STUDY ON NEW ANTIBIOTICS

The arms race against bacteria

The world urgently needs new antibiotics. Wageningen researchers are looking for inspiration and for promising substances in deep-sea sponges, soil bacteria and plant sprouts.

TEXT RIK NIJLAND **PHOTO** ANP

In the brand-new Laboratory for Microbiology on Wageningen campus, Anak Indraningrat holds up two glass test tubes containing bright orange and muted red pieces of sponge. Indra was on board when the submersible from the Curaçao Sea Aquarium plucked these specimens from the Caribbean seabed at a depth of over 200 metres. 'In Wageningen I am going to study the extracts from these sponges for their anti-microbial effects,' explains the Indonesian PhD candidate. 'Against *E.coli* for example.'

All around the world, researchers are searching for new compounds with which to keep bacteria in check. Overuse of antibiotics is making more and more pathogenic bacteria resistant to these drugs. As a consequence diseases which are still treatable at the moment, such as pneumonia and bladder infections, could become life-threatening. So new antibiotics are more than welcome.

CHEMICAL WARFARE

For several decades now, scientists have been studying sponges as potential sources of medical drugs. Sponges make certain unusual bioactive compounds with which to defend themselves against attackers. Sponges are primitive multicellular organisms without an immune system and they cannot get away if they are attacked. So they depend on chemical warfare to defend themselves. A handful of the compounds which these sea creatures use have already been registered as drugs for treating cancer. But scientists suspect there are many more interesting candidates for use in human medicine. Indra and his supervisor Detmer Sipkema published an article in *Marine Drugs* in May with an overview of the antibacterial and antiviral compounds discovered in sponges over the past few decades. They hope to be able to add a few new candidates themselves.

Sipkema did his PhD in Wageningen on the cultivation of sponges, but during a postdoc period at Berkeley he turned his attention to bacteria. It has now become clear that most of the bioactive compounds are made not by the sponge itself but by bacteria it harbours.

Since then Sipkema has studied the interaction between bacteria and sponges, still uncharted territory. 'In the submersible we go deeper than 30 metres, the usual depth limit for submersibles. Below that we are in a whole new world with new sponges, as well as familiar species which are just different in colour in deeper waters. That means there are probably different bacteria in them and other substances too. That has never been studied.'

KILOMETRES DEEP

He is currently working with sponges from Curacao, from as deep as about 250 metres below sea level, but he will soon be tackling specimens from even greater depths than that. Sipkema participates in the Norwegian project SponGES, EU-funded to the tune of 10 million euros, which researches sponge fields at depths of between one and seven kilometres on the Mid-Atlantic Ridge. 'They occur in places where scarce minerals may be mined in future. We want to try to get a better understanding of those ecosystems,' says Sipkema. 'It is my role to find out what kinds of micro-organisms live in those biotopes, and what interesting substances they produce.'

'Some of the compounds from plant sprouts are just as powerful as conventional antibiotics'

Among the subjects Sipkema's team studies are the anti-microbial effects of the substances, and which genes are involved in their production. If a suitable potential antibiotic is identified, he will hand over the substance to a research group in Sweden which specializes in clarifying chemical structures.

But even then, pharmaceutical companies will not be raring to get involved. The quantities of bioactive substances made by sponges are negligible, and to get enough to carry out tests, pharmaceutical companies would practically have to exhaust the ocean's supply. Yet chemical synthesis of these usually complex bioactive compounds is extremely expensive or even impossible, says Sipkema. 'As long as we don't have a method of cultivating enough material to at least do clinical tests, no pharmaceutical company is going to be keen.'

So elsewhere in Wageningen, work has been going on

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The search is on for new antibiotics in soya sprouts (above), which manufacture bioactive substances when they encounter a fungus, and in fungal filaments (below), which make these substances when competing with other fungus species.

for years on cultivating sponges or sponge cells in tissue culture. It remains a tricky process. Sipkema focuses primarily on breeding the bacteria involved. If only you have enough of them, you can manufacture enough of an antibiotic. But how do you do that? How do you find out the preferences of these prima donnas that are accustomed to the depths of the oceans and to high pressure? Trial and error produced an occasional success but the bacteria from the sponges are not usually amenable to being isolated and cultured. This also made it impossible to study their DNA for hints about the conditions they require.

PUZZLES

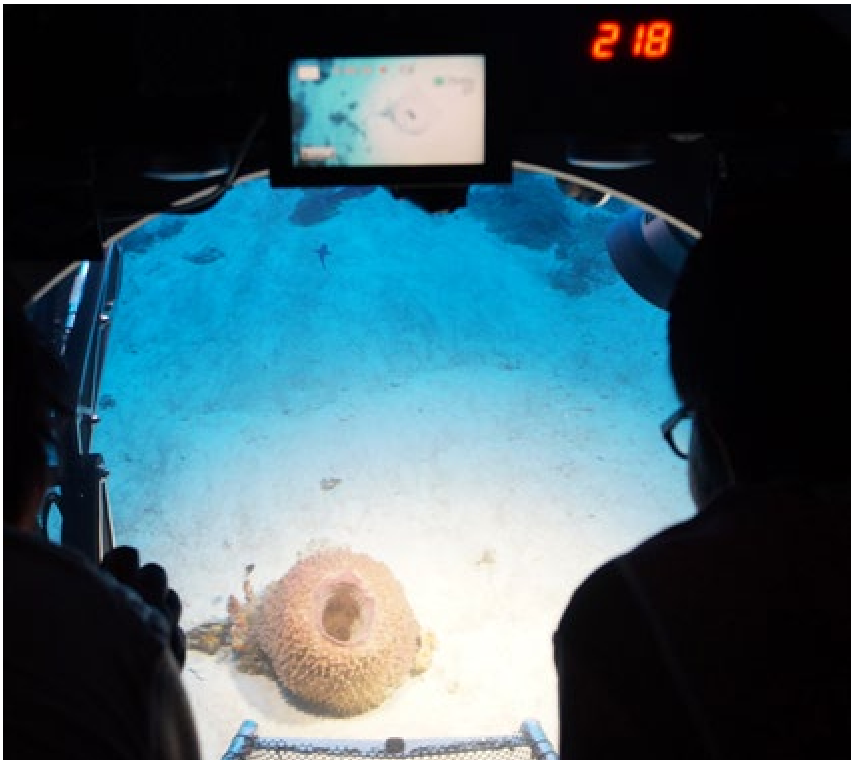
Sipkema has recently started using a new trick to get around this chicken-and-egg problem: reverse engineering. 'For this we sequence all the DNA, both of the sponge and of the bacteria living in it. Out of that mixed bag of DNA fragments we try to reconstruct the genome of an individual bacterium. That is like having a lot of puzzle pieces but not knowing how many different puzzles they are part of. But it works pretty well. Once you have the genome of a bacterium you can say something about the breeding conditions it requires.'

Using this approach Sipkema has found out in the case of one sponge bacterium that it grows extremely slowly and with the help of carbon dioxide. 'No wonder we didn't find that one in our breeding experiments. It needs more time and uses only CO_2 as a source of carbon. This new technique makes me optimistic. Maybe we will eventually be able to manufacture enough bioactive material in the lab.'

The researcher sees the ocean as by far the most promising potential source of new bacteria. 'Marine organisms have not been studied nearly as much as those on land. Last year it was found that at least 90 percent of the genes of DNA fragments fished out of the sea at 500 metres down were unknown. There's a much bigger chance of finding really new antibacterial mechanisms in the sea than on land.'

THOROUGH SCREENING

But Wietse de Boer, a researcher at the Dutch Institute for Ecology (NIOO-KNAW) in Wageningen, has by no means lost interest in the possibilities on land. Although micro-organisms and fungi have been thor- >



PHOTO'S WUR, SHUTTERSTOCK

Sponges photographed from the Curaçao Sea Aquarium's mini-submarine Curasub. Pictured above right is the submarine itself with PhD candidate Indra (left).

oughly screened for bioactive compounds in recent decades, De Boer does not think their potential has been exhausted. 'That screening is often done one test at a time. You have an organism, a fungus perhaps, and you test it against a pathogen. If nothing happens, that species is ruled out.' To demonstrate that this is a premature conclusion, De Boer shows a sawn-off birch trunk in his office. Two kinds of fungus, a tinder and a birch polypore, have moved into the tree. Where the fungal filaments touch each other, chemical warfare breaks out between the competing fungi. The frontlines can easily be made out because they are marked by extra melanin – a dark pigment which protects them against chemical attack – produced by the fungi.

In the mushroom which grows as the fungus's fruiting body on the bark of the tree, however, nothing happens. No special compounds are manufactured there. The arsenal is only activated if danger threatens. 'I want to use that knowledge to find out whether we can find new antibiotics like this, on the basis of the ecological reality that species compete,' says De Boer. He hopes to get research funding to do this. At the end of September the Dutch minister of Public Health Edith Schippers announced that her ministry would be investing an extra 6 million euros in research on new antibiotics.

De Boer, who is also special professor of Microbial Soil Ecology at Wageningen University & Research, has experience with a comparable approach to bacteria. 'We discovered six years ago that a *Pseudomonas soil* bacterium which does not manufacture any antibiotics on its own does do so when combined with other bacteria,' explains De Boer. 'They only produce antibiotics when necessary. They only shoot when they have an enemy in their sights.'

LITTLE INTEREST

Olaf Tyc got his PhD in Wageningen last month for a screening method for antibiotics in which bacteria are tested together. A number of those combinations of bacteria have delivered new components which have been tested successfully on pathogenic bacteria such as E. coli and *Staphylococcus.*

The chemical characteristics of these compounds are currently being investigated in Germany. At this stage De Boer is extremely cautious about the chances of success. 'In the past we've had a promising substance that was so unstable that it changed in appearance after every stage of refinement. Because of that uncertainty, the pharmaceutical industry is showing little interest at the moment. And then there is the fact that a new antibiotic

'There's a much bigger chance of finding really new antibacterial mechanisms in the sea than on land'

would probably only be prescribed in emergency cases and therefore only rarely, in order to prevent resistance developing. So it is not an interesting prospect financially.'

PRODUCING ANTIBIOTICS

Researcher Jean-Paul Vincken and his colleagues at the Wageningen Laboratory for Food Chemistry, headed by professor Harry Gruppen, have already taken the first step towards producing antibiotics. Working with scientists from Singapore, they are developing a method of producing new antibiotics that could be attractive to the industry.

The candidates for this were discovered in Wageningen, says Vincken. 'We've been doing research on isoflavonoids in soya for ten years. At first we focused mainly on the estrogenic effects of these molecules. These seemed promising for use in nutritional supplements that are anticarcinogenic or prevent hot flushes, for example. Four years ago we started looking into what else these substances do,' says Vincken.

'We had already noticed that soya bean sprouts which are affected by a fungus during germination manufacture ten times more isoflavonoids. And these compounds have a slightly different structure. It didn't take long to join the dots: they must have antimicrobial properties. It seems there is a defence mechanism in the sprouts which we can switch on through stress, in this case by introducing the fungus. Although plants have been extensively studied, these kinds of stress responses have hardly been looked at yet.'

In order to grow sprouts in the laboratory, Food Chemistry bought a malting machine: a stainless steel appliance the size of a large washing machine. Breweries use XXXL versions of this machine to germinate barley for beer production. The machine at Food Chemistry does the same with a maximum of five kilos of soya beans, and seeds of other plant species too. A spoonful of fungal spores is added as an ingredient. 'The conditions have to be precisely right,' says Vincken. 'The temperature and humidity must be such that the seeds germinate and the fungus thrives too.'

Potential antimicrobial substances are extracted from the sprouts and tested against bacteria. Next spring, Carla Araya-Cloutier will present her PhD thesis on the initial results. She scrutinized the isoflavonoids in soya bean sprouts as well as those from related plant species such as other beans, lupin and liquorice. Together with her colleague Milou van de Schans, she refined and described about 30 substances, and tested their effectiveness against *E. coli*, *Listeria* and MRSA, a resistant *Staphylococcus*.

'Some of our compounds are just as powerful as conventional antibiotics such as vancomycin or ampicillin,' says Vincken. The new components turn out to be effective against what are known as gram-positive bacteria. Most of these isoflavonoids probably bore holes in the cell membrane of the bacterium.

Gram-negative bacteria have a protective mechanism against being punctured. However, if the researchers simultaneously added a substance which switches off that protection, those bacteria bite the dust too. Two more PhD candidates are now studying sprouts of various brassicas and grains, looking at their effectiveness against bacteria.

The sprouts that come out of the small malting machine on the campus produce at the most a few tens of milligrams of potential antibiotic. That is not enough to be of interest to the pharmaceutical industry. With a view to being able to produce the newly discovered antibiotics on a large scale, Food Chemistry is working with a research group at Nanyang Technical University in Singapore on a yeast which can manufacture the isoflavonoids. In the pharmaceutical industry, biotechnological production in yeast is a tried and tested strategy for producing medical drugs.

To achieve this, all the plant genes required for the entire production process need to be built into yeast. 'We have now managed to take the first steps,' says Vincken.

If it really works, and the yeast turns out to be capable of making a new antibiotic on a large scale, Vincken expects some interest from the pharmaceutical industry. But that is some way off yet, he warns. 'The crucial question is of course: won't the yeast – itself a microorganism – die too if it produces an antimicrobial substance?' ■

Jean Paul Vincken is looking for additional funding for the research on new antibiotics. If you want to know more about this project or contribute to finding new antibiotics, visit the website: www.wur.eu/new-antibiotics