# Testing Antimicrobial Activity of Protein Filament

## Part 1: Evaluating Methods of Sterilising Filament and Slime

*Date: 8/7/14*

## Method

### YPD Plates

1. Filament samples were placed aseptically on LB agar plates as follows:
    a. Freeze dried (stored in non-sterile water)
    b. Treated with Formic Acid (90%)
2. Controls were also set up as follows:
    a. Positive Control – touched with unwashed fingers
    b. Negative Control – unopened plate
3. These plates were incubated overnight at 37 degrees.

### LB Plates

1. Filament samples were placed aseptically on LB agar plates as follows:
    a. Freeze dried
    b. Natural slime treated with DTT and Sodium Citrate
    c. Freeze dried & treated with Formic Acid (90%)
2. Controls were also set up as follows:
    a. Positive Control – touched with unwashed fingers
    b. Negative Control – unopened plate
3. Plates were incubated overnight at 37 degrees.

## Results

**Table 1: Results of Sterility Testing on YPD Plates**

| Plate | Result | Comment |
|---|---|---|
| Positive Control | Growth | Plates required being left for two nights at 37 degrees before growth was seen. |
| Negative Control | No growth | |
| Freeze dried fibre | Growth | Had been stored in non-sterile water which may have caused growth. Suggests that fibre is not antimicrobial. |
| Fibre treated with Formic Acid | No growth | |

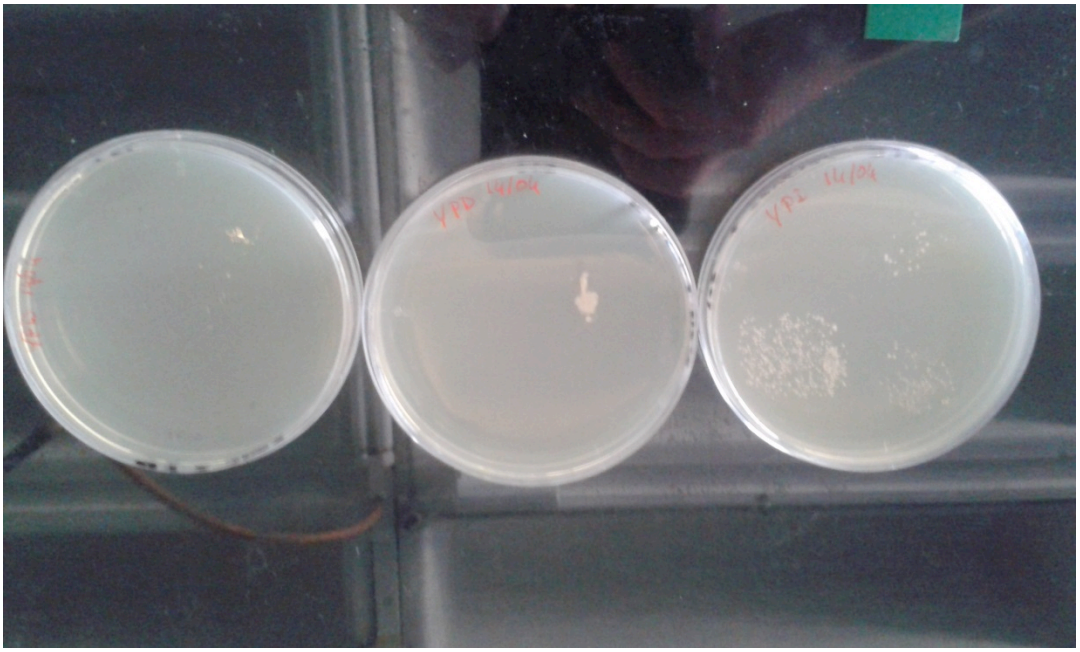

**Figure 1: No growth on Formic Acid treated Fibre (left), Growth on freeze dried fibre (middle), Growth on positive control (right)**

**Table 2: Results of Sterility Testing on LB Plates**

| | Plate | Result | Comment |
|---|---|---|---|
| 1 | Negative Control | No growth | |
| 2 | Positive Control | Growth | |
| 3 | Freeze dried fibre (sterile handling) | Growth | |
| 4 | Slime in buffer (DTT + Sodium Citrate) | Growth | |
| 5 | Fibre freeze dried & treated with Formic Acid | No growth | |

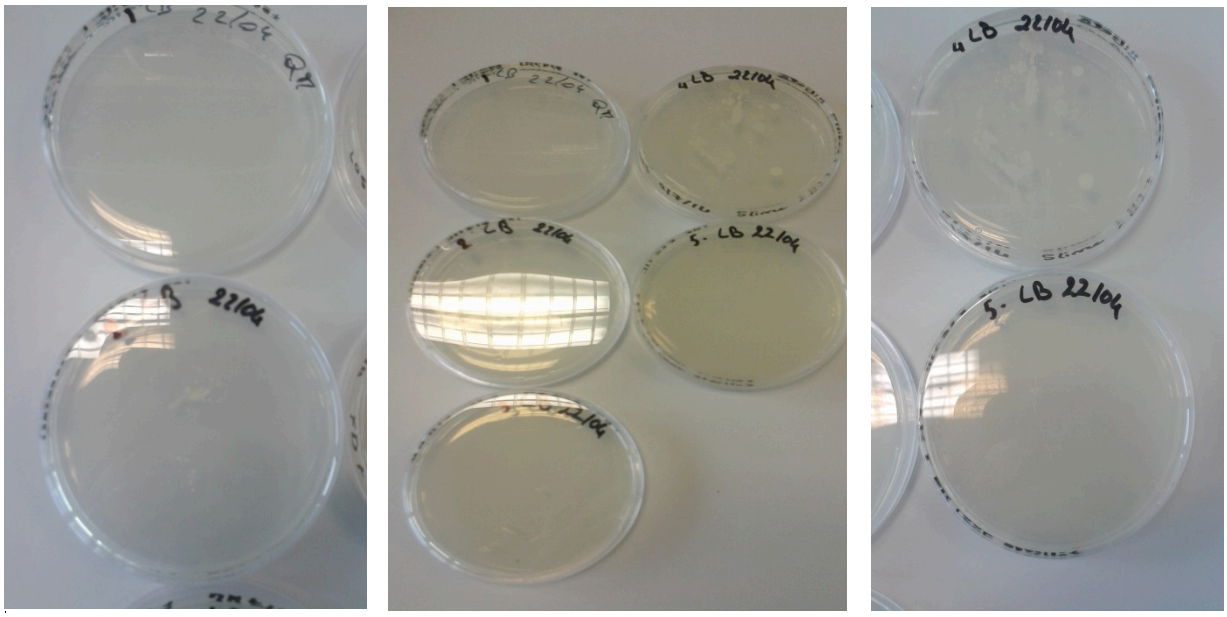

**Figure 2: Plates 1 & 2 (left), Plates 1-5 (middle), Plates 4 & 5 (right)**

# Conclusion

Treatment of sample with formic acid appears to sterilise filament without damaging structure.

# Part 2: Evaluating Methods to Assess Antimicrobial Activity

*Date : 15/7/17*

# Method

**Test 3: Filter Paper Disc Method**

1. Sloppy agar was inoculates with E. coli and S. aureus cells and then poured onto TSA plates and allowed to solidify.
2. Filter paper circles were cut with a diameter of 1 cm.
3. These were sterilised in the autoclave.
4. The discs were aseptically dipped into samples.
5. Excess liquid was allowed to drip from the discs before being placed onto sloppy agar TSA plates.
6. Plates were incubated overnight at 37 degrees.

**Test 4: MIC using Well Plate**

1. 50ul of TSB were pipetted into all 8 wells of row 4
2. An additional 40ul of TSB and 10ul of sample (Formic Acid + Protein) were added to well 1
3. 50ul from well 1 were pipetted into well 2 and mixed by pipetting.
4. This was repeated between wells 2 and 3, 3 and 4 etc. to create a serial dilution of protein.
5. This procedure was repeated on row 2 using TSB + E. coli cells and row 3, using TSB + S. aureus.
6. Well plate was incubated overnight at 37 degrees.

**Results**

| **Test** | **Cells** | **Sample** | | | | |
|---|---|---|---|---|---|---|
| | | **Chloromphenicol** | **Protein + Formic Acid + $MgCl_2$** | **Protein + Formic Acid** | **$MgCl_2$** | **Formic Acid 90%** |
| 1 | **E. coli** | Inhibition | Inhibition | inhibition | No inhibition | Inhibition |
| | **S. aureus** | Inhibition | Inhibition | inhibition | No inhibition | Inhibition |
| 2 | **E. coli** | Inhibition | Inhibition | inhibition | No | Inhibition |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | | | inhibition | |
| | **S. aureus** | Inhibition | Inhibition | inhibition | No inhibition | Inhibition |
| 3 | **E. coli** | Inhibition | No inhibition | No inhibition | No inhibition | No inhibition |
| | **S. aureus** | Inhibition | No inhibition | No inhibition | No inhibition | No inhibition |
| | **Conrol** | No growth | No growth | No growth | No growth | No growth |
| 4 | **E. coli** | Inhibition (++)* | Inhibition (++++) | Inhibition (D=6.4cm) | Inhibition (+) | Inhibition (D=8cm) |
| | **S. aureus** | Inhibition (++) | Inhibition (++++) | Inhibition (D=6.8cm) | No inhibition | Inhibition (D=6cm) |

* Diameter of zone of inhibition : (+) - (++++)

**Results of MIC**

| | **Well** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Row** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| A. Control | - | - | - | - | - | - | - | - |
| B. E. coli | Protein seen | - | - | - | - | - | - | + |
| C. S. aureus | Protein seen | - | - | - | - | - | + | + |

Growth = + // No growth = -

**Conclusion**

Zones of inhibition seen on tests 1-3 were too large to measure accurately. Dilutions of samples would have to be made. The disc assay (test 4) gave the clearest results – zones of inhibition were not overlapping on plate and could be measured. This assay could be used in future experiments to determine antimicrobial activity. For MIC o give results, correct concentration of protein must be known, which had not yet been determined in this experiment.

# Part 3: Determination of Antimicrobial Activity of Natural Protein and of Gold Nanoparticles

**Method**

1. Sloppy agar was inoculated with E. coli, S. aureus and no cells (control)
2. The agar was poured onto TSA plates and allowed to solidify.
3. Sterile filter paper discs of 1cm diameter were dipped in samples (Formic Acid 90%, Gold nanoparticles in solution?, Protein + formic Acid (estimated 21.9 mg/ml), Chloromphenicol 21.9 mg/ml)

4. These were placed on plates using sterile forceps and incubated overnight at 37 degrees

**Preparation of Protein and Chloromphenicol Dilutions**

| Sample | Calculation | Result |
|---|---|---|
| Chloromphenicol (50mg/ml) | C1V1=C2V2<br>50 mg/ml (V) = 21.9 mg/ml (1ml)<br>V = 21.9 / 50<br>V = 0.438 ml<br>V = 438 ul Chloromphenicol<br><br>438ul sample + 562ul ethanol | 21.9 mg/ml |
| Protein (21 mg/ml) | Dry mass determined using 4 point balance and dissolved in 1 ml Formic Acid (90%) | 21mg/ml |

**Results**

| | Sample | | | |
|---|---|---|---|---|
| **Plate** | **2. Gold Nanoparticles** | **3. Chloromphenicol** | **4. Formic Acid 90%** | **5. Protein + Formic Acid** |
| **Control (no cells)** | No growth | No growth | No growth | No growth |
| **E. coli** | No inhibition | Inhibition (D=3.1cm) | Inhibition (D=2.8cm) | Inhibition (D=3.5cm) |
| **S. aureus** | No growth | No growth | Inhibition (D=3.5cm) | Inhibition (D=3.8cm) |

# Conclusion:

Samples 2 and 3 on the S. aureus plate showed no growth, likely due to error. This may have been due to inoculating cells with a loop that was too hot, killing cells. Also cells may have been inoculated into agar that had not cooled sufficiently. However, for E. coli and S. aureus plates, protein + formic acid appeared to have greater inhibition than just formic acid or chloramphenicol. This suggests that the natural hagfish slime protein has antimicrobial activity.

# References

1. **http://www.scielo.br/pdf/bjm/v38n2/v38n2a34.pdf**

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**SCREENING METHODS TO DETERMINE ANTIBACTERIAL ACTIVITY OF NATURAL PRODUCTS**

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