Current Science Reports

Arctic Bacterial Diversity

Terrestrial and fjord sediments

Bacterial diversity orchestrates the remineralization of organic matter in the Arctic. Bacterial communities are adapted to the cold. Any climatic or environmental change would immediately impact the microbiota.

Recently, researchers from the National Centre for Polar and Ocean Research, Goa, and the Mahatma Gandhi University, Kerala reported investigating sediment samples from the Arctic.

Glacier front sediment has lower total organic and inorganic carbon content than glacier snout sediment. In fjord sediment, total organic carbon content reduces from the outer to the inner fjord, whereas inorganic carbon shows a reverse trend.

The metabolic profiles of microbes from terrestrial sediment showed that they tend to use amino acids, amines and amides. Fjord communities, however, prefer carbohydrates and polymers.

The total number of microorganisms in fjord sediment was two-fold higher than that in terrestrial sediment. However, bacterial diversity and richness were higher in terrestrial sediment.

Besides carbon sources, geochemical properties were also significantly correlated to bacterial communities. Inorganic carbon, nickel, cadmium and zinc were positively correlated with the terrestrial bacterial community. Trace elemental concentrations, except for lead and mercury, were higher in terrestrial sediment. But lead and mercury content were closely associated with fjord sediment.

'Atmospheric circulation transports metals from global and local sources and precipitates them in Arctic glaciers. But ocean currents carry metals from low and middle latitudes to the Arctic,' explains K. P. Krishnan, NCPOR, Goa.

Bacterial isolates from terrestrial sediment at 4°C were four times more than those from sediments at 20 degrees. But differences in bacterial isolates from fjord sediment at different temperatures were not that significant. Most bacterial isolates could grow at about zero degrees. Only three isolates grew between 15 and 20 degrees.

More than half the bacterial isolates were adapted to cold environments and UV radiations.

The researchers used 16 rRNA sequencing and found a total of 2376 distinct varieties. They could identify 1103 bacteria. This microbial diversity, adapted to Arctic conditions, holds promise for various useful products, but is threatened by climatic and environmental changes. Such studies help us actualize promises from the Arctic.

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Mapping Mine Water Seepage

Jharia coalfields, Jharkhand

The Jharia coalfield mines are mostly underground. Underground mine galleries access the deep-seated coal deposits. To support the roof, some barrier pillars are left untouched during tunnelling. But water seeping through the pillars can flood the tunnels. Many fatalities occur due to such flooding in underground coal mines. Can we predict the seepage?

Recently, Rajwardhan Kumar, Sanjit K. Pal and Praveen K. Gupta from IIT Dhanbad set out to map seepage in an underground coalmine barrier pillar at Jogidih, Jharia coalfields. By measuring naturally occurring electric potential differences in the rocks, using a voltmeter, they determined the self-potential of the barrier pillar. Self-potential indicates weak zones caused by weathering.

As coal gets porous due to fracturing, it holds more water and resistivity decreases. So the researchers assessed electrical resistivity to characterize seepage in the pillars using electrical resistivity tomography. Thus the team delineated seven low resistivity anomaly zones that indicate water saturated or fractured coal strata in the pillar. In the barrier pillar, low resistivity values slowly reduced from bottom up.

'Mine water stored in pits percolates into the lower part of the pillar faster than to the upper part due to higher hydrostatic pressure,' explains Sanjit K. Pal.

The researchers analysed seepage water and found high sulphate concentration due to sulphide leaching through the pillar. Water seepage makes the material underground more porous.

Mine safety departments can use such techniques to infer hidden seepage paths and water-saturated fractured zones. Monitoring seepage in mines can reduce the chances of fatalities.

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Carnatic Carp

Stock identity revealed

The Carnatic carp is only endemic in rivers originating in the Western Ghats regions of Karnataka, Tamil Nadu and Kerala. Geographical barriers between the rivers induce differences between the fish stocks there.

Recently, scientists from the Bengaluru and Barrackpore centres of the Central Inland Fisheries Research Institute and the Central Institute of Fisheries Education, Mumbai collected Carnatic carp samples from four sites.

Three groups were from the Cauvery – one from Tamil Nadu, one from Karnataka and one was from farm-reared fish. The fourth group was from the Chalakudy River, Kerala.

The researchers photographed the fish and identified 15 points representing key morphometric features. They connected the points using a network of lines that created quadrilaterals. Then they digitized the points and lines.

The digitized landmarks were encrypted to extract values that represent the morphometrics of fish from different sites. From a statistical analysis of the morphometry of the four groups, they found that Carnatic carp from Kerala and Tamil Nadu resembled farm-reared Carnatic carp. So, the Carnatic carp from Karnataka was a different stock.

'Understanding the stock structure of endemic fish species helps develop conservation programmes like introducing artificially reared populations into the wild,' says B. K. Behera, CIFRI Barrackpore.

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Bamboo Feed for Shrimps

Making bacteria non-toxic

Vibrio parahaemolyticus infection damages shrimp pancreas and liver. The resultant mortality causes loss to shrimp farmers.

Vikash Kumar and team from the Central Inland Fisheries Research Institute, Barrackpore found that *V. parahaemolyticus* tends to transform from a virulent strain to one that does not cause disease as a response to the environment.

Changing environmental or nutritional conditions induce bacterial biofilm formation. Something involved in the process of forming the biofilm seemed to render the bacteria harmless.

Vikash teamed up with scientists from Belgium and Sweden to uncover what made the bacteria harmless and helped shrimps evade death. They knew that bamboo powder helps the bacteria form biofilm.

'We hypothesized that, if the powder is used to feed shrimp, we can reduce infection by vibrio species,' says Vikash, ICAR-CIFRI.

The team fed bamboo powder at doses of up to 100 mg per litre to the larvae of germ-free brine shrimp, *Artemia franciscana*. The feed was non-toxic.

The researchers then took five strains of Vibrio bacteria and checked which was most lethal. The variety M0904 was the most lethal, causing around 90% mortality.

The team used the strains to infect shrimp larvae fed with bamboo powder. Bamboo powder protected shrimps from two of the most virulent strains. The higher the bamboo powder concentration, the better the survival.

But what caused the bacteria to change? The researchers isolated and examined the bacterial proteins. With bamboo powder, the bacteria did not secrete toxin proteins. Instead, there was a new protein, identical to alkaline phosphatase.

Shrimp farmers can now reduce losses caused by at least some Vibrio species by modulating bacterial virulence.

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Grapefruit Fungus

Tackling green mould

Grapefruit shelf life is severely affected by fungal infections. The green mould, *Penicillium digitatum*, devours fruit nutrients, causing rot post-harvest.

Nithesh Naik, Manipal Institute of Technology recently collaborated with Chinese and American researchers to test a strain of yeast that occurs naturally on healthy grapefruit against the green mould infection. To improve the effect, they mixed the strain with carboxy-methyl-cellulose, known to protect fruits from infections by inducing defence reactions.

The researchers washed microbes off healthy fruits, and cultured them to see how effective they were in suppressing green mould growth. Of the five strains found, one was the best: *Cryptococcus laurentii*. Carboxymethyl-cellulose is not used as carbon source by some fungi. But *C. laurentii* could form a biofilm in a 1% solution of the polymer.

The team prepared a thin film of the fungus, *P. digitatum*, extracted from infected Rio Red grapefruit and tested the mixture of carboxy-methyl-cellulose and *C. laurentii* in different proportions. Alone, both had suppressive effects on the virulence of green mould. Together, they had a synergistic effect.

Under a scanning tunnelling microscope, the researchers observed that *P. digitatum* hyphae were surrounded by carboxymethyl cellulose, causing shrivelling, and that *C. laurentii* attached itself to the growing parts of the hyphae, causing deformations.

When the researchers coated grapefruit with the mixture, they observed that enzymes like chitinase were produced and these break down protecting layers of chitin in fungi, and beta-glucanase breaks fungi cell walls. The mixture enhanced peroxidase activity, improving disease resistance. Fruits treated with the mixture lost the least weight and retained most nutrition for 28 days.

There are questions that remain. *P. digitatum* infects other fruits. Can this mixture work on them? And how about other fungal infections in fruit?

While researchers tackle these questions, fruit farmers, transporters and traders wait anxiously for reliable and safe methods to improve fruit storage.

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Hospital Infections

Bed sheets spread

Hospital bed sheets capture pathogens. Some of these pathogens become resistant to antibiotics. And infections by such organisms are difficult to treat. These nosocomial infections affect millions the world over, claiming thousands of lives every year.

Deepti Gupta and her team from IIT Delhi recently analysed bacterial load on bed sheets in a healthcare unit in Delhi. Hospitals use a polyester-cotton blend fabric.

'The fabric regulates air and moisture permeability and percolation. This makes it easy to determine the microbial load,' says Shilpi Sharma, IIT Delhi.

In the first and last weeks of the month, the researchers stitched sterilized fabric patches onto three freshly washed bed sheets used in the healthcare unit. The patches were removed every five days and processed to isolate bacterial groups. The researchers found seven different groups. *Acinetobacter* spp. was dominant. They continued the experiment from May to November since temperature and humidity impact microbial growth.

'Most bacterial groups are higher in June when temperatures are highest and relative humidity low,' says Deepti Gupta. 'In winter, the bacterial load gradually decreases.'

The bacterial strains were processed for antibiotic susceptibility. *Enterococcus faecalis* and *Staphylococcus* spp. were much more resistant than the others. Ampicillin resistance was highest.

'Season-dependent disinfection protocols can be implemented to prevent drug-resistant load in health-care units,' says Swati Varshney, IIT Delhi.

'Choosing fabric that restricts bacterial growth can also save patients from nosocomial infections,' suggests Deepti Gupta.

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Sensitive Bacterial Detection

By measuring impedance

When bacteria grow or die in a medium, the electrical properties of the system change. So, impedance spectroscopy – impedance measurements with a spectrum of alternating current frequencies – can tell us a lot about bacterial behaviour in a medium. However, the phosphate buffer saline used in growth media is highly conductive, complicating inferences from impedance spectroscopy. And diluted phosphate buffer saline puts osmotic pressure on the bacteria.

Shalini Gupta, Gaurav Goel and team from IIT Delhi have now come up with a solution to this problem: zwitterions. Zwitterions contain both positive and negative functional groups on the same molecule and are thus electrically neutral. In contrast to ionic electrolytes, a zwitterion's net neutral molecules decrease a solution's conductivity.

The researchers theorized how electrolytes behave in an aqueous solution and chose an aminosuphonic acid derivative, HEPES. They found that HEPES zwitterions are involved in intra- and cross-ionic interactions. Due to large ionic sizes, the ions interact even in dilute conditions. Thus, in contrast to the phosphate buffer saline, the HEPES buffer has low conductivity at any concentration. But is this zwitterionic environment suitable for bacteria?

The team hypothesized that positively charged nitrogen atoms in HEPES interact with negatively charged bacterial cell walls to aid stabilization. They tested impedance signals in this favourable buffer environment. They lysed bacterial cells in zwitterionic buffers by heating and noted the impedance change. The HEPES buffer performed best with high impedance signal and signal-to-noise ratio, say the researchers.

Manufacturers of point-of-care diagnostics can now opt for zwitterionic buffers to improve the sensitivity of impedance biosensors to detect bacterial death or growth.

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Vitamin D and Arterial Stiffness

Disease signatures

Rigid artery walls are an independent predictor of cardiovascular issues in diabetes and in chronic kidney patients. Studies show that such patients also have vitamin D deficiency. Does the deficiency indicate arterial stiffness?

Scientists from JIPMER and the Sri Manakula Vinayagar Medical College and Hospital, Puducherry tested vitamin D levels in blood samples from 120 patients with type 2 diabetes and intermediate chronic kidney disease. They measured vitamin D levels in blood and segregated the patients into normal, insufficient and deficient. They also examined vascular parameters.

'Heart beats generate an electrical waveform, a proxy measure of blood flow and pressure. People with healthy hearts and flexible arteries have normal blood pressure and flow as indicated by the waveform,' explains Saibal Das, JIPMER.

But almost 75% of patients with the deficiency had significantly higher blood pressure and faster blood flow than patients with insufficient and normal vitamin D levels.

'Vitamin D levels in type 2 diabetes patients with intermediate chronic kidney disease can help identify arterial stiffening for early intervention,' notes Sandhiya Selvarajan, JIPMER.

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Chitosan-coated Skim Milk

Protects probiotics

Probiotics are live microorganisms that improve microbial diversity in the gut and help improve health. However, most probiotics taken orally are susceptible to damage in the gastrointestinal tract.

To protect the microbes, they need to be encapsulated with the right material. Alginate, a naturally derived polysaccharide, is useful for microencapsulation. But it is sensitive to the stomach's acidic conditions. To overcome this, it is usually mixed with other polymer compounds.

Skim milk improves the survival rate of probiotics in acidic conditions. Can combining with alginate increase probiotic efficiency? Packaging in chitosan also helps microbes survive their journey to the colon.

Preetha and her team from the SRM Institute of Science and Technology, Tamil Nadu tried different combinations to compare the effectiveness: only alginate, skim milk with alginate, and chitosan-coated skim milk with alginate.

The team harvested probiotic cells and encapsulated them through extrusion, a technique where polymers are forced through pores to produce microspheres of fixed size.

Chitosan-coated skim milk alginate microspheres retained the viability of the harvested probiotic cells during microencapsulation.

'This may be attributed to chitosan's strong cationic nature in relation to the anionic alginate,' says Preetha, SRM Institute of Science and Technology.

'The microspheres survived damage by bile salt concentrations,' adds Padhmavathi, her colleague.

Higher concentrations of skim milk and chitosan coatings improved the survival of probiotics in artificial gastrointestinal fluid. The microencapsulation seemed to be useful to deliver viable bacterial cells to the colon.

The team analysed the microspheres' storage stability for a month.

'The counts of viable cells were higher in the microspheres. So it improves shelf life,' says Shruthy, SRM Institute of Science and Technology.

Pharma industries need to pitch in to take the findings to the next level and ultimately to the market.

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Grain-based Carbonated Beverage

A nutritious alternative

Beverage premixes with dry carbonated powder provide refreshing fizzy drinks when mixed with water. The powder form increases shelf life and is easy to handle. However, commercial beverage premixes are rarely nutritious. Can we prepare a nutritious beverage premix without compromising on taste?

Anjali Thakur and her team from IIT, Kharagpur set out to prepare a grain-based beverage premix with maize, Bengal gram and finger millet.

They roasted the grains at varying timings and temperatures using a hot-air roaster, to optimize roasting conditions. To retain nutritional values, maize was heated for 10 minutes at 180°C. Bengal gram needed 27 minutes and finger millet 30 minutes at

110°C. These variations are due to differences in grain structures, say the researchers.

They then ground and sieved the roasted grains to form the premix. Sugar and pea protein were added to enhance taste and nutrition and the premix's water solubility was analysed.

'It dissolves easily in water,' says Chandrakant Genu Dalbhagat, IIT Kharagpur.

'The premix had 16 per cent protein and several minerals including iron, calcium and magnesium,' says Pooja Pandey, IIT Kharagpur.

The researchers added varying concentrations of carbonation powder to the premix and made beverages by adding different water ratios. A team of 15 trained judges analysed the taste of the beverages.

'A mixture with 8 per cent carbonated powder at a 1 : 4 ratio of premix and water turned out to be the best,' says Hari Niwas Mishra, IIT Kharagpur.

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Quinoline Fluorescent Probe

Detecting lead in milk

Lead exposure in dairy cattle leads to lead contamination in milk. This toxic heavy metal can affect brain and nervous system development. In children, it leads to reduced intelligence, memory loss and behavioural issues. Often, physical growth is also impaired.

Researchers from the Karunya Institute of Technology and Sciences, Coimbatore, and the Anna University, Chennai in collaboration with universities in China recently developed a sensitive and selective fluorescent probe to detect lead in milk.

To prepare the probe, they selected quinoline and morpholine scaffolds. Quinoline acts as a fluorophore and has been extensively used to construct highly selective fluorescent probes to detect target metal ions. Morpholine sequesters lead ions and donates electrons to quinoline, increasing fluorescence intensity. The researchers harvested the potential of both compounds by preparing a quinoline–morpholine conjugate probe as a chemosensor.

The probe emitted a higher blue fluorescent signal in the presence of lead ions.

'Even very low levels of lead can be detected,' says Raju Nandhakumar, Karunya Institute of Technology and Sciences.

The probe selectively detected lead ions in alkaline earth or in a mixture of ions like cadmium, mercury and zinc which interfere with the conventional sensor's detection of lead.

'The probe is easily recyclable. Treating with ethylenediaminetetraacetic acid removes lead ions from the probe,' says Velmurgan, Karunya Institute of Technology and Sciences.

The researchers used the probe to detect lead ions in commercial milk and red wine samples. They also used it to detect lead in living cells using live imaging.

The probe has environmental and biomedical applications as well. It can be used to detect lead in household items, say the researchers.

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Electrodes for Supercapacitors

Banana peels find use

The fast charging and discharging of supercapacitors depend on their electrodes. Carbon provides the best electrode material. With higher surface area and more pores, performance improves. To produce such carbon, biowaste is a good and cheap source. But most biowaste yields carbon with narrow porous structure and low surface area.

Mani Shanmugam, Institute of Aeronautical Engineering, Hyderabad collaborated with Indian and Korean researchers considered trying banana peels – a naturally porous biowaste.

To carbonize the peels, they dried and pyrolysed them in the presence of an inert gas. Carbonization leaves doped atoms behind on the substrate. Chemical analyses confirmed the presence of oxygen and nitrogen doping on the carbon's surface. Doping increased the material's effective surface area.

Electron microscopy showed that the banana peel carbon had disordered porous shapes. Pores with diameters around 10 nanometres were interconnected micropores and mesopores.

'This structure provides enough space for ions to access electrolytes,' says M. Shanmugam, Institute of Aeronautical Engineering.

'The higher the number of mesopores, the larger the surface area,' says T. Somanathan, Vels Institute of Science, Technology and Advanced Studies, Chennai.

To check electrochemical behaviour, the team measured the carbon's capacitance and resistance potential. They used the carbon as electrode in an aqueous solution of 1 molar sulphuric acid. A good flow of current was seen in the voltmeter.

After 10,000 charges and discharges, a capacity of 94% was maintained.

'Nitrogen and oxygen atoms doping the carbon surface are responsible for increasing the capacity,' adds T. Somanathan.

Such experiments inch us closer to the best structural and material design of carbon electrodes for supercapacitors.

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Reports by: **D. C. Jhariya, Ravi Mishra, G. Sharath Chandra, Tahera Arjumand, Manish Kumar Tekam, Ravindra Jadav**, **Aradhana L. Hans, K. Sri Manjari, Shwetakshi Mishra, Archana Singh, Sileesh Mullasseri,** and **Khuban Buch**

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scienceandmediaworkshops@gmail.com