

Science Last Fortnight

Microbial Biofilms in Burgers

Flavonoid inhibitor in action

Some microbes combat unfavourable environments by forming biofilms. They assemble together, adhere to a surface and enclose themselves in a capsule composed of amyloids, exopolysaccharides and adhesive fimbriae. This makes them resistant to physical or chemical elimination.

Biofilm formation by foodborne pathogens is a major concern for the food industry: it is the basis for disease outbreaks. Multiple handling and processing steps make ready-to-eat fast foods more susceptible to contamination by foodborne pathogens.

Last fortnight, scientists from the Aligarh Muslim University collaborated with scientists from Saudi Arabia to report isolating foodborne pathogens from regularly consumed fast foods: burgers and shawarma. They explored the inhibitory effect of rutin on biofilm formation. Rutin, a flavonoid found in the citrus family, peaches and buckwheat, has extensive pharmacological properties.

The researchers first isolated bacterial strains from fast food samples. They found that, of the 72 strains recovered, 20 were resistant to most antibiotics. For their inhibition studies, they selected six strains showing strong biofilm growth.

The team then performed assays with sub-lethal doses of rutin and optimized the minimal concentrations of rutin needed to inhibit biofilm formation. They established that rutin, at sub-minimal inhibitory concentration levels, inhibits exopolysaccharide formation by bacterial colonies in a dose-dependent manner. These exopolysaccharides are a major component of biofilms.

Using scanning electron microscope images, the scientists demonstrated that rutin inhibits even multi-species biofilms formed by selected drug-resistant strains. Thus, they claim that rutin can be used in the food industry, especially in equipment and food contact surfaces.

Antimicrobial peptides are often used to prevent biofilm formation. However, it is safer to use plant-based inhibitors,

especially in the food industry, since these are relatively less harmful.

Food Control, **79**: 325–332

Healthy Ageing

Ashwagandha augments longevity

Ageing is becoming older or more mature. Intriguingly, everyone wants to become mature – but without ageing. The reason could be the association of ageing with chronic diseases and increased mortality. With anti-ageing products selling at a premium, scientists have been trying to find solutions to slow down the process.

Recently, a team of researchers from AIIMS, New Delhi, reported that an ethanolic extract from a medicinal plant, Ashwagandha – *Withania somnifera* – enhances the longevity of human cells by modulating the expression levels of two proteins: Forkhead Box protein O3A – FOXO3A and Sirtuin3 – SIRT3.

The role of FOXO3A and SIRT3 in healthy ageing is well documented. Both proteins increase the antioxidant capacity of cells. Reactive oxygen species accumulation is one of the main factors causing oxidative stress in cells. Excessive formation of reactive oxygen species accelerates ageing. SIRT3 regulates the function of FOXO3A by a physical interaction which detoxifies the reactive oxygen species.

The team studied the levels of FOXO3A and SIRT3 proteins and observed that the expression of both proteins declined with increasing age. There was no difference in the protein levels between male and female.

The team then assessed the effect of the Ashwagandha extract on the expression of these proteins. The levels of the two proteins increased on treatment with the plant extract. This result suggests that Ashwagandha can be an excellent agent for healthy ageing.

Ashwagandha can be a better alternative than synthetic anti-ageing compounds since it is a natural product. With the growing demand to lead a long and healthy life, this information might benefit the pharmaceutical industries.

Exp. Gerontol., **95**: 9–15

Gallstones: South and North

Variations in India

Minute stones that build-up in the gall bladder might turn out to be very painful, sometimes requiring surgery. Imbalance in the chemical composition of bile leads to the formation of gallstones. Precaution or treatment can be made effective only with a better understanding.

A team of researchers from the Anna University, the AMET University and the University of Duisburg-Essen analysed the chemical and structural composition of gallstones. They tested representative gallstones from North and South India. The researchers used several combinations of physicochemical methods like X-ray diffraction, FTIR and NMR spectroscopy, CHNS analysis, infrared spectroscopy and thermal analysis. Common chemicals found in both the types were carbon, nitrogen, oxygen, calcium, sulphur, sodium, chloride and magnesium.

The team found that the stones obtained from North Indians categorized as *Cholesterol gallstones* consisted of cholesterol monohydrate and anhydrous cholesterol and appeared yellowish and platy. On the other side, the ones from South Indians, the *Pigment gallstones* appeared globular and black and consisted of cholesterol and calcium bilirubinate having higher nitrogen and low carbon content.

These differences could be due to different food habits and habitat. Geological location, components in bile such as glycoproteins, fatty acids, and liver associated diseases may also be some important factors.

The composition varies in different parts of the world. Cholesterol is dominant in the western hemisphere and pigment/mixed type in south Asian countries. Cholesterol gallstones are predominant in northern, eastern and western parts of India. Pigment gallstones are prominent in South India. It is found that *Tamarindus indica*, high in iron content, may be an important risk factor for pigment gallstone formation. The mechanism responsible for this pattern is yet to be investigated.

One hypothesis has been the altered composition of bile. Supersaturated bile remains stable for many days and during gallstone formation, this metastability is lost and within a few hours, crystal formation starts. Further growth occurs either by deposition of additional insoluble precipitants or by agglomeration of cholesterol crystals.

Based on these characteristic features of gallstones, can pharmacologists find new effective remedies?

Mater. Sci. Eng. C., **78**: 878–885

Hope for HIV Vaccine

Deciphering the unchanging code

Mother-to-child transmission is one of the major routes of HIV infection. This can be prevented if pregnant HIV positive mothers undergo antiretroviral therapy before delivery. However, many babies do become positive because of lack of awareness of this preventive treatment.

Preventing HIV infections using vaccines has, therefore, been a public health requirement. However, the virus undergoes rapid changes from generation to generation and there are many strains now. Finding a target protein common to all strains as a target to develop a vaccine is difficult. Any vaccine must have multiple targets to be effective. Designing and testing vaccines will have to take into consideration the geographical diversity of the HIV.

A team of researchers from Jamia Millia Islamia and the National Centre for Disease Control, New Delhi, recently genotyped the HIV-1 *nef* gene in two age groups of less than 6 and between 6–18 months. The negative regulatory factor or *nef* protein appears abundantly in early phases of viral replication. It down-regulates the expression of host cell histocompatibility antigens as well as the CD4 molecules on the T cells.

The team analysed 50 out of 57 PCR positive samples belonging to the Indian subtype C *nef* sequence. They found insertion of 10 residues in variable regions of the protein and deletion of 13 residues in one of the infants. Higher genetic variability was observed in functional motifs in infants of acute age group compared to early age group infants of less than 6 months. This variability may be due to getting infected with a heterogeneous virus population from their mothers.

However, when they examined the peptide motif, which binds with the histocompatibility antigens, they found an epitope, a section of the protein which is common in more than 80% of the sequences – a potential target for vaccine development.

Besides enhancing our understanding of the genetic diversity in the Indian strains of HIV, the study gives us a clue about the association of genetic variations to progression of the disease in infants. Moreover, it is a major step towards a better designed AIDS vaccine, for subtype C strains in the Indian subcontinent.

J. Medical Virol., **89**(9): 1606–1619

Ofloxacin Resistant Tuberculosis

Mycobacterium tuberculosis infections remain a challenging public health issue. Ofloxacin, a potent fluoroquinolone, is a popular choice for treatment. It inhibits the bacterial enzyme, DNA gyrase, halting the supercoiling of DNA and thus disrupting bacterial reproduction.

Fluoroquinolones have been used as antituberculous agents since the 1980s. And ofloxacin resistant *M. tuberculosis* has emerged due to mutations in the DNA gyrase. The challenge now is to understand the mechanism of resistance.

Recently, researchers from the Panjab University, IIT Delhi, JNU, Banastali University and TERI University joined hands to investigate the molecular principles involved in *M. tuberculosis* resistance to ofloxacin.

Sequencing results from previous studies had revealed that 50–90% of the resistant strains of *M. tuberculosis* carry mutations in the *gyrA* gene and 7% in the *gyrB* gene. Double mutants in both *gyrA* and *gyrB* have also been identified. Using molecular dynamics simulation and protein ligand docking studies, the scientists have now found that the interaction of the wild type DNA gyrase and ofloxacin have high affinity. Whereas double mutant DNA gyrase–ofloxacin interaction exhibited poor affinity. The team found lesser hydrogen bonds in the mutant DNA gyrase–ofloxacin complex due to the changes in the amino acids, which explains the lower affinity.

The team report that the binding area and volume of the wild type DNA

gyrase is much less than the double mutant DNA gyrase. Wild type DNA gyrase had high binding free energy compared to double mutant DNA gyrase. The results explain the lowered antituberculous effect of ofloxacin in strains with double mutants DNA gyrase: the drug cannot inhibit DNA replication because it cannot bind to the double mutant DNA gyrase.

Such *in silico* studies reveal the structure of the active sites of the double mutant protein. This can help designing drugs against drug resistant-DNA gyrase mutants. So there is hope that these findings will help us win the next battle in the war against *M. tuberculosis*.

J. Cell. Biochem., **118**(9): 2950–2957

Sharks and Rays

DNA barcoding reveals new species

The word shark evokes fear. But it is they who are now endangered. Sharks belong to the family of fish that have cartilaginous skeletons. They are adapted to a wide range of aquatic environments. Some shark species like bull sharks are even known to swim in salt, brackish and fresh waters.

Shark fin soup is a delicacy in South-East Asia. The increased global demand for their fins and meat is reducing their numbers. And rays, relatives of sharks, are also in the same boat due to bycatch in commercial fishery.

Human exploitation has spread across land, coastal seas and the open ocean. This has transformed ecosystems by eliminating many species. In the past half century, fishing fleets have expanded rapidly exploiting large marine predators.

Rays and sharks are slow growing fishes. So overfishing threatens them more than other fishes. The decreasing population of sharks, top predators in this ecosystem, makes the marine ecosystem unstable. Therefore, different government and private agencies are concerned about their conservation and management. However, accurate data on these fishes is needed to take informed decisions.

To address the issue, Bineesh and team from the Central Marine Fisheries Research Institute collaborated with researchers from Australia, and collected 111 species of sharks and rays from all along the Indian coast and did DNA

barcoding of a mitochondrial gene, cytochrome c oxidase subunit 1.

The DNA barcoding technique helped the researchers identify the species. Interestingly, among the 111 species identified by this method, 11 were found to be first records from Indian waters. Ten may turn out to be new to science and further research is needed to determine their designations.

The study reveals that the species diversity of sharks and rays in India is underestimated. The researchers point out that, previously, more than 150 species of this group have been reported in Indian waters. They draw attention to the need to properly document the extant species.

The scientists have now developed a database of genetic information on sharks and rays. This can be used for testing a piece of fish to identify fish products in commercial trade. This will help regulate illegal trade of protected species.

Mitochondr. DNA, **28**(4): 458–472

Bacterial Bioluminescence

Reporter for heavy metal pollution

Globalization and industrialization have increased heavy metal pollution. Even low concentrations of heavy metal pollutants in soil, water, air and plants raise concerns because of bioaccumulation: the concentration of the metals increases with the food chain. To resolve this problem, several researchers are trying to develop reporter assays to detect and monitor heavy metal pollutants discharged from different industries.

Recently, a team of researchers from the Pondicherry University developed a heavy metal reporter to detect copper, mercury and zinc in wastewater. They used the principle of bioluminescence to design this sensor.

Bioluminescence – light emitted by several marine, terrestrial, and some freshwater organisms – has two protein components: luciferin and luciferase. A chemical reaction between luciferin and oxygen in the presence of the luciferase enzyme generates oxyluciferin that emits light. The emitted spectra fall between 400 to 700 nm and we see green, yellow, red blue and pink.

Bioluminescence is used for interspecies signalling, alarming predators, lur-

ing prey and camouflaging. Unlike macro bioluminescent organisms, bacterial bioluminescence has been extensively studied. So the scientists homed in on *Vibrio harveyi* – efficient bioluminescent bacteria – to detect heavy metals *in vivo*.

The researchers assessed the heavy metal sensing efficiency of this bacterium by inoculating copper, mercury and zinc at 1, 2 and 3 mM concentrations in a broth containing luminescent *V. harveyi* grown overnight. They found a reduction in *V. harveyi* bioluminescence with increasing concentrations of heavy metals.

The team also isolated an extracellular bioflocculant molecule – a thermally stable polysaccharide from a bacterium, *Bacillus cereus*, associated with the marine sponge, *Dendrilla nigra*. This polysaccharide adsorbs heavy metals in culture media effectively and, thereby, enhances bioluminescence. The scientists suggest that these bioflocculant polysaccharides, along with *Vibrio harveyi* bioluminescence, can be used in wastewater treatment and drinking water purification.

The bioluminescent assay will help public health authorities monitor heavy metal discharge from food and chemical industries. The team claim that bacterial bioluminescence can also be used to develop whole-cell biosensors for detecting other toxicants in the environment and contaminants in food.

Environ. Pollut., **228**: 118–127

Greening Coal Mine Soil

Increasing nutrient stock

The Ramagundam area in Telangana has a rich deposit of coal, estimated to be more than 20,000 metric tonnes. To get to the coal, the surface is stripped and dumped elsewhere. Loose rock fragments without organic matter and nutrients create small hills in the area. This mine soil degrades the environment. To reclaim such coal mine overburden, scientists have tried out various afforestation strategies. But the problem with Ramagundam is the low rainfall that makes reforestation difficult. And if it does rain, the slopes get eroded and plants find it hard to survive in the loose soil.

Now, scientists from the Indian School of Mines, Dhanbad have come

up with a solution to reclaim such areas: *Prosopis juliflora*. This plant can grow even in nutrient poor conditions. The scientists took eight sites afforested with *P. juliflora* in eight consecutive years for their evaluation. They collected soils of these sites from three different depths and compared them to the soils from different depths of a Sal forest.

They found that the fine fraction of the soil increases in time probably due to erosion of larger fragments through the natural process of weathering. The bulk density of soil also increases with years of afforestation. Here, the plant root plays a part.

Analysis of the soils revealed that the carbon and nitrogen content in the first 20 centimetres improved significantly over years and reached almost 60% of the levels found in the topsoil of Sal forests. The nitrogen content of the afforested area will probably catch up with the levels found in Sal forests in another few years, say the scientists.

However, increase in phosphorus content, though significant, was slower. The improvement of the lower parts of the soil in terms of these nutrients was much slower. To reach the levels found in Sal forests may take decades.

Prosopis juliflora is considered an invasive species in semi-arid regions. But scientist claim that this nitrogen fixing plant can be used to reclaim overburden from sandstone coal mines. Other soil bacteria and fungi too have played a role in this success story, say the scientists.

Catena, **156**: 42–50

Chimney Flue

Composite membrane captures CO₂

Scientists across the globe face a challenge in finding methods to curb carbon dioxide. A research team at the IIT Guwahati demonstrated a method to efficiently capture carbon dioxide from a 20 : 80 mixture of carbon dioxide and nitrogen.

They used a composite membrane consisting of chitosan, tetraethylenepentamine and poly(ether sulphone). Polymeric membranes such as cellulose acetate, polysulphone and polyimides have been traditionally utilized to separate gas mixtures. However, they lack better separation and thermal stability. On the other hand, a poly(vinyl alcohol)

membrane blended with polyethylimine is more thermally stable. Another biopolymer, chitosan, also shows better thermal stability. Its ability to capture carbon dioxide and its thermal efficiency increase when it blends with either of trimesoyl chloride, arginine salt, triethanolamine, poly(ethersulfone), a metallic organic framework or a mixed matrix membrane.

The research team conducted the study because there was no other study on the carbon dioxide capturing capacity of chitosan with tetraethylenepentamine. They report that the carbon dioxide capture capacity of chitosan blended with a 30% (w/w) tetraethylenepentamine (CS70) composite membrane increases by twofold unlike the case with just a chitosan membrane. It also shows thermal stability till 120°C. To prepare a Chitosan70 optimum weight ratio, they used thermogravimetric analysis, Fourier transform infrared spectroscopy, X-ray diffraction, an atomic force microscope and a field emission scanning electronic microscope. They claim that the Chitosan70 membrane can be tested for the separation of other fuel from gases. Such methodology provides avenues to reduce the global warming caused by carbon dioxide.

J. Appl. Poly. Sci., **134**: 45206(1)–45206(9)

Perovskite Solar Cells

Stability versus efficiency

Organic-inorganic metal halide perovskite solar cells have received considerable attention due to their excellent power conversion efficiency. However, these cells degrade very fast and, hence, are not suitable for commercial purposes.

Recently, Pankaj Kumar and co-workers from the National Physical Laboratory, New Delhi reported their studies on the factors that impact the stability of mixed halide perovskite solar cells. They prepared solar cells using different device structures with different concentrations of the parent perovskite. They used UV-visible spectroscopy and found that the spectral coverage did not change with concentrations. However,

absorbance increased with the concentration of perovskite. X-ray diffraction measurements show the formation of similar structural profiles of the perovskite. The current density–voltage plot indicated that power conversion efficiency increases with an increase in the concentration. This is further supported by external quantum efficiency data.

Scanning electron microscopy images show that perovskite thin films with well-defined boundaries have a different surface morphology. In other words, the surface grain size or substrate coverage depends linearly on the precursor concentration. These results corroborate the X-ray diffraction data.

Kumar and co-workers observed that a higher concentration of the parent perovskite leads to larger crystalline grains which resulted in higher power conversion efficiency.

Stability studies show that degradation of the solar cells with concentration is nonlinear. Under exposure to dark and light conditions, the efficiency of solar cells varies linearly with degradation.

The results show that increasing the larger grain size to improve efficiency of a mixed halide perovskite solar cell has a limit due to faster degradation.

J. Mater. Sci., **52**(18): 10886–10897

Supply Chain and Product Promotion

Win-win-win strategy

The objective of any commercial business is to make profit. In today's competitive markets, decisions taken in a supply chain should benefit all players in the supply chain. In treating the problem as a profit maximization problem, researchers from the Vidyasagar University, West Bengal, in 2017, developed a hybrid algorithm to maximize the profit of the retailer as well as the wholesaler in a supply chain under various promotional activities in the supply chain.

Suppliers, wholesalers and retailers adopt various strategies to sell their product as promotional activity. The researchers consider a three level partial trade credit – credit period offered on a fraction of the total purchased amount –

offered by the supplier, wholesaler and retailer to the wholesaler, retailer and customers. The wholesaler and retailer also obtain a freight charge discount based on quantity ordered. In addition, quantity discounts are also provided to the retailer by the wholesaler. The retailer introduces a promotional cost to the customers which is shared by the wholesaler and retailer. Demand is influenced by the retailer selling price, customer's credit period and partial credit amount.

Due to the uncertainty and vagueness of different costs related to the inventory control system, the researchers developed the inventory model in fuzzy and rough environments. They consider different costs as fuzzy and rough variables. Combining the features of particle swarm optimization and simulated annealing, a hybrid algorithm has been proposed to find the most appropriate strategies for both retailer and wholesaler.

The algorithm established that if the wholesaler contributes some portion of the promotional cost, the individual as well as the joint profit of the wholesaler and retailer increases.

Applying this algorithm will enable wholesalers and retailers in a manufacturing supply chain to take better decisions to ensure profitability. The limitation of the model is that it assumes that all end customers follow business ethics and clear their dues just after the allowed delay period.

Appl. Soft Computing, **58**: 553–575

Reports by: Ashutosh Gupta, Monika Jaggi, Biraja Kumar Sahu, Srividhya, Saurabh Dewan, V. Anu, Azhwar Raghunath, Manabendra Sarma, Chatragadda Ramesh, S. Priya and Jai Benjamin

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scienceandmediaworkshops@gmail.com