



Jan 10, 2025

🌐 Isolation of bacteria, microalgae and fungi from single sea anemone holobionts for phenotyping and culture-dependent functional -omics applications

DOI

<https://dx.doi.org/10.17504/protocols.io.n92ldrzx8g5b/v1>

Laura Bulson¹, Claudia Pogoreutz²

¹Ecole Polytechnique Fédérale de Lausanne; ²Université de Perpignan Via Domitia



Claudia Pogoreutz

Université de Perpignan

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.n92ldrzx8g5b/v1>

Protocol Citation: Laura Bulson, Claudia Pogoreutz 2025. Isolation of bacteria, microalgae and fungi from single sea anemone holobionts for phenotyping and culture-dependent functional -omics applications. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.n92ldrzx8g5b/v1>

License: This is an open access protocol distributed under the terms of the **[Creative Commons Attribution License](#)**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: November 30, 2024

Last Modified: January 10, 2025

Protocol Integer ID: 113359

Keywords: symbiosis, marine host-microbe interactions, Cnidaria, Aiptasia, microbial cultivation, aseptic technique, marine microbe, endosymbiotic algae, members of the cnidarian microbiome, cnidarian microbiome, single sea anemone holobionts for phenotyping, exchange symbiosis with the cnidarian host, photosymbiotic cnidaria, single sea anemone holobiont, microbial members from different kingdom, cnidarian holobiont health, algae, such as algae, microbial member, complex holobiont, isolation of bacteria, microbial isolate, functional study of symbiodiniaceae, certain coral, bacteria, significant challenge to functional microbiology effort, symbiodiniaceae, availability of microbial isolate, holobiont, functional microbiology effort, exchange symbiosis, fungi, sea anemone, microalgae, other protist, jellyfish, cnidarian host

Funders Acknowledgements:

Agence Nationale de la Recherche

Grant ID: ANR-22-CPJ2-0113-01

Swiss National Science Foundation

Grant ID: 212614

Abstract

Photosymbiotic cnidaria such as certain corals, sea anemones and jellyfish are complex holobionts that consist of an animal host and a diversity of viruses, prokaryotes (bacteria and archaea) and microeukaryotes such as algae, fungi, and other protists (Rohwer et al. 2002, Cárdenas et al. 2022, Bonacolta et al. 2023). While considerable efforts have been channeled into the functional study of Symbiodiniaceae - endosymbiotic algae that engage in an intimate nutrient-exchange symbiosis with the cnidarian host, the remaining members of the cnidarian microbiome remain highly understudied from a functional point of view (Roik et al. 2022; Pogoreutz & Ziegler 2024; Voolstra et al. 2024). Importantly, while culture-independent -omics applications are increasingly providing valuable insight into the complex inter-kingdom interactions that govern cnidarian holobiont health and functioning (Vega Thurber et al. 2007, Wegley-Kelly et al. 2009, Amend et al. 2012, Cárdenas et al. 2022, Keller-Costa et al. 2021, 2022, Hochart et al. 2023; and many more), mechanistic work heavily depends on the availability of microbial isolates. Well-known limits to the cultivation of marine microbes hence constitute a significant challenge to functional microbiology efforts. Hoping to stimulate an increase in culture-dependent functional work on cnidarian (and other marine) holobionts, such as microbial co-cultures, we here provide a comprehensive protocol on the isolation of microbial members from different kingdoms of life – from the same host individual.



Guidelines

Heterotrophic bacteria, microalgae, and fungi not only occupy separate branches on the tree of life, but also exhibit diverse and divergent lifestyles, metabolisms, and interactions. As such, there is no single growth media that will permit the simultaneous co-isolation of a diversity of all these organisms at the same time. If isolation of such diverse groups from the same host or even individual is desired, the following conditions must be met:

- 1) different media suitable to sustain the growth of these respective groups have to be prepared in advance,
- 2) sufficient host material for inoculation has to be collected and processed as soon as possible after collection,
- 3) media have to be incubated at conditions suitable to sustain the growth of the target organisms (e.g., photosynthetic organisms such as microalgae or cyanobacteria will require light in the appropriate spectrum to grow).

Materials

Media preparation

Media (a selection):

- Difco 2216 Marine Agar,
- Half-Strength Difco 2216 Marine Agar,
- R2A,
- sea salt-supplemented LB or Nutrient Agar,
- sea salt-supplemented Guillard's F/2,
- Daigo's IMK,
- Modified Malt Extract Agar (MMEA),
- Potato-Dextrose-Agar (PDA),
- Artificial sea salts (microbiology grade).
- MilliQ water.

Consumables:

- Sharpie (ultra fine tip)
- Sterile, vented petri dishes (90 mm diameter)

Sample collection:

- 70 % ethanol,
- Clean nitrile gloves,
- Kim wipes,
- Forceps,
- Collection tools (hammer, pestle),
- Pre-labelled falcon tubes (50 ml), one for individual samples
- **Optional:** Underwater camera.
- **Optional:** pre-labelled and pre-filled tubes for additional samples for histology (containing appropriate fixative in appropriate volumes) or sequencing (containing appropriate sample preservation buffer, such as DNA/RNA shield, or RNAlater).

Sample processing, inoculation, purification of isolates:

- Hand-held homogenizer or motorized pellet pestle,
- If applicable: sterile, autoclavable pestle,
- Sharpie (ultra fine tip),
- 10, 100, 1000 ml micropipettors,
- 10, 100, 1000 ml barrier tips, sterile.
- Sterile and nuclease-free 1.5 ml ultracentrifuge tubes,
- Sterile (autoclaved) artificial sea water (for serial dilutions and rinsing of samples),
- 70 % ethanol
- 1 ul inoculation loops,
- Sterile tooth picks,
- Sterile forceps.

Cryopreservation:

- Sharpie (ultra-fine tip),
- Pre-labelled sterile 1.5 ml cryotubes,
- 80 % glycerol,
- 10, 100, 1000 ml micropipettors,
- 10, 100, 1000 ml barrier tips, sterile.
- Pre-grown microbial culture at desired density,
- Appropriate broth for organisms that do not grow well in suspension,
- Cryobox for storage in -80C.

Identification of isolates:

- Appropriate DNA extraction reagents (e.g., Qiagen Blood and tissue or PowerSoil Pro kit),
- PCR strips or plates,
- Adherent PCR foil,
- Appropriate primers:
 - Heterotrophic bacteria: targeting the full length of the 16S rRNA gene: 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTACGACTT-3') (Heuer et al. 1997),
 - Symbiodiniaceae: targeting the psbA non-coding region on the chloroplast mini-circle of dinoflagellates: psbAFor_1 (5'-GCA GCT CAT GGT TAT TTT GGT AGA C-3') and psbARev_1 (5'-AAT TCC CAT TCT CTA CCC ATC C-3') (LaJeunesse et al. 2021); for optional primers when working with temperate anemone-associated Symbiodiniaceae (Philozoon), refer to LaJeunesse et al. (2021).
 - Cyanobacteria: targeting a fragment of the 16S rRNA gene: CYA106F: 5'-CGG ACG GGT GAG TAA CGC GTG A-3' (Nübel et al. 1997) and 530R: 5'-CCG CNG CNG CTG GCA C-3' (Usher et al. 2014).
 - Marine-derived fungi: For an initial identification, primers targeting the 18S region NS1 (5'-GTAGTCATATGCTTGTCTC) and NS8 (5'-TCCGCAGGTTTACCTACGGA) and primers targeting the ITS regions ITS1 (5'-TCCGTAGGTGAACCTGCGG) and ITS4 (5'-TCCTCCGCTTATTGATATGC) can be used (Galkiewicz et al. 2012). In general, marine fungi identification might require morphological identification in combination with PCR targeting specific regions with group-specific primers (Marchese et al. 2021).
- PCR reagents (polymerase, dNTPs, or ready-made master mix),
- Nuclease-free, sterile water,
- PCR clean-up reagents (e.g., ExoProStar).

Safety warnings

Remarks: Microbial culturing

1. This is not an introductory protocol for users who have no prior experience with aseptic technique. It is recommended that inexperienced users receive a thorough training in aseptic technique prior to being introduced to this advanced protocol.
2. The isolation of algae without antibiotic-supplemented media may result in the co-isolation of cyanobacteria and heterotrophic (especially agarolytic) bacteria. Serial passaging of algae can help reduce bacterial loads, but use of a KAS antibiotic cocktail (50 $\mu\text{g} \cdot \text{mL}^{-1}$ kanamycin, 100 $\mu\text{g} \cdot \text{mL}^{-1}$ ampicillin, 50 $\mu\text{g} \cdot \text{mL}^{-1}$ streptomycin; Soffer et al. 2008) can be added to the growth medium at a later stage to render the algae axenic. Please note that at least the first passages in antibiotic-supplemented growth media might result in overall slower growth rates of algae.
3. The purpose of the here provided protocol is, in part, to give an idea of the logistics behind the isolation of diverse organisms from different kingdoms of life from the same host. As such, here suggested media and growth conditions are not exhaustive. Rather, users are encouraged to explore a greater diversity of media and conditions to select for specific taxonomic or functional groups. Here, adaptation of existing media for marine organisms (e.g., addition of sea salts) or development of new media (derived from sterilized marine substrates or host extracts) may constitute exciting new avenues for functional work and is explicitly encouraged.
4. Blue light rather than white light spectrum might help increase algal growth yield.
5. After purification, antibiotics can be omitted for long-term cultivation of fungal isolates. Note that some isolates might change their phenotype upon omitting the antibiotics.
6. In addition to standard PPE, consider wearing an FFP2 mask / K95 respirator when handling uncharacterised environmental fungal cultures.

Remarks: Disposal of contaminated waste and microbial cultures

Contaminated waste and cultures to be disposed of should be inactivated before being discarded. In the absence of an autoclave (or pressure cooker / sterilizer), the use of bleach is recommended.

Ethics statement

Make sure that sampling permits appropriate for the organism sampled and sampling area are in place.

Before start

Preparation of different microbial growth media

Heterotrophic bacteria

Unless specific functional groups are targeted that require selective media or growth conditions (e.g., diazotrophs, DMSP degraders, etc.), a good starting point to cover a broad diversity is the use of media supporting a broad selection of organisms, such as

- Difco 2216 Marine Agar,
- Half-Strength Difco 2216 Marine Agar,
- R2A,
- sea salt-supplemented LB or Nutrient Agar.

For an excellent overview of culture media previously used for the isolation of bacteria from reef-building corals, we recommend looking into Sweet et al. (2021).

Marine microalgae

For marine algae, specialized growth media are available (<https://roscoff-culture-collection.org>). Two common media which support the growth of tropical and temperate Symbiodiniaceae from corals and sea anemones are:

- sea salt-supplemented Guillard's F/2,
- Daigo's IMK.

A number of modifications (e.g., use of different antibiotics to suppress bacterial growth) can be performed to maximize the diversity of Symbiodiniaceae isolated from photosymbiotic Cnidaria. An excellent starting point for beginners is Xiang et al. (2013). We further recommend reading the recommendations of the Roscoff Culture Collection to learn more about the intricacies of marine microalgae cultivation (<https://roscoff-culture-collection.org/protocols>).

Note: The isolation of algae without antibiotic-supplemented media may result in the co-isolation of cyanobacteria and heterotrophic (especially agarolytic) bacteria. Serial passaging of algae can help reduce bacterial loads, but use of a KAS antibiotic cocktail (50 $\mu\text{g} \cdot \text{mL}^{-1}$ kanamycin, 100 $\mu\text{g} \cdot \text{mL}^{-1}$ ampicillin, 50 $\mu\text{g} \cdot \text{mL}^{-1}$ streptomycin; Soffer et al. 2008) can be preferred at a later stage to render the algae axenic.

Marine fungi

Marine fungi are incredibly diverse and possess significant chemodiversity. Unless you are interested in specific functional groups, media which can be used by a diversity of organisms include:

- Modified Malt Extract Agar (MMEA)
- Potato-Dextrose-Agar (PDA)

Supplementation with sea salts and chloramphenicol to suppress the growth of heterotrophic bacteria is recommended. For a broader choice of growth media previously used for the isolation of marine fungi and

fungi-like organisms from coral reef environments, we recommend reading Raghukhumar (2012) and (2017).

General considerations:

- Determine plate replication and number of growth conditions for each individual media. Prepare an appropriate number of agar plates accordingly. Take into consideration major differences in growth rates (e.g., microalgae will grow more slowly than most heterotrophic bacteria). After preparation of sterile agar plates, store them upside down at 4 °C until use.
- Determine the amount of host material needed. Depending on the size of the host, it might be necessary to collect entire specimens (e.g. Aiptasiidae), or it might suffice to collect a few tentacles only (e.g., Anemoniidae; large tropical sea anemones). In the latter, it might be possible to re-visit the same host at a later time if location is well documented.
- Prepare filter-sterilized, autoclaved artificial seawater for rinsing of samples to remove loosely associated cells, and for serial dilutions of inoculum.
- If it is important to match individual hosts and isolates to microbial community data, histology, etc., appropriate buffers or preservatives should be prepared in appropriately pre-labelled tubes and cryoboxes for storage in advance for field sampling.
- Prepare sample list for the field collection.
- Prepare tools, reagents and consumables for field sampling.

Sampling

- 1 Collect samples using gloves and clean tools (tweezers, hammer and chisel).
- 2 Clean tweezers with 70 % ethanol. Transfer samples into clean 50 ml falcon tubes. If it is important to match the exact host individual with specific plates for inoculation, do not place more than one host into each tube.
- 3 Clean tweezers and razor blades (used to cut tentacles or entire animals in a clean receptacle) in between each sample.
- 4 If it is important to match individual hosts and isolates to microbial community data, histology, etc., samples should be preserved in appropriate buffers or preservatives upon collection in the field.
- 5 Keep samples cool and transport them back to the lab as soon as possible.
- 6 Process samples on the same day.

Inoculation of growth media for isolation of bacteria and microalgae

- 7 Pre-label your growth media. Relevant information should include: Type of media, date of inoculation, information on host (e.g., Av = indicating host species *Anemonia viridis*), dilution, individual plate number, initials of researcher performing the work.
- 8 During preparation and inoculation, keep open 'control' plates in your sterile working area to keep track of potential contamination.
- 9 For heterotrophic bacteria and algae, fractions of host material are rinsed thoroughly in sterile artificial seawater and then homogenized in an appropriate volume, which may depend on means of homogenization (e.g., hand-held motorized pellet pestle vs. UltraTurrax, Polytron, or other system). For instance, pieces of tentacles from larger anemones can be homogenized in a volume of 250 – 500 µl, depending on biomass and means of homogenization.
- 10 Prepare a serial dilution of host homogenate for inoculation (undiluted, 1:10, 1:100, 1:1000).

- 11 Place aliquot of each homogenate on agar plates, and gently spread around on plate using a clean spreader or drigalski spatula.
- 12 Close plate and let it rest facing bottom-up for 10 minutes.
- 13 Seal plate with clean parafilm and place it upside-down in incubator.
- 14 **Note:** while inoculated plates intended for the isolation of heterotrophic bacteria will be placed in the dark, preferably a few degrees centigrade below ambient seawater temperature, plates intended for the isolation of microalgae will require light for growth (e.g., ideally a light-controlled environment, such as a phytotron or plant growth chamber with 12:12 h light:dark conditions; isolation on a lab bench next to a window works just as well, but might take longer depending on light and temperature environment). As microalgae grow more slowly than heterotrophic bacteria, temperatures may be maintained at a similar temperature to in situ conditions upon collection.
- 15 Keep monitoring plates for growth. Heterotrophic bacteria will form colonies faster than microalgae at the same temperature. Please note that inoculated media to support the growth of marine microalgae might also support the growth of autotrophic cyanobacteria (these will grow faster than microalgae – more similar to heterotrophic bacteria).
- 16 **Note:** pick colonies with a range of different morphologies and different growth rates to maximise diversity of strains at the end of the isolation process.
- 17 Keep monitoring growth on plates. Pick and purify colonies using appropriate aseptic techniques (e.g., quadrant streak) with a sterile toothpick or 1 ul loop before they start merging together.
- 18 **Note:** depending on temperature and light conditions for microalgae, it might take 2-8 weeks until you see growth on plates. Temperate algae might grow more slowly than tropical algae.
- 19 After several passages, identify organisms by Sanger sequencing using appropriate sets of primers.
 - Heterotrophic bacteria: targeting the full length of the 16S rRNA gene: 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTACGACTT-3') (Heuer et al. 1997),
 - Symbiodiniaceae: targeting the psbA non-coding region on the chloroplast mini-circle of dinoflagellates: psbAFor_1 (5'-GCA GCT CAT GGT TAT TTT GGT AGA C-3') and psbARev_1 (5'-AAT TCC CAT TCT CTA CCC ATC C-3') (LaJeunesse et al. 2021); for

optional primers when working with temperate anemone-associated Symbiodiniaceae (Philozoon), refer to LaJeunesse et al. (2021).

- Cyanobacteria: targeting a fragment of the 16S rRNA gene: CYA106F: 5'-CGG ACG GGT GAG TAA CGC GTG A-3' (Nübel et al. 1997) and 530R: 5'-CCG CNG CNG CTG GCA C-3' (Usher et al. 2014).

- Marine-derived fungi: For an initial identification, primers targeting the 18S region NS1 (5'-GTAGTCATATGCTTGTCTC) and NS8 (5'-TCCGCAGGTTACCTACGGA) and primers targeting the ITS regions ITS1 (5'-TCCGTAGGTGAACCTGCGG) and ITS4 (5'-TCCTCCGCTTATTGATATGC) can be used (Galkiewicz et al. 2012). In general, marine fungi identification might require morphological identification in combination with PCR targeting specific regions with group-specific primers (Marchese et al. 2021).

- 20 For phenotyping, enumeration and experimentation, it will be more practical to grow the organisms in suspension (liquid media, i.e. broth) rather than on agar plates. Be aware that transitioning of microbial cultures, especially microalgae from agar plates into liquid cultures might take adjustment time and sometimes requires trouble-shooting (e.g., supplementation of media with additional nutrients or amino acids).
- 21 Cryo-preserve organisms that you wish to keep using standard protocols and store at appropriate temperatures (e.g., glycerol stocks for bacteria and fungi at -80°C; MeOH-DMSO-Gly-EG-PG-sucrose preservation for microalgae, storage at -140°C or in LN₂; Chong et al. 2016).
- 22 Bacterial and microalgal isolates can be subsequently used for phenotyping, genomic and chemical characterization, and microbial co-culturing experiments.

Inoculation of growth media for the isolation of marine-derived fungi

- 23 **Note:** While fungi can also be isolated from host homogenates, morphological diversity and numbers of colonies obtained may be greater when using unhomogenized, surface-sterilized host samples. Important: As terrestrial fungi and their spores can easily end up in the ocean due to dust deposition and terrestrial run-off, surface-sterilization of samples in 70 % ethanol prior to inoculation of growth media is strongly recommended (Kjer et al. 2010). Nonetheless, fungal spores are hardy. Hence, fungal isolates should be considered 'marine-derived' rather than 'marine'.
- 24 During preparation and inoculation, keep open 'control' plates in your sterile working area to keep track of potential contamination.
- 25 Cut intact host sample into pieces (1 cm², or larger pieces of individual anemones; in case of small anemones, do not cut into smaller pieces).

- 26 Using clean forceps, dip and rinse sample in sterile artificial sea water to remove loose surface-associated cells.
- 27 Using clean forceps, dip sample into 70 % ethanol for 60 – 90 s.
- 28 Remove sample from ethanol and gently remove excess liquid without touching contaminated surfaces or tools.
- 29 Prepare 'control' agar plates for surface sterilization: Using clean forceps, smear surface-sterilized samples all over the agar plate.
- 30 Dissect sample into smaller pieces using clean forceps and razor blades.
- 31 Using clean forceps, place cut pieces of host tissues onto fungal growth-supporting media. Make sure that cut internal surfaces are in direct contact with the surface of the growth media.
- 32 Close agar plate and let it rest with bottom facing up for 10-15 min.
- 33 Seal inoculated plates and place them upside down in a light incubator, or in the light on a benchtop.
- 34 Keep monitoring plates for growth. Pick and purify colonies with a sterile toothpick or loop using appropriate aseptic techniques before they start merging together.
- 35 Please note that fungi can exhibit very diverse growth forms and behaviors. Yeasts might resemble and handle similarly to heterotrophic bacteria. Filamentous fungi may form spores and should hence be handled carefully to avoid persistent contamination.
- 36 Purification and passaging of heterotrophic fungi might have to be adapted depending on the organisms. For some organisms, it may be feasible to scrape off mycelium from the edge with a loop and use this to inoculate a fresh plate. For other fungi, it might be necessary to carefully cut a piece of agar containing the fungal crust and carefully transfer this agar 'plug' face-down onto a fresh plate for passaging.
- 37 Fungal isolates can be subsequently used for phenotyping, genomic and chemical characterization, and microbial co-culturing experiments.

Protocol references

- Amend, A. S., Barshis, D. J., & Oliver, T. A. (2012). Coral-associated marine fungi form novel lineages and heterogeneous assemblages. *The ISME Journal*, 6(7), 1291-1301.
- Bonacolta, A. M., Weiler, B. A., Porta-Fitó, T., Sweet, M., Keeling, P., & del Campo, J. (2023). Beyond the Symbiodiniaceae: diversity and role of microeukaryotic coral symbionts. *Coral Reefs*, 42(2), 567-577.
- Cárdenas, A., Raina, J. B., Pogoreutz, C., Rädcker, N., Bougoure, J., Guagliardo, P., ... & Voolstra, C. R. (2022). Greater functional diversity and redundancy of coral endolithic microbiomes align with lower coral bleaching susceptibility. *The ISME journal*, 16(10), 2406-2420.
- Chong, G., Tsai, S., Wang, L. H., Huang, C. Y., & Lin, C. (2016). Cryopreservation of the gorgonian endosymbiont Symbiodinium. *Scientific Reports*, 6(1), 18816.
- Galkiewicz, J. P., Stellick, S. H., Gray, M. A., & Kellogg, C. A. (2012). Cultured fungal associates from the deep-sea coral *Lophelia pertusa*. *Deep Sea Research Part I: Oceanographic Research Papers*, 67, 12-20.
- Heuer, H., Krsek, M., Baker, P., Smalla, K., & Wellington, E. (1997). Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. *Applied and environmental microbiology*, 63(8), 3233-3241.
- Hochart, C., Paoli, L., Ruscheweyh, H. J., Salazar, G., Boissin, E., Romac, S., ... & Galand, P. E. (2023). Ecology of Endozoicomonadaceae in three coral genera across the Pacific Ocean. *Nature Communications*, 14(1), 3037.
- Keller-Costa, T., Kozma, L., Silva, S. G., Toscan, R., Gonçalves, J., Lago-Lestón, A., ... & Costa, R. (2022). Metagenomics-resolved genomics provides novel insights into chitin turnover, metabolic specialization, and niche partitioning in the octocoral microbiome. *Microbiome*, 10(1), 151.
- Keller-Costa, T., Lago-Lestón, A., Saraiva, J. P., Toscan, R., Silva, S. G., Gonçalves, J., ... & Costa, R. (2021). Metagenomic insights into the taxonomy, function, and dysbiosis of prokaryotic communities in octocorals. *Microbiome*, 9, 1-21.
- Kjer, J., Debbab, A., Aly, A. H., & Proksch, P. (2010). Methods for isolation of marine-derived endophytic fungi and their bioactive secondary products. *Nature protocols*, 5(3), 479-490.
- Marchese, P., Garzoli, L., Young, R., Allcock, L., Barry, F., Tuohy, M., & Murphy, M. (2021). Fungi populate deep-sea coral gardens as well as marine sediments in the Irish Atlantic Ocean. *Environmental Microbiology*, 23(8), 4168-4184.
- Nübel, U., Garcia-Pichel, F., & Muyzer, G. (1997). PCR primers to amplify 16S rRNA genes from cyanobacteria. *Applied and environmental microbiology*, 63(8), 3327-3332.

LaJeunesse, T. C., Wiedenmann, J., Casado-Amezúa, P., D'ambra, I., Turnham, K. E., Nitschke, M. R., ... & Suggett, D. J. (2022). Revival of Philozoon Geddes for host-specialized dinoflagellates, 'zooxanthellae', in animals from coastal temperate zones of northern and southern hemispheres. *European Journal of Phycology*, 57(2), 166-180.

Lane, D. J., Pace, B., Olsen, G. J., Stahl, D. A., Sogin, M. L., & Pace, N. R. (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proceedings of the National Academy of Sciences*, 82(20), 6955-6959.

Pogoreutz, C., & Ziegler, M. (2024). Frenemies on the reef? Resolving the coral-Endozoicomonas association. *Trends in Microbiology*, 32(5), 422-434.

Raghukumar, S. (2017). *Fungi in coastal and oceanic marine ecosystems* (Vol. 378). New York, NY, USA:: Springer.

Raghukumar, C. (Ed.). (2012). *Biology of marine fungi* (Vol. 53). Springer Science & Business Media.

Rohwer, F., Seguritan, V., Azam, F., & Knowlton, N. (2002). Diversity and distribution of coral-associated bacteria. *Marine Ecology Progress Series*, 243, 1-10.

Roik, A., Reverter, M., & Pogoreutz, C. (2022). A roadmap to understanding diversity and function of coral reef-associated fungi. *FEMS Microbiology Reviews*, 46(6), fuac028.

Roscoff Culture Collection. (<https://roscoff-culture-collection.org/protocols>). Last visit 29 November 2024.

Soffer, N., Gibbs, P. D. L., & Baker, A. C. (2008, July). Practical applications of contaminant-free Symbiodinium cultures grown on solid media. In *Proc. 11th International Coral Reef Symposium* (pp. 159-163).

Sweet, M., Villela, H., Keller-Costa, T., Costa, R., Romano, S., Bourne, D. G., ... & Peixoto, R. (2021). Insights into the cultured bacterial fraction of corals. *Msystems*, 6(3), 10-1128.

Usher, K. M., Kaksonen, A. H., & MacLeod, I. D. (2014). Marine rust tubercles harbour iron corroding archaea and sulphate reducing bacteria. *Corrosion Science*, 83, 189-197.

Vega Thurber, R., Willner-Hall, D., Rodriguez-Mueller, B., Desnues, C., Edwards, R. A., Angly, F., ... & Rohwer, F. (2009). Metagenomic analysis of stressed coral holobionts. *Environmental microbiology*, 11(8), 2148-2163.

Voolstra, C. R., Raina, J. B., Dörr, M., Cárdenas, A., Pogoreutz, C., Silveira, C. B., ... & Peixoto, R. S. (2024). The coral microbiome in sickness, in health and in a changing world. *Nature Reviews Microbiology*, 1-16.

Wegley-Kelly, L., Edwards, R., Rodriguez-Brito, B., Liu, H., & Rohwer, F. (2007). Metagenomic analysis of the microbial community associated with the coral *Porites astreoides*. *Environmental microbiology*, 9(11), 2707-



2719.

Xiang, T., Hambleton, E. A., DeNofrio, J. C., Pringle, J. R., & Grossman, A. R. (2013). Isolation of clonal axenic strains of the symbiotic dinoflagellate *Symbiodinium* and their growth and host specificity¹. *Journal of Phycology*, 49(3), 447-458.