European Environment Agency

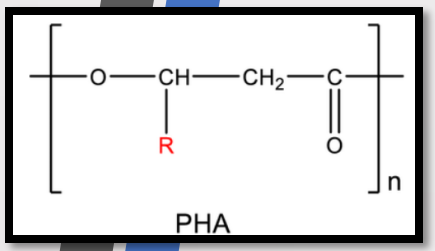
ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

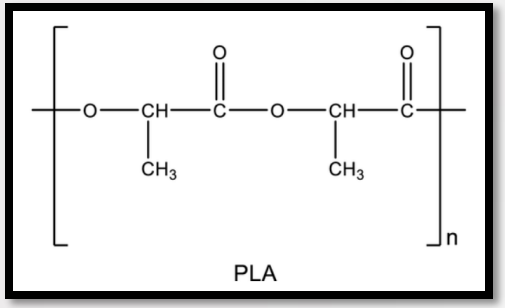
Hybrid Genome Assembly and Multi-Omics Analysis of Composting-Residue Isolates Reveal Bioplastic Degradation Potential

Martina Castellucci | A.Y. 2024/2025

MSc Bioinformatics

Applied Genomics Project





Polyhydroxyalkanoates

-Microbial polyester
-Bioplastics, medical
-Faster degradation

Polylactic Acid

-Plant polyester
-Packaging, biomedical
-Slow degradation

Scientific Context & Study Rationale

Background

- **PLA & PHA**: persistent bioplastics.
- Fungi → **hydrolytic enzymes** (esterases, cutinases, lipases).
- **Compost** = hotspot for degraders.
- **20 isolates**; several active.

Rationale

- Reveal **genes & enzymes** for degradation.
- Integrate **genome + transcriptome**.
- Basis for **bioplastic valorization**.

Goal

- **High-quality genome + RNA-Seq**.
- Link **genotype ↔ expression**.
- Identify **candidate enzymes**.

Research Workflow Overview

1) **Sample collection** from PLA/PHA-enriched compost

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2) **Isolation & identification** (Rhodamine B assay + ITS sequencing)

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3) **DNA/RNA extraction & QC**

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4) **Hybrid sequencing:** Illumina NovaSeq + Oxford Nanopore GridION

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5) **Genome assembly & polishing**

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6) **Functional annotation** (CAZymes, BGCs, degradative enzymes)

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7) **Comparative genomics & phylogenetic analysis**

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8) **Transcriptomic profiling** under PLA/PHA conditions

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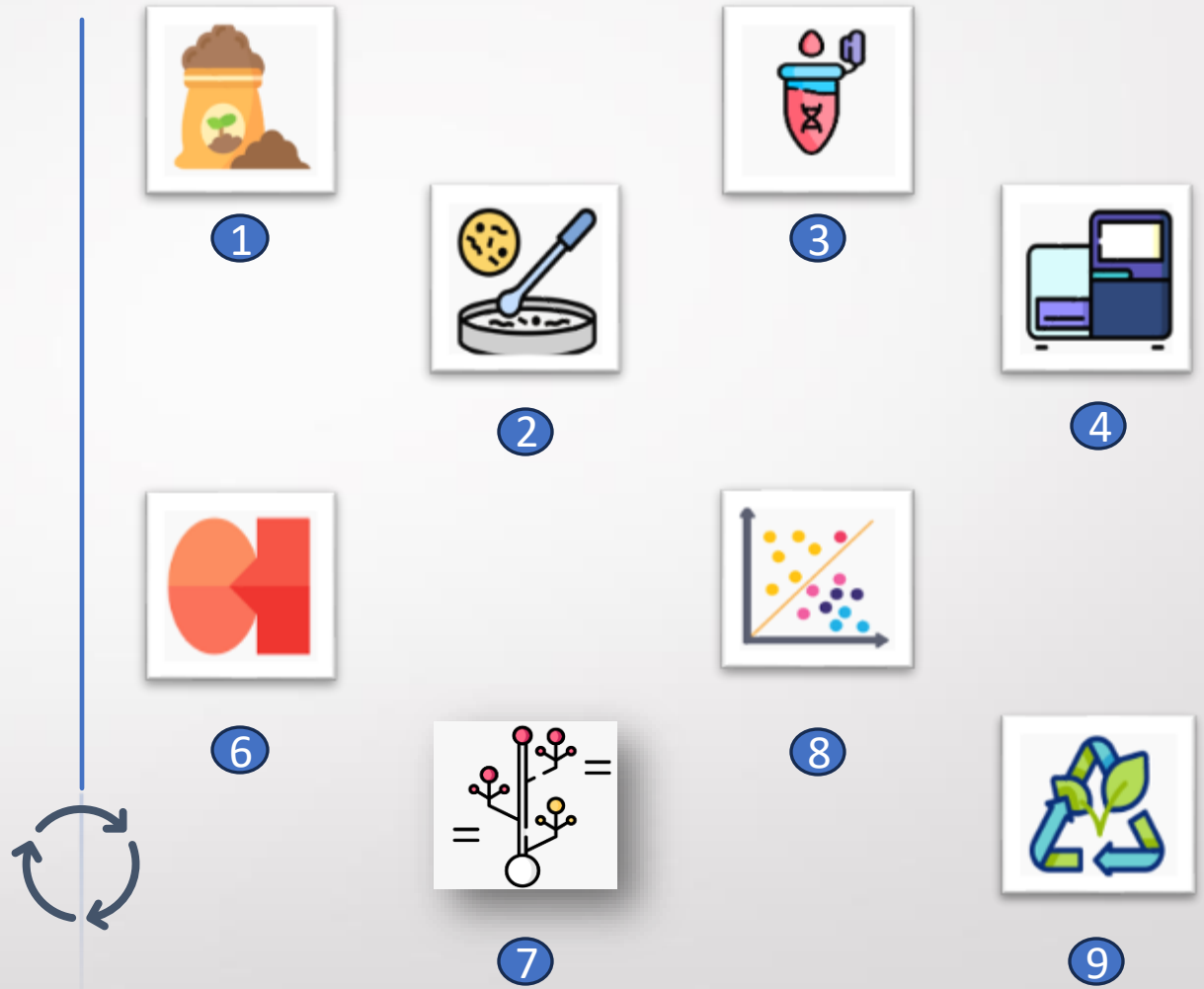
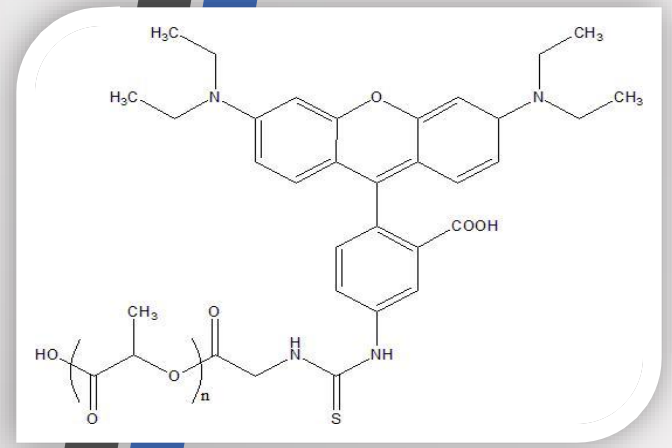
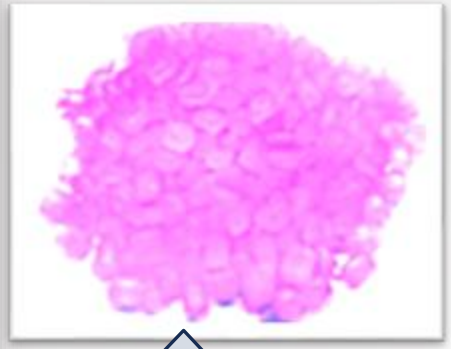
9) **Integration of multi-omics** for enzyme discovery



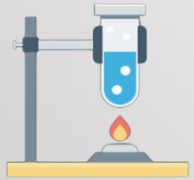
Collection and Identification

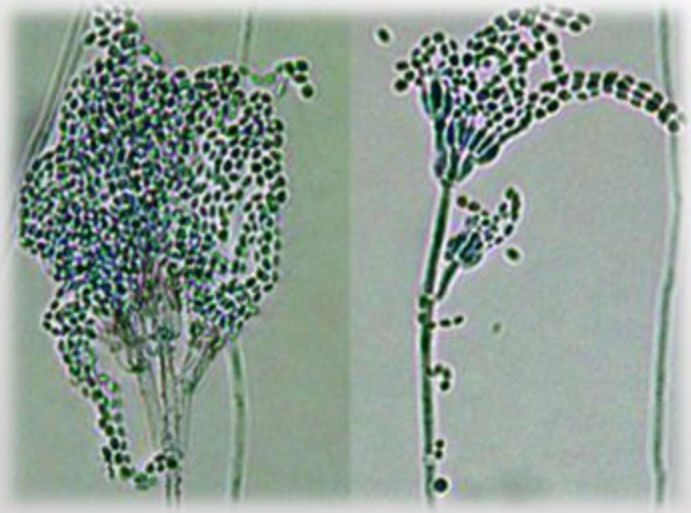


PLA-Rhodamine B – Ruixibiotech



PLA granules coated with **Rhodamine b**



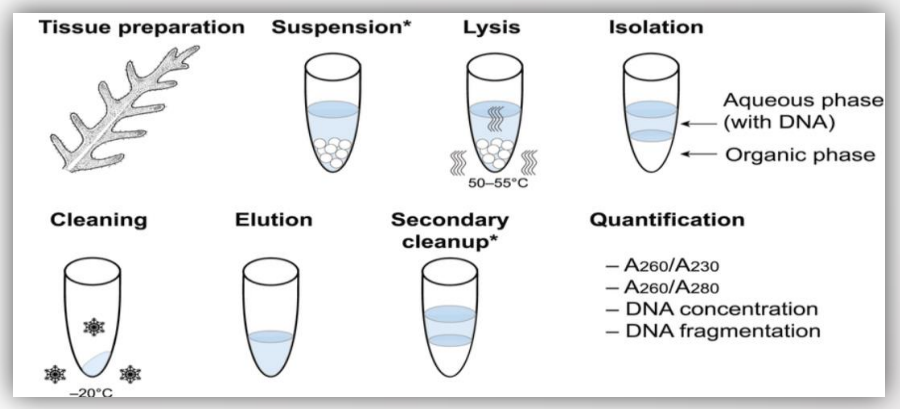


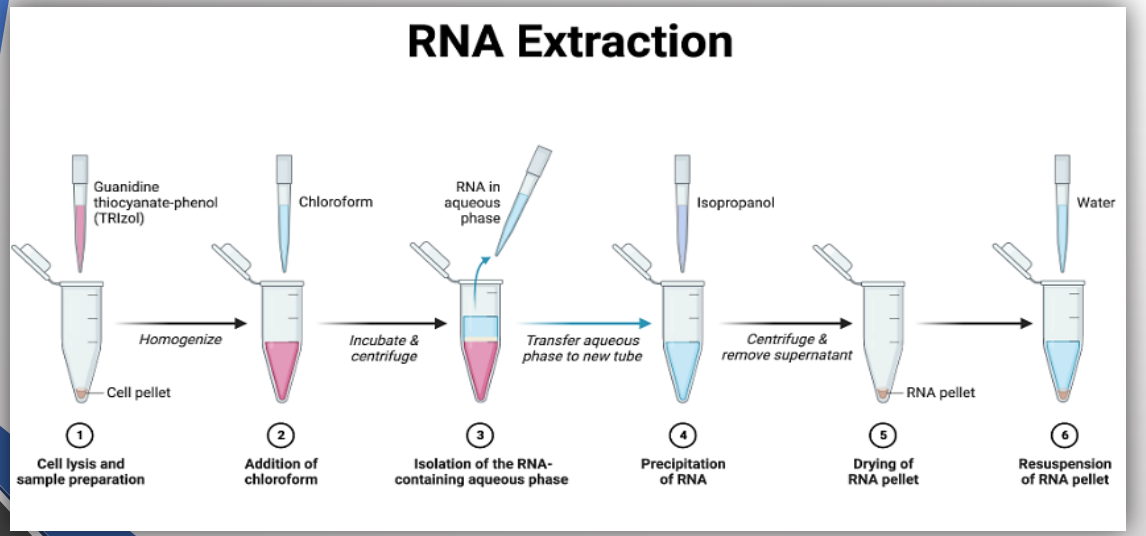
- Compost from **PLA/PHA-rich organic waste** yielded **20** isolates; **5** were selected as PLA/PHA degraders.
- Cultured on **PDA + 0.5% PLA + Rhodamine B** → visible **fluorescent halos** = esterase activity.
- Microscopy showed **septate hyphae with conidia,** serving as the first step in taxonomic identification.
- **ITS rDNA sequencing** confirmed species-level identity.

DNA Extraction & RNA Quality Control

Genomic DNA

CTAB extraction protocol → high-molecular-weight DNA (>20 kb).
QC: **Qubit** (yield), **Nanodrop** (purity), **gel electrophoresis** (integrity).
Essential for **Nanopore long-read sequencing**.





Total RNA

Extraction: **TRIzol + silica column purification**.
QC: **Bioanalyzer** (RIN > 8), **Nanodrop** (A260/280).
Libraries: **12 RNA-Seq samples** (Control, PLA, PHA, Blank × 3 replicates).
Ensures **reliable transcriptome profiling**.

Hybrid Genome Sequencing Strategy

Illumina NovaSeq 6000

Oxford Nanopore GridION

*Combine **Illumina accuracy** with **Nanopore contiguity** → **polished, complete genome***

Feature	**Illumina NovaSeq 6000**	**Oxford Nanopore GridION**
Read type	Short, paired-end	Long, single-molecule
Read length	150 bp (PE150)	Up to 20 kb
Coverage in project	~up to 30×	~35×
Accuracy	>99.9% (Phred > Q30)	~90–95% (after polishing)
Strengths	High base accuracy, SNP detection, RNA-Seq	Resolves repeats, structural variants, improves contiguity
Main role in project	Error correction, polishing, transcriptome mapping	Primary genome scaffolding, structural resolution

Hybrid Assembly & CAZyme Profile

Assembly & QC

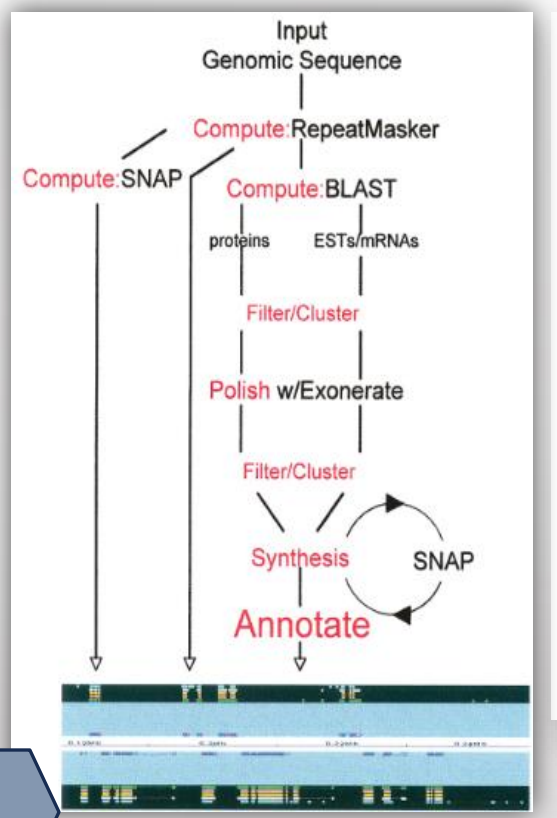
- **Flye** (Nanopore-first assembly) + **Pilon** (Illumina polishing)
- **QC**: QUAST (contiguity), **BUSCO >90% expected** (completeness)

Annotation

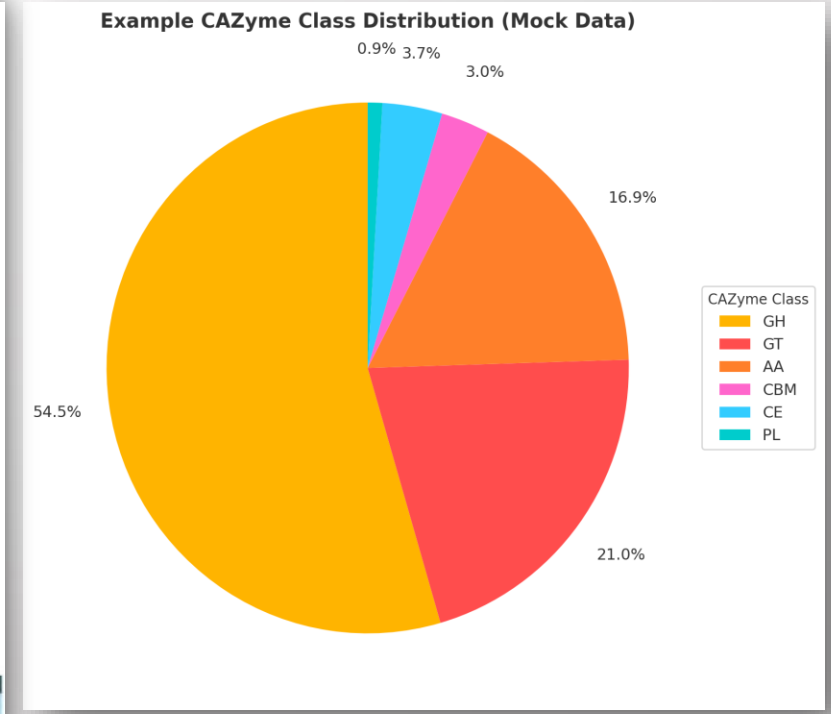
- **MAKER3 pipeline** (Augustus, GeneMark-ES)
- **RNA-Seq evidence** for gene models
- **dbCAN3**: CAZyme annotation
- **Geneious**: manual curation

Expected Key Results

- ✓ **Genome assemblies**: high contiguity + completeness
- ✓ **Broad CAZyme repertoire** predicted, including:
  - ✓ Esterases
  - ✓ Cutinases
  - ✓ Lipases

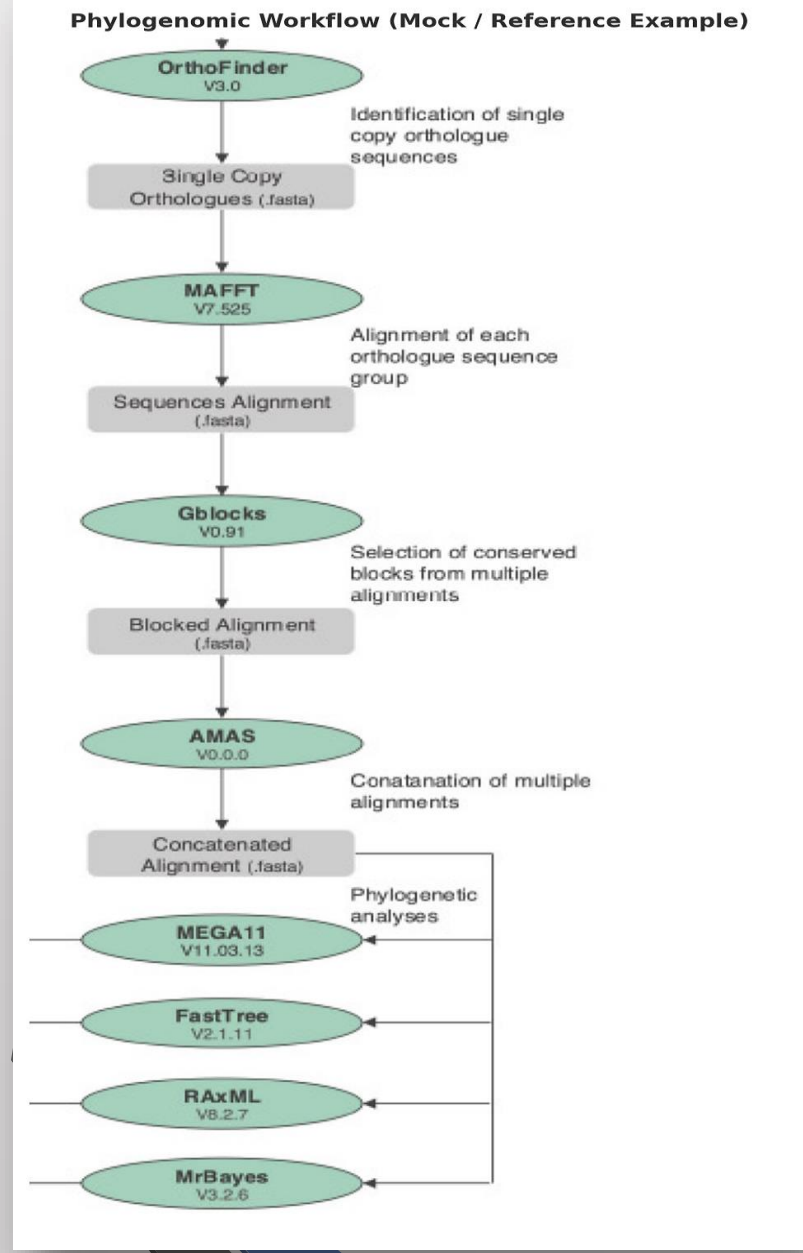


MAKER Overview



BUSCO dbCAN3

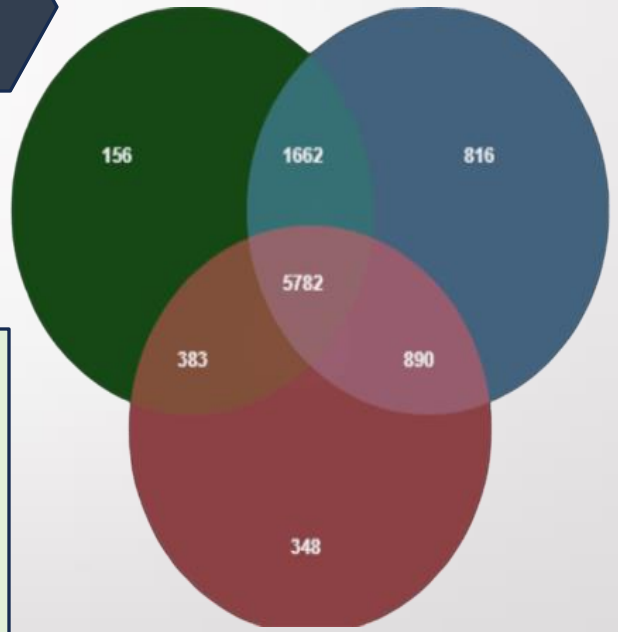
Genomic Comparison & Evolutionary Context



Approach →

- Phylogenomics integrates degraders and non-degraders to resolve evolutionary placement. **OrthoFinder** identifies orthogroups, distinguishing shared vs unique genes. **MCScanX / MAUVE** assess genome collinearity, synteny, and structural rearrangements across related fungi.

Mock example jvenn (Orthofinder)

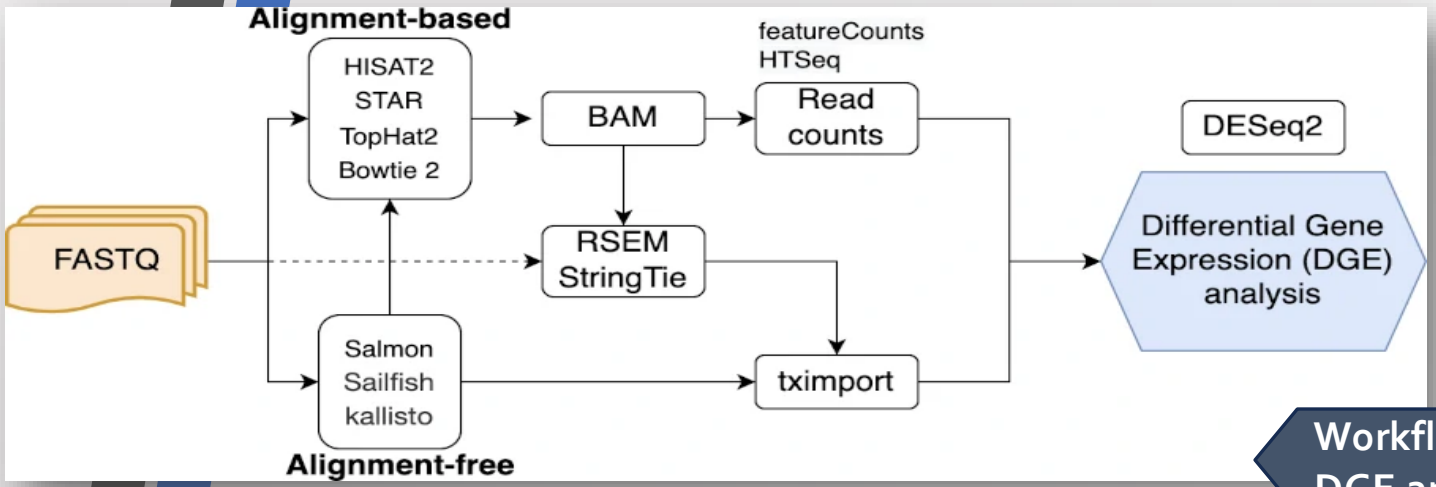


Expected Results:

- **Unique orthogroups** in degraders → candidate degradation genes.
- **Synteny breaks / rearrangements** linked to adaptation.
- **Evolutionary placement** of degraders within related fungi.
- **Integration** pinpoints gene clusters for PLA/PHA degradation.

OrthoFinder MCScanX

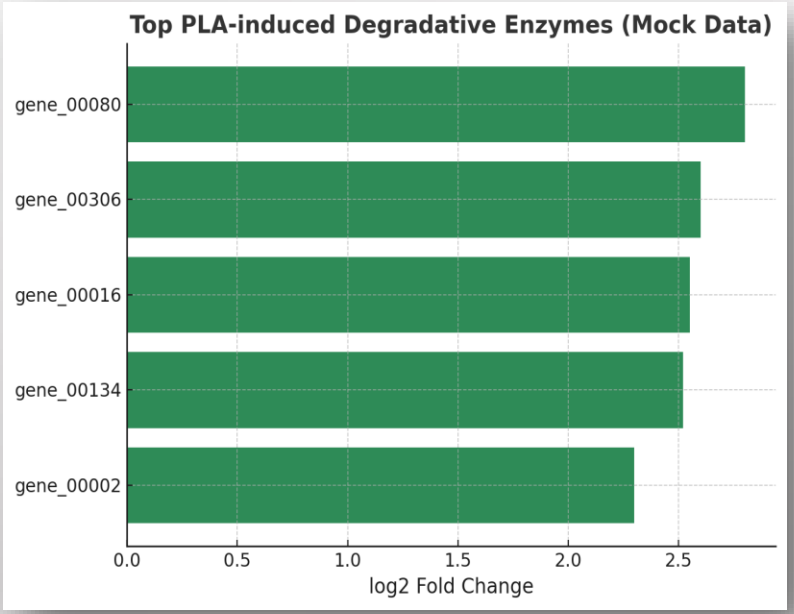
Gene Expression Response to Bioplastics



Workflow for DGE analysis

Expected DEGs

PLA → ↑ cutinases, esterases, lipases
PHA → ↑ PHA depolymerase-like, lipases
Shared → ↑ oxidoreductases, transporters
Integration → overlap with CAZymes & orthogroups



Transcriptomic Analysis

RNA-Seq design

- 12 libraries: Control, PLA, PHA, Blank × 3 replicates
- PLA/PHA sampled at **12 h, 24 h, 36 h**; Control = baseline

Quantification & DEG analysis

- **Salmon**: transcript quantification
- **tximport**: gene-level aggregation
- **DESeq2**: DEG detection (FDR ≤ 0.05, $|\log_2 FC| \geq 2$)
- **Thousands of DEGs** identified, with **plastic-specific signatures**

From Genome to Green Solutions

Strengths	Challenges
Targeted enzyme discovery enabling efficient PLA/PHA degradation	**Scaling from lab to industrial composting facilities** required
Multi-omics integration for mechanistic insights	Enzyme stability and efficiency under **real environmental conditions**
Alignment with **EU Green Deal** & **Circular Economy Action Plan**	Regulatory and safety assessments for field applications
Potential for **industrial composting** and **waste valorization**	Economic feasibility vs. conventional waste management
Creation of an **open-access dataset** for biotechnology R&D	

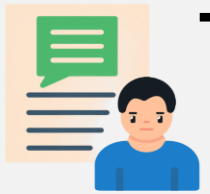
European Environment Agency (2020).
EEA Briefing No. 09/2020.
DOI: 10.2800/552241

Estimated Project Budget

Project budget: €200,000

Personnel	Activity	Estimated Cost (€)	Description
Wet lab Postdoc salary		45,000	One-year contract for lab activities
	Sampling & Isolation	3,000	Compost collection & fungal culturing
	DNA Extraction & QC	2,000	CTAB reagents, plasticware, gel materials
	Illumina PE150 Sequencing	30,000	Short-read library preparation & sequencing
	Oxford Nanopore Sequencing	50,000	Long-read flow cells & library kits
	RNA extraction (12 libraries)	1,000	TRIzol & silica column kits
	RNA library preparation (12 libraries)	1,500	Illumina TruSeq RNA kits
	RNASeq Illumina (12 libraries)	2,500	PE150 sequencing
Bioinformatics Postdoc		45,000	One-year contract for analysis & reporting
	Genome Assembly & Polishing	0	Flye + Pilon (open-source)
	Functional Annotation	0	MAKER3, dbCAN3, Geneious (manual curation)
	Comparative Genomics	0	OrthoFinder, MAUVE, MCScanX
	Non-open source softwares	5,000	Geneious & other licensed tools
	Reports, Dissemination	**15,000**	**Publications, conferences, outreach**
		200,000	

Selected References & Tools



Scientific Articles

- Menicucci, A., Iacono, S., Ramos, M., Fiorenzani, C., Peres, N. A., *et al.* (2025). Can whole genome sequencing resolve taxonomic ambiguities in fungi? The case study of *Colletotrichum* associated with ferns. *Frontiers in Fungal Biology*, 6, 1540469.
- Ekanayaka, A. H. et al. (2025). Linking metabolic pathways to plastic-degrading fungi: a comprehensive review. J. Fungal Biol.
- Prasad, P., Varshney, D., & Adholeya, A. (2015). *Whole genome annotation and comparative genomic analyses of bio-control fungus Purpureocillium lilacinum*. BMC Genomics, 16(1), 1004.

Main Tools Used

- **Assembly**: Flye (long reads), Pilon (polishing with Illumina).
- **QC**: QUAST + BUSCO (assembly completeness).
- **Annotation**: MAKER3 (gene models, repeat masking), dbCAN3 (CAZyme annotation).
- **Comparative Genomics**: OrthoFinder (orthogroups), MCScanX (synteny/collinearity).
- **Transcriptomics**: Salmon (quantification), DESeq2 (DEG analysis).
- **Visualization**: R / Python (plots), Geneious (manual curation).