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WHAT'S NEW?

INTERVENTION OF BIOTECHNOLOGY IN FORENSIC ENTOMOLOGY

Mysterious and unnatural criminal cases are accelerated significantly in past decades around the globe. Nowadays, suicides and intentional crimes have become front page information in a number of online news portals. To unmask the timing, reason and sites of mysterious deaths, legal agencies are using several branches of biomedical sciences. Among them forensic entomology, though less known but is the more relevant one. Forensic entomology deals with the study of morphology, life cycle, behavior and molecular architecture of insects that colonize on dead bodies of human and other animals. Such a study can be of great value for crime scene scientists and other legal agencies in saving time and ruling out the cause and time since death. When the time of death has exceeded more than 72 hours, entomological time line becomes an integral part of death investigations.

About Insects

Insects belong to a vastly abundant taxa and are both land and aquatic inhabitants. Different insect species have a unique timeline for development of stages such as egg, larva, pupa and adult. Eggs after certain period hatch out into larvae. The intermediate developmental stages of larvae are described as instars. The post feeding larvae isolate themselves into the soil to convert themselves into pupae. The last metamorphosed stage is the adult that emerge out from the pupal sheath by a process of eclosion. In forensic entomology, various developmental stages and respective durations are usually represented through isomegalen and isomorphen plots.

Adult insects have an amazing sense of smell that enables them to detect food sources. Hidden dead bodies escape the sight of human beings but flesh-eating insects can precisely localize the site owing to the smell of gases and organic fluids. Insects lay eggs on decomposing bodies and thereafter they run the cycle of development. Since the developmental durations are of fixed periods, their identification provides the clue for time, reason and site of the crime.

Flesh eating Insects and Entomological post mortem interval:

Fresh dead matter attracts Blow flies (family: Calliphoridae) mainly in the first 3 months. They lay eggs that resemble rice particles. Dead body when it starts to putrefy, releases a pungent smell in the first couple of months that attracts flesh flies (family: Sarcophagidae). If the fat goes rancid in the dead organic matter in next 3-6 months, it keeps on attracting Dermestid beetles (family: Dermestidae). Mites can also be recovered in 1-12 months. If the dead remains go completely dry in 1-3 years, there is still the possibility to discover Dermestid beetles, which are found even after 3 years of death. Therefore, identifying these species and their developmental stages can provide clues regarding the post mortem interval in case of mysterious deaths.

Biotechnology aids accuracy in forensic entomology

Until the 20th century, studies were limited to the traditional morphological identification of insects with relevance to their predictable life cycles according to their class, order and species that made entomology lengthy and time-consuming in forensics. But fortunately, with advancement in biotechnology, forensic entomological data have become more realistic and reliable. DNA typing and barcoding techniques led to accurate identification of insect species and therefore encouraged legal investigators to rely on entomological data more than in previous times.

Species identification is carried out using certain genetic markers such as STRs and minisatellite DNA. The non-repetitive sites on mtDNA are the promising targets for species identification. Moreover, study on differential gene expression can help to predict the ages of insects on dead bodies. Expression of genes like bicoid, slalom and chitin synthase help to unravel the correct developmental stages.

Age of larvae can be estimated through naked eyes but that for pupal stage is not possible. Interestingly, with the help of real time PCR, differential expression of two genes viz. actin and arylphorin receptors can be evaluated. Expressions of these genes are age dependent and therefore useful in predicting pupal age on corpses. Moreover, cuticle contains hydrocarbons whose chemical composition alters with age. These alterations can be monitored through gas chromatography to determine age of larvae, pupae and adults. In addition, cytochrome oxidase subunits 1 and 2 are actively used to identify species of flesh-eating insects.

Conclusion

Entomological time line is very useful in determining post mortem interval, site, circumstances and causes of unnatural/mysterious deaths. Study at the molecular level can help to congregate more accurate and reliable data for forensic investigations.

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MACHINE LEARNING IN EPIDEMIOLOGY

Epidemiology is the study of distribution of health conditions (physical and/or mental), the factors causing or affecting these conditions and the risk associated. This study is conducted over a defined population. It aims to find and establish patterns in the spread of diseases in particular groups and come up with solutions that will prove to be the most appropriate with respect to the nature of the disease in question. Epidemiology plays a very important role in the formulation of health policies by assessing the needs of a population. This is followed by delivering interventional procedures to the population; which could be a drug, as in the case of polio prevention or a guidance system as in the various awareness campaigns conducted for people. These activities make epidemiology an

interdisciplinary field involving biostatistics, management, technology as well as policy making expertise.

The study and subsequent prediction is carried out using available techniques that include biostatistical tools of measurement like proportion, rates and ratio. However, attributing to the recent advancements in the field of Artificial Intelligence, constant efforts are being made to implement technologies like Machine Learning (ML) for assisting in the prediction and prevention of various negative health outcomes. This is done by processing and analysing data gathered from the sample population.

The capability of ML to solve complex tasks with dynamic parameters and knowledge has contributed to its popularity in the field of public health. Off late, Data Analytics is being included as well. Data Analytics, when used in epidemiology, caters to the aspect where the data collected is cleaned and organised; and imbalanced data is normalized. The use of highly precise computational models for processing and performing the required operations to come up with the required results have been in the talks for some time now. In ML models, the factors that cause/ affect a particular condition become the features that act as the independent variable which the models take as an input to return the predicted value/class label.

All this while, lack of availability of large scale data was the main issue for testing these models. In recent times, however, various online platforms that claim to help patients with self-assessment by collecting their medical history and details, global surveys conducted that people willingly partake in, numerous health and fitness tracking apps, etc. have led to an increased availability of automated patient historical data. This has made it possible for Machine Learning and its applications to be implemented for intelligent and improved systems of prediction. With the help of the available data, Machine Learning algorithms like artificial neural networks, support vector machines, Bayesian neural networks, decision trees and others can be employed to solve the given problem statement. A great example is the case study by Carnegie Mellon University's Computational Data Science Labs called "Using Machine Learning for Epidemiological Forecasting", wherein the researchers have used machine learning to develop epi-forecasting successfully for diseases like influenza and dengue.

Considering how the COVID- 19 pandemic has adversely affected all walks of life globally, the need for a system to be in place cannot be more highlighted; a system that can predict situations like these beforehand and in case of outbreaks, help with identifying and thus warning the population which is most prone to infection. This identification can be done based on the data available, using which, trained models can detect patterns in the spread of the disease and can give meaningful insights regarding the people/community most affected by it. This helps to categorize the patients and provide them with necessary care pertaining to the severity of their condition. Coming up with containment strategies for the spreading disease and guidelines for the at-risk patients will follow.

It is a great time to realize the vast potential of Machine Learning in the field of epidemiology and to implement it in times of crises and otherwise. The ability of these models to become more and more accurate with increasing data reinforces the idea of its importance. The agility of such

systems to assist in coming up with relevant solutions is a very powerful tool that can be a game-changer in the way epidemics and their aftermaths are dealt with.

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NEXT-GENERATION SEQUENCING

Massive parallel sequencing or next-generation sequencing is a revolutionary technology that has made it possible for us to sequence a whole human genome in a single day. The key principle behind NGS technology that makes it so revolutionary is that the sequencing of millions of DNA fragments is done in parallel unlike Sanger sequencing techniques. Bioinformatics analyses are used to piece together these fragments by mapping the individual reads to the human reference genome.

While the global market for NGS is still in its introductory phase marking a $7.81 billion market output, it is expected to have a $24.4 billion market at a 20.9% CAGR by 2025. While the technology is still limited, the future seems very bright since NGS offers the world a whole new window into the advent of personalised medicine. In addition, a Whole Genome Sequence can give you loads of information about risks, traits and tendencies that can change the lives of people.

Whole genome sequencing, an extremely innovative application of Next-Generation Sequencing technology allows us to sequence an entire genome from a swab or a drop of blood within a day. That is revolutionary and very chronologically effective.

Whole genome sequencing involves sequencing an organism's entire genome including non-coding regions and epigenetic modifications which uncover a lot of information. Advanced bioinformatics tools and technology are needed to make this information humanly understandable with the help of supercomputing codes.

As of today, Sanger Sequencing is still the most commonly used technique and dominates the sequencing market in research and in commercial biotechnology. However, disruptive innovation has made NGS a lot cheaper than earlier and hence a lot more accessible to the world. Some benefits NGS provides over existing technology:

1. NGS captures a wider array of mutations in your genome than other sequencing technology.
2. NGS allows the detection of mosaic mutations- Mosaic mutations are ones that are spread across the genome with variable frequency. NGS provides the sensitivity and detail to detect them giving a massive boost especially in cancer diagnostics.

- NGS gives a lot more depth in terms of risk analysis based on inherited genes and mutational occurrences.

While NGS boasts of many advantages, the biggest limitation is the requirements of tech heavy infrastructure which is costly and require lot of capital, human and land investments.

However, the future of NGS looks very bright especially in the growing Asia-Pacific market segment where numerous start-ups are competing with the big diagnostic and pharma players to provide this ground-breaking technology to the consumer. NGS offers personalised medicine a whole new outlook, it will help patients get the **right treatment** at the **right time** which can be instrumental especially with cancer therapy. A genome analysis can tell a patient what kind of treatment/therapy would be best suited for his physiology which is greatly beneficial since side-effects outweigh benefits many times.

It took scientists 15 years to complete the human genome project. Today, we can do an even better job in half-a-day, it's a great time to be in the field of biotechnology. Innovation is the key to progress and constant progress has just marked the beginning of the biotechnology revolution.

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ISOLATION, SCREENING AND CHARACTERIZATION OF FUNGAL XYLANASE OBTAINED FROM AGRO-WASTES: A GREEN STRATEGY TOWARDS WASTE VALORIZATION

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INTRODUCTION

The linear polysaccharide, β-1, 4-xylan (hemicellulose), is a major component of plant cell walls. The enzyme xylanase hydrolyses β-1, 4-xylan into xylose, thereby making it useful for preparation of different value added products having wide industrial applications. Xylose is the second most abundant sugar present in lignocellulosic biomass after glucose. Xylanases are produced by diverse genera and species of bacteria, actinomycetes and fungi. However, filamentous fungi are the principal producer of xylanase. Xylanase plays a major role in micro-organisms thriving on plant and agricultural sources for the degradation of plant matter into usable nutrients, increasing the sustainability of biomass. Several efforts are underway to achieve an efficient as well as commercially viable process development for xylose production and purification. Xylanase can be explored in various industrial applications such as paper-pulp industries, textile, food and bakery industries as a greener and eco-friendly approach.

METHODOLOGY AND RESULTS

In this work, a Three Phase Partitioning (TPP) method has been employed for isolation of this enzyme from *Agaricus bisporus* (basidiomycete). This is a novel bio separation approach for isolating industrially important enzymes with the enhancement of catalytic power of the enzyme. TPP technique is widely adopted for the purpose of commercial downstream processing of enzymes using agro-residues that too in a cost effective manner. The agro-wastes harbour huge amount of microbial population over them especially fungal.

- These fungal spores were isolated, cultured and fermented in laboratory, to derive xylanase.
- The isolated enzyme extract wasassayed for calculating xylanase activity.
- The enzyme extract was made to undergo optimisation under variable experimental conditions such as ammonium sulphate concentration, ratio of culture filtrate to tertiary butanol (v/v) and pH.

Experimentally it was found that all three variables influence the degree of enzyme production as well as its activity. Maximum enzyme activity was obtained at 50 % ammonium sulphate saturation (w/v) when the other conditions were kept constant. In the same way, at 1:2 ratio of culture filtrate to *t*-butanol (v/v) and at pH 6 keeping constant the remaining experimental variables individually, maximum enzyme activity was achieved.

Thus, it can be concluded that the xylanase enzyme isolated using TPP method can be implemented in paper-pulp and other industries that might pave the path towards a clean, environmentally safe and sustainable perspective.

- The fungus *Agaricus bisporus* was made to undergo submerged fermentation to produce xylanase using Potato Dextrose Agar media and Tamarind Kernel Powder was used as the carbon source.
- Three phase partitioning uses *t*-butanol and ammonium sulphate to precipitate xylanase from aqueous solution at the intermediate phase.
- For optimizing best three-phase partitioning result of endo-xylanase isolation from culture filtrate, effect of various process parameters such as percent saturation of ammonium sulphate, crude extract to *t*-butanol ratio and pH of the culture medium were analyzed.

After carrying out the respective optimisation experiments, it was concluded that the combination of 50 %(w/v) ammonium sulphate saturation with 1:2 ratios of xylanase to *t*-butanol (v/v) at ph 6.0 was optimal for attaining the best recovery of xylanase.

The data obtained is conclusive to state that TPP is a scalable and quite efficient as an initial step of bio separation of xylanase. This enzyme might be effectively used in a number of industrial applications as a substitute of harsh chemical-based reagents thereby embarking in the pathway of non-polluting, viable and ecologically sound strategy.

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DIVERSITY OF *lin* GENES IN THE STRAINS ISOLATED FROM HCH CONTAMINATED SOIL

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INTRODUCTION

Lindane also known as benzene hexachlorocyclohexane (BHC) is an organochlorine chemical variant of hexachlorocyclohexane (HCH) that has been used as agricultural insecticide previously. Technical HCH comprises four isomers (α, β, γ, δ) out of which only gamma (γ) isomer has insecticidal property. Gamma isomer of HCH can be extracted and purified by photochlorination of benzene under UV light which takes the form, commonly known to as lindane. Lindane enters our food chain through agricultural water system. Lindane causes neurotoxin that interferes with GABA neurotransmitter function by interacting with the $GABA_A$ receptor-chloride channel complex at the ficrotoxin binding site. It affects the nervous system, liver and kidneys leading to cancer hence, it is the need of the hour to treat HCH contaminants that enters our food chain and causes diseases. Therefore, scientists have found a strain of bacteria *Sphingobium indicum B90A* that has biodegradative and biosynthetic capabilities. Sphingomonas are gram negative bacteria, rod shaped, aerobic contains glycosphingolipids (GSLS) instead of LPS and they produce yellow pigmented colonies when grown in LB plates. Sphingomonas are subdivided into four genera: *Sphingobium, Novosphingobium, Sphingosinicella, and Sphingopyxis.* This bacteria has got immense application in bioremediation. They are also extensively used in food industries for the production of extracellular polymers such as sphingans.

Among all other bacterial strains isolated from HCH contaminated soils, *Sphingobium indicum B90A* appeared to have better potential for HCH degradation. This strain contains *lin* genes which maybe scattered or organized in different operons. The transcriptional units that encode for the lindane degradation pathway are lin A, lin B, lin C, lin DER, lin E. However, bioremediation for decontamination of HCH by S. indicum B90A has got some disadvantages which we will discuss later in this article.

METHODOLOGY

Techniques used to screen different strains of microbes from HCH dumpsite samples are-

- Plate pouring method
- Serial dilution and spread plate
- Streaking method
- Genomic DNA isolation by small scale supercos method
- Nanodrop spectrophotometer to find out the concentration and absorbance of DNA
- Gel electrophoresis method
- Colony PCR

PLATE POURING METHOD-

- We need to make LB media for 300 ml so weigh 6 grams LB in weighing balance.
- In a reagent bottle transfer the LB and weigh 4.5 grams Agar-Agar type I and transfer it into the bottle.
- Measure 300 ml distilled water and transfer it into the bottle.
- Autoclave it.
- After cooling add nystatin (600 µl for 300 ml) and streptomycin (180µl for 300 ml).
- Pour the contents in the petri plates and keep it overnight.

SERIAL DILUTION AND SPREAD PLATE-

- Take six test tubes and label it.
- Weigh 0.9 gm NaCl.
- Measure 100 ml MQ/DW and put the weighed 0.9 gm Nacl into it in a 250ml bottle.
- Put 5 ml freshly prepared saline in each test tube.
- Put cotton plugs in each test tube and autoclave it.
- Now for the serial dilution take six MCT tubes inside the laminar and mark it.
- Take 900µl saline in every MCT.
- Weigh 1 gm HCH soil sample and 10 ml saline and take 100µl of this sample and transfer it in the first marked MCT. Then again take 100µl sample from the first MCT and transfer it into the second MCT.
- Take the agar plates and put one drop of the saline and soil sample mixture in it and do spread plate.
- After doing spread plate put paraffin around the agar plate and incubate it in the room temperature.

STREAKING-

- Label the plates at the bottom.
- Sterilize the inoculating loop in the bunsen burner by putting the loop into the flame until it is red hot. Allow it to cool.
- Pick out pure colony from the previously streaked plates with the sterilized loop and lift the petri plate at an angle of 60°C.
- Streak from side to side in parallel lines.
- First streaked line should be the continuation for the second streaking then third and fourth. Last streaked line should not be in continuation with the first streaked line.
- After streaking is complete put paraffin around the plates.
- Incubate at 37°C
- After incubation for 24 hours we can observe growth along the streaked lines.
- We record the growth of individual colonies, morphology, colour.

GENOMIC DNA ISOLATION BY SMALL SCALE SUPERCOS METHOD-

- 5 ml culture pellet/ colonies from a fresh plate was taken.
- Prepare lysis buffer and adjacent and aliquot of 200µl of it in each eppendrof. Lysis buffer consists of (5M Nacl, 1M Tris Hcl, 0.5M EDTA, and adjust the volume with MQ)
- Dissolve the pellet in lysis buffer and add 2µl of proteinase k (20mg/ml) to each.

- Add 10-20µl SDS (10%) and keep at 50℃ waterbath for 10 minutes (check lysis in regular interval).
- Once lysis has taken place solution becomes viscous and slurry.
- Make up the volume to 1 ml by adding MQ.
- Add 1 ml phenol chloroform (equal volume), lower layer in the bottle should be taken (phenol).
- Vortex nicely and till solution becomes white.
- Centrifuge 10,000 rpm for 15 minutes at 12℃.
- Take upper layer in fresh Eppendorf and add 1 ml chloroform.
- Vortex and centrifuge at 10,000 rpm/10 minutes at 12℃.
- Take the upper layer in fresh ependrof and add equal volume of absolute alcohol and mix nicely (cut the tips) so that during transfer cell experience less friction.
- 20µl of 5M Nacl is added and invert mixed.
- Centrifuge at 10,000 rpm for 10 minutes at 12℃.
- Decant the supernatant and add 700µl of 70% ethanol.
- Centrifuge at 10,000 rpm for 10 minutes at 12℃ spool out the DNA.
- Decant supernatant and air dry the pellet.
- Dissolve in 500µl MQ and keep it at 37℃.
- Store at 4℃.

NANODROP SPECTRPHOTOMETER METHOD-

- First we open the software ND 1000 and go to blank setup where we add 2µl MQ to the lower pedestal of the nanodrop instrument.
- Then we go to nucleic acids and add one drop of the DNA to be measured an then click on the measure button.
- Then we get the absorbance and concentration of the DNA sample.

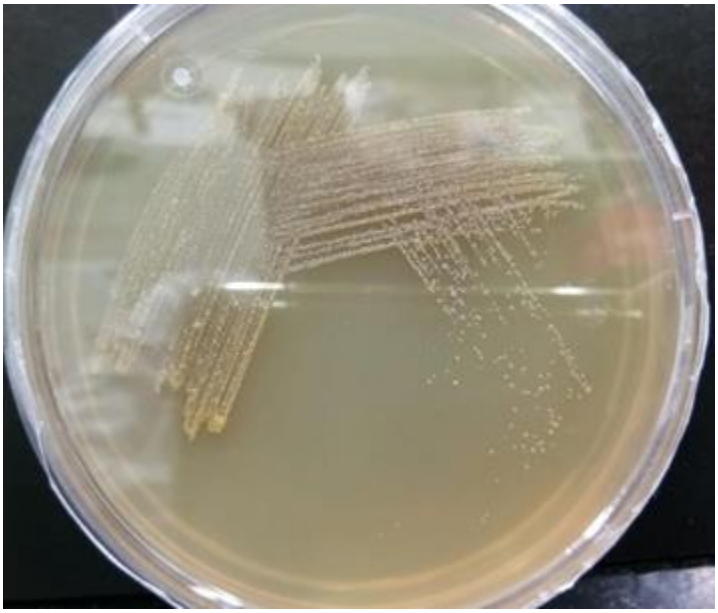
GEL ELECTROPHORESIS METHOD-

- Weigh 0.8 grams agarose in weighing machine and transfer it in gel bottle.
- Add 100 ml 0.5X TBE in the gel bottle.
- Heat it in the oven for nearly two minutes then with silicon gloves take it out from the oven, keep it in room temperature for sometime then add ethidium bromide to it and mix well.
- Then cast the gel in the gel apparatus and wait for 30 minutes to solidify.
- After 30 minutes remove the comb slowly from the apparatus and load the DNA samples into the well using loading dye for visibility.
- Run the gel at 100V and after running it for few hours when the samples come out of the wells then run it at 70V for proper resolving.
- Then observe it in gel documentation and analyse the result comparing it with DNA ladder and take image of the gel.

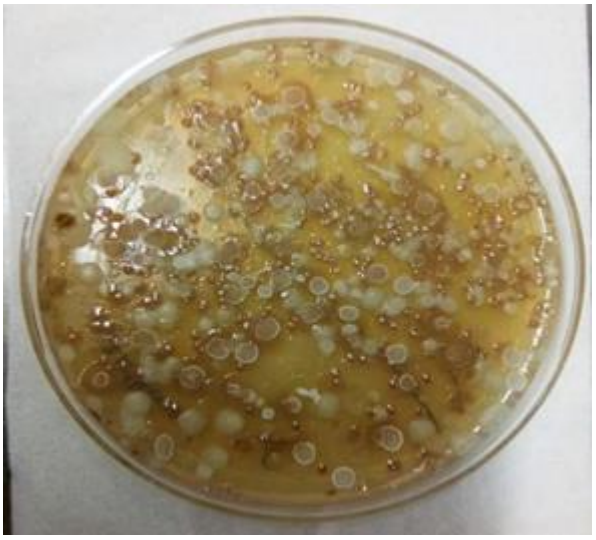
COLONY PCR METHOD-

- Pick up 14 cell isolates collected from HCH dumpsite and one B90A cell as positive control they are labelled as (MSM2, MSM3, MSM4, DS9, DS11, DS12, DS1, DS3, DS8, S1, DS15, DS14, DS19, B90A) from the streaked or restreaked plates.
- Suspend in 40µl MQ in MCT tube inside the laminar.
- Boil for 10 minutes, snap chill for 5 minutes on ice.
- Flash spin the MCT tubes.
- Take out 30µl of supernatant and add 70µl MQ in fresh MCT.
- Use this as DNA for PCR.
- Prepare the master mix for PCR which includes the following-DNA soup(15µl, taq buffer(5µl), DMSO(1.5µl), taq polymerase(0.5µl), dNTPS(3µl), forward primer(1.5µl), reverse primer(1.5µl) and MQ(22µl).
- Then set the programming in PCR machine and run it.

RESULT-



Streak plate- We observed brown color colonies, with slimy growth, this is a B90A strain.



Spread plate technique- Colonies on agar plate after spread plate technique.

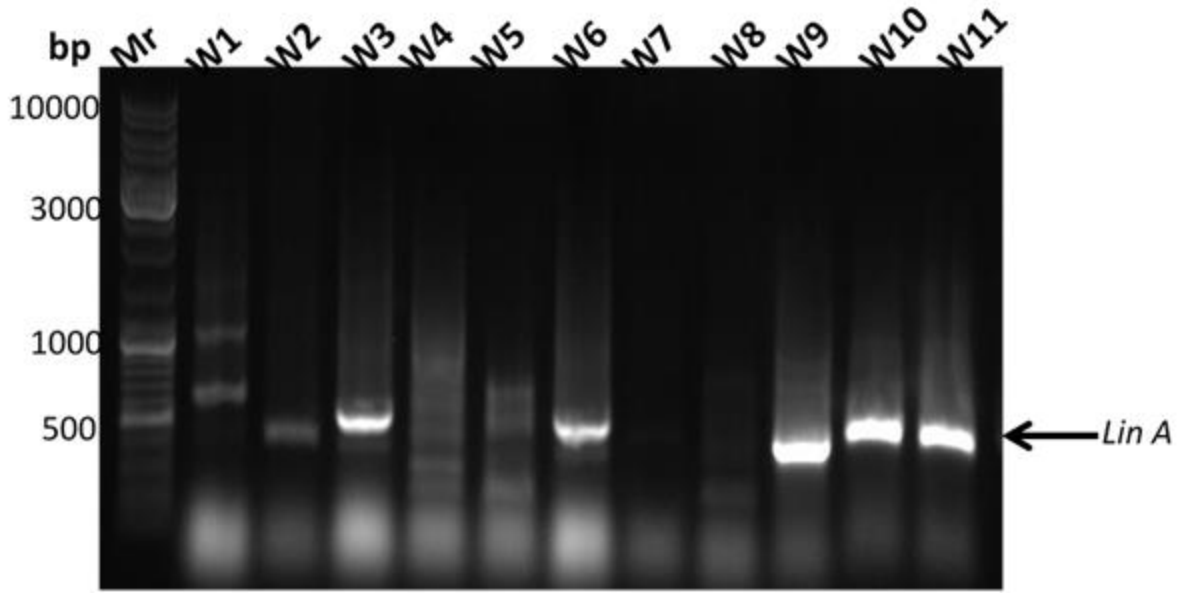


Figure : 0.8% Agarose gel electrophoresis picture showing amplification of *linA* from bacterial isolates.

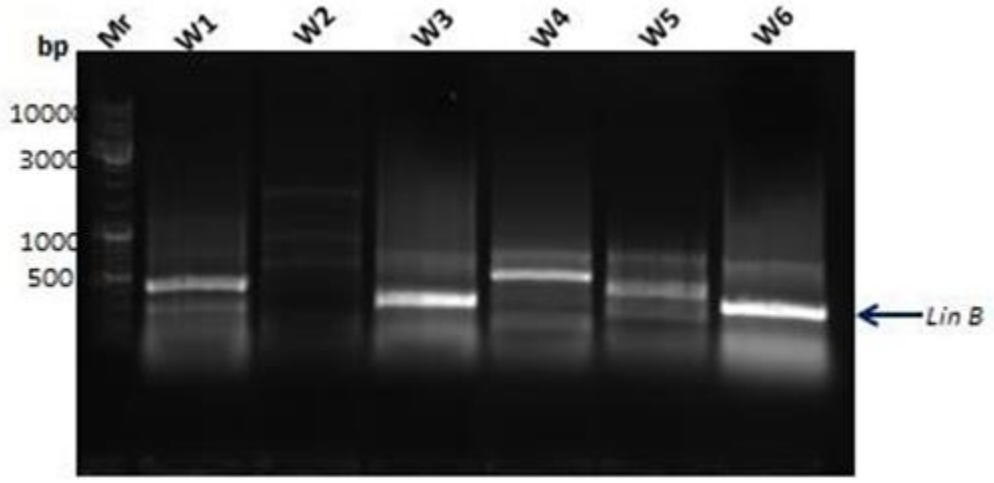


Figure : 0.8% Agarose gel electrophoresis picture showing amplification of *linB* from bacterial isolates W1-W6. DNA ladder is from Thermoscientific.

DISCUSSION-

HCH degradation has become a very important issue for the environmentalist. Though in India HCH usage is banned, still there are some parts where poisonous HCH is used as an agricultural insecticide which enters our tropic chain. This issue has become a very big challenge to the scientific community. Studies on HCH degradation by aerobic means have become a great success but there are very few research and results found for anaerobic degradation. Also HCH isomer complexity adds on to the problem. *Sphingobium indicum* B90A strain has got greater potential for HCH degradation but this has also got some limitations when applied to large scale. The significance of genetic variations in *lin* genes should be exploited more to gather more knowledge on HCH degradation.

CONCLUSION-

We screened different strains of microbes from HCH dumpsite but we came to the conclusion that no *lin* genes were present in any of the strains. Therefore, in the strains that we selected do not have biodegrading capability and thus we need to look for other strains.

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OPINIONS

COVID-19 CHALLENGES IN VACCINE DEVELOPMENT-I

Conducting High-Quality COVID -19 Vaccines

Healthcare workers and regulatory authorities have put into severe pressure by the COVID -19 pandemic to swiftly make the vaccine more rapidly. During this crisis it is a heroic but difficult task for conducting clinical trials, as clinicians have to provide patient care while they are at risk of encountering infection. As the name suggests, the virus is novel, therefore humans have no natural immunity to it, and researchers must start to develop a vaccine to educate the immune system to defend itself from the virus. Numerous pharma and academic institutes are racing to develop a vaccine against SARS-CoV-2 across the world, including India. There are a few major hurdles to overcome to create a vaccine against COVID-19.

Vaccine development has played a hugely important role in combating infectious diseases. The successful eradication of smallpox globally became a new era for the vaccine world. Today, the term "vaccine" applies to all biological compositions, processed from living organisms that amplify immunity against disease and either prevent or in some cases, medicate disease. Vaccines are administered in the liquid form either by injection, by oral, or by intranasal routes. There are many types of vaccines developed for a variety of diseases around the world at certain times when it is needed. Some of them are mentioned below:

Types of Vaccines	**Examples**
Live attenuated	Measles, Mumps, Rubella
Inactivated	Hepatitis-A, Influenza
Recombinant subunit	Hepatitis-B
Toxoid	Tetanus, Diphtheria
Conjugate-polysaccharide-protein	Pneumococcal, Meningococcal

Vaccines help to develop immunity by initiating an infection. This type of infection never causes illness, but it does cause the immune system to produce T-lymphocytes and antibodies. However, it typically takes a couple of weeks for the body to supply T- lymphocytes and B- lymphocytes after vaccination. Therefore, the possibility that a person infected with a disease just before or just after vaccination could develop symptoms and gets a disease, because the vaccine doesn’t have enough time to provide protection. Vaccine development efforts make us enlightened for operating swiftly, and several major vaccine platforms are moving toward clinical evaluation. These include traditional recombinant protein, replicating and non-replicating viral vectors, and macromolecule DNA and mRNA approaches. Each of these vaccine platforms has advantages and drawbacks.

The principal attributes include speed and versatility of manufacture, safety and reactogenicity, the longevity of immunity, scale and price of producing, vaccine stability, and cold chain requirements. No single vaccine or vaccine platform alone is likely to meet the global need, and

so a strategic approach to the multi-pronged endeavor is critical. Traditional recombinant protein technology is often wont to express the spike protein (e.g., Sanofi, Novavax). Moreover, it takes much time to determine cell lines needed for manufacturing than for macromolecule vaccines-there's a strong commercial experience with protein and protein particle vaccines.

Janssen Pharmaceuticals for COVID-19 developed the replication-defective adenovirus 26 (rAd26), which was recently shown to be safe and immunogenic in preventing Ebola virus infection. A recombinant chimpanzee Ad vector (ChAdOx1), developed by the University of Oxford and AstraZeneca, and had also entered clinical trials. Similar versions of ChAd vaccine products are tested in prior clinical trials and shown to be safe and immunogenic. These manifold perspectives provide the potential for modular production required for widespread population use.

Corona Virus Vaccine Challenges

There are certain ultimatums for COVID-19 pandemic:

- **Ensuring vaccine safety**: - Several vaccines for SARS have been tested in animals. Most of the vaccines improved the animal's survival but didn't prevent infection. Some vaccines also caused complications, such as lung damage. A COVID-19 vaccine will need to be thoroughly tested to make sure it's safe for humans.
- **Providing long term protection**: - After infection with coronavirus, re-infection with the same virus- though usually mild and only happening in a fraction of people- is possible after months or years. An effective COVID-19 will need to provide people with long-term infection protection.
- **Protecting old people: -** People older than age 60 are at higher risk of severe COVID-19. But older people usually don't respond to vaccines as well as younger people. An ideal COVID-19 vaccine would work well for this age group.
- **Highly Mutagenic: -** The COVID-19 is highly mutagenic as it frequently changes strain. Many reports suggest it might change its strain from person to person.

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COVID-19 CHALLENGES IN VACCINE DEVELOPMENT-II

Recent Development of Corona Virus Vaccine

Many countries are in the race to introduce the first-ever novel coronavirus vaccine. Six months into the global outbreak and trials are underway in laboratories around the world with several companies and governments doubling their efforts to find a persistent solution for the deadly virus.

In May, US pharmaceutical major Pfizer and its German partner BioNTech claimed that they have already initiated human trialing in the USA as well as in Germany. On the flip side, Remdesivir is a broad-spectrum antiviral medication developed by the biopharmaceutical company Gilead Sciences which is being tested as a specific treatment for COVID-19 infections. The drug has been approved for emergency use in countries like the USA and Japan with a severe manifestation of coronavirus infection. Later, pharmaceutical companies like Regeneron revealed that its 'anti-body' treatment drug could also be available.

Several other biopharmaceutical companies like Moderna, Johnson & Johnson have also geared up attempts to design a COVID-19 vaccine. mRNA-1273 developed by Moderna based on prior studies of related coronaviruses such as those that cause severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS). It showed successfully neutralizing the antibody titers in 8 participants who received either 25 µg or 100 µg doses. Moderna began operating mRNA-1273 to patients in their Phase 2 trial on 29 May. The protocol is being finalized for a Phase 3 trial of 30,000 volunteers, expected to start in July which is being funded by Operation Warp Speed.

Unlike Moderna, Johnson & Johnson is working with Biomedical Advanced Research and Development Authority (BARDA) and the company notified it had started pre-clinical testing on various candidates in Boston and later disclosed that it had selected its lead vaccine candidate, with two back-ups. Scientists at the Jenner Institute of Oxford University have affirmed to have made a potential vaccine for coronavirus and the vaccine is being progressed with collective partners including the Serum Institute of India.

A drug developed by biotech company Synairgen, Interferon Beta, has been administered in patients as part of initial trials and the results of the Interferon Beta injection will be delivered by June. Sanofi is leading upfront for developing a vaccine collaborating with Translate Bio under its recombinant DNA manifesto using work from a previous SARS vaccine and measures that could have both an instant and long-term impact. The company funded by BARDA plan to volunteer patients in a Phase 1/2 trial in September.

Most recently India also shifted its gear in making the COVID 19 vaccine. India’s leading vaccine development company, Bharat Biotech in collaboration with the Indian Council of Medical Research (ICMR) and National Institute of Virology (NIV) has declared that India will probably design an inactivated vaccine for COVID 19 candidate which they called as Covaxin expected to begin in July. With the approval of DCGI (Drug Controller General of India) the indigenous, inactivated vaccine is developed and manufactured in Bharat Biotech's BSL-3 (Bio-Safety Level 3) high containment facility. Bharat Biotech is a reputed company and developed many vaccines like rotavirus, hepatitis, Zika, Japanese encephalitis. The director-general of ICMR has lightened on the fact by saying that the vaccine can be launched in the market for public use latest by August 15 after the vaccine completes all the mandatory clinical trials. Besides Covaxin, Bharat Biotech is operating on two other vaccine candidates: one with the University of Wisconsin–Madison, and FluGen, and the other with Thomas Jefferson University. As the virus threatens community spread, there is a drastic need to ameliorate infrastructure, develop novel vaccine, assemble human resources, support vanguard health workers and search for a remedy until the battle is accomplished.

What Is The Way Out?

Many vaccines work by eliciting a neutralizing Antibody (Abs) that prevents infection. However, for some infectious agents, it has not been possible to create an efficacious vaccine, and for others, the protection provided by vaccines is strain specific. When we are infected with any highly mutating virus just like COVID-19, most of the antibodies we make are directed against the most common epitope. Amino acids on these might vary from year to year, necessitating frequent reformulation of the vaccine. In contrast, amino acid residues on the conserved domains are highly conserved. Infected hosts rarely make antibodies against these domains. Nevertheless, broadly neutralizing antibodies against these domains have been isolated that can block infection with many different strains of the same viruses.

If we could design a vaccine that induces antibodies against these conserved motifs, it might confer protection for many years. Strategies that aim to direct the immune system towards a particular region of protein are referred to as “immunofocusing". But these strategies have diverse drawbacks too as they are not easily generalizable. Also, these immunofocussing techniques have low resolution and difficulty to maintain the 3D structure of the epitope which is challenging. A comprehensive understanding of the specific epitope recognized by the antibody is valuable not only for antibody engineering but has also proven valuable knowledge for vaccine design.

Presently, there is no immunogen available that can influence COVID-19 neutralizing antibodies. So, there is a striking need to manufacture and dispense enough safe and effective vaccines that can immunize an exceptionally huge number of individuals to protect the universal coterie from the continued threat of medical issues and mortality from SARS-CoV-2. There is a global need for vaccines to develop; and relating to the diversity of the pandemic, it requires more than one effective vaccine approach.

The collaboration will be crucial among biotechnology and pharmaceutical companies, many of which are bringing forward a variety of vaccine approaches. The full development pathway for an effective vaccine for SARS-CoV-2 will require that industry, government, and academia join forces in unparalleled ways, each adding their strengths. This mechanism aims to initiate essential safety and virtue data for several candidate vaccines in parallel, to quicken the endorsement and distribution of multiple vaccine platforms and vaccines to protect against COVID-19 (coronavirus disease 2019).

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HYDROPONICS-THE FUTURE FOOD MARKET

HYDROPONICS as the name suggests is the system of growing plants without a soil system, simply by using mineral nutrient solutions and water solvent. It does not use soil, instead the root system is supported using inert medium such as perlite, rockwool, clay pellets, peat moss or vermiculture. Despite the inert media, roots can cause changes to the rhizosphere and the root exudates can change the rhizosphere biology.

The nutrient solutions used in the system can come from a variety of different sources, including duck manure, fish excrement, chemical fertilizers or artificial nutrient solutions. Some plants have revealed capacities to grow in hydroponic systems fed with wastewater, reducing the pollutant loads. Thus, the hydroponic systems constitute a promising future in biotechnology for food production and wastewater treatment.

There are many advantages of the hydroponic system, the most important being reduced water usage in agriculture and thus lesser evaporation. Because of the lack of water needed to grow fresh produce, it would be possible in future for harsh environments, which don't have much accessible water, to be able to grow their own food.

Another advantage is increased rate of growth in the plants. With an appropriate setup, plants will mature 25% faster and produce 30% more than the same plants grown in soil. Plants will grow bigger and faster because they will not have to work as hard to obtain the nutrients. Even a small root system will provide the plant exactly what it needs, so the plant will focus more on growth than expanding its root system.

There are different techniques of the hydroponic system depending on home-grown or commercial application. Irrespective of where it is applied, hydroponic system widely uses plastic containers (although other materials have been used too) and must exclude light to prevent algal and fungal growth in the nutrient solution.

Hydroponics is better for the environment because it reduces waste and pollution from soil runoff and hence is a promising solution for future where crisis of water and scarcity of food appear to be accelerating.

Despite all the advantages, there are a few disadvantages of this system. It needs a supply of fresh water failing which the plants may die. On a commercial scale, the setup may take a lot of time, a pump failure will result in the death of all plants and the cost is more than its soil counterpart.

Hydroponics is yet an excellent choice for all types of growers. It is a great choice because it gives you the ability to meticulously control the variables that affect how well your plants grow. A fine-tuned hydroponics system can easily surpass a soil based system in plant quality and amount of produce yielded.

If you want to grow the biggest, yummiest and juiciest plants you can possibly imagine, then hydroponics is the right choice for you. It may seem intimidating at first with all the equipment and work involved, but it will all seem simple once you get a hang of the basics

Start small, keep it simple and your hydroponics system will never cease to amaze you.

Kadambini Alva P.
Ex-Faculty
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BACTERIAL CROWN ROT OUTBREAK IN PAPAYA IN TAMIL NADU

Papaya (*Carica papaya*) is one of the most important fruit crops in the world and is mostly cultivated in tropical and subtropical regions. Mostly, it has been cultivated due to its sweet nature with medicinal properties (papain) and nutritional values *viz*., proteins, edible fibers, vitamins (B_2, niacin and C) and minerals. Across the world, India is represented as the top producer of papaya followed by Brazil, Nigeria, Indonesia and Mexico. In India, papaya is cultivated around an area of 1.39 m. ha with a production of 5831.0 MT. Of this production, Andhra Pradesh occupies the highest production with 1288.5 MT. Popular varieties like, Red lady, Sinta and Co 8 were mostly cultivated in a total area of 1.76,000 ha with moderate production of 403.1 MT from Tamil Nadu.

A vast survey was conducted for papaya growing in the western Ghats districts of Tamil Nadu. During this period a new disease incidence was recorded, especially in the Coimbatore district. Based on symptomatic and molecular analysis it was confirmed to be bacterial crown rot (BCR) caused by *Erwinia papayae*. In the field, the whole plant was infected at an age of 5 months being completely drooped, and recorded an incidence of up to 60%. The cultivar Red Lady was highly infected as compared to other local elite varieties like Co7 and Co8. The infected trees, whole shoot, branches, leaves, fruits and young crown also had typical symptoms of water-soaked lesions in fruits, crowns with pale white lesions, emitting a foul odor. When split the infected stem region showed pale brownish discoloration with bacterial outburst. It was caused by a gram negative, rod shaped, peritrichous, single colonizing phytopathogenic bacteria called as *Erwinia papaya (*2-3 mm diameter) that survived on infected fruits and seeds, mostly predominant in and spread by infected seeds as nature.

Analysis of the eleven collected strains (CCRP1 – CCRP11) through biochemical methods proved as gram-negative phytopathogenic and sequencing through 16S rRNA gene (MT322147 to MT322823)-it showed a similarity of >95% with *E. papayae* from a strain of Malaysia.

Analysis of ORFs in all strains, 3 to 10 ORFs were contributed in gene sequences and the maximum ORFs (10) being present in strains *viz*., MT322147 (Leaf), MT322787 (Stem) and MT322792 (Branch). Out of these eleven strains, the least number of ORFs (3) were in strain MT322823 (Leaf). Hence, it proved to be of an adaptive nature and showed diversification in phylloplane hosting pathogenic bacteria. In bases analysis of all strains, adenine (A) and thiamine (T) were majorly playing a vital role in ratio at (3:2). This was confirmed by pathogenic bacteria thriving and surviving through nitrogenous synthesis and accumulation nature in eco-system. That has helped in survival and pathogenic expression.

The outbreak of bacterial crown rot disease seems to be new and is conditioned as an emerging threat to farmer in Tamil Nadu. It has been more severe in local elite cultivars like, Co 7, Co 8 and Red Lady. As a result, prior to cultivation, it is important for plant protection officers to follow practices that are essential for management of this bacterial disease. I hope this article helps you gain clarity to the disease outbreak of bacterial crown rot (BCR) in papaya (*Carica papaya* L.) and in gathering knowledge to the students, researchers in future.

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INTERVIEW WITH DR. DIVYASHREE NAGESWARAN

Dr. Divyashree C. Nageswaran has conducted research on multiple projects in Plant Breeding. At such a young age, she has a phenomenal profile with a 6 page long resume that is a concise version of the original. She completed her education at prestigious universities around the world- Univeristy of Cambridge in UK, Cornell University in USA, Tamil Nadu Agricultural University in India-gaining a truly global experience in Biotechnology. With multiple publications in prestigious journals and fellowships and awards acclaiming her talent, she currently works as a Scientific Advisor at a firm in UK.

We were pleased to interview ma'am for our magazine. Her dynamic contributions to the field of Biotechnology as a scientist and her journey as a student in three countries truly inspired us. Read her interview where she talks about her projects, journey and ideologies!

1. You completed your PhD in Plant Sciences from University of Cambridge. Can you tell us about your research project on Genetic and Epigenetic inheritance in plants?

I joined the research lab which primarily works on identifying genetic and epigenetic factors, its underlying mechanisms that control DNA recombination or crossovers during meiosis in plants. My PhD project was to perform a forward genetic screen using chemical mutagenesis to identify genes that regulate meiotic recombination in the model plant *Arabidopsis thaliana*.

Before explaining the aim of my project, I would like to explain some biology in simple words about meiosis and recombination. Meiosis is a specialized biological process of cell division, which is fundamentally essential for sexually reproducing organisms to propagate. Organisms vary in chromosome number and ploidy level. For example, humans are diploid (2n) individuals with 23 pairs of chromosomes, similarly, the model plant *Arabidopsis* is also a diploid with 5 chromosome pairs.

Meiotic recombination promotes genetic variation by reciprocal exchange of DNA producing such novel allelic combinations of genes that influence important traits/characters, say agronomic traits in crops. Exploiting meiotic recombination in agriculture has a great potential to speed-up crop improvement via breeding methods.

Several genes/proteins involved in the meiotic recombination pathway have been found in model organisms. For e.g., SPO11 endonuclease initiates DNA double stranded breaks in the pathway across all eukaryotes, which may be repaired into crossovers (recombinant) or non-crossovers (parental or gene conversions). Unlike *SPO11*, which is functionally conserved across sexually reproducing organisms, genes/modifiers acting downstream of the meiotic recombination pathway have species-specific roles. Hence, I realized there's more scope to identify novel genes/modifiers of recombination specific to *Arabidopsis thaliana*. Finally, this turned out to be my 4-year PhD project. The identified gene(s) and its function in the context of recombination will soon be out

online in the form of a scientific publication. Once it's available for view, I would be able to talk in detail about the outcomes of my research work.

2. What are your responsibilities as a scientific advisor at your current place of employment?

Soon after my PhD graduation, which was in July 2019, I officially joined the Tango Group International (TGI) Ltd., UK as a Scientific Advisor. TGI was founded by Mr Robert Smith, an exceptional businessman and basically a Cambridge University graduate. TGI primarily manufactures knit-wear garments in Europe and Bangladesh in their own factories. In recent years, TGI started investing in agriculture-based R&D in growing crops like Hemp for its natural fibre to develop sustainable and eco-friendly clothing products. As a Plant geneticist/breeder, I provide technical inputs to the R&D team, which is located in Romania for the selection and breeding of high-yielding varieties in terms of its fibre biomass. In order to mobilize EU funds for all our R&D works, I'm technically assisting TGI in attracting EU grants. TGI aims to set up an independent research institute in Romania, Europe in the near future. I'm actively coordinating to execute this goal in bringing/bridging potential collaborations for conducting world-class translational research in the field of Agriculture and Life sciences.

3. You have completed double masters in plant breeding and biotechnology from Cornell University and Tamil Nadu Agricultural University. You worked on extensive research projects during both degrees. Can you share your experiences in both places- the difference in terms of resources, facilities and research in India and USA?

Ideally, to do a PhD, you don't require a masters degree in countries like USA and UK. The USA offers an integrated PhD program for a minimum period of 5 years. Despite the fact, I chose to take up this Dual masters program at Cornell University and TNAU funded by the Ratan Tata Foundation. This was purely my desire to experience world-class education and research at one of the top-ranking universities in the world. I was able to spot a sea of differences between both the countries 8 years ago in terms of education & research standards, resources, student support & guidance, facilities, career opportunities, general work culture, ethics, gender equality & dignity at work and many more. To elaborate a few of my experiences in the United States, I had the choice to choose inter-disciplinary courses I wanted to study apart from the mandatory ones. The courses offered were of exceptional standards, its assignments and test evaluations gave us the space to enhance our lateral thinking abilities. I had the opportunity to choose the lab I wanted to work, and I was given all the research support and guidance. I was given sufficient funds by the university to participate in conferences and workshops relevant to our area of research. I was given surplus lab resources and facilities to conduct my research project. I was stunned to see the enormous Mann Library at Cornell that was exclusively dedicated to the College of Agriculture and Life Sciences (CALS). Most importantly, I had uninterrupted access to all the scientific journals and books throughout my studies. I was exposed to various career opportunities and learnt the fact that research skills can be made transferable when aspiring for non-science or non-academic careers at some point and that still requires a decent masters or a doctorate degree. Indeed, TNAU laid the necessary foundation to step up higher in my career. Among many academic institutions in India, TNAU until today stands one of the best in terms of teaching and research. I hope to see significant transformations in the years to come.

4. Your expertise in various domains of biotechnology is commendable. Do you think it is important for scientists to be all-rounders in their field of study, or mastering skills pertaining to their specific domain is better? Basically, from a career perspective, how often do you get to employ the skills you have mastered in your work and is this what employers are looking for?

It's essential to be aware and have a piece of basic knowledge on various domains of biotechnology. In my opinion, I don't think a scientist should be a jack of all skills, and no one can be. It's always important to master the skills specific to an area of your research interest. I worked on mastering skills relevant to every research project I was involved until now. We often find our research skills or techniques not matching to 100% of the posted job roles by the employers. We will not be 100 % fit to any given job role, but it's all about impressing and convincing the employer that you are capable of handling all the assigned tasks under the job requirements. Once you bagged the job and entered the organisation, then its vital to understand the concerned project, update yourself with the required skills and employ your transferable skills to execute the project efficiently.

5. Covid-19 has created an uproar in the world. Are you directly/indirectly working on any projects dealing with pandemic?

I'm not currently working on any projects related to the Covid-19 pandemic. During these times, I gathered some knowledge on Drug Discovery through *in silico* methods. With my close association with few academic institutions in India, we have some plans to write projects on the identification of potential drug molecules from plant sources, especially medicinal plants.

6. What inspired you to choose Biotechnology? Where do you see our field going post the pandemic?

I was exposed to biotechnology for the very first time when I was doing my high school. I was in awe by the magic what biotechnology does with cells *in vitro.* I was fascinated by the technology used to understand the cellular machinery of living organisms. Hence, this attracted me to explore into this field further.

Biotechnology has advanced over the years in various areas of biological sciences, especially in tackling dreadful diseases of the past like Ebola, SARS, etc. In the present situation, biotechnology as an application has been providing solutions on diagnostics, therapeutics, vaccine development and drug discovery. Post the pandemic, the field of biotechnology, bioinformatics and artificial intelligence tools will offer solutions in terms of drug discovery and development to tackle similar coronaviruses.

EDITORS' PAGE

EDITOR'S ARTICLE

FAST-TRACKING PRODUCTION OF BIOLOGICS: A PROCESS OF THE FUTURE

During this covid-19 pandemic, the world has come to recognize biotechnology and its potential. However, we are so used to equipping ourselves with one click-solutions, that waiting for a vaccine is becoming increasingly difficult. With scientists around the world working tirelessly to fast-track this process for us, we have taken for granted the timeline that goes into development of a biological product. There are billions of lives at stake when a biological product of such urgent need is introduced in the world. We are literally using future's research to control the present crisis. This article introduces biologics and their development and underscores why the current biotech industry is raw and brutal.

The process of drug development spans 5 extensive steps

1. discovery and development

2. preclinical research

3. clinical research

4. FDA review

5. FDA post market safety monitoring

From in-vitro, in-vivo, animal testing to multiple phases of human clinical trials, the complexity of releasing the final product to the consumer has increased manifolds over the past 50 years. With over a billion-dollar monetary investment and research spread across 12-15 years, the risk of your drug failing persists even after consumers use it. Moreover, getting monopoly over the market as a blockbuster drug before your competitors launch their replica is another ball game altogether.

And yet, this remains the story of conventional drugs synthesized by chemical means. Biologics, on the other hand, are composed of complex combinations of these proteins, nucleic acids and sugars, or may be living entities such as cells and tissues. Isolated from humans, animals or microorganisms, they are produced using complex biotechnology tools or cutting-edge technology. Gene-based and cellular biologics are often at the forefront of bioengineering research and provide treatments for complex medical conditions. Basically, they are much more complicated to develop, with unpredictable susceptibilities to different populations and rigorous in terms of money and research.

They have a wide product range- monoclonal antibodies, therapeutic vaccines, blood transfusion products, gene and cell therapies- medicines of the future indeed. With increased understanding of genetic bases and protein interactions, novel targeted therapeutic approaches can be employed to combat the bigger players in the disease arena. The first biologic, genetically engineered synthetic human insulin, marketed in 1982 opened doors to an entire new industry- biopharma.

Moreover, manufacturing complexities of biologics are major. With their ultra-sensitive nature to temperature, pH, co-factors and cellular conditions- not only their production but also administration becomes critical and tedious. Clinical studies aren't as straightforward as for conventional drugs. Their safety, purity and potency need to be verified with more scrutiny.

Diving back to the vaccine we need- it is yet another product synonymous to a biologic. Despite already going extensive research in genetic engineering, bioinformatics, molecular targeting- any biological product is limited by its complexity and novelty. Moreover, we are developing a novel product for an entirely novel organism. It is a long and costly endeavor with low success rates- in a fairly new industry. The industry is doing their work to compress a 15+ year long and rigorous process into a one-year project. It's time we do ours of being patient and supportive.

Deepakshi Kasat

NETFLIX SERIES/FILM REVIEW

LIVING WITH YOURSELF

How often do you wish that you were better at something? If you could just suddenly become a better version of yourself, you would be capable of solving all of your problems and achieve anything you want, life would be so much easier.

The Netflix original 'Living with yourself' is based on a similar concept. Miles Elliot (Paul Rudd) undergoes a mysterious treatment that promises him the allure of a better life, but he then discovers that he has been replaced by a cloned version of himself. So, the clone is not only his exact copy but also is much better at everything.

In reality, cloning a human embryo is very much possible but no one has managed to make one yet. Ever since the first successful cloned mammal- A sheep named Dolly was born in 1996, the discussion turned to cloned humans. The governments of the world immediately banned human cloning. Despite that, there were a few false claims of successfully cloned human embryos. A cult called Raëlians who believe that humans are the clones of aliens claimed in 2002 that they had cloned a human but there was no proof. Similarly, false claims of successfully cloned human embryos were made by a group of Korean scientists in 2004. But in 2018, a Chinese group reported births of the first Monkey clones. It was not an efficient process: About 80 cloned embryos led to

six pregnancies and two live births. Even so, reproductive cloning had succeeded for the first time in a primate. So there is solid proof that human embryos can be cloned.

Most scientific, governmental and religious organizations oppose reproductive cloning. Besides being ethically questioned, cloning humans has no commercial motive. Cloning animals, on the other hand, is rather normalized. Many organisations offer cloning services for pets like dogs, cats, even livestock to some extent. In fact, the World's champion polo team has used cloned ponies for several years. Advocates of human therapeutic cloning believe the practice could provide genetically identical cells for regenerative medicine, and tissues and organs for transplantation. Such cells, tissues, and organs would neither trigger an immune response nor require the use of immunosuppressive drugs. The value of using cloned human embryos to produce stem cell lines from an adult has been cast into doubt by competition from induced pluripotent stem cells (iPSCs). These stem cells can, like embryonic cells, produce all the cell types of a living human from cells carrying that individual's own DNA. Thus, there was no profit involving cloning humans. And once the potential use of CRISPR for DNA editing was discovered, it was possible to edit embryos and make designer babies. Why settle for a mere genetic copy of a living person when one could try to make a new and improved version?

In 'Living with yourself' Miles visits a "spa" where they manage to clone him within a couple of hours as he is unconscious. In reality, if someone managed to lure you into such a scheme and planned to clone you, it would take them at least a lifetime to replace you with your clone. The result of cloning an adult human can only be an embryo as the DNA from a somatic cell of the 'donor'(in this case- you) will be transferred to an oocyte, that has its own DNA containing nucleus removed. The embryo will then be implanted in the uterus of a surrogate mother who will give birth to your clone. They would then have to wait for the baby to grow up which will obviously take plenty of years. Moreover, the possibility of the clone being an exact copy of you is as good as none. So if a Spa promises you a 'better version of yourself' it's probably just a really good Spa.

Bhairavi Savur

GET TO WORK!

Solve the crossword and test your knowledge on enzymes.

Enzymes are proteins that act as biological catalysts. They act on substrates in living organisms regulating the rate at which chemical reactions proceed without itself being altered in the process. From digestion in the alimentary canal to transport around the body to replication and defense mechanisms- enzymes are present everywhere.

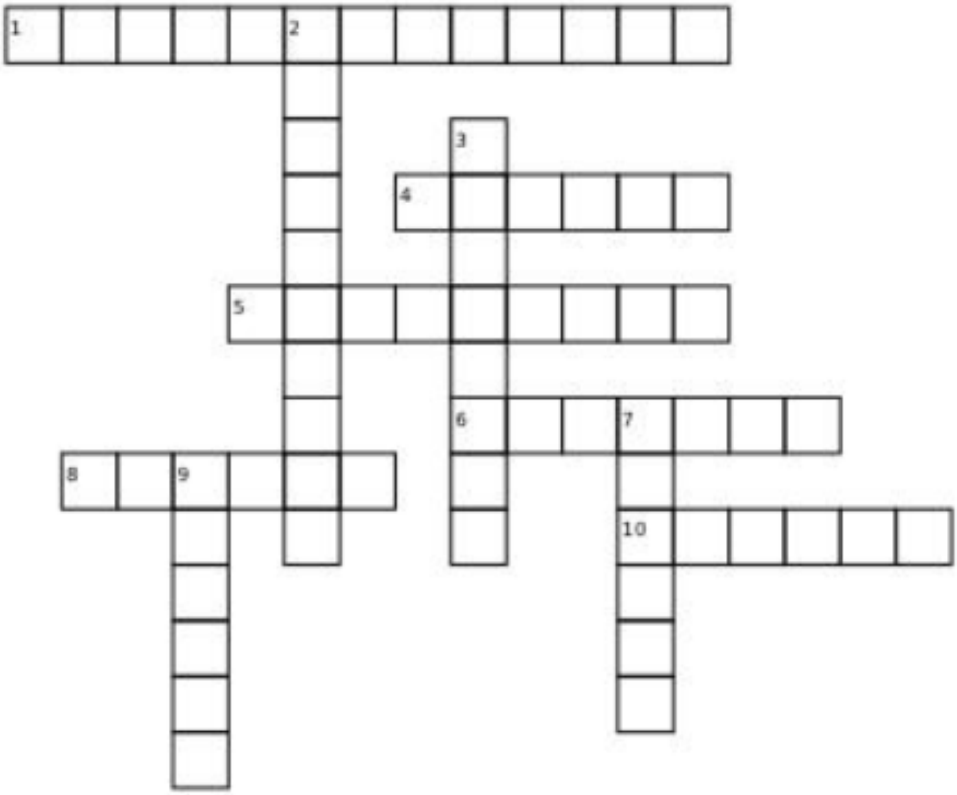
Find these enzymes in the crossword given below based on the following clues:

Across:

1. used in the amplification stage in PCR
4. found in the stomach used to break down proteins
5. used commonly to clear up wines and fruit juices
6. found in human saliva it helps convert starch into sugars
8. can connect two strands of a DNA
10. found in papayas and used as a meat tenderizer

Down:

2. makes fireflies glow
3. separates the strands DNA
7. breaks down fats
9. unwinds the DNA



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