TOJSR
The Oxford Journal of Science and Research

ISSN No.: 2581-9380
TOJSR, 2019, 2(1), 43-47

Effect of Different Food Media on the gut Microbiota of the Larvae of Wild Type *Drosophila melanogaster*

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KEYWORDS: *gut microbiota, serial dilutions, colony forming unit*

ABSTRACT: There is an immense importance of the gut microbiota and its effects on the host system. In order to study the same, there is a need to understand the composition of the microbiome. It is a well-known fact that the influence of these resident microorganisms is profound, altering many aspects of host physiology, especially digestive and immune functions. However, the host organism and its diet too alter the microbiome residing in its gut. The host diet gives different growing environment to the organisms. Here, we show that *D. melanogaster* exhibits numerically varied gut microbes when they were grown in different types of food media.

INTRODUCTION

Microbes are naturally found in gut of various organisms. The gut-associated microbial communities usually have a strong effect on host physiology and development. This association between host and the microbial diversity, brings about a stable environment to the host gut. Formationof a healthy gut microbiome depends on the diet of the host (1)

Drosophila has been used extensively as a model organism to study the gut microbes-host interactions. The gut of Drosophila melanogaster is generally dominated by two types of bacteria belonging to the group of Firmicutes and Proteobacteria. These are *Lactobacillus* and *Acetobacter*(2)

Gut microbiota in Drosophila has been reported to affect its development and olfactory behavior Qiao, 2019). Recent studies have also proposed that the chemosensory response influences flies' behavior for favouring some diet over other. These responses are probably dictated by the gut microbiome (3)

The diversity of gut flora is usually associated with the kind of diet of the host organism. Not only the diet leads to growth of certain bacteria in the gut but are also responsible for the maintenance of the same (4).

In this study we have tried to assess the importance and contributions of varied food media in developing the gut flora of third instar larvae of *Drosophilamelanogaster*.

MATERIALS USED AND METHODOLOGY

GROWTH MEDIA

To determine the effect of various food media on gut microbiota in wild type *Drosophila melanogaster*, the flies were cultured in bottles and composition of different media are as follows:

PREPARATION OF LAB MEDIA USED AS CONTROL (WITHOUT YEAST)

Requirements-

- 20 g of Semolina
- 20 g of jaggery,
- 2 g of agar
- 2 ml of propionic acid
- Water

PREPARATION OF LAB MEDIA USED AS CONTROL (WITH YEAST)

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Media was prepared exactly as before except few drops of yeast suspension were added to the culture vials containing media and left untouched overnight. The next day flies were introduced into the vials.

Other media used for this study

- Banana

Banana was used as a natural media to capture and rear the flies. For this, a mashed banana were kept in a vial and once the flies start to feed on them the vial was plugged with the cotton loosely, and the flies captured were used for the experiment purpose.

- Guava

Guava was used as a natural media. The ripe pieces of freshly bought guava was cut and transferred into sterilized culture vial and once the flies start to feed on them the vial was plugged with the cotton.

- Pumpkin

Pumpkin was another media source used for our experiment. Pieces of ripe pumpkin were grated and crushed and kept in a vial. Flies were then introduced into it.

- Grapes

Black Grapes were also used as another food source. Freshly bought black grapes were kept in a vial and flies were introduced into it.

2. PREPARATION OF LB MEDIA AND LA PLATES

PREPARATION OF LB MEDIA

100 ml of Luria Broth (LB) media was prepared as per instruction manual of Himedia.

PREPARATION OF LA MEDIA

A 250 ml conical flask was used to prepare the media. The components used were Luria broth and Agar (LA). 6.25 gm of Luria broth and 3.75 gm of agar was weighed in a weighing balance. LB was first dissolved using distilled water in a beaker. Then agar was added, and the volume was made up to 250 ml using distilled water

3. REARING OF FLIES

Newly emerged male and female flies were introduced into each vial containing different media and allowed to breed for 5-6 days.

4. COLLECTION OF LARVAE

After 4-5 days of rearing of flies, 3rd instar larvae were collected from each vial.

5. DISSECTION OF LARVAL GUT

Six 3rd instar larvae were taken from each media sample one by one using a 1mm brush. The larva taken was placed on a clean glass slide with a drop of sterile saline and the slide was placed under a dissecting microscope. Using two clean dissecting needles the whole gut which appears to be a prominent line in the centre of the larva was aseptically and carefully collected.

6. PREPARATION OF SAMPLE

The collected gut of six 3rd instar larvae for each sample was transferred to a centrifuge tube containing 200 microlitre of sterile LB media. The mixture in the vial was homogenized aseptically in the LAF chamber using a sterile micropipette tip. This was considered as stock solution and it was used for serial dilution for plating onto the LA petri plates.

7. SERIAL DILUTION AND PLATING

A serial dilution is the stepwise dilution of substance in solution. This is carried out in the LAF chamber. 100 microliter of stock solution was taken in vial using sterile microtips and 900 microliter LB media is added to it (10^{-1}dilution). It is mixed well. 100 microliters of solution were taken from 10^{-1}dilution and added to 900 microliters of fresh LB media in the third vial(10^{-2}dilution). It was also mixed well. Both serial dilutions along with 100 microliters of stock were plated for each sample separately into the petri plates (total- 3 LA plates were used for each sample)

Plating was done by spread plate technique in the LAF chamber using sterile L-Shaped glass rod. Each petri plate was marked with the dilution and media information.

The petri plates were left undisturbed for 24 hrs at 37°C and then they were observed for growth of bacterial colonies.

8. GRAM STAINING

Gram staining is usually done to distinguish between Gram positive and Gram-negative bacteria. Gram positive bacteria takes up violet stain due to the presence of thick layer of peptidoglycan in their cell walls, which retains the crystal violet during staining. The Gram-negative bacteria takes up the pink stain due to its thin peptidoglycan wall, this will not retain the crystal violet during decolourising process.

In this experiment the colonies were randomly picked and stained according to following protocol:-

Method:

1. The slide to be stained is prepared by taking a loop full of bacteria from the center of the colony found on the petri plates. It was heat fixed.
2. crystal violet was then added to the slide and incubated for 1 minute. The slide was rinsed with distilled water to remove the extra stain.
3. Gram's iodine was then added, and the slide was left undisturbed for 1 min. It was then rinsed with water (iodine fixes the crystal violet to the bacterial cell wall).
4. Around 1-2 drops of 95% alcohol was added and after 30 seconds it was rinsed with water. (the alcohol decolourises the sample if it is Gram negative, removing crystal violet).
5. Safranin drops was then added and kept for 1 minute. It was thoroughly rinsed with distilled water.
6. The slide was allowed to dry and then observed under microscope for the presence of violet or pink coloured bacteria. The shape of the bacteria can also be observed.

RESULTS AND DISCUSSION

1. The colonies obtained by dissecting the gut of third instar larvae of *D. melanogaster* are tabulated as follows.

Table 1- at the end of the paper

The results are shown graphically (Fig-1)

2. Thecolonies thus found were then Gram stained to understand the bacterial population diversity of gut of third instar larvae (Table-2)

Table-2

Discussion-

The experimental study is conducted to see the effect of different food sources ongut microbiome of larvae of wild type *Drosophila melanogaster*. It is highly essentialto interpret the presence of gut microbiome and its composition. It can be deducedfrom the experiment, that Pumpkin is the favourable media for gut microbiome (Table 1, Fig-1)

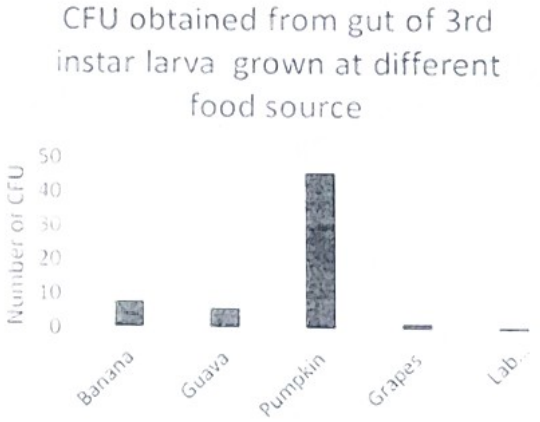

Fig-1. Number of bacterial colonies obtained from the gut of third instar larvae of *D. melanogaster*.

Also, it favours growth of Gram-negative family such as *Acetobacteraceae* which, has been evaluated with the help of Gram-staining (Table 2). Guavafavours the growth of Gram-positive family such as *Lactobacillaceae*. Banana andGrapes promote the growth for both, but Banana is seen to be more favourable for*Lactobacillaceae*and Grapes for *Acetobacteraceae*. This variation is probably seenbecause of the acetic acid and lactose content in the food media. As deducedfrom the experiments, the majority of microbiome in *Drosophila*gutcomprises of *Acetobacter* and *Lactobacillus* although more work needs to be done to confirm the same.

There are some well known studies about the microbial interactions. Consideringthe fact that there is a variety of microbiota present in the gut, there is a strong possibility of interspecies and intraspecies interactions within the gut (5).

Probability of microbiome gettinginvolved in the molecular & cellular processes are also explored (6).However more confirmatory results need to be obtained to reach a conclusive decision. This will only open possibilities of exploiting the gut-host interaction studies in more details infuture.

ABBREVIATIONS

CFU- Colony forming unit, TNTC- Too numerous to count

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FOOD MEDIA	100 microlitre stock	10^{-1}dilution plate	10^{-2}dilution plate
Banana	TNTC*	71	08
Guava	TNTC	28	06
Pumpkin	TNTC	TNTC	46
Grapes	304	59	02
Lab media (without yeast)	22	04	01
Lab media (with yeast)	TNTC	TNTC	~1488

Table 1- Number of colonies obtained after the larvae were grown in different food media.

Table-2-

MEDIA	Colony 1	Colony 2	Colony 3	Colony 4
Banana	Positive	Positive	Negative	-
Guava	Positive	Positive	Positive	Positive
Pumpkin	Negative	Negative	-	-
Grapes	Negative	Positive	Negative	-
Lab media (without yeast)	Negative	Negative	Negative	-
Lab media (with yeast)	Negative	Positive	Negative	-