Acta Veterinaria (Beograd), Vol. 47. No. 2-3, 135-140, 1997.

UDK 619:616.34:638.1

A STUDY OF THE MICROBIAL FLORA OF THE BEE INTESTINE

NADA DUGALIĆ-VRNDIĆ

The Scientific Institute for Veterinary Medicine of Serbia

(Received, 14. March 1997.)

The isolation and identification of the microbial flora of the intestine of 3000 honey bees from 150 bee societies are presented in the paper. A total of 19 bacterial species were identified, the most frequent being Klebsiella pneumoniae (71,30%), Enterobacter aglomerans (43,30%) and Enterobacter cloacae (35,30%) while Citrobacter freundii (28,00%), Klebsiella ozenae (14,00%) and Enterobacter aerogenes (16,60%) were found less frequently. The rarest isolates were Staphylococcus saprofiticus (1,3%), Escherichia coli (1,33%), Salmonella biotype C (1,33%), Proteus sp. (0,66%) and Pseudomonas sp. non oxidizer (0,16%). Among anaerobic species, the only isolate belonged to Clostridium perfringens (5,33%).

Key words: honey bee, bacteria, intestine, enterobacteria, gram-negative bacteria

INTRODUCTION

The normal intestinal microflora in adult honey bees has proved to be rich compared to other insects. Trilenko, and Federova 1963 isolated a total of 7 Bacillus species from the intestine of adult honey bees. Gilliam, and Valentine 1976 prepared homogenates from the intestinal contents and brain heart agar (Difco) and using these nutrient media (nutrient agar and brain heart agar) identified only bacteria belonging to the genus Bacillus.

Gilliam, 1989 identified gramvariable pleiomorphic bacteria of the Bacillus sp. and enterobacteria as the most frequent intestinal bacteria in bees. Gram-positive coccae, Staphylococcae and Streptococcae were found rarely.

Gilliam, 1978 investigated the presence of bacteria in samples of intestinal haemolymph, sperm and ovaries of the queen bee. After preparing homogenates from the specimens and enriching the nutrient media (eugon agar BBL) and

nutrient agar (Difco), she identified some bacteria as belonging to the genus Bacillus.

Smolska-Szymeczewska 1989, used the intestines of young bees, homogenized them and enriched the nutrient agar with 5 percent ovine blood. Bacterial growth was passaged to solid media according to Champan and Levin. Bacteria from the family of Enterobacteria were identified by using the API 20E system. On that occasion Bacillus, Enterobacter, Escherichia, Micrococcus, Staphylococcus and Streptococcus species were identified.

MATERIALS AND METHODS

Samples. A total of 3000 honey bees from 150 colonies from the municipal territory of Kraljevo were examined. The insects originated from healthy bee societies, sampled early in the morning. From each honey society a total of 10 bees flying out of the society were caught end placed in cages similar to those provided for honey bee queen transportation. The cages were transported to the laboratory, separated one from another by a plain paper.

Materials. The whole digestive tract of a vital bee was taken for the examination (the rectum, large intestine, small intestine and stomach with the honey bag).Previously to taking the material, the bees were kept in the freezing chamber of a refigerator for 10 minutes.

Preparation of the materials and media. Digestive tracts of bees from one bee society were put into a sterile tube containing 2 ml of distilled water. Using a sterile glass stick the contents were mixed to make a suspension from the intestinal contents and distilled water. The suspension was used for the enrichment of nutrient media: selenite broth, Kitt-Tarozzi broth, 10% blood agar, endo agar, brilliant green agar and Zeissler blood agar.

Isolation and identification. After incubation all the bacterial growth was checked and characteristic colonies were picked out and identified using the API 20E system for entero and other gramnegative bacteria. API 20E was also used for the identification of anaerobic bacteria, while other bacteria were identified by employing standard bacteriological procedures.

RESULTS AND DISCUSSION

A number of bacterial species was isolated from the digestive tracts of selected bees and were regarded as permanent or temporary occupants of the digestive tract of bees in general.

Table 1. Bacterial strains present in the digestive tract of bees

Serial number	Bacterial strain	Number of isolates	%
1	Klebsiella pneumoniae	107	71,30
2	Enterobacter aglomerans	65	43,30
3	Enterobacter cloace	53	35,30
4	Citrobacter freundii	42	28,30
5	Klebsiella ozenae	21	14,00
6	Enterobacter aerogenes	25	16,60
7	Streptococcus sp.	21	14,00
8	Flavobacterium sp.	26	17,30
9	Aeromonas hydrophila	15	10,11
10	Enterobacter haphniae	7	4,60
11	Clostridium perfringens	8	5,33
12	Acin. calc. var. lwoffi	10	6,66
13	Bacillus sp.	9	6,00
14	Staphylococcus saprofticus	2	1,33
15	Pseudomonas fluorescens	17	11,30
16	Escherichia coli	2	1,33
17	Proteus sp.	1	0,66
18	Salmonella biotype C	2	1,33
19	Pseudomonas sp. non oxidizer	1	0,66
	Total number of examined bee societies = 150		

According to Table, a total of 19 bacterial strains was isolated. The most frequent bacteria were Klebsiella pneumoniae and Enterobacter genus (Enterobacter aglomerans et cloacae). Citrobacter freundii, Klebsiella ozenae and Enterobacter aerogenes were found less frequently. Other species were detected only occasionally and the rarest were Staphylococcus saprofiticus, Escherichia coli, Salmonella biotype C, Proteus sp. and Pseudomonas sp. non oxidizer. Among anaerobic strains only Clostridium perfringens was isolated.

All the isolated bacteria (according to Bergey's Manual of Determinative Bacteriology – IX edition) were classified into 7 families, 14 genuses and 19 species.

The isolation procedures differed between authors depending on the isolation media used (Gilliam et al. 1976, 1989. and 1990a i b; Smolska-Szymeczewska, 1989). Our choice of media was not in accordance to the aforementioned workers, whereas the preparation and isolation procedures of the bee intestine were similar. The identification of enterobacteria and other bacteria in this paper was completely in accordance with the work of Smolska-Szymeczewska 1989.

Some authors such as Gilliam et al. 1989. and Smolska-Szymeczewska (1989) described enterobacteria, but only as a genus without identifying the corresponding species. Such findings led us to examine and identify gramnegative bacteria and enterobacteria isolated from bee intestines in more detail.

Table 2. The isolated species classified according to Bergey's Manual

Section	Family	Genus	Species
N°4	Neisseriaceae	Acinetobacter	Acin. calc. var. lwoffi
N°4	Pseudomondaceae	Pseudomonas	P. fluorescens P. sp. non oxidizer
N°4	Other genuses	Flavobacterium	Flavobacterium sp.
N°5	Enterobacteriaceae	Citrobacter	C. freundii
		Klebsiella	Kl. pneumoniae Kl. ozenae
		Enterobacter	E. agglomerans E. cloacae E. aerogenes E. hafniae
		Proteus	Proteus sp.
		Salmonella	Salmonella biotyp C
		Escherichia	E. coli
N°5	Vibrionaceae	Aeromonas	A. hydrophila
	By VIII edition of Bergey		
	Streptococcae	Streptococcus	Streptococcus sp.
	Micrococcaceae	Staphylococcus	St. saprofiticus
	Bacillaceae	Bacillus	Bacillus sp.
		Clostridium	C. perfringens

During the study the most frequent bacterium found was Klebsiella pneumonae, which was, not mentioned as an isolate from the bee intestine in the consulted literature. According to the obtained results from the tested bee intestines the most frequent bacteria were: Kl.pneumonae, Enterobacter cloacae and Enterobacter agglomerans. Citrobacter freundii, Flavobacterium sp. Pseudomonas fluorescens, Aeromonas hydrophila and Acin. calco. vari woffi were isolated less frequently and could be regarded as temporary occupants of the bee digestive tract from their rate of occurrence. Other isolated bacteria could be regarded as rare, such as Staphylococcus saprofiticus, Escherichia coli, Salmonella biotype C. Proteus sp., Pseudomonas non oxidizer, which confirms the results of Gilliam, 1989.

REFERENCES

1. *Bergey's* 1984. *Manual of Determinative Bacteriology 8th edition.*
2. *Gilliam M., Valentine K. D.,* 1976. Bacteria isolated from the intestinal contents of foraging work honey bees, Apis mellifera: The Genus Bacillus. *Journal of Invertebrate, Pathology 28,* 275-276.
3. *Gilliam M. et al* 1989. Microbes from aparian sources: molds in Frass from Larvae of the greater wax moth, Galleria mellionella *Journal of Invertebrate Pathology 54,* 406-408.
4. *Gilliam M.* 1988. Microbial Ecology of Honey Bees. The XXXI st International Congress of Apiculture, Warsaw, Poland, 217-220. *Apimondia Publishing House Buchurest, Romania.*
5. *Gilliam M.* 1978. Bacteria belonging to the genus Bacillus isolated from selected organs of queen honey bees, Apis mellifera. *Journal of Invertebrate Pathology 31,* 389-391.

6. *Gilliam M. et al.* 1990.Microorganisms associated with pollen, honey and broad provisions in the nest of a wringless bee Melipona facciata. *Apidology 21,* 89-97.
7. *Gilliam M. et al.* 1990b. Bacteria belonging to the genus Bacillus associated whith three species of solitary bees. *Apidology 21,* 99-105.
8. *Smolska-Szymeczewska B.* 1989. Influence de quelques chiamiotherapeutigeuest save la flore intestinale de labeille mellifere. *Aplacta XXIV,* 71-79.
9. *Trilenko V. S.* 1965. The microflora of the bee intestine XX Yubilenyi Merzhdumolodyi Kongre. Pchelvodstan Moskva 292-298.
10. *Trilenko V. A., Federova G. M.,* 1963. Naucho Konf. *Leningrad Vet. Inst. 12,* 131-133.

ISPITIVANJE BAKTERIJSKE FLORE DIGESTIVNOG TRAKTA PČELA

NADA DUGALIĆ-VRNDIĆ

SADRŽAJ

U radu je izvršena izolacija i identifikacija prisutnih bakterija u digestivnom traktu (3000 pčela iz 150 pčelinjih zajednica). Tom prilikom identifikovano je 19 vrsta bakterija, a najčešći izolat su bila Klebsiella pneumoniae (71,30%), Enterobacter aglomerans (43,30%) i Enterobacter cloacae(35,30%). Zatim u manjem broju Citrobacter freundii (28,00%), Klebsiella ozena (14,00%) i Enterobacter aerogenes (16,60%).

Najređe su bile izolovane bakterije Staphylococcus saprofiticus (1,3%), Escherichia coli (1,33%), Salmonella biotip C (1,33%), Proteus sp. (0,66%), Pseudomonas sp. non oxidizer (0,16%). Od anaerobnih bakterija izolovana je samo jedna vrsta Clostridium perfringens (5,33%).