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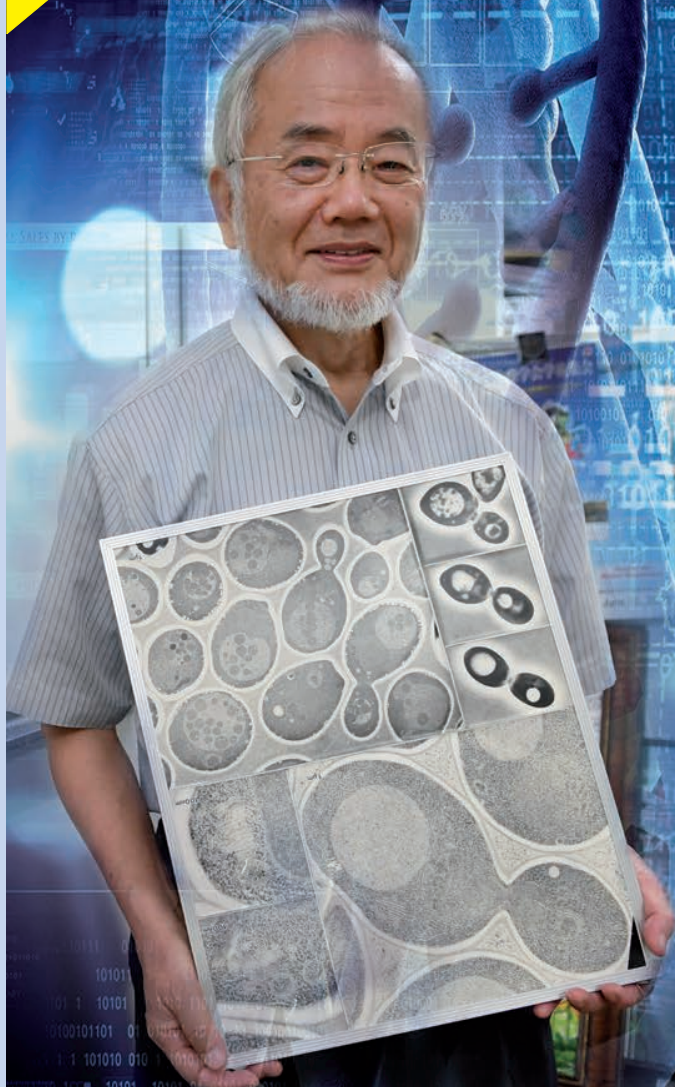
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2016 Nobel Prizes in Science

How Cells 'Eat Themselves'



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... think scientifically, act scientifically... think scientifically, act scientifically... think scientifically, act...

The Blood Rain Mystery



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Blood rain is a weird, but natural, phenomenon in which raindrops appear

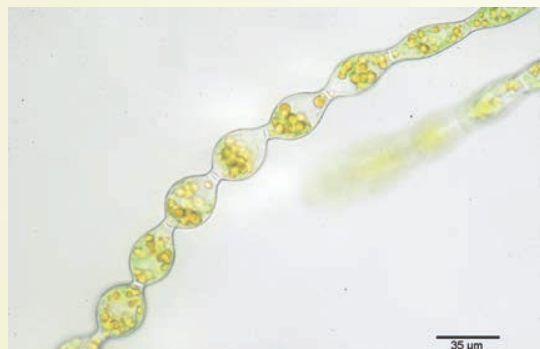


Fig. 1 Microscopic morphology

red, sometimes dark red, resembling the human blood. Spells of this extraordinary phenomenon had been reported since ancient times. Mention of blood rain can be found even in the *Iliad*—an ancient Greek epic set during Trojan war believed to have been authored by Homer around 700 BC. This phenomenon has been reported almost every year from South India – especially from the state of Kerala, and Sri Lanka; with the latest episode reported during December 2013. According to witnesses, rain droplets were so red that the sun-dried white laundry turned dark-red after drenching in the rain.

Since then some purported causes for this mysterious phenomenon have been circulating, including divine spell (god and goddesses from the heavens caused it) and alien involvement (extra-terrestrial aliens caused it). For example, an American newspaper *The Huffington Post* reported in 2012 that the blood rain was caused by extraterrestrial life (aliens). Our group at the Central University of Punjab was interested in this phenomenon and decided in 2013 to work on it, using evidence-based scientific methodology. This write-up presents an overview of ‘behind the scene’ works done by our team that ultimately lead to the resolution of this mystery. First, we needed the sample of blood rain, for which we collaborated with scientists from Kerala, Dr. Nataraja Panikkar and Dr. Jakson Achankunju. Under the optical microscope, it was confirmed that samples contained

beautiful beaded-necklace-like organisms, mostly green but sometimes red (Fig 1). It did look like microscopic algae, but we needed a reliable confirmation, so we decided to sequence its DNA.

Deoxyribonucleic Acid (DNA) is a double-helical molecule found in the nucleus of cells in which genetic information is coded in the form of unique letters called nucleotides. For reading the DNA letters or its so-called “sequence”, we rely on chemical reactions, followed by an analysis of products of those reactions by spectroscopy. In a typical DNA sequencing, DNA molecules are

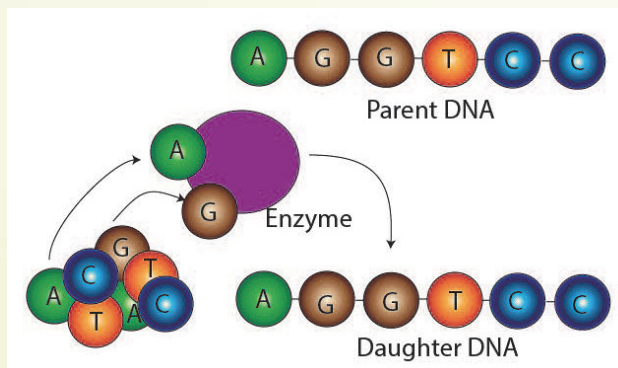


Fig. 2 Amplification of DNA by DNA Polymerase enzyme (purple) in Polymerase Chain Reaction

multiplied by a technique called polymerase chain reaction (PCR) in which enzyme DNA polymerase ‘reads’ the parent DNA and makes an exact copy of it, the daughter DNA, like a Photostat copy (Fig. 2). This process is repeated 30 or 40 times, each ‘cycle’ resulting in the doubling of DNA molecules. For example, one molecule to 2 molecules by the end of the 1st cycle; 2 to 4 by the end of the 2nd cycle; and so on. By the end of the 40th cycle, we will have 34 billion molecules, all looking exactly the same. Typically we start not with just one molecule, but millions of it, so you can imagine the number of molecules we get after 40 cycles!

In DNA sequencing, “sequencing PCR” is performed in which normal nucleotides are mixed with fluorescently labelled nucleotides, such that when

these fluorescently ‘capped’ nucleotides incorporate, amplification terminates. This results in products with different lengths, each bearing a cap of four different types, depending on the sequence at particular positions (Fig. 3).

The molecules are then separated by a technique called ‘capillary gel electrophoresis’ and analysed using spectroscopy. We used a genome sequencing machine in our lab to sequence a section of the genome of the mysterious organism found in red-coloured rain. The DNA sequence was found to be around 1,000-letters long. To confirm the identity, we made use of a database called GenBank, offered by the Government of USA, and is accessible via the internet free of charge. GenBank informed us that the organism in the blood rain was the microscopic green algae, *Trentepohlia annulata*. Our initial suspicion that it was a microalgae got confirmed, but we were surprised to know about this particular alga, as it had never been reported from the Indian subcontinent earlier. In fact,

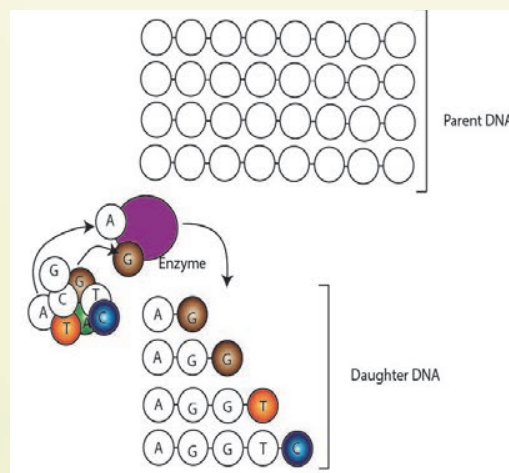


Fig. 3. DNA sequencing reaction (Sequencing PCR).

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Joint pains

If you develop persistent joint pain, you may benefit from use of NSAIDs, corticosteroids, or physiotherapy.

Protect yourself from mosquito bites

If you have chikungunya, prevent mosquito bites for the first week of your illness. During the first week of infection, chikungunya virus can be found in the blood and can be passed from an infected person to a mosquito at the time a mosquito bites. An infected mosquito can then spread the virus to other people.

The preventive drill

No vaccine or preventive drug is available. The best way to prevent chikungunya virus infection is to avoid mosquito bites.

Avoid travel

Travellers at increased risk for more severe disease, including travellers with underlying medical conditions and women in their late pregnancy, may consider avoiding travel to geographic areas which prone to a chikungunya outbreak. If travel is unavoidable, adequate protective measures against mosquito bites must be taken.

Protective clothing and nets

Basic precautions should be taken by people travelling to risk areas and these include use of wearing long sleeves and pants and ensuring rooms are fitted with screens to prevent mosquitoes from entering. For those who sleep during the daytime, particularly young children, or sick or older people, insecticide-treated mosquito nets afford good protection.

Mosquito repellents, coils and insecticide vaporisers

Repellents can be applied to exposed skin or to clothing. Repellents should contain DEET (N, N-diethyl-3-methylbenzamide), IR3535 (3-[N-acetyl-N-butyl]-aminopropionic acid ethyl ester), or icaridin (1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-1-methylpropylester). Mosquito coils or other insecticide vaporisers may also reduce indoor biting.

Community prevention and control

The proximity of mosquito vector breeding sites to human habitation is a significant risk factor for chikungunya. Prevention and control relies heavily on reducing the number of natural and artificial water-filled container habitats that support breeding of the mosquitoes. This requires enlistment of affected communities in effecting adequate steps by all concerned. During outbreaks, insecticides may be sprayed to kill flying mosquitoes, to treat water in containers to kill the immature larvae, and applied to surfaces in and around containers where the mosquitoes land.



Prof Yatish Agarwal is a physician and teacher at New Delhi's Safdarjung Hospital. He has authored 47 popular health-books. ■

The Blood Rain Mystery (continued from page 25)

Trentepohlia annulata is a European species, reported previously only from Austria.

The next step in our investigation was to compare the microscopic morphology of our isolate with that from Austria, for which we collaborated with Prof. Stocker-Wörgötte and Dr. Christina Hemetner from Salzburg University in Austria. Both of the isolates had similar, indistinguishable morphological features, with exact taxonomically pertinent characters, thereby confirming the identity. Our study sought a scientific explanation for blood rain and confirmed that the blood rain is nothing but a mechanism employed by this alga to disperse its spores (similar to plant seeds) to a very large area, so that it can quickly colonise a large area. The red colour of the algal spores is due to the pigment beta-carotene – the same pigment that gives colour to carrots. *T. annulata* is harmless, and the “blood” rainwater is perfectly potable (suitable for human consumption) even for vegetarians! The study was published in February 2015 issue of the journal

Phylogenetics and Evolutionary Biology (DOI: 10.4172/2329-9002.1000144)

We used molecular phylogenetics to compare the evolution of our sample with that of *T. annulata* from Austria. Our results suggest that the isolate from Kerala is, in fact, a recently introduced species from Austria. How could an alga come all the way from Austria to India? Our research confirmed the likelihood that the introduction happened through clouds over the ocean – a phenomenon of intercontinental species dispersal previously reported for bacteria and fungi, but first time for algae. Clouds-over-ocean dispersal is analogous to the intercontinental flights; spores of this alga from Europe get transported to India via clouds that drift across the Arabian Sea. The spores might have got carried first to the clouds for its dispersal. How exactly these lower stratospheric clouds containing algal spores got to Kerala remains unknown. It might be related to the South-West monsoon, as Kerala is the first state the South-West

monsoon strikes. Again, trade winds (SE and NE) converge at a region called Inter-Tropical Convergence Zone (ITCZ), which is located close to Kerala and Sri Lanka, which might be another clue for this puzzle. Our next steps would be the analysis of intercontinental clouds using high-efficiency particulate air filters, using the similar DNA sequence-based technique called “metagenomics”, which would reveal the entire microbial diversity of these clouds. Who knows what other kinds of microbes the clouds are being transported! It could, for example, transport potent human or plant pathogens, no one has investigated in these lines yet.

Felix Bast holds a Ph.D. in Molecular Phylogenetics from MEXT, Japan and works as Assistant Professor at the Central University of Punjab. He is a regular writer for Indian popular science magazines including *Resonance* and *Science Reporter* and has published a well-received book on Indian animals, *Creatures of India*. ■