New Medicines from Microbes of the Oceans

Miss: the sea gives us fish for food and water for swimming; do we get other useful things from the ocean?

Planet Ocean

Lone Gram

Department of Biotechnology and Biomedicine, Technical University of Denmark

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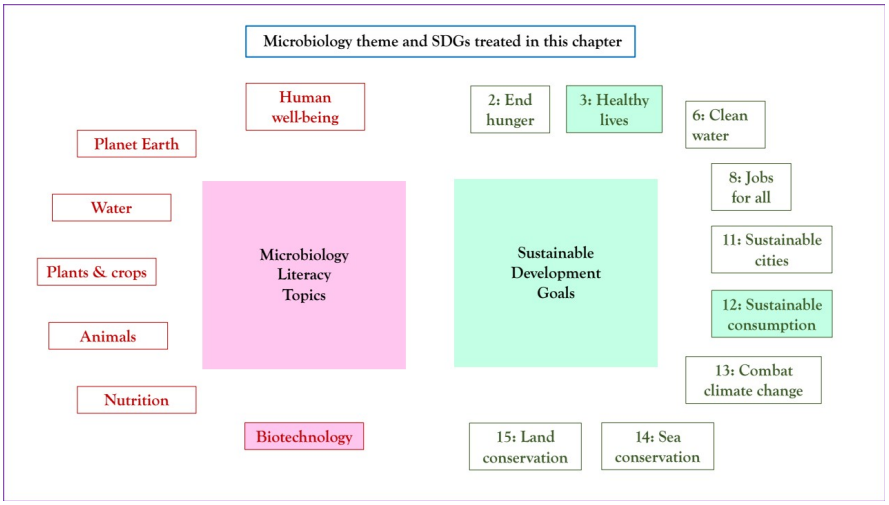
Storyline

In 1928, Alexander Fleming discovered that a microorganism, a filamentous fungus, produced a chemical that could kill bacteria. Such compounds, called antibiotics, have since been found in many other microorganisms and plants, and have revolutionized our treatment of infectious diseases. Also, other medicines e.g. for treating cancer can be produced by microorganisms and plants. During the decades following 1928, a multitude of new antibiotics were identified and most were produced by microorganisms. Unfortunately, bacteria are smart. When they are met with unpleasant conditions, they modify their genetic code and slowly, sometimes rapidly, evolve mechanisms to survive and grow when antibiotics are present; they become resistant. This may mean that in the future, simple infections can no longer be treated and we therefore constantly need to find and develop new antibiotics. Soil dwelling microorganisms, such as the bacterium *Streptomyces* or filamentous fungi, have historically been our major source of novel antibiotics. Unfortunately, we seem to be re-discovering the same chemicals by continuous screening of these sources. Therefore, exploring microorganisms from other environments than soil may lead to discovery of truly new antibiotic compounds. Oceans cover 70% of the globe and make up 95% of the biosphere — and we could say that we live on planet Ocean not planet Earth. Microorganisms in the ocean differ from those in soil as they have evolved to tolerate salt and often high pressure and low nutrient conditions. Also, oceans are rich in halogens such as bromide and iodine. Therefore, some of the chemicals produced by bacteria from the ocean are likely different from the chemicals produced by terrestrial bacteria, and exploring marine bacteria as sources of novel antibiotics and other drugs is a rapidly growing research and commercial area.

The Microbiology and Societal Context

The microbiology: the marine microbiome and its roles; microorganisms as producers of secondary metabolites; antibiotic resistance; antibiotic discovery, bioassay guided fractionation, genome mining, biotechnology and production. *And peripherally for completeness of the story*: infectious disease treatment; strategies of pharma companies; policy decisions on antibiotic development; regulatory issues related to antibiotic approval. *Sustainability issues*: Nagoya Protocol on Access and Benefit Sharing (ABS); exploitation of the seas; reduction in use of antibiotics.



New Medicines from Microbes of the Oceans: the Microbiology

1. ***Microorganisms as producers of antibiotics.*** In 1928, Alexander Fleming discovered that a microorganism, a filamentous fungus, produced a chemical that could kill bacteria. He had cultured staphylococcal bacteria on a nutrient plate and, by accident, it was contaminated with a *Penicillium* fungus. Fleming noticed that the area around the fungal colony was cleared of bacteria (Figure 1). The fungus produced penicillin and Fleming was awarded the Nobel Prize in physiology and medicine in 1945 for this discovery.

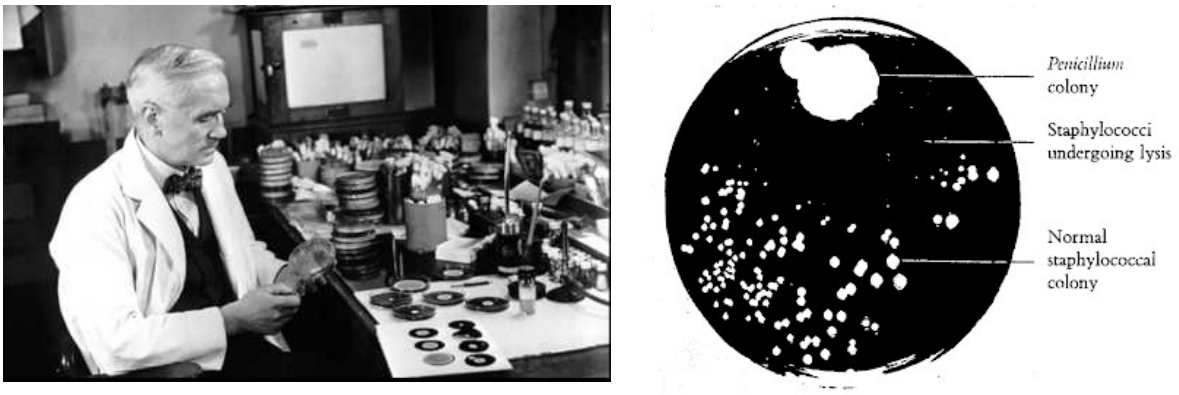


Figure 1. Dr. Alexander Fleming in his laboratory (left) and the original plate with staphylococcal bacteria and the penicillin producing fungus (right)

Following Fleming's discovery, other microorganisms were searched (see section 2) for their ability to produce antibiotics, and series of new compounds were discovered, especially from soil bacteria belonging to the phylum Actinobacteria, and especially from the genus *Streptomyces*. Also, several antibiotics were discovered from filamentous fungi. Antibiotics can either be bacteriostatic – that is, they *inhibit* the growth of a bacterium, – or they can be bacteriocidal – that is, they *kill* bacteria. Different antibiotics have different chemical structures; there are several different classes, and their mode-of-action (they way they inhibit or kill) differs. They can inhibit the cell wall synthesis, destroy the cell membrane, inhibit protein biosynthesis, inhibit nucleic acid (RNA and DNA) synthesis, or inhibit metabolic pathways.

Many bacteria and fungi produce antibiotics, but some genera are more potent producers than others. Many species of the Gram-positive genus *Streptomyces* are prolific producers of antibiotics, and between 10 and 15% of their genetic code encode activities related to the production of such compounds. Since these bacteria also have relatively large genomes (for

bacterial standards), each organism typically has the potential to produce between 20 and 40 different compounds. However, also bacteria with smaller genomes, such as some species of the marine genus *Pseudoalteromonas*, produce a battery of antibiotics, and also dedicate 10-15% of their genetic code to antibiotic production-related functions, being capable of producing 15-25 antibiotic compounds.

2. *How do we find antibiotic producing microorganisms?* Fleming's original discovery in essence became one of the methods we use today to search for antibiotic compounds. Classically, potential producing microorganisms will be isolated from any kind of environment – soil, plants, food, lakes and oceans – and then cultured on a plate in which target bacteria (pathogens) are also grown. If the target bacterium does not divide because of antibiotic production, a zone of inhibition will be seen. This only tells us that there is an activity, but not what the chemical compound is. When searching for antibiotics from natural sources, we then continue by a so-called *bio-assay guided fractionation*. Here, the producing microorganism is grown and all the compounds it has produced (the metabolome) are extracted with organic solvents. The extract is then split into different fractions (for instance, dependent on molecular size of the many different compounds) using liquid or gas chromatography (LC or GC) and each fraction is then tested for antibiotic activity. Fractions with activity are further split, tested and this goes on and on until a single compound that exhibits activity inhibiting bacteria is left. This can then be characterized chemically in detail and its structure determined by Nuclear Magnetic Resonance (NMR) (Figure 2).

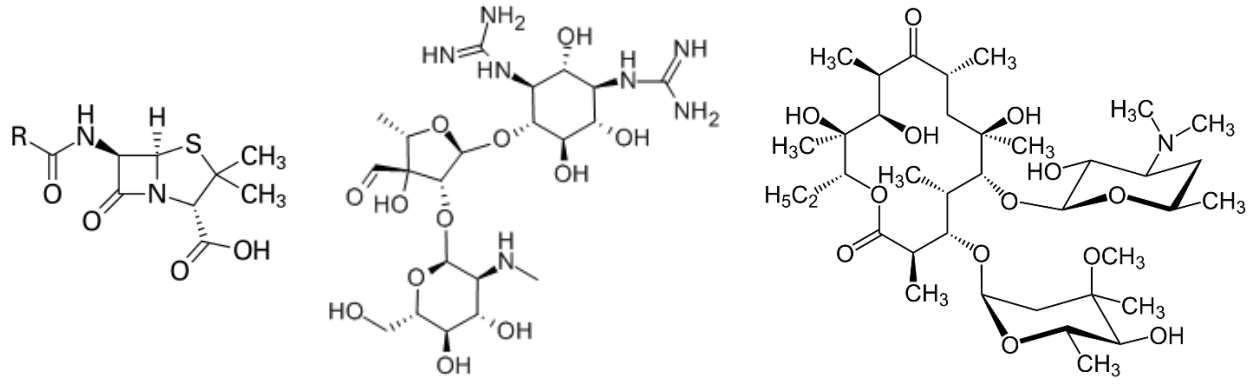


Figure 2. Three examples of antibiotics, from left to right: penicillin (produced by a filamentous fungus, *Penicillium*), streptomycin (from the Gram-positive bacterium *Streptomyces*) and erythromycin (produced by the Gram-positive bacterium, *Saccharopolyspora*).

Antibiotics are chemically complex molecules and are typically synthesized through a series (20-30 steps) of chemical reactions each involving specific enzymatic steps. Whilst antibiotics are chemically diverse, many of them share core structures of modular nature, and hence the genetic code for core enzymatic reactions is conserved across orders, genera and species. With the explosion in gene sequencing, nowadays, it is possible for many laboratories to sequence the genomes of bacteria, often hundreds or even thousands in a short time. In parallel, scientists have developed mathematical tools (algorithms) that allow searching of the sequences of genomes for specific patterns, including those patterns typical of antibiotic production. Such "genome mining" is today an integral part of antibiotic discovery, before any chemical analyses is done, as it can point to microorganisms with a large potential for producing antibiotics.

3. ***The antibiotic resistance crisis: what can we do?*** Bacteria multiply by growing and dividing (1→2→4→8→16→→→1,000,000,000… and more) and in each cell division, the genome of the bacterium is copied (replicated). Tiny errors (mutations) occur during this replication and, whilst most of them have no effect on the bacteria, some confer the organisms an advantage under changing growth conditions. Thus, if bacteria are exposed to antibiotics, some will mutate and develop mechanisms that allow them to circumvent the action of the antibiotic, e.g. degrading the antibiotic or altering the antibiotic target. They have become resistant. In some bacteria, the genes coding for the resistance are located on small pieces of DNA that can be transferred to other bacteria, so they can share the resistance. The more we have used antibiotics, both in the clinic and also in the food production industry, the more bacteria have become resistant and today we may face an era where we will not be able to treat even simple infections.

Many strategies are needed to counter antibiotic resistance: limit the use of antibiotics to only the situations where they work and are essential and ensure good hygiene and management practices. However, even with strict measures, we will need new antibiotics. As discussed, most of the antibiotics we use today have been found in soil bacteria and whilst there are likely more to be discovered in these bacteria, we should also search for microorganisms and compounds from completely new, not explored environments. Different microorganisms will live and thrive under different conditions, and we assume that the chemicals they produce differ, according to the chemical and physical nature of the ecosystem. Thus, searching in environments different from soil is a good strategy.

4. ***Microorganisms in the ocean, what do they do?*** Oceans are one of the hitherto unexplored environments where we search for microorganisms that can produce new antibiotics. Oceans cover 70% of the planet and, because of the depth, they make up for 95% of the actual biosphere (the area in which living organisms can exist). The sea is visibly full of plants (sea weed) and animals (thinks of fish, dolphins, and shrimp), but it is also the home of billions and billions of microorganisms. Actually, there are one billion bacteria in just one liter of seawater. Marine bacteria are essential for all life on earth. They degrade and grow on organic material, such as dead plants and animals, becoming food for algae, that are then eaten by copepods, that are eaten by shrimp, that are eaten by fish, that are eaten by mammals: the food-web of the ocean (Figure 3). Also, many marine bacteria are photosynthetic, so they use light as energy source and capture CO_2 from the atmosphere, thereby acting as a sink for an important greenhouse gas that causes global warming and simultaneously providing us with much of the oxygen we breathe. Some of the ocean bacteria are also pathogenic, meaning they can infect other organisms causing disease. Some cause disease in algae or in whales and some cause infection in humans.

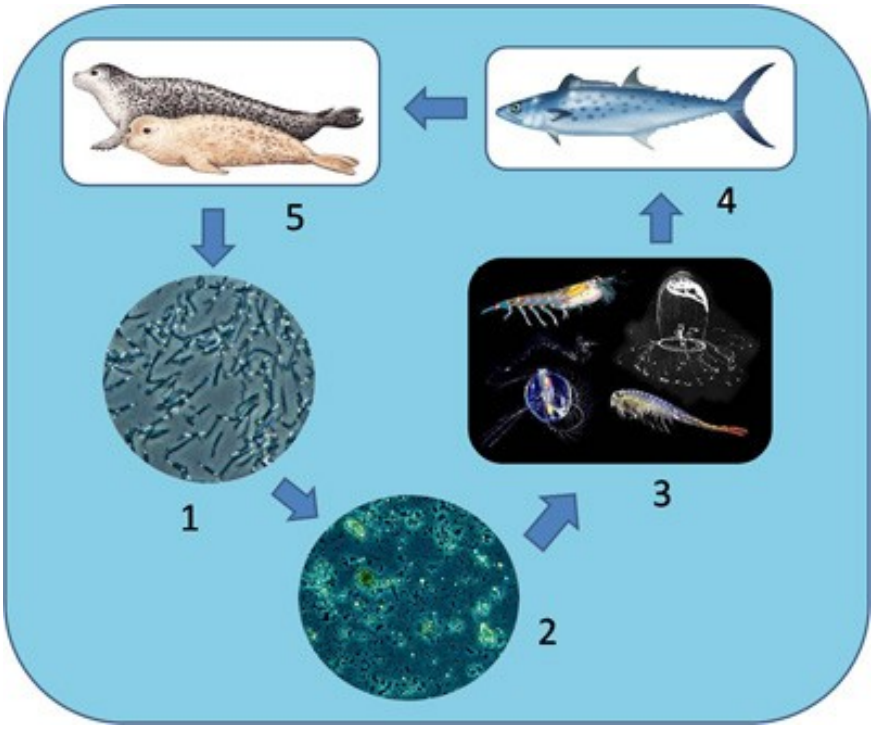


Figure 3. The marine food web. Microorganisms (1) are eaten by plankton (2) that rea eaten by crustaceans and larvae (3) that are eaten by fish (4) that are eaten by mammals (5). From https://seos-project.eu/oceancolour/oceancolour-c02-p03.html

5. *Marine bacteria as source of antibiotics – and other medicines.* In addition to being essential parts of the so-called biogeochemical cycle (cycling of chemical compounds), or being pathogenic, we have during the past decades learned that marine bacteria can also be very useful for us from a pharmaceutical and biotechnological perspective. In other words: many marine bacteria produce very useful compounds. Some produce useful enzymes, proteins that can speed up chemical reactions, for instance degrading insoluble compounds such as chitin from shrimp shells into small soluble useful nutrients. Others produce gelling compounds that can be used in the food industry. And yet others produce chemicals that have antibiotic activity. Several of these compounds with antibiotic activity have unique chemical components that are typical of the marine environment. For instance, some species of the genus *Pseudoalteromonas* produces several compounds with a lot of bromine, a halogen typical of oceans (Figure 4).

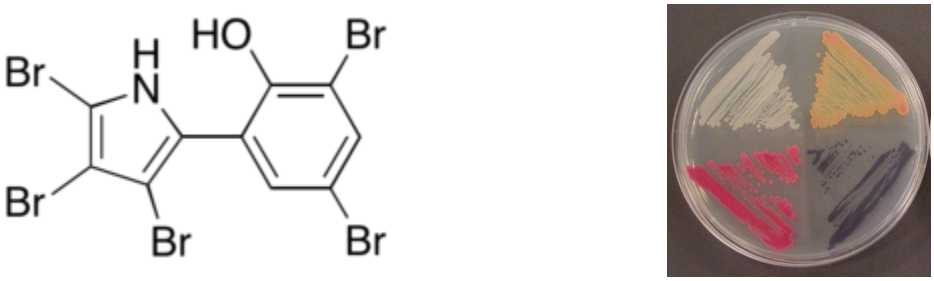


Figure 4. Pentabromopseudilin (left) produced by several pigmented *Pseudoalteromonas* species (right)

Marine bacteria not only produce compounds with antibiotic activity, but several also produce compounds with anti-cancer activity. For instance, the Spanish company, PharmaMar, is dedicated to exploring marine environments for anticancer agents. One example is the product called Yondelis that is used to treat soft tissue cancer and which originally was discovered in a tunicate (a small invertebrate animal), but is actually believed to be produced by one of the symbiotic bacteria of the tunicate (Figure 5). Also, the marine *Streptomyces*, *Salinospora*, produces a molecule called salinosporamide A that can act on cancers of plasma cells. This molecule is currently being tested in patients.

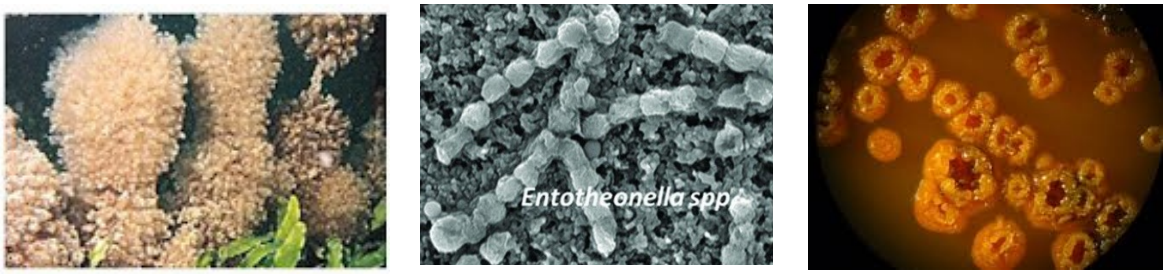


Figure 5. The tunicate *Ecteinascidia turbinata* (left) from which the anticancer drug Yondelis was first isolated and a bacterium (middle) that is likely the true producer. Colonies of *Salinospora* (right) that produce a cancer drug, Salinosporamide A, currently in clinical trials. Photo of *Endoecteinascidia* from Trinidad et al. 2015

Relevance to Sustainable Development Goals and Grand Challenges

- ***Goal 3.*** **Ensure healthy lives and promote well-being for all at all ages.** According to the WHO, antibiotic resistance is one of the major challenges facing humanity. To enable treatment of infectious diseases in the future, new antibiotics must be discovered. Hence, exploring new environments for antibiotics can facilitate finding novel chemistry and compounds, and enable treatment of infectious diseases in the future.
- ***Goal 12.*** **Ensure sustainable consumption and production patterns.** Quite a number of medicaments, including antibiotics, have been isolated from larger organisms (plants, animals) and, whilst some compounds can be chemically synthesised, others require constant harvesting and extraction, potentially causing extinction of the organism. Therefore, exploiting marine microbes for drug production is a sustainable process, since the native producer can typically be grown on marine polymers or the genes encoding antibiotics can be transferred to well-known host-cells (bacteria, fungi, mammalian cells) for large scale production not requiring constant harvesting from the wild.

Pupil Participation

1. ***Class discussion***
   - **a.** What kind of living organisms live in the sea?
   - **b.** Can you see the bacteria in the ocean?
   - **c.** Why do you think bacteria produce antibiotics; compounds that kill other bacteria?

d. Have you been treated with antibiotics?
e. Can you – and should you - use antibiotics against all infectious diseases?

2. ***Pupil stakeholder awareness***
   a. How will antibiotic resistance affect your life?
   b. How can we, as society, reduce antibiotic resistance?
   c. How can we find and produce new antibiotics?

3. ***Exercises***
   a. What options are there for using less antibiotics?
   b. Explain why antibiotics produced by marine bacteria could be different from antibiotics produced by soil bacteria.

The Evidence Base, Further Reading and Teaching Aids

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Trindade, M., L.J. van Zyl, J. N. -Fernández and A. Abd Elrazak 2015. Targeted metagenomics as a tool to tap into marine natural product diversity for the discovery and production of drug candidates. *Front. Microbiol.* 6: article890 https://doi.org/10.3389/fmicb.2015.00890

Glossary

Antibiotic: is a type of antimicrobial substance active against bacteria. It is the most important type of antibacterial agent for fighting bacterial infections, and antibiotic medications are widely used in the treatment and prevention of such infections.

Anticancer drug is a medicine that is effective in the treatment of malignant, or cancerous, disease. There are several major classes of anticancer compound; these include alkylating agents, antimetabolites, natural products, and hormones.

Bacteriocidal: an antimicrobial compound that kills bacterial cells

Bacteriostatic: an antimicrobial compound that prevents growth of bacterial cells

Bio-assay-guided fractionation: An experimental protocol to isolate a pure chemical agent (with a particular bioactivity) from natural origin. It consists of a step-by-step separation of extracted components based on differences in their physico- chemical properties, and assessing the biological activity, followed by next round of separation and assaying.

Gas Chromatography: a process in analytical chemistry where compounds that can be vaporized without decomposition are separated and analyzed. Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture.

Liquid Chromatography: also a technique in analytical chemistry used to separate a sample (an extract with many chemical components) into its individual parts. This separation occurs based on the interactions of the sample with the mobile and stationary phases.

Resistance: bacteria or other infectious agents that have evolved to tolerate antibiotics (or other antimicrobials) and can survive treatments that otherwise would kill or inhibit the agent.