14 2026 SOCRATIC LECTURES

ISOLATION AND PROFILING OF EXTRACELLULAR VESICLE-ASSOCIATED RNA FROM *VERTICILLIUM NONALFALFAE*

Miona Kovachevikj[1], Taja Jeseničnik[1], Veronika Kralj-Iglič[2], Jernej Jakše[1]
[1]Biotechnical faculty, Departement of agronomy, University of Ljubljana
[2]Faculty of Health Sciences, University of Ljubljana
Contact: Miona Kovachevikj, miona.kovachevikj@bf.uni-lj.si

UNIVERZA V LJUBLJANI BF Biotehniška fakulteta

BACKGROUND

Verticillium nonalfalfae is a soil-borne phytopathogen that causes Verticillium wilt, a vascular disease affecting numerous economically important crops. Successful fungal infection requires a complex molecular interaction between the fungus and its host. Therefore, understanding the mechanisms that underlie the host-pathogen interaction is essential to develop new combat strategies. Extracellular vesicles (EVs) have emerged as potential mediators of cross-kingdom communication, as they enable the transport of biologically active molecular cargo, including RNA. Recent studies suggest that fungal pathogens, may exploit RNA delivery pathways, such as vesicle-mediated RNA transfer to promote infection.

METHODS

Growth media
- Czapek-Dox medium (CDA)
  or
- Xylem simulating medium (XSM)

Isolation of extracellular vesicles (EVs)
- Differential ultracentrifugation

Characterization
- Interferometric light microscopy (Videodrop, Myriade)

RNA isolation
- Monarch Total RNA Miniprep kit
- mirVana miRNA Isolation kit

Preliminary sequencing of EV-associated RNA
- IonChef™

RESULTS

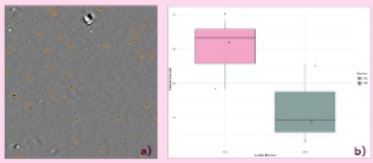


Figure 1: a) Visualization of isolated particles by interferometric light microscopy (Videodrop, Myriade). b) Distribution of particle sizes of vesicles isolated from CDA or XSM medium. A one-way ANOVA indicated no statistically significant difference in particle size between the two media, $F(1, 4) = 3.63$, $p = 0.129$.

	Total sequences	Mapped sequences (%)
small CDA 1	1.20 M	51.66
small CDA 2	1.42 M	68.39
small XSM 1	6.07 M	82.29
small XSM 2	5.29 M	75.05
total CDA 1	2.16 M	81.52
total CDA 2	2.12 M	83.92
total XSM 1	1.50 M	81.85
total XSM 2	1.86 M	86.91

Table 1: Overview of the sequencing output and mapping efficiency to reference fungal genome for each sample. The table shows the total number of raw reads and the percentage of reads that successfully mapped to the reference genome. The values provide an assessment of sequencing depth and data quality prior to downstream analysis.

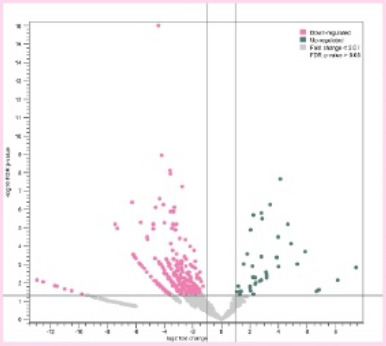
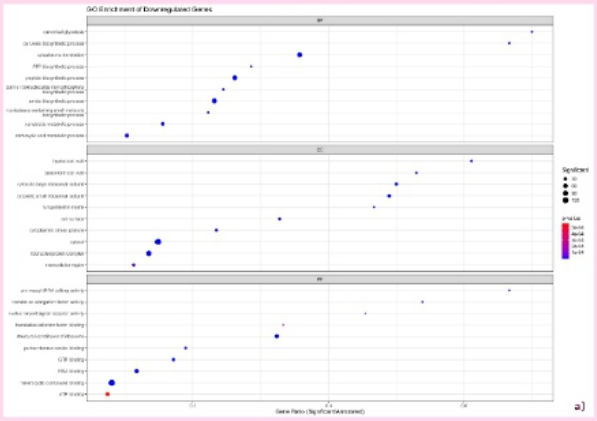


Figure 2: Differential expression analysis of transcripts from total RNA detected in fungal extracellular vesicles. Transcripts significantly downregulated in EVs isolated from the XSM medium compared to the CDA medium are shown in pink (Log2fold change ≤ -2, FDR-p≤0.05), while the significantly upregulated transcripts are shown in green (Log2fold change ≤ 2, FDR-p≤ 0.05).



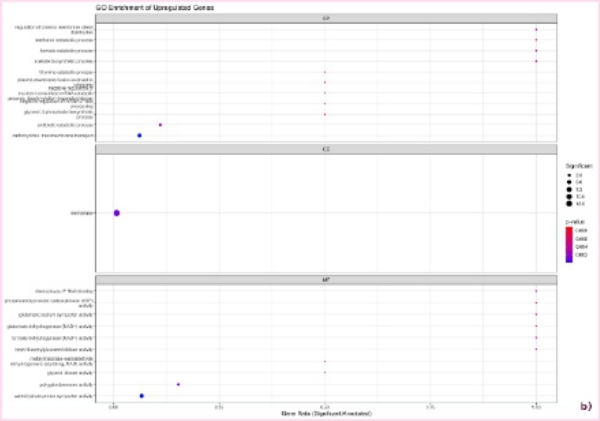


Figure 3: Gene Ontology (GO) enrichment analysis of transcripts detected in extracellular vesicles from preliminary total RNA sequencing data. The graphs highlight the significantly overrepresented biological processes, cellular components and molecular functions associated with: a) transcripts downregulated in EVs isolated from the XSM medium compared to EVs isolated from the CDA medium. b) transcripts upregulated in EVs isolated from the XSM medium compared to EVS isolated from the CDA medium.

CONCLUSION

The particles isolated from both media exhibited sizes within the 100-300 nm range, consistent with the characteristics dimensions of fungal extracellular vesicles. The results demonstrate that the composition of fungal extracellular vesicles is influenced by the growth medium, with the differential expression analysis revealing distinct sets of transcripts up-and downregulated in EVs from XSM compared to CDA. These findings suggest that EV cargo responds to environmental cues and may play a role in host interaction. Future work will expand these analyses by comparing EV-associated RNA with intracellular fungal RNA to assess selective RNA packaging, alongside comparative profiling of V. nonalfalfae strains with contrasting virulence to identify pathogenicity-related RNA signatures.

FUNDING

This research was supported by the Slovenian Research and Innovation Agency (ARIS), grant number P4-0077 and ARIS grant to young researcher Miona Kovachevikj.