What is the best strategy to cryopreserve marine microbiomes ?

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Marine microbiome preservation

The EU-funded MICROBE project aims at developing approaches to enable access to microbiome samples and associated data.

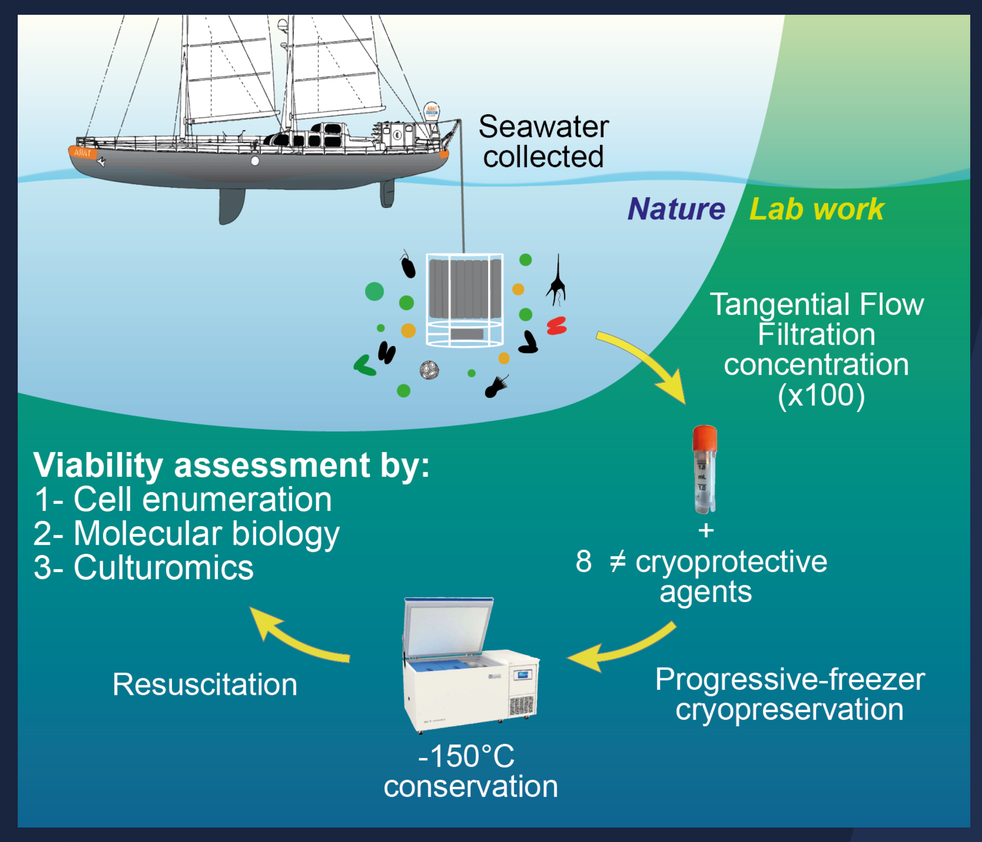

Figure 1. The strategy for collecting, cryopreserving, storing and reviving complex natural microbiome from marine waters

1. Challenges

- Cryopreservation of diluted complex microbial communities.
- Molecular diversity analyses from small volumes.
- Isolation of phototrophs after resuscitation.

2. Methods

- Microalgae are fragile, so are best concentrated using TFF (Fig 1).
- DNA/RNA are extracted in parallel using single-cell approach protocol. Universal primers (Parada *et al.*, 2016) are used to amplify the 16S/18S rRNA marker gene to identify the whole microbial communitiy (Fig 2).

3. Results

- Metabarcoding is an effective method if there are at least 100 active cells (Table 1).
- Bacterial, eukaryotic (and plastidial) molecular markers are traceable.
- 4,500 isolates yielded only 6 eukaryotic phototroph cultures. We easily grew hundreds of heterotrophic bacterial strains.

4. Perspectives

- Other CPA treatments under analysis.
- Test RNA/DNA ratios to assess viability.
- Test other culture methods to improve yield.

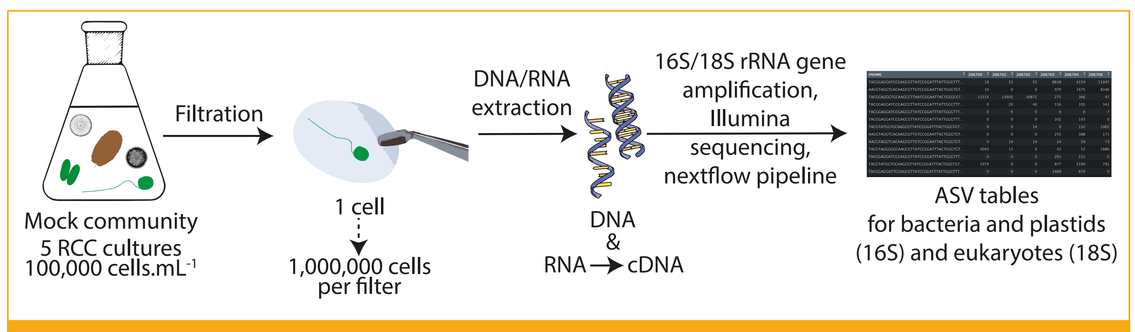

Figure 2. Mock community experiment to assess the minimal number of active cells needed to be detected by DNA/RNA metabarcoding

	0 ¢	1 ¢	10 ¢	100 ¢	1,000 ¢	10,000 ¢	100,000 ¢	250,000 ¢	1,000,000 ¢
DNA	-	+ (2/5)	+ (4/5)	✓	✓	✓	✓	✓	✓
RNA	-	+ (2/5)	+ (3/5)	✓	+ (4/5)	✓	✓	✓	✓
Viability (RNA/DNA >1)	-	+ (1/5)	+ (1/5)	+ (2/5)	+ (2/5)	+ (3/5)	+ (3/5)	+ (3/4)	✓

- Not detected ✓ All strains detected + (# strains detected/# strains in the mock)

Table 1. Cell detection threshold for the presence of Mock strains by DNA and RNA metabarcoding, and if, for each strain, the RNA/DNA ratio is above 1

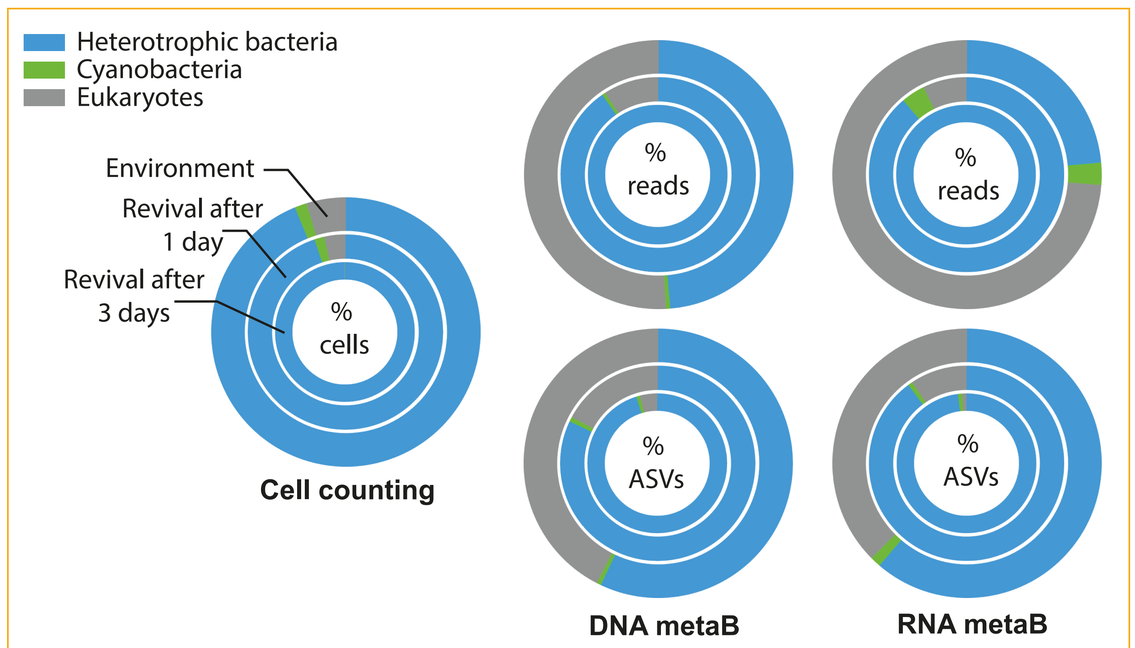

Figure 3. Proof-of-concept results of the molecular approach to describe the microbial community after cryopreservation (here without CPA)

References

Parada, Alma E., David M. Needham, et Jed A. Fuhrman. (2016) Every Base Matters: Assessing Small Subunit rRNA Primers for Marine Microbiomes with Mock Communities, Time Series and Global Field Samples ». Environmental Microbiology 18, 5: 1403-14.

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