



Exploring Soil Microbiota's Impact on *Aedes aegypti* Larvae: Prospects for Vector Management

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Abstract

Soil microbiota play a pivotal role in shaping the development, immunity, and vectorial capacity of *Aedes aegypti* larvae, the primary vector of dengue virus. This review synthesizes recent evidence on how soil-derived bacterial communities influence larval biology through mechanisms such as toxin production, immune priming, and metabolic support. Larvae acquire their microbiota from the aquatic environment, selectively retaining bacterial taxa that modulate their gut microbiome and impact adult traits, including virus susceptibility and insecticide resistance. Experimental studies demonstrate that exposure to specific soil bacteria can induce high larval mortality, alter microbial diversity, and reduce dengue virus dissemination in adults. These findings underscore the potential of soil microbiota as eco-friendly biocontrol agents and highlight the importance of habitat manipulation in vector management. However, the complexity of microbial communities, environmental variability, and concerns about non-target effects present significant challenges. Future research directions include metagenomic studies to identify key microbial players, field trials to validate efficacy under natural conditions, and the development of synthetic microbiomes and regulatory frameworks for safe deployment. Leveraging the interactions between soil bacteria and *Ae. aegypti* larvae offers a promising, sustainable approach to reducing mosquito populations and mitigating the global burden of mosquito-borne diseases.

Keywords: *Aedes aegypti*; Soil Bacteria; Microbiota; Vector Control; Larval Development; Biocontrol; Dengue; Microbial Ecology

Introduction

Vector-borne illnesses pose a major threat to global public health, impacting nearly 30% of the world's population [1]. Among these, arboviruses transmitted to humans through insect vectors are of particular concern due to their rapidly rising incidence and expanding geographic range. Dengue (DENV), Zika

(ZIKV), and chikungunya (CHIKV) are currently the most medically significant arboviruses, necessitating continuous and robust epidemiological monitoring [2]. Dengue, the most widespread viral disease in tropical and subtropical regions, affects an estimated 100 to 400 million individuals annually, with its incidence increasing by more than 30 times in recent decades [3]. Meanwhile, both ZIKV

and CHIKV have emerged strongly since the early 2000s, triggering major outbreaks across Asia and the Americas [4]. The primary vectors for human transmission of DENV, CHIKV, and ZIKV are the highly competent mosquitoes *Aedes aegypti* and *Aedes albopictus* [5]. *Aedes aegypti* is a mosquito vector that is widely distributed globally, particularly in tropical and subtropical environments, and is closely linked to urban areas and regions with environmental disturbances [6]. In contrast, *Ae. albopictus* has a larger geographical expansion, as it colonizes all five continents. Even with vector control measures, a rise in the geographical spread of *Aedes* spp. has been observed in recent years, attributed to factors linked to climate change, globalization, urbanization, and resistance to various insecticides. Consequently, these vector species are viewed as a significant threat [3], undermining the efficacy of preventive strategies, control initiatives, and management efforts related to the diseases they affect [4]. The threat posed by these diseases is being intensified by climate change, which facilitates the expansion of mosquito habitats and increases the geographic spread of disease vectors [7].

In recent years, changes in public health policy and social factors, along with reports of resistance in both vector mosquitoes and the pathogens they carry, have led to a resurgence in the incidence of mosquito-borne diseases [8,9]. Vector control plays a crucial role in managing these diseases. In India, the main approaches to vector control include the widespread use of long-lasting insecticide-treated nets (LLINs), indoor residual spraying of insecticides in rural regions, and anti-larval measures in urban areas. Targeting mosquito larvae can be especially effective in areas aiming to eliminate or eradicate vector-borne diseases, as it helps reduce the mosquito population before they mature into adults [10,11].

Even though there's a push for new ways to control mosquitoes, chemicals are still a big part of the strategy. However, using insecticides to kill mosquitoes has caused significant issues [12]. Mosquitoes have become resistant to these chemicals, and the insecticides themselves are harmful to people, animals, and the environment. For instance, human exposure to these chemicals has been linked to problems with the immune and nervous systems, different types of cancer, birth defects, liver damage, and fertility issues [13]. Because of these negative effects, scientists have been looking for safer alternatives. One promising approach is using microbial control agents, which have proven effective against young mosquitoes of both *Anopheles* and *Culex* species. Biolarvicides

based on the bacteria *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* are now widely used worldwide in mosquito control programs. These bacteria have been thoroughly studied at the microscopic and molecular levels. Consequently, various effective and well-tested commercial products based on these bacteria are available, such as powders, slow-release granules, briquettes, tablets, and liquid concentrates. These products are often used as a key part of integrated vector management strategies, which are recommended by the World Health Organization and adopted by countries where mosquito-borne diseases are common. This article aimed to review and synthesize current knowledge on the influence of soil microbiota on *Aedes aegypti* larvae, focusing on the mechanisms through which soil bacteria shape larval development, immunity, and vector competence. By exploring experimental evidence, practical applications, and future research directions, the article seeks to highlight the potential of soil bacteria as eco-friendly tools for mosquito control and to identify key challenges and knowledge gaps in leveraging microbial communities for sustainable vector management.

The ecology of *Aedes aegypti* Larvae

Life cycle overview

Aedes aegypti undergoes complete metamorphosis, progressing through four distinct developmental stages: egg, larva, pupa, and adult (Figure 1). The life cycle typically spans approximately 8–12 days under optimal conditions, although environmental factors can influence its duration [14].

Egg stage

Female *Ae. aegypti* lay their eggs individually on the inner walls of containers just above the waterline (Figure 1). These eggs are highly resilient, capable of surviving desiccation for several months and hatching only when submerged by water, such as after rainfall or flooding. This adaptation enables the species to persist through dry periods and rapidly exploit new water sources [14].

Larval stage

Upon hatching, larvae—commonly called “wigglers”—are entirely aquatic and undergo four instar stages, each marked by molting (Figure 1). The larval period lasts about 6–8 days, during which larvae feed actively on organic matter, microorganisms, and algae present in the water. Larvae breathe at the water surface using a specialized siphon and are highly sensitive to environmental

conditions such as temperature, pH, and salinity. Growth and development rates are influenced by water quality, food availability, and competition [14-16].

Pupal stage

After completing the fourth larval instar, *Ae. aegypti* enters the pupal stage, which is also aquatic but non-feeding and lasts approximately 1–2 days (Figure 1). During this time, the transformation into an adult mosquito occurs [14].

Adult stage

Adult mosquitoes emerge from the pupae and rest briefly before becoming active (Figure 1). Females require a blood meal to develop eggs, while males feed on nectar and do not bite humans. Females can lay multiple batches of eggs throughout their lifespan, contributing to rapid population growth under favorable conditions [14].

the water [17]. While these taxa represent a minor fraction of environmental microbial diversity, they dominate larval midguts, reflecting selective retention driven by host factors and habitat type. Water chemistry-including pH, salinity, and nutrient levels-further shapes microbial composition, with urban containers often harboring stress-tolerant bacterial communities due to higher temperatures and lower nitrogen availability [17,18]. Soil-derived bacteria play dual roles in larval development: decomposing organic matter to release nutrients essential for growth (e.g., *Chryseobacterium* and *Paenibacillus*) and priming immune pathways (e.g., Toll/IMD) through exposure to taxa like *Escherichia coli*, which enhances antiviral defenses in adult mosquitoes [17]. The species' ecological adaptability is evident in its ability to thrive in both natural and artificial habitats, with African populations exploiting these interchangeably, reflecting a generalist strategy in microbial resource use. This habitat-microbe interplay offers biocontrol opportunities, such as introducing soil bacteria like *Bacillus thuringiensis* or *Chromobacterium* spp. for larvicidal effects or competitively suppressing pathogens. Additionally, modifying larval habitats to favor antipathogenic bacteria through detritus management or water chemistry adjustments presents a sustainable approach to reducing vector competence [19]. Key factors like habitat type, organic inputs, and water pH critically influence microbial exposure, underscoring the potential for microbiome-targeted interventions to disrupt dengue transmission cycles.

Bacterial communities in soil

Soil bacterial communities are dominated by Proteobacteria, Actinobacteria, Acidobacteria, Bacteroidetes, and Firmicutes, which collectively account for over 70% of microbial abundance in most ecosystems. Proteobacteria (e.g., *Alphaproteobacteria*, *Gammaproteobacteria*) are ubiquitous and play critical roles in nitrogen fixation and organic matter decomposition, while Actinobacteria specialize in degrading complex organic compounds and producing antibiotics. Acidobacteria exhibit niche specialization, with subgroups like Gp1-3 thriving in acidic soils and Gp4-6 preferring neutral pH. Bacteroidetes contribute to polysaccharide hydrolysis, particularly in nitrogen-rich environments, whereas Firmicutes (e.g., *Clostridium*) dominate in nutrient-poor or disturbed soils, displaying stress tolerance through spore formation [20].

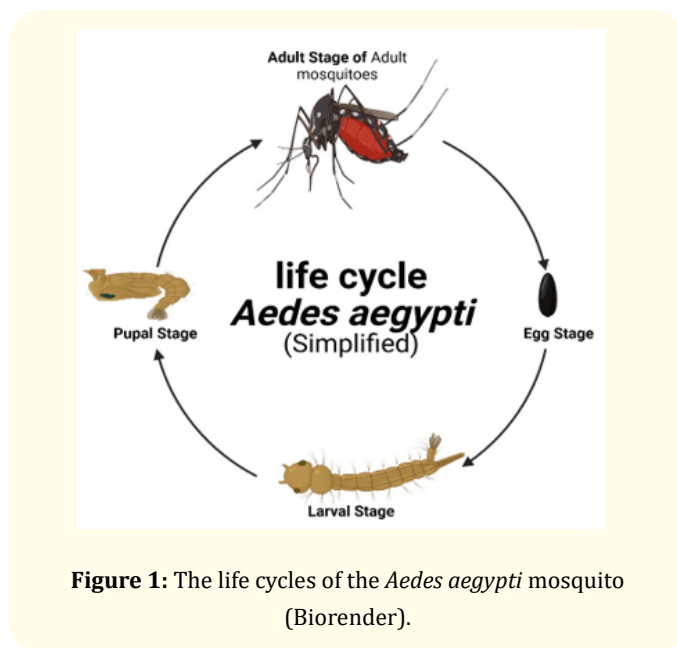


Figure 1: The life cycles of the *Aedes aegypti* mosquito (Biorender).

Larval habitats and exposure to soil microbes

Aedes aegypti larvae inhabit diverse aquatic environments, including natural containers like tree holes and rock pools, as well as artificial receptacles such as discarded tires and plastic containers. These habitats receive organic inputs like leaf litter, detritus, and soil particles, which introduce bacteria such as *Elizabethkingia*, *Corynebacterium*, and Enterobacteriaceae into

Mechanisms of Interaction Between Soil Bacteria and *A. aegypti* Larvae

The interaction between soil bacteria and *Aedes aegypti* larvae is multifaceted, involving direct and indirect mechanisms that influence larval survival, development, and ultimately, the mosquito's capacity to transmit disease. One prominent mechanism is toxin-mediated larvicidal activity. Certain soil bacteria, such as *Brevibacillus centrosporus* and *Cytobacillus* species, produce potent bioactive compounds that can cause rapid larval mortality, often by disrupting the integrity of the larval midgut or interfering with essential physiological processes [21]. Similarly, *Bacillus thuringiensis israelensis* (Bti), a well-known biocontrol agent, produces Cry toxins that destabilize the larval digestive system and alter the composition of the gut microbiota [22].

Beyond direct toxicity, soil bacteria play a crucial role in shaping the larval microbiome. As larvae feed and grow in aquatic habitats rich in organic matter and soil-derived microbes, they selectively acquire and retain certain bacterial taxa—such as *Elizabethkingia* and *Corynebacterium*—while excluding the majority of environmental bacteria [23]. This selective process is influenced by competitive interactions among microbes; for example, native gut bacteria can inhibit the colonization of potential pathogens, and the introduction of specific strains like *Escherichia coli* has been shown to reduce dengue virus dissemination in adult mosquitoes. Conversely, the presence of bacteria such as *Staphylococcus aureus* can disrupt the native microbiota, leading to a state of dysbiosis characterized by reduced microbial diversity and diminished populations of beneficial symbionts [24].

Another important mechanism is immune priming. Exposure to diverse soil bacteria during the larval stage activates the mosquito's immune pathways, such as the Toll and IMD pathways, resulting in increased production of antimicrobial peptides [25]. This immune activation can persist into adulthood, making mosquitoes less susceptible to dengue virus infection. Additionally, soil bacteria contribute to nutrient cycling within the larval habitat by decomposing organic matter and releasing essential nutrients, which support larval growth and development. The larvae, in turn, modify their environment through feeding and excretion, enriching the water with ammonia and other by-products that further influence microbial community structure and water chemistry [23,24]. Collectively, these interactions underscore the dynamic relationship between soil microbiota and *Aedes aegypti*

larvae, highlighting the potential for microbiome manipulation as a strategy for mosquito control and disease prevention.

Experimental evidence of soil bacteria impact on larvae

Extensive experimental research has demonstrated the profound influence of soil bacteria on *Aedes aegypti* larvae, both in terms of survival and developmental outcomes. Laboratory studies have shown that exposure to soil-derived bacterial strains, particularly those from the genera *Bacillus* and *Brevibacillus*, can cause significant larval mortality. For example, one *Brevibacillus halotolerans* strain, isolated from soil, induced 100% mortality of *A. aegypti* larvae within 48 hours of exposure. Similarly, eleven *Bacillus* strains were found to kill 100% of larvae within 24 hours, mirroring the efficacy of the well-known larvicide *Bacillus thuringiensis israelensis* (Bti) [26]. Intriguingly, the larvicidal effect of some bacteria persists even after the removal of live cells, indicating that bioactive metabolites are responsible for toxicity rather than the presence of the bacteria themselves [27].

Beyond direct mortality, soil bacteria also modulate larval development and adult traits. Controlled experiments using gnotobiotic larvae (those exposed to single bacterial isolates) have revealed that different bacterial exposures during the larval stage can result in significant variation in pupation rates and adult body size, although not necessarily in adult lifespan [28]. Exposure to certain Enterobacteriaceae isolates during larval development, for instance, has been shown to reduce antibacterial activity in adult hemolymph and decrease dengue virus dissemination titers in adults, suggesting a carryover effect of larval microbial environment on adult vector competence [28].

A study conducted by Naif (2022) revealed that 2 out of 66 bacterial isolates (3.03%) caused mortality in *Aedes aegypti* larvae. Among these, *Bacillus velezensis* and *Priestia megaterium* led to 100% mortality within 24 hours. In another study also carried out by Naif (2022), two additional isolates *Brevibacillus centrosporus* N8 and *Cytobacillus* species N7 were found to cause 100% larval mortality within the same time frame. Furthermore, two *Escherichia coli* isolates (N3 and N4) resulted in at least 70% mortality. All bacterial isolates in both studies were collected from environments in Saudi Arabia [21,29].

According to Prasad., *et al.* (2012) [30], several bacterial species have been reported for their larvicidal activity against various

mosquito species. *Bacillus thuringiensis* is effective against *Culex* and *Anopheles* mosquitoes, specifically targeting the larval stage. Similarly, *Bacillus sphaericus* exhibits larvicidal activity against *Culex* and certain *Anopheles* species. Other bacteria, such as *Bacillus alvei* and *Bacillus brevis*, are effective against *Culex fatigans*, *Anopheles stephensi*, and *Aedes aegypti*. *Bacillus circulans* has been shown to be toxic to *Cx. quinquefasciatus* and *Anopheles gambiae*, and is reported to be 107 times more toxic to *Aedes aegypti*. *Brevibacillus laterosporus* also demonstrates larvicidal activity against *Cx. quinquefasciatus* and *Aedes aegypti* [30]. In addition, *Bacillus subtilis* is effective against *Cx. quinquefasciatus*, while *Clostridium bifermentans* targets *Anopheles maculatus*. Notably, *Pseudomonas fluorescens* has shown toxicity not only to the larvae but also to the pupae of *Anopheles stephensi*, *Cx. quinquefasciatus*, and *Aedes aegypti*, indicating its broad-spectrum efficacy against multiple mosquito species and developmental stages [30].

Applications in vector management

Soil bacteria as biocontrol agents

Soil bacteria, particularly *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus*, have emerged as highly effective and environmentally safe biocontrol agents for mosquito larvae. These naturally occurring bacteria produce toxins that specifically target the gut of mosquito larvae, causing rapid mortality without harming humans, mammals, or non-target organisms [31]. Field trials and laboratory studies have demonstrated that Bti and *B. sphaericus* can achieve high larval mortality rates, with Bti often causing 100% mortality in *Aedes aegypti* larvae under controlled conditions [31]. These microbial larvicides are now widely used in integrated vector management programs worldwide, providing a sustainable alternative to chemical insecticides, especially in areas facing insecticide resistance [30,31]. Ongoing research continues to isolate and characterize novel soil bacteria with larvicidal potential, further expanding the range of available biocontrol options [30].

Environmental manipulation

Environmental manipulation involves modifying larval habitats to favor the growth of beneficial soil bacteria or to suppress vector populations. Strategies include altering water chemistry, organic matter content, and habitat structure to create conditions that either directly inhibit larval development or promote the proliferation of naturally occurring larvicidal bacteria [21,30,31].

For example, managing detritus and leaf litter in breeding sites can influence bacterial community composition, potentially increasing the abundance of bacteria that are toxic to larvae or that outcompete pathogenic microbes. Such habitat-based interventions are particularly attractive in urban and peri-urban settings, where artificial containers serve as primary breeding grounds for *A. aegypti* [19,30,31].

Integration with other control strategies

The integration of soil bacteria-based biocontrol with other vector management strategies enhances the overall effectiveness and sustainability of mosquito control programs. Combining microbial larvicides with physical measures (e.g., source reduction, habitat modification), biological controls (e.g., predatory fish, entomopathogenic fungi), and community engagement initiatives can lead to more robust and resilient vector control systems [30,32]. This integrated approach not only reduces reliance on chemical insecticides but also addresses the challenges of insecticide resistance and environmental contamination. Furthermore, the use of soil bacteria as part of a broader integrated pest management (IPM) framework is supported by international guidelines and has been endorsed by organizations such as the World Health Organization [31,32].

Challenges and knowledge gaps microbial community complexity

The complexity of soil and aquatic microbial communities presents a significant challenge in understanding and predicting their impact on *Aedes aegypti* larvae. Soil harbors a vast diversity of bacteria, each with unique functional roles and interactions, making it difficult to isolate the specific microbial drivers of larval development or vector competence. Even within a single habitat, the composition and abundance of bacteria can fluctuate rapidly due to changes in organic matter, water chemistry, and environmental conditions [33]. This complexity complicates efforts to engineer or manipulate larval microbiota for biocontrol, as the introduction of specific bacterial strains may not yield consistent results across different ecological contexts [30,33].

Environmental variability

Environmental variability—including differences in temperature, humidity, rainfall, and land use—further complicates the application of microbial-based control strategies. Microbial communities in

mosquito breeding sites are highly dynamic, responding to both natural and anthropogenic disturbances. For example, urban and rural habitats can host distinct bacterial profiles, and even minor changes in water pH or nutrient levels can alter the effectiveness of biocontrol agents. This variability means that strategies developed in controlled laboratory settings may not always translate to real-world conditions, necessitating site-specific adaptations and ongoing monitoring [30,34].

Non-target effects

Although microbial larvicides like *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus* are generally considered safe for humans and most non-target organisms, concerns remain about their broader ecological impacts. Studies have shown that these agents can persist in the environment and may affect non-target aquatic invertebrates, such as chironomids, which play important roles in ecosystem functioning [35]. The long-term and large-scale application of microbial agents could alter the structure and function of aquatic microbial communities, potentially disrupting nutrient cycling and food webs. Assessing and minimizing these non-target effects is essential for the sustainable deployment of microbial-based vector control strategies [35].

Future directions

Advancing our understanding of the interactions between soil microbiota and *Aedes aegypti* larvae will require a multifaceted research agenda. Metagenomic studies are essential for identifying the key microbial players involved in larval development and vector competence. By characterizing the diversity and functional profiles of microbial communities in different larval habitats, researchers can pinpoint specific bacterial taxa and metabolic pathways that influence mosquito biology. This knowledge will inform the development of targeted interventions.

Field trials are needed to validate the efficacy of soil microbiota-based control strategies under natural conditions. Laboratory studies often provide promising results, but real-world factors such as environmental variability, competing microbial species, and habitat heterogeneity can significantly affect outcomes. Large-scale field trials will help determine the feasibility, durability, and scalability of microbiome-based interventions.

The design and deployment of synthetic microbiomes represent an innovative approach to vector management. By engineering

microbial communities with defined compositions, researchers can create consortia that suppress larval development, enhance immune priming, or outcompete pathogenic bacteria. This approach offers the potential for highly specific and adaptable control solutions.

Finally, the development of robust regulatory frameworks is critical to ensure the safe and responsible use of microbial biocontrol agents. As new products and strategies emerge, guidelines must be established to assess ecological risks, monitor non-target effects, and prevent unintended consequences. Collaborative efforts among researchers, policymakers, and public health agencies will be essential to translate scientific advances into effective and sustainable vector control programs.

Conclusion

Soil bacteria play a crucial role in shaping the development, immunity, and disease-transmitting potential of *Aedes aegypti* larvae. These microbes can kill larvae directly, boost their immune defenses, and influence their gut microbiome, ultimately affecting their ability to spread dengue virus. Using beneficial soil bacteria as biocontrol agents—often alongside habitat modifications and other control strategies—offers a promising, eco-friendly approach to mosquito management. However, challenges remain due to the complexity of microbial communities, environmental variability, and potential impacts on non-target species. Future research should focus on identifying key bacteria, testing these approaches in the field, designing synthetic microbial solutions, and developing regulations to ensure their safe and effective use. Overall, leveraging the interactions between soil microbiota and mosquito larvae presents a powerful new avenue for sustainable vector control and public health protection.

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