



The Chironomid Microbiome Plays a Role in Protecting Its Host From Toxicants

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Organisms are assemblages of the host and their endogenous bacteria, which are defined as microbiomes. The host and its microbiome undergo a mutual evolutionary process to adapt to changes in the environment. Chironomids (*Diptera*; *Chironomidae*), are aquatic insects that grow and survive in polluted environments; however, the mechanisms that protect them under these conditions are not fully understood. Here we present evidence that the chironomids' microbiome enables them to survival in polluted environments. It has been demonstrated that about 40% of the microbiota that inhabit *Chironomus transvaalensis* egg masses and larvae has the potential to detoxify different toxicants. Metagenomic analysis of *Chironomus ramosus* larvae demonstrated the presence of genes in the insects' microbiome that can help the insects to survive in hostile environments. A set of experiments demonstrated that short exposure of *C. transvaalensis* larvae to metals significantly changed their microbiota composition in comparison to unexposed larvae. Another experiment, that followed Koch's postulates, demonstrated that disinfected *C. transvaalensis* larvae can survive toxic lead and chromium exposure when they are recolonized with bacteria that can detoxify these toxic metals. This accumulating research, points to the conclusion that the chironomid microbiome plays a role in protecting its host from toxicants.

Keywords: *Chironomus*, microbiome, microbiota, toxicants, holobiont

INTRODUCTION

All organisms host microbial assemblages that reside inside or on the surfaces of their body. These endogenous microorganisms, and the genes that are encoded in their chromosomes, are called the microbiome. Rosenberg and Zilber-Rosenberg (2013), proposed that an organism should be defined together with its microbiome as a holobiont. Moreover, they suggested that the host and the microbiota that inhabit it undergo joint evolutionary processes. One of these mutual evolutionary processes may be the adaptation to survive under hostile environments.

This review discusses how chironomids can survive and proliferate in stressful environments. We will highlight the chironomids' abilities to endure toxic heavy metals and demonstrate which microbes and genes may be involved in protecting the insects under stressful conditions.

CHIRONOMIDS

Chironomids (*Insecta; Diptera; Chironomidae; Chironomus* spp.), are insects that undergo a full metamorphosis of four life stages: eggs, larvae, pupae (that develop in the water) and flying adults. In contrast to mosquitoes, chironomid adults do not feed or bite. They are globally distributed in various aquatic environments. It has been demonstrated that chironomids are natural hosts of *V. cholerae* and *Aeromonas* spp. (Broza and Halpern, 2001; Senderovich et al., 2008; Laviad and Halpern, 2016; Laviad-Shitrit et al., 2020).

The larvae of *Chironomus* spp. live at the bottom of freshwater habitats and form one of the most important functional groups in these ecosystems (Pinder, 1986). Aquatic sediments act as a sink for naturally occurring wastes and tend to be contaminated with toxic inorganic and organic compounds (Salmons et al., 1987). Chironomids live in almost every type of aquatic habitat and can tolerate extreme temperatures, pH, salinity, depth, current velocity and even dehydration and ultraviolet (UV) and gamma radiations (Armitage et al., 1995; Datkhile et al., 2009a,b, 2015; Thorat and Nath, 2015). As a group, they are considered pollution tolerant and are known to thrive under adverse environmental conditions (Armitage et al., 1995; Wright and Burgin, 2009). They were found to dominate the fauna in polluted areas of streams, while in unpolluted sections of the same streams, they formed only a minor part of the fauna (Winner et al., 1980; Richardson and Kiffney, 2000; Watanabe et al., 2000). Tolerance of chironomids to pollution is well documented; however, the protective mechanisms they employ in contaminated and sometimes extreme environments are not fully understood.

CHIRONOMIDS' MICROBIOME

Several studies of *Chironomus transvaalensis* endogenous microbiota that used both culturable and unculturable methods identified a list of bacterial species that comprised *C. transvaalensis* microbiome. Among the microbiota that were identified from *C. transvaalensis* microbiome there were species from the following genera: *Acidovorax*, *Acinetobacter*, *Aeromonas*, *Aquabacterium*, *Bacillus*, *Brevundimonas*, *Cetobacterium*, *Chryseobacterium*, *Citrobacter*, *Clostridium*, *Comamonas*, *Deinococcus*, *Delftia*, *Desulfovibrio*, *Exiguobacterium*, *Hydrogenophaga*, *Klebsiella*, *Oceanobacillus*, *Paracoccus*, *Pseudomonas*, *Rheinheimera*, *Shewanella*, *Stenotrophomonas*, *Vogesella*, *Yersinia*, and *Vibrio* (Halpern et al., 2007a; Halpern, 2012; Senderovich and Halpern, 2012, 2013; Halpern and Senderovich, 2015; Sela and Halpern, 2019; Sela et al., 2020). Four isolates that were cultured and identified from *Chironomus* egg masses were identified as novel bacterial species: *Oceanobacillus chironomi*, *Rheinheimera chironomi*, *Leucobacter chironomi*, and *Brachymonas chironomi* (Halpern et al., 2007b, 2009a,b; Raats and Halpern, 2007).

Sela et al. (2020) found significant differences between the microbiota compositions of *C. transvaalensis* egg mass, larva, pupa and adult life stages. In each developmental

stage, some genera were relatively dominant. In the egg mass stage, *Hydrogenophaga*, *Deinococcus*, *Rheinheimera*, and *Aeromonas*; for larvae, *Cetobacterium*, *Vibrio*, *Aeromonas*, and *Acinetobacter*; in pupae, *Aeromonas*, *Aquabacterium*, and *Clostridium*; and in adults, *Aeromonas*, *Acinetobacter*, and *Vogesella*. Interestingly, *Aeromonas* species were detected in all life stages of *C. transvaalensis*, suggesting that chironomids are reservoirs of *Aeromonas* species and they may serve as symbionts (Senderovich et al., 2008; Sela et al., 2020).

EVIDENCE FOR THE PROTECTIVE ROLE OF THE ENDOGENOUS MICROBIOTA

It has been demonstrated that chironomids' larvae microbiota may play a role in protecting their host from toxic metals (Senderovich and Halpern, 2012, 2013). Senderovich and Halpern (2012), isolated and identified different bacterial species from *C. transvaalensis* egg masses and larvae that were able to grow on selective media with high metal concentrations. The following bacterial species were isolated and identified from egg masses and demonstrated resistance to toxic metals; *Citrobacter freundii*; *Citrobacter youngae*; *Enterobacter ludwigii* were found to be resistant to copper while *Bacillus horneckiae*; *Bacillus stratosphericus*; *Exiguobacterium indicum* were identified as resistant to hexavalent chromium. Resistance to zinc was found in *Pseudomonas geniculata* and *Stenotrophomonas maltophilia*, while *Yersinia nurmii* and *Exiguobacterium profundum* demonstrated resistance to lead.

When Senderovich and Halpern (2013) analyzed the bacterial communities of *C. transvaalensis* egg masses and larvae they found that about 40 and 25% of all the genera that were identified in the egg masses and larval bacterial communities, respectively, may potentially have detoxifying abilities (Table 1). For example; *Aeromonas* species (3.3 and 1.6% prevalence in the larvae and the egg masses, respectively) were documented as species with the ability to detoxify toxic substances including heavy metals like Pb, Cr, Cu, Zn, and tributyltin (TBT) (Cruz et al., 2007; Senderovich and Halpern, 2012, 2013; Laviad and Halpern, 2016; Table 1). Further confirmation for the potential abilities of chironomid microbiota to detoxify a wide variety of toxicants was reported by Sela and Halpern (2019), who found that 43.3% of the genera that were identified from egg masses that were sampled from natural environments have the potential to detoxify or degrade different toxicants (Table 1; Sela and Halpern, 2019).

Leucobacter chironomi and *Brachymonas chironomi* (Halpern et al., 2009a,b), that were isolated from chironomid egg masses, were found to be resistant to heavy metals and other toxicants. The whole genome sequences of these two species (Genbank ID; ATXU00000000 and ARGE00000000, respectively) (Laviad et al., 2015a,b), revealed that their genomes include genes with the potential to detoxify toxic substances (Table 2).

A metagenomic study on *Polypedilum vanderplanki* (Chironomidae) larvae, that can endure desiccation, revealed that microbiota that inhabit this larval species can also withstand desiccation and radiation. However, it is not clear if these microorganisms that are resistant to desiccation

TABLE 1 | A list of bacterial genera that were identified in *C. transvaalensis* egg masses, larvae or pupa and that have the potential to detoxify toxic substances.

Class/Genus	Known detoxifying activity	References
Betaproteobacteria		
<i>Hydrogenophaga</i>	Biodegradation of polychlorinated biphenyls	Lambo and Patel, 2007
<i>Acidovorax</i>	Degradation of polychlorinated biphenyls	Ohtsubo et al., 2006
<i>Dechloromonas</i>	Degradation of benzene, toluene, ethylbenzene, and xylene	Chakraborty et al., 2005
<i>Comamonas</i>	Catabolism of biphenyl or chlorobiphenyl; steroid degradation	Sylvestre, 1995; Horinouchi et al., 2014
<i>Diaphorobacter</i>	Degradation of pyrene	Klankeo et al., 2009
<i>Burkholderia/Paraburkholderia</i>	Crystal violet biodegradation; xenobiotic pollutants degradation	O'Sullivan and Mahenthiralingam, 2005; Gan et al., 2014
Gammaproteobacteria		
<i>Pseudomonas</i>	Aromatic hydrocarbon oxidation; bioremediation of polluted areas; phenol degradation	Williams and Sayers, 1994; Jõesaar et al., 2017
<i>Acinetobacter</i>	Biodegradation of chlorinated phenols	Lee et al., 1994
<i>Aeromonas</i>	Tributyltin degradation	Cruz et al., 2007
<i>Rheinheimera</i>	Degradation of phenolic lignin-related compounds	Virk et al., 2012
<i>Halomonas</i>	Arsenic detoxification	Wu et al., 2018
<i>Stenotrophomonas</i>	Utilization of aromatic compounds; tolerance to heavy metals	Aslam et al., 2018
<i>Escherichia-Shigella</i>	Detoxification of silver ions; nitric oxide detoxification	Franke et al., 2001
<i>Thiothrix</i>	Sulfur oxidization	Flot et al., 2014
Deltaproteobacteria		
<i>Desulfomicrobium</i>	Chromate bioremediation	Michel et al., 2001
<i>Desulfovibrio</i>	Chromate reduction	Michel et al., 2001
<i>Smithella</i>	Degradation of alkanes	Tan et al., 2014
Bacilli		
<i>Exiguobacterium</i>	Bio-removal of hexavalent chromium from water; arsenic tolerance	Okeke, 2008; da Costa et al., 2018
Flavobacteriia		
<i>Flavobacterium</i>	Bioremediation of polycyclic aromatic hydrocarbons	Samanta et al., 2002
<i>Fluviicola</i>	Phenol and thiocyanide degradation	Liu G. X. et al., 2017
<i>Cloacibacterium</i>	Heavy metal resistance	Jayanthi et al., 2017; Liu H. et al., 2017
Actinobacteria		
<i>Aeromicrobium</i>	Hydrocarbon-degrading bacteria isolated from petroleum-polluted soil	Chaillan et al., 2004
<i>Propionibacterium</i>	Dechlorination of tetrachloroethylene- and cis-1, 2-dichloroethylene	Chang et al., 2011
<i>Leucobacter</i>	Heavy metal tolerance	Hou et al., 2018
Anaerolineae		
<i>Longilinea</i>	Vanadium reduction	Liu H. et al., 2017
Caldisericia		
<i>Caldisericum</i>	Sulfur oxidation and/or sulfate reduction reactions	Aida et al., 2014
Cyanophyceae		
<i>Cyanobacterium</i>	Cr VI removal; phenol degradation	Sood et al., 2015
Alphaproteobacteria		
<i>Rhodobacter</i>	Adaptation to metal stress; cadmium bioremediation	Bai et al., 2008; Volpicella et al., 2014

Data were summarized from Senderovich and Halpern (2013), Sela and Halpern (2019), and Sela et al. (2020). Classes names are in bold letters.

contribute to the resistance of the larvae to desiccation (Shaikhutdinov et al., 2020).

METAGENOMIC STUDY OF *Chironomus ramosus* LARVAE

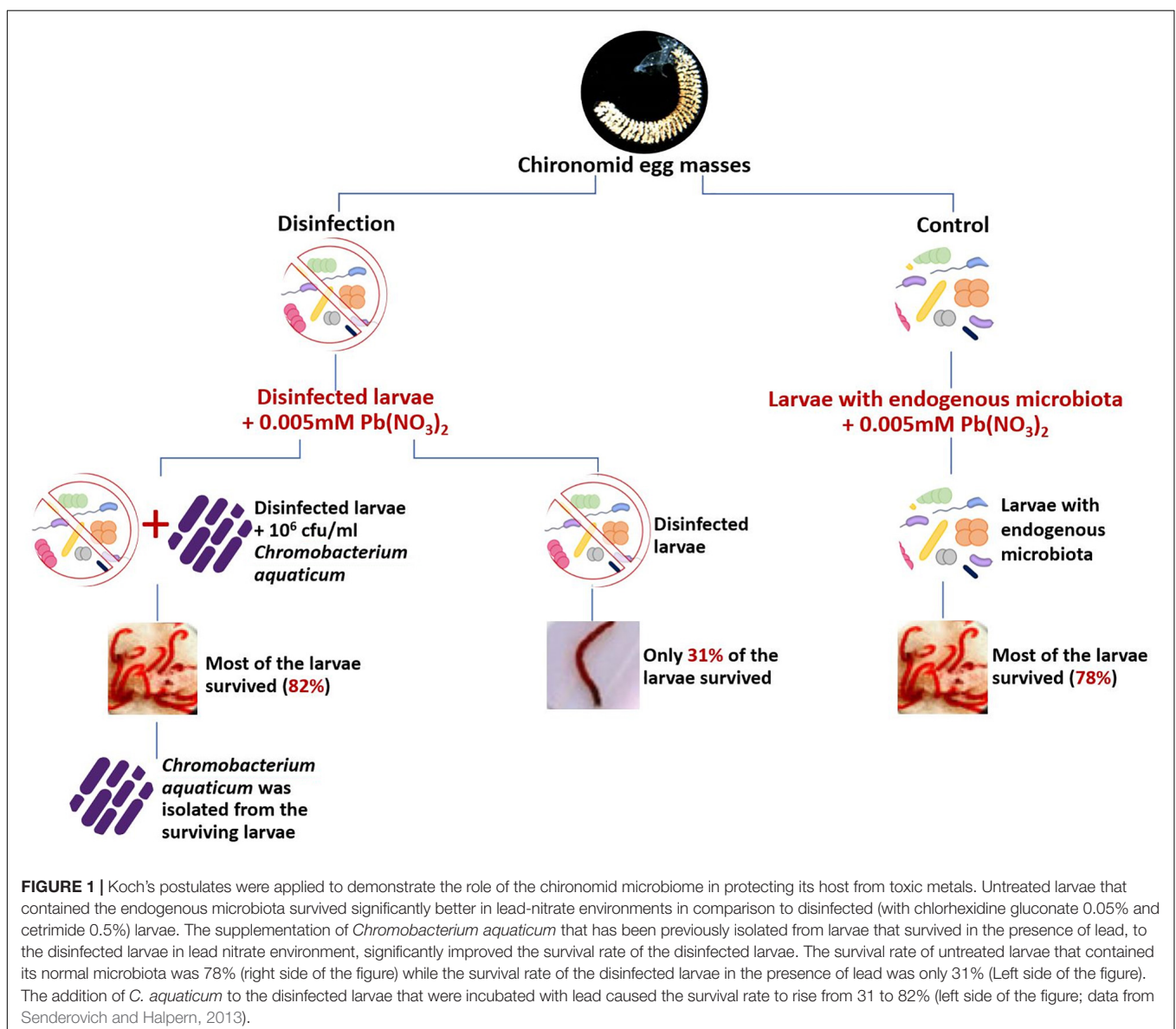
Sela et al. (2021) analyzed three *Chironomus ramosus* larval microbiomes using metagenomics. Genes for detoxifying toxic metals like, copper (*pcoD*; *copC*; *pcoC*), Zinc (*zraP*), lead (*cadC*;

smtB), and others, were detected in the metagenomic data. Furthermore, the presence of genes for resistance to antibiotics, UV radiation and others that were related to stress tolerance were also identified in the *C. ramosus* larval microbiome. Overall, 115,959 bacterial functional genes and 445 different pathways were detected in the *C. ramosus* larval microbiome (Sela et al., 2021).

One of the pathways that was detected in the larval microbiome was the toluene degradation pathway. Toluene, known to cause harm to the central nervous system

TABLE 2 | A list of genes encoding enzymes with predicted resistance to toxic substances (data from Laviad et al., 2015a,b).

Bacterial species	Arsenic resistance	Copper resistance	Other heavy metals	Organic solvents
<i>Brachymonas chironomi</i> (Genbank ID; ARGE000000000)	ArsR ; Arsenical resistance operon trans-acting repressor, ArsD ; Arsenite efflux ATP-binding protein, ArsA ; A hypothetical arsenic resistance protein (ACR3 family). A gene for arsenate reductase (ArsC family) is present in a different operon.	Copper resistance protein D, CopD ; copper chaperone, copper-resistance protein, CopA ; copper (or silver) translocating P-type ATPase		Organic solvents ; two genes encoding the ABC-type transport system involved in resistance to organic solvents
<i>Leucobacter chironomi</i> (Genbank ID; ATXU000000000)	Arsenical resistance protein (ArsB); arsenite efflux pump ACR3 and related permeases.	Copper chaperone; copper (or silver) translocating P-type ATPase	Heavy metal-(Cd/Co/Hg/Pb/Zn)-translocating P-type ATPase and transcriptional regulator (ArsR family) involved in stress-response to heavy metal ions	



(Beller et al., 1992), is a waste product in different industrial processes and as such, is defined as an environmental contaminant (Heydarnezhad et al., 2018). It was found that species of *Bacillus* and *Pseudomonas* genera are able to degrade toluene and use it as their sole carbon source (Hamzah et al., 2011). These genera were identified in *C. ramosus* metagenomic analysis (Sela et al., 2021). Another relevant pathway identified is the atrazine degradation pathway (Sela et al., 2021). Atrazine is herbicide (Steinberg et al., 1995) extensively used to prevent the growth of wild plants in agriculture worldwide (Graymore et al., 2001). High concentrations of atrazine are detected in waterbody sediment (Graymore et al., 2001), where chironomids flourish. A variety of Gram-negative and Gram-positive bacterial genera were reported to degrade atrazine, including *Pseudomonas* (Mandelbaum et al., 1995), *Rhodococcus* (Behki et al., 1993), *Acinetobacter* (Mirgain et al., 1993), and *Arthrobacter* (Cai et al., 2003). All these genera were identified in the metagenomic data. Remarkably, high abundances of *Acinetobacter* and *Pseudomonas* were identified in *C. ramosus* larvae (Sela et al., 2021). These data provided evidence for the ability of the *C. ramosus* microbiome to protect the insect in hostile environments (Sela et al., 2021).

EXPERIMENTAL PROOF OF THE ROLE OF THE MICROBIOTA IN PROTECTING CHIRONOMIDS

To determine the role of chironomids' endogenous bacteria in the survival of chironomids in polluted environments, a bioassay based on Koch's postulates was performed (Figure 1; Senderovich and Halpern, 2013). In a preliminary experiment, they isolated two bacterial strains; *Chromobacterium aquaticum* and *Shewanella decolorationis*, that were identified from *C. transvaalensis* larvae that survived exposure to toxic lead and hexavalent chromium, respectively. These species were found to reduce 94% of toxic lead and 100% of hexavalent chromium, respectively. When these species were added to disinfected larvae that were incubated in lead or chromate containing environments, respectively, they significantly increased survival of the larvae in these toxic environments, compared to disinfected larvae without the addition of *C. aquaticum* and/or *Sh. decolorationis*. Thus, it has been demonstrated that these endogenous bacteria enable their host to survive in lead-nitrate and hexavalent chromate containing environments (Senderovich and Halpern, 2013).

In another study, Laviad-Shitrit et al. (2021) examined larval survival and the change in the larval microbiota composition after 6 days of exposure to different concentrations of toxic copper and hexavalent chromium. A shift in the bacterial microbiota composition was observed in the larvae that were exposed to the different metals (Figure 2). Moreover, this shift was specific to the contaminating metal, suggesting that microbial composition changes—specifically proliferation of species with metal-reducing properties—in response to metal exposure may protect the larvae from the toxic metals. A proof for this hypothesis is the fact that there were no significant differences

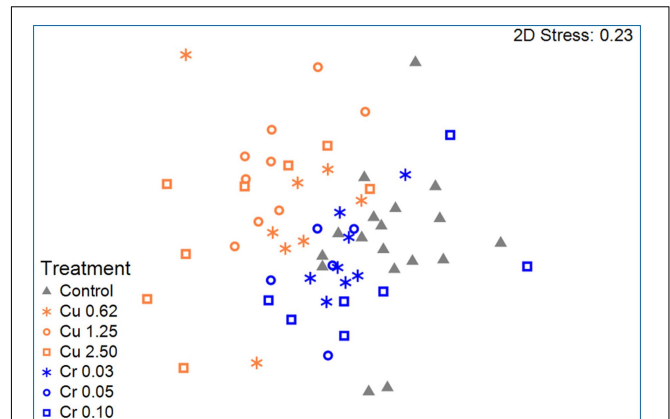


FIGURE 2 | An NMDS (non-metric multidimensional scaling) plot of the bacterial community composition of larvae that were exposed to copper or hexavalent chromium vs. the control (stress value = 0.23). Significant differences were found between the bacterial communities of the control, the hexavalent chromium, and the copper treated larvae (ANOSIM: $R = 0.476$, $p = 0.001$). Adopted with permission from Laviad-Shitrit et al. (2021).

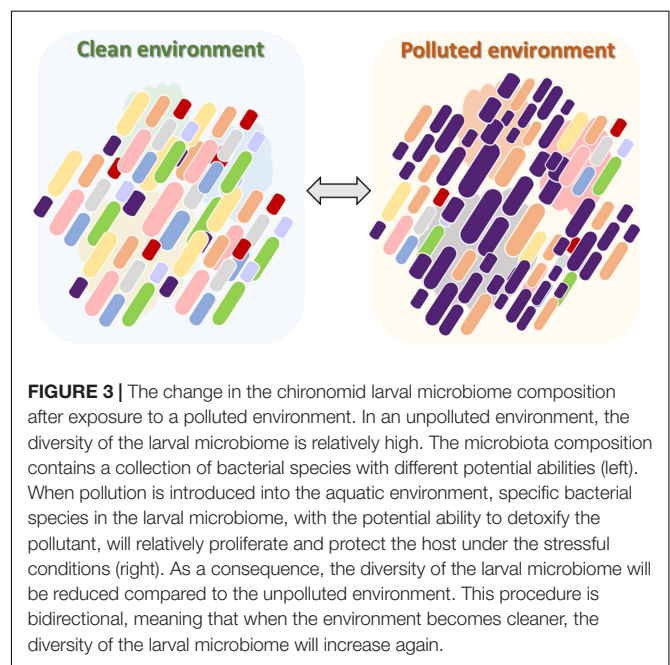


FIGURE 3 | The change in the chironomid larval microbiome composition after exposure to a polluted environment. In an unpolluted environment, the diversity of the larval microbiome is relatively high. The microbiota composition contains a collection of bacterial species with different potential abilities (left). When pollution is introduced into the aquatic environment, specific bacterial species in the larval microbiome, with the potential ability to detoxify the pollutant, will relatively proliferate and protect the host under the stressful conditions (right). As a consequence, the diversity of the larval microbiome will be reduced compared to the unpolluted environment. This procedure is bidirectional, meaning that when the environment becomes cleaner, the diversity of the larval microbiome will increase again.

in the larval mortality between the control and the metal exposed larvae (Laviad-Shitrit et al., 2021; Figure 2). Furthermore, at the genus level, *Yersinia* and *Acinetobacter* abundances increased in the copper treated larvae, while *Yersinia*, *Dysgonomonas*, *Delftia*, and *Enterococcus* abundances increased after the larvae were exposed to hexavalent chromium, compared to the control. The change in the larval microbiota composition was rapid and metal-specific. Laviad-Shitrit et al. (2021) concluded that *C. transvaalensis* larvae host a bacterial consortium that proliferates differently and specifically under each environmental

change and thus, protects the insect in various harsh conditions (Laviad-Shitrit et al., 2021).

CONCLUDING REMARKS AND FUTURE DIRECTIONS

The majority of the endogenous microbiota in chironomid egg masses and larvae is unculturable (Senderovich and Halpern, 2012). In this review we presented evidence that *C. transvaalensis* and *C. ramosus* microbiomes play a role in the survival of their host under toxic metal conditions. It is likely that the relative abundance of the different endogenous species that inhabit the insect changes according to environmental changes. As shown in this review, under high toxic metal concentrations, specific bacterial species with the potential ability to detoxify these metals will relatively proliferate and protect the host (Figure 3). More studies are needed to explore the mechanisms of this phenomenon in different *Chironomus* species and in

different toxic and extreme environments. We assume that this phenomenon of the protective nature of the microbiota in the chironomid holobiont unit is probably only the tip of the iceberg. Further study of the role that microbiomes play in protecting different organisms is needed to understand the role of endogenous microbiota in protecting their hosts.

AUTHOR CONTRIBUTIONS

RS and MH wrote the manuscript. Both authors contributed to the article and approved the submitted version.

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