Identification normal external and internal bacteria and fungi in larvae and pupae *Papilio polyetes*

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Abstract. Interaction between insects and microorganism has been occurring thousands years ago. The numerous ones are bacteria that live inside insect, but there are possibility also to finding other microorganisms like fungus. It can be becoming a good atmosphere. It is also indicating healthy of an insect. If there were existing foreign microbiota, it can be concluded that the insect was sick. The Methods of this research are examining bacteria external and internal with Nutrient Agar (NA) as Media under following the method of Caoili (2003) with investigating external, fore gut, mid gut and hind gut. The result showed that weather in larvae 5th of *Papilio polyetes* and its pupae on external examine. The appearance of bacteria gram + were more numerous than gram – one. While in the fore gut, mid gut and fore gut were dominated by bacteria gram+, its correlated with the fact that its alkaline. Their presence influenced by habitat, morphology and feeding habits. The conclusion the symbiosism existence between *P. polyetes* with external and internal microfloral appear to assist from protection and metabolism process.

1. Introduction

An insect is a place for various habitats for microorganisms. The most reside in habitats for microbial colonists is cuticle and the gut. Microorganisms that can break the exoskeleton or gut wall can gain a way into the hemocoel and a further set of habitats provided by insect cells. As a human-like human, an insect can be colonized by commensal and advantage of microorganism and this microorganism place 10 % of insect biomass [1]

The first physical barrier to microbial infection in an insect is chitin and it is a substrate of so many microorganisms. Such as on Drosophila melanogaster more than 1,000 culturable bacterial cells interact with the body surface of, two orders of magnitude fewer than are borne internally by flies. Some factors limiting microbial populations on the insect cuticle can include physical disturbance (ecdysis and grooming behavior) as well as antimicrobial secretions (the meta-pleural glands of ant) [2]. The extent to which cuticle-associated bacteria can proliferate and form stable communities, as occurs on the human skin, is largely unknown [3].

Some properties of the insect intestine are favorable occupancy by microorganisms, including ease of access for food for microbial cells, availability of nutrients, and protection from various stresses of the external environment. Nevertheless, the insect gut poses multiple challenges for microorganisms ingested with the food, including unfavorable physicochemical conditions (e.g., oxygen content, pH, redox potential) in the gut lumen, secreted digestive enzymes and immune-related compounds, physical disturbance caused by peristalsis of gut contents, and loss of habitat at insect molts and
metamorphosis [4]. The gut habitat for microorganisms vary among insect groups and with the life stadia of the insect and region within the gut, describing the great variation in the insect gut.

2. Methods
Imago of *P. polyetes* obtained from rearing place that was taken from Subang and Lembang Bandung Indonesia and Brought to Reserach laboratory in Biology FPMIPA UPI Bandung from February – June 2017.

2.1. *P. polyetes* Collection
Eggs were collected from citrus Plant in Subang UPI area. Eggs thus obtained were put on the leaves of the host plant. They have then transferred into a container jar already containing the hostplants. Eggs that have been emerging started to consume the host plants. Larvae were provided citrus leaves to allow them to become pupae later.

2.2. Identification of fungal habitat’s in *P. Polyetes*
*P. polyetes* were place in petri disc and examined cuticular, foregut, mid gut and hind gut. Each butterfly was separated in to its parts. Afterward they removing the cuticular with forceps, the bodies were disected and separated. They were transfer to PDA medium and observe for 3-5 days [5].

2.3. Identification of bacteria habitat’ in *P. Polyetes*
Place imago in a jar, with a flock of cotton impregnated with ethyl ether for 1 or 2 minutes, or longer, if necessary, for anesthesia. Remove the imago from the jar and close its oral and anal openings with sewing silk or cotton thread. Dip the imago for 5 minutes in germicide solution. Shake well during this time Rinse the larva or pupae or imago in the three changes of sterile distilled water, Dip the larva in a culture tube, containing fluid thioglycollate medium, shake, then remove the insect using a flame wire loop. The insect may be stored in a sterile Petri dish, in the refrigerator, if desired. Place the specimen in a sterile Petri plate and cut the integument along a longitudinal. Incubate the medium at room temperature. Record the change in the medium after 24, 48 and 72 hours. Repeat (1-6) using a second treatment [6].

3. Results and Discussion
The bacterial colony which was obtained from external surface and from the mid-gut of *P. polyetes* instar 5 and pupae, almost had similar characteristics even though they varied in number. There are three kinds of colonies found on the external surface and in the mid-gut, hindgut and for gut respectively (figure. 1). These colonies show the similar characteristics in the density, chromogenesis and in the elevation which only one internal bacterial colony shows dissimilarity which is flat while others are convex. Nevertheless, if we compare all of the characteristics of the colonies we can find that they are different from each other (Table 1 and Table 2).
Figure 1. Bacterial colony on surface and in intestines’ *P. polyetes* on instar and pupae stage

Table 1. Characteristics of bacteria of *P. polyetes* on fifth instar

| Insect stage | Date of isolation | Culture medium | Source of bacteria and strain | Form and size | Elevation | Surface | Margin | Density | Chromogenesis | Number | Motility | Form and arrangement | Gram stain/sporulation |
|--------------|------------------|----------------|-----------------------------|---------------|-----------|---------|--------|---------|--------------|--------|---------|----------------------|------------------------|
| Instar 5     | March 5, 2017    | Nutrient agar  | External surface 1          | Circular      | Convex    | Smooth  | Entire | Opaque  | creamy       | 2      |         |                      | Gram variable           |
|              |                  |                | Mid-gut                    | Circular      | Convex    | Smooth  | Smooth/entire | Opaque     | Yellow    | 2      |         |                      | Gram variable           |
|              |                  |                | Hind-gut                   | Circular      | Flat      | Smooth  | Smooth/entire | Opaque     | Creamy    | 3      |         |                      | Gram variable           |
|              |                  |                | Fore-gut                   | Circular      | Flat      | Smooth  | Smooth/erose  | Opaque     | White     | 1      |         |                      | Gram variable           |

The Gram variable bacteria were found predominantly both on the external surface and in the mid-gut. The Gram staining is based on the ability of bacteria cell wall to retaining the crystal violet dye during solvent treatment. The length of decolorizing is critical in differentiating the Gram-positive bacteria from the Gram-negative bacteria. A prolonged exposure to the decolorizing agent will remove all the stain from both types of bacteria. Some Gram-positive may lose the stain easily and therefore appear as a mixture of Gram-positive and Gram-negative bacteria (Gram-variable) [7].

In many insects, the hindgut is the gut region bearing the largest microbial populations. In particular, the ileum (the region between the proximal pylorus and distal rectum) is a relatively benign environment, in that it lacks the digestive enzymes of the midgut and, for many terrestrial insects, the desiccation stress of the distal hindgut, where water is actively resorbed from the lumen into insect tissues.
Table 2. Characteristics of bacteria of *P. polyetes* of pupae

| Insect stage | Date of isolation | Culture medium |
|--------------|------------------|----------------|
| Pupa         | March 5, 2017    | Nutrient agar  |

| Source of bacteria and strain | Form and size | Elevation | Surface | Margin | Density | Chromogenisis | Number | Motility | Form and arrangement | Gram stain/sporulation |
|-------------------------------|---------------|-----------|---------|--------|---------|---------------|--------|----------|----------------------|------------------------|
| External surface 1            | Circular      | Convex    | Smooth  | Entire | Opaque  | creamy        | 2      |          |                      | Gram variable          |
| Mid-gut                      | Circular      | Convex    | Contoured | Smooth/ entire | Opaque  | Yellow        | 2      |          |                      | Gram variable          |
| Hind-gut                     | Circular      | Flat      | Smooth  | Smooth/ entire | Opaque  | Creamy        | 3      |          |                      | Gram variable          |
| Fore-gut                     | Circular      | Flat      | Smooth  | Smooth/ erose | Opaque  | White         | 1      |          |                      | Gram variable          |

Microbial function and growth may also play a role as a symbiotic relationship with the hindgut in the filtrate from the Malpighian tubules. In many insects, the ileum displays no evident morphological or physiological adaptations to maintain microorganisms. In many insect taxa, the cuticle of the hindgut is thrown into spines and plates, and microorganisms can preferentially adhere to these structures [4].

Table 3. Characteristics of fungi of larvae *P. Polyetes*

| Insect stage | Date of isolation | Culture medium |
|--------------|------------------|----------------|
| Larvae and Pupae | March 5, 2017    | Potatoes dextrose Agar |

| Source of Fungi | Shape | Color | Diameter | Hyphae | Asexual spore | Genus | Species  |
|-----------------|-------|-------|----------|--------|---------------|-------|----------|
| Instar 5        | A loose texture | White | 4.3 cm | Sporangiospore | segmented | Beauveria | B. bassiana |
|                 |       |       | 4.1 cm | Sporangiospore | segmented | Aspergilus | Aspergilus sp |
|                 |       |       | 4.3 cm | Sporangiospore | segmented | Penicilium | Penicilium sp |
|                 |       |       | 4.1 cm | Sporangiospore | segmented | Penicilium | Penicilium sp |
|                 |       |       | 4.0 cm | Sporangiospore | segmented | Beauveria | Beauveria sp |
|                 |       |       | 4.4 cm | Sporangiospore | segmented | Aspergilus | Aspergilus Sp |
|                 |       |       | 4.3 cm | Sporangiospore | segmented | Penicilin | Penicilin Sp |
|                 |       |       | 4.3 cm | Sporangiospore | segmented | Penicilin | Penicilin Sp |
| Pupae           | A loose texture | Greenish green | 4.2 cm | Sporangiospore | segmented | Aspergilus | Aspergilus sp |
The fungi colony which was obtained from external surface instar 5 and pupae of *P. polyetes* (Table 3), almost different in number of instar 5 and pupae. On instar 5 it has more fungi than pupae due higher immune respond on older stage. It assume that the existense fungi act also as a protection.

**Figure 2.** Cuticular External fungal mikrosopis dan makroskopis of fifth instar of *P. Polyetes* (a) and (b) *B. Bassiana*, (c) and (d) *Penicium* sp, (e) and (f) *Aspergillus* sp

Cuticular structures that show colony by certain microorganisms that live in many insects (Figure 2). For xexample, the mycangia, i.e., invaginations intestines can be occupied by fungi in adult insects,
that can be considered as culture habitat in which fungi required by the insect's offspring are live and protected against abiotic factors and contamination by other microorganisms [8].

4. Conclusion
The existence of normal microflora in P. polyetes indicate correlation with some aspects such as food and protection, On external cuticle most finding fungi and bacteria’s function act as protection while in the internal organ such as foregut, midgut and hindgut’s role as assist metabolism process and protection as well.

5. References
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