Isolation, Identification and Assessment of the Antimicrobial Activities of a Thermophilic *Actinomycetes* Strain Extracted from the Soil.

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Introduction

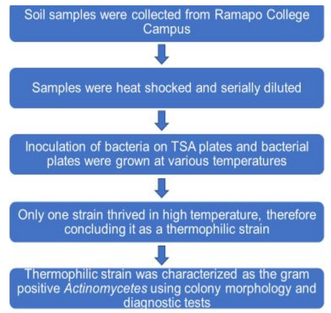
Pseudomonas aeruginosa is an opportunistic bacterium that often results in serious infections in health-care settings especially in immunocompromised patients. *Pseudomonas* growing in biofilms exhibit adaptive resistance to essentially all antibiotics and there is an urgent need to work on new methods to supplement our current antibiotics. Much of our current research is aimed at a long term goal of developing anti-biofilm agents targeting biofilm forming and the planktonic forms of the bacteria *Pseudomonas aeruginosa*.

One of the most important groups of soil bacteria, recognized as a source of commercially important enzymes and antimicrobials, is the *Actinomycetes* and the diversity of *Actinomycetes* is largely underestimated. Considering the potential of discovering *Actinomycetes* that can produce enzymes and antimicrobials with industrial and medical applications, this study is aimed to isolate *Actinomycetes* from soil samples collected from selected sites of Ramapo campus soils. Several strains were isolated and screened using the standard Microbiology and Biochemical diagnostic tests. One of the several strains isolated was identified as a thermophile and based on the colony morphology, gram staining and the diagnostic tests it was identified to be a gram positive spore forming bacteria belonging to the genus *Actinomycetes*.

The extracellular crude extract from the cell free supernatant of the thermophile exhibited anti-bacterial and anti-fungal activity assessed by agar disk diffusion and agar well diffusion method. The thermophilic extract also showed anti-biofilm activity against the bacterial biofilm formed by the bacteria *Pseudomonas aeruginosa*. Addition of Proteinase-K to the extract showed a reduction in the antimicrobial activity of the crude extract which indicated that the cell-free supernatant has antimicrobial peptides (AMP) inhibiting the test microbes. The novel thermophile shows a promising anti-bacterial, anti-fungal and anti-biofilm activity against *Pseudomonas aeruginosa* and other microbes. The future goal is to further purify the AMPs from the strain and examine the synergistic effects of the peptides with the commercial antibiotics on *Pseudomonas aeruginosa* biofilm inhibition. A combined treatment of antibiotics with antimicrobial peptides may offer a very potent treatment of both biofilm and planktonic infections resulting in novel adjuvant therapies.

Isolation, Characterization & Screening

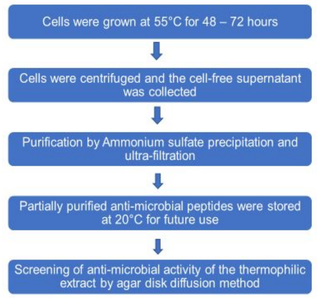
Ammonium sulphate at a 60% saturation (w/v) was used to precipitate the extracellular AMP from the crude cell-free extract. To further purify and remove the ammonium sulphate, ultra-filtration technique (using Amicon filters) was used. The concentration of the partially purified AMP was measured using the Bradford Assay. Using the disk diffusion method, the antimicrobial activity was measured using the zones of inhibition against the gram positive and gram negative bacteria, *Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli.* The antifungal activity was measured against *Candida albicans.* The results showed that the extract had both anti-bacterial, anti-fungal and anti-biofilm activity against *Pseudomonas aeruginosa* biofilms.



Biochemical Testing

Starch Hydrolysis	MR-VP	Gelatinase Testing	Catalase
+	+	+	+

Purification and Antimicrobial Activity



Results

Figure 1: The thermophilic extract exhibited anti-bacterial and anti-fungal activity

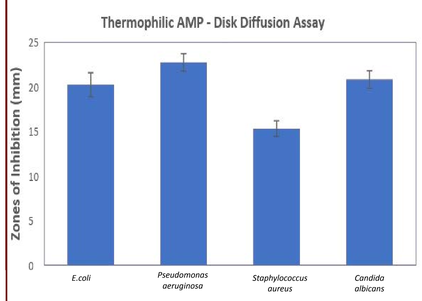
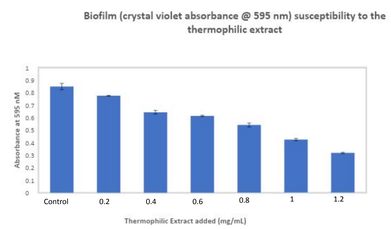


Figure 2: The thermophilic extract also exhibited anti-biofilm activity against *Pseudomonas aeruginosa*



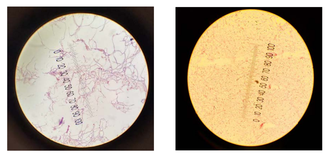
Diagnostic Tests and Results

Starch Hydrolysis Test

Gelatinase Testing

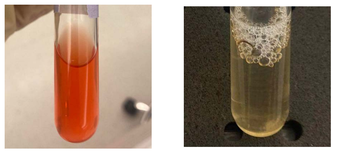
Catalase Test

Staining



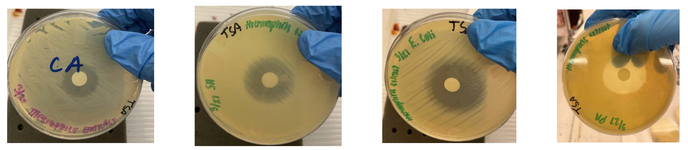
Gram Stain Endospore Stain

Glucose Fermentation Test



MR Test VP Test

Agar Disk Diffusion method



Candida Albicans *Staphylococcus aureus* *E. coli* *Pseudomonas aeruginosa*

Summary

- A thermophilic strain was extracted from the soil.
- The strain was identified as gram positive spore forming *Actinomycetes* through colony morphology and diagnostic tests.
- The extracellular antimicrobial peptides extracted from the thermophilic strain exhibited antibacterial, antifungal, and antibiofilm activities.

Future Work

- The thermophilic extract needs to be further purified using various protein purification methods.
- Antimicrobial activities then should be further assessed.
- Further identification of the species should be done using the standard 16srRNA sequencing method.

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