A Glow in the Waste: Advancing Food Safety with Transgenic Fluorescent Black Soldier Fly Larvae

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Framing the problem

The agrifood system as we know it has been affecting human, animal and environmental health

Search for new sustainable protein sources

Meet the black soldier fly larvae (BSFL): high protein content; less greenhouse gas emissions; less consumption of water; less occupation of land

Campbell, B. M., Beare, D. J., Bennett, E. M., Hall-Spencer, J. M., Ingram, J. S. I., Jaramillo, F., Ortiz, R., Ramankutty, N., Sayer, J. A., & Shindell, D. (2017). Agriculture production as a major driver of the Earth system exceeding planetary boundaries. *Ecology and Society*, *22*(4). https://doi.org/10.5751/es-09595-220408

Surendra, K., Tomberlin, J. K., Van Huis, A., Cammack, J. A., Heckmann, L. L., & Khanal, S. K. (2020). Rethinking organic wastes bioconversion: Evaluating the potential of the black soldier fly (Hermetia illucens (L.)) (Diptera: Stratiomyidae) (BSF). *Waste Management*, *117*, 58-80. https://doi.org/10.1016/j.wasman.2020.07.050

Framing the problem

1.05 billion tonnes

Of food were wasted globally in 2022

Food waste is a **public health problem** – contributing to food insecurity, propagation of infectious agents and to environmental issues

Food waste is also **undermining** the **resilience** and **sustainability** of our **agrifood** systems

United Nations (2023). *International Day of Awareness on Food Loss and Waste Reduction 29 September*. https://www.un.org/en/observances/end-food-waste-day
United Nations Environment Programme (2024). *Food Waste Index Report 2024. Think Eat Save: Tracking Progress to Halve Global Food Waste*. https://wedocs.unep.org/20.500.11822/45230.

Turning challenges into opportunities

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BSFL can perform **bioconversion**

A process in which BSFL can transform organic substrates into value added products (e.g.: animal feed).
Sustainable protein source.

BSFL can perform **bioremediation**

A process in which BSFL can remove or reduce to acceptable levels contaminants present on several organic substrates (e.g.: food waste; animal manure; residual water).
Sustainable alternative to waste management.

Lopes, I. G., Lalander, C., Vidotti, R. M., & Vinnerås, B. (2020). Reduction of bacteria in relation to feeding regimes when treating aquaculture waste in fly larvae composting. *Frontiers in Microbiology, 11*. https://doi.org/10.3389/fmicb.2020.01616
Singh, A., & Kumari, K. (2019). An inclusive approach for organic waste treatment and valorisation using Black Soldier Fly larvae: A review. *Journal of Environmental Management, 251*, 109569. https://doi.org/10.1016/j.jenvman.2019.109569

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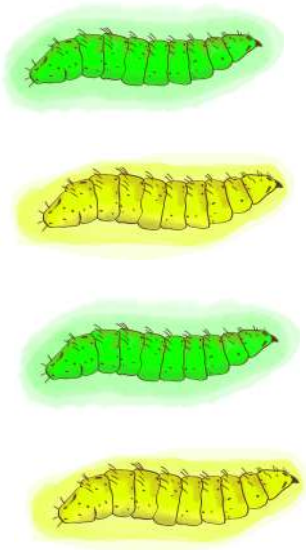
Fluorescent BSFL: illuminating food safety

The problem

Larvae performing **bioremediation cannot** enter the **agrifood chain** (e.g.: cannot be used to produce feed)

The goal is for **industries** to have larvae performing **bioconversion** and **bioremediation** at the same time



The solution

Production of a transgenic **fluorescent** line of black soldier fly (*Hermetia illucens)*

Traceability of larvae during all the process; **identification** of bioremediation larvae
Improve food safety measures

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How to: Fluorescent Larvae

29th January 2025

Starting with a transposon-mediated transgenesis approach…

The plan

1	DNA extraction from BSF eggs + *H. illucens* actin promoter amplification
2	Cloning of the promotor into the pGem®-T Easy vector system
3	Construction of the donor plasmids – restriction enzyme + T4 DNA ligase cloning (with yellow and green fluorescent proteins)
4	Injection of the plasmids in the embryos – microinjection system
5	Functional studies with transgenic BSF

Results so far…
(Unpublished)

Amplification of the *H. illucens* actin promotor (HiActin) through Polymerase Chain Reaction (PCR) and ran through agarose gel electrophoresis

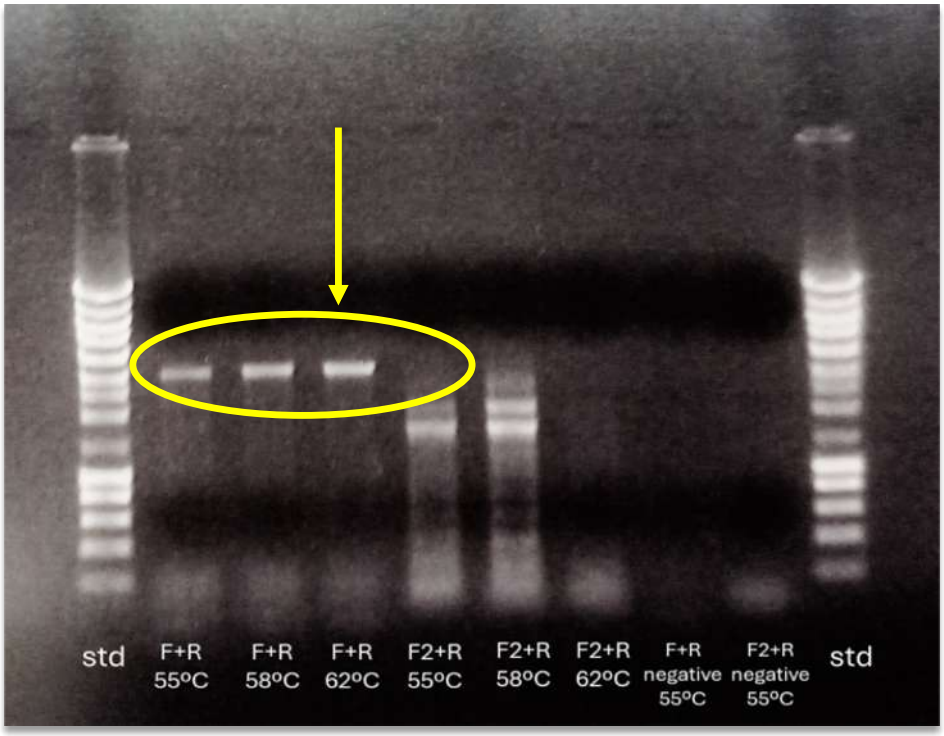

Figure 1- Hiactin fragments on agarose gel electrophoresis (original; unpublished)

Results so far…
(Unpublished)

Cloning of the promotor into pGEM®-T Easy vector (Promega, Madison, WI, USA)

Restriction enzyme digestion + ligation with T4 DNA ligase;

Transformation with competent cells NZY5α (NZYtech, Lisbon, Portugal) + plasmid DNA extraction;

Plasmid linearization with restriction enzyme;

Sanger sequencing (Stabvida, Almada, Portugal).

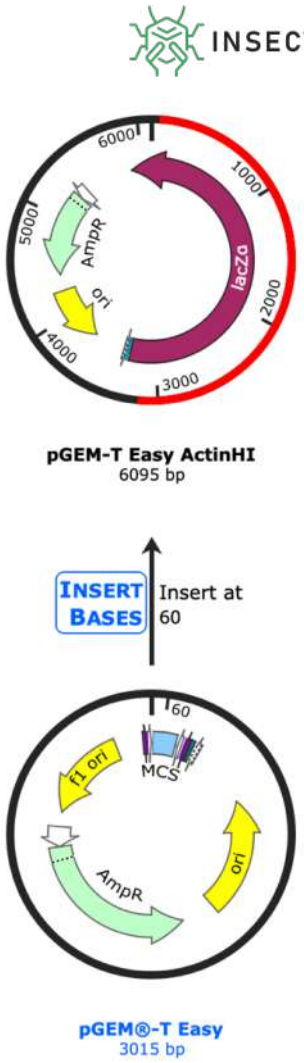

Figure 2 - pGEM®-T Easy Hiactin (original; unpublished)

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Results so far…
(Unpublished)

Donor plasmid construction:

pBac{3XP3::EYFP,attP} (David Stern, Addgene plasmid # 86860; http://n2t.net/addgene:86860; RRID:Addgene_86860) ;

pBac{3XP3::EGFP, Pactin::Ptrsps} (David Stern, Addgene plasmid #86861; http://n2t.net/addgene:86861; RRID:Addgene_86861).

3XP3 Induces expression in the eyes: we needed to remove it (BSFL do not have eyes) and we need to insert the Hiactin promotor

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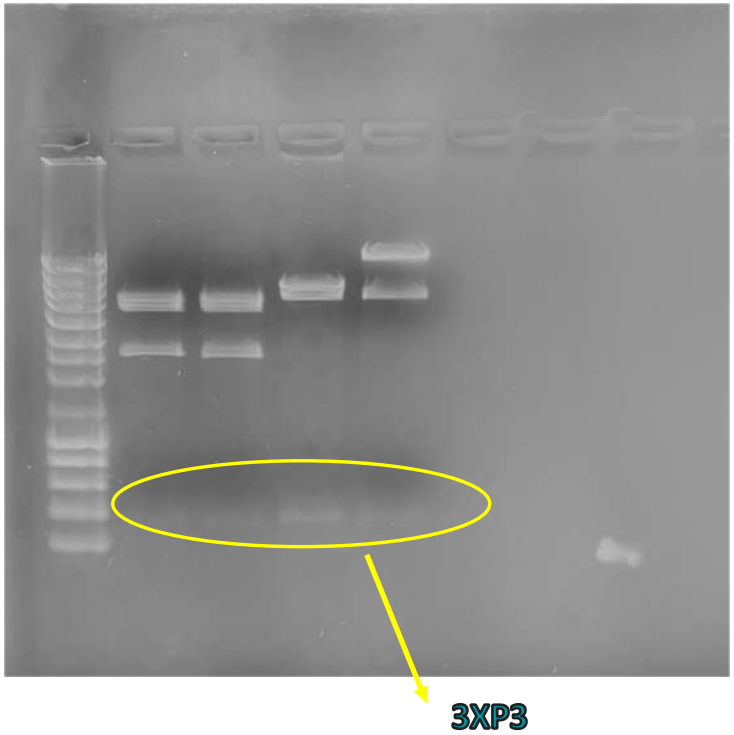
Results so far…

(Unpublished)



We have successfully removed the 3XP3 plasmid and are now enhancing protocols for inserting the Hiactin promotor into the plasmids

Next steps

Injection of the plasmids in the embryos – microinjection system

Functional studies with transgenic BSF

Next steps

Developing a CRISPR transgenesis protocol

Conclusion

With this approach, we exploit the potential of genetics to monitor the entire production process, guaranteeing efficiency and safety in the new agrifood systems

29th January 2025

THANK YOU!

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