Arthrospira – a multitalented microalga

Dr-Ing Paul Janssen discusses the challenges and opportunities inherent to the genome project of Arthrospira sp. PCC 8005. This multicellular cyanobacterium is cultivated worldwide as food and feedstock but has many other important (bio)technological applications.

Could you provide a brief introduction to your research on Arthrospira?

Arthrospira is of particular interest because it is so versatile. Its biomass is not only highly nutritious, but it also has a very wide range of technological applications. The Microbiology Unit (MIC) at the Belgian Nuclear Research Centre (SCK•CEN) started to work with Arthrospira some 12 years ago in the frame of the Micro Ecological Life Support System Alternative (MELiSSA) project funded by the European Space Agency. It is ideal for life support in closed environments, such as spacecraft or space stations, as it fixes CO₂, produces oxygen, and serves as an edible, nutritious and health-promoting end product.

Why did the characterisation of the genome sequence of Arthrospira prove to be so challenging?

The first hurdle, about five years ago, was the preparation of suitable genomic DNA from our strain, PCC 8005. Arthrospira species produce a wide variety of endonucleases that chop up DNA upon cell lysis. Dr Nicolas Morin, then a PhD student in our group and now at the French National Institute for Agricultural Research (INRA), established methods to prevent Arthrospira DNA degradation so that sequencing libraries with large enough inserts could be constructed. Sequence analysis by GenoScope led to a first genome assembly, in 2010, of 119 contigs in 16 unordered scaffolds, encompassing more than 6 million basepairs (Mb) of DNA.

However, many ambiguities remained and proper assembly proved difficult owing to the highly repetitive nature of the genome. Eventually, improved versions were obtained by GenoScope using novel technologies, including paired-end sequencing and new assembly software. The January 2014 version is one scaffold of six ordered contigs totalling 6.23 Mb, harbouring about 6,000 genes, and is used by us for genome comparisons and bioinformatic analyses.

How can Arthrospira’s tolerance of high salinity, alkalinity and extreme radiation be most effectively exploited?

Arthrospira can grow in seawater. While its growth has also been tested in various industrial effluents, the use of such waters can be cost-saving and supports application in remediation schemes. Its optimal growth at high pH (9.5–11) is an added bonus as it can outcompete most contaminant species in open ponds.

The radiotolerance of Arthrospira was discovered by another PhD student at MIC, Hanène Badri. She subjected PCC 8005 to acute doses of gamma radiation as high as 5,000 gray (Gy). To put this into perspective, an 8 Gy dose kills an adult person and 150 Gy will kill most bacteria. While it is reassuring to know that Arthrospira can easily withstand the radiation dose rates of space, it is also important to study chronic exposure of Arthrospira to space radiation of various types. Therefore, we will run long-term low-dose irradiation experiments mimicking space radiation and will carry out an extended mission (the ArtEMISS project) to the International Space Station in 2015.
So much more than just a superfood

Microbiologists at the Belgian Nuclear Research Centre SCK•CEN investigate topics ranging from life support systems in space to industrial waste recycling and alternative energy. They are eager to exploit the biotechnological potential of the cyanobacterium, Spirulina

Are there particular challenges that must be overcome to work with Arthrospira?

Using a unique tiling array we are pursuing large-scale gene expression analyses for a wide variety of growth conditions since much genetic and metabolic information can be gained this way. The major technical drawback of working with Arthrospira is that it has extraordinary defences against incoming DNA, so it currently cannot be genetically manipulated. The development of a genetic system allowing us to replace or knock out genes, tops our to-do list. Also, Arthrospira cannot be stored, making continuous subculture under standard growth conditions a strict necessity – an issue in space if the bioreactor needs a restart. So we are investigating cryoprotective agents and preservation methods.

Do you plan to widen the scope of your research on Arthrospira?

Arthrospira species produce bioactive compounds such as polysaccharides, lipids, proteins, vitamins, sterols, enzymes, pharmaceuticals, cosmetics and a range of other fine chemicals. Additionally, owing to their filamentous growth and extracellular polymeric substance production, they can be used in tissue engineering.

Next to its feed- and food-related uses, Arthrospira is of immediate interest to us in terms of technological applications. These include the bioremediation of heavy metals and radionuclides from the environment and carbon mitigation schemes: Arthrospira grows on high CO₂ and is tolerant to levels of sulphur and nitrogen oxides typical of flue gases. It was recently reported that a 30 cubic metre Arthrospira bioreactor can easily fix 2 tonnes of CO₂ per annum. The beauty of microalgal carbon mitigation is that the biomass generated can be used to produce hydrogen or chemical precursors for biofuels. We are following such energy production developments closely.

CYANOBACTERIA, ALSO KNOWN as ‘blue-green algae’, are prokaryotes. Responsible for the original oxygenation of the Earth’s atmosphere 2-3 billion years ago, it is thought that some were engulfed by eukaryotic cells, which then gave rise to the plastids that enable photosynthesis in plants and algae.

Originally classified within the Spirulina taxa of cyanobacteria, Arthrospira is a distinct genus of helical-shaped filamentous cyanobacteria that mainly bloom on alkaline carbonate-rich lakes – ‘soda lakes’ – in warm countries. It is a highly efficient photosynthesiser capable of producing oxygen in harsh environments, such as extremely salty or high pH water, and in high concentrations of CO₂ and other noxious gases.

Used extensively for feedstock in agriculture and fish farming, Arthrospira is also marketed as a food supplement for human use. Its promise as a key component in solutions for future food security, as well as evidence of its health benefits, has led to the establishment of large farms with shallow raceway ponds for commercial Spirulina production around the world – estimated to exceed 100,000 tonnes by the year 2020.

SUPERFOOD

It is thought that the harvesting of Arthrospira as a source of human nutrition dates back many thousands of years, though its earliest recorded use is in 9th Century Chad. Today, it is still collected from Lake Chad, sundried in biscuit form and sold in markets under the name Dihé. “The nutritious value of Arthrospira was likely discovered in ancient times when it enabled human survival during famines,” muses Dr-Ing Paul Janssen of the Belgian Nuclear Research Centre (SCK•CEN). “Its consumption may have developed as a preventive measure against famine-related blindness caused by vitamin A deficiency, since it contains high levels of beta-carotene.”

In addition to beta-carotene, species of Arthrospira produce high amounts of chlorophyll, digestible proteins, gamma linoleic acid, alpha-tocopherol, vitamins B (including B12), E, C and K, phycocyanin, antioxidiants and important minerals. They are therefore widely deemed highly effective nutraceuticals. “Marked benefits include anticancer, antiviral and antibacterial activities, in addition to immunomodulation. Positive effects of Arthrospira against malnutrition, diabetes, obesity, inflammatory allergy, anaemia, cytotoxicity and radiation damage have also been observed,” states Janssen. Research carried out by the Belarus Institute of Radiation Medicine following the Chernobyl nuclear disaster, showed that when Spirulina was administered to local children at 5 grams per day over a 20-day period, radioactivity levels in their urine reduced by 50 per cent.

Janssen is a Research Scientist in the Microbiology Unit (MIC) of SCK•CEN. He works together with other SCK•CEN research units on projects ranging from genomics to bioremediation and the biological effects of radiation, and collaborates with Professor Ruddy Wattiez’ group at the University of Mons, Belgium, in proteomics investigations. MIC has now established a large body of knowledge from long-term research into Arthrospira in many contexts: “We collaborate with groups in Germany, Switzerland, Spain, France, Canada, The Netherlands, the UK, Finland and Italy,” declares Janssen.

SUPPORTING LIFE IN SPACE AND ON EARTH

So far, the genome sequences of four strains of Arthrospira have been largely derived, and Janssen is the project leader of one of these genome projects, carried out in collaboration with the French National Sequencing Centre, GenoScope. The strain studied by Janssen and colleagues at SCK•CEN is PCC 8005 which, in addition to possessing all the usual Arthrospira qualities, is resilient to extremely high doses of gamma radiation. In tests at SCK•CEN’s irradiation facilities, PCC 8005 was able to grow normally even after being subjected to 1,600 gray (Gy), and recovered growth at up to 6,400 Gy, despite some morphological damage. This feature has confirmed the suitability of PCC 8005 as the principal organism selected by the European Space Agency for converting waste products, including urine and faeces, into oxygen and directly-consumable food.
**ARTHROSPIRA**

**RESEARCH @ SCK•CEN**

**OBJECTIVES**

- To integrate the cyanobacterium *Arthrospira* in the MELiSSA life support system as an efficient oxygen producer and edible end-product.
- To investigate its applicability for the biosorption of radionuclides and its high tolerance to ionising radiation.
- Other fields of interest include CO₂ mitigation, cell factories, water purification, biosensors and biofuels.

**KEY COLLABORATORS**

- Dr Valérie Barbe, GenoSope, CEA, France • Dr Claudine Médigue, MaGe Platform, CNRS & CEA, France • Dr Christophe Lasseur, MELiSSA, European Space Agency, The Netherlands • Professor Rudy Wattiez, University of Mons, Belgium • Additional members of the MELiSSA, University of Mons, European Space Agency, The Netherlands • CEA, France • Dr Christophe Lasseur, MaGe Platform, CNRS & Claudine Médigue

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**FURTHER PROSPECTS**

In terms of bioremediation, *Arthrospira*’s ability to fix CO₂ and its tolerance of high concentrations of pollutants in air renders it a strong candidate for removing sulphur and nitrogen oxides from flue gases emitted by industrial boilers, power stations and oil refineries. In terms of phycoremediation, it can remove or transform heavy metals, such as lead and cadmium, in grey water and air; and it could be used to remove uranium from reactor cooling water after nuclear accidents. It also has potential use as biomass for energy generation as an alternative to fossil fuels.

Dr Felice Mastrolorenzo at MIC, who studies the effects of simulated microgravity on bacterial cultures using random positioning machines, has recently tested the effects of cosmic radiation on another MELiSSA bacterium, *Rhodospirillum rubrum*, by comparing gene expression before and after exposure. Next year, the head of MIC, Dr Natalie Leys, will manage experiments on *Arthrospira* in the ISS to explore its photosynthetic efficiency, carbon fixation, cell division, oxygen evolution and genetic stability in that environment.

Unfortunately the means of genetically manipulating *Arthrospira*, and so being able to precisely tailor its activities, has proved elusive. With this in mind, MIC is now inspecting *Arthrospira* more closely, using bioinformatics, flow cytometry and the -omics techniques (a tiling array designed by MIC is the first of its kind for *Arthrospira*), to understand how its genomic information translates into its exceptional properties, metabolism and activities – including the specific factor that confers its high tolerance to gamma radiation. They are additionally conducting comparative genomics studies to establish how PCC 8005 differs from the other three sequenced strains, C1, NIES-39 and CS-328.

Janssen plans to publish a paper on the current version of the PCC 8005 genome; he is also looking for opportunities to contribute MIC’s *Arthrospira* expertise to multidisciplinary and international collaborations in programmes such as Horizon2020 or in European Science Foundation COST Actions: “We are keen to apply our knowledge of *Arthrospira* and its treasure box of genomic information via collaborative endeavours,” enthuses Janssen.