AN INVESTIGATION INTO THE ANTIBACTERIAL PROPERTIES OF *LUCILIA CUPRINA* FLIES

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Summary

In this study, we investigated the antibacterial properties of the body fluids of *Lucilia cuprina* flies. The body fluid of the flies was extracted via centrifugation and screened against 4 different kinds of bacteria. The results showed that the body fluid do indeed have antibacterial properties as there was a significant decrease in the number of colonies of bacteria growing on the sample plates.

We believe that this discovery could be incorporated in the production of inhalants aimed at treating infections of the respiratory tract. It could also have possible industrial applications in the form of detergents.

1. Abstract

The use of Maggot Debridement Therapy (MDT) has increased over the years for the debridement of chronic wounds and for the enhancement of wound healing. In it, the bodily secretions of maggots were revealed to contain some form of natural defence against bacteria, such that treatment of wounds against infection in patients was possible. Research carried out in this field mainly focused on larval therapy and barely any study has been conducted on the adult flies to investigate for possible anti-bacterial properties.

After hatching, female *Lucilia cuprina* need a source of protein for their reproductive organs to mature, and also another feed of protein before they lay their eggs. The common sources of protein for *Lucilia cuprina* include carcasses and manure.[i] [ii] As microorganisms are found in abundance amongst these, we have concluded that in order to feed on the rotting flesh of carcasses and manure, *Lucilia cuprina* must have some antibacterial and antimicrobial properties in their bodies to counteract the bacteria and fungi found in their diet.

Thus, this motivated the project team to investigate the anti-bacterial properties of the bodily fluids of *Lucilia cuprina* adult flies and compare them to that of maggots from the same species.

The body fluids of *Lucilia cuprina* flies were extracted and screened against four strains of bacteria: *Escherichia coli*, *Staphylococcus epidermidis*, *Pseudomonas putida* and *Micrococcus luteus* through serial dilution. Our study conducted showed that the body fluid of Lucilia cuprina flies indeed had antibacterial properties against all the above bacteria apart from *Escherichia coli*.

2. **Materials and Methodology**

2.1 Apparatus:

Petri dishes
Centrifuge tubes
Glass rods
Inoculating loops
Sterile 0.45µm syringe filter
Micropipettes
Micropipette tips
Disposable droppers
Disposable spreaders

2.2 Materials

Lucilia cuprina Adult Flies
Sterile Water
Bacteria: *Escherichia coli*
Staphylococcus epidermidis
Pseudomonas putida
Micrococcus luteus

2.2 Preparation to test for Anti-bacterial Properties of Lucilia cuprina Fly Fluid

2.2.1 Preparation of nutrient agar and nutrient broth

1. Nutrient agar was prepared by mixing 14g of nutrient agar in powdered form with 500ml of water.
2. Nutrient broth was prepared by mixing 3.25g of nutrient broth in powdered form with 250ml of water.
3. A total of four 500ml-bottles containing nutrient agar and four 250-bottles containing nutrient broth were made and autoclaved at 121˚C for 15 minutes.
4. The nutrient agar is then poured into 66 petri dishes and allowed to set before being stored for later use.

2.2.2 Preparation of *Lucilia cuprina* fly fluid

1. 750 *Lucilia cuprina* adult flies in a cage was frozen and taken out of the cage.
2. 30 dead flies were placed into each centrifuge tube and squashed using a glass rod.
3. 1ml of sterile water was transferred using a micro-pipette into each centrifuge tube and the flies were further squashed by the glass rod.
4. The 25 centrifuge tubes obtained were then sent for centrifugation at 7000 rpm for 10 minutes.
5. The supernatant in each centrifuge tube was obtained and collated using disposable droppers and then sent for further centrifugation till no sediment can be observed.
6. The final obtained supernatant was filtered through a sterile 0.45µm syringe filter.

2.2.3 Preparation of bacteria broth cultures

1. 8ml of nutrient broth was transferred using a 10ml pipette into a centrifuge tube. Four such tubes were prepared, each for the respective bacteria: *Escherichia coli*, *Staphylococcus epidermidis*, *Pseudomonas putida* and *Micrococcus luteus*.
2. Using the inoculating loop, single colonies on the respective bacteria master plates were selected and placed into the nutrient broth in the respective centrifuge tubes for overnight culture.

2.2.4 Serial dilution

1. 49 centrifuge tubes were prepared and each of them contained 9ml of nutrient broth.
2. Serial dilution was carried out. For Tube A, 1ml was transferred from the sample tube incubated overnight into A-1. The tube A-1 was shaken and 1ml was transferred from A-1 to A-2. The tube A-2 was shaken and 1ml was transferred from A-2 to A-3 and so on. The same was repeated for the other tubes.

2.2.5 Plating

1. After serial dilution, the 66 agar plates were dried and ready for plating.
2. 100µl was transferred from each respective centrifuge tube using a micro-pipette, onto the agar plate and spread across the entire surface of the agar plate using a disposable spreader.
3. Three replicates were prepared for each dilution.
4. The agar plates were then incubated at 37°C overnight.

3. Results

Overall Experimental Results

9 tubes were prepared, each containing the following:

Tubes	Reagents (ml)						
	Nutrient broth	Sterile Water	Fly fluid	*Escherichia coli*	*Staphylococcus epidermidis*	*Pseudomonas putida*	*Micrococcus luteus*
A	8.0	1.0	-	1.0	-	-	-
B	8.0	1.0	-	-	1.0	-	-
C	8.0	1.0	-	-	-	1.0	-
D	8.0	1.0	-	-	-	-	1.0
E	8.0	1.0	1.0	-	-	-	-
F	8.0	-	1.0	1.0	-	-	-
G	8.0	-	1.0	-	1.0	-	-
H	8.0	-	1.0	-	-	1.0	-
I	8.0	-	1.0	-	-	-	1.0

Number of *Lucilia cuprina* flies involved: 640 (separated into tubes of 30)
Amount of fly fluid obtained after centrifugation and filtering: 8.5ml

Tube/ agar Plate	Colony Count Observations (CFU/ml)			
	Replicate 1	Replicate 2	Replicate 3	Average
A	8.9×10^7	9.6×10^7	8.1×10^7	8.9×10^7
B	1.9×10^7	2.0×10^7	1.8×10^7	1.9×10^7
C	2.1×10^7	2.3×10^7	1.9×10^7	2.1×10^7
D	1.3×10^7	1.3×10^7	1.3×10^7	1.3×10^7
E	$<1 \times 10^5$	$<1 \times 10^5$	$<1 \times 10^5$	$<1 \times 10^5$
F	1.1×10^7	1.4×10^7	1.1×10^7	1.2×10^7
G	7.5×10^1	5.0×10^1	2.0×10^1	4.8×10^1
H	3.3×10^1	6.7×10^1	4.0×10^1	4.7×10^1
I	6.2×10^1	4.5×10^1	6.3×10^1	5.7×10^1

Due to unforeseen experimental errors throughout the course of the laboratory process, nutrient agar plates with the contents of Tubes A, B, C and D were contaminated with another bacteria type (*Serratia marcescens*).

However, there was a significant difference in the *Serratia marcescens* colonies between the agar plates containing culture from the control tubes and the sample tubes, with the sample tubes having a much lesser count that the colonies originally present on the control plate. This led the project team to conclude that the body fluids of *Lucilia cuprina* flies also prove to be very effective against *Serratia marcescens*.

The experimental results show that the negative control Tube A had a colony count of 8.9×10^7 CFU/ml on average when plated on nutrient agar. Encouragingly, Tube F, which contains exactly the same contents as Tube A except that sterile water was replaced with the test sample of fly fluid, only had an average count of 1.2×10^7 CFU/ml on plated agar culture. This indicates that the number of colonies of *Escherichia coli* capable of growth in the presence of fly fluid was greatly reduced.

Similarly, the negative control Tube B had a colony count of 1.9×10^7 CFU/ml on average when plated on nutrient agar. However, plates carrying samples from Tube G which contains exactly the same contents as Tube B except that the 1ml of sterile water was replaced by 1ml of fly fluid, only had an average of 4.8×10^1 colonies. This indicates that the growing ability of *Staphylococcus epidermidis* was greatly inhibited by presence of *Lucilia cuprina* fly fluid.

Also, the negative control Tube C had a colony count of 2.1×10^7 CFU/ml on average when plated on nutrient agar. Tube H, which contains exactly the same contents as Tube C except that the 1ml of sterile water was replaced by 1ml of fly fluid, only had an average of 4.7×10^1 colonies on each agar plate. This indicates that the number of colonies of *Pseudomonas putida* capable of growth in the presence of fly fluid was greatly reduced.

Lastly, the negative control Tube D had a colony count of 1.3×10^7 CFU/ml on average when plated on nutrient agar. Tube I, which contains exactly the same contents as Tube D except that the 1ml of sterile water was replaced by 1ml of fly fluid, only had an average of 5.7×10^5 colonies. This indicates that the growing ability of *Micrococcus. luteus* was greatly inhibited by presence of *Lucilia cuprina* fly fluid. This indicates that the number of colonies of capable of growth in the presence of fly fluid was greatly reduced.

4. Analysis and discussion

4.1 Discussion and application of results

One problem that we encountered whilst conducting our study was that the agar plates plated with culture inoculated with samples of fly fluid did not show any growth. One probable reason for this could be that the period of incubation of the agar plates was too long (overnight), and all the bacteria present in the plates were eliminated by the presence of the fly fluid during this time period. One possible method to overcome this would be using culture from a lower dilution from plating, or to find out the Minimum Inhibitory Concentration of the active compound in the body fluid of *Lucilia cuprina* flies, which is the lowest concentration of an antibiotic that is able to sufficiently kill bacteria effectively.[iii] This could be done by varying the concentration of fly fluid in the sample tubes during repeats of the experiment.

The body fluids of *Lucilia cuprina* are proven to have antibacterial properties as there was a significant difference between the control and the sample tubes as shown in the discussion of the results. However, one interesting result that we have noted is that the body fluid of *Lucilia cuprina* fly fluid is more effective against gram positive bacteria. One study conducted by a team of Hwa Chong Institution (High School) students reported that the body fluids of *Lucilia cuprina* maggots do have a greater effectiveness against gram positive bacteria, but this may be due to different chemical compounds present in the immune system of the flies as compared to maggots as the compounds synthesized can change in accordance to the diet of the flies.

The difference between gram positive and gram negative bacteria is that gram positive bacteria have several peptidoglycan layers making up the cell wall, whereas gram negative bacteria may have one or a few layers of peptidoglycan on the inside of the cell, and the bacterial cell is further surrounded with a lipid membrane.

One possible suggestion for this phenomenon occurring is that there is an active compound, similar to that of natural penicillin in the body fluids of *Lucilia cuprina* adult flies that specifically inhibits the synthesis of the peptidoglycan cell walls by binding with the transpeptidase enzymes that repairs the cell wall as new peptidoglycan monomers are added during bacterial cell growth. This blocks the transpeptidase enzymes from cross-linking the sugar chains in the cell wall to form the mesh structure, thus resulting in a weak cell wall, causing the bacteria to lyse due to uncontrolled entry of water. [iv] Another possible suggestion would be that it inhibits the transglycosylase enzyme, as both enzymes are crucial to the formation of the peptidoglycan cell wall. It has been found that penicillin, which has a stronger effect against gram positive bacteria, works by inhibiting the transpeptidase enzyme. [v]

Although this mechanism would affect gram negative bacteria to a certain extent, research has shown that this mechanism would have less effect against gram negative bacteria as compared to gram positive strains. This is because gram negative bacteria would still retain a part of their cell walls even after having been treated with penicillin, and it would be probable for them to grow and undergo binary fission.[vi] However, gram positive bacteria lose their cell walls completely after having been treated with penicillin, thus rendering them incapable to survive and thus undergo lysis.

One possible application of this is to isolate this compound and incorporate it inside inhalers. This would prove to be effective as *Serratia marcescens* colonies are commonly found in the human respiratory tract and has been proven to cause respiratory infections. Incorporation of this compound in the inhalants would no doubt prove to be a quick method of treating infections. Alternatively, it could be included in bandages to ensure quicker healing due to its antibacterial properties.

4.2 Future work

Also, since *Lucilia cuprina* flies are scavengers that feed on rotting carrion by nature, besides antibiotic properties being present in their body fluids, proteases would also be present. These proteases could be identified and isolated using high performance liquid chromatography (HPLC). One possible industrial application of these proteases would be in the manufacture of detergents as these would probably prove to be effective in removing stubborn stains. In the different growth stages of flies, different classes of proteases may be synthesized due to the changes in their diet. For example, *Lucilia cuprina* maggots produce great quantities of collagenase, but this enzyme may not be present in *Lucilia cuprina* flies at such high concentrations. [vii] Thus, different proteases may be isolated from *Lucilia cuprina* flies at different stages of their life cycle, broadening the industrial applications of the body fluid of *Lucilia cuprina* flies as different kinds of proteases may be incorporated into different detergents.

5. Acknowledgements

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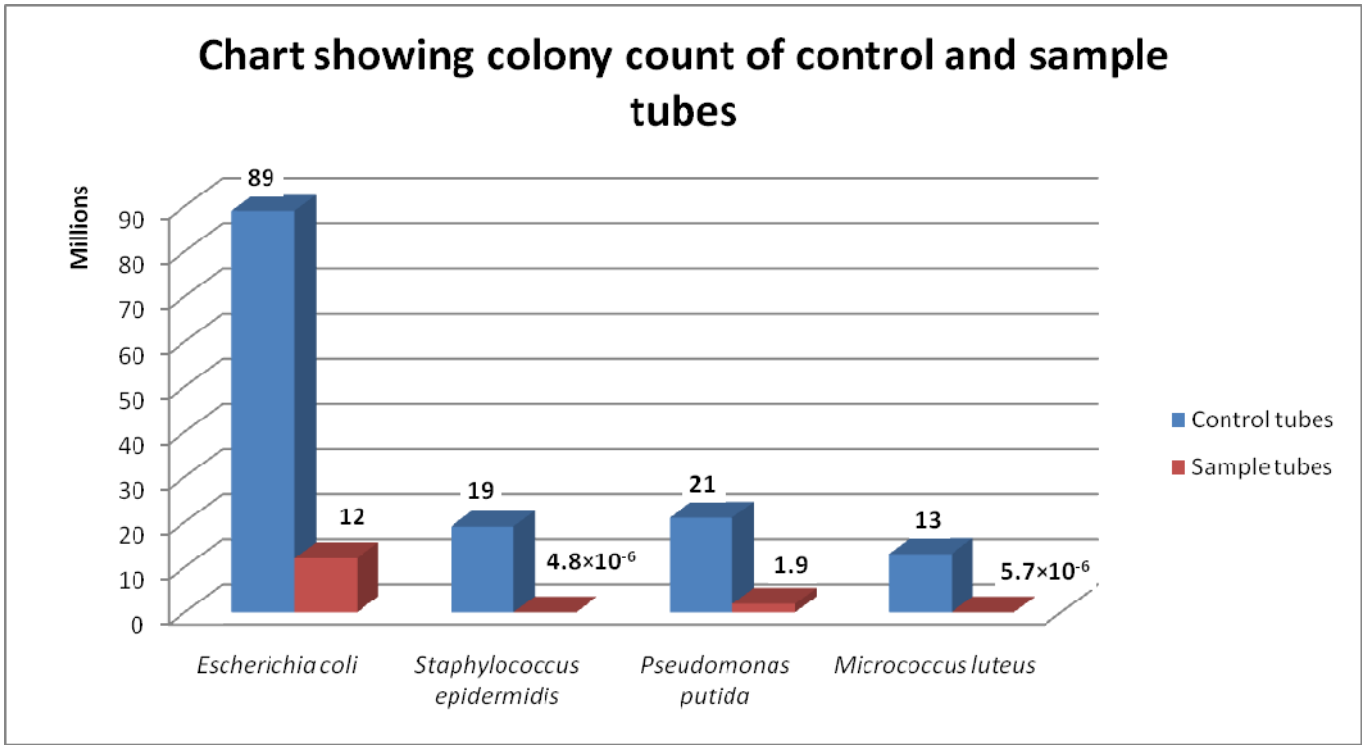
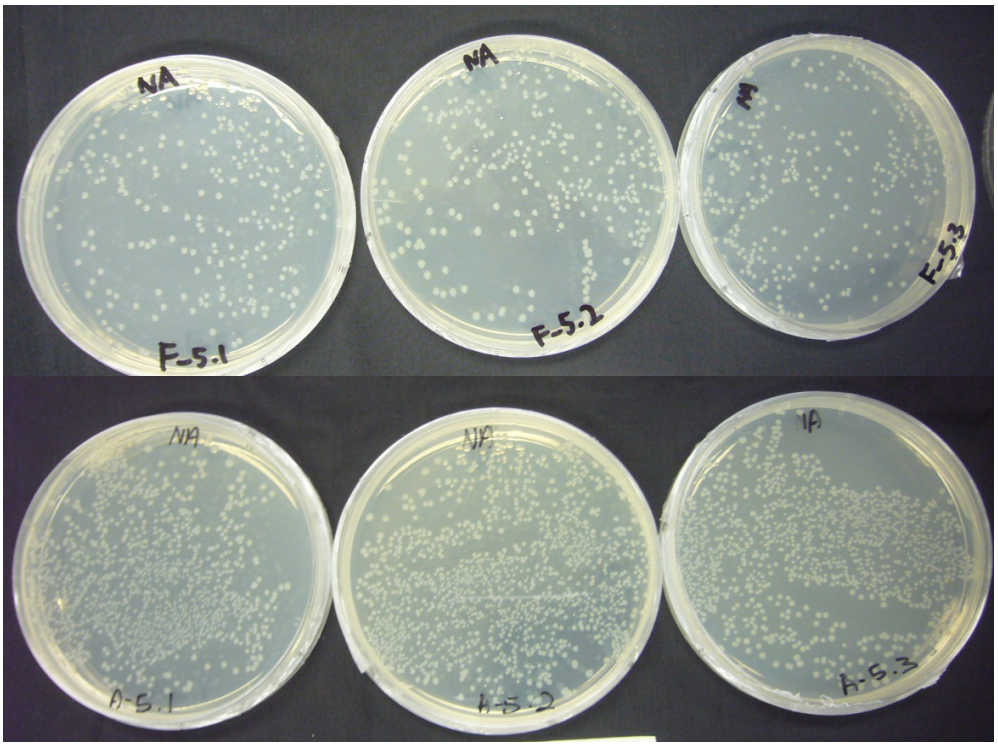
Appendix

Appendix I



Appendix II

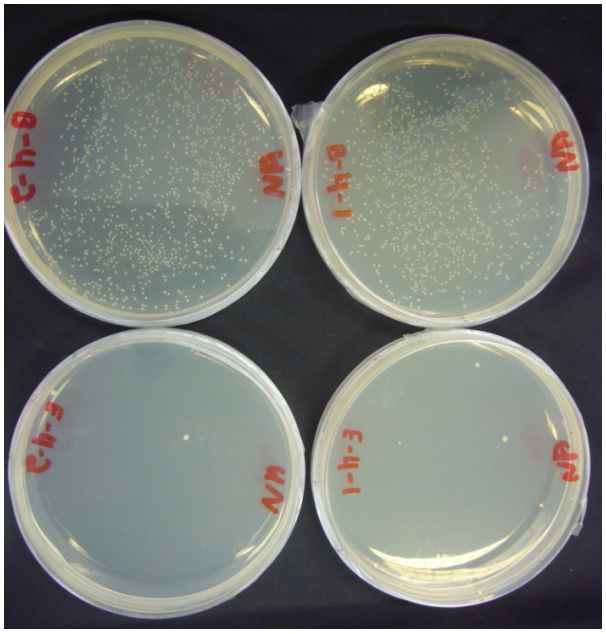
Pictures of experimental results



Control plates: *Escherichia coli* only

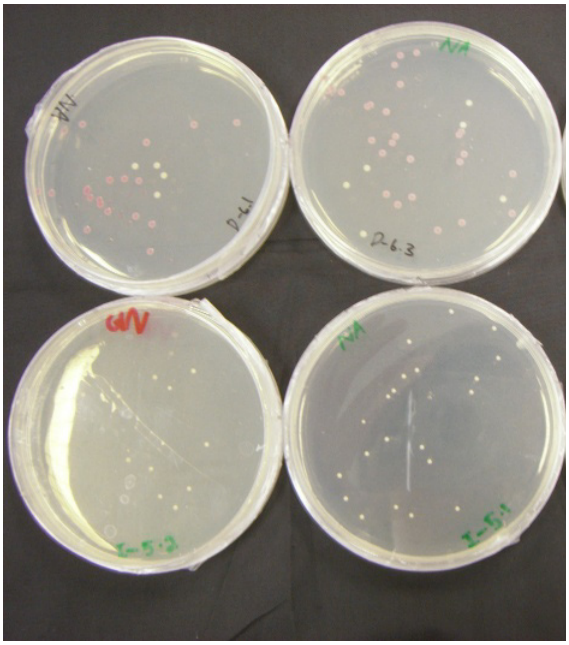
Sample plates: *Escherichia coli* and body fluid of *Lucilia cuprina* flies

Fig 1. Agar plates plated with *Escherichia coli* culture from sample and control tubes



Control plates: *Staphylococcus epidermidis* only

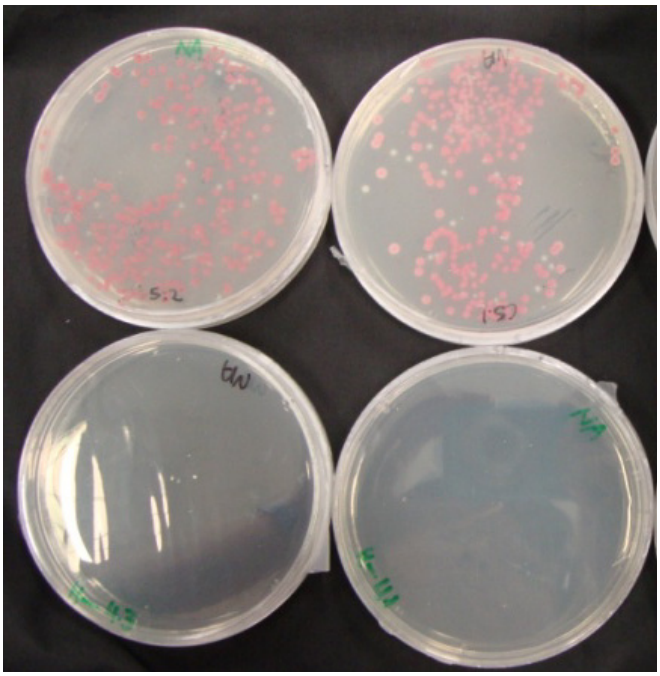
Sample plates: *Staphylococcus epidermidis* and body fluid of *Lucilia cuprina* flies



Control plates: *Pseudomonas putida* only

Sample plates: *Pseudomonas putida* and body fluid of *Lucilia cuprina* flies

Fig 3. Agar plates plated with *Pseudomonas putida* culture from sample and control tubes (with *Serratia marcesens* contaminant)

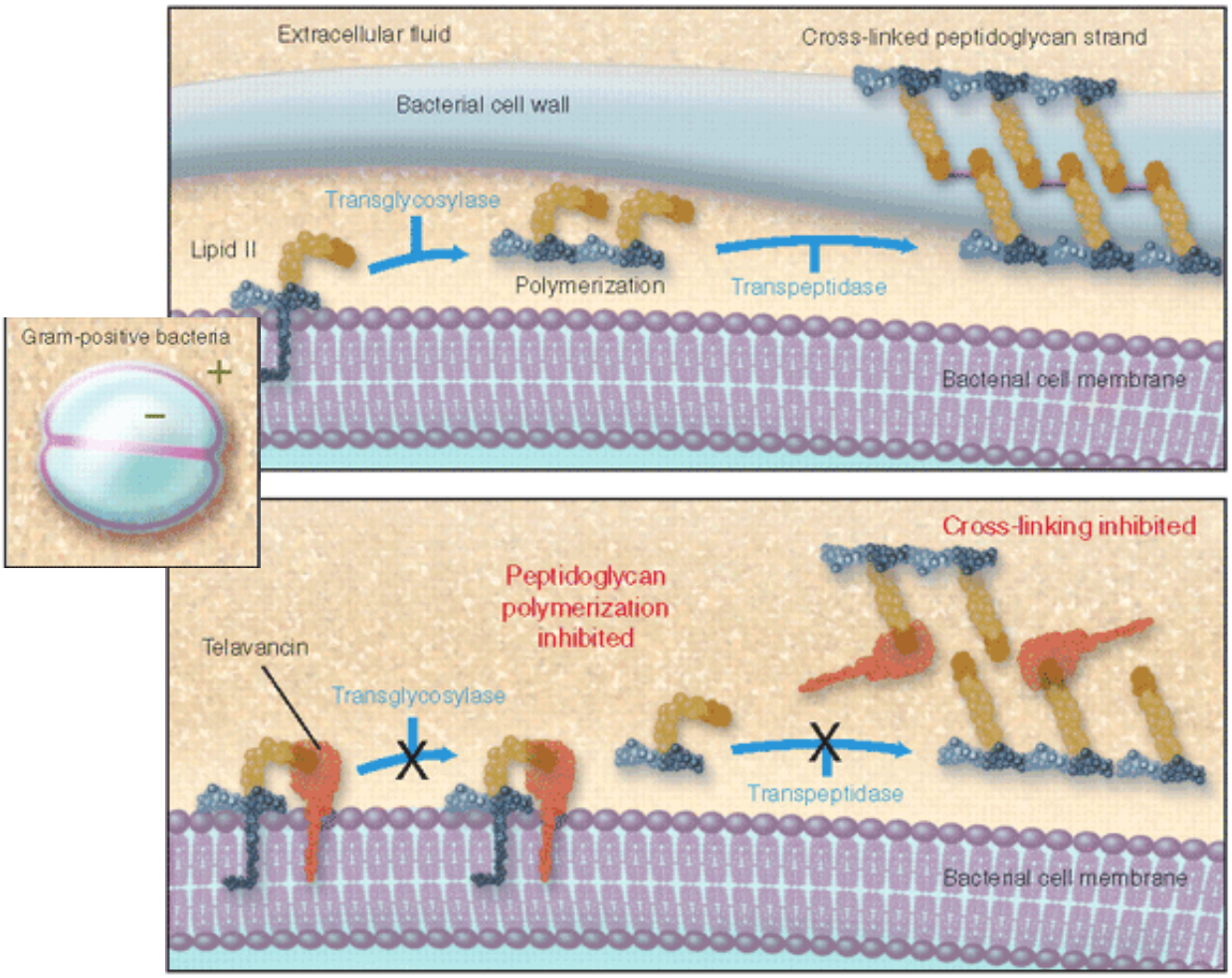


Control plates: *Micrococcus luteus* only

Sample plates: *Micrococcus luteus* and body fluid of *Lucilia cuprina* flies

Fig 4. Agar plates plated with *Micrococcus luteus* culture from sample and control tubes

Appendix III



Picture taken from: http://www.medscape.com/viewarticle/566883_2 [viii]

As can be seen from the above diagram, the transpeptidase enzyme is responsible for the cross linking in the peptidoglycan cell wall. If the transpeptidase enzyme has been inhibited by the active compound in the fly fluid, the cross-linking in the cell wall will not be established, causing the cell wall formed to be weak and in turn cause the bacteria to undergo osmotic lysis.

References

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