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**Microflora of the Larvae of *Achroea grisella* F a b.**

Mikroflora larw *Achroea grisella* F a b.

Микрофлора личинок *Achroea grisella* F a b.

The larvae of the wax-moth *Achroea grisella* F a b. require a more varied food for growth than do those of the big wax-moth *Galleria mellonella* L. This is demonstrated among others by the fact that the larvae of *Achroea* grow and feed over the whole life cycle on bee cerumen with addition of many partly indeterminate substances contained in wax impurities. The authors found it very difficult to obtain and cultivate a culture of this organism. Bee honeycombs are generally contaminated by *Galleria mellonella*, which controls environment relatively easily. In the available literature there is lack of data on the microflora of *Achroea grisella*; therefore an attempt has been made to study the intestinal microflora of the larvae of this organism.

MATERIALS

1. Larvae of *Achroea grisella* F a b. were obtained from the Department of Apiculture, Agricultural Academy, Lublin.
2. Media: nutrient agar, blood agar, Sauton medium.
3. Strains: acid-fast saprophytic bacilli (*Mycobacterium smegmatis*, *Mycobacterium phlei*, *Mycobacterium* 279 and *Mycobacterium* 607) and *Sarcina lutea* from Museum of Strains of the Department of Plant Physiology, Maria Curie-Skłodowska University, Lublin.

## METHODS

1. Inoculation of strains: *Achroea larvae*, 0.5—0.8 cm long, were kept in 70% ethyl alcohol for 2 min. and then ground with 1 ml of physiological saline in a bacterial mortar. Each time three larvae were taken for isolation. A droplet of the suspension obtained was transferred on to a Petri dish with nutrient and blood agar. The suspension was distributed on the surface of the agar, then incubated at 37°C for 24—48 hrs. Then the material taken from single colonies was transferred on slants with nutrient agar.

2. Isolation: The strains were stained according to Gram's method. Three morphological groups were isolated: cocci, bacilli and bacteria.

3. Classification of cocci: In preliminary classification of cocci the following differentiation tests were used: O-F reaction (Hough-Leifson medium), test for catalase and peroxydase (Bogen reaction), production of coagulase, hemolysis, growth on Pike's medium with potassium tellurite (3). Additional biochemical, morphological and physiological studies of bacteria belonging to cocci were carried out.

4. Classification of bacilli: Classification and determination of the species of these bacteria were based on the scheme proposed by Smith and Gordon (quot. 4) with the use of the following differentiation tests: the test for lecithinase, the reaction on medium with ammonium and glucose, the Voges-Proskauer reaction and the test for catalase and peroxydase. The authors also determined a number of biochemical, morphological and physiological features which are taken into consideration in determining the species within the genus *Bacillus* according to Bergey's (1): the test for ammonium, the decomposition of gelatin, the test with litmus milk, the decomposition of sugars, and the hydrolysis of starch.

5. From *Achroea larvae* were isolated a Gram-negative strain and four fungi which are not dealt with in this paper.

6. Antibiotic properties of the strains isolated: The antibiotic properties of the strains isolated from *Achroea larvae* were studied in relation to *Sarcina lutea* and acid-fast saprophytic bacilli. In these studies Gratia's method was used (2).

## EXPERIMENTS AND RESULTS

68 strains were isolated from the alimentary canal of the larvae of *Achroea grisella* F a b. Among the three morphological groups distinguished the largest one numbering 35 strains — was that of the cocci, which made 51.47% of the total number of the strains isolated. 28 strains belonged to bacilli (41.18%), beside there were one strain of bacterium (1.42%) and 4 strains of fungi (5.88%). The biochemical and physiological features of the cocci and the bacilli are presented in Tables 1 and 2.

Cocci: streptococci and staphylococci were found in the group of cocci. Strains Nos. 31, 64 and 74 showed a negative test for catalase and grew on Pike medium which indicated that they were bacteria of the genus *Streptococcus*. However, they were excluded from the group of faecal streptococci because they did not grow on the medium with potassium tellurite (Table 3).

Table 1. Biochemical and physiological properties of cocci

Strain No.	Raffinose	Glucose	Mannitol	Inuline	Peroxydase	MR	Starch hydrolysis	H <sub>2</sub> S	Nitrates reduction	Glycerol	Litmus milk
1	+	-	-	-	+	-	-	-	-	+	CA
3	-	-	+	-	+	+	-	+	+	+/-	R, CA
4	-	+	-	-	+	+	-	-	-	+	R, CA
7	-	-	-	+/-	+	+	-	-	-	-	P, CA
10	-	-	-	-	+	-	-	-	+	+/-	CA
12	-	-	+/-	+	+	+	-	-	-	+/-	R, CA
21	+/-	-	+	+	+	+	-	+	-	-	R, CA
24	-	-	-	+	+	+	-	+	-	+/-	CA
26	-	-	-	+	+	+	-	+	+	+/-	R, CA
27	-	+	-	+	+	+	-	-	+	+/-	R, CA
30	-	-	-	-	+	+	-	+/-	+	+/-	R, CA
31	-	+	-	-	+	-	-	-	-	+/-	R, CA
32	-	-	-	-	+	+	-	-	-	+/-	CA
35	+/-	-	+/-	-	+	+	-	-	-	-	CA
40	+	-	-	-	+	+	-	+	-	-	CA
42	-	+/-	-	-	+	+	-	-	-	-	R, CA
44	-	-	-	-	+	+	-	-	-	-	R, CA
47	-	+/-	+/-	+	+	+	-	-	+	-	R, CA
50	-	+/-	+	+	+	+	-	-	+	-	CA
51	-	-	+/-	+	+	+	-	+	+	-	R, CA
53	-	+	-	-	+	+	-	-	+	-	CA
55	-	+	-	-	+	+	-	-	-	-	R, CA
56	+/-	-	-	-	+	-	-	-	-	-	R, CA
58'	-	+	-	-	+	+	+	+	+	+/-	CA
60	-	-	-	-	+	+	-	-	+	+/-	R, CA
61	-	-	+	+	+	+	-	-	-	+	CA
63	-	-	+	+	+	+	-	-	-	-	R, CA
64	+	+	-	-	-	+	-	-	-	+	R, CA
67	-	-	-	-	+	+	-	+	+	+/-	CA
69	-	-	+	+	+	-	-	-	-	+	CA
70	-	-	+	+	+	+	-	+	-	+/-	CA
72	-	-	-	+/-	+	+	-	+	-	+/-	CA
73	-	+	+	-	-	+	-	+	-	+	CA
75	-	+/-	-	-	+	+	-	+	+	+	R, CA
76	+	-	+	-	+	+	-	+	-	+/-	CA

All cocci decomposed saccharose, maltose, fructose, lactose; liquefied gelatin and produced ammonium. Negative reaction to indol.

Explanations: + positive reaction, - negative reaction, +/- variable reaction, CA acid coagulation, R reduction, P peptonization.

Staphylococci were distinguished from streptococci using Bergey's (1) differentiating tests. Gram-positive cocci producing and fermenting glucose under anaerobic conditions can be included in the genus *Staphylococcus*. Strains Nos. 4, 27, 53, 55 and 58' were classified as streptococci. These strains showed a negative test for mannitol and for coagulase. They were included in *Staphylococcus epidermis* (Tables 4).

Some isolated strains of *S. epidermis* differed from the standard *S. epidermis* (1). Thus, strain No 27 decomposed inuline, whereas its

Table 2. Biochemical and physiological properties of bacilli

Strain No.	Broth with glucose in anaerobic conditions	M.R.	Starch hydrolysis	H <sub>2</sub> S	Nitrates reduction	Mannitol	Arabinose	Xylose	Saccharose	Lactose	Fructose	Glycerol	Litmus milk
5	growth	+	-	+	-	+	-	+	+	+	+	+/-	R
6	growth	+	-	+/-	+	+	-	+	+	+	+	+	R, CA
8	no growth	+	-	+	-	+	-	-	+	-	+	-	R, P
9	growth	+	+	-	-	+	+/-	-	+	+	+	+	R
13	growth	+	+/-	+	+	+	+	+/-	+	+	+	+	CA, P
15	growth	+	-	-	+	-	-	+	+	+	+	+	R
17	growth	+	+/-	+	-	-	-	+/-	+	+	+	+	R, CA
22	growth	+	+	+/-	+	+	+	-	+	+	+	+	R
23	no growth	+	-	+/-	+	-	-	+/-	+	+	+	+	R, P
29	growth	+	-	+	-	+/-	-	+/-	+	+	+	+	R, CA
33	growth	+	-	+	+/-	+/-	-	+	+	+	+	+	R, CA
34	growth	+	-	+	+	+	-	+	+	+	+	+	R, CA
37	growth	+	-	+	+	+	+/-	+	+	+	+	+	R
38	growth	+	+	+	-	+	-	-	+	+	-	-	R, CA
39	growth	+	+	-	-	+	+/-	-	+	+	+	-	CA, P
41	growth	-	-	+	-	+	-	+/-	+	+/-	+	-	R, P
43	growth	+	+	+	-	-	-	+/-	+	+/-	+	-	R, CA
45	growth	+	+	+	-	+/-	-	+/-	+	+	+	+	R, CA, P
46	no growth	+	-	-	-	-	+/-	+	+	+	+	-	R
49	no growth	+	+	+	-	+/-	-	+/-	+	+	+	+/-	CA
52	growth	+	-	-	+	-	-	+/-	+	+/-	+	+	R, CA
54	growth	+	+	+/-	+	-	-	+/-	+	+	+	+	R, CA
58	growth	+	+	+	+	-	-	+	+	+	+	+	R, CA
59	growth	+	+	+	+	-	-	+	+	+	+	+	R, P
62	no growth	+	-	+	-	-	-	+	+	+	+	+	R, P
68	growth	+	-	+	+	-	-	-	-	+	+	-	R, P
71	growth	+	+	-	-	-	-	+/-	+	+/-	+	+	R, P
74	growth	+	+	-	-	-	-	+	+	+/-	+	-	R, CA

All bacilli decomposed glucose and maltose gave, positive reaction to peroxidase and V.P., showed gelatin liquefaction and produced ammonium. Negative reaction to indol, was also found in all strains.

Explanations: + positive reaction, - negative reaction, +/- variable reaction, CA acid coagulation, R reduction, P peptonization.

Table 3. Streptococci

Test Strain No.	Catalase	Growth on the medium		Hemolysis
		of Pike	with potassium tellurite	
31	-	+	-	-
64	-	+	-	α
73	-	+	-	α

Table 4. Staphylococci

Test Strain No.	Catalase	Mannitol	Coagulase	Glucose fermentation on Hugh-Leifson medium anaerobically
4	+	-	-	+
27	+	-	-	+
53	+	-	-	+
55	+	-	-	+
58	+	-	-	+

+ positive reaction, — negative reaction.

typical form does not: all strains classified in this paper as *S. epidermis* caused, beside fermentation, also coagulation of milk whereas its typical form caused only milk fermentation. Strains 4, 27 and 55 did not reduce nitrates.

Bacilli: Determination of bacilli was carried out according to Smith and Gordon's methods (quot. 4). It was shown in preliminary studies that isolated bacilli grew abundantly on nutrient agar, produced catalase and were aerobic. They were gram-positive bacteria forming cylindrical spores with a thin or thick membrane. Among bacteria of this group strains of swollen sporangia were found. These bacteria were included, after Smith and Gordon, in group I or II. No bacteria of group III of spherical spores were found. Within the particular species in which the analyzed strains were included, some of the strains deviated from standard ones. These divergencies, however, do not concern the basic features. On the basis of additional differentiation tests it was found that strains Nos. 28, 33, 43, 74, 45 and 49 and 54, 58 and 59 were identical (Table 5).

#### Antibiotic properties

Out of 63 isolated strains 14 appeared antibioticly active against *Sarcina lutea* (9 strains classified into cocci and 5 into bacilli). Antibiotic action of all cocci and bacilli in relation to saprophytic acid-fast bacteria was also tested. Five strains showed a slight activity in relation to *M. smegmatis* (three cocci and two bacilli). Maximum inhibition zone of the growth of *M. smegmatis* was 0.8 mm for strain No. 31. However, the ability of inhibition of all the active strains towards *M. smegmatis* was not stable and disappeared during further subcultures.

Table 5

Strain No.	Standard strain	Differences in properties in standard strains according to Bergey	
6	<i>B. cereus</i>	Does not hydrolyze starch. Decomposes xylose and mannitol	
8	<i>B. subtilis</i>	Does not hydrolyze starch. Lack of nitrates reduction. Does not decompose xylose, arabinose and glycerol	
38	<i>B. licheniformis</i>	No reduction of nitrates. Positive M.R. reaction. Produces H <sub>2</sub> S. Does not decompose xylose and arabinose	
17	<i>B. stearothermophilus</i>	Gelatin liquefaction. V.P. (+)	
29		Gelatin liquefaction. V.P. (+). Does not hydrolyze starch	
33		Gelatin liquefaction. V.P. (+). Does not hydrolyze starch	
52		Gelatin liquefaction. V.P. (+). Does not hydrolyze starch. Lack of nitrates reduction	
5	<i>B. coagulans</i>	Lack of starch hydrolysis. Does not decompose arabinose	
13		Does not decompose xylose	
9		Reduces nitrates	
15		Lack of starch hydrolysis. Reduces nitrates. Does not decompose arabinose and mannitol	
22		Reduces nitrates. Does not decompose xylose	
23		Does not hydrolyze starch. Reduces nitrates. Does not decompose arabinose, glycerol and mannitol	
34		Does not hydrolyze starch. Reduces nitrates. Does not decompose arabinose	
37		Does not hydrolyze starch. Reduces nitrates	
39		Does not decompose xylose and glycerol	
41		Does not hydrolyze starch. Does not decompose arabinose and glycerol	
43		Does not decompose arabinose, glycerol and mannitol	
45		Does not decompose arabinose	
46		Does not hydrolyze starch. Decomposes glycerol and mannitol	
49		Does not decompose arabinose	
54		}	Reduces nitrates. Does not decompose arabinose and mannitol
58			
59			
62			
68		Does not hydrolyze starch. Does not decompose arabinose, xylose, saccharose, mannitol, and glycerol. Reduces nitrates	
71		Does not decompose arabinose and mannitol	
74	Does not decompose arabinose, glycerol and mannitol		

## CONCLUSIONS

1. In the intestinal flora of the larvae of *Achroea grisella* F a b. dominated cocci and bacilli, mostly gram-positive. Fungi were also repeatedly isolated.

2. Streptococci and staphylococci were found among the cocci. Five strains were included in the staphylococci and were classified as *Staphylococcus epidermis*. All streptococci found were excluded from the group of faecal streptococci.

3. The following bacilli species were determined: *Bacillus cereus* — 1, *Bacillus subtilis* — 1, *Bacillus licheniformis* — 1, *Bacillus coagulans* — 17 and *Bacillus stearothermophilus* — 3.

4. Relative anaerobes were found.

5. Some of the strains isolated from the intestinal flora of the larvae of *Achroea grisella* showed antibiotic properties in relation to *Sarcina lutea*, but they were inactive towards the saprophytic acid-fast bacilli.

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#### STRESZCZENIE

Z przewodu pokarmowego larw *Achroea grisella* F a b. wyizolowano 68 szczepów. W obrębie wyróżnionych trzech grup morfologicznych najliczniejszą grupę (35 szczepów) stanowiły ziarniaki i laseczki (28 szczepów). Izolowano również stałe grzyby.

W obrębie ziarniaków wyróżniono paciorkowce i gronkowce. Do gronkowców zaliczono pięć szczepów, klasyfikując je jako *Staphylococcus epidermis*. Paciorkowce wykluczono z grupy paciorkowców kałowych. Oznaczono następujące gatunki laseczek: *Bacillus cereus* — 1 szczep, *Bacillus subtilis* — 1 szczep, *Bacillus licheniformis* — 1 szczep, *Bacillus coagulans* — 17 szczepów i *Bacillus stearothermophilus* — 3 szczepy. Wśród wyizolowanych bakterii oprócz tlenowców stwierdzono także względnie beztlenowce.

Niektóre szczepy wyizolowane z flory jelitowej larw *Achroea grisella* wykazują własności antybiotyczne w stosunku do *Sarcina lutea*, natomiast nie wykazują ich w stosunku do saprofitycznych prątków kwasoopornych.

#### РЕЗЮМЕ

Из пищевода личинок *Achroea grisella* F a b. были изолированы 68 штаммов. В пределах трех выделенных морфологических групп наиболее многочисленную группу составляли кокки (35 штаммов) и бациллы (28 штаммов). Были также изолированы постоянные грибы.

В пределе кокков выделены стрептококки и стафилококки. К стафилококкам отнесены 5 штаммов, классифицируя их как *Staphylococcus epidermis*. Стрептококки исключались из группы каловых стрептококков. Были определены следующие виды бацилл: *Bacillus cereus* — штамм, *Bacillus subtilis* — 1 штамм, *Bacillus licheniformis* — 17 штаммов и *Bacillus stearothermophilus* — 3 штамма. Кроме аэробов в изолированных бактериях были также найдены факультативные анаэробы.

Некоторые из штаммов, изолированных из кишечной флоры личинок *Achroea grisella* обнаруживают антибиотические свойства по отношению к *Sarcina lutea*, в то же время не обнаруживая их по отношению к кислотоустойчивым микро-бактериям.