Sabuncuoglu Serefeddin Health Science (SSHS)

ISSN: 2667-6338, 2022/Vol.4:3/1-13

INVESTIGATION OF THE EFFECT OF PROBIOTIC USE ON ORGANISM BEHAVIORS IN *DROSOPHILA MELANOGASTER*

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Research Article

Received: 09/11/2022; Accepted: 14/12/2022

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Abstract

Microbiota; Microbiome refers to the genes carried by microorganisms living commensally with humans. Recent studies have shown that gut microbiota plays an important role in shaping the health and fitness of animals, including humans. Probiotics, known as live microorganisms that, when taken in sufficient quantities, are beneficial to the health of the organism they inhabit, have been used traditionally by people for centuries in different ways. This study, it was aimed to investigate the effect of modified gut microbiota of *Drosophila melanogaster* individuals fed on probiotic-supplemented media and standard media on mate selection, which is one of the behavioral characteristics. For this purpose, probiotic food containing 11 different bacterial species was added to the medium of *D. melanogaster* for 6 generations to reshape the fly microbiota. Flies grown on standard *Drosophila* medium and flies fed probiotic food were placed on the cell plates as 1 male and 1 female mixed. The mate choices of individuals carrying different markers were tracked and recorded. Obtained results were evaluated statistically. As a result, it was observed that nutrition, and therefore microbiota, was effective in the selection of mates of flies mixed in cell plates. In particular, it was determined that individuals fed with probiotics chose the opposite sex fed with probiotics in the selection of mates for mating. It has been concluded

that the effects of microbiota, the importance of which has been better understood in recent years, on living things should be clarified by different studies.

Key Words: Probiotic, Mate Selection, Microbiota, *Drosophila melanogaster*

Özet

Mikrobiyota; insanlarla birlikte yaşayan mikroorganizmaların tamamını, mikrobiyom ise insanlarla kommensal olarak yaşayan mikroorganizmaların taşıdıkları genleri ifade etmektedir. Bağırsak mikrobiyotasının, insanlar da dahil olmak üzere hayvanların sağlığını ve formunu şekillendirmede önemli bir rol oynadığı yapılan son çalışmalarla ortaya konmuştur. Yeterli miktarda alındığında, yerleştiği organizmanın sağlığına faydalı olan canlı mikroorganizmalar olarak bilinen probiyotikler, yüzyıllardır insanlar tarafından geleneksel olarak farklı şekillerde kullanılmaktadır. Bu çalışma ile probiyotik ilaveli besiyerinde ve standart besiyerinde beslenen *Drosophila melanogaster* bireylerinin değişikliğe uğramış bağırsak mikrobiyotalarının davranış özelliklerinden biri olan eş seçimi üzerine etkisini araştırmak amaçlanmıştır. Bu amaçla 11 farklı bakteri türü içeren probiyotik besin, *D. melanogaster*'in 6 nesil boyunca besiyerine eklenerek sinek mikrobiyotasının yeniden şekillenmesi sağlanmıştır. Standart *Drosophila* besiyerinde yetiştirilen sinekler ve probiyotik gıda ile beslenen sinekler hücre plaklarına 1 erkek 1 dişi olacak şekilde karışık olarak yerleştirilmiştir. Farklı işaretleyiciler taşıyan bireylerin eş seçimleri takip edilmiş ve kaydedilmiştir. Elde edilen sonuçlar istatistiksel açıdan değerlendirilmiştir. Sonuç olarak hücre plaklarında karışık olarak bulunan sineklerin eş seçiminde beslenmenin, dolayısıyla mikrobiyotanın etkili olduğu görülmüştür. Özellikle probiyotik ile beslenen bireylerin çiftleşmek için eş seçiminde yine probiyotik ile beslenen karşı cinsiyeti seçtiği tespit edilmiştir. Son yıllarda önemi daha da iyi anlaşılan mikrobiyotanın canlılar üzerindeki etkilerinin yapılacak farklı çalışmalarla aydınlatılması gerektiği sonucuna ulaşılmıştır.

Anahtar Kelimeler: Probiyotik, Eş Seçimi, Mikrobiyota, *Drosophila melonagaster*

1. Introduction

Human gut: It includes a variety of microorganisms such as bacteria, fungi, parasites, viruses, and more than 100 million bacteria, 10-100 times the number of eukaryotic cells in our body, reside in the human gastrointestinal tract. Human body: After years of co-development, it has become a mutually beneficial symbiotic relationship with gut bacteria (Zhu et al., 2017). In recent years, the terms microbiome and microbiota have been used frequently. Microbiota: microbiome refers to the genes carried by microorganisms living commensally with humans (Yılmaz and Altındiş, 2017).

While the word microbiome was first used as a title in three articles in 2006, this number reached 1087 in 2016. The "human microbiome project" initiated by NIH/NHGRI (National Health/Human Genome Research Institute) in the USA in 2008 and the "human gut metagenomics" (MetaHIT) project launched in Europe after a short time is significant in terms of showing the importance of the subject. The history of research in the same scope in veterinary medicine is more rooted, especially due to studies examining the rumen and intestinal flora. However, it is relatively new to see articles published with the term microbiome in the literature and covers various domestic and wild animal species, especially poultry (Deusch, et al, 2015).

Different animal species may have different populations of microbes or microbe communities in animals may differ from their environment (Hanning, 2015). In addition, this difference can be seen between individuals or genotypes of the same species and in the developmental stages of the animal over time (Levy, Blacher, & Elinav, 2017). This community, also called the gut microbiome (microbiota), not only finds a habitat for itself but also contributes to many physiological events, from the host's metabolic and energy homeostasis to immunity.

Beneficial microorganisms, which are called probiotics and can colonize the host and have a positive effect on the health of the host, provide the protection of the natural microflora in the intestines by helping the digestion of food, vitamin production, inhibition of pathogenic bacteria with the help of the substances they produce (Oelschlaeger, 2012). The microorganisms most commonly used as probiotics are Lactobacilli and Bifidobacteria, which can survive in the intestines (Alp and Aslım, 2009).

Among the positive effects of probiotic bacteria on human health, the antimicrobial peptides (bacteriocins) they produce are of great importance. Probiotic bacteria can regulate and protect the natural microflora of the host cell. In addition, the antimicrobial toxins they produce have the effect of competing with unwanted or pathogenic microorganisms and forming colonies in the

intestines. The effect of the gut microbiota on the immune system, brain development, behavior has been the focus of attention in recent years. It is thought that microorganisms living in the intestines are in communication with the intestinal epithelium and immune system cells, and thanks to this communication, they play a role in the formation of many neuropsychiatric and metabolic disorders, especially autoimmune diseases. Clinical observations and animal experiments have provided ample evidence of a strong connection between the brain and the gut. This connection starts in the intrauterine period and continues throughout life (Borre et al, 2014).

Probiotics, which are used as supplements and contain many different non-pathogenic microorganisms, are the most effective agents in the regulation or differentiation of microbiota. In our study, probiotics were used as supplementary food to reshape the microbiota.

In our study, probiotic food containing 5 different bacterial species, which is used as a food supplement sold in pharmacies in our country, was used. *Drosophila melanogaster* was used as a model organism in the study. The behavior of flies fed probiotic food and flies fed standard Drosophila medium was observed. Drosophila is emerging as a valuable model for microbiome research, by combining genetic and genomic resources with simple protocols to manipulate the microbiome, microbiologically sterile flies and flies carrying a standardized microbiome can be easily produced in large numbers. Studying Drosophila has the potential to increase our understanding of how its microbiota influences host traits and allow for hypothesis testing of microbial effects on human health. Drosophila: It has been used to investigate aspects of host-microbe interactions, including metabolism, immune system, and behavior. Drosophila offers a valuable alternative to rodent and other mammalian microbiology research models for the fundamental exploration of microbiome function and provides better research cost-effectiveness and benefits for animal welfare.

This model has two important advantages (genetic measurability and traceability of large-scale experiments). In addition, the fruit fly has the potential to be established as an important system *in vivo* study of the gut microbiome. Early studies revealed that the gut microbiome of this creature is relatively simple, consisting of ~20 species (Woyke et al, 2006; Wong, Ng, Douglas, 2011). The aerobic of *D. melanogaster* gut microbiome components (this organism's gut is permeable to oxygen) may facilitate experimental intervention in this community. This creature has the potential to emulate human biology, thanks to its gut microbiome and fascinating aspects of host-microbial interaction. For example, Acetic acid produced by *Acetobacter pomorum* in the microbiota of *D. melanogaster* plays a role in insulin signaling (Shin et al, 2011). However,

Acetobacter spp. It is not a prominent member of the human gut microbiome. Acetic acid is a central metabolic component in the human gut (Julian and Marchesi, 2014). In addition, gut bacteria found in *D. melanogaster* have been associated with mating preference (Sharon, 2010). This suggests that different members of the human gut microbiota may play a similar role, just as some members of the microbiome play a role in insulin signaling.

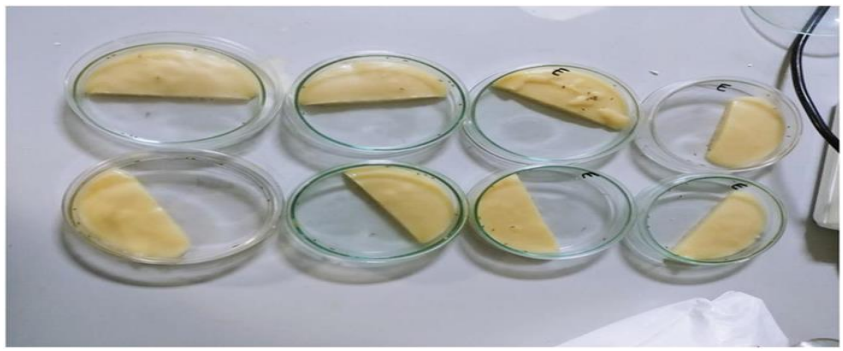
Over the years, various researchers have investigated how Drosophila interacts with non-pathogenic microorganisms, which has paved the way for increased research interest in Drosophila as a model for the science of microbiology (Douglas, 2018). The body cavity and cells of the insect the outer surface of Drosophila also often carries a microorganism similar to the gut microbiota but in ten times less abundance. In terms of the above-mentioned features, Drosophila is an ideal organism both for genetic research and because it is an easy creature to manipulate microbiota.

D. melanogaster, which was first used in experimental studies by Thomas Morgan in 1911, has many advantages in terms of use. In addition, it is an important model organism that allows the investigation of the genotoxic, cytotoxic and mutagenic activities of many unknown chemicals or herbal extracts (Fidan et al. 2020; Fidan and Ayar, 2021a, b, c; Fidan and Ayar, 2022).

In this study, the effect of modified gut microbiota of *Drosophila melanogaster* members fed on probiotic supplemented media and standard media on mate selection, which is one of the behavioral characteristics, was investigated. Drosophila used for this purpose; It is emerging as a valuable model for microbiome research, whereby manipulating the microbiome and sterile flies and flies carrying a standardized microbiome can be produced in large numbers and easily.

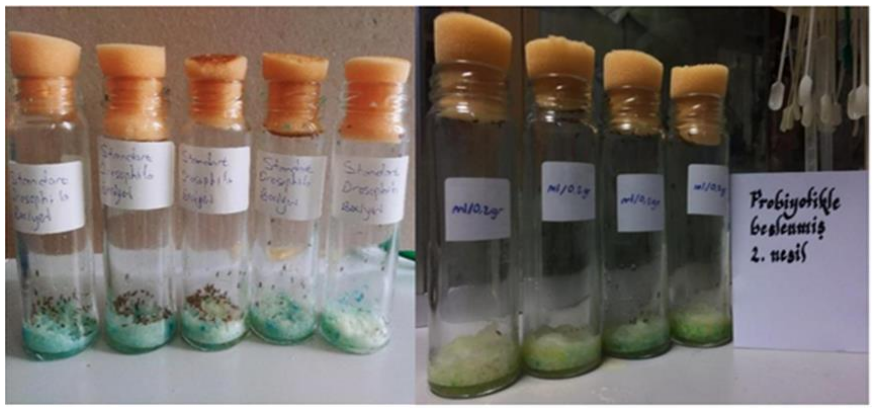
2. Material and Methods

In the study, standard Drosophila medium was prepared in petri dishes to obtain adult flies fed with standard medium and probiotic food (Picture 1). Eggs obtained from here were kept in 0.6% hypochlorite solution to be sterilized.



Picture 1. Media prepared in petri dishes to obtain Drosophila eggs

Eggs obtained from Petri dishes were kept in an environment containing 0.6% hypochlorite to be sterilized, then added to media containing standard and probiotic food. In the study, *D. melanogaster* grown in four 50 ml bottles for 6 generations in standard media and environments containing probiotics was used (Picture 2). All materials used during the experiments were sterilized in a dry air sterilizer at 180 °C. Attention was paid to the fact that the only difference between the fly growing in the standard medium and the growing in the medium containing probiotic food was the food added to the medium.



Picture 2. Flies reared on standard and probiotics diets

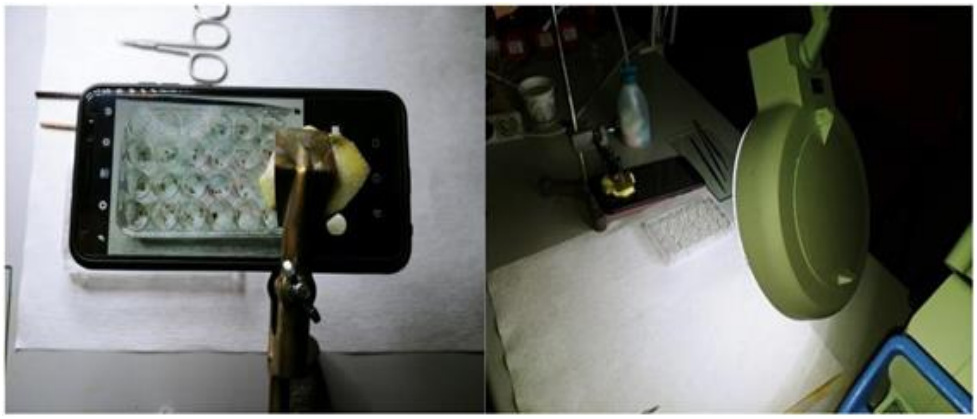
The probiotic used in the study was obtained from a private company. It is sold in the market as NBL Probiotic ATP. The probiotic food we used in the research is available as a powder in the form of sachets. The bacterial species contained in the fortification food containing a total of 11

species and 5 billion active probiotics are given below (Table 1).

Table 1. Types of probiotic bacteria used in the study

No	Probiotic Microorganisms
1	*Lactobacillus acidophilus*
2	*Lactobacillus rhamnosus*
3	*Bifidobacterium longum*
4	*Bifidobacterium infantis*
5	*Bifidobacterium lactis*
6	*Bifidobacterium bifidum*
7	*Lactobacillus plantarum*
8	*Streptococcus thermophilus*
9	*Lactobacillus reuteri*
10	*Lactobacillus bulgaricus*
11	*Lactobacillus paracasei*
12	*Lactobacillus breve*

Male and female flies obtained from flies reared in the same media for 6 generations were placed in 24-cell plates as 1 male and 1 female. Etherization was not performed during the placement of the flies in the wells. The flies were placed in the prepared wells with the help of an aspirator. Since the mating behavior of the flies would be observed, the flies that reached maturity in the standard medium and the medium containing probiotics were collected every 8 hours mating was prevented. Each cell contains 4 flies. The wings of those fed with probiotic food were clipped with micro-scissors so that these flies placed in the same environment would not mix. The clipping process provides a very clear separation of the flies under the camera. It has been previously tested that wing clipping does not cause any negative effects on fly life and mating behavior (Dodd and Powell, 1985; van den Berg, 1984). The flies in the 24-cell plate were recorded every hour for 4 minutes (Picture 3).



Picture 3. Video recording of flies fed with probiotic and standard media

In the experiments conducted, besides the fact that flies fed with similar foods preferred each other (probiotic ♂ x standard medium ♀), (standard medium ♂ x probiotic ♀) pairings were also realized (Table 3). In addition, the SII index was used to determine whether there was mating isolation depending on the foods in the observations. An SII index greater than zero indicates positive mating isolation between flies due to food (Sharon et al., 2010).

$\boldsymbol{SII} =$ (Mating number of individuals with the same characteristics- Number of mating of individuals with different characteristics) / n (Total Mating Number)

$$SEM\ of\ SII = \sqrt{\frac{(1 - SII^2)}{n}}$$

3. Results

In the study, the effect of supplementary food, which is used as a probiotic, *on Drosophila melanogaster* larvae was investigated. For this purpose, different proportions of probiotics were added to the standard Drosophila medium. Standard Drosophila medium was prepared as the control group. 100 larvae were placed in these prepared media and the effect of the probiotic food we used on the mortality rate was investigated (Table 2).

Table 2. Mortality rates of foods containing different amounts of probiotics on *D. melanogaster* larvae

Medium	Number of Larvae	Number of Adult Individuals
Standard *Drosophila* Medium	100	95
0,02g/ml probiotics	100	96
0,04g/ml probiotics	100	98
0,06g/ml probiotics	100	93
0,08g/ml probiotics	100	94

As can be seen in Table 3, no negative effects of the supplementary food used as probiotics were found on flies. When the data we obtained were examined, the highest number of adult individuals was reached in the medium with 0.04g/ml probiotic added.

A total of 10 experiments were conducted in our study. In the first experiment, 30 matings were detected. While 14 of them were among those fed with probiotics, 10 of them were among the flies fed on a standard medium. In the second experiment, there were 9 matings between the flies fed with probiotics and 11 matings between those fed with the standard medium. In our third experiment, there were 12 matings among the flies fed with probiotics and 9 matings between the flies fed with the standard medium. In our 4th experiment, there were 14 matings among those fed with probiotics and 12 matings between those fed with standard medium. In our 5th experiment, 9 matings were observed between those fed with probiotics and 11 matings between those fed with standard medium. In our 6th experiment, there were 15 matings among those fed with probiotics and 14 matings between flies fed with standard medium. In our 7th experiment, 11 matings were observed between the flies fed with probiotics, and 9 matings were observed between the flies fed with a standard medium. In our 8th experiment, 16 matings were observed between the flies fed with probiotics, 14 matings were observed between the flies fed with the standard medium. In our 9th experiment, 9 matings were observed between the flies fed with probiotics, and 9 matings were observed between the flies fed with the standard medium. In our last experiment, 11 matings were observed between the flies fed with probiotics, 13 matings were observed between the flies fed with the standard medium (Table 3), (Figure 1).

Table 3. Mating experiments of *Drosophila melanogaster* fed probiotic food and standard diet

Experiment Numbers	Pb ♂ X Pb♀	SM♂X SM♀	Pb♂ X SM♀	SM♂ X Pb♀	SII ± SEM
1	14	10	2	4	0,6 ± 0,1
2	9	11	6	5	0,3 ± 0,2
3	12	9	6	5	0,3 ± 0,2
4	14	12	4	4	0,4 ± 0,2
5	9	11	5	7	0,25 ± 0,2
6	15	14	7	7	0,3 ± 0,1
7	11	9	6	6	0,25 ± 0,2
8	16	14	9	6	0,3 ± 0,1
9	9	9	6	7	0,2 ± 0,2
10	11	13	5	7	0,3 ± 0

SII: Mating isolation index, SEM: Standard error of the mean, SM: Standard Medium, PB: Probiotic

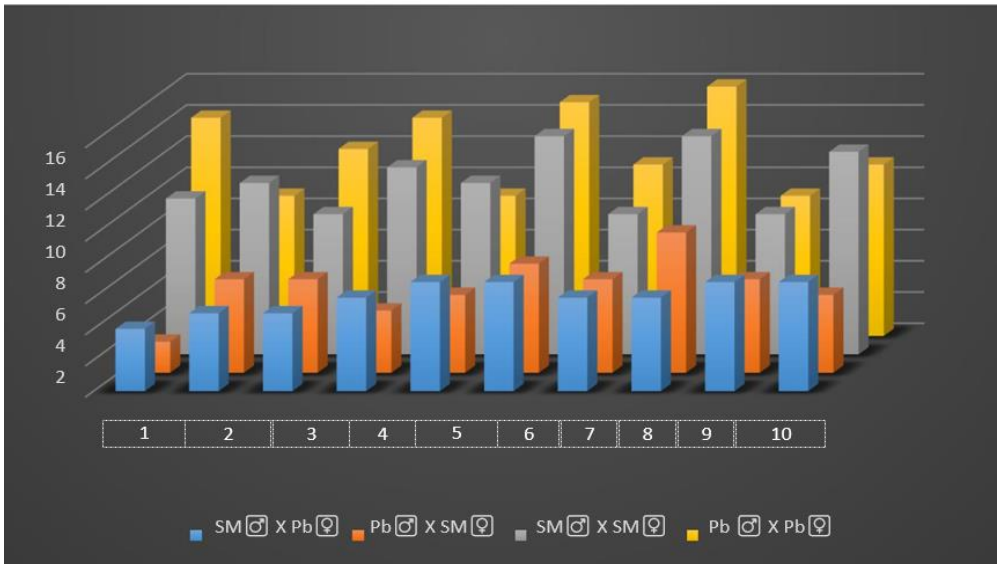


Figure 1. Results plot of mating experiments (Number 1-10; Numbers of experiment)

4. Conclusion and Discussion

The gut microbiome is increasingly recognized to play an important role in shaping the health and fitness of animals, including humans. Drosophila is emerging as a valuable model for microbiome research, by combining genetic and genomic resources with simple protocols to

manipulate the microbiome, microbiologically sterile flies and flies carrying a standardized microbiome can be easily produced in large numbers.

As seen in the results obtained in our study, nutrition and the intestinal microbiota shaped depending on nutrition can be effective in behaviors. In our study, the gut microbiota, which is shaped by nutrition, can affect our life significantly as well as be effective in mate selection. Similar to our study, Sharon et al. (2010) observed the mating behavior of flies fed on starch-based medium and flies reared on molasses-containing medium, and the flies fed on the same food preferentially preferred each other. Again, in the continuation of the study, it was shown that the use of antibiotics eliminated the mating preference. Similarly, in the study conducted by Rosenberg and Zilber-Rosenberg (2011), it was stated that vinegar (fruit) flies (*Drosophila melanogaster*) control their sexual behavior by changing the level of *Lactobacillus plantarum* sex pheromones, a bacterium living in their intestines.

After a normal birth, the gut microbiome of the offspring is modeled after the maternal genital tract, whereas the gut microbiome of the offspring born by cesarean section is more similar to the composition of the skin. In addition, while the gut microbiome is stable in the absence of significant nutritional and environmental changes in adulthood, the microbiome structure follows a dynamic course from newborn to adulthood. During this period, there are significant changes in the relative densities of the main bacterial groups that carry out different metabolic functions. While some of these changes are attributed to diet and physiology changing with age, unpredictable environmental influences play an important role in shaping the microbiome. In our study, the intestinal microbiota of flies grown in an environment containing probiotic food for 6 generations underwent changes. The gut microbiota and the effects of bacteria on human health seem to be the focus of attention in neuroscience for the next decade. There are also authors presenting microorganisms as a new group of drugs called “psychomicrobiotics” in the treatment of psychiatric disorders (Fond, 2014).

Changing microflora due to various environmental factors is the root cause of many diseases. It is suggested that protection against various chronic diseases, including obesity, can be achieved by making the microflora composition healthy. Even such a possibility would be a huge step forward for the treatment of chronic diseases that shorten human lifespan, have high mortality rates, prolong dependent life expectancy, and increase healthcare costs. Many studies have been conducted to examine changes in life span in *Drosophila melanogaster* individuals depending on nutrition (Ayar et al,2021).

The microbiota has started to be evaluated as a new organ in line with current research. Its relationship with many diseases (cancer, obesity, nervous system diseases) needs to be revealed by further research.

Conflicts of interest

The authors declare that there are no potential conflicts of interest relevant to this article.

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