SPECTRUM

SCIENCE AND TECHNOLOGY

A Peer Reviewed Research Journal

Vol. 1, 2014

St. Anthony's College
Shillong, Meghalaya, India
www.anthonys.ac.in

: EDITORIAL BOARD :

ISSN :.....

EDITORIAL

The inaugural issue of Spectrum: Science and Technology contains two review articles on information technology and eleven on biological sciences. One of the information technology articles explains how computers can be used to detect events that occur in the system and to respond intelligently to them. The other describes software that translates information to one's mother tongue. These are some of the most recent applications of information technology. The authors need to be commended for using the journal as a medium for presenting these concepts.

Among the biological science two articles deal with stem cell research and explain the recent advances on this area. There are also a couple of articles on medicinal properties of plants found commonly in India, particularly North East India. These articles share the same platform with two other articles dealing with pharmaceutical applications of laboratory synthesized compounds, thus providing an opportunity to readers to make a comparative assessment on medical compounds from natural and chemically synthesized sources. The review on an endophytic fungus explains plant-microbe interaction lucidly and will induce readers to think about a new approach to the development of biofertilizers.

The article on genetics of Beta-Thalassemia provides the much-needed information on this important human genetic disease and will draw the attention of readers interested in working on human molecular genetics and gene therapy.

One article on electropollution and another on hydrocarbon degrading microorganisms address the issue to pollutants vis-à-vis modern day demands and lifestyle of humans; they are interesting reads.

This first issue of Spectrum: Science and Technology materialized due to the diligence and sincerity of the members of the editorial board and also reviewers all of whom magnanimously spared time from their busy schedules to contribute to quality enhancement of the manuscripts.

One of the special features of the journal is that several of the articles are co-authored/ solely authored by post graduate students and research scholars who got opportunities to present topics from the perspectives of next generation of researchers, teachers and policy makers.

Spectrum: Science and Technology is a manifestation of our motto: Better More Better Ever. Long Live St. Anthony's College!

M. A. Laskar

CONTENTS

MEDICINAL USES AND BIOLOGICAL ACTIVITIES OF *CLERODENDRUM COLEBROOKIANUM*

Linu John[a] and Laishram Indira Singha[b]

ABSTRACT

C. colebrookianum is credited with innumerable medicinal activities like anti-hypertensive, antioxidant, hypolipidemic, anti-peroxidative, antimicrobial, anticancer, anti-helmintic, anti-asthmatic, anti-inflammatory, anti-stress and analgesic etc. Further investigations are required to authenticate all biological activities and to characterise the active principles before it is can be considered for clinical use. However the plant carries a great potential to be developed as drug by pharmaceutical industry. In this paper general medicinal uses and various biological activities of the plant have been reviewed.

Keywords: *Clerodendrum colebrookianum*, antihypertensive, anti-inflammatory, ethnomedicine, biological activity, hypolipidemic

INTRODUCTION

Plants and plant based compounds has been used by mankind from time immemorial for treating various diseases. The human use of plants as a source of medicine dates back to the middle Paleolithic age around 60,000 years ago (Solecki & Shanidar 1975). Plants are the basic source of knowledge of modern medicine and over 50% of all modern clinical drugs are of natural product origin. India's use of plants for health care dates back close to 5000 years (Singh, Khare, Iyer, Sharwan, and Tripathi 2012). *Clerodendrum colebrookianum* is an example of a medicinal plant with several therapeutic qualities validated by modern science and used since ancient times. *Clerodendrum colebrookianum* belongs to family Verbenaceae consisting of 400 – 500 specific and subspecific taxa which are widely distributed in tropical and subtropical regions of the world (Shrivastava and Patel 2007a). About 23 species of the genus are found in India (Srivastava and Choudhary, 2008). A number of species from this genus were documented in traditional systems of medicine and used as folk medicine by various tribes in countries like India, China, Korea, Thailand and Japan (Shrivastava and Patel 2007a). *Clerodendrum colebrookianum* has been considered as the most important medicinal species which is used in the treatment of hypertension by different tribes of north east India (Kalita, Sureshkumar and LatifKhan 2012).

[a] *Dept. of Bio-technology, St. Anthony's College, Shillong, Meghalaya*
[b] *Dept. of Bio-technology, St. Anthony's College, Shillong, Meghalaya*

PLANT DESCRIPTION AND DISTRIBUTION

Clerodendrum colebrookianum Walp. is a synonym of *Clerodendrum glandulosum* Lindl.).It is known as Dieng-ja-kaugum in Khasi, Kaombi in Lepcha, Orematong in Nagaland and Banbhait, napigacha in Tripura. It is distributed in South and South-east Asian countries like Bangladesh, Bhutan, China, India, Indonesia, Malaysia, Myanmar, Nepal, Sri Lanka and Vietnam (Yang *et al*, 2000). In India, the species is confined to North east region including West Bengal and Sikkim (Kalita *et al*, 2012). It is an evergreen shrub which generally grows well in moist and shady habitat at higher altitudes (Gupta, Mazumder and Das 1998). Clerodendron colebrookianum Walp. Khasi - Dieng-ja-kaugum, Lepcha - Kaombi, Lushai - Aripuni, Nagaland - Orematong; Tripura - Banbhait, napigacha *Clerodendrum colebrookianum* is an evergreen shrub which grows up to 4 - 8 ft. height with quadrangular stem, robust branches with sparsely pubescent corky internodes. Leaves are often 9 inch diameter, opposite, broad-ovate, acute, entire, petiolate, small lateral veins (6-9) with a few glands clustered at the petiole and scattered beneath. Inflorescence is terminal, compact, corymbose cymes. Flowers are white or rose coloured with broad terminal compact corymbose cymes, numerous, bracteates, pedicelate (2-4 cm), drupes bluish green. Bracts are lanceolate or narrowly ovate, caduceus with one bract for each flower and glands are present on lower surface. Calyx is gamosepalous, persistent, sepaloid, campanulate with several peltate glands, sepals (5) and glandular. Calyx-teeth are short triangular, reddish purple. Corolla is gamopetalous, petals (5), white in colour, tube nearly glabrous, stamens (4), didynamous, filiform. Gynoecium is of exerted style and shorter than stamen with four loculi. Anthers are reddish or maroon, introse. Fruit is drupe, subglobose, glossy and bluish green in colour that turns on black on drying. *Clerodendrum colebrookianum* is distinguished by having broadly ovate or cordate leaf blade with large peltate glands or glands on the abaxial surface of the leaf base and corymb thrysoid inflorescence (Fig 1 a-c). The taxonomical classification of the plant is given in table 1 (Kalita *et al*, 2012). In India C. *colebrookianum* is known by more than thirty vernacular names among 20 different tribes and communities of north eastern region (Kalita *et al*, 2012).

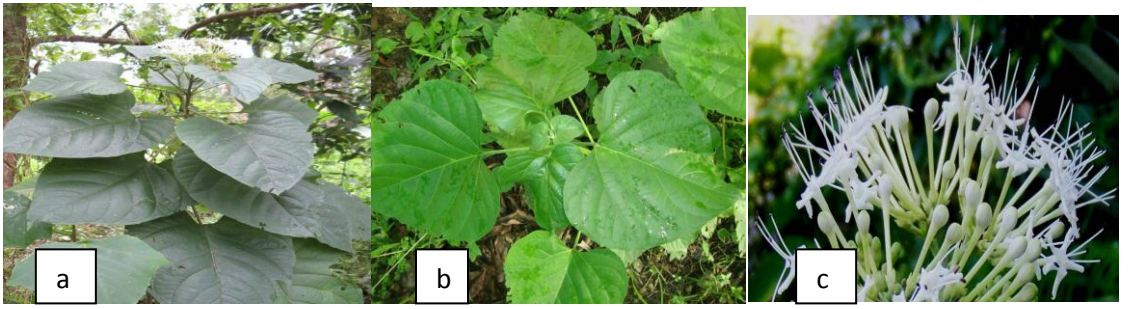


Fig 1a- Mature plant, b- seedling, c- Flowers (Kalita *et al*, 2012)

Table 1:- Taxonomical Hierarchy

Taxonomical Hierarchy	**Name**
Phylum	Magnoliophyta
Class	Magnoliopsida
Order	Lamiales
Family	Verbenaceae
Genus	*Clerodendrum* L.
Species	*Clerodendrum colebrookianum* Walp

CHEMICAL CONSTITUENTS

C. colebrookianum is reported to contain chemical constituents like phenols, alkaloids, saponins, flavonoids, polyphenols, steroids, tannins, terpenes, minerals and various other compounds like 2-methyleicosa 2,9-diene,10,11,32-trimethyletratricontanol, pentatriacontane, palmitic acid etc which are responsible for its biological activities such as anthelmintic, antibacterial, anti-fungal, hypolipidemic, anti-peroxidative antioxidant and antihypertensive properties (Devi, Singh and Salam 2014; Shrivastava *et al*, 2007a, 2007b). Majaw and Nongbet (2013) reported flavonoids with good antioxidant activity from TLC fractions of the leave extracts. Presence of twenty eight compounds was indicated in the GC MS analysis of *C. colebrookianum* hexane extracts. Joshi, Singh and Mehra (1979) reported terpenes like triacatane, clerodin and clerodendrin A. Five new steroids, colebrin A-E and colebroside were also isolated from the aerial parts of the species. Other steroidal compounds like β-sitosterol, clerosterol and taraxerol were extracted from leaves of *C. colebrookianum* using hexane solvent (Singh *et al*, 1995). Furthermore presence of mineral elements like calcium, phosphorous, potassium, magnesium and trace elements of iron, zinc, copper, manganese, cobalt and sulphur were detected in the plant (Majaw *et al*, 2013; Majaw and Moirangthem 2009; Kotoky, Dasgupta and Dekka 2005).

MEDICINAL USES

C. colebrookianum is reported to be used for treatment and cure of more than 16 different diseases and ailments like hypertension, rheumatism, diabetes, malarial fever, diarrhoea, dysentery, heart trouble, gastric disorders etc. Though the use of young and tender leaves as decoction for antihypertensive purpose is the most common form of ethnomedicinal application for the species of *C .colebrookianum*, it is also used as a

remedy for abdominal pain, cough, obesity, colic in infants and also as an antidote and blood purifier (Kalita *et al*, 2012). Generally the leaves are used as bitter tonic, vermifuge, laxative and cholagogue. In north eastern region of India the root, leaf and bark are used in malarial fever and the leaves are also used against hypertension (Nath *et al*, 1991). Fresh leave juice is introduced in the rectum for the removal of ascarids and also believed to possess distinct anthelmintic properties. Leaves and roots are used for external applications on tumors (Devi *et al*, 2014). The roots are reported to cure bronchial asthma, gastrointestinal tract disorders, syphilis and gonorrhea and several hematological disorders (Kotoky, 2005). Leaves are the most used part of the plant; however preparation from roots and other parts are also used in the form of decoction, juice, extract, raw, soup, boiled, infusion etc for various treatments.

BIOLOGICAL ACTIVITIES

C. colebrookianum is reported to have several biological activities like anti-hypertensive, antioxidant, hypolipidemic, anti-peroxidative, antimicrobial, anticancer, antihelmintic, antiasmatic, anti-inflammatory, anti-stress and analgesic properties. The traditional claims are validated by many studies carried out using various animal models and *in vitro* assays.

Anti- Inflammatory Activity: - Studies with methanolic plant extracts on mice and rat models demonstrated strong anti- inflammatory activity and central and peripheral analgesic activity. The strong anti-inflammatory suggests that they could have acted by affecting kinnin, prostaglandin, bradykinin and lysozyme synthesis. These activities are attributed to the terpenes, sterols, glycosides and other polar bioactive components of the plant (Kotoky *et al*, 2005). Studies on leaves extracts of the plant also reported protective effect on acute and chronic stages of inflammation in rats. The observations from the study suggested that the flavonoids in the leaves could be the anti-inflammatory agents (Deb *et al*, 2013).

Hypolipidemic Activity: - A study using leaf extracts on Wistar albino rats confirmed the hypolipidemic activity by causing a significant reduction of lipid peroxidation in plasma and tissues. From the quantification of total polyphenols, tannins and flavonoids contents in the plant sample it was suggested that hypolipidemic activity is due to the presence of the valuable polyphenolic compounds (Devi *et al*, 2011).

Anti-Hypertensive:- *Evaluation of n*-butanol (*n*BFCc), Ethyl-acetate (EtFCc) and Chloroform fractions of *aqueous* leaves extracts revealed antihypertensive potential of plant on fructose-induced hypertension model in rats and in isolated frog heart. The findings confirmed that the antihypertensive effect is mediated by cholinergic action and through the inhibition of ROCK – II, PDE-5 enzymes of *C. colebrookianum (*Lokesh_and Amitsankar 2012*).* This finding justifies the traditional claim of C. *colebrookianum* as a medicinal plant with potent antihypertensive activity (Lokesh *et al*, 2012).

Antioxidant activities: - Organic and aqueous extracts of *C. colebrookianum* showed significant inhibition of lipid peroxidation *in vitro* and *in vivo* induced by $FeSO_4$-ascorbate in rats. Aqueous extracts showed strongest inhibitory activity over organic extracts. TLC fractions of the leave extracts revealed flavonoids with good antioxidant activity .This lends scientific support to the therapeutic use of the plant leaves claimed in tribal medicine (Majaw *et al* 2013; Shrivastava and Patel 2007a)

Anti-Stress Effects: - Anti-stress studies using the leaf extract of *C. colebrookianum* revealed reduction in plasma corticosterone level in cold-restraint mice. The findings from the study suggest that the extract might contain principle(s) that possibly exert multiple actions involving different mechanisms in exerting anti-stress effects (Majaw *et al,* 2008).

Anti-obesity Potential:- In vitro study using aqueous leaf extract reported significant anti-adipogenic effect and decreased adipogenesis by down regulation of PPARγ-2 related genes and Lep expression thus validating its traditional therapeutic use in controlling obesity (Jadeja *et al,* 2011a).

Hepatoprotective and Cardioprotective Effect :- Aqueous leaf extracts of *C. colebrookianum* also manifested hepatoprotective effect on Swiss albino mice against CCl(4)-induced hepatic damage by modulating activity levels of enzymes and metabolites governing liver function and by helping in maintaining cellular integrity of hepatocytes (Jadeja *et al,* 2011b). Studies on lipid profile in rats using organic and crude extracts of *C. colebrookianum* significantly lowered the serum lipid levels suggesting that it has cardioprotective potential (Devi and Sharma 2004).

CONCLUSION

Ethnomedicinal applications and biological activities of *C. colebrookianum* are diverse and numerous. However a thorough phytochemical investigation is recommended for identification, isolation, purification and characterization of active principle(s) of the plant for its therapeutic utilization and drug discovery.

REFERENCES

- Deb, L., Dey, A., Sakthivel, G., Bhattamishra, S. K. and Dutta, A. 2013. ”Protective Effect of *Clerodendrum colebrookianum* Walp., on Acute and Chronic Inflammation in Rats”. *Indian Journal of pharmacology* 45(4):376-380.
- Devi, M. N., Singh, S. R. and Salam, J. S. 2014. “Antioxidant, Mineral and Phytochemical
- Composition of *Clerodendrum colebrookianum* Walp, A Well Known Home Remedy Herbal for Humankind”. *World Journal of Pharmaceutical Research* 3(2):2667-2676.
- Devi, R., Boruah, D. C., Sharma, D. K. and Kotoky, J. 2011. “Leaf Extract Of *Clerodendron colebrookianum* Inhibits Intrinsic Hypercholesterolemia and Extrinsic Lipid Peroxidation”.
- *Inernational Journal of Pharm Tech Research* 3(2): 960-967.

- Devi, R. and Sharma, D. K. 2004. "Hypolipidemic Effect of Different Extracts of *Clerodendron colebrookianum* walp in Normal and High-fat Diet Fed Rats". *Journal of Ethnopharmacology* 90:63-68.
- Gupta, M., Mazumder, U. K. and Das, S. 1998. "Effect of Leaf Extract from *Clerodendron colebrookianum* on CNS Function in Mice". *Indian Journal Experimental Biology* 36:171–174.
- Jadeja, R. N., Thounaojam, M. C., Ramani, U. V., Devkar, R. V. and Ramachandran, A.V. 2011a. "Anti-Obesity Potential of *Clerodendron glandulosum*.Coleb Leaf Aqueous Extract". *Journal of Ethnopharmacology.* 135(2):338-43.
- Jadeja, R. N., Thounaojam, M. C., Jadav, S. V., Patel M. D., Patel D. K., Salunke S. P., Padate G. S., Devkar R. V. and Ramachandran A. V. 2011b. "Toxicological Evaluation and Hepatoprotective Potential of *Clerodendron glandulosum*.Coleb Leaf Extract*". Human and Experimental Toxicology.* 30(1):63-70.
- Joshi, K. C., Singh, P. and Mehra, A. 1979."Chemical Investigation of the Roots of Different *Clerodendron* Species". *Planta Medica* 37:64-66.
- Kalita, J., Sureshkumar, S. S, and LatifKhan, M. 2012. "*Clerodendrum colebrookianum* Walp: A Potential Folk Medicinal Plant of Northeast India". *Asian Journal of Pharmaceutical and Biological Research* 2(4): 256-261.
- Kotoky, J., Dasgupta, B. and Deka, N. 2005. "Pharmacological Studies of *Clerodendron colebrookianum* Walp, a Potent Hypotensive Plant". *Indian Journal of Physiology Pharmacolgy* 49 (3):289–296.
- Lokesh D. and Amitsankar D. 2012. "Evaluation of Mechanism for Antihypertensive Action of *Clerodendrum colebrookianum* Walp, Used by Folklore Healers in Northeast India". *Journal of Ethnopharmacolgy* 143(1):207-212.
- Majaw, S., Kurkalang, S., Joshi, S. R. and Chatterjee, A. 2008. "Effect of *Clerodendron colebrrokianum* walp. Leaf Extract on Cold-Restraint Stress in Mice". *Pharmacologyonline* 2: 742-753.
- Majaw, S. and Moirangthem, J. 2009. "Qualitative and Quantitative Analysis of *Clerodendron colebrookianum* Walp. Leaves and *Zingiber cassumunar* Roxb. Rhizome". *Ethnobotanical Leaflets* 13:578-89.
- Majaw, S. and Nongbet, A. 2013. "Evaluation of Antioxidant Activity of TLC Fractions of *Clerodendron colebrookianum* Leaf Extract Containing Flavonoids". *International Journal of Pharmacy and Pharmaceutical Sciences* 5(2):90-94
- Nath, S.C, and Bordoloi, D.N. 1991: "*Clerodendrum colebrookianum*, a Folk Remedy for the Treatment of Hypertension in Northeastern India". *Pharmaceutical Biology* 29(2): 127-129.

- Singh, M., Chaudhuri, P. K., Sharma, R. P. and Jain, S. P. 1995. Constituents of the leaves of *Clerodendrum colebrookianum*". *Indian Journal of Chemistry* 34(B): 752-754.
- Srivastava, R. C. and Choudhary, R. K. 2008. "Species Diversity and Economic Importance of the Family Verbenaceae in Arunachal Pradesh". *Bulletin of Arunachal Pradesh Research* 24(1&2):1-21.
- Shrivastava, N. and Patel, T. 2007a. *"Clerodendrum* and Healthcare: An overview". *Medicinal Aromatic Plant Sci. Biotech* 1(1): 142-150
- Shrivastava, N. and Patel, T. 2007b. "*Clerodendrum* and Healthcare: An overview- Part II. Phytochemistry and Biotechnology". *Medicinal Aromatic Plant Science Biotechnology* 1(2): 209-223.
- Solecki, R.and Shanidar, V. 1975. "A Neanderthal Flower Burial in Northern Iraq". *Science,* 190: 880-881.
- Yang, H., Wang, J., Hou, A., Guo, Y., Lin, Z. and Sun, H. 2000. "New Steroids from *Clerodendron colebrookianum.* Fitotirapia".*Journal of Asian Natural Products Research* 71: 641–648.

EVENT DRIVEN ARCHITECTURE: A REVIEW

Joby Joseph[a] and C. Jothi Venkateswaran[b]

ABSTRACT

There are various models of computing that are in use. These models have evolved keeping to the need of more recent developments in the area of resources and computer architectures. The characteristics and stages involved in the buildup to the event-driven architecture is an interesting focus of study. An event is s seen as the smallest tick in the chain that follows which can lead to higher degree of scalability and parallelism. This is a review that attempts to look into these building blocks of such a system.

Keywords: Events, Event Driven Architecture, Event Processing, Event Computing

1.1. INTRODUCTION

Events are in every aspect of life. Everything that happens around is an event that has its cause and effect on subsequent sections. One of the emerging modes of computing is the Event Driven Architecture, where computation takes place as response to events that occur in the system (Taylor et al. 2011). This review is a detailed study on this model of computing. The review begins with a detailed study of the fundamentals in the field of event processing, their characteristics, prerequisites and go on to analyse in detail an architectural layout that is useful in an Event Driven model.

1.2. BASICS OF EVENT DRIVEN COMPUTING

An Event Driven model of computing presents a modern, dynamic and reconfigurable processing solution to the computing world. An Event Driven computing is one that can detect the various *events* that occur in the system and respond intelligently to it. In order to understand the proper intricacies of an Event Driven system, the term *event* needs to be defined. An event can be defined as a notable action that happens inside or outside the system. An event in the computing scenario can signify an occurrence or non-occurrence of an action (Niblett, Luckham and Etzion 2010).

Any event has three aspects associated with it that needs to be clarified in the realm of the computing model. The first part is that –an event occurs as notable action in the system and the second aspect is that there is the definition for an event that classifies the action as an event and finally there are the details of the specific event. Hence it is important to understand these aspects of an *event:* viz., Event Notification,

[a] *Assistant Professor, St. Anthony's College, Shillong, Meghalaya.*
[b] *Head, Dept. of Computer Science, Presidency College (Autonomous), Chennai.*

Event Definition and Event Specification while considering an EDA model (Sriraman 2005). These are seen in detail in the coming section of the review.

1.3. CHARACTERISTICS OF EVENT DRIVEN COMPUTING

Any event driven model of processing exhibits the following computing properties:

- **Observation of Events:** This feature implies that the EDA model is actively monitoring the system and any occurrence of an event initiates the necessary alerts to the system (Taylor et al. 2011; Sriraman 2005).
- **Information Dissemination:** This aspect of the EDA model is to deliver the right information to the right component at the right intensity to the proper respondents (Taylor et al. 2011).
- **Dynamic Operational Behaviour:** Every event driven computing system needs dynamic and quick interaction to respond to the report of an event (Taylor et al. 2011) (Kowalewski, Bubak and Balis 2009).
- **Active Diagnostics:** Every event driven system should also be able to diagnose a problem if it occurs based on the events observed (Taylor et al. 2011; Yang, Wu and You 2008).
- **Predictive Processing:** In an efficient EDA model, the style is not merely *report-respond* model of processing, there should also be methods to prevent erroneous events that can cause fault in the process (Taylor et al. 2011; Yang et al. 2008).

The conceptual diagram incorporating all these features is shown in **Figure 1**.

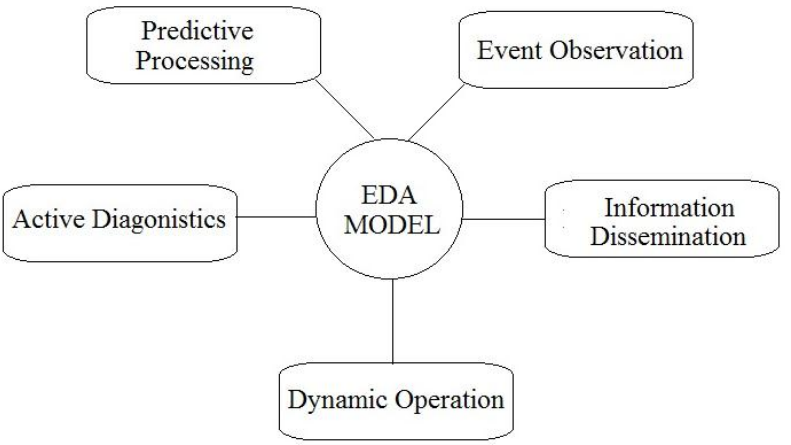


Figure 1: Event Driven Architecture Characteristics

1.3.1. EVENT PROCESSING REQUIREMENTS

An Event Driven Architecture needs certain functional capabilities that allow the system to work with optimum throughput. Some of these requirements are:

- ***Event Input/Output*:** Most event driven applications need some way to consume the events. These can come from other input devices or applications that are internal or external to the system. Similarly, applications also need a way to output an event as a response (Taylor et al. 2011; Li 2005).
- ***Data Reduction:*** This involves the process of extracting information from the ingested input events. These include the steps of filtering, projection and aggregation (Taylor et al. 2011; Li 2005).
- ***Reasoning:*** This is the process of extracting the desired event from the set of raw events through the stages of transformation, aggregation and pattern detection (Taylor et al. 2011; Li 2005).
- ***Analysis*:** The process of analysis involves the stages of detailed observation of extracted event in comparison to existing event details that are kept in logs (Li 2005).

- ***Prediction*:** Based on the analysis of the event and the log data, predictive actions can be made regarding the system and possible events in the future can be anticipated (Kowalewski et al. 2009; Li 2005).
- ***Learning*:** As events are observed, analysed and predictive plans are marked; an EDA system does a lot of inherent learning and adaptation for the future (Kowalewski et al. 2009; Li 2005).
- ***Distribution*:** The last task of the EDA system has the function of passing the events received to the appropriate module so that proper event processing can take place (Li 2005).

1.4. EVENT PROCESSING

Most event processing models have a computing process that responds to event objects. The event processing mostly includes creating, reading, deleting, transforming and responding to such objects. Such systems will mostly comprise of two major components: First, a Sensor or a source that senses any event and emits event objects. Secondly, a Responder, that receives and responds to such event objects. Both these actions occur at two layers of the model (Hohpe 2006). Any EDA model will consist of multiple levels of sensors and responders. Each acts in response to a request from the other. The mode of processing that occurs in most EDA is explained in the sections that follow (Michelson 2006).

1.4.1. EVENT PROCESSING STYLE

In most EDA, there are three general styles of event processing. They are Simple, Stream and Complex.

1.4.1.1. *Simple Event Processing*

In such a model an event happens and it initiates many downstream actions. In most real time flow of business systems, such models are used. Simple event processing is the base of an EDA system and it makes up the base component that initiates the event processing style in the computing realm (Taylor et al. 2011; Dagstuhl Seminar Proceedings 2010).

1.4.1.2. *Stream Event Processing*

In such a model, there are both ordinary and notable events happening. This model is commonly used to drive the real time flow of information in and around an enterprise, or a business or computing models, making decision making in time (Hohpe 2006). In the stream event mode of processing there is the combination of ordinary events that make up a chain to have them in the stream format. Such events can be as the cause or effect of other preceding events that happen elsewhere in the system (Dagstuhl Seminar Proceedings 2010).

1.4.1.3. *Complex Event Processing*

Complex Event Processing deals with evaluating a combination of events and then taking the action. There is a high degree of event-correlation possible here. This model will need high degree of matching and processing techniques. This is commonly used in models that need high degree of adaptability, responses and inter-dependencies (Li 2005; Dagstuhl Seminar Proceedings 2010).

In all these levels of interaction and information flow, there is a need of high degree of data interchange and interconnect. The architectural design of the models will have to be one that supports this at the processing and design levels of the model. The architecture needs systems that detect, transfer and process the events appropriately while maintaining efficiency and agility at each stage. These are performed at the various layers that occur in an EDA model (Dagstuhl Seminar Proceedings 2010). These are explained in the sections that follow.

EVENT FLOW LAYERS

Every event in the model starts with an event being generated at some level and proceeds to any down-stream layers to meet its activity. The stages from the occurrence to the action done in response to the event can be classified in various layers. The layering of an event makes it easy to understand and to troubleshoot it at the within the right time and place. Figure 2 is a structural diagram for the layers. This involves the following logical layers (Taylor et al. 2011; Hohpe 2006; Michelson 2006).

1.4.1.4. *Event Generator*

This is the source that generates an event. The source may be an application, service or a request of any type. In most cases, not all events are generated in the required format for processing. In such cases, they have to be transformed to a format prior to depositing the event into the next stage. The generated event is as raw data, it is

to be pruned, processed prior to being processed. An EDA model consists of such applications that perform these pre-processing tasks (Hohpe 2006; Michelson 2006).

1.4.1.5. *Event Channel*

The event channel typically is the messaging backbone, which transports standard formatted events between the event generators, the processing systems and the downstream layers. An event that happens at one level of the system needs to be transferred to the proper level(s) for its subsequent processing or for initiating other successive event generation. The channel takes care of the flow of an event to such applications in the EDA model (Taylor et al. 2011; Hohpe 2006).

1.4.1.6. *Event Processing Layer*

At this layer, once the events are received, they are evaluated against the set of rules for processing and appropriate span out actions are initiated. The actions may include invoking a service, initiating a process, publishing the event to another system, generating another new event or capturing the event for a later process. A lay out diagram of the three stages of the layers is shown in Figure 2 (Taylor et al. 2011; Hohpe 2006; Michelson 2006).

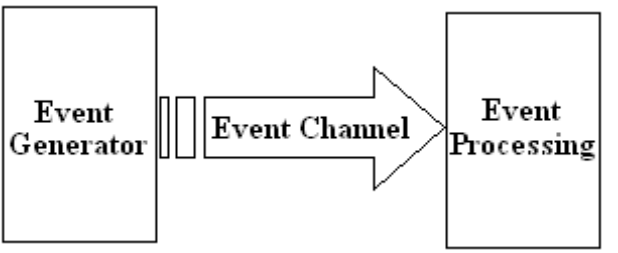


Figure 2: Event Flow Layers

1.5. EVENT DRIVEN ARCHITECTURE

A layout diagram for the EDA model in the context of a Cloud paradigm is shown in Figure 3. The basic nature of such a model is that the system is triggered by the occurrence or nonoccurrence of an *even*t (Taylor et al. 2011; Chandy 2006).

The system is as a single unit inherent in the Cloud set up and the whole set up is to be seen as a constituent part of the Cloud. The events that occur in the cloud are sensed by the Event Observer and it passes the events thus observed to the Event Manager. This is mostly a middleware that acts as a control and coordination units prior to passing the events to the next unit of Event Processor.

In the Event Processor, the input events are filter, correlated and all pruning is done to isolate and collect meaningful data from the raw input of events. The output of this unit is the notification to the proper Event Application within the EDA model. This initiates the proper *response* from the Event Application to the Event Response System.

The Event Response system initiates proper actions and to the required unit within the cloud set up.

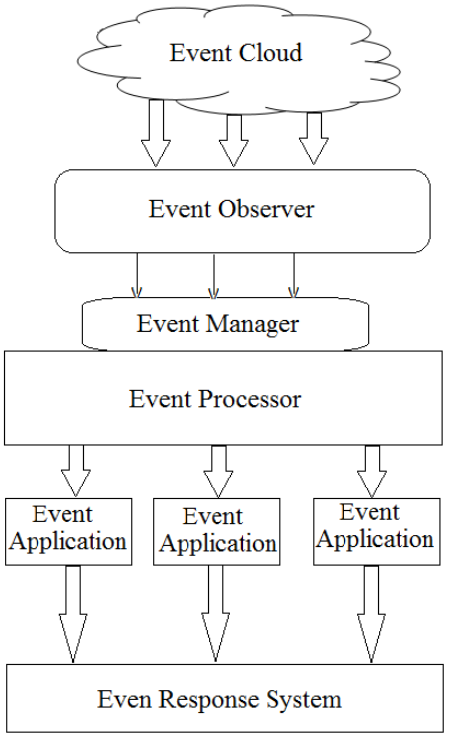


Figure 3: Proposed Event Driven Architecture for the Cloud Model

1.6. UNIQUENESS OF EVENT DRIVEN ARCHITECTURE

Event Driven mode of computing is one that is gaining advance in the computing models where large scale distributed computing or service oriented computing takes place. In the sections that follow, an analysis is done to highlight the features of the EDA model that single it out from other emerging models of computing (Michelson 2006; Levina and Stantchev 2009; Eisenhauer et al.2009; Dagstuhl Seminar Proceedings).

- ***Broadcasting Events:*** The occurrence of an event is broadcasted to all the units in the system that are actively participating in the architecture and any one system or multiple systems can listen to an event and take up the process so initiated. This allows a degree of parallelism as well as dynamism in response.
- ***Asynchronomy:*** The constituent systems in an EDA publish events as they occur instead of storing them and delaying for a process cycle. This incorporates a lot of asynchronous process style in responding and attending to input events. This style of process can make the EDA mode of information process with optimum use of the resources for performance as they are done on the fly.

- ***Ontology of Events:*** An EDA can have built in rules to classify and event or to group a collection of events into an ordered hierarchy. Hence, once an event is published, the receiving system can respond to the event based on its hierarchy or handle a category of them in a like manner. This brings in a priority mode of processing into the EDA model.
- ***Complex Events:*** Many events that happen within the system have inter-relation to higher level of events and can cause a pattern of lower level events. Hence, in an EDA model, there can be an advantage of event aggregation or event causality. Both these features together allow requests and responses in the system more scalable and parallel.
- ***Event Replay:*** The EDA has another important and unique characteristic. If all the interaction within the system takes place as events, then the same can be recreated by replaying the events all over again. The advantage is that as events are recreated, some of these can be replayed with modifications. The replay feature is good to analyse and make future correction for the EDA model.

1.7. RESEARCH TRENDS IN EVENT PROCESSING

This section makes listing of the emerging research trends in the field of Event Driven processing. The field of Event processing itself is new and there are many areas of application and related research that can be anticipated. Some of the outstanding ones are listed below:

- ***Towards Decentralization:*** The Event Driven computing has found wide area of coverage. It is incorporated into many fields of application as Event Processing Technical Society (EPTS) has noted. This has brought about new programming and architectural challenges for research. The event driven model brings a shift from the monolithic architectures that were so commonly used to architectures that are diversified. The shift is from a centralized architecture to a distributed model keeping in line with the current mode of parallel computing. This diversification comes with variety of functions, quality of services and a variety of platforms over which such models can be tested and tried (Taylor et al. 2011).
- ***Greater Standardization:*** As Event Processing becomes more popular and is implemented into diverse applications, there is the new research trend of incorporating a standardized mode of conceptualization and implementation. The various fields of standardization that the research focus is on are: Event structure and related metadata representation, event distribution standards, event processing language, event assembling model and event rules that can guide the system. A common norm and rule can bring about a greater level of interoperability in the processing arena (Taylor et al. 2011; Li 2005).
- ***Business Model:*** Current area of focus is much on business process management using the event model of data management and process. The EDA model is heavily used in business workflow systems (Niblett et al. 2010).

- ***Embedded Models:*** Lots of current research is active into the area of embedded event models. This involves event functions being embedded into the packaged applications or into other middleware applications. In all these the focus of the current trend is to switch gear from having a reactive model of computing to a proactive model of processing (Taylor et al. 2011; Li 2005).
- ***Other Areas:*** There is lot of research on to realize EDA models of virtual platforms. These include hardware application platforms, embedded platforms etc. Hardware application platforms with multicore processors, embedded platforms like robotics or other gateways where EDA models are areas of related research (Taylor et al. 2011; Kowalewski et al. 2009).

1.8. CONCLUSION

The uniqueness of the Event Driven architecture is a promise to many emerging models of computing. The EDA seems to promise lot of processing style in Service Oriented Architectures. The Cloud model of computing is one where there are applications that are service driven. In such model, the computing power in the model can be better utilized and thus served if the EDA model is incorporated into the Cloud. This opens a span of area for incorporating such a model of computing into the cloud model.

REFERENCES

- Chandy, K. M. In *Event-Driven Applications: Costs, Benefits and Design Approaches*, Proceedings of Gartner Application Integration and Web Services Summit 2006, Sao Paulo, Brazil.
- Dagstuhl Seminar Proceedings 10201. In *The Event Processing Manifesto*, Proceedings of Dagstuhl Seminar on Event Processing, Dagstuhl, May 16 – 21, 2010. (http://www.dagstuhl.de/10201).
- Eisenhauer, G.; Abbasi, H.; Wolf, M.; Schwan, K. 2009. In *Event-based Systems: Opportunities and Challenges at Exascale*, 3rd ACM International Conference on Distributed Event-Based Systems July 6-9, 2009 (DEBS'09) - Nashville, TN, USA.
- Hohpe, G. 2006."Programming Without a Call Stack- Event-driven Architectures", http://www.eaipatterns.com.
- Kowalewski, B.; Bubak, M.; Balis, B. 2009. *An Event-Based Approach to Reducing Coupling in Large-Scale Applications.* Krakow, Poland.
- Levina, O.; Stantchev, V. In *Realizing Event-Driven SOA*, Proceedings of 2009 Fourth International Conference on Internet and Web Applications and Services (ICIW '09), Los Alamitos, CA, USA.
- Li, C.S. In *Real-Time Event Driven Architecture for Activity Monitoring and Early Warning*, Proceedings of Emerging Information Technology Conference, 2005, 15-16 Aug. 2005

- Michelson, B. M. 2006."Event-Driven Architecture Overview", Patricia Seybold Group and Elemental Links, Inc.
- Niblett, P.; Luckham, D.; Etzion, O. 2010. *Event Processing In Action.* New Delhi, Manning Publications.
- Sriraman, B. 2005. *Event Driven Architecture Augmenting Service Oriented Architectures*. Sun Microsystems Inc.
- Taylor, H.; Yochem, A.; Phillips, L.; Martinez, F. 2011. *Event-Driven Architecture: How SOA Enables The Real-Time Enterprise.* New Delhi, Pearson.
- Yang, J.; Wu, J.; You, Y. 2008. In *A Web- based, Event Driven Management Architecture*, Proceeding of Fifth Asia-Pacific Conference on Communications and Fourth Optoelectronics and Communications Conference, 1999. APCC/OECC '99.

UNL MULTILINGUAL CONVERSION ARCHITECTURE FOR LEARNING REGIONAL LANGUAGES

A. Clementking[a]

ABSTRACT

Technology in the Learning process is a tool effective for its methodology and process. The teaching learning efficiency can be increased via technology as a tool for learning. Learning process leads to gain knowledge. Learning process leads to knowledge gain. Knowledge is represented in different languages. The learning process and acquiring knowledge will be easy if the learner uses their own mother tongue. This paper addresses the conventional architecture method form one language to regional language via Universal Networking Language. The Conversional architecture and the proposed teaching and learning tools are discussed in the paper.

Keywords: UNL, Learning using languages, Languauge converter

INTRODUCTION

The Indian educational system achieved mass literary rates and knowledge innovation through the use of regional languages. Indian teaching and learning pedagogy are based on the regional culture and life style. The teaching learning process always provides the path to extract the knowledge from where it is available. Various conventional methods are used to learn the knowledge and innovation which is available in other languages and different parts of the world. Various conventional and innovative methods used in the learning process, are available in different languages and in different parts of the world. But the conventional methods have their own limitation of time and the lack of impact of the conventional authors' language. To overcome the limitations and provide the standard with zero time delay, language convention process UNL (Universal Networking Language) needs to be initiated. This paper addresses the conventional tool architecture that can be utilized for the regional language in the teaching-learning process.

UNL SYSTEM

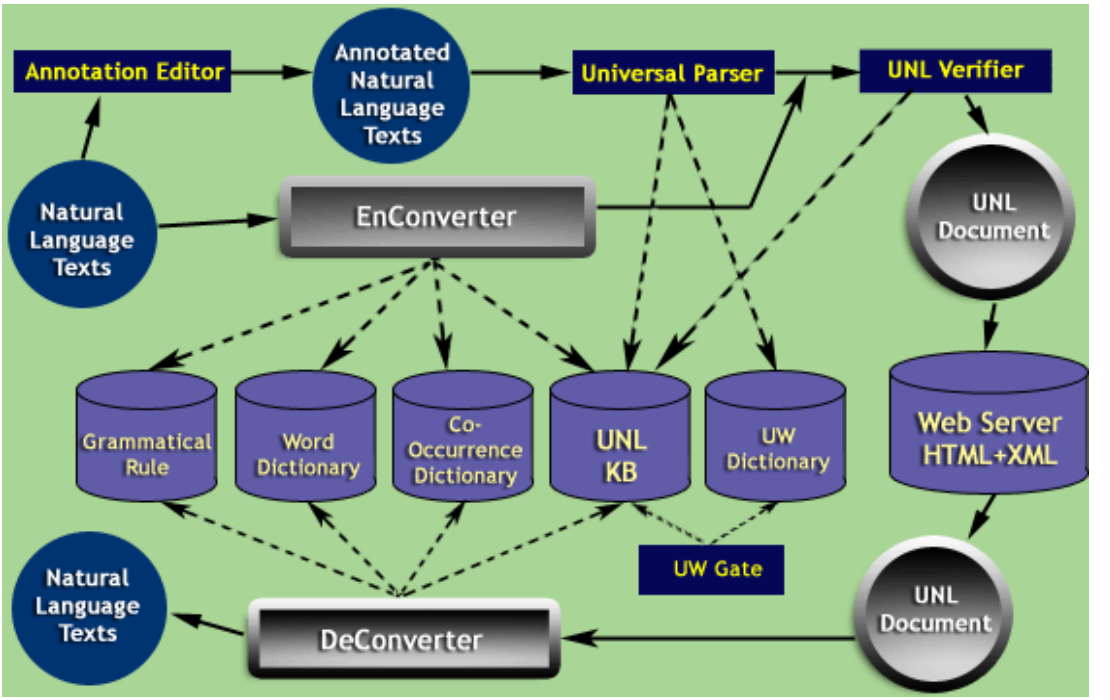
UNL is a system which is used to convert a language from one to another using common Knowledge base. The intermediate code can be generated using the encoder and decoder which converts into another language .The UNL System consists of three major components: language resources, software for processing the language resources and tools and systems for maintaining and operating the language processing software.

[a] College of Computer Science, King Khalid University, Kingdom of Saudi Arabia.

Language resources are divided into language dependent parts and language independent parts. Linguistic knowledge on concepts that is universal to every language is considered language independent and is stored in the common database UNLKB. Language dependent resources like word dictionaries and analysis and generation rules, as well as the software for language processing, are stored in each language server. Language servers are connected through the Internet. Supporting tools for producing UNL documents can be used in a local PC. Such supporting tools operate with consulting language servers through the Internet. Verification of UNL documents can be carried out through the Internet or in a local client.

MECHANISM OF CONVERSION OF UNL EXPRESSIONS

. The EnConverter and DeConverter are the core software in the UNL system. The EnConverter converts natural language sentences into UNL expressions. The Universal Parser (UP) is a specialized version of the EnConverter. It generates UNL expressions from annotated sentences using the UW dictionary without using grammatical features. All UNL expressions are verified by the UNL verifier. The DeConverter converts UNL expressions to natural language sentences.



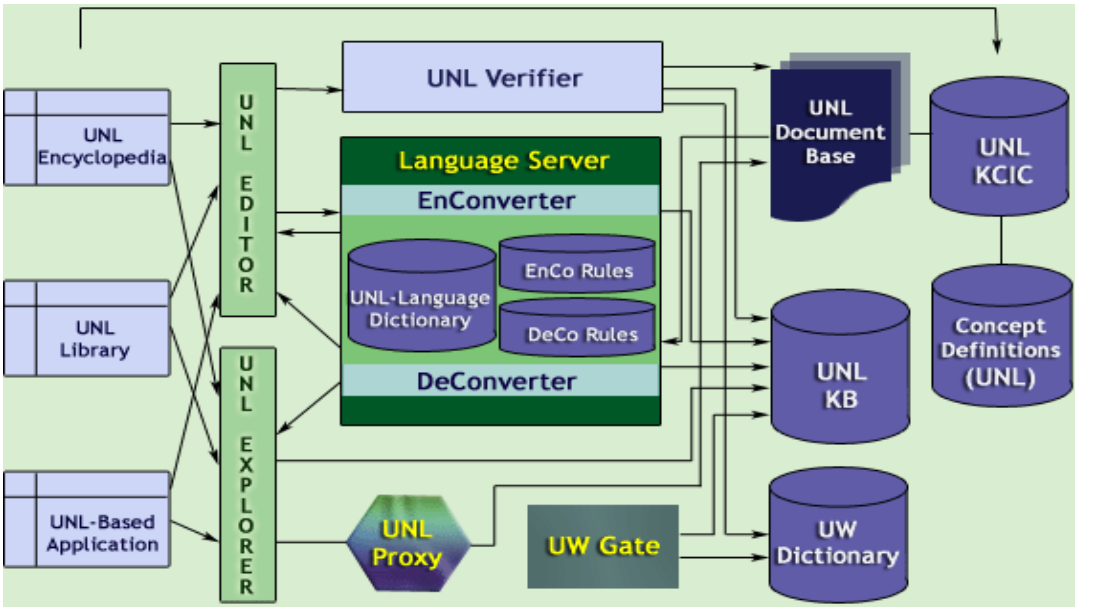
Source: UNL-UNDL

Fig. 1. Mechanism of conversion of UNL

Above diagram shows the mechanism how a UNL document is made and how a UNL document is converted into natural languages in the UNL system. Arrows in solid line show dataflow, arrows in broken line show access.

STRUCTURE OF THE UNL SYSTEM

.



Source: UNL-UNDL

Fig. 2. Structure of the UNL System

UNL Documents mean the documents in which UNL expression is described for each sentence of natural language. A UNL document can be made of a plain text file or an UNL-embedded html file. A UNL document base is a collection of UNL document files. The purposes of UNL documents are to provide information and knowledge in UNL expression.

UNLKB is a semantic network comprising every directed binary relation between UWs. These binary relations are the possible relations that each UW can have with others. Such relations are established based on the UW System. Such UNLKB has the following functions: 1) define semantics (concepts) of UWs, and 2) provide linguistic knowledge of concepts.

UW Dictionary stores and provides the interface between UWs and words of natural languages.

Concept Definitions are the collection of UNL documents made for the sentences of definitions of UWs. These definitions of concepts provide the knowledge of concepts in connection with other concepts that can specify the concepts. This knowledge is indispensable for reasoning in information retrieval, etc.

UNLKCIC stores information of Key Concept in Context (KCIC) about UNL documents. The UNLKCIC is made for every binary relation of UNL documents. This information is used for searching related UNL expressions of a UNL expression. Through UNLKCC, every UW of UNLKB is linked to the UNL documents each UW involved, and all UWs included in the UNL documents are also stored in the UNLKB and linked to corresponding UNL documents. UNLKCIC expands the knowledge in UNLKB from merely linguistic knowledge such as binary relations between context independent concepts to real world knowledge such as binary relations between context sensitive concepts restricted by other concepts knowledge.

UNL Verifier verifies whether a UNL expression is correct syntactically, lexically and semantically. The syntax check of a UNL expression is carried out against the UNL Specifications. In lexical check, whether all UWs of a UNL expression are defined in the UNLKB are checked. In semantic check, whether each binary relation of a UNL expression is defined as possible is certified with consulting the UNLKB.

UNL Language Servers (LSs) are located in the Internet to carry out the conversions between natural languages and UNL expressions. Each LS contains an EnConverter and a DeConverter of a language. EnConverter converts natural language sentences to UNL expressions. DeConverter convert UNL expressions to natural language sentences.

UW Gate is a tool for people to access the UNLKB and the UW dictionary through the Internet.

UNL Proxy Server works in a local computer to communicate with language servers. It functions as a filter to check whether a web page that a user requires, is written in UNL or not. If UNL expressions are included in the web page, it communicates with an appropriate language server in the Internet for deconverting the UNL expressions into desired language sentences and provides the Internet browser with the results to display.

UNL Editor is a tool which helps to produce UNL documents. It includes an EnConverter and a DeConverter. Each of them can be selected according to language. EnConverter converts natural language sentence into UNL expressions. DeConverter provides generated results as feedback for checking correctness of UNL expressions.

UNL Explorer provides the basic means to knowledge infrastructure. It manages UNLKB, UNLKCIC and UNL documents and provides knowledge or information through UWs. The UNL Explorer can be used in two ways. For human, it

allows users or developers to view or to develop the UNL Knowledge System such as the UNL Encyclopedia. For computers, it provides information or knowledge on UWs.

The UNL Explorer uses UNLKB for navigating information stored in UNL database. It has two windows: the hierarchy of UWs (UW System) of UNLKB is shown in the left window. UWs of the UNLKB are keys for information stored in UNL database. Information on UWs is shown in the right window through navigation, through the UW System. Information on UWs is described in UNL documents. All UWs used in the UNL documents are included in the left window of UNLKB and are keys for further information.

The UNL Explorer allows users to search for information using UWs or words of natural languages. It shows the information in UNL or a desired natural language by accessing UNL Language Servers. It also provides functions for developers to add information to, or modify information of the UNL database in their native languages.

Information about a UW is stored in a file. Location of the file is linked with the UW. This architecture of the UNL Knowledge System allows its development to be carried out by a wide range of developers from different languages and cultures. Such a database can provide a wealth of up-to-date information on various aspects of information and knowledge from all over the world.

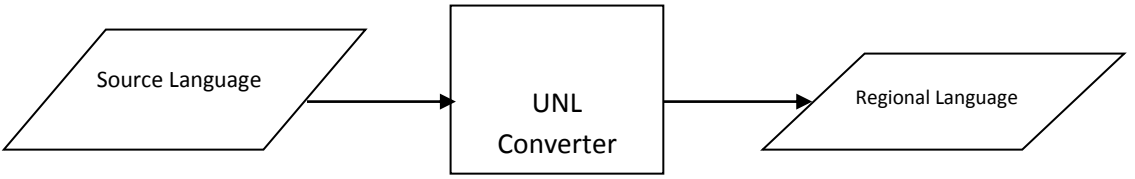
UNL Encyclopedia is a collection of UNL documents describing various knowledge or information. The merit of the UNL Encyclopedia is that it enables each people to use its native language to share knowledge or information with other peoples in their respective languages, through UNL Explorer, for example. The UNL Encyclopedia basically describes ordinary encyclopedias in UNL and integrates those descriptions (UNL documents) into the UNLKB by linking the UNL documents to corresponding UWs. It is a multilingual encyclopedia navigating through the UNLKB for human, with the knowledge description necessary for computers to process knowledge.

PROPOSED SYSTEM FOR TEACHING LEARNING PROCESS

The language converter is used to convert from one language to another language. Other than the language grammar, all the contents can be converted using UNL converter. Using the converted content, learner can think and explore his/her learning process in their mother tongue.

The following diagram represents the proposed idea of conversion. The source language content is to be an input file, from the viewed content or the selected area. That content is an input file to the UNL converter. In the Converter, using the UNL parser and the Encoder the intermediate code is generated. The intermediate code will

be converted using the decoder with the support of target language UNL Knowledgebase.



The given language expression from the input sentence extracted with independent grammatical annotations. The parser generates UNL expression , the target meaning representations by interrupting inserted tags for annotation.

CONCLUSION

This UNL converter system can be incorporated with developing learning tools; it will convert the content from one language to another. This architecture of the UNL Knowledge System allows its development to be carried out by a wide range of developers from different languages and cultures. Such a database can provide a wealth of up-to-date information on various aspects of information and knowledge from all over the world.

REFERENCE

- Gaurishankar, H. Oza. 1977. *Prachin Bharatiya Lipimala* (L. Narayan, Trans.). Bharatiya Sahitya Academy, New Delhi.
- Miller, George A. 1995. WordNet: A Lexical Database for English, Communications of the ACM Vol. 38, No. 11: 39-41.
- Uchida, H., Zhu, M. and Della Senta, T,. 2005. *The Universal Networking Language.* 2nd ed. UNDL Foundation.
- Uchida, H., Zhu, M. and Della Senta, T. 1999 *A gift for a Millenium*. Tokyo: IAS/UNU.
- *Indian Script Code for Information Interchange – ISCII* (ISCII-91 or IS13194:1991) (http://brahmi.sourceforge.net/docs/iscii91.pdf).
- Joshi, R.K. 1994. *"Traditional Knowledge and the role of computer technology in the context of 'TEXT and BEYOND"*. International Seminar on Language and Knowledge, CIIL, Mysore.
- Khan Md. A. S., Uchida, H. and Nishino, T. 2011*"How to Develop Universal Vocabularies Using Automatic Generation of the Meaning of Each Word*", 7th International Conference on Natural Language Processing and Knowledge Engineering (NLPKE'11), Tokushima, Japan. ISBN: 978-1-61284-729-0. Page 243 – 246.

- Iwanska, L. M. and Shapiro, S. C. 2006. *Natural Language Processing and Knowledge Representation,* Universities Press.
- Joshi, R.K. 1976. *"Deshanagari – a common script for all Indian languages"*. Royal College of Arts, London.
- Shanbhag, S., Rao, D. and Joshi, R.K. 2003 *"An intelligent multi-layered input scheme for phonetic scripts"*. SmartGraphics'02, Hawthorne, New York.
- Steven Pinker. 2007. *The Stuff of Thought: Language As a Window Into Human Nature*. Viking: New York, USA.
- *The Unicode Standard,* version 3.0 (http://www.unicode.org); Addison Wesley Longman, Inc. Reading, Massachusetts.
- Wallace, M. 1984.*Communicating with databases in natural language*. Ellis Horwood series in Artificial Intelligence (John Campbell) John Willey and Sons.

A REVIEW ON THE ACTIVE CONSTITUENTS AND BIOLOGICAL EFFECTS OF *MORUS INDICA* L: THE WONDER TREE

Ivee Boiss[a] and Laishram Indira Singha[b]

ABSTRACT

Morus indica L., White Mulberry is a commonly growing tree in Indian subcontinent. It contains a large amount of secondary metabolites like phenols, flavonols and flavonoids which impart anti-oxidant property on the plant. Various parts of the plant are used for different remedies. The present paper aims to review available information on Morus indica L. in the last 10 years.

Keywords: Phenolic, flavonoids, flavonols, anti-hyperglycemic, anti-oxidant

INTRODUCTION

Morus indica of the family Moraceae is commonly known as the white Mulberry, 'Shahtoot' in hindi, 'Nuni' in assamese, 'Tut' in bengali and marathi, 'Shetur' in gujrati 'Soh lyngdkhur' in khasi and 'Hipnerli' in kannada (Kelkar *et al*,1996) . It is a fast growing deciduous woody perenial plant with a deep root system. According to earlier literature, the species found in India were designated as *M. alba*, *M. indica,* Linn., *M. atropurpurea* Roxb., *M. nigra*, *M. serrata,* and *M. laevigata*. Later authors have considered the first three as merely synonyms or varieties of *M. alba*, while the other three are kept distinct. Besides these, a large number of mulberry types have been introduced into India from China and Japan mainly for rearing silk-worms. The introductions are reported to belong to two species, *M. multicaulis* Perr. and *M. latifolia* Poir., but some authorities regard them as a variety of *M. alba* (*M. alba* var. multicaulis Loud. (Rehder, 1949, 147).

The leaves are simple, alternate, stipulate, petiolate , entire or lobed (fig 1). The number of lobes vary from 1-5. Mulberry tree is generally dioecious. Inflorescence is catkin with pendant or drooping penduncle bearing unisexual flowers. The inflorescence is always auxillary (fig 2). Male catkins are usually longer than female catkins. Male flowers are loosely arranged and the inflorescence dries and fall offs after shedding the pollen. The flower contains 4 stamens. On the other hand the female inflorescence is short and the flowers are compactly arranged. The number of perianth lobes is 4 and persistent. The ovary is one celled and bifid. The fruit is a sorosis and the colour is violet black. The sweet fruits can be used for making jam, jelly and refreshing juice. The fruits may be a potential source for the formulations of nutraceuticals.

[a] *Department of Biotechnology, St. Anthony's College, Shillong, Meghalaya*
[b] *Department of Biotechnology, St. Anthony's College, Shillong, Meghalaya*

Fig 1. Mulberry leaves

Fig 2. Catkin inflorescence

(Source: http://tree-species.blogspot.in/2011/04/white-mulberry-spring-flowers.html)

Table 1. Taxonomic Classification

Kingdom	Plantae
Order	Rosales
Family	Moraceae
Genus	*Morus*
Species	*Indica*
Binomial name	*Morus indica*

Fig 3. Silkworm feeding on Mulberry leaves

(Source: http://faculty.ucc.edu/biology-ombrello/pow/mulberry_tree.htm)

Mulberry is appropiately known as 'Kalpa Vriksha' as all the parts of the plant has multiple uses. It is essential to sericulture as the foliage constituent is the sole feed of mulberry silkworm '*Bombyx mori*'(fig 3). It produces a very large amount of

renewable biomass in the form of branches, shoots, leaves and fruits. Farmers also use the twigs as their fuel source after pruning of the garden. Mulberry can also be exploited by raising it as energy plantation in cultivable / wasteland/ low lying areas / canal bunds etc under various afforestation, watershed development and soil conservation programmes.

The various parts of the mulberry plant have several uses in ayurvedic preparation. The leaves have diaphoretic and emollient effects. It is used for making a decoction which can be used as a gargle to get relief from throat inflammation. The fruits are used to treat sore throat, depression, high fever. It is both a coolant and laxative. The root extract possess hypoglycemic effect. The bark of the root is used as an antihelminthic, purgative and vermifuge. The milky latex is used as a plaster for sores and for preparation of dermal creams (Sarker *et al*,2000).

Fig 4. Harvested Mulberry fruits

Fig 5. Mulberry fruits on the tree (Source: http://theshakespeareblog.com/2013/08/shakespeares-mulberries/)

Table 2. Chemical composition of leaf (Sarkar et al 2000).

Leaf composition	Range (%)
Moisture	65-78
Protein	19-25
Minerals	10-15
Reducing sugars	1.2-19
Sugars	10-15

Nutritional composition: - The black, ripe fruits are known for their ravishing taste (fig 4 & 5) and have been reported to contain high moisture content, crude fat, crude fibre, crude protein and carbohydrate. The nutritional composition of the fruits were estimated with shade dried, pulverized fruits. The ash content which is an index of mineral content was estimated by heating in a muffle furnace till the weighed became constant.

The reported ash content was 8.73± 0.02% and these can be favourably compared with the values reported for some commercial fruits as shown in the Table 3 (Gopalan et al., 2004). The fruits of Morus contains minerals like sodium, potassium, calcium, manganese, iron, zinc, and cooper with appreciable quantities of calcium, copper and manganese and a good amount of iron (Table 4) (Seal,2011a and Gopalan et al,2004). Thus the consumption of Indian mulberry in sufficient amount could provide adequate protection against malnutrition which is a common problem in India (Seal, 2011b).

Table 3. Comparison of nutritional parameters of different fruits(Gopalan et al., 2004 and Seal,2011b).

Nutritional parameter	Mulberry	Apple	Mango
Ash (%)	8.73±0.02	1.2	1.1
Moisture (%)	90.36±0.14	84.60	81.00
Crude Fat (%)	5.07±0.05	0.30	0.40
Protein (%)	1.04±0.05	0.20	0.60
Crude Fibre (%)	1.10±0.03	3.40	0.70
Available Carbohydrate (%)	84.4±0.12	13.70	16.90
Nutritive value (kcal/100g)	386.00±0.30	58.00	74.00

Table 4. Comparison of Mineral contents of different fruits. (Gopalan et al., 2004 and Seal,2011b).

Mineral content	Mulberry	Apple	Mango
Na	1.01±0,01	28	26
K	10.86±0.01	75	205
Ca	9.75±0.11	10	14
Mn	0.102±0.0002	1.88	0.13
Fe	0.309±0.0015	0.66	1.30
Cr	Not traceable	0.008	0,006
Zn	0.326±0.0016	0.06	0.27
Cu	0.007±0.0001	0.10	0.11

The aqueous methanol and acetone extract of the ripe fruits was analyzed for total phenolic content, flavanoids and flavanols by measuring the absorbance at 765 nm, 420 nm, and 440 nm respectively. Both the fruit extracts contain good amounts of total phenol (24.94 ±0.58 and 9.21±0.23.) respectively. Total flavonoid content of the extracts was also appreciable. The aq. methanol extracts contain a very good amount of flavonoid (7.04±0.06 mg/g). The same also contains appreciable quantity of flavonol.

These compounds can absorb and neutralize free radicals that are generated during aerobic respiration (Florence *et al*, 2011). Both flavonols and flavonoids show antioxidant activity through scavenging and chelating process (Pourmorad *et al*, 2006). Besides they also inhibit enzymes like prostaglandin synthase, lipoxygenase and cycloxygenase whose activity is closely related to tumorigenesis and may induce detoxifying enzymes like glutathione S- transferase (Karadeniz *et al*, 2005). Consequently the extracts also showed reducing activity by reducing Fe^{+3}/ferricyanide complex to the ferrous form. The same study also proved that the fruits of this tree possess strong antioxidant activity (Seal, 2011). Therefore the fruit could be exploited as antioxidant additives or as nutritional supplements.

Pharmacology

Medicinal plants possess pharmacological actions on animals due to the presence of secondary metabolites.The pharmacological aspect of this tree with immense applications has been well studied for its efficacy and wide utility on different animal models (Swiss albino mice and Wistar rats). These pharmacological activities prove the traditional utilization of this tree scientifically (Sarwar *et al*, 2011).

Antihypoglycemic and antioxidant activities

Diabetes is a worldwide major health problem with approximately 5% of the world's population suffering from the disease. The effective control of blood glucose is the key in preventing or reversing diabetic complications and improving the quality of life for both type I and type II diabetic patients. Although different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, none offers complete glycemic control (Jiang *et al*, 2003).

Kumar *et al*, 2010, reported the antidiabetic and antioxidant activity of the leaves of *Morus indica* L. along with *Asystasia gangetica* both individually and synergistically in alloxan induced male wistar rats. The study clearly showed reduced levels of blood glucose with increased glycolysis and glycogenesis and reduced glucogenesis. Moreover the effect of combination of both the plants leaf ethanolic extract on carbohydrate metabolism was found to be similar to that of glibenclamide. The same study also reported the increased levels of the antioxidant enzymes like Superoxide dismutase, Catalase and Glutathione in the pancreas of the sacrificed rats. Further they also reported a decrease in the lipid peroxidation levels.

Delouee and Urooj, 2007, also evaluated the *in vitro* antioxidant potential of mulberry leaves in different extracts –methanol, acetone and water. All the extracts showed antioxidant activity which was again proportional to the dose. The methanolic extract contained the largest amount of phenolics and consequently was the most potent antioxidant amongst the three extracts.

Devi and Urooj,2007, also studied the potent hypoglycemic effect of the leaves of *M. indica* L. and *Costus igneus* on streptozotocin induced diabetic male wistar rats. Oven dried leaf powders of both the plants showed a prominent decrease in the blood

glucose level. However the decrease was more significant in case of Morus leaves stating its effectiveness in reducing blood sugar.

Anticancer activity

Cancer is a major cause of death at present times. The number of new cases as well as the number of individuals living with cancer is expanding continuously. Despite the successful utilization of a few phytochemicals like taxol and vincristine into mainstream cancer chemotherapy, commercial plant-derived anticancer formulations represent only one-fourth of the total repertoire of the available treatment options (Spiridon, 2006).

The methanolic leaf extract was found to suppress skin tumourigenesis in Swiss albino mice that was induced by 7,12-dimethylbenz(a)anthracene and croton oil. Application of the leaf extract prior to the application of croton oil showed inhibitory effects on tumour promotion in terms of a reduction in the number of tumours/mouse and percentage of mice with tumours. This shows that the *Morus* extract holds promise has a therapeutic agent for cancer control.

CONCLUSION AND FUTURE PROSPECT

Hence *Morus indica* L.is an important medicinal tree. Bioactive compounds of the tree have several pharmacological activities like antihyperglycemic, antioxidant and anticancer. There are strong prospects for the commercial utilization of this tree. However most of the studies are carried out on the leaves and flowers. Therefore the pharmacological potential of the other parts constitute a potential scope for research in future. The need of the hour is to work on this bioactive compounds and its proper utilization as plants have a very significant role in health care system of humans.

REFERENCES

- Delouee, S. A and Urooj, A. 2007. Antioxidant properties of various solvent extract of mulberry (*M. indica* L.) leaves. *Food Chemistry.* 102 (4):1233-1240.
- Devi, V, D.and Urooj, A. 2008. Hypoglycemic effect of *Morus indica* L. and *Costus igneus* Nak.-A preliminary study. *Indian journal of Experimental Biology*. 46:614-616.
- Florence, O. J., Adeolu, A. A., Anthony, J. A. 2011. Comparison of the nutritive value, antioxidant and antibacterial activities of *Sonchus asper* and *Sonchus oleraceus* . *Rec. Nat. Prod*. 5 (1):29-42.
- Gopalan, C., Shastri, B., Balasubramanium, S.C. 2004. Nutritive value of Indian Fruits. National Institute of Nutrition, Indian Council of Medical Research, Hyderabad. India. 2-58.

- Jiang, Z., Zhen-Dan, H., Ren-Wang, J., Wen-Cai, Y., Hong-Xi X., Paul, P.B. 2003. Antiviral flavanoids from the roots bark of *Morus alba* L. *Phytochemistry.* 62:1235-1238.
- Karadeniz, F., Burdurlu, H. S., Koca, N., Soyer, Y. 2005. Antioxidant activity of selected fruits and vegetables grown in Turkey. *Turk J. Agric For.* 29: 297-303.
- Kelkar, S. M., Bapat, V. A., Ganapathi, T. R., Kaklij, G.S., Rao, P. S., Heble, M. R. 1996. Determination of hypoglycemic activity in *Morus indica* L (Mulberry) shoot cultures. *Current Sc.* 71:71-72.
- Kumar, P. R., Sujatha, D., Saleem, M. T., Chetty, C. M. Ranganayakulu, D. 2010. Potential antidiabetic and antioxidant activities of *M. indica* and *Asystasia gangetica* in alloxan induced diabetes mellitus. *J. of Experimental Pharmacology*. 2:29-36.
- Pourmorad, F., Hosseinimehr, S.J., Shahabimajd, N. 2006. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *Afr. J. Biotechnol*. 5 (11):1142-1145.
- Prasad, L. Khan, T. H., Sehrawat, A., Sultana, S. 2004. Modulatory effect of *Morus indica* against two-stage skin carcinogenesis in Swiss albino mice: possible mechanism by inhibiting aryl hydrocarbon hydroxylase. *J. Pharm Pharmacol.* 56 (10):1291-1298.
- Rehder, A. 1949. *Bibliography of cultivated trees and shrubs hardy in the cooler temperate regions of the Northern Hemisphere.* Jamaica Plain, Mass., Arnold Arboretum of Harvard Univ.
- Sarkar, A., Jalaja S.Kumar and Datta, R.K.. 2000. Gradual improvement of mulberry varieties under irrigated condition in South India and the optimal programme for varietal selection in the tropics. *Sericologia,* 40(3):449-461.
- Sarwar, M., Attitalla, H. I., Abdollahi, M. A. 2011. A review on the recent advances in Pharmacological studies on Medicinal plants; Animal studies are done but Clinical studies needs completing . *Asian J. Animal Vet Adv*. 6(8):867-888
- Seal, T. 2011a. Evaluation of antioxidant activity of some wild edible fruits of Meghalaya state in India. *International J. of Pharmacy and Pharmaceutical Sc.* 3(4):233-236.
- Seal, T. 2011b. Nutritional conposition of wild edible friuts in Meghalaya state of India and their Ethno-Botanical Importance. *Research journal of Botany*. 6(2):58- 67.
- Spiridon, E. K. (2006). Terrestrial plant-derived anticancer agents and plant species used in anticancer research. *Critical Reviews in plant sciences.* 25:79-113.

THE GENETICS AND INHERITENCE OF BETA-THALASSEMIA

Tapu Ghosh[a] and Laishram Indira Singha[b]

ABSTRACT

Thalassemia is a group of genetic blood disorders characterized by anemia due to enhanced red blood cell destruction. In thalassemia one of the two proteins that are constituents of hemoglobin in red blood cells is deficient. Alpha and beta-thalassemia are the two different types of thalassemia. Beta thalassemia is one of the most prevalent autosomal disorders in the world especially in developing countries. Beta-thalassemias are a group of hereditary blood disorders characterized by anomalies in the synthesis of the beta chains of hemoglobin which can results in severe anemia and also clinically asymptomatic individuals. Three different types of beta thalassemia were reported-major, intermediate and minor. Different techniques are available for diagnosis of beta thalassemia. Treatment is mostly done by transplantation of bone marrow. Symptomatic treatment involves regular blood transfusion and the use of iron chelating drugs to remove the excess iron that may result from transfused blood.

Keywords: Thalassemia, Beta thalassemia, Effects, Diagnosis, Treatment

INTRODUCTION

The term thalassemia is derived from the Greek, *thalassa* (sea) and *haima* (blood) (Galanello and Origa 2010). The thalassemia are a group of inherited disorders of hemoglobin, first independently reported by the United States and Italy in 1925. There was a mistaken belief that these disorders were confined to the Mediterranean region. It was only later that it was discovered that they are the most common genetic disorder and have a widespread distribution in many countries of the world (Figure 1). The thalassemia were among the first diseases to be characterized at the molecular level, which provided some indications of the repertoire of mutations that underlie human genetic disease. This led to a better understanding of their clinical features and so much can be done to treat the disease. The thalassemia result from inherited defects in the synthesis of the globin chains of hemoglobin. Humans have different hemoglobins at various stages of development. Normal adults have a major hemoglobin (Hb) called HbA, comprising about 90% of the total, and a minor component, HbA_2, which accounts for 2–3%. The main hemoglobin in fetal life is HbF, traces of which are found in normal adults. There are three embryonic hemoglobins. All these different hemoglobins are tetramers of two pairs of unlike globin chains. Adult and fetal hemoglobins have α chains associated with β (HbA, $\alpha_2\beta_2$), δ (HbA_2, $\alpha_2\delta_2$), or γ chains

[a] *Department of Biotechnology, St. Anthony's College, Shillong, India*
[b] *Department of Biotechnology, St. Anthony's College, Shillong, India*

(HbF, $\alpha_2\gamma_2$), whereas in the embryo there are different α-like chains called ζ chains and distinct β-like chains called ε chains. Each individual globin chain has a heme moiety attached to it, to which oxygen is bound (Weatherall et al 2001). Most of the thalassemia result from mutations in either α or β globin genes.

ALPHA-THALASSAEMIA

The genetics of a thalassaemia is complicated as normal humans receive two α genes from each parent, a genotype can be written as αα/αα. There are two main classes of α thalassaemia. First, the α^0 thalassemia, in which both α genes are deleted; that is, all parts of the gene is missing. The homozygous state is written --/ --, and the heterozygous state --/αα. On the other hand, in the second class that is in the α^+ thalassemia only one of the α genes is lost; the homozygous and heterozygous states are designated as - α/αα and - α/- α, respectively. Sometimes α^+ thalassaemia may results from a mutation that inactivates the α globin gene and not from deletion of genes. In this case the heterozygous state is written α^Tα/αα (Table 1).

BETA-THALASSAEMIA

More than 180 different mutations of the β globin genes have been found in patients with β thalassaemia. They may affect gene function at any level between transcription, processing of the primary messenger ribonucleic acid transcript, translation, or post-translational stability of the gene product. β thalassaemia, like α thalassaemia, may result from a partial or complete deletion of the β globin gene but it is rare. Some of these mutations cause an absence of β-chain production and the resulting disease is called β^0 thalassaemia, whereas others result in a reduced output of β chains, β^+ thalassaemia. Some of the latter forms are extremely mild and it is difficult to identify in carriers; most heterozygotes for β thalassaemia have mild anemia and a raised level of HbA_2 (Weatherall et al 2001).

EPIDEMIOLOGY of BETA-THALASSEMIA

Beta-thalassemia is prevalent in Mediterranean countries, the Middle East, Central Asia, India, Southern China, and the Far East as well as countries along the north coast of Africa and in South America. The highest carrier frequency is reported in Cyprus (14%), Sardinia (10.3%), and Southeast Asia (Flint et al.1998). The high gene frequency of beta-thalassemia in these regions has been found to be related to the selective pressure from *Plasmodium falciparum* malaria (Flint et al.1998). Migration of population and intermarriage between different ethnic groups has introduced thalassemia in almost every country of the world, including in countries like Northern Europe where thalassemia was not reported earlier. It has been estimated that about 1.5% of the global population (around 90 million people) are carriers of beta thalassemia, with about 60,000 symptomatic individuals born annually, the great majority in the developing world. The total annual incidence of symptomatic individuals is estimated at 1 in 100,000 throughout the world and 1 in 10,000 people in

the European Union. However, accurate data on carrier rates in many populations are not available. According to Thalassemia International Federation, only about 200,000 patients suffering from thalassemia major are alive and registered to receive treatment around the world. The most common combination of beta-thalassemia with abnormal Hb or structural Hb variant with thalassemic properties is HbE/betathalassemia which is most prevalent in Southeast Asia where the carrier frequency has been reported to be around 50% (Galanello and Origa 2010) (Table 2).

CLINICAL DESCRIPTION of BETA-THALASSEMIA

Beta-thalassemia can be classified into three types which are, thalassemia major, thalassemia intermedia and thalassemia minor. Thalassemia intermedia patients do not require regular transfusion. Except in the rare dominant forms, heterozygous beta-thalassemia results in the clinically silent carrier state. HbE/beta-thalassemia and HbC/beta-thalassemia exhibit a great range in terms of diversity of phenotypes and spectrum of severity**.**

Beta-Thalassemia Major

Thalassemia major occurs between 6 and 24 months of age. Individuals with thalassemia major usually come to medical attention within the first two years of life and patients are required to take regular RBC transfusions to survive. Affected infants fail to thrive and become progressively pale. Feeding problems, diarrhea, and irritability, recurrent bouts of fever, and enlargement of the abdomen caused by spleen and liver enlargement may occur. In some developing countries like in south-east Asia, where due to the lack of resources patients are untreated or poorly transfused, thalassemia major is mostly characterized by growth retardation, pallor, jaundice, poor musculature, genu valgum, hepatosplenomegaly, leg ulcers, development of masses from extramedullary hematopoiesis, and skeletal changes resulting from expansion of the bone marrow. Some skeletal changes are also seen which includes deformities in the long bones of the legs and changes like bossing of the skull, prominent malar eminence; depression of the bridge of the nose, upper teeth is exposed. If a regular transfusion program that maintains a minimum Hb concentration of 9.5 to 10.5 g/dL is initiated, growth and development tends to be normal up to 10 to 12 years. Other complications are hypersplenism, chronic hepatitis (resulting from infection with viruses that cause hepatitis B and/or C), HIV infection, venous thrombosis, and osteoporosis. The risk for hepatocellular carcinoma is increased in patients with liver viral infection and iron overload (Pignatti et al. 2004). Compliance with iron chelation therapy mainly influences frequency and severity of the iron overload-related complications. Individuals who have not been regularly transfused usually die before 20-30 years of age. Survival of individuals who have been regularly transfused and treated with appropriate chelation may extend beyond the age of 40 years. Cardiac disease caused by myocardial siderosis is the most important life-limiting complication of iron overload in beta-thalassemia. It has been seen that cardiac complications are the cause of the deaths in 71% of the patients with beta-thalassemia major (Pignatti et al. 2005).

Beta-thalassemia intermedia

Thalassemia intermedia occurs at an age later than that of thalassemia major, patients suffering from this have milder anemia and do not require or only occasionally require transfusion. The patients who are between the ages of 2 and 6 years are capable of surviving without regular blood transfusion, growth and development are retarded. In some cases it has been seen some of the patients are completely asymptomatic until adult life with only mild anemia. Hypertrophy of erythroid marrow with the possibility of extramedullary erythropoiesis, a compensatory mechanism of bone marrow to overcome chronic anemia, is common. Its consequences are characteristic deformities of the bone and face, osteoporosis with pathologic fractures of long bones and formation of erythropoietic masses that primarily affect the spleen (enlargement of the spleen), liver, lymph nodes, chest and spine. Extramedullary erythropoiesis may cause neurological problems such as spinal cord compression with paraplegia and intrathoracic masses. As a result of ineffective erythropoiesis and peripheral hemolysis, thalassemia intermedia patients may develop gallstones, which occur more commonly than in thalassemia major (Glanello et al. 2001). Patients with this kind of thalassemia frequently develop leg ulcers and have an increased predisposition to thrombosis as compared to thalassemia major. Although individuals with thalassemia intermedia are at risk of iron overload secondary to increased intestinal iron absorption, hypogonadism, hypothyroidism and diabetes are not common (Sanctis et al. 1998). Patients who are pregnant may have successful spontaneous pregnancies. However, if blood transfusions are necessary during pregnancy, those never or minimally transfused are at risk of developing hemolytic alloantibodies and erythrocyte autoantibodies. Cardiac involvement in thalassemia intermedia mainly results from a high-output state and pulmonary hypertension, while systolic left ventricle function is usually preserved (Aessopos et al. 2005; Galanello and Origa 2010). Some of the mutations which are common in beta-thalassemia interrmedia are mutation at codon 19 of β-globin gene (AAC to AGC, Asn to Ser) etc.,(Panja et al., 2012).

Beta-thalassemia Minor

Thalassemia minor is most common form of beta-thalassemia, and is also known as the 'thalassemia trait', in which affected individuals are asymptomatic (Cao et al.2005; Rund et al. 2005). In this case patients are typically heterozygous for beta-thalassemia since they carry one normal *HBB* allele and one thalassemia allele, either B^0 or B^+ (Thein et al., 2005). Asymptomatic patients are usually detected through routine hematologic testing, but in some newly diagnosed patients are observed to have mild anemia and small RBCs (Bunn et al., 1984). The primary caution for individuals with thalassemia minor is a potential risk of having children suffering from more serious thalassemia if their partner is also a carrier of thalassemia minor (Thein et al., 2005). Beta-thalassemia associated with other Hb anomalies. The interaction of HbE and beta-thalassemia results in thalassemia phenotypes ranging from a condition indistinguishable from thalassemia major to a mild form of thalassemia intermedia (Galanello and Origa, 2010) (Figure 2).

Depending on the severity of symptoms three categories may be identified (Table 3):

HEREDITARY TRANSMISSION OF BETA-THALASSEMIA

The beta-thalassemias are inherited in an autosomal recessive manner. The parents of an affected child are obligate heterozygotes and carry a single copy of a disease causing beta globin gene mutation. At conception, there is a 25% chance of having a thalassemic child if the parents are heterozygotes parents, 50% chance of being an asymptomatic carrier, and 25% chance of being unaffected and not carrier (Galanello and Origa, 2010). .

DIAGNOSIS

Diagnosis can be done by the following methods:

CLINICAL DIAGNOSIS

As mentioned above thalassemia major is usually suspected in infants younger than two years of age with severe microcytic anemia, mild jaundice and hepatosplenomegaly. Thalassemia intermedia can be seen at a later age with similar but milder clinical findings. Carriers are usually asymptomatic, but sometimes may have mild anemia.

HEMATOLOGIC DIAGNOSIS

It has been seen that RBC indices show microcytic anemia. Thalassemia major is characterized by reduced Hb level (<7 g/dl), mean corpuscolar volume (MCV) > 50 < 70 fl and mean corpuscolar Hb (MCH) > 12 < 20 pg. Thalassemia intermedia is characterized by Hb level between 7 and 10 g/dl, MCV between 50 and 80 fl and MCH between 16 and 24 pg. Thalassemia minor is characterized by reduced MCV and MCH, with increased Hb A2 level (Galenello et al., 1979).

PERIPHERAL BLOOD SMEAR

Affected individuals show RBC morphologic changes (microcytosis, hypochromia, anisocytosis, poikilocytosis (spiculated tear-drop and elongated cells)), and nucleated RBC (erythroblasts). The number of erythroblasts is related to the degree of anemia and is markedly increases after splenectomy. Also carriers have less severe RBC morphologic changes than affected individuals. Normally erythroblasts are not seen.

QUALITATIVE AND QUANTITATIVE HB ANALYSIS BY CELLULOSE ACETATE ELECTROPHORESIS AND HPLC)

This technique identifies the amount and type of Hb present. The Hb pattern in beta-thalassemia varies according to beta-thalassemia type. In $beta^0$ thalassemia, homozygotes HbA is absent and HbF constitutes the 92-95% of the total Hb. In $beta^+$ thalassemia homozygotes and $beta^+$/ $beta^0$ genetic compounds HbA levels are between 10 and 30% and HbF between 70-90%. HbA_2 is variable in beta thalassemia homozygotes and it has been seen that it is enhanced in beta thalassemia minor. Hb electrophoresis and HPLC also detect other hemoglobinopathies that may interact with beta-thalassemia.

MOLECULAR GENETIC ANALYSIS

The prevalence of a limited number of mutations in each population has greatly facilitated molecular genetic testing. Commonly occurring mutations of the beta globin gene are detected by PCR-based procedures (Vrettou et al., 2003). The most commonly used methods are reverse dot blot analysis or primer-specific amplification, with a set of probes or primers complementary to the most common mutations in the population from which the affected individual originated.

Beta globin gene sequence analysis can be used to detect mutations in the beta globin gene if targeted mutation analysis fails to detect the mutation (Galanello and Origa, 2010).

CONTROL AND TREATMENT

All the thalassemia can be identified in the carrier state, and most forms can be diagnosed in the fetus, and thus it is possible to offer counselling and prenatal diagnosis for parents who wish to terminate pregnancies carrying babies with severe forms of the disease. This approach has resulted in a major reduction in the birth of new cases in some of the Mediterranean islands and in some other countries. Bone marrow transplantation is the only definitive form of treatment for thalassaemia, but is possible only when there is a matching donor relative. Symptomatic treatment involves regular blood transfusion and the use of iron chelating drugs to remove the excess iron that may result from transfused blood. Children with β thalassaemia who are adequately transfused and chelated grow and develop normally, and in some cases are now able to have children of their own. They need expert care as they are prone to a variety of complications, such as blood borne infections, notably hepatitis C and human immunodeficiency virus, endocrine damage leading to growth retardation and bone disease, and the side effects of chelating agents. Future therapeutic efforts are being aimed at trying to stimulate the production of fetal hemoglobin production, or developing somatic gene therapy, directed at replacing defective α or β globin genes (Weatherall et al., 2001).

CONCLUSION

Beta thalassemia is highly prevalent in developing countries like India where diseases like malaria is still a matter of concern. So this suggests that relatively cost-effective dedicated carrier screening methods could be implemented in these areas. Prenatal diagnosis or other preventative approaches may be the most important strategy to control the clinical problems arising from beta-thalassemia. But as with the invention of various molecular techniques some of which are PCR-based study to identify, diagnose and finally to treat beta thalassemia may be useful.

REFERENCES

- Aessopos A, Farmakis D, Deftereos S, Tsironi M, Tassiopoulos S, Moyssakis I, Karagiorga M. 2005. "Thalassemia heart disease: a comparative evaluation of thalassemia major and thalassemia intermedia"**.** *Chest*,127:1523-1530.

- Bunn HF FB. 1984. “Hemoglobin”: Molecular, Genetic and Clinical Aspects.W.B. Saunders Company.
- Borgna-Pignatti C, Vergine G, Lombardo T, Cappellini MD, Cianciulli P, Maggio A, Renda D, Lai ME, Mandas A, Forni G, Piga A, Bisconte MG. 2004.”Hepatocellular carcinoma in the thalassemia syndromes”**.** *Br J Hemeatol*, 124:114-117.
- Borgna-Pignatti C, Cappellini MD, De Stefano P, Del Vecchio GC, Forni GL, Gamberini MR, Ghilardi R, Origa R, Piga A, Romeo MA, Zhao H, Cnaan A. 2005. “Survival and complications in thalassemia”**.** *Ann N Y Acad Sci*, 1054:40-47.
- Cao A GR. Beta-Thalassemia. Gene Reviews 2005.cited 16/11/2007; Available from: www.genetest.org.
- De Sanctis V, Tangerini A, Testa MR, Lauriola AL, Gamberini MR, Cavallini AR, Rigolin F. 1998. “Final height and endocrine function in Thalassemia Intermedia”**.** *J Pediatr Endocrinol Metab*, 11:965-971.
- Flint J, Harding RM, Boyce AJ, Clegg JB. 1998. “The population genetics of the hemoglobinopathies**.** *Bailliere's Clinical Hematology*, 11:1-50.
- Galanello R, Piras S, Barella S, Leoni GB, Cipollina MD, Perseu L, Cao A. 2001.”Cholelithiasis and Gilbert's syndrome in homozygous betathalassemia”**.***Br J Hemeatol*, 115:926-928.
- Galanello R, Melis MA, Ruggeri R, Addis M, Scalas MT, Maccioni L, Furbetta M, Angius A, Tuveri T, Cao A. 1979. “Beta^0 thalassemia trait in Sardinia”**.** *Hemoglobin*, 3:33-46.
- Galanello R, Origa R.2010. “Beta thalassemia”. *Galanello and Origa Orphanet Journal of Rare Diseases*, 5:11.
- Lahiry P,. Al-Attar SA and Hegele RA. 2008. “Understanding Beta-Thalassemia with Focus on the Indian Subcontinent and the Middle East”. *The Open Hematology Journal*, 2: 5-13.
- Panja A, ghosh TK, Basu A. 2012. ‘Genetics of thalassemia in Indian population”.*Journal of community Nutrition and Health*, Vol. 1, Issue 1.
- Rund D, Rachmilewitz E. 2005. Beta-thalassemia. *New Eng J Med*, 353(11): 1135-46.
- Thein SL.2004. “Genetic insights into the clinical diversity of beta thalassaemia”. *Br J Hemeatol*, 124(3): 264-74.
- Weatherall D, John Radcliffe Hospital. 2001. ‘Thalassemia”. *Encyclopedia of life sciences. Nature Publishing group.*

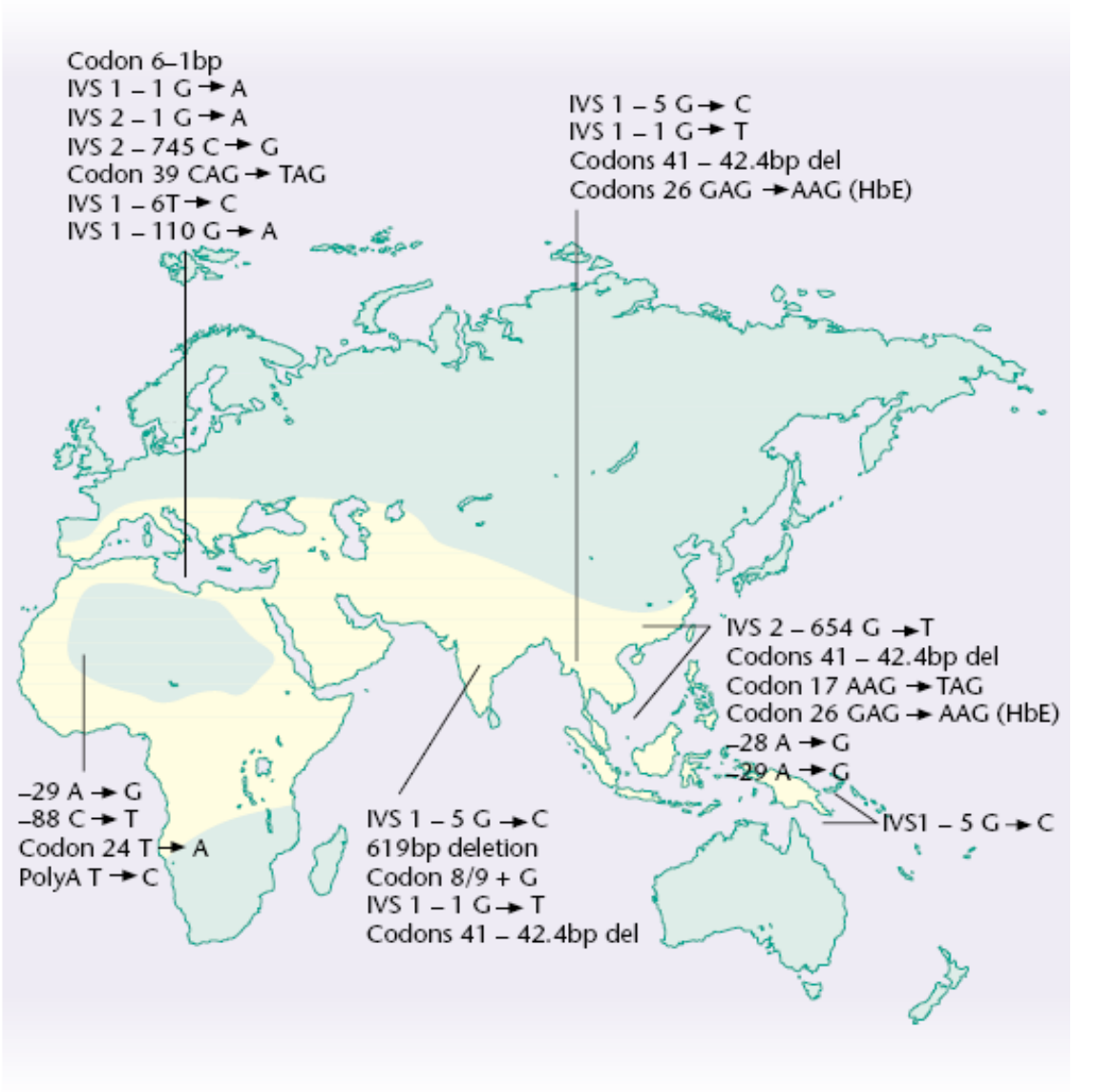


Figure 1: World distribution of the beta thalassemia. Each population has a different set of mutations. These are described either by the nucleotide base position in introns (IVS 1 or 2) or in the particular codons in exons. Mutations that are given the prefix are those in the 5' noncoding regions of the β globin genes. Those marked polyA are mutations in the 3' noncoding regions. bp, Base pair(Weatherall et al. 2001).

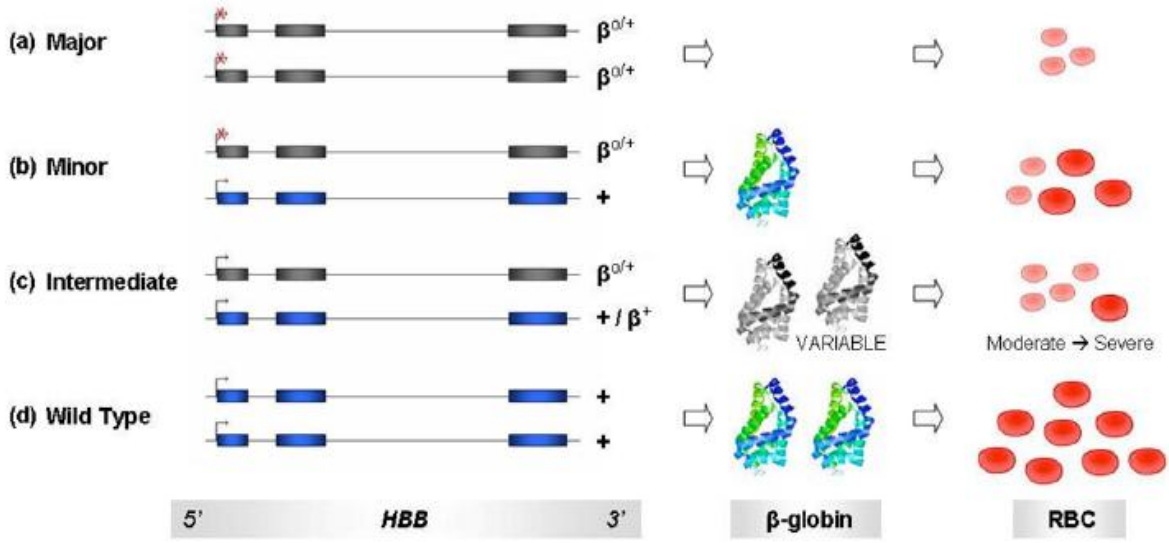


Figure 2:. Schematic representation of inherited beta-globin variants and related beta-chain and red blood cell (RBC) phenotype.

The *HBB* variants are represented in grey exons while the wild type alleles are represented in blue exons. Production of beta-globin from a single/ double wild type alleles are represented by one/two colored schematic of the beta-globin protein respectively. Grey colored beta-globin diagrams refer to below-normal synthesis levels of the protein, created by mutant *HBB* variants. Bright red-colored RBCs represent normal cell phenotype, while pink colored ones represent microcytic, hypochromic cells characteristic of beta-thalassemia phenotype. Relative number of RBC reflects relative levels of anemia amongst the three classes of beta-thalassemia and in comparison to the wild type RBC pool (Lahiry et al 2008).

Table1: Different types of α-thalassemia based on the deletion of entire α gene (Panja et al. 2012).

Gentypes	Description	Symptoms
αα/ αα	Normal-all four α alleles are present	Normal hematological profile
-α/αα	Only one α allele is absent,	Clinically asymptomatic, they can be diagnosed during antenatal screening
-α/-α or --/αα	Two α alleles are deleted	Mild hypochromic, microcytic anemia (iron deficiency)
--/-α	Three α alleles are deleted	Moderate anemia, folic acid deficiency, ulcers, jaundice
--/--	Total absence of α chain, Hydrops fetalis	Intrauterine death, skeletal deformities, cardiovascular problems, improper brain growth, enlarged placenta, hypatosplenomegaly

Table 2: Common types of beta-thalassemia: severity and ethnic distribution (Galanello and Origa 2010).

Population	**β-gene mutation**	**Severity**
Indian	-619 del	β^0
Mediterranean	-101 CTT	β++
Mediterranean; African	-87 CTG	β++
Japanese	-31 ATG	β++
African	-29 ATG	β++
Southeast Asian	-28 ATC	β++
Mediterranean; Asian Indian	IVS1-nt1 GTA	β0
East Asian; Asian Indian	IVS1-nt5 GTC	β0
Mediterranean	IVS1-nt110 GTA	β+
Chinese	IVS2-nt654 CTT	β+
Mediterranean	codon 39 CTT	β0
Mediterranean	codon 5 –CT	β0
African-American	AATAAA to AACAAA	β++
Mediterranean	AATAAA to AATGAA	β++
Southeast Asian	codon 79 G>A (Hb E)	β++

(IVS= intervening sequence i.e. the specific nucleotide sequences present in gene that intervene between exons.)
β^0:complete absence of beta globin on the affected allele
β^+:residual production of beta globin (around 10%)
β^{++}:very mild reduction in beta globin production

Table 3:

Categories	**Hb level**	**Treatment**
Mild HbE/beta-thalassemia	Patients maintains Hb levels between 9 and 12 g/dl and usually does not develop clinically significant problems.	No treatment is required.
Moderately severe HbE/beta-thalassemia	The Hb levels remain at 6-7 g/dl	Transfusions are not required unless infections precipitate further anemia
Severe HbE/beta-thalassemi	The Hb level can be as low as 4-5 g/dl	Patients in this group manifest symptoms similar to thalassemia major and are treated as thalassemia major patients

(Galanello and Origa 2010).

BIOLOGICAL AND PHARMACEUTICAL APPLICATIONS OF PYRAZOLOPYRIMIDINES

Melboureen E Sunn[a] and Laishram Indira Singha[b]

ABSTRACT

This review aims at outlining the definition and biological effects of pyrazoles and pyrimidines. When heterocyclic rings of pyrazoles and pyrimidines are fused, pyrazolopyrimidines are formed. Pyrazoles and their derivatives are used as antitumor, antibacterial and antifungal, antiviral, antiparasitic, antitubercular and insecticidal, anti-inflammatory, antidiabetic, anesthetic, analgesic and antileukemic agents. Pyrimidine derivatives have been developed as antineoplastics, anticancer, antifolates, antibacterial, antiprotozoal, antiviral, anti-AIDS, antibiotic, anthelmentics, sedative, hypnotics, antiepileptic, antitubercular, diuretics, uricosurics, anaesthetics, antihistaminic, analgesics, NSAIDS drugs and cardiac agents. Hence, pyrazolopyrimidines has therefore been developed as antineoplastics, anticancer, antifolates, antibacterial, antiviral, anti-AIDS, antibiotic, hypnotics, antitubercular, anaesthetics, analgesics, NSAIDS drugs, cardiac agents and many more.

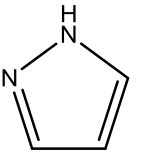
Keywords: Pyrazole, Pyrimidines, Pyrazolopyrimidines

INTRODUCTION:

The simple doubly unsaturated compound containing two nitrogen and three carbon atoms in the ring is known as **pyrazoles** (Singh *et al.*, 2011). Pyrazoles (figure1) are also known as 1, 2-diazoles due to the presence of two neighbourhood nitrogen atoms. Pyrazoles and their derivatives had been reported as important biological agents. In particular, they are used as antitumor, antibacterial and antifungal, antiviral, antiparasitic, anti-tubercular and insecticidal agents, anti-inflammatory, anti-diabetic, anesthetic and analgesic properties (Sharshira *et al*., 2012). In addition, some fused pyrazole derivatives were reported to induce various antileukemic, antitumor and antiproliferative activities (Faidallah *et al*., 2010).

Some of the pyrazole possessing drugs like *allopurinol* (figure 3), *butazoline, phenylbutazone, oxyphenbutazone, novalgin , pyrazofurin, ramifenazone ,indisteron , apixaban, fipronil, rimonabant* and many more are already in market (Arora *et al*., 2013**)**.

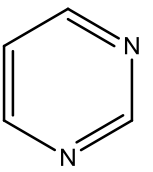
[a] *Department of St. Anthony's college, Shillong, Meghalaya*
[b] *Department of St. Anthony's college, Shillong, Meghalaya*



Pyrazole

Figure 1

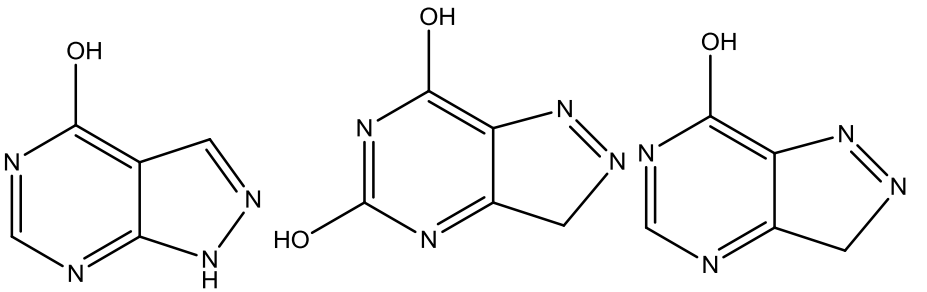
Pyrimidines are the most important six membered heterocyclic compounds containing two N- atoms on 1, 3 positions (figure 2). Cytosine, uracil and thymine are pyrimidines found in nucleic acids (Singh *et al*., 2011). Pyrimidines have a long and distinguished history extending from the days of their discovery as important constituents of nucleic acids to their current use in the chemotherapy of AIDS. The pyrimidine ring is found in vitamins like thiamine, riboflavin and folic acid. Barbituric acid, the first barbiturate hypnotic sedative is a pyrimidine derivative. A pyrimidine antimetabolite which shows excellent antitumour activity against murine solid tumours is Gemticabine and against Hodgkin's lymphoma and disseminated testicular cancer is Bleomycin. Bacimethrin, tubercidine are pyrimidine derivatives used as antibiotics. 2-Thiouracil and its alkyl analogue, thiobarbital are effective drugs against hyperthyroidism. 5-Iododeoxyuridine is an antiviral agent of high selectivity. A metabolite 5-fluorouracil which is a pyrimidine derivative exhibits some useful antineoplastic activities. Pyrimidine derivatives have therefore been developed as antineoplastics, anticancer, antifolates, antibacterial, antiprotozoal, antiviral, anti-AIDS, antibiotic, anthelmentics, sedative, hypnotics, antiepileptic, antitubercular, diuretics, uricosurics, anaesthetics, antihistaminic, analgesics, NSAIDS drugs and cardiac agents (Jain *et al*., 2006).



Pyrimidine

Figure 2

When heterocyclic rings of pyrimidine and pyrazole are fused, it resulted in formation of **pyrazolopyrimidine** and its derivatives (fig 3). Pyrazolopyrimidine derivatives have high impact in the field of pharmaceutical and biotechnological sciences with vast spectrum of biological activities such as analgesics, antibacterial, anticancer, antiinflammatory, antiasthmatic, CNS stimulant etc. (Singh *et al*., 2011).



Allopurinol Oxypurinol Hypoxanthine

Figure 3

Antimicrobial activity:

Aly (2011) reported the synthesis and antibacterial activity of imidazo[1,2-c] pyrazolo[4,3-e] pyrimidines, imidazo[1,2-c] pyrazolo[4,3-e] triazines, pyrazolo[4,3-e] triazolo[1,5-c] pyrimidines and pyrazolo[3', 4': 4, 5) pyrimido[1, 6-b] triazines. The anti- bacterial activity of 3-Methyl-N,1-diphenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine and 3-Methyl-1-pheny-N-o-tolyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine against *Escherichia coli* was reported by Rahmouni *et al.,* 2014. Vishwakarma *et al*., 2009 reported the synthesis and antibacterial activity of 1,2,3,4-Tetrahydro-3-benzyl-1-methylpyrimidin5-yl)(pyridine-4-yl)methanone, 1,2,3,4-Tetrahydro-1,3-dibenzylpyrimidin 5-yl)(pyridine-4-yl) methanone, 1,2,3,4-Tetrahydro-1,benzyl-3-methyl-pyrimidin5-yl)(pyridine-4-yl) methanone and 1,2,3,4-Tetrahydro-1, methyl-3-phenyl-pyrimidin 5-yl)(pyridine-4-yl) methanone against gram positive bacteria. Patel *et al*., 2010 synthesized pyrazole derivatives and reported the anti-tubercular activity of 5-(substituted phenyl)-N, 3-diphenyl-4, 5-dihydropyrazole-1-carbothioamide derivatives. The *in vitro* screening of 2-[(N-Methylindodyl)methyl]-4H-5-cyanomethyl-8-methyl-1,2,4-triazolo[4',3':2,3]pyr-azolo[5,4-d]pyrimidine, 2-[(N-Methylindolyl)methyl]-5-amino-4H-8-methyl-1,2,4-triazolo[4',3':2,3]pyrazolo [5,4-d]pyrimidine, 2-[(N-Methylindolyl)methyl]-4H-6-hydrazino-7-methylpyrazolo[3,4-d]pyrimidine , 2-[(N-Methylindolyl)methyl]-4,6-dihydro-5-oxo-8-methyl-imidazo[3',2':2,3]pyrazolo[5, 4-d]pyrimidine, 2-[(N-Methylindolyl)methyl]-4H-6-[3-hydroxy-5-methyl-pyrazolo-1-yl] pyrazolo [3,4-d]pyrimidine, 2[(NMethylindolyl)methyl]-4H-6-[3-amino-1H-5-oxo-pyrazolo-1-yl]pyrazolo[3,4-d] pyrimidine, 2-[(N-Methlyindolyl)methyl]-4,7-dihydro-5,6-dioxo-9-ethyl[1,2,4]triazino[3',4':2,3] pyrazolo[5,4-d]pyrimidine, 2[(NMethylindolyl)methyl]-4H-5-cinnamoylhydrazino-8-methyl[1,2,4]triazolo[4',3':2 ,3]pyrazolo[5,4-d]pyrimidine against four species of bacteria (*Bacillus cereur, E. coli, Staphylococcus aureus, and Serratia marcescens*) and species of fungi (*Aspergillus flavus, Aspergillus niger, Candida albicans, Geotrichum candidum, Scopulariopsis breuicaulis, and Trichophyton rubrum*) were done and were reported that all the screened compounds were found to be active against all the tested bacterial species in comparison to chloramphenicol and terbinafine which are standards (Bashwan *et al.,* 2010). Reddy (2011) synthesized new

4-(aryl/hetaryl)-6-(3, 5-dimethyl-1-phenyl-1H-4-pyrazolyl)-2-pyridinamine and tested for their antimicrobial activity against bacteria and fungi. Compounds containing 4-nitrophenyl, 2-furyl and 2- thiazolyl at 6- position of pyrimidine ring are highly active against all the organisms employed. Singh *et al*., 2011 had done a review on pyrazolopyrimidines and reported about a series of pyrazolopyrimidine derivatives synthesized and studied by many workers. In particular, compounds pyrazolo[4´,3´:5,6]pyrido[2,3-d]pyrmidine-dione , pyrazolo[3,4-d]pyrimidine derivative, a series of 5-substituted 6-methyl-1-[8-(Trifluoromethyl)quinolin-4-yl]-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-ones, a series of compound 4-amino-1-[8-(trifluoromethyl) quinolin-4-yl]-1*H*-Pyrazolo [3,4-d]pyrimidine, a series of compound 6-(5,6-diphenyl-1,2,4-triazin-3-yl)-3,8-dimethylpyrimido[4´,5´:3,4]pyrazolo[5,1-c][1,2,4]triazine-4,10-6H,9H)-dione, a series of compound (1S) -1-C-(8,9-*Dihydronaphtho*[1´2´:4,5] thieno-[3,2-e][1,2,4]triazolo[1,5-c] pyrimidine-2-yl) polyols, series of compound 9-aryl-7-(4-nitrophenyl)-7H-pyrazolo[4,3-e][1,2,4] triazolo [1,5-c] pyrimidine-2(3H)-Thiones were reported to have antimicrobial activity against a list of tested organisms. Cieplik *et al.,* 2011 reported the synthesis of 1, 2, 3, 7-tetraaryl-1, 2, 3, 4 tetrahydropyrimido[4,5d] pyrimidines and their antibacterial activity was tested against 9 selected strains. This study showed that changes in chemical structure of these compounds enhance microbiological activity. It was confirmed that aromatic residues in the hydrogenated pyrimidine ring constitute a significant element influencing antibacterial activity. Sanjeeva (2011) synthesized new 4-(aryl/hetaryl)-6-(3, 5-dimethyl-1-phenyl-1H-4-pyrazolyl)-2-pyridinamine and tested for their antimicrobial activity against bacteria and fungi. Compounds containing 4-nitrophenyl, 2-furyl and 2-thiazolyl at 6- position of pyrimidine ring are highly active against all the organisms employed. Sharma *et al*., 2011 synthesized some novel pyrimidine-2, 4-diones. Novel pyrimidine-2,4-(1H,3H)-diones were then screened for their antimicrobial activity and reported that compounds 3-**(**4-hydroxyphenyl)-6-(4-methoxyphenylamino) pyrimidine-2,4(1*H*, 3*H*)-) dione,3-(4-hydroxyphenyl)-6-(4hydroxyphenylamino) pyrimidine-2,4(1*H*, 3*H*)-dione had better activity against tested *B. subtilis* (Gram-positive) whereas 6-(3-ethyl-4-methylphenylamino)-3-(4-hydroxyphenyl)pyrimidine-2,4(1*H*, 3*H*)-dione showed good activity against *E. coli* (Gram-negative) and anti-fungal activity against *Aspergillus niger* & *Penicillium marneffei*. Some novel pyrimidine-2, 4-diones were synthesized and screened for their antimicrobial activity against tested gram-positive organism, anti-fungal activity against *Aspergillus niger* & *Penicillium marneffei*. This study showed that 3-(4-methoxyphenyl)-6-(6-hydroxy benzothiazol-2-ylamino) pyrimidine-2,4(1H, 3H)-dione, 3-(4-methoxyphenyl)-6-(6-methoxy benzothiazol-2-ylamino) pyrimidine-2,4(1H, 3H)-dione possessed good activity against gram positive bacteria while another derivative 3-(4-methoxyphenyl)-6-(pyridine-2-ylamino) pyrimidine-2, 4(1H, 3H)-dione possessed good antifungal activity against *Aspergillus niger* and *Penicillium marneffei* (Sharma *et al*. 2012). The synthesis and antimicrobial test of some pyrazoles derivatives against four test organisms, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* using rifampicin and

ampicillin as standard drugs were reported (Sharshira *et al.,* 2012). Azam *et al*., 2013 reported that compounds 1-(1,3-benzothiazol-2-yl)-3-methyl-4-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine , 1-(1,3-benzothiazol-2-yl)-4-(4-chlorophenyl)-3-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidine and 1-(1,3-benzothiazol-2-yl)-3-methyl-4-substituted phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidines showed significant inhibitory activity against *P.aeruginosa* whereas compounds 1-(1,3-benzothiazol-2-yl)-4-(2-chlorophenyl)-3-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidine ,2-[1-(1,3-benzothiazol-2-yl)-3-methyl-1*H*-pyrazolo[3,4*d*]pyrimidin4yl]phenol , 1-(1,3-benzothiazol-2-yl)-4-(3,4-dimethoxyphenyl)-3-methyl-1*H*-pyrazolo[3,4*d*]pyrimidine,4-[1-(1,3-benzothiazol-2-yl)-3-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl]-*N,N*-dimethylaniline and 1-(1,3-benzothiazol-2-yl)-3-methyl-4-[2-phenylvinyl]-1*H*-pyrazolo[3,4-*d*]pyrimidine were found to be active against *C. albicans.* Some of these synthesized compounds were evaluated for their *in vivo* acute toxicity, analgesic, anti-inflammatory and ulcerogenic actions. The tested compound 4-[1-(1, 3-benzothiazol-2-yl)-3-methyl-1*H*-pyrazolo [3, 4-*d*]pyrimidin-4-yl]-*N,N*-dimethylaniline exhibited maximum analgesic and anti-inflammatory activities. Compound 1-(1, 3-benzothiazol--2-yl)-3-methyl-4-(3-nitrophenyl)-1*H*-pyrazolo [3, 4-*d*]pyrimidine showed a significant gastrointestinal protection compared to the standard drug, diclofenac sodium.

Anticancer activity:

Celano *et al*., 2008 reported the cytotoxic effect of N-benzyl-1-(2-chloro-2-phenylethyl)-6-(methylthio)-1H-pyrazolo[3,4-d]pyrimidin-4-amine where it exerted its cytotoxic effects even at lower doses and after shorter incubation time either in ARO or other thyroid cancer cell lines.

The review on the anticancer activities of a series of pyrazolopyrimidine derivatives done by many workers was reported by Singh *et al*., 2011. Some of these compounds are a series of novel 1-arylmethyl-3-aryl-1*H*-Pyrazole-5-carbohydrazide, 1-alkyl(aryl)-4-substitutedpyrazolo[3,4-*d*]pyrimidines and 1-alkyl(aryl)-4-substituted-aminopyrazole[3,4-*d*]pyrimidine. The report showed that 1-methyl-4-aminopyrazolo[3,4-*d*]pyrimidine , 1-methyl-4-methylamino pyrazolo[3,4-*d*]pyrimidine exhibited activity against adenocarcinoma and leukemia. 6-alkyl-1,4-disubstituted pyrazolo[3,4-*d*]pyrimidines and 6-alkyl-4-N-substituted-pyrazolo[3,4-*d*] pyrimidines were reported to not show any antitumor activity but inhibited the growth of *Neurospora crassa*. Pyrazolo[4,3-*e*]1,2,4-triazolo[1,5-*c*]pyrimidines was reported to be a potent adenosine receptor antagonists and a series of (1-aryl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)aryl hydrazones were reported as GSK-3 (Glycogen Synthase Kinase) inhibitors and was determined to bind in a competitive manner with ATP.

Fathalla *et al.,* 2009 synthesized and reported the antitumor activity of novel 6-(2-methylphenyl)-4-oxo-2-thioxo-1, 2, 3, 4- tetrahydropyrimidine-5-carbonitrile derivatives against liver cancer (HEPG2) tumor cell line. This drug was given in varying quantities in comparison to the known anticancer drugs 5-Flurouracil and Doxorubicin. Gouhar *et al*., 2013 synthesized novel substituted pyrazole derivatives and

their anticancer activity was evaluated against MCF-7, HCTH-6, HePG-2 carcinoma cell lines using Doxorubicin as a reference drug. The data showed that moderate cytotoxic activity against both MCF-7 and HCTH-6 carcinoma cell lines was gained by 3,5-diaminopyrazolyl derivative and, 4,6-diaminopyrimidinyl derivative exhibited moderate effect against HePG-2 carcinoma cell line. A very recent report given by Weitensteiner *et al.,* 2013 was on the anti-angiogenic activity of novel trisubstituted pyrazolopyrimidines. The report showed that all the tested compounds inhibited endothelial cell proliferation, interestingly, not due to cytotoxicity as none of them showed acute cytotoxic effects at a concentration of 10µM. In particular, it was reported that three compounds, 7- Benzylamino-5(R) –[(1-hydroxymethyl) propylamino] -3-isopropyl-1(2) H-pyrazolo[4,3-d] pyrimidine, 5-[(2-E/Z-aminocyclohexyl) amino]-7-benzylamino-3-isopropyl-1(2) H-pyrazolo[4,3-d] pyrimidine and 5-[(4-E-aminocyclohexyl) amino]-7- benzylamino-3-isopropyl-1(2) H-pyrazolo[4,3-d] pyrimidine were found to be most potent as they could inhibit cell migration, chemotaxis and tube formation at a non toxic concentration of 10µM. Angiogenesis was found to be reduced *in vivo* in the CAM assay. These compounds were reported to inhibit cdk2, cdk5 and cdk9 and hence are highly attractive anti-angiogenics whose effects largely depend on their cdk5 inhibiting properties. Parmar (2013) reported that the characterized heterocyclic compounds containing pyrazole ring were subjected for antimicrobial screening with gram positive, gram negative bacteria and fungi and the result indicated that when 4-OCH_3- C_6H_4 group , 4-Br- C_6H_4 were used, it gave better antibacterial activity . However, the report also showed that the introduction of 4-NO_2 group did not increase antibacterial activity but on comparison with 4-Cl- C_6H_4 and 2, 4-di-Cl-C_6H_3, the latter compound showed better antibacterial activity. The halogen substituted benzene derivatives was ineffective antifungals.

Antiinflammatory and analgesic activity:

Burguette *et al.,* 2007 reported the synthesis, anti-inflammatory and antioxidant activities of novel ring substituted 3-phenyl-1-(1, 4-di-N-oxidequinoxalin-2-yl)-2-propen-1-one derivatives and of their 4, 5-dihydro-(1H)-pyrazole analogues. The tested compounds inhibited the carrageenin-induced rat paw edema and hence showed important scavenging activities. Nitulescu *et al.,* 2010 synthesized compounds, N-(1-methyl-1Hpyrazole-4-carbonyl)-thiourea derivatives, and tested their analgesic and sedative effects. In their experimental conditions, they reported that the substances analyzed did not manifest an analgesic effect, except compound N-[(1-methyl-1H-pyrazole-4-yl) carbonyl]-N'-phenylthiourea which reduced the locomotor activity, indicating a sedative effect. Singh *et al.,* 2011 reviewed on the anti-inflammatory activity of a series of compounds reported by other workers. Compound 1-thiocarbamoyl-3, 5-diphenyl-2-pyrazolines was found to exhibit analgesic and anti-inflammatory activity as compared to standard drug Pargyline. Compound 5-(2, 6, 6-Trimethyl-2-cyclohexen-1-yl) ethenyl-1H-pyrazole was reported to be potent inhibitors of neutrophilchemotactic responsiveness. Pyrazolotriazolopyrimidine derivatives when

screened for anti-inflammatory analgesic activity were found to show good anti-inflammatory activity associated with non-narcotic analgesic property with remarkable systemic and gastric tolerance. The synthesis, analgesic and anti-inflammatory activity of novel substituted pyrazolediazenylpyrimidine derivatives was reported by Nofal *et al.,* 2011. Compounds 6-[(3,5-Diamino-1H-pyrazol-4-yl)diazenyl]pyrimidine-2,4-(1H,3H)-dione ,6-[(3,5-Dimethyl-1-phenylpyrazol-4-yl)diazenyl]pyrimidine-2,4-(1H,3H)-dione, 6-[(3-Amino-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazol-4-yl)diazenyl]pyrimidine-2,4-(1H,3H)-dione were reported to have the most significant analgesic effects among synthesized moieties. Choudhary *et al.,* 2011 reported the synthesis and analgesic activity of 6- Bromo- 3-(6-(4-Chlorophenyl)-2-(morpholinomethylamino) pyrimidin- 4-yl)-2H-chromen-2-one derivatives. Kota *et al*. 2011 synthesized and evaluated the biological activity of some novel anilide derivatives of pyrazolo [3,4-d]pyrimidines . These compounds were screened for anti-inflammatory activity by carrageenan induced edema model. Among the compounds tested for anti-inflammatory activity, compounds containing electron withdrawing groups such as chloro and fluoro groups showed appreciable results when compared with the standard drug indomethacin. However, compounds bearing electron releasing groups such as methyl and amino groups showed inferior activity. Khanage *et al.,* 2012 synthesized 6-(substituted aryl)-4-(3, 5-diphenyl-1H-1,2,4-triazol-1-yl)-1, 6-dihydropyrimidine-2-thiol and reported their activity as beneficial antimicrobial, anticonvulsant and antitumour agents. Khanage *et al*., 2013 tested the compounds 1, 2, 4-triazole clubbed with pyrazoles, tetrazoles, isoxazole and pyrimidines. The report showed that these compounds can act as analgesic agents.

Compound 4-[1-(1, 3-benzothiazol-2-yl)-3-methyl-1*H*-pyrazolo [3, 4-*d*] pyrimidin-4-yl]-*N*, *N*-dimethylaniline exhibited maximum analgesic and anti-inflammatory activities. Compound 1-(1, 3-benzothiazol--2-yl)-3-methyl-4-(3-nitrophenyl)-1*H*-pyrazolo [3, 4-*d*]pyrimidine showed a significant gastrointestinal protection compared to the standard drug diclofenac sodium (Azam *et al*., 2013). Saleh *et al*., 2013 reported the anti-inflammatory activity of novel triazolo- and tetrazolopyrimidine derivatives when compared with a known drug, indomethacin. Khanage *et al*., 2013 tested the compounds 1, 2, 4-triazole clubbed with pyrazoles, tetrazoles, isoxazole and pyrimidines. The report showed that these compounds can act as analgesic agents.

Antiviral activity:

Pelling and Shipman (1976) reported that 2,3-Dihydro-lH-imidazo[l,2-blpyrazole (IMPY), a known inhibitor of DNA synthesis, which is a useful drug for the synchronization of mammalian cells in culture may also possess significant antiviral activity against herpes simplex virus (HSV) type 1.

The anesthetic and anti-arrhythmic activity of the new pyrazolys-acetanilides derivatives is currently under investigation (Zalaru *et al.,* 2008). El-Etrawy and Abdel Rahman (2010) synthesized and reported the promising antiviral effects of 6-(1H-1, 2,

3-triazol-1-yl) pyrimidine-2, 4(1H,3H)-dione derivatives against Hepatitis A virus (HAV, MBB-cell culture adapted strain) and Herpes simples virus type-1 (HSV-1).

Miscelleneous activity:

A variety of new pyrazolo[1,5-*a*]pyrimidines have been prepared as potential drugs for the treatment of insomnia (Behbehani *et al*., 2010). 6- (substituted aryl)-4-(3,5-diphenyl-1H-1,2,4-triazol-1-yl)-1, 6-dihydropyrimidine-2-thiol were reported as beneficial antimicrobial, anticonvulsant and anticancer agents (Khanage *et al.*, 2012).

REFERENCES

- Aly, A. A and El- Karim, I. A. G. 2011. "Facile synthesis of new pyrazolopyrimidine derivatives of potential biosignificant interest". *Journal of the Korean chemical society, 55(5):*781-786.
- Arora, H. A and Jain, S. 2013. "The synthetic development of pyrazole nucleus: From reflux to microwave". *Der Pharmacia Letter, 5(1)*:340-354.
- Azam, M. A., Dharanya, L, Mehta, C. C, and Sachdeva, S. 2013. "Synthesis and biological evaluation of some novel pyrazopyrimidines incorporating a benzothiazole ring system". *Acta Pharm, 63*:19-30.
- Bashwan, S. A., Fayed, A. A. and Amer, A. A. 2010. "A convenient synthesis of some new pyrazolopyrimidine derivatives with potential biological activity". *Nature and Science, 8(9)*:86-91.
- Behbehani, H., Ibrahim, H. M. and Makhseed, S. 2010. "Synthesis of 7-substituted pyrazolo[1,5-a] pyrimidine-3-carboxamides as potential non benzodiazepine hypnotics". *Arkivoc, 2010(2)*:267-282.
- Burguette, A., Pontiki, E., Hadjipavlou-Litina, D., Villar, R., Vicente, E., Solano, B.,…..,. and Monge, A. 2007. "Synthesis and anti-inflammatory/antioxidant activities of some new ring substituted 3-phenyl-1-(1,4-di-N-oxidequinoxalin-2-yl)-2-propen-1-one derivatives and of their 4,5-dihydro-(1H)-pyrazole analogues". *Bioorganic &Medicinal Chemistry Letters, 17(2007):*6439-6443.
- Celano, M., Schenone, S., Cosco, D., Navarra, M., Puxeddu, E., Racanicchi, L.,...., and Russo, D. 2008. " Cytotoxic effects of a novel pyrazolopyrimidine derivative entrapped in liposomes in anaplastic thyroid cancer cells in vitro and in xenograft tumours in vivo". *Endocrine- related Cancer, 15*:499-510.
- Chaudhary, A., Sharma, P. K., Verma, P., Dudhe, R. 2011. " Synthesis of novel pyrimidine derivative and its biological evaluation". *Analele Universitanii din Bucuresti, 20(2)*:123-140.
- Cieplik, J., Stolarczyk, M., Pluta, J., Gubrynowicz, O., Bryndal, I., Lis, T., & Mikulewicz, M. 2011. "Synthesis and antibacterial properties of pyrimidine derivatives". *Acta Poloniae Pharmaceutica- Drug Research, 68(1):*57-65.

- El-Etrawy, A. Sh and Abdel-Rahman, A. A. H. 2010. "Synthesis and antiviral evaluation of 1, 3-dimethyl-6-(1H-1, 2, 3-triazol-1-yl) pyrimidine-2, 4(1H, 3H)-dione derivatives". *Chemistry of Heterocyclic Compounds, 46(9)*:1105-1108.
- Faidallah, H. M., Rostom, S. A. F., & Al-Saadi, M. S. 2010. "Synthesis and biological evaluation of some new substituted fused pyrazole ring systems as possible anticancer and antimicrobial agents". *Journal of King Abdulaziz University: Science, 22(1)*:177-191.
- Fathalla, O. A., Zeid, I.F., Haiba, M. E., Soliman, A. M., Abd-Elmoez, Sh. I., and El-Serwy, W.S. 2009. " Synthesis, antibacterial and anticancer evaluation of some pyrimidine derivatives". *World Journal of Chemistry, 4(2)*:127-132.
- Gouhar, R. S., Fathalla, O. A., and El-Karim, S.S. Abd. 2013. "Synthesis and anticancer screening of some novel substituted pyrazole derivatives". *Der Pharmica Chemica, 5(6):*225-233.
- Jain, K. S., Chitre, T.S., Miniyar, P.B., Kathiravan, M.K., Bendre, B. S., Veer, V.S., Shahane, S. R., and Shishoo, C. J. 2006. " Biological and medicinal significance of pyrimidine". *Current Science, 90(6):*793-803.
- Khanage, S. G., Raju, A., Mohite, P. B., and Pandhare, R. B. 2013. "Analgesic activity of some 1, 2, 4- triazole heterocycles clubbed with pyrazole, tetrazole, isoxazole and pyrimidine". *Advanced Pharmaceutical Bulletin, 3(1):*13-18.
- Khanage, S. G., Raju, S. A., Mohite, P. B., & Pandhare, R. B. 2012. "Synthesis and pharmacological evaluation of some new pyrimidine derivatives containing 1, 2,4- triazole". *Advanced Pharmaceutical Bulletin, 2(2)*:213-222.
- Kota, R. K., Kompelly, K. K., Surampudi, R., and Kulakarni, R. 2011. "Synthesis and antiinflammatory activity of novel pyrazolo [3, 4-d] pyrimidines". *Journal of Chemical & Pharmaceutical Research, 3(4)*:848-853.
- Nitulescu, G. M,. Paunescu, H., Draghici, C., Missir, A. V., Coman, O. A., and Fulga, I. 2010. "Synthesis and pharmacological evaluation of some new pyrazole derivatives". *Farmacia, 58(2):*190-197.
- Nofal, Z. M., Fahmy, H. H., Zarea, E. S., and El-Eraky, W. 2011. " Synthesis of new pyrimidine derivatives with evaluation of their anti-inflammatory and analgesic activities". *Acta Poloniae Pharmaceutica- Drug Research, 68(4):*507-517.
- Parmar, K. C., Vora, J. J., and Vasava, S. B. 2013. "Synthesis, spectral and microbial studies of some novel schiff base derivatives of 2-amino pyrimidine*". Journal of Chemical and Pharmaceutical Research,* 5(9):222-226.
- Patel, V. I., and Patel, B. 2010. "Synthesis and study of some pyrazole derivatives as antitubercular agent". *International Journal of Pharma and Biosciences, 1(4):*453-458.
- Pelling, J. C., and Shipman, C. 1976. "Antiviral activity of 2, 3-dihydro-1H-imidazo[1,2-b] pyrazole in herpes simplex virus type1-infected mammalian cells". *Biochemical Pharmacology , 25:*2377-2382.

- Rahmouni, A., Romdhane, A., Ben Said, A., Majouli, K, and Ben Jannet, H. 2014. "Synthesis of new pyrazole and antibacterial pyrazolo pyrimidine derivatives". *Turkish Journal of Chem*istry, *38(2)*:210-221.
- Reddy, Ch. S., Devi, M. V., Rajesh, G., Rao, L. S., and Nagaraj, A. 2011. " Synthesis and antimicrobial activity of linked heterocyclines containing pyrazolo-pyrimidine rings". *Indian Journal of Chemistry, 50B:*1181-1186.
- Sharma, O. P., Singhla, R. K., Shrivastava, B., Bhat G, V., Shenoy, G.G., Jayashree, B.S., and Sreenivasan, K. K. 2012. " Synthesis, spectral characterisation & antimicrobial evaluation of some novel pyrimidine-2, 4(1H, 3H) –diones". *Indo Global Journal of Pharmaceutical Sciences, 2(1)*:70-75.
- Sharma, O., Shrivastava, B., Singhla, R. K., and Bhat G, V. 2011. "Synthesis & antimicrobial activity of some novel pyrimidine-2, 4(1H, 3H) –diones". *Indo Global Journal of Pharmaceutical Sciences, 1(3)*:252-257.
- Sharshira, E. M., and Hamada, N. M. M. 2012. "Synthesis and antimicrobial evaluation of some pyrazole derivatives". *Molecules, 17*:4962-4971.
- Singh, S., Sharma, P. K., Dudhe, R., and Kumar, N. 2011. "A review: pyrazolopyrimidine moiety". *Pharma Science Monitor, 2(4):*131-144.
- Vishwakarma, J.N., Dutta, M. C., Chanda, K., Das, B., Laskar, M. A., and Nongkhlaw, R. N. 2009. " Synthesis and anti-bacterial activities of novel 5-isonicotinoyl-1, 2, 3, 4- tetrahydropyrimidines and bis-(5-isonicotinoyl-1, 2, 3, 4- tetrahydropyrimidines)". *Arkivoc, 2009(xiii):*131-141.
- Weitensteiner, S. B., Liebl, J., Krystof, V., Havlicek, L., Gucky, T., Strnad, M.,……, and Zahler, S. 2013. " Trisubstituted pyrazolopyrimidines as novel angiogenesis inhibitors". *Plos One, 8(1):*1-12.
- Zalaru, C., Dumitrascu, F., Tarcomnicu, I., and Neata, M. 2008. " New pyrazole derivatives with potential local anesthetic activity". *Revue Roumaine De Chimie, 53(4):*267-271.

ADAMANTANE DERIVATIVES: FUTURE PHARMACOLOGICAL APPLICATIONS

Revinus Nongkynrih[a] and Laishram Indira Singha[b]

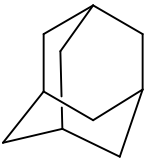
ABSTRACT

Adamantane is a saturated hydrocarbon which is white crystalline solid at room temperature. In the 1960s admantane derivatives- amantadine and remantadine were found to have inhibitory effect on influenza A viruses. Because of the anti-viral effect of amantadine and remantadine thousandsof adamantane derivatives were synthesised and tested for biological activities.In this review we will discuss a few examples of adamantane derivatives as anti-viral,anti-microbial agents against Gram-negative and Gram-positive bacteria as well as against fungus,the anti-inflammatory property,anti-HIV and some other biological activities. Because of the symmetrical structure, its hydrophobic nature and non-toxic to living organisms adamantane get the attention of many researchers to use it in drug designing and discovery.

Keywords: Adamantane, amantadine and rimantadine

INTRODUCTION

Adamantane (tricyclo[3.3.1.1^{3,7}]decane)is a saturated hydrocarbon which is the first and simplest member of diamondoids group. It is a white crystalline solid at room temperature. Adamantane can exist in gas, liquid and two solid crystalline states. Its odour is similar to that of camphor. It is stable and non-biodegradable compound. It is not hazardous or toxic to living organisms (Mansoori, 2007). Adamantane is found in trace amount in petroleum. Its molecular formula is $C_{10}H_{16}$. It was first isolated from petroleum by Landa and Machacek in 1933. Adamantane was first synthesised by Prelog and Seiwerth in 1941 using bicyclo-[3,3,1]-nonane as the starting material (Kallay, 1996).



adamantane

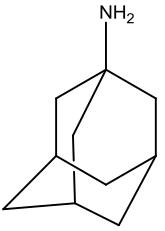
In the early 1960s adamantine derivatives - amantadine and rimantadine have been identified by traditional biological screening and shown to have inhibitory effect on different strains of influenza A viruses in cell culture and in animal model. The anti-Parkinsons effect of amantadine was also discovered during the prophylaxis of

[a] *Department of Biotechnology, St. Anthony's College, Shillong, Meghalaya*
[b] *Department of Biotechnology, St. Anthony's College, Shillong, Meghalaya*

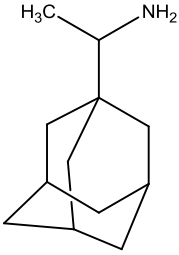
influenza A viruses in the late 1960s (Hayden, 2006). At present amantadine in combination with other preparation is being studied as a cure for Hepatitis C in cases where other methods are ineffective (Bazyleva *et al.,* 2008). Because of the anti-viral activity against influenza A viruses of amantadine and rimantadine thousands of adamantane derivatives are designed, synthesized and tested for their biological activities.

Adamantane derivatives as anti-viral agents:

Amantadine hydrochloride (Symmetrel®) was the first drug to show promising effect as a practical anti-influenza agent (Hornick *et al*, 1969). Amantadine has been found to have anti-viral activity against A2/Hong Kong influenza (Nafta *et al*, 1970) and both amantadine and rimantadine have been found to be effective against influenza A/Alaska/6/77 (H3N2) and A/Bangkok/1/79 (H3N2) viruses when tested in organ culture of ferret tracheal ciliated epithelium (Burlington, Meiklejohnand Mostow, 1982). In 1966 amantadine has been approved in the United States to use clinically for A/H3N2 and subsequently in 1976 for all influenza A infection. Whereas rimantadine was widely developed and used in the Soviet Union, and in 1993 the United States approved its use (Hayden, 2006). The incidence of adamantane resistance among influenza A (H3N2) have been identified worldwide between 1995 and 2005 and the use of amantadine and rimantadine as anti-influenza has been limited because of the rapid emergence of virus-drug resistance (Clercq, 2006).

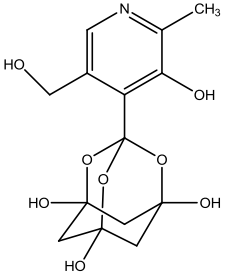


Amantadine

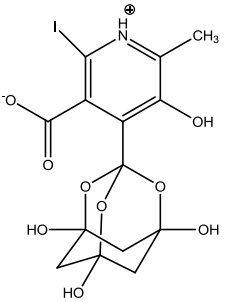


Rimantadine

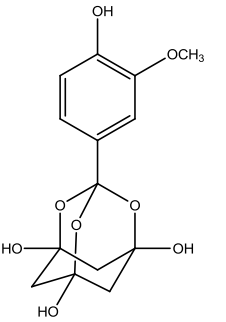
Tanner *et al* (2005) synthesised adamantane-derived bananins and tested for their biological activity against SARS coronavirus and they found thatbananin, iodobananin, vanillinbananin and eubananin were effective inhibitor of ATPase activity of the SARS coronavirus (SCV) helicase.



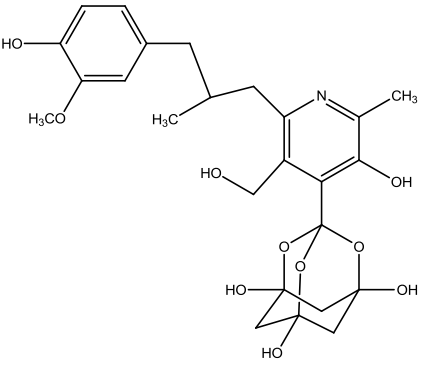
bananin



iodobananin



vanillinbananin

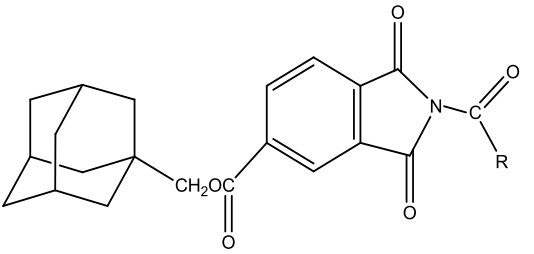


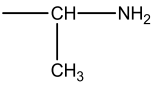
eubananin

In 2008, Zoidis *et al* reported the synthesis of new adamantyl derivatives and their biological testing against influenza A virus (H3N2).They also reported that from the compounds tested one adamantanopyrrolidine derivative and one adamantanopyrrolidine derivative exhibit potent anti-viral activity.

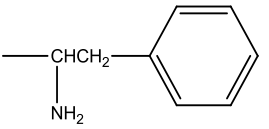
Adamantane derivatives as anti-microbial agents:

Orzeszko *et al* (2000) synthesized a series of fourteen adamantane derivatives and tested for anti-microbial activity against Gram-positive bacteria- *Staphylococcus aureus*, *Micrococcus flavus*, *Enterococcus faecium* and Gram-negative bacteria *Bordellabronchiseptica*, *Pseudomonas aeruginosa* as well as fungus *Candida albicans*. They found that strong anti-microbial activity which is comparable to clinically used antibiotic was exhibited by both the isomers of 4-(Adamant-1-ylmethoxycarbonyl)-N-(alanyl) phthalimide and 4-(Adamant-1-ylmethoxycarbonyl)-N-(phenylalanyl) phthalimide.



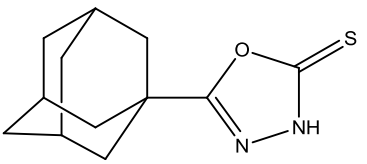
R = 

4-(adamant-1-ylmethoxycarbonyl)-N-(alanyl)phthalimide

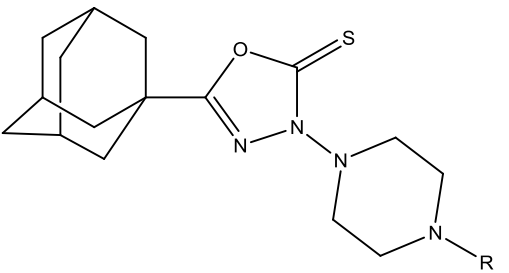


4-(adamant-1-ylmethoxycarbonyl)-N-(phenylalanyl)phthalimide

In 2004,El-Emam *et al* reported the synthesis and the anti-microbial and anti-HIV-1 activity of the compounds 5-(1-adamantyl)-2-substituted thio-1,3,4-oxadiazoles and 5-(1-adamantyl)-3-substituted aminomethyl-1,3,4-oxadiazoline-2-thiones. They found that the compounds exhibit varying degree of anti-microbial activity and among them 5-(1-adamantyl)-1,3,4-oxadiazoline-2-thione and two derivatives of 5-(adamantly)-3-(4substituted-1-piperazinylmethyl)-1,3,4-oxadiazoline-2-tionesexhibit broad spectrum activity.



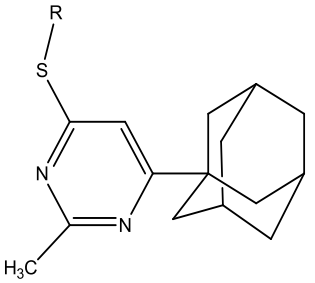
5-(1-adamantyl)-1,3,4-oxadiazoline-2-thione



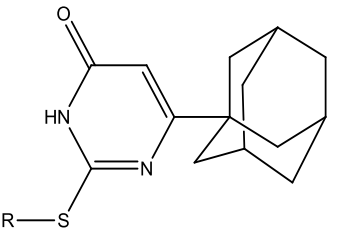
5-(adamantyl)-3-(4substituted-1-piperazinylmethyl)-1,3,4-oxadiazoline-2-tiones

R = CH_3 and R = 4-FC_6H_4

Orzeszko *et al* (2004) tested the anti-bacterial activity of adamantyl substituted pyrimidines and found that the derivatives of 6-(adamant-1-yl-2-methyl-3H-pyrimidine-4-thione and 6-adamant-1-yl-2-tioxo-2,3-dihydro-1H-pyrimidin-4-one were strongly active against *Bacillus subtilis, Staphyloccocus aureus* and *Bacillus stearothermophilus* and also against the fungi *Candida albicans* and *Candida parapsilosis.*

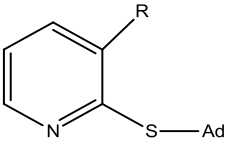


6-adamant-1-yl-2-methyl-3H-pyrimidine-4-thione
R = Br

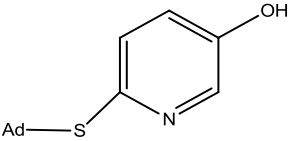


6-adamant-1-yl-2-thioxo-2,3-dihydro-1H-pyrimidin-4-one
R = Br

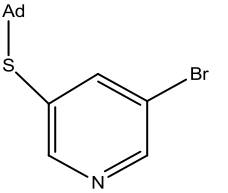
Prachayasittikul *et al* (2008) have reported the anti-microbial activity of 1-adamantylthio derivatives of 3-substituted pyridines particularly N-acetyl-2-(1adamantylthio)-3-acetamidopyridine, 2-(1-adamantylthio)-5-hydroxypyridine and 3-(1-adamantylthio)-5-bromopyridine which displayed complete inhibition of β-hemolytic *Streptococcus*.



N-acetyl-2-(1adamantylthio)-3-acetamidopyridine
R = NAc_2

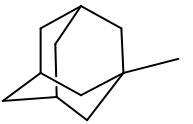


2-(1-adamantylthio)-5-hydroxypyridine

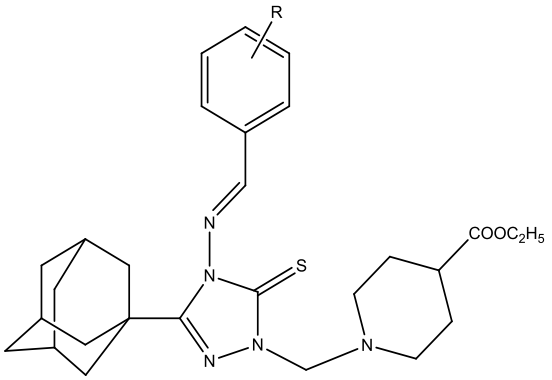


3-(1-adamantylthio)-5-bromopyridine

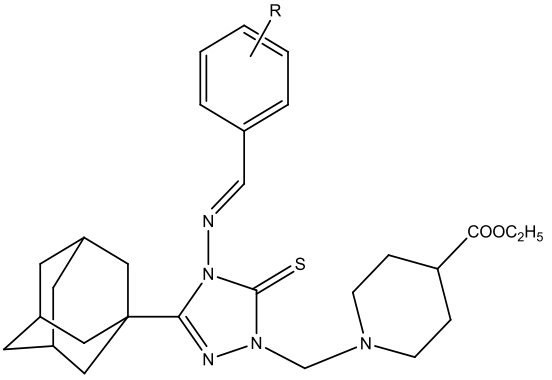
Ad =



A novel compound 5-adamantyl-4-arylideneamino-3-mercapto-1,2,4-triazoles and related derivatives have been synthesised and the anti-microbial activityof these compounds wereperformed and found that 5-(1-adamantyl)-4-(4-hydrobenzylideneamino)-2-(4-ethoxycarbonyl-1-pipetridylmethyl)-1,3,4-triazoline-3-thione and 5(1-adamantyl)-4-(3,4-dimethylbenzylideneamino-2-(4-ethoxycarbonyl-1-piperidylmethyl)-1,2,4-triazoline-3-thione displayed strong broad spectrum anti-microbial activity (Al-Omar *et al*, 2010)



5-adamantyl-4-(4-hydrobenzylideneamino)-2-(4-ethoxycarbonyl-1-pipetridylmethyl)-1,3,4-triazoline-3-thione
R = 4-OH

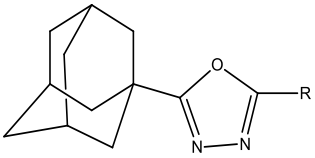


5-adamantyl-4-(3,4-dimethylbenzylideneamino-2-(4-ethoxycarbonyl-1-piperidylmethyl)-1,2,4-triazoline-3-thione
R = 3,4-$(CH_3O)_2$

Recently El-Emam *et al* (2012) reported that the anti-microbial activity of the newly synthesized N'-heteroarylidene-1-adamantylcarbohydrazide derivatives depends on the heterocyclic nucleus.

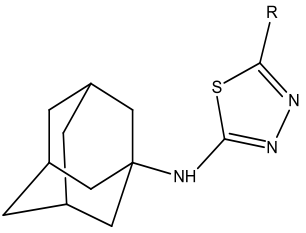
Adamantane derivatives as anti-inflammatory agents:

Kadi *et al* (2007) have reported the synthesis, antimicrobial, and anti-inflammatory activities of novel 2-(1-adamantyl)-5-substituted-1,3,4-oxadiazoles and 2-(1-adamantylamino)-5-substituted-1,3,4-thiadiazoles derivatives and found theoxadiazole derivatives and to a less extent the thiadiazole derivatives showed significant dose-dependent anti-inflammatory activity.



Oxadiazole derivative

R = 4-ClC_6H_5; 3,4-$(OCH_3)_2C_6H_3$; 2-Thienyl and 1-Adamantyl

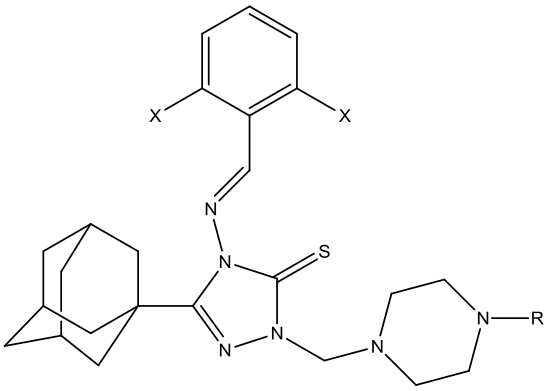


Thiodiazole derivative

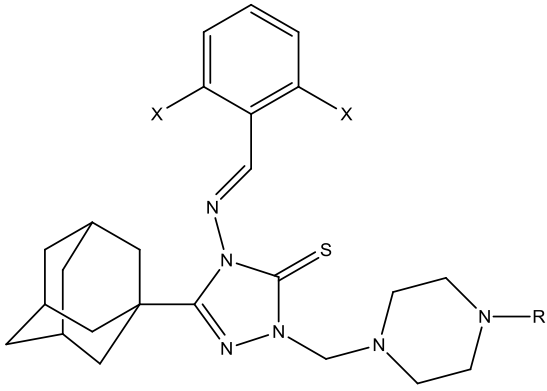
R = C_6H_5; 4-FC_6H_4; 4-ClC_6H_4; 2-Thienyl; 1-Adamantyl

The synthesis, anti-microbial and anti-inflammatory activity ofnovel 5-(1-adamantyl)-4-arylideneamino-3-mercapto-1,2,4-triazoles and related derivatives have been carried out by Al-Omar *et al* (2010). They found that the highest activity was exhibited by 5-(1-adamantyl)-4-(2,6-dichlorobenzylideneamino)-2-(4-phenyl-1-piperazinylmethyl)-1,2,4-triazoline-3-thione which is dose dependent and compound 5-(1-adamantyl)-4-(2,6-difluorobenzylideneamino)-2-(4-phenyl-1-piperazinylmethyl)-1,2,4-triazoline-3-thione and 5-(1-adamantyl)-4-(2-chlorobenzylideneamino)-2-(4-ethoxycarbonyl-1-

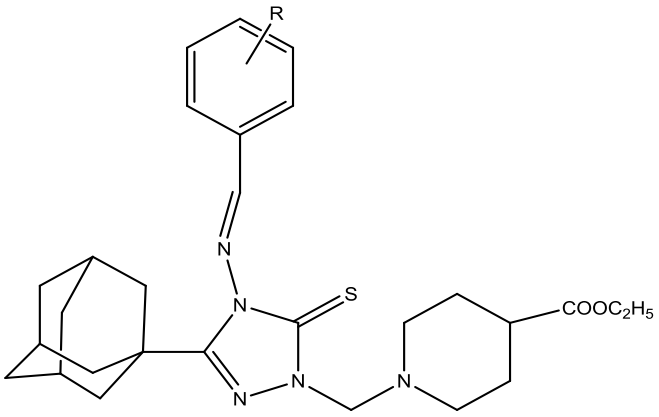
piperidylmethyl)-1,2,4-triazoline-3-thione showed moderate activity at 20 & 40 mg/kg dose.



5-adamantyl-4-(2,6-dichlorobenzylideneamino)-2-(4-phenyl-1-piperazinylmethyl)-1,2,4-triazoline-3-thione
X = Cl and R = C_6H_5



5-adamantyl-4-(2,6-difluorobenzylideneamino)-2-(4-phenyl-1-piperazinylmethyl)-1,2,4-triazoline-3-thione
X = F and R = C_6H_5

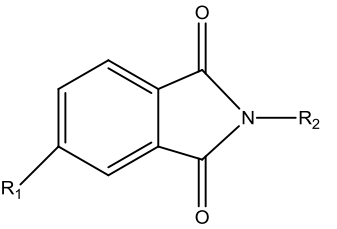


5-adamantyl-4-(2-chlorobenzylideneamino)-2-(4-ethoxycarbonyl-1-piperidylmethyl)-1,2,4-triazoline-3-thione

R = 2-Cl

Adamantane derivatives as an anti-HIV:

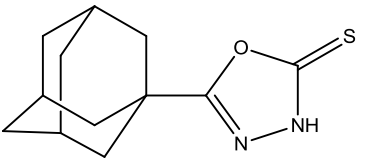
In 1997 Depoorten *et al* reported synthesis of a novel N-1-adamantyl-4-aminophthalimide and tested for anti-HIV activity. The N-1-adamantyl-4-aminophthalimide compound was found to possess anti-HIV-1 and anti-HIV-2activity in CEM cell cultures.



N-1-adamantyl-4-aminophthalimide derivative

R_1 = NH_2 R_2 = Adamantyl

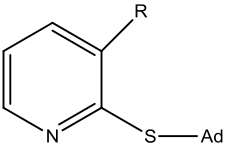
Burstein *et al* (1999) have reported the inhibition of HIV-1 replication by adamantane containing polyanionic agents at an early stage. El-Emam *et al* (2004) also have reported the synthesis, antimicrobial, and anti-HIV-1 activity of certain 5-(1-adamantyl)-2-substituted thio-1,3,4-oxadiazoles and 5-(1-adamantyl)-3-substituted aminomethyl-1,3,4-oxadiazoline-2-thiones and found that 5-(1-Adamantyl)-1,3,4-oxadiazoline-2-thioneproduced 100% inhibition of viral replication.



5-adamantyl-1,3,4-oxadiazoline-2-thione

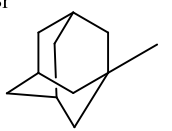
Other biological activities of adamantane derivatives:

In 1971, Barbeau *et al* have reported the effectiveness of amantadine in the treatment of Parkinson's disease in their doubled-blind study. Hareng *et al* (2006) reported the three fold enhancement in production of tumour necrosis factor alpha (TNF-α) by several human ovarian cancer cell lines when stimulated with 2-(1-adamantylamino)-6-methylpyridine.The anti-microbial and anti-oxidative property of 1-adamantylthio derivatives of 3-substituted pyridines have been reported by Prachayasittikul *et al* (2008).They found thatamong all the tested compounds, 2-(1-adamantylthio)-3-bromopyridineshowed the highest anti-oxidative activity.



2-(1-adamantylthio)-3-bromopyridine

R = Br

Ad = 

Naik *et al,* (2011) have reported that adamantane derivatives are potent Multidrug Resistance (MDR) reversal agents. Recently Spilovska *et al,* (2013) have reported that 1-adamantyl-3-(2-(7-methoxy-1,2,3,4-tetrahydroacridin-9-yl-amino)pentyl)thiourea 2,3-dihydroxysuccinate is a suitable novel lead compound for further evaluation for the development of novel Alzheimer's Disease (AD) drugs.

Pharmacology of adamantane derivatives:

The comparative pharmacokinetics of amantadine hydrochloride and rimantadine hydrochloride was performed in youngandelderly adult, and it was found that rimantadine differed significantly from amantadine in peak plasma concentration and plasma half-life (Hayden *et al,* 1985). Absorption of rimantadine occurs rapidly after oral administration and the maximum plasma concentration occurred at less than 0.5 hr. Two rimantadine metabolites have been identified and they appeared to be the ring-substituted isomers of hydroxyrimantadine (Hoffman *et al,* 1988). The mechanism of the anti-viral activity of amantadine is not clearly understood but it appears to prevent the delivery of viral nucleic acid into the cell by interfering in the transmembrane domain of the viral M2 protein (www.accessdata.fda.gov/drugsatfda_docs/label/2009/01623s041,018101s016lbl.pdf).

CONCLUSION

Afteradamantane was first synthesised chemically in the laboratory, and remantadine was found to be effective against influenza A virus, thousands of adamantanederivatives have been synthesised and tested for their biological activities and a large number of them were found to have antimicrobial activity, anti-inflammatory, anti-HIV and other biological properties. Because of the symmetrical structure and hydrophobic nature adamantane is a good backbone for drug designing and discovery. Through combinatorial chemistry adamantane can be attached to other functional groups or molecules or to known compounds with specific biological activity to improve the activity of the compounds.

REFERENCES

- Al-Omar M. A., Al-Abdullah E. S., Shehata I. A., Habib E. E., Ibrahim T. M. and El-Emam A. A. (2010). "Synthesis, anti-microbial, and anti-inflammatory activities of novel 5-(1-adamantyl)-4-arylideneamino-3-mercapto-1,2,4-triazoles and related derivatives"[a]. *Molecules* 15, 2526-2550.
- Barbeau A., Mars H., Botez M. I. and Joubert M. 1971. "Amantadine-HCl (Symmetrel) in the management of Parkinson's disease: a double-blind cross-over study". *Canadian Mecical Association Journal* 105:42-46.

[a] Corresponding author: laishramsingha@gmail.com, contact number: +919862091916.

- Bazyleva B. A., Blokhin B. A., Kabo G. A., Kabo G. J., Emel'yanenko V. N. and Verevkin S. P. (2008). "Thermodynamics properties of 1-aminoadamantane". *Journal of Chemical Thermodynamics,* 40:509-522
- Burlington D. B., Meiklejohn G. and Mostow S. R. 1982."Anti-influenza A virus activity of amantadine hydrochloride and rimantadine hydrochloride Ferret tracheal ciliated epithelium". *Anti-microbial Agents and Chemotherapy* 21:794-799.
- Burstein M. E., Serbin A. V., Khakhulina T.V., Alymova I. V., Stotskaya L. L., Bogdan O. P., Manukchina E. E., Jdanov V. V., Sharova N. K. and Bukrinskaya A. G. 1999. "Inhibition of HIV-1 by newly developed adamantane-containing polyanionic agents". *Antiviral Research* 41:135-144.
- Clercq E. D. 2006. "Anti-viral agents active against influenza A viruses". *Nature Reviews*, 5:1015-1025.
- Depoorten K. V., Balzarini J., Clercq E. D. and Poupaert J. H. 1997."Anti-HIV activity of N-1-adamantyl-4-aminophthalimide". *Biomedicine and Pharmacotherapy* 51:464-468.
- El-Emam A. A., Al-Deeb O. A., Al-Omar M. and Lehmann J. 2004. "Synthesis, antimicrobial, and anti-HIV-1 activity of certain 5-(1-adamantyl)-2-substituted thio-1,3,4-oxadiazoles and 5-(1-adamantyl)-3-substituted aminomethyl-1,3,4-oxadiazoline-2-thiones". *Bioorganic & Medicinal Chemistry* 12:5107-5113.
- El-Emam A. A., Alrashood K. A., Al-Omar M. A. and Al-Tamimi A. S. 2012. "Synthesis and antimicrobial activity of *N′*-heteroarylidene-1-adamantylcarbohydrazides and (±)-2-(1-adamantyl)-4-acetyl-5-[5-(4-substituted phenyl-3-isoxazolyl)]-1,3,4-oxadiazolines". *Molecules* 17:3475-3483.
- Endopharmaceuticals.Symmetrel®.Retrieved from www.accessdata.fda.gov/drugsatfda_docs/label/2009/01623s041,018101s016lbl.pdf on 22/06/2013.
- Hareng L., Lasek W., Switaj T., Sienko J., Jakubowska A. B., Nowaczyk M., Kasprzycka M. and Kazimierczuk Z. 2006. "TNF-α production-enhancing activity of 2-(1-adamantylamino)-6-methylpyridine (AdAMP) in cultures of human normal and neoplastic cells". *Anticancer Research* 26:1209-1216.
- Hayden F. G., Minocha A., Spyker D. A. and Hoffman H. E. 1985."Comparative single-dose pharmacokinetics of amantadine hydrochloride and rimentadine hydrochloride in young and elderly adults". *Antimicrobial Agents and Chemotherapy* 28 (2), 216-221.
- Hayden F. G. 2006. Anti-virals for influenza: Historical perspectives and lessons learned. *Antiviral Research,* 71:372-378.
- Hoffman H. E., Gaylord J. C., Blasecki J. W., Shalabi L. M., and Whiteney C. C. 1988. "Pharmacokinetics and metabolism of rimantadine hydrochloride in mice and dogs". *Antimicrobial Agents and Chemotherapy* 32 (11):1699-1704.

- Hornick R. B., Togo Y., Mahler S. and Iezzoni D. 1969."Evaluation of amantadine hydrochloride in the treatment of A2 influenzal disease". *Bulletin of the World Health Organisation* 41:671-676.
- Kadi A. A., El-Brollosy N. R., Al-Deeb O. A., Habib E. E., Ibrahim T. M. and El-Emam A. A. 2007. "Synthesis, antimicrobial, and anti-inflammatory activities of novel 2-(1-adamantyl)-5-substituted-1,3,4-oxadiazoles and 2-(1-adamantylamino)-5-substituted-1,3,4-thiadiazoles". *European Journal of Medicinal Chemistry* 42:235-242.
- Kallay N. (1996). :Surprise festschrift in honour of Professor Vladimir Prelog". *Croatica Chemica Acta* 69 (2):379-739.
- Mansoori G. A. 2007. "Diamondoid molecules". *Advances in Chemical Physics*, 136:207-258.
- Naft I., Turcanu, A. G., Braun I., Companetz W., Simionescu A., Brit E. and Florea V. (1970)." Administration of amantadine for prevention of Hong Kong influenza". *Bulletin of the World Health Organisation* 42:423-427.
- Naik R., Jeon C., Min H., Choi H. K., Min K. H. and Lee K. 2011. "Synthesis and bioactivity of novel adamantyl derivatives as potent MDR reversal agents". *Bulletin of Korean Chemical Society*, 32 (12):4444-4446.
- Orzeszko A., Gralewska R., Starosciak B. J. and Kazimierczuk Z. 2000. "Synthesis and anti-microbial activity of new adamantane derivatives I". *ActaBiochimicaPolonica 47*:87-94.
- Orzeszko B., Laudy A. E., Starosciak B. J., Orzeszko A. and Kazimierczuk Z. 2004. 'Synthesis and anti-bacterial activity of adamantyl substituted pyrimidines". *ActaPoloniaePharmaceutica-Drug Research* 61 (6):455-460.
- Prachayasittikul S., Suksrichavalit T., Isarankura-Na-Ayudhya C., Ruchirawat,S. and PrachayasittikulV. 2008. "Anti-microbial and anti-oxidative activities of 1-adamantylthio derivatives of 3-substituted pyridines". *Excli Journal* 7:63-77.
- Spilovska K., Korabecny J., Kral J., Horova A., Musilek K., Soukup O., Drtinova L., Gazova Z., Siposova K. and Kuca K. 2013. "7-Methoxytacrine-adamantylamine heterodimers as cholinesterase inhibitors in Alzheimer's disease treatment — Synthesis, biological evaluation and molecular modelling studies". *Molecules* 18:2397-2418.
- Tanner A. J., Zheng B., Zhou J., WattM. R., Jiang J., Wong K., Lin Y., Lu L., He M., Kung H. and Kesel J. A. 2005. "The adamantane-derived bananins are potent inhibitors of the helicase activities and replication of SARS coronavirus". *Chemistry and Biology* 12:303-311.
- ZoidisG., Tsotinis A., Kolocouris N., Kelly M. J., Prathalingam R. S., Naesens L. and Clercq D. E. 2008. "Design and synthesis of bioactive 1,2-anullated adamantane derivative". *Organic and Biomolecular Chemistry* 6:3177-3185.

PIRIFORMOSPORA INDICA- THE COSMOPOLITAN AND BENEFICIAL PLANT ROOT-COLONIZING-ENDOPHYTIC FUNGUS

Pyniarlang Lyngdoh Nongbri[a]

ABSTRACT

Amongst abiotic factors, nutrients and light determine and greatly impact the growth, health and yield of the plants. Microbes present in the soil facilitate nutrient uptake from the soil into the plants via root systems. Mycorrhizal fungi colonizing over 80% of land plants constitute a predominant group of the root-interacting microbiota and plants tremendously benefit from their associations with fungi. However, the inability of mycorrhizal fungi grow in axenic culture has become a hindrance for basic researches and their biotechnological applications. Piriformospora indica, is beneficial growth-promoting root-endophytic fungus belonging to Basidiomycota which mimics the capabilities of mycorrhizal fungi. Unlike mycorrhizae, P. indica can be cultured in axenic medium and has a broad host range and is capable of colonizing and promoting growth of the host plants tested so far. It can be applied as a biological hardening agent for plant tissue cultured plantlets in the field. Recent studies demonstrated that it mobilizes insoluble phosphates and facilitates an energy-dependent uptake of phosphorus by the host. Besides growth-promoting ability, P. indica also influences the performance of the colonized host plants by rendering tolerance to abiotic stresses and enhances resistance to insect attacks and pathogenic infections.

Keywords: *Piriformospora indica*, *Arabidopsis thaliana*, microbes, genes, symbiosis, Glucosinolates, resistance

Introduction

In nature, all living organisms such as plants, animals and microbes engage in intimate relationships which abound due to evolution, climate, competition and symbiosis. Therefore, studies on the ecosystem on earth have shown to be involved ofcomplex and highly interactive systems. Soil microbes are indispensible agents in most ecosystems and play key roles in influencing a large number of important processes such as nutrient acquisition (Smith and Read 1997; Sprent 2001), nitrogen cycling (Tiedje 1988; Kowalchuk and Stephen 2001), carbon cycling (Hogberg *et al.*, 2001) and soil formation (Rillig and Mummey 2006). Being microscopic in size, soil microbes have been the unseen majority in soil and comprise one of the largest genetic diversities on Earth (Whitman *et al.*, 1998). Estimations showed that in one gram of soil as many as 10^{10}-10^{11} bacteria (Horner-Devine *et al.*, 2003), 6000-50,000 bacterial species (Curtis *et al.*, 2002), and up to 200 m fungal hyphae (Leake *et al.*, 2004) have been reported. Despite this overwhelming number and their essential roles in

[a] *Department of Biochemistry, St. Anthony's College, Shillong, Meghalaya.*

biogeochemical cycles, little has been studied concerning the impact these microbes have on plant productivity and diversity.

Rhizosphere-soil microbes interaction determines plant fitness

Due to the sessile and soil-dependent characteristics of the plants, they have developed sophisticated mechanisms to interact and withstand biotic (living) and abiotic (non-living) stresses. Soil microbes play important roles in nature which greatly impact plant productivity either directly through root-associations with mutualistic and/or pathogenic microbes or indirectly *via* the free-living microbes that alter rate of nutrient supply and mobilize the resources (van der Heijden *et al.,* 2008). Among plant-microbe symbiotic associations nitrogen (N)-fixing bacteria that convert atmospheric N into ammonium-N (Sprent 2001) and arbuscular mycorrhizal (AM) fungi that provide phosphates in exchange for carbohydrates (Marschner and Dell 1994; Smith and Read 2008) are perhaps the best studied. Plants are unable to fix the free nitrogen present in the atmosphere and cannot assimilate the insoluble phosphates in the soil that limits plant productivity (Chapin 1980).

Role of N-fixing bacteria have been thought to be prominent in tropical savannah, grasslands and tropical forests that are dominated by legumes which contribute up to 20% of all plant N that is annually acquired by the vegetation (Cleveland *et al.,* 1999; Van der Heijden *et al.,* 2006a). Bond (1983) reported that around 400 shrubs that associate with actinomycetes such as *Casuarina*, *Myrica*, *Hippophae* and *Alnus* are able to form beneficial interaction with N-fixing bacteria. Other plant species harboring N-fixing endophytes such as cyanobacteria includes approximately 150 cycad and 65 Gunnera species (Rai *et al.,* 2000).

Mycorrhizal fungi with host range of more than 80% land (terrestrial) plant species (Smith and Read 1997) are widespread and represent another important group of plant symbionts. They promote plant growth and enhance productivity by supplying the limiting nutrients. Furthermore, they can provide resistance to disease and confer drought tolerance. Mycorrhizal fungi comprise of three most abundant and important groups: the arbuscular mycorrhizal (AM) fungi, the ecto-mycorrhizal (EM) fungi and the ericoid mycorrhizal (ERM) fungi. However the geographical abundance and ecological distribution vary among the three groups. AM fungi are abundantly associated with many grasses, herbs, tropical trees and shrubs growing in grassland, savannah and tropical forests (Read and Perez-Moreno 2003). On the other hand, EM fungi have been reported to interact with 6000 tree species and are abundant in temperate and boreal forests and in some tropical forests (Alexander and Lee 2005). While, ERM fungi associate with members of Ericaceae that are mostly abundant in heath land (Smith and Read 1997).

P.indica- the wonder plants' symbiotic fungal partner influencing many responses in hosts

P. indica was first isolated from the rhizosphere of xerophytic woody shrubs *Prosopis juliflora* (Swartz) DC. and *Zizyphus nummularia* (Burm. fil.) Wt. & Arn. in the sandy desert soils of Rajasthan, India (Verma *et al.,* 1998, Varma *et al.,*1999). The spores produced by this fungus are pear-like in shape. Therefore, the name, *Piriformospora indica* was selected because of the shape and the geographical origin of the first collection of this fungus (Verma *et al.,* 1998, Varma *et al.,*1999, 2001). It has very important characteristics such as its easy culturing method on artificial media, lacking of host specificity and root colonizing abilities. It is classified as a member in the newly created order Sebacinales and has an extremely versatile lifestyle which enables to form a broad mycorrhizal type of association and its growth promoting ability in the colonized plants. Root colonization by *P. indica*usually does not produce any symptoms, and its host range includes members of bryophytes, pteridophytes, gymnosperms and angiosperms (Oelmüller *et al.,* 2009; Peškan-Berghöfer *et al.,* 2004; Pham *et al.,* 2004; Fakhro *et al.,* 2009; Prasad 2008; Shahollari *et al.,* 2005; Varma *et al.,* 2001). Inside the host, *P. indica* grows inter- and intracellularly and produces pear-shaped spores within the cortex and rhizodermis but has never been observed to invade the vasculature and aerial parts of the plants (Deshmukh *et al.,* 2006). *P. indica* genome was successfully sequenced and annotated and its genome size is 25-Mb (Zuccaro *et al.,* 2009, 2011). One of the important findings following the sequencing of *P. indica* genome concerns its colonization, it relates to two sequential lifestyles, a biotrophic followed by a saprophytic nutrition allocation during colonization of plant roots.

Colonization does not cause any negative impact to the host plants, as it was observed to last several months in soil-grown barley plants (Waller *et al.,* 2005). Interestingly, the fungus leads an initial biotrophic lifestyle 3 days post inoculation which trigger cell death of the colonized host (Deshmukh *et al.,* 2006; Jacobs *et al.,* 2011; Qiang *et al.,* 2012). Deshmukh *et al.,* (2006) showed that the protein BAX-Inhibitor 1 which controls cell death is also involved in controlling the level of fungal colonization during the first three weeks post inoculation. Furthermore, hormone signaling pathways are essential for colonization and establishment of beneficial interaction and induction of defense. It was reported that ethylene signaling pathways were important in restricting the level of *P. indica* colonization, as well as for induction of host plant growth by the fungus (Camehl *et al.,* 2010). Colonization also altered expression of genes involved in biosynthesis andresponses to gibberellic acid (GA) and abscisic acid (ABA) which were shown to be induced at specific stages of colonization (Schäfer *et al.,* 2009). *P. indica* can synthesize the auxin indole-acetic acid (IAA) inisolated culture (Sirrenberg *et al.,* 2007) and influences auxin-induced genes in barley roots (Schäfer *et al.,* 2009).

A beneficial interaction between *P. indica* and host plant requires chemical signals recognition, induction of signaling cascade which subsequently effect responses in the host. It has been demonstrated that a diffusible signal from *P. indica* cell wall extract

showed induced-growth promotion in cabbage and *Arabidopsis thaliana* (Lee *et al.*, 2011).

Arabidopsis thaliana - *P. indica* symbiosis: unraveling the signaling pathways involved

The *Arabidopsis thaliana*/*P. indica* interaction system is a good model system which has been used in the study of beneficial and non-benefical traits in a symbiosis. *A. thaliana* has been extensively used as a model organism for studying plant biology. It is a small flowering plant and is grouped in the mustard (Brassicaceae) family which together includes cultivated species such as cabbage and radish. Unlike its group members, *Arabidopsis* does not have agronomic importance; however, it has special characteristics which offer important advantages for basic research in genetics and molecular biology. Scientists working with mycorrhizal fungi were disappointed with the failure of mycorrhizal fungi to form symbiosis with the model plant organism *Arabidopsis*. However this problem was overcome by *P. indica* which can associate to form symbiosis with *Arabidopsis* (Peškan-Berghöfer *et al.*, 2004). Johnson *et al.*, (2011a, 2012) established co-cultivation systems for studying *Arabidopsis* - *P. indica* interaction at different conditions. Now a number of research groups have taken a great deal of interest in this *A. thaliana* - *P. indica* interaction system and what follows is the identification of a list of *Arabidopsis* genes involved to respond to *P. indica.*

This section reviews how beneficial interaction between *Arabidopsis* and *P. indica* is initiated and how long term symbiosis establishes to allow both the symbionts to live together in harmony. A good number of literature has indicated that defense processes are activated very early in the symbiosis, such as those leading to reactive oxygen species (ROS) production (Pozo and Azcón-Aguilar 2007; Salzer *et al.*, 1999), defense gene activation (Blilou *et al.*, 2000; Molina and García-Olmedo 1993;Li *et al.*, 2006; Morandi 1996; Larose *et al.*, 2002), and callose deposition (Cordier *et al.*, 1998; Hamiduzzaman *et al.*, 2005). However, in the later phases of the symbiosis repression of defense gene expression was noticed (Gianinazzi-Pearson *et al.*, 1996; Harrison and Dixon 1993; Lambais and Mehdy 1993; Harrison 2005). No experiments have clearly explained the origin or the cause of these processes; either triggered by signals from the fungus or from the plant itself. However, it was postulated that oce nutrient exchange starts to take place between the two symbiotic partners defense gene activation declines (Harrison, 1999).

Arabidopsis/*P. indica* symbiosis requires the participation of defense genes and their expression because mutations in *Arabidopsis* genes involved in different and unrelated defense responses do not respond to the fungus (Camehl and Oelmüller 2010; Camehl *et al.*, 2010; Sherameti *et al.*, 2008; Nongbri *et al.*, 2012b; Jacobs *et al.*, 2011; Nongbri *et al.*, 2013c). These mutants, unlike the wild-type *Arabidopsis* which grow bigger in presence of the fungus, are smaller in size thereby indicating the loss of beneficial interaction with the fungus. Over-colonization was generally observed in these mutants and as a result of the stressful situations generated in the roots

consequently induces the expression of stress-related genes. Wild-type *Arabidopsis* plants are able to thwart attempts of *P. indica* to overcolonize the roots by activating defense genes which are otherwise impaired in the corresponding mutants. *P. indica* also affects upstream signalling pathways involving the versatile secondary messenger Ca^{2+}, and with the help of aequorin technology elevation of cytosolic calcium concentration $[Ca^{2+}]_{cyt}$ was measured in response to *P. indica* treatment (Johnson *et al.*, 2011b; Sherameti *et al.*, 2010).

Therefore, symbiotic beneficial interaction ultimately is established by a mild and constitutive activation of defense responses. Due to the involvement of the different and unrelated defense processes in *Arabidopsis thaliana* - *P. indica* beneficial interaction it was therefore thought that plants interact with friends and foes alike but integrate/discriminate their signals to balance defense gene activation and development (Camehl and Oelmüller 2010; Camehl *et al.*, 2010; Sherameti *et al.*, 2008; Nongbri *et al.*, 2012a,b; Nongbri *et al.*, 2013b).

Role of sulfur metabolism and enzymes of glucosinolate metabolism in *Arabidopsis*/*P. indica* symbiosis

Sulfur (S) is the least abundant amongst the macroelements found in plants but is incorporated into essential components that are involved in catalytic or electrochemical processes. S is generally present in the soil as sulfate. Plants assimilate sulfate into reduced forms and incorporated into S-containing compounds which are required for plant performance and productivity (Leustek *et al.*, 2000; Saito *et al.*, 2004). Reduced sulfate assimilated by plants therefore supply S-containing amino acids and proteins to animals that are unable to reduce sulfate. S is also incorporated in many plant metabolites including vitamins, coenzymes, volatiles, and defense compounds (Grubb and Abel 2006; Halkier and Gershenzon 2006; Leustek *et al.*, 2000; Saito *et al.*, 2004). Sulfur metabolism in *Arabidopsis* has been observed to be strongly affected by *P. indica* (Nongbri and Oelmüller 2013c). Plants in the order Brassicales including *Arabidopsis* produced glucosinolate secondary metabolites which can store upto 30% of the total plant S; these S-rich glucosinolates play essential roles in plants defense against pests and pathogens (Falk *etal*. 2007). Two groups (Bednarek *et al.*, 2009 and Clay *et al.*, 2009) have separately reported the importance of the glucosinolate metabolism for antifungal defense and innate immune response. Biologically active compounds present in intact glucosinolates are released by hydrolytic cleavage with myrosinase enzymes. These hydrolytic active compounds have been reported to play significant functions in plant/microbe interactions. One such myrosinase enzyme PEN2 restricts pathogen entry into leaf cells (Bednarek *et al.*, 2009). Coincidently and interestingly, PEN2 share a striking sequence similarity with a root abundant enzyme PYK10 that restricts root colonization by *P. indica* (Sherameti *et al.*, 2008). Our group have demonstrated the role of tryptophan-derived indole glucosinolates and camalexin in short term and long term interaction, respectively (Nongbri *et al.*, 2012b). Their basic and significant function is to prevent fungal propagation by restricting colonization

during initial and later phases of the interaction.Investigation of the processes that control S metabolism would be a big incentive for sectors in agriculture, horticulture and medicine as S deficiency causes serious problems in crop productivity and S-rich glucosinolate degradation products affect plant fitness (Walker and Booth 2003; Svanem *et al.,* 1997; Schnug and Haneklaus 1993; Nikiforova *et al.,* 2003).

P. indica -a multipurpose biological agent for agriculture

A. Induction of resistance response of host against pathogens

P. indica chlamydospores only moderately induced a transient upregulation of the defense-related transcripts in the roots (Schäfer *et al.,* 2009)followed by repression *via* jasmonic acid-dependent signaling (Jacobs *et al.,* 2011). In contrast, barley colonized with *P. indica* induces faster and stronger defense responses against the biotrophic leaf pathogen *Blumeria graminis* and enhances defense responses against powdery mildew fungus *Golovinomyces orontii* in *Arabidopsis* leaves (Waller *et al.,* 2005; Deshmukh *et al.,* 2006). Also observed was the induced resistance of barley roots by *P. indica* colonization against infection by the root pathogen *Fusarium culmorum* (Waller *et al.,* 2005). It has also been demonstrated that *P. indica* regulates the biosynthesis of aliphatic glucosinolates that are known to play a significant role in resistance against insect hervbivores *Mamestra brassicae* (Beekwilder *et al.,* 2008). Colonized *Arabidopsis* plants are protected against infection by a necrotrophic pathogenic fungus *Alternaria brassiceae* in roots, as well as enhance the systemic resistance in the leaves by inducing the expression of MYB transcription factors that regulate aliphatic glucosinolates (Nongbri 2013a).

B. Imparting tolerance under stressful abiotic conditions

The fungus mobilizes the resources in the rhizosphere and promotes nutrient uptake by the roots, enhances tolerance of plants to survive under water, temperature and salt stresses, and confers systemic resistance to toxins, heavy metal ions, insects and pathogenic organisms (Das *et al.,* 2012a). It was reported that the fungus stimulates biomass production, induce early flowering, increased seed production and a potential biological agent for imparting hardening to tissue culture-raised plantlets (Das *et al.,* 2012b; Verma *et al.,* 1998; Yadav *et al.,* 2010). Micropropagated *Chlorophytumborivilianum* upon biotization with *P. indica* significantly improves the survival rate of plantlets (Gosal *et al.,* 2010). Seed germination under extreme low temperature has been reported to be influenced by *P. indica* treatment (Murugan 2011; Varma *et al.,* 2012).

Conclusion and Outlook

This review briefly introduces the importance of microbes-soil-plants continuum and the changes associated with the system. Abiotic factors such as climate, nutrient availability and water are long known to have a major influence on plants fitness. The thrust to investigate the role of biotic factors have gain considerable attention in last few years. Soil microbes can influence various biogeochemical processes which shape the

diversity and richness of ecosystem on earth. Prominent effects of soil microbes on plant productivity were for those plants growing under nutrient poor areas where the supply of growth nutrients N, P and S has become limiting. Fungal or bacterial species regulate plant productivity *via* different mechanisms and depending on the lifestyle and biology of the interacting partners a positive and/or negative interaction may occur. Negative effects might occur due to competitions between the microbes for nutrients and space inside the host or when there is no exchange of resources between the microbes and the hosts. Transforming nutrients into forms inaccessible by plants can also happen by non-beneficial microbes. Investigating microbial communication with plants and how they influence plant diversity and productivity is a broad area which demands intricate questions to be answered. First we need to understand where, and what initiates the signals and how it regulates the downstream events. Secondly, how the signals are perceived by the other partner, probably by cell surface receptors. This endeavor to address these questions requires dissection involving development of experimental set-ups capable of filtering unrelated factors from contaminating the system under study.

On the other hand, having a model system which is accessible to genetic, biochemical and molecular analysis will greatly impact our present understanding of this intricate area of research. *P. indica* is one of the favorite plant-interacting fungi. It is easy to culture in synthetic artificial media and its genome has been sequenced which therefore allows the dissection of genes and their functions to understand the biology of the interaction. And with the recently accomplished annotation of the *P. indica* genome research efforts will be immensely helpful not only to *P. indica* related research but also to other mutualistic plant-colonizing fungi. As far as agriculture is concerned, *P. indica* and its Sebacinales relatives have considerable potential as plant growth promoters and biopesticides. It has been successfully exploited for biotechnological purposes in other areas such as forestry, arboriculture and flori-horticulture. *P. indica* has been extremely helpful in the investigation of the relationship between colonization and beneficial traits observed in the host plant. Furthermore, the fungus is a valuable system to understand the regulation of innate defense in roots. *Arabidopsis thaliana - P. indica* symbiosis has increased our understanding and provides an alternative system to elucidate and explore aspects from both the symbionts during the interaction. We may be able to have a better insight into events like how plants discriminate between signals coming from the surrounding which help them to distinguish friends from enemies.

References

- Alexander, I.J. and Lee, S.S. (2005) "Mycorrhizas and ecosystem processes in tropical rain forest: implications for diversity." *In*: *Biotic Interactions in the Tropics: Their Role in the Maintenance of Species Diversity* (eds Burslem, D.F.R.P., Pinard, M.A. & Hartley, S.E.). *Cambridge University Press, Cambridge, 165-203.*

- Bednarek, P., Pislewska-Bednarek, M., Svatos, A., Schneider, B., Doubsky, J., Mansurova, M., Humphry, M., Consonni, C., Panstruga, R., Sanchez-Vallet, A., Molina, A. and Schulze-Lefert, P. (2009) "A glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense." *Science*, 323:101-106.
- Beekwilder, J., van Leeuwen, W., van Dam, N.M., Bertossi, M., Grandi, V., Mizzi, L., Soloviev, M., Szabados, L., Molthoff, J.W., Schipper, B., Verbocht, H., de Vos, R.C., Morandini, P., Aarts, M.G. and Bovy, A. (2008) " The impact of the absence of aliphatic glucosinolates on insect herbivory in *Arabidopsis*." *PLoS One*, 3:e2068.
- Blilou, I., Ocampo, J.A. and Garcia-Garrido, J.M. (2000) "Induction of Ltp (Lipid transfer protein) and Pal (Phenylalanine ammonia-lyase) gene expression in rice roots colonized by the arbuscular mycorrhizal fungus *Glomus mosseae*." *J Exp Bot*, 51:1969-1977.
- Bond, G. (1983) Taxonomy and distribution of non-legumenitrogen-fixing systems. *In*: *Biological Nitrogen Fixation in Forests: Foundations and Applications* (eds Gordon, J.C. & Wheeler, C.T.). Martinus Nijhoff/Dr W. Junk Publ, *The Hague*
- Camehl, I., Sherameti, I., Venus, Y., Bethke, G., Varma, A., Lee, J. and Oelmüller, R. (2010) "Ethylene signalling and ethylene-targeted transcription factors are required to balance beneficial and nonbeneficial traits in the symbiosis between the endophytic fungus *Piriformospora indica* and *Arabidopsis thaliana." New Phytol*, 185:1062-1073.
- Camehl, I. and Oelmüller, R.(2010) "Do ethylene response factors 9 and 14 repress PR gene expression in the interaction between *Piriformospora indica* and *Arabidopsis*?" *Plant Signal Behav*, 9:5-8.
- Chapin, F.S. (1980) The mineral nutrition of wild plants. *Annu Rev Ecol Syst*, 11:233-260.
- Clay, N.K., Adio, A.M., Denoux, C., Jander, G. and Ausubel, F.M.(2009) "Glucosinolate metabolites required for an *Arabidopsis* innate immune response." *Science*, 323:95-101.
- Cleveland, C.C., Townsend, A.R., Schimel, D.S., Fisher, H., Howarth, R.W., Hedin, L.O., Perakis, S.S., Latty, E.F., Von Fischer, J.C., Elseroad, A. and Wasson, M.F. (1999) "Global patterns ofterrestrial biological nitrogen (N-2) fixation in natural ecosystems." *GlobalBiogeochemCycles*, 13:623-645.
- Cordier, C., Pozo, M.J., Barea, J.M., Gianinazzi, S. and Gianinazzi-Pearson, V.(1998) " Cell defense responses associated with localized and systemic resistance to *Phytophthora parasitica* induced in tomato by an arbuscular mycorrhizal fungus." *Mol Plant-Microbe Interact*, 11:1017-1028.
- Curtis, T.P., Sloan, W.T.andScannell, J.W.(2002) "Estimating prokaryotic diversity and its limits." *Proc Natl Acad Sci USA*, 99*:*10494-10499.
- Das, A., Sheramati, I. and Varma, A. (2012a) Contaminated soils: physical,chemical and biological components. *In*: Varma A, Kothe E(eds) *Bio-geo interactions in metal-contaminated soils. Springer, Heidelberg,*

- Das, A., Kamal, S., Shakil Najam, A., Sherameti, I., Oelmüller, R., Dua, M., Tuteja, N., Johri Atul, K. and Varma, A. (2012b) "The root endophytefungus *Piriformospora indica* leads to early flowering, higherbiomass and altered secondary metabolites of the medicinal plant,*Coleus forskohlii*." *Plant Signal Behav*,7:1-10.
- Deshmukh, S., Hückelhoven, R., Schäfer, P., Imani, J., Sharma, M., Weiss, M., Waller, F. and Kogel, K-H. (2006) "The root endophytic fungus Piriformospora indica requires host cell death for proliferation during mutualistic symbiosis with barley." *Proc Natl Acad Sci USA*, 103:18450-18457.
- Fakhro, A., Andrade-Linares, D.R., von Bargen, S., Bandte, M., Büttner, C., Grosch, R., Schwarz, D. and Franken, P. (2009) Impact of*Piriformospora indica* on tomato growth and on interaction withfungal and viral pathogens. *Mycorrhiza*,20, 191-200.
- Falk, K.L., Tokuhisa, J.G. and Gershenzon, J. (2007) The effect of sulfur nutrition on plant glucosinolate content: physiology and molecu-lar mechanisms. *Plant Biol*, 9, 573-581.
- Gianinazzi-Pearson, V., Dumas-Gaudot, E., Gollotte, A., Tahiri-Alaoui, A. and Gianinazzi, S.(1996) Cellular and molecular defence-related root responses to invasion by arbuscular mycorrhizal fungi. *New Phytol*, 133, 45-57.
- Gosal, S.K., Karlupia, A., Gosal, S.S., Chhibba, I.M and Varma, A. (2010)Biotization with *Piriformospora indica* and *Pseudomonas fluorescens*improves survival rate, nutrient acquisition, field performanceand saponin content of micropropagated *Chlorophytum*sp. *Indian J Biotechnol*, 9, 289-297.
- Grubb, C. and Abel, S. (2006) Glucosinolate metabolism and its control. *Trends Plant Sci*, 11, 89-100.
- Halkier, B.A. and Gershenzon, J. (2006) Biology and biochemistry of glucosinolates. *Annu Rev PlantBiol*, 57, 303-333.
- Hamiduzzaman, M.M., Jakab, G., Barnavon, L., Neuhaus, J.M. and Mauch-Mani, B.(2005) β-Aminobutyric acid-induced resistance against downy mildew in grapevine acts through the potentiation of callose formation and jasmonic acid signaling. *Mol Plant-Microbe Interact*, 18, 819-829.
- Harrison, M.J.(2005) Signaling in the arbuscular mycorrhizal symbiosis. *Annu Rev Microbiol*, 59, 19-42.
- Harrison, M.J. (1999)Molecular and cellular aspects of the arbuscular mycorrhizal symbiosis. *Annu Rev Plant Physiol Plant Mol Biol*, 50, 361-389.
- Harrison, M.J. and Dixon, R.A.(1993) Isoflavonoid accumulation and expression of defense gene transcripts during the establishment of vesicular arbuscular mycorrhizal associations in roots of *Medicago truncatula*. *Mol Plant-Microbe Interact*, 6, 643-659.
- Hogberg, P., Nordgren, A., Buchmann, N., Taylor, A.F.S., Ekblad, A., Hogberg, M.N., Nyberg, G., Ottosson-Löfvenius, M. and Rea, D.J.(2001)Large-scale forest

girdling shows that current photosynthesis drives soil respiration. *Nature*, 411, 789-792.

- Horner-Devine, M.C., Leibold, M.A., Smith, V.H.andBohannan, B.J.M.(2003)Bacterial diversity patterns along a gradient of primary productivity. *Ecol Lett*, 6, 613-622.
- Jacobs, S., Zechmann, B., Molitor, A., Trujillo, M., Petutschnig, E., Lipka, V., Kogel, K,-H. and Schäfer, P. (2011) Broad-spectrum suppression of innate immunity is required for colonization of *Arabidopsis* roots by the fungus *Piriformospora indica*. *Plant Physiol*,156, 726-740.
- Johnson, J.M., Sherameti, I., Nongbri, P.L. and Oelmüller, R. (2012) Standardized conditions to study beneficial and non-beneficial traits in the *Piriformospora indica*/*Arabidopsis* interaction. *In:* Varma, A., Kost, G. and Oelmüller, R. (eds) *Piriformospora indica: Sebacinales and their biotechnological application. Springer, Heidelberg, Vol. 33.*
- Johnson, J.M., Sherameti,I., Ludwig, A., Nongbri, P.L., Sun, C., Lou, B., Varma, A. and Oelmüller, R. (2011a) Protocols for *Arabidopsis thaliana* and *Piriformospora indica* co-cultivation - A model system to study plant beneficial traits. *Endocyt Cell Res*, 101-113.
- Johnson, J.M., Nongbri, P., Sherameti, I. and Oelmüller, R.(2011b) Calcium signaling and cytosolic calcium measurements in plants. *Endocyt Cell Res*, 64-76.
- Johnson, J.M. and Oelmüller, R. (2009) Mutualism or parasitism: life in an unstable continuum. What canwe learn from the mutualistic interaction between *Piriformospora indica* and *Arabidopsis thaliana*? A review. *Endocyt Cell Res*, 19, 81-111.
- Kowalchuk, G.A.andStephen, J.R.(2001)Ammonia-oxidizing bacteria: a model for molecular microbial ecology. *Ann Rev Microbiol*,55, 485–529.
- Lambais, M.R. and Mehdy, M.C.(1993) Suppression of endochitinase, β-1,3-endoglucanase, and chalcone isomerase expression in bean vesicular-arbuscular mycorrhizal roots under different soil phosphate conditions. *Mol Plant-Microbe Interact*, 6, 75-83.
- Larose, G., Chenevert, R., Moutoglis, P., Gagne, S., Piche, Y. and Vierheilig, H.(2002) Flavonoid levels in roots of *Medicago sativa* are modulated by the developmental stage of the symbiosis and the root colonizing arbuscular mycorrhizal fungus. *J Plant Physiol*, 159, 1329-1339.
- Leake, J.R., Johnson, D., Donnelly, D.P., Muckle, G.E., Boddy, L.andRead, D.J.(2004)Networks of power and influence: the role of mycorrhizal mycelium in controlling plant communities and agroecosystem functioning. *Can J Bot*,82, 1016-1045.

- Lee, Y-C., Johnson, J.M., Chien, C-T., Sun, C., Cai, D., Lou, B., Oelmüller, R. and Yeh, K-W. (2011)Growth promotion of Chinese cabbage and*Arabidopsis* by *Piriformospora indica* is not stimulated by mycelium-synthesized auxin. *Mol Plant-Microbe Interact*, 24, 421-431.
- Leustek, T., Martin, M.N., Bick, J.A. and Davies, J.P. (2000) Pathways and regulation of sulfur metabolismrevealed through molecular and genetic studies. *Annu Rev Plant Physiol Plant Mol Biol*, 51, 141-165.
- Li, H.Y., Yang, G.D., Shu, H.R., Yang, Y.T., Ye, B.X., Nishida, I. and Zheng, C.C.(2006) Colonization by the arbuscular mycorrhizal fungus *Glomus versiforme* induces a defense response against the root-knot nematode *Meloidogyne incognita* in the grapevine (*Vitis amurensis* Rupr.), which includes transcriptional activation of the class III chitinase gene VCH3. *Plant Cell Physiol*, 47, 154-163.
- Marschner, H. and Dell, B. (1994) Nutrient uptake in mycorrhizal symbiosis. *PlantandSoil*,159, 89-102.
- Molina, A. and García-Olmedo, F.(1993) Developmental and pathogen-induced expression of three barley genes encoding lipid-transfer protein. *Plant J*, 4, 983-991.
- Morandi, D.(1996) Occurence of phytoalexins and phenolic compounds on endomycorrhizal interactions and their potential role in biological control. *Plant Soil*, 185, 241-251.
- Murugan, P. (2011) Enhancing winter vegetable production ingreenhouse of cold arid desert of Ladakh by inoculation of *Piriformosporaindica*. *DRDO Technology Spectrum*, 164-170.
- Nikiforova, V., Freitag, J., Kempa, S., Adamik, M., Hesse, H. and Hoefgen, R. (2003) Transcriptome analysis of sulfur depletion in *Arabidopsis thaliana*: interlacing of biosynthetic pathways provides response specificity. *Plant J*,33, 633-650.
- Nongbri, P.L. (2013a) The role of glucosinolates in the *Arabidopsis*/*Piriformospora indica* interaction. *Ph.D Thesis, Friedrich-Schiller Universität Jena, Germany*. (http://www.db-thueringen.de/servlets/DerivateServlet/Derivate-28048/UrMEL/P.L.Nongbri.pdf).
- Nongbri, P.L., Vahabi, K., Mrozinska, A., Seebald, E., Sun, C., Sherameti, I., Johnson, J.M. and Oelmüller, R.(2013b) Balancing defense and growth-Analyses of the beneficial symbiosis between *Piriformospora indica* and *Arabidopsis thaliana. Symbiosis*, 58, 17-28.
- Nongbri, P.L. and Oelmüller, R. (2013c) Role of *Piriformospora indica* in Sulfur Metabolism in *Arabidopsis thaliana*. *In*: Varma, A., Kost, G. and Oelmüller, R. (eds) *Piriformospora indica: Sebacinales and their biotechnological application. Springer, Heidelberg, Vol. 33.*
- Nongbri, P.L. (2012a) Plant defense responses in beneficial plant/microbe interactions. *Endocyt Cell Res*, 1:70-81.
- Nongbri, P.L., Johnson, J.M., Sherameti, I., Glawischnig, E., Halkier, B.A. and Oelmüller, R.(2012b) Indole-3-acetaldoxime-derived compounds restrict root

colonization in the beneficial interaction between *Arabidopsis* roots and the endophyte *Piriformospora indica*. *Mol Plant Microbe Interact*, 25:1186-1197.

- Oelmüller, R., Sherameti, I., Tripathi, S. and Varma, A. (2009) *Piriformospora*
- *indica*, a cultivable root endophyte with multiple biotechnologicalapplications. *Symbiosis*,49:1-17.
- Peškan-Berghöfer, T., Shahollari, B., Giong, P.H., Hehl, S., Markert, C.,Blanked, V., Kost, G., Varma, A. and Oelmüller, R. (2004) Association of*Piriformospora indica* with *Arabidopsis thaliana* roots representsa novel system to study beneficial plant–microbe interactions andinvolves early plant protein modifications in the endoplasmic
- reticulum and at the plasma membrane. *Physiol Plant*, 122:465-477.
- Pham, G.H., Kumari, R., Singh, A., Sachdev, M., Prasad, R., Kaldorf, M., Buscot, F., Oelmüller, R., Hampp, R., Saxena, A.K., Rexer, K-H., Kost, G. and Varma, A. (2004) Axenic cultures of *Piriformospora indica*. *In*: Varma, A., Abbott, L., Werner, D., Hampp, R. (eds) *Plant surface microbiology. Springer, Germany.*
- Pozo, M.J. and Azcón-Aguilar, C. (2007) Unraveling mycorrhiza-induced resistance. *Curr Opin Plant Biol*, 10:393-398.
- Prasad, R.P. (2008) "Studies on interaction between symbioticfungus (*Piriformospora indica*), rhizobacteria and selected plants. *Ph.D Thesis, Merrut University, India*.
- Qiang, X., Zechmann, B., Reitz, M.U., Kogel, K-H. and Schäfer, P. (2012) "The mutualistic fungus Piriformospora indica colonizes *Arabidopsis* roots byinducing an endoplasmic reticulum stress-triggered caspase-dependentcell death." *Plant Cell*,24, 794-809.
- Rai, A.N., Soderback, E. and Bergman, B. (2000) "Cyanobacterium-plantsymbioses." *New Phytol*, 147:449-481.
- Read, D.J. and Perez-Moreno, J. (2003) "Mycorrhizas and nutrient cycling in ecosystems - a journey towards relevance?" *New Phytol*, 157:475-492.
- Rillig, M.C.andMummey, D.L.(2006) "Mycorrhizas and soil structure*." New Phytol*,171:41-53.
- Saito, S., Soga, T., Nishioka, T. and Tomita, M. (2004) "Simultaneous determination of the mainmetabolites in rice leaves using capillary electrophoresis mass spectrometry and capillaryelectrophoresis diode array detection." *Plant J*, 40:151-163.
- Salzer, P., Corbiere, H. and Boller, T. (1999) "Hydrogen peroxide accumulation in *Medicago truncatula* roots colonized by the Arbuscular Mycorrhiza-forming fungus *Glomus intraradices*." *Planta*, 208:319-325.
- Schäfer, P., Pfiffi, S., Voll, L.M., Zajic, D., Chandler, P.M., Waller, F., Scholz, U., Pons-Kühnemann, J., Sonnewald, S., Sonnewald, U. and Kogel, K-H. (2009) "Manipulation of plant innate immunity and gibberellin as factor ofcompatibility in the mutualistic association of barley roots with *Piriformospora indica*." *Plant J*, 59:461-474.

- Schnug, E. and Haneklaus, S. (1993) "Physiological backgrounds of different sulphur utilization in *Brassica napus* varieties. *Aspects Appl Biol*,34:235-242.
- Shahollari, B., Varma, A. and Oelmüller, R. (2005) "Expression of areceptor kinase in *Arabidopsis* roots is stimulated by the basidiomycete*Piriformospora indica* and the protein accumulates inTriton X-100 insoluble plasma membrane microdomains." *J Plant Physiol*,162:945-958.
- Sherameti, I., Johnson, J.M., Nongbri, P. and Oelmüller, R. (2010) "The central roles of iron and calcium for plant/microbe interaction and shaping microbial communities in the soil." *Albanian JAgri Sci*, 9:1-24.
- Sherameti, I., Venus, Y., Drzewiecki, C., Tripathi, S., Dan, V.M., Nitz, I., Varma, A., Grundler, F.M. and Oelmüller, R. (2008) "PYK10, a beta-glucosidase located in the endoplasmatic reticulum, is crucial for the beneficial interaction between *Arabidopsis thaliana* and the endophytic fungus *Piriformospora indica*." *Plant J*, 54:428-439.
- Sirrenberg, A., Göbel, C., Grond, S., Czempinski, N., Ratzinger, A., Karlovsky, P., Santos, P., Feussner, I. and Pawlowski, K. (2007) "*Piriformospora indica* affects plant growth by auxin production." *Physiol Plant*,131:581-589.
- Smith, S.E. and Read, D.J. (2008) Mycorrhizal Symbiosis. *London*:*AcademicPress*.
- Smith, S.E.andRead, D.J.(1997)*MycorrhizalSymbiosis*, 2nd Ed. *Academic Press, London, UK.*
- Sprent, J.I.(2001)*NodulationinLegumes. Royal Bot. Gardens, Kew, UK.*
- Svanem, P.J., Bones, A.M. and Rossiter, J.T. (1997) "Metabolism of [α-14C]-desulphophenylethylglucosinolate in *Nasturtium officinale*." *Phytochem*,44:1251-1255.
- Tiedje, J.M.(1988) Ecology of denitrification and dissimilatory nitrate reduction to ammonium. *In: Biology of Anaerobic Microorganisms*(ed.Sehnder, A.J.B.). *Wiley, New York.*
- van der Heijden, M.G.A., Bardgett, R.D. and van Straalen, N.M. (2008) "The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems." *Ecol. Lett*, 11, 296-310.
- Van der Heijden, M.G.A., Bakker, R., Verwaal, J., Scheublin, T.R., Rutten, M., van Logtestijn, R. and Christian Staehelin. (2006a). "Symbiotic bacteria asa determinant of plant community structure and plant productivityin dune grassland." *FEMS Microb Ecol*, 56:178-187.
- Varma, A., Tripathi, S., Prasad, R., Das, A., Sharma, M., Bakshi, M.,Arora, M., Rastogi, K., Agrawal, A., Kharkwal, A.C., Tsimilli-Michael, M., Strasser, R.J., Bagde, U.S., Bisaria, V.S., Upadhyaya, C.P., Malla, R.,Kost, G., Joy, K., Sherameti, I., Chen, Y., Ma, J., Lou, B. and Oelmüller, R. (2012) The symbiotic fungus *Piriformospora indica*: update. *In*:Hock B (ed) *The Mycota XXL. Springer, Berlin, in press.*
- Varma, A., Singh, A., Sudha, M., Sahay, N.S., Sharma, J., Roy, A.,Kumari, M., Rana, D., Thakran, S., Deka, D., Bharti, K., Hurek, T.,Blechert, O., Rexer, K-H.,

Kost, G., Hahn, A., Maier, W., Walter, M.,Strack, D. and Kranner, I. (2001) *Piriformospora indica*: a cultivable

- mycorrhiza-like endosymbiotic fungus. *In*: Hock B (ed) *The Mycota IX. Springer-Verlag, Berlin, Germany.*
- Varma, A., Verma, S., Sudha, Sahay, N., Buttehorn, B., Franken, P. (1999) "*Piriformospora indica*, a cultivable plant-growth-promotingroot endophyte." *Appl Environ Microbiol*,65:2741-2744.
- Verma, S., Varma, A., Rexer, K., Hassel, A., Kost, G., Sarbhoy, A., Bisen, P., Bütehorn, B., Franken, P. (1998) *Piriformospora indica*, gen. et sp. nov., a new root-colonizing fungus. *Mycologia*,90:896-903.
- Walker, K.C. and Booth, E.J. (2003) Sulphur nutrition and oilseed quality. *In*: Abrol YP, Ahmad A. (eds.) *Sulphur in Plants. Kluwer Academic Publishers Dordrecht.*
- Waller, F., Achatz, B., Baltruschat, H., Fodor, J., Becker, K., Fischer, M., Heier, T., Hückelhoven, R., Neumann, C., von Wettstein, D., Franken, P. and Kogel, K.-H. (2005) "The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield." *Proc Natl Acad Sci USA*, 102:13386-13391.
- Whitman, W.B., Coleman, D.C.&Wiebe, W.J.(1998) "Prokaryotes: the unseen majority." *Proc Natl Acad Sci*, 95*:* 6578-6583.
- Yadav, V., Kumar, M., Deep, D.K., Kumar, H., Sharma, R., Tripathi, T.,Tuteja, N., Saxena, A.K., Johri, A.K. (2010) "A phosphate transporterfrom the root endophytic fungus *Piriformospora indica* plays a role in phosphate transport to the host plant." *J Biol Chem*, 285:26532-26544.
- Zuccaro, A., Lahrmann, U., Güldener, U., Langen, G., Pfiffi, S., Biedenkopf, D., Wong, P., Samans, B., Grimm, C., Basiewicz, M., Murat, C., Martin, F. and Kogel, K.H. (2011) "Endophytic life strategies decoded by genome and transcriptome analyses of the mutualistic root symbiont *Piriformospora indica*." *PLoS Pathog*,7:e1002290.
- Zuccaro, A., Basiewicz, M., Zurawska, M., Biedenkopf, D. and Kogel, K.H. (2009) "Karyotype analysis, genome organization, and stable genetic transformationof the root colonizing fungus *Piriformospora indica*." *Fungal Genet Biol*,46:543-550.

ELECTRO- POLLUTION AND ITS EFFECTS: A REVIEW

Nibir Ranjan Parasar[a]

Introduction

We are living in an age when there is an ever increasing global concern over different forms of pollution that are creating havoc to the environment. Apart from the common forms of pollution like air pollution, water pollution, noise pollution, soil pollution, light pollution etc., another relatively new form of pollution that is a growing cause of concern is Electro-pollution. Electro-pollution, also known as dirty electricity is the increased levels of non-ionizing electromagnetic radiation (EMR) in the atmosphere.

Electromagnetic radiation (EMR) - both from man-made and natural sources - has been present in the environment for years. However, in the last few decades, because of the exponential growth of cellular telecommunication technology and the growing popularity of other wireless technologies, the levels of non-ionizing EMR in the environment has increased alarmingly. Studies conducted in different places around the world has established beyond doubt, that, the present day generation, more so in the urban areas, is living in a sea of electromagnetic energy. This has led to growing international concern about the possible adverse effects on flora and fauna particularly in human beings where many health issues have been linked to over exposure to EMRs. Studies have been conducted on the possible link between different forms of cancer – particularly those of the brain, eye, ear and leukaemia to EMR exposure. Electro-pollution has been identified as a probable cause for the increasing incidences of miscarriages and birth defects. Also, conclusive evidences have been found which has established that continuous exposure to low energy EMR can cause sleep disorders, dizziness, palpitation, lack of concentration, anxiety, loss of memory, chronic stress, autism etc. Children and pregnant women are found to be most vulnerable to the ill effects of exposure to EMR. Apart from that the vanishing of bees, insects, butterflies, sparrows etc. have been linked to the increased levels of EMR in the atmosphere. Various studies have been also conducted as to the effects of EMR in plants. As a result, there have been efforts by different countries to tackle the problem of electro-pollution and limit its possible adverse effects. The International Commission on Non-Ionizing Radiation Protection (ICNIRP) was established in the year 1992. This organisation, formally recognised by World Health Organisation as its partner in dealing with non-ionizing EMR was set up with an aim to address the issue of possible adverse effects of non-ionizing EMR on human health. The International Commission on Non Ionizing Radiation Protection (ICNIRP), has set limits to the maximum permissible exposure to electromagnetic radiation.

[a] *Department of Biotechnology, St. Anthony's College, Shillong, Meghalaya.*

In India too, electro-pollution is on the rise, more so with the growth of cellular telecommunication devices in the last decade. Studies have been done to estimate the radiation energy levels in cities like Mumbai [Prof. G. Kumar, 2010, Neha Kumar & Girish Kumar, 2009], Chandigarh [A. K. Dhami, 2012] and Delhi [V. S. Tanwar, 2006]. Also the government has set limits to the amount of EMR energy levels permissible in the atmosphere.

Effects of non-ionizing Electromagnetic radiations

All Electromagnetic radiations possess energy. When it is incident on matter it transfers energy to the body on which it is incident. Exposure to energetic ionizing radiations like X-rays and Gamma rays is harmful to living beings and can cause immediate effects like burns and radiation sickness whereas long term effects like cancer and genetic damage also takes place.

The main emphasis of this article is on the effects of non-ionizing radiation. The range of frequency of non-ionizing EMRs is not exactly defined and is quite broad. However for determining the levels of electro-pollution in the atmosphere, EMRs up to the frequency of about 300 GHz are considered. The levels of EMRs in the atmosphere in this range of frequency has risen alarmingly in the last few decades primarily due to the boom in cellular and wireless communication technologies which primarily uses Radiowaves and Microwaves having frequencies up to 300 GHz.

The human body consists of millions of cells which communicate among themselves to carry on the vital life processes. These communications are in the form of low frequency electromagnetic signals as well as through biochemical reactions. Continuous exposure to electromagnetic radiation can distort these intercellular communications. This may lead to abnormal biological processes resulting in various complications.

Innumerable studies have been done on the possible adverse effects of non-ionizing on flora and fauna. In the following sections we present a brief review of some of the possible adverse effects of non-ionizing EMRs.

Genotoxic Effects

Genetic materials in living organisms play the most vital role in the various biological processes occurring in them. For example, deoxyribonucleic acid (DNA) carries information in the form of genetic codes which are responsible for performing the different biological processes in a desired manner. However, some agents known as gentoxins may be present and tamper with the genetic material and in the process lead to different complications. Genotoxicity is thus toxicity manifested in the genetic material of cells. For example, sudden change of genetic codes in DNA or mutations because of some agents can lead to cancer or other genetic abnormalities. The broader definition of genotoxicity includes both direct and indirect effects in DNA: (1) the induction of mutations (gene, chromosomal, genomial, recombinational) that at the molecular level are similar to events known to be involved in carcinogenesis, (2) indirect surrogate events associated with mutagenesis (e.g., unscheduled DNA synthesis

(UDS) and sister chromatid exchange (SCE), or (3) DNA damage (e.g., the formation of adducts), which may eventually lead to mutations.

Different types of genotoxins have been identified like different chemical agents. However with the rapid increase in the level of electro-pollution all over the world, the genotoxicity of low energy EMRs is getting considerable focus. Various studies have been done which have proved the genotoxicity of low energy EMR particularly that of the Radiofrequency radiation that is used for cellular/mobile telecommunication and other wireless communication technologies.

Radiofrequency radiation (RFR) and DNA damage

The following is a summary of the research data that reported DNA damage, in literature-

Markova et. al., in 2005 reported that GSM signals affected chromatin conformation and gama-H2AX foci that co-localized in distinct foci with DNA double strand breaks in human lymphocytes. Mice were also exposed to 900-MHz RFR at a specific absorption rate (SAR) of 0.09 W/kg for 7 days at 12 hr per day. They observed DNA damage in caudal epididymal spermatozoa which was assessed by quantitative PCR (QPCR) as well as alkaline and pulsed-field gel electrophoresis post exposure. Gel electrophoresis revealed no significant change in single- or double-DNA strand breakage in spermatozoa. However, QPCR revealed statistically significant damage to both the mitochondrial genome ($p < 0.05$) and the nuclear β-globin locus ($p < 0.01$). Increase in DNA strand breaks and micro-nucleation in lymphocytes obtained from cell phone users was reported by Gandhi [2005]. It was also reported that a low and transient increase in DNA double strand breaking mouse embryonic stem cells after acute exposure to 1.7- GHz field. There was a report of an increase in DNA single strand breaks in human lens epithelial cells after 2hrs of exposure to1.8 GHz field at 3 and 4 W/kg. Here the DNA damages caused by 4 W/kg field were irreversible. Human fibroblasts and rat granulosa cells were exposed to mobile phone signal (1800 MHz; SAR 1.2 or 2 W/kg; different modulations; during 4, 16 and 24 h; intermittent 5 min on/10min off or continuous). RFR exposure induced DNA single- and double-strand breaks as measured by the comet assay. The Effects occurred after 16 h exposure in both cell types and after different mobile-phone modulations. The intermittent exposure showed a stronger effect in the than continuous exposure. Moreover, changes in DNA synthesis and structure in Chinese hamster cells after various durations of exposure to 7.7 GHz field at30 mW/cm2 was reported by Garaj-Vrhovac et. al., [1990].It was found that there was an increase and decrease in DNA strand breaks in cells exposure to various forms of cell phone radiation. In certain experiments (Narasimhan and Huh [1991]) also reported changes in lambda-phage DNA suggesting single-strand breaks and strand separation. An increase in DNA damage in human lens epithelial cells at 0 and 30 min after 2 hrs of exposure to 1.8 GHz field at 3 W/kg was reported. It was also reported a low and transient increase in DNA double strand breaking mouse embryonic stem cells after acute exposure to 1.7- GHz field.

Cytogenetic Effects

Cytogenetics is the branch of biology linking the study of genetic inheritance with the study of cell structure, especially for human chromosome analysis for the detection of inheritable diseases.

Radiofrequency radiation (RFR) and Chromosome and genome effects

The following are the chromosome and genome studies that reported effects-

Belyaev et. al., [2006] investigated whether exposure of rat brain to microwaves of global system for mobile communication (GSM) induces DNA breaks, changes in chromatin conformation and in gene expression at a specific absorption rate (SAR) of 0.4 mW/g for 2 hr. Data showed that GSM MWs at 915 MHz did not induce DNA double stranded breaks detectable by pulsed-field gel electrophoresis or changes in chromatin conformation, but affected expression of genes in rat brain cells. A significant increase in di-centric chromosomes in blood cells among mobile users who were smoker–alcoholic as compared to non-smoker–non-alcoholic; the same held true for controls of both types. Garaj-Vrhovac et. al., [1990] exposed V79 Chinese hamster cells to a continuous-wave of 7.7GHz RFR at power density of 30 mW/cm2 for 15, 30, and 60 min. It was observed that the radiation causes changes in the synthesis as well as in the structure of DNA molecules. Garaj-Vrhovac et. al., [1991] exposed V79 Chinese hamster fibroblast cells to continuous wave 7.7 GHz radiation at power density of 0.5 mW/cm2 for 15,30 and 60 minutes. There was a significantly higher frequency of specific chromosome aberrations such as di-centric and ring chromosomes in irradiated cells. It was found that human peripheral blood lymphocytes exposed to continuous 830-MHz electromagnetic fields (1.6-8.8 W/kg for 72 hr) showed a SAR-dependent chromosome aneuploidy, a major "somatic mutation leading to genomic instability and thereby to cancer. The aneuploidy was accompanied by an abnormal mode of replication of the chromosome 17 region engaged in segregation (repetitive DNA arrays associated with the centromere), suggesting that epigenetic alterations are involved in the SAR dependent genetic toxicity. The effects were non-thermal. Ono et. al., (2004) exposed pregnant mice intermittently at a whole-body averaged specific absorption rate of 0.71 W/kg (10 seconds on, 50 seconds off which is 4.3 W/kg during the 10 seconds exposure) for 16 hours a day, from the embryonic age of 0 to 15 days. At 10 weeks of age, mutation frequencies at the *lacZ* gene in spleen, liver, brain, and testis were examined. Quality of mutation assessed by sequencing the nucleotides of mutant DNAs revealed no appreciable difference between exposed and non-exposed samples.It was also reported that exposure to microwaves of 895-915 MHz at 5.4 mW/kg resulted in statistically significant changes in condensation of chromatin in human lymphocytes. Effects are similar to stress response, differ at various frequencies, and vary among donors. Garaj-Vrhovac et. al., [1991] exposed V79 Chinese hamster fibroblast cells to continuous wave 7.7 GHz radiation at power density of 0.5 mW/cm2 for 15, 30 and 60 min. There was a significantly higher frequency of specific chromosome aberrations such as di-centric

and ring chromosomes in irradiated cells. Belyaev et. al., [2005] investigated response of lymphocytes from healthy subjects and from persons reporting hypersensitivity to microwaves from GSM mobile phone (915 MHz, specific absorption rate 37 mW/kg), and power frequency magnetic field (50 Hz, 15 microT peak value).Changes in chromatin conformation were measured with the method of anomalous viscosity time dependencies (AVTD). Exposure at room temperature to either 915 MHz or 50 Hz resulted in significant condensation of chromatin, shown as AVTD changes, which was similar to the effect of heat shock at 410C.No significant differences in responses between normal and hypersensitive subjects were detected.

From this literature survey, it is apparent that radiofrequency radiation exposure could induce genetic damages/changes in cells and organisms. Moreover, it is observed that during cell phone use, a relatively constant mass of tissue in the brain is exposed to the radiation at relatively high intensity (peak SAR of 4-8 W/kg). Apparently, several studies reported DNA damage at lower than 4 W/kg. This questions the wisdom of the IEEE Committee in using 4 W/kg as the threshold of effect for exposure-standard setting. Furthermore, since critical genetic mutations in one single cell are sufficient to lead to cancer and there are millions of cells in a gram of tissue, it is inconceivable that the base of SAR standard was changed from averaged over 1gm of tissue to 10 gm. (The limit of localized tissue exposure has been changed from 1.6 W/kg averaged over 1gm of tissue to 2 W/kg over10gm of tissue. Since distribution of radio frequency energy is non-homogenous inside tissues, this change allows a higher peak level of exposure). What actually needed is a better refinement of SAR calculation to identify 'peak values' of SAR inside the brain, genetic damages/changes in cells and organism.

The interpretation and understanding of experimental data from bio-electromagnetic research is that via free radical formation inside cells the electromagnetic fields affects the biological system. The free radicals kill cells by damaging macromolecules, such as DNA, protein and membrane. Moreover, several reports have indicated that electromagnetic fields (EMF) enhance free radical activity in cells [e.g., Lai and Singh, 1997a, b; 2004], particularly via the Fenton reaction [Lai and Singh, 2004]. The Fenton reaction is a catalytic process of iron to convert hydrogen peroxides, a product of oxidative respiration in the mitochondria, into hydroxyl free radical, which is a very potent and toxic free radical.

Neuronal Damage

The blood-brain barrier (BBB) is a dynamic interface that separates the brain from the circulatory system and protects the central nervous system from potentially harmful chemicals while regulating transport of essential molecules and maintaining a stable environment. The Blood-Brain Barrier originates from endothelial cells that line brain capillaries and functionally these transduce signals from the vascular system and from the brain. Moreover studies on young rats have shown that radio-frequency from mobile phones causes opening of the Blood-Brain Barrier and consequently leakage of albumin from blood vessels. Besides, in the exposed brains dark spots are observed and no such spots or leakage of albumin was noticed in the un-exposed animal brains

(control).Damage to this barrier can be detected by the presence of albumin in the brain. Moreover, exposed animals had scattered and grouped dark neurons often shrunken with loss of internal cell structures. The blood brain barrier and neurons are same in rats and human being.

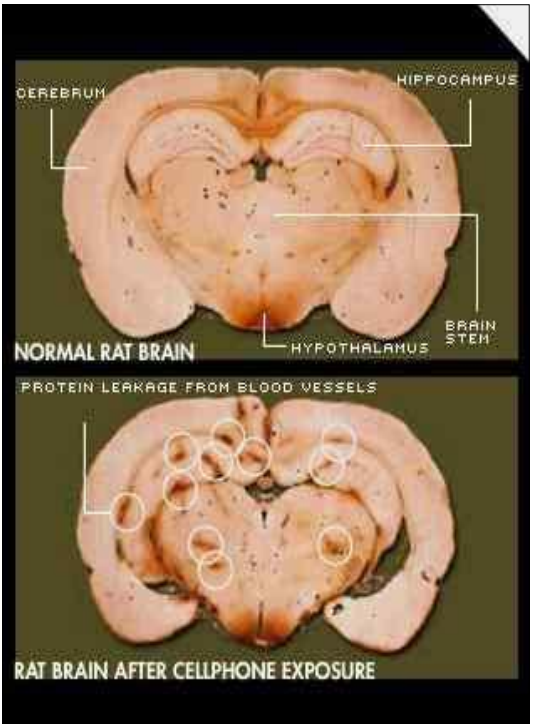


Fig:1- THE BLOOD-BRAIN BARRIER OF A NORMAL RAT BRAIN AND A RAT BRAIN EXPOSED TO EMR
Photo source-www.norad4u.com

Irreversible Infertility

According to recent studies, it has been confirmed that mobile phone radiation adversely affects male fertility. Moreover, studies done by the American Society for Reproductive Medicine (2006) reported that use of cell phones by men leads to degradation of semen quality, sperm count, motility, viability and normal morphology .Men who are continually exposed to mobile phone radiation have a sperm count of 59 million sperm per millilitre of seminal fluid compared to 83 million for men not continually exposed to mobile phone radiation. Likewise, motility of the sperm is inversely related to the exposure time to non-ionizing electromagnetic radiation. Studies have also the effects of the electromagnetic radiation passes on from one generation to another. In mice, after five generations were exposed to mobile phone radiation, they were unable to reproduce.

High Risk of Cancer and Melatonin Inactivation

Consistent use of mobile phones can cause cancer. Different studies have reported that the risk of brain cancer is highly increased due to the use of mobile phones beyond ten years. The risk is highest for ipsilateral (on the same side of the head where the instrument is held) exposure. As compared to the adults, children and teenagers below the age of 20 are five times more vulnerable to brain cancer, as their brain is not fully developed and radiation penetration is much deeper. Moreover, salivary gland tumors, lymphoma, facial nerve tumors, skin, blood, testicular and breast cancer have also been reported due to exposure to electromagnetic radiations. Heavy users of mobile phones (> 20 minutes per day) for a period of 10 years or more have been found with a significantly increased risk of some brain tumors.

Melatonin is a special hormone, which play a vital role in regulation of human growth and also display anti-cancer properties. The pineal gland is the primary source of this hormone. Moreover, melatonin is a powerful antioxidant, antidepressant and immune system enhancer that regulates our circadian rhythm. Melatonin levels rises everyday when we go to sleep. The free radicals in the cell are scavenged by this hormone which protects the DNA and prevents the cells from becoming carcinogenic. The daily sleep/wake cycle, blood pressure and heart rate cycle, metabolic rate and thermal regulation, hormone production and immune system activity all have a daily cycle regulated by melatonin directly or indirectly through the autonomic system. It has been proved that exposure to EMR reduces the levels of melatonin in animals and humans. Daily cellular telephone use of >25 minutes over years may lead to reduced melatonin production. Studies with animals show a reduction in melatonin levels following radiofrequency radiation exposure from cell phones and cell sites. Turning off the transmitters resulted in a significant increased melatonin levels within few days. Reduced melatonin is also associated with increased DNA damage and increased risk of cancer, arthritis, seasonally affective disorder (SAD), schizophrenia, increased eye stress, renal impairment), and increased risk of childhood leukemia.

Effects of EMR on Pregnant Woman and Children

Children are more vulnerable as compared to adults because of the following reasons

a. They have an immature immune system as compared to adults and moreover they are less effective against countering cancer growth.

b. Children's cells reproduce more quickly as compared to adults and consequently this makes cancers more deadly.

c. They absorb more energy than adults from the same phone owing to their smaller head and brain size, thinner cranial bones and skin, thinner, more elastic ears, lower blood cell volume, as well as greater conductivity of nerve cells and the energy penetrates more deeply. Tumors in the mid brain are more deadly than in the temporal lobe.

d. Children have longer exposure time.

A particular radiation hits head it penetrates the skull and in case of children the skull are thinner as compared to adults. A radiation penetrates the skull of an adult (25%), 10 year old (50%) and a 5 year old (75%).So, the younger the individual deeper the penetration of radiation. Children below sixteen are more prone to brain tumor. Brain tumor is the biggest causes of death amongst children. A research conducted in 2009 in Sweden reported 420 percent increase in getting tumors from cell phone radiation.

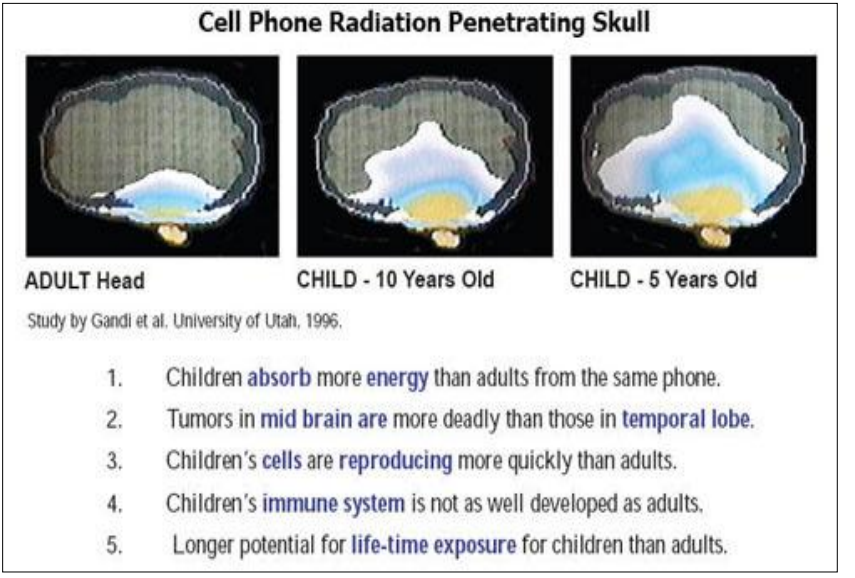


Fig:2- CELL PHONE RADIATION PENETRATING THE SKULL OF ADULT HEAD, CHILD (10 years old) and CHILD (5 years old)
Photo source- Study by Gandi et al. (1996)

The foetus of a pregnant woman is more vulnerable as the non-ionizing electromagnetic radiation continuously reacts with the developing embryo. Moreover, studies have shown that these non-ionizing radiations causes damage to the placental barriers (the membrane which prevents the passage of some materials between the maternal and the foetal blood protecting the foetus, implying that pregnant woman should avoid cell phone or use during emergency. Some of the other adverse effects on the pregnant woman are spontaneous abortion, congenital malformations and behavioural problems in their children.
The Russian National Committee on Non-Ionizing Radiation Protection says that use of the phones by both pregnant women and children should be "limited". It concludes that children who talk on the handsets are likely to suffer from "disruption of memory, decline of attention, diminishing learning and cognitive abilities, increased irritability" in the short term, and that long-term hazards include "depressive syndrome" and "degeneration of the nervous structures of the brain".

Conclusion

As one cannot see it or smell it, and its effects on health is noted after a long period of exposure, therefore the seriousness of the health hazards caused by electromagnetic radiation from cell phones and cell towers has not been realized by the common man. Consequently, the majority of the common man is reluctant for their personal protection. The policy makers are expected to enforce stricter radiations norms across the globe. Air pollution by automobiles is controlled using CNG driven vehicles, hybrid vehicles. Likewise, the solution to electro-pollution is the use of radiation shield, which absorbs around 10% to 50% of the radiation depending upon its placement and the direction of the source of radiation. Some radiation shield can absorb radiation up to 80% to 90%.

Acknowledgement

I acknowledge the contribution of Dr. Shantu Saikia, Assistant Professor, Department of Physics, St. Anthony's College, Shillong.

References

- Agarwal et al, "Relationship between cell-phone use and human fertility: an observational study", Oasis, The Online Abstract Submission System, 2006.
- Fucic , S. D. Horvat, V. Garaj-Vrhovac The correlation between the frequency of micronuclei and specific chromosome aberrations in human lymphocytes exposed to microwave radiation in vitro. *Mutat Res* 281(3):181-186, 1992.
- *K. Dhami, "Study of Electromagnetic Radiation Pollution in an Indian City", Environmental Monitoring Assessment (Springer), 184:6507–6512 (2012).*
- Rodriguez et. al., Regulation of antioxidant enzymes: a significant role for melatonin, J Pineal Res. Jan; 36(1):1-9, 2004 - http://www.ncbi.nlm.nih.gov/pubmed/14675124
- Gandhi G., Singh P.; Cytogenetic damage in mobile phone users: preliminary data. *Int J Hum Genet* 5(4):259-265, 2005.
- Gandhi et al., IEEE Transactions on Microwave Theory and Techniques, 1996
- Y. Belyaev, V.S. Shcheglov, Y.D. Alipov, V.A. Polunin, Resonance effect of millimeter waves in the power range from 10(-19) to 3 x 10(-3) W/cm2 on Escherichia coli cells at different concentrations. *Bio-electromagnetics* 17(4):312-321, 1996.
- I.Y. Belyaev, C.B. Koch, O. Terenius, Roxstrom-Lindquist K, L.O. Malmgren, L.G Salford, B.R Persson, H Sommer W, "Exposure of rat brain to 915 MHz GSM microwaves induces changes in gene expression but not double stranded DNA breaks or effects on chromatin conformation". *Bioelectromagnetics.* 27:295-306, 2006.
- ICNIRP- International Commission on Non-Ionizing Radiation Protection-Guidelines for limiting exposure to time-varying electric, magnetic, and

electromagnetic fields (up to 300GHz), Health Phys, 1998, 74:494-522.- http://www.icnirp.de/documents/emfgdl.pdf.

- Independent Expert Group on Mobile Phones, Report of the Group (The Stewart Report), Mobile Phones and Health, First issued 11 May 2000 - http://www.iegmp.org.uk/report/text.htm.
- Lai H., Singh N.P., Acute low-intensity microwave exposure increases DNA single-strand breaks in rat brain cells. *Bio-electromagnetics*16(3):207-210, 1995.
- Lai, H., Singh N.P., Single- and double-strand DNA breaks in rat brain cells after acute exposure to radiofrequency electromagnetic radiation. *Int. J. Radiat. Biol.* 69(4):513-521, 1996.
- Lai, H., Singh N.P., Melatonin and a spin-trap compound block radiofrequency electromagnetic radiation-induced DNA strand breaks in rat brain cells. *Bioelectromagnetics* 18(6):446-454, 1997a.
- Lai, H., Evidence For Genotoxic Effects (RFR AND ELF Genotoxicity), PhD Department of Bioengineering University of Washington Seattle, Washington USA, July 2007.
- Leif G. Salford., Effects of mobile phone radiation upon the blood-brain barrier, neurons, gene expression and cognitive function of the mammalian brain,2009,http://www.icems.eu/docs/brazil/Salford_abstract.pdf.
- M. Simkó, "Cell type specific redox status is responsible for diverse electromagnetic field effects", *Current Medicinal Chemistry*, 14, 1141-1152, 2007.
- M.A. Carino, Lai H, NP Singh , Naltrexone blocks RFR-induced DNA double strand breaks in rat brain cells. *Wireless Networks* 3:471-476, 1997.
- N. Sakuma, Y. Komatsubara, H. Takeda, M. Sekijima, T. Nojima, J. Miyakoshi, H. Hirose .DNA strand breaks are not induced in human cells exposed to 2.1425 GHz band CW and W-CDMA modulated radiofrequency fields allocated to mobile radio base stations. *Bioelectromagnetics.* 27:51-57, 2006.
- Narasimhan V, Huh WK, Altered restriction patterns of microwave irradiated lambdaphage DNA. *Biochem. Int.* 25(2):363-370, 1991.
- Neha Kumar and Prof. Girish Kumar, "Biological effects of cell tower radiation on human body", ISMOT/09/C/318 (12^{th} International Symposium on Microwave and Optical Technology;).
- Prof. Girish Kumar, "Report on Cell Tower Radiation", Submitted to Secretary, Department of Telecommunication, Govt. Of India, on December, 2010.
- V. S. Tanwar "Living dangerously in Indian cities: An RF radiation pollution perspective", Proceedings of the 9th International Conference on electromagnetic interference and compatibility (INCEMIC - 2006) 458-466.
- World Health Organization, ELF Health Criteria Monograph. Neurodegenerative Disorders, p. 187, 2007.

MICROSATELLITES: A USEFUL TOOL FOR GENETIC VARIATION AND CONSERVATION GENETICS STUDIES IN PLANTS

Bharati Jalan[a]

ABSTRACT

Advancement in molecular biology, have lead the scientific community to find the new ways of conserving the endangered species of plants using the microsatellite markers. The Simple Sequence Repeats (SSRs) microsatellite markers are co-dominant, hypervariable, abundant and uniformly distributed in the genome of every individual. Microsatellites are present in the non-coding region of the genomethat is highly susceptible to accumulate mutations. These mutations can produce a new allele at that locus. The habitat fragmentation, inbreeding depression and hybridization can result in the loss of the alleles at a particular position. Loss in the allele number of microsatellite helps the scientists to identify the endangered species and carry out the conservation programs to protect the endangered species. This type of study is highly informative to delineate effective strategies for genetic conservation, population inbreeding, phylogenetic analysis and genetic linkage mapping. In this review paper, we will discuss about the microsatellite marker system and its utilization in identification of polymorphic alleles among the individual of the population under study. Citrus species are extensively studied for their genetic diversity among the individual using microsatellite markers in order to conserve the different species of Citrus present in small regions of India.

Keywords: Conservation, microsatellite, homozygous alleles, heterozygous alleles, short simple repeats, inbreeding depression, genetic variation

INTRODUCTION

Advancement in the field of molecular biology has led the scientist to show their interest in the conservation of the endangered species of plants using the conservation genomics. The consequences of habitat fragmentation lead to decrease in the size of the population remaining, which results in the inbreeding depression and increase interpopulation distance. These result in decrease in gene flow among the population. The inbreeding can result in the loss of genetic variation, that is, the decrease in average number of alleles per locus (Keller *et al*. 2002). This leads to the increase in the homozygosity among the populations. As a consequence of the loss of genetic diversity owing to inbreeding depression, the evolutionary potential of the population to adapt to the changing environmental conditions is reduced resulting in the increased probability of extinction of the particular population (Ouborg *et al*. 2006). Therefore, genetic status

[a] Department of Biotechnology, St. Anthony's College, Shillong, Meghalaya.

of the population is an important parameter to be taken in consideration in conservation approach of the endangered species.

Genetic variations are result from both natural and anthropogenic impact on the environment. The genetic variation of populations and their mechanism of variation maintenance provide a powerful tool for detection of relationship among the species of the population under study. For the protection of biodiversity, the conservation genetics is of primary importance to avoid the extinction of most endangered species (Antiqueira, 2013).

CONSERVATION GENETICS

The main goal of Conservation Genetics is to give the information about the conservation of microsatellite alleles in the population which result in better understanding of population and evolutionary processes relevant to the conservation of endangered species. The knowledge about presence of genetic variation among the individual of the population are of great value in designing effective strategies for genetic conservations.

The conservation genetics can be used as tool for protection of endangered species. The studies of inbreeding depression and variation maintenance are important to achieve the goals of conservation genetics (Hedrick and Miller et al. 1992).

Genetic diversity in a population is a naturally occurring phenomenon which occurs due to mutation, recombination and gene flow and genetic drift, inbreeding and most types of natural selection (Keller *et al*. 2002).

The main tool to assess how genetic variability is distributed within and between populations is the molecular marker. A molecular marker is any molecular phenotype derived from an expressed gene or from a specific DNA segment corresponding to regions of the genome which may or may express as a phenotypic change (Antiqueira, 2013). The most widely accepted molecular marker for the study of conservation genetic of endangered species is microsatellite molecular markers.

MICROSATELLITES

Microsatellites are short tandem repeats consisting of 1-6 bases repeated in the nuclear genome of all eukaryotic cells. These microsatellitesarealso known as Simple Sequence Repeats (SSRs) or Short Tandem Repeats (STRs). Themicrosatellite markers can be analyzed by amplifying the regions containing simple short tandem repeats using the specific pairs of primers by Polymerase Chain Reaction (PCR) (Litt *et al*. 1989). The primer pairs used are complementary to the unique sequence (20-30 base pairs) flanking the microsatellites. The primer pair usedare forward primer (binds in 5'to 3' direction of one of the strand of the DNA duplex) and the reverse primer (binds in 5'to 3'of the opposite strand of the same DNA duplex) as shown in Figure1 below. In some of the species, unique flanking region of the microsatellite to which the complimentary primers binds are conserved across the species belonging the same family (Kijas *et al*. 1995).

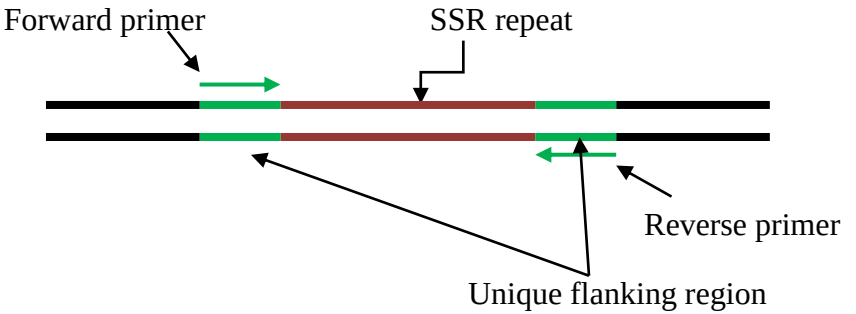


Figure1. Representation of SSR marker amplification using forward and reverse primers (green arrows) which base pair with the unique flanking regions (green region on DNA band) present at upstream (5'-3'region) of the simple repeat sequences (red region of DNA) (example: Dinucleotide repeats $(CA)_n$).

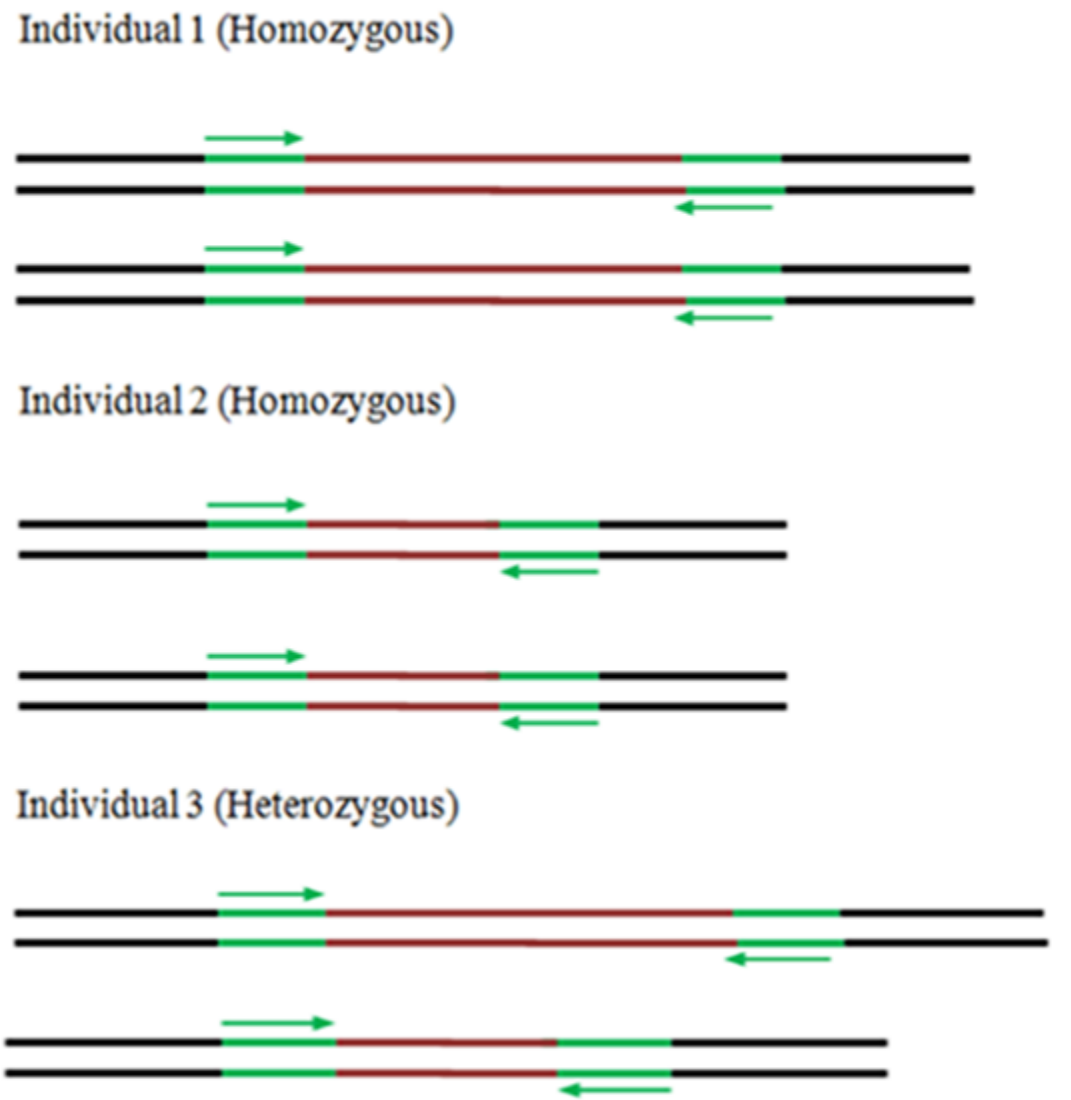
Microsatellite markers can be used as a DNA marker only if they are inherited in simple Mendelian fashion (Varshney *et al*. 2005). Each amplified segment has a different size and corresponds to a different allele from the same locus (Litt *et al*. 1996). They are abundant, codominant, hypervariable and uniformly distributed in the genome. Microsatellites are present in the non-coding regions of the genome and therefore are highly susceptible to the mutations forming the polymorphic genetic loci.The variation in the length of the repeat base pair is the basis of the study of microsatellite polymorphism and presence of heterozygosity among the population (Ellegren *et al*. 2004).

Polymorphic Loci give rise to Genetic Diversity

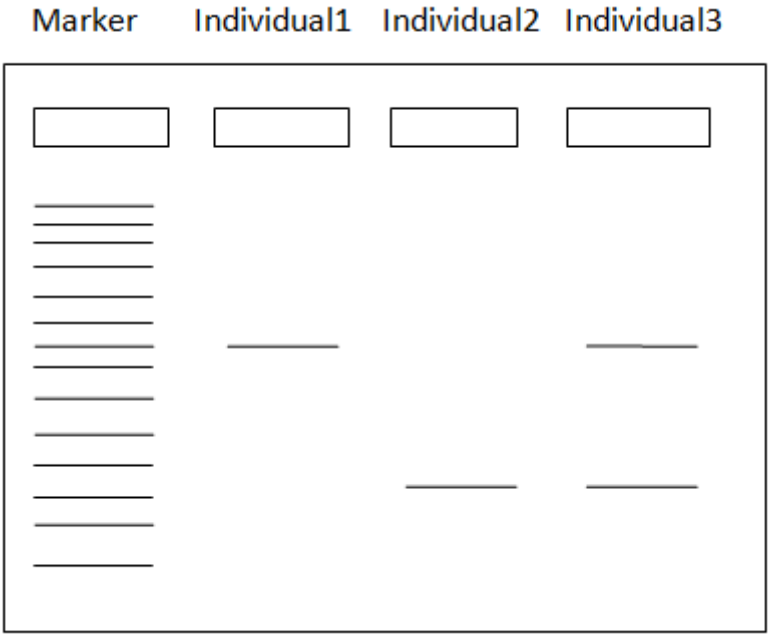
The microsatellite islands are unstable regions of the genome as they always susceptible to mutational changes like addition or deletion of the repeats due to replication slippage, gene flow, inbreeding depression and various anthropogenic activities (Schlotterer *et al*. 1992). The addition or deletion of short simple sequence repeats results in the difference in the length of the microsatellite repeats in terms of base pair. This difference in the repeat length produces a new locus (when the change in repeat length produce is homozygous in nature) or allele (when the change in repeat length produce is heterozygous in nature) at that position (Chase *et al*. 1996). Presence of more than one alleles for each locus is the result of genetic variations occurred among the population. The loss of these alleles is an important criterion to study the loss of genetic variation and presence of inbreeding depression in the population (Willi *et al*. 2006).

The rate of mutation can reach up to 1 per 1000 pairs of bases (Zucchi *et al*. 2003). This instability generates a large number of polymorphic loci, which is useful for

the study of populations and gene mapping, thus favouring a more complete coverage of the genome.



(A)



(B)

Figure 2. (A) The representation shows the detection of polymorphism present in the genome of all the three individuals using microsatellite as a marker. All the three individual mention above are have difference in their number of simple short sequence repeats and thus, have difference in their banding pattern and number and size (in Kb) ofthe band observed in the gel (figure B). (B) It represents the banding pattern of PCR amplified region of microsatellites. According to the size and number of the repeats in the amplified region these DNA bands varies in their distance from the well as the large number of repeats will be bigger in size (in Kb) and can travel a short distance then others and but the DNA fragment with low number of repeat are smaller in size and can travel long distance from the wells. These difference shows the polymorphism and diversity among the samples tested.

According to Frankel and Soule (1981:3), study of the genetic variability employing microsatellite markers must consider two different aspects of diversity: allelic richness (measured by the total number of SSRs alleles present in the population) and evenness in the frequency of different alleles in the population (measured by the coefficient of gene diversity).

According to Weir B.S and Cockerham, C.C (1984:1362), other indices which also can be taken in the consideration for are : (i) observed heterozygosity (indicator of genetic diversity), since each heterozygote carries different alleles andbest represents the variation in autogamous and allogamous populations; (ii) the percentage of polymorphic loci that is employed together with the number of alleles per locus, in

order to characterize and compare the levels of genetic variation in populations; (iii) and Wright's fixation index, which measures the excess or deficiency of heterozygote's.

Polymorphic Information Content (PIC)

The polymorphic information content (PIC), indicates the quality of the marker used. The PIC value ranges from 0 (monomorphic profile) to 1 (highly polymorphic profile). Markers with PIC values greater than 0.5 are consider more informative, whereas values between 0.25 and 0.50 indicate a moderate informative level. Values under 0.25 are considered less informative (Botstein *et al*, 1980).

According to Crandall *K*.A , Bininda-Emonds O.R.P, Mace G.M, Wayne R.K *et al*. (2000:393), the results obtained after the study of the PIC values present in the population are:(i) the identification and understanding of the sexual and reproductive system, (ii) the assessment of possible occurrences of hybridization or introgression in populations, (iii) the estimation of genetic risks of extinction, (iv) the definition of strategies of translocation/reintroduction of species in recovery programs, (v) the identification of vulnerable or conservation priority populations, (vi) the estimation of the effective population size, (vii) the determination of the minimum viable area of reserves and (viii) the identification of Evolutionary Significant Units and Management Units.

***Citrus* species are most extensively studied for their Genetic Diversity and Conservation Genetics**

Over 116 million tons of citrus fruit is produced every year. It is an extremely important crop worldwide. It is commonly grown in the tropical, subtropical, and borderline subtropical/temperate (Kahn *et al*. 2001). The genus *Citrus* L. belongs to the subtribe Citrineae, the tribe Citreae within the subfamily Aurantioideae of the Rutaceae family (Swingle *et al*. 1967). The three true species of citrus are citron (*Citrus medica*), pummelo (*Citrus maxima*), and mandarin (*Citrus reticulate*). Other important types of *Citrus* species such as *Citrus sinensis, Citrus limon,* are believed to originated from one or more generation of hybridization between these ancestral genera (Scora *et al*. 1975).

Currently,*Citrus* fruits have high level of morphological variations and various different fruit characteristic because of inter and intraspecific interaction. The genetic diversity within *Citrus* groups is relatively high because of the presence of adventitious nucellar embryo, and mode of sexual hybridization and cross pollination (Fusurato, 1957). The true species of *Citrus* like mandarins, pummelos, and citrons have higher levels of genetic diversity. Other species within the *Citrus* subgenus are hybrids derived from these true species, species of subgenus Papeda or closely related genera (Scora *et al*. 1975).

The loss of increasingly large numbers of plant species through habitat destruction threatens the availability of a diverse plant germplasm base which will be needed to feed future generations. Increase in the endangered species of Citrus had made the ecologist to employ *ex-situ* method of conservation.This method of conservation includesprocesses like seed storage, field gene banks and botanical gardens where the plants are conserved.The *in situ* method of conservation using

microsatellitemarkers maximizes the probability of success of the conservation programs, as this program uses the information present in the genome of the targeted species. The *in situ* method of conservation helps in the identification of the genetic diversity present in the genome of the targeted species (Izuno *et al*. 2012).

Understanding genetic diversity that is present in collections is required for the better management of conserved germplasm. Microsatellite SSR markers are very efficient in identifying the polymorphic alleles between the individuals of the *Citrus* species (Kijas *et al*. 1995 and Barkley *et al*. 2006). It will help us in determining which species to conserve as well as where to conserve, and it will also improve our understanding of the taxonomy and origin and evolution of plant species of interest.

CONCLUSION

Genetic variation of species is essential for population survival over generations, and the understanding of the distribution patterns of this variability allows us to comprehend the effects of habitat fragmentation, mutations, hybridization and inbreeding depression that put species at risk of extinction. Investigations of genetic variation using microsatellite markers have increased in recent years despite being severely limited by the high costs involved in the construction of genomic libraries and development of microsatellite markers for each of the targeted population under study. This technique has been proved beneficial by allowing the adoption of broad approaches for the understanding of genetic variation of species. In summary, the use of microsatellite markers has provided subsidies for conservation and management programs, thus allowing a sustainable use of resources.

The populations of endangered plant species have less genetic diversity than wild populations due to the founder bottleneck effect and/or other adverse effects (Robichaux *et al*. 1997; Krauss *et al*. 2002). In the endangered species, the numbers of the microsatellite alleles are lost due to inbreeding depression due to habitat fragmentation.

However, the application of microsatellites in plant species has been limited due to the associated difficulties such as technical details, cost, and the relatively time-consuming nature of obtaining these markers. Recently, different isolation protocols have been developed, and the cost and time involved in their isolation have been reduced (Zane *et al*. 2002). Thus, it is now possible for us to isolate microsatellite DNA. In order to prevent the extinction of critically threatened species, implementation of conservation measures such as restoration of habitat, establishment of *ex situ* populations, and reintroduction are required along with the *in situ* conservation programs (Guerrant *et al*. 2004; Vergeer *et al.* 2010).

The *in situ* method of conservation clearly indicate that both genetic and ecological information are essential for developing effective conservation programs for critically endangered species (Izuno *et al*. 2012). Analysis of the resulting genotypic data is likely to provide valuable information, such as the exact amount of genetic diversity in both wild and ex situ populations and the extent of inbreeding and

outbreeding (Kaneko *et al*. 2013). This information can help conservation managers to identify individuals that are of particular importance economically and maintain and recover the genetic diversity of the target species in order to conserve them.

REFERENCES

- Barkley N.A, Roose M.L, Krueger R.R, Federici C.T (2006). Assessing genetic diversity and population structure in a citrus germplasm collection utilizing simple sequence repeat markers (SSRs). *Theorotical and Applied Genetics* 112: 1519-1531.
- Botstein, D., *et al*. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *The American Journal of Human Genetics* 32(3):314-31.
- Chase M, Kesseli R and Bawa K (1996). Microsatellite markers for population and conservation genetics of tropical trees. *American Journal of Botany* 83(1): 51-57.
- Crandall K.A, Bininda-Emonds O.R.P, Mace G.M, Wayne R.K *et al*. (2000). Considering evolutionary processes in conservation biology. *Trends in Ecology and Evolution* 17:390-395.
- Ellegren, H (2004). Microsatellites: simple sequences with complex evolution. *Nature reviews genetics* 5 (6):435-445.
- Frankel, O.H, Soulé, M.S (1981). Conservation and evolution. Cambridge: *Cambridge University Press.*
- Fusurato K. (1957). Studies on polyembryony in Citrus. *Journal of Kihara Inst. Biol. Res*., 8:40–48.
- Guerrant E.O, Havens K, Maunder M (2004*). Ex situ* plant conservation: supporting species survival in the wild. *Island Press*, Washington,DC 1:504
- Hedrick P.W and Miller P.S (1992). Conservation Genetics: Techniques and Fundamentals. Ecological Applications, *Ecological Society of America* 46 (2):30-46.
- Izuno A, Takamiya M, Kaneko S, Isagi Y (2012). Genetic variation and structure of the endangered Lady Fern Athyrium viridescentipes based on ubiquitous genotyping. *Journal of Plant Res* 125:613−618.
- Kahn T.L, Krueger R.R, Gumpf D.J, Roose M.L, Arpaia M.L, Batkin T.A, Bash J.A, Bier O.J, Clegg M.T, Cockerham S.T, *et al*. (2001). Citrus genetic resources in California: Analysis and recommendations for long-term conservation. *Genetic Resources Conservation Program* 22:1-58.
- Kaneko S, Abe T, Isagi Y (2013). Complete genotyping in conservation genetics, a case study of a critically endangered shrub, Stachyurus macrocarpus var. prunifolius (Stachyuraceae) in the Ogasawara Islands, Japan. *J Plant Res* 126 (5):635-642.
- Keller L.F, Waller D.M (2002). Inbreeding effect in wild populations. Trends Ecology Evolution 17: 230−241.
  Kijas J.M.H, Fowler J.C.S and Thomas M.R (1995). An evaluation of sequence tagged microsatellite site markers for genetic analysis within *Citrus* and related species. *Genomes* 38:349-355.

- Krauss S.L, Dixon B, Dixon K.W (2002). Rapid genetic decline in a translocated population of the endangered plant Grevillea scapigera. *Journal of Conservation Biology* 16:986−994.
- Lia Maris Orth Ritter Antiqueira (2013). Application of microsatellite molecular markers in studies of genetic diversity and conservation of plant species of Cerrado. *Journal of Plant Sciences* 1:1-5.
- Litt, M, and Luty, J (1989). A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. *American Journal of Human Genetics* 44(3): 397-408.
- Liu, J., Sun, Z., Chen, Y. and Jiang, J. (2012). Isolation and characterization of microsatellite loci from an endangered tree species, *Toonaciliata* var. pubescens. *Genetics and Molecular Research* 11 (4):4411-4417.
- Ouborg N.J, Vergeer P, Mix C (2006). The rough edges of the conservation genetics paradigm for plants. *Journal of Ecology* 94:1233−1248.
- Robichaux R.H, Friar E.A, Mount D.W (1997). Molecular genetics consequences of a population bottleneck associated with reintroduction of the Mauna Kea silversword (Argyroxiphium sandwicense ssp. sandwicense [Asteraceae]). *Journal of Conservation Biology* 11:1140−1146.
- Schlotterer C, Tautz D (1992). Slippage synthesis of simple sequence DNA, *Nucleic Acids Res* 20(2): 211–215.
- Scora R.W (1975). On the history and origin of *Citrus*. *Bull Torr Bot Club* 102:369–375.
- Swingle W.T and Reece P.C (1967). The botany of *Citrus* and its relatives. The Citrus industry. *University of California Press, Berkeley* 1:190-430.
- Varshney R.K, Graner A and Sorrells M.E (2005). Genic microsatellite markers in plants: features and applications. *Trends in Biotechnology* 23(1):1-8.
- Vergeer P, Ouborg N.J *et al.* (2010). Habitat fragmentation, climate change, and inbreeding in plants. *Annals of the New York Academy of Sciences* 77: 84-98.
- Weir B.S, Cockerham, C.C (1984). Estimating F-statistics for the analysis of population structure. *Evolution* 38(6):1358-1370.
- Willi Y, Van Buskirk J, Hoffmann A.A (2006) Limits to the adaptive potential of small populations. *Annual Revolution Ecology Evolution* 37:433−458.
- Zane L, Bargelloni L and Patarnello T (2002). Strategies for microsatellite isolation: A review. *Molecular Ecology* 11:1-16.
- Zucchi, M.I *et al*., (2003). Genetic structure and gene flow in *Eugenia dysenterica* DC in the Brazilian Cerrado utilizing SSR markers, *Genetics and Molecular Biology* 26(4)1:449-457.

STEM CELL: A POTENTIAL APPLICATION IN HUMAN REGENERATIVE MEDICINE

Asmita Singha[a]

ABSTRACT

After the first isolation of mouse embryonic stem cells, it is now possible to isolate and culture the stem cells from embryos and adult tissues of many species, including humans. Aiming at facilitating the body to form new functional tissue to replace the lost or defective tissues, the use of stem cells as regenerative medicine has a potential whose main purpose would be to provide therapeutic treatment for conditions where the presently available therapies are inadequate or sometimes do not have the power to overcome the concerned condition. Inspite of the rapid progress that we have achieved in this field, several important questions in this area pertaining to embryonic and adult stem-cell research are still to be answered. Certain issues such as, what combination of molecules confers differentiation to specific cell types? How can we isolate stem cells from different tissues? And how will knowledge of the mechanisms that underlie self-renewal and differentiation help us to develop patient-specific therapies, need to be addressed through thorough investigation.

Keywords: Stem cell, cell based therapy, regenerative medicine, tissue engineering and Pluripotency

Introduction

Self-renewal and capacity of maintaining long-term proliferation are the two major properties of stem cells [Bajada *et. al*. 2011]. Human embryonic stem (hES) cells can proliferate extensively in culture and can differentiate into representatives of all three embryonic germ layers in vitro and in vivo [Lebkowski, J., Gold, J., Xu, C., Funk, W., Chiu, C. and Carpenter, M. (2000)]. When they are provided with suitable specific conditions, they are capable of differentiating into a diverse population of mature and also functionally specialized cell types. Embryonic stem (ES) cells are pluripotent and they can differentiate into all germ layers, whereas, non-Embryonic stem cells are multipotent that means their potential to differentiate into different cell types is more limited. The most important properties in stem cell that are must for it to be a regenerative medicine are the ability for potency, the ease of isolating these cells and the possibility to generate generations to expand them.

Regenerative medicine aims at facilitating the body to form new functional tissue to replace lost or defective ones. The main objective of this would be to provide therapeutic treatment for conditions where the currently available therapies are inadequate or sometimes lack the power to overcome the concerned condition.

[a] *Department of Biotechnology, St. Anthony's College, Shillong, Meghalaya.*

Regenerative medicine includes science pertaining to both tissue engineering and stem cell technology. When we talk about tissue engineering, the concept of Y.C. Fung who first used this term in 1985 comes to our mind, which gradually drew the world's attention towards 'tissue' being the fundamental level of investigating living organisms, between cells and organs. Langer and Vacanti in an article published in *Science* wrote "Tissue engineering is an interdisciplinary field that applies the principle of engineering and the life sciences toward the development of biological substitutes that restore maintain or improve tissue function."

Now we are aware of the fact that the ability to propagate pluripotent ES cells present unique opportunities for experimental analysis of gene regulation and function during self-renewal, cell commitment, and differentiation. Therefore, when harnessed effectively, ES cell differentiation can provide defined cell populations for pharmacological testing and cellular transplantation. Progress is also being made towards understanding and in turn controlling lineage- and/or cell-type-specific differentiation of ES cells in vitro.

Stem Cell Derivatives and Their Uses for Cell-Based Therapies

For ages, therapeutics has been limited only to the surgical removal of damaged tissues or treatment with pharmacological therapies to ameliorate symptoms and fight infection. Cellular insufficiency or deficiency, due to dysfunction or degeneration, respectively, is the root of diseases such as heart failure, neurodegenerative disorders, diabetes, bone marrow failure, and spinal cord injury [Yabut *et al*., 2011, Bernstein *et al*., 2011]. Therefore, the concept of restoring the function of the tissues is considered to be a better step rather than replacing the damaged or the missing cells with new functional cells. That is when we look up to the ES cells. Thus, the first step would be to isolate and derive specific cell populations from human embryonic stem cells (hESCs) that could serve in either replacing the damaged or non-functional cells to function normally. Success in such case would ultimately lead us to a very promising strategy. With the successful derivation of hESCs, it would therefore be possible to produce 'lineage-restricted' progenitors that are capable of differentiating into specialized cell types such as cardiomyocytes, pancreatic islet cells, chondrocytes, hematopoietic cells, or neurons. An addition to this, these cells no matter how many times they divide indefinitely, they are inexhaustible large scale source of specific progenitors [Yabut *et al.*, 2011, Bernstein *et al.*, 2011].

hESCs in Tissue Engineering

hESCs provide much promise in tissue engineering and regeneration since hESCs can act as an inexhaustible in vitro source of differentiated cell type. The potential use of hESCs in tissue engineering include organ substitutes, vascularisation, and ex vivo cartilage/bone construction. In regard to cell transplantation, successful tissue engineering would depend on the generation of the appropriate cell type that is able to provide normal cellular function. Use of hESCs to treat lung injury has been an

area of active investigation. A significant step toward directed differentiation of lung-specific cells was reported by Wang et al., in which genetically modified hESCs carrying lung-specific reporters under the control of promoters from tissue-specific genes such as surfactant protein C, aquaporin 5, and T1α, resulted in the purification of type I and type II alveolar epithelial cells. When these cells were grafted into a mice suffering from acute lung injury, these cells terminally differentiated in vivo into type I and type II alveolar epithelial cells and exhibited functional properties that include the capacity for gas exchange.

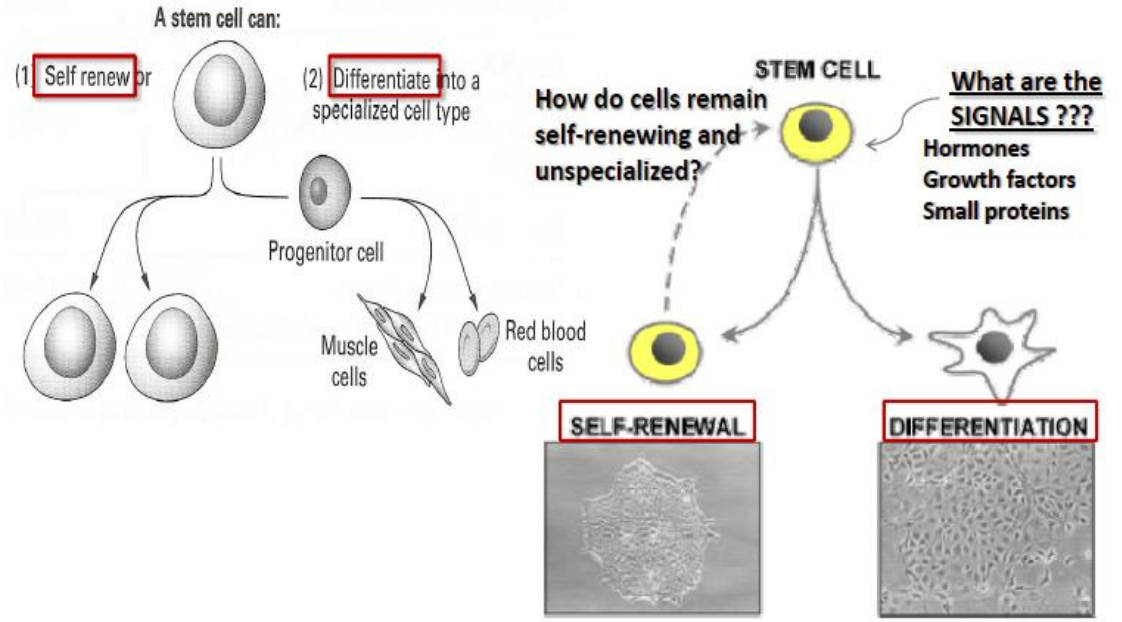


Fig 1. Basic characteristics of Stem Cells
[Source: Adapted from Stem Cells and Cloning by Kelly A. Hogan]

Bone defects due to congenital and acquired causes such as trauma, surgery and tumours may lead to extensive bone loss and defects which require transplantation of bone tissue or substitutes to restore structural integrity and function at the same time. The current 'gold standard' is the use of bone grafting [Meister, K., Segal, D. and Whitelaw, G. (1990); Bajada *et. al* 2011]. However, the supply of suitable bone is limited especially in osteoporotic, paediatric and oncological patients and also these grafting results in additional morbidity at the site, leading to pain, or infection. Since, bone grafting is not so effective in all cases, experimental and clinical evidence, that is supportive of the efficacy of MSCs in enhancing bone formation and healing of bone defects has been carried out.

Pluripotent cells can differentiate into neural tissue which includes neurons, astrocytes and oligodendrocytes. An inherent capacity for regeneration is well suggested by the presence of endogenous stem cells in the mammalian spinal cord. Axonal

regeneration and functional recovery after spinal cord injury has been shown in certain animal models under research. Akiyama *et al*. 2002 found that Mesenchymal Stem Cells can remyelinate spinal cord axons after direct injection into the lesion.

A strategy to increase axonal regeneration could involve transplantation of stem cells into the injured spinal cord. A clinical study on five patients with acute SCI was conducted by Park *et. al.*, these were treated by bone marrow derived cells and granulocyte-macrophage colony stimulating factor (GM-CSF). GM-CSF is a signalling molecule that induces proliferation and differentiation of bone marrow cells. Perhaps it also leads to propagation of endogenous neural stem cells. There was no complication observed and improvement in the sensory and motor function was shown. However, even with these upcoming research work from different parts of the world, controlled clinical trials are still needed to understand the complete process of stem cell therapy in case of spinal cord damages.

Conclusion

Today even with so much work on stem cells and with the discovery of the huge potential that hESCs have, the international scientific society needs to answer questions pertaining to the use of stem cell as a regenerative medicine. Not to forget the ethical issues concerned with the use of hESCs and the scientific challenges together with legislative issues have to be addressed. However, even with these challenges it is very encouraging to see the various clinical trials carried out and the ones that are still undergoing with hESCs. Bajada *et. al*. 2011 in his paper said that current laboratory and animal trials are studying the possibility to introduce stem cell therapy to clinical practice for regeneration in muscular dystrophy, cerebral infarcts and transplantation medicine. Therefore, these studies and the one already done will floor a way towards shaping the therapeutic assistance of hESCs in regenerative medicine.

References

- Akiyama, Y., Radtke, C. and Kocsis, J. (2002). Remyelination of the rat spinal cord by transplantation of identified bone marrow stromal cells. The journal of neuroscience, 22(15), pp.6623--6630.
- Bajada, S., Mazakova, I., Ashton, B.A, Richardson, J.B. and Ashammakhi, N. 2008.Stem Cells in Regenerative Medicine.Topics in Tissue Engineering, Vol. 4.
- Lebkowski, J., Gold, J., Xu, C., Funk, W., Chiu, C. and Carpenter, M. (2000). Human embryonic stem cells: culture, differentiation, and genetic modification for regenerative medicine applications. Cancer journal (Sudbury, Mass.), 7, pp.83--93.
- Meister, K., Segal, D. and Whitelaw, G. (1990). The role of bone grafting in the treatment of delayed unions and nonunions of the tibia. Orthopaedic review, 19(3), pp.260--271.
- Yabut, O. and Bernstein, H. S. 2011.Human Embryonic Stem Cells in Regenerative Medicine. *Springer*, pp. 17--38.

H. OBOKATA AND CO-WORKERS DISCOVER "ACID WASH" TECHNIQUE TO TURN REGULAR CELLS INTO STEM CELLS

Arnab Das[a]

ABSTRACT

Low pH stress (pH 5.7) reprogrammed CD45^{+} cells which were isolated from lymphocyte fractions of postnatal spleens of C57BL/6 mice carrying Oct4-GFP transgene. Exposure to near lethal stress resulted in cellular reprogramming and expression of cellular pluripotency related markers such as Oct4, Nanog, Sox2 etc. This phenomenon is called stimulus-triggered acquisition of pluripotency (STAP), hence they are called STAP cells. STAP cells when injected in mice embryos also resulted in birth of chimeras, hence showing germline transmission and epigenetic regularity.

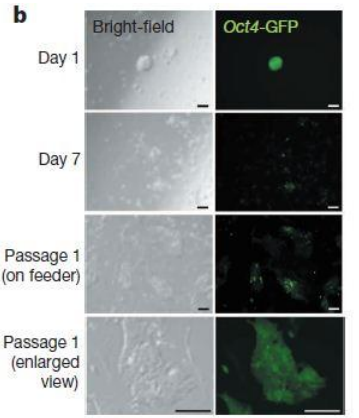
Keywords: Stimulus-triggered acquisition of pluripotency (STAP), Low pH, Oct4, Nanog, E-Cadherin

A new line of stem cells was generated by a unique cellular reprogramming phenomenon called *stimulus-triggered acquisition of pluripotency* (STAP), by a team of researchers in *RIKEN, Center for Developmental Biology, Kobe, Japan*.

The main question under investigation was whether somatic cells can reprogramme themselves simply in response to some strong external stimuli without the need of nuclear manipulation. Obokata in an interview with nature stated that her inspiration to induce pluripotent cells from regular somatic cells came from a plant, the carrot. In dissociated carrot cells, mature somatic cells convert into immature blastema cells, from which a whole plant can develop in the presence of auxins. A challenging question for Obokata and her team was whether animal somatic cells behaved in the same manner.

This novel method required neither nuclear transfer, nor introduction of several transcription factors. Various methods were utilized by Obokata and her team to physically alter the environment of the somatic cells in culture. This included squeezing the cells through a pipette, starving the cells for a long time etc., until low pH exposure strategy caught the attention of the research team.

[a] *Department of Biotechnology, St. Anthoy's College, Shillong, Meghalaya.*



Fgf4 treatment promoted the generation of flat cell clusters that expressed Oct4-GFP at moderate levels (right). Top and middle: days 1 and 7 of culture with Fgf4, respectively. Bottom: culture after the first passage. (Source: Nature 505 (7485), pp. 641—647)

Strong external stimuli such as low pH stressor (pH 5.7) reprogrammed the $CD45^{+}$cells which were isolated from the lymphocyte fraction of postnatal spleens of 1 week old C57BL/6 mice carrying an *Oct4-gfp* transgene. When examined for activation of Oct4 promoter after culture in suspension the surviving cells showed positive signals for *Oct4-gfp*, suggesting that they have been reprogrammed into stem cells, rather STAP cells as called by the researchers. These cells showed a distinctive small cell size with petite cytoplasm and also showed a distinct fine structure of the nucleus as compared with parental $CD45^{+}$ cells. A week after the low pH exposure these cells expressed many pluripotency related protein markers such as *Oct4, SSEA1, Nanog, E-cadherin* and gene markers such as *Oct4, Nanog, Sox2 ,Ecat1, Esg1, Dax1 and Rex1* as seen in Embryonic Stem (ES) cells.

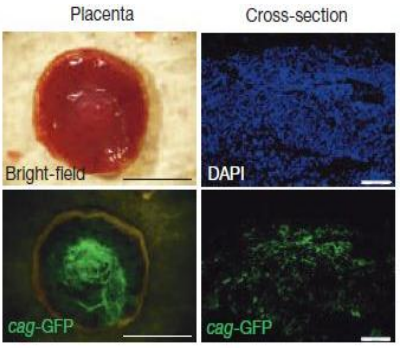


Figure: Placental contribution of Fgf4-induced stem cells (FI-SCs) (genetically labelled with constitutive GFP expression). In addition to placental contribution, Fgf4-induced stem cells contributed to the embryonic portion at a moderate level. (Source: Nature 505 (7485), pp. 641—647)

The STAP cells derived from $CD45^+$ cells of neonatal mice were also injected in mice embryos for blastocyst injection assay. Chimeric progenies were born at a considerable rate and developed normally. These off springs also demonstrated germline transmission and epigenetic regularity. In a supplemental video it was also demonstrated that these STAP cells have the developmental capacity to differentiate into all somatic cell lineages as well as germ line cells, thus having higher flexibility than ES cells and adult stem cells. In conclusion, STAP cell discovery holds a great promise in making regenerative medicine and stem cell therapies cheaper and effective.

To mention the stir caused by Obokata et al., the paper came under attack by the scientific community a few months after the paper was published in *Nature*. Obokata was accused for scientific misconduct and plagiarism for the work she published. *RIKEN* set up an investigation committee to probe into the matter and in a preliminary report cleared off the scientists, as they found no proof of any scientific misconduct. Many of the senior authors of the paper, most notably Charles A. Vacanti, of *Brigham and Women's Hospital, Havard Medical School* still supported the paper and its authors saying the work done is authentic and there is no reason to retract the paper from the journal. It still remains to be seen whether the allegations are true or just an effort to taint the image of *RIKEN* scientists.

References

- Blogs.nature.com. 2014. *Confusion as institute releases report on controversial acid-bath stem-cell papers: Nature News Blog*. [online] Available at: http://blogs.nature.com/news/2014/03/confusion-as-institute-releases-report-on-controversial-acid-bath-stem-cell-papers.html [Accessed: 20 Mar 2014].
- Obokata, H., Sasai, Y., Niwa, H., Kadota, M., Andrabi, M., Takata, N., Tokoro, M., Terashita, Y., Yonemura, S., Vacanti, C. A. and others. 2014. Bidirectional developmental potential in reprogrammed cells with acquired pluripotency. *Nature*, 505 (7485), pp. 676-680.
- Obokata, H., Wakayama, T., Sasai, Y., Kojima, K., Vacanti, M. P., Niwa, H., Yamato, M. and Vacanti, C. A. 2014. Stimulus-triggered fate conversion of somatic cells into pluripotency. *Nature*, 505 (7485), pp. 641-647.
- Times, L. 2014. *Institution to discuss probe into STAP stem cell controversy*. [online] Available at: http://www.latimes.com/science/sciencenow/la-sci-sn-riken-to-discuss-investigation-into-stap-cell-controversy-20140313,0,7779979.story [Accessed: 20 Mar 2014].

HYDROCARBON DEGRADING MICROORGANISMS AND OIL SPILLS

•**Nibir.R.Parasar, Mitusella Basumatary and Amarjyoti Saikia**

ABSTRACT

Microorganisms used to reduce or minimize the hazardous waste concentration from contaminated sites. Presently accepted disposal methods of incineration or burial insecure landfills turn out to be expensive when amounts of contaminants are large. Mechanical and chemical methods generally used to remove hydrocarbons from contaminated sites are limited effective and expensive. But the Bioremediation is now used extensively for the biodegradation of hydrocarbon contamination resulting from the activities related to the petrochemical industry, which may refer to complete mineralization of organic contaminants into carbon dioxide, water, inorganic compounds, and cell protein or transformation of complex organic contaminants to other simpler organic compounds, by biological agents like microorganisms .Many indigenous micro-organisms in water and soil are capable of degrading hydrocarbon contaminants. This paper emphasizes on the mechanism by which micro-organisms degrades hydrocarbons.

Keywords: Microorganisms, Hydrocarbons, Hazardous Waste Concentration

Introduction

In the modern world petroleum and petroleum products play an inseparable part in our day to day lives. With the increase in pollution level and harmful effects of various constituents towards the environment need has arose to overcome harmful effects that arise from oil spills to unattended petroleum reservoirs. In such conditions, an eco-friendly process to degrade such harm are the microbial army, 'OIL EATING MICROBES'. When we talk about microbes that are able to clean up oil, we are talking primarily about bacteria and fungi. Bacteria can break down oil to carbon dioxide and water. Different compounds that make up oil can only be biodegraded by communities of microorganisms acting in concert. Some bacteria can degrade several hydrocarbons or a class of hydrocarbons. Crude oil (petroleum) is a highly complex mixture of organic compounds of which some 1.3 million litres enters the environment each year. More than anything else, oil-shipping disasters, such as of the *Exxon Valdez*(1989),*the Erika* (1999) and *the Prestige*(2003),have captured the public attention to this environmental problem. However, these accidents account for only a small part of the annual global release of crude oil, as most enters the environment from deliberate discharge and processing sites. Around three million tons of oil enters the sea each year, of which about 20% originates from oil-pumping operations, transport and refining activities and 25% from non-tanker shipping and natural seepages. More than half

• *Department of Biotechnology, St. Anthony's College, Shillong, Meghalaya.*

(55%) originates from illegal activities that include the dumping of ballast water and oil residues as well as accidents (Golyshin et al., 2003).Hydrocarbons are also produced continuously by living cells as natural oils and fats (de Lorenzo, 2006).The observation that the oceans are not covered with an oily layer is a testimony to the activity of the hydrocarbon degrading micro-organisms (Head et al., 2006).Several bacteria are even known to feed exclusively on hydrocarbons (Yakimov et al., 2007). For these (facultative) hydrocarbon degraders the occasional super-tanker oil spill forms an occasional carbon banquet. They play an important role in the clean-up after an oil spill and form the biological basis for the natural oil-degrading capacity of the eco-system. In this article, we deal with the special class of hydrocarbon degrading bacteria and their mechanisms.

1. **Chemical Composition of Crude Oil**

Crude Oil or Petroleum, is simply unprocessed oil found deep beneath the earth's surface. It can range in colour from clear to black and can be found as a liquid or solid. Overall properties of crude oils are dependent upon their chemical composition and structure. Crude oil is pumped and stored in barrels for future refinement. The refinement process may involve filtering, addition of additives, and specialized separation techniques to create specific crude oils and crude oil products. Crude oils are made up of both hydrocarbon compounds (accounting 50-98% of the total composition) and non-hydrocarbon compounds (containing Sulfur,oxygen, nitrogen and trace elements).The main hydrocarbons found in crude oil are Aliphatics, Alicyclics, and Polycyclic Aromatic Hydrocarbons (PAH) for example- Napthalene,Asphaltene,etc. Petroleum components may be classified into four major groups based on their differential solubility inorganic solvents-

A. Saturated Hydrocarbons- These include normal and branched alkanes with structures of C_nH_{2n+2} (aliphatics) and cyclic alkanes with structures of C_nH_{2n} (alicyclics),which range in chain length from one carbon to over 40 carbons. Saturates usually are the most abundant constituents in crude oils.

Aliphatics and Alicyclics Properties:

a) Quickly broken down by natural processes.
b) Residence time in environment is less than a day.
c) Straight chain or ring carbon structures with weak bonds.
d) Low fluorescence characteristics .

B. Aromatic Hydrocarbons- These Include monocyclic aromatics (e.g., benzene, toluene, and xylenes) and polycyclic aromatic hydrocarbons (PAHs)(e.g., naphthalene, anthracene, and phenanthrene),which have two or more fused aromatic rings. PAHs are of particular environmental concern because they are potential carcinogens or may be transformed into carcinogens by microbial metabolism.

Polycyclic Aromatic Hydrocarbons (PAH) Properties-

a) Most abundant of the main hydrocarbons found in crude oils.
b) Many are toxic.

c) Can be carcinogenic to plants and animals.
d) Difficult to separate from water using regular filtering techniques making them a potential human health hazard s.
e) 6-sided carbon rings which contain strong bond .
f) Prolonged breakdown by natural processes.
g) Highly fluorescent aromatic characteristics allow researchers to easily detect PAH's using fluorescence techniques.

These Polycyclic Aromatic Hydrocarbons(PAH) have to be degraded to overcome the harmful consequences resulting from oil spills in marine and terrestrial enviroment.

C. Resins- These include polar compounds containing nitrogen, sulfur, and oxygen (e.g., pyridines and thiophenes).They are often referred to as NSO compounds.

D. Asphaltenes- These consist of poorly characterized high molecular weight compounds that include both high molecular weight and poorly characterized hydrocarbons and NSOs. Metals such as nickel, vanadium, and iron are also associated with asphaltenes.

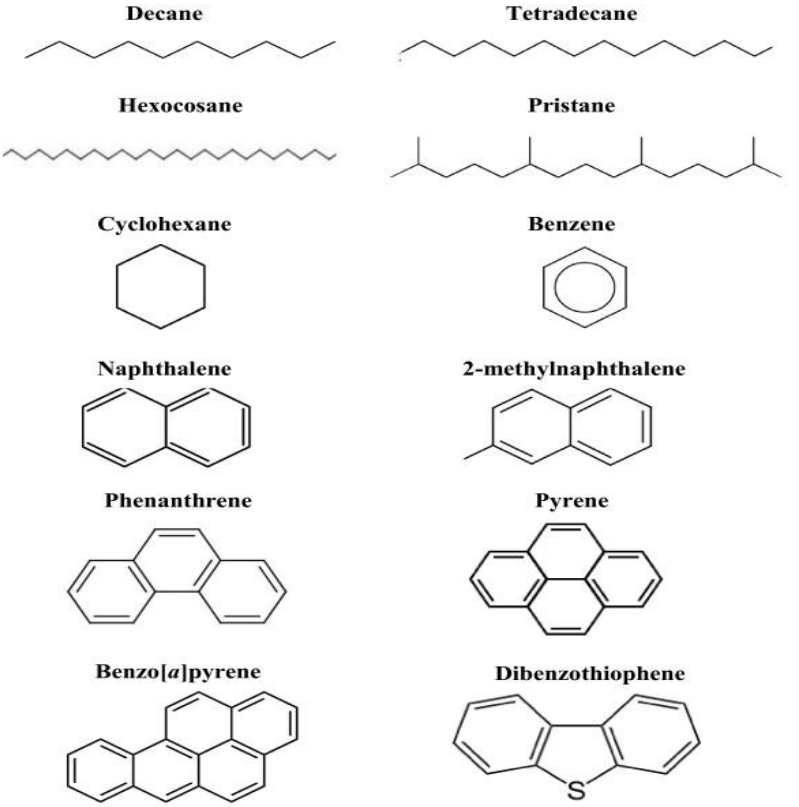


Fig-1:Structure of Aliphatic hydrocarbons (Decane,Tetradecane,Hexocosane,Pristane), Aromatic Hydrocarbons(Benzene),Polycyclic Aromatic Hydrocarbon (Naphtalene,2methylnaphthalene,Phenanthrene,Pyrene,Benzo(a)pyrene,Dibenzothiopene) And Alicyclic Hydrocarbon (Cyclohexane).(Source-www.fas.org)

2. **Bio-Degradation**

"Transformation of a substance into new compounds through biochemical reactions or the actions of microorganisms such as bacteria." - U.S. Geological Survey, 2007.

According to the Office of Technology Assessment (OTA, 1990),current mechanical methods typically recover no more than 10-15 percent of the oil after a major spill.Bioremediation has emerged as one of the most promising secondary treatment options for oil removal since its successful application after the 1989 *Exxon Valdez* spill (Bragg et al. 1994; Prince et. al. 1994). Bioremediation has been defined as "the act of adding materials to contaminated environments to cause an acceleration of the natural biodegradation processes" (OTA, 1991).This technology is based on the fact that large percentage of the oil components are readily biodegradable in nature.The success of oil spill clean-up technology depends on the ability to establish and maintain conditions that favor enhanced oil biodegradation rates in the contaminated environment.The two main approaches to oil spill bioremediation are-

- Bioaugmentation- Here known oil-degrading bacteria are added to supplement the existing microbial population.
- Biostimulation- Here the growth of indigenous oil degraders is stimulated by the addition of nutrients or other growth-limiting cosubstrates, and/or by alterations in environmental conditions (e.g. surf-washing, oxygen addition by plant growth etc.)

Table-1:**Representative microorganisms capable of degrading petroleum hydrocarbons**

BACTERIA	FUNGI
Achromobacter	*Aspergillus*
Acinetobacter	*Candida*
Alcaligenes	*Cladosporium*
Arthrobacter	*Penicillium*
Bacillus	*Rhodotorula*
Brevibacterium	*Sporobolomyces*
Cornybacterium	*Trichoderma*
Flavobacterium	
Nocardia	
Pseudomonas	
Vibrio	

3. **Mechanism of Oil Bio-Degradation: A Microbial Perspective–**

Hydrocarbons are only degraded in water. Bacteria and fungi do not grow in hydrocarbons, they grow on water in or surrounding the hydrocarbons. All hydrocarbons are soluble to some extent. This is why the process of degradation is able to start and then continue. Without the presence of water, oil is not degraded. Emulsion

formation is an important part of hydrocarbon degradation. There are two kinds of emulsion-

A) Oil-in-water emulsion- These are common and occur when small quantities of oil are present in large quantities of water. Droplets of oil are suspended in water.

B) Water-in-oil emulsion- These are less common and occur when large quantities of oil are present in water.

The damage caused by oil spills in marine or freshwater systems is usually caused by the water-in-oil emulsion. A thick layer of crude oil on the surface of water will take up about 50% water by weight and remain a free-flowing, oily liquid. It can spread over the water surface to form an oily film. Eventually, it will disperse and be degraded. When the water content reaches about 80% by weight, the consistency of the emulsion changes - it becomes a thick semi-solid mass with a grease-like consistency. It is often called "chocolate mousse" at this stage because of both its consistency and its brown colour. This process does not readily occur with fuel oils such as diesel oil and kerosene, but occurs easily with light and heavy crude oils - the usual cargoes of oil tankers.

TABLE-2: Enzymes Involved In Degradation Of Petroleum Hydrocarbons-

ENZYMES	SUBSTRATES	MICROORGANISMS
Soluble Methane Monooxygenase	C_1–C_8 alkanes alkenes and cycloalkanes	*Methylococcus,Methylosinus,Methylocystis Methylomonas*
Particulate Methane Mono-oxygenases	C_1–C_5 (halogenated) alkanes and cycloalkanes	*Methylobacter, Methylococcus*
AlkB related Alkane Hydroxylases	C_5–C_{16} alkanes, fatty acids, alkyl benzenes, cycloalkanes and so forth	*Pseudomonas,Burkholderia,Rhodococcus,Mycobacterium*
Eukaryotic CytochromeP450	C_{10}–C_{16} alkanes, fatty acids	*Candida maltose,Candida tropicalis,Yarrowia lipolytica*
Bacterial P450 oxygenase system	C_5–C_{16} alkanes, cycloalkanes	*Acinetobacter, Caulobacter, Mycobacterium*
Dioxygenases	C_{10}–C_{30} alkanes	*Acinetobacter sp.*

[**Cytochrome P450-**The key difference between eukaryotic and prokaryotic cytochromes P450 is that the former enzymes are integral membrane proteins which are anchored by an N-terminal transmembrane helical segment whereas the latter are generally soluble, cytoplasmic enzymes. Although *E.coli* does not possess its own cytochrome P450, it has been found that the eukaryotic P450 is heterologously expressed in the bacterium are catalytically active due to electron transfer from a bacterial redox system.]

4. **Biochemistry of Hydrocarbon Degradation-** The common types of hydrocarbon degradation reactions involved are:

A). **Hydroxylation at C1-** In this reaction the substrate n-alkane is oxidized to the corresponding alcohol by substrate-specific terminal mono-oxygenases or hydroxylases. The alcohol is further oxidized to corresponding aldehyde, and finally converted into a fatty acid which is conjugated to CoA and subsequently processed by β-oxidation to generate acetyl CoA.

B). **Hydro-peroxidation-** In this reaction aliphatic hydrocarbons undergo oxidation to peroxides, which are further oxidized to carboxylic acids. This reaction is catalyzed by mono-oxygenases.

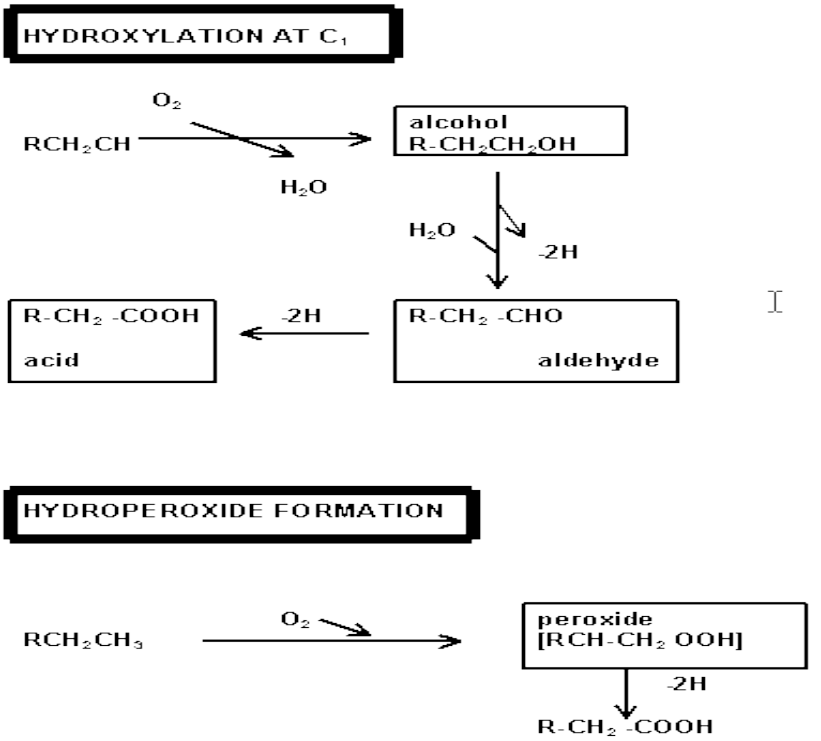


Fig-2-: Hydroxylation At C_1 and Hydro-peroxidation

C)**. Sub-terminal reactions-** Sub-terminal oxidation has also been described for both short and long-chain alkanes. Initial sub-terminal oxidation in most of the microorganism are catalyzed by enzymes viz. -: (1) propane monooxygenase (C3), (2) different classes of butane monooxygenase (C2-C9), (3) CYP153 monooxygenases (C5-C12), (4) AlkB-related non-heme iron monooxigenase (C3-C10 or C10-C20), (5) flavin-binding monooxigenase AlmA (C20-C36), (6) flavin-dependent monooxygenase LadA (C10-C30), (7) copper flavin-dependent dioxygenase (C10-C30) .

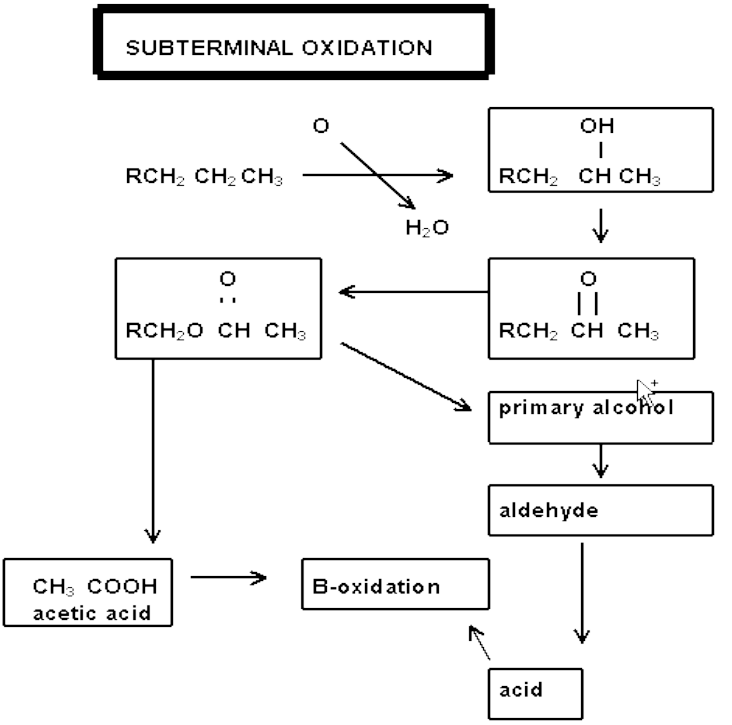


Fig-3:Sub-terminal reactions

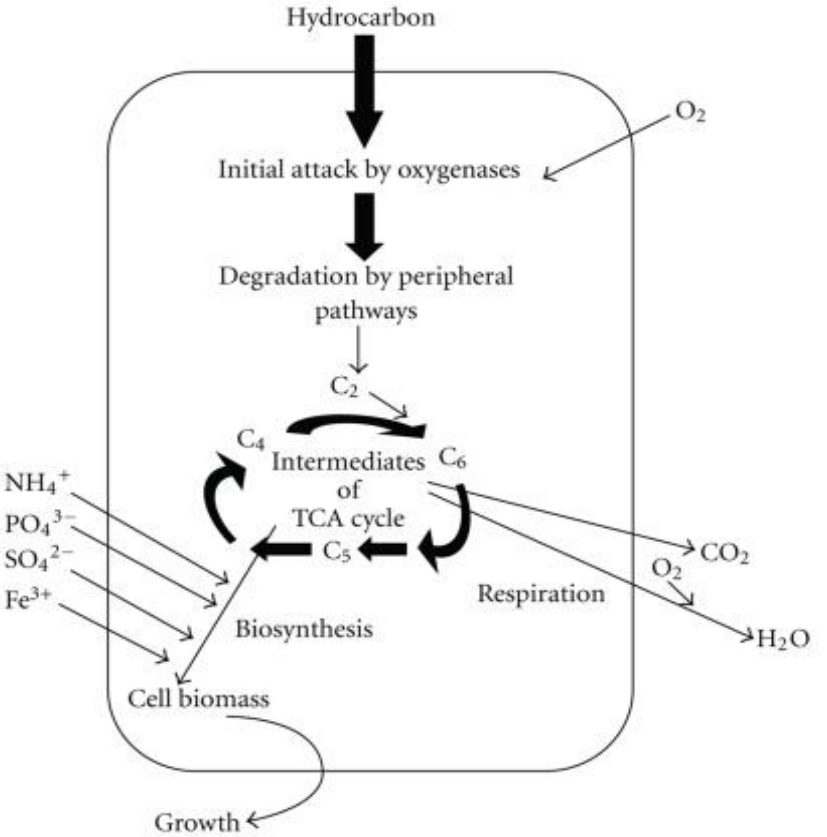


Fig-4:The general scheme of aerobic hydrocarbon degradation process involved in the micro-organism (Diagram Source-www.fcgi.com)

The initial intra-cellular reaction of organic pollutants is an oxidative process and oxygenases and peroxidases are the key enzymes involved in the activation and the activation and incorporation of oxygen. Organic pollutants are converted step by step into intermediates of the central intermediary metabolism through peripheral degradation pathway, for example, the tri-carboxylic acid cycle. Central precursor metabolites viz. acetyl-CoA, succinate, pyruvate are utilized for biosynthesis of cell biomass. Gluconeogenesis synthesizes the sugars required for various biosynthesis and growth.

5. **Uptake of Hydrocarbon by Bio-surfactants**

Bio-surfactants are heterogeneous group of surface-active complex chemical compounds produced by a wide variety of microorganisms. Surfactants enhance solubilizations and removal of contaminants. The rate of Biodegradation is also enhanced by surfactants due to increased bioavailability of pollutants. These organic bio-surfactants, released by microorganisms, when come in contact with oil forms

micelles by a process called emulsification (fig-5). Consequently, the oil surface area for bacteria to utilize the oil is increased by the bio-surfactants. *Pseudomonas* are the best known bacteria capable of utilizing hydrocarbons as carbon and energy sources and producing bio-surfactants. Among *Pseudomonads*, *P.aeruginosa* is widely studied for the production of glycolipid type biosurfactants. However, glycolipid type bio-surfactants are also reported from some other species like *P.putida* and *P.chlororaphis*.

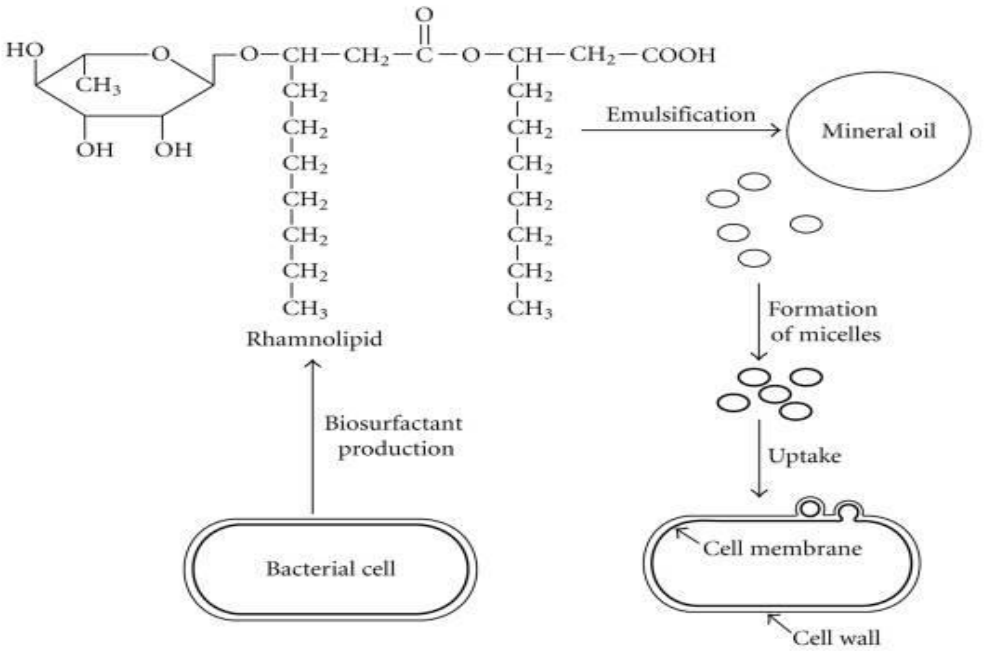


Fig-5: Role of Bio-surfactants and Micelle Formation (Diagram source www.redorbit.com)

6. **Genetically Modified Bacteria**

Genetically modified bacteria display high degradation capacity. GEM is not yet tested in the field because of certain environmental and ecological concerns. GEM can be applied for bioremediation process monitoring, stress response, strain monitoring, toxicity assessment and end-point analysis.

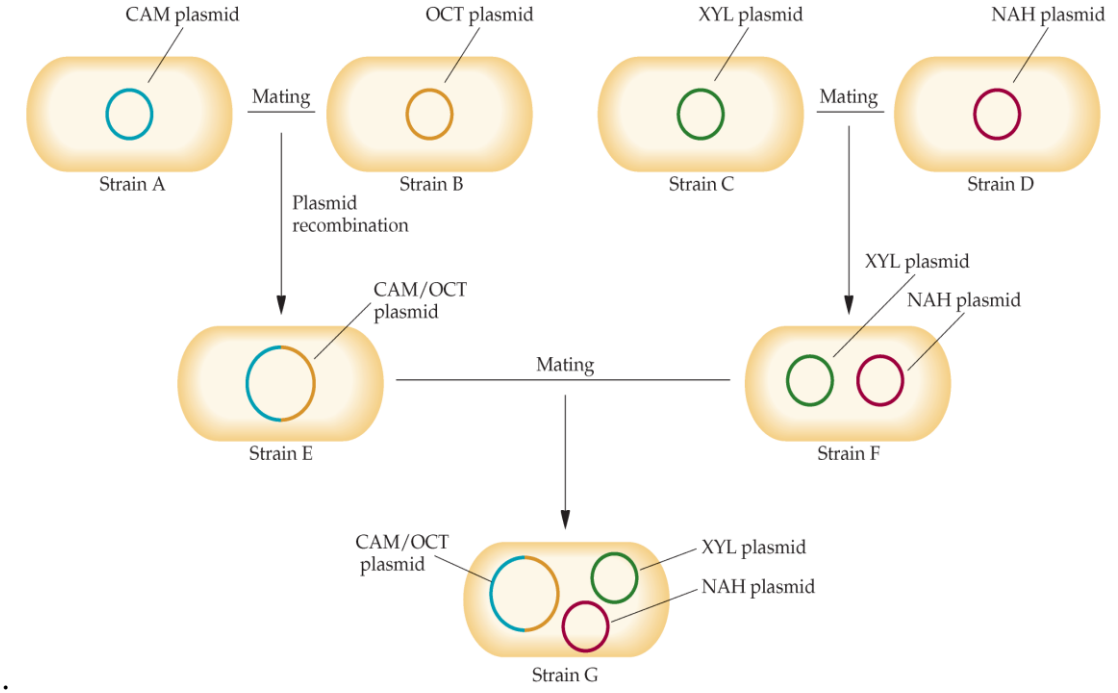


Fig-6: Chakrabarty et al. (1980) developed and patented a "superbug" that degraded petroleum (camphor, octane, xylene, and naphthalene) by plasmid transfers. (source-www.scielo.br)

As shown in fig-5, four strains of *Pseudomonas putida* having plasmids (containing genes for) viz. Xylene degrading (XYL),naphthalene-degrading (NAH),octane-degrading (OCT) and camphor-degrading (CAM) are `conjugated so as to create a single strain of Pseudomonas with expanded degradative capabilities allowing degradation of xylene, naphthalene, octane and camphor. Chakrabarty et al. introduced plasmids from different strains of *Pseudomonas* into single cell and the resulting bacterium was capable of consuming the four types of hydrocarbons present in oil. This strain of *Pseudomonas* with enhanced degradation capabilities is named as "Super-bug".

7. Conclusion

Cleaning up of petroleum hydrocarbons in the subsurface environment is a real world problem. A better understanding of the mechanism of biodegradation has a high ecological significance that depends on the indigenous microorganisms to transform or mineralize the organic contaminants. Microbial degradation process aids the elimination of spilled oil from the environment after critical removal of large amounts of the oil by various physical and chemical methods. This is possible because microorganisms have enzyme systems to degrade and utilize different hydrocarbons as a source of carbon and energy.

The use of genetically modified bacteria represents a research frontier with broad implications. The potential benefits of using genetically modified bacteria are significant. The contribution of Dr.Ananda Mohan Chakravarty in the field of oil degrading superbug is commendable in this field although it has not been commercially used so far.

Therefore, based on the present review, it may be concluded that microbial degradation of petroleum components can be considered as a key component in the clean-up strategy for petroleum hydrocarbon remediation.

References

- September, 2001,U.S. Environmental Protection Agency Office of Research and Development National Risk Management Research Laboratory Land Remediation and Pollution Control Division 26 W. Martin Luther King Drive Cincinnati, OH 45268. *Guidelines for the bio-remediation of the marine shore lines and fresh water wetlands.*
- P.Jokuty, Whiticar, S.P., Z.Wang, M. Fingas, P. Lambert , B Fieldhouse , and J.Mullin (2000) *A Catalogue of Crude Oil and Oil Product Properties*. Environmental Protection Service,Environment Canada, Ottawa, ON. http://www.etcentre.org/spills/oil_intr.html.
- Jones, J.G. and Edington, M.A. (1968) *An ecological survey of hydrocarbon oxidizing microorganism*. J. Gen. Microbiol., 52, 381-390.
- Hose, J. E., McGurk, M. D., Marty, G. D., Hinton, D. E., Brown, E. D., and Baker, T. T. (1996).Sublethal effects of the *Exxon Valdez* oil spill on herring embryos and larvae: morphological, cytogenetic, and histopathological assessments, 1989-1991. *Canadian Journal of Fisheries and Aquatic Science*, 53, 2355-2365.
- Hommel, R.K. (1990) Formation and physiological role of biosurfactants produced byhydrocarbon-utilizing microorganisms: biosurfactants in hydrocarbon utilization. *Biodegradation*, 1, 107-119.
- Jahangeer and Kumar Vikram , International Journal of Engineering and Technical Research (IJETR) ISSN: 2321-0869, Volume-1, Issue-8, October 2013 . *An Overview on Microbial Degradation of Petroleum Hydrocarbon Contaminants*
- Mahmound, Aziza Y.,Abdeltif A., and Rachida M., *Biosurfactant production by Bacillus strain injected in the petroleum reservoirs*, Journal of Industrial Microbiology & Biotechnology, vol. 35, pp. 1303–1306, 2008.
- U. K. Lee, Tremblay G.H, J.Gauthier, S. E. Cobanli, and M.Griffin, ―Bioaugmentation and biostimulation: a paradox between laboratory and field results,‖ in Proceedings of the International Oil Spill Conference, pp. 697–705, American Petroleum Institute,WashingtonDC,USA,1997.

- Scheuer,T.Zimmer,D.Becher,F.Schauer, and W.H.Schunck,-*Oxygenation cascade in conversion of n-alkanes to α,ω-dioic acids catalyzed by cytochrome* P45052A3,Journal of Biological Chemistry, vol. 273,no.49,pp.32528–32534, 1998.
- Pornsunthorntawee, Wongpanit P., S. Chavadej, M. Abe, and R. Rujiravanit, ―*Structural and physicochemical characterization of crude biosurfactant produced by Pseudomonas aeruginosa SP4 isolated from petroleum—contaminated soil,Bioresource Technology*, vol. 99, no. 6, pp. 1589–1595,2008
- J.W Rob Brooijmans, I.Margreet Pastink, J.Roland Siezen1,3,5;Laboratory of Microbiology, Wageningen University,6703HB Wageningen, The Netherlands 5 CMBI, Radboud University Nijmegen, 6500HB Nijmegen, The Netherlands.[a]BiotechnolResInt.2011;2011:941810. Publishedonline,sep13,2010.doi:10.4061/2011/941810 *Hydrocarbon-degrading bacteria: the oil-spill clean-up crew*

About the Journal :

Spectrum: Science and Technology is a peer reviewed open access research journal of St. Anthony's College, Shillong, India. It is published annually by the Research Cell of the college in both print and e-print.

The primary goal of the journal is to publish double blind peer reviewed articles from the fields of Science and Technology. The journal looks for articles that can share knowledge, new, modified or systematically arranged, and promote substantive dialogue among the research scholars of the world.

The journal follows the styles of citation and referencing as per the instructions laid down in the college website. Manuscripts should be prepared strictly as per the specifications of the journal. The complete specifications of the journal are available at www.anthonys.ac.in. The journal accepts only soft copy of the articles for reviewing and publications which can be sent at ***spectrum.st@anthonys.ac.in***

All other correspondence related to the journal may be addressed to:

The Chief Editor
Spectrum: Science and Technology
St. Anthony's College
Shillong, Meghalaya, India
PIN - 793001

SPECTRUM

Science and Technology

St. Anthony's College, Shillong, established in 1934, became the first institute of higher education of the Don Bosco Society world-wide. The college was accredited by NAAC with the rank of Five Stars in 2000. It was also awarded the status of College with Potential for Excellence by UGC in 2006 and the same was renewed in 2011. The college was re-accredited with Grade 'A' (3.60/4.00 CGPA) by NAAC in 2009. With more than 75 years of experience and expertise, St. Anthony's College, Shillong excels as one the top colleges in the country.

SPECTRUM: SCIENCE AND TECHNOLOGY Vol. 1, 2014