

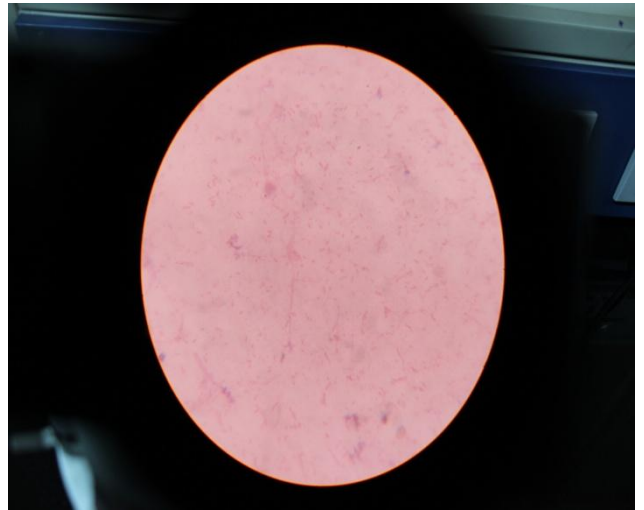
Results:

our test organism were chosen to be *Staphylococcus aureus* and *Pseudomonas aeruginosa*. They were isolated and confirmed with various biochemical test and sequencing.



Pseudomonas aeruginosa plate.

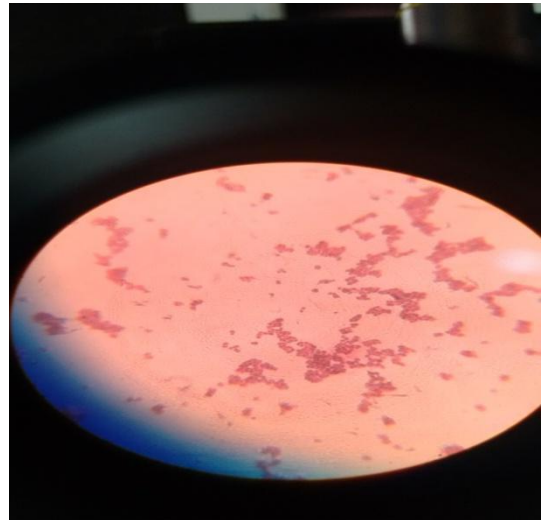
Gram stain image of *Pseudomonas aeruginosa*.





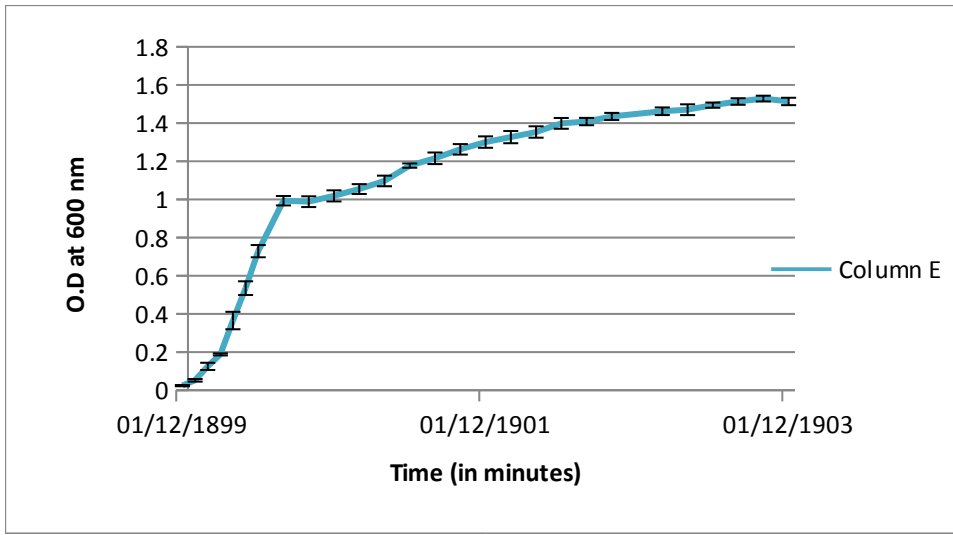
Staphylococcus aureus plate

Gram stain of Staphylococcus aureus.

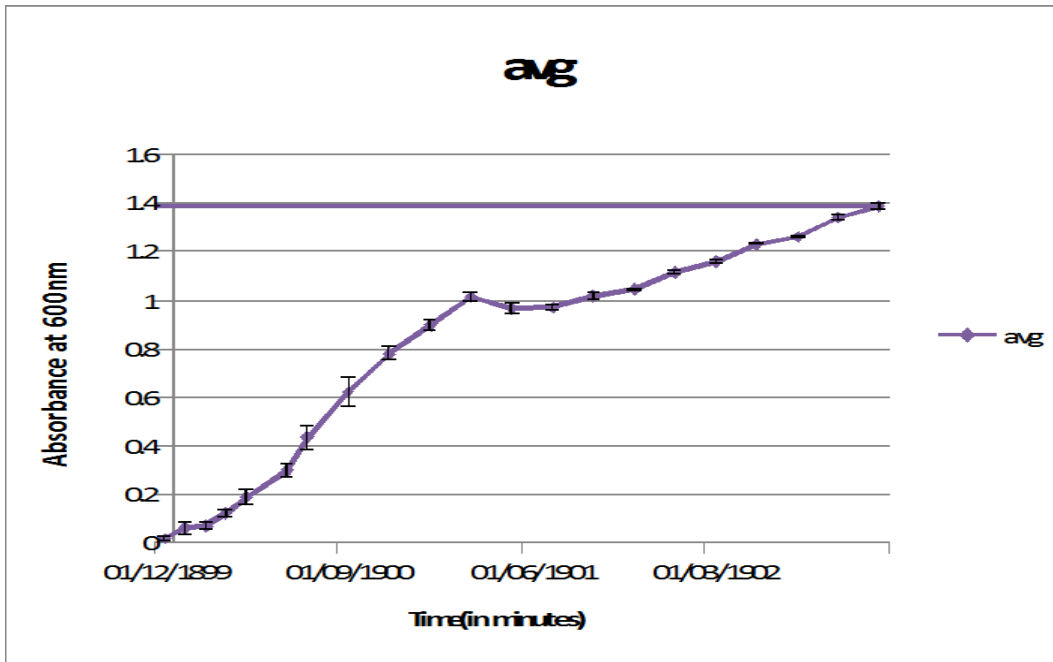


Growth kinetics was done for *Staphylococcus aureus* and *Pseudomonas aeruginosa* for about 25 hours and the results were analyzed.

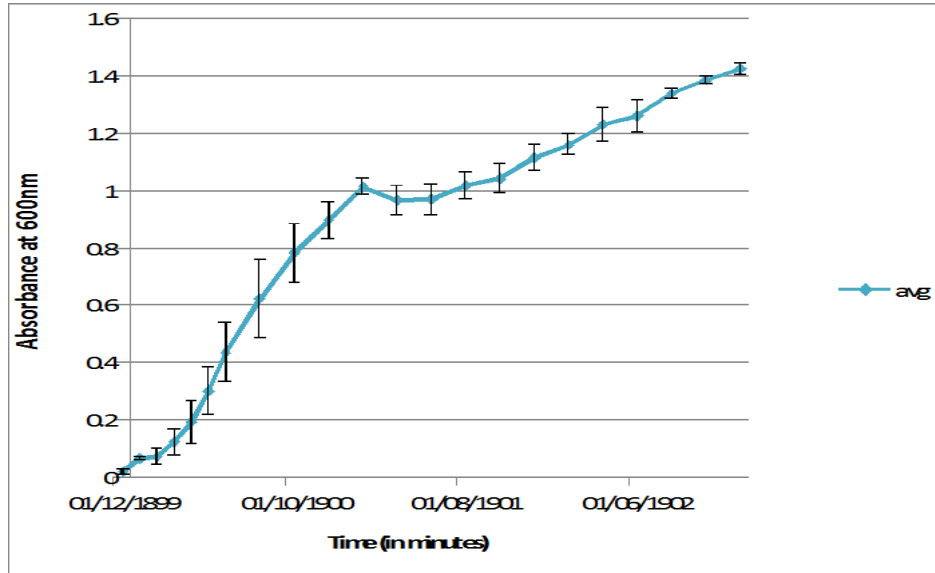
Growth curve of *Staphylococcus aureus*



Growth curve of *BL21*:



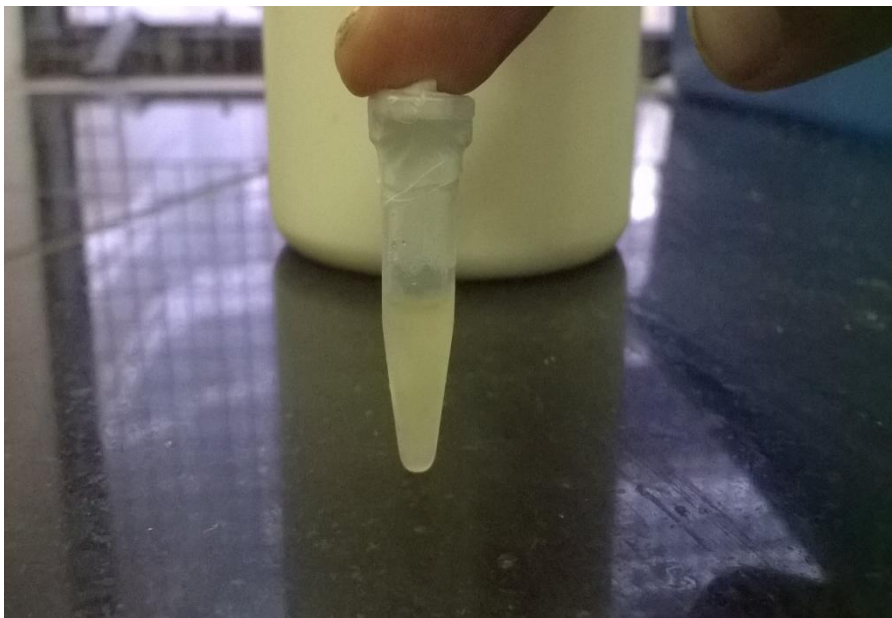
Growth curve of *Pseudomonas aeruginosa*:



For production of minicells we choose a plasmid containing T7 promoter, which is pET24a. Plasmid isolation for pET24a was done using optimized protocols. Since Ecoli BL21 has T7 polymerase it regulates minicell production, this strain was chosen as a host organism. BL21 was confirmed by sequencing.

Competent cell preparation of E.coli BL21 was performed and pET24a was transformed. Its

transformation
efficiency was
calculated.



Competent cells preparation

pSB1C3 which is used for the submission of parts was also transformed and efficiency was calculated.

Concentration	Transformation Efficiency
0.5pg	$1.55 \times 10^{10} / \mu\text{g DNA}$
5pg	$2.366 \times 10^9 / \mu\text{g DNA}$
10pg	$1.6566 \times 10^9 / \mu\text{g DNA}$
20pg	$4.812 \times 10^8 / \mu\text{g DNA}$
50pg	$3.531 \times 10^8 / \mu\text{g DNA}$

Step by step cloning of FtsZ was done. The gBlock amplification results were not promising and we were not able to trouble shoot. Therefore minicell production was not done.

PCR amplification and cloning of Bactofencin A and Thuricin S was performed and the parts was submitted to the registry. Due to time constraint the functionality assay were not performed but will be completed and resented in the Giant Jamboree.