9. The Antimicrobial Activity of Medicinal Maggots

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Bacterial infection of wounds is a serious and growing issue and contributes to a delay in wound healing. Whilst debridement is often the primary motivation for the clinical use of maggot therapy, there is accumulating evidence that the therapy has other therapeutic properties. In particular, larvae have a significant antibacterial effect on the wound surface through the antimicrobial action of their excretions and secretions and the disruption of microbial biofilms that are common in chronic wounds. This chapter describes the principles and mechanisms that allow medicinal maggots to successfully shape and control the microbial environment of the chronic wound.

Introduction

Bacterial infection of wounds is a serious and growing issue and contributes to a delay in wound healing. Progression of healing is said to be dependent on both bacterial count and microbial species present [1], so disinfection of the wound-site is vital to enable the wound to heal. Whilst debridement is often the primary motivation for the clinical use of maggot therapy, there is accumulating evidence that the therapy has other therapeutic properties. In particular, larvae have a significant antibacterial effect on the wound surface, not only through the removal

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of infected tissue, but also through the antimicrobial action of their excretions and secretions. When the American military surgeon William S. Baer encountered seriously wounded soldiers colonised by wild maggots during WW1, he observed the remarkably good condition the men were in and the absence of sepsis [2]. He considered the action of maggots to be that of scavengers sucking up bacteria and consuming dead tissue. He also noted the presence of excretions and secretions in the wound and believed that "some biological reaction" was responsible for helping the wound to heal, though the nature of this biochemical substance was something that he was not able to uncover.

In their natural environment, blowfly larvae exploit decaying carrion, which is a microbe-rich food source. Thus, it is intrinsic to their survival that they adapt to this environment and evolve strategies to cope with and control microbes [3]. It was this ability to control infection that motivated William S. Baer to eventually pioneer maggot therapy in his own peace-time clinical practice for the treatment of patients with osteomyelitis [2]. This chapter explains how medicinal maggots control wound infection.

Historical Investigations into the Antimicrobial Activity of Maggots

It had long been suspected that larvae possessed an antimicrobial quality. In the 1920s it was theorised that larvae were capable of destroying bacteria taken into their gut after the "remarkable sterility" of the gut contents of certain fly species was noted [4]. This was later expanded on by Robinson and Norwood [5, 6], who found that bacteria were destroyed as they passed through the digestive system of medicinal maggots. As well as examining the destruction of bacteria in the gut, early studies also investigated the antimicrobial properties of larval excretions and secretions. Examining these "elimination products", Simmons [7, 8] demonstrated the presence of a potent antibacterial entity within the biological material. He also found that the use of non-disinfected larvae (compared to the use of the same material from disinfected organisms) increased the potency of the antibacterial activity, with a 5- to 10-minute incubation sufficient to prevent the growth of *Staphylococcus aureus* [7, 8]. Further research also determined the presence of a heat-stable

antibacterial agent which could be partially purified using paper chromatography [9].

Resurgent Interest in Antibacterial Bioactivity from Maggots

More recently, there has been particular interest in understanding and identifying the therapeutic antimicrobial properties of maggot excretions and secretions, the main drive of this being the use of larvae as a source of novel antibiotics and anti-infectives, especially with the rise of drug-resistant forms of pathogenic bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) [10].

The accumulation of evidence for maggot antimicrobial activity has been slow. Most of the compelling evidence on the nature of the therapeutic antimicrobial effects has come from scientific laboratory findings using maggot excretions and secretions. There have been numerous investigations into the types of bacteria vulnerable to maggot excretions and secretions. In one of the first modern investigations into the antimicrobial activity of maggot excretions and secretions, Thomas and colleagues [11] described variable bactericidal activity against different species and strains of bacteria, with marked activity against Gram-positive strains such as *Streptococcus* (group A, and group B) and *S. aureus*. Less marked activity was seen against MRSA and the Gram-negative *Pseudomonas* species, with no evidence of inhibition against the Gram-negative *Escherichia coli* and *Proteus* species.

Several tests have served to reaffirm the notion that maggot excretions and secretions are effective in destroying a broad range of Gram-positive bacteria. However, there is less consistency in results regarding Gram-negative species. For example, in a turbidometric assay using excretions and secretions from disinfected larvae, Bexfield and colleagues [12] observed significant antibacterial activity against Gram-positive species *S. aureus* (including MRSA), and *Bacillus thuringiensis*. In contrast to Thomas and colleagues [11], however, significant activity was also observed against Gram-negative species including *E. coli*, *Pseudomonas aeruginosa*, and *Enterobacter cloacae*. Findings in other investigations have also been inconsistent, with reports of maggot activity against both Gram-positive and Gram-negative species [13, 14], or activity against only Gram-positive and not against Gram-negative species [15].

Other investigators, too, noted antimicrobial activity against both Gram-positive and Gram-negative species, but observed that perhaps the activity against Gram-negatives was less pronounced. Using a colony forming units bioassay, it was demonstrated that maggots exhibited antibacterial activity against both S. aureus and E. coli, though the effectiveness of the activity was markedly less against E. coli than S. aureus [16]. A similar finding was reported in a clinical study, which noted that maggot therapy was more effective for Gram-positive-infected wounds than it was for Gram-negative-infected wounds [17]. A further investigation into the interaction of larvae with P. aeruginosa, a potent Gram-negative bacterium, found that the bacterium was harmful to the larvae, leading to a reduction in maggot food intake and their movement away from areas of contamination [18]. The authors found that this negative effect was caused by the action of specific quorum-sensing molecules that are released by bacteria in order to communicate with each other—usually prior to forming a biofilm. No bactericidal effect was detected against P. aeruginosa in this case [18].

Interestingly, a study by a Dutch group of clinicians and researchers found no antibacterial activity of maggot excretions and secretions at all, against either Gram-positive or Gram-negative species. However, recognising the clinical successes with maggot therapy and their own contradictory results in comparison with other reports, the authors suggested that the method of maggot secretion collection may account for the difference in results, not only in their own study, but also in that of others. This was expanded on by Barnes *et al*. [19] who, recognising the variety of methods employed in previous investigations and the variation in results, advocated for the standardisation of liquid culture assays used to quantify the antibacterial effectiveness of maggot excretions and secretions. In their own test, the authors found that different concentrations of maggot excretions and secretions and the presence of additional nutrition influenced the growth of tested bacteria (*E. coli*, *S. aureus*, and *P. aeruginosa*), and therefore would have contributed to the variation in previously reported results. Addressing this issue, the authors noted that whilst it is important to use media sufficiently high in nutritional content to enable bacterial growth, it should also not be detrimental to the antibacterial activity exhibited by the maggot excretions and secretions. Incidentally, in this test, the

antibacterial effectiveness of maggot excretions and secretions was most potent against *E. coli* and less so against *S. aureus* [19].

These studies indicate the presence of antibacterial activity in maggot excretions and secretions but also demonstrate that care must be taken with regard to the method by which the excretions and secretions are collected, the choice of the bioassay used to assess the activity, and the need to establish sufficient control experiments to generate valid results from such studies. Regardless of the inconsistency of results related to the activity against Gram-negative bacteria, it is now widely accepted that there is an antibacterial entity present in the excretions and secretions of *L. sericata*. Indeed, excretions and secretions from medicinal maggots are used as positive controls in the study of antibacterial compounds found in excretions and secretions from other organisms [20].

Evidence for the Antimicrobial Activity of Medicinal Maggots

Maggots Can Destroy Ingested Bacteria

As well as investigations into the antibacterial action of larval excretions and secretions, there has also been some interest regarding the destruction of ingested bacteria in the maggot gut. By feeding larvae with *E. coli* that produce a fluorescent green protein and tracking this protein's movement through the alimentary tract, an Israeli team tracked the fate of ingested bacteria [21]. They showed that the majority of bacteria were destroyed in the mid-gut, with the remainder being destroyed in the hindgut, so that maggot faeces were either sterile or contained only a greatly reduced number of viable bacteria. In contrast, a subsequent study found that after ingestion of Methicillin-sensitive *Staphylococcus aureus* (MSSA) and Methicillin-resistant *Staphylococcus aureus* (MRSA), the strains remained viable within the maggot and were excreted into the environment [13]. However, it was noted by the authors that the larvae were exposed to a very large quantity of bacteria, which would not necessarily correspond to the bioburden of a wound, and therefore the capability to destroy bacteria in the gut may have been overwhelmed in this instance.

Clinical Evidence of Antibacterial Activity from Maggots

Whilst most of the evidence for the antimicrobial efficacy of *L. sericata* larvae comes from *in vitro* investigations, a small number of tests have also been conducted *in vivo*. In one such study swabs were tested from the wounds of 16 patients before and after treatment with maggot therapy and the chance of culturing Gram-positive and/or Gram-negative bacteria was determined [17]. For example, if three wound cultures were taken and two showed the growth of Gram-positive bacteria, the chance of culturing Gram-positive bacteria was given as 0.66. The results showed a reduction (although statistically not significant; $p=0.07$) in the chance of culturing Gram-positive bacteria after treatment with maggot therapy and a significant increase in the chance of culturing Gram-negative bacteria. The authors argued that the results indicated lower antibacterial effectiveness of maggot therapy in Gram-negative-infected wounds and suggested that treatment with a larger number of larvae may be necessary when treating a wound infected with Gram-negative bacteria.

A subsequent investigation similarly monitored the bacterial diversity before and after treatment with maggot therapy in 30 patients. The study found vast reductions in the species of bacteria present, though some species, mainly Gram-negative, were unaffected or increased in numbers, particularly *Proteus* species. As a result, the authors concluded that maggot therapy would be most appropriate in treating wounds infected with Gram-positive bacteria and advocated the need for special precautions when treating Gram-negative infections [16]. Incidentally, *Proteus* species is a frequent and natural commensal of *L. sericata* and previous laboratory studies [12] have also shown that it is unaffected by the antimicrobial activities of maggot excretions and secretions.

Randomised Controlled Trials and Antimicrobial Effects of Maggot Therapy

Randomised controlled trials are believed to be the gold standard for clinical studies. A recent randomised controlled trial was carried out with 50 patients who had a diabetic foot ulcer [22]. Patients were randomly assigned to a 'control' group, which received a conventional treatment of surgical debridement, antibiotic therapy, and offloading,

or to a 'treatment' group, which received maggot therapy in addition to the conventional therapy. A swab culture was collected before and after each maggot application and analysed for the presence of *S. aureus* and *P. aeruginosa*. In the treatment group, the number of patients whose wound was infected with *S. aureus* significantly reduced after 48 hours of treatment, with a further reduction after a second application of larvae. The number of patients whose wound was infected with *P. aeruginosa*, meanwhile, did not reduce significantly after a single 48-hour treatment, but did after a second treatment. In the control group without maggot therapy, no significant reduction in the number of patients with a wound infected with either species was observed [22].

Animal Models and Antimicrobial Effects of Maggot Therapy

Tests of the antibacterial capability of larvae have also been conducted using a rat model [23]. Wounds were created in the rats and contaminated with a mixed population of Gram-positive and Gram-negative bacteria. The rats were then placed in one of four groups: 1) non-treated control, 2) antibiotic-treated, 3) maggot-treated, and 4) combined treatment with antibiotic and maggots. Results revealed that maggot-treated wounds reduced bacterial bioburden significantly faster compared to the control and antibiotic-treated groups, allowing for faster wound contraction and healing. The results of treatment with only maggots and the combined maggot and antibiotic treatment were similar [23].

Together with the clinical evidence, these few studies give an indication of the antibacterial capability of maggot therapy *in vivo* and serve to verify the findings of previous investigations conducted *in vitro*. The clinical evidence is still limited, however, and further work into the antibacterial effectiveness of larvae in clinical practice would be useful. However, despite the relative lack of clinical evidence, the idea that effective antibacterial molecules are contained in the excretions and secretions of *L. sericata* larvae is, at this point, widely accepted [24].

The Antimicrobial Compounds in Maggot Excretions and Secretions

Constitutive versus Inducible Antibacterial Activity

One key issue regarding the antibacterial activity is whether bioactivity is constitutive or inducible. In other words, are the antibacterial properties produced by larvae at a constant level, or is production stimulated upon bacterial attack? This is a potential issue for maggot therapy as the use of disinfected or medical-grade larvae is essential for its implementation in modern medicine, in part to meet regulatory requirements and to eliminate the risk of introducing new pathogens into the wound. The inducible nature of larval antibacterial properties could therefore have ramifications for their effectiveness in wound treatment or during *in vitro* experimentation.

Early investigation into the antimicrobial properties of *L. sericata* larvae originally suggested that non-disinfected maggots appeared to produce more bioactive excretions and secretions [7, 8]. Subsequent data produced using whole body extracts and haemolymph noted a three- to six-fold increase in the comparable bioactivity seen when using maggots removed from chronic wounds compared to disinfected maggots [14]. The study also found haemolymph-related activity increased 16-fold when disinfected maggots were injured with a needle containing bacteria. An additional study found that certain genes within the *L. sericata* genome would be differently expressed in second-instar larvae in response to receiving a septic wound (punctuated dorsolaterally with a needle that was contaminated with a lipopolysaccharide solution containing 10 mg/mL crude preparation of *E. coli*). This included genes that encode for signalling proteins, proteinases, homeostasis proteins, and potential antimicrobial peptides, indicating that the production of these factors was induced by the infection event [25]. Further investigations showed that homogenised whole-body extracts of larvae incubated with a bacterial suspension had greater antibacterial activity than extracts from disinfected larvae [26], and that preincubation of third-instar larvae in concentrations of *P. aeruginosa* resulted in the production of excretions and secretions that were significantly more effective than those from disinfected larvae in preventing and degrading biofilm [27].

A separate study found that the level of bacterial contamination had no bearing on the antibacterial potency of larval excretions and secretions [28]. Although this seems to contradict the observations described earlier, the authors draw attention to the fact that their study tested excretions and secretions while the earlier-mentioned studies tested internal haemolymph [25] and/or whole-body extracts [14, 26]. This would suggest that antibacterial factors of excretions and secretions are produced constitutively, whilst antibacterial properties of haemolymph may only be expressed when induced by the presence of bacteria. This idea was further corroborated after finding that a large antimicrobial peptide, the *Lucilia* defensin lucifensin (see below), was produced in the salivary glands and fat body of larvae and that certain infectious environments increased expression in the fat body, but had no effect on its expression of excretion and secretion products [29]. Indeed, many previous studies reporting on the *in vitro* antibacterial activity of collected excretions and secretions did so with disinfected larvae [11, 12, 16, 30, 31], demonstrating that this insect is able to produce and secrete constitutive antibacterial factors without prior exposure to bacteria.

Identification of Maggot-derived Antimicrobial Compounds

With the knowledge that maggot excretions and secretions contain antibacterial properties came an interest in discovering the identities of the compounds responsible. A study by Kerridge and colleagues [15] noted the antibacterial properties of maggot excretions and secretions and found that when extracted they were highly stable as a freeze-dried preparation. They went on to suggest these extractions could be used as a source of novel antibiotic-like compounds, which could be used for infection control. Indeed, the potential for these compounds to be used in the development of novel treatments is driving considerable research into the identification and characterisation of individual antimicrobial compounds or molecules. A summary of this research is presented in this section along with a summary table listing activities, characteristics, and modes of action (Table 9.1 available at https://hdl.handle.net/20.500.12434/2f7d13xl).

Partial characterisation of some small molecular antibacterial compounds was completed by Bexfield and colleagues [12, 30].

Particular attention was paid to a <500 Da fraction that showed broad-spectrum antibacterial activity, including activity against a range of MRSA strains [30]. This fraction was later identified as $C_{10}H_{16}N_6O_9$ and it was registered as the antibiotic Seraticin [32, 33]. Work on uncovering the mode of action of Seraticin indicates it may be due to inhibition of septal formation and cell division (Nigam, unpublished data). Another report also identified low molecular weight compounds that exhibited antimicrobial activity, including phydroxybenzoic acid (138 Da), phydroxyphenylacetic acid (152 Da) and proline diketopiperazine (194 Da), all of which showed activity against *Micrococcus luteus* and/or *P. aeruginosa*, and even more pronounced effects when used in combination [34].

Čeřovský and colleagues [35] later extracted and purified a defensin from the body tissues of *L. sericata* larvae which they believed to be the key antimicrobial component of maggot excretions and secretions. The compound was named "*Lucilia* defensin" or "lucifensin". This defensin was found to show an antibacterial effect against a range of *Staphylococcus* and *Streptococcus* species, though no effect was shown against Gram-negative species [36]. Its mode of action was also described, and involves a process of oligomerisation within the bacterial membrane, forming channels that result in membrane permeabilisation resulting in cell leakage and death [37, 38].

Lucifensin was successfully sequenced and chemically synthesised. The synthetic defensin is active against Gram-positive bacteria, but not Gram-negative strains such as *E. coli*, corroborating previous findings [38]. Additionally, lucifensin is produced in the salivary glands and fat body and certain infectious environments increase expression in the fat body, but not in excretion and secretion products [29]. The structure and characteristics of lucifensin were later described [39], and the potential for manufacturing an antibiotic-like pharmaceutical using a synthesised lucifensin was explored [37]. An almost identical defensin, named "lucifensin II", has also been isolated and described from a closely related blowfly species, *Lucilia cuprina* [40].

Zhang and colleagues [41] described the isolation and purification by ultrafiltration of an antimicrobial protein which they named MAMP. The <10 kDa fraction showed antimicrobial activity against standard and antibiotic-resistant strains of *S. aureas in vitro* and *in vivo*. The

authors described possible mechanisms of action by interaction with the bacterial cell membrane and destruction of the cell surface structure. Pöppel and colleagues [10] used RNA sequencing to characterise the transcriptomes of various organs that contribute to the synthesis of antimicrobial peptides and found larvae capable of producing a broad spectrum of antimicrobial peptides. The group identified 47 genes encoding putative antimicrobial peptides, of which 23 were produced as synthetic analogues. These displayed antimicrobial activity against a range of pathogens including *P. aeruginosa*, *Proteus vulgaris*, and *Enterococcus faecalis*, though they found mostly additive effects against *E. coli* and *M. luteus*.

A cecropin antimicrobial peptide named "Lucilin" was identified and partially characterised, and showed activity against a number of Gram-negative bacteria [42]. A variant was also identified in the species *Lucilia exiamia*, which displayed similar properties [43]. In addition, researchers characterised two cationic antimicrobial peptides from *L. sericata*, LA-sarcotoxin and LS-stomoxyn [44]. These showed selective activity against a range of Gram-negative species. Pharmacological profiling indicated no cytotoxicity or cardiotoxicity, and no acute toxicity in experiments with mice, making them lead candidates for the development of novel antibiotics. Having said that, pharmacokinetic properties need to be improved for oral and systemic administration [44].

Table 9.1 Overview of antibacterial molecules/compounds. Inspired by an overview table of maggot bioactivates by Yan and colleagues [45, Table 1]. https://hdl.handle.net/20.500.12434/2f7d13xl.

Mechanism of Maggot Action on Bacterial Biofilms

A bacterial biofilm is an assemblage of microbial cells embedded in a complex self-produced polymeric matrix, which adhere to each other and/or to a surface [46]. Chronic wounds are highly prone to developing biofilm as necrotic tissue allows for bacterial attachment and the wound may be susceptible to infection due to impaired immune response [47–49]. Free-living planktonic bacteria easily attach to the fibrin surface, switching to create a strong, slowly metabolising, walled environment. This results in the depletion of nutrients which in turn

causes starvation-induced growth arrest, thought to be a key mechanism in producing antibiotic tolerance in biofilm-forming bacteria [50]. This resistant, stable biofilm then serves to keep the wound in a chronically infected, non-healing state [51, 52]. Biofilms pose a serious problem to wound healing as they are widely recognised as being highly resistant to antibiotics as well as host immune responses [53]. Maggots, however, can tackle bacteria in this more resistant form, and various studies have sought to determine the effect of maggot excretions and secretions both on the ability of bacteria to form biofilm communities, and as an agent to disrupt existing bacterial biofilms.

Initial investigations into the effect of maggot excretions and secretions against biofilm found that different species of bacteria were impacted to varying degrees. The formation of biofilms composed of *S. aureus* was blocked by freeze-dried maggot excretions and secretions, whilst the formation of biofilms by *P. aeruginosa* was initially enhanced by the addition of maggot excretions and secretions before the biofilm collapsed after 10 hours. Against preformed biofilms, excretions and secretions were able to degrade *S. aureus*, whilst ten-fold more was required to degrade *P. aeruginosa* which only began 10 hours after application [31]. This difference in activity against different species was also observed in a subsequent investigation. Maggot excretions and secretions significantly reduced biofilm formation by *S. aureus* and *E. cloacae*, while growth of *P. mirabilis* was unaffected and even stimulated [54]. These results suggest that maggot ES may act selectively against different strains, rather than combatting a broad spectrum of bacteria.

Maggot excretions and secretions were also observed to disrupt biofilm formation of two different strains of *Staphylococcus epidermidis* (1457 and 5179-r1) that exhibit different mechanisms of bacterial adhesion and subsequent biofilm formation, thus corroborating the idea that more than one bioactive entity or mechanism present in maggot excretions and secretions may be involved in the prevention of biofilm formation [55]. The authors provided further support for this theory when they demonstrated differing effects of an *L. sericata*-derived recombinant chymotrypsin on bacterial adhesion of multiple *Staphylococcus* strains. They concluded that chymotrypsin was unlikely to represent a standalone agent. Rather, maggots secrete a variety

of bioactive antibiofilm agents, of which chymotrypsin is only one component [56].

In a separate investigation which examined biofilm formation on surfaces commonly used in a medical setting, maggot excretions and secretions were found to prevent biofilm formation and disrupt existing biofilms of *P. aeruginosa*, with more effective excretions and secretions being produced by third-instar maggots than first-instar maggots. In a subsequent study, the research group also observed antibiofilm activity against *S. aureus* and *S. epidermidis* [57]. An *in vitro* experiment on dermal pig skin explants found that maggots were effective in combatting biofilms of *S. aureus* and *P. aeruginosa* [58]. Interestingly, results from an investigation into the effect of incubating maggots with *P. aeruginosa* bacteria, and then washing and collecting excretions and secretions from these bacteria pre-treated maggots, suggested that excretions and secretions from maggots previously exposed to *P. aeruginosa* were more effective in degrading biofilm of that species than those from disinfected maggots [27]. Additionally, fatty acid extract from dried *L. sericata* larvae was found to prevent biofilm formation of *S. aureus* and *Streptococcus pneumonia* and to eradicate preformed biofilms of these bacteria [59].

As well as whole extracts of larvae, isolated molecules derived from maggot excretions and secretions have also been found to display antibiofilm properties. The recombinant Chymotrypsin 1, a serine proteinase, was found to be effective in degrading macromolecules containing microbial surface components that recognise adhesive matrix molecules (MSCRAMMs). MSCRAMMs play an important role in the initial attachment of bacteria prior to biofilm formation and by degrading these molecules, Chymotrypsin 1 may work to impede colonisation and subsequent biofilm formation [60]. Further work corroborated this notion, finding that the recombinant Chymotrypsin can interfere with bacterial adhesion and disrupt protein-dependent bacterial biofilm-formation mechanisms [56]. Additionally, another molecule, a purified DNAse isolated from maggot excretions and secretions was found to degrade extracellular bacterial DNA in *Pseudomonas* biofilms [61]. Bacteria need to acquire extracellular DNA (either from host tissue itself or from their own bacterial sources) in order to help construct a biofilm, but maggot DNAse appeared capable of digesting all extracellular and

bacterial sources of DNA, and thus inhibited the ability of bacteria to form a biofilm [61].

A common observation from biofilm investigations is that whilst maggot excretions and secretions are able to degrade and break down the biofilm of various species, the bacteria which are released from these biofilms are not destroyed [27, 31, 54, 55]. This was explored further by a group of researchers who noted that biofilms resisted antibiotics alone, but found that combining a treatment of maggot secretion and antibiotics (vancomycin, daptomycin or clindamycin) resulted in both the break-down of *S. aureus* biofilm and the elimination of the resulting bacteria [62]. This introduces a promising approach to the treatment of biofilm-infected chronic wounds, whereby use of a combination of maggot excretions and secretions and antibiotics could result in a more successful treatment than the use of a single method alone. Other findings also support this idea. A study investigating the combined use of maggot excretions and secretions with ciprofloxacin showed enhanced antimicrobial activity compared to the use of either individually [63]. Two other commonly used antibiotics, gentamicin and flucloxacillin, also showed enhanced synergistic antibacterial activity with maggot excretions and secretions [64].

Antifungal Activity of Maggot Excretions and Secretions

Within the scope of investigating antimicrobial activity of *L. sericata* larvae, research has primarily focussed on antibacterial compounds. The study of selective antifungal agents, meanwhile, has received much less attention. In an initial report, *L. sericata* larvae were found to be able to ingest yeasts, and ES collected from these maggots showed moderate antifungal activity against *Trichophyton terreste* mycelium [65]. This suggested the possibility of using maggot therapy in the treatment of wounds with fungal infections and superficial fungal infections. The authors went on to suggest that alkaline compounds in maggot excretions and secretions (such as ammonium carbonate, allantoin, and urea), may be partially responsible for this antifungal activity. Subsequent separate investigations also noted the potent antifungal activity of maggot excretions and secretions against *Candida*, *Aspergillus*, *Geotricum*, and *Saccharomyces* species [66, 67]. The antifungal component

was characterised and found to be heat-stable and resistant to freeze-drying. Following ultrafiltration of maggot excretions and secretions into three main fractions (>10, 10–0.5, and <0.5 kDa), it was revealed that the greatest level of anti-*Candida* activity was observed in the <500 Da fraction, suggesting that maggots were capable of producing a very small, but very active, antifungal molecule [67]. In addition, a larger antibacterial molecule, Lucifensin, also showed slight antifungal activity against *Candida albicans* [38].

Characterisation of one of the maggot antifungal compounds was also achieved in a 2014 study which managed to produce a recombinant form of the discovered peptide. Named "Lucimycin", the novel antifungal peptide showed activity against a range of phyla including Ascomycota, Basidiomycota, and Zygomycota, as well as the oomycete plant pathogen *Phytophtora parasitica* [68]. This shows potential for the use of antifungal peptides isolated from *L. sericata* not only in human medicine for the treatment of fungal infections, but also in agriculture for crop protection. The possibility of producing transgenic plants capable of expressing lucimycin is also postulated [68].

Additionally, maggot therapy has been used successfully in the treatment of wounds with mycotic infection. In one described case study, maggots in biobags were used for the treatment of a complex hand injury that was infected with *Absidia corymbifera* [69]. Significant improvement in wound condition was reported after just two 72-hour applications of maggots. Maggots effectively removed necrotic tissue and the mycotic infection was successfully eradicated [69].

Summary

Dating back to the 1920s, the antimicrobial potential of medical maggots has been recognised and explored. In more recent years, the interest in understanding and identifying the antimicrobial properties of maggot excretions and secretions has been driven by the potential to use medical maggots as a source of novel antibiotics and anti-infectives. This is particularly relevant at this time considering the rise of drug-resistant forms of pathogenic bacteria such as MRSA.

There is mounting evidence for the antimicrobial activity of maggot ES against a range of bacteria and fungi. This includes efficacy against

antibiotic-resistant bacterial strains, with greater activity generally observed against Gram-positive species and less so against Gram-negative bacteria. However, some studies have shown contradictory results in this regard, which may be a result of differing methods that have been used to test the antimicrobial effects of maggot excretions and secretions. Consequently, a greater consensus in testing methodology may be useful to produce more consistent and comparable results. Evidence has also shown action of maggot excretions and secretions against biofilms, to which chronic wounds can be prone, and which pose a significant problem to wound healing due to their resistance to antibiotics. This is therefore an important and promising area of investigation. Much of the evidence relating to the antimicrobial properties of maggots has relied on investigations conducted *in vitro*. A small number of clinical reports and case studies have been described, but their scope and scale has generally been limited, so the clinical evidence is still lacking. Further investigations would be useful to more conclusively demonstrate the antimicrobial properties of maggot therapy clinically.

As well as understanding the properties of maggot excretions and secretions, there is also great interest in identifying the compounds responsible with a view to developing new treatments. So far, a number of antibacterial molecules have been identified with varying structures, mechanisms, and activities against Gram-positive and Gram-negative bacteria, and susceptible and resistant strains. Some antibiofilm and antifungal molecules have also been described. The various research and discoveries in this area highlight the versatility of larval excretions and secretions as sources of effective antimicrobial molecules. It is anticipated that ongoing efforts in this field will advance our understanding of maggot therapy and its therapeutic principles and that this will lead to the development of new therapies. Many of these secreted factors are currently being isolated and investigated for the development of new antimicrobial drugs and treatments. However, when whole maggots are placed on a chronic and infected wound, they excrete not just a single antimicrobial compound, but a cocktail of such compounds thus providing an effective and unique antimicrobial environment. It is therefore unclear whether it will ever be possible to match the multiple therapeutic benefits conveyed by whole-organism maggot therapy with drugs based on individual active compounds of maggot excretions and secretions.

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