Quicker and cheaper toxicity checking of mussels

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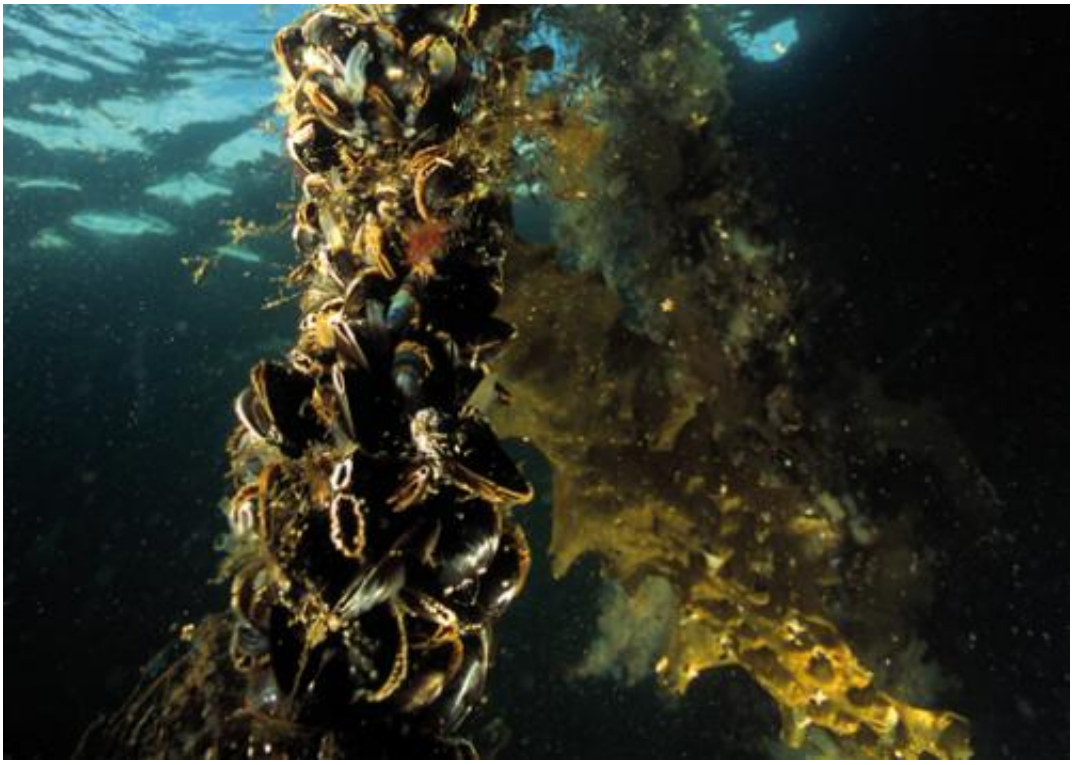

Safer self-harvesting: With the new toxicity check, people who harvest mussels on their own will have a quicker answer to whether the mussels are edible or not. Credit: Seafood, Per Eide Studio

A new discovery made at the University of Oslo, Norway, can make it far easier to check whether mussels are poisonous.

Poisonous mussels contain the extremely dangerous and paralyzing

neurotoxin saxitoxin. This neurotoxin is the cause of paralytic shellfish poisoning (PSP). The first symptoms include numbness in the mouth and lips, spreading to the face and neck. Then, the discomforts come in abundance: headache, dizziness, slurred speech and reduced motor function. The most serious poisonings can lead to fatal paralysis.

The toxin is 1000 times more potent than sarin nerve gas. Each year, 2 500 people worldwide are affected by this ominous poisoning. Mortality ranges from 2 to 14 per cent. Most cases are mild and pass without incident in three to four days.

The toxin stems from certain dangerous algae in a group known as dinoflagellates, including a sub-group called Alexandrium. The toxin accumulates in shellfish, since they feed by filtering seawater that occasionally contains poisonous algae.

Unfortunately for us humans, most shellfish are resistant to this toxin. If the number of algae surpasses a certain level, the mussels are defined as hazardous, although they may remain poisonous for several weeks after the poisonous algae have disappeared. Some claim that the mussels can store the toxin for up to two years, the explanation being that it takes time to be break down.

"The problem is that the toxin will not disappear even when the mussel is cooked. The toxin is flavourless. Since it takes a week to obtain a response, eating fresh, self-harvested mussels is something of a gamble," warns Russell Orr, researcher at the Department of Biosciences, University of Oslo. After five years of research, in which he was joined by Professor Kjetill S. Jakobsen and post-doc researcher Anke Stüken, they have developed a quick method that can determine within a few hours whether mussels are poisonous.

More dangerous abroad

In hot climates there are two extremely poisonous species of Alexandrium.

"The problem is especially great in tropical areas such as Indonesia, the Philippines, Thailand and Malaysia."

Moreover, they have no systems for checking whether the mussels are hazardous to eat. In addition, deaths have been recorded in Alaska, New Zealand, Guatemala, South Africa, Japan and the USA. In Spain they use mice to test whether the mussels are poisonous. This is the method approved by the EU.

"But even if Alexandrium is detected in water samples, this does not have to mean that the toxin is present. Not all species of Alexandrium produce the toxin, but it is extremely difficult to distinguish them from poisonous species."

The UiO researchers have also detected the evolutionary correlation between all species of Alexandrium.

Using DNA analysis, they can distinguish between poisonous species and harmless ones.

"The poisonous ones are the most common. Some of them are less toxic, whereas others are extremely toxic. We have also discovered a group of species that are toxic, but with today's methods are defined as non-toxic. In other words, the current methods are not completely safe. However, the amount of toxin produced by these algae is so small that they still cannot be deemed as poisonous to humans."

Five of the species are so similar that they cannot be distinguished morphologically.

"So it might be that some of them are not poisonous, even though the morphological method will indicate that they are. It's important to be able to determine this, so as to avoid issuing unnecessary warnings," says Russell Orr.

The poison gene has been found

In cooperation with the University of New South Wales in Sydney, UiO has identified the gene responsible for the production of the toxin.

"Testing of water samples from the Oslo fjord, Spain and Australia shows that our method has a higher sensitivity than the methods that are used today. The new method permits a reduction in costs, combined with more frequent examinations in a higher number of test stations along the Norwegian coast to prevent poisonings and risks in eating seafood," says Kjetill S. Jakobsen, Professor at the Department of Biosciences.

And the DNA test leaves no doubt as to the source of the toxin: it comes from the algae, not from bacteria inside the mussel.

"Previously, there were many theories about the origin of the toxin. Many believed that the toxin was produced by small bacteria inside the mussel, but this is untrue. We have discovered that the genes producing the toxin stem from a eukaryotic species," Russell Orr says.

Life on Earth can be divided into prokaryotic and eukaryotic species. Prokaryotic species, to which bacteria belong, are the simplest form of living organisms. They have no organelles inside their cells. Eukaryotic species, on the other hand, such as algae, mussels and humans, have such membranes.

Prokaryotic and eukaryotic species have different genes. The genes of eukaryotic species have a special sequence on one side of the gene. In

addition, Alexandrium algae have a special sequence at the beginning of the gene.

"Genes from bacteria lack this. We can therefore deduce that the toxin was made by the alga. But to complicate things further: bacteria are found also on the Alexandrium algae. Many are of the opinion that these bacteria cause Alexandrium to produce the toxin. Even though the UiO researchers have discovered part of the genetic code in the alga that produces this dreadful toxin, they still do not know how many genes the alga has."

The genome of Alexandrium is approximately 30 times larger than that of humans, but the number of active genes is lower than in the human genome.

The point is that they have discovered the particular gene found only in poisonous Alexandrium algae. Or to be even more specific: the code to the toxin is found in only a small part of a certain gene.

Evolutionary enigma

One of the questions that Russell Orr and his colleagues have set out to answer is why the alga started to produce the toxin in the first place.

"Alexandrium developed this ability 100 million years ago when they stole this skill from a bacterium, but we still don't know why. Some of their descendants lost this ability along the way. In the course of evolution, some species lost the ability to produce this toxin. Some believe that the toxin is a defence mechanism, while others claim that they use the toxin to communicate with each other, but no one has yet determined this for certain."

Now that the poison gene has been found, it is a simple matter to

determine whether a mussel is poisonous. Even though the mussel has digested the alga and not a single whole strand of DNA is left inside the mussel, many small pieces of this strand are left – including the part of the DNA sequence that produces the toxin in the mussel.

"Now, we can immediately establish whether the toxin is present. We would have liked to develop the test further to study the potency of the toxin. The drawback of our method so far is that the test can yield too rigid results for mussel farms if the mussels are not dangerously toxic," Russell Orr explains.

While the current labour-intensive laboratory test in Norway requires four days and costs USD 2000, the new test can be undertaken in a few hours at a cost of USD 35.

The research work has taken five years. To break the DNA code of the poison gene, the researchers have used several thousand hours of high-performance computing on one of the university's fastest computers.

A patent is secured

The innovation company Inven2, which belongs to UiO and the university hospitals, has patented the method. The patent is valid for seven years – worldwide.

Today, the world spends several hundred million dollars annually to check mussels. The researchers therefore believe that there are good opportunities to earn some money on this.

"Changing the testing methods is difficult. Current mussel tests are regulated by the EU. In order to have our method approved, lobbying is needed," Russell Orr says.

The Australian company Diagnostic Technologies has bought the patent.

Director Mark Van Asten of Diagnostic Technologies believes that the Norwegian invention can monitor and predict development of toxins in the ocean and reduce the dependency on costly and time-consuming tests. Moreover, the method can give a more rapid assessment of when the peak of the poison production has been reached and the concentration of toxin starts to decline. This is important for timing the restart of the mussel harvest.

The test kit they are making includes a molecular test that identifies the presence of the gene that is required to produce saxitoxin. The test will measure the amount of toxin to reveal the degree of hazard that it poses.

One of the questions they are asking themselves is whether the poison in the mussels may also be caused by other toxins than saxitoxin. Mark Van Asten hopes to have the test ready within a year, and foresees that the market will be in the range of USD 10–12 million annually.

PS: If you wonder whether Russell Orr eats mussels, the answer is: "Yes, they're delicious!"

Provided by University of Oslo