UNIVERSITY OF MANITOBA

CANADIAN PRAIRIE BIOSCIENCES
BIOFIBE2015

POLYHYDROXYALKANOATES (PHAS):

BIODEGRADABLE POLYMERS FOR INDUSTRIAL APPLICATIONS

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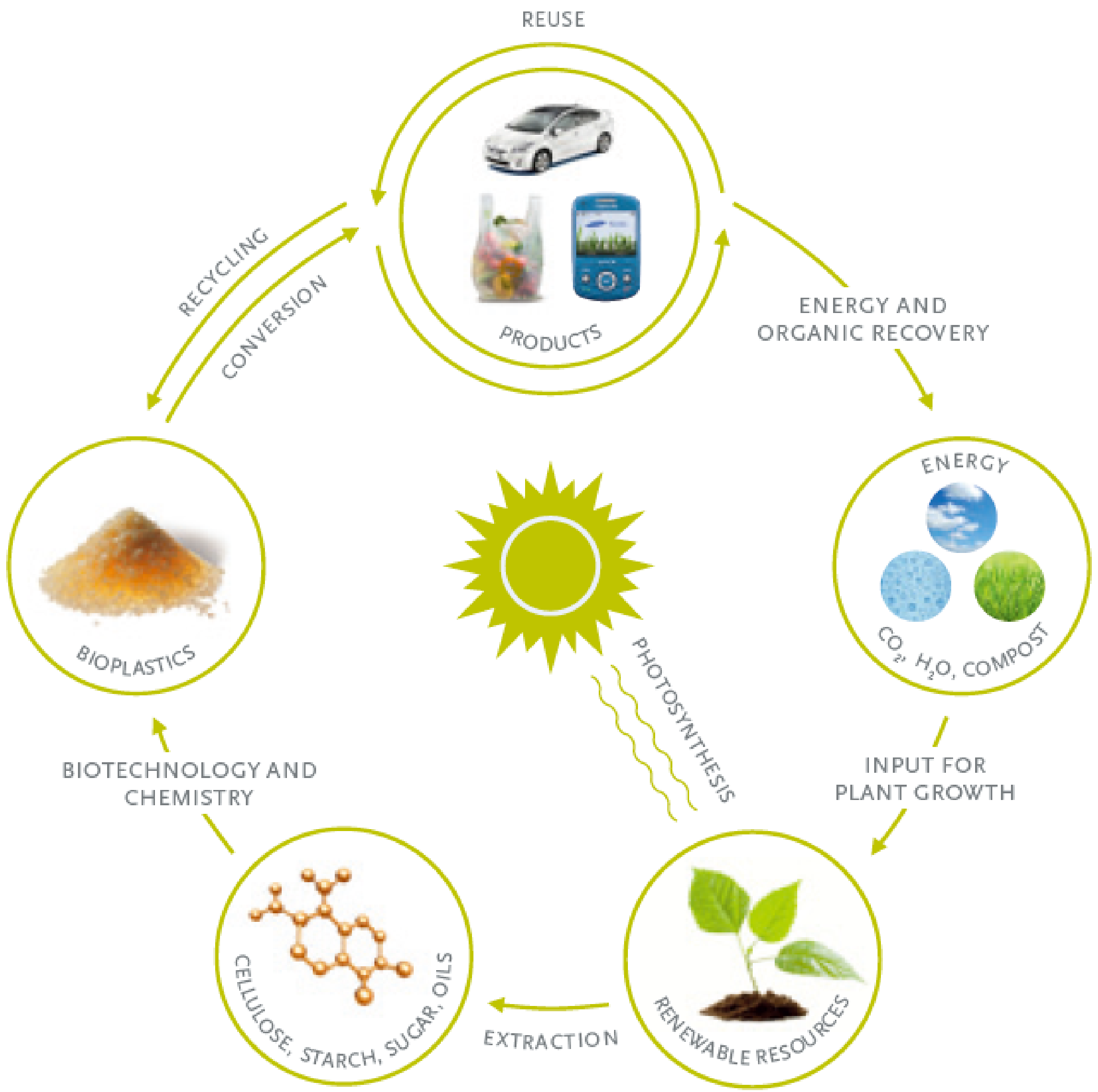
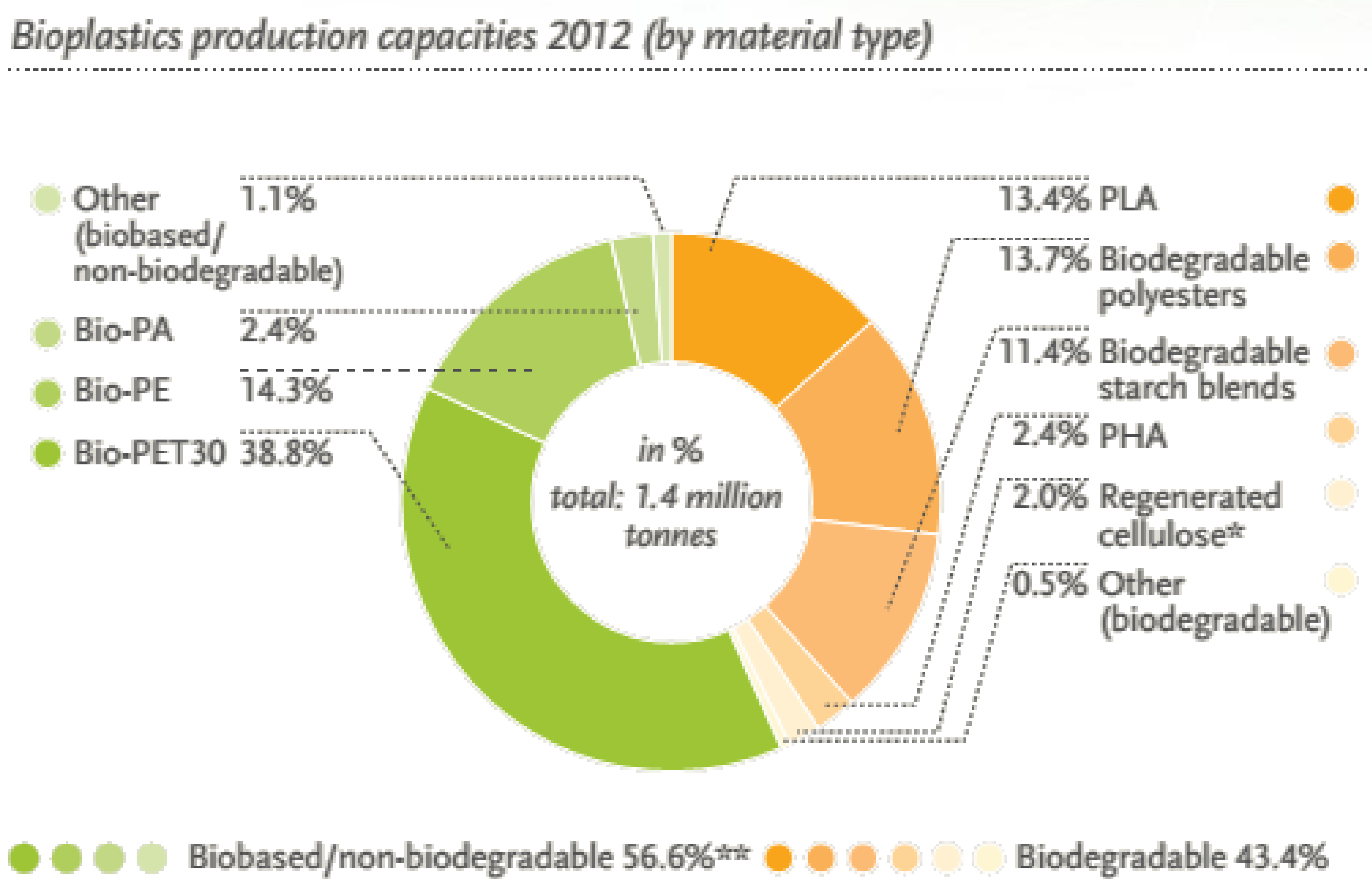
BIO-BASED PLASTICS

"Human-made or processed organic macromolecules derived from **biological resources** and **used for plastic** and fibre applications"

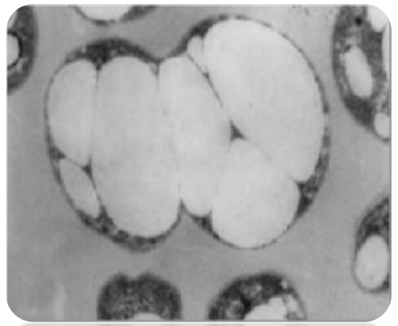


Types of Bioplastic



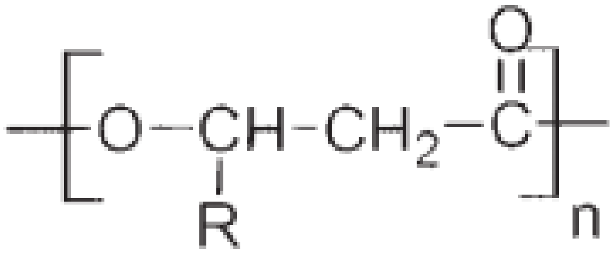
PA, **Polyamide; PE, Polyethylene; PE,: Polyethylene terephthalate; PLA, polylatic acid; PHA, Polyhydroxyalkanoates**

Polyhydroxyalkanoate (PHA): Biodegradable Plastics from Bacteria



- **PHA are natural polyester polymers synthesized by bacteria**
- **100% biodegradable,**
- **Physical properties are similar to conventional petro-plastics.**
- **Polymer composition can be tailored into different industrial applications**
- **Can be produced from agro-industrialwaste streams**
- **Bacterial can be genetically modified to enhance the polymer production.**

- **Represent a potential platform for bioplastic,bioresins fine chemicals, and biocomposite materials**



Poly(hydroxyalkanoates), PHA

PHYSICAL PROPERTIES OF PHA POLYMERS

The differences in R-groups greatly affect the final physical, mechanical, and thermal properties of PHA polymers

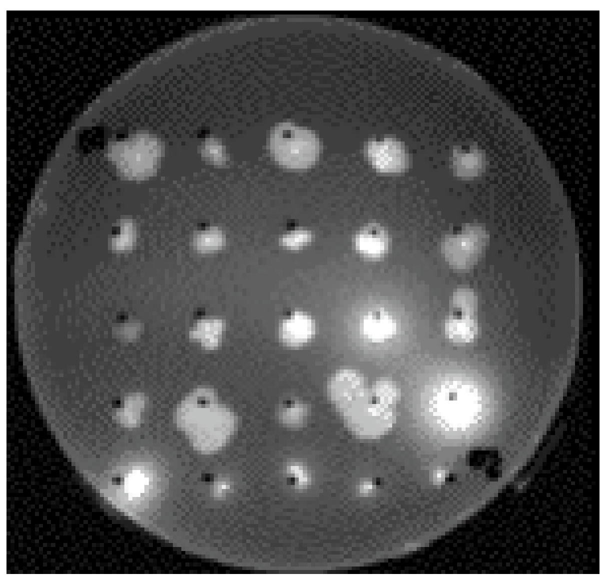
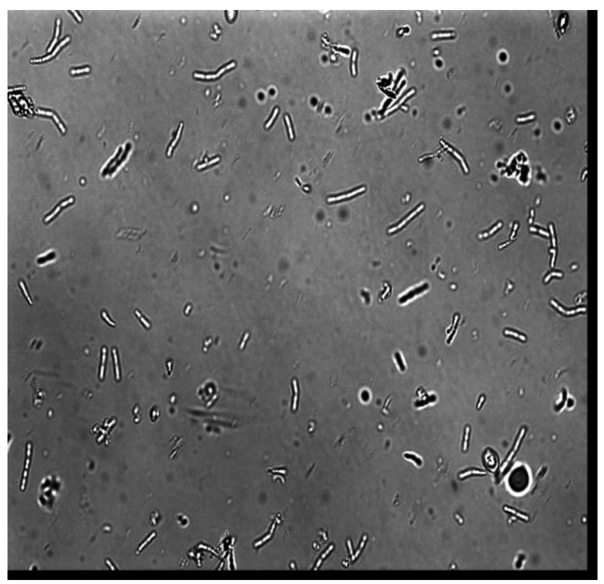
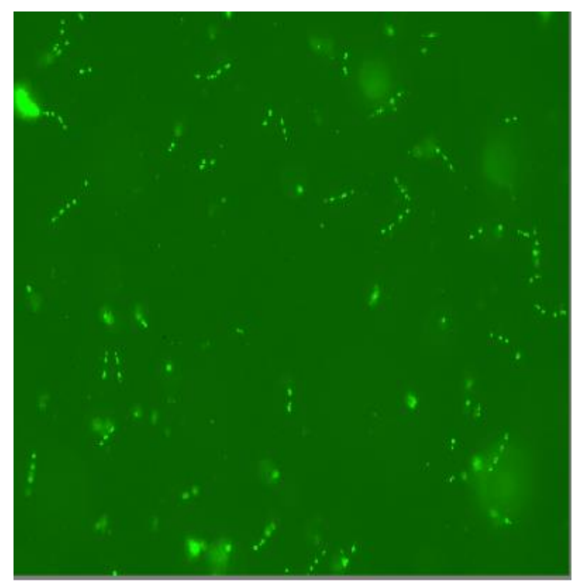
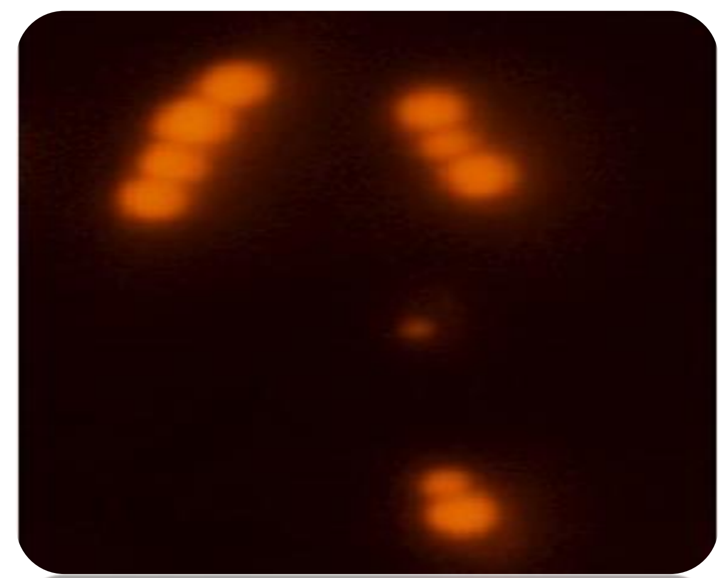
	PHA			Poly(propylene)
	scl-PHA	mcl-PHA	lcl-PHA	
Crystallinity [%]	40–80	20–40	?	70
Melting point [°C]	80–180	30–80	–	176
Density [gcm^{-3}]	1.25	1.05	?	0.91
Extension to break [%]	6–10	300–450	–	400
UV light resistance	good	good	good	poor
Solvent resistance	poor	poor	poor	good
Biodegradability	good	good	good	none

Screening for PHA Producing Bacteria

Method: Enrichment

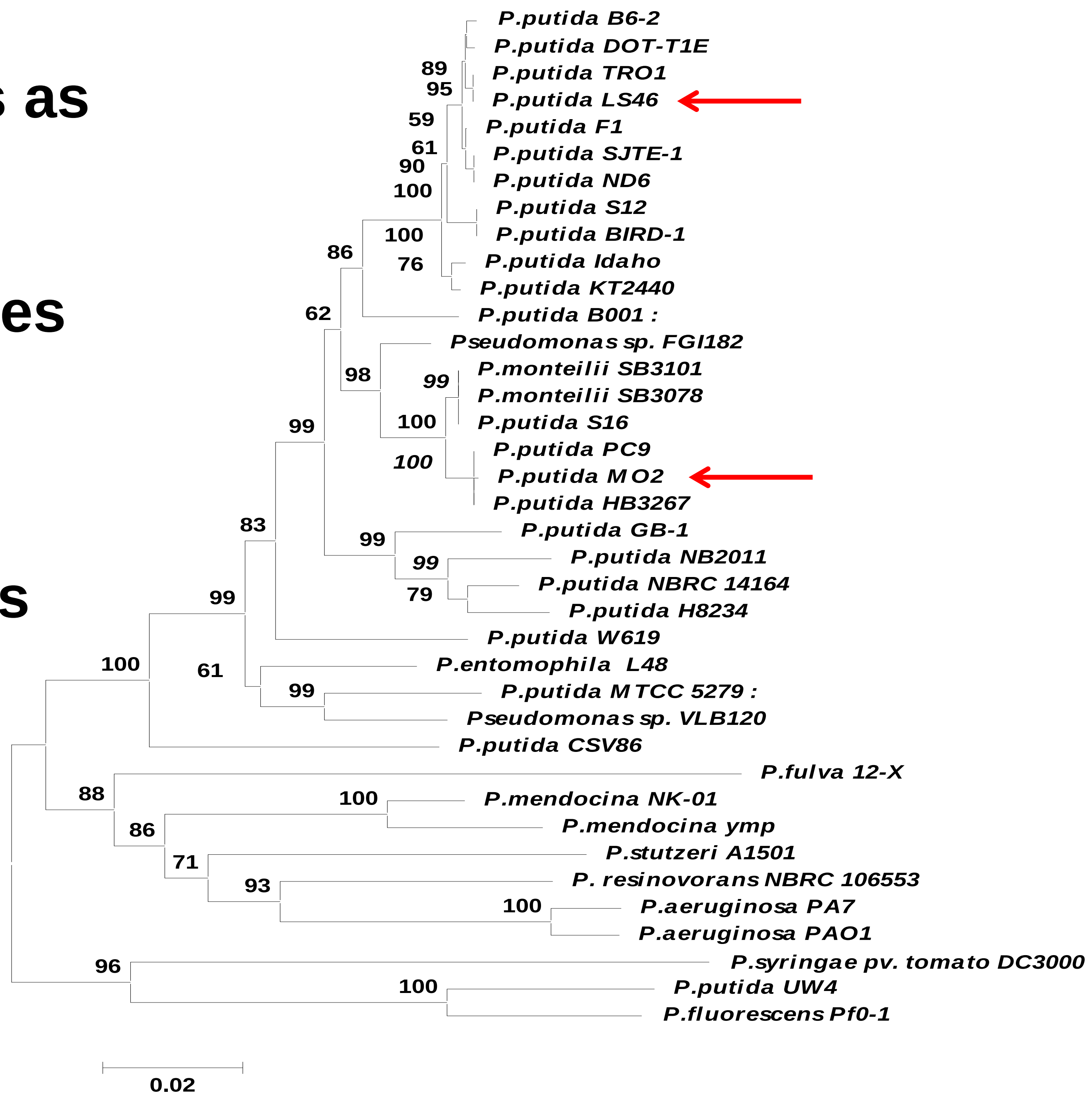
Inocula: Sewage sludge and hog barn wash
Medium: Thin slurry, wet cake or DDGS as sole a carbon source

Results: 45 isolates screened on plates containing Nile Red dye
Observed under UV light. PHA producers gave bright fluorescence

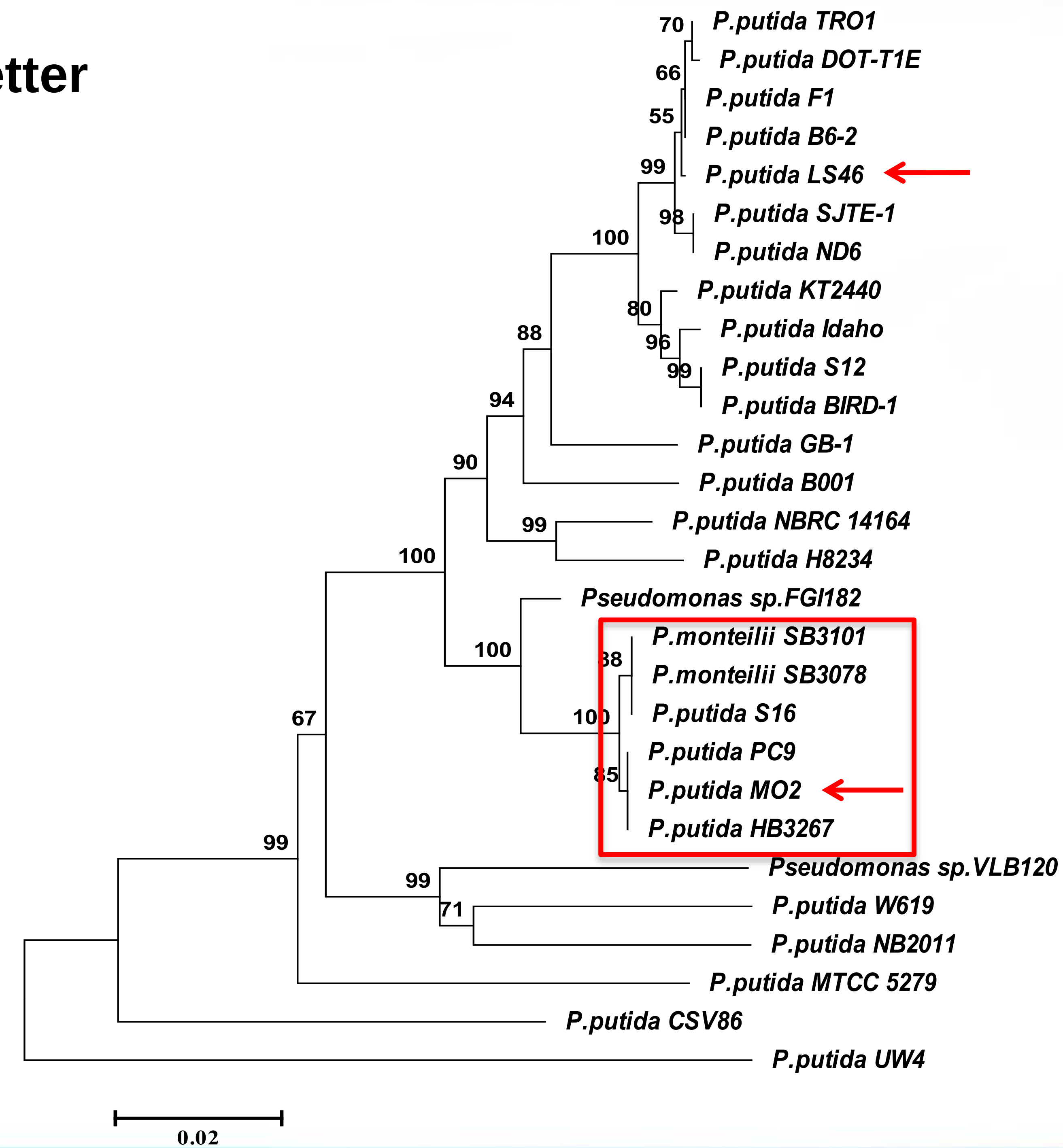
Characterization of *P. putida* isolates

- **16S rDNA analysis identified several isolates as *Pseudomonas putida***
- **Phylogenetic analyses of 16S rDNA sequences revealed relationships among isolates and known species**
- **Two strains with high levels of PHA synthesis are indicated by red arrows**



Phylogenetic Analysis of *P. putida* strains using *cpn60*

- *cpn*60 can differentiate closely related strains better than 16S rDNA
- Phylogenetic analyses of *cpn*60 sequences revealed that several strains previously considered to belong to *P. putida* are actually more closely related to *P. monteilli* strains
- *P. putida* MO2, therefore, now called *P. monteilli* MO2
- Phylogenetic analyses with several other genetic targets confirmed this result: *rpo*A, *rpo*D*, gyr*A, *gyr*B, *dna*J, *pha*C1, and concatinated genes of *cpn60, dnaJ*, *gyrA rpoA,* and *rpoD*

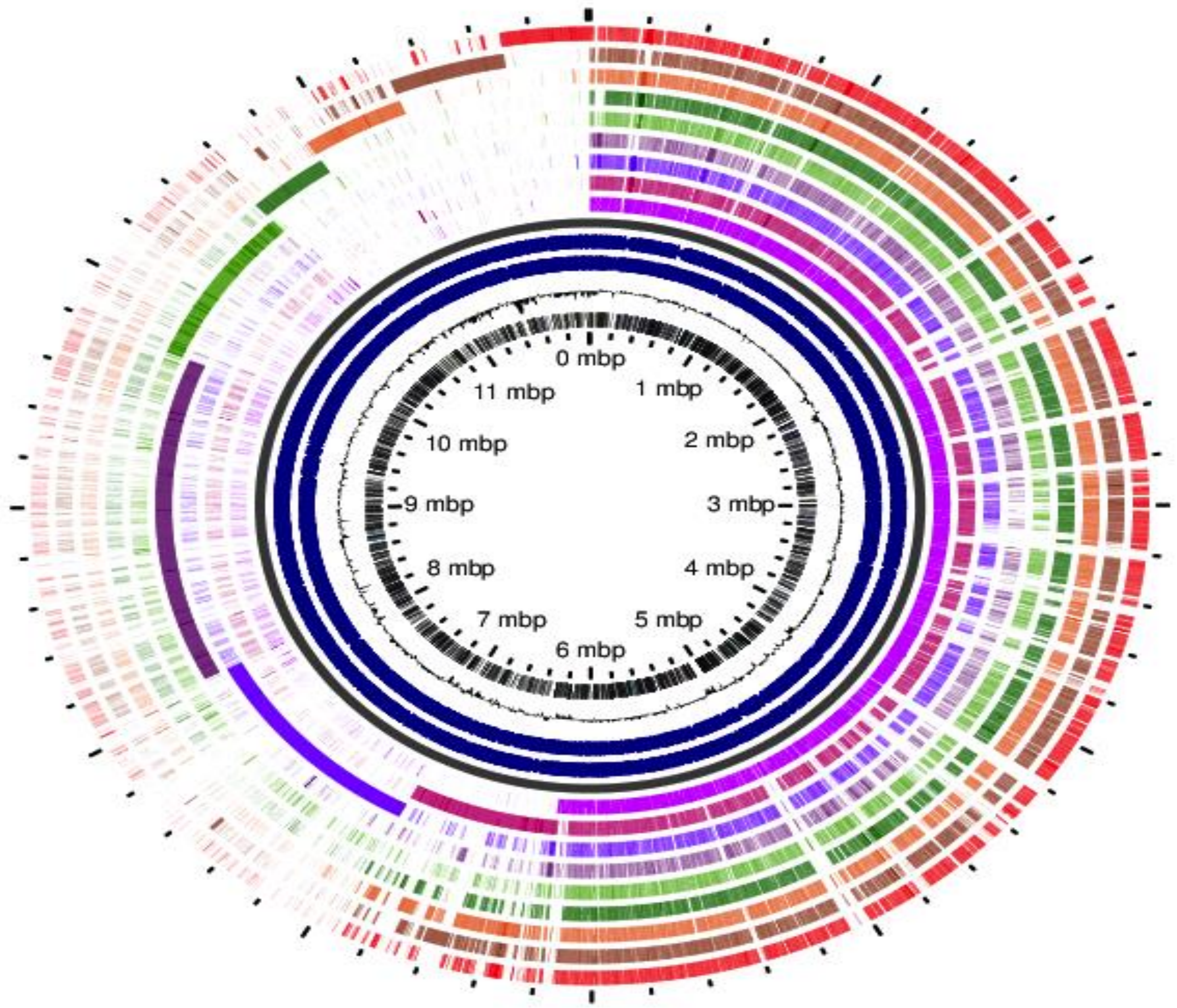


Genome Sequence Analysis of *P. putida* LS46

Genomes of Strains LS46 and MO2 where sequenced

Strain	*Pseudomonas putida* LS46	*Pseudomonas* sp. MO2
Genome Size (bp)	5,862,556	6,240,608
% GC Content	61.69 %	61.96%
# Genes	5,316	5,970
# CDS	5,219	5,895
# 5S RNA genes	8	2
# 16S RNA genes	7	1
# 23S RNA genes	8	1
# tRNA genes	74	69

Pangenomic Analysis of 10 *P. putida* strains



Pangenome analysis of 9 *P. putida* strains with *P. putida* KT2440 as a reference.

Characterization of *P. putida* isolates

P. putida LS46

- **Mcl-PHA production by *P. putida* LS46 grown on different substrates**
- **Cell mass, mcl-PHA content, and monomer composition from *P. putida* LS46 grown on different low cost carbon sources in bench-scale batch culture experiments.**
- **Polymers of different monomer composition synthesized when the bacteria are grown on different carbon sources**

Substrates	*Mcl-PHA (wt%)*	*Biomass (g/L)*	*Monomer composition (% mol fraction)*				
			C6	C8	C10	C12	C14
Glucose	20.5	3.3	1.1	14.7	68.9	6.3	N.d.
Glycerol	17.4	3.6	5.1	28.6	60.3	5.3	0.9
Biodiesel-derived Glycerol	15.3	3.3	9.2	28.2	57.1	4.9	0.5
Decanoic acid	33.7	1.5	4.5	47.5	46.6	1.3	N.d.
Biodiesel-derived Free Fatty Acids	28.8	4.6	4.9	58.5	28.5	6.2	0.7
Waste Fryer oil	17.7	2.8	5.2	60.5	23.8	6.9	2.7
Octanoic acid	50.2	2.3	6.5	88.1	3.8	1.6	N.d.

Potential Applications: Tailor made PHAs

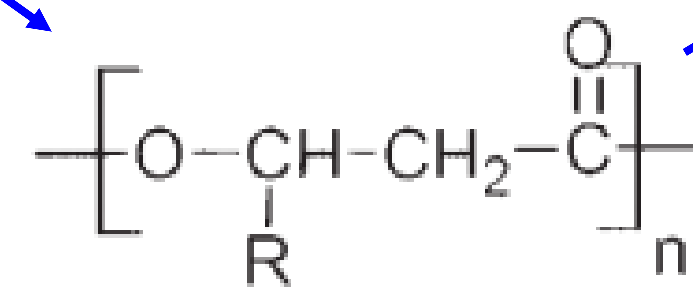
Monomer composition and molecular weight of PHA determine the thermal and mechanical properties of the polymers

Many factors influence polymer composition:

Bacteria:
C. necator: Class I PHA Synthase
P. putida: Class II PHA synthase

Carbon sources:
Functional side chains

Novel bacteria:
New isolates or genetic modifications



Poly(hydroxyalkanoates), PHA

scl-PHAs (R = 1)
mcl-PHAs (R = 1 to 11)

Unsaturation; Aromatic
Halogens; Carboxy
Hydroxy; Phenoxy; Epoxy;
Methyester, Etc…

Novel PHAs: scl-mcl-PHA (Co-polymers)

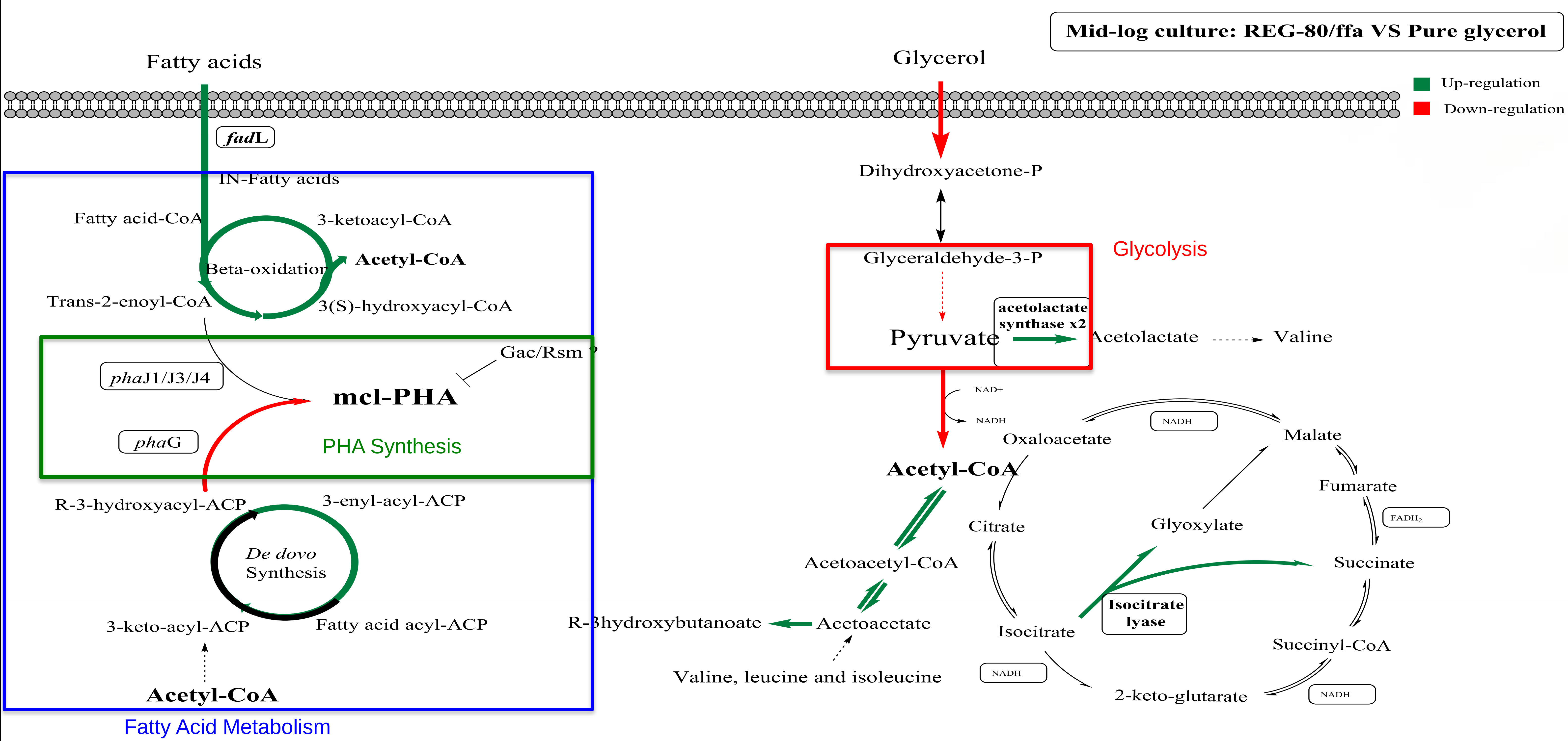
Potential Applications: Tailor made PHAs

Presence of unsaturated subunits suggest potential for crossing-liking

Cross-linking will increase tensile strength and melting temperature

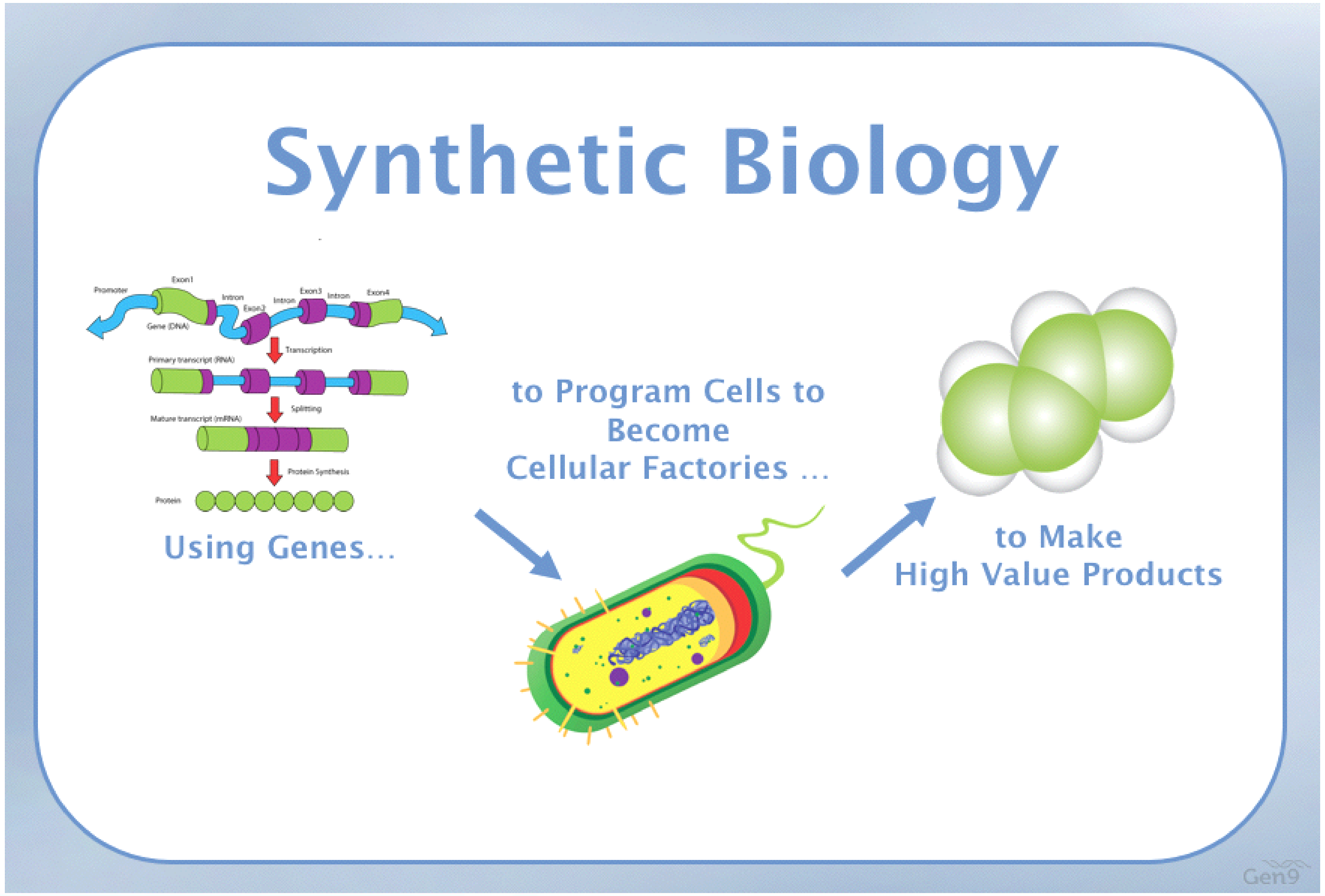
Substrate	Monomer composition						
	% C6	% C8	% C10	% C12	% C14	% C12-1	% C14-1
Waste Fryer Oil	6.1 ± 0.1	54.9 ± 0.7	27.6 ± 0.8	4.5 ± 0.4	3.1 ± 0.9	1.7 ± 0.3	2.1 ± 0.7
Canola Oil	6.7 ± 1.2	51.8 ± 2.8	29.7 ± 0.9	5.6 ± 0.7	2.5 ± 0.3	1.6 ± 0.1	1.9 ± 0.6
Corn Oil	9.5 ± 3.9	52.7 ± 1.7	26.4 ± 2.1	3.4 ± 0.4	0.9 ± 0.3	4.3 ± 1.0	3.0 ± 1.3
Bacon Fat	8.3 ± 2.2	53.1 ± 5.2	28.0 ± 3.8	5.4 ± 1.7	1.9 ± 1.3	1.6 ± 0.7	1.6 ± 0.5
Soybean Oil	10.0 ± 0.8	52.1 ± 1.6	25.8 ± 1.0	3.7 ± 0.7	0.9 ± 0.2	4.5 ± 0.5	3.0 ± 0.9
Biodiesel-derived Waste Glycerol	4.0 ± 0.4	32.8 ± 2.0	57.4 ± 1.3	2.3 ± 0.9	0.2 ± 0.1	3.3 ± 0.7	n.d.

Changes in Gene Product Expression Levels in *P. putida* LS46 grown on FFAs vs Waste Glycerol



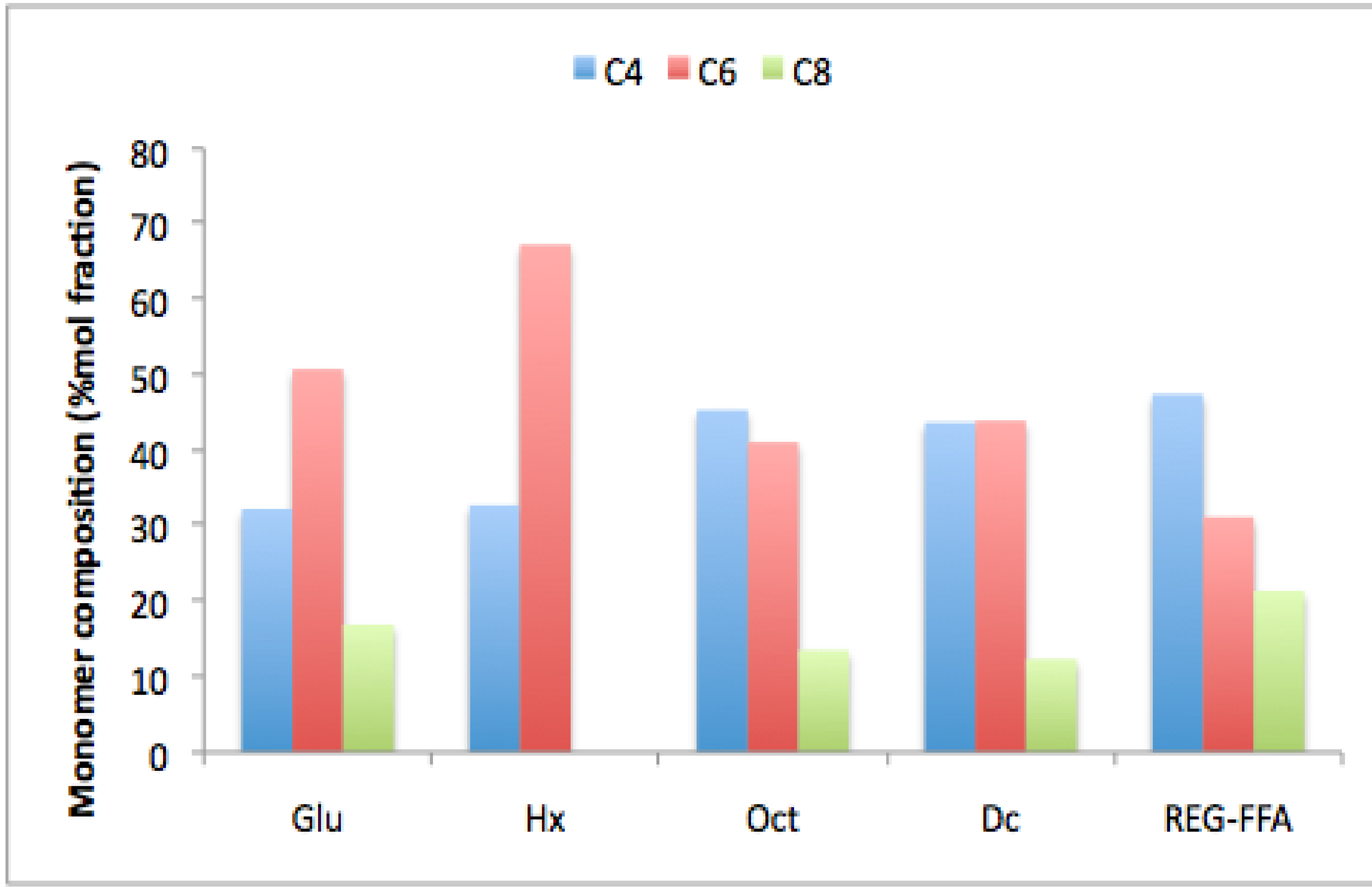
Potential Applications: Tailor made PHAs

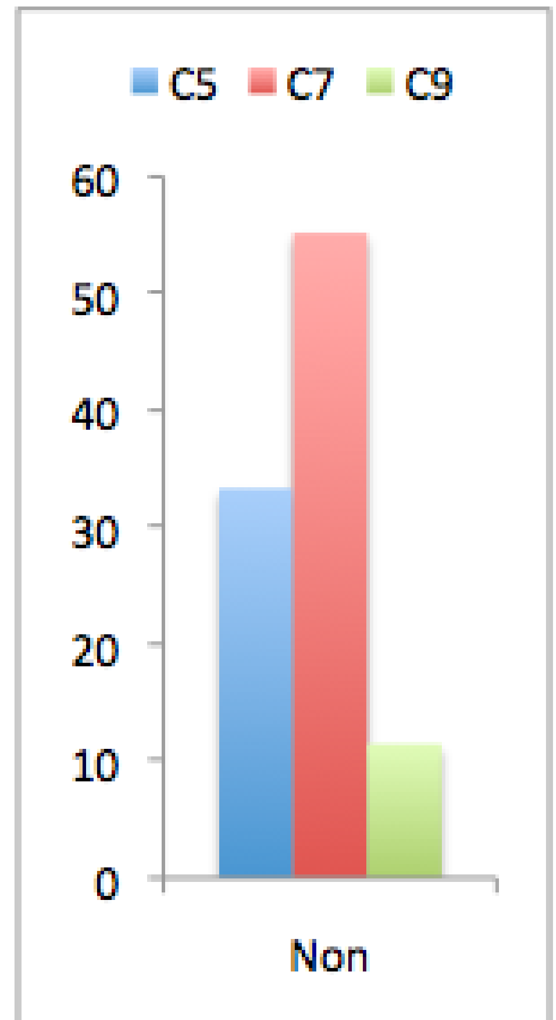
Genetic modifications can enhance yields and modify monomer composition of PHA polymers



Potential Applications: Tailor made PHAs

Cloning and expression of novel *pha*C genes in *P. putida* LS46 →PHA polymers with novel monomer compositions





Glu, glucose; Hx, hexanoic acid; Oct, Octanoic acid; Non, Nonanoic acid; Dc, Decanoic acid; REG-FFA, biodiesel-derived free fatty acids.

Thank-you

Questions?