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## Bioinformatic Analysis of Biofilm from Different Wastewater Treatment Plant Samples in East Texas

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Background and Significance

- ☐ Bacillus thuringiensis (Bt), a gram positive, biofilm-forming bacteria, was used to grow biofilms in chlorine treated wastewater from San Augustine WWTP, Nacogdoches WWTP, sterilized water, and tap water.
- ☐ Biofilm a community of microbial cells that form chains and attach to surfaces by secreting extracellular polymeric substances (EPS) like DNA, proteins, and polysaccharides
- ☐ Scanning electron microscopy (SEM) was used to study the extent of biofilm growth as well as its structure.
- □ Novogene: a global biotechnology company specializing in next-generation sequencing (NGS), transcriptomics, RNA-sequencing, and other bioinformatic services.
- □ RNA-Sequencing (RNA-seq): A technique used to measure gene expression by identifying and quantifying RNA transcripts, showing which genes are active and how their activity varies under different conditions.

## Objectives

- ☐ Determine RNA expression profiles of the biofilm produced by Bacillus thuringiensis in the different treated wastewater samples.
- Assess the correlation between transcriptomic variations and the quantity of biofilm produced by the different samples.

#### **Future Studies**

- □ Analyze KEGG Enrichment Analysis from Novogene report.
- □ Isolation and characterization of genomic DNA and biofilm specific protein
- Analyze Circos Plots from Novogene report.

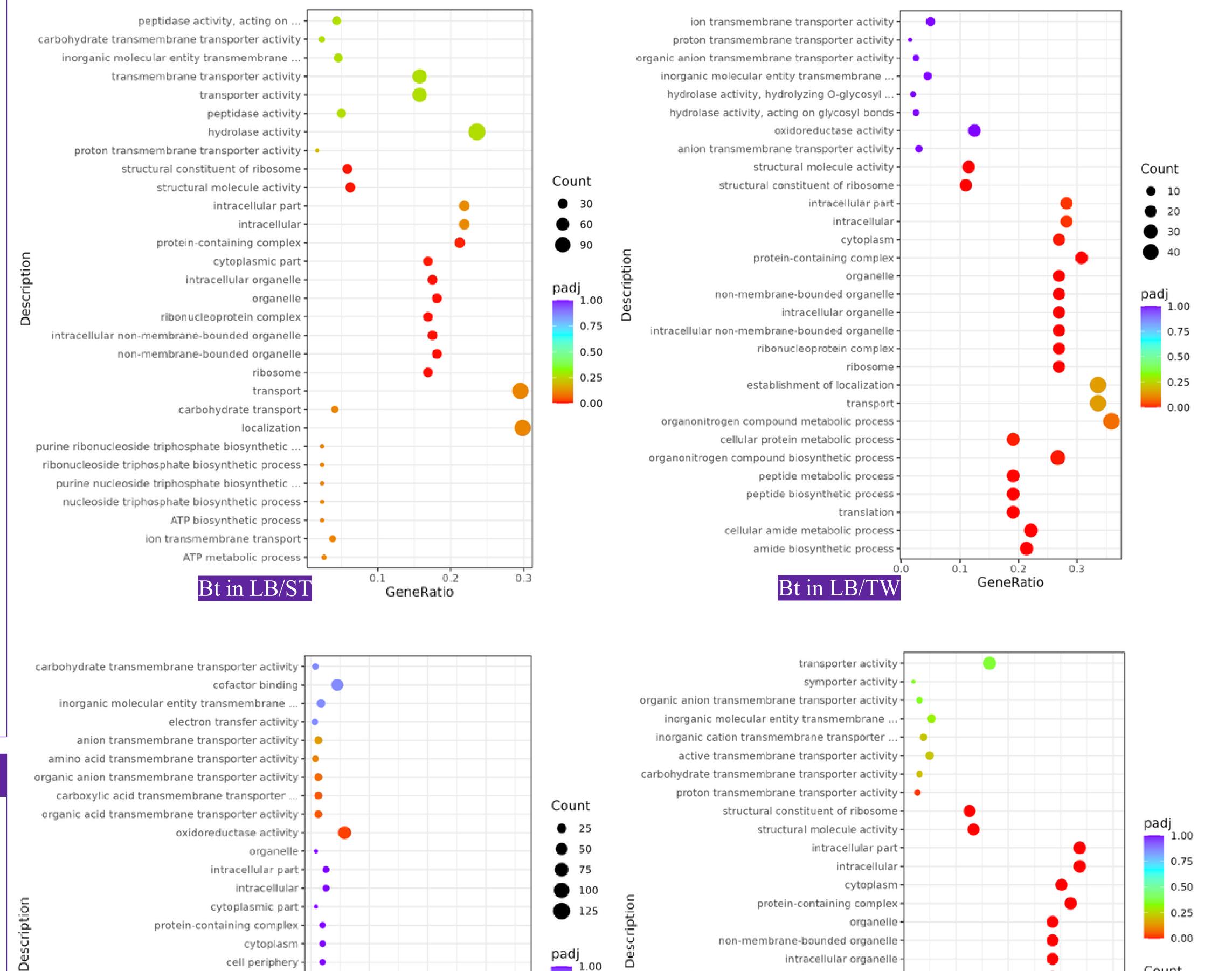


Figure 1: Gene Ontology Enrichment Analysis of Biofilms

## References

0.75

0.50

0.25

plasma membrane -

catalytic complex -

membrane

anion transport

amino acid transport

organic anion transport 🕂

organic acid transport

Bt in LB/SAC

localization

0.50

GeneRatio

0.25

0.75

nitrogen compound transport

establishment of localization

[1] Sengupta, Bidisha & Sinha, Sudarson & Garner, Bianca & Arany, Istvan & Corley, Christa & Jr, Keith & Brown, Elrica & Ray, Paresh. (2016). Influence of Aptamer-Enclosed Silver Nanocluster on the Prevention of Biofilm by Bacillus thuringiensis. Nanoscience and Nanotechnology Letters. 8. 10.1166/nnl.2016.2243. [2] Chattopadhyay, I.; J, R. B.; Usman, T. M. M.; Varjani, S. Exploring the Role of Microbial Biofilm for Industrial Effluents Treatment. Bioengineered 2022, 13 (3), 6420–6440. [3] Scialla, S.; Martuscelli, G.; Nappi, F.; Singh, S.S.A.; Iervolino, A.; Larobina, D.; Ambrosio, L.; Raucci, M.G. Trends in Managing Cardiac and Orthopaedic Device-Associated Infections by Using Therapeutic Biomaterials. Polymers 2021, 13, 1556.

cellular nitrogen compound biosynthetic process

organonitrogen compound biosynthetic process

protein metabolic process

peptide metabolic process

Bt in LB/NC

amide biosynthetic process

peptide biosynthetic process

cellular protein metabolic process

cellular amide metabolic process

# Gene Ontology Enrichment Analysis and Imaging Studies

20

60

0.3

GeneRatio

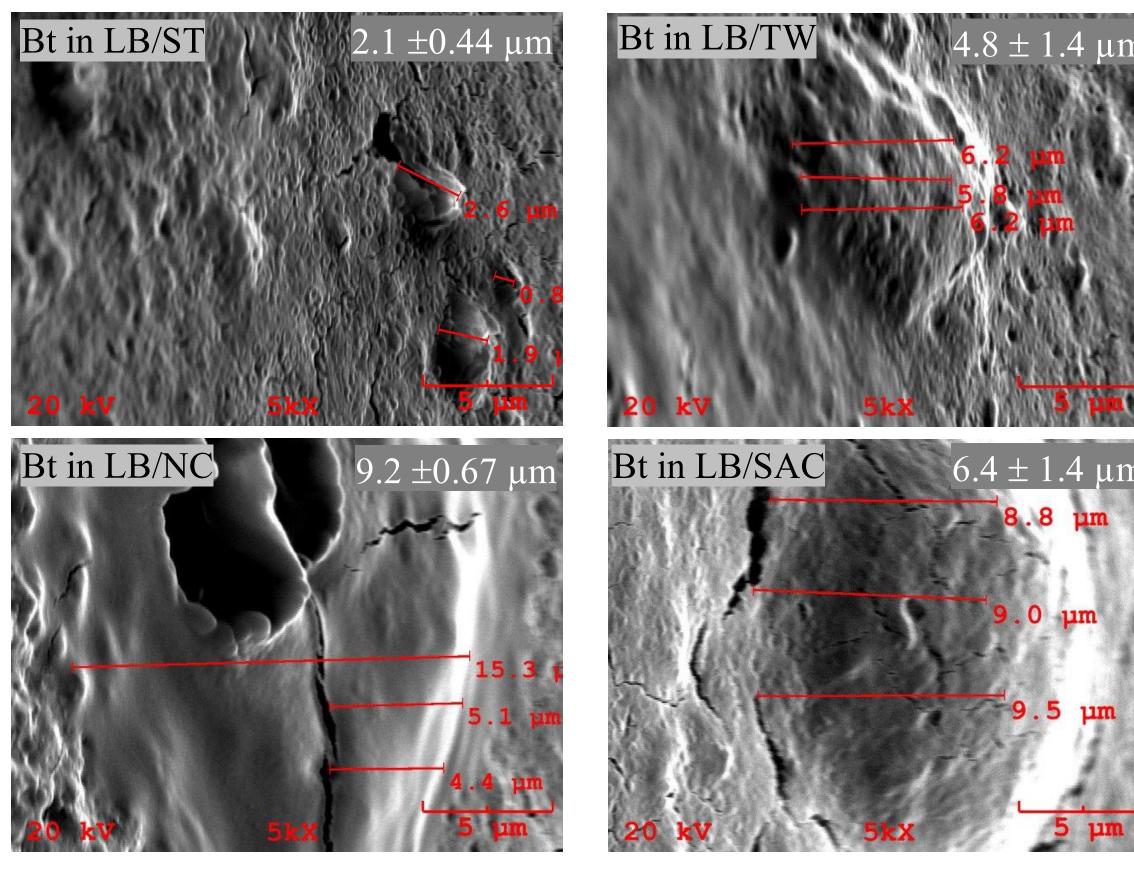
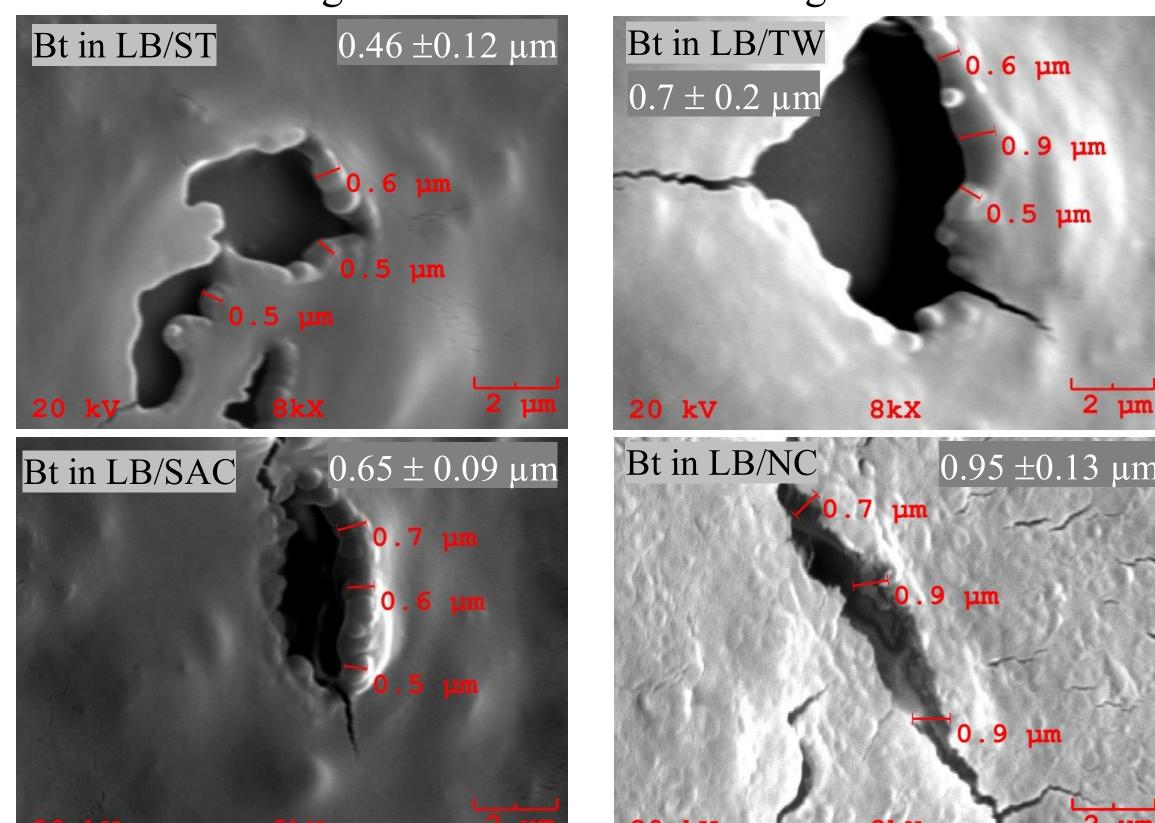


Figure 2: Height of the Biofilm hills measured at 5000x magnification tilted at 65° angle



**Figure 3:** Thickness of Biofilms measured at 8000x magnification tilted at 45° angle

### **Observations and Conclusions**

- ☐ The composition of chlorine treated wastewater does induce genomic alterations in bacteria.
- ☐ SEM images show the height and surface structure of the biofilm.
- ☐ There is significant enrichment in genes related to the formation of biofilm in all samples.
  - ☐ LB/TW, LB/NC, and LB/ST all have significant enrichment in genes related to the synthesis of ribosomes and proteins.
  - ☐ LB/SAC has significant enrichment in genes related to nutrient distribution and uptake.

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