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TRANSCRIPTOMIC ANALYSIS OF THE *Pseudomonas aeruginosa* BIOFILM EXPOSED TO ConA-UNBOUND PROTEIN FRACTION OF *Melipona beecheii* HONEY

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Introduction

Honey produced by the stingless bee *Melipona beecheii* is highly valued in Mayan communities for its medicinal properties [1]. It is traditionally used to treat respiratory diseases, strengthen the immune system, and manage eye infections and wounds. Recently, it has been reported that ConA-unbound protein fraction in this honey exhibit antimicrobial activity and inhibit biofilm formation by *Pseudomonas aeruginosa*, affecting its cell integrity, flagellar gene expression, and reducing exopolysaccharide matrix formation [2]. In this study, the transcriptomic response of *P. aeruginosa* ATCC 27853 biofilms exposed to ConA-unbound protein fraction from *M. beecheii* honey was analyzed using RNA-seq.

Methodology

1. Honey Sampling

Meliponary: Maní, Yucatán, México

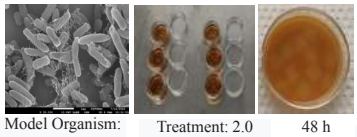
Honey of *M. beecheii*

2. Honey Protein Extraction & Fractionation

Ultrafiltration Amicon ultra 3K

Affinity Chromatography Con-A Sepharose 4B

3. Biofilm Formation on Glass

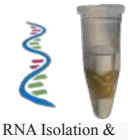


Model Organism: *P. aeruginosa* ATCC 27853

Treatment: 2.0 µg/mL ConA-unbound protein fraction

48 h Incubation

4. RNA Extraction



RNA Isolation & Purification:
- RNeasy Mini Kit (Qiagen)
- RiboZero Plus rRNA Depletion Kit, Illumina

5. RNA-seq library prep

Kit: KAPA RNA HyperPrep

6. Sequencing

Sequence Libraries: Illumina NovaSeq 6000, 150 PE (Paired-End)

7. Data analysis

Quality Control: NGS QC Toolkit v2.3.3
Mapping: HISAT2
Assembly & Expression: StringTie2 v2.2.0

Results

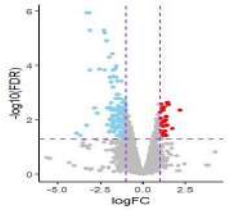


Figure 1. Volcano plot of differentially expressed genes (DEGs) of untreated and ConA-unbound protein fraction-treated *P. aeruginosa* ATCC 27853 biofilm.

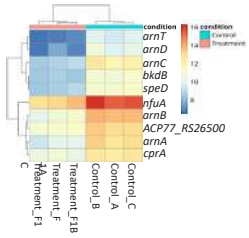


Figure 2. Heat map of the 10 most affected genes differentially expressed between the three replicates of untreated (control) and ConA-unbound protein fraction-treated *P. aeruginosa* ATCC 27853 biofilm.

Table 1. Differentially expressed genes in *P. aeruginosa* ATCC 27853 biofilm with the ConA-unbound protein fraction-treated.

Gene ID	Gene name	log2 *FC	p-value	Up/Down Regulated	Description
ACP77 RS31925	*rnpB*	1.70309859	9.96594E-09	Up	RNase P RNA component class A
ACP77 RS06765	*CAZ10_20305*	1.470209761	0.000957126	Up	Antibiotic biosynthesis monooxygenase
ACP77 RS30940	*olsA*	1.392081536	5.34314E-08	Up	Lyso-ornithine lipid O-acyltransferase
ACP77 RS01535	*hflC*	1.292586537	2.83312E-06	Up	Protease modulator HflC
ACP77 RS20750	*tagT*	1.187694779	0.000484114	Up	Type IV secretion associated ABC transporter ATP-binding protein TagT
ACP77_RS06270	*arnD*	-3.10600235	1.58433E-26	Down	4-deoxy-4-formamido-L-arabinose-phosphoundecaprenol deformylase
ACP77 RS28020	*oprH*	-2.563437398	5.90758E-24	Down	Outer membrane beta-barrel protein
ACP77 RS06250	*PA2022*	-1.79431309	8.42135E-13	Down	UDP-glucose 6-dehydrogenase
ACP77 RS16275	*PA4781*	-1.553378506	7.13933E-19	Down	Cyclic di-GMP phosphodiesterase
ACP77 RS21195	*oprD_10*	-1.551257856	1.98314E-07	Down	OprD family porin
ACP77 RS28470	*fliC*	-1.072595423	3.96393E-05	Down	Flagellin

RNA-seq analysis revealed that treatment with ConA-unbound protein fraction from *M. beecheii* honey altered the transcriptomic profile of *P. aeruginosa* ATCC 2785 biofilms. Of the 6,423 genes analyzed, 27 were upregulated and 143 were downregulated (|log2FC| > 1.0 and FDR < 0.05) (Figure 1). A set of 10 genes showed significant expression changes between the Control and Treatment groups, including a reduction in *arn* genes, which impacts the Cationic Antimicrobial Peptide (CAMP) resistance pathway (Figure 2). Upregulated genes may be associated with adaptive or defensive strategies during biofilm formation, whereas downregulated genes included those encoding enzymes involved in the regulation of the second messenger c-di-GMP (cyclic di-guanosine monophosphate) and in the biosynthesis of exopolysaccharides (Table 1).

Conclusions

ConA-unbound protein fraction from *Melipona beecheii* honey predominantly negatively affected the regulation of genes that are key to *Pseudomonas aeruginosa* biofilm formation. These findings provide key insights into the mechanism of action of these honey-derived proteins and highlight their potential as natural antibiofilm agents, particularly against antibiotic-resistant pathogens.

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References

[1] Moo-Huchin, V. M., G. A. Gonzalez-Aguilar, J. D. Lira-Maas, *et al*., (2015. Physicochemical Properties of *Melipona beecheii* Honey of the Yucatan Peninsula. *J. Food Res*. 4:25

[2] Pool-Yam, L., Ramón-Sierra, J., *et al*., (2024). Effect of conA-unbound proteins from *M. beecheii* honey on the formation of *P. aeruginosa* ATCC 27853 biofilm. *Arch. Microbiol*, *206* (1), 54.

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Transcriptomic analysis of the Pseudomonas aeruginosa biofilm exposed to ConA-unbound protein fraction of Melipona beecheii honey

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Melipona beecheii honey has been used since ancient times for its medicinal attributes. It has recently been reported that the ConA-unbound proteins from this honey present antimicrobial activity against various pathogenic strains of clinical interest. In addition, they inhibit the formation of the Pseudomonas aeruginosa biofilm, affecting its cellular integrity, flagellar gene expression and decreasing the formation of the exopolysaccharide matrix. This bacterium is classified by the WHO as high priority on its list of bacterial pathogens, therefore, the study of the mechanisms on the antibiofilm capacity of the honey proteins in P. aeruginosa is of interest. In this work, the effect of the crude protein extract of M. beecheii honey and its ConA-unbound fraction on the biofilm transcriptome of P. aeruginosa ATCC 27853. The crude protein extract of M. beecheii honey was obtained by ultrafiltration and the ConA-unbound (F1) fraction was obtained by affinity chromatography. Transcriptomic analysis was carried out by RNA-seq analysis. The results of the RNA-seq analysis of the P. aeruginosa biofilm showed a total of 6,404 of which genes with p-value ≤ 0.05 and fold change ratio (log2) ≥ |1| were identified as DEG (genes statistically most affected), obtaining 35 differentially expressed genes; 7 overexpressed and 28 underexpressed in the presence of ECP. In the case of the F1 fraction, 231 genes were differentially expressed; 33 overexpressed and 198 underexpressed. Several of the affected genes were related to ribosome biogenesis, affecting the stability of the 50 and 30s subunits. It also affected genes involved in lipopolysaccharide metabolism. On the other hand, the RNA-seq analysis showed an effect on the function of certain genes related to stress and the formation of the biofim of P. aeruginosa ATCC 27853, affecting genes such as phoQ, arnC, oprD, and fliC. Therefore, the ConA-unbound proteins from M. beecheii honey presented an effect on several pathways that are related to the formation of the P. aeruginosa biofilm. This basic research about the proteins from M. beecheii honey, could give added value to this product, increasing its harvest and sustainable marketing. Additionally, promoting the meliponiculture positively can impact biodiversity and the local economy.