Reprinted from
PHARMACEUTICAL ENGINEERING
THE OFFICIAL TECHNICAL MAGAZINE OF ISPE
MAY/JUNE 2014, VOL 34, NO 3

www.PharmaceuticalEngineering.org

To Investigate the Antimicrobial Potential of *Lucilia sericata* Larvae

by Qin Xiang Ng

This article presents a research project that investigates the antimicrobial effects of excretions/secretions and gas flatulence produced by *Lucilia sericata* larvae on *Escherichia coli*, *Staphylococcus epidermidis,* and *Micrococcus luteus*. It was presented at the 2013 ISPE Annual Meeting as part of the student poster competition.

According to a study done by the Center for Disease Control and Prevention (CDC) in the United States alone, food-borne diseases account for 76 million diseases, 325,000 hospitalizations, and 5,000 deaths each year;[1] and bacterial infections make up for approximately 5 million of these diseases.[1]

Modern medicine depends heavily on the use of antibiotics to kill pathogenic bacteria. Antibiotics are classified according to their structure and mechanism of action, e.g., the inhibition of metabolic processes which are vital for bacterial growth and replication. The main mechanisms of antibiotics are inhibition of cell wall, protein, important enzymes, nucleic acid synthesis and disruption of cell membrane;[2] however, in the recent years, the emergence of antibiotic-resistant bacteria (superbugs) has become a global concern as these "superbugs" are resistant to even the most powerful of modern antibiotics.[3]

Worryingly, there has not been a new class of antibiotics discovered since the 1980s.[4] The World Health Organization (WHO) has warned that the world is heading toward a "post-antibiotic era" and "many common infections will no longer have a cure and, once again, could kill unabated."[4]

The limitations of antibiotic treatment and the rapid emergence of antibiotic-resistant pathogenic bacteria have renewed interest in efforts to find alternative antimicrobial therapeutics. Maggot Debridement Therapy (MDT) is an unconventional therapeutic treatment involving the introduction of live, sterile larvae into the non-healing skin and soft tissue wounds of a patient for the purpose of cleaning out the necrotic tissue within a wound and disinfection. Maggot debridement therapy is reportedly effective for wounds infected by methicillin-resistant Staphylococcus aureus (MRSA) and "flesh-eating bacteria."[5] As limited studies have been done to investigate the exact antiseptic mechanisms, this project aimed to investigate the antimicrobial effects of excretions/secretions and gas flatulence produced by *Lucilia sericata* larvae on *Escherichia coli, Staphylococcus epidermidis,* and *Micrococcus luteus*.

Materials and Methods

Lucilia sericata larvae were reared on a diet of ad libitum pig's liver; third-instar larvae (three-day-old) were used for all experiments. Briefly, overnight Excretions/Secretions (ES) were collected from 10 g of third-instar larvae, centrifuged to remove particulate material and filter-sterilized before use. 1000 µl of 10^3 colony forming units (cfu)/ml bacterial broth culture was combined with 100 µl of sterile ES extract, antibiotic ampicillin or sterile water. Subsequently, 10 µl of the mixture was plated onto a new sterile Luria Bertani (LB) agar plate for enumeration of bacterial colonies after overnight incubation at 37°C.

To investigate the possible antimicrobial effects of larval flatulence, an air-tight setup was constructed. The entire experiment was carried out within a laminar flow hood to ensure sterility. Gas flatulence produced by 500 third-instar

larvae was applied to bacterial culture plates (plated with 10 µl of 10^3 cfu/ml of *E. coli, S. epidermidis,* or *M. luteus*) and an accompanying control plate (with no bacteria plated) for an hour. Unidirectional flow of air was ensured by using a suction pump set at 80 Pa. The plates were then removed from the exposure box and incubated overnight at 37°C for enumeration of bacterial colonies.

Results and Discussion

As seen in Figure 1, the larval excretions/secretions showed significant inhibitory effects against both Gram-positive and Gram-negative bacteria tested in this study. When compared to the control (sterile water), the difference in the average number of bacterial colonies counted was confirmed to be significant using two-tailed unpaired t-test. Furthermore, we can see that the antimicrobial effects of larval excretions/secretions were comparable to that of ampicillin (30 mcg/ml); in fact, it exhibited a more pronounced antimicrobial effect against *S. epidermidis* than ampicillin, causing a remarkable 89.21% decrease in the number of *S. epidermidis* colonies as compared to the control.

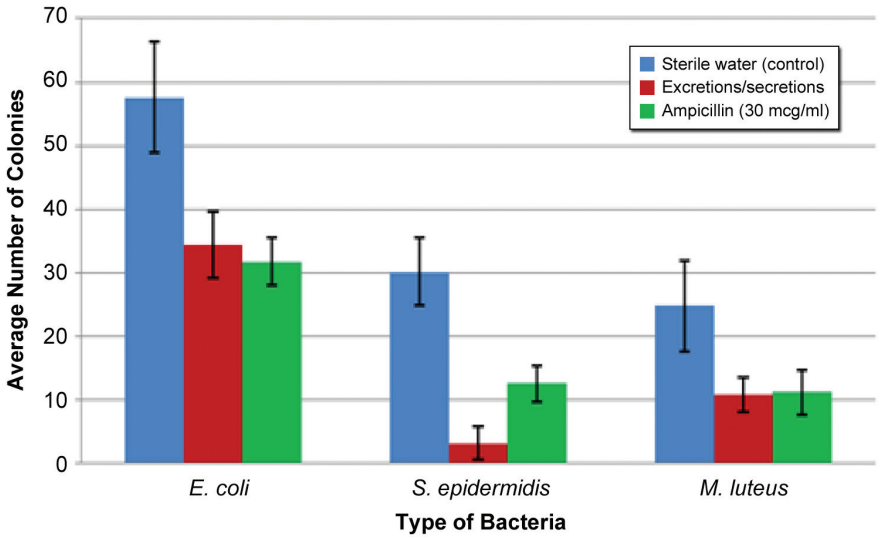

Figure 1. Antimicrobial effects of larval excretions/secretions on *E. coli, S. epidermidis,* and *M. luteus* (error bars showing ±1 standard deviation, n = 30).

The larval excretions/secretions were tested with a pH probe and showed to be alkaline in nature (pH 9). Its antibacterial effects and alkaline nature can be attributed to the presence of ammonia, ammonium bicarbonate, urea, allantoin and various proteolytic enzymes, e.g., chymotrypsins.[6]

As for the larval flatulence, it also has significant antimicrobial properties when comparing the average number of bacterial colonies counted to the control setups (i.e., $p < 0.05$). This can be seen in Figure 2. Two controls were used in this experiment. As the larval maggots were fed with pig's liver, another control setup was prepared with just pig's liver and no maggots present to rule out the effect of any gases that may be released by microorganisms found in the non-sterile pig's liver.

Preliminary chemical analysis of the gas flatulence has been done using gas chromatography-mass spectrometry (GC-MS) and results showed that compounds like aldehydes, aliphatic esters, ethers, ketones, phenols and derivatives, alcohol and siloxane are present in the flatulence produced by the larvae. The presence of these organic compounds creates an environment unfavourable for bacterial growth as some of them are bactericidal in nature. However, 28% of the compounds remain unidentified. GC-MS is also unable to analyse non-volatile and thermally fragile compounds, further analysis should be done using HPLC-MS.

Conclusion

In conclusion, the results showed that natural products from *L. sericata* larvae hold great promise for development of potent antimicrobial therapeutics.

The larval excretions/secretions, being liquids, could be

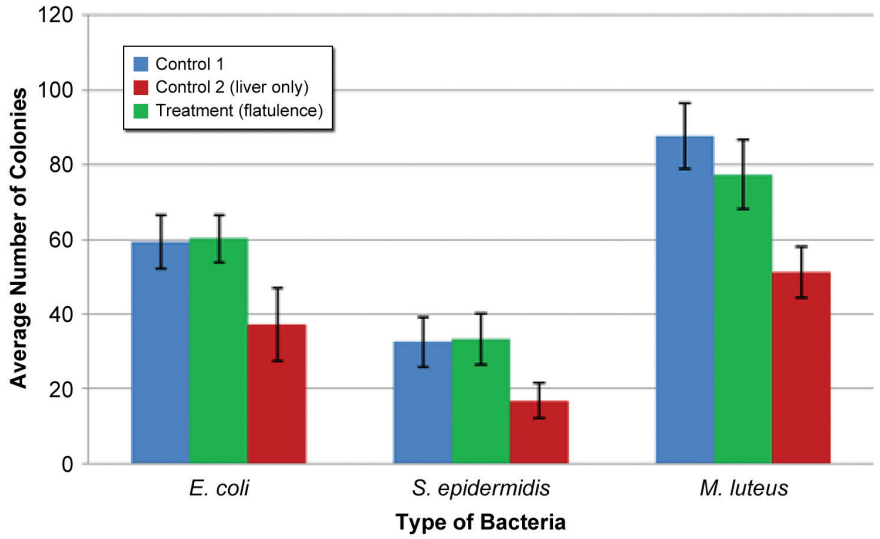

Figure 2. Antimicrobial effects of larval flatulence on *E. coli, S. epidermidis,* and *M. luteus* (error bars showing ±1 standard deviation, n = 30).

freeze-dried to prolong its shelf life. And these larval excretions/secretions could be further analyzed using high-performance liquid chromatography to identify and isolate the bioactive compounds present.

The gas flatulence could be further refined and made into an inhalant for treating lung infections, such as tuberculosis, a terrible disease that affects an estimated one third of the world's population, with new infections occurring at a rate of about one per second.[7] Antibiotic resistance is a growing problem in Multiple Drug-Resistant Tuberculosis (MDR-TB) infections. Normally, tuberculosis is treated using oral antibiotics and there is no targeted delivery. If the larval flatulence is made into an inhalant, targeted delivery is possible as the medication is directed straight to the lungs. Gases and volatile drugs may be inhaled and absorbed through the pulmonary epithelium and mucous membranes of the respiratory tract. Access to the systemic circulation is rapid by this route because the lung's surface area is large (pulmonary absorption is rapid) and first-pass metabolism by the hepatobiliary system is avoided.[8]

Future work entails testing the larval excretions/secretions and flatulence on a greater variety of bacteria, particularly antibiotic-resistant strains, and further chemical analyses of these natural products. In addition, for an antimicrobial agent to be considered effective, not only must it possess good antibacterial efficacy, it also must be selectively toxic. Being selectively toxic means that they must target only the disease-causing bacteria and have minimal or no toxicity to the host cells. Hence, the possible cytotoxic and genotoxic effects of the proposed treatment methods also must be evaluated (by testing on human cell lines so as to ensure that these natural products are safe for oral consumption or topical application).

Also, previous studies have suggested that excretions/secretions from aseptically raised larvae were much less potent than those collected from non-sterile maggots.9 This is an exciting area for future study as this possibly implies that the antimicrobial efficacy of these extracts (and maggot therapy) can be enhanced by pre-inoculating larvae with non-pathogenic strains of antibiotic-resistant bacteria in order to prime the gut with antimicrobials.

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