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Hidden Depths - Secondary metabolites of deep-sea fungi are a promising source of novel antimicrobials

Laurence Meslet-Cladière

Metabolism is the set of chemical reactions that take place within an organism to enable it to stay alive, to reproduce, to develop and to respond to stimuli from the environment. Primary metabolism is essential in living organisms. In contrast, secondary metabolism is not essential but the products of these pathways (known as the secondary metabolites) often have important biological properties that we can exploit, for example as antimicrobials (antibiotics, antivirals or antifungals) anticancer molecules or antispasmodics.

Perhaps one of the best-known secondary metabolites is 'penicillin' a molecule produced by the fungus *Penicillium.* The antibacterial activity of this fungus was identified in 1928 but the active compound was only isolated in the 1940s. This antibiotic is still used worldwide, however, as many bacteria have developed resistance to antibiotics we must find new molecules. One solution is to find novel sources of antibiotic-producing organisms by exploring new environments. Oceans cover about 70% of the Earth's surface. These are unexplored environments because some areas beneath the ocean are difficult to access, such as sub-seafloor sediments, hydrothermal vents or the abyssal zone (the zone at depths of 4000-6000 m). In these marine ecosystems there is a great diversity of marine organisms, including fungi. One obvious and exciting question is "Are these marine fungi able to produce antibiotics like terrestrial fungi?" To answer this question researchers follow a specific workflow (**Figure 1**). First oceanographic cruises are conducted to collect samples. For example, the International Ocean Discovery Program (https://www.iodp.org/) which explores the earth under the sea. The microorganisms extracted from these samples are then taken to research laboratories to be isolated and studied.

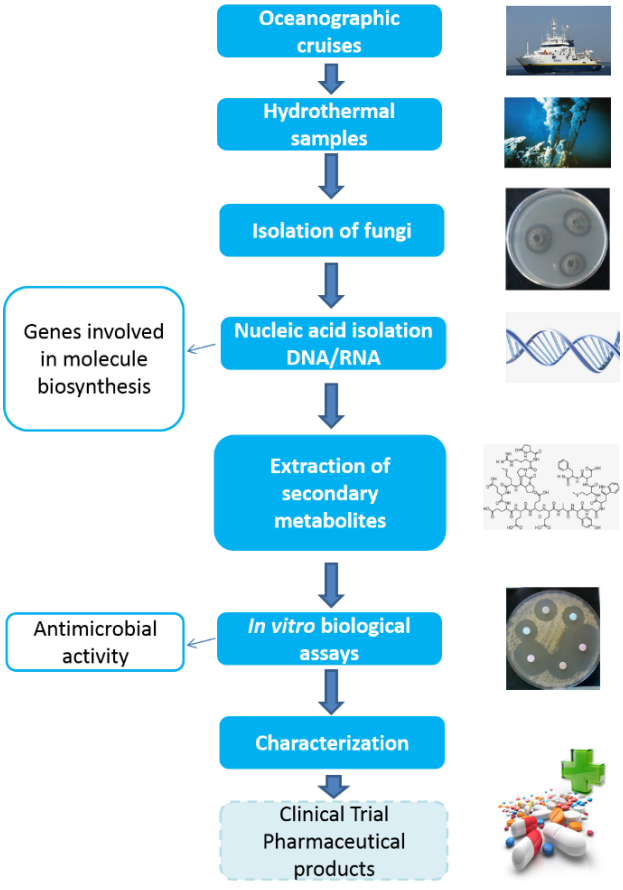

Figure 1. Schematic diagram depicting the methodology for complete screening of marine fungal strains, from isolation to the development of a pharmaceutical product. The entire process from discovery to use in the clinic can take decades. Image of fungal growth on plate kindly provided by Gaëtan Burgaud.

In my laboratory we study deep-sea fungi isolated from sediments that extend up to 2000 metres below the sea floor. Among these are species such as *Penicillium*, *Fusarium* or *Aspergillus*. These species can also be found in terrestrial environments but we are especially interested in the marine strains as they might produce novel secondary metabolites. Despite their deep-sea origins we are able to grow many of these strains in the laboratory by varying the temperature and including sea salt in the growth medium. Once grown, we extract the nucleic acids (DNA or RNA) of the fungi and then, using DNA sequencing, we are able to obtain the sequence of their entire genome (genetic material). These sequences provide us with insight into their similarity to terrestrial fungi. In addition, using our knowledge of the DNA sequences that code for components of metabolic pathways in other organisms, we can identify whether these marine fungi can synthesize secondary metabolites.

Next, we try to purify these metabolites. First we have to understand what type of molecules the fungi produce in order to identify the best protocol for extracting and purifying them. Refining the protocols can be a long and difficult task and often there are molecules we cannot purify. In this case, we try to find ways to synthesise them *in vitro*. Purified molecules or fungal extracts are then analysed to determine whether they have antibacterial activity. This is done using standardised tests called 'disc diffusion', where the secondary metabolites are added to paper discs, and these are placed onto bacteria on agar plates. Antibacterial activity is observed as a zone of no-bacterial growth around the disc (see **Figure 1**).

Using this approach, we have found that 33% of the marine fungi we have studied can synthesize bioactive compounds with activities against at least one disease-causing bacterial species, such as *Escherichia coli*, *Staphylococcus aureus* or *Pseudomonas aeruginosa*. Our challenge now is to identify whether any of these antimicrobial molecules are novel. Our hope is that novel compounds with promising antimicrobial activity may be taken through the long and rigorous process involved in the development of new antibiotics, and that they may one day be used to combat resistant microbes.

In summary, marine fungi represent an interesting, untapped resource with immense biotechnological potential. To access and exploit this potential requires a dedicated team of scientists with different expertise (*e.g*. chemistry, microbiology, biochemistry, pharmacology) working together to unlock the secrets of these fascinating organisms.

REFERENCE: Silber *et al*., From Discovery to Production: Biotechnology of Marine Fungi for the production of New Antibiotics. (2016) Mar. Drugs. 14: 137. doi: 10.3390/md14070137

AUTHOR PROFILE

Laurence Meslet-Cladière is an Associate Professor at the Laboratoire Universitaire de Biodiversité et d'Ecologie Microbienne (LUBEM) in Brittany, France. She obtained her PhD in 2003 and subsequently worked in Paris studying Archaea and algae. In 2010 she moved to Brittany, to continue her work on algae. Her current work aims to characterise the secondary metabolic pathways of marine fungi, and identify their potential for use in biotechnology or medicine.