Uncovering bioplastic production potential in marine host-associated bacteria using comparative genomics

ana.s.fernandes@tecnico.ulisboa.pt

Ana M. Fernandes[1,2], M. Teresa Cesário[1,2], Rodrigo Costa[1,2]

[1] iBB-Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa – Portugal

[2] Associate Laboratory i4HB—Institute for Health and Bioeconomy, Instituto Superior Técnico, Universidade de Lisboa – Portugal

Motivation & Background

Polyhydroxyalkanoates (**PHA**) are biopolyesters that can be used by a wide range of industries, from agricultural, to biomedical or automotive.

PHAs are **carbon reservoirs** produced by bacteria under stress conditions, namely **nutrient limitation**, when **excess carbon** is present.

Seaweed residues from hydrocolloid extraction-industries contain polysaccharides that **after hydrolysis release simple sugars** that can be utilised by bacteria for the production of PHAs.

Marine host-associated bacteria are an **underexplored niche** as possible producers of biopolymers.

There is a possibility of finding more polyhydroxyalkanoates producers isolated from similar environments.

?

This study aims to perform genotypic and phenotypic screening for PHA biosynthesis on a panel of 86 marine bacteria with complete genome sequences available.

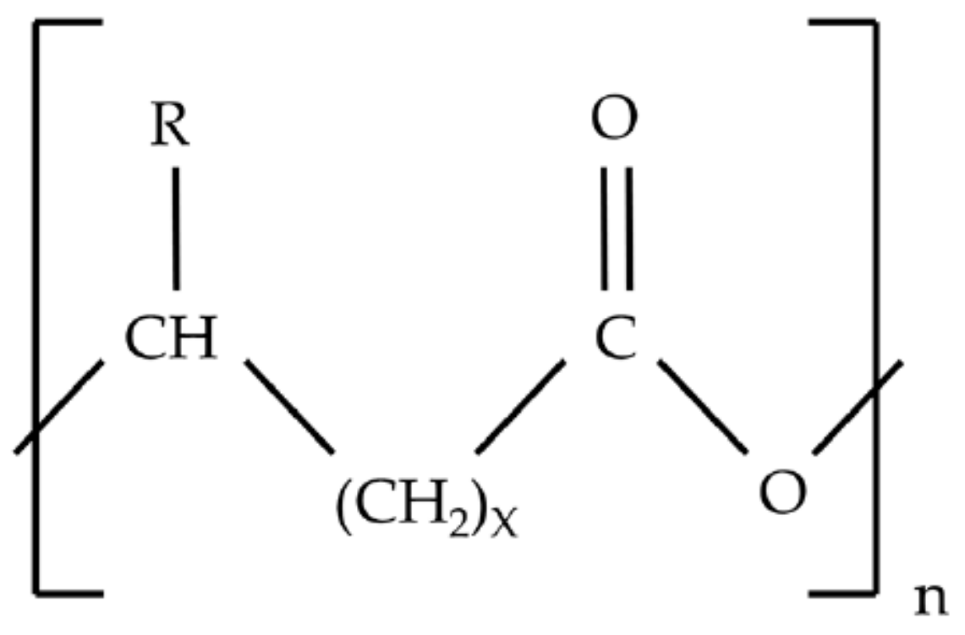


Fig. 1: General structure of polyhydroxyalkanoate monomers. R represents groups C1 to C13 and X represents methylene repetitions within the monomer (up to 8).

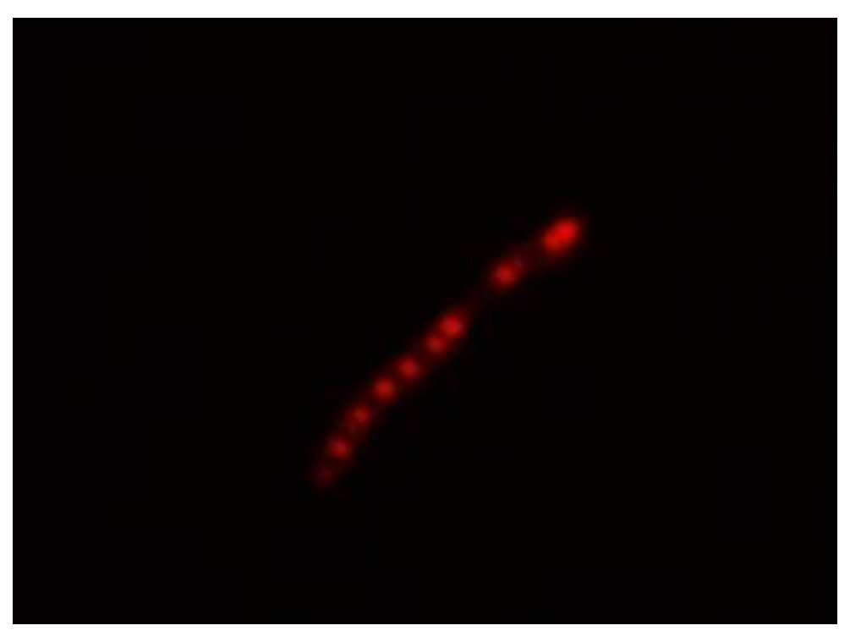


Fig. 2: PHA granules in *Halomonas boliviensis*

Results & Discussion

Phenotypic Screening

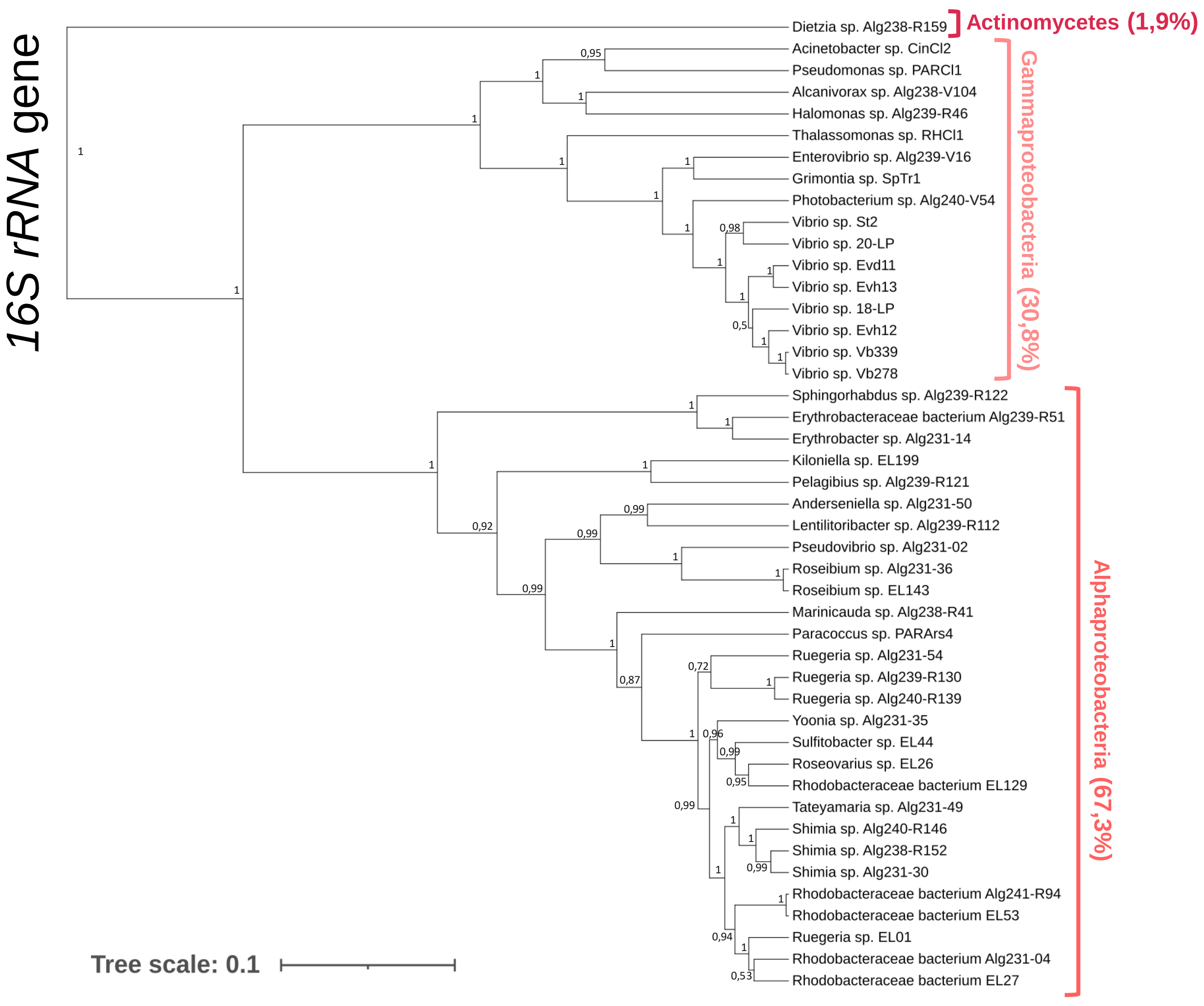


Fig. 3: Phylogenetic tree built based on 16S rRNA partial sequences, resorting to BEAST (Bayesian Inference). Nodes are labelled with posterior probabilities > 0,7.

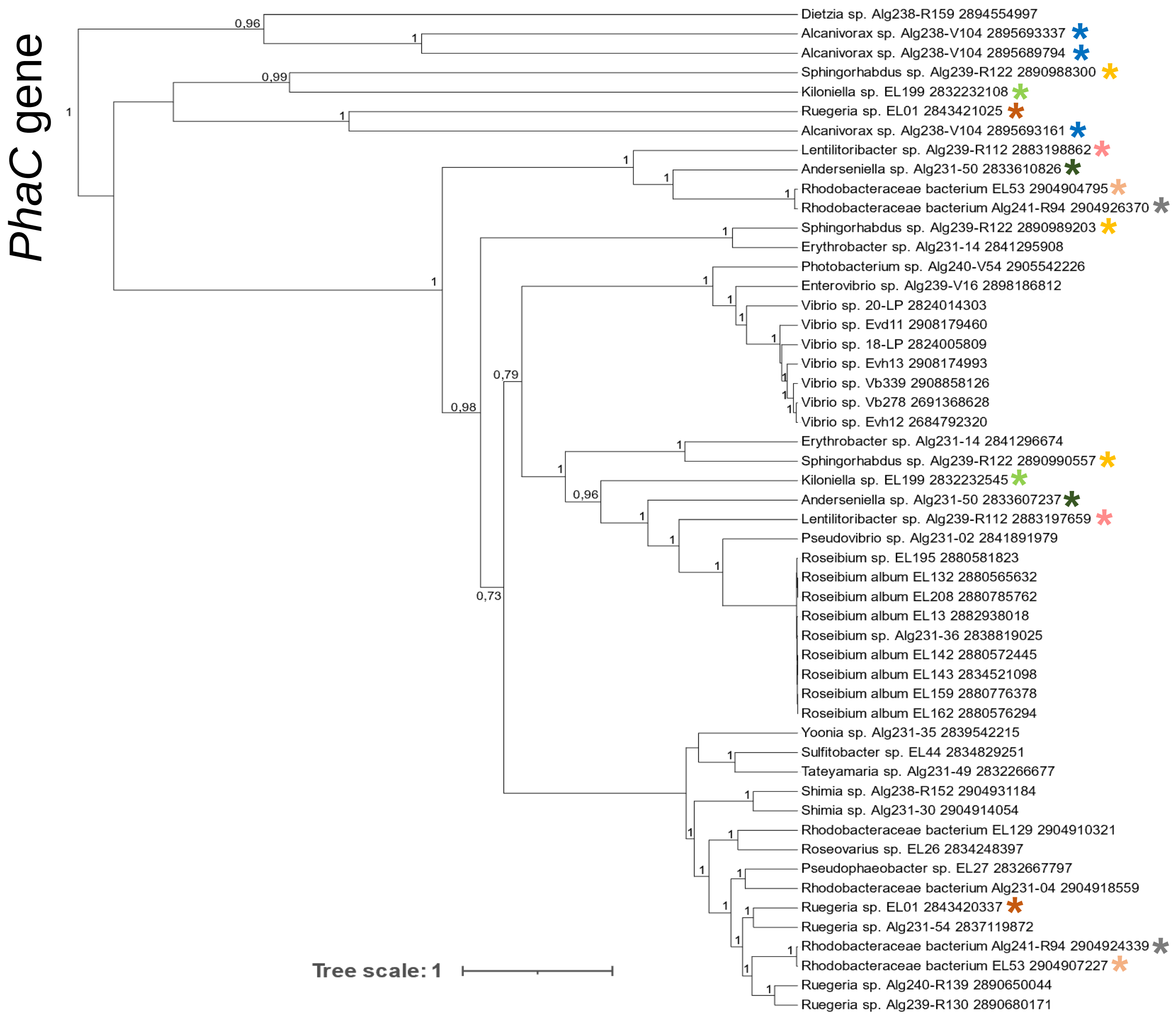


Fig. 4: Phylogenetic tree built based on PhaC-encoding genes (COG3243 and PF07167), resorting to BEAST (Bayesian Inference). Nodes are labelled with posterior probabilities > 0,7. Duplication of genes marked with *.

Different tree topology

Division of bacterial isolates according to taxonomic class is not evident.

Horizontal gene transfer?

Duplication of *PhaC* gene

From a panel of 86 bacterial isolates, **52 are potential PHA producers** (*PhaC* gene is present).

Genotypic Screening

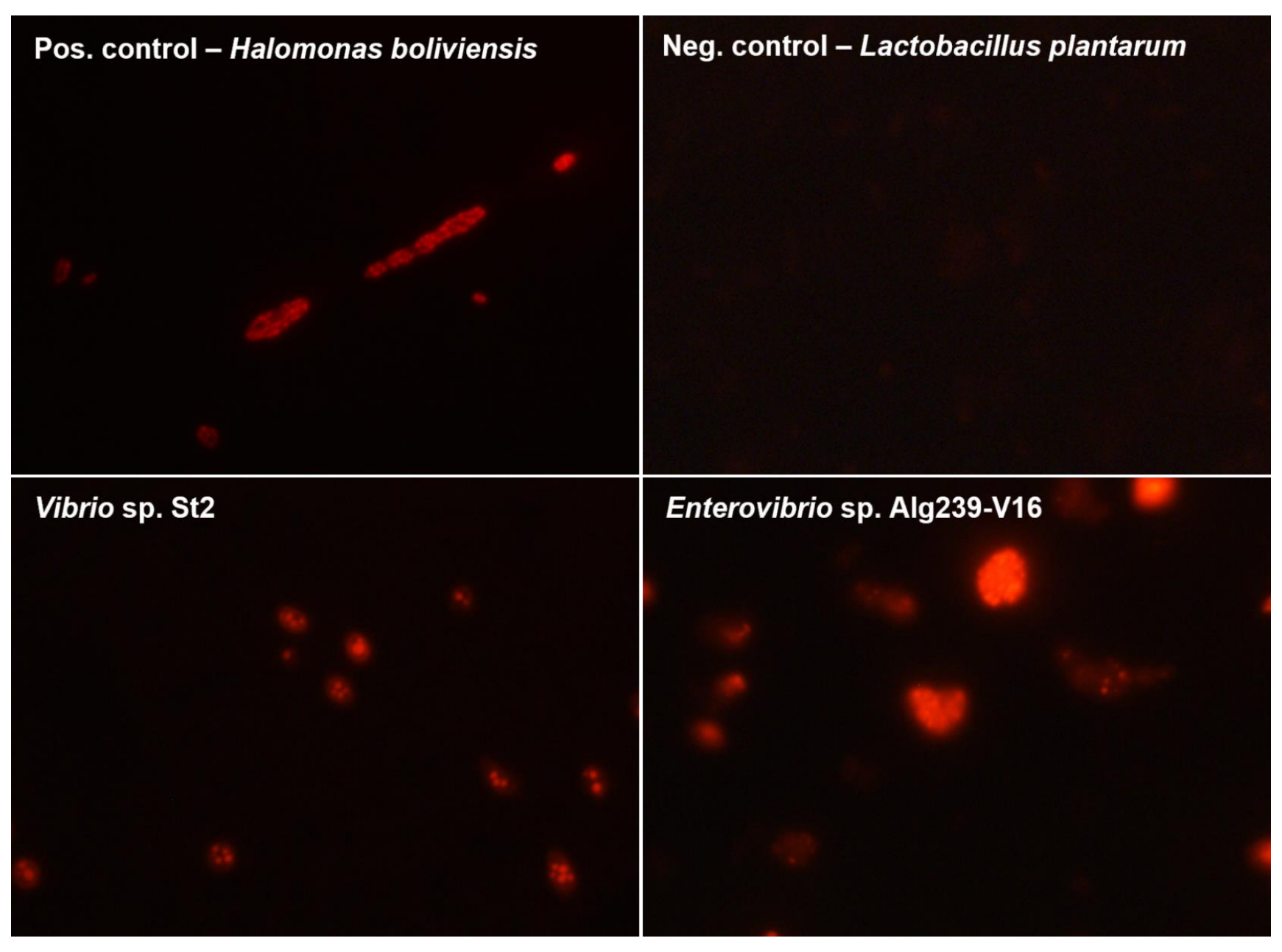


Fig. 5: Fluorescence microscopy imaging after Nile Red staining

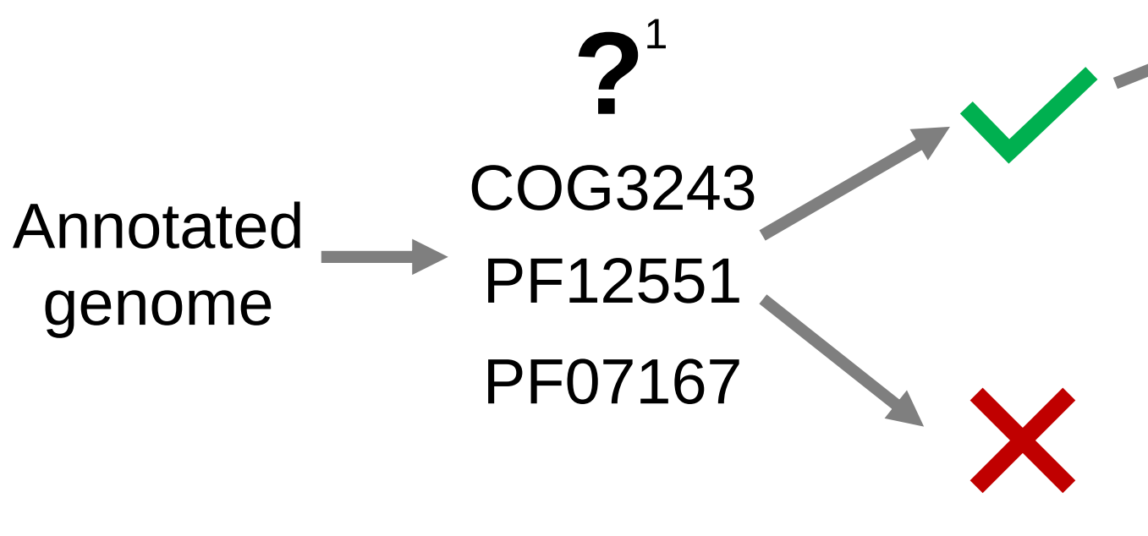
Only **19 bacterial isolates** were able to grow on nutrient-limited medium containing **20 g/L glucose**.

16 isolates show fluorescence

Confirm phenotype via quantification of PHA with gas chromatography

Experimental Design

Genotypic screening

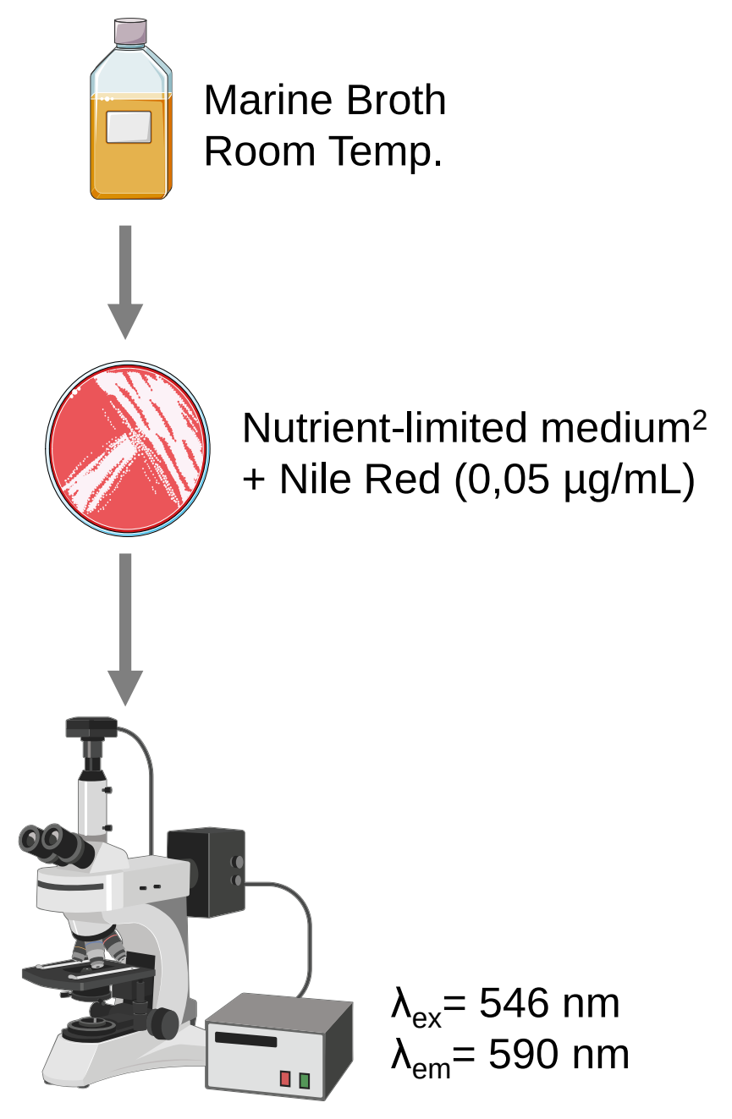


Annotated genome → ?[1] COG3243 PF12551 PF07167

[1] Search for COG and Pfam annotation identifiers that correspond to genes encoding for PhaC, the key-enzyme in polyhydroxyalkanoates biosynthesis, in the already annotated genomes.
COG3243: Poly(3-hydroxyalkanoate) synthase
PF07167: Poly-β-hydroxybutyrate polymerase, N-terminus
PF12551: Poly-β-hydroxybutyrate polymerase N-terminal

Phenotypic screening

Nile Red Staining (determination of phenotype) → Gas Chromatography (confirmation of phenotype)



Marine Broth
Room Temp.

Nutrient-limited medium[2]
+ Nile Red (0,05 µg/mL)

λ_{ex}= 546 nm
λ_{em}= 590 nm

[2] Nutrient-limited medium:

NaCl	25 g/L
Na_2PO_4	1,71 g/L
KH_2PO_4	1,09 g/L
$MgSO_4$	1,22 g/L
$(NH_4)_2SO_4$	0,5 g/L
Yeast Extract	0,1 g/L
Peptone	0,5 g/L
Glucose	20,0 g/L *OR* 4,0 g/L
Trace Elements Sol.	1 mL/L

Conclusions

- There is potential in marine-host associated bacteria to produce bioplastics.
- Tree topology suggests evolution might have occurred with gene transfer events.
- Two groups of bacteria with different glucose tolerance indicate that only part of the PHA producers may be of interest for upscaling.

Acknowledgements: This study was supported by the "BLUE BIOECONOMY PACT" (Project Nº. C644915664-00000026), co-funded by Next Generation EU European Fund, under the incentive line "Agendas for Business Innovation" within Component Funding Scheme 5 - Capitalization and Business Innovation of the Portuguese Recovery and Resilience Plan (RRP). Further support was provided by the Portuguese Foundation for Science and Technology (FCT) in the scope of the projects UIDB/04565/2020 and UIDP/04565/2020 of iBB and the project LA/P/0140/2020 of i4HB.

PROJETO Nº C644915664-00000026