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The antifreeze fish teaching us about cryopreservation

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Cryopreservation aims to prevent the formation of ice crystals in the freezing process and prevent damage to cell walls. Image: Wikimedia Commons, Marrabbio2

Cryopreservation, in which organic material is stored at extremely low temperatures, may not yet have reached the science fiction dream of placing people in suspended animation, but technology inspired by hard-to-freeze fish is helping make it an effective way of preserving genetic plant material for future use.

One of the biggest challenges of cryopreservation is the formation of ice during the freezing process, as the crystals can rupture cell walls and damage the frozen organism. To investigate how these crystals can be prevented, the EU-funded CRYOPRESERVATION project has turned to fish that live in sub-zero temperatures to find out how specialised antifreeze proteins prevent their blood from turning to ice.

'Their blood is super cooled – there should be lethal ice inside them,' said Dr Ido Braslavsky at the Hebrew University of Jerusalem, who recevied a EUR 1.5 million grant from the European Research Council (ERC) for the project. 'But any ice crystals that do form in their body grow no further and don't harm the fish.'

Dr Braslavsky and his team are studying these antifreeze proteins to see if they can be used for food preservation and for the preservation of living organisms in general. So far, the proteins have been found in many fish which live in cold conditions, as well as in insects, plants, algae and bacteria, in which they prevent ice growth and the crystallisation of organic fluid matter.

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The researchers are studying the detail of the interactions between antifreeze proteins and ice crystals at the molecular level by labelling the proteins with fluorescent tags to make them visible with a fluorescence microscope. They are looking in particular at hyperactive antifreeze proteins – highly active forms of the protein which haven't yet been widely investigated.

‘We’re trying to find a good way to produce and purify them,’ said Dr Braslavsky. ‘The first step is to produce enough for research and then the next step will be to ramp up the production to a higher level, but we’re not there yet.’

> ‘With cryopreservation, at -196°C, the temperature is low enough to halt all metabolic, physical, and chemical processes.’
>
> *Dr Bart Panis, Bioversity International, Catholic University of Leuven, Belgium*

There is currently only one commercial application of antifreeze proteins. Unilever has been using the proteins since 2009 to produce low-fat ice cream that remains smooth and creamy. Originally sourced from a North Atlantic fish known as an eelpout and now commercially manufactured by the company, this added ingredient changes the shape of the ice crystals to form needle-like structures, which stabilise the structure of the ice cream.

Researchers believe that knowing more about how antifreeze proteins work would enable the technology to be used for many more applications than ice cream. Further advances in understanding antifreeze proteins could be the catalyst for a cryopreservation revolution, helping to perfectly preserve the genetic diversity of foodstuffs and improve the quality of frozen and chilled food products.

Preserving genetic diversity

Future genetic diversity is also being protected through the long-term freezing of plants and seeds. At the Svalbard Global Seed Vault in the Arctic, 4.5 million seed samples from the world’s crop collections are frozen and stored at -18°C. The vault is designed to secure global food sources by acting as a back-up to seed banks in case specimens are lost through war, accidents or natural disasters.

However, not all plant material can be preserved at this temperature, which is where cryopreservation comes in.

‘Seed storage is quite easy for many plants,’ said Dr Bart Panis, whose lab at Bioversity International, based at the Catholic University of Leuven, Belgium, specialises in cryopreserving bananas. ‘You dry out the seed a little, and then you can store it at around -20°C. But at this temperature, there is still some deterioration, and the seed can age in as little as 20 years.

‘With cryopreservation, at -196°C, the temperature is low enough to halt all metabolic, physical, and chemical processes. Whether you store it in liquid nitrogen for one minute or 100 years, it should remain the same.’

However, it is a labour intensive process. One technician working in Dr Panis’ lab when trained ‘very well and working very efficiently’ can cryopreserve about 50 plants a year.

It is therefore only used for those cases in which seeds cannot be stored, such as sterile plants which don’t produce seeds, plants in which the seed itself isn’t storable - for example, rubber and oil palm - or plants that are vegetatively propagated by tubers and stems such as potato and cassava. However, if the CRYOPRESERVATION project is successful, this technique could become more widespread.

Dr Panis and his colleagues care for the world’s biggest banana collection – a tricky crop to freeze because of its high water content and preference for tropical climates. To solve the problem, his lab has pioneered a cryopreservation technique called droplet vitrification, which turns liquids into a glass-like substance, free from ice crystals.

‘You need to extract enough water so that the cells vitrify when you expose them to liquid nitrogen,’ he said. ‘Vitrification means the solidification of a liquid without the formation of ice crystals. You need high concentrations of the solution and a rapid freezing rate. That’s the trick.’

Droplet vitrification is used to freeze the banana’s meristem tip, the one millimetre-wide growth centre of the plant, which can be regenerated to create an entire new plant. The same technique is now used for the cryopreservation of 20 to 30 plant species, including Peru’s potato collections and Colombia’s cassava collections.

Until faster technology is developed, the main bottleneck to preserving the world's plants is time. So far, Dr Panis’ lab has 900 banana accessions successfully stored in liquid nitrogen – an effort that represents 18 working years – and there are another 600 to go.

More info

CRYOPRESERVATION

Svalbard Global Seed Vault

Bioversity International