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Original article

Microflora in the honeybee digestive tract: counts, characteristics and sensitivity to veterinary drugs

V Rada[1]*, M Máchová[2], J Huk[3], M Marounek[4], D Dušková[4]

[1] *Czech University of Agriculture in Prague, Prague 6, Suchdol 165 21;*
[2] *Bee Research Institute, Dol 252 66, Libčice nad Vltavou;*
[3] *Research Institute of Antibiotics and Biotransformations, Roztoky u Prahy 252 63;*
[4] *Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Uhříněves 104 00, Czech Republic*

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Summary — Experiments were carried out to enumerate and characterize the microorganisms in the midgut and rectum of the honeybee. Counts of aerobic microorganisms were distinctly lower than counts of anaerobes (10^4–10^5 viable cells per gram of intestinal contents versus 10^8–10^9 per gram). Total numbers of anaerobic microorganisms were almost identical with counts of anaerobic Gram-positive acidoresistant rods. These bacteria represent the principal groups of microorganisms in the bee digestive tract. Anaerobic and aerobic microorganisms, lactobacilli, coliforms, staphylococci, *Bacillus* sp, and yeasts were found in all bees. Only one out of 31 isolates (*Bifidobacterium asteroides*) was identified at the species level. Fluvalinate, fumagillin and nystatin significantly increased mortality of bees. Treated bees kept in cages contained more yeasts than control bees in the beehive. The veterinary drugs tested significantly increased counts of yeasts in comparison with the control.

***Apis mellifera* / associated microflora / *Bifidobacterium* / veterinary drugs**

INTRODUCTION

The symbiotic microflora of the digestive tract of mature honeybees (*Apis mellifera* L) consists of Gram-negative, Gram-positive and Gram-variable bacteria, moulds, and under some conditions also yeasts (Gilliam, 1987). The normal microflora is obtained from consumption of pollen, other food, and through contacts with older bees in the colony (Poltěv, 1969; Glinski and Jarosz, 1995). Early work in this field states

* Correspondence and reprints
Tel: (420) 2 24 38 27 61; fax: (420) 2 20 92 03 12; e-mail: rada@af.czu.cz

that typical honeybee gut microbes are *Lactobacillus rigidus apis, L constellatus* and *Bacillus influzoides apis* (White, 1921). Also bifidobacteria were often isolated from the honeybee digestive tract (Scardovi and Trovatelli, 1969; Scardovi 1986). Their quantitative significance, however, remains unclear. According to more recent studies, unidentified Gram-variable pleomorphic bacteria and bacteria belonging to the genus *Bacillus* and the family Enterobacteriaceae are the most numerous microbes of the honeybee gut (Gilliam, 1987; Gilliam et al, 1988; Gilliam and Taber, 1991). The intestinal flora of the honeybee is susceptible to various chemotherapeutics and its species composition varies seasonally (Smolska-Szymczewska, 1989). Our knowledge of the microbial ecosystem in the honeybee digestive tract is far from complete. Future research should determine the composition of the microflora of honeybees more exactly, and specifically with host–microbe and microbe–microbe interactions. The aim of our study was to estimate counts of several specific groups of microorganisms in the midgut and rectum of summer and winter honeybees and to isolate, characterize, and identify some typical gut bacteria. We also investigated effects of several veterinary drugs on microbial counts and mortality of bees.

MATERIAL AND METHODS

Counting of microorganisms

Workers of *A mellifera* from the Bee Research Institute (Libčice, Czech Republic) were used for experiments. For the experiment, 25 winter and 25 summer bees were taken from the honey combs from the same healthy colony. The workers were decapitated and their midgut and rectum were weighed and aseptically transferred into tubes containing sterile Wilkins-Chalgren broth (Oxoid). The tubes were flushed with O_2-free CO_2 and closed by rubber stoppers. The same broth was used for serial dilutions of all samples. The 0.1-mL aliquots were plated on

Table I. Media and cultivation conditions used for enumeration of microorganisms.

Microbial groups	*Medium*	*Length of incubations (h)*	*Cultivation method*	*Temperature (°C)*
Total anaerobes	Wilkins-Chalgren agar	72	anaerobic	37
Gram-positive anaerobic acidoresistant roads	Rogosa agar with cysteine (0.05% w/v)	72	anaerobic	37
Lactobacilli	Rogosa agar	72	microaerophilic	37
Total aerobes	yeast extract agar with glucose (1% w/v)	72	aerobic	37
Coliforms	endo agar	24	aerobic	37
Staphylococci	staphylococcus medium	24	aerobic	37
Enterococci	Slanetz-Bartley medium	48	aerobic	37
Bacillus spp	nutrient agar	24	aerobic	37
Yeasts	Czapek-Dox agar	72	aerobic	25
Moulds	Czapek-Dox agar	72	aerobic	25
Pseudomonas spp	Pseudomonas agar	48	aerobic	37

All media were purchased from Oxoid.

various agar media and incubated until a good microbial growth was noted (table I). Anaerostats (Anaerobic Plus System, Oxoid) and CO_2/H_2 (10/90%) atmosphere were used for anaerobic cultivations. The same equipment filled with a $CO_2/O_2/N_2$ (10/6/84%) atmosphere was used for microaerophilic cultivation. Colonies were counted and microbial counts expressed as $\log_{10}$ cfu per gram of the gut contents.

Differences among winter and summer bees and among midgut and rectum counts were analyzed using *t*-test procedure.

Characterization of bacterial isolates

Bacteria grown on the Rogosa agar modified by addition of cysteine hydrochloride (0.05% w/v) were the most numerous groups observed and therefore were presumptive characterized. One-hundred colonies arising on the plates with modified Rogosa agar inoculated with midgut or rectum contents were picked off and examined microscopically after being Gram stained.

Seventeen isolates from the midgut and 14 isolates from the rectum, obtained from plates with Wilkins-Chalgren agar for total anaerobes, were examined. The following characteristics were compared: Gram staining, catalase (Levett, 1991), and oxidase (commercial kit, Lachema Brno, Czech Republic). In order to determine the end-products of glucose fermentation, the bacteria were grown in M10 broth (Caldwell and Bryant, 1966). Lactate was determined by gas chromatography on a column of DB-Wax Megabore (J & W, USA), acetate and propionate by means of a column with 4% Carbowax 20M on Carbopack B-DA (Supelco, USA). The fructose-6-phosphate phosphoketolase (EC 4 1 2 22) was assayed in all Gram-positive rods producing acetate and lactate (Scardovi, 1986). Phosphoketolase-positive strains were assigned to the genus *Bifidobacterium* and identified according to their fermentation characteristics (Scardovi, 1986; Biavati et al, 1992; Mitsuoka, 1992) using the API 50 CHL tests (BioMérieux, France). Inoculated plates were incubated anaerobically in CO_2/H_2 atmosphere at 37 °C for 48 h. Other strains were characterized by means of commercial tests produced by Lachema Brno (Anaerotest, Enterotest 1 and 2, Nefermtest, Streptotest). Gram-positive, catalase, oxidase and nitrate reductase-negative, nonmotile rods producing mainly lactic acid were assigned to the genus *Lactobacillus*. The susceptibility of isolates toward metronidazole was tested with antibiotic discs (5 μg), purchased from Oxoid (UK).

Effect of veterinary drugs

Thirty-two laboratory cages (130 × 130 × 60 mm; four cages per group) with 150–200 bees per cage were utilized. Three cages were used for the determination of LT_{50}, one for microbiological analyses. Bees were fed pollen for 10 days and sucrose syrup with or without a drug. Some drugs were evaporated, fumigated or offered in the form of impregnated wood (table II). The applied concentrations were with respect to the number of bees in a cage (100 times lower than 20000 bees, which is considered as mean strong colony). Both temperature (26 °C) and humidity (60%) were controlled. Enumeration of rectum microorganisms was performed weekly, using the methods described above for bees aged 3, 4, 5 and 6 weeks. Control bees were also sampled at the age of 53 days.

For all treated bees a comparison was made at the end of the experiment of the state of construction and the area of constructed cells on the cage foundation with the foundation construction for control bees. 100% indicates fully finished construction of the comb.

Differences among data were analysed using an *f*-test procedure.

RESULTS AND DISCUSSION

Table III offers counts of main groups of microorganisms in the honeybee digestive tract. Anaerobic and aerobic microorganisms, lactobacilli, coliforms, staphylococci, *Bacillus* sp, and yeasts were found in all bees. On the other hand, enterococci, pseudomonads and moulds were found only in 6, 8 and 28% of the bees examined, respectively. Their counts varied from 2 to 6 log cfu per gram.

The midgut of winter bees contained more anaerobic microorganisms and yeasts than the midgut of summer bees ($P < 0.01$). On the contrary, the midgut and rectum of

Table II. Scheme of experiments with veterinary drugs.

	Drug						
Trade name of the medicament	*Gabon PA 92*	*M-1*	*Tylan 200*	*Nystatin*	*Fumagilin*	*Taktivar FUM*	*Formidol*
Active substance	*Acrinathrin*	*Fluvalinate*	*Tylosin*	*Nystatiin*	*Fumagilin*	*Amitraz*	*Formic acid*
Supplier	Bee Research Institute, Dol, Czech Republic	Bee Research Institute, Dol, Czech Republic	Bioveta. Ivanovice, Czech Republic	Sigma	Sanofi, Bratislava, Slovak Republic	Bee Research Institute, Dol, Czech Republic	Bee Research Institute, Dol, Czech Republic
Method of administration	contact with impregnated wood	contact with impregnated foundation	sugar syrup	sugar syrup	sugar syrup	fumigation	evaporation
Concentration of the active substance per bee colony	2.4–3.4 mg	0.25%	400 mg	45 mg	140 mg	25 mg	34 mL
Length of exposure (days)	30	30	21	21	7	1 h	4
Initial age of bees (days)	11.5	11.5	13.5	13.5	13.5	17.5	17.5

Table III. Microbial counts (log cfu/g) in the midgut and rectum of winter and summer honeybees.

	Midgut		*Rectum*	
Microbiological groups	*Winter bees*	*Summer bees*	*Winter bees*	*Summer bees*
Total anaerobes	$8.62 \pm 0.42^{A*}$	$8.17 \pm 0.63^{B*}$	9.62 ± 0.43^{A}	9.49 ± 1.05^{B}
Gram-positive anaerobic acidoresistant rods	8.01 ± 0.55^{C}	7.71 ± 0.70^{D}	9.60 ± 0.51^{C}	9.40 ± 1.37^{D}
Lactobacilli	7.24 ± 0.67^{D}	7.01 ± 0.63^{E}	8.66 ± 0.73^{D}	8.37 ± 1.99^{E}
Total aerobes	5.63 ± 1.50	5.42 ± 1.04^{F}	5.00 ± 1.36	4.42 ± 1.40^{F}
Coliforms	2.50 ± 2.63	3.57 ± 1.14	3.77 ± 1.40	3.95 ± 1.70
Staphylococci	3.22 ± 0.54	3.81 ± 1.03	3.48 ± 0.77	3.75 ± 1.28
Bacillus sp	$2.44 \pm 0.52^{G*}$	$3.72 \pm 0.69*$	3.59 ± 0.87^{G}	3.45 ± 0.60
Yeasts	$5.48 \pm 1.41^{H*}$	$3.92 \pm 1.03*$	3.80 ± 1.02^{H}	3.41 ± 0.90

* Differences among winter and summer samples ($P < 0.01$).

A–H Capital letters indicate differences among the midgut and rectum data ($P < 0.01$). All counts are means from 25 determinations.

Table IV. Biochemical characteristic of pure bacterial strains isolated from the midgut (Nos 1–17) and rectum (Nos 18–30).

	Midgut																*Rectum*												
	1	*2*	*3*	*4*	*5*	*6,7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*	*29*	*30*
Mannitol	-	+	-	-	-	-	-	-	w[c]	-	-	-	-	-	-	-	+	+	-	+	-	+	-	-	-	-	-	-	+
Glycerol	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sorbitol	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
Inositol	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Adonit	-	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dulcit	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Xylose	+	+	-	+	+	+	+	+	-	-	+	+	+	+	+	-	+	+	-	+	-	+	+	-	+	-	-	+	-
Fructose	+	+	+	-	-	+	+	+	+	+	+	w	+	+	+	+	-	+	-	+	-	-	+	+	+	+	+	+	+
Galactose	-	+	+	+	-	+	+	+	+	-	+	-	w	+	+	+	-	+	-	-	-	-	+	+	-	+	+	+	+
Rhamnose	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
Maltose	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
Lactose	+	-	+	-	-	-	-	-	+	-	-	-	+	+	+	-	+	+	-	-	-	+	+	-	-	-	+	-	+
Sucrose	+	-	-	-	+	+	+	+	+	-	-	+	-	+	+	+	-	+	-	+	-	-	-	-	-	+	+	-	+
Trehalose	+	+	-	-	+	-	-	-	w	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+
Cellobiose	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Melezitose	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+
Raffinose	+	-	+	+	-	+	+	+	-	w	-	+	-	+	+	+	-	+	-	+	-	-	-	+	-	-	+	-	+
Inulin	-	-	-	-	-	+	+	-	-	-	-	w	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arginin dihydrolase	+	-	+	+	+	+	+	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-
Ornithine decarboxylase	-	-	+	-	+	+	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-
Phenylalanin deaminase	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydrogen sulfide	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urease	+	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	+	-	-	-	-	-	-	-	+	+	-	+
Phosphatase	-	+	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-	+	+	+	-	+	+	-
ONPG test	-	-	-	+	-	-	-	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	-	w	-
NaCl-aesculin	+	+	-	+	-	+	+	+	w	+	+	+	-	+	-	+	-	+	-	+	-	-	-	+	-	-	-	+	+
Bile-aesculin	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	-	+	-	-	+	+	+	+	+	+	+
Simmons citrate	-	-	-	+	+	+	+	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	+	-	-
Nitrate reduction	+	-	+	-	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+
Catalase	-	-	-	-	-	+	-	-	-	-	+	+	-	+	+	+	-	-	-	+	-	-	-	-	+	-	+	-	-
Products from glucose[a]	LA	AL	LA	LA	L	AL	AL	AL	AL	L	L	NT	L	ALP	A	NT	L	L	LA	AL	LA	AL	LA	LA	L	NT	LA	LA	L
Morphology[b]	R	R	R	C	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	C	R	R	R	R	R	R	R	R	R

[a] A, acetate; L, lactate; NT, not tested; P, propionate; [b] C, coccus; R, rod; [c] w, weak reaction.
All strains fermented glucose and salicin. No strain fermented arabinose and sodium malonate. All strains hydrolyzed aesculin. The following reactions were negative among all the strains tested: lysine decarboxylase, Voges-Proskauer, oxidase, and lipase (tween 80). All strains were gram positive, nonmotile, nonsporulating and resistant to metronidazole (5 µg).

summer bees harboured more coliforms and staphylococci. Counts of aerobes were distinctly lower than counts of anaerobes. Numbers of aerobes varied more than the numbers of anaerobes. Total numbers of anaerobic microorganisms were practically identical with bacterial counts found on Rogosa agar with cysteine under anaerobic atmosphere. These bacteria can be simply characterized as anaerobic Gram-positive acidoresistant rods. The classification was confirmed by Gram stain and by the ability to grow in the presence of acetic acid (pH 5.4), which is routinely added to the Rogosa medium. Counts of lactobacilli were ca 7–8 log cfu per gram of midgut and rectum contents. Counts of the most numerous groups of microorganisms were significantly ($P < 0.01$) higher in the rectum in comparison to midgut counts.

Only one isolate (*Bifidobacterium asteroides*) was identified at the species level. Other isolates were Gram-positive rods (24 strains), or cocci (two strains) fermenting glucose and salicin and able to hydrolyze aesculin. All strains were resistant to metronidazole. Attempts to identify lactobacilli (strain Nos 11, 14, 18 and 19) at the species level failed. Biochemical characteristics of isolates are shown in table IV.

In previous reports, the principal bacteria of the bee digestive tract were described as Gram-variable pleomorphic rods with uncertain taxonomy (Gilliam, 1987; Gilliam et al, 1988; Gilliam and Taber, 1991). In our experiments, the most numerous microorganisms were Gram-positive bacterial rods of rather regular shape. Several authors mentioned the presence of bifidobacteria among organisms isolated from the bee gut (Scardovi and Trovatelli, 1969; Scardovi, 1986; Biavati et al, 1992). In our opinion, bifidobacteria belong to typical but not dominant bacterial species in the bee gut. The most numerous organisms seem to be Gram-positive rods related to the genus *Lactobacillus*.

Table V summarizes the results of the effect of some veterinary drugs on the bee gut microflora. Again, anaerobic bacteria

Table V. Microbial counts in the rectum of honeybees after exposure to veterinary drugs.

Drug	*Total anaerobes*	*Gram-positive anaerobic acidoresistant rods*	*Lactobacilli*	*Total aerobes*	*Yeast*
Gabon PA 92 (acrinathrin)	10.01 ± 1.22	10.00 ± 1.30	9.50 ± 0.89	5.46 ± 0.34	6.67 ± 0.25*
M-1 (fluvalinate)	10.00 ± 1.00	9.92 ± 1,25	9.13 ± 0.61	3.98 ± 0.63	6.99 ± 0.88*
Tylan 200 (tylosin)	9.50 ± 0.78	9.68 ± 0.99	8.84 ± 0.53	5.68 ± 0.30	6.84 ± 0.53*
Nystatin	9.98 ± 1.00	9.87 ± 0.85	9.04 ± 0.56	5.26 ± 0.59	7.40 ± 1.11*
Fumagillin	10.02 ± 1.21	10.06 ± 0.91	9.36 ± 0.58	4.96 ± 0.72	6.79 ± 0.56*
Taktivar FUM (amitraz)	10.09 ± 0.92	9.91 ± 0.87	9.27 ± 0.71	4.56 ± 0.99	7.87 ± 1.13*
Formidol (formic acid)	9.99 ± 0.94	9.99 ± 1.19	9.36 ± 0.78	NT[a]	NT
Control (cages)	9.97 ± 0.98	9.95 ± 1.00	9.24 ± 0.83	4.88 ± 1.13	6.10 ± 1.20
Control (beehive)	9.96 ± 0.67	10.05 ± 0.82	9.07 ± 0,27	5.24 ± 1.32	4.32 ± 0.99

[a] NT, not tested. Values are means ($\log_{10}$) ± SD per gram for quadriplicate determination.
* Differences among treated and control (beehive) bees ($P < 0.01$).

Table VI. Mortality of bees expressed as LT_{50}[a] (days) and percentage building of cage foundation.

Drug	n[a]	LT_{50} *(days)*[b]	*SD*	*Building of cages foundations* (%)	*SD*
Gabon PA 92 (acrinathrin)	3	51.7	10.6	33.3	5.8
M-1 (fluvalinate)	3	35.7*	3.5	53.3	20.8
Tylan 200 (tylosin)	3	43.7	4.7	20.0	26.5
Nystatin	3	35.0*	2.6	10.0	10.0**
Fumagillin	3	36.0*	4.0	16.7	15.3*
Taktivar FUM (amitraz)	1	44.0	NT[c]	50.0	NT
Formidol (formic acid)	3	43.3	6.6	40.0	10.0
Control	3	43.0	1.7	50.0	10.0

[a] Number of cages per group; [b] LT_{50}, survival time corresponding to 50% mortality of bees; [c] NT, not tested.
* Differences among treated and control groups ($P < 0.05$); ** differences among treated and control groups ($P < 0.01$).

were the most numerous microorganisms (9–10 log cfu/g). Treated bees kept in cages contained more yeasts and yeast-like organisms than control bees in the beehive ($P < 0.01$). Gilliam et al (1977) and Gilliam (1987) found high counts of yeasts in stressed bees kept in cages, fed a diet deficient in some nutrients or fed a diet contaminated with pesticides. Counts of total anaerobes, anaerobic Gram-positive acidoresistant rods and lactobacilli were lower, and those of aerobes were higher in tylosin-fed bees than in bees of other groups. Fluvalinate M-1 decreased counts of aerobic microorganisms and amitraze (Taktivar Fum) caused proliferation of yeasts. But all these differences were not significant. Fumagillin and nystatin had generally little influence on microbial counts (table V). Smolska-Szymcewska (1989) observed lower counts of coliform bacteria in bees fed fumagilin. Our results are in line with findings of Gilliam (1986) and Prabucki and Górski (1987) who noticed an increase in numbers of yeasts caused by fumagillin.

Veterinary drugs had a harmful effect on bees as evidenced by mortality data (table VI). Since the variability in mortality data was high, only the effect of fluvalinate, fumagillin, and nystatin was significant ($P < 0.05$). Subsequently, lower production of cage foundation was observed. Aerobic bacteria *Bacillus cereus*, *Achromobacter* sp, and *Pseudomonas fluorescens* degrade fluvalinate in vitro (Maloney et al, 1988). These bacteria are present in the honeybee digestive tract, but their numbers, however, are very low as follows from our results. In conclusion, veterinary drugs for the honeybees tested in this study more or less changed the composition of the honeybee gut microflora and often increased the mortality of bees. Previous studies on interactions of drugs with the honeybee gut microbes utilized aerobic bacteria, eg, bacilli (Drobníková and Bacílek, 1982). These bacteria, however, were not dominant organisms of the bee digestive tract in our study.

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Résumé — La microflore de l'appareil digestif de l'abeille *(Apis mellifera)* : quantification, caractéristiques et sensibilité aux médicaments vétérinaires. Nous avons fait des expérimentations pour déterminer le nombre et les caractéristiques des microoorganismes appartenant aux différents groupes physiologiques et taxinomiques, qui sont présents dans l'estomac et le rectum de l'abeille *(Apis mellifera)* (tableau I). Dix-sept souches microbiennes isolées de l'estomac et quatorze souches du rectum ont été caractérisées en utilisant les tests suivants : coloration de Gram, tests biochimiques et détermination des produits terminaux de métabolisme du glucose (tableau IV). L'effet de sept médicaments vétérinaires sur la microflore intestinale et sur la mortalité d'abeille a été aussi étudié.

Presque toutes les bactéries anaérobies étaient des bâtonnets Gram-positifs acido-résistants. Ces bactéries forment le groupe majeur des microorganismes de l'appareil digestif de l'abeille (10^9/g). Le nombre de lactobacilles était de 10^7–10^8/g (tableau III). Chez toutes les abeilles on a trouvé des bactéries anaérobies et aérobies, des lactobacilles, des bactéries coliformes, des staphylocoques, *Bacillus* sp, et des levures. Des enterocoques, *Pseudomonas* sp, et des moisissures n'ont été décelés que dans 6, 8 et 28 % des isolats, respectivement. Nous n'avons réussi à identifier l'espèce que dans un seul isolat (*Bifidobacterium asteroides*). Les autres isolats étaient des bâtonnets Gram-positifs (24 souches) ou des coques (deux souches), capables de fermenter le glucose et la salicine et d'hydrolyser l'aesculine. Toutes les souches étaient résistantes au métronidazole. Nous n'avons pas réussi à identifier au niveau de l'espèce les lactobacilles (souches numéro 11, 14, 18 et 19). Les caractéristiques biochimiques d'isolats sont données dans le tableau IV.

Les médicaments vétérinaires ont eu un effet défavorable sur l'abeille, comme le montre les taux de mortalité (tableau VI). Le fluvalinate, la fumagilline et la nystatine appliquées aux abeilles encagées durant 3 semaines ont augmenté significativement la mortalité ($p < 0,05$). Les abeilles encagées contenaient plus de levures que les témoins ; le même effet a été observé après application des médicaments vétérinaires (tableau V).

Des études précédentes sur l'interaction entre les médicaments vétérinaires et les microorganismes intestinaux, les bactéries aérobies, comme par exemple *B cereus*, ont été utilisées en majorité. Les médicaments vétérinaires testés ont eu un effet réduit sur la composition de la microflore intestinale.

***Apis mellifera* / microflore associée / *Bifidobacterium* / médicament véterinaire**

Zusammenfassung — Die Darmflora der Honigbienen (*Apis mellifera* L): Zählungen, Eigenschaften und Empfindlichkeit auf veterinäre Arzneimittel. Anzahl und Eigenschaften einiger physiologischer und taxonomischer Gruppen von Mikroorganismen im Magen und Enddarm der Honigbienen *(Apis mellifera)* wurden untersucht. Durch Gramfärbung, biochemische Teste und Bestimmung der Endprodukte des Glukosestoffwechsels wurden 17 Stämme aus dem Magen und 14 aus dem Enddarm isoliert (Tabelle IV). Außerdem wurde der Einfluß von sieben Veterinärarzneimitteln auf Darmflora und Bienensterblichkeit untersucht (Tabelle II).

Fast alle anaeroben Bakterien waren anaerobe, grampositive und säureresistente Stäbchen. Sie bilden demnach die Hauptgruppe der Mikroorganismen im Verdauungstrakt der Bienen (10^9/g). Die Anzahl der Laktobazillen betrug etwa 10^7–10^8/g (Tabelle III). Bei allen Bienen wurden anaerobe und aerobe Bakterien, Laktobazillen, coliforme Bakterien, Staphylokokken, *Bacillus* sp und Hefepilze gefunden. Enterokokken, Pseudomonaden und Schimmelpilze

wurden nur bei 6 bzw 8 und 28% der Isolate nachgewiesen. Nur bei einem Isolat ist es gelungen, die Art zu bestimmen: *Bifidobacterium asteroides*. Die übrigen Isolate bestanden aus grampositiven Stäbchen (24 Stämme) oder Kokken (zwei Stämme), die Glukose und Salicin fermentierten und Aesculin hydrolysierten. Alle Stämme waren gegen Metronidazol resistent. Versuche, die Laktobazillen auf dem Artniveau zu identifizieren (Stämme Nr 11, 14, 18 und 19), schlugen fehl. Die biochemischen Eigenschaften der Stämme sind in Tabelle IV aufgelistet. Die Behandlungsmittel hatten einen ungünstigen Effekt auf die Bienen, wie die Sterblichkeitsraten in Tabelle VI zeigen. Fluvalinate, Fumagilline und Nystatin, die Bienen in kleinen Käfigen appliziert wurden, erhöhte die Mortalität ($P < 0,05$). Alle Bienen in Käfighaltung hatten im Vergleich zu den Kontrollbienen aus Normalvölkern mehr Hefepilze. Auch die getesteten Arzneimittel erhöhte die Anzahl der Hefepilze im Vergleich zu den Kontrollbienen (Tabelle V). In früheren Studien über eine Wechselwirkung zwischen Arzneimittel und Darmbakterien wurden vorwiegend aerobe Bakterien wie zB *Bacillus cereus* getestet. Die Mittel, die wir getestet haben, hatten nur einen geringen Einfluß auf die Zusammensetzung der Darmflora.

***Apis mellifera* / assoziierte Microflora / *Bifidobacterium* / Veterinärarzneimittel**

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