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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Aim of this work

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
Acartia tonsa: microbial hotspot

Shoemaker M. K. et al., 2019
Functionality of microbial communities associated to *A. tonsa* carcasses

Newly formed carcasses

Degradation → Remains of carcasses

- **Protease activities**
- **Lipase activities**
- **Chitinase activities**
poly(butylene succinate-co-butylene adipate)(PBSA)

Shah A.A. et al., 2014

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

**Acartia tonsa** culture

**Rearing conditions:**
- Filtered seawater $\rightarrow 0.22 \, \mu m$
- Salinity $\rightarrow 30$ psu
- Photoperiod $\rightarrow 14:10$ h light: dark
- Temperature $\rightarrow 20 \pm 1 ^\circ C$

**ISPRA Laboratory culture**

*R. reticulata* monoalgal diet

**culture independent approach**

- New formed adults (VG0R)
- Seven-day adults (VG7R)
- New formed carcasses (CG0R)
- Four-day carcasses (CG4R)
- Thirty-three-day carcasses (CG33R)

**culture dependent approach**

- Adults, artificially induced death (carcasses)

**metabarcoding** rDNA16S and predictive functional profiling

- Bacterial and fungal morphotype isolation from PBSA

**7 mm**
Results of *Culture-independent* approach

**α-diversity**

**β-diversity**
Results of *culture-independent* approach: Taxonomic profiling of bacterial community

**Heatmap Phyllum level**
- **d_Bacteria/Proteobacteria** (54.96%)
- **d_Bacteria/Bacteroidia** (12.4%)
- **d_Bacteria/Actinobacteriota** (10.29%)
- **d_Bacteria/Planctomycyctota** (3.49%)
- **d_Bacteria/Chloroflexi** (3.47%)
- **d_Bacteria/Acidobacteriota** (2.12%)
- **d_Bacteria/Verrucomicrobiiota** (1.88%)
- **d_Bacteria/Mycococcota** (1.67%)
- **d_Bacteria/Firmicutes** (1.08%)
- **d_Bacteria/Gemmatimonadota** (0.71%)
- **d_Bacteria/Bdellovibrio&rrubrobacterota** (0.44%)
- **d_Bacteria/Desulfovibrio** (0.35%)
- **d_Bacteria/Armatimonadota** (0.18%)
- **d_Bacteria/Nitrospirota** (0.13%)
- **Unassigned/Uncl.** (0.11%)
- **d_Bacteria/Methylomirabilibioti** (0.11%)
- **d_Bacteria/Uncl.** (0.099%)
- **d_Bacteria/Entotheonellaeota** (0.06%)
- **d_Bacteria/WPS-2** (0.05%)
- **d_Bacteria/Latescibacterota** (0.04%)
- **d_Bacteria/RCP2-54** (0.04%)
- **d_Bacteria/NB1-1j** (0.03%)
- **d_Bacteria/Sphirochaeta** (0.03%)
- **d_Bacteria/Nitrospina** (0.02%)
- **d_Archaee/Crenarchaeota** (0.02%)
- **d_Bacteria/Patescibacteria** (0.01%)
- **d_Bacteria/SAR324_clade (Marine_group_B)** (0.01%)
- **d_Bacteria/Fibrobacterota** (0.01%)
- **d_Bacteria/Adblibacterota** (0.01%)

**Heatmap Family level**
- **Proteobacteria**: Rhodobacterales/Rhodobacteraceae (9.42%)
- **Bacteroidota**: Flavobacteriales/Flavobacteriaceae (8.34%)
- **Proteobacteria**: Alteromonadales/Altermonadaceae (7.02%)
- **Proteobacteria**: Alteromonadales/Pseudoalteromonadaceae (4.02%)
- **Proteobacteria**: Burkholderiales/Comamonadaceae (3.04%)
- **Proteobacteria**: Oceanospirillales/Marinomonadaceae (2.63%)
- **Proteobacteria**: Rhizobiales-Stappiaceae (2.53%)
- **Proteobacteria**: Sphingomonadales/Sphingomonadaceae (2%)
- **Proteobacteria**: Vibrionales/Vibrionaceae (1.98%)
- **Proteobacteria**: Pseudomonadales/Pseudomonadaceae (1.91%)
- **Proteobacteria**: Aeromonadales/Aeromonadaceae (1.68%)
- **Planctomycetota**: Phylaraphales/Phylophagaerae (1.52%)
- **Actinobacteriota**: Solirubrobacterales/67-14 (1.17%)
- **Proteobacteria**: SAR11_clade/Clade I (1.14%)
- **Bacteroidota**: Chitinophagales/Saprospiraceae (1%)
- **Proteobacteria**: Cellvibrionales/Haliicaceae (0.97%)
- **Proteobacteria**: Rhizobiales/Devisiaceae (0.96%)
- **Actinobacteriota**: Propionibacteriales/Nocardoidaceae (0.95%)
- **Proteobacteria**: Burkholderiales/Methylphilaecae (0.95%)
- **Actinobacteriota**: Gaiellales/uncultured (0.87%)
- **Proteobacteria**: Rhizobiales/Rhizobiaceae (0.86%)
- **Proteobacteria**: Azospirilla/Azospirillaceae (0.95%)
- **Actinobacteriota**: Rubrobacterales/Rubrobacteriaceae (0.81%)
- **Proteobacteria**: Rhizobiales/Beijerinckiaeae (0.77%)
- **Actinobacteriota**: Solirubrobacterales/Solirubrobacteraceae (0.76%)
- **Proteobacteria**: Caulobacteriales/Hyphomonoaceae (0.71%)
- **Actinobacteriota**: Vicinibacteriales/Vicinibacteriaceae (0.69%)
- **Bacteroidota**: Chitinophagales/Chitinophagaceae (0.95%)
- **Actinobacteriota**: Micrococcales/Microbacteriaceae (0.65%)
- **Actinobacteriota**: Micrococcales/Micrococcaceae (0.64%)
- **Proteobacteria**: Thalassobacales/Thalassobacteraceae (0.62%)
- **Bacteroidota**: Cytophagales/Fammeaeovirgaceae (0.6%)
- **Proteobacteria**: Burkholderiales/Coxibacteriacae (0.6%)
- **Gemmatimonadota**: Gemmatimonadales/Gemmatales (0.58%)
- **Planctomycetota**: Tepidiphaerales/WD2101 sol_goup (0.57%)
- **Proteobacteria**: Kordimonadales/uncultured (0.57%)
- **Planctomycetota**: Pirellulales/Pirellulaceae (0.57%)
- **Chloroflexi**: Anaerolineales/Anerolineaceae (0.57%)
- **Proteobacteria**: Cellvibrionales/Cellvibrionaceae (0.56%)
Results of *culture-independent* approach: *predictive functional profiling* of bacterial communities

- **Carboxylic-ester hydrolase activities**
  - Carboxylesterase
  - Triacylglycerol lipase
  - Cutinase

→ PBSA degradation

**Streptomycetales** → **Phytophagaceae** → **Bacillales** → **Pseudomonadales** → **Comamonadaceae**
Results of culture-dependent approach: Vibrio sp.01 promotes the degradation of PBSA
Results of *culture-dependent* approach: *Vibrio* sp.01 promotes the degradation of PBSA

IR spectrum from non-inoculated granule
Cristalline regions

IR spectrum from 82 days inoculated granule

Amorphous regions

FTIR-ATR

<table>
<thead>
<tr>
<th></th>
<th>Carbonyl index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninoculated granule (82 days) (mean ± sd)</td>
<td>15±0,3</td>
</tr>
<tr>
<td>Inoculated granule with <em>Vibrio</em> sp. 01 (82 days) (mean ± sd)</td>
<td>13,4±0,5</td>
</tr>
<tr>
<td>t-test (independent samples)</td>
<td>p=0,0085</td>
</tr>
</tbody>
</table>
Results of *culture-dependent* approach: *Cladosporium* sp. 01 promotes the degradation of PBSA

![Image of fungal growth and measurement](image_url)

- **p = 0.0001**
- **ns**
- ***
- ****

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Initial mass of PBSA granules (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Non-inoculated</td>
</tr>
<tr>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>60</td>
<td>80</td>
</tr>
<tr>
<td>90</td>
<td>40</td>
</tr>
<tr>
<td>125</td>
<td>20</td>
</tr>
</tbody>
</table>

Graph showing degradation over time with statistical significance.
Results of *culture-dependent* approach: IR spectrum of PBSA granule inoculated with *Cladosporium* sp.01

<table>
<thead>
<tr>
<th>Carbonyl index</th>
<th>t-test (independent samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14,3±0,1 (mean ± sd)</td>
<td>11,6±0,8 (mean ± sd)</td>
</tr>
</tbody>
</table>

Uninoculated granule (60 days)

Inoculated granule with *Cladosporium* sp. 01 (60 days)
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.
Conclusions and perspectives

**Copepod**

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

*nursery* of microorganisms that show enzymatic activities of interest
degradation of biopolymers with potential applications in the marine environment
Thank you for your attention
Introduction
Acartia tonsa: life cycle overview

- Adults stage 12 ÷ 20 giorni (20°C)
- Continuous production of eggs
- Easy maintenance of the eggs at 4°C

Laboratory cultures
A. tonsa: Core microbiome

Intestine

pH

O₂

Exoskeleton

Chitin

[Chemical structure of Chitin]
Marine feeding chain position and POM and DOM release during feeding of A. tonsa
Metods
Culture-independent approach: metabarcoding rDNA 16S and Predictive functional profiling

- Metagenomic DNA extraction
- Metabarcoding of V4-V5 16S rDNA
- Reads analysis → ASV
- Predictive functional profiling

PICRUSt2
*Culture-dependent* approach: isolation of bacterial and fungal morphotypes that use PBSA as the only carbon source

- Growth medium
  - PBSA film
  - 5 fungal morphotype
  - PBSA
  - Minimal medium
  - Minimal medium
  - 11 bacterial morphotype
  - PBSA as the only carbon source
  - Minimal medium

*debris of A. tonsa* carcasses
Culture-dependent approach: Screening of carboxylic-ester hydrolase and lipase activities of isolates and FITR-ATR analysis on inoculated PBSA

Pre-screening of carboxylic-ester hydrolase activities

Screening of lipase activities
**Rhinomonas reticulata cultures**

- Filtered seawater (0.45 µm)
- Sterilization cycle with Sodium Hypochlorite and Sodium Thiosulphate
- Salinity (30 psu)
- Photoperiod: 14 h; 10 light; dark
- Temperature = 20°C

---

**Culture medium f2 (Guillard et al 1975)**

<table>
<thead>
<tr>
<th>Salts</th>
<th>Quantity (mg)</th>
<th>Final concentration to 1.0 l of culture medium (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Macronutrients</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaNO₃</td>
<td>75</td>
<td>883</td>
</tr>
<tr>
<td>NaH₂PO₄*H₂O</td>
<td>5</td>
<td>36.3</td>
</tr>
<tr>
<td><strong>Micronutrients</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na₂EDTA⁺</td>
<td>4.36</td>
<td>11.7</td>
</tr>
<tr>
<td>FeCl₃*6H₂O⁺</td>
<td>3.15</td>
<td>11.7</td>
</tr>
<tr>
<td>CuSO₄*5H₂O</td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>ZnSO₄*7H₂O</td>
<td>0.022</td>
<td>0.08</td>
</tr>
<tr>
<td>CoCl₂*6H₂O</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>MnCl₂*4H₂O</td>
<td>0.18</td>
<td>0.9</td>
</tr>
<tr>
<td>Na₂MoO₄*2H₂O</td>
<td>0.006</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Vitamins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tiamin*HCl</td>
<td>0.1</td>
<td>/</td>
</tr>
<tr>
<td>Biotin</td>
<td>5*10⁻⁴</td>
<td>/</td>
</tr>
<tr>
<td>B₁₂ vitamin</td>
<td>5*10⁻⁴</td>
<td>/</td>
</tr>
</tbody>
</table>

Up to 1.0 l with the filtered seawater (0.22 µm)
Filtration of the Egg and Naupliar stages

Methods: *Acartia tonsa* culture maintenance

- Density of *Rhinomonas reticulata* cultures = 27000 cells/ml
- Density of *A. tonsa* <1000 copepod/l of culture

Monitoring the density of *Rhinomonas reticulata* cultures

Monitoring the developmental stages of *A. tonsa*
Metagenomics analysis

Raw sequences processed with Trimmmomatic and FastQC to remove sample barcodes and evaluate the goodness of the sequence

Denoising with DADA2, reads assembling, chimera check for both Forward and Reverse sequences

Taxonomic assignment with RDP classifier and Greengenes codes for bacterial taxa.
Predictive functional metagenomic profiling \(\rightarrow\) PICRUSt2

«it is possible to predict the gene content of a non-annotated genome of which the 16S rDNA sequence is known starting from the gene content of an annotated genome with the shortest phylogenetic distance»
Culture-dependent approach: Isolation and identification of bacterial morphotypes from debris of *A.tonsa* carcasses

1. gDNA extraction
2. ARDRA
3. Amplification of 16S rDNA
4. Sanger sequencing

**Vibrio sp.01**

Debris of carcasses (artificially induced death)

7 bacterial morphotypes

Growth medium (MYE)

**Debris of carcasses** (natural death)

4 bacterial morphotypes

Growth medium (MYE)

Transfer in Agar plates with Minimal Medium (MM)

- ✓ 42 days
- ✓ 20°C

Transfer in Agar plates with Minimal Medium (MM)

- ✓ 42 days
- ✓ 20°C

PBSA as the only carbon source

- ✓ 20°C
- ✓ 48h

Not yet identified

Growth medium (MYE)
*Culture-dependent* approach: Isolation and identification of fungal morphotypes from *debris* of *A.tonsa* carcasses

**Debris of carcasses** (natural death)

- PBSA film + MM
  - ✓ 20°C
  - ✓ 12 days

4 fungal morphotypes

**Debris of carcasses** (artificially induced death)

- PBSA film + MM
  - ✓ 20°C
  - ✓ 12 days

Cladosporium sp.01

1. gDNA extraction
2. ITS amplification
3. Sanger sequencing

Not yet identified

Transfer in Agar plates with Minimal Medium (MM)

- ✓ 35 days
- ✓ 20°C

Transfer in Agar plates with Minimal Medium (MM)

- ✓ 20 days
- ✓ 20°C

1 fungal morphotypes

PBSA film + MM

- ✓ 20°C
- ✓ 12 days

Cladosporium sp.01

- ✓ 20°C
- ✓ 13 days

1 fungal morphotypes

- ✓ 20°C
- ✓ 13 days
PBSA degradative mechanism

- α-β fold domain $\rightarrow$ catalytic triad $\rightarrow$ Ser-Glu-His
- Lid domain $\rightarrow$ hydrophobic aminoacid residues $\rightarrow$ substrate adesion $\rightarrow$ conformational change $\rightarrow$ exposure of the catalytic triad to the substrate

Modified from (Shah et. al 2014).
Results
**α-Diversity: Coverage e Hill index**

- **Coverage**
- **Hill-Shannon**

The samples are normalized for the same degree of completeness and not for size.

**General equation**

\[
D_q = \frac{1}{\sum_{i=1}^{S} p_i q} \left( \frac{1}{1-q} \right)
\]

**Hill index**

\[
qD = \left( \sum_{i=1}^{S} p_i q \right)^{1/(1-q)}
\]

**Shannon-Weiner**

\[
limit_{q \to 1} qD = \exp \left( -\sum_{i=1}^{S} p_i \log p_i \right)
\]

**Hill-Shannon**

\[
q = 2
\]

**Simpson**

\[
d = 1 - \frac{\sum_{i=1}^{S} p_i^2}{\sum_{i=1}^{S} p_i}
\]

*modified from (Roswell et al., 2021).*
β-Diversity: Weighted UniFrac

Unweighted UniFrac

\[ u = \sum_{i} b_i \left| \frac{A_i}{A_{tot}} - \frac{B_i}{B_{tot}} \right| \]

\[ D = \sum_{j} d_j \left( \frac{A_j}{A_{tot}} + \frac{B_j}{B_{tot}} \right) \]

WeightedUniFrac_{AB} = \frac{u_{AB}}{D_{AB}}

**UniFrac Phylogenetic distance**

**PCoA: Principal Coordinate Analysis**

<table>
<thead>
<tr>
<th>Samples</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>0</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>S2</td>
<td>0.47</td>
<td>0</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>S3</td>
<td>0.84</td>
<td>0.64</td>
<td>0</td>
<td>...</td>
</tr>
<tr>
<td>S4</td>
<td>0.96</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

b_i = lunghezza ramo i
A_i e B_i = numero di sequenze nel ramo i per le comunità A e B
A_{tot} e B_{tot} = numero totale di sequenze per le comunità A e B
d_j = distanza della sequenza j dalle radici dell’albero filogenetico
A_j e B_j = conteggi della sequenza j nelle comunità A e B
**Culture-independent approach:**

culture of *A. tonsa*

**A. Tonsa adults**

14 days

**New formed adults (VG0R)**

- 80°C

100 adults/replicate

3 X

**7 giorno**

**New formed carcasses (CG0R)**

- 80°C

100 carcasses/replicate

3 X

**30 thirty-three-day carcasses (CG33R)**

- 80°C

100 carcasses/replicate

3 X

**Four-day carcasses (CG4R)**

- 80°C

100 carcasses/replicate

3 X

**Carcasses (artificially induced death)**

14 days

**Experimental culture of *A. tonsa***

**New formed adults (VG0R)**

- 80°C

100 adults/replicate

3 X

**7 giorno**

**New formed carcasses (CG0R)**

- 80°C

100 carcasses/replicate

3 X

**30 thirty-three-day carcasses (CG33R)**

- 80°C

100 carcasses/replicate

3 X

**Four-day carcasses (CG4R)**

- 80°C

100 carcasses/replicate

3 X

**culture water**

*filtration 50 µm*
Results of culture-independent approach: Predictive functional profile of bacterial communities

Carboxylesterase (EC 3.1.1.1)

Cutinase (EC 3.1.1.74)

<table>
<thead>
<tr>
<th>Carboxylesterase</th>
<th>Cutinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteobacteria: Burkholderiales/Comamonadaceae/Uncl. (18.9%)</td>
<td>Proteobacteria: Marinomonadaceae/Marinomonas (62.23%)</td>
</tr>
<tr>
<td>Firmicutes: Bacilliaceae/Rodobacter (8.43%)</td>
<td>Actinobacteria: Nocardioidaeae/Nocardioales (6.8%)</td>
</tr>
<tr>
<td>Actinobacteria: Nocardioidaeae/Nocardioales (6.8%)</td>
<td>Actinobacteria: Micrococcales/Micrococcaceae/Uncl. (6.66%)</td>
</tr>
<tr>
<td>Actinobacteria: Micranthaceae/Anthuraceae/Uncl. (3.82%)</td>
<td>Chloroflexi: Anaeromonadaceae/UTCFX1 (3.82%)</td>
</tr>
<tr>
<td>Actinobacteria: Streptomycetaceae/Streptomyces (3.53%)</td>
<td>Chloroflexi: Roseiflexaceae/Uncl. (2.7%)</td>
</tr>
<tr>
<td>Chloroflexi: Roseiflexaceae/Uncl. (2.7%)</td>
<td>Actinobacteria: Geosporisporacaeae/Blastosaccus (2.41%)</td>
</tr>
<tr>
<td>Myxococcales: Myxococcaceae/Myxococcales/Uncl. (2.19%)</td>
<td>Actinobacteria: Propionibacteriales/Nocardioidaeae/Uncl. (2.19%)</td>
</tr>
<tr>
<td>Actinobacteria: Propionibacteriales/Nocardioidaeae/Uncl. (2.19%)</td>
<td>Proteobacteria: Hyphomicrobiaceae/Hyphomicrobiaceae (2.17%)</td>
</tr>
<tr>
<td>Chloroflexi: PG30-KF-CM45/PG30-KF-CM45 (2.07%)</td>
<td>Chloroflexi: JG30-KF-CM45/JG30-KF-CM45 (2.07%)</td>
</tr>
<tr>
<td>Germinimonadota: Germinimonadaceae/Uncl. (1.92%)</td>
<td>Chloroflexi: Actinomycetales/Uncl. (1.83%)</td>
</tr>
<tr>
<td>Chloroflexi: Actinomycetales/Uncl. (1.83%)</td>
<td>Actinobacteria: Propionibacteriales/Micromonosporaceae (1.82%)</td>
</tr>
<tr>
<td>Actinobacteria: Propionibacteriales/Micromonosporaceae (1.82%)</td>
<td>Chloroflexi: Anaerobiaceae/Uncl. (1.69%)</td>
</tr>
<tr>
<td>Actinobacteria: Nocardioidaeae/Nocardioales (1.69%)</td>
<td>Actinobacteria: Thermomonosporaceae/Thermomonospora (1.16%)</td>
</tr>
<tr>
<td>Actinobacteria: Thermomonosporaceae/Actinoalloricia (1.16%)</td>
<td>Chloroflexi: Uncl. (1.01%)</td>
</tr>
<tr>
<td>Actinobacteria: Thermomonosporaceae/Actinoalloricia (1.16%)</td>
<td>Germinimonadota: Germinimonadaceae/Uncl. (0.96%)</td>
</tr>
<tr>
<td>Actinobacteria: Thermomonosporaceae/Selenomonas (0.96%)</td>
<td>Proteobacteria: Comamonadaceae/Remisibacter (0.94%)</td>
</tr>
<tr>
<td>Actinobacteria: Thermomonosporaceae/Selenomonas (0.96%)</td>
<td>Firmicutes: Bacillales/Planococcaceae/Uncl. (0.93%)</td>
</tr>
<tr>
<td>Actinobacteria: Actinobacterales/Actinobacteria/Uncl./Uncl./Uncl. (0.96%)</td>
<td>Firmicutes: Actinobacteriales/Selenomonas (0.89%)</td>
</tr>
<tr>
<td>Actinobacteria: Actinobacterales/Actinobacteria/Uncl./Uncl./Uncl. (0.96%)</td>
<td>Germinimonadota: Germinimonadaceae/Uncl. (0.96%)</td>
</tr>
<tr>
<td>Chloroflexi: Actinomycetales/Uncl. (0.63%)</td>
<td>Chloroflexi: SBR1031/SBR1031 (0.62%)</td>
</tr>
<tr>
<td>Chloroflexi: SBR1031/SBR1031 (0.62%)</td>
<td>Proteobacteria: Hyphomicrobiaceae/Pedomycesplicium (0.56%)</td>
</tr>
<tr>
<td>Proteobacteria: Hyphomicrobiaceae/Pedomycesplicium (0.56%)</td>
<td>Actinobacteria: Pseudomonadaceae/Actinophytoica (0.54%)</td>
</tr>
<tr>
<td>Actinobacteria: Pseudomonadaceae/Actinophytoica (0.54%)</td>
<td>Firmicutes: Staphylococcaceae/Staphylococcus (0.53%)</td>
</tr>
<tr>
<td>Firmicutes: Staphylococcaceae/Staphylococcus (0.53%)</td>
<td>Proteobacteria: TRAS-35/TRAS-35 (0.46%)</td>
</tr>
<tr>
<td>Proteobacteria: TRAS-35/TRAS-35 (0.46%)</td>
<td>Proteobacteria: Rhodobacteraceae/Uncl. (0.46%)</td>
</tr>
<tr>
<td>Proteobacteria: Rhodobacteraceae/Uncl. (0.46%)</td>
<td>Firmicutes: Bacillales/Planococcaceae/Uncl. (0.45%)</td>
</tr>
<tr>
<td>Firmicutes: Bacillales/Planococcaceae/Uncl. (0.45%)</td>
<td>Actinobacteria: Kineosporiaceae/Kineosporia (0.44%)</td>
</tr>
<tr>
<td>Actinobacteria: Kineosporiaceae/Kineosporia (0.44%)</td>
<td>Proteobacteria: Xanthomonadaceae/Stenomphalaceae (0.44%)</td>
</tr>
<tr>
<td>Proteobacteria: Xanthomonadaceae/Stenomphalaceae (0.44%)</td>
<td>Actinobacteria: Sporichthyaceae/Uncl. (0.43%)</td>
</tr>
<tr>
<td>Actinobacteria: Sporichthyaceae/Uncl. (0.43%)</td>
<td>Chloroflexi: SJA-15/SJA-15 (0.41%)</td>
</tr>
<tr>
<td>Chloroflexi: SJA-15/SJA-15 (0.41%)</td>
<td>Firmicutes: Peptostreptococcaceae-Tissierellales/Peptostreptococcaceae/Uncl. (0.4%)</td>
</tr>
</tbody>
</table>
| Firmicutes: Peptostreptococcaceae-Tissierellales/Peptostreptococcaceae/Uncl. (0.4%) | Cg33B | CgG3R | VgFR | CgFR | VgGR | Z-score | Cg33B | CgG3R | VgFR | CgFR | VgGR | Z-score
Results of culture-independent approach: Predictive functional profile of bacterial communities
Results of *culture-independent* approach: unstratified ANCOM-BC with carboxyl-ester hydrolase activities of bacterial communities
IR spectrum of the superficial regions of PBSA granule and second derivative
Carbonyl index = A/B

(carbonyl index = Peak intensity at 1715 cm⁻¹ / Peak intensity at 1473 cm⁻¹)
In progress: PBSA degradation → *in vivo* experiments

Inoculated with *Cladosporium* sp.01 (with fungal biomass)

Inoculated with *Cladosporium* sp.01 (without fungal biomass)

Uninoculated PBSA