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zakisaati@usal.es

A different point of view of plant-bacterial interactions: RNA-Seq analysis of a PGP bacterial endophyte colonizing rapeseed plants

Zaki Saati-Santamaría[1,2*], Daniel Kumazawa Morais[3], Vojtěch Tláskal[3], Oldřich Benada[3], Olga Kofroňová[3], Alejandro Jiménez-Gómez[1], Peter Baldrian[3], Raúl Rivas[1,2,4], Paula García-Fraile[1,2,4]

1. Microbiology and Genetics Department. University of Salamanca. Doctores de la Reina SN, 37007 Salamanca, Spain
2. Spanish-Portuguese Institute for Agricultural Research (CIALE), Salamanca, Spain
3 Laboratory of Environmental Microbiology, Institute of Microbiology of the Czech Academy of Sciences, Videnska 1083, 14220, Praha 4, Czech Republic
4 Associated R&D Unit, USAL-CSIC (IRNASA), 37008 Salamanca, Spain

Some microbes are important players in plant's fitness, contributing to their nutrients acquisition and protection against diverse biotic and abiotic stresses[1]. Despite the vast knowledge acquired during the last decades about the effects in plants of plant growth promoting (PGP) bacteria[2], apart from those of the legume-rhizobial interactions[3], not much is known about the response of bacteria to the interaction with plant. Thus, we aim to decipher the transcription profile (RNA-Seq) of a *Pseudomonas* strain with plant growth promotion activities over *Brassica napus* in its interaction with the plant.

MATERIALS AND METHODS

Brassica napus (rapeseed) seeds were outer sterilized (2' EtOH (70%), 10' NaClO, followed by sterile water washing) and placed on water-agar plates. Germinated plants were transferred to square Petri dishes with Hoagland's medium and inoculated with a PGP *Pseudomonas* strain able to protect the plant against biotic and abiotic stresses. SEM microscopy was carried out over 11 days post-inoculation (dpi) roots to observe the capability of the bacterium to colonize the plant roots at that moment. At the same timepoint, roots were sonicated in order to get bacterial cells colonizing the seedlings' roots its RNA was extracted. RNA from free living cells in (Hoaglands Petri dishes + 0.1% glucose) was used as control. Total RNA was sequenced through Illumina NovaSeq PE150 platform. Raw reads were processed with Khmer, trimmomatic and fastx tools. Bowtie2 was employed for mapping reads back to the bacterial genome. Transcript counts per CDS was made through the FADU program. Differential expression analyses were carried out with DESeq2.

RESULTS AND DISSCUSION

Our analyses allowed us to identify 1378 bacterial genes differentially expressed (log2 fold change > 2; adjusted p value < 0.05) (Fig. 1c,d). Most overexpressed genes in the interaction are related to biofilm formation (which is in concordance with SEM microscopy; Fig. 2), bacterial immunity and infection and bacterial survival to antimicrobial compounds -likely excreted by the plant-. However, genes implicated in PGP traits which had been previously demonstrated *in vitro* for this strain, appeared to be not significantly overexpressed, suggesting a latter PGP action in the interaction.

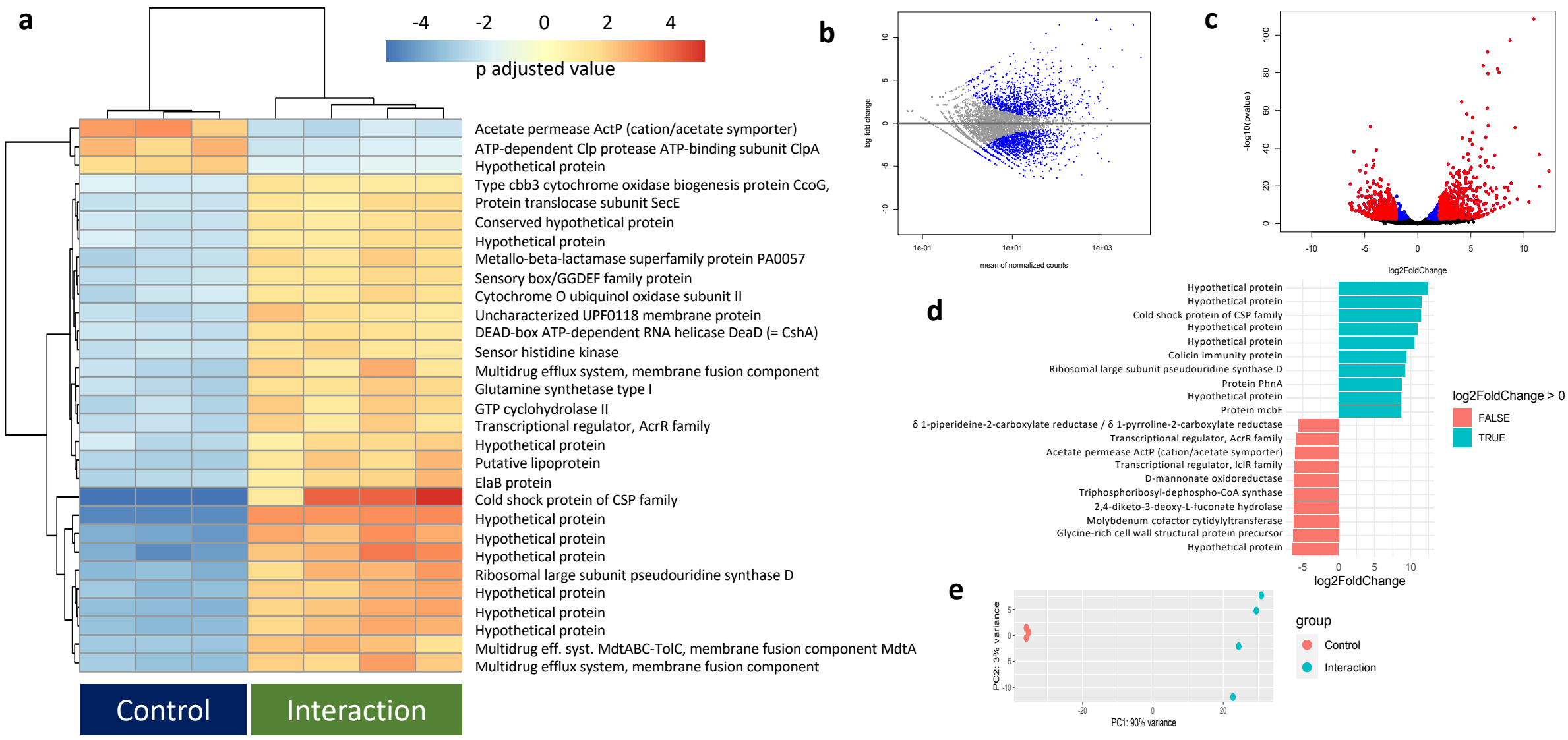

Figure 1. a) Heatmap representing CDS differentially expressed with the smallest p adjusted value. CDS are labelled according to RAST gene annotations. **b)** Plot representing the mean of normalized counts per CDS **c)** Volcano plot representing the Log2 fold change (X axis) of CDS expression values vs its p value (Y axis). **d)** Divergent bar plot depicting 10 CDSs with greater Log2 Fold Change value and 10 CDSs with the lower Log2 Fold Change value. **e)** PCA plot of RNA-Seq samples.

CONCLUSSIONS

RNA-Seq is a Good technique to study genes implicated in symbiotic interactions.
Based on this RNA-Seq experiment, our results shed light into bacterial mechanisms to effectively colonize plant roots, to survive to plant defense mechanisms as well as to promote plant immunity.

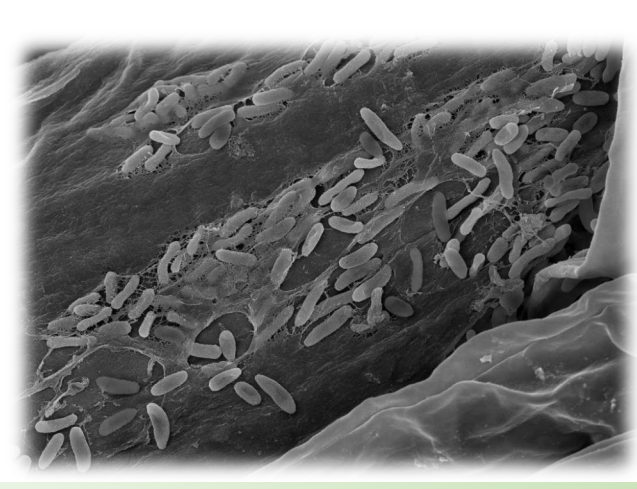

Figure 2. SEM image of the *Pseudomonas* strain colonizing *B. napus* roots

References:
[1]Menéndez, E., & Garcia-Fraile, P. (2017). *AIMS microbiology*, 3(3), 502. [2]Hayat, R., et al., (2010). *Annals of microbiology*, 60(4), 579-598. [3]Oldroyd, G. E., et al., (2011). *Annual review of genetics*, 45, 119-144.

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