



European  
Environment  
Agency



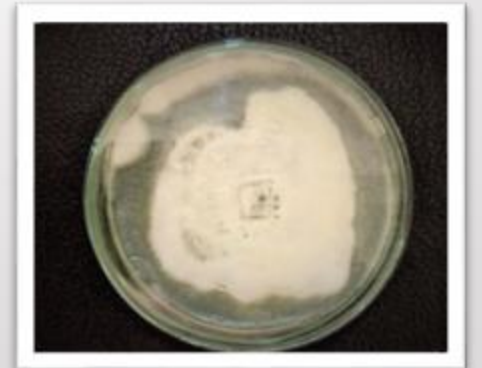
ALMA MATER STUDIORUM  
UNIVERSITÀ DI BOLOGNA

# 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



# Scientific Context & Study Rationale

## Background

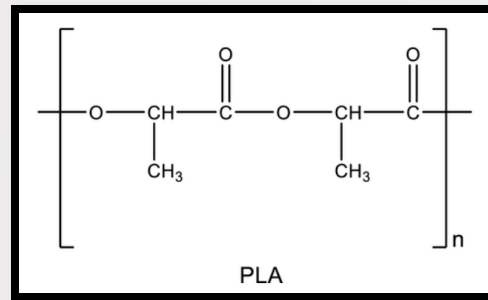
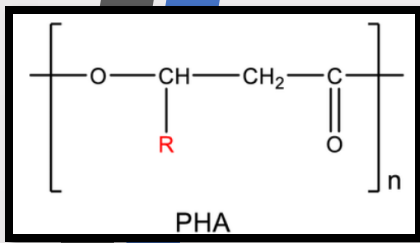
- **PLA & PHA**: persistent bioplastics.
- Fungi → **hydrolytic enzymes** (esterases, cutinases, lipases).
- **Compost** = hotspot for degraders.
- **20 isolates**; several active.

## 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**.

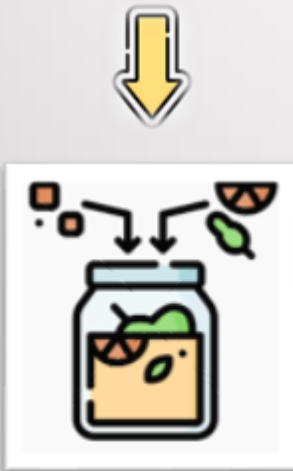
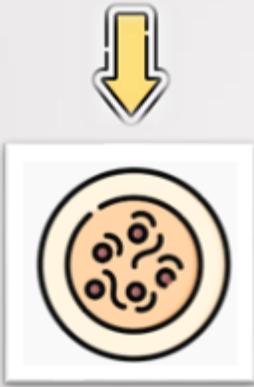


## Polyhydroxyalkanoates

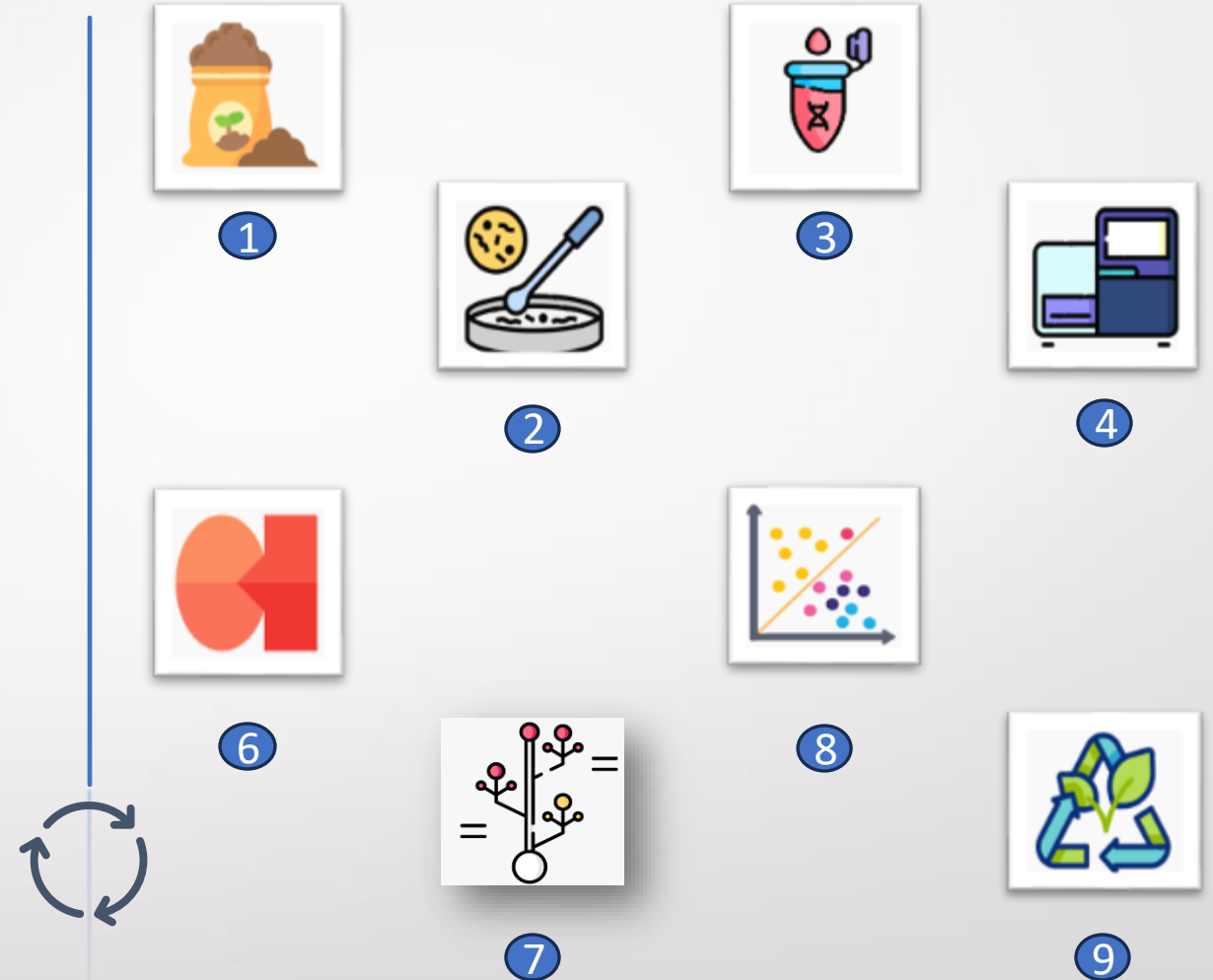
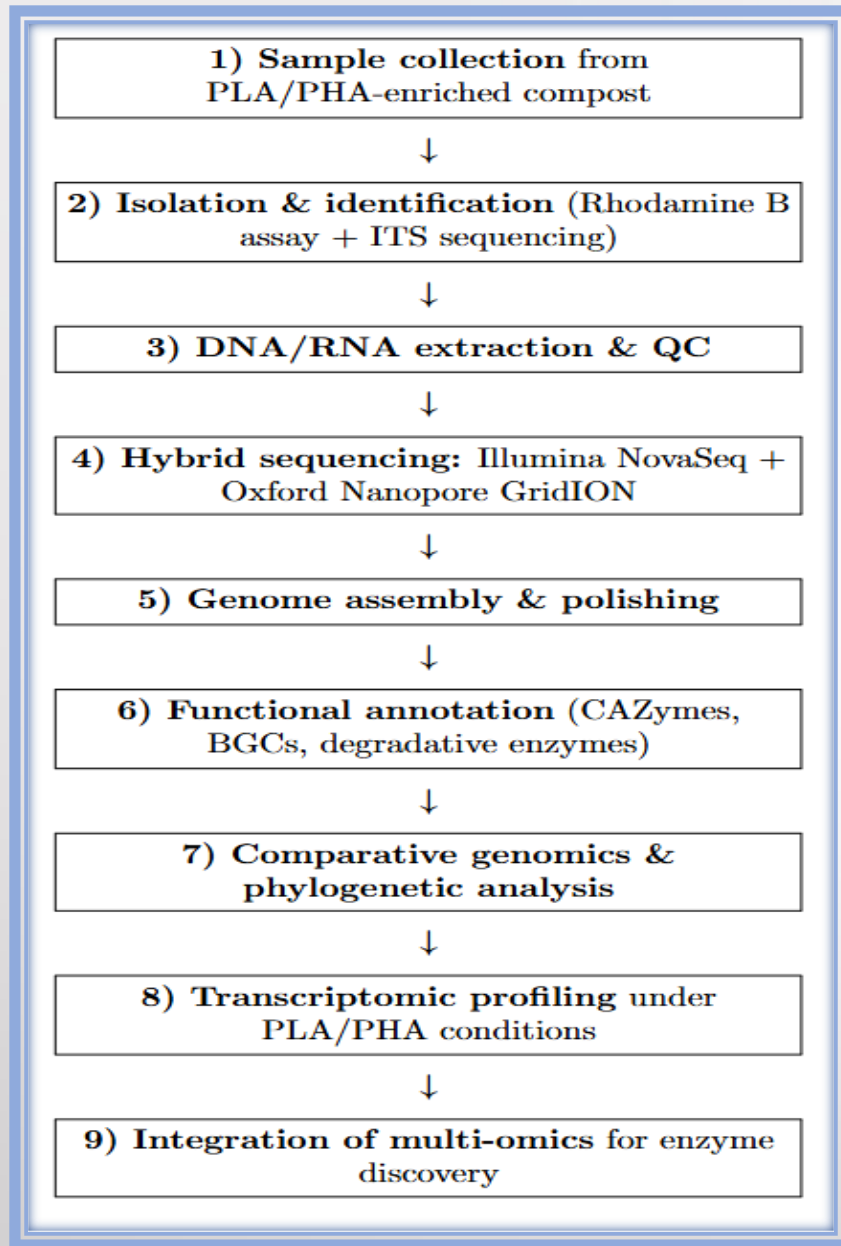
-Microbial polyester  
-Bioplastics, medical  
-**Faster degradation**

## Polylactic Acid

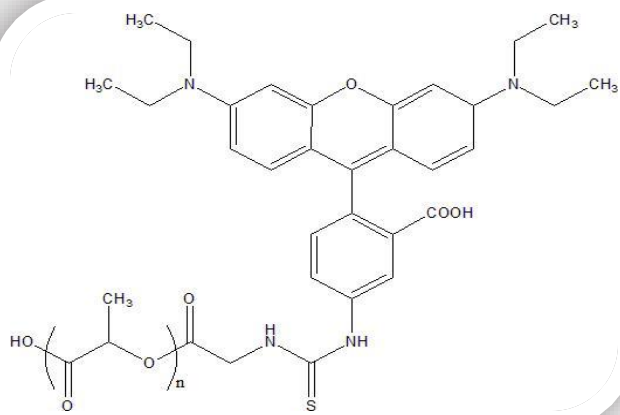
-Plant polyester  
-Packaging, biomedical  
-**Slow degradation**



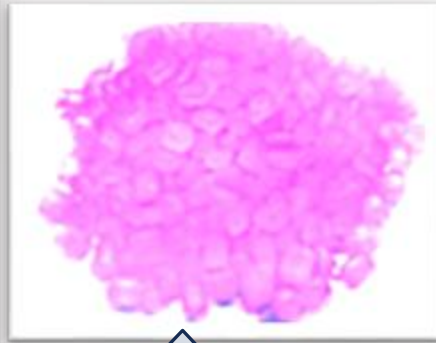
# Research Workflow Overview



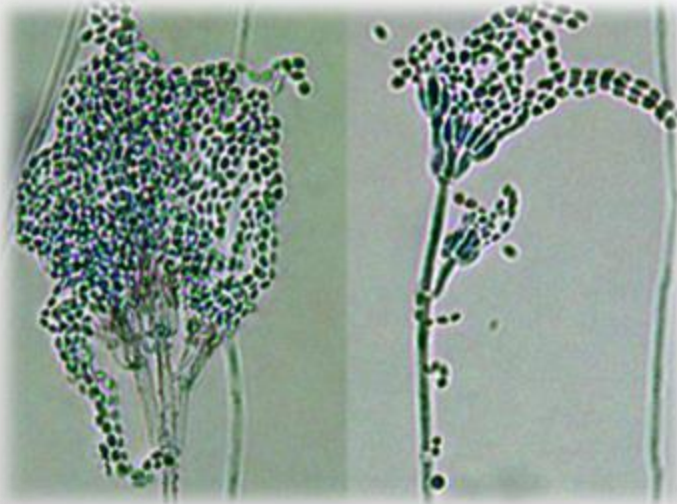
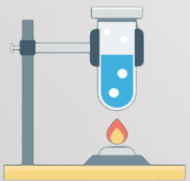
# Collection and Identification



*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



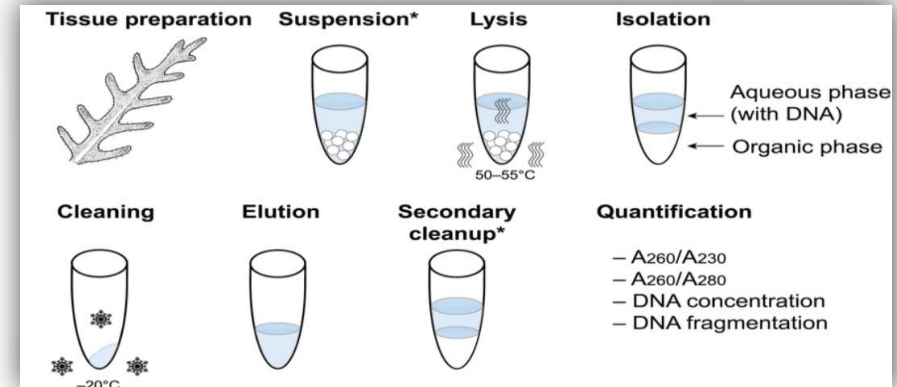
## Genomic DNA



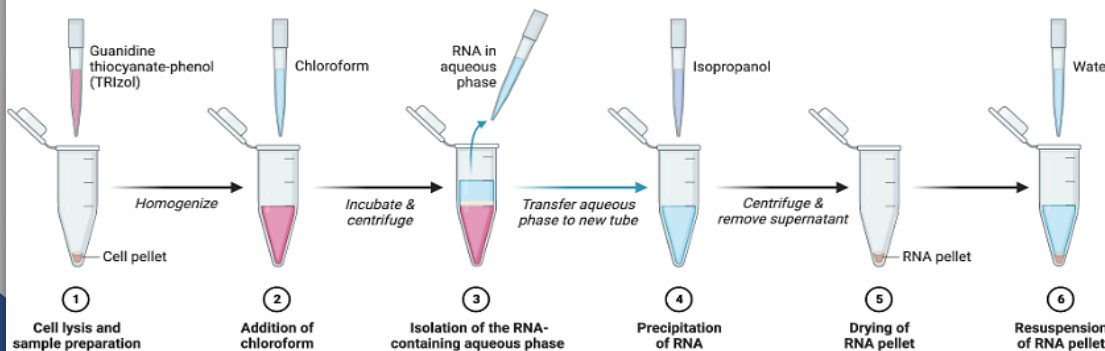
**CTAB extraction protocol** → high-molecular-weight DNA (>20 kb).

QC: **Qubit** (yield), **Nanodrop** (purity), **gel electrophoresis** (integrity).

Essential for **Nanopore long-read sequencing**.



## RNA Extraction



## 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**.

Illumina NovaSeq 6000



Oxford  
Nanopore  
GridION

# Hybrid Genome Sequencing Strategy



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

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



## Assembly & QC

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

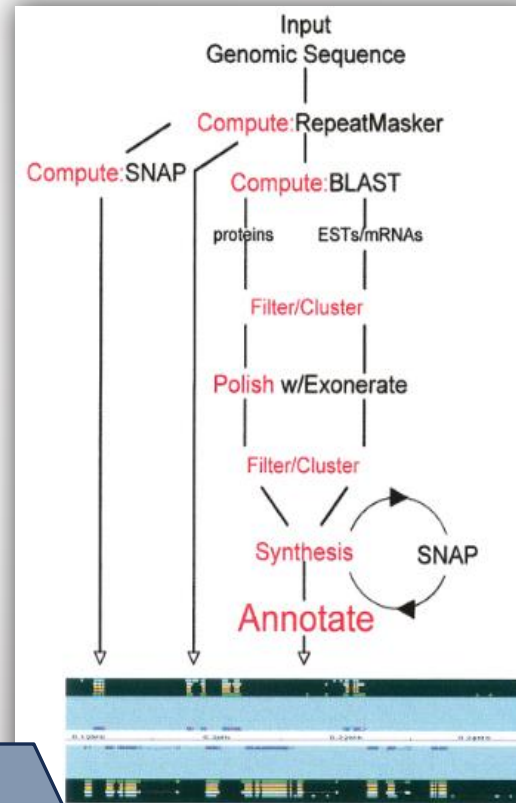
## Annotation

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

## Expected Key Results

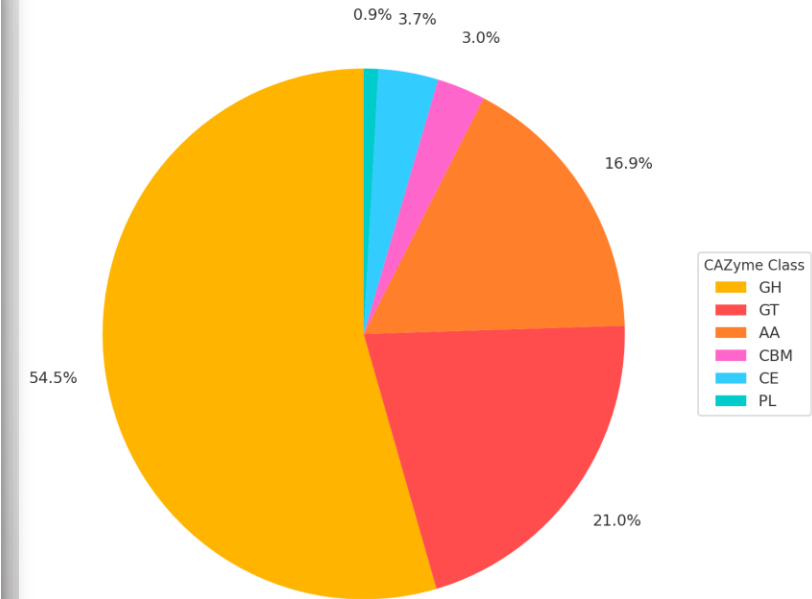
- ✓ **Genome assemblies**: high contiguity + completeness
- ✓ **Broad CAZyme repertoire** predicted, including:
  - ✓ Esterases
  - ✓ Cutinases
  - ✓ Lipases

## MAKER Overview

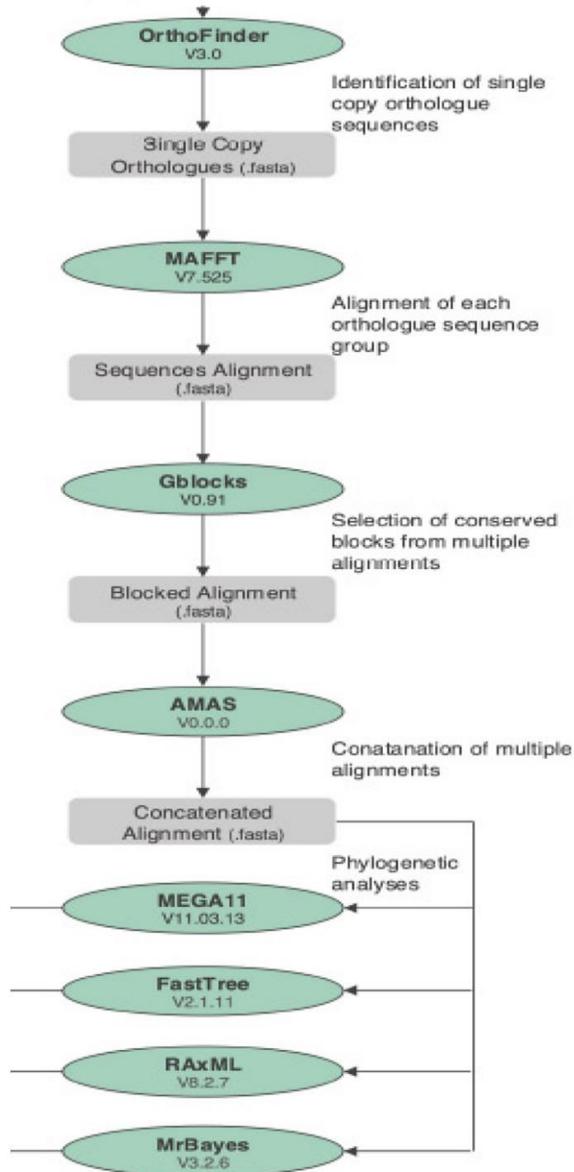


# Hybrid Assembly & CAZyme Profile

Example CAZyme Class Distribution (Mock Data)



## Phylogenomic Workflow (Mock / Reference Example)



## Approach →

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

## 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.

# Genomic Comparison & Evolutionary Context

Mock example  
jvenn  
(Orthofinder)



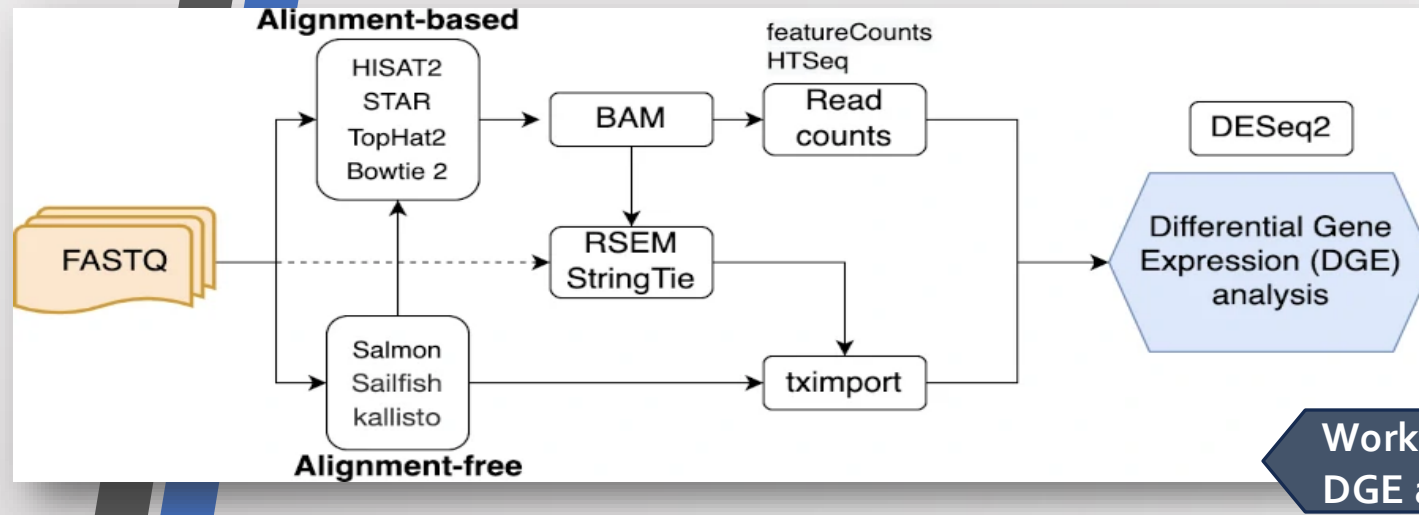
OrthoFinder



MCSanX

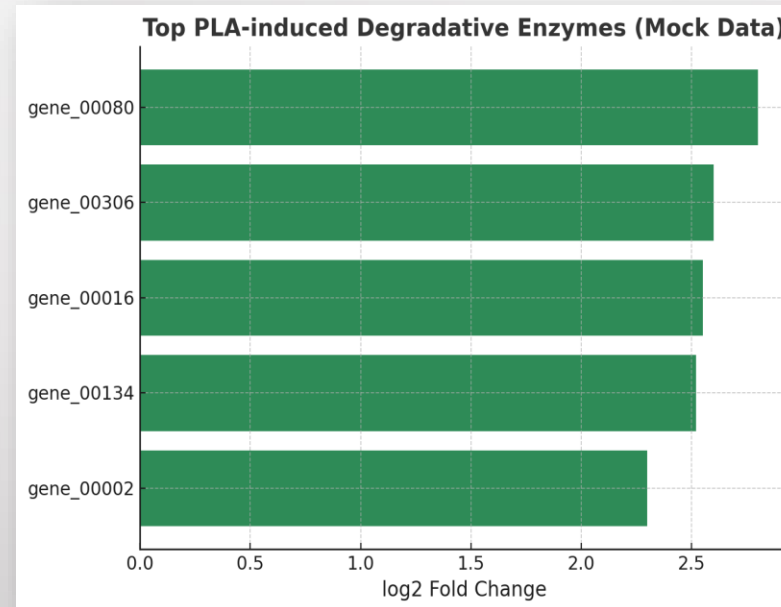


# Gene Expression Response to Bioplastics



## 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 ( $\text{FDR} \leq 0.05$ ,  $|\log_2\text{FC}| \geq 2$ )
- **Thousands of DEGs** identified, with **plastic-specific signatures**

# From Genome to Green Solutions

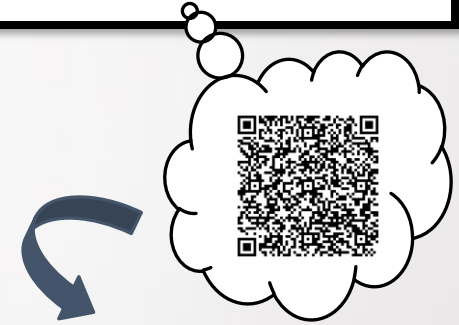


Resource efficiency

**Biodegradable and compostable plastics**  
— challenges and opportunities



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



**European Environment Agency (2020).**

EEA Briefing No. 09/2020.

DOI: 10.2800/552241

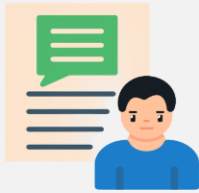
# Estimated Project Budget



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
	<b>Reports, Dissemination</b>	<b>15,000</b>	<b>Publications, conferences, outreach</b>
		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).