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Effect of different concentrations of dietary yeast on the behaviour and the gut microbiota in the third instar larvae of wild type *Drosophila Melanogaster*

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Abstract: *Drosophila melanogaster* is a good model organism to understand the physiology of gut and its interactions with microorganisms. Hence the effect of gut microbiome in *D. melanogaster* can be studied to understand the importance of nutrition on its physiology. In this study the effect of nutrient in the diet was studied in the third instar larvae of *D. melanogaster*. Yeast is a key part of the diet of *D. melanogaster* and has been known to influence the gut microflora. With our study we propose that a diet enriched with yeast improves the crawling behaviour as well as the gut flora of the third instar larvae.

Keywords: *Gut Microbiota, yeast, Bacteria, Foraging and Locomotion, Crawling Behaviour*

INTRODUCTION

The common fruit fly, D. melanogaster is most used model organisms for biological sciences. The fly has become indispensable for research as it has much similarity with genomes of higher organism. Numerous molecular tools have only made it proficient in understanding various mechanisms regulating physiological function of this organism. It has been extensively used as a robust model organism in genetics, developmental biology, aging, and other areas of biomedical research [1]. Nutritionists have begun to consider *Drosophila* as a useful organism even in food and nutrition research [2,3].

It has long been proved that microorganisms play a key role where nutrition of animals are concerned. Some of these microorganisms are in obligate symbiotic relationship with its host and such microbes include both bacteria and fungi. Most of the microbes inhabit the insect digestive tract and aid in digestion as well as detoxification. Essential amino acids, vitamins and other components can also be synthesized by these microbes within their host. It has also been suggested that yeasts provide a source of dietary protein which usually is absent in ripening fruit and even though the sugar present in these fruits are important for obvious nutrition purpose but protein too play a role in improving fitness, fecundity and life span in *Drosophila*. Behavioral studies conducted suggests that *Drosophila* larvae show preference for protein rich diet which can be provided by yeast probably to maintain their fitness and growth [4,5]

The gut of *Drosophila* like any other organism plays a significant role not only in digestion and absorption of nutrients but also in maintaining physiological homeostasis. The larvae of *Drosophila* like adults are particular about their food and the olfactory sense seems to dominate in its foraging behaviour.[6]

Drosophila is known to consume microorganism laden food and show special liking for yeast rich food [7,8]. Third instar larvae of *D. melanogaster* usually show two typical behavior patterns related to foraging and pupation [9].

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In this study we have attempted to analyze the effect of yeast as a protein rich source of diet which can influence the foraging behavior in the *Drosophila* by studying the larval locomotion. We have also analyzed the gut microbiome of larvae to assess the quantitative change in microorganisms and to relate whether such change can be reflected in the physiological behavior of the organism.

METHODS

1.PREPARATION OF LAB MEDIA USED AS CONTROL (WITHOUT YEAST)

About 20 gm of semolina was weighed and added in 200 ml of boiling distilled water taken in a beaker to make a slurry. It was continuously stirred with a glass rod. 20 gm of jaggery was then added to the mix. After getting the right consistency 2 gm of agar was added. At last the heat source was removed and 2 ml of propionic acid was added. The prepared media was then cooled down and transferred into sterilized vials carefully. The excess moisture at side of the vials was wiped out using a sterile cotton and forceps.

PREPARATION OF LAB MEDIA USED AS CONTROL (WITH DIFFERENT CONCENTRATION OF YEAST)

The media was prepared similarly as mentioned above. However various concentration of yeast solution, viz. 1%, 2.5%, 5% and 10% were added to this culture media.

2. PREPARATION OF LB MEDIA AND LA AND MRS AGAR PLATES

The Luria Broth, Luria agar and De Man, Rogosa and Sharpe agar were prepared according to the manufacturer (Himedia) instructions. MRS is a selective culture medium designed to favor the luxuriant growth of *Lactobacilli*.

3. REARING OF FLIES

Newly emerged male and female flies were introduced into each vial containing media with different concentration of yeast and allowed to breed for 10-12 days.

4. COLLECTION OF LARVAE

After around 10 days of rearing of flies, 3rd instar larvae were collected from each vial.

5. DISSECTION OF LARVAL GUT

Ten 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 solution 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 center of the larvae was aseptically and carefully collected.

6. PREPARATION OF SAMPLE

The collected gut of ten 3rd instar larvae for each sample was transferred to a centrifuge tube containing 200 micro litres 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

About 100 microliter of stock solution was taken in vial using sterile microtips and 900 microlitre LB media is added to it (10^{-1} dilution). Then 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). The process of serial dilution was repeated till dilution of 10^{-4} was reached. Among all serial dilutions, 100 microliters from 10^{-2} and 10^{-4} dilutions was plated for each sample separately into the petri plates. However only 100 micro litres from 10^{-1} dilutions was plated for each sample separately into the petri plates containing MRS agar.

The petri plates were left undisturbed at 37^{o}C for 24 hours for LA plates and 48 hours for MRS plates and then they were observed for growth of bacterial colonies.

8. GRAM STAINING

Gram staining process that was followed was essentially as described (10)

9. BEHAVIOURAL ASSAY

For behavior assay of third instar larvae of *Drosophila melanogaster*, 15 cm petriplates

were used. 2% and 2.3% of agar were poured into the plates for crawling assays.

I. Behavior assays

a. Larval Crawling assay

- The assay was performed as essentially described by Nichols et al, 2012 [11].
- 15 cm Petri dish containing 2% agarose (previously poured and allowed to harden) was placed over graph paper with a 0.2 cm^2 grid.
- The 3rd instar larvae were transferred with the help of brush one at a time. Count number of grid lines crossed in 1 minute.
- At least 5 larvae were used from each experimental vial and average value was calculated
- The experiment was repeated thrice

b. Foraging and locomotion assay

- The assay was performed as described by Min and Condron, 2005 [12] with few changes
- The apparatus was a 100 mm 15 mm dish composed of 2.3% agar with a circular hole dug out in the center. A small amount of cold water-yeast pastes (50:50) was spread along the edges of the hole prior to running the assay.
- In addition, the larvae were gathered and put onto a spatula for transfer onto the plate with a brush.
- At the start of the assay, the larvae were washed in distilled water, placed and spread out 5 mm from the edge of the plate.
- The assay was run for 60 min.
- The larvae were scored by counting at least half the number to reach the edge of the yeast
- The experiment was repeated twice

RESULTS AND DISCUSSION

Behavior studies- Foraging and locomotion result implied that nutrition is important factor to stimulate olfactory sensory organs in case of organisms to obtain food. Our result suggests that an optimum amount of yeast (1%) is required for best crawling movement (Fig-1).

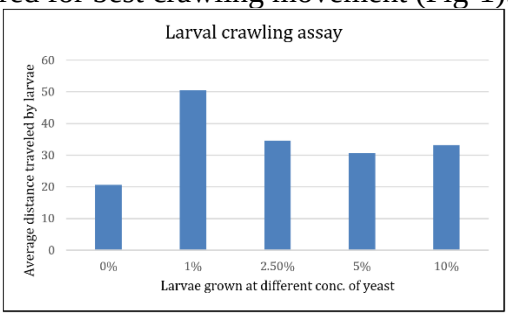

Fig. 1 – Average distance traveled by third instar larvae of *D. melanogaster* when grown in different concentration of yeast

In another behaviour assay we found that larvae reared without yeast showed the maximum crawling behaviour to reach to the yeast source faster than the larvae already fed with it (Fig-2).

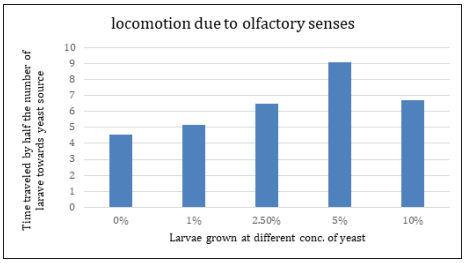

Fig. 2 – Time taken by third instar larvae of *D. melanogaster* (grown in different concentration of yeast) to reach the yeast source (see text for more detail)

Our result suggest that yeast starved larvae showed heightened sense of olfaction allowing it to crawl faster towards the yeast source compared to the larvae which were reared in various concentrations of yeast. This indicates that sensory organs play a significant role in foraging behaviour specially in case of larvae of *D. melanogaster*. Hence it can be deduced from these experiments that an optimal quantity of

yeast can improve the crawling behaviour which is related to foraging and pupation. Also, olfactory senses do help larvae to find a preferred food source, in this case a protein rich yeast.

Quantity of microorganisms in the gut- Gut microbiome assessment was conducted to assess the effect of yeast on the enrichment of gut microbes in third instar larvae. Interestingly it was seen that larvae grown with 5% yeast showed an abundance of bacteria in their gut (Table 1). Hence, we conclude that yeast in the diet allows the gut microbes to flourish.

Table 1- The number of bacterial colonies obtained in the serially diluted plates

MEDIA WITH DIFFERENT CONC. OF YEAST	CFU IN 10^{-2} DILUTION PLATE	CFU IN 10^{-4} DILUTION PLATE
0%	TNTC	77
5%	TNTC	216
10%	TNTC	37

On further investigations we found that it is the Gram-positive bacteria which dominates the flora of the gut. However, Gram negative bacteria were also found. The result was proved by using MRS agar plates which are specific for *Lactobacillus*, a Gram-positive bacterium (result not shown).

It is the requirement that further in-depth studies need to be taken up to understand the relationship between behavior of an organism and its food source. Also, it will indeed be interesting to unravel more of the mysteries behind the diet and gut microbiota using various other model organisms for genetic studies.

Acknowledgement

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ABBREVIATIONS

TNTC - Too numerous to count; CFU - Colony forming Unit

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