Select Bacterial Species Establish and Maintain Functionally Essential pH Gradient Profile within the Mosquito Larval Gut

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ABSTRACT

Mosquitoes are known to harbor a highly functional gut microbiota, which strongly influence its vector competence through regulating nutrition, development, immunity, fecundity, reproduction, resistance to insecticides, etc. Therefore, a thorough understanding of the function of gut microbiota is required to develop novel and more effective vector-control tactics. With an aim to investigating the role of gut associated bacteria in the larval physiology, we conducted experiments using axenic and gnotobiotic larvae of Aedes albopictus. Our results show that gut associated bacteria along with an intact Carbonic Anhydrase (CA) activity is essential for establishment and maintenance of the characteristic gut pH profile of larvae, which in turn is essential for its digestion. Further, we documented that the requirement of 'live' bacteria for establishment of gut pH profile cannot be replaced by either bacterial cell-free supernatant or lysate. Interestingly, we observed that not all the members the gut microbiome has the capability to establish gut pH profile, suggesting that different members of the microbiome possibly have different roles in the larval physiology. Our findings thus reveal an important mechanism through which bacteria modulate different phenotypes in mosquito, which in turn impacts their life-history traits.

KEYWORDS

Aedes, gut microbiota, gut pH, axenic, gnotobiotic,

INTRODUCTION

Insects are considered the most diverse and adapted life forms on the earth. Certain insects have evolved strategies for maximal exploitation of available resources through switching of ecological niche and feeding habits at various stages of their life cycle (Douglas, 2011; Gullan & Cranston, 2010). The natural ability of insects to feed upon and digest a wide variety of substrates available in different habitats has been linked to the unique physiology and microbiology of its gut; and thus, insect gut has been a subject of intense research for long (Serrato-Salas & Gendrin, 2023; Schmidt & Engel., 2021; Martinson & Strand, 2021; Gullan & Cranston, 2014). According to feeding habits, gut milieu in herbivorous insects is generally more alkaline as compared to carnivorous insects (Harrison, 2001; Yonge, 1937). Surprisingly, in larvae of certain endopterygote insects belonging to the orders Diptera, Lepidoptera, Coleoptera and Trichoptera, gut pH levels reach exceptionally high (pH 9.0-12.0), while, extremely low pH have been documented in several others (Gullan & Cranston, 2014; Clark, 1999a; Dow, 1984; Berenbaum, 1980; Dadd et al., 1975; Waterhouse, 1949). Like all other organisms, acid-base homeostasis in insects is indispensable for various biochemical and physiological processes including digestion, membrane transport of nutrients, enzyme-substrate reactions, vitellogenesis, secretion and functioning of hormone, excretion of nitrogen compounds, pesticide metabolism, pathogen infection etc., thereby disturbance in acid-base homeostasis severely affects survival, growth, development, and vector competence (Harrison, 2001).

Among other insects, mosquitoes have remained at the centerstage of vector research, owing to their significance in public health. Larval stages have been extensively studied, as a potential target for vector-control strategies, which has led to revelation of several intriguing physiological characteristics of its gut (Dow, 1984; Dadd et al., 1975; Yonge, 1937). Mosquito larvae belonging to various genera display a wide longitudinal pH gradient profile within a short span of the gut, that too without any morphological barrier (Boudko et al., 2001; Dadd et al., 1975). Mosquito larval midgut includes an extremely alkaline (pH~10.0-12.0) anterior midgut (AMG), immediately flanked by neutral/ near-neutral gastric cecum (GC) and posterior midgut (PMG), on either side [**Figure 1**]. The level of alkalinity seen in the AMG of these larvae are rarely met in any other living organism (Corena et al., 2002). Furthermore, this gastric arrangement is remarkably contradictory to other Dipterans (including Drosophila) and metazoans, where optimal digestion initiates at an acidic to highly acidic environment (Overend et al., 2016; Smith

et al., 2007; Yonge, 1937). The characteristic gut pH gradient profile in mosquitoes (also in silkworms) has been shown to provide suitable ionic conditions for sequential functioning of digestive enzymes (with different pH optima), necessary for efficient digestion of complex feed materials (Douglas, 2015; Gullan & Cranston, 2010; Berenbaum, 1980; Dadd et al., 1975; Horie et al., 1963).

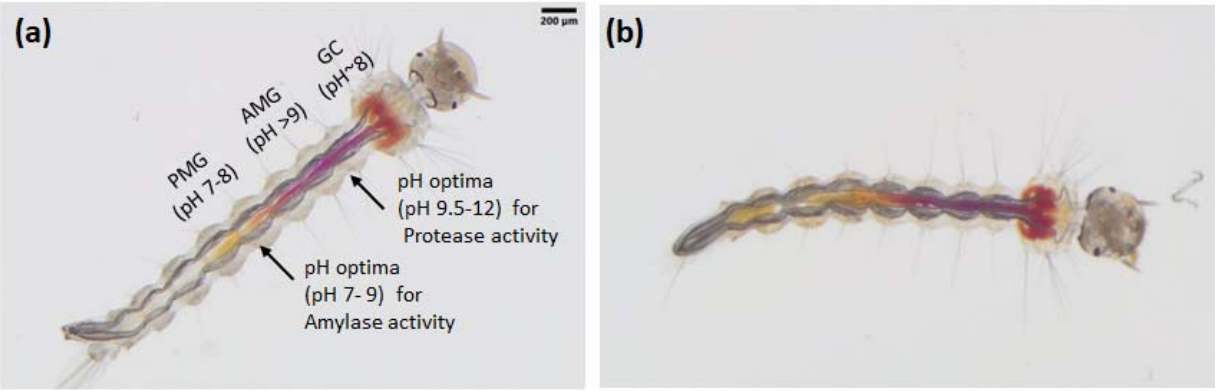


Figure 1. Characteristic gut pH profile as seen in (a) monoculture (Bacillus) associated gnotobiotic and (b) conventionally reared *Ae albopictus* larva, fed with Phenol Red pH indicator dye. Functional significance of the distinct pH regions of the midgut is shown (following Dadd, 1975) in panel (a).

Conversely, it is also well known for long that mosquito larvae harbor diverse microbiota within their gut, which essentially modulate a wide variety of phenotypes that are associated with the evolutionary success of the host (Schmidt & Engel., 2021; Strand, 2017; Douglas, 2015; Chao & Wistreich, 1960; Buddington, 1941; Yonge, 1937). Although, an imperative and positive role of bacteria in larval survival, growth and development is principally accepted, exclusive requirement of 'live' bacteria for implementation of such functions has been seriously debated (Schmidt & Engel., 2021; Steven et al., 2021; Coon et al., 2020; Correa et al., 2018; Strand, 2018; Coon et al., 2017; Coon et al., 2014). While a group of researchers claim that 'live' bacterial signals larval growth and molting, others have challenged the proposition (Correa et al., 2018; Valanzia et al., 2018a, b; Coon et al., 2017). In addition, other researchers have also suggested a vital role of bacteria in providing the larvae with vital nutrients, which the larvae cannot synthesize (Serrato-Salas & Gendrin, 2023; Douglas, 2009; Buddington, 1973; Dadd, 1973). Based upon extensive review of literature, insect microbiota has been proposed to carry out two core functions, namely- nutrition and defense (Douglas, 2011).

Nevertheless, comparative studies conducted on axenic and gnotobiotic cultures have demonstrated that mosquito larvae grown under bacteria-free or antibiotic treated conditions along with nutritionally complete diet, may survive for limited periods, but growth and development in such larvae is either stalled at an early stage or is severely delayed; whereas several bacterial species of the gut microbial community or non-community members could individually rescue normal growth and development of axenic larvae, even without additional nutrient supplements (Wu et al., 2023; Romoli et al., 2023; Martinson & Strand, 2021; Steven et al., 2021; Valzania et al., 2018a, 2018b; Correa et al., 2018; Strand, 2018; Strand, 2017; Coon et al., 2017; Coon et al., 2014; Chouaia et al., 2012; Buddington, 1941). These observations clearly suggested that larval digestion, growth, and development, are tightly regulated through an evolutionary conserved mechanism, essentially involving gut-bacteria (Schmidt & Engel., 2021; Strand, 2018; Strand, 2017). However, in the context of exponentially growing volumes of scientific literature on genetic diversity of mosquito larval gut microbiota and its association with diverse phenotypic consequences, it is quite surprising that information available on mechanism of mosquito-gut microbiota association is extremely scanty. Considering the accumulating evidence on the role of gut microbiota in development, reproduction, fecundity, vectorial capacity etc., elucidation of functional significance of mosquito-gut microbiota interaction in larval physiology and metabolism are essential for controlled manipulation of microbiota as a potential vector-control strategy (Arellano et al., 2023; Gomez et al., 2022; Cansado-Utrilla et al., 2021; Gao et al., 2020; Huang et al., 2020; Scolari et al., 2019).

While studying the effects of bacteriophage treatment on monoculture gnotobiotic *Aedes albopictus* larvae (gut colonized with *Bacillus* sp.), we incidentally observed that the characteristic pH gradient profile in larval gut is severely disturbed after treatment with lytic bacteriophages. In sharp contrast, pH gradient remained intact in mock treated (no bacteriophage) larvae [**Figure 2**]. This observation led us to hypothesize a direct involvement of gut bacteria in larval gut pH profile. Subsequently, we tested our hypothesis using an *Ae. albopictus* axenic/ gnotobiotic experimental culture model.

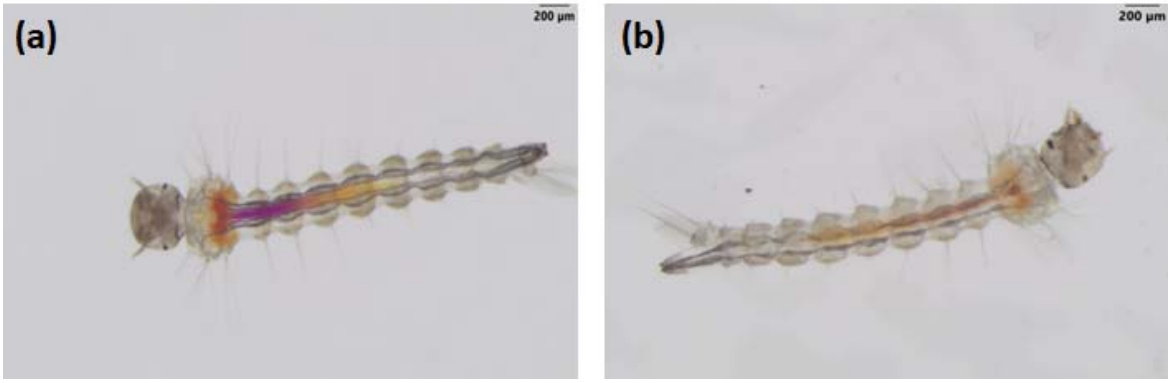


Figure 2. Gut pH profiles in (a) mock treated (no bacteriophage) mono-associated gnotobiotic larvae; (b) bacteriophage treated gnotobiotic larvae.

Our research reveals that gut bacteria plays a key role in establishment and maintenance of the characteristic pH profile in larval gut, which in turn is related to larval digestion, growth, and development. In this communication, we discuss the significance of our findings under the light of available literature and propose probable mechanisms for this aspect of host-microbe interaction.

MATERIALS & METHODS

Generation of axenic, gnotobiotic larvae and gut pH visualization

Axenic larvae were obtained from surface sterilized eggs, following an earlier protocol with slight modifications (Romoli & Gendrin, 2020). Sterility of axenic larvae were verified by culturing specimen larval homogenates on NA plates for 48 hrs. A subset of axenic larvae was then used to generate monoculture gnotobiotic larvae by colonization with a strain of *Bacillus* sp. (GenBank Accession No. OR513030), which we previously isolated from laboratory reared *Ae. albopictus* larvae. Colonization of bacteria in gut was carried out by adding fresh bacterial culture (@ 10^6 CFU mL^{-1}) to the axenic larval culture plate-wells. After overnight incubation, larvae were collected, washed thoroughly with sterile water, and transferred to fresh plate-wells holding sterile water. Stable bacterial colonization was confirmed by culturing homogenates from thoroughly washed specimen gnotobiotic larval on LA plates for 48 hrs, followed by morphological examination of the colonies.

All larval culture experiments were carried out at 27±2°C with 80±10% RH. All bacterial manipulations were performed inside class II (A2) biological safety cabinet, and standard clean laboratory procedures were strictly followed.

Larval gut pH profile was visualized using 0.1 % w/v phenol red (PR) pH indicator dye, as described previously (Overend et al., 2016; Boudko et al., 2001; Dadd et al., 1975) with minor modifications. PR was added to larval culture plate-wells and larvae were allowed to ingest the dye for at least 15 mins before visualization.

Study of gut pH profile in experimentally treated larvae

Gut pH profiles in axenic, gnotobiotic (monoculture, Bacillus), antibiotic (gentamycin @ 20 µg/mL) treated gnotobiotic, and acetazolamide (@ 100 µM) treated gnotobiotic larvae were documented using a stereomicroscope (Olympus SZX16). Larvae from *Ae. albopictus* culture, conventionally reared in open tray under standard laboratory conditions were included as natural controls.

Subsequently, to examine if continual presence of gut bacteria is needed for maintenance of already set-up gut pH profile, we studied the gut pH profiles before and after treatment with antibiotics (gentamycin @ 20 µg/mL).

We also studied the gut pH profiles in axenic, axenic with bacterial culture supernatant, axenic with bacterial cell lysate and gnotobiotic larvae to verify if component(s) from bacterial cultures (supernatant or bacterial lysate) could substitute the requirement of 'live' bacteria for establishment of gut pH profile. Larval length was documented to compare the variation in growth in larvae from different experimental groups.

Gut pH profile in gnotobiotic larvae colonized with different bacteria

We studied the gut pH profile in various monoculture gnotobiotic larvae, separately colonized with *Bacillus sp.* (GenBank Accession No. OR513030), *Aeromonas sp.* (OR513036), *Microbacterium sp.* (OR513035), *Leucobacter sp.* (OR513031), *Lysinibacillus sp.* (OR513032), to examine if any member of the gut-microbiota could establish pH gradient profile or certain selected members have the capability. Larval length was documented to compare the variation in growth in larvae from different experimental groups.

RESULTS & DISCUSSION

Our results show that, in sharp contrast to the typical gut pH profile seen in conventionally reared and gnotobiotic larvae, no such profile was observed in axenic, antibiotic treated, or acetazolamide treated larvae [**Figure 3**]. While antibiotic treated larvae presented the bactericidal effects of gentamicin on gut colonized bacteria, acetazolamide treatment presented the effects of carbonic anhydrase (CA) inhibition in presence of gut bacteria.

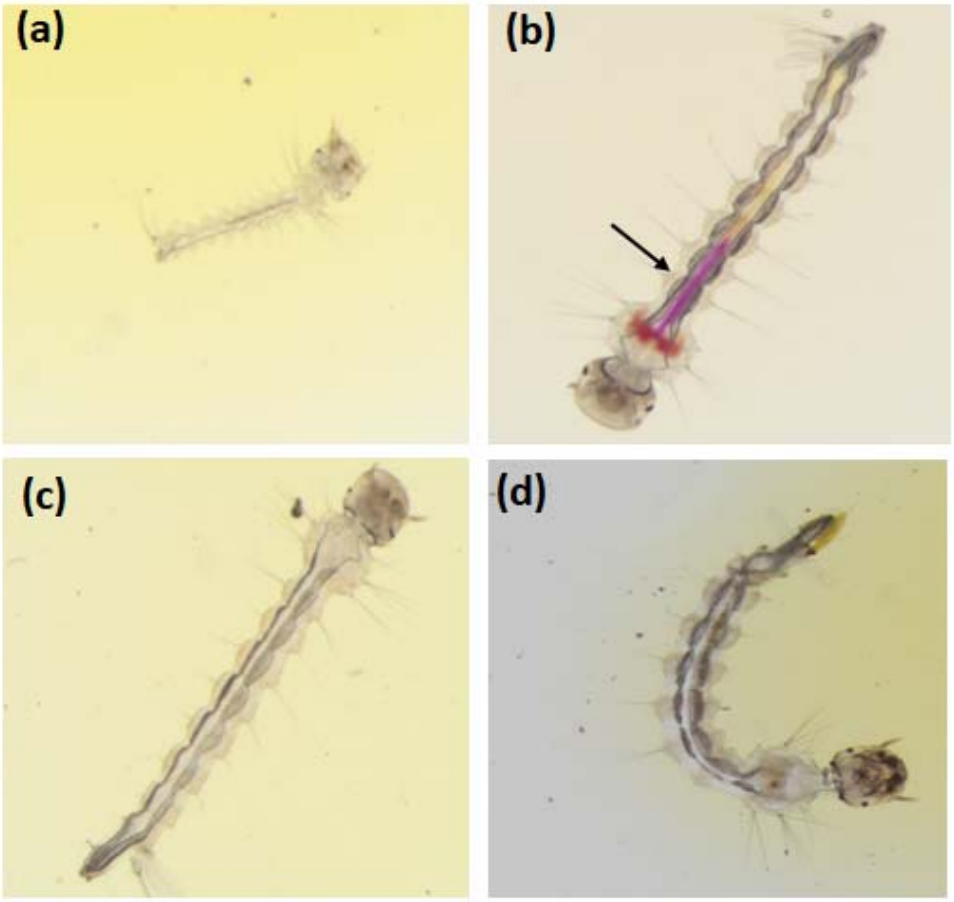


Figure 3. Gut pH profiles in (a) axenic; (b) gnotobiotic; (c) acetazolamide treated gnotobiotic and (d) antibiotic (Gentamycin) treated gnotobiotic larvae.

We further noted that well-established gut pH profile in gnotobiotic larvae is completely abolished after treatment with antibiotic, suggesting that establishment and maintenance of gut pH profile by gut bacteria is not a typical '*hit and run*' phenomenon, rather requires continual presence of bacteria [**Figure 4**].

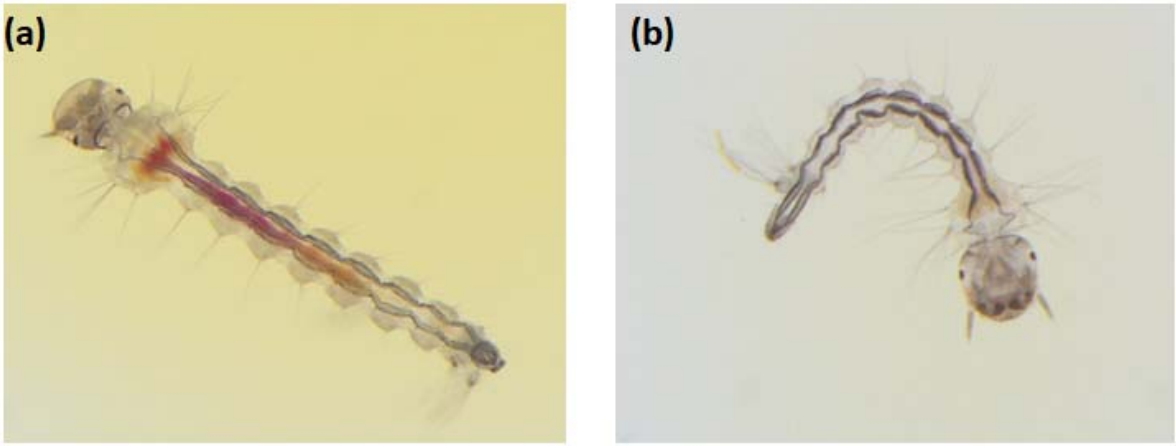
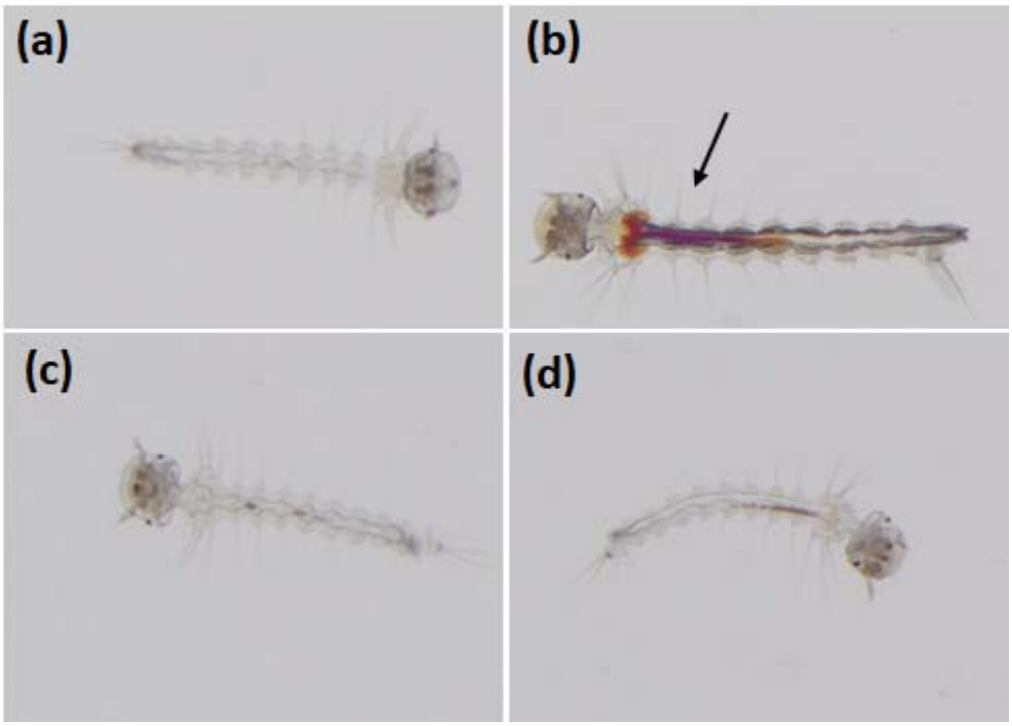


Figure 4. Gut pH profiles in (a) gnotobiotic larvae (before antibiotic treatment); (b) gnotobiotic larvae 24 hrs after treatment with antibiotic (Gentamycin).

Our results also demonstrate that the requirement of live bacteria for establishment of gut pH profile cannot be replaced by either bacterial culture supernatant or bacterial cell lysate [**Figure 5**].



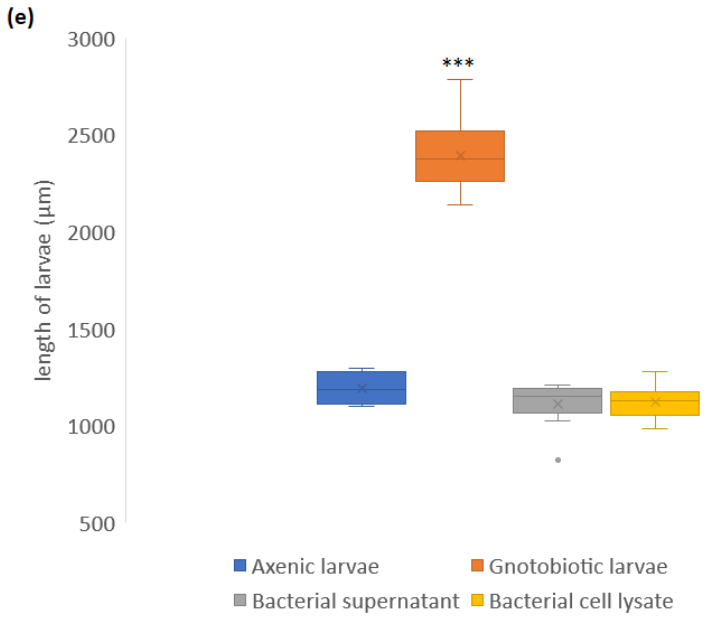
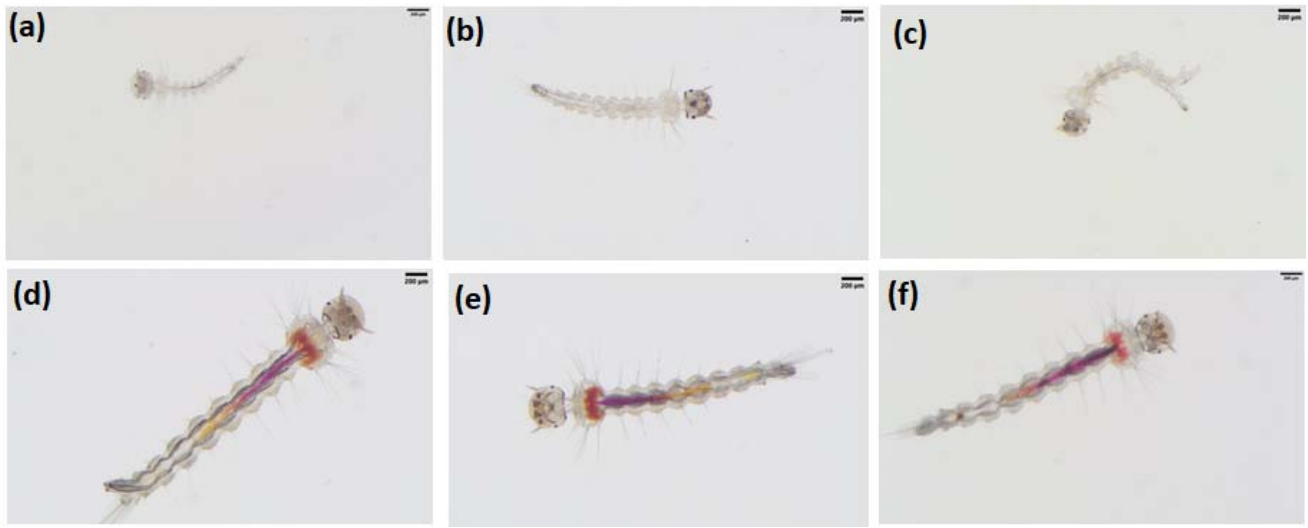


Figure 5. Gut pH profiles in (a) axenic larvae; (b) axenic larvae grown in presence of live bacteria (gnotobiotic); (c) axenic larvae grown in presence of bacterial cell-free culture supernatant; (d) axenic larvae grown in presence of cultured bacterial cell lysate; (e) graph showing comparison of larval length from each experimental group (n≥10 in each group).

Further, we also show that among the members of the gut microbiota included in the experiments, bacteria from 2 different families- *Bacillaceae (Bacillus sp.* and *Lysinibacillus sp.)*, *Aeromonadaceae (Aeromonas sp.*) could set up and maintain the gut pH profile, while bacteria from the family *Microbacteriaceae* (*Microbacterium sp.*, *Leucobacter sp.*) could not [**Figure 6**].



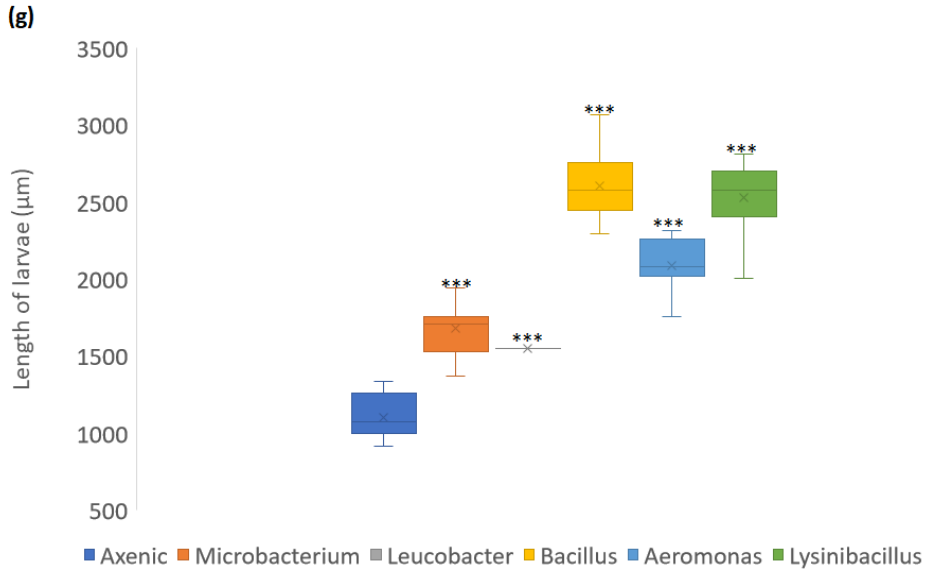


Figure 6. Gut pH profiles in (a) axenic larvae and monoculture gnotobiotic larvae, individually colonized with different bacteria- (b) Microbacterium; (c) Leucobacter; (d) Bacillus; (e) Aeromonas; (f) Lysinibacillus; and (g) graph showing comparison of larval length from each experimental group.

Collectively, our results showing the requirement of a certain family of live bacteria in the larval gut, along with intact CA activity for establishment and maintenance of functional gut pH gradient profile is a novel finding.

Our observation of comparable gut pH profiles in conventionally grown larvae (harboring a complex microbial community) and in select monoculture gnotobiotic larvae (*Bacillus*, *Aeromonas*, *Lysinibacillus*) suggest that this aspect of host-microbe interaction may not be stringently taxon-specific [**Figure 1, Figure 6**]. Several of the bacterial species associated with larval gut may individually set up pH gradient profile, but not all the members could do so. Our results showing a functional pH gradient in monoculture gnotobiotic larvae colonized with *Aeromonas*, but not in monoculture gnotobiotic larvae colonized with *Microbacterium* and *Leucobacter*, strikingly agrees with earlier growth and development studies showing that larvae reared with *Aeromonas* sp. survive, grow, and develop normally, while those reared with *Microbacterium* or *Leucobacter* do not (Coon et al., 2016; Coon et al., 2014). Taken together, these results suggest strong connotation between bacteria driven gut pH gradient profile, survival, growth, and development; signifying an evolutionarily conserved mechanism of gut associated microbiota in larval growth and development.

Additionally, non-establishment of normal pH profile in acetazolamide treated gnotobiotic larvae reconciles with earlier studies signifying an imperative role of CA enzymic activity in establishment of functional pH profile in larval gut (Linser et al., 2009). Present results from experiments with bacterial supernatant/ lysate also support earlier studies, strongly arguing the requirement of live bacteria for larval growth and development (Coon et al., 2014). Our findings therefore provide direct evidence in support of earlier hypotheses on influence of microorganisms in regulation of gut pH and enzymic activity, in certain insects (Overend et al., 2016; Coon et al., 2014; Yonge, 1937). Our results also firmly challenge earlier notion that colonization or decolonization of gut microbiota in mosquito larvae is a function of gut pH (Schmidt & Engel., 2021; Coon et al., 2017; Dillion & Dillion, 2004). Rather our results show that the association works the other way, where certain members of the gut microbiota set up and sustain the larval gut pH.

Earlier electrophysiological and biochemical studies have shown an intrinsic role of CA along with the participation of various ionic homoeostasis mechanisms in AMG alkalinization (Linser et al., 2009). The direct importance of gut associated CA activity in survival is supported by an earlier study showing toxic effects of CA inhibitor fed mosquito larvae (Francis et al., 2016; Corena et al., 2004). However, in localization studies, expression patterns of CA in larval tissues did not correlate with the gut alkalinization profile (very high CA expression in near-neutral GC and PMG region; absent or very low expression in highly alkaline AMG), based on which, involvement of 'soluble extracellular CAs' in AMG alkalinization was anticipated (Linser et al., 2009; Onken et al., 2008; Corena et al., 2005; Seron et al., 2004; Corena et al., 2002). Considering our finding of concurrent requirement of gut bacteria and CA activity for establishment of functional gut pH profile, we assume that the proposed 'soluble extracellular CAs' might be either secreted by certain gut associated bacteria themselves or by host cells under signal cues from bacteria.

Earlier experiments demonstrating the capability of live larvae (conventionally reared larvae, hence presumably with normal gut microbiota) to generate high luminal pH and its significant modulation in response to various CA inhibitors; while a drastic loss in capacity to generate high luminal pH and response to CA inhibitors in dissected-out *in vitro* bathed and perfused (gut bacteria presumably washed out due to bath and perfusion) gut preparations (Onken et al., 2008;

Onken et al., 2004a), appear to support our assumption. Even though a bacterial CA has not yet been formally characterized from larval gut, various studies have documented secretive CA in gut lumen; CA localization within the ectoperitrophic fluid; and expression of CA belonging to certain classes (e.g., α & β CA classes, also known to be encoded by prokaryotes) in larval gut (Vullo et al., 2018; Syrjanen et al., 2010; Smith et al., 2007; Fisher et al., 2006; Corena et al., 2005; Seron et al., 2004; Corena et al., 2002), which tend to indicate the involvement of bacterial CA activity in larval gut. It is noteworthy that expression of 'immunologically CA-like' protein have been documented in several bacteria, including those commonly found in mosquito midgut (Wu et al., 2019; Nafi et al., 1990).

Interestingly, detailed analyses of CA sequence have revealed that β-CA sequences found in genome of various insects including mosquitoes, have originated from endosymbiotic prokaryotes, and integrated into insect genome through movement of mobile genetic element (MGE including β-CA gene along with integrase, resolvase, transposase, and conjugation complex protein genes), in the distant past (Emameh et al., 2016). Considering the deep evolutionary prokaryotic origin of β-CA gene sequences within the insect genomes, one can assume that expression control of these genes might be regulated by signal cue(s) from bacteria, analogous to that previously demonstrated for other physiological processes related to development in mosquito larvae (Coon et al., 2014; Vogel et al. 2017; Valanzia et al., 2018 a, b). Such an evolutionary gene expression arrangement could then rationalize our assumption of bacteria stimulated expression of CA from host cells (at least MGE associated β-CA genes).

In a previous study conducted on dissected, bathed, and perfused gut preparations (gut bacteria presumably washed out), treated with CA inhibitors, experimental stimulation with serotonin reactivated HCO_3^- secreting cells and induced 'faster and more pronounced' alkalinization in AMG region, which was comparable to AMG alkalinization seen in intact larvae; whereas AMG alkalinization was either 'absent or developed slowly' in absence of serotonin (Onken et al., 2008; Onken & Moffett, 2009). *Prima facie*, these observations seemingly indicate at least two independent mechanisms for generation of HCO_3^-, essential for gut alkalinization in insect gut- (i) CA activity mediated conversion of CO_2 into HCO_3^- and (ii) serotonin induced secretion of HCO_3^- from gut epithelial cells. However, when we reviewed the results of the earlier studies from the perspective of gut bacteria, it appears to us that stimulation with external serotonin is

needed for AMG alkalinization only in cases where bacteria are absent due to bathing and perfusion of the gut tissues (Onken et al., 2008; Onken & Moffett, 2009). This tempts us to hypothesize another possible mechanistic relationship between gut bacteria, serotonin and gut alkalinization, where gut bacteria secreted serotonin or gut bacteria induced host serotonin probably signals target gut epithelial cells to secrete HCO_3^-. A previous study has reported different subcellular localization of serotonin receptors in the cells of AMG (nucleus and nucleolus of principal cells) and PMG (plasma membrane of principal, regenerative and enteroendocrine cells), which suggest probably different modes of action (nuclear in AMG, cytoplasmic in PMG) of serotonin on gut cells, corroborating with another study demonstrating that serotonin induce strong alkalinization in the AMG, while reacidification in the PMG (Petrova & Moffett, 2016; Jagadeshwaran et al., 2010; Onken et al., 2009).

Therefore, from the perspective of earlier findings, our results showing the characteristic pH profile in AMG and PMG in presence of gut bacteria seem to show an intricate interrelationship between gut bacteria, serotonin, and gut pH profile. Interestingly, putative serotonin receptors have been detected in different components of the alimentary canal (GC, AMG, PMG, Malpighian tubules) including enteroendocrine cells of PMG, and role of serotonin in a wide array of functions such as growth, reproduction feeding behavioral patterns, metabolism, digestion, nutrient processing, intestinal motility, homeostasis of gut microbiome, gastrointestinal physiology, etc. have been reported in different insects including mosquitoes, coincidentally most of which have also been discretely associated with the gut microbiota (Strand, 2017; Zeng et al., 2022; Ling & Raikhel, 2018; Petrova & Moffett, 2016; French et al., 2014; Kinney et al., 2014; Orchard, 2006; Onken et al., 2004 JEB). Similar modulation of immune response, gastrointestinal physiology, digestive processes by gut microbiota through secretion of serotonin/ serotonin receptor inhibitors (Phenylethylamides derivatives) or through signaling host serotonin-secreting cells (primarily intestinal enteroendocrine cells), have been reported in various organisms (Everett et al., 2022; Arora et al., 2021; Gonçalves et al., 2022; Hasan et al., 2019; Yano et al., 2015; Shahkolahi & Donahue, 1993). Collectively, these observations provide yet another potential mechanism of host-microbe interaction in gut pH modulation, which needs further exploration.

Even though the relationship between gut pH profile and its role in digestion has been studied in detail by early researchers, their relationship with gut bacteria has remained unexplored. The fact that midgut proteases and other enzymes from certain Dipteran and Lepidopteran insects have remarkably high pH optima and that larvae of these insects support a remarkably high alkaline midgut, is known since long (Eguchi & Iwamoto, 1976; Dadd, 1975; Yang & Davis, 1972; Gooding, 1966; Horie et al., 1963). Through exhaustive physiological and biochemical studies on larvae belonging to three distinct mosquito genera (*Aedes*, *Culex*, *Anopheles*), Dadd (1975) proved the functional significance of gut pH profile in enzymic activity related to digestion, growth, and development. He showed that the characteristic gut pH profile remained intact throughout different instar stages; irrespective of the presence or absence of food in gut and that pH optima of important digestive enzyme complexes are remarkably similar across the tested genera, suggesting gut pH profile as a distinguishing evolutionarily conserved taxonomical feature, at least in lepidopterans as well as culicids (Dadd, 1975). Further, based on observed range of pH optima of amylase (7.0-9.0) and protease (9.5-12.0), he concluded that protein digestion is strictly restricted to the highly alkaline AMG milieu, while starch digestion is limited to the neutral to moderate alkaline PMG (Dadd, 1975). These observations clearly underline the crucial role of the distinct larval gut pH profile in dissociation and coagulation of ingested protein complexes, regulation of digestion reactions, neutralizing toxic materials, all of which contribute towards survival, nutrition, and development of the larvae (Gullan & Cranston, 2010; Berenbaum, 1980; Dadd et al., 1975; House, 1974).

Another interesting fact about microbial contribution to larval digestion has come to our notice while reviewing relevant literature on enzyme activities in silkworm (*Bombyx mori*, Lepidoptera) larval gut. In silkworm, functionally distinct proteolytic activities in digestive juices and in membrane bound midgut tissues (Intracellular distribution) have been documented, where the former have a higher pH optimum (~11.0-11.5); higher enzymic activity; is strongly inhibited by $HgCl_2$ and Di-isopropyl fluorophosphate (DFP) (Eguchi & Iwamoto, 1976; Horie et al., 1963). Moreover, unlike the reported conversion of midgut alkaline phosphatase into digestive fluid phosphatase, midgut membrane associated protease could not be converted into digestive fluid protease (Eguchi & Iwamoto, 1976; Eguchi & Iwamoto, 1975). Horie and colleagues (1963) described digestive juices hydrolyzed high molecular proteins into peptides at pH 10.0, followed by hydrolyzation of peptides into amino acids by midgut tissue associated strong peptidases at

pH 7.0. Remarkably, the distinctive proteolytic activity of digestive juices was found to rapidly decline near the end of 5th instar, almost vanishing in the pharate pupal stage (Eguchi & Iwamoto, 1976; Horie et al., 1963). Interestingly, in holometabolous insects, during pupation, gut is replaced due to radical reorganization, causing major turnover of the gut microbiota, often complete removal of the gut microbiota, decoupling the larval gut microbiota from the later adult stages (Manthey et al., 2022; Rolff et al., 2019; Hammer & Moran, 2019). Considering the observed sensitivity of the digestion fluid associated proteolytic activity to heat and DFP (a serine protease inhibitor), quite similar to that reported for bacterial proteinases (Fuke, 1963); $HgCl_2$ (a well-known antiseptic/ disinfectant), inability to be converted from tissue associated protease and its abrupt disappearance during pupation, strikingly coinciding with replacement of gut and its microbiota during metamorphosis in holometabolous insects, encourage us to assume that among the diverse enzymes present in the larval midgut, serine protease(s) activity crucial for larval protein metabolism is actually contributed by its gut associated microbiota. In fact, in an earlier comparative study on transcriptional profiles between axenic and gnotobiotic larvae, significant downregulation of peptidase genes was seen, based on which significant alteration of protein and amino acid metabolism in axenic larvae was suggested (Correa et al., 2018; Vogel et al., 2017). These facts along with an earlier study suggesting prominent role of a possible alkaline-active chymotryptic part in gross proteolytic activities in mosquito larval gut (Dadd, 1975), we believe that the candidate alkaline-active chymotrypsin is of microbial origin. Our belief is strongly supported by another study showing strong mosquito larvicidal activity of a plant origin trypsin inhibitor, which exerted its lethal effect through killing of midgut bacteria (Pontual et al., 2014). Similarly, inhibition of blood meal digestion, protease and other enzymic activity after antibiotic treatment has been shown in other insect larvae (velvetbean caterpillar), adult mosquitoes, and arachnids (O Gaio et al., 2011; Visotto et al., 2009; Yang & Davis, 1972; Gooding, 1966; Terzian, 1958).

CONCLUSIONS

Results of our study reveal an important and fascinating aspect of host-gut microbiota interaction in mosquito larvae and its implication in larval digestion, growth, development, and related physiological processes. We have correlated and discussed our findings in the light of available knowledge on the role of gut bacteria in regulation of larval digestion physiology. Further, we also propose possible mechanisms of such interaction based on our results and review of relevant literature. We firmly believe that our findings will pave new avenues for future research towards better understanding of the role of gut microbiota in vector biology and in developing more efficient, eco-friendly vector-control strategies. Additionally, it would be interesting to examine if the findings of the present study have similar implications in other animals including humans.

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AUTHOR CONTRIBUTIONS

JS and SD conceptualized the study. JS performed all the experiments. JS and SD analyzed data, wrote, reviewed, and edited the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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