

Genomic and Proteomic Analysis of Functional Gene in *P. infestans* DSM 5139 for Nutrient Acquisition and Ecosystem Functioning

Chahira Zerouki, Suvi Kuittinen, Ari Pappinen, Ossi Turunen



Introduction

The current study investigates the ability of *P. infestans* to tolerate **freezing temperatures** and **acquire nutrients from pine needles**, which are known to exhibit antifungal properties (Butin & Söderholm, 1984).

Methods

- **Genomic DNA** purification and PacBio Sequel II sequencing.
- Genome assembly (Flye 2.6) and annotation (Augustus and COMPANION tools).
- Functional annotations and pathway reconstruction
- **Mass spectrometry** analysis of *P. infestans* methanol extracts obtained by fungal cultivations at **22 °C** , at **-3 °C**.

Results

- **573 carbohydrate-active enzymes** and approximately **400 genes** were linked to **secondary metabolite biosynthesis**.
- Presence of phytoalexins-detoxifying enzymes, acyclic terpene synthase proteins, and drug-efflux pumps to maintain cell integrity.
- Utilization of the needle's cell wall constituents.

Results

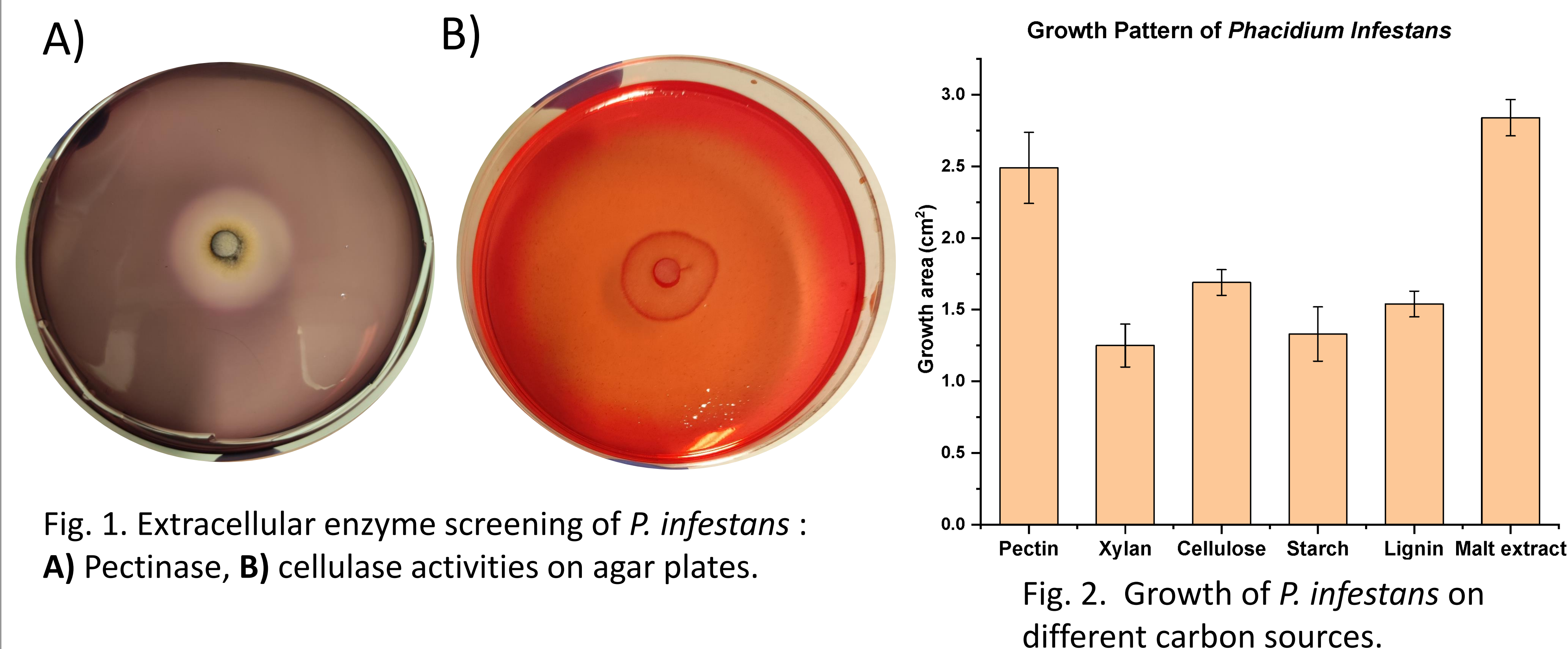


Table 1. **Secretion of cryoprotectants at -3 °C** (Zerouki et al., 2023).

Monoisotopic mass	Formula	Compound (annotation level)	- 3 °C average area, ± standard deviation	22 °C average area, ± standard deviation	Control ^a (area)
226.10	C10 H14 N2 O4	Carbidopa (level 2b)	1,603,064 ± 370,612	521,044 ± 262,797	9976
237.09	C9 H11 N5 O3	Sepiapterin (level 2b)	6,875,365 ± 1,891,958	2,373,722 ± 1,371,746	2076
253.08	C9 H11 N5 O4	Neopterin (level 2b)	7,637,449 ± 2,402,794	3,599,159 ± 2,471,979	3872
117.08	C5H 11N O2	Betaine (level 1)	1,654,148,902 ± 478,694,055	1,481,382,680 ± 355,974,675	2,336,773
161.11	C7H15 N O3	Carnitine (level 1)	2,154,586 ± 1,622,666	1,568,248 ± 681,028	41,428
342.12	C12 H22 O11	Hexose dimer (level 1)	3,431,904 ± 2,713,679	6,026,936 ± 5,548,518	422,496
152.07	C5 H12 O5	Sugar alcohol (level 1)	9,687,053 ± 20,331,693	11,545,816 ± 10,995,786	20,757

^aUnit used is relative area (areas under the curve/ chromatographic peak)

^bCalculated from the difference between mean values of the - 3 °C and 22 °C extracts

Presence of **two ice-binding proteins**, a complete trehalose pathway, and several desaturases, contributing to the fungus's cold tolerance.

Results

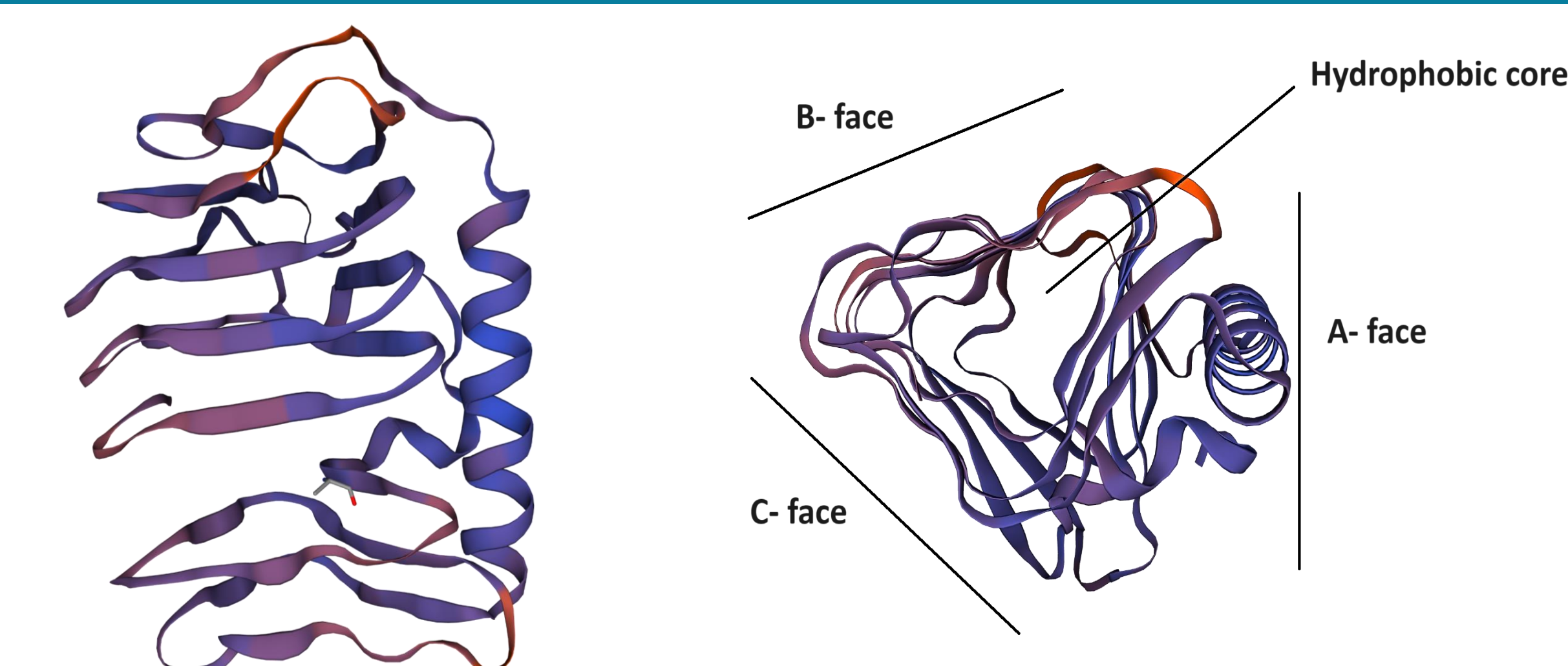


Fig.3. SWISS-MODEL structure of the ice-binding protein Phain_OT5_Proseq10963 based on the crystal structure 7bwx.1.A of *Antarctomyces psychrotrophicus*.

Conclusion

This is the first whole-genome sequence of *P. infestans* available on the databases (98.6% core genome completeness), with a size of ~36.8 Mb assembled in 44 contigs. This study offers valuable insights into the adaptation of *P. infestans* to its ecological niche.

References

- Butin, H., & Söderholm, U. (1984). Über *Phacidium fennicum* sp. nov. und verwandte Arten auf Kiefernadeln. *Sydowia. Fungal Phyla*, 21, 21–28. (in German)
- Zerouki, C., Chakraborty, K., Kuittinen, S., Pappinen, A., & Turunen, O. (2023). Whole-genome sequence and mass spectrometry study of the snow blight fungus *Phacidium infestans* (Karsten) DSM 5139 growing at freezing temperatures. *Molecular Genetics and Genomics*, 298, 1449–1466. <https://doi.org/10.1007/s00438-023-02073-7>