Life science Programme 5th semester, Batch 2019
Biotechnology Research Lab Visit to ICGEB

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INTRODUCTION

The International Centre for Genetic Engineering and Biotechnology - ICGEB - is an inter-governmental organization with the unique mandate of fostering research, capacity building and technology transfer in Life Sciences, with the ultimate purpose of promoting sustainable global development. With 64 member states and over additional 20 signatory countries, the ICGEB forms an interactive network of internationally recognized scientists and state-of-the-art laboratories in its Components in Trieste, New Delhi and Cape Town. ICGEB was established within the United Nations Common System as a special project of UNIDO in 1983, and has been an independent international organization since 1994. Its Headquarters are based in Trieste, Italy. Housing state-of-the-art laboratories where advanced research in Life Sciences is performed makes ICGEB unique amongst Intergovernmental Organizations. These laboratories offer a scientific environment of top international standard for both basic and applied research. In the three Components, cutting-edge instrumentation, specialized facilities and advanced services are available to the ICGEB investigators. In 2018, almost 600 scientists representing more than 47 nationalities were on board in the ICGEB laboratories, undertaking research across five macro-areas (Infectious Diseases, Non-Communicable Diseases, Medical Biotechnology, Industrial Biotechnology and Plant Biology and Biotechnology). The Delhi component is located within the ICGEB Campus in South Delhi, which comprises an area of approximately 16 acres. It is situated alongside the Jawaharlal Nehru University and the Sanjay Van in a bush forest area.

Dr. Navin Khanna

We were welcomed and attended by Dr. Navin Khanna at ICGEB. He is a Senior Scientist and Group Leader of the Recombinant Gene Products Laboratory at the International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi. He is also an adjunct Professor at the Translational Health Sciences and Technology Institute (THSTI), Faridabad. Dr. Khanna

holds a Doctoral Degree in Biochemistry from All India Institute of Medical Sciences (AIIMS), New Delhi. He was an Alberta Heritage Foundation Fellow at University of Calgary, Canada. He worked as a Post Graduate Research Biologist at the Centre for Molecular Genetics, University of California San Diego (UCSD), and later as a Research Assistant Professor at University of California Irvine (UCI). For the past twenty years, Dr. Khanna has been working on genetically engineered bio-molecules of medical use at ICGEB, New Delhi. Several diagnostics kits have been successfully commercialized through his research efforts. At present, his major research activities are focused towards the development of experimental Dengue tetravalent subunit vaccines in yeast.

ICGEB Campus (International centre For Genetic Engineering and Biotechnology)

EDUCATIONAL VISIT OF BIOTECHNOLOGY RESEARCH LAB WITH ZOOLOGY DEPARTMENT TEACHERS

We all Life Sciences 5th semester students gathered in the college campus in the early morning of 16th September 2019 for an educational trip to ICGEB (JNU, New Delhi). The trip was organised by Dr. KiranBala and Mr. Rajkumar in growing aspects of biotechnology. The morning was become more cheerful and enthaustic when we board the bus and reached there at 10:30 am.

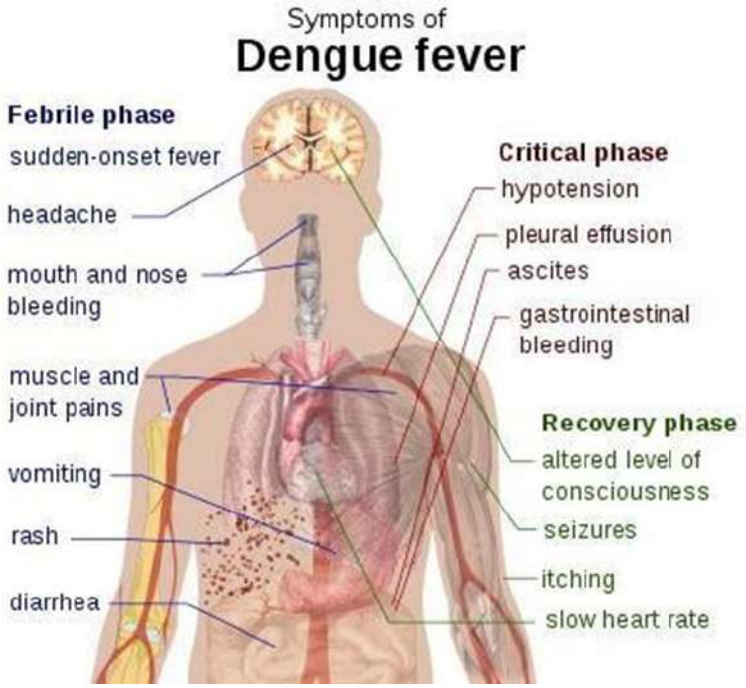
Dr. Navin Khanna, Dr. Kiran Bala & Mr. Rajkumar Assistant Professor
with B.Sc Life Sciences (P) 5th semester students

We are happily welcomed by Dr. Navin Khanna, a Senior scientist and group leader of the Recombinant Gene Products at the international centre for genetic engineering and biotechnology research (ICGEB). We were asked to assemble in seminar hall then and there Dr. Navin Khanna who delivered the informative talk on "THE DENGUE FEVER". Dr. Khanna briefed us about ICGEB and gave us a lecture on how along with his lab he was able to prepare an extremely affordable diagnostic test kit for dengue. He also told us about the prospects of drugs and vaccines for it.

Dr. Navin Khanna addressed the students, how Ayurveda can cure dengue fever?

Students attending the lecture on dengue

Dengue fever is a mosquito- borne tropical disease caused by the **dengue** virus (*Flavivirus).* Dr Khanna told us almost every other person who is suffering from fever and headache could be having **Primary dengue** and **Silent Dengue**. However, it is the **Secondary dengue** which is severe and problematic. This occurs due to several serotypes of the virus. After Primary Dengue the antibodies formed should help to fight the virus but they hide the virus when they attack the second time.



Symptoms of Dengue

This is the reason why secondary dengue is dangerous?. There are three forms of dengue – first: dengue fever; second: dengue hemorrhage fever and third: dengue shock fever. The first form is easily treatable while in the shock fever the patient dies within 24 hours. In hemorrhage fever, the patient initially loses plasma through leaky capillaries into interstitial fluid, platelets follow. This leads to swelling in some cases. The critical part begins when the fever goes down, blood concentration increases as a result of the exit of plasma to the interstitial fluid which causes the blood to become thick. This leads to low blood pressure and decrease in the movement of blood. The administration of I.V. fluid at this stage should be done in small amounts as after 48 hours the plasma in the interstitial fluid returns to the capillaries automatically. Infusion of platelets or high amounts of I.V. fluid can lead to increase in blood volume and eventually high blood pressure which results in rupture of blood capillaries leading to hemorrhage (bleeding/ loss of blood). He told us about how our former president Dr. A. P. J. Abdul Kalam encouraged ICGEB to develop a diagnostic test kit as there was a lack of an affordable and reliable one in the Indian market. It took almost four years and many Ph.D. students at ICGEB to achieve this. The diagnostic test kit was to be made with objectives to identify whether a person suffers from dengue, to distinguish between Primary and Secondary dengue and whether the person has ever suffered from dengue. These objectives were achieved on the basis of the measure of antibodies present in the person. The ratio of the two types of antibodies produced as a response- IgG (Immunoglobin G) and IgM (Immunoglobin M) are considered. If the percentage of IgM is greater than IgG it is indication of Primary Dengue and if IgG is greater than IgM it indicates Secondary Dengue. The other basis of this test was the Dengue Antiagent NS-1 (Non- Structural Protein of the virus). This test is highly sensitive as it can detect dengue even before 40 hours (when first symptom appears). Other commercially successful tests imported in India from USA, Korea and Australia picked up the test after 3 or 4 days. While this “Homemade Garbage” as sarcastically quoted by Dr. Khanna himself, gave results on the very first day of infection. Even the most well founded PCR test is negative for a person suffering from dengue but the NS-1 test comes out positive. This is an advantage as the NS-1 test has a test window of a month. This is explained by the fact that dengue virus is an ssRNA virus which mutates itself extremely fast. Thus, the standard primer used in PCR does not bind to the dengue RNA and fails to give a positive test. The proteins of the RNA virus, however, do not change over time (as proteins do not mutate as opposed to RNA which do so due to them being a self- replicating and catalytic molecule) which is why NS-1 detects it, gives a positive test and is more efficient. Hence, this test was launched in the market as “Dengue Day-1 Test” kit. Initially it was launched at 700 rupees but is now available at a mere

70 rupees. This has resulted in this test being exported to several countries. This test was modified to a rapid test called “dengue finger test” which requires only a finger prick and drop of blood. This test is very useful at time of accidents and for pregnant women. Another modification of this test which works on the detection of NS-1 protein is used by municipalities and other local authorities to check whether the mosquitoes in an area carry the dengue virus by quashing the mosquitoes and testing them. A vaccine produced earlier by pharmaceutical company **Sanofi** used in the vaccination programme in Philippines led to severe cases of dengue for uninfected people. Thus, this vaccine is now banned and ICGEB has now come up with a possible solution which will be undergoing trial. When a weakened microbe (principle of immunization) is introduced, the body forms antibodies which are both beneficial (non-crosslinking/ non-interacting) and detrimental (crosslinking/ interactive). As sir quoted “less is more,” researchers at ICGEB used only those immunogens against which non-crosslinking antibodies are formed in the body. The zika virus in the presence of antibodies against dengue virus in pregnant woman interferes with the brain development of the fetus. This can cause significant decrease in the size of the brain of the fetus, a condition known as microcephaly. This happens because the detrimental antibodies produced against dengue crosslink with Zika.
Research Scholars at ICGEB doing combined research on biotechnology with ayurveda and identified a plant *Cissampelos pareir* extracted an active compound against dengue and modified it as an antiviral ingestible medicine which will be launched soon by **SunPharma**.

Cissampelos pareira plant used for preparing the dengue drug

Lab Visit with Dr. Navin Khanna

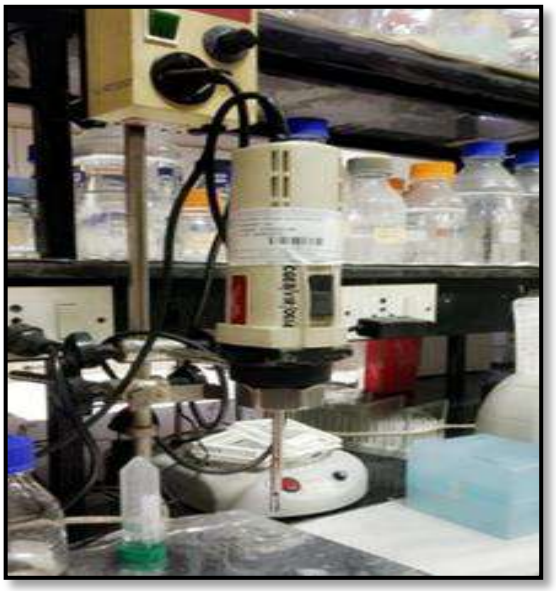
After the lecture, He took all students around the lab where he displayed as a variety of instruments used in the research laboratory.

Research lab

VARIOUS RESEARCH INSTRUMENTS

HOMOGENIZER:

A homogenizer is a piece of laboratory or industrial equipment used for the homogenization of various types of material, such as tissue, plant, food, soil, and many others. Many different models have been developed using various physical technologies for disruption. The mortar and pestle, already used for thousands of years, is a standard tool even in modern laboratories. More modern solutions are based on blender type of instruments (also known in the kitchen), bead mills, ultrasonic treatment (also sonification), rotor-stator mechanical, high pressure, and many other physical forces.



Homogenizer

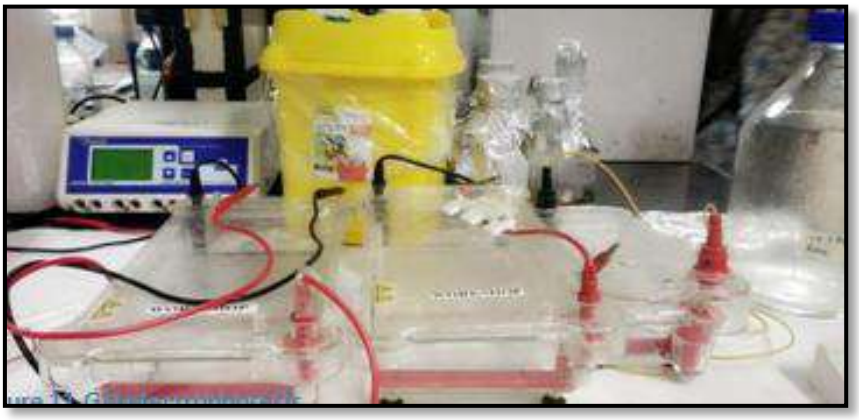
SHAKERS:

Shaker is a piece of laboratory equipment used to mix, blend, or agitate substances in a tube or flask by shaking. It is mainly used in the fields of chemistry and biology. A shaker contains an oscillating board that is used to place the flasks, beakers, or test tubes. Although the magnetic stirrer has lately come to replace the shaker, it is still the preferred choice of equipment when dealing with large volume substances or when simultaneous agitation is required. An incubator shaker (or thermal shaker) can be considered a mix of an incubator and a shaker. It has an ability to shake while maintaining optimal conditions for incubating microbes or DNA replications. This equipment is very useful since, in order for a cell to grow, it needs oxygen and nutrients that require shaking so that they can be distributed evenly around the culture.

Orbital Shaker

GEL ELECTROPHORSIS:

Gel electrophoresis is a method for separation and analysis of macromolecules (DNA, RNA and proteins) and their fragments, based on their size and charge. It is used in clinical chemistry to separate proteins by charge or size (IEF agarose, essentially size independent) and in biochemistry and molecular biology to separate a mixed population of DNA and RNA fragments by length, to estimate the size of DNA and RNA fragments or to separate proteins by charge.



Apparatus of agarose gel electrophoresis

CRYO-CUBE:

Laboratory refrigerators are used to cool samples or specimens for preservation. They include refrigeration units for storing blood plasma and other blood products, as well as vaccines and other medical or pharmaceutical supplies. They differ from standard refrigerators used in homes or restaurant because they need to be totally hygienic and completely reliable. Laboratory refrigerators need to maintain a consistent temperature in order to minimize the risk of bacterial contamination and explosions of volatile materials. To achieve a high degree of accuracy the refrigerator needs air to circulate and a fan to maintain an even temperature at all times. The fan turns off when the door is open to prevent cold air from blowing out of the unit. Laboratory refrigerators feature separate compartments to prevent cross contamination and can hold specific medical supplies, such as blood or vaccines.

CryoCube

ELISA MICROPLATE READER:

An elisa plate reader is also referred to the microplate reader, which essentially perform a number of functions including measuring fluorescence and luminescence where the chemical dyes fluoresce or emit one wavelength when exposed to light. The amount of reflection, absorption, and the color are then used to identify and measure the amount of a substance. Initially, the elisa plate readers were designed to measuring antibody tests, but lately, these instruments have been adapted to perform other advanced functions are aforementioned above. What makes an elisa plate reader preferable at times is because it can measure more samples in a short time frame. Researchers use the elisa plate readers for protein and enzyme assays.

Elisa Microplate Reader

INCUBATOR:

Incubator is a device used to grow and maintain microbiological cultures or cell cultures. The incubator maintains optimal temperature, humidity and other conditions such as Carbon dioxide (CO2) and oxygen content of the atmosphere inside. Incubators are essential for a lot of experimental work in cell biology, microbiology and molecular biology and are used to culture both bacterial as well as eukaryotic cells.

Incubator

AUTOCLAVING FERMENTER SYSTEM:

Fermenters simulate a natural biochemical environment for the optimum growth of cells or tissues in microbial and cell culture. Bioreactor types range from small, <1-L bench top units to 10,000-L systems for large-scale industrial applications, and can be batch type or continuous flow, in which materials flow constantly through the unit. Tight control over parameters such as temperature, moisture, pH, oxygen, and stirring rate will produce the most satisfactory results—namely, maximized cell growth and productivity.

Single-use, autoclavable, or in situ sterilizable options for process development and production are offered, as well as adapter kits that allow existing controllers to be used with single-use vessels. Additional features to keep in mind:

a) Integrated pumps and control of dozens of parameters
b) Multiple gas mixing and multiple thermal mass flow controllers
c) Intuitive touch screen interface
d) Modular designs for flexibility
e) Advanced process management features for research or GMP manufacturing

Autoclaving fermenter

CENTRIFUGE:

A centrifuge is a piece of equipment that puts an object in rotation around a fixed axis (spins it in a circle), applying a force perpendicular to the axis of spin (outward) that can be very strong.

Centrifuge

The centrifuge works using the sedimentation principle, where the centrifugal acceleration causes denser substances and particles to move outward in the radial direction. At the same time, objects that are less dense are displaced and move to the center. In a laboratory centrifuge that uses sample tubes, the radial acceleration causes denser particles to settle to the bottom of the tube, while low- density substances rise to the top.

UV TRANS ILLUMINATOR:

UV- trans illuminators are used in molecular biology labs to view DNA (or RNA) that has been separated by electrophoresis through an agarose gel. During or immediately after electrophoresis, the agarose gel is stained with a fluorescent dye which binds to nucleic acid. Exposing the stained gel to a UVB light source causes the DNA/dye to fluoresce and become visible. This technique is used wherever the researcher needs to be able to view their sample, for example sizing a PCR product, purifying DNA segment after a restriction enzyme digest, quantifying DNA or verifying RNA integrity after extraction.

UV- trans illuminators

ALPHA – IMAGER:

AlphaImager® gel documentation systems and gel imaging systems give you high-performance gel imaging for a wide range of UV fluorescent and colorimetric applications.

Alpha Imager

DYNOMILL:

Dyno Mill is an agitator bead mill with a horizontal grinding container for dispersion and finest wet grinding in a completely enclosed system. The dyno mill is appropriate for a wide range of items from low to exceptionally gooey pump able items. Uniquely composed instigator circles, mounted symmetrically on a pole, exchange the vitality required for scattering and wet granulating to the round crushing globules. An outside pump feeds the product into the mill.

Dyno mill

CONCLUSION:

It was a highly enriching experience for all the students. Students came to know about a lot of things that were unknown to the students especially about dengue. Studenst also saw a variety of biotechnological laboratory apparatus that were taught in the theory classes.

ACKNOWLEDGEMENT

We would like to thank our principal Dr. Rajiv Aggarwal and Dr. Varsha Baweja, Techer in charge, Department of Zoology to allow the students for this informative visit. We are grateful to Dr. Kiran Bala and Mr. Rajkumar for their efforts in making this visit possible. We would also like to acknowledge Dr. Navin Khanna for his valuable time and for imparting knowledge to the students. In the Last, we would like to thanks our batch mates for their cooperation.