Regulatory Roles of Novel Small Non-Coding RNAs in Antimicrobial Resistance, Biofilm Formation, Bacterial Survival, and Stress Response in Strong Biofilm-Forming *Acinetobacter baumannii*

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BACKGROUND

- *Acinetobacter baumannii* is a multidrug-resistant nosocomial and WHO's critical priority pathogen for new antibiotic research. [1]
- It causes a wide range of human infections such as ventilator-associated pneumonia, septicemia, catheter-associated urinary tract infections, meningitis, and skin and soft tissue infections. [2]
- Its ability to form robust biofilm on host tissues and abiotic surfaces exacerbates the critical concerns regarding antimicrobial resistance.[3]
  - Biofilm inherently withstands environmental stressors, including antimicrobial agents, and therefore, they present a significant medical challenge.
  - The structure of biofilm and the physiological features of bacteria in the biofilm provide the cells with an inherent resistance to antimicrobial agents. [4]
- Non-coding small RNAs (sRNAs) regulate genes involved in antimicrobial resistance, biofilm formation, and virulence of various bacterial species, including *A. baumannii*. [5 – 10]
- Objective: To investigate the molecular mechanisms underlying strong biofilm formation and reduced carbapenem susceptibility in biofilm cells of *A. baumannii* ST10 clinical isolate.

METHODS

- In our lab, 225 *A. baumannii* isolates were assessed for antimicrobial susceptibility and biofilm-forming ability.
- Among isolates originally derived from human subjects, *A. baumannii* ST10 (IC8) exhibited the strongest biofilm-forming ability.
  - ✓It also showed broad planktonic antimicrobial susceptibility and reduced carbapenem (imipenem) susceptibility in biofilm.

Transcriptome profiling in biofilm and planktonic cells

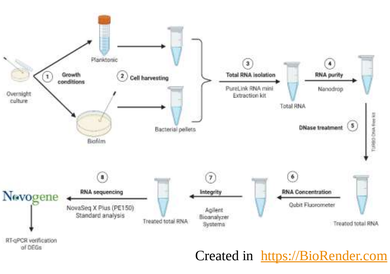

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Figure 1. Schematic diagram showing the work-flow of the RNA samples preparation And sequencing in planktonic and biofilm cells of *A. baumannii* ST10.

Table 1. RNA quality assessment report for samples used in the transcriptome sequencing.

Conditions	Sample name	Concentration (ng/µl)	Purity (Nanodrop) $OD_{260/280}$	Purity (Nanodrop) $OD_{260/230}$	RIN
Biofilm	B1	621	2.05	2.06	8.50
	B2	623	2.03	2.01	8.10
	B3	547	2.00	2.00	8.10
Planktonic	P1	1200	2.06	2.04	9.20
	P2	1455	2.04	2.18	9.10
	P3	1220	2.04	2.14	9.40

RESULTS

Differentially expressed genes (DEGs)

- Clean bases of ~2G across all replicates in both biofilm and planktonic groups, with Q20 >98%, Q30 >96%, and an error rate of <0.01% were obtained.
- Average mapping rates of >70% and multiple mapping rates <10% were obtained for both biofilm and planktonic cells.
- Quantification of DEGs showed that the expressions of 1164 genes were significantly altered.

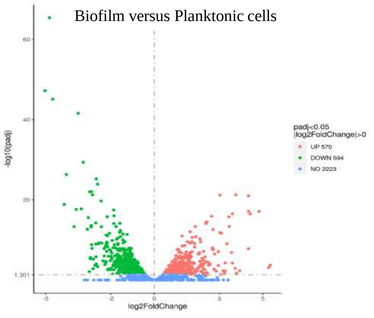

Figure 2. Volcano plot for differentially expressed genes between biofilm cells and planktonic cells of *A. baumannii ST10*.

Discovery of novel sRNAs

- A total of 22 novel sRNAs were predicted from the transcriptome data.
- sRNA00007 and sRNA00048 were significantly upregulated in biofilm cells.
- In silico target prediction using IntaRNA indicated that sRNA00007 could potentially interact with a number of biofilm-associated genes.

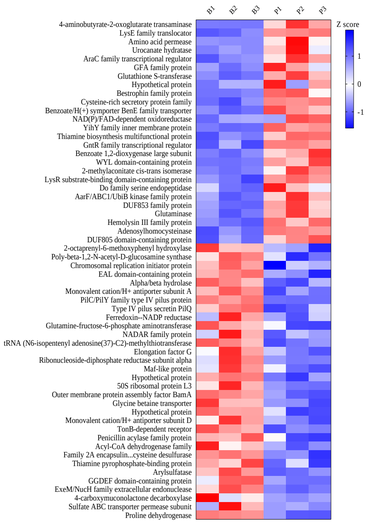

Figure 3. Expression patterns of some genes that were predicted to be potential targets of sRNA00007.

Downregulated targets of sRNA00007

- ***GABA transaminase:*** It is involved in GABA catabolism.
  - ✓The GABA metabolism bypass leads to the accumulation of GABA in bacterial cells.
    - This is crucial in microbial response to environmental stresses such as desiccation, osmotic stress or acid stress. [11]

RESULTS…

- ***LysE translocator:*** It is a group of transmembrane transport proteins involved in exporting amino acids. [12]
  - ✓LysE is an essential component of bacterial growth; the absence of these exporters could result in slow growth. [13]
  - ✓Biofilm cells have retarded growth compared to their planktonic counterparts. [4] This could be a part of a strategy to conserve energy or to alter the intracellular concentration of amino acids that are essential for biofilm maintenance and development.
- ***AraC family transcriptional regulator:*** It typically controls the catabolism of sugars and amino acids. [14]
  - ✓Its downregulation in biofilm cells could be associated with retarded growth in biofilms.

Upregulated targets of sRNA00007

- ***TonB-dependent receptor:*** It is a surface protein that plays an important role in the transportation of iron in Gram-negative bacteria. [15]
  - ✓ It is important for iron acquisition to promote biofilm formation.
- ***ExeM/NucH family extracellular endonuclease:*** It plays multiple roles, including nutrient acquisition, biofilm formation, and natural transformation of biofilm cells. [16]
  - ✓It plays a central role in the modification or degradation of eDNA in biofilm.
  - ✓It is also important in inducing biofilm dispersion.
- ***GGDEF domain-containing protein:*** It acts as diguanylate cyclase for the synthesis of bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP).
  - ✓C-di-GMP is a signalling molecule (intracellular second messenger) that serves as the central biofilm regulator in Gram-negative species.
  - ✓The accumulation of c-di-GMP promotes biofilm formation. [17]

CONCLUSION AND FUTURE DIRECTIONS

- DEGs, including sRNAs in the biofilm cells, may contribute to bacterial survival under stress, enhance biofilm formation, and promote antimicrobial resistance.
- The regulatory roles of sRNA00007 and sRNA00048 in antimicrobial resistance, biofilm formation, survival, and stress response in *A. baumannii* ST10 will be further investigated.

SELECTED REFERENCES (scan the QR code)

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