SNAPSHOTS

Eating up CO_2
Scientists have engineered a strain of *E. coli* bacteria to eat carbon dioxide and grow rather than eat sugars and other organic matter. The report is published in the journal *Cell*. This is a breakthrough and can be used to make several organic molecules in the lab which can be used as biofuels or food.

Animal bridges
Animals such as penguins, carrying sensors could help humans monitor the oceans according to a study which assessed 183 species. When monitoring oceans, large areas still remain under-sampled, leaving gaps in our knowledge. Using animals can bridge this gap, the study found.

Amazon burning
The scale of fires in the Amazon rainforest drew global attention earlier. However, the concerns were countered by the Brazilian government's claims that the fires were of the "normal" amount. A new study by an international team of scientists says that the number of active fires in August was three times higher than in 2018 and was the highest since 2010.

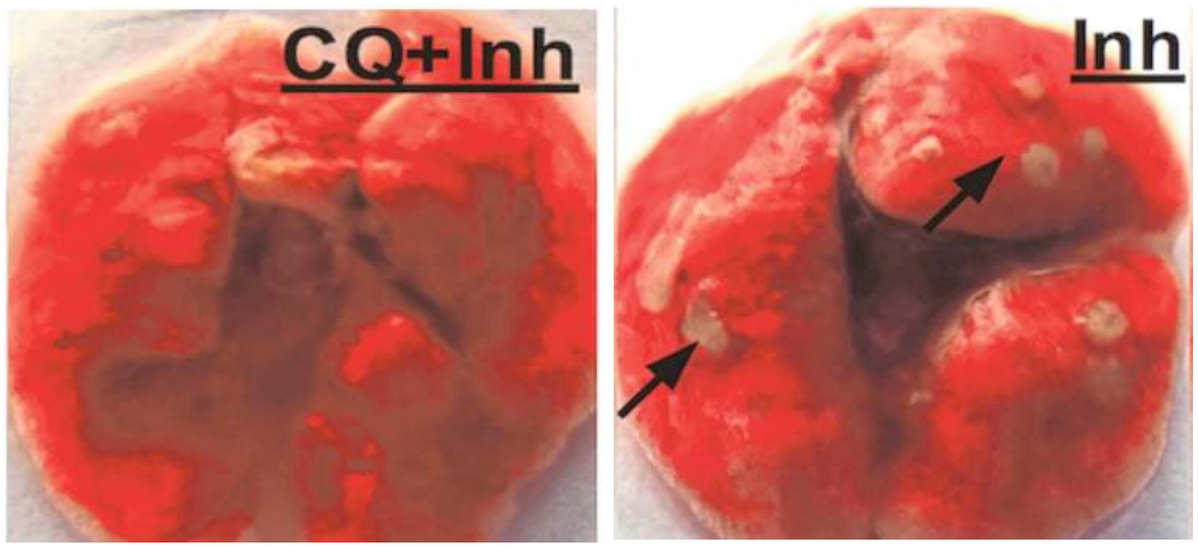
Plant defence
Grey mould caused by fungus devastates soft fruit crops. A study published in *Nature Communications* found strawberry plants had a bacteria *(Streptomyces globisporus)* in its vascular tissues and pollen that rendered them immune. Pollinating bees gained by ingesting the pollen and helped spread immunity.

Combination therapy using malaria drug quickly clears TB

The treatment led to near-complete clearance of the bacteria from the mice lungs in just two months

R. PRASAD

Researchers from Bengaluru have made an important discovery of the mechanism used by TB bacteria to tolerate TB drugs, which necessitates longer treatment of six-nine months. They have also demonstrated that a drug combination that prevents the bacteria from inducing this mechanism leads to almost complete clearance of the bacteria from the mice lungs in just two months of therapy. If further studies and trials show similar results, a shorter treatment regimen might be sufficient to treat drug-sensitive TB.



Potent combination: TB lesions (white patches) are absent in lungs of mice treated with chloroquin plus isoniazid (left) while these can be seen in the lungs of mice treat with isoniazid alone (right) as indicated by arrows.

The common notion is that only the non-replicating or slowly metabolising TB bacteria become tolerant to anti-TB drugs. But the team led by Amit Singh from the Department of Microbiology and Cell Biology, and Centre for Infectious Disease Research at the Indian Institute of Science (IISc) found a fraction of the bacteria inside the macrophages was able to tolerate anti-TB drugs even when actively multiplying.

The researchers found that using an already approved anti-malaria drug chloroquine in combination with a TB drug isoniazid can almost clear all the bacteria from the lungs of mice and guinea pigs in just eight weeks. In addition, the drug combination also reduces the chances of TB relapse. The results were published in the journal *Science Translational Medicine*.

Reducing the pH to make it acidic is the first-line of defence by macrophages when infected with pathogens. But the researchers found that instead of controlling the TB bacteria, the mildly acidic pH was actually facilitating a fraction of the bacteria to continue multiplying and develop drug tolerance.

Counter oxidative stress

"We used a biosensor which we had developed a few years ago to see the amount of oxidative stress inside the TB bacteria during infection. We found that anti-TB drugs induce oxidative stress to kill bacteria inside macrophages. However, the drug tolerant bacteria have a remarkable ability to counter oxidative stress," says Prof. Singh. "The bacteria used the acidic pH of macrophages as a cue to specifically increase its capacity to deal with oxidative stress." Also, the drug-tolerant bacteria induce efflux pumps to expel antibiotics as an additional mechanism to reduce antibiotic efficacy.

The drug-tolerant bacteria were found in macrophages that were more acidic (pH 5.8) while the drug-sensitive bacteria were seen in macrophages that were less acidic (pH 6.6).

"We hypothesised that reverting the pH within macrophages to its normal state could probably make the bacteria sensitive to antibiotics," Prof. Singh says. "The chloroquine drug does just that – it neutralises the pH within the macrophages. This prevented the bacteria from inducing the mechanism to protect themselves from oxidative stress. So no drug-tolerant TB bacteria emerged." Once the pH is neutralised, the isoniazid drug was able to eradicate TB from animals.

While the two-month treatment was able to completely sterilise mouse lungs, a near-complete eradication was observed from the lungs of guinea pigs. "The combination was shown to reduce TB bacteria load in both mice and guinea pigs," says Richa Mishra from IISc and the first author of the paper.

Many times effective

In the case of *in vitro* studies using cell lines and mice macrophages, the ability of the combination drug therapy to reduce TB load was found to be three- to fivefold higher than when treated only with TB drugs. "Reduction in bacteria load was more when we combined chloroquine with isoniazid," says Mishra. "We observed threefold reduction when we combined chloroquine with rifampicin and fivefold reduction when we used chloroquine-isoniazid combination."

To determine TB relapse following treatment, mice infected with TB were completely rid of bacteria using the drug combination. Eight weeks later, the immune system of mice was suppressed using a drug. While all the five mice treated with only isoniazid relapsed with high bacterial load, three of the five mice treated with the combination drug showed very little presence of bacteria. "This shows that the drug combination reduces the chances of TB relapse," says Mishra.

The work was carried out in collaboration with researchers from Bengaluru's National Centre for Biological Sciences and Foundation for Neglected Disease Research.

Question Corner

Light and shade in photosynthesis

Q ***Is there less photosynthesis taking place in leaves on lower branches of a tree and in permanent shade from direct sunlight?***

A **ARUN K. SHANKER,**
CRIDA, Hyderabad

Yes, photosynthesis will be less in leaves that are in shade. Usually they will not be in permanent shade, there will some light falling on them at some time of the day, also due to wind the leaves will get light.
The leaves in shade will adapt to shade. One of the adaptions of these leaves that are in shade is to have larger area to capture more light for photosynthesis.
These leaves are thinner than the leaves that are open to the Sun. The shade leaves are dark green in colour and also the chloroplasts and the light harvesting complex moves within the cells to places where there can be more light interception.
The shade leaves are more efficient in photosynthesis, the problem is that they lose more water by transpiration at the same temperature and humidity conditions.
The shade leaves also have low dark respiration rates and hence fewer light compensation points - which is the light intensity on the light curve where the rate of photosynthesis exactly matches the rate of cellular respiration - this is low for leaves in shade.
These adaptations take place during the development of the leaves. Leaves in shade also have short and long term strategies to count fluctuating light intensity.
The short term strategy is rearrangement of antennae system in photosystems in the leaves so that they can make use of the available light effectively.
This is done by movement of the mobile pool of the light harvesting complex in the photosystems.
The long term strategy of the leaves in the shade is to overexpress some of the genes of Photosystem I - psaA, psaB and Photosystem II - psbA.

This week's questions

How does a fully charged mobile phone lose its charge when left unused for several days together?

Why and how does the blue light of light emitting diodes affect sleep while yellow promotes sleep?

How do some aircraft avoid detection by radar? Can clouds hamper detection?

BHAVANI GIDDU,
New Delhi

These questions will be answered in subsequent weeks in this column.

Readers may send their questions / answers to questioncorner@thehindu.co.in

Fossil reveals how middle ear evolved

Extinct rodent lived 145-66 mya in what is now northeastern China

PRESS TRUST OF INDIA

Researchers have unearthed well-preserved middle ear bones from a new species of an extinct rodent that lived 145-66 million years ago in what is now northeastern China, an advance that may lead to better understanding of the evolution of hearing.

The study, published in the journal *Nature,* looked at fossils of the extinct rodent-like mammal – *Jeholbaatar kielanae* – at Jehol Biota of China, and noted that these animals had a middle ear that is distinct from those of its relatives.

The researchers, including those from the Chinese Academy of Sciences, said the evolution of the rodent's bones and muscles involved in hearing may have been driven by specialisation for hearing. They said the fossil clues provide solid evidence of the morphology and formation of the inner ear bones, which are fully detached from the lower jaw.

Unique configuration

According to the researchers, the bones reveal a unique configuration with more complete components than those previously reported in these creatures.

The new fossil, they said, reveals a transitional stage in the evolution of the surangular – a "reptilian" jawbone.

Based on the new findings, the scientists speculated that in a class of extinct mammals, the joints connecting the middle ear to the eardrum, and those connecting bones in the cheek region to the lower jaw may have evolved in tandem, allowing a distinct jaw movement while chewing.

They suggested that in these extinct mammals, the evolution of the middle ear may have probably been triggered by functional constraints on the bones and muscles involved in feeding.

Oxygen bars are surely not a solution for pollution

No medical community has come forward to spread awareness on this captivating yet unscientific business

SUBHABRATA MOITRA

New trend: A user breathes in oxygen mixed with perfume at an oxygen bar in New Delhi. ▪ REUTERS

The popularity of packaged air began around four years ago when a Canadian company launched 'canned air' for people in China when air pollution in many cities became alarmingly high. The newer addition – oxygen-bar – a recreational parlour or cafe which serves 'pure oxygen' is becoming a more attractive destination, particularly in cities with dangerous levels of air pollution. At times, the oxygen comes in different scented flavours.

In cities with highly polluted air, the business of 'canned oxygen' or 'oxygen-bar' is flourishing. The recent launch of such a recreational oxygen parlour in Delhi amidst the city's infamously bad air condition has caught significant media attention. But how safe are they and are any benefits at all?

First, do we really need this extra oxygen? The simplest answer is no. Unlike conventional oxygen therapies used in respiratory conditions that is administered for a short or long period in hospital or at home, people take oxygen for an ultra-short period in these bars (30 minutes or less). As per the standard clinical procedure, oxygen supplementation can be administered only in case of hypoxemia (lowering of oxygen saturation in the arterial blood below 95%) and it does not have any consistent beneficial effect on non-hypoxemic patients.

Placebo, at best

It must and should be remembered that the oxygen level does not alter in the air even when the pollution level is high. The same applies to our health – oxygen saturation in blood remains unchanged in healthy people in normal conditions, and such recreational oxygen cannot provide any health improvement. It can at best have a placebo effect. Though users and proponents of purified oxygen claim several benefits such as relieving stress, headache and migraine, and help in achieving better energy and mood, there is no clinical evidence available so far in support of the beneficial effects of recreational oxygen use.

Most importantly, the use of scented oxygen might not be safe. To add scent to oxygen, the oxygen is bubbled through a liquid containing scented additives or aroma oil. Users will seldom know the properties of the oils or the components of the additives used. Scented oxygen can be harmful to people, particularly to those with allergies and lung diseases. Fragrant materials very often contain aromatic hydrocarbons, many of which are potential allergens and can trigger asthma and allergic symptoms.

Moreover, the aromatistion of oxygen generates ultrafine droplets of essential oils which, when inhaled with oxygen, get deposited in the lungs and accumulate in the alveoli leading to a respiratory condition known as "lipoid pneumonia". In this condition, deposited oil droplets can cause severe inflammation, damage alveolar septa (thin single cell lining between two adjacent alveoli) and interstitium (the area between an alveolus and its adjacent capillary) and lead to fibrosis. Long-term exposure to such exogenous oil substances may cause chronic lipoid pneumonia in which the patients remain asymptomatic and are often diagnosed at a very late-stage, and that too, incidentally, due to other illnesses. Among people with a lung condition, even a short-term acute exposure to such exogenous fragrance or oils can be life-threatening.

It must be borne in mind that oxygen-bars are sole-proprietorship ventures and are not legalised to administer oxygen for therapeutic purposes. These bars are not endorsed by local or federal healthcare systems and are not obliged to follow clinical bylaws, and thus cannot be held liable for any unwarranted health effect or an acute medical condition that occurs in the bars. Moreover, there are no statutory warnings or guidelines available at these bars about the potential adverse effects, particularly applicable to vulnerable population such as children, aged and person with allergies or lung conditions.

Captivating yet unscientific

It is unfortunate that no medical community has come forward to spread awareness among people for this increasingly captivating yet unscientific business with no known or established clinical benefit. It definitely calls for serious vigilance by the clinicians and policy makers to ensure the safety issues associated with recreational oxygen use, particularly flavoured oxygen in such bars, parlours and spas.

The writer is a European Respiratory Society Research Fellow at ISGlobal, Barcelona, Spain, and associate member of the Royal Society for Public Health, United Kingdom.

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CCMB team uses *E. coli* to study bacterial cell wall development

The cell wall of the bacteria is made up mostly of one large molecule called peptidoglycan

SHUBASHREE DESIKAN

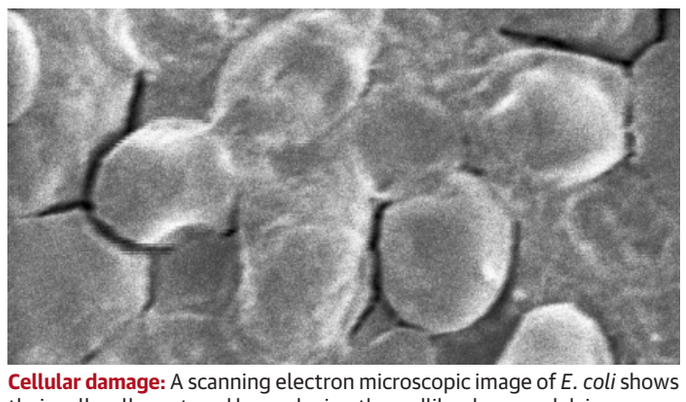
Researchers from Hyderabad have identified an enzyme that plays a crucial role in the enlargement and growth of bacteria, by studying *E. coli*. The enzyme MepK helps in cutting a particular class of bonds that connect the peptidoglycan, which is a sac-like molecule that envelops the cell. This action allows more material to be added to the cell wall, making a larger compartment for the cell to reside in.

One of the most important features of a bacterium is its cell wall which protects it from external environmental conditions and also internal pressure and keeps it in shape. Harming the cell wall causes irreversible damage to the bacterium and eventually kills it.

Crucial factor

For example, *E. coli* are rod shaped bacteria often experimented with in the lab. The bacteria die when the integrity of the cell wall is destroyed. Its crucial role in maintaining the wellbeing of the bacterium makes the cell wall a target of study, especially by scientists interested in developing new drug strategies to combat the bacteria.

In this context, understanding how the bacterial cell wall develops during growth and division of cells is an important question being addressed in Manjula Reddy's lab at the Centre for Cellular and Molecular Biology (CSIR-CCMB) in Hyderabad for a decade now.



Cellular damage: A scanning electron microscopic image of *E. coli* shows their cell walls ruptured hence losing the rodlike shape and dying. ▪ CCMB

In an earlier work, done in 2012, Dr Reddy's group showed that opening the cell wall by hydrolysing enzymes is crucial for the new material to be incorporated into it, leading to the cell's expansion and elongation.

The cell wall is made up mostly of a single net-like molecule (peptidoglycan). This consists of many sugar polymers interconnected by short peptides. It encloses the bacterial cytoplasmic membrane very much like a jute bag. The peptides connecting the baglike structure are cross-linked in several ways. Of significance to this work are the links between particular amino acid residues located on adjacent peptide chains. This is a rare component present only in bacterial cell walls and is known as mDAP for short..

Vital enzyme

In a paper published recently in the *Proceedings of National Academy of Sciences (PNAS),* the group identified an enzyme (MepK) which helps in breaking down the bond between two mDAP residues. This leads to cutting the molecular mesh and thus aiding the growth (or enlargement) of the cell. "By cleaving these cross-links, MepK [along with other known enzymes] contributes to growth and enlargement of sac-like peptidoglycan... This emphasises the fundamental role of cross-link cleavage in bacterial cell wall synthesis," says Pavan Kumar Chodisetti, from CSIR-CCMB and the first author of the paper.

"The class of enzymes reported in this paper was not known earlier, and identifying this enzyme [MepK] gave us lot of excitement," says Dr. Reddy. "[The study] has higher significance in organisms like *Clostridia* and *M. tuberculosis* because cell walls of these bacteria have very high levels of mDAP-mDAP type of cross-links. Therefore, MepK-like enzymes will be very important for the growth of these bacteria."

These cross-links constitute approximately 10% of total cross-links in Gram-negative bacteria like *E. coli* and *Pseudomonas*. However, they are predominant in many Gram-positive bacteria such as *Mycobacteria* and *Clostridia* (occur up to 80% of total cross-links)

The next step according to Dr. Reddy is "identifying small-molecule inhibitors for this class of enzymes and also to understand the molecular mechanisms by which the cell wall growth is initiated".