5—10^6$	$10^6—5\times10^6$	$5\times10^6—10^7$
Larvae						
1st week	22	66	10	0	0	2
3rd week	10	24	34	8	18	6
4th week	10	42	20	13	8	7
Pupae						
1st day	20	13	13	27	27	0
3rd day	27	20	33	7	13	0
5th day	27	27	20	20	6	0
7th day	20	20	33	7	20	0
9th day	13	33	27	13	13	0
Imago						
1st day	53	47	0	0	0	0

The distribution of bacteria in the alimentary tract of a larva, in relation to its length, is uniform. The anterior part constitutes 14 per cent, the medial part — 50 per cent, and the posterior part — 36 per cent of the length of the total intestine. Respectively, 14—18 per cent of the total number of bacteria were found in the anterior part of the intestine, 61—65 per cent in the medial intestine, and 21—22 per cent in the posterior intestine (Table 7).

Table 7. Distribution of bacteria in the intestines of the larvae of *Galleria mellonella* L.

	Number of bacteria/intestine section			Total number of bacteria in %			Length of section in % of the intestine
	Week			Week			
	first	third	fourth	first	fourth	third	
Anterior intestine	$\frac{2.3\times10^4}{0—9\times10^5}$	$\frac{2.3\times10^5}{0—2.9\times10^6}$	$\frac{1.1\times10^5}{0—1.2\times10^6}$	14	18	13	14
Medial intestine	$\frac{1.1\times10^5}{0—4.1\times10^6}$	$\frac{7.8\times10^5}{0—1\times10^7}$	$\frac{5.7\times10^5}{0—6.4\times10^5}$	64	61	65	50
Posterior intestine	$\frac{3.7\times10^4}{0—1.4\times10^6}$	$\frac{2.7\times10^5}{0—3.4\times10^6}$	$\frac{1.9\times10^5}{0—2.1\times10^6}$	22	21	22	36

DISCUSSION OF RESULTS

Studies of the microflora of laboratory cultures of wax-moth growing on beeswax showed that the microorganisms occurring in the alimentary tract of larvae, pupae and imagines belong in 98.8 per cent to *Eubacteriales*. Actinomycetes and fungi were isolated in a few cases.

The bacteria observed are represented almost exclusively (98.4 per cent) by Gram-positive microorganisms genera with a marked dominance of cocci (79.6 per cent). Cocci of group D, and among them *Streptococcus faecalis*, were the most frequently and numerously occurring bacteria. This microorganism was found in all the forms of the insect's ontogeny and frequently it was the only microorganism encountered. Only in a small number of larvae, especially in the first week of life, no *Streptococcus faecalis* was found

It seems that the spread of streptococci through the wax-moth population takes place by a contamination of the medium with excreta. Before administration, food was not infected, but samples taken from the culture always showed the presence of *Streptococcus faecalis*. These observations seem to indicate that the wax-moth organism is an environment conducive to the growth of this microorganism.

The results obtained are in good agreement with those of Stephens (60), Bucher and Williams (6) and Bucher (5). Regardless of the artificial medium (6), or beeswax sterilized with steam (60), on which the cultures of *Galleria mellonella* grew, *Streptococcus faecalis* was encountered in all ontogenetical stages. On the other hand, no streptococci multiplication was observed in the medium, and frequently before the experiments the medium had not contained any streptococci.

Besides streptococci, we found microorganisms representing other groups during the whole feeding period of the larvae as well as in the pupae and imagines.

In a small percentage of larvae the only microorganisms found were those from the genera *Bacillus, Staphylococcus* and *Micrococcus*. In single individuals they are less numerous than streptococci.

Bucher and Williams (6) include spore-forming bacteria of the genus *Bacillus* among the organisms which accompany insects in laboratory cultures in several generations (persistent organisms). In the same group they also include such microorganisms as *Serratia mercescens*, genera *Staphylococcus* and *Micrococcus*, yeasts and fungi. To the group of rare species they include *Proteus* and *Pseudomonas*, as well as *Bacillus licheniformis*, *Gaffkya* and *Lactobacillus*.

The high percentage of larvae containing uniform populations of *Streptococcus faecalis* and its marked dominance in mixed populations seems to be caused by two factors. As the observations of Bucher and Williams (6) have shown, *Streptococcus faecalis* inhibits the growth of other bacteria. Rinsing eggs with sterile water or a mild disinfectant, before placing them in a fresh medium, clearly reduced the number of larvae in which spore-forming bacteria, less sensitive to the action of an antiseptic, developed; and, at the same time, it diminished the number of individuals containing streptococci.

Streptococcus faecalis shows its lytic action especially against Gram-positive bacteria. In the filtrates of cultures, the presence of lysozyme has been mentioned (9). The resistance of insects to bacterial infections depends to a large extent on the level of this enzyme.

The other factor inhibiting the growth of the bacteria is the *pH* of the alimentary tract. In *Lepidoptera*, the *pH* of the medial intestine is strongly alkaline (8.9—10.4) (20, 23, 62). With such a high *pH* the growth of spore-forming bacilli is very slow, or it does not occur at all. Elimination of antagonistic flora as well as the appropriate medium reaction seem to account for the constant presence of *Streptococcus faecalis* in the alimentary tract of the insect in an unsatisfactory way. It must find easily available sources of energy and carbon that allow intensive multiplication in the medium.

There is also a great diversity of isolated strains that seem to support the weak growth of aerobic bacilli in the wax-moth organism. Most probably, the feeding larvae get constantly infected with still new species occurring incidentally in the wax. The number of these bacteria in wax before feeding, and in medium samples, does not undergo any significant changes. It seems to indicate the fact that they do not find an appropriate environment for development, either in the organisms or in the medium. Thus aerobic bacilli are hardly ever passed on to subsequent wax-moth generations. They can, however, survive the whole period of metamorphosis and then appear in the imago. Most probably they survive this period in the form of spores.

The frequency of occurrence of fungi seems to depend, to a large extent, on medium humidity. This phenomenon is especially marked in artifical media which are more hygroscopic than wax (6).

Results of wax analysis for the contents of fungi, carried out before and after administration to the culture, proved that the fungi are destroyed in the organism of larvae. It is also confirmed by the fact that no fungi have been isolated either from pupae or from imagines.

Similarly, Gram-negative bacteria and actinomycetes do not find appropriate conditions for multiplication either in the organism or in the medium. However, their occurrence in the larvae, pupae and imagines indicates that they may survive the metamorphosis. Gram-negative bacteria constitute a group of microorganisms which dominate in other insects. They include *Dendrolimus sibiricus* Tschtv. (47), *Aedes aegypti* (68) and termites (27).

Streptococcus faecalis has been isolated from quite a number of other insects. Eaves and Mundt (17) isolated that microorganism from twenty-six genera of insects occurring on cultivated fields. Steinhaus (55) found its presence in the medial intestines of *Blatella germanica, Thyridopteryx ephemeraeformis, Tibbicen linnei* and *Hyphantria cunea*. They were also isolated from *Apis melifera* (3) and *Musca domestica* (63).

In some cases, *Streptococcus faecalis* may be pathogenic for wax-moth larvae (8, 57, 58).

The numerousness of bacterial populations in *Galleria mellonella* of various age shows great variation. Similar variation has also been observed by Bucher (5) and Bucher and Williams (6). The highest degree of bacteria numerousness in one individual was observed in the third week of life of the larvae. It is the period of the most intensive feeding. Before the transformation into a pupa, the number of bacteria in the alimentary tract of the larva diminishes. However, complete sterilization of the alimentary tract does not take place. Such a sterilization does take place in *Diptera* (37).

Changes in the number of bacteria occurring in the pupal stage indicate that in the period of the most intensive transformation (till the seventh day) the growth of bacteria is inhibited and their number decreases very markedly. In this period the highest percentage of sterile individuals was observed. On the seventh day the insect is almost completely formed. At the same time there occurs an increase in the number of bacteria.

Among adult insects examined on the first day of life, the highest percentage of sterile individuals was found, and the bacterial populations in the imagines of the alimentary tract were the least numerous. Such a result could be expected on the basis of Bucher's observations (5) of the imago which, after getting out of the cocoon or even when leaving it, excretes biestings and most of the bacteria with them. Besides, imagines of *Galleria mellonella* do not take in any food, and their alimentary tract is greatly reduced.

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STRESZCZENIE

Drobnoustrojami charakterystycznymi dla mikroflory *Galleria mellonella* są bakterie właściwe reprezentowane niemal wyłącznie przez gatunki gramdodatnie, gramujemne oraz promieniowce i grzyby występują sporadycznie.

Mikroorganizmami towarzyszącymi w całym okresie rozwoju, dominującymi ilościowo i przekazywanymi z pokolenia na pokolenie są paciorkowce grupy D, głównie *Streptococcus faecalis*. Obecność innych bakterii zależy w dużej mierze od ich występowania w pokarmie.

Charakterystyczna jest także ogromna zmienność liczebności drobnoustrojów, nawet wtedy gdy pochodzą one z tej samej kultury i są w tym samym wieku. Najliczniejsze średnio populacje bakteryjne występują w okresie najintensywniejszego żerowania larw. Liczebność bakterii w poczwarkach, początkowo taka sama jak w gąsienicach bezpośrednio przed oprzędem, spada w okresie metamorfozy. Najuboższa pod względem liczebności a także reprezentowanych gatunków mikroorganizmów jest mikroflora postaci dorosłych.

Drobnoustroje są równomiernie rozmieszczone w przewodzie pokarmowym. Żaden z gatunków bakteryjnych nie wykazuje specyficznej lokalizacji w poszczególnych odcinkach jelita.

РЕЗЮМЕ

Характерными для микрофлоры *Galleria mellonella* микроорганизмами являются бактерии, представленные почти исключительно грамположительными видами. Грамотрицательные бактерии, а также актиномицеты и грибы выступают очень редко.

Микроорганизмами, сопутствующими *Galleria mellonella* на протяжении всего периода развития, количественно преобладающими и переходящими из поколения в

поколение, являются стрептококки из группы Д, в основном *Streptococcus faecalis*. Присутствие других бактерий зависит от их выступления в корме.

Большое количественное изменение микроорганизмов у особей насекомых характерно даже тогда, когда они происходят из той же культуры и имеют одинаковый возраст. Больше всего бактериальных популяций выступает в период интенсивной кормежки личинок. Количество бактерий в куколках, в начале такое же как в гусеницах непосредственно перед коконом, уменьшается в период метаморфоза. Более бедная флора в отношении количества микроорганизмов и их видов выступает у взрослых особей.

Микроорганизмы размещаются в пищеварительном тракте равномерно и не имеют определенного положения в отдельных частях кишечника.