Characteristic of Species Composition of Fungi Involved in the Formation of Mycobiota of Honey Bees in Azerbaijan

Aysel F. Isayeva¹, Guler M. Seyidova², Sahla A. Abdullayeva³, Matanat S. Novruzova¹, Panah Z. Muradov¹

¹Institute of Microbiology of the NAS of Azerbaijan, Baku, Azerbaijan
²Azerbaijan Medical University, Baku, Azerbaijan
³Azerbaijan State Pedagogical University, Baku, Azerbaijan
Email: mpanah@mail.ru

Abstract
The purpose of the presented work was dedicated to identifying the species composition of the mycobiota of honey bees in Azerbaijan condition. From the samples taken from bees, materials became clear that in the formation of mycobiota those materials (from bees, from where they live and their products) in generally participate 52 species of fungi. Among the recorded fungi, species take part such as Alternaria alternata (Fr.) Keissl., Aspergillus flavus Link, Candida albicans (C.P. Robin) Berkhout, Cladosporium herbarum (Pers.) Link, Penicillium cuclopium Westling, P. granulatum Bainier and etc. which carry features conditionally pathogenicity, toxicity, allergens and danger to biological productivity of bees and as well as to pollution of their products. It is known for a long time to scientists that these species are dangerous for human health. For this reason, preparation of normative documents that reflect the principles of microbiological safety of bee products is a necessary task.

Keywords
Honey Bees, Mycobiota, Toxigenes, Askosferoz, Aspergillosis

1. Introduction
As known, beekeeping is one of the ancient and very important fields of farming. So that for honey products, firstly honey is not only the energy source for people, but also the source of various biologically active substances (bioflavo-
noids, phytosterols, indoles, etc.). For many years honey has been used to remove many diseases. People before successfully used chemicals; mixtures of various natural herbs were used and honey was prepared to eliminate the disease [1] [2].

As it is noted, beekeeping is closely related with environment, so that, the development and productivity of the bee family not only depends on climate factors, but also on the wild or cultural flora of the area. Formation of flora, to use the various chemical compounds for improving the phytosanitary condition of the area, expansion of urbanizationas [3] and an element of biogeocenosis also has a serious impact on bees and completely changes their living conditions [4] [5] [6]. This changeability can be dangerous to human beings who use honey product for the traditional prophylactic treatment. In addition, there are also numerous data available about pollution of honey bee products, as well as their biological supplements with pathogenic microorganisms, including with toxic fungi [7] [8]. The complicated aspect of this issue is that, microbiological insecurity of honey is not regulated by regulatory documents.

After the use of products contaminated with fungi, especially their toxigenic species, falling its total load into the human body may not be so great, but its role in the occurrence of myogenic allergies, violation of the normal microbiota of the gastrointestinal tract, occurring mycosis and toxicosis in the people with weak immune system today, is not clear in the full sense. For this reason, clarification of these issues is one of the actual research directions of the modern era.

Moreover, according to a number of researchers, the interest in the problems of mycotoxin is growing [9] [10] and it is part of the global pollution of the biosphere. At any stage of the trophic chain of “Soil-water-entomophilous plants—powder, nectar, bee, beekeeping products”, contamination with mold fungi can occur and therefore the fungus spores in any product of honey bees can be collected and stored for a long time. A danger aspect of this is that, in the case of favorable conditions for mold fungi, most of which are saccharolytic, they can grow and be enriched with bee products by secondary metabolites [5] [11], including mycotoxins.

Taking into account the weakness of the study of all the above-mentioned issues the present work was dedicated to the determination of the mycobiota of honey bees and its toxigenic species.

2. Material and Method

Samples for research have been taken from honey bees stored in different regions (Greater Caucasus, Small Caucasus, Khur-Araz and Lankaran-Astara) of Azerbaijan (Figure 1). During taking sampling were used the bees themselves (mostly new dead and the workers bee who do not fulfill their function properly), as well as from the places where they were stored (an apiary) and their materials (specially prepared frame for bee where products were formed by them). The analysis of
samples was carried out on the basis of known by mycological methods [12] [13] and obtained pure cultures was identify up to the species level. In designating pure cultures were used determinants [14] [15] [16] compiled according to cultural morphological and some physiological features of fungi, which, represents the entire population distributed in a particular area. During naming of fungi were used from information given on the official website of the International Mycology Association [17].

As a nutrient medium was used agar followed cereal juice, Chapek medium and cultivation was carried out at 26˚C - 28˚C.

3. The Results and Discussion

As a result of the analysis 130 fungi culture from 250 samples taken from 2016 up to date were taken to the pure culture which 118 of them were identified up to the species level. It became clear that, in the formation of mycobiota of bee and various materials belonging to the bees, involveds 49 species of fungi and their taxonomic structure was noted in Table 1. As seen, 5 species from registered fungi belong to the division of Zygomycota, but 44 species to the division of Ascomycota. Also have been found most of the latest relates to the anamorphs of sack fungi (38 species).

In studies conducted to date were identified that nearly all of registered fungi involved in the formation of mycobiota of senoses of soil, water and plant in Azerbaijan [4]. Although among them are not encountered true biotrophs according to ekolo-trophic relations was identified that species which does not
Table 1. Numerical characteristics of taxonomic structure of fungi involved in the formation of mycobiota of bee and place where they storage.

| Kingdom   | Division   | Class | Order | Family | Genus (species) |
|-----------|------------|-------|-------|--------|----------------|
| Mycota    | Zygomycota | 1     | 1     | 2      | 2 (5)          |
|           | Ascomycota | 4     | 7     | 10     | 21 (44)        |
| Total     |            | 2     | 5     | 8      | 12 23 (49)     |

have character of true sapotrophy or biotrophy are in the majority. So that, nearly all of registered fungi are suits to this characteristics and many of them are capable of causing various pathologies in different living things, including on the insects.

It is known that some fungi that are spread in the nature are causes this or another pathology on the honey bees and in the course of research were found similares to them, which following in below:

1) *Aspergillus flavus* Link, Magazin der Gesellschaft Naturforschenden Freunde Berlin 3 (1): 16 (1809) [MB#209842].

This fungi, along with toxigenity (synthesis aflatoxin, which is dangerous for human health), same time mainly causes disease of aspergilliosis in honey bees. The danger side of this is that it is widely spread throughout the ecologically different regions of the Republic of Azerbaijan [9] and among its settlements, plants, especially floral plants used by honey bees, are quite high.

2) *A. fumigatus* Fresen., Beiträge zur Mykologie 3: 81 (1863) [MB#211776].

Syn.: *Aspergillus cellulosae* Hopffe (?) [MB#490561].

This fungus belongs to both toxigens and opportunists, that is, causes aspergilliosis on the honey bees. True, this fungi is not so widely spread in Azerbaijan condition, but it also has the ability to synthesize mycotoxins [16] that have toxic effects and to have the ability to spread on plants and soil, which is why it is important to keep this fungus in the spotlight.

3) *A. nidulans* (Eidam) G. Winter, Rabenhorst’s Kryptogamen-Flora, Pilze-Ascomyceten 1(2): 62 (1884) [MB#182069].

This fungus for the spread is considered as a rare species in Azerbaijan, but its participation in the formation of mycobiot of bees has been found in studies, which, this case meets only in bees kept in the Gabala region. Fungi belong to toxigens and can synthesis toxin called sterigmatocystin [5] which has kansorogenic properties.

4) *A. niger* Tiegh., Annales des Sciences Naturelles Botanique 8: 240 (1867) [MB#284309].

This fungus, one of the most widely spread species of the mycobiota of the nature of Azerbaijan. They participate in the formation of mycobiota of both the honey bees and of the apiary. It is synthesized toxic compounds such as ochratoxin and aflatoxin [5]. These fungi also have the ability to synthesize antibiotics called aspergilin which has antibacterial activity.

5) *Aureobasidium pullulans* (de Bary) G. Arnaud, Annales de l’École Natio-
This species, which is cosmopolitan for the distribution causes the disease of melanosis in the bees. Although not widely spread in bees kept in Azerbaijan, almost was found in all of samples taken from regions.

6) Candida albicans (C.P. Robin) Berkhout, De schimmelgeslachten Monilia, Oidium, Oospora en Torula: 44 (1923) [MB#256187].

This fungi is one of most active the species involved in the formation of mycobiota of Azerbaijan and participates to the causes the pathology in bees. At the same time belonging of the fungi to the toxigens and the synthesis of dangerous metabolites for human health to gives a reason to note that they dangerous.

Along with the species registered in research also meets species such as Alternaria alternata, Cladosporium herbarum, Penicillium cyclopium, Penicillium granulatum and oth.

Although these fungi do not causes any pathology in bees, they are all toxigens [5] [9] [10] and synthesizes toxins dangerous for human health, which is the facts that have been confirmed.

4. Conclusion

Thus, from the research it became clear that, bees are also one of the places where fungi are populated and those fungi involved in the formation of their mycobiota cause pathology which reduces biological productivity of bees and synthesizes toxic substances. Products that have their dangerous metabolites are transported to the human body when used. Preventing of this and determination of permissible concentrations of substances having a toxic effect synthesized by fungi is one of the important issues and it would be advisable to conduct researches in this direction.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Adimasu Abeshu, M. and Geleta, B. (2016) Medicinal Uses of Honey. Biology and Medicine, 8, 2. https://doi.org/10.4172/0974-8369.1000276

[2] Ayoub Meo, S., Ahmad Al-Asiri, S., Latief Mahesar, A. and Ansari, M.J. (2017) Role of Honey in Modern Medicine. Saudi Journal of Biological Sciences, 24, 975-978. https://doi.org/10.1016/j.sjbs.2016.12.010

[3] McDonald, R.I., Marcotullio, P.J. and Güneralp, B. (2013) Urbanization and Global Trends in Biodiversity and Ecosystem Services. In: Elmqvist, T., et al., Eds., Urbanization, Biodiversity and Ecosystem Services Challenges and Opportunities A Global Assessment, Springer, Dordrecht, 31-52. https://doi.org/10.1007/978-94-007-7088-1_3

[4] Asensio, I., Vicente-Rubiano, M., Muñoz, M.J., Fernández-Carrion, E., Sánchez-Vizcaino, J.M. and Carballo, M. (2016) Importance of Ecological Factors and Colony Handling for Optimizing Health Status of Apiaries in Mediterranean Ecosystems. PLoS ONE, 11, e0164205. https://doi.org/10.1371/journal.pone.0164205
[5] Chekriga, G.P. (2013) Factors Determining Microbial Contamination of Products of Honeybees. Sibir Herald. Agricultural. Science, 2013, 32-39.

[6] Nyunza, G. (2018) Anthropogenic and Climatic Factors Affecting Honey Production: The Case of Selected Villages in Manyoni District, Tanzania. Journal of Agricultural Biotechnology and Sustainable Development, 10, 45-57. https://doi.org/10.5897/JABSD2017.0292

[7] Kashyap, D., Pandey, H., Jaiswal, K. and Mishra, S. (2019) Fungal Diseases of Honey Bees: Current Status and Future Perspective. In: Gupta, A. and Singh, N., Eds., Recent Developments in Fungal Diseases of Laboratory Animals, Fungal Biology, Springer, Cham, 7-27. https://doi.org/10.1007/978-3-030-18586-2_2

[8] Lopes, L.Q.S., et al. (2014) Fungal Infections in Honey Bees. Fungal Genomics & Biology, 4, 1. https://doi.org/10.4172/2165-8056.1000118

[9] Bakshaliyeva, K.F. (2017) Ecobiological Features of Toxic Fungi Spread in Azerbaijan. Abstract of Dissertation. Baku, 45. http://www.aak.gov.az/avtoref_to_mudaf/pdf_to_mudaf/bio/bio_d_bkf_30_10_17.pdf

[10] Wang, L., Liu, B., Jin, J., Ma, L., Dai, X., Pan, L., Liu, Y., Zhao, Y. and Xing, F. (2019) The Complex Essential Oils Highly Control the Toxigenic Fungal Microbiome and Major Mycotoxins During Storage of Maize. Frontiers in Microbiology, 10, 1643. https://doi.org/10.3389/fmicb.2019.01643

[11] Ayansola, A.A. (2012) Fungal Isolates from the Honey Samples Collected from Retail. Journal of Biology and Life Science, 3, 189-199. https://doi.org/10.5296/jbils.v3i1.1974

[12] Jensen, A.B., Aronstein, K., Flores, J.M., Vojvodic, S., Palacio, M.A. and Spivak, M. (2013) Standard Methods for Fungal Brood Disease Research. Journal of Apicultural Research, 52, 1-20. https://doi.org/10.3896/IBRA.1.52.1.13

[13] FAO (2006) Handbook of Mycological Methods-Project GCP/INT/743/CFC. http://www.fao.org/fileadmin/user_upload/agns/pdf/coffee/Annex-F.2.pdf

[14] Kirk, P.M., Cannon, P.F., Minter, D.W., Stalpers, J.A., et al. (2008) Ainsworth & Bisby’s Dictionary of Fungi. CAB International, Oxfordshire, 771. https://doi.org/10.1079/9780851998268.0000

[15] Klich, M.A. (2002) Identification of Common Aspergillus Species. Utrecht, CBS, 116 p.

[16] Sutton, D. Fothergill, A. and Rinaldi, M. (2001) The Determinant of Pathogenic and Conditionally Pathogenic Fungi. M: The World, 468.

[17] http://www.mycobank.org/MycoTaxo.aspx