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RECYCLES WORKSHOP

Metagenomics and metabarcoding approaches to describe ecological systems and infer their development

5th, 6th & 7th of July 2022

Study of the microbial communities associated with cultures of *Acartia tonsa* (Copepoda, Calanoida) and involved in the degradation of poly(butylene succinate-co-butylene adipate) (PBSA)

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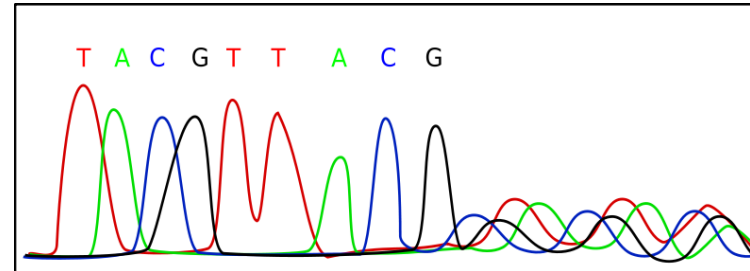
³ISPRA-Italian Institute for Environmental Protection and Research, Livorno, Italy



GA: 872053 — H2020 - MSCA - RISE-2019

Taxonomic and functional characterization of microbial communities associated with cultures of the marine calanoid copepod *Acartia tonsa*:

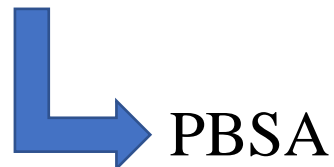
❖ *Culture-independent* approach



❖ *Culture-dependent* approach



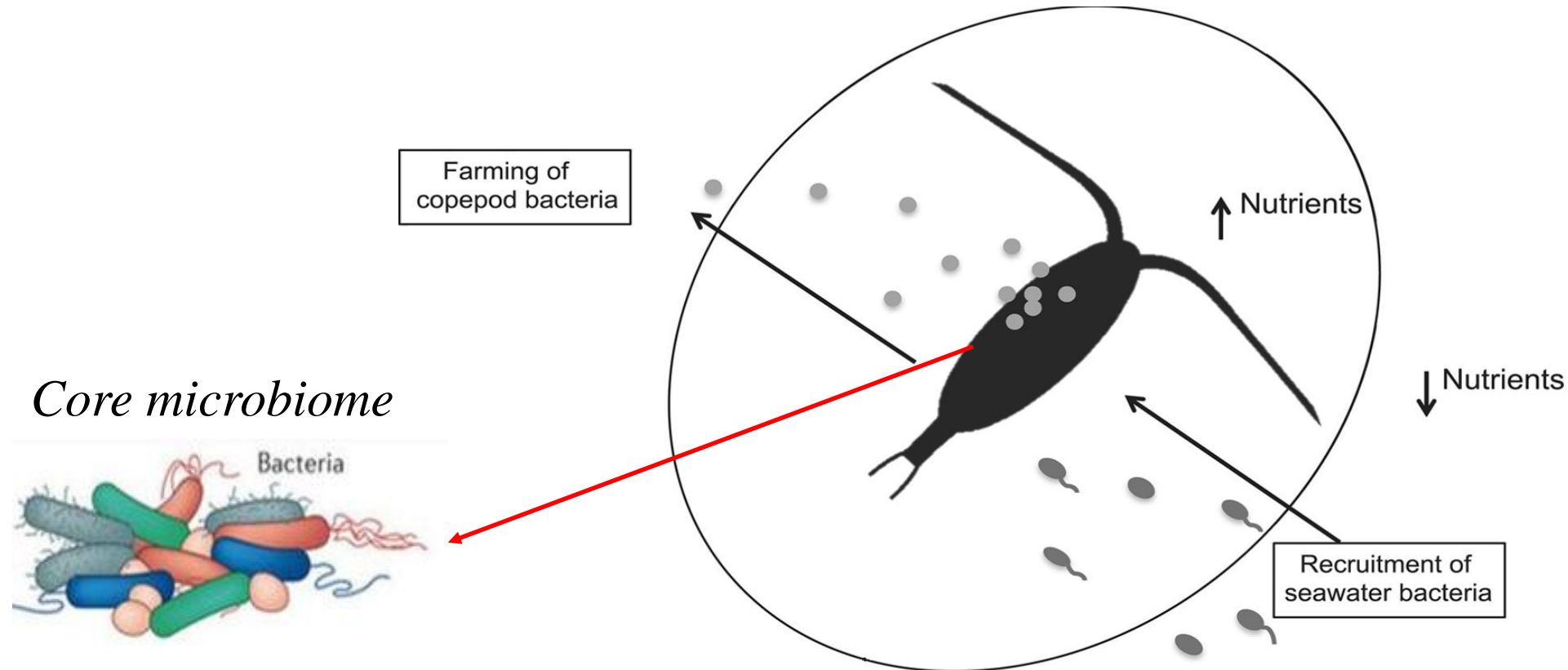
❖ *Culture-independent* and *culture-dependent* approaches



1. *Acartia tonsa*: microbial hotspot



Zoosphere

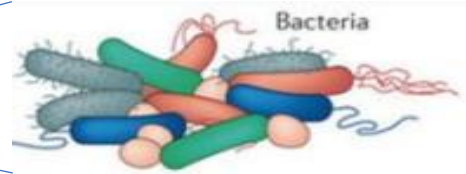


Shoemaker M. K. *et al.*, 2019

2.The functionality of microbial communities associated with *A.tonsa* carcasses

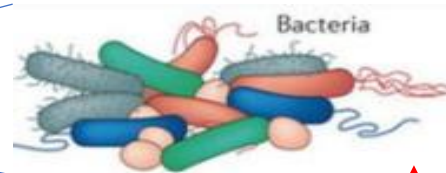


Newly formed carcasses



Degradation ↓

Remains of carcasses



Protease activities

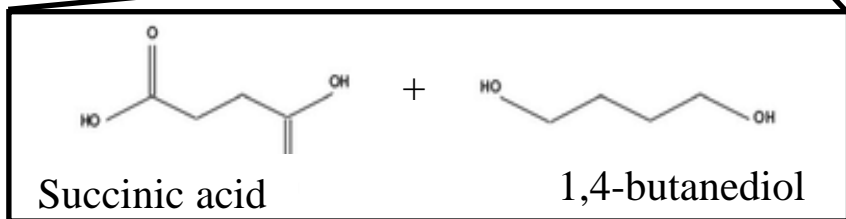
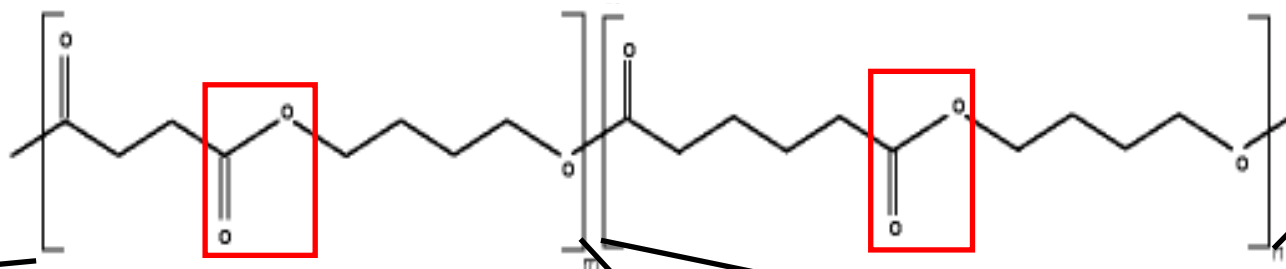


Lipase activities

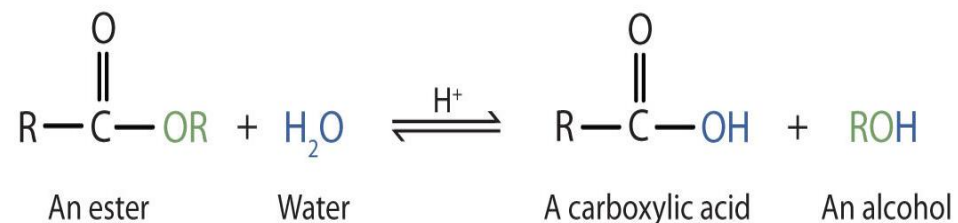


Chitinase activities

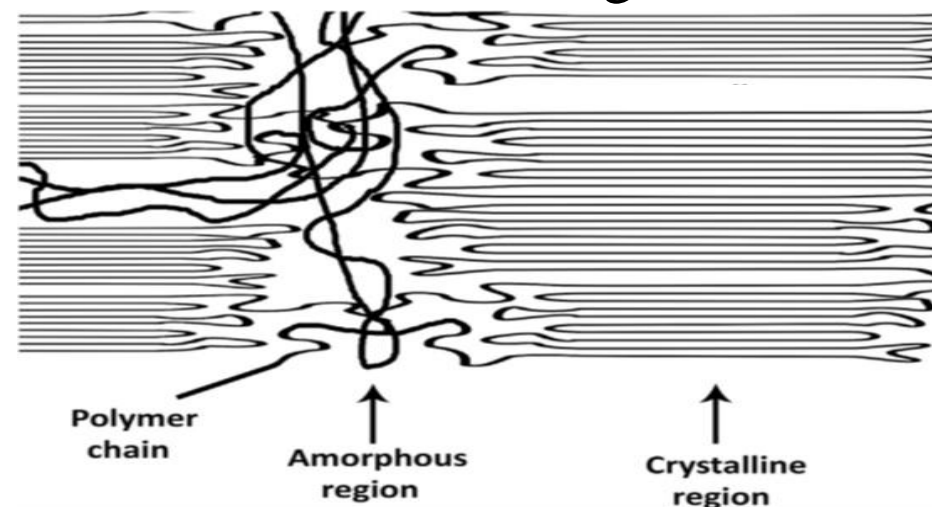
3.poly(butylene succinate-co-butylene adipate)(PBSA)



Carboxyl-ester hydrolase



Three-dimensional organization



Crawford C. B. *et al.*, 2017

4.Methods: *Acartia tonsa* culture



ISPRA
Laboratory
culture

R. reticulata
monoalgal diet



Culture-independent
approach

“alive” Adults



- **New formed adults (VG0R)**
- **Seven-day adults (VG7R)**

Adults,
artificially
induced death
(carcasses)



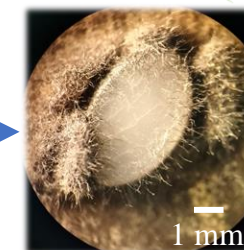
- ✓ **New formed carcasses (CG0R)**
- ✓ **Four-day carcasses (CG4R)**
- ✓ **Thirty-three-day carcasses (CG33R)**

Culture-dependent
approach

debris of
A.tonsa
carcasses

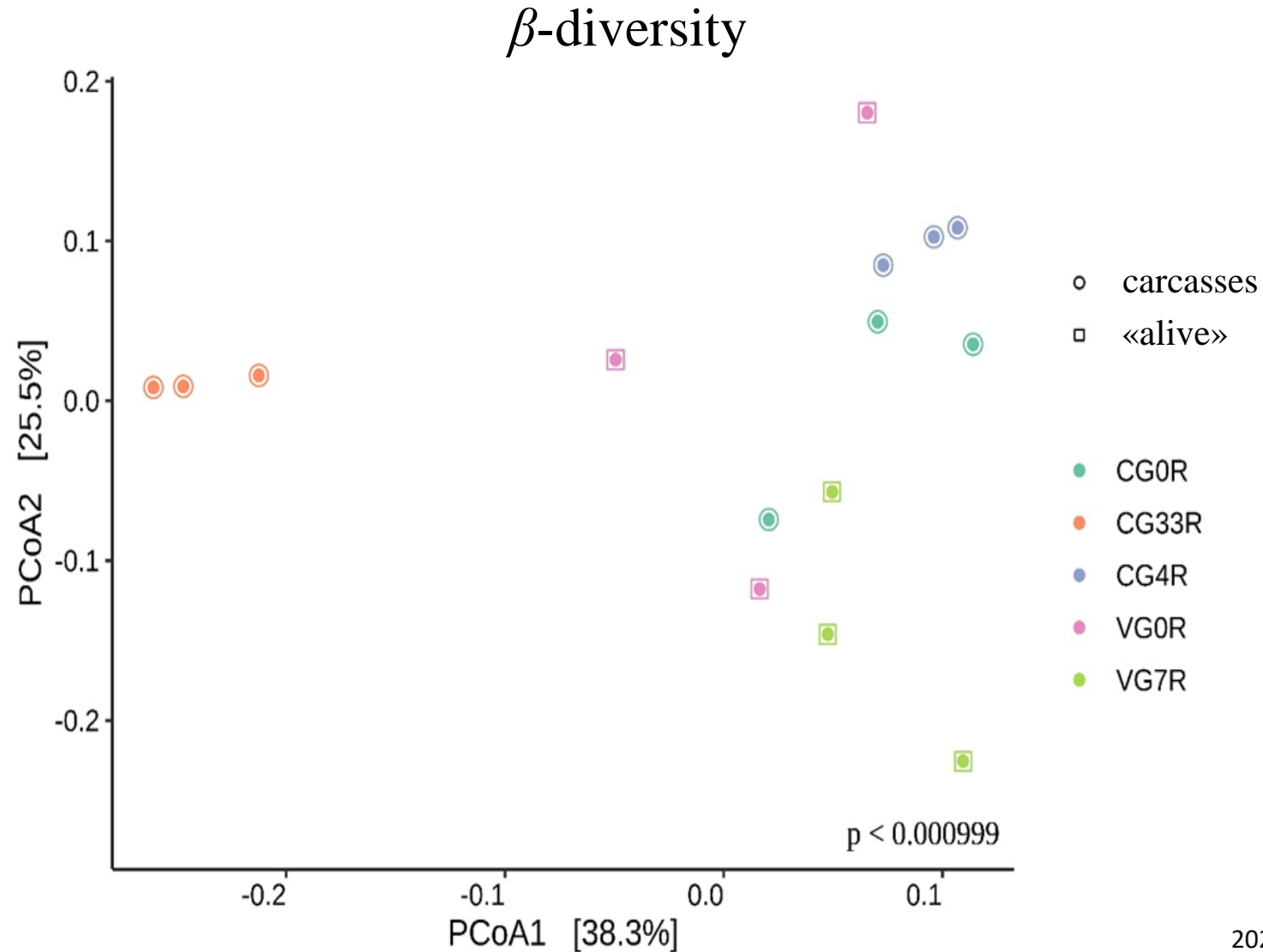


Bacterial and fungal morphotype isolation
from PBSA

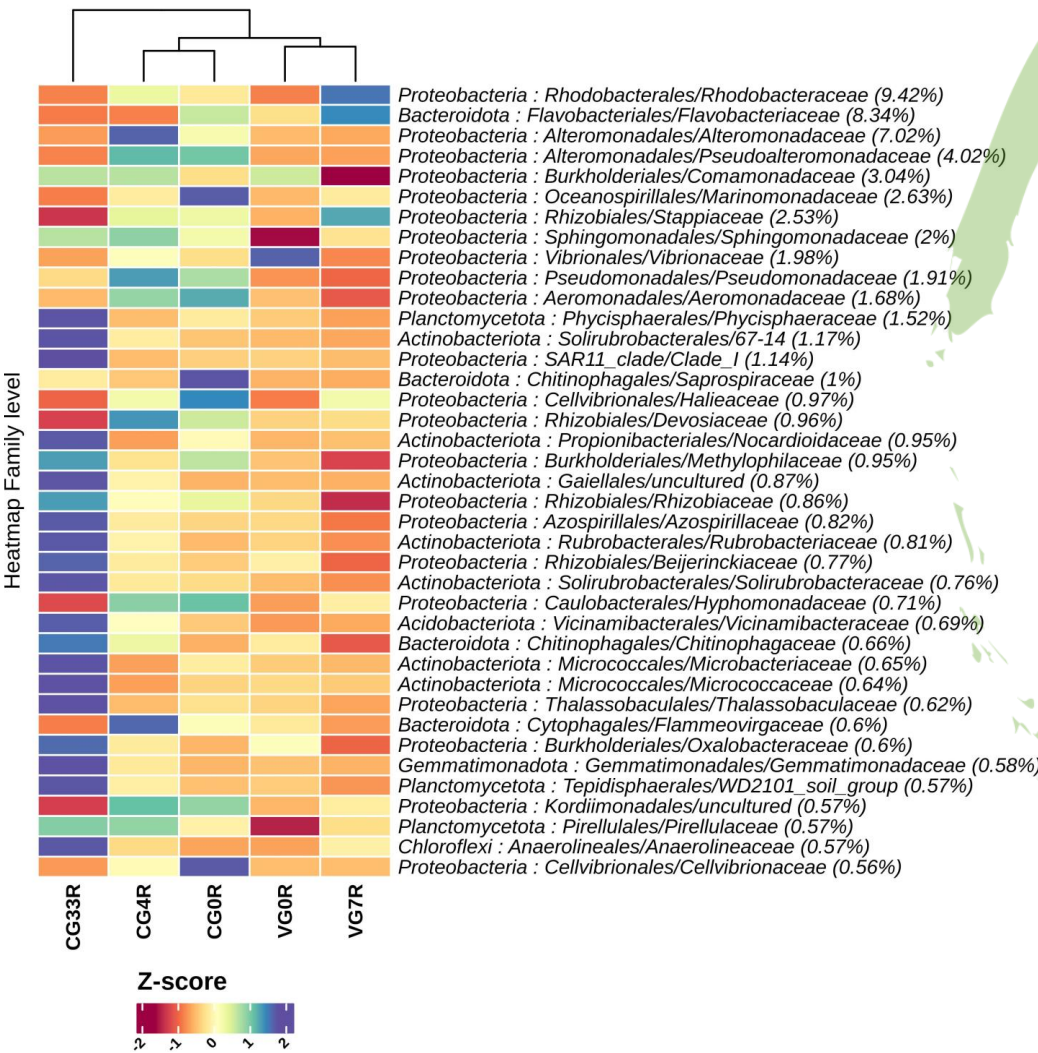
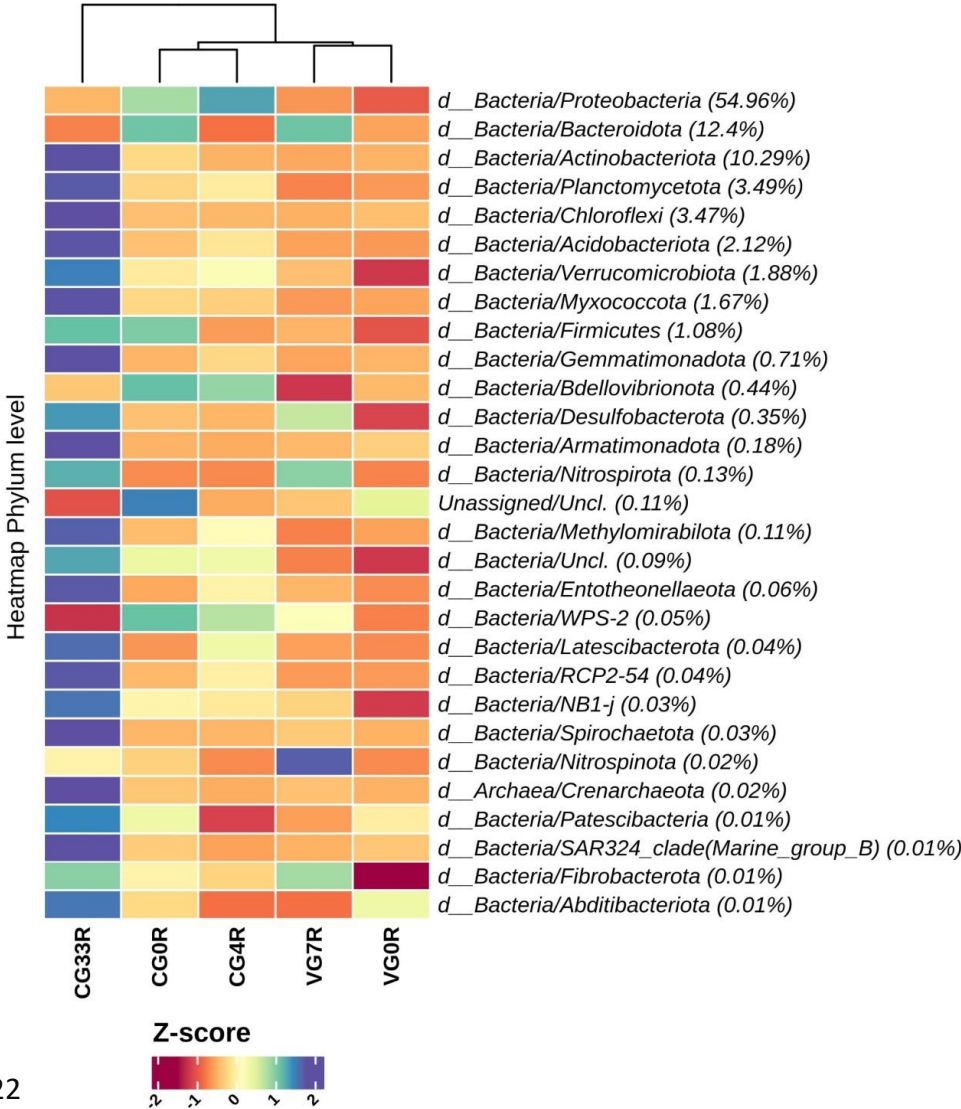


metabarcoding rDNA16S and
predictive functional profiling

5. Results of culture-independent approach



6.Results of culture-independent approach: Taxonomic profiling of bacterial community



7.Results of culture-independent approach: predictive functional profiling of bacterial communities



Carboxylic-ester hydrolase activities



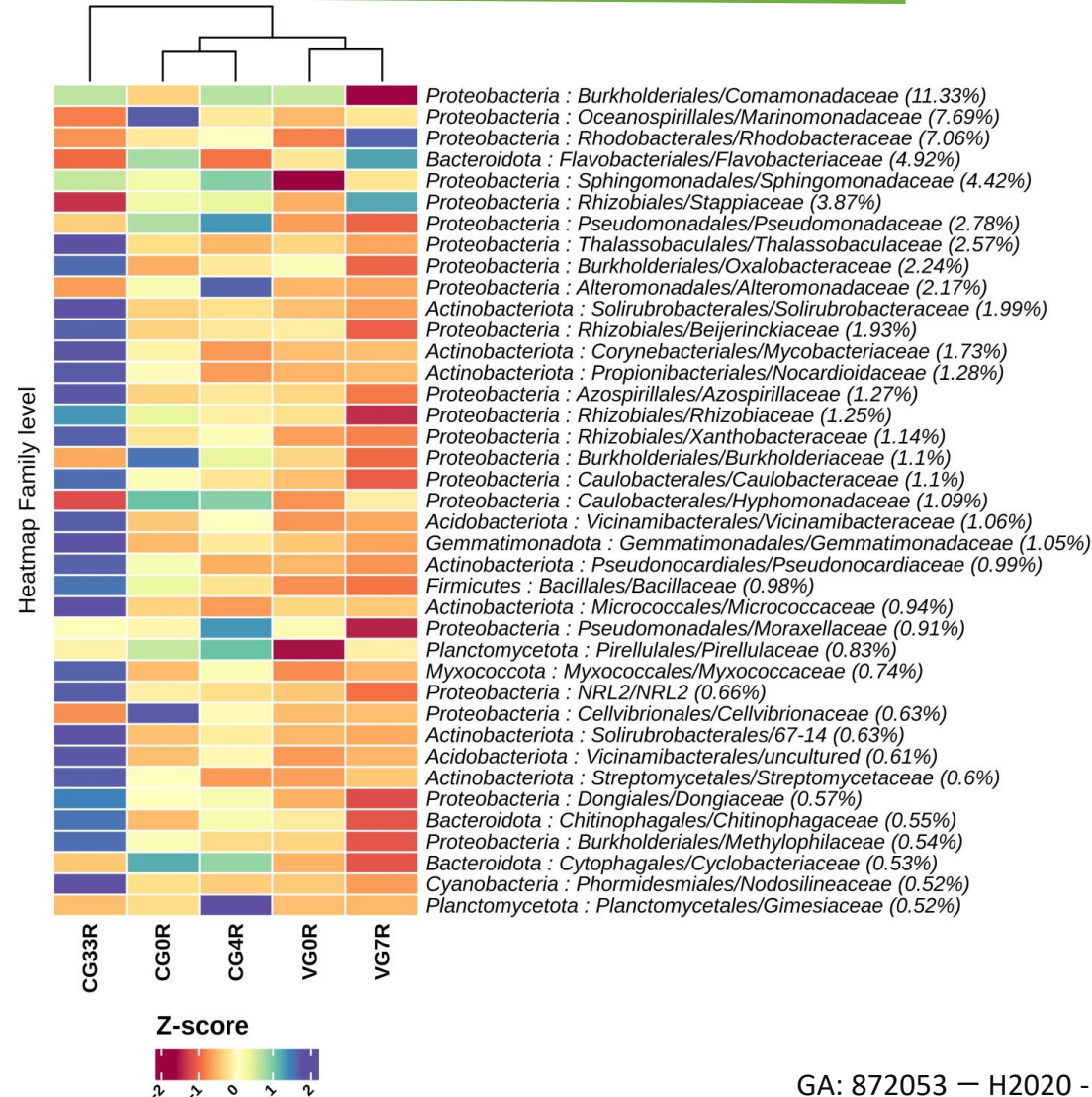
→ Carboxylesterase

→ Triacylglycerol lipase

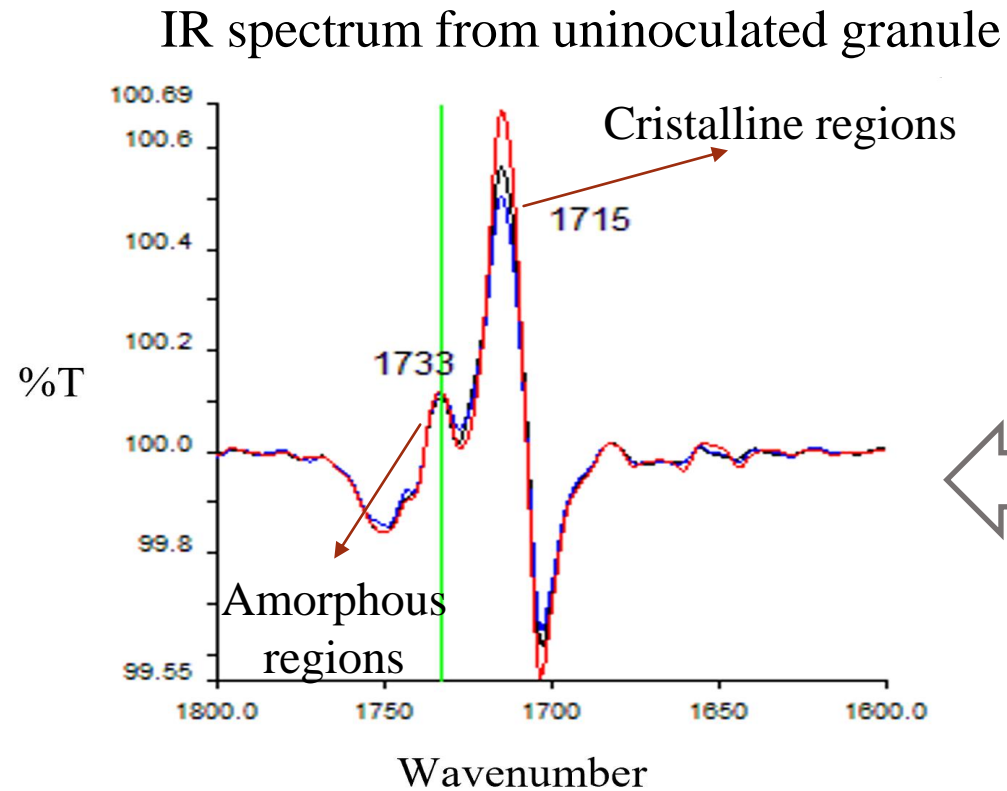
→ Cutinase



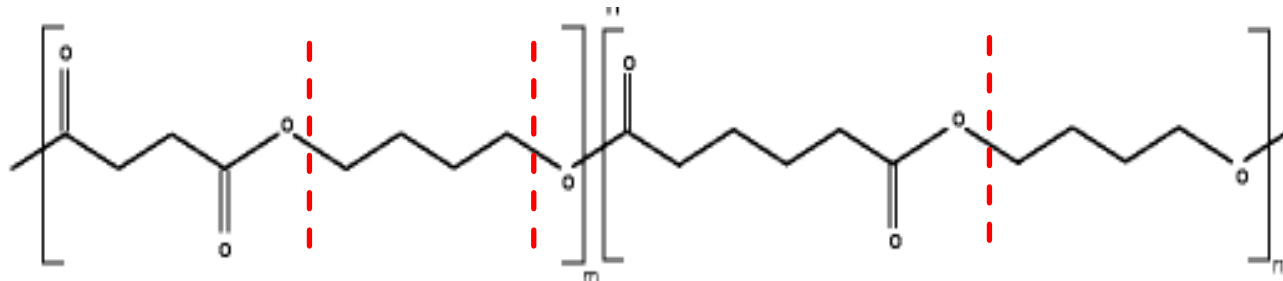
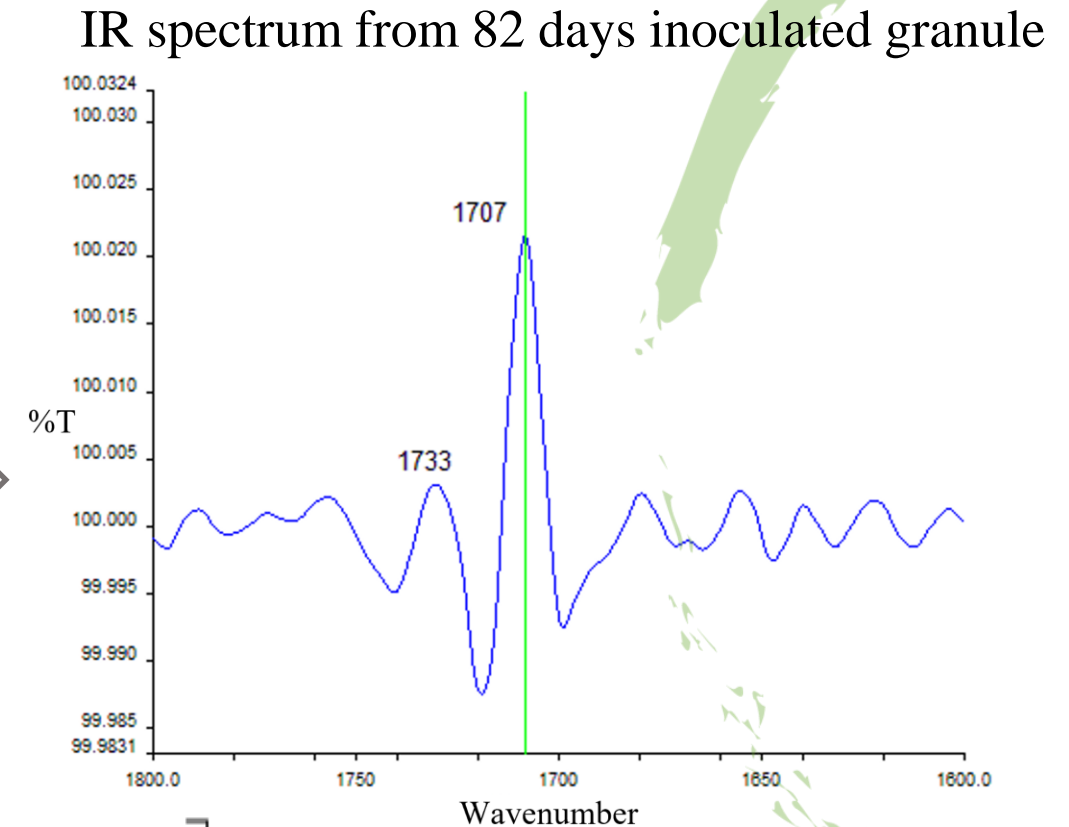
PBSA degradation



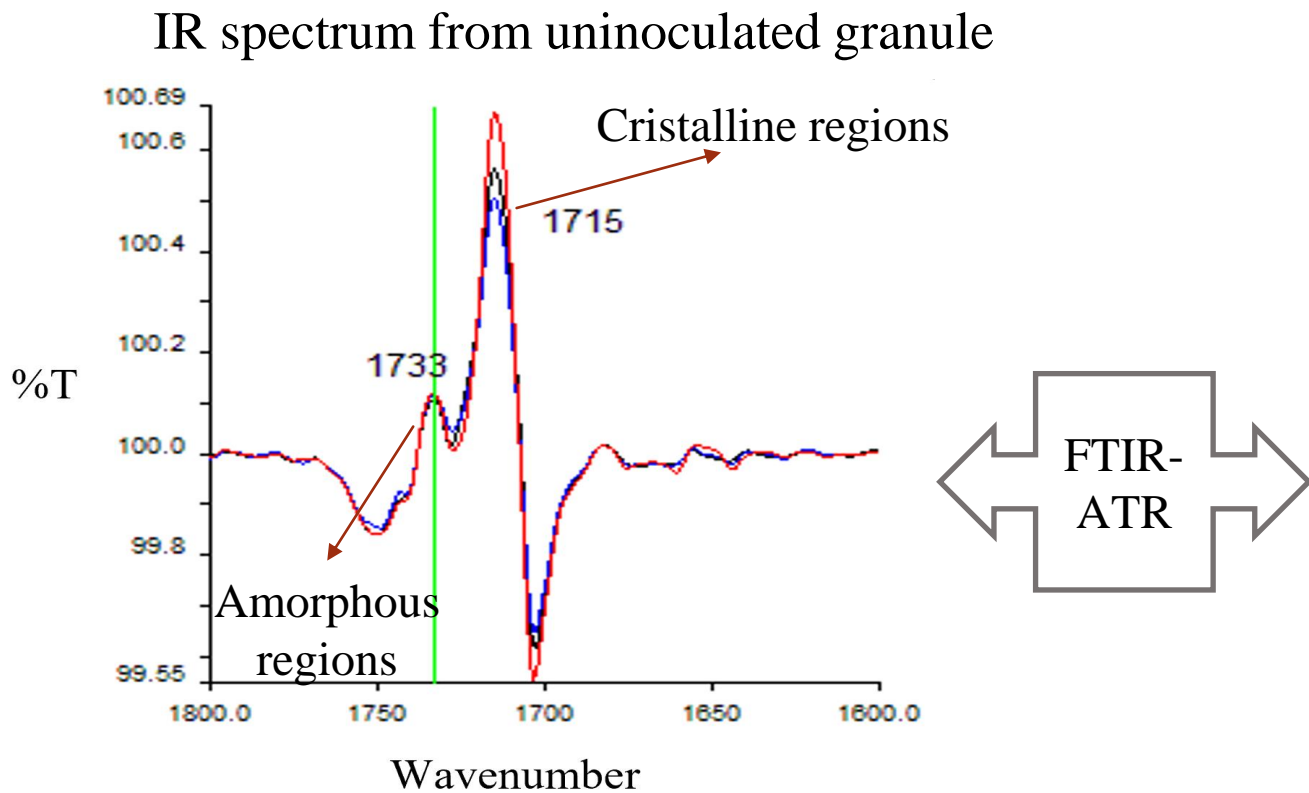
8. Results of culture-dependent approach: *Vibrio* sp.01 promotes the degradation of PBSA



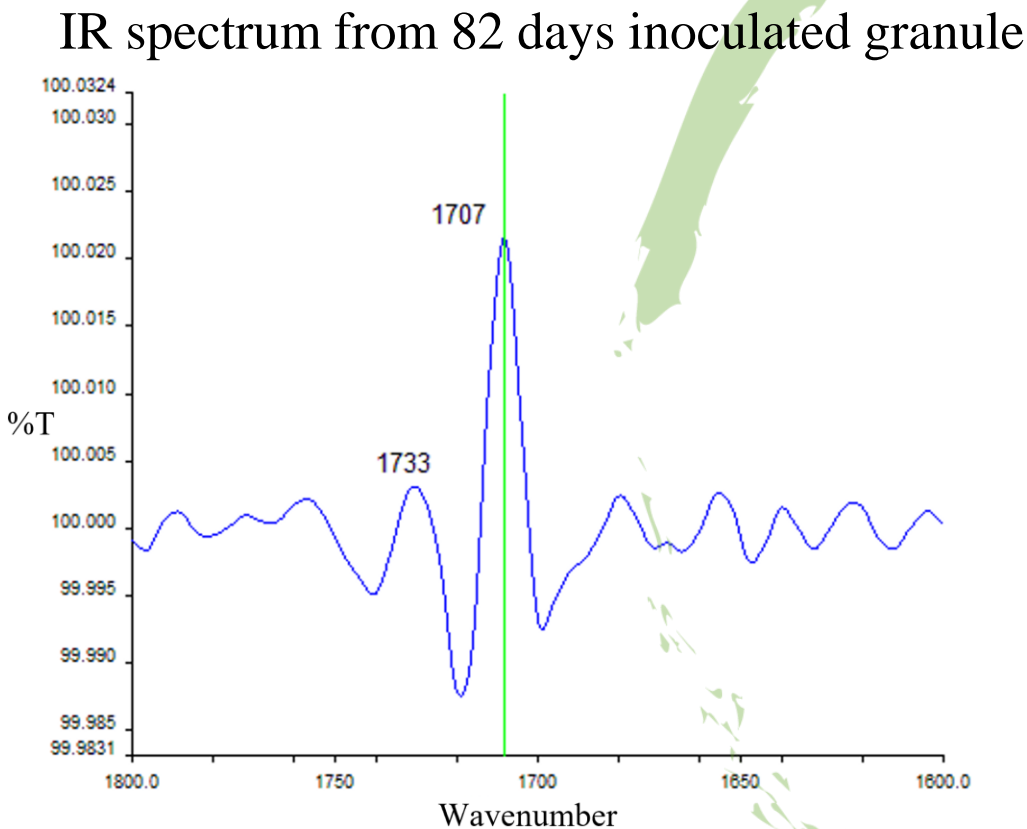
FTIR-ATR



9.Results of culture-dependent approach: *Vibrio* sp.01 promotes the degradation of PBSA

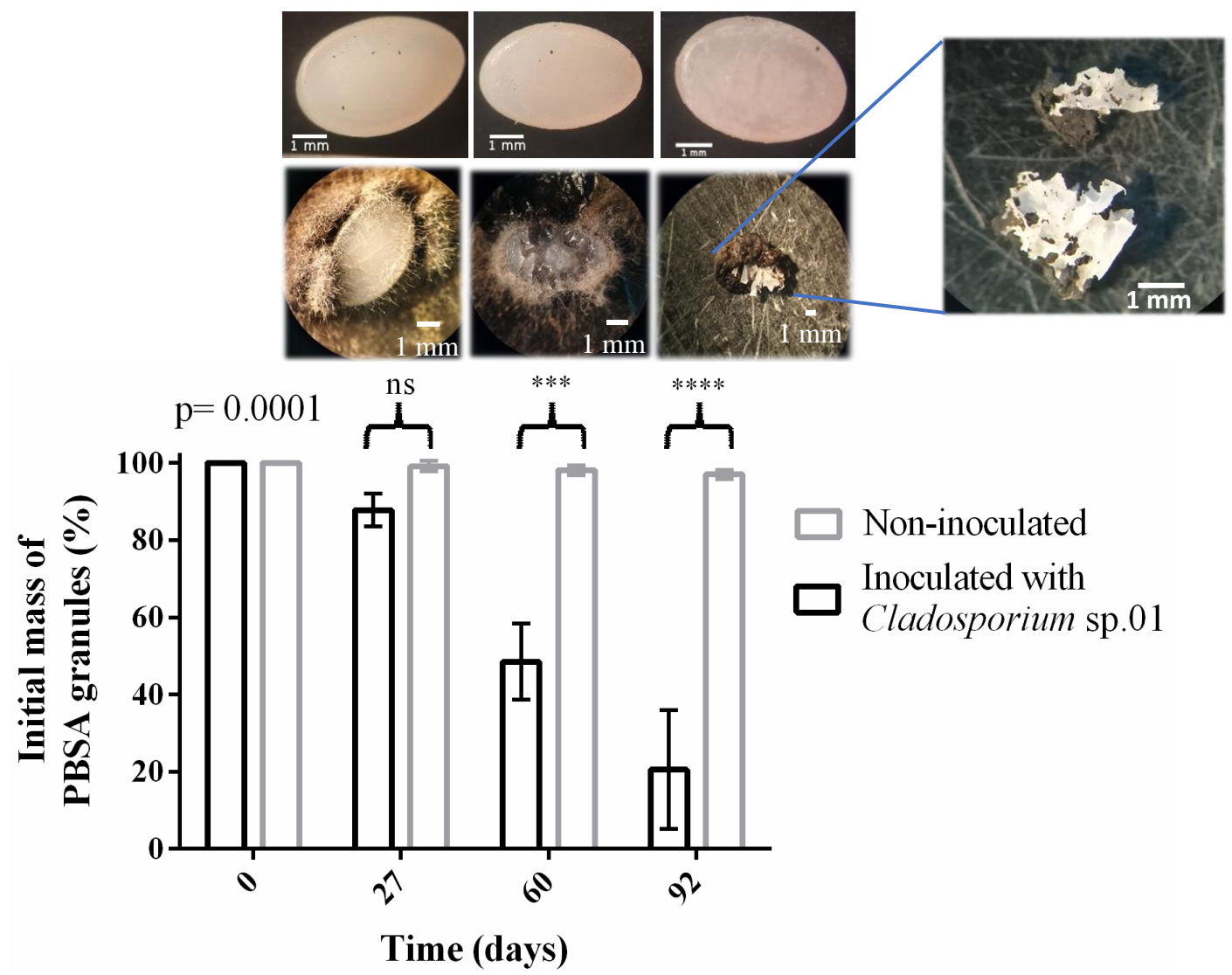


FTIR-ATR

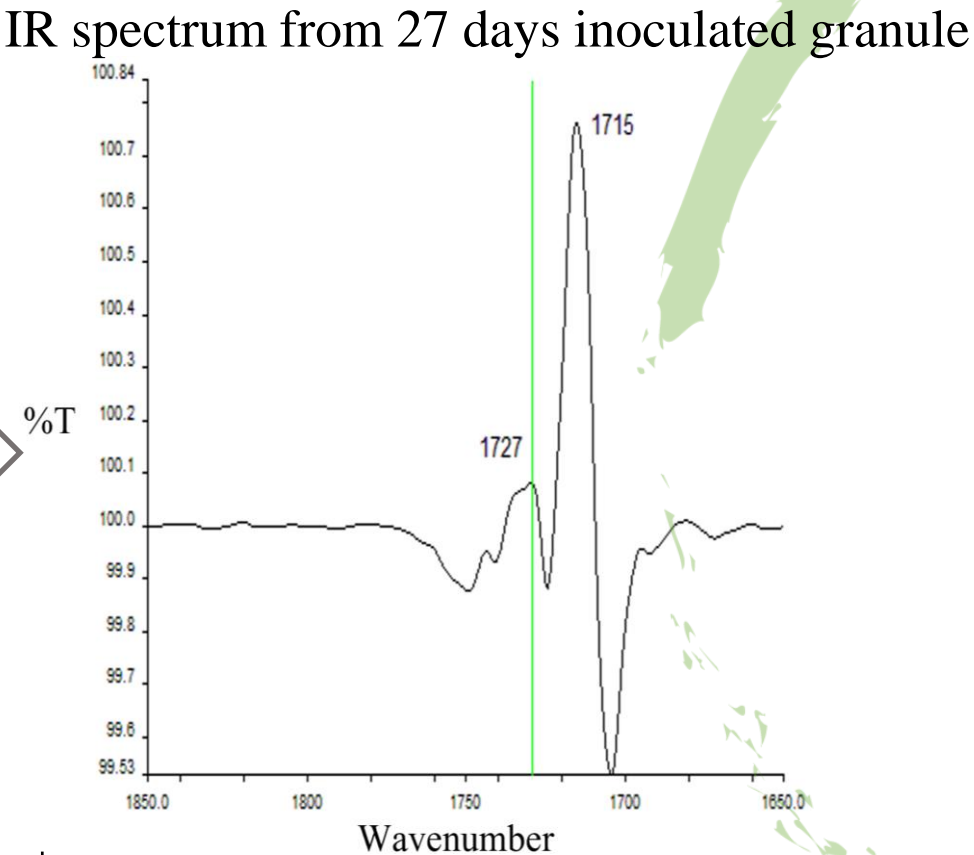
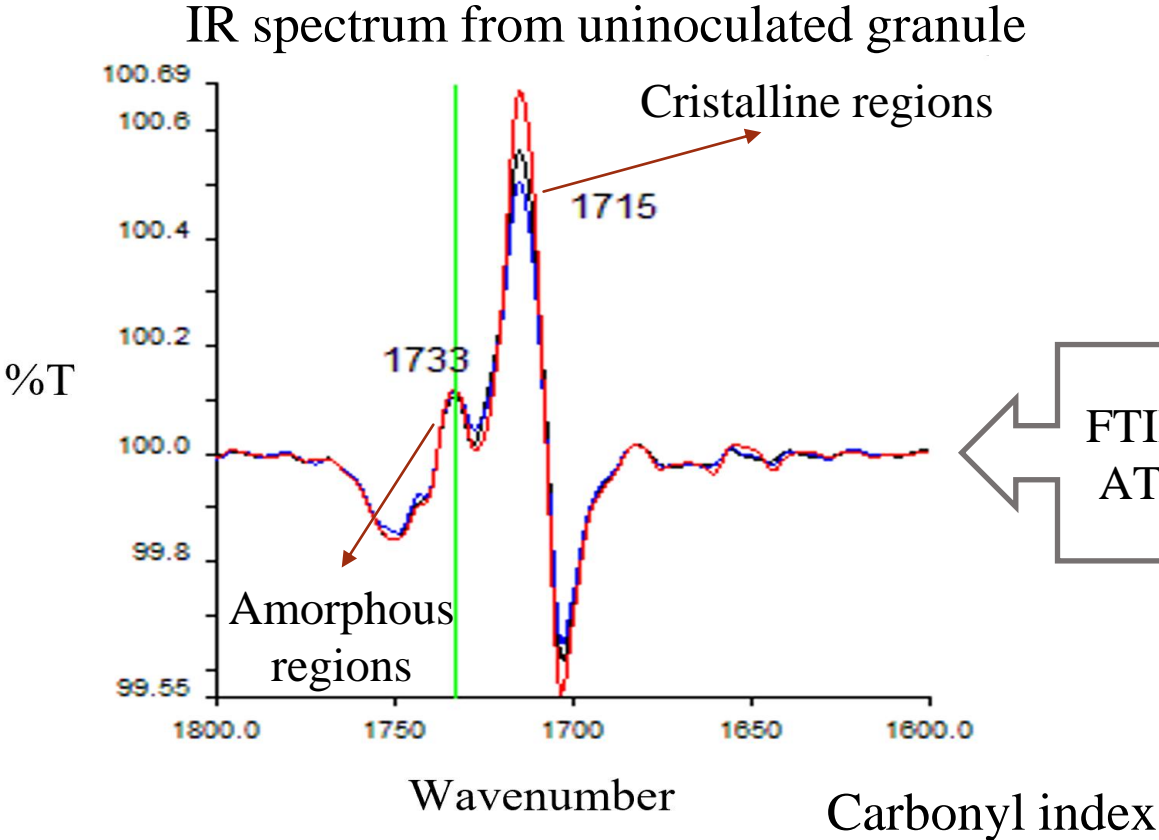


Carbonyl index		
Uninoculated granule (82 days) (mean ± sd)	Inoculated granule with <i>Vibrio</i> sp. 01 (82 days) (mean ± sd)	t-test (independent samples)
15±0,3	13,4±0,5	p=0,0085

10.Results of culture-dependent approach: *Cladosporium* sp. 01 culture-dependent promotes the degradation of PBSA



11.Results of culture-dependent approach: IR spectrum of PBSA granule inoculated with *Cladosporium* sp.01



12.Results overview



Culture-independent
approach



- ✓ «alive» adults → transient associations
- ✓ Carcasses → stable associations and equidistribution of contribution to the PBSA degradation

Culture-dependent
approach

PBSA as the only
carbon source



- I. *Vibrio* sp.01 → hydrolysis of ester bonds in the surface regions of PBSA
- II. *Cladosporium* sp.01 → hydrolysis of ester bonds throughout PBSA and reduction of PBSA granule mass.

13. Conclusions and perspectives



Copepod



nursery of microorganisms that show enzymatic activities of interest



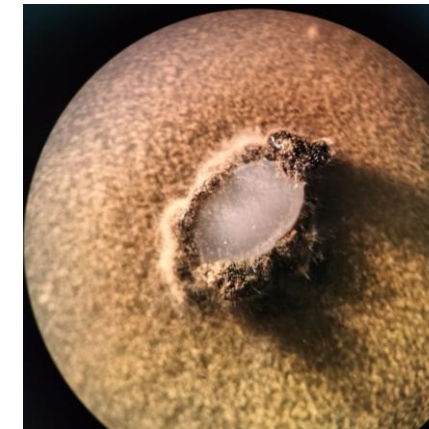
degradation of biopolymers with potential applications in the marine environment

- ➔ Genome sequencing of bacterial and fungal isolates.
- ➔ Set-up of petroleum-derived plastics experiments
- ➔ Refining *culture-dependent* approaches
- ➔ Extend a *culture-independent* approaches to fungi

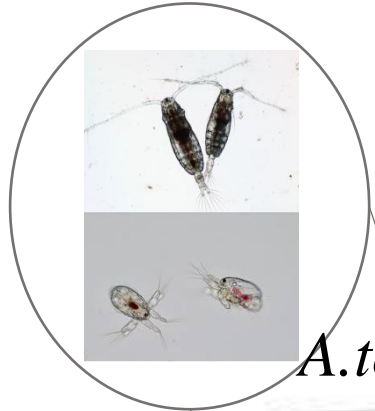
Fungal and bacterial isolates identification



Fungal isolates	Percentage of identity (%)	Fungal species with the highest similarity	Gene Bank Accession number
<i>Cladosporium</i> sp.01	100	<i>Cladosporium psychrotolerans</i>	MF473224.1
<i>Cladosporium</i> sp.02	100	<i>Cladosporium cladosporioides</i>	MT466517.1
<i>Cladosporium</i> sp.03	99	<i>Cladosporium austrohemisphaericum</i>	MF472935.1
<i>Cladosporium</i> sp.04	100	<i>Cladosporium subuliforme</i>	ON795077.1
<i>Cladosporium</i> sp.05	100	<i>Cladosporium</i> sp. isolate CSB_F136	KU574680.1
<i>Cladosporium</i> sp.06	99	<i>Cladosporium sphaerospermum</i>	MN202689.1



In progress: PBSA degradation → “in vivo” experiments



A. tonsa culture

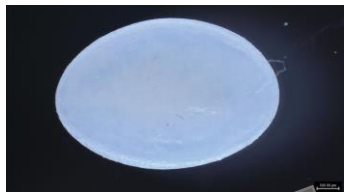
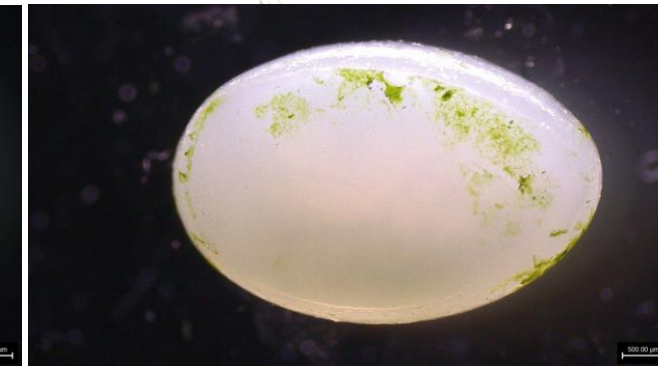
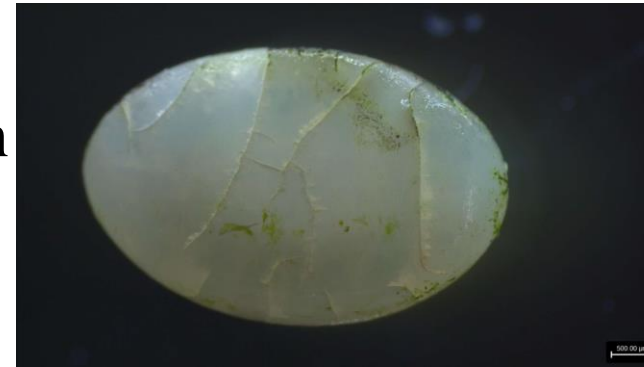
Inoculated with
Cladosporium
sp.01

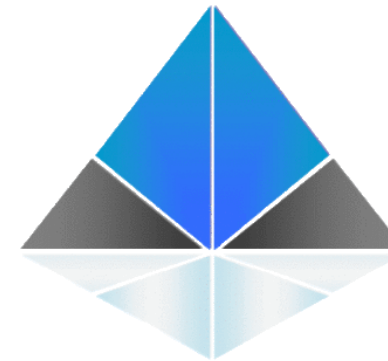


Uninoculated
PBSA

30 days

60 days





Thank you for your attention