Microbes under the microscope

What tools do bacteria in the sea or in soil use to break down organic pollutants? This is the type of question the research group General and Molecular Microbiology led by Ralf Rabus addresses. The scientists are studying the proteome – the entire set of proteins present in a cell – to gain a better understanding of how certain microbes function. A journey from tiny to even tinier

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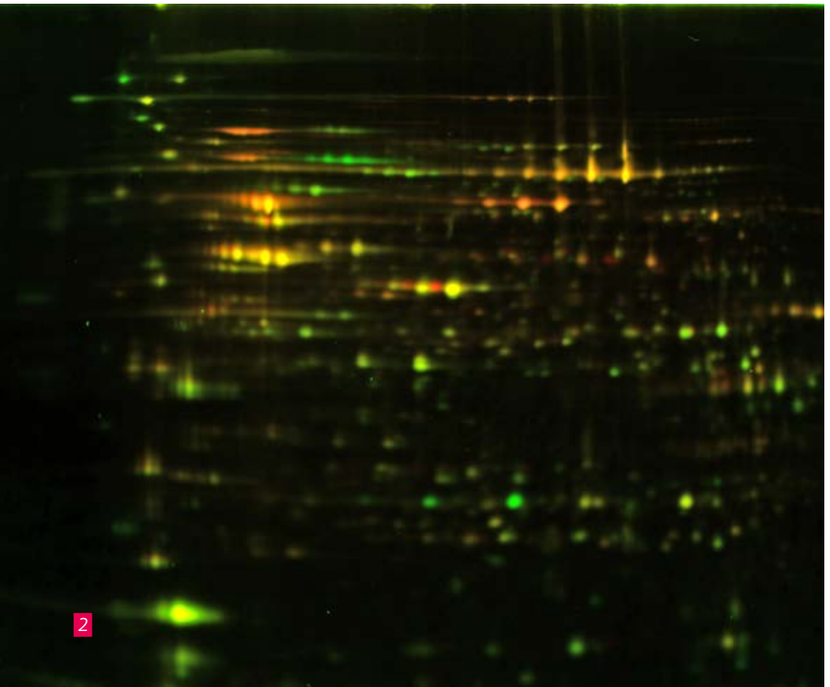

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1 Bacteria are no more than a few thousands of a millimetre in size. Yet these tiny cells can do all the things larger organisms do – for example breathe, take up and use nutrients, and excrete metabolic waste.

2 At least a thousand different proteins bring bacteria to life – here in the image they have been separated and appear as fluorescent dots on a separating gel. The green-coloured protein spots are produced in larger amounts by the cells under the conditions studied.

3 The bacteria that the researchers are interested in live in the sea, in oxygen depleted zones of the seabed or in the soil. Lab technician Christina Hinrichs presents two samples from the Janssand sandbank near Spiekeroog.

4 Many bacteria are difficult to culture in the lab, in particular those that naturally live in oxygen-free conditions. The researchers have to heat special syringes in order to treat culture vials with nitrogen under sterile conditions. This allows the bacteria to grow without oxygen in the laboratory.

5 The bacteria grow under controlled conditions in the stainless steel bioreactor. Feeding them specific substances makes it possible to determine which proteins are used in biodegradation processes. Microbiologist Dr. Daniel Wünsch checks how much cell mass has formed. The number of newly isolated environmental bacteria generally doubles within a few hours or days.

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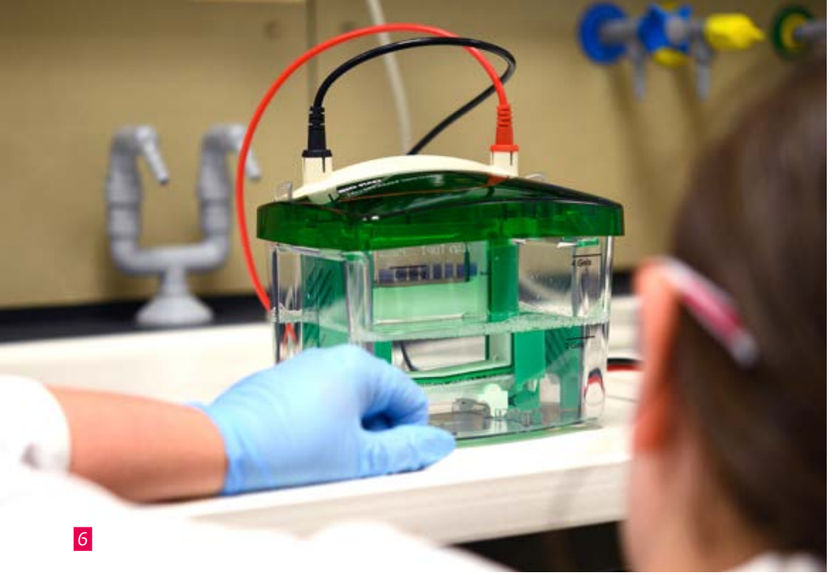

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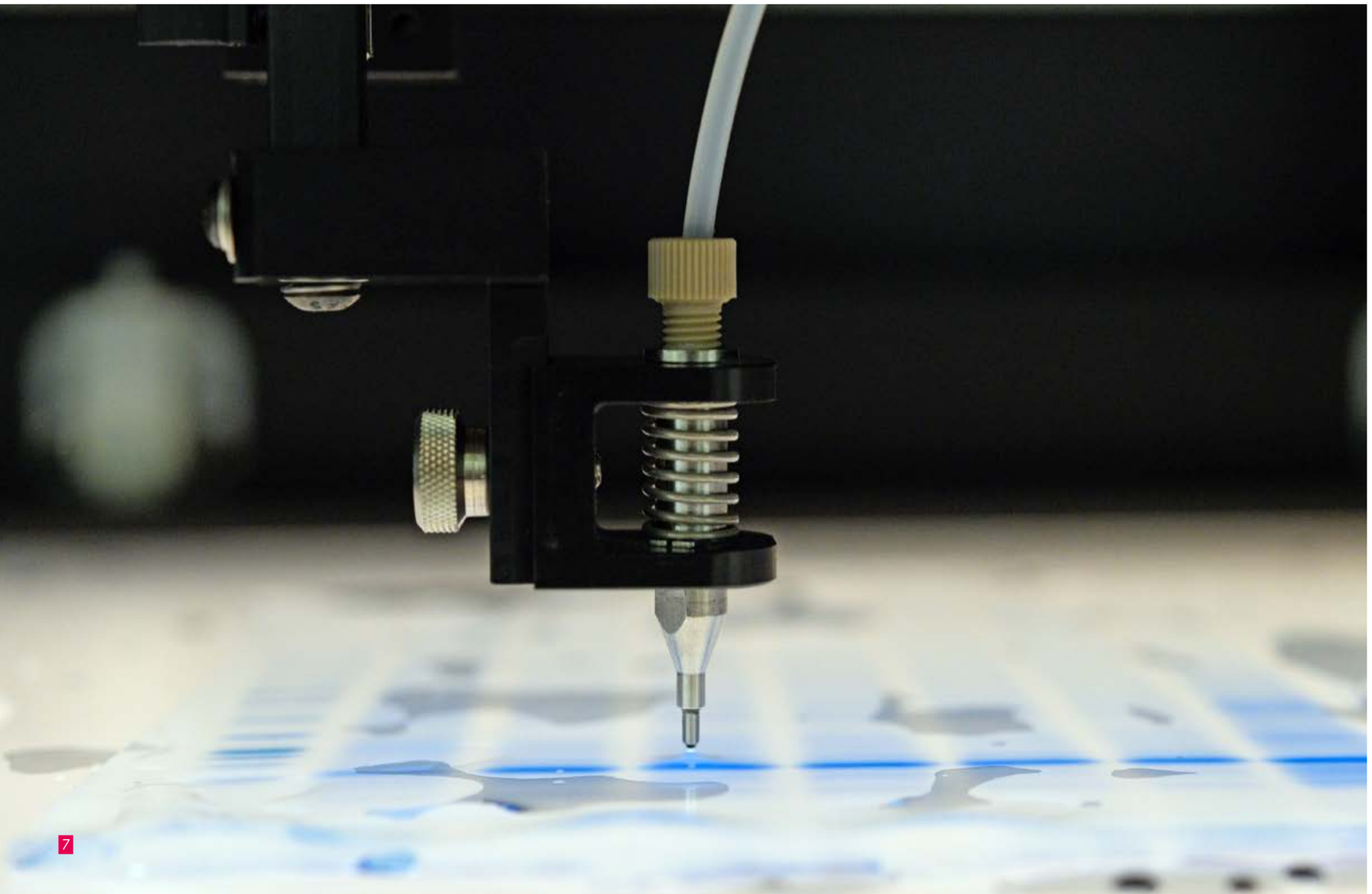

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6 A few steps later, the researchers have isolated the proteins from the cells. The resulting liquid contains one to two thousand different proteins from the cultured cells. The process of gel electrophoresis separates the proteins using an electric field. Within two to three hours the protein molecules that have been dyed blue have moved towards the positive electrode within the gel. This produces thin blue bands with proteins of similar size and charge.

7 Large image: A separating gel formed using this method, is placed on the light table. A robot arm cuts pinhead-sized pieces from the gel before inserting the individual pieces into the indentations of a microtiter plate – a plastic tray with multiple "wells" used as small test tubes. Each of these gel pieces can contain from just a few to more than hundred proteins.

8 In order to sort the proteins even more precisely, the Oldenburg microbiologists use a nano ultrahigh-performance liquid chromatography system. The pre-sorted proteins are cut up very precisely using molecular scissors, then dissolved in a liquid and passed through miniature columns under high pressure. The chromatograph slowly releases tiny droplets containing the fragments of just a few proteins.

9 The final step on the path to deciphering the proteome is the mass spectrometer in the research group's lab. Once inside, the protein fragments are vaporised, electrically charged and accelerated into a vacuum pipe. The large molecules fly slowly through the pipe, the smaller ones more quickly. This allows the device to calculate the mass of the separated fragments with such precision that each individual protein can be identified.