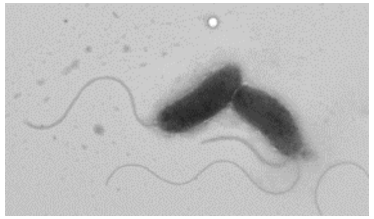
MARPLAST - Marine microorganisms for bioplastics production



Unexploited biomass (waste-/by-products) → Bioplastics (polyhydroxyalkonates)

The project aims to:

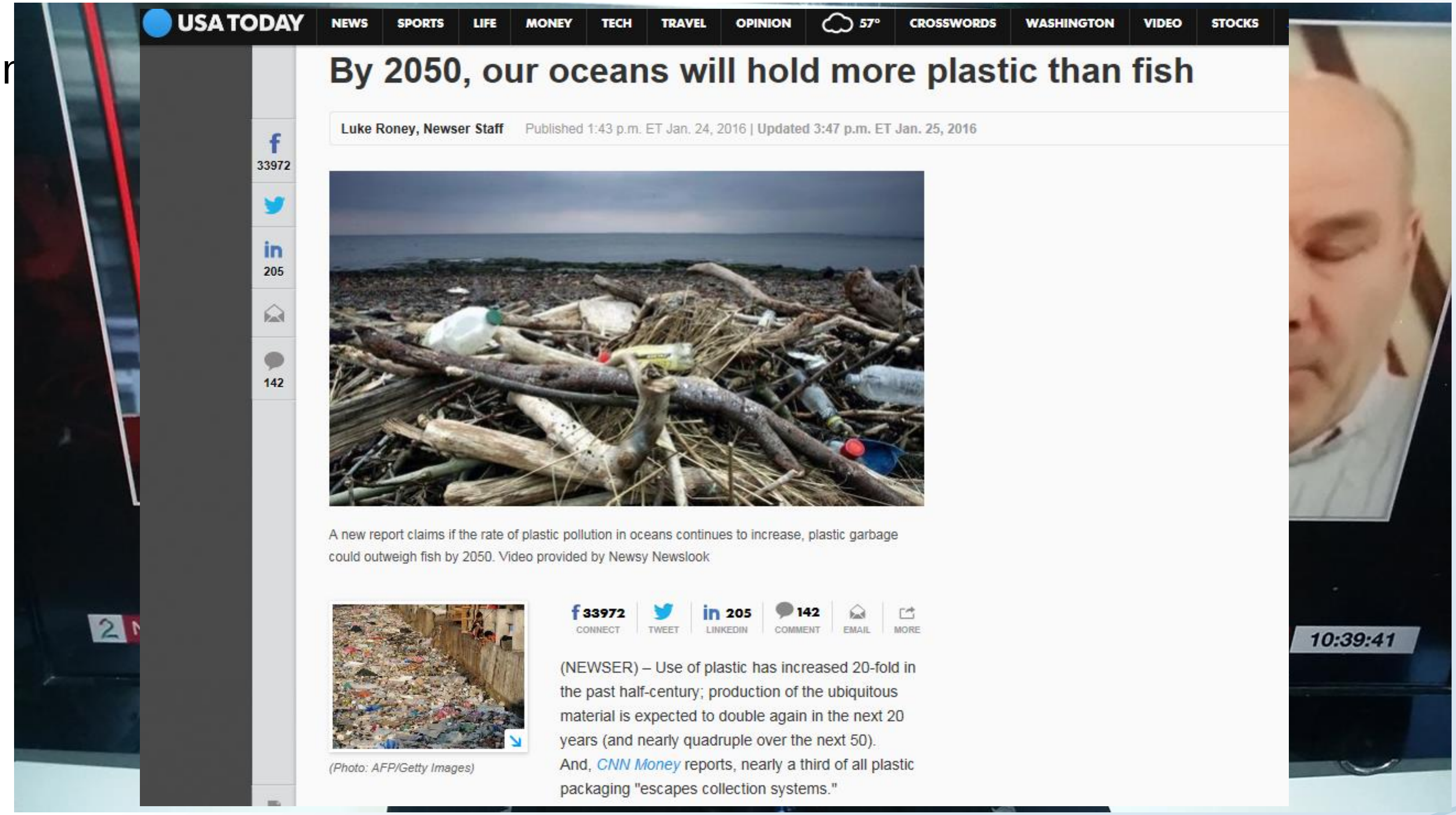
Develop and provide tools (bacteria, enzymes, and pathways) to enable efficient production of sustainable and biodegradable bioplastics from low-cost unexploited biomass.

Focus will be on PHA-producing cold-adapted marine bacteria, which have a range of properties that make them especially suitable for industrial applications.

Make important progress and contributions to the transition to a bio-based European economy.

Challenges related to our plastic consumption

- Accumulation of plastic wastes in landfills and in natural habitats
- Increasi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By 2050, our oceans will hold more plastic than fish

Luke Roney, Newser Staff Published 1:43 p.m. ET Jan. 24, 2016 | Updated 3:47 p.m. ET Jan. 25, 2016

A new report claims if the rate of plastic pollution in oceans continues to increase, plastic garbage could outweigh fish by 2050. Video provided by Newsy Newslook

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(Photo: AFP/Getty Images)

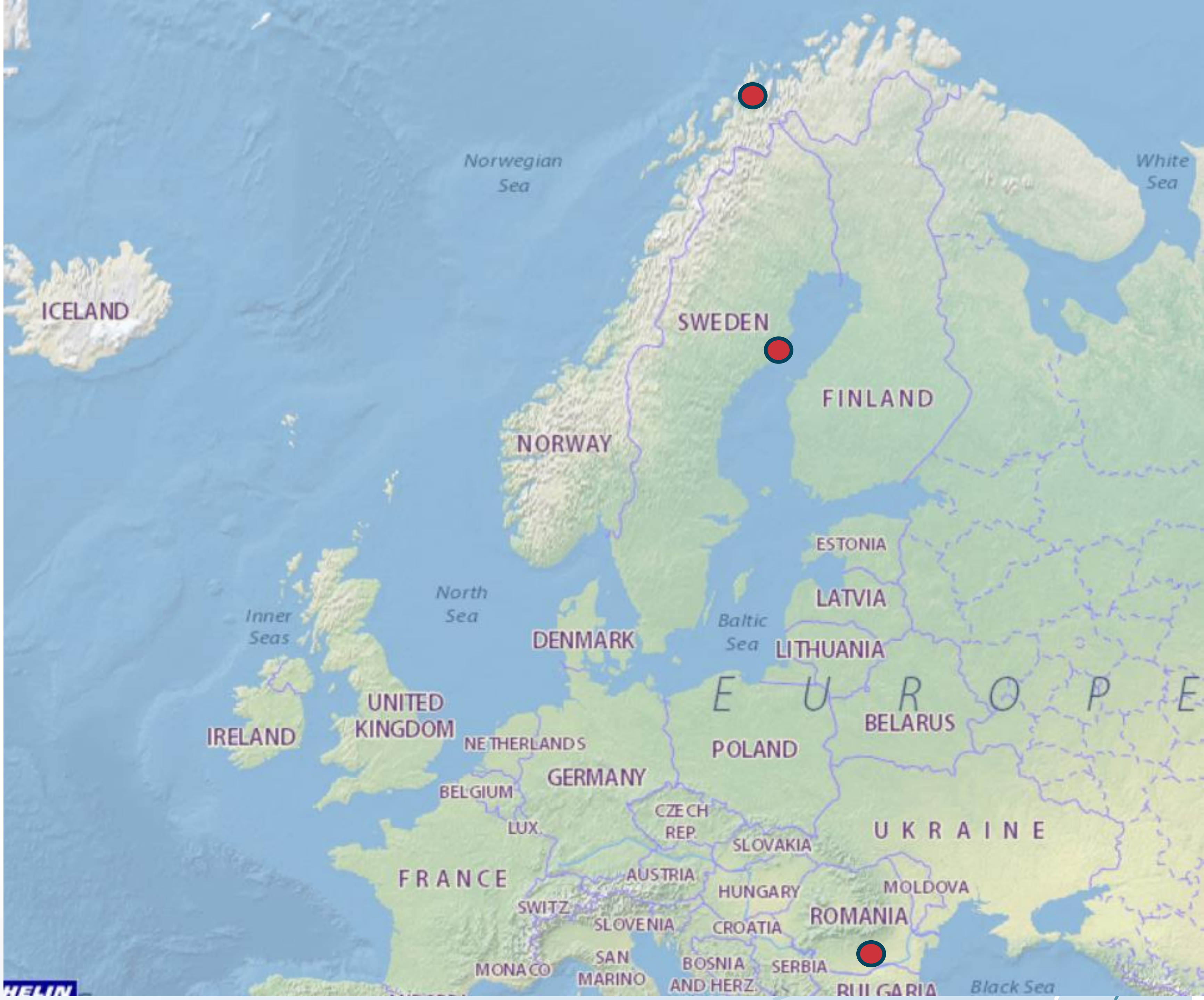
(NEWSER) – Use of plastic has increased 20-fold in the past half-century; production of the ubiquitous material is expected to double again in the next 20 years (and nearly quadruple over the next 50). And, *CNN Money* reports, nearly a third of all plastic packaging "escapes collection systems."

Consortium of 3 universities from 3 European countries:

UiT – the Arctic University of Norway
Norway

Umeå University
Sweden

University of Bucharest
Romania



MarineBiotech

MarPlast is organized in 5 Work Packages

WP1 Identification and analysis of PHA producing microbes

WP2 Extraction and chemical analysis of PHAs

WP3 Optimization of growth conditions

WP4 Enzyme characterization

WP 5 Management and dissemination

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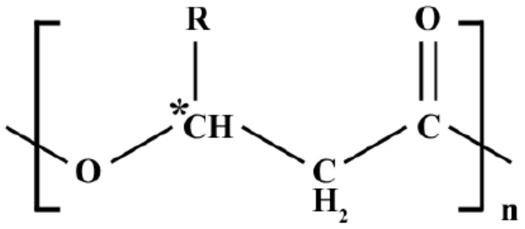
Microbial bioplastics

- Polyhydroxyalkanoates (PHA)
  - 1927; granules containing poly(3-hydroxybutyrate) were discovered in *B. megaterium*
  - Late 1970ies; investigation of the commercial potential of PHA
  - Today; over 300 bacteria are known to produce PHA

- Are made when bacteria grows in excess carbon compared to other nutrients
- Intracellular carbon and energy storage
- Only family of “bioplastics“ entirely produced and degraded by living cells

- Physical properties similar to polyethylene, polystyrene and synthetic polyesters

PHA chemical structure



Poly(3-hydroxyalkanoate)

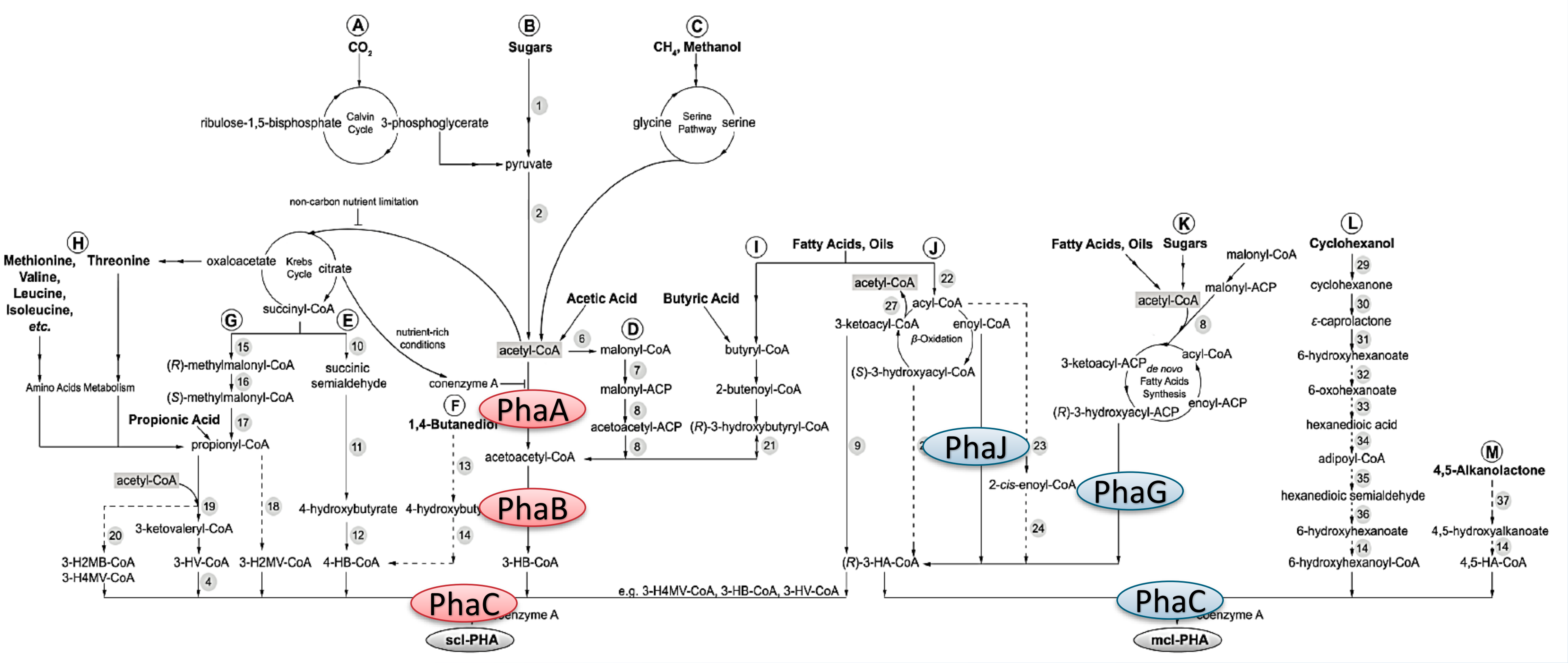
R group	Carbon no.	PHA polymer	
methyl	C_4	Poly(3-hydroxybutyrate)	Short-chain length PHA (scl-PHA)
ethyl	C_5	Poly(3-hydroxyvalerate)	
propyl	C_6	Poly(3-hydroxyhexanoate)	Medium-chain length PHA (mcl-PHA)
butyl	C_7	Poly(3-hydroxyheptanoate)	
pentyl	C_8	Poly(3-hydroxyoctanoate)	
hexyl	C_9	Poly(3-hydroxynonanoate)	
heptyl	C_{10}	Poly(3-hydroxydecanoate)	
octyl	C_{11}	Poly(3-hydroxyundecanoate)	
nonyl	C_{12}	Poly(3-hydroxydodecanoate)	
decyl	C_{13}	Poly(3-hydroxytridecanoate)	
undecyl	C_{14}	Poly(3-hydroxytetradecanoate)	
dodecyl	C_{15}	Poly(3-hydroxypentadecanoate)	Long-chain length PHA (lcl-PHA)
tridecyl	C_{16}	Poly(3-hydroxyhexadecanoate)	

R = unsaturated, branched and substituted alkyl groups

PHA polymer typically consists of 600 – 35 000 monomer units

Approx. different 150 PHA monomers identified; diverse material properties

P(3-HB) is synthesized by only three key enzymes; PhaA, PhaB and PhaC

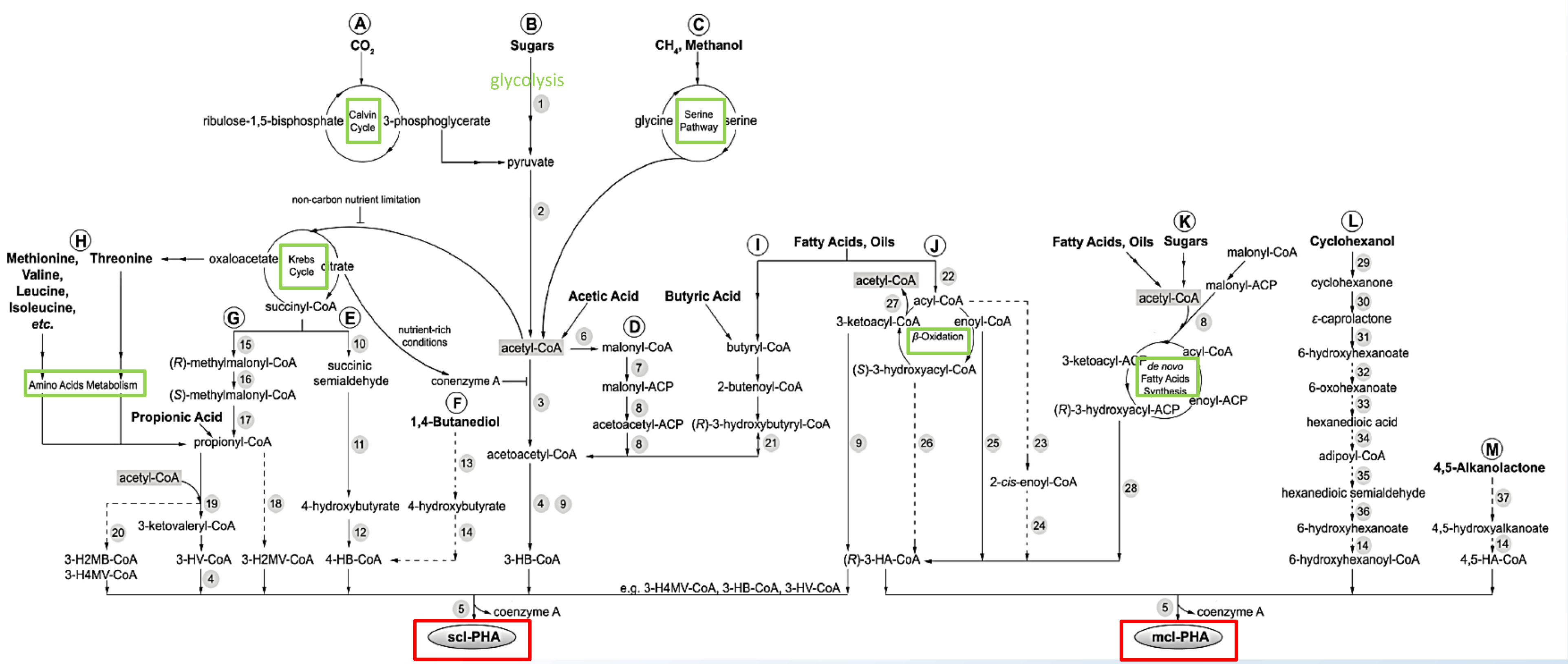


3-ketothiolase

NADPH-dependent acetoacetyl-CoA reductase

PHA synthase (polymerase)

PHA biosynthetic pathways are intricately linked with the bacterium’s central metabolism



Tan et al.,2014

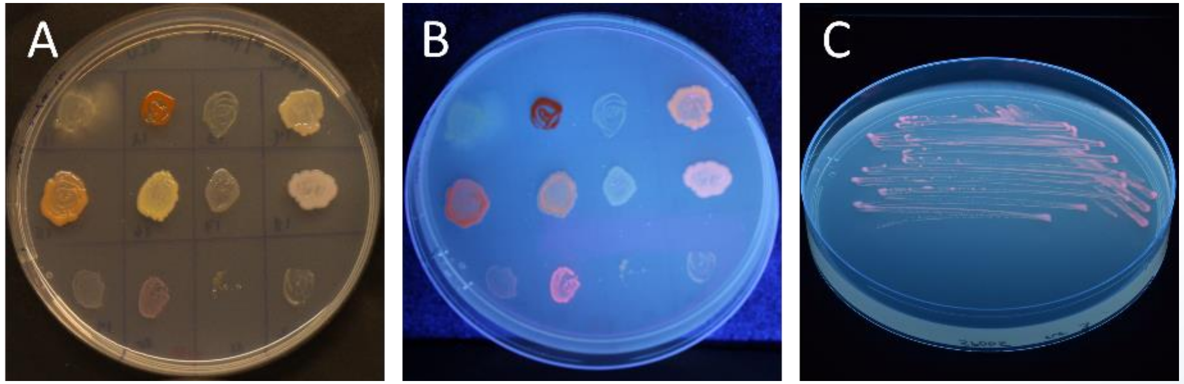
Genetic background of the bacteria and carbon source determine which PHA may be produced

Status

- More than 300 isolates from the in-house collections at UiT and UB, are so far screened
- ~10 isolates are selected as apparently “highly potent” PHA-producers
- Development of extraction and analysis protocols of the PHAs, are in progress
- 3 PHA synthesizing enzymes are recombinantly produced and subjected to characterization
- Selected marine biomass by-products are collected and preliminary analyzed

Screening

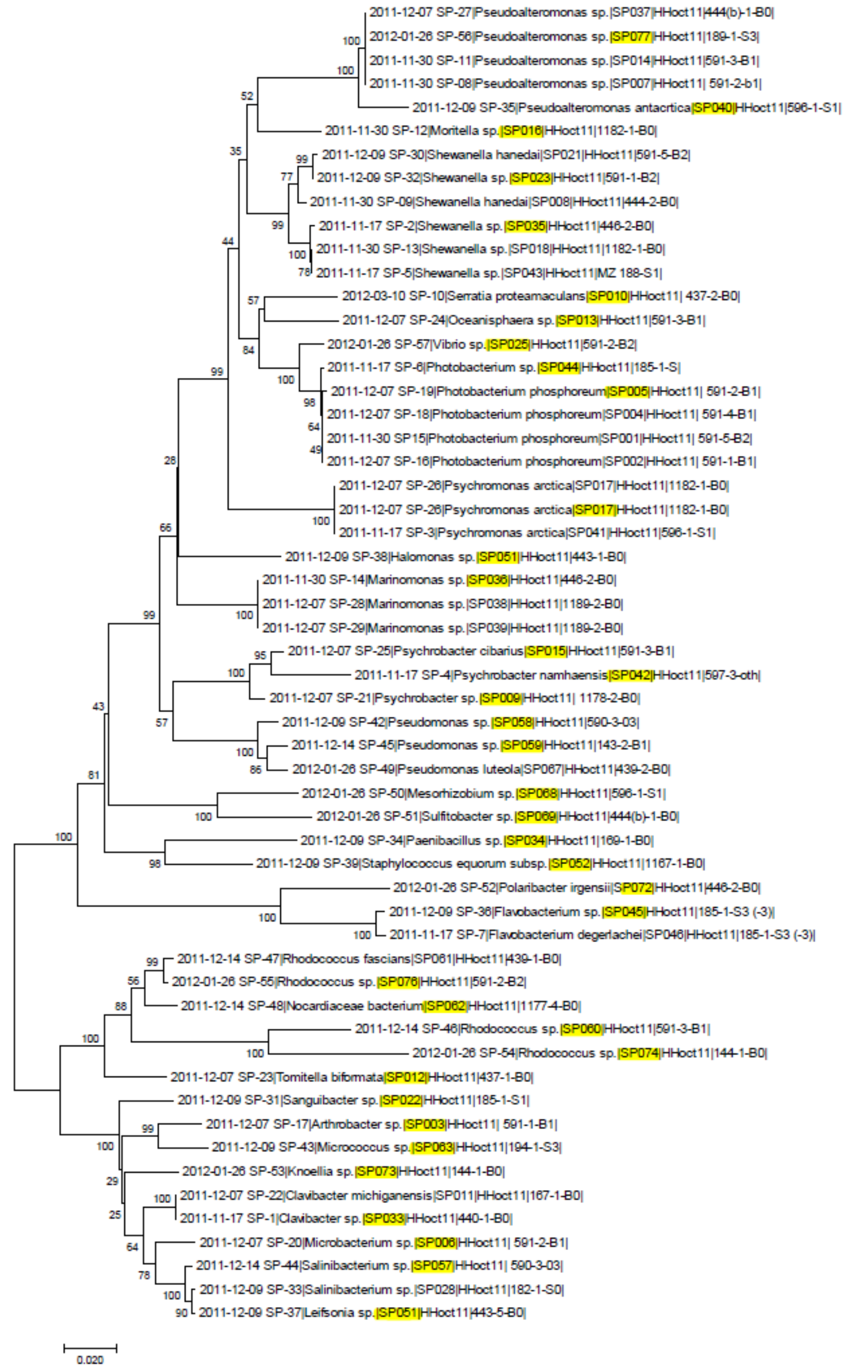
Strains are selected for screening based on 16s RNA phylogeny to represent the diversity of the different bacteria collections



A) Visual light

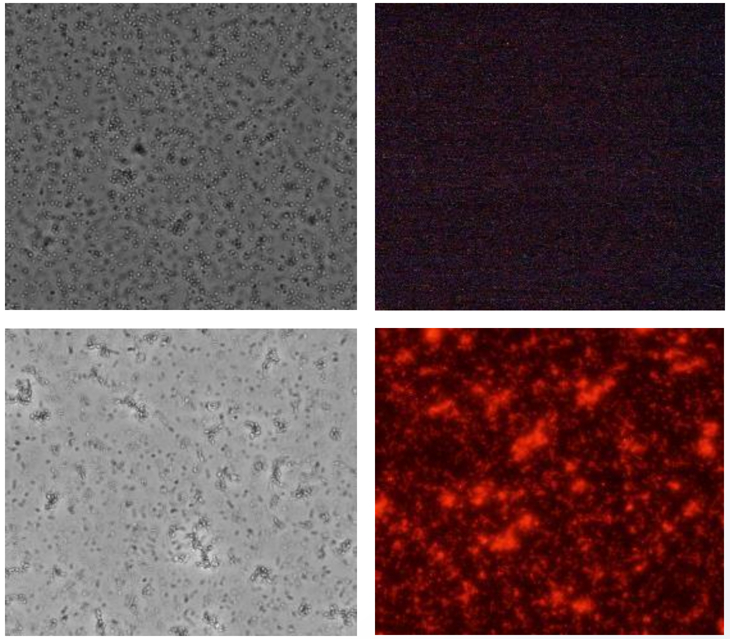
B and C) UV light

Potential PHA producers should be pink under UV light

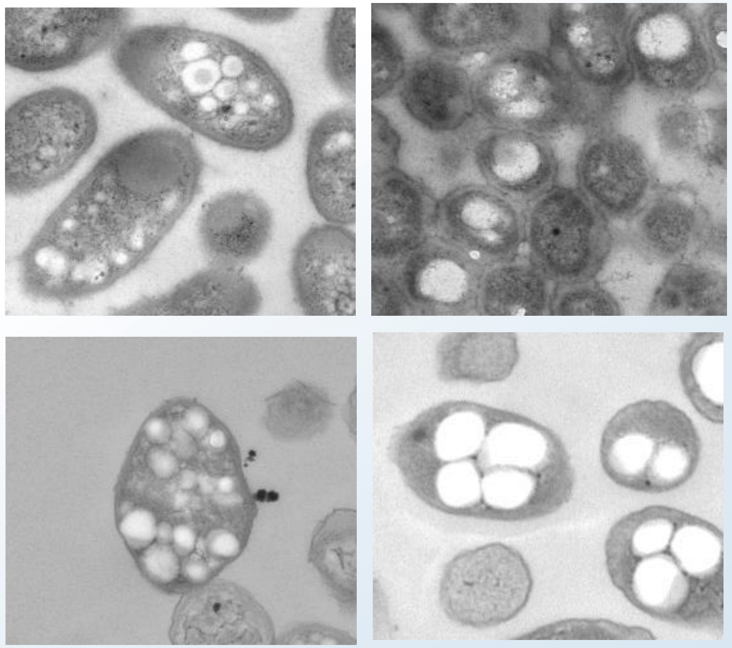


Microscopy

Visible light microscopy Fluorescens microskopy

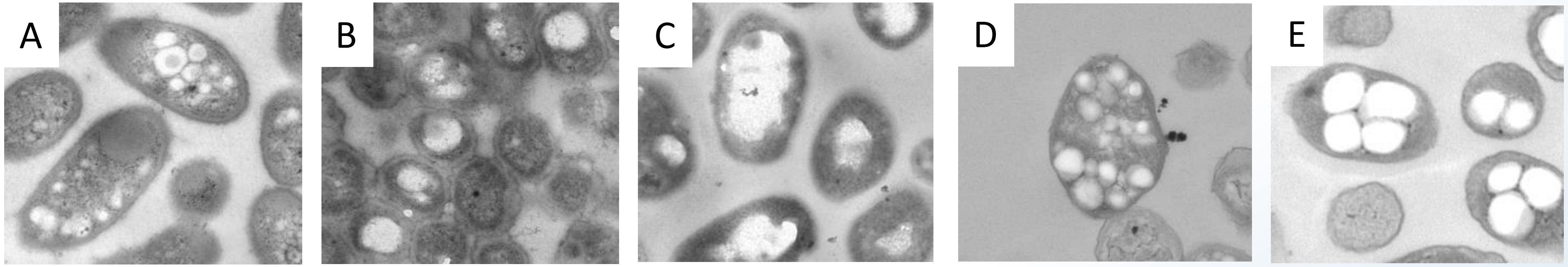


Electron mikroskop



PHA granules identified in marine bacteria

Electron microscopy



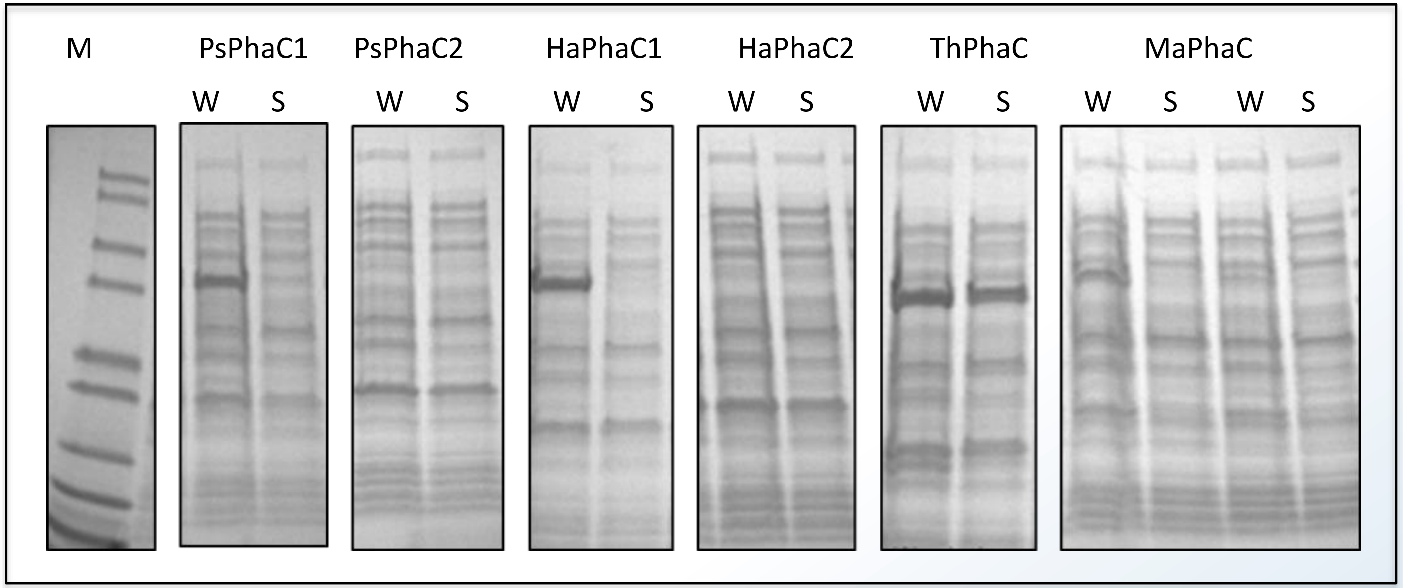
A) *Pseudomonas sp.*, B) *Halomonas sp.*, (C) *Photobacteria sp*. and D) *Roseobacter sp.* grown on a agar with a low C/N ratio. E) *Roseobacter* grown on a minimal media with a **high** C/N ratio.

Gas chromatography/Mass spectrometry

After extraction, all PHA production will be verified by GC/MS (qualitatively and quantified)

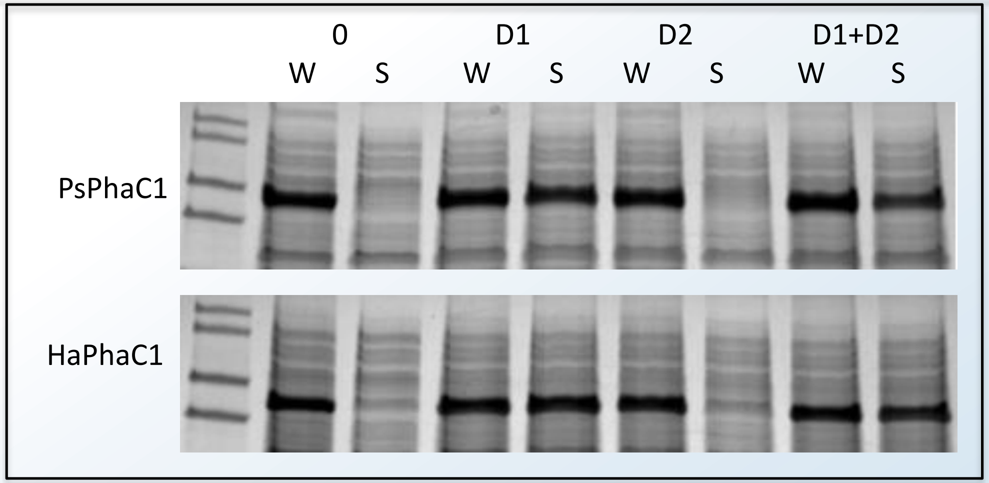
PHA-synthases (PhaCs)

Expression and solubility experiments



E. coli
BL21 (DE3)

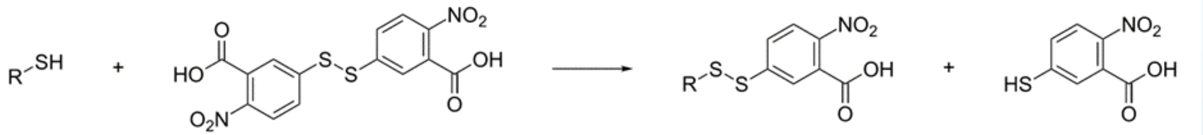
D: Methyl 6-O-(N-heptylcarbamoyl)-α-D-glucopyranoside (hecameg)

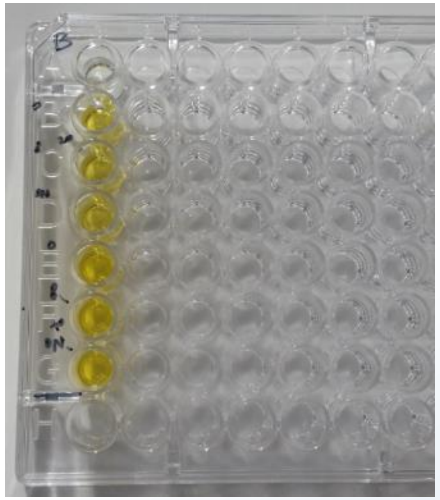


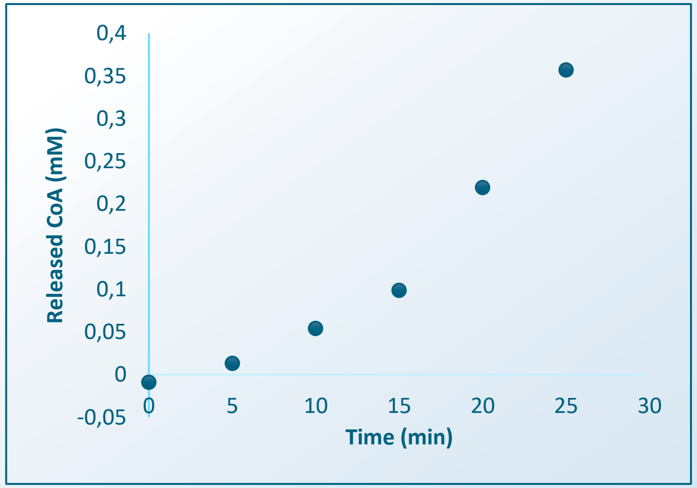
D1: 1 % sarcosyl
D2: 1% Triton-X 100

Enzyme assay

- The reaction of thiols with the chromogenic DTNB (5,5'-dithiobis-2-nitrobenzoate) -yellow dianion of 5-thio-2-nitrobenzoic acid (TNB)







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