CAPSULE

Roots of empathy
Sharing another's emotional state has a neural basis. A study published in *Current Biology* found that special neurons named mirror neurons are activated in rats when they feel pain and also when they observe others who are in pain. When mirror neurons are not active, the animals no longer share others' pain, the study found.

Mending hearts
Zebrafish and salamanders can normally regenerate their hearts unlike humans and many mammals. If exposed to excess of thyroid hormones, zebrafish lose this ability. The study published in *Science* infers that lack of heart regenerative ability in humans may be due to a trade-off for increase in metabolism, to stay warm.

JNCASR's molecule improves recovery after spinal cord injury

In mice and rat models with spinal cord injury, the molecule led to recovery of sensory, motor functions

R. PRASAD

Spinal cord injury can now be repaired using a small molecule (TTK21) synthesised by a team led by Tapas Kumar Kundu from the Molecular Biology and Genetics Unit at Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Bengaluru, a study has found. The small molecule tested both on mice and rat models promoted regeneration and growth of new sensory and motor axons leading to recovery of sensory and motor functions in the animals with spinal cord injury.

Since the small molecule cannot cross the blood-brain barrier and enter the brain, the researchers used 400 nanometre-size carbon nanospheres made using glucose, which is self-fluorescent, and attached the molecule to its surface. The non-toxic nature of the small molecule has already been demonstrated in animals.

When the spinal cord is injured, the tails (axons) of nerve cells that stretch up and down the spine are either damaged or even completely cut. So signals from the brain can no longer travel beyond the site of injury when axons are severed, leading to paralysis.

Cbp-mediated acetylation level is important in making new neurons and extending the length of the axons connecting injured neurons, says Tapas Kumar Kundu,

According to anecdotal evidence, people with an active lifestyle have greater chances of recovering after spinal cord injury compared with those who are not active. Researchers tested this on animals by providing them with a larger cage, more mice to interact with, exercise (running) wheel, unidentified objects before inflicting an injury to the spinal cord. The environmental, physical or social stimuli were priming the cells and boosting their potential to regenerate such that five weeks after spinal injury, the damaged nerve fibres regenerated at the site of injury. Some axons regenerated so well that they expanded beyond the lesion site.

This finding in animal models prompted the researchers to investigate the underlying molecular mechanism to identify a therapeutic target to achieve recovery after spinal injury. They found that post spinal cord injury, animals that were earlier exposed to different stimuli expressed changes in the Cbp enzyme-mediated acetylation. This change brought about by the enzyme caused an increase in the expression of a set of genes associated with regeneration and growth of axons.

"Modification in the level of Cbp-mediated acetylation plays an important role in several biological phenomena including memory recovery, making new neurons as well as extending the length of the axons which connect the injured neurons," says Prof. Kundu, presently the Director of CSIR-Central Drug Research Institute, Lucknow.

"Mimicking the regenerative effect of environmental stimuli, we wanted to test if our small molecule could activate the Cbp enzyme and promote axon regeneration and recovery," says Akash K. Singh, a PhD student at JNCASR and co-author of a paper published in the journal *Science Translational Medicine*.

In trials carried out in mice and rats, the small molecule injected four hours after the injury and once a week for five weeks resulted in regeneration and growth of axons at the site of injury. "The extent of regeneration and functional recovery of axons was nearly the same in both mice and rats. This proved our small molecule has a therapeutic effect," says Dr. Sarmistha H. Sinha, post-doctoral fellow from JNCASR and co-author of the paper.

Behavioural tests

"Behavioural tests showed the mice could walk with fewer falls and slips due to imbalance. The number of times the animals slipped reduced with time. And on the floor, mice were also able to walk normally on the floor without limping," says Prof. Kundu. "And the mice were able to quickly sense and remove the adhesive stuck to the hindpaws indicating recovery. When animals are paralysed they fail to sense the presence of the tape."

"Along with the Imperial College London, we are exploring the possibility of conducting pre-clinical trials and jointly develop the molecule for therapeutic use in humans," Prof. Kundu says.

A coil in the stomach for better TB treatment

The coil eliminates the need for daily drug administration and brings down cost

ASWATHI PACHA

The treatment of tuberculosis is becoming more difficult as most patients don't adhere to the treatment regimen which includes six to nine months of daily antibiotics. This also contributes to emergence of multidrug-resistant bacteria.

Now, researchers from Massachusetts Institute of Technology, U.S., have developed a coil loaded with antibiotics that can stay in the stomach for up to a month and release the necessary drugs in the required doses. This eliminates the need for daily administration and also brings down the cost of treatment. The coil is made of a nickel-titanium alloy (nitinol) and looks like a small slinky toy. Just like adding beads on a string, the researchers added 600 pills (4 mm height and diameter) of different formulations to the coil. The drugs used included doxycycline hyclate, isoniazid, ethambutol, pyrazinamide, moxifloxacin, and rifampicin. The pills were spray coated with a special polymer which enabled controlled release of the drug. The final coil measures 32 mm in thickness and when stretched out is around 2 metre in length.

Lab studies

Laboratory studies showed that the coated pills were able to release the drugs slowly for up to one month.

For *in vivo* studies, the researchers stretched out the coil and inserted it through the nose into a pig's stomach.

When asked why nasogastric route was used instead of oral, Malvika Verma, the first author of the study said: "As of now, the nasogastric tube is the feasible way to deliver this device since the largest swallowable capsule can only hold 1 gram of drug at most. This tube enables delivery of more than 10 grams of drugs."

After reaching the stomach, the tube coiled up, stayed in the stomach and released drugs for 28 days. Endoscopic evaluation showed that having such a large coil in the stomach did not cause any injury or ulcer. There was also no weight loss or limitations in the passage of food and water.

The researchers added 600 pills of different formulations to the coil made of a nickel-titanium alloy. ▪ MALVIKA VERMA

The coil can be retrieved via the same nasogastric route by inserting a tube which attaches to the magnet on the coil. Ms. Verma added that further work is being carried out to understand the acceptability and feasibility of the system during insertion and retrieval. The team anticipates initial human trials to begin in the next five years.

The researchers spoke to 300 tuberculosis patients and over 100 TB health providers in India and noted that most of them were open to the idea of a monthly administration using this coil.

"In many cases, this was preferred by the patients, who could come to a health-care setting every two weeks or every four weeks instead of having to be seen by a health care provider every day," says Prof. Giovanni Traverso from MIT's Department of Mechanical Engineering in a release. He is one of the corresponding authors of the paper published in *Science Translational Medicine*. The team also analysed the economic impact of this new treatment regimen and the paper notes that the cost could be reduced by "more than $8,000 per patient"

What drives tiger dispersal

Terrain affects dispersal in different ways in the Western Ghats and central India

AATHIRA PERINCHERY

Tigers in India traverse long distances to find mates and new territories. But the movement depends on roughness of the terrain and human disturbance in the area. The terrain affects tiger dispersal differently in the Western Ghats and central India, two strongholds of wild tiger populations in the country, finds a new study.

The central Indian landscape is highly fragmented with high densities of people, while the Western Ghats has lesser human disturbance and is home to the world's largest contiguous tiger population. A study in 2017 by a team including Anuradha Reddy (of Hyderabad's CSIR-Centre for Cellular and Molecular Biology) revealed that roughness of terrain and human footprint drove tiger gene flow in central India: tigers moved across ridges and rough topography to avoid the presence of people. Do similar landscape features drive tiger gene flow in the Ghats?

Varied samples

Another team including Dr. Reddy studied this across 30,000 sq km in the Western Ghats in Kerala, Karnataka and Tamil Nadu. They collected tiger faeces in forests including Bhadra Tiger Reserve and Nilgiri Biosphere Reserve, and used forensic samples that came to CSIR-CCMB between 2011 and 2015 to obtain genetic data of 115 individual tigers. They complemented this with overlays of land cover and land use categories, using maps showing terrain, road networks, developed areas (reflecting human disturbance) and historical maps (from the 1960s, to see how vegetation cover changed over the decades).

Role of gene flow

Though the team did not find strong correlations between current genetic structure and historical landscape in the Ghats, comparing the data with the team's earlier study in central India (after standardising the methods for comparisons) revealed an interesting pattern – the relationship between terrain and gene flow is "inverted" in both regions. While gene flow correlated with rough terrain in central India, it was linked with smooth forest terrain containing minimal human disturbance in the Ghats, finds the team's study published in *Animal Conservation*.

This pattern is mainly due to differing levels of human disturbance, Dr. Reddy said in an email. While Central India has more fragmented forests and higher human disturbance, the Ghats have relatively larger, connected forest patches and lesser human disturbance, facilitating tiger movement across lower and smoother areas, she added.

ICGEB's novel yeast strain increases ethanol production

The strain produces ethanol by fermenting rice and wheat straw

R. PRASAD

Compared with currently available strains, a robust yeast strain (*Saccharomyces cerevisiae* NGY10) that can produce up to 15.5% more ethanol when glucose or lignocellulose biomass – rice and wheat straw – is fermented has been isolated by researchers from the International Centre for Genetic Engineering and Biotechnology, DBT-ICGEB Centre for Advanced Bioenergy Research, Delhi.

In India, ethanol production is mostly by fermenting molasses to meet the annual target of 5% blending of petrol with ethanol. But with India setting a target of blending petrol with 10% of biofuel by 2022, other sources such as rice and wheat straw have to be considered. Fermenting lignocellulose efficiently to generate more ethanol than what is currently possible is therefore necessary. To that end, the strain isolated by ICGEB becomes important.

The team led by Dr. Naseem A. Gaur from the Yeast Biofuel Group at ICGEB isolated 500 yeast-like colonies from different natural habitats – distillery waste, dairy waste, hot springs, sewage and algal bloom. After screening, 25 yeast-like colonies were chosen and an additional nine yeast strains from the National Culture collection of Industrial Microorganisms (NCIM), Pune, were included for evaluation. Of these, one strain was found to suitable for fermenting rice and wheat straw. The results were published in the journal *Biotechnology for Biofuels*.

Lignocellulose is comprised of lignin, cellulose and hemicellulose. While cellulose is rich in hexose or C6 (glucose) sugar, hemicellulose, which accounts for about 30% of the composition, is made mostly (more than 90%) of pentose or C5 (xylose and arabinose).

Three challenges

Ethanol production by fermenting lignocellulose biomass faces three challenges. During the fermentation process, the temperature increases from about 30 degree C to 40 degree C. Since the commercially available yeast strains are good at fermenting at 30 degree C, the fermentor has to be cooled down when the temperature increases. This increases the cost of ethanol production. Second, lignocellulose biomass (rice and wheat straw) contains a mixture of hexose and pentose sugars.

Though yeast can ferment glucose (hexose sugar), it cannot ferment pentose sugar (xylose and arabinose) that make up 30% of the composition. Finally, the pretreatment of lignocellulose (to breakdown the recalcitrant structure of the biomass) results in the production of three main inhibitors (furfural, 5-HMF and acetic acid). These inhibitors reduce the fermentation performance of yeast, leading to reduced ethanol production.

Functional superiority

Unlike currently available, commercially used yeast strains, the strain (NGY10) isolated by the ICGEB team has been found to be thermotolerant and can continue to ferment the biomass even when the temperature increases to 40 degree C. "The strain (NGY10) displayed a negligible reduction in the growth even in the presence of all three fermentation inhibitors at 40 degree C. And it produced more ethanol than currently available industrial yeast strains," says Dr. Gaur. "But the NGY10 strain was not able to ferment the pentose sugar (xylose and arabinose)."

"Our strain showed better efficiency than the industrial strains now available in producing ethanol from lignocellulose. Also, the NGY10 isolate produced 11.1 % and 15.49 % more ethanol compared with the industrial yeast (Angel yeast) when glucose and pretreated lignocellulose were fermented, respectively," says Ajay Kumar Pandey from ICGEB and first author of the paper.

Engineering the strain

The NGY10 strain can be metabolically engineered so it can ferment both hexose and pentose sugars leading to increased production of ethanol using lignocellulose.

This will increase the quantity of ethanol produced from lignocellulose biomass but also reduce the cost of ethanol production.

"We have almost engineered the strain to make it capable of fermenting both pentose and hexose sugars," Dr. Gaur says. "The productivity and ethanol yield from pentose sugars after metabolic engineering are encouraging and comparable with yield obtained with glucose (hexose sugar) fermentation."

'Keep the cell wall shut' model to fight bacterial infection

Even as we discover drugs and molecules to fight them, bacteria quickly mutate, resist

SPEAKING OF SCIENCE

D. BALASUBRAMANIAN

Bacteria have been on Earth for the last 3.8 to 4 billion years. And they have depended on whatever the environment has provided them for carrying on with their lives and reproduction. We humans are late-comers on Earth, roughly about 66 million years ago. Today there are about 7 billion of us (7 followed by nine zeros) while the total number of bacteria is astounding – five million trillion trillion (a five with 30 zeros after it); there are far more bacteria on Earth than there are stars on the universe.

And many of these bacteria feed on us. While some of them are "safe" and even useful for us (the human gut hosts about 100 trillion bacteria, helping us in our growth and development), many others make us ill and even kill us. And we humans have tried various ways to fight these infections using herbs and drugs since ancient times. Dr Rustam Aminov writes in his paper: "A brief history of the antibiotic era: Lessons learned and challenges for the future" (*Frontiers in Microbiology*, 8 Dec 2010) that ancient Egyptians tried using poultices of moulded bread against infection and ancient Sudanese skeletons had traces of the antibiotic tetracycline – obviously from some herb they would have used against microbial infections.

A recent course

The day of modern medical treatment is recent. Dr. Hara discovered the compound arsphenamine to fight syphilis in 1909 and Dr. Bertheim synthesised it and called it salvarsan in 1910. And in 1928, Alexander Fleming discovered penicillin, which could kill a large number of infecting bacteria.

Even as we discover more and more drugs and molecules to fight them, bacteria quickly change their genetic composition by mutation and resist the action of the drug. It has thus been a tug of war between scientists and bacteria. We have now come to realise that unless we understand the basic biological steps involved in bacterial infection, this fight cannot be won by us.

It is towards this challenge that microbiologists have been studying the molecular biology of bacteria using the species called *Escherichia coli* (*E. coli* for short) as the model organism in the laboratory. We now know that bacterial cells are surrounded by a protective cell wall made up of a large sac-like structure called peptidoglycan or PG. The PG that bacteria use to build their cell walls is specific to them alone, and not found anywhere else on earth.

The PG is a baglike structure which is made of sheets of two sugar molecules, NAG and NAM, linked together as long chains. These sheets are cross-linked or stapled together to form a continuous layer around the bacterial cells. Therefore, as the bacterium grows in size, this PG bag also has to expand. That means the stapler has to be opened, new material incorporated and the bag stapled again into compactness for successful bacterial growth.

Key step

An important step towards this has been made by Dr. Manjula Reddy and her colleagues at the Centre for Cellular and Molecular Biology (CCMB) at Hyderabad. Her group has been studying the basic biology behind how the bacterium builds its cell wall, how the bag opens for growth, and what molecules help in opening the bag. Her group has identified a particular class of enzymes, which are responsible for unstapling the PG bag (see their publications: Singh et al., *Mol. Microbiol.*, 2012; 86 (5), 1036-1051; Singh et al., *PNAS*, 112, 10956-10961; Chodisetti et al., *PNAS*, April 2, 2019; https://doi.org/10.1073/pnas.1816893116). They further showed that if any or all of these enzymes are removed from the bacterium (using genetic engineering methods), the PG bag does not open, starving the bug to death.

What does this mean? If we can find molecules or methods to inhibit these enzymes, and thus arrest the infecting bacterium from making its protecting cell wall, we will have found a way to overcome infection and offer safety.

A differing approach

Incidentally, the classical antibiotic, penicillin, inhibits the enzymes which help in re-stapling the once-opened cell wall, thus weakening the bug and killing it. While this approach is a "do not close the wall" one, the CCMB approach is a "keep the cell wall shut and never open it" one. The currently popular class of antibiotics, called the fluoroquinolones (such as ciprofloxacin), acts not on the cell wall, but inhibits the enzymes that allow the DNA of the bacterium to open up and replicate itself. These drugs thus inhibit the reproduction and repair of genes of the infecting bacteria.

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