Microbial Stress Resistance of *Eristalis tenax* Rat-Tailed Maggots

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**Abstract**
The maggots of the drone fly *Eristalis tenax* (Diptera) have survived in habitats with extreme microbial contamination. Despite this polluted environment, they avoid infection by microorganisms. We have investigated the first physical barrier, the cuticle surface of *E. tenax* maggots collected from Alakrasha dain, Egypt, using scanning electron microscopy which revealed an array of high density and dimensions of nano and microstructures that narrow to sharp points appear to make it difficult for bacteria to colonize its surface which interferes with the formation of biofilms and potentially acts as a defense against bacterial infection. This investigation leading us to more examine the antibacterial properties of the whole maggot extract naturally without any previous simulation, the results were promising against *Staphylococcus aureus* ATCC 6538, *Staphylococcus mutans* ATCC 25175, *Escherichia coli* ATCC 25922, *Salmonella enterica* Serotype Typhimurium ATCC 14028 bacteria compared to Nitrofurantoin antibiotic. Such antibacterial properties of both the maggot cuticle surface and the whole maggot extract have applications in many different fields, including antibacterial surfaces and biofilms besides the future isolating and developing of antimicrobial peptides from the maggot crude extract which could be a breakthrough against antibiotic resistance.

**Introduction**

*Eristalis tenax*, the common drone fly, family Syrphidae (Diptera), with over 5,000 described species (Rotheray, 1993). In contrast to the flower-feeding habitats of adult syrphids, their immature stage is usually referred to as a rat-tailed maggot which is found in a very diverse array of habitats. They prefer living in stagnant aquatic environments with high organic and microbial contamination (Altincicek & Vilcinskas 2007). Because of the preference of *E. tenax* larvae for dirty waters with anaerobic conditions, they are reliable and prominent indicators in the biological assessment of water quality for extremely high pollution with organic material (Chapman, 1996). Although Rat tailed maggots are closely associated with filthy and highly polluted water, they're actually remarkably resistant to infection from all the bacteria that surround them, the case which attracts the attention of scientists to survive in such aquatic habitat which
is usually not colonized by other dipteran larvae or animals. Insects possess antimicrobial properties and substances which are produced on the surface or within their haemolymph to prevent microbial infection (Hazlett and Wu 2011), these antimicrobial properties and substances may be utilized for various defense purposes, which contain various antimicrobial peptides (AMPs) as effective inhibitory substances against diverse pathogens (Brown et al., 2008). AMPs, which are synthesized by the fat body and hemocytes and then secreted into the hemolymph, are an essential part of the immune defense (Tsakas & Marmaras 2010). AMPs are promising candidates as alternatives to conventional antibiotics, thanks to their low toxicity to eukaryotic cells and their broad spectrum of action against bacteria, mycobacteria, fungi, viruses and cancer cells. AMPs with insects being among the richest sources more than any other organism, due to insects' high biodiversity and their extremely varied living environments (Tsakas & Marmaras 2010). Recently Two cecropin-like peptides (EtCec1-a and EtCec2-a) and a diptericin-like peptide (EtDip) were identified under simulated physiological conditions which showed antibacterial activity against multidrug-resistant Gram-negative bacteria. However, the antibacterial activity of Rat-tailed maggot extract in natural conditions without any simulation has not been reported yet. Therefore, the aim of this study is to investigate the adaptation against incredibly high microbial pressure environment beginning from its surface as a first physical barrier (Rady et al., 2018), we have examined larvae of the drone fly by scanning electron microscopy (SEM) and have identified an array of micro-scale spikes on the surface of the insect cuticle. Also, antimicrobial activity of the maggot extract in natural condition without any induction have been screened against two species of Gram-positive and another 2 species of Gram-negative bacteria.

**MATERIALS AND METHODS**

**Insects:** Rat-tailed maggots were collected in sufficient numbers (about a hundred), (Fig 1), between March and April (2019), from an open drain in Alakrasha, Al Khankah city, Al Qalyubia Governorate, using a standard aquatic D-frame hand net. Using a practical key for the determination of hoverfly larvae, we identified third instar larvae of the drone fly *Eristalis tenax*. As typical characters, we observed the absence of setae along the lower lateral margins and the last pair of prolegs with most of the outsized primary crochets opposite towards the lateral margins of the body. Primary crochets broad, strong, distinctly bent, their length barely exceeding their width at base; distal 2/5 of crochets intensely pigmented (Rotheray, 1993).

![Fig.1: Larva of the Rat-tailed maggot, Eristalis tenax (Linnaeus).](image)
Bacterial Strains:
We used the following human pathogenic bacterial strains, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922, *Salmonella enterica* serotype Typhimurium ATCC 14028, and *Streptococcus mutans* ATCC 25175, were purchased from the American Type Culture Collection.

Transmission Electron Microscopy:
Five Rat-tailed maggot samples were fixed, washed three times in phosphate buffer, osmicated, washed, and dehydrated into alcohol. The samples were then gently resuspended into hexamethyldisilazane (reagent grade >99%, Aldrich chemicals) and placed onto SEM stubs in a fume-hood. The volatile hexamethyldisilazane evaporated rapidly and preserved the delicate membranous structure of fragile specimens (Hayes et al., 2016).

Preparation of Rat-Tailed Maggot’s Crude Extract:
Insect samples were washed thoroughly in clean sterile water, the whole bodies of 30 larvae were homogenized using a hand homogenizer. The homogenate was applied as a crude extract without any solvent.

Antibacterial Assay:
The selected human bacterial pathogens were subjected for antibacterial screening and their susceptibility patterns to Rat-tailed maggot extract using the standard disc diffusion method. In which holes with a diameter of 11 mm were punched into the agar and filled with 100 μl of crude extract. The diameters of the clear zones were measured after 24 h of incubation at 37°C, a standard antibiotic was used for comparison (Nitrofurantoin 300 μg) according to the standard procedures of the CLSI, 2020 and British Society for Antimicrobial Chemotherapy (BSAC) disc diffusion method [CLSI, 2020]. The experiment was done in triplicate and the mean diameter of a radius of clear inhibition of zones (mm) was calculated.

RESULTS AND DISCUSSION

-Antimicrobial Susceptibility Testing:
The results of the antimicrobial susceptibility testing for the Rat-tailed maggot extract of the whole body as a crude extract show a high level of antibacterial activity against both Gram-positive and Gram-negative bacterial tested strains, zones of inhibitions are observed as indicators of antimicrobial activity. The obtained results revealed that the highest antibacterial activity after 24 hrs post-treatment against *Staphylococcus mutans* (46mm) followed by *Staphylococcus aureus* ATCC 6538 (40mm), *Escherichia coli* ATCC 25922 (38mm), *Salmonella Enterica Ser. Typhi* ATCC 14028 (35) compared to Nitrofurantoin 300 mcg (11mm) for Gram positives and 15 mm for Gram-negatives, Table 1 and Figure 2(A-D). All tested strains are resistant strains to Nitrofurantoin 300 μg except for *Salmonella Enterica* Ser. Typhi ATCC 14028 which not Applicable yet according to CLSI, 2020 interpretation.

Table 1. The antibacterial results of Rat-tailed maggot extractives/ mm.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Inhibition zone diameter (mm) on agar plate</th>
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<tbody>
<tr>
<td></td>
<td>Staphylococcus aureus ATCC 6538</td>
</tr>
<tr>
<td>Rat-tailed maggot extract</td>
<td>40</td>
</tr>
<tr>
<td>Nitrofurantoin 300 μg</td>
<td>11</td>
</tr>
<tr>
<td>Interpretation to Nitrofurantoin 300 μg (CLSI, 2020)</td>
<td>Resistant if ≤ 14</td>
</tr>
</tbody>
</table>


The antibacterial activity results showed that, tested extracts evoked a high activity against both Gram-positive and Gram-negative comparing to many insects crude extract. (Leem et al., (1999), Thomas et al., (1999), Yamauchi,(2001) and  Amer et al., (2019). Positive bacterial strains were more sensitive to the tested maggots’ extracts than Gram-negative bacterial strains. The obtained antibacterial activity of the crude extract in natural conditions without any simulation help to explain the amazing interdependency of ecological adaptation.

However, vitro induction to immune transcripts in Eristalis tenax maggots was performed by Altincicek & Vilcinskas (2007) and followed by Hirsch et al., (2020). Altincicek & Vilcinskas (2007) identified thirty novel genes of E. tenax that were induced in response to septic injury including novel putative AMPs.Hirsch et al., (2020) tested the antibacterial activity under simulated physiological conditions, a bacterial lipopolysaccharide was injected into Eristalis tenax maggots to enhance immunity-related AMPs that are synthesized in response to the injection, they are identified twenty-two AMPs and selected nine for larger-scale synthesis to test their activity against multidrug-resistant Gram-negative bacteria. A diptericin-like peptide (EtDip) and two cecropin-like peptides (EtCec1-a and EtCec2-a) displayed strong activity against the pathogens.

The ecological niche with this extremely harsh environment enable Eristalis tenax immune system to overcome infection by a general and rapid response to different invading organisms, similarly, the larvae of the black soldier fly, Hermetia illucens, shows an extraordinary ability to live in harsh environments, as it forages on decaying substrates, which are rich in diverse microbial colonies, and is one of the most promising sources for AMPs. The related physicochemical properties were assessed and allowed to identify 57 active peptides appropriate for later experimental validation studies (Moretta et al., 2020).

Transmission Electron Microscopy of Rat-Tailed Maggot Surface:

Rat-tailed maggot surface observed by SEM exhibited with specific micron and nanoscale features like pillars, spikes and strips with various topographies Figure 3 (a, b, c & d), these features exhibited a significant overall reduction of bacterial adhesion compared to the flat surfaces (Wu, et al., 2018). The topography patterns that are larger than bacteria in every dimension influence the development of multicellular structures on surfaces. Depending on the size and the shape of these larger surface patterns, it is possible to favour or hinder bacterial adhesion and hence biofilm formation. Even if bacteria could fit between micro-structures and attach to the surface; the presence of

Fig.2: Antibacterial activity of Rat-tailed maggots crude extracts against (A)Escherichia coli ATCC 25922, (B) Salmonella Enterica Serotype Typhimurium ATCC 14028 (C)Staphylococcus aureus ATCC 6538, (D) Staphylococcus mutans ATCC 25175.
neighbouring microstrips Figure 3c may interfere with biofilm creation as there is no enough room for the bacteria to successfully divide or form productive interactions with other colonizing microbes.

Nanopillars are grouped together in lattice-like arrays on the surface of the maggot cuticle each about 10 μ projection from the insect cuticle as shown in Figure 3 (a &b) . Areas of the maggot cuticle on which the nanopillars are highly abundant are completely free of adherent bacteria, fungi, and algae Figure 3 (a & b) (Hayes et al., 2016). At the side where there are no nanopillars, a chain of bacilli bacteria is able to adhere to the surface beside the center-to-center gap Figure 3a. The center-to-center gap measuring 120μ represents a separation track between nanopillars. A closer analysis of the mechanism reveals that nanopillars with their tapered pointed ends penetrate the membranes of adherent bacterial and rupture the cells, leading to their departure. Flat surfaces cannot penetrate the bacteria, and thus lack antibacterial activity (Wu et al., 2018). Spines and nanopillars are almost entirely absent from the siphon Figure 3 e. This may be due to scratching especially; the origin of each previous spine is present. There are truncated nanopillars on the surface of the cuticle (Hayes et al., 2016).

All surface projections present random size, shape, and spatial distribution Figure 3 (a,c,d) resulting in more than one form of Topography, roughness and shape. More work is required to establish an actual correlation between surface topography and bacterial adhesion. It was concluded that the topography and chemistry of the surface are the critical aspects to consider in achieving the bactericidal surfaces, and the slope presented by these surfaces can allow machine learning to design functional Antibacterial surfaces, (Dickson, et al., 2015).

Wings of several species of cicada, dragonfly, as well as a damselfly, have been discovered to possess mechano-bactericidal nanopillar or nanospike topographies (Mainwaring, et al., 2016). Clanger cicada (Psaltoda claripenni), an insect whose wings possess nanopillars (Pogodin et al, 2013). The wings serve as a natural defense mechanism against bacteria, so it was hypothesized that manmade nanopillars would exhibit similar antibacterial properties. To this end, a study by (Dickson et al.,2015) showed that after E. coli cells were cultured on the nano-pillared surface, the nanopillars negatively impact the growth of the E. coli cells. Their study showed that nanopillar surfaces killed more bacterial cells than flat surfaces did.
Fig. 3: SEM image showing Rat-tailed maggot cuticle surface (a): Nanopillar grouped together in lattice-like arrays, the white arrows refer to a chain of bacilli bacteria at side areas lacking nanopillars. (b): A higher enlargement image to Nanopillars, (25KV 150X) the diameter at the base and top, 100 and 10 μ respectively, the height of nanopillars is 120 μm. (c): Different topographies in the same section demonstrating Micro stripes (length of 50 μm and thickness of 2-10 nm and other projections, scale bar 152. 50 μm. (d): SEM image showing Strong Topography (microscale) spike-like microstructure with pointed tapering ends. Scale bar 158.75 μ m. (e) The siphon with truncated nanopillars. Scale bar 145.83 μm.
Conclusion
Screening the antibacterial activity of *E. tenax* maggot crude extract and the antimicrobial properties of their cuticle surface demonstrating that *E. tenax* maggot prove useful in the fight against invading pathogenic bacteria and can potentially be a source of novel antibiotic-like compounds for infection control beside inspiration to artificial antimicrobial surfaces.

REFERENCES


CLSI (Clinical and Laboratory Standards Institute). (2020). Performance standards for antimicrobial susceptibility testing. CLSI document M100. 30th ed. CLSI.


