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CURRENT RESEARCH TRENDS IN WEST BENGAL COLLEGES VOLUME I

EDITORS
Dr. Arunima Biswas
Dr. Madhumita Majumder
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EDITORS

Dr. Arunima Biswas

Assistant Professor, Department of Microbiology, Raidighi College

Dr. Madhumita Majumder

Assistant Professor, Department of Botany, Raidighi College

Dr. Shakuntala Ghorai

Assistant Professor, Department of Microbiology, Raidighi College

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CHIEF ADVISOR

Dr. Sasabindu Jana

Principal, Raidighi College

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PREFACE

This book, partly sponsored by Raidighi College and edited by faculty members of Raidighi College, aims to focus on the recent areas of interest that are being explored by the different research laboratories located in various colleges across West Bengal. Most of these colleges severely lack modern research facilities and adequate funding; but the enthusiastic and undaunted researchers are overcoming all hurdles to study traditional as well as emerging and trending topics that range from basic to applied science and cover contemporary and cross-cutting issues like environment, bio-diversity, sustainability, as well as health, nutrition, modern agriculture and medicine. Raidighi College, itself, is a rural institute located in the remote, backward region of Sundarbans in West Bengal. In spite of being an undergraduate college, it has been tirelessly trying to foster a strong research culture and to enhance capacity building, with constant support and encouragement from Principal Dr. Sasabindu Jana, the Governing body and the Internal Quality Assurance Cell of the college. With financial support from the Department of Higher Education, Science and Technology and Biotechnology, Government of West Bengal & from Raidighi College, an extremely successful one-day National Seminar was organized on "Recent Trends and Scopes of Modern Biology" which brought into light the highly potential and productive research activities going on in several colleges of the state. The decision was taken to bring together through a series of books all such brilliant ideas, investigations. discoveries and their future prospects that are stemming from the state colleges. This is the first volume of the series.

ACKNOWLEDGEMENT

Financial support from the Department of Higher Education, Science and Technology and Biotechnology, Government of West Bengal & from Raidighi College is gratefully acknowledged. Constant motivation, guidance and support of chief patron, Principal Dr. Sasabindu Jana are greatly appreciated. Heartfelt thanks are due to the Governing Body, IQAC and Research Committee of the college. Warm gratitude is expressed to all contributing authors and to Bhumi Publishing House. Sincere gratitude is conveyed to each and every individual who has been associated directly or indirectly with the publication of this book.

Editors

FROM THE EDITORS

Among the developing countries that are racing to achieve scientific excellence in this age, India is a leading contestant today. Soon after its independence, the country set out to gradually build a strong foundation in modern science. Initially, Biology did not have an equal footing with Physical Science, Mathematics, Medicine and Engineering, which were considered as higher scientific endeavours than studying general Life Sciences. But the past few decades have seen tremendous changes in the Bioscience sector in India, as it continually provided a more complete understanding of complex biological systems and an ability to manipulate such systems for human benefit. Appreciating the important role that biology is predicted to play in the near future, the Indian Government started expanding and setting up several biological research institutes, thus progressively decreasing the earlier large disparity in funding, and support facilities between the top research institutes in India and the western countries. Sadly, the situation is quite different in state colleges and most universities where faculty members often have to combat crumbling basic infrastructure, such as lack of reliable electricity, running water, high speed internet, etc. Insufficient incentive for setting up a successful research program, in addition to mandatory teaching and administrative duties, are discouraging factors. There is hardly any scope of research in undergraduate colleges which is extremely de-motivating for its faculty members having doctoral degrees and post-doctoral experiences. Inadequate faculty strength, negligible funding, unsatisfactory laboratory and technological facilities add to the challenges. In spite of these hurdles, many college and university teachers from the state of West Bengal are actively involved in modern biological research. This book is the first of a series highlighting the recent study interests and research trends in West Bengal colleges. This current volume is a collection of a few research papers that reflect high-quality science seeking to elucidate the molecular and cellular basis of biological processes ranging from prokaryotic to complex higher eukaryotic systems and also addresses issues like biodiversity and environmental challenges and health and nutrition.

We are grateful to our Principal Dr. Sasabindu Jana who motivated us to take up this initiative and to our funding agencies and to all the authors.

TABLE OF CONTENT

SPORE-POLLEN DIVERSITY RECORDED FROM HOLOCENE SEDIMENTS OF LOWER BENGAL BASIN, INDIA IN DEDUCING DEPOSITIONAL ENVIRONMENT

Argha Sarkar
Department of Botany, Bangabasi College, Kolkata
*Corresponding author E-mail: argha.sarkar09@gmail.com

Abstract:

A rich assemblage of spores and pollen have been recovered from C^{14} dated Holocene sediments collected at close intervals of one profile each from Kumirmari (KUI), Dakshin Harishpur (DHI), Taldi (TDI) and Canning (CNI) of Indian Sundarbans area in Lower Bengal basin and on this basis, pollen diagrams of each section have been constructed. Six Regional Pollen (P) assemblage zones of Lower Bengal basin, India (LBb) have been established from the pollen diagrams viz. LBb. P-I to LBb. P-VI. These regional pollen assemblage zones reveal six environmental phases of deposition i.e. Phase I Mixed brackish water and fresh water tidal mangrove with regular inundation (c. 11, 000 – c. 9271 yr BP), Phase II Tidal mangrove with regular inundation (9271±41 - 7687±38 yr BP), Phase III *Phoenix* dominated mangrove upland (7687±38 – c. 6000 yr BP), Phase IV Swampy mangrove vegetation (4450±170 – c. 3500 yr BP), Phase V Brackish water mixed fresh water *Heritiera* forest (c. 3500 – c. 3000 yr BP) and Phase VI Supra-tidal fresh water swamp with abundance of *Acrostichum aureum* (c. 3000 – 1970±80 yr BP).

Keywords: Holocene; lower bengal basin; pollen assemblage zone; depositional environment

Introduction:

As early as in 1918, Von Post (1) established the climatic change corresponding to glacial and interglacial phases by percentage pollen analysis through initiation of the science of Palynology and examining the Quaternary peat bog sediments of Scandinavia.

But much earlier in 1906, Hutington (2) studied the palynological aspect of Indian Quaternary sediments of interglacial phase from Pangong lake, Ladak. The Quaternary palaeoclimatic condition related to glacial, interglacial phases in the Karewa of Kashmir was also interpreted by Wodehouse (3) through study of pollen.

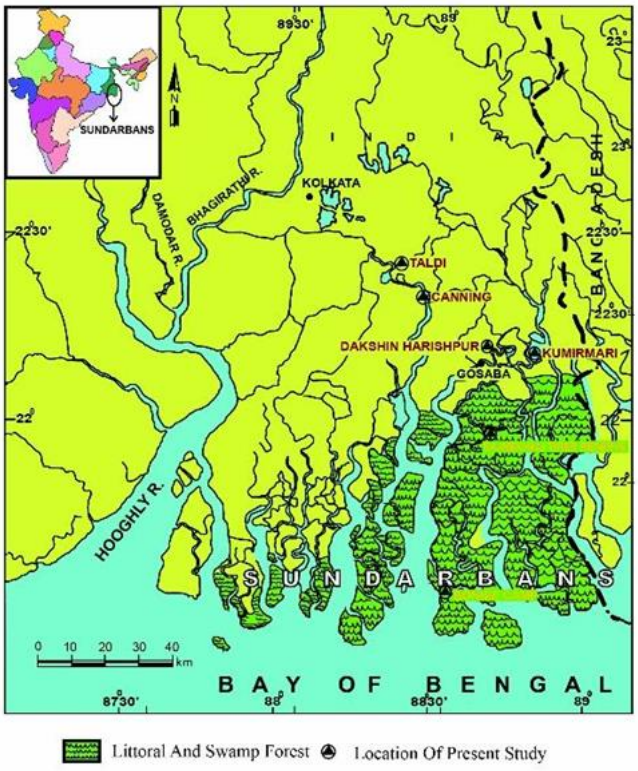
Subsequently and in recent years extensive study of the Pleistocene and Holocene sediments throughout the world have been made to unravel the history of the origin and migration of the forest and climatic history of the immediate past in South America (4; 5; 6; 7; 8; 9; 10), North and Central America (11; 12; 13), Australia (14; 15; 16; 17; 18), Europe (19; 20),

New Zealand (21; 22), Egypt (23), Africa (24, 25), Asia (26; 27; 28; 29a,b; 30; 31), Bengal basin (32a,b; 33; 34; 35; 36, 37; 38; 39;40; 41;42).

The present study deals with a rich assemblage of spores and pollen recovered through analysis of samples collected at close intervals of one profile each from Kumirmari (KUI), Dakshin Harishpur (DHI), Taldi (TDI) and Canning (CNI). The samples are from known depth and C^{14} dated. The results have been plotted to understand the trend of biosphere-eco-climate and phytogeographical changes since late Quaternary (9271±41 to 1970±80 yr BP) in Lower Bengal basin.

Area of investigation:

The location of present investigation viz. Kumirmari near Bagna forest ($22^0$12’18.5148” N Lat. $88^0$56’11.7528” E Long.), Dakshin Harishpur, Kachukhali, near Gosaba ($22^0$13’3.3204” N Lat. $88^0$49’11.1468” E long.), Taldi ($22^0$12’40.104” N Lat. $88^0$23’35.214” E Long.) and Canning (22^0 18' 37.3422" N Lat. 88^0 39' 28.4292" E Long.), south-east of Kolkata, West Bengal of Lower Bengal basin are 20 to 60 Km. inland from the seashore and ±1 m above mean sea level (Fig. 1).



Text fig. 1: Area of investigation

Material:

The material for the study of microscopic biological remains viz., pollen and spores are collected from freshly excavated lithosections. All possible precautions are taken to avoid contamination. The sample interval has been suitably reduced for peat and peaty clay (10 cm)

and increased for clay sediments (20-30 cm). Sample position along with C^{14} data of sediments collected from the sections at Kumirmari (KUI), Dakshin Harishpur (DHI), Taldi (TDI) and Canning (CNI) are enumerated in Text figs. 2-5.

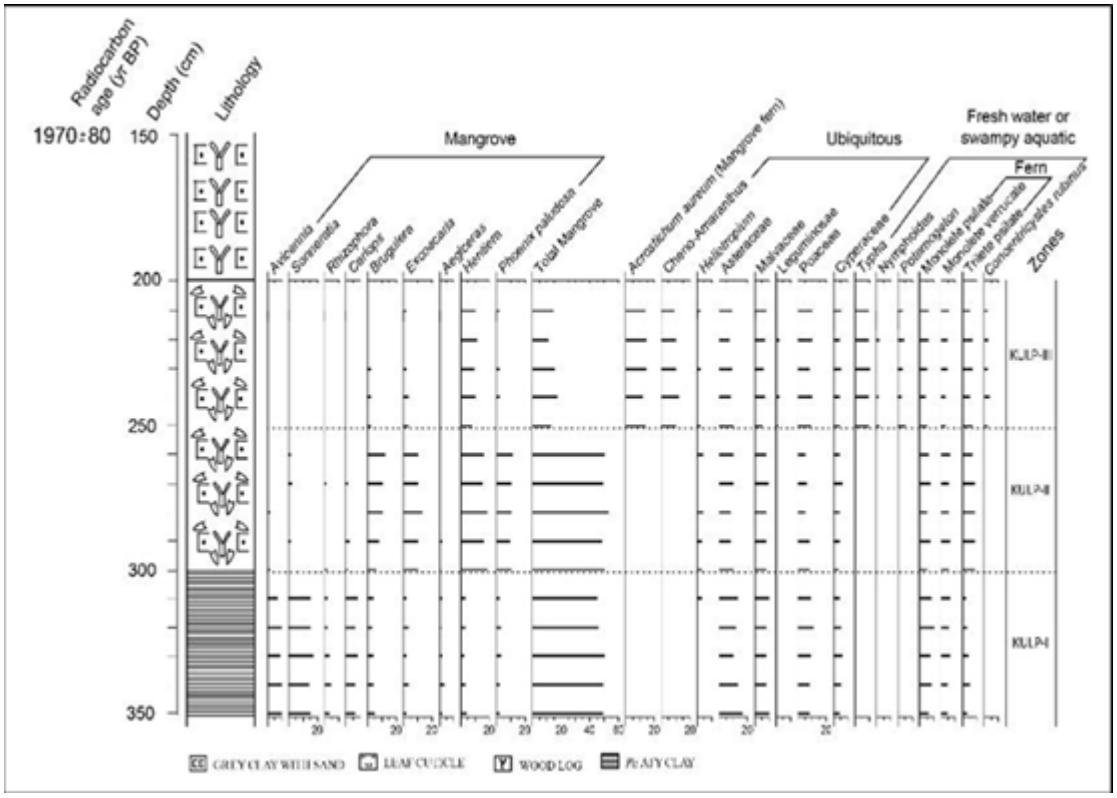
Methodology:

Laboratory technique:

Pollens and spores are recovered from the sediments following standard acetolysis treatment (43). All microscopic biological remains, including pollen grains and spores are identified by comparison with reference material held in Dept. of Botany, Bankim Sardar College, South 24 Parganas, West Bengal, India.

Pollen diagram:

All pollen diagrams (Text figs. 2-5) are created using the spreadsheet program TILIA and graphing counterpart TILIAGRAPH (44). So far obtained radiocarbon records from Lower Bengal basin are listed in Table 1. On the basis of ecological characteristics viz. mangrove, ubiquitous, aquatic or swampy and fresh water, taxa of the respective assemblages are categorized. The assemblage zones are proposed according with the Code of the International Subcommission on Stratigraphic Classification (47).



Text fig. 2: Pollen percentage diagram of Section Kumirmari (KUI) plotted against depth and age showing Local Pollen Assemblage Zone

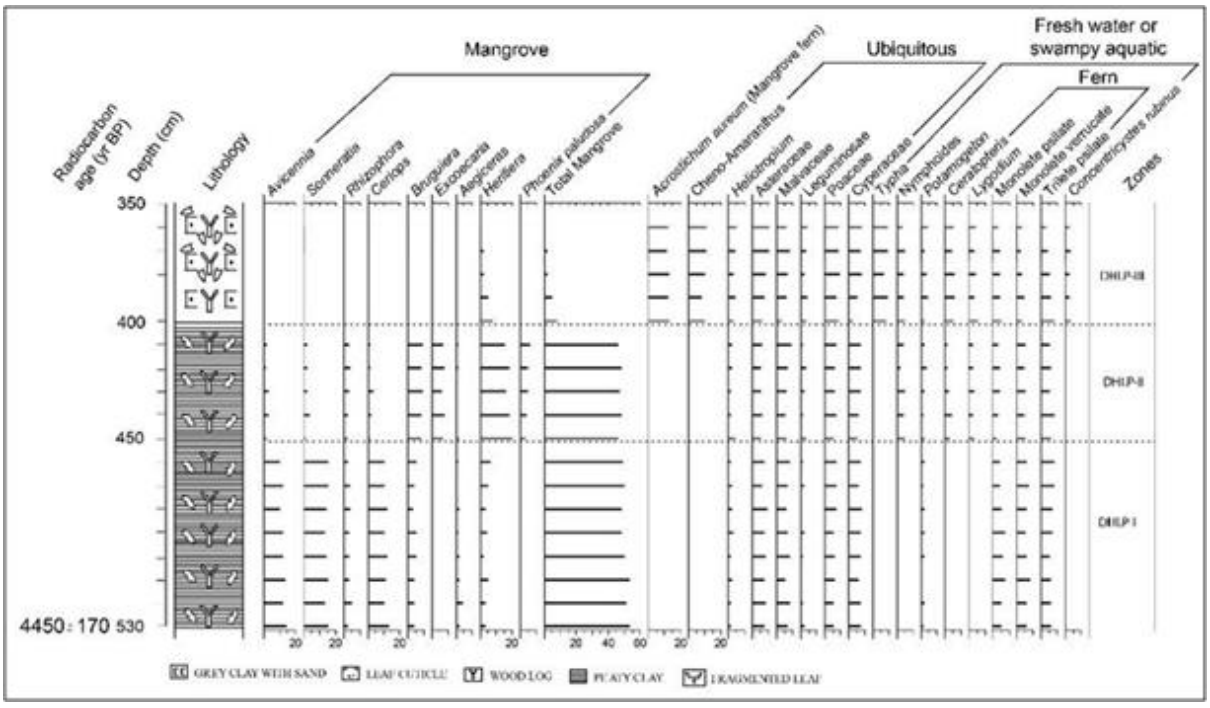
Table 1: C^{14} data recorded from Late Quaternary sediments of Lower Bengal basin, India

Location	Geographical coordinates		Sample No.	Nature of the dated material	Depth from Surface (cm.)	^{14}C Age (yr BP ± error)	Refer-ence
Kumirmari	$22^0$12’18.5148”N	$88^0$56’11.7528”E	BS-3435	E- Clay	325	810±70	Data from present study
Kumirmari	$22^0$12’18.5148”N	$88^0$56’11.7528”E	BS-3449	M-Wood	150	1970±80	Data from present study
Dakshin Harishpur	$22^0$13’3.3204”N	$88^0$49’11.1468”E	BS-3434	E- Clay	530	4450±170	Data from present study
Dakshin-Harishpur	$22^0$13’3.3204”N	$88^0$49’11.1468”E	BS-3430	E- Clay	410	410±70	Data from present study
Dakshin Harishpur	$22^0$13’3.3204”N	$88^0$49’11.1468”E	BS-3443	M-Wood	250	590±70	Data from present study
Taldi	$22^0$19’52.7”N	$88^0$35’59.0”E	BB-1	M- Peat	465	9271±41	Data from present study
Taldi	$22^0$19’52.7”N	$88^0$35’59.0”E	BB-2	M- Peat	425	7687±38	Data from present study
Namkhana	$21^0$46’11.8164”N	$88^0$13’53.4468”E	GrN7137	E- Clay	175	3170±70	34
Bakkhali	$21^0$33’47.268”N	$88^0$15’34.2072”E	BS-1159	M-Wood	838	4710±12	45
Bakkhali	$21^0$33’47.268”N	$88^0$15’34.2072”E	BS-1191	E- Clay	4100	6165±195	45
Diamond Harbour	$22^0$12’5.562”N	$88^0$12’30.5568E	PRL 1779	E- Clay	2800	14460±350	45
Canning	$22^0$18’37.3422”	$88^0$39’28.4292”E	BS-1160	M- wood	3168	6250±140	45
Pakhiralaya	$22^0$8’6.8568’N	$88^0$24’5.7744”E	BS-1156	Wood fragments	2230	7530±100	45
Pakhiralaya	$22^0$8’6.8568’N	$88^0$24’5.7744”E	BS-1190	Organic rich mud	4980	8800±135	45

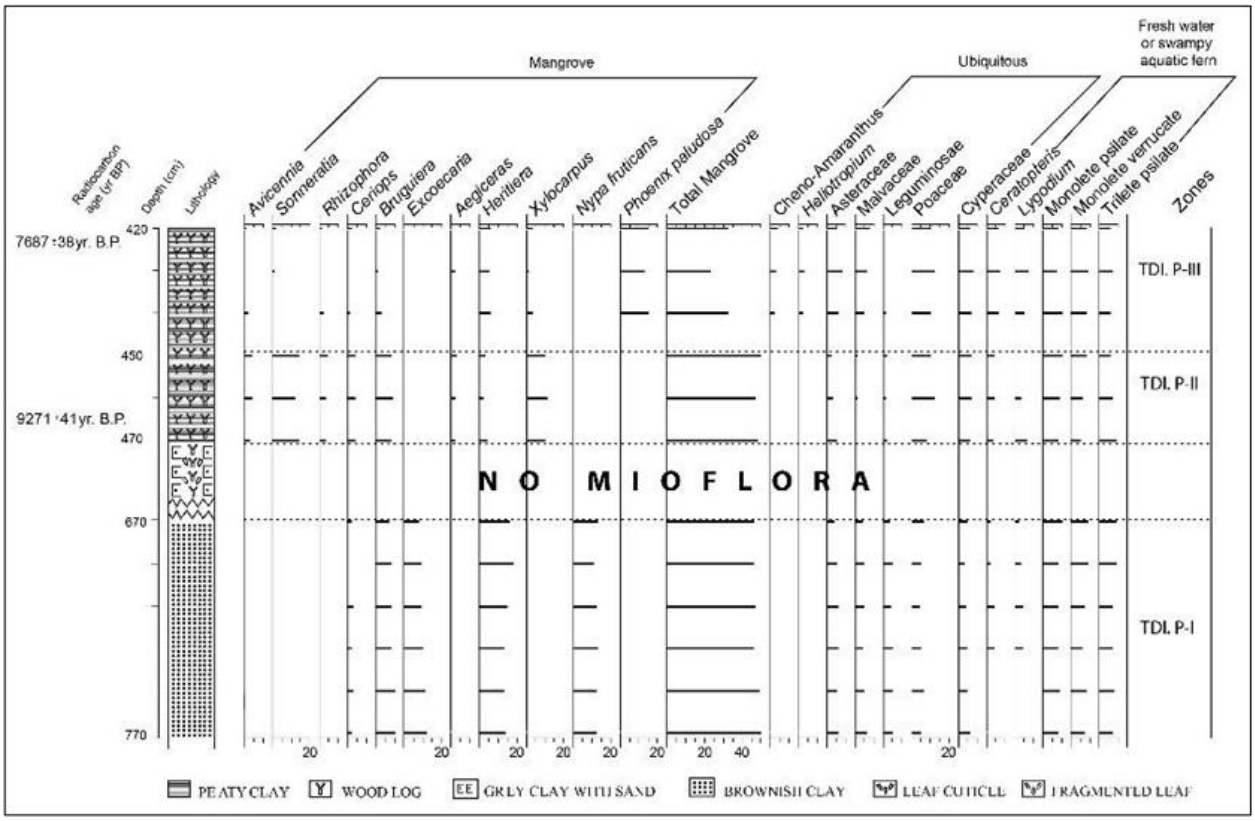
Pakhiralaya	$22^0$8'6.8568'N	$88^0$24'5.7744"E	OS-17287	Wood fragments	490	4250±40	39
Pakhiralaya	$22^0$8'6.8568'N	$88^0$24'5.7744"E	OS-18427	Wood fragments	2115	7150±70	39
Pakhiralaya	$22^0$8'6.8568'N	$88^0$24'5.7744"E	OS-17064	Wood fragments	4130	8250±60	39
Raidighi	$22^0$0'4.302"N	$88^0$26'7.296'E	OS-17063	Wood fragments	2173	7260±50	39
Raidighi	$22^0$0'4.302"N	$88^0$26'7.296'E	BS-1394	Organic rich mud	2340	7960±170	39
Raidighi	$22^0$0'4.302"N	$88^0$26'7.296'E	BS-1389	Organic rich mud	2350	9420±235	39
Raidighi	$22^0$0'4.302"N	$88^0$26'7.296'E	OS-17283	Wood fragments	2700	7430±45	39
Ganga Sagar	$21^0$38'56.4792"N	$88^0$4'31.5084"E	-------	E- Clay	90	2920±20	46

(M= Mangrove, E=Estuarine)

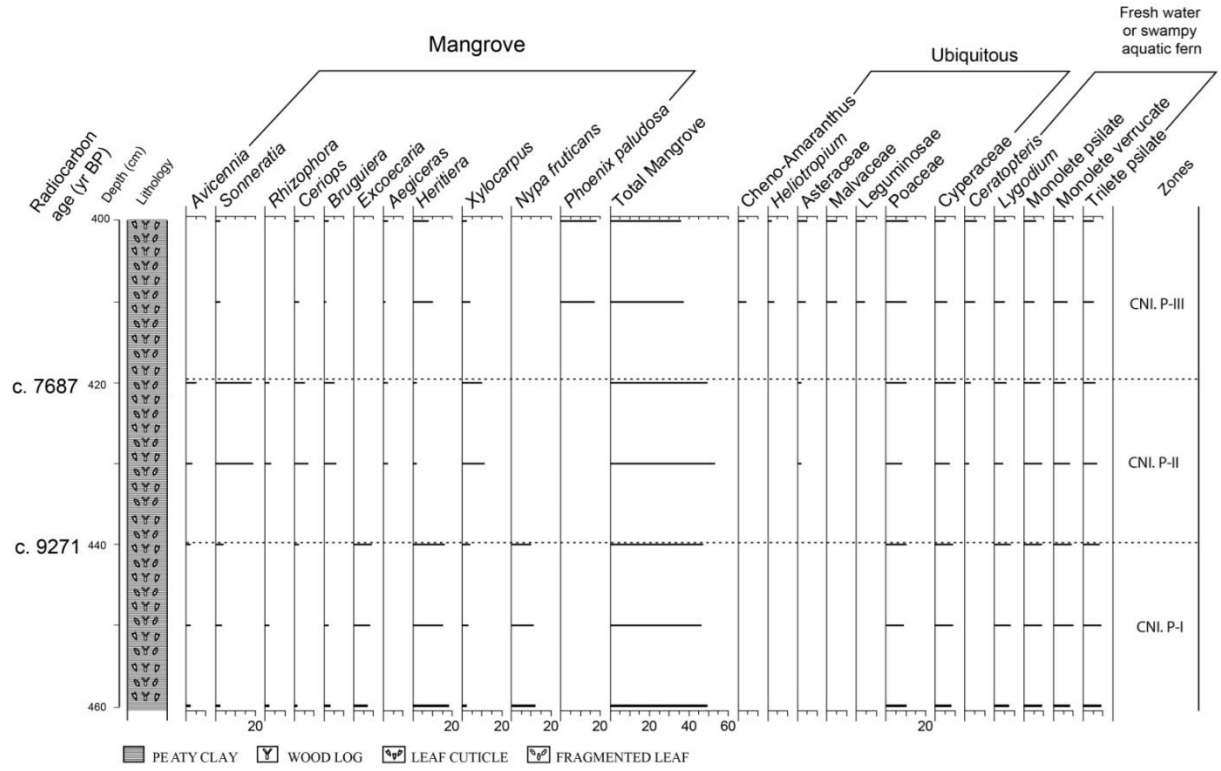
Notes: C^{14} dating Laboratory- BS = Birbal Sahni Institute of Palaeobotany, Lucknow, India, BB = Bengal Basin (Geological Survey of India, Kolkata, India), PRL = Physical Research Laboratory, Ahmedabad, India, GrN – Groningen, The Netherlands and OS = Study by Stanley and Hait, 2000.



Text fig. 3: Pollen percentage diagram of Section Dakshin Harishpur (DHI) plotted against depth and age showing Local Pollen Assemblage Zone



Text fig. 4: Pollen percentage diagram of Section Taldi (TDI) plotted against depth and age showing Local Pollen Assemblage Zone



Text fig. 5: Pollen percentage diagram of Section Canning (CNI) plotted against depth and age showing Local Pollen Assemblage Zone

Findings or Recovery

The recovered pollen grains and spores are described as follows (Plate 1, Figs. 1-16; Plate 2, Figs. 17-31):

A. Mangrove Plant Pollen

(Taxa arranged according to the mangrove ecosystem succession of Sundarbans, India)

Avicennia sp., *Sonneratia* sp., *Rhizophora* sp., *Ceriops* sp., *Bruguiera* sp., *Excoecaria* sp., *Aegiceras* sp., *Heritiera* sp., *Xylocarpus* sp., *Nypa fruticans, Phoenix paludosa*

B. **Ubiquitous Pollen**

Suaeda type / *Amaranthus* type, *Heliotropium* sp., *Acacia* sp., Malvaceae, Asteraceae, Poaceae, Cyperaceae

C. **Swampy / Aquatic Environment Indicator Pollen (Fresh Water to Brackish Water)**

Typha, *Potamogeton* sp., *Nymphoides* sp., *Acrostichum aureum*, *Ceratopteris* sp., *Lygodium* sp., Trilete psilate, Monolete psilate, Monolete verrucate

D. **Fresh Water Microplankton -** *Concentricystes rubinus*

Regional Pollen Assemblage Zones during Holocene Period through Correlation of Local Pollen Assemblage Zones

Pollen analysis of sections KUI, Kumirmari (Fig. 2), DHI, Dakshin Harishpur (Fig. 3), TDI, Taldi (Fig. 4) and CNI, Canning (Fig. 5) have explored three (KUI. P-I – P-III), three (DHI. P-I – P-III), three (TDI. P-I – P-III) and three (CNI. P-I – PIII) Local Pollen Assemblage Zones respectively. Their correlation is inferred from almost identical pollen assemblages and correspondence of the available chronological data (Fig. 2). Correlation of the Local Pollen Assemblage Zones described from the four sections viz., KUI, DHI, TDI and CNI have been explored on the basis of pollen assemblages recognized in the sections, corresponding chronological data and lithocharacters (Table 2 and 3, Fig. 6). The minor differences observed in the assemblages may be due to local site variations.

Six distinct Regional (R) Pollen Assemblage Zones of Lower Bengal basin (LBb) established from four sections are LBb. P-I, LBb. P-II, LBb. P-III, LBb. P-IV, LBb. P-V and LBb. P-VI (Fig., 6, Table 3).

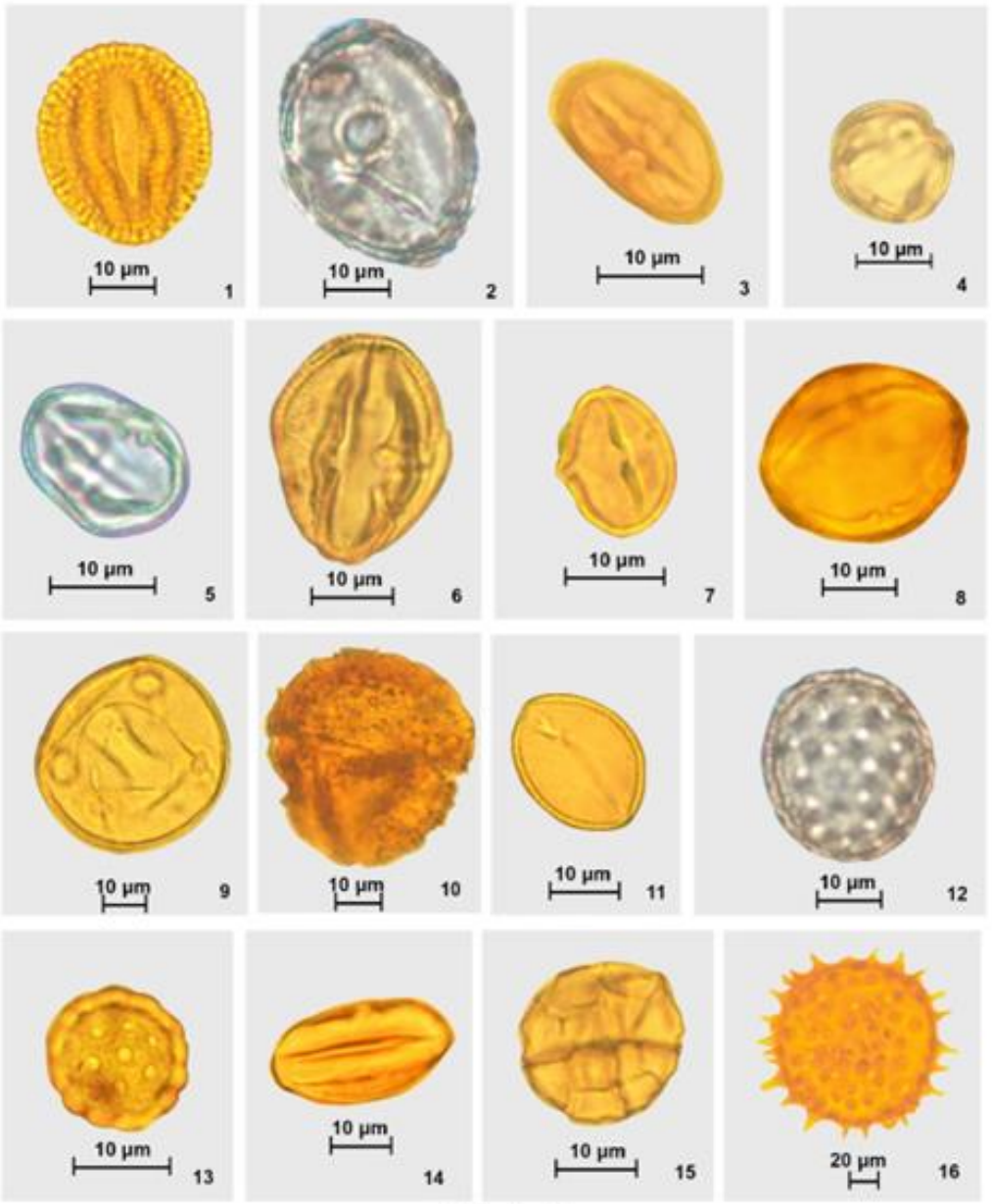


Plate 1

Fig. 1 Pollen grain of *Avicennia* sp.
Fig. 2 Pollen grain of *Sonneratia* sp.
Fig. 3 Pollen grain of *Rhizophora* sp.
Fig. 4 Pollen grain of *Ceriops* sp.
Fig. 5 Pollen grain of *Bruguiera* sp.
Fig. 6 Pollen grain of *Excoecaria* sp.
Fig. 7 Pollen grain of *Aegiceras* sp.
Fig. 8 Pollen grain of *Heritiera* sp.
Fig. 9 Pollen grain of *Xylocarpus* sp.
Fig. 10 Pollen grain of *Nypa fruticans.*
Fig. 11 Pollen grain of *Phoenix paludosa.*
Fig. 12,13 Pollen grains of Cheno-Amaranthus type.
Fig. 14 Pollen grain of *Heliotropium* sp.
Fig. 15 Pollen grain of Leguminosae.
Fig. 16 Pollen grain of Malvaceae.

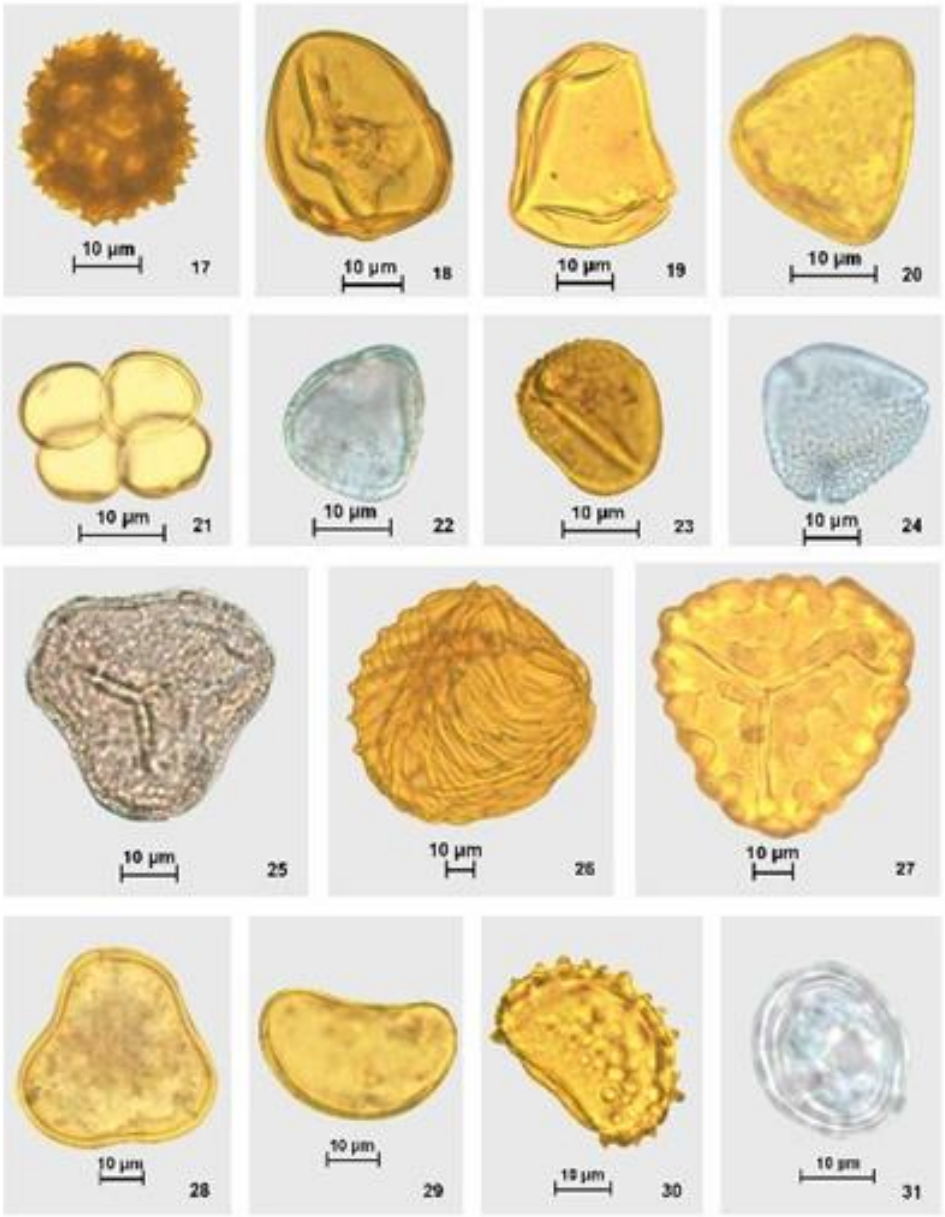


Plate 2

Fig. 17 Pollen grain of Compositae.
Fig. 18 Pollen grain of Poaceae.
Fig. 19 Pollen grain of Poaceae.
Fig. 20 Pollen grain of Cyperaceae.
Fig. 21 Pollen grain of *Typha* sp.
Fig. 22 Pollen grain of *Typha* sp.
Fig. 23 Pollen grain of *Potamogeton* sp.
Fig. 24 Pollen grain of *Nymphoides* sp.
Fig. 25 Spore of *Acrostichum* sp.
Fig. 26 Spore of *Ceratopteris* sp.
Fig. 27 Spore of *Lygodium* sp.
Fig. 28 Spore of Trilete psilate.
Fig. 29 Spore of Monolete psilate.
Fig. 30 Spore of Monolete verrucate.
Fig. 31 *Concentricystes rubinus* (splitted half).

Table 2: Lithology of sediments, corresponding chronological data, the microscopic palynomorphs recovered and Local Pollen (P) Assemblage Zone with environment of deposition

Location	Lithology and depth from surface (cm)	C^{14} date (yr BP)	Ecologically significant and dominant microscopic palynomorphs recovered	Local pollen assemblage zone	Environment of deposition
Kumirmari	Grey clay with sand, 250-200	c. 3000 – 1970±80	*Heritiera-Acrostichum*-Cheno- Amaranthus-*Typha*-Poaceae- Fern	KUI. P-III	Fresh water grassland
	Grey clay with sand, 300-250	c. 3500 – c. 3000	*Heritiera-Excoecaria-Phoenix paludosa-Bruguiera*-Asteraceae-Malvaceae -Fern	KUI. P-II	Brackish water mixed fresh water *Heritiera* forest
	Peaty clay, 350-300	c. 4450 – c. 3500	*Sonneratia-Avicennia-ceriops- Bruguiera-Heritiera*-Fern	KUI. P-I	Swampy mangrove vegetation
Dakshin Harishpur	Grey clay with sand 400-350	c. 3000 – c. 2000	*Heritiera-Acrostichum*-Cheno- Amaranthus-*Typha*-Poaceae- Fern	DHI. P-III	Fresh water grassland
	Grey clay with sand,` 450-400	c. 3500 – c. 3000	*Heritiera-Excoecaria-Phoenix paludosa-Bruguiera*-Asteraceae-Malvaceae –Fern	DHI. P-II	Brackish water mixed fresh water *Heritiera* forest
	Peaty clay, 530-450	4450±170 – c. 3500	*Sonneratia-Avicennia-ceriops-Bruguiera-Heritiera*-Fern	DHI. P-I	Swampy mangrove vegetation
Taldi	Peat, 450-420	7687±38 – c. 6000	*Phoenix paludosa-Heritiera- Xylocarpus*-Poaceae-Fern	TDI. P-III	*Phoenix* dominated mangrove upland
	Peat, 470-450	9271±41 – 7687±38	*Sonneratia-Xylocarpus-Bruguiera-Heritiera*-Fern	TDI. P-II	Tidal mangrove with regular inundation
	Grey clay, 670-770	c. 11000 – c. 10000	*Heritiera-Nypa fruticans-Bruguiera-Excoecaria*-Fern	TDI. P-I	Mixed brackish water and fresh water tidal mangrove with regular inundation

Canning	Peat, 420-400	c. 7687 – c. 6000	*Phoenix paludosa-Heritiera-Xylocarpus-* Asteraceae-Malvaceae-Fern	CNI. P-III	Phoenix dominated mangrove upland
	Peat, 440-420	c. 9271 – c. 7687	*Sonneratia-Xylocarpus-Ceriops-Bruguiera-Heritiera-* Fern	CNI. P-II	Tidal mangrove with regular inundation
	Peat, 460-440	c. 10000 – c. 9271	*Heritiera-Nypa fruticans-Excoecaria-* Fern	CNI. P-I	Mixed brackish water and fresh water tidal Mangrove with regular Inundation

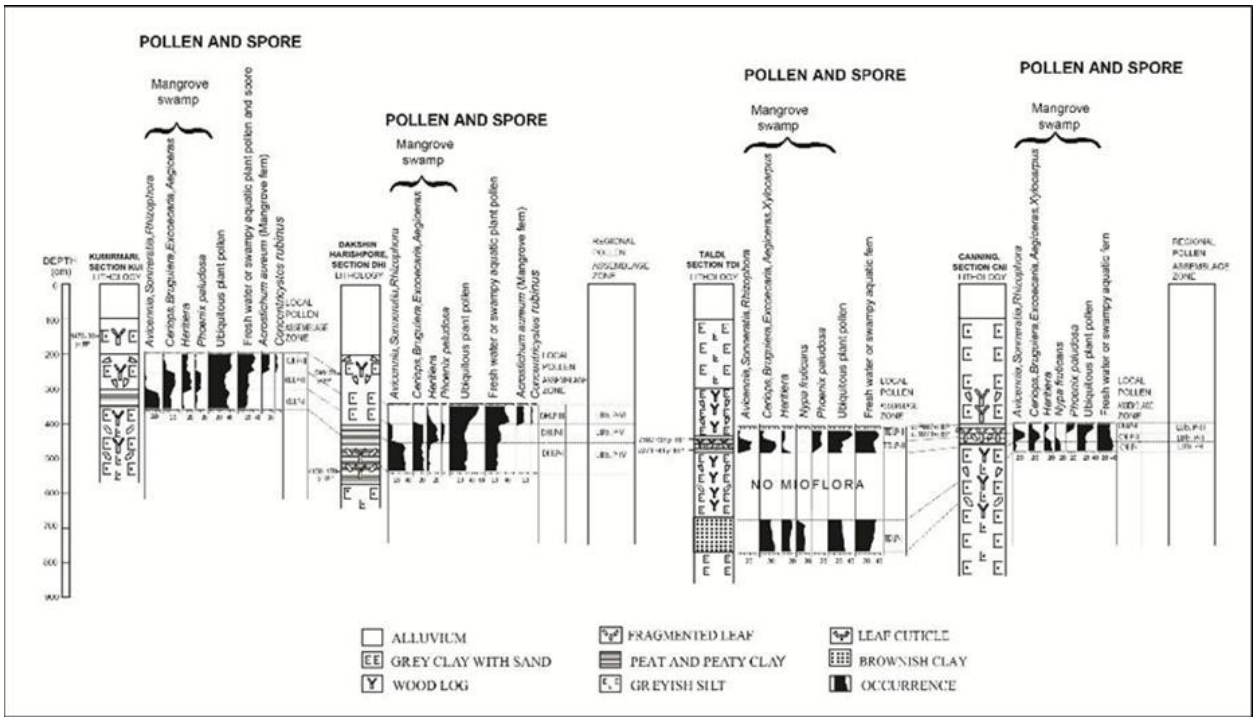
Phases of Depositional Environment Explored Through Palyno- Stratigraphic Zones of Lower Bengal Basin during Holocene

Six distinct depositional environments have been explored in the Lower Bengal basin during c.11, 000 – 1970±80 yr BP through six palynostratigraphic zones. The changes of depositional environment and the factors controlling the changes are also revealed from the analysis of Regional Pollen Assemblage Zones (Table 3).

Table 3: Regional Pollen Assemblage Zones of Holocene sediments of Lower Bengal basin, India (LBb) and corresponding phases of deposition in chronological sequence

C14 dates (yr BP)	Regional Pollen Assemblage Zone	Phases of deposition
c. 3000 - 1970±80	LBb. P-VI *Heritiera-Acrostichum*-Cheno-Amaranthus-*Typha*-Poaceae-Fern	Phase VI Fresh water grassland
c. 3500 – c. 3000	LBb. P-V *Heritiera-Excoecaria-Phoenix-Bruguiera-* Asteraceae-Malvaceae-Fern	Phase V Brackish water mixed fresh water *Heritiera* forest
4450±170 – c. 3500	LBb. P-IV *Sonneratia-Avicennia-Ceriops-Bruguiera-Heritiera*-Fern	Phase IV Swampy mangrove vegetation

7687±38 – c. 6000	LBb. P-III *Phoenix-Heritiera-Xylocarpus*-Poaceae-Fern	Phase III *Phoenix* dominated – mangrove Upland
9271±41 – 7687±38	LBb. P-II *Sonneratia-Xylocarpus-Bruguiera-Heritiera*-Fern	Phase II Tidal mangrove with – regular Inundation
c. 11000 – c. 9271	LBb. P-I *Heritiera-Nypa fruticans-Bruguiera-Excoecaria*-Fern	Phase I Mixed brackish water and fresh Water tidal mangrove with Regular inundation



Text Fig. 6: Lithology of sediments, corresponding chronological data, the microscopic palynomorphs recovered, Local Pollen Assemblage Zone and Regional Pollen Assemblage Zone of Holocene sediments of Lower Bengal basin, India

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References:

1. Von Post, L., 1918. Skogstradpollen i Sydsvenska torfmosselager foljder. Forth. 16. Skand. Naturforskerm, 433.
2. Wodehouse, R.P., 1935. The Pleistocene Pollen of Kashmir with an introductory note by H.de Terra. Mem. Connecticut Acad. Arts. Sci. 9, 1-18.
3. Hutington, E., 1906. Pangong, a glacial lake in Tibetan Plateau. Jour. Geol. 14, 599-617.
4. Van der Hammen,T, Hooghiemstra, H., 2000. Neogene and Quaternary history ofvegetation, climate and plant diversity in Amazonia. Quaternary Science Reviews 19, 725–742.
5. Behling, H., 2002. Carbon storage increases by major forest ecosystems in tropical SouthAmerica since the Last Glacial Maximum and the early Holocene. Global and PlanetaryChange 33, 107-116.
6. Behling, H., Cohen, M.C.L., Lara, R.J., 2001. Studies on Holocene mangrove ecosystemdynamics of the Braganca peninsulain north-eastern Para, Brazil. Palaeogeogr.Palaeoclimatol. Palaeoecol. 167, 225-242.
7. Lorscheitter, M. L., 2003. A Concentração Polínica no Estudo da Vegetação dos Últimos Milênios no Sul do Brasil. In: Luiz Mauro Barbosa & Nelson Augusto dos Santos Junior (orgs.) A Botânica no Brasil. Pesquisa, ensino e políticas públicas ambientais. 1ª ed.São Paulo: Sociedade Botânica do Brasil 1, 463-466.
8. De Oliveira, P.E., Behling, H., Ledru, M.P., Barberi, M., Bush, M., Salgado-Labouriau,M.L., Garcia, M.J., Medeanic, S., Barth, O.M., Barros, M.A., Scheel-Ybert, R., 2005. Paleovegetação e paleoclimas do Quaternário do Brasil. Capítulo 3. In: Souza CRG, Suguio K, Oli-veira AMS, De Oliveira PE, editors. Quaternário
9. Leal, M. G., Lorscheitter, M. L., 2007. Plant succession in a forest on the Lower NortheastSlope of Serra Geral, Rio Grande do Sul, and Holocene palaeoenvironments, SouthernBrazil. Acta Botanica Brasilica 21, 1-10.
10. Macedo, R.B., Souza, P.A., Bauermann, S.G.,Bordignon S.A.L., 2010. Palynologicalanalysis of a late Holocene core from Santo Antônio da Patrulha, Rio Grande do Sul, Southern Brazil. Anais da Academia Brasileira de Ciências 82 (3), 731-745.

11. Macintyre, I.G., Toscano, M.A., Lighty, R.G., Bond, G.B., 2004. Holocene history of themangrove islands of Twin Cays, Belize, Central America. Atoll Research Bulletin 510, 1-16.
12. González, C., Urrego, L.E., Martínez, J.I., 2006. Late Quaternary vegetation and climatechange in the Panama Basin: Palynological evidence from marine cores ODP 677B andTR 163-38. Palaeogeography, Palaeoclimatology, Palaeoecology 234, 62-80.
13. Smidth, D.P., 2008. A palynological and stratigraphic analysis of mangrove sediments atPunta Galeta, Panama. Proquest dissertation and theses, 189 pages.
14. Schulmeister, J., 1992. A Holocene pollen record from lowland tropical Australia. TheHolocene 2 (2), 107-116.
15. Barham, A.J., 1999. The local environmental impact of prehistoric populations on SaibaiIsland, northern Torres Strait, Australia: enigmatic evidence from Holocene swamplithostratigraphic records. Quaternary International 59, 71-105.
16. Hashimoto, T.R., Saintilan, N., Haberle, S.G., 2006. Mid-Holocene Development ofMangrove Communities Featuring Rhizophoraceae and Geomorphic Change in the Richmond River Estuary, New South Wales, Australia. Geographical Research 44 (1), 63-76.
17. Woodroffe, C.D., Keneddy, D.M., Rasmussen, C.E., Smithers, S.G., 2000. Holocene reefgrowth in Torres Strait. Marine Geology 170, 331-346.
18. Rowe, C., 2007. A palynological investigation of Holocene vegetation change in TorresStrait, seasonal tropics of northern Australia. Palaeogeogrpahy, Palaeoclimatology,Palaeoecology 251, 83-103.
19. Laval, H., Medus, J., Roux, M., 1991. Palynological and sedimentological records of Holocene human impact from the Etang de Berre, southeastern France. The Holocene1 (3), 269-272.
20. Kalis, A.J., Merkt, J., Wunderlich, J., 2003. Environmental changes during the Holoceneclimatic optimum in central Europe - human impact and natural causes. QuaternaryScience Reviews 22, 33–79.
21. Newnham, R.M., Lowe, D. J., Wigley, G. N. A., 1995. Late Holocene palynology andpalaeovegetation of tephra-bearing mires at Papamoa and Waihi Beach, western Bay of Plenty, North Island, New Zealand. Journal of The Royal Society of New Zealand25 (2), pp. 283-300.0
22. Dunbar, G.V., McLea, B., Goff, J.R., 1997. Holocene pollen stratigraphy and sedimentation, Wellington Harbour, New Zealand. New Zealand Journal of Geology and Geophysics 40 (3), 325-333.

23. Kholeif, S.E.A., 2007. Palynology of mangrove sediments in the Hamata Area, Red SeaCoast, Egypt: vegetation and restoration overview. Coastline Reports 7, pp. 5-16.

24. Lézine, A-M., 1997. Evolution of the West African Mangrove During the Late Quaternary:A Review. Géographie physique et Quaternaire 51 (3), 404-414.

25. Hooghiemstra, H., Le´zine, A-M., Leroy, Suzanne, A.G., Dupont, L., Marret, F., 2006. LateQuaternary palynology in marine sediments: A synthesis of the understanding of pollendistribution patterns in the NW African setting. Quaternary International 148, 29-44.

26. Vishnu-Mittre, Gupta, H.P., 1971. The origin of Shola forest in the Nilgiris, South India.Palaeobotanist 19(1), 110-114.

27. Gupta, H.P., 1973. Quaternary vegetational history of Ootacamund, Nilgiris, South India. I. Kathathope and Raes Corner. Palaeobotanist 20(1), 74-90.

28. Somboon, J.R.P., 1990. Palynological study of mangrove and marine sediments of the Gulf of Thailand. Journal of Southeast Asian Earth Sciences 4 (2), 85-97.

29a. Kumaran, K.P.N., Shindikar, M.R., Mudgal, T.R., 2004a. Floristic composition, palynology and sedimentary facies of Hadi mangrove swamp (Maharashtra). Journal of IndianGeophysical Union 8, 55-63.

29b. Kumaran, K.P.N., Shindikar, M., Limaye, R.B., 2004b. Mangrove deposits as archives ofpalaeomonsoon records: the palynological assessment of the Late Quaternary depositsof West Coast of India. Pollen 14, 246-247.

30. Mao, L., Jhang, Y., Bi, H., 2006. Modern Pollen Deposits in Coastal Mangrove Swamps from Northern Hainan Island, China. Journal of Coastal Research 22 (6), 1423-1436.

31. Rugmai, W., Grote, P.J., Chonglakmani, C., Zetter, R., Ferguson, D.K., 2008. A LatePleistocene palynoflora from the coastal area of Songkhla Lake, southern Thailand.ScienceAsia 34, 137-145.

32a. Mukherjee, B.B, 1972a. Quaternary pollen analysis as a possible indicator of prehistoricagriculture in deltaic part of West Bengal, India. J. Palynol. 8, 144-151.

32b. Mukherjee, B.B., 1972b. Pollen analysis of a few quaternary deposits of Lower Bengalbasin. In A.K.Ghosh et al. (Eds.) – Proceeding of the Seminar on palaeopalynologyand Indian Stratigraphy, 1971. Department of Botany, Calcutta University, Calcutta,357-374.

33. Vishnu-Mittre, Gupta, H.P., 1972a. Pollen analytical study of Quaternary deposits in Bengalbasin. Palaeobotanist 19 (3), 297-306.

34. Gupta, H.P., 1981. Paleoenvironments during Holocene time in Bengal Basin as reflected bypalynostratigraphy. Paleobotanist 27, 138-160.

35. Banerjee, M., Sen, P.K., 1986a. Late Holocene organic remains from Calcutta Peat In: Bimalk.Samanta (Editor), Proceeding XI Indian Colloquium on micropalaeontology andStratigraphy, Bulletin Geological Mining Metallurgical Society of India 54, 272-284.
36. Barui, N.C., Chanda, S., Bhattacharya, K., 1986. Late Quaternary vegetational history of the Bengal basin. Proc. XI Colloquium on micropalaeontology and stratigraphy. Bull. Geol. Mic. Met. Soc. India 54, 197-201.
37. Sen, P.K., Banerjee, M., 1990. Palyno-plankton stratigraphy and environmental changesduring theHolocene in the Bengal Basin, India. Review of Palaeobotany and Palynology 65 (1-4), 25-35.
38. Barui, N.C., Chanda, S., 1992. Late Quaternary pollen analysis in relation to palaeoecology,biostratigraphy and dating of Calcutta peat. Proc. Indian Natl. Sci. Acad. B58 (4), 191-200.
39. Stanley, D.J., Hait, A.K., 2000. Holocene depositional patterns, Neotectonics and Sundarbanmangroves in the western Ganges-Brahmaputra delta. J Costal Res 16, 26-39.
40. Hait, A.K., Behling, H., 2009. Holocene mangrove and coastal environmental changes in the western Ganga-Brahmaputra Delta, India. Veget. Hist. Archaeobot. 18, 159-169.
41. Das, S., 2009. Palaeoecology of the Lower Bengal basin, Calcutta, India, during the Late Quaternary. Feddes Repertorium 120 (5 – 6), 333– 342.
42. Das, S. 2014. Palaeo-palynology of late Quaternary peat deposit from Lower Bengal basin, India: A Palaeoecological approach. Quaternary International 325, 197-204.
43. Erdtman, G., 1952. Pollen Morphology and plant Taxonomy of angiosperms. Stockholm and New York.
44. Grimm, E., 1999-2002. TILIA Program, Ver.2.0 B4. Illinois Museum, Springfield.
45. Chanda, S., Hait, A.K., 1996. Aspects and appraisal of late Quaternary vegetation of lowerBengal Basin, Palaeobotanist 45, 117-124.
46. Chakraborty, S. C. 1991. Some Aspects of Coastal Dynamics of Bakkhali and GangasagarBeach, W.B., unpublished annual progress report, Geological Survey of India, (FieldSession 1989- 90), 1-24.
47. Hedberg, D.H. 1976. International stratigraphic Guide. New York.

BROMELAIN IS A SOURCE OF MANY MEDICINALLY ACTIVE COMPOUNDS

Sampa Debnath* and Gourab Shome

Department of Microbiology, Vijaygarh Jyotish Roy College, Kolkata

*Corresponding author E-mail: deb_sampa19@yahoo.com

Abstract:

Bromelain is a crude, aqueous extract from the stems and fruits of pineapples (*Ananas comosus*) derived from Bromeliaceae family. It contains a mixture of different proteases as well as phosphatase, glucosidases, peroxi- dases, cellulases and glycoproteins. It has been studied that bromelain shows beneficial activities in medicinal aspects. The aim of our study was the phytochemical analysis of bromelein. Our experimental study has revealed that bromelain contains many important compounds e,g. carbohydrate, saponins, quininone, terpenoids, phenols, steroids, vitamin C and protein. These constituents contributes to many medicinal properties e.g antimicrobial activity, anti inflammatory activities etc. Therefore bromalein is good source of many medicinally important compounds.

Keywords: Pineapple, Bromelain, Phytochemical Compounds

Introduction:

Recent trend is to explore novel medicine in plants besides the existence of traditional medicine. Different plants have been identified for having some medicinal properties. Among those plants pineapple plant is one of them. During the sixteenth and seventeenth centuries, the pineapple plants were introduced to Asia Pacific and became the first commercial crop (1). Several studies have revealed that Bromelain from pineapple contains different bioactive compounds (2, 3). Bromelain is present in all parts of the pineapple plant, but the stem is the most common commercial source. Bromelain is a complex natural mixture of proteolytic enzymes derived from pineapple (Ananas cosmosus) and possesses notable therapeutic properties. Interest in bromelain is increasing gradually as bromelain has been used as folk medicine for several years. Several studies have been done regarding the potential therapeutic value of bromelain (4,5). Our study is related to identification of different medicinally active compounds of bromelain of stem part

Methods:

Preparation of bromelain extract:

Homogenate of stem part of pineapple was prepared by homogenising chopped stem in (0.1M) Sodium-Acetate buffer (pH : 4.5). The extract was prepared by centrifugation of filtrate of homogenate at 6000 rpm for 10min. The extract was used different phytochemical tests.

Phytochemical tests:

Test for carbohydrates: To 2 ml of bromelain extract, 1 ml of Molisch's reagent and few drops of concentrated sulfuric acid were added. The presence of purple or reddish indicates the presence of carbohydrates [6].

Test for tannins: To 1 ml of bromelain extract, 2 ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins [7].

Test for saponins: To 2 ml of bromelain extract, 2 ml of distilled water was added and shaken in a graduated cylinder for 15 minutes lengthwise. Formation of 1 cm layer of foam indicates the presence of saponins [8].

Test for flavonoids: To 2 ml of bromelain extract, 1 ml of 2 N sodium hydroxide was added. The presence of yellow indicates the presence of flavonoids [9].

Test for alkaloids: To 2 ml of bromelain extract, 2 ml of concentrated hydrochloric acid was added. Then, few drops of Mayer's reagent were added. The presence of green or white precipitate indicates the presence of alkaloids [10].

Test for quinones: To 1 ml of extract, 1 ml of concentrated sulfuric acid was added. Formation of red indicates the presence of quinones [11].

Test for glycosides: To 2 ml of bromelain extract, 3 ml of chloroform and 10% ammonia solution was added. Formation of pink indicates the presence of glycosides [12].

Test for cardiac glycosides: To 0.5 ml of extract, 2 ml of glacial acetic acid and few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated sulfuric acid. Formation of brown ring at the interface indicates the presence of cardiac glycosides [9].

Test for terpenoids: To 0.5 ml of extract, 2 ml of chloroform was added and concentrated sulfuric acid was added carefully. Formation of red-brown at the interface indicates the presence of terpenoids [9].

Test for phenols: To 1 ml of the extract, a few drops of phenol Ciocalteu reagent were added followed by few drops of 15% sodium carbonate solution. Formation of blue or green color indicates the presence of phenols [11].

Steroids and phytosteroids: To 1 ml of bromelain extract, an equal volume of chloroform is added and subjected with few drops of the concentrated sulfuric acid appearance of brown ring indicates the presence of steroids and appearance of bluish-brown ring indicates the presence of phytosteroids [13].

Phlobatannins: To 1 ml of bromelain extract, few drops of 2% HCL were added the appearance of red precipitate indicates the presence of phlobatannins [9].

Anthraquinones: To 1 ml of bromelain extract, few drops of 10% ammonia solution were added, appearance pink precipitate indicates the presence of anthraquinone [9]

Results:

Observations of different tests for identification phytochemical compounds have been tabulated in table 1.

Table: 1: Different tests for identification phytochemical compounds

Name of the compounds	Observation
Tanins	-
Phytosteroid/ Steroid	-/+
Alkaloids	-
Saponin	+
Carbohydrate	+
Quinones	+
Anthraquinone	-
Phlobatannins	-
Phenols	+
Terpenoids	+

Discussion:

Our study revealed that the constituents found in the bromelain extract were saponins, phenol, terpenoids, steroids, carbohydrates and quinones. Saponins lowers plasma cholesterol levels in humans Phenol prevents the oxidative damage by acting over the reactive species. Quinone shows antimicrobial activity, flavonoids, phenols, coumarins, steroids, terpenoids, and quinones. Secondary metabolites such as alkaloids, quinones, and phenols, present in pineapple extract showed anticancer potential. The presence of phenols suggests the antioxidant activity of the extract. Bromelain has been used for wide range of therapeutic applications for last four decades. It can be used in inhibition of platelet aggregation. Use of natural products is increasing nowadays to avoid the various side effects caused by different drugs. The potential to develop bromalein extract as drug against different life-threatening diseases is a thrust area for future research in drug designing industry.

References:

1. Morton J., Julia F. Morton, Miami, FL: (1987) Fruits of Warm Climates Pineapple; pp. 18–28.
2. Vidhya R, Noorjahan B A,[2] Subramaniam S,[3] Samikannu K,[4] and Rajendran R[5] (2016) Potential role of bromelain in clinical and therapeutic applications.Biomedical Rep Sep; 5(3): 283–288.

3. Menon A *, Vishnu P V2, Gayatri R2 (2016) Preliminary Phytochemical Analysis And Cyto toxicity Potential Of Pineapple Extract On Oral Cancer Cell Lines, Asian Journal of pharmaceutical and clinical research,Vol 9, Suppl. 2, pp.140-143
4. Scopes RK.(1982) Protein Purification: Principles and Practice. 2nd. Springer-Verlag; New York, NY: pp. 41–65.
5. Arnon R, Shapira E.(1967) Antibodies to papain. A selective fractionation according to inhibitory capacity. Biochemistry;6:3942–3950. doi: 10.1021/bi00864a041. [PubMed]
6. Sofowora A. (1993). Screening plants for bioactive agents. Medicinal Plants and Traditional Medicinal in Africa. 2nd ed. Ibadan, Nigeria: Spectrum Books Ltd., Sunshine House; p. 134-56.
7. Harborne JB. (1973) Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. New York: Chapman and Hall;. p. 279.
8. Smolenski SJ, Silinis H, Farnsworth NR. (1969) Alkaloid screening. V. Lloydia 1974;37(3):506-36.
9. Kapoor LD, Singh A, Kapoor SL, Srivastava SN.(1974) Survey of Indian plants for saponins, alkaloids and flavonoids. I. Lloydia ;32(3):297-304.
10. Jana S, Shekhawat GS.(2010) Phytochemical analysis and antibacterial screening of in vivo and in vitro extracts of Indian medicinal herbs: Anethum graveolens. Res J Med Plants;4(4):206-12.
11. Ayoola GA, Coker HA, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, et al. (2008) screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Trop J Pharm Res;7(3):1019-24.
12. Sureshkumar CA, Varadharajan R, Muthumani P, Meera R, Devi P, Kameswari B. (2009) Pharmacognostic and preliminary phytochemical investigations on the stem of Saccharum spontaneum. J Pharm Sci Res;1(3):129-36.
13. Boxi M, Rajesh Y, Rajakumar V, Praveen B, Mangamma K. (2010); Extraction, phytochemical screening and in-vitro evaluation of anti-oxidant properties of commicarpuschinesis (aqueous leaf extract). Int J Pharm Bio Sci 1(4):975-6299.

GUT MICROBIOTA ON OBESITY: RECENT ADVANCEMENTS

Sreosi Chakraborty, Subhajit Nath, Sayak Dolai and Shilajit Barua*

Department of Microbiology,

Vijaygarh Jyotish Ray College, 8/2, Bejoygarh, Jadavpur. Kolkata: 700032

*Corresponding author E-mail: shilajitj82@gmail.com

Abstract:

Obesity is a common disorder nowadays with multifactorial causation and clinical significance. In addition to common cause of its rising prevalence like, urbanization of the world's population, increased availability of food supplies, and reduction of physical activity, recent studies have shown that gut microbial population plays an important role in obesity. The relationship has been found to be co-dependent, i.e, obesity alters gut microbial population and gut microbial population influences obesity. Human gut has been reported to be inhabited by diverse microbial population that maintains a symbiotic relationship with the body. Some helps to produce some growth factors and some contributes to the energy harvesting in the body. Many studies have indicated the association of gut microbes with obesity. Inoculation of gut microbiota in germ free lean mice showed a significant increase in body mass and insulin resistance. Changes in gut microbiota have been reported during absorption, storage and metabolism of dietary lipids, which might have contributed to obesity. Studies proved that obese mice can harvest more energy and intake more calorie than lean individual. Bacteroidetes and Firmicutes are the main two abundant microbial phyla in the gut that has been reported to regulate obesity. Many experiments revealed that a significant increase in Firmicutes and decrease in Bacteroidetes population occurs in obese individuals in contrast with the lean individuals. However, difference in human and mice gut microbial population leaves scope for further studies and research of gut micro biota and its influence on obesity in human.

Keywords: Obesity, Obese mice, Gut Microflora, Bacteroides, Firmicutes.

Introduction:

Obesity is referred to as a medical condition in which the body fat gets accumulated in the adipose tissues of the body and cause an elevated value of BMI (body mass index) i.e, measurement calculated by dividing a person's weight by square of the person's height, than normal (25-30 kg/m^2) (1). This caused several disorders like heart disease, obstructive sleep apnea, certain types of cancer and osteoarthritis (2). As often reported, the common reasons behind obesity are a combined result of excessive calorie intake, less physical activity and genetic vulnerability (3). However, studies have shown that obese individuals carry different

microbial ecology in their gut that influence the efficient extraction or storage from the diet (4). The human body harbours more than 10 trillion microbes that represent its normal microbial flora (5). Some organisms in the population establish a permanent relationship with the body, while others are more transient and are present for limited periods of time (6). Gastrointestinal tracts also harbour such resident and transient microbial population (7). Physiological factors are major regulators for growth of certain microbes, like- in stomach due to high acidity it acts as a chemical barrier for most of the microorganisms to get entry in the gut (8). However, organisms such as *Helicobacter pylori* can colonise the stomach wall (9). Bacterial genera commonly found in the small intestine include *Lactobacillus*, *Clostridium*, Staphylococcus, Streptococcus, and Bacteroides, among others (10) and *Bacteroides, anaerobic gram-positive cocci, such as Peptostreptococcus sp., Eubacterium sp., Lactobacillus sp., and Clostridium sp* etc are found in large intestine (11).

Most of the microorganisms maintain a symbiotic relationship with the body. They are provided with nutrition and habitat from the host and in return they contribute to some metabolic pathways and production of certain growth factors, like, production of vitamins like Vitamin K, Vitamin B12 (12). Also, they help in generating energy from some polysaccharides which are otherwise indigestible, since humans do not possess genomes for the product like glycoside hydrolases and polysaccharide lyases, which the gut microbes do (13). Further studies have shown that the microbial population regulate the expression of a circulating inhibitor of lipoprotein lipase: fasting induced adipocyte factor (FIAF) (14). Microbial population in the gut ferments some dietary polysaccharides to monosaccharides (13) which intern is converted to short chain fatty acids in the distal gut (14). These short chain fatty acids get adsorbed subsequently to stimulate de novo synthesis of triglycerides in the liver (15). Thus, reduced level of circulating LPL inhibitor, increased LPL activity in adipocytes and enhanced storage of liver derived triglycerides in fat cells are the result of microbial suppression of FIAF in the gut epithelial cells (16).

In addition, gut microbiota of genetically obese mice and lean mice as well as obese and lean human volunteers has shown distinct nature and populations type (17). Mainly two major types of organisms have been found in abundance in the gut their relative content has been correlated with obesity: Bacteroidetes and Firmicutes (18).

Studies to prove the relationship between gut microbiota and obesity:

Microbial population can regulate the host metabolism and develops low grade inflammation (19). Several experiments and studies proved that microbes in gut play an important role in alteration of energy homeostasis and development of fat mass.

This was evident from several experiment wherein germ free (GF) mice i.e, the mice was grown in absence of any microorganism and the other conventional (CONV) mice contained different microbiota since birth, were studied and compared. Earlier reports revealed that germ free mice were leaner compared to the microorganism harboured mice. Further, the germ free mice altered by inoculation of gut microbiota showed an increased level of fat mass and insulin resistance (20). Influence of western diet feeding on GF and CONV mouse has been compared recently been reported. Both groups exhibited glucose tolerance, increased adipose tissue inflammation, repressed hepatic AMP-protein kinase (AMPK) activity, fatty liver and elevated hepatic triglycerides. However, enhanced fatty acid oxidation and higher CPT1 activity in the GF mouse compared to their CONV counterparts was opined to be responsible for resistance to diet induced obesity (DIO) (21).

Studies have shown that different individuals carry different gut microbiota and the microbiota also varies between an obese individual and a lean individual (22). Even a human baby microbiota and a mice microbiota exhibited difference and it was revealed that gut microbiota regulate absorption, storage and metabolism of dietary lipids. It was also found that gut microbiome is responsible for the co-dependency between metabolic patterns and induction of obesity with high fat diet in rats or mice (23). Changes in gut microbiota also disrupted choline metabolism, which eventually developed into insulin resistance and non-alcoholic fatty liver disease in a mouse strain that has been given high-fat diet and genetically predisposed to these disorders (24). Many other studies have contributed to prove the importance of gut microbiota in host metabolism. It was found that difference in gut microbial population in genetically homogenous individual exerts diversity in metabolic phenotypes (25). Surprisingly, identical twins also had significant diversity in their gut microbial populations although they share a lot of similarity between them (26). So, it was opined that microbiota uniqueness in gut plays a significant role in host metabolism and at the whole organism level on host genetics and pathophysiology (27).

Gut associated microbes in obese individuals:

Different experiments have shown that gut microbial population varies from one individual to another with the presence or absence of obesity. Present day gut microbiota composition and diversity could be associated with the pathology of obesity (21,23). There may be changes in plenty at the level of species, genus or phyla of microbial population in gut. Previously, it has been reported in genetically obese mouse (ob/ob), have 50% decreased *Bacteroidetes* and increased *Firmicutes* in contrast with their leaner siblings (+/+) (28). Diversity of microbes in gut decreased significantly in obese people compared to non-obese controls (29).

However, in two separate reports, unlike previous studies no significant effect of firmicutes: bacteroides (F/B) ratio on obesity phenotype in human was observed and was opined that only gut F/B ratio could not be confirmly used as a marker of obesity (30,31). In a mouse model-based study, gut microflora has been reported to be responsible for controlling apetite by regulating grelin and leptin (elevation in leptin and depression in grelin decreases the level of apetite satiety and increases hunger) levels by production of short chain fatty acids (SCFA). The abundance of several bacterial genera like *Mucispirillum, Lactococcus*, *Bifidobacterium*, and *Lactobacillus*, in particular was found to be positively correlated with circulating leptin concentrations in mice, while other bacterial genera, including *Allobaculum, Clostridium, Bacteroides*, and *Prevotella*, negatively correlated with leptin levels. As far ghrelin is cocerned, predominance of *Bifidobacterium*, *Lactobacillus*, and *B. coccoides, Eubacterium rectale* groups was negatively correlated; instead, Bacteroides and Prevotella positively influenced its levels. Increase in hunger results in more food intake thereby increasing body weight (32). In addition to *Firmicutes* and *Bacteroides* that accounts to 64% and 23% of gut microflora respectively, *Proteobacteria* (8%) which included gram-negative bacteria (i.e., *Escherichia coli* and *Helicobacter pylori*), *Fusobacteria, Verrucomicrobia*, and *Actinobacteria* (3%) that include genera such as *Bifidobacterium* were also reported (33). Role of SCFA in prevention and treatment of obesity-associated insulin resistance has been extensively reported. Mainly, propionate and butyrate suppress the activity of orexigenic neurons that express neuropeptide Y in the hypothalamus and the modulates signaling mediate by the ghrelin receptor. Experimental shift in the gut microbiome in mice toward increased production of butyrate resulted in production of higher level of GLP-1, as well as hypothalamic expression of pro-opiomelanocortin, thereby influencing the hunger-satiety cycle (34). Gut bacteria are responsible for production of principally three types of SCFA: acetate, propionate and butyrate. Enteric bacteria, including *Ruminococcus spp*., *Prevotella spp.*, *Bifidobacterium spp*., and *Akkermansia muciniphila* are suggested to be the main acetate-producing bacteria in gut (35). Propionates are mainly produced by *Bacteroidetes* and *Negativicutes* (Firmicutes phylum), and *Lachnospiraceae*, while butyrate is principally produced by *Faecalibacterium prausnitzii, Eubacterium rectale, Eubacterium hallii, Coprococcus comes* and *Coprococcus eutactus* (36). As far as childhood obesity is concerned genus *Bifidobacterium* was found to be higher and *Staphylococcus spp*. was lower in normal weight children although loss of abundance and diversity of gut microbiota in obese individuals was principally evident (37).

Experiments carried out to establish the relationship between gut microbe and obese host:

Role of gut microbiota in establishment of metabolic disease such as obesity and type 2 diabetes has been extensively reported in recent studies.

Base on the fact that impaired leptin signalling resulted in metabolic disease like obesity and type 2 diabetes, gut microflora of genetically obese (Ob/ob) (biologically inactive leptin encoding gene due to systemic nonsense) and genetically diabetic (db/db) (mutated receptor encoding sequence of leptin hence inactive intracellularly) mice was studied and compared (38). Total metagenomic DNA was isolated from these two groups and high throughput next generation sequencing was carried out targeting highly variable V3-V4 region of the 16S rRNA gene was using universal primers 341F (5′-CCTACGGGRSGCAGCAG-3′) and 806R (5′-GGACTACVSGGGTATCTAAT-3′). Clustering analysis of obtained sequence data identified identified 14,292 operational taxonomic units amongst which *Bacteroidetes* and *Firmicutes* dominated the microbial composition in ob/ob mice, and exhibited relative abundance of 39.28% and 52.86%. The same in db/db mice was 46.06% and 46.58%, respectively. Significant difference in gut microbial population between both group of mice at different age and between the two groups was also reported. In fecal microbiota of 12-week-old ob/ob mice Bacillales was significantly high which exhibited a shift to abundance of *Moraxellaceae* in same mice of 18 weeks age. In fecal microbiota of db/db mice, abundance of *Coriobacteriaceae, Ochrobactrum*, and *Bradyrhizobiaceae* at 8 weeks of age shifted to abundance of *Desulfovibrio* after 12 weeks and after 18 weeks abundance of *Brachybacterium, Pseudonocardia* and *Bacteroidales* was observed. Difference in microbial composition of fecal samples between two types of mice of same age was observed also observed. At 8 weeks of age, fecal microbiota of ob/ob mice was dominated by Bacteroidaceae, Desulfovibrionaceae, Pasteurellaceae, and Anaeroplasmataceae family of microbes in contrast to Propionibacteriaceae, Prevotellaceae, Dehalobacteriaceae, Ruminococcaceae, Sphingomonadaceae, Eterobacteriaceae, and Verrucomicrobiaceae family in db/db mice. It was concluded that since microbial composition showed a similar fluctuation pattern with that of the blood glucose values in ob/ob and db/db mice with age. This reflects that the gut microbiota, especially the taxa with a relatively higher abundance at a specific age, may be involved in glucose homeostasis.

In another study, gut microflora of 21 adult obese human subjects was studied by 16S rRNA gene sequence-based analysis and was compared with gut flora sequences of non-obese (n=21) subjects obtained from Microbial Genome Database System. Total genomic DNA was obtained from the stool samples of obese individuals, amplified V3-V5 region of 16S rRNA gene and the amplicons were subjected to next generation sequencing (39). The study in total

compared 1,943,896 sequences (39,477 16S rRNA genes of each sample) using the widely used bioinformatics software Mothur. After removal of low quality sequences, this software was used to calculate community richness, evenness and diversity index analysis. Differences between the gut flora composition in obese and non obese control samples were assessed using Analysis of Molecular Variance (AMOVA) in Mothur. Prediction of microbiome function in the same study was carried out by another bioinformatics tool called PICRUSt2. The alpha diversity calculated revealed that the gut microbiota of obese individuals had lower richness (ACE and Chao index), lower evenness and diversity (based on Shanon Index) in comparison to that of non-obese individuals. Based on AMOVA test, decrease in abundance of phylum firmicutes and decrease in abundance of phylum bacteroides was observed in the gut microflora of obese individuals, when compared to that of non-obese healthy subjects. At family level, *Prevotellaceae, Veillonellaceae, Ruminococcaceae, Fusobacteriaceae, Porphyromonadaceae and Rikenellaceae* of which three were *Bacteroides* were found to different among obese and healthy individuals. Significantly, the concentration of *Bacteroides uniformis* was reduced while *Blautia* and *Prevotella copri* increased in gut microflora of obese individuals, which inturn could be corelated to SCFA content in feaces, a major indicator of obesity. The abundance of *Bifidobacterium* (which was previously reported to exhibit anti-obesity effect (39)) was reduced in obese individuals (0.33% in comparison to 1.21% in non obese individuals), while that of the genus *Prevotella* increased in obese individuals (30.57%) with respect to non obese subjects (7.22%). Referring to previous reports it was concluded that that the genus *Prevotella*, due to their physiological characteristics like production of succinate, triglycerides and highly sensitive C- reactive protein, could be positively co-related to obesity.

Body mass index (BMI) has been considered as a major indicator of obesity and as per classification by WHO, MI < 18·5 kg/m^2 for lean, BMI = 18·5 – 24·9 kg/m^2 for normal weight, BMI = 25 – 29·9 kg/m^2 for overweight and BMI >30 kg/m^2 for obesity (40). As per Chinese BMI classification (more or less similar to WHO BMI classification), a study group was classified into 3 classes- lean, normal and overweight and their feacal microbiota was analyzed by 16Sr RNA gene based next generation sequencing. Based on the obtained results, taxonomic classification and determination of α and β-diversity indices was carried out and was corelated with BMI of the subjects under study (41). Like other reports, the phylum Bacteroidetes and Firmicutes dominated in the fecal microflora, but no significant variation in Firmicutes:Bacteroidetes ratio was observed among different groups. BMI exhibited negative correlation with gut microbiota Shanon and Simpson indices indicating decrease in gut microbial diversity with increase in BMI. Microbiome signatures among lean and obese students was reported in the same study. In obese group, the genus *Parasutterella* was dominant while the

genus *Blautia, Megasphaera, Klebsiella,* uncultured *Lachnospiraceae* and *Anaerotruncus* were enriched in the fecal samples of lean group.

Conclusion:

Results and observations from different experiments showed that gut microbial population both in experimentally studied mice system as well as human population typically could be corelated with several metabolic disorders like type 2 diabetes and obesity. In all the studies reviewed herein, it was generally concluded that obese phenotype could be typically correlated with decrease in gut microbial diversity. SCFA producing bacterial phenotypes were found to control obesity by modulating hunger-satiety balance while *Bifidobacterium* that has been widely reported to possess anti-obesity effect typically decreased in gut of obese individuals. However, Firmicutes:Bacteroidetes ratio, which was typically considered as a marker of diversity was opined not to be considered in conformity and requires further analysis. With the advent of next generation sequencing and availability of several bioinformatics tools, it has been possible to successfully carry out microbial community analysis based on molecular signature markers and species richness, evenness and diversity indices of gut microflora has been successfully determined. All the analysis and reports unifiedly opined that loss in diversity of gut microflora could be a major reason behind obesity.

References:

1. Donnelly, J. E., Hillman, C. H., Castelli, D., Etnier, J. L., Lee, S., Tomporowski, P., ... & Szabo-Reed, A. N. (2016) Physical activity, fitness, cognitive function, and academic achievement in children: a systematic review. Med. Sci. Sports. Exerc. 48(6), 1197
2. Ramya, A., & Rohini, K. (2022). Implications Of Associated Diseases And Its Factors In Predicting The Obesity Disorder. Elementary Education Online, 20(6), 3381-3381.
3. Bouchard, C. (2021). Genetics of obesity: what we have learned over decades of research. Obesity, 29(5), 802-820.
4. Tseng, C. H., & Wu, C. Y. (2019). The gut microbiome in obesity. Journal of the Formosan Medical Association, 118, S3-S9.
5. Fagoonee, S., & Pellicano, R. (2019). Does the Microbiota Play a Pivotal Role in the Pathogenesis of Irritable Bowel Syndrome?. Journal of Clinical Medicine, 8(11), 1808.
6. Van Meter, K. C., & Hubert, R. J. (2015) Microbiology for the Healthcare Professional-E-Book. Elsevier Health Sciences. pp. 182
7. Hall, A., & Versalovic, J. (2018). Microbial metabolism in the mammalian gut: molecular mechanisms and clinical implications. Journal of pediatric gastroenterology and nutrition, 66, S72-S79.

8. Sahu, S., Jaysingh, P., & Mishra, M. (2022). As an in vivo model for the investigation of host-microbiota interaction. In Prebiotics, Probiotics and Nutraceuticals (pp. 275-300). Springer, Singapore.
9. Sharndama, H. C., & Mba, I. E. (2022). Helicobacter pylori: An up-to-date overview on the virulence and pathogenesis mechanisms. Brazilian Journal of Microbiology, 1-18.
10. Kastl Jr, A. J., Terry, N. A., Wu, G. D., & Albenberg, L. G. (2020). The structure and function of the human small intestinal microbiota: current understanding and future directions. Cellular and molecular gastroenterology and hepatology, 9(1), 33-45.
11. Dekaboruah, E., Suryavanshi, M. V., Chettri, D., & Verma, A. K. (2020). Human microbiome: an academic update on human body site specific surveillance and its possible role. Archives of microbiology, 202(8), 2147-2167.
12. Hadadi, N., Berweiler, V., Wang, H., & Trajkovski, M. (2021). Intestinal microbiota as a route for micronutrient bioavailability. Current opinion in endocrine and metabolic research, 20, 100285.
13. Song, Q., Wang, Y., Huang, L., Shen, M., Yu, Y., Yu, Q., ... & Xie, J. (2021). Review of the relationships among polysaccharides, gut microbiota, and human health. Food Research International, 140, 109858.
14. Cunningham, A. L., Stephens, J. W., & Harris, D. A. (2021). A review on gut microbiota: a central factor in the pathophysiology of obesity. Lipids in Health and Disease, 20(1), 1-13.
15. Liaqat, I., Durrani, A. I., Zafar, U., Rubab, S., Faheem, M., Mubin, M., ... & Aftab, N. (2021). Role of modified diet and gut microbiota in metabolic endotoxemia in mice. Archives of Microbiology, 203(8), 5085-5093.
16. Jian, Z., Zeng, L., Xu, T., Sun, S., Yan, S., Zhao, S., ... & Dou, T. (2022). The intestinal microbiome associated with lipid metabolism and obesity in humans and animals. Journal of Applied Microbiology.
17. Stanislawski, M. A., Dabelea, D., Lange, L. A., Wagner, B. D., & Lozupone, C. A. (2019). Gut microbiota phenotypes of obesity. NPJ biofilms and microbiomes, 5(1), 1-9.
18. Tseng, C. H., & Wu, C. Y. (2019). The gut microbiome in obesity. Journal of the Formosan Medical Association, 118, S3-S9.
19. Wang, P. X., Deng, X. R., Zhang, C. H., & Yuan, H. J. (2020). Gut microbiota and metabolic syndrome. Chinese medical journal, 133(07), 808-816.
20. Bäckhed, F., Ding, H., Wang, T., Hooper, L. V., Koh, G. Y., Nagy, A., ... & Gordon, J. I. (2004). The gut microbiota as an environmental factor that regulates fat storage. Proceedings of the national academy of sciences, 101(44), 15718-15723.

21. Moretti, C. H., Schiffer, T. A., Li, X., Weitzberg, E., Carlström, M., & Lundberg, J. O. (2021). Germ-free mice are not protected against diet-induced obesity and metabolic dysfunction. Acta Physiologica, 231(3), e13581.
22. Sun, L., Ma, L., Ma, Y., Zhang, F., Zhao, C., & Nie, Y. (2018). Insights into the role of gut microbiota in obesity: pathogenesis, mechanisms, and therapeutic perspectives. Protein & cell, 9(5), 397-403.
23. Li, M., Wang, B., Zhang, M., Rantalainen, M., Wang, S., Zhou, H., ... & Zhao, L. (2008). Symbiotic gut microbes modulate human metabolic phenotypes. Proceedings of the National Academy of Sciences, 105(6), 2117-2122.
24. Jennison, E., & Byrne, C. D. (2021). The role of the gut microbiome and diet in the pathogenesis of non-alcoholic fatty liver disease. Clinical and Molecular Hepatology, 27(1), 22.
25. Li, M., Wang, B., Zhang, M., Rantalainen, M., Wang, S., Zhou, H., ... & Zhao, L. (2008). Symbiotic gut microbes modulate human metabolic phenotypes. Proceedings of the National Academy of Sciences, 105(6), 2117-2122.
26. Turnbaugh, P. J., Quince, C., Faith, J. J., McHardy, A. C., Yatsunenko, T., Niazi, F., ... & Gordon, J. I. (2010). Organismal, genetic, and transcriptional variation in the deeply sequenced gut microbiomes of identical twins. Proceedings of the National Academy of Sciences, 107(16), 7503-7508.
27. Rowland, I., Gibson, G., Heinken, A., Scott, K., Swann, J., Thiele, I., & Tuohy, K. (2018). Gut microbiota functions: metabolism of nutrients and other food components. European journal of nutritio
28. Ley, R. E., Bäckhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., & Gordon, J. I. (2005). Obesity alters gut microbial ecology. Proceedings of the national academy of sciences, 102(31), 11070-11075.
29. Duan, M., Wang, Y., Zhang, Q., Zou, R., Guo, M., & Zheng, H. (2021). Characteristics of gut microbiota in people with obesity. Plos one, 16(8), e0255446.
30. Houtman, T. A., Eckermann, H. A., Smidt, H., & de Weerth, C. (2022). Gut microbiota and BMI throughout childhood: the role of firmicutes, bacteroidetes, and short-chain fatty acid producers. Scientific Reports, 12(1), 1-13.
31. Lv, Y., Qin, X., Jia, H., Chen, S., Sun, W., & Wang, X. (2019). The association between gut microbiota composition and BMI in Chinese male college students, as analysed by next-generation sequencing. British Journal of Nutrition, 122(9), 986-995.

32. Neuman, H., Debelius, J. W., Knight, R., & Koren, O. (2015). Microbial endocrinology: the interplay between the microbiota and the endocrine system. FEMS microbiology reviews, 39(4), 509-521.
33. Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., ... & Relman, D. A. (2005). Diversity of the human intestinal microbial flora. science, 308(5728), 1635-1638.
34. Silva, Y. P., Bernardi, A., & Frozza, R. L. (2020). The role of short-chain fatty acids from gut microbiota in gut-brain communication. Frontiers in endocrinology, 11, 25.
35. Nogal, A., Louca, P., Zhang, X., Wells, P. M., Steves, C. J., Spector, T. D., ... & Menni, C. (2021). Circulating levels of the short-chain fatty acid acetate mediate the effect of the gut microbiome on visceral fat. Frontiers in microbiology, 1943.
36. Deleu, S., Machiels, K., Raes, J., Verbeke, K., & Vermeire, S. (2021). Short chain fatty acids and its producing organisms: An overlooked therapy for IBD?. EBioMedicine, 66, 103293.
37. Chen, X., Sun, H., Jiang, F., Shen, Y., Li, X., Hu, X., ... & Wei, P. (2020). Alteration of the gut microbiota associated with childhood obesity by 16S rRNA gene sequencing. PeerJ, 8, e8317.
38. Duan, M., Wang, Y., Zhang, Q., Zou, R., Guo, M., & Zheng, H. (2021). Characteristics of gut microbiota in people with obesity. Plos one, 16(8), e0255446.
39. Chang, C. S., Ruan, J. W., & Kao, C. Y. (2019). An overview of microbiome based strategies on anti-obesity. The Kaohsiung Journal of Medical Sciences, 35(1), 7-16.
40. Zhou B. F. (2002). Effect of body mass index on all-cause mortality and incidence of cardiovascular diseases--report for meta-analysis of prospective studies open optimal cut-off points of body mass index in Chinese adults. Biomedical and environmental sciences : BES, 15(3), 245–252.
41. Lv, Y., Qin, X., Jia, H., Chen, S., Sun, W., & Wang, X. (2019). The association between gut microbiota composition and BMI in Chinese male college students, as analysed by next-generation sequencing. British Journal of Nutrition, 122(9), 986-995.

BIODIVERSITY AND CONSERVATION OF THE WORLD'S LARGEST MANGROVE ECOSYSTEM- THE SUNDARBANS

Arvinda Shaw
Department of Food & Nutrition, Raidighi College, West Bengal.
Corresponding author E-mail: arvindajswl844@gmail.com

Abstract:

The Sundarbans- the most important mangrove ecosystem on the Earth, meaning the "beautiful forest" derives its name from the Sundari trees found here. The Sundarbans is intersected by a complex network of tidal waterways, mudflats and little islands of salt-tolerant mangrove forest formed by the super confluence of the rivers- Ganges, Brahmaputra and Meghna in the Bay of Bengal. This unique diverse ecosystem is understood for its wide selection of flora and fauna -the most famous being the man-eating Royal Bengal Tigers besides numerous species of birds, spotted deer, crocodiles, snakes etc. Due to its extremely rich diversity and uniqueness, Sundarbans has been declared as a UNESCO World Heritage Site. Over the centuries, these mangroves have been extensively exploited for timber, fish, prawns or converted for paddy and aquaculture, as a result, it is now facing the intense challenge for its existence. The constantly growing human population, global climate changes particularly the ocean level rise are some of the main threats to this vast magnificent biodiversity. Further loss of this precious mangrove forest will ultimately result in the reduced protective biological shield against disastrous cyclones and tsunamis which would not only greatly endanger its diverse flora and fauna but also put the encompassing coastal communities at high risk of survival. Therefore strict measures should be taken to conserve this valuable biodiversity and decrease the man-animal conflict.

Keywords: Sundarbans, mangrove, ecosystem, biodiversity, conservation

Introduction:

The Sundarbans- the world's most important and largest mangrove ecosystem, meaning the "beautiful forest" derives its name from the Sundari trees found here.[1]The Sundarban Mangrove ecosystem is a unique and highly productive ecosystem within the sea-land interphase with the conglomerations of plants, animals and microorganisms acclimatized within the fluctuating environment of the tropical intertidal zone and protect the coastal areas against natural hazards like cyclones and tsunamis.[2-5] It is intersected by a complex network of tidal waterways mudflats and little islands of salt-tolerant mangrove forests This ecosystem is highly valued in terms of economy, environment and ecology.[6-12]

Area:

The Sundarbans is found within 21°32′ to 22°40′N and 88°05′ to 89°51′E and covers an area of roughly 10,000 km of which 62% lies within Bangladesh and only 38% in India.[13]

Formation of sunderban delta:

This ecosystem has been formed by the super confluence of the rivers-Ganges Brahmaputra and Meghna. [1]The mighty Indian river, the Ganges and its associated estuaries like Muriganga, Saptamukhi, Bidyadhari, Haribhanga, Thakuran etc, open into the Bay of Bengal having a north-south direction of water flow. The silt and loam carried by these estuaries were deposited on the salt marsh eventually resulting in the formation of a mosaic of 102 deltaic islands of which 54 are reclaimed for human habitation. Rivers within the Sundarbans are meeting places of saltwater and freshwater [13-14] actually it's a region of transition between the freshwater of the rivers originating from the Ganges and the saline water of the Bay of Bengal.[14]

Uniqueness:

This unique diverse ecosystem is understood for its wide range of flora and fauna -the most famous being the man-eating Royal Bengal Tigers besides numerous species of birds, spotted deer, crocodiles, snakes etc. Extremely rich biodiversity and uniqueness of Sundarbans has earned it the prestigious tag of UNESCO World Heritage Site and natural wonder of the planet. [1,13]

Biodiversity:

This ecosystem harbours thousands of flora and fauna in its diversified habitats along with the highest number of mangrove tree species which accounts for one-third of the global total, high biodiversity within the Sundarbans is additionally represented by other groups with more than 200 additional plant species, more than 400 species of fish, over 300 species of birds, 35 species of reptiles, 42 species of mammals, also countless invertebrates, bacteria, fungi, etc.[15,16]

Importance of this ecosystem:

The environment of the Sundarbans mangroves—both in India and Bangladesh—is densely populated. This population being impoverished depends heavily on the forests for their livelihood and sustenance. [17-19] This ecosystem maintains the agricultural economy by providing timber & fuelwood, faunal resources like fishes, honey etc, protects the coast from erosion, mitigates flood and maintains estuarine flow. Mangrove trees are used for timber and construction material (e.g. for houses, boats, traps) also as for fuel and charcoal production. Apiculture is widespread within the Sundarbans mangrove forests and provides honey and wax. A large number of people are engaged in beekeeping within the Indian Sundarbans, producing

approximately 90% of the entire natural honey production in India.[13] Apart from these useful resources, mangrove trees also provide tannins for leather production and are home to a wide array of medicinal plants. Innumerable varieties of sea creatures like crabs, molluscs, shrimps and fish are caught within the brackish waters [1] which also acts as nursery grounds for several commercially important fish species. One of the most important beneficial effects of this mangrove is that it acts as buffers against cyclones, storms etc. The 2004 tsunami could have caused more destruction of lives and property had the buffering capacity of the forest been not present.[20] Furthermore, in May 2009, much of the momentum of cyclone Aila was absorbed by the mangroves, saving the city of Kolkata from clutches of greater loss.

Threats to this biodiversity & ecosystem:

During the past three decades, large parts of the remaining Sundarbans are protected for wildlife conservation, particularly tigers, through the creation of several sanctuaries and biosphere reserves. However, the biodiversity and basic fabric of ecosystem functioning are being threatened due to several reasons like [1]: reclamation of the deltaic island for human use, deforestation, erosion and unwanted accretion, embankment (polder) erosion/waterlogging salinity invasion,[21] freshwater reduction from the north (eg. Farraka barrage), [1,22,23] overexploitation of natural resources, illegal shrimp fry collection, hunting and tree felling. Top dying in Sundri (*Heritiera fomes*) trees [22,23], construction of embankments,[24] ecotourism [25] pollution & sea-level rise, global climate change and heating[26] have aggravated the matter.

Conclusion:

Although, significant conservation measures are being taken by the government of both the countries (Bangladesh and India) to preserve the magnificent biodiversity of the Sundarbans, yet the mangrove is slowly being destructed due to heating, climate change, human interference, man and animal conflict, rampant deforestation etc. Conservation of this ecosystem is urgently required because it's not only the World's most valuable mangrove which provides food, water, other natural resources and occupation to millions for their livelihood and sustenance; habitat for several species of plants, birds, reptiles, insects, animals, including the protected Royal Bengal tigers but also protects its inhabitants and adjoining areas from the worst effects of cyclones and tsunamis.

References:

1. Wikipedia – the Sundarbans. Available at: https://en.wikipedia.org/wiki/Sundarbans
2. Dahdouh-Guebas, F., Jayatissa, L.P., di Nitto, D., Bosire, J.O., Lo Seen, D. and Koedam, N. (2005) How effective were mangroves as a defence against the recent tsunami? Curr. Biol. 15, 443–447

3. Danielsen, F., Sørensen, M.K., Olwig, M.F., Selvam, V., Parish, F., Burgess, N.D., Hiraishi, T., Karunagaran, V.M., Rasmussen, M.S., Hansen, L.B. et al. (2005) The Asian Tsunami: A protective role for coastal vegetation. Science, 310, 643
4. Kathiresan, K. and Rajendran, N. (2005) Coastal mangrove forests mitigated tsunami. Estuar. Coast. Shelf Sci. 65, 601–606
5. Williams, N. (2005) Tsunami insight into mangrove value. Curr. Biol. 15, 73
6. Costanza, R., Farber, S.C. and Maxwell, J. (1989) The valuation and management of wetland ecosystems. Ecol. Econ. 1, 335–361
7. Costanza, R., de Groot, R., Sutton, P., van der Ploeg, S., Anderson, S.J., Kubiszewski, I., Farber, S. and Turner, R.K. (2014) Changes in the global value of ecosystem services. Glob. Environ. Change. 26, 152–158
8. Bann, C. (1997) The Economic Valuation of Mangroves: A Manual for Researchers; International Development Research Centre: Ottawa, ON, Canada; p. 54
9. Barbier, E.B. (2007) Valuing ecosystem services as productive inputs. Econ. Policy. 49, 177–229
10. Walters, B.B., Rönnbäck, P., Kovacs, J.M., Crona, B., Hussain, S.A., Badola, R., Primavera, J.H., Barbier, E. and Dahdouh-Guebas, F. (2008) Ethnobiology, socio-economics and management of mangrove forests: A review. Aquat. Bot. 89, 220–236
11. Salem, M.E. and Mercer, D.E. (2012) The economic value of mangroves: A meta-analysis. Sustainability. 4, 359–383
12. Russi, D., ten Brink, P., Farmer, A., Badura, T., Coates, D., Förster, J., Kumar, R. and Davidson, N. (2013) The Economics of Ecosystems and Biodiversity for Water and Wetlands; IEEP: London, UK; p. 84
13. Spalding, M., Kainuma, M. and Collins, L. (2010) World Atlas of Mangroves; Earthscan: London, UK; p. 319
14. Wahid, S.M., Alam, M.J. and Rahman, A. (17–18 June 2002). "Mathematical river modelling to support ecological monitoring of the largest mangrove forest of the world – the Sundarbans". Proceedings of First Asia-Pacific DHI software conference.
15. Gopal, B. and Chauhan, M. (2006) Biodiversity and its conservation in the Sundarban mangrove ecosystem. Aquat. Sci. 68, 338–354
16. IUCN. (2001) International Union for the Conservation of Nature—Bangladesh: The Bangladesh Sundarbans: A Photo Real Sojourn; IUCN Bangladesh Country Office: Dhaka, Bangladesh.

17. Banerjee, A.K. (1964) Forests of Sundarbans, Centenary Commemoration Volume, West Bengal Forests. Planning and Statistical Cell, Writer's Building; Calcutta: Bengal, India; p. 188
18. Iftekhar, M.S. (2008) An overview of mangrove management strategies in three South Asian countries: Bangladesh, India and Sri Lanka. Int. For. Rev. 10, 38–51
19. Shams-Uddin, M., Shah, M.A.R., Khanom, S. and Nesha, M.K. (2013) Climate change impacts on the Sundarbans mangrove ecosystem services and dependent livelihoods in Bangladesh. Asian J. Conserv. Biol. 2, 152–156
20. Saenger, P. (2011) Mangroves: Sustainable management in Bangladesh. In Tropical Forestry 8, Silviculture in the Tropics; Günter, S., Weber, M., Stimm, B., Mosandl, R., Eds.; Springer: Berlin, Germany, pp. 339–347
21. Hazra, S., Ghosh, T., Dasgupta, R and Sen, G. (2002) Sea level and associated changes in the Sundarbans. Science and Culture. 68, 9-12:309-321
22. Rahman, M.A. (1990) A comprehensive report on sundry (Heritiera fomes) trees with particular reference to top dying in the Sundarbans. In Seminar on Top Dying of Sundri (*Heritiera fomes*) Trees; Rahman, M.A., Khandakar, M.A., Ahmed, F.U., Ali, M.O., Eds.; Bangladesh Agricultural Research Council: Dhaka, Bangladesh; p. 256.23. Zaman, S., Bhattacharyya, S.B., Pramanick, P., Raha, A.K., Chakraborty, S. and Mitra, A. (2013) Rising water salinity: A threat to mangroves of Indian Sundarbans. InWater Insecurity: A Social Dilemma; Abedin, M.A., Habiba, U., Shaw, R., Eds.; Emerald Group Publishing Limited: Bingley, UK; pp. 167–183
23. Chaudhuri, A. B. and Choudhury, A. (1994). Mangroves of the Sundarbans. Vol I, 1-247
24. Dinda, A. (2007) Evaluation of eco-tourism activity: A case study of Sundarban tiger reserve. In Man in Biosphere: A Case Study of Sundarban Biosphere Reserve; Gyan: New Delhi, India observing satellite phased array type L-Band SAR (ALOS PALSAR) to inform the conservation of mangroves: Sundarbans as a case study. Remote Sens, 5, 224–237
25. Raha, A., Das, S., Banerjee, K. and Mitra, A. (2012) Climate change impacts on Indian Sunderbans: A time series analysis (1924–2008). Biodivers. Conserv. 21, 1289–1307

STUDIES ON INTERACTION OF tRNA AND UNFOLDED PROTEIN WITH RIBOSOME

Arunima Biswas

Department of Microbiology,

Raidighi College, Raidighi, South 24 Parganas, Pin 743383, West Bengal

Corresponding author E-mail: mou.aru@gmail.com

Abstract:

Ribosome from all sources can act as general protein folding modulators. In bacteria, the active site for protein folding overlaps with the peptidyl transferase centre (PTC) in the domain V of *Escherichia coli* 23S rRNA. The same set of conserved nucleotides in the PTC interacts identically with full length nascent protein and chemically unfolded proteins both *in vivo* and *in vitro* to make them folding competent. During *in vitro* protein folding, the unfolded protein dissociates the 70S ribosome and sequesters the 50S subunit to access the PTC in order to attain a folding competent state. This folding competent protein can then slowly achieve its tertiary active conformation with the help of other factors like the chaperones. For efficient protein folding, the PTC needs to be fully open and accessible to the folding protein and also be free of any other PTC binding factors. The transfer RNA (tRNA) is a crucial member of the translation team. It interacts with a number of sites on the ribosome. Preliminary *in vitro* findings suggest that deacylated tRNA molecules, bound to P and E sites of domain V, inhibit 70S from participating in the folding process. The refolding ability of the 50S subunit, 23S rRNA and domain V rRNA, when studied separately, is also found to be significantly affected by the presence of bound tRNA. Such inhibition seems to be very specific requiring precise interaction between P loop of domain V rRNA and 3′-CCA end of tRNA.

Keywords: protein folding, ribosome, tRNA

Introduction:

It has been shown that the active site of protein folding in bacteria is the domain V of 23S rRNA on the 50S ribosomal subunit. The domain V has been split into two fragments. One part, called RNA1 (comprising of mainly the central loop that overlaps the PTC), binds the denatured protein. The rest of domain V, called RNA2, helps in releasing the protein into the “on pathway” of folding. Only a conserved set of nucleotides of domain V rRNA binds specifically to unfolded proteins both *in vivo* and *in vitro* to make them folding competent (1-3). Earlier studies show that during *in vitro* protein folding, the unfolded protein dissociates the 70S ribosome and sequesters the 50S subunit to access the PTC (4-6) in order to attain a folding competent state which can then slowly achieve its tertiary active conformation with the help of other factors like the

chaperones. The folding process thus seems to require that the PTC be fully open and accessible to the folding protein and most likely also be free of any other PTC binding factors that might interfere with the process. Antibiotics which inhibit protein synthesis by binding to PTC, can also block protein folding *in vitro* and *in vivo* (1). Many of the conserved nucleotides on PTC that are crucial for ribosome - mediated protein folding overlap with regions for binding of A, P and E site tRNA molecules during translation. The main functional interactions between 23S rRNA and peptidyl-tRNA in the P site of the large ribosomal subunit are apparently limited to a Watson-Crick base pair between G2252 and C74, a non-canonical (possibly, triple-nucleotide) interaction of U2506 and U2585 with A76, and a contact between A2451 and the amino-acyl moiety of peptidyl-tRNA. Protection of some additional bases (e.g. G2251, G2253, U2584) also occur (7). This work shows that tRNA molecules bound to P and E sites on PTC can indeed inhibit ribosome-mediated protein folding *in vitro*.

Materials and Methods:

Preparation of ribosome and tRNA:

Wild type 70S ribosome and its subunits were prepared from *E.coli* MRE 600 (RNase I^-) cells, as described before (8). *E. coli* conditional mutant strains harbouring mutant ribosomes were kindly gifted by Prof. A.E. Dahlberg of Brown University, RI, USA. Wild type 23S rRNA, domain V rRNA and RNA 1 and RNA 2 were prepared as described before (9). Plasmid pSKb2 containing the gene for $tRNA^{Gln}$ and plasmid pHTk1 containing the gene for $tRNA^{Lys}$, were kindly gifted by Prof. Samit Adhya of IICB, Kolkata, along with the protocol for their preparation (10). Plasmid pKR15 containing the gene for $tRNA^{Glu}$ was a kind gift from Prof. Siddhartha Roy of IICB, Kolkata. $tRNA^{Glu}$ isolation was done as described before (11).

Study of ribosome mediated protein folding and its inhibition by tRNA:

For protein folding studies, a 31kD monomeric protein Bovine Carbonic Anhydrase (BCA) was used, that can be assayed quickly and conveniently. BCA was unfolded in 6M guanidine hydrochloride for 3 hours at 25ºC. The loss of secondary structure of the protein was confirmed by CD spectral analysis. Deacylated tRNA, in absence of any mRNA, is reported to bind to E site of 70S ribosome at 20mM Mg^{++} concentration and to both P site and E sites at a higher Mg^{++} (25mM) concentration (12). So, the refolding buffer was prepared accordingly. The refolding buffer was divided into a number of sets – one was used as control, and to the others ribosome (wild type or mutant) or rRNA was added such that its concentration would be same as that of the denatured protein that would be added to the same buffer (i.e. 1:1 molar ratio). To a number of these sets, tRNA (wild type or mutant) was added in increasing concentrations, and these sets were pre-incubated at 37ºC for 30 minutes to allow tRNA to bind to domain V rRNA

region, before the denatured protein was added to them. The denatured BCA was 100 times diluted in the refolding buffer to reduce guanidine hydrochloride concentration below the level which would be inhibitory to refolding reaction or enzyme assay. Refolding was done for 30 minutes at 25ºC after which each set was diluted 2-fold in assay buffer. The activity of the refolded enzyme was assayed by adding 500µM paranitrophenyl acetate to each set and measuring the increase in A_{400nm} with time. The extent of refolding was calculated by taking the ratio of the activity of the refolded enzyme to the activity of the same amount of native enzyme. While plotting graph, the level of self folding was taken as the baseline.

Filter Binding Assay:

Filter binding assay was performed by using cellulose nitrate filter paper with a pore size of 0.45 micron through which small molecules like RNA1 (337 nucleotides), RNA2 (425 nucleotides), or tRNA (~100 nucleotides) can pass easily, but would fail to pass if the RNA is bound by protein or some other large molecule like ribosome. If the RNA is radio-labeled, its binding to the filter can be measured.

Result and Discussion:

In order to study the interaction of tRNA and unfolded protein with PTC *in vitro*, refolding of unfolded protein was initially carried out by 70S ribosome with deacylated tRNA bound to its P and/or E site(s) at higher Magnesium concentrations in a poly-U independent manner (12). The study was then extended to 50S subunit, 23S rRNA, domain V rRNA and finally to the RNA1 and RNA 2 species.

Ribosome-tRNA complex formation was verified by filter binding assay (Fig. 1).

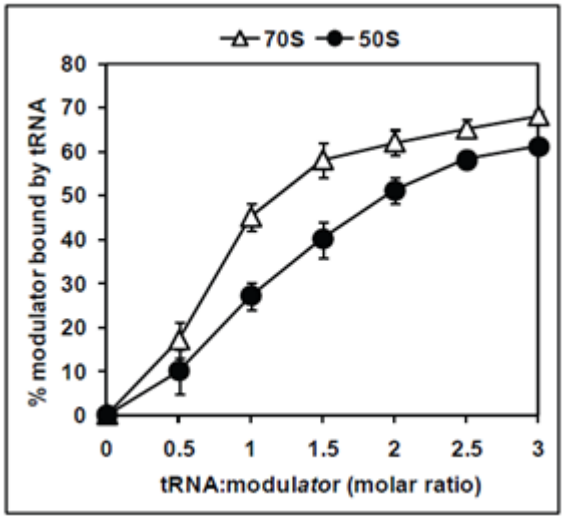

Figure 1: Deacylated tRNA binds to ribosome at high magnesium

Effect of tRNA on protein folding by wild type 70S ribosome, its 50S subunit and its rRNA:

In agreement with previous data, it was found that when allowed to self fold, unfolded BCA could gain back only 20-25% of its native activity whereas ribosome assisted folding increased the activity to 65% or more (1-3). But, deacylated tRNA bound to the E site of 70S ribosome at 20mM Mg^{++}, reduced the protein folding activity of that population of ribosome from 65% to 40% while deacylated tRNA bound to both P site and E sites of the 70S ribosome at 25mM Mg^{++}, lowered its protein folding activity from 65% to 28% at an added molar stoichiometry of ribosome : tRNA 1:3 (Fig. 2, panel A).

Although the tRNA binding conditions are optimum for 70S binding, studies were extended to 50S subunit of ribosome (Fig.2, panel A), its 23S rRNA and domain V rRNA (Fig. 2, panel B), and then finally to RNA 1 and RNA 2 (Fig.2, panel C). In each case, we found reproducible and significant inhibition of refolding by these folding modulators when bound to tRNA. Also, the extent of inhibition was found to be more pronounced at 25mM Mg^{++} concentration than in 20mM Mg^{++} concentration, especially for the complete 70S particle (Fig.2, panels A-C). It is to be noted that in case of the rRNA modulators, a comparatively higher tRNA concentration was needed to ensure enough complex formation between tRNA and rRNA molecules *in vitro*. Also, standardizing the tRNA binding conditions separately for all the folding modulators (50S onwards) may actually give even better folding inhibitory effect in each case.

Effect of tRNA on protein folding by mutant 70S ribosomes:

Single base change at position 2251 or 2252 of P loop of domain V rRNA eliminates its ability to interact with 3'-CCA end of P site tRNA. We obtained a large number of conditional ribosome-mutants in domain V region of rrnB operon in a plasmid derived from pKK3535 from Prof. A.E. Dahlberg of Brown University, USA. It should be noted that the mutant ribosomal preparations invariably were contaminated with some chromosomally encoded wild type 70S ribosomes, although the mutants were the predominant species. We worked with three different mutant species defective in P-site tRNA binding that carried G2251A, G2251U and G2252A mutations respectively in 70S ribosome. Refolding studies carried out with these mutant 70S ribosomes on unfolded BCA revealed that they act as very efficient folding modulators. It was also found that these mutant ribosomes fully retain their folding activity under P and E site tRNA binding conditions (Fig. 2, panel D). On the other hand, mutant U2555A 70S recognizes non-cognate tRNA at the A site, but is not defective in tRNA binding at the P site. In this mutant, tRNA inhibited the folding of denatured BCA under P site tRNA binding conditions, similar to the wild type 70S ribosome (positive control) (Fig. 2, panel D). This suggests that the conserved

nucleotides in P loop of domain V rRNA are necessary for inhibition of protein folding by tRNA.

Effect of tRNA with and without 3′-CCA end on protein folding by 70S ribosome:

Watson-Crick base pairing is reported between G2252 base in the P loop of domain V rRNA and C-74 of 3′-CCA end of tRNA. Folding inhibition studies were carried out with *E. coli* $tRNA^{Glu}$ and human $tRNA^{Lys}$, both having intact 3′-CCA ends and with *Leishmania tarentolae* $tRNA^{Gln}$ with 3′-CCA end deleted. BCA was unfolded as mentioned and allowed to refold by itself, by wild type 70S ribosome and by 70S ribosome pre-incubated separately with each of these tRNA molecules. It was found that protein folding by 70S ribosome was inhibited by wild type tRNA with intact 3′-CCA ends, while tRNA depleted of 3′-CCA end failed to show any inhibitory effect, presumably due to failure in tRNA-70S complex formation in the latter case (Fig. 3, panel A).

Effect of tRNA with 3′-UCA end on protein folding by 70S ribosome carrying G2252A mutation in P loop:

In ribosomes carrying G2252A mutation, the base pairing with C-74 of tRNA is lost, resulting in dominant lethal mutants that are defective in P site tRNA binding.

In an attempt to suppress such loss of interaction, the 3′-CCA end sequence of human $tRNA^{Lys}$ was mutated to UCA. It was incubated in increasing concentrations with ribosomes carrying G2252A mutation, under P site tRNA binding conditions at 37ºC for 30 minutes. Refolding reaction was carried out with this folding mix on unfolded BCA and assayed for recovery of enzyme activity. It was seen that the protein folding activity of the mutant 70S still remained unaffected (Fig. 3, panel B). This suggests the importance of tRNA CCA-end specificity in ribosome interaction, as loss of inhibition is not restored by mutation of CCA end of tRNA to UCA. Thus, this result once again establishes the importance of interaction between the universally conserved 3′ end sequence of the acceptor arm of tRNA and the conserved hairpin loop, the P loop, of 23S rRNA. It should be emphasized here that the binding of deacylated tRNAs to the E-site of ribosome is dependent on an intact CCA end. Although there are many contacts between the 23S rRNA and the tRNA, somehow the CCA end controls seventeen out of twenty contacts between the two.

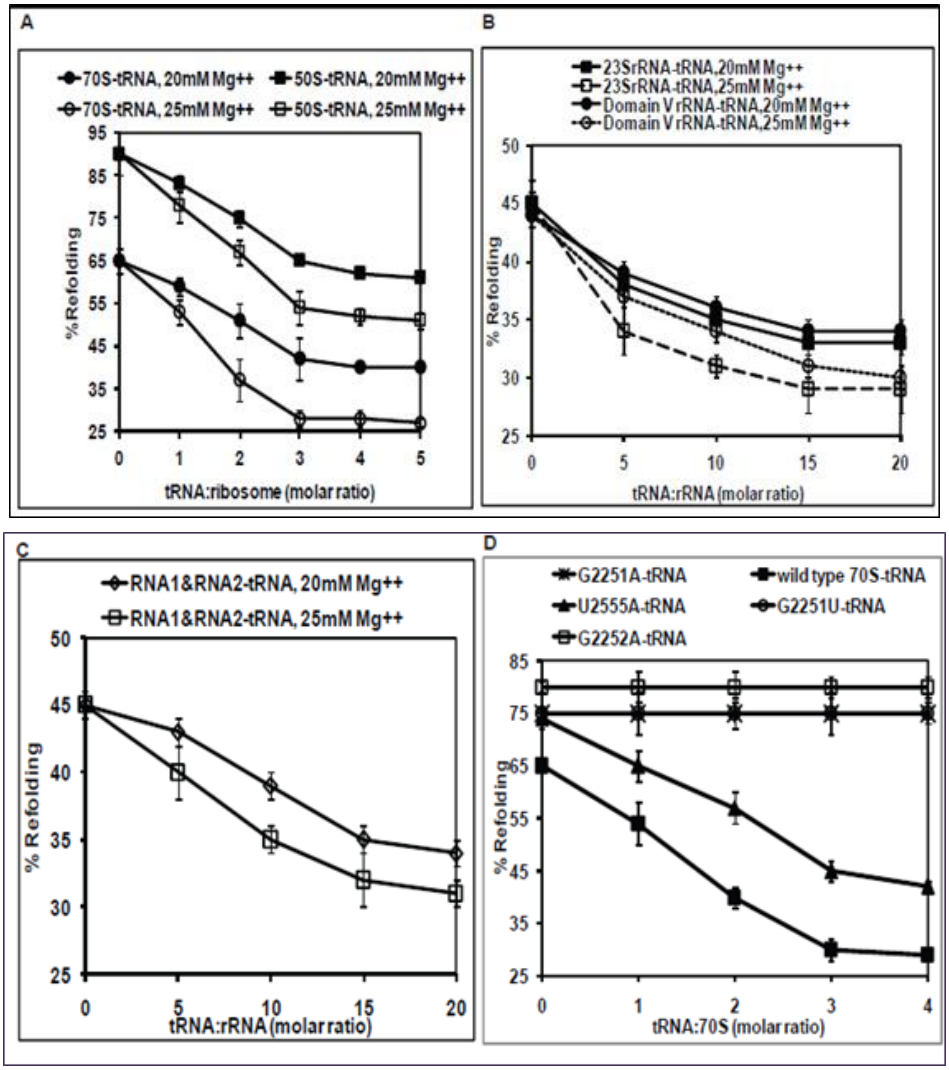

Figure 2 (A-D): tRNA bound to wild type ribosome and its rRNA inhibits protein folding. Loss of interaction restores protein folding ability of ribosome (described in details above). The level of self folding of protein has been taken as the baseline

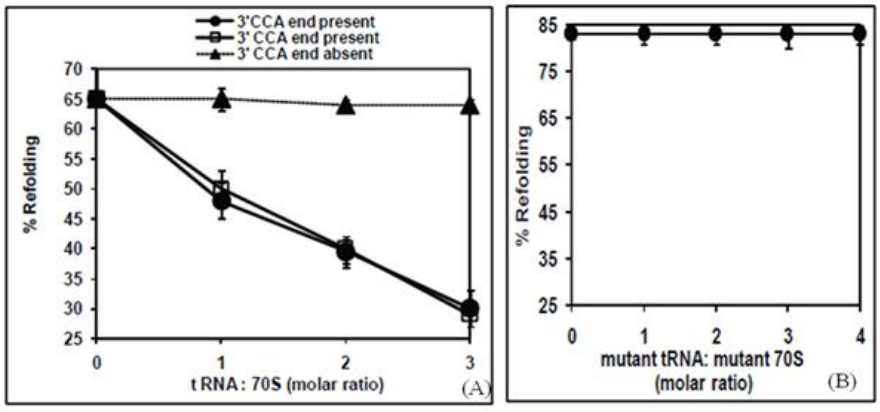

Figure 3 (A, B): Only wild type tRNA with 3′-CCA end can bind to ribosome and interfere with the protein folding process

Conclusion:

Prokaryotic ribosome takes an active and crucial part in protein folding, thus helping in the formation of the correct tertiary conformation of all proteins it synthesizes. This has been well substantiated by *in vitro* and *in vivo* experiments (1-3). Therefore, the effect of any interaction of ribosome with the accessories involved in its peptidyl transferase activities on its protein folding property is worth studying. Such studies will help establish a novel paradigm for the role of ribosome in protein folding. The transfer RNA is probably the most important candidate in this search. It interacts with a number of sites on the ribosome on one hand and with a large number of translation accessories on the other. Moreover, it evolved as a link between transcription and translation with its outstanding codon reading properties. Therefore, the way it modulates protein folding is a crucial information which is worth collecting. In this study we tried to find out whether 70S ribosome can refold unfolded proteins while its domain V is occupied by tRNA. As described above, our preliminary findings suggest that deacylated tRNA molecules, bound to P and E sites of domain V, inhibit 70S from participating in the folding process. The refolding ability of the large subunit, 23S rRNA, domain V rRNA and RNA 1& 2 is also significantly affected by the presence of bound tRNA. Such inhibition seems to be very specific requiring precise interaction between P loop of domain V rRNA and 3′-CCA end of tRNA.

Acknowledgement:

I am extremely grateful to my Ph. D. mentor Prof. Chanchal Dasgupta and my former colleague Dr. Arpita Bhattacharyya and to the Department of Biophysics, Molecular Biology and Genetics, Calcutta University where most of this work was carried out. I would also like to thank Dr. Sasabindu Jana, Principal, Raidighi College, and my colleagues Mr. Prabir Sahoo and Dr. Shakuntala Ghorai in the Department of Microbiology, Raidighi College, for their support. For financial assistance, I am grateful to CSIR, India.

References:

1. Samanta, D. *et al.,* (2009) Mechanism of ribosome assisted protein folding: a new insight into rRNA functions. Biochem Biophys Res Commun. 384, 137-140.
2. Das, D., *et al.,* (2010) Ribosome: The Structure-Function Relation and a New Paradigm to the Protein Folding Problem. Israel Journal of Chemistry. 50 (1), 109-116.
3. Samanta, D., *et al.,* (2011) Ribosome Assisted Protein Folding: Some of its Biological Implications. Protein Folding**.** pg 377-400. ISBN: 978-1-61728-990-3.
4. Das, D., *et al.,* (2017) A Possible Role of the Full-Length Nascent Protein in Post-Translational Ribosome Recycling. PLOSONE. 12 (1): e0170333. doi: 10.1371/ journal.pone.0170333.

5. Basu, A, *et al.,* (2008) Protein folding following synthesis *in vitro* and *in vivo*: Association of newly synthesized protein with 50S subunit of *E. coli* ribosome. Biochemical and Biophysical Research Communications. 366 (2), 592–597.
6. Basu, A, *et al.,* (2008) *In vitro* protein folding by *E. coli* ribosome: unfolded protein splitting 70S to interact with 50S subunit. Biochemical and Biophysical Research Communications. 366 (2), 598–603.
7. Samaha, R. R., *et al.* (1995) A base pair between tRNA and 23S rRNA in the peptidyl transferase center of the ribosome. Nature. 377 (6547), 309-314.
8. Chattopadhyay S, *et al.,* (1994) Refolding of denatured lactate dehydrogenase by Escherichia coli ribosomes. Biochem J. 300, 717-721.
9. Samanta, D., *et al.,* (2008) Protein folding by Domain V of E. coli 23S r RNA: Specificity of RNA-Protein interactions. Journal of Bacteriology. 190 (9), 3344-3352.
10. Goswami, S., *et al.,* (2003) Allosteric regulation of tRNA import: interactions between tRNA domains at the inner membrane of *Leishmania* mitochondria. Nucl. Acids Res. 31, 5552-5559.
11. Sylvers, L.A., *et al.,* (1993) A 2-thiouridine derivative in $tRNA^{Glu}$ is a positive determinant for amino-acylation by *Escherichia coli* glutamyl-tRNA synthetase. Biochemistry. 32, 3836–3841.
12. Joseph, S. and Noller, H. F. (1996) Mapping the rRNA neighborhood of the acceptor end of tRNA in the ribosome. EMBO J. 15 (4), 910-916.

BIO CULTIVATION: TURNING BACK TO PAST

Rupa Acharya* and Swarnaditya Mondal,
Department of Botany, Dhruba Chand Halder college, Dakshin Barasat, West Bengal
*Corresponding author E-mail: rupaacharya69@yahoo.co.in

Abstract:

Domestication of plants or "Agriculture" is a process developed by human race as a means of their survival strategy. Historic evidence of agriculture has been found to be recorded as long as at least 20000 B.C. Agriculture was implemented as a holistic natural approach exploiting natural processes and resources. Cultivars learned the skills of maintaining good soil health and plant growth. In the 18th century, Industrial Revolution initiated urbanization and caused occupational changes of people in different parts of the world. More recently, after 1950s, Green Revolution was thought to be the great device to cope up the hunger of gradual increasing world population by manipulating the soil health and plant character. Introduction of chemical fertilizer for greater crop production was done during World War I. Initially they were cheap and powerful. A similar advance in chemical pesticides was occur during 1940s. Use of these chemical fertilizer and pesticides become an integral part of Green revolution. But gradual accumulation of these agrochemicals acted as silent killer of microbes and macrobes directly and / or indirectly destroying several ecosystems in the soil, water and plant surface including accumulation of heavy metals in the food chains. Lots of microbes, plants and animals become extinct, rare and endangered. To recover the whole system, concept of Biological Firming has been considered which is nothing but reintroduction of conventional method of cultivation with some modern technological advances that would restore the natural soil biology and crop physiology along with food and health security of human race and conservation of other life forms.

Keywords: Organic farming, Indian agriculture, sustainable agriculture,

Background:

Agriculture may be defined as the domestication of plants in a monoculture manner for procurement of necessary food in an easier way during the course of civilization when human kind was approaching from hunter to gatherer and so ahead. History of agriculture is intermingled with the history of humankind's development which is probably more than 10,000 years back as indicated in the archaeological data. It is evidenced from the history of India that wheat, pulses, sesame seeds, barley, cotton etc was in use since 6,000BC during Indus Valley civilization. People built dam and drainage system by that time for agriculture and domestic use.

In the journey of civilization, the communities of farmers increased faster than those of the hunter-gatherers, villages developed; crafts, painting and writing evolved. The plants also

developed further through natural selection: wheat, barley, flax and peas produced larger seedlings with a better yield. Thus symbiosis and co-evolution gradually took place in different parts of the world. By the early 1800s, agricultural practices, was rapidly evolved through selection of hardy strains and cultivators, so improved that yield many folds. Experiments on Plant Hybridization started in the late 1800s and subsequently, the development of hybrid crops. However, increasing dependence upon monoculture crops lead to famines and food shortages, most notable example being the Irish Potato Famine (1845–1849). After World War II U.S. Government, the philanthropies and the sciences turned their attention to the rest of the world. Agriculture became an instrument for grabbing the global power. In the process, the U.S. exported agricultural technology and kept billions of people alive. Green Revolution came into the scenario.

The Green Revolution in India commenced in the early 1960s by the Government to ensure food security that led to an increase in food grain production, especially in Punjab, Haryana, and Uttar Pradesh. The Green Revolution in India refers to a period when Indian agriculture was converted through industrial system due to the adoption of modern methods and technology such as the introduction of high yielding variety (HYV) seeds, tractors, pesticides, fertilizers etc which was responsible for gradual displacement of several local varieties of crops, conventional and ethnic system of agriculture. Green revolution also invited indiscriminate use of agrochemicals which severely damaged the soil health and water quality of the field area. The ultimate negative impact was imposed on the plant health, crop quality and its productivity. It was found by early 90s that extreme dependence on chemical fertilizers and other agrochemicals caused loss of fertility of soil, alteration of physical properties of soil particles, death and damage of soil inhabiting microbes and other life forms. The cumulative effect of all the conditions caused increased cost of farming, farmers are falling into the trap of money lenders and even many of them committed suicide. In this situation, both the scientists and farmers tried to shift back to organic farming in our country as well as throughout the world. Green revolution provided a temporary quantitative development of agriculture products but we need a qualitative and sustainable agriculture system (Gahukar, 2009). Bio-cultivation or organic farming is the probable measure of it.

Consequences of green revolution:

Although Green Revolution increased crop production for a particular period of time, it facilitated the development of Agro-industries and different Multinational seed companies. Agriculture then became dependent on industries and thus required a huge amount of investment for crop production. Poor farmers were unable to afford it. Again, there was a reduction in agriculture labour absorption for the adoption of new technology and thus reduced the employment opportunity in the rural area. The most important effect of this system was on soil

and water where both the physical and biological health of soil and both the surface and ground water level was contaminated with excess agrochemicals as well as heavy metals. This situation potentially damaged the sustainability of agriculture. This scenario alerted the scientists to resolve the situation. The package of methods to compensate the damage and to improve the present situation towards sustainability is described here as organic farming or bio- cultivation.

How to compensate the damage:

1) By maintaining long term fertility of soil through effective use of natural resources:

Soil fertility is the ability of soil to sustain plant growth and optimize crop yield. Integrated soil fertility management is the best way in this situation to maximizing the efficiency of the soil. Integrated Soil Fertility Management (ISFM) is defined as: A set of soil fertility management practices that necessarily include the use of fertilizer, organic inputs, and improved germplasm combined with the knowledge on how to adapt these practices to local conditions in aim of maximizing the agronomic use efficiency of the applied nutrients and improving crop productivity (Vanlauwe *et al.*, 2010). The main nutrients for the plants are considered to be NPK and other micro and macro nutrients. Instead of using chemical fertilizers the nutrients could be obtained from organic sources. The nutrient facts of organic matter (Grachene et al, 2003) have been shown in the table 1.

Table 1: Nutrient facts of organic matter

Organic material	Nitrogen %	Phosphate %	Potash %
Cow manure	0.4-0.6	0.2	0.2-0.5
Horse manure	0.5-0.7	0.3	0.6
Goat manure	1.4	0.2	0.3-1.0
Sheep manure	0.7	0.3	0.4
Human waste	2.0	1.0	0.2
Pig manure	0.5	0.3-0.4	0.5-0.8
Poultry manure	1.1-1.5	0.8-1.3	0.5-2.7
Rabbit manure	1.1-2.4	1.2-1.4	0.6
Compost (household)	0.5	0.2	0.8
Bean trash	0.8	0.07	1.57
Banana stalks	0.73	0.18	4.10
Sugar cane trash	0.47	0.06	1.23
Banana leaves	1.3	0.1	1.72
Coffee husks	1.63	0.14	4.45
Sweet potato vines	1.73	0.48	6.63
Napier grass	1.97	0.14	3.85
Lantana camara	2.5	0.26	1.93

2) By re-establishing soil's physical and biological health:

As both the physical and biological health of the soil has been damaged, reestablishment of the soil structure is one important consideration in this matter. To rectify the physical structure application of Phosphate solubilizing bacteria (PSB), Mychorrhiza, *Rhizobium*, *Azospirillum*, different Plant Growth Promoting Bacteria (PGPR), different other bio-fertilizers are advisable. Use of Green manures, composts, vermicompost, *Azolla* etc. are able to convert the damaged soil into an amiable habitat for introduced microbes. It is necessary to maintain the pH of the soil before application of the beneficial microbes. Use of lime is important to handle the situation.

The amount of lime to apply depends on the pH and texture of the soil. Acidic soil needs more lime. Clayey soils and soils with high organic matter need also to be limed. To increase the pH of the different soil types, the following quantities of agricultural lime are required as shown in the table 2.

Table 2: Quantities of lime used in agriculture field

Soil Texture	**pH 4,5-5,5 t/ha**	**pH 5,5-6,5 t/ha**
Sand to sandy loam	0.5	0.75
Sandy loam	1.0	1.5
Loam	1.5	2.0
Silt loam	2.5	3.0
Clay loam	3.0	4.0

3) By cultivation of local varieties of crops and practice of crop rotation:

Cultivation and culture of local varieties of crop and other plants are extremely important. Presently, we have lost several local varieties of crops which are naturally selected in a particular area having a good relation with that geo-climatic condition as well as with a significant range of resistant power. Thus conservation and cultivation of local varieties of crops significantly restore the genetic pool in the biome. In comparison with hybrid or exotic crops local varieties are more potent to support food security.

Practice of Crop rotation and intercropping are also a good method for crop productivity and balanced use of soil nutrients by the plants.

***4) Cultural practices*:**

Every country and every state have has its own conventional cultivation practices most of which now-a-days has been replaced by different agrochemical technologies and chemicals. Re establishment of such techniques like use of trap crop, light trap, ploughing, burning of crop wastes in the field etc are much more helpful and advisable.

Conclusion:

Organic farming is a very native concept of India. It can protect the soil maintaining the level of organic matter, encouraging biological activity of the soil, provides nutrients through microbial action, supplement of the nitrogen requirements through legumes, recycling of crop materials and crop residues in the field, management of diseases in conventional ways and maintenance of the quality of environment and crop are the characteristics of this method.

The Government of India has implemented schemes for promotion of organic cultivation through several schemes. The scheme for promotion of Bio-fertilizers has been adopted since the 7th Five Year Plan. Even though bio-fertilizers are superior to chemical fertilizers, immediate and complete replacement of them is not possible. Presently, gradual replacement by balanced use of bio and chemical fertilizers are in practice (Sundara *et al.*, 2002).

In our country, organic cultivation is being increasingly adopted by farmers. Profitability of organic farming is remarkable. Profit increases by (a) As cost of farm input is reduced by the use of animal residues, organic wastes as bio-fertilizers. (b) Market value and demand of product is high in comparison with available products. Although, use of more man power, time interval taken for conversion of soil quality for bio cultivation is considered to be the disadvantages for poor farmers.

In conclusion it could be said that India is going through a safe venture to establish the system as fully functional. Organic farming is highly necessary to develop sustainable agriculture in a vast country like India.

References:

1. Gachene, C.K.K. and Kimaru, G. (2003). Soil Fertility and Land Productivity - A guide for extension workers in the eastern Africa region. Technical Handbook No.30. Regional Land Management Unit (RELMA) / Swedish International Development Cooperation Agency (Sida). ISBN: 9966-896-66-X.
2. Gahukar, R. T. (2009) Sustainable agriculture in India.: Current situation and future needs. International Journal of Agriculture Science, (5)1: 1-7
3. Sundara, B.; Natarajan,V. and Hari, K. (2002) Influence of Phosphorus solubilizing bacteria on the change in soil available. Field Crop research, Hyderabad, 77(10):43-49.
4. Vanlauwe, B.; Chianu, J.; Giller, K.E.; Merck, R.; Mokwenye, U.; Pypers, P.; Shepherd, K.; Smaling, E.; Woomer, P.L.; Sanginga, N. (2010). Integrated Soil Fertility Management: Operational definition and consequences for implementation and dissemination. Outlook on Agriculture 39:17-24

A NUMERICAL REPRESENTATION OF DNA SEQUENCES TO PREDICT CODING AND NON-CODING REGIONS IN EUKARYOTIC GENES USING DIGITAL FILTERS

Subhajit Kar[1,2], Saptarshi Das[2] and Madhabi Ganguly*[3]

[1]Monirtat No 2 School, Joynagar, West Bengal- 743349

[2]Department of Electronics, West Bengal State University, Barasat, Kolkata - 700126

[3]Department of Electronics, West Bengal State University, Barasat, Kolkata – 700126

* Corresponding author E-mail: ray_madhabi@yahoo.co.in

Abstract:

Bioinformatics is a very powerful tool which combines Computer science with Biology to unleash many unexplored areas of Biological science application. Such data are very useful in understanding the genetic basis of many diseases like Cancer, Arthritis, Anemia and all the genetic disorders and thus provide new theories to cure them. In this paper a given DNA sequence is converted into three separate numerical representations based on two bit binary representation according to well-known chemical properties of nucleotide bases Adenine(A), Cytosine(C), Guanine(G), Thymine(T). In a DNA primary sequence the four bases A, C, T and G can be classed into groups, purine{A,G}/pyrimidine{C,T}, amino{A,C}/keto{G,T} and weak-H bond{A,T} /strong- H bond{C,G}. In this way the converted sequence will not only reflect the DNA structures of strings but also their chemical properties. Digital signal processing tools are then applied to the numerical sequence to predict the exon regions in gene. Two staged digital filter comprises of a band pass filter which essentially extracts the period three components from the sequence and secondly a low pass filter to eliminate high frequency noise present in the output spectra due to long range correlation of nucleotide bases are used. Employing the above coding rule, all three numerical representations are compared to reflect well known 'period three property' in gene F.56F11.4a on chromosome III of *Caenorhabditis elegans*.

Keywords: Biomedical signal processing, DNA, Mutation, DIT-FFT, IIR Filter.

Introduction:

Bioinformatics in today's science has provided major breakthrough in medical research. Use of bioinformatics data such as genomic sequence analysis has great potential to detect any abnormality in human genome and hence paved way for their rectification using very recent biological technologies. Recently, DSP techniques are found to be very useful in every research area of bioinformatics for diagnosis and disease management [1 – 4]. It has the advantage that it can analyze a great amount of data within a few milliseconds and compare these data with given

reference set to find the abnormality in gene. The data thus obtained can be stored very easily. Thus medical diagnosis of any genetic abnormalities can be found easily using DSP algorithms. DSP techniques can identify hidden periodicities, nucleotide distribution and features that cannot be revealed easily by conventional methods such as DNA symbolic and graphical representation otherwise it will require much tedious, expensive, time consuming biological process. DNA is a Deoxyribonucleic acid that contains the genetic instructions used in the development and functioning of all known living organisms. The information in DNA is stored as a code made up of four chemical bases: adenine (A), guanine (G), cytosine(C), and thymine (T).

The four base pairs present in DNA string (A,C,T,G) can be divided into three functional groups according to their chemical properties.

Purine/Pyrimidine: Purines and Pyrimidines are the two families of nitrogenous bases that make up nucleic acids. Adenine and guanine belong to purine family whereas cytosine and thymine are from pyrimidine family. Any substitution of purine or pyrimidine in gene results in transition and transverse mutations.

Amino/Keto: Amino and ketostructures are isomers that differ in the positions of their atoms and in the bonds between the atoms to each other. A keto structure can be seen in adenine and cytosine whereas an amino structure in guanine and thymine. The replacement in tautomer of one of the standard bases miss pair can cause a mutation in the course of DNA replication.

Weak-H bond/strong-H bond: Hydrogen bonding is a non-covalent type of bonding between molecules or within them. There are two hydrogen bonds between adenine and thymine but three hydrogen bonds between cytosine and guanine. Hence adenine and thymine produce weak H-bond comparing to the cytosine and guanine.

Figure 1 describes a mutation on DNA sequence by deletion of a single base. A eukaryotic gene with multiple exons is shown in Figure 2.

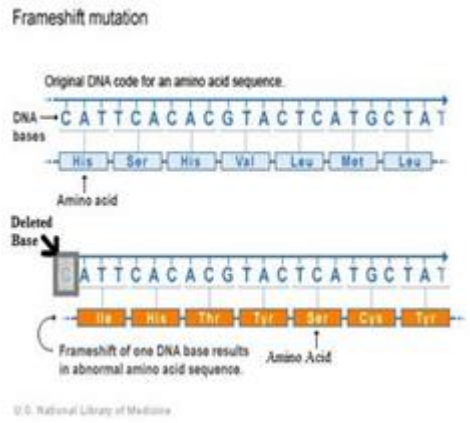

Figure 1: Mutation in DNA sequence

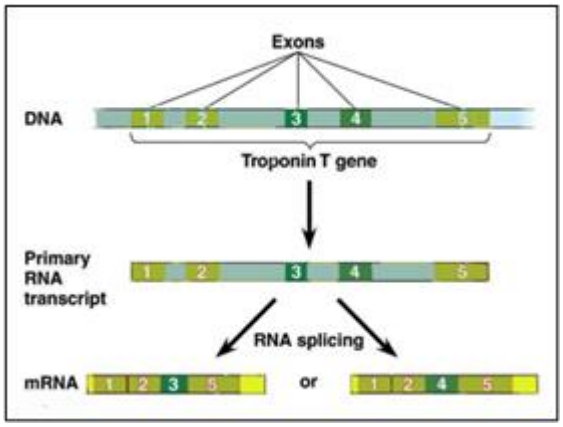

Figure 2: Eukaryotic gene producing protein

Mutation in gene can lead to serious disease because a mutated exon can code for a faulty protein which essentially disturbs the biological process of body. Figure 3 describes the process of Coding faulty protein [5].

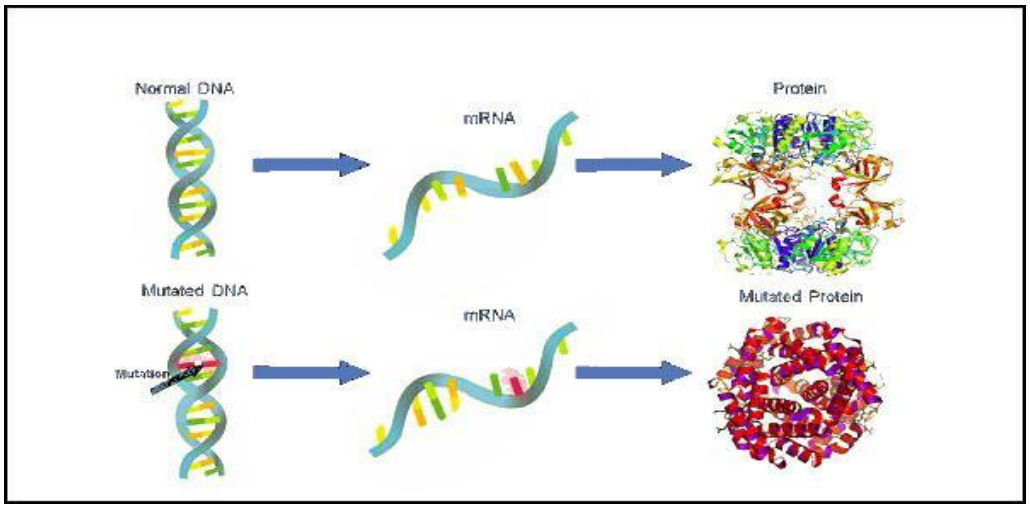

Figure 3: Production of faulty protein due to mutation

To find the exonic regions present in the gene we have employed three-base periodicity factor which refers to the sharp peak at frequency f =1/3 in the Power spectrum. The property arises in exon region due to short-term correlation present in the bases and codon bias [6]. Most of the GSP algorithms are based on the 3 base periodicity property, as this feature is very prominent in most of the genes.

Materials and Method:

Datasheet

DNA sequence of gene F.56F11.4a on chromosome III of Caenorhabditis elegans is taken from the GenScan test database (accession number AF099922 from the NCBI website). It has five distinct exons, relative to nucleotide position 7021 according to the NCBI database. These regions are 3156-3267, 4756-5085, 6342-6605, 7693-7872 and 9483-9833 [7].

Proposed numerical representation

In order to apply different digital signal processing tools to a DNA sequence to analyze different properties the sequence must be converted into a numerical sequence. There are so many types of numerical representations which includes ─ Electron Ion Interaction scheme (EIIP) [8], Voss representation [9], Complex number representation [10], Paired numeric representation [11], Genetic code context [12], two bit binary representation [13]. Previous study shows that two bit binary number representation scheme greatly enhances prediction accuracy of DNA protein coding region.

In this study the DNA sequence is converted into three unique sequences according to the chemical property ─ (i) Purine{A,G}/Pyrimidine{C,T} (ii) Amino{A,C}/Keto{G,T} and (iii) weak-H bond{A,T}/strong-H bond{C,G} [14] based on two bit binary representation technique. According to the first classification corresponding to Purine{A,G}/Pyrimidine{C,T} it is assumed that A ⊕ G = 11, C ⊕ T = 11. So, one of the coding rules satisfying the above formulae may be ─ A = 01, G= 10, C = 00, T = 11. For the second classification the two bit binary value of the four bases are ─ A = 01, C = 10, G = 00, T = 11 which satisfy the formula A ⊕ C = 11, G ⊕ T = 11. For the third classification corresponding to strong-H bond/Weak- H bond the respective value of four bases are assumed to be ─ A = 01, T = 10, C = 00, G = 11 satisfying the formulae A ⊕ T = 11, G ⊕ C = 11.

Based on this rule DNA sequence is converted in the following way ─

A C T G A T G C A A T G C

01 00 10 11 01 10 11 00 01 01 10 11 00

The proposed numerical can find mutations in a gene sequence in the following way.

Example 1 : [Mutation take place between same class]

A single mutation took place in 3rd position of the sequence where base A is changed to base G.

C T A G A T C ─ Original sequence

C T G G A T C ─ Mutated sequence

Corresponding to first classification rule above two sequence become

00 11 01 10 01 11 00─ Original sequence (1)

00 11 10 10 01 11 00─ Mutated sequence (2)

To find out the mutation in the given sequence an operating rule based on EX-OR operation has been introduced [15]. The operating rules are given in Table 1. Logic diagram of EX-OR operation is given in Figure 4.

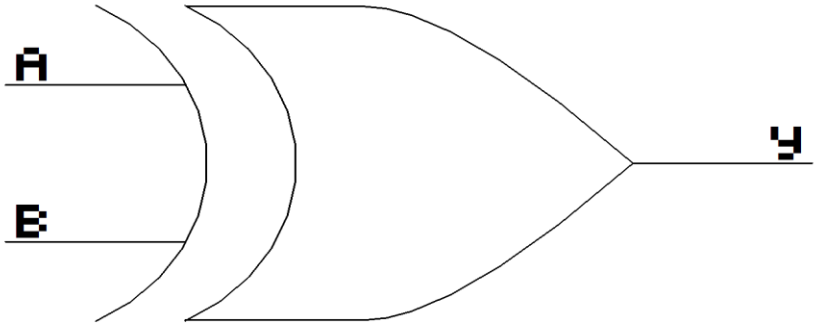

Figure 4: Circuit symbol of logical two input EX-OR gate

Table 1: Operating rules to detect mutation in exons

A	B	Y=A⊕ B
00	00	00
00	01	01
00	10	10
00	11	11
01	00	01
01	01	00
01	10	11
01	11	10
10	00	10
10	01	11
10	10	00
10	11	01
11	00	11
11	01	10
11	10	01
11	11	00

Using the operating rules in the sequence (1) and (2) a new sequence is obtained as-

00 00 **11** 00 00 00 00

The analysis of this sequence will find out the mutation in the given sequence. Here the subsequent zeros imply no change in the mutated sequence with respect to the original sequence. The bold portion indicates a change in the sequence and it is originated from same class. As the original sequence contains 'A' in 3rd position so it can be surely said that it is changed to 'G' and vice-versa as both belong to the same class purine. In similar way it can be definitely said that if the original sequence contains 'C' in that position then it must have been altered to 'T' and vice versa as both of them belong to the same class pyrimidine.

Example 2 : [Mutation take place between different class]

A single mutation took place in 5th position of the sequence where base A is changed to base T.

A T C A A T T G ─ Original sequence

A T C A T T T G ─ Mutated sequence

Corresponding to the second classification rule above two sequences become,

01 11 00 01 01 11 11 10 ─ Original sequence (3)

01 11 00 01 11 11 11 10 ─ Mutated sequence (4)

Applying the operating rules in sequence (3) and (4) a new sequence is obtained below.

00 00 00 00 **10** 00 00 00

The bold portion of the above sequence indicates a mutation between different class i.e., base 'A' may change to base 'C' or base 'T'. In order to find out the correct mutation other two classifications must be considered. Thus corresponding to the second classification the two sequences become,

01 11 10 01 01 11 11 00 ─ Original sequence

01 11 10 01 11 11 11 00 ─ Mutated sequence

Applying the operating rules in sequence (5) and (6) we obtain the following sequence.

00 00 00 00 **10** 00 00 00

The bold portion of the above sequence indicates base 'A' that may be altered to base 'G' or base 'T'. Since the previous discussions conclude that the mutation cannot be between 'A' and 'G', and then surely 'A' must be changed to 'T'. The third classification rule is certainly required if base 'A' is changed to 'C'.

Design of digital filter

Choosing appropriate filter is very much essential becausedigital filters have the potential to reduce or enhance certain properties of a signal [16]. We have utilized IIR elliptical band pass filter with appropriate parameters to extract the three base properties expected in exonic regions of eukaryotic genes.The design specification for Elliptical filter used in the experiment is given below:

Filter order N=3, the lower & upper passband edge frequencies [0.664, 0.672], the lower & upper stop band edge frequencies [0.659, 0.678], the maximum passband attenuation=0.4dB, the minimum stop band attenuation=20dB.

The output PSD contains high frequency noise which is expected due to long range correlation present in the nucleotide bases [17]. To remove noise we have incorporated a moving average filter and allowed the signal to pass through it. Finally we get a smooth PSD with peaks identifying the exons present in the sequence. We have implemented the weighted moving average filter using Gaussian window of length 30 which gives very good results.

The Filters are designed using FDA tools in Matlab environment. We have used Matlab 'filtfilt' command instead of 'filter' command to filter the input sequence. 'filtfilt' performs zero phase digital filtering by processing the input data in both the forward and reverse directions [18]. This will result in zero phase distortion at output.

Results and Discussions:

According to the proposed representation a DNA sequence " ACATGAC……" would result in a single sequence representation given as: x[n] = {01000111100100……} corresponding to the Purine/Pyrimidine classification. Here *n* represents the base index. This proposed representation utilizes a useful DNA structural property inrepresentation, in addition to reducing complexity in subsequent processing. The spectral content of the signal can be measured using the given formula: PSD[k] = |X[k]|^2. Where, X[k] is the DFTs for the indicator sequence *x*[*n*] [19].

The numerical sequences obtained using three classification rules are applied through FIR band-pass filter and weighted moving average filter respectively to get the resulted power spectral content diagram. The paper measured the efficiency of the three proposed coding rules to detect exons in eukaryotic gene. The output power spectral density plot for the three separate coding rules is shown in Figure 5, 6 and 7 respectively.

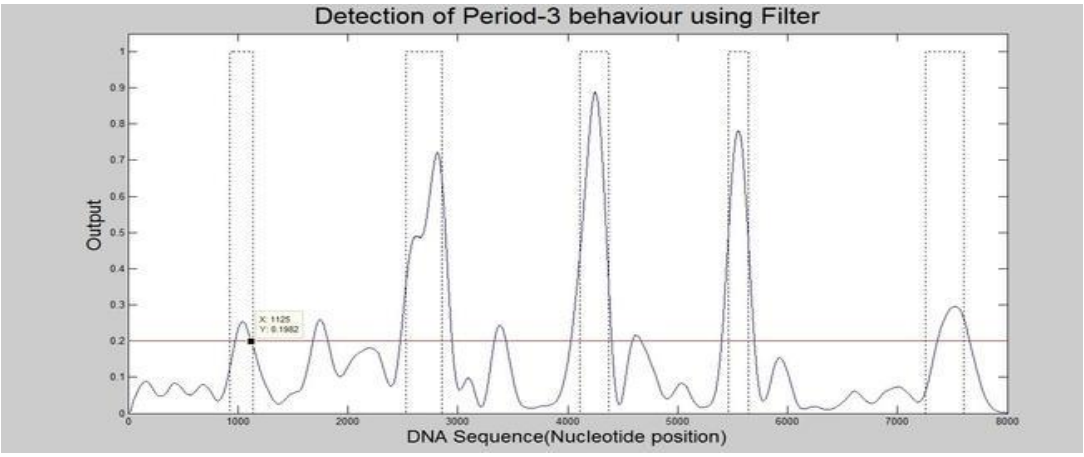

Figure 5: PSD obtained using Purine/Pyrimidine structure

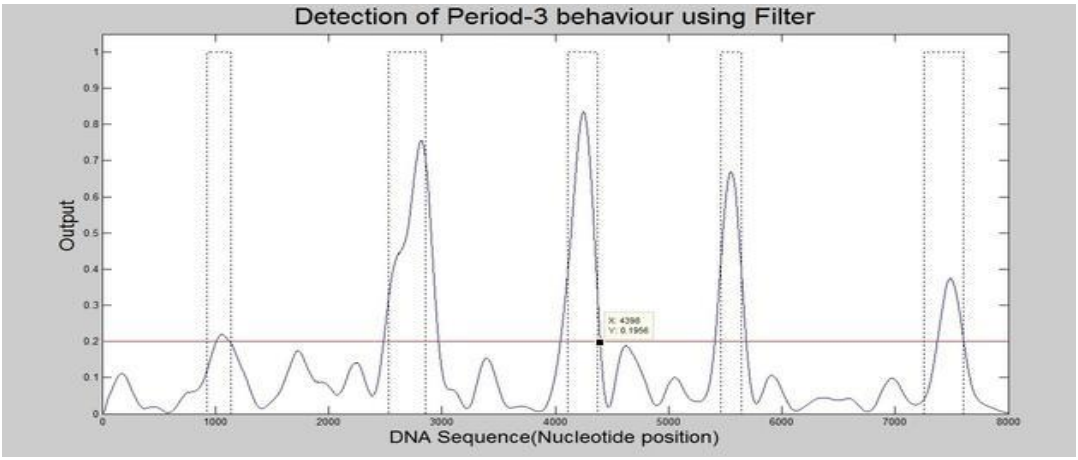

Figure 6: PSD obtained using Amino/Keto structure

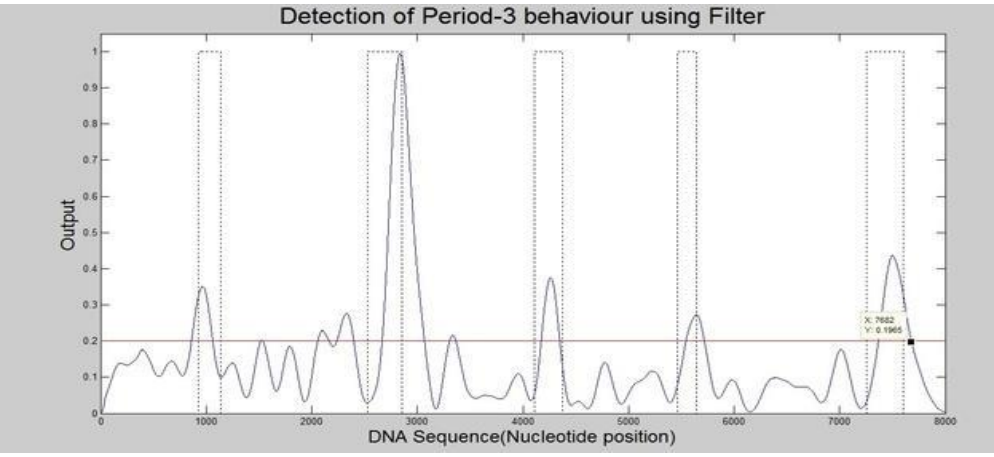

Figure7: PSD obtained using strong/weak Hydrogen bond

We have summarized the predicted introns and exons locations in Table 2. The results suggest our experimental findings to be very close to NCBI ranges. The figures in the brackets indicate the length nucleotides in each exon.

Table 2: Nucleotide ranges of exons and introns as obtained from experiment

Coding Schemes	Exon 1	Exon 2	Exon 3	Exon 4	Exon 5
Corresponding the first classification: Purine{A,G}/Pyrimidine{C,T}	974-1125 (151)	2476-2948 (476)	4027-4401 (374)	5400-5699 (298)	7361-7659 (226)
Corresponding the second classification: Amino{A,C}/Keto{G,T}	1006-1134 (128)	2483-2971 (488)	4045-4398 (353)	5409-5688 (279)	7372-7608 (236)
Corresponding the third classification: Weak H bond{A,T}/Strong H bond {G,C}	860-1064 (204)	2666-3069 (403)	4171-4351 (180)	5544-5720 (176)	7366-7682 (316)
NCBI ranges	928-1138 (210)	2527-2856 (329)	4113-4376 (263)	5464-5643 (179)	7254-7605 (351)

Conclusion:

The proposed work is a part of larger scientific cause. After the successful completion of the human genome project almost all the human genes and their structures are available. Using proposed algorithm we can find out the exonic regions responsible for coding of protein and finding single or multiple mutations taking place in nucleotide sequence. Further improvement in

the algorithm can be made to design effective software that can easily scan a DNA sequence to detect any abnormalities in gene which will be simple, less time consuming and cost effective comparing to the current technologies. Cancer is the most common human genetic disease caused by mutations in a number of growth controlling genes. Development in bioinformatics can greatly contribute in the cancer treatment by pointing out the exact locations of mutation in gene. Extension of the proposed work is needed to find out if insertion or deletion took place in mutation. Next challenge to be addressed in this regard is to reconstruct the original structure of the gene so that the abnormal protein is replaced by conventional one.

References:

1. Barman, S., Roy, M., Biswas, S., Saha, S.: Prediction of cancer cell using digital signal processing, Annals of Faculty Engineering Hunedoara. Int. J. Eng., 91–95, Tome IX 1584–2673 (2011).
2. S. Aydin, "Determination of autoregressive model orders for seizure detection",TUBUTAK, Turk J Elec Eng& Comp Sc, 18, (1), pp. 23-29,2010.
3. K.B. Ramesh, P. Shankar, B.P. Mallikarjunaswamy, and E.T. Puttaiah. Genomic signal processing (GSP) of rheumatic arthritis (RA) using different indicator sequences. International Journal of Computer Science and Mobile Computing, 2(5):332-337, 2013.
4. Vaidyanathan, P.P. and Yoon, B.J. 2004. The role of signal-processing concepts in genomics and proteomics.J.Franklin Inst. 341: 111-135.
5. Bielinska-Waz, P. Waz, T. Clark, Similarity Studies of DNA Sequences Using Genetic Methods, *Chem. Phys. Lett.* 445 (2007) 68−73.
6. S. Tiwari, S. Ramachandran, A. Bhattacharya, S.Bhattacharya, and R. Ramaswamy, "Prediction of probablegenes by Fourier analysis of genomic sequences," *Comput.Appl.Biosci.*, vol. 13, pp. 263–270, 1997.
7. D.P.Snustad, M.J.Simmons, Principles of genetics, John Wiley & Sons Inc.
8. Achuthsankar S. Nair and Sreenadhan S. Pillai, "A coding measure scheme employing electron-ion interaction pseudo potential (EIIP),"Bioinformation, vol. 1, pp. 197-202, October 2006.
9. R. F. Voss, "Evolution of long-range fractal correlations and 1/ *f* noise in DNA base sequences," *Phys. Rev. Lett.*, vol.68, no. 25, pp. 3805–3808, 1992.
10. P. D. Cristea, "Conversion of nucleotides sequences into genomic signals," J. Cell. Mol. Med., vol. 6, pp. 279-303, April-June 2002.

11. M. Akhtar, J. Epps, and E. Ambikairajah, “On DNA numerical representations for period-3 based exon prediction,” in Proc. of IEEE Workshop on Genomic Signal Processing and Statistics (GENSIPS),June 2007, pp. 1-4.
12. Kwan HK, Arniker SB, Numerical representation of DNA sequences, *Electro/Information Technology, IEEE International Conference*, 2009.
13. Ranawana R, Palade V, A neural network based multi-classifier system for gene identification in DNA sequences, *Neural Computing & Applications*, pp. 122-131, 2005.
14. Y. Yao, X. Nan, T. Wang, A new 2D graphical representation-classification curve and the analysis of similarity/dissimilarity of DNA sequences *J. Mol. Struct.(Theochem)*,764,2006 101−108.
15. WeiyangChen, Bo Liao, Yanshu Liu, Wen Zhu, Zhizhong Su, A Numerical Representation of DNA Sequences and Its Applications, *MATCH Communications and Mathematical and in Computer Chemistry*, Vol. 60 (2008), pp. 291-300.
16. R. Kakumani and V. Devabhaktuni, “Prediction of Protein Coding Regions in DNA Sequence using a model based approach”, IEEE explore DOI: 978-1-4244-1684-4/08, pp 1918-1920, 2008.
17. RF. Voss, "Evolution of long-range fractal correlations and 1/f noise in DNA base sequences", *Phys. Rev. Lett.*, vol. 68, pp. 3805-3808, 1992.
18. Wassfy HM, AbdElnaby MM, Salem ML, Mabrouk MS, Zidan AA, Eukaryotic gene prediction using advanced DNA numerical representation schemes, Proc Fifth IntConf Advances in Applied Science and Environmental Engineering (ASEE), Kuala Lumpur, Malaysia, 2016.
19. Anastassiou, D. 2001.Genomic signal processing.*IEEE Sign. Proc. Mag.* 18: 8-20.

NUTRITION AND NUTRIGENOMICS: THE FUTURE OF DIET

Sutripta Sarkar
Post Graduate Department of Food & Nutrition,
Barrackpore Rastraguru Surendranath College, 85, Middle Road, Barrackpore- 700120
Corresponding author E-mail: sarkar.sutripta@gmail.com

Abstract:

Nutrigenomics is the application of high-throughput genomics tool in nutrition research. It is the area of nutrition that uses molecular tools to search, access and understand the several responses obtained through a certain diet applied between individuals or population groups (Cozzolino & Cominetti, 2013) and explains interactions between genes and nutrients at a molecular level. Components of a particular diet may alter the expression of genes by binding with transcription factors. This binding enhances or interferes with the ability of transcription factors on interacting with elements that will lead to the binding control of RNA polymerase (Cozzolino & Cominetti, 2013).

Some researchers have found that vitamins A and D have the ability to activate nuclear receptors and induce gene transcription (Dauncey, 2012). Besides vitamins, macronutrients such as glucose interact with the transcription factors like upstream stimulatory factor, sterol-responsive-element binding protein, and carbohydrate responsive binding protein to modulate gene expression. The discovery of more such gene –nutrient interactions definitely help in the prescription of customized diet according to an individual's genotype. The Human Genome Project has revealed the molecular signatures of several genes that have particular relevance to our diet (Lucock, 2014). It has also been found that certain genes predispose us to diseases, however, they can be modified in their effect by dietary nutrients.

Nutrigenomics also includes the studies on nutritional factors that act by protecting the genome. This field of study is important in developing an understanding on the influence of dietary nutrients on the Genome, Trancriptome, Proteome and Metabolome. The current review is an attempt to highlight the different aspects of Nutrigenomics.

Keywords: Nutrition, Nutrigenomics, gene-nutrient interactions, customized diet`

Introduction:

Food is an integral part of our being. It is very essential for our survival as it provides the nutrition our body requires to grow, and for maintenance. Since time immemorial humans knew about edible and non-edible foods. With the advent of scientific thinking in society foods were classified based on their chemical and structural characteristics, requirement etc. Macro nutrients

included carbohydrates, proteins and fats whereas micronutrients comprised of vitamins and minerals. Concepts of ideal weight and food requirements began to take shape. Development in Biochemistry lead to deciphering of metabolic pathways and food was related to the metabolites produced in the body. Modern genomics studies the impact of food at the molecular level.

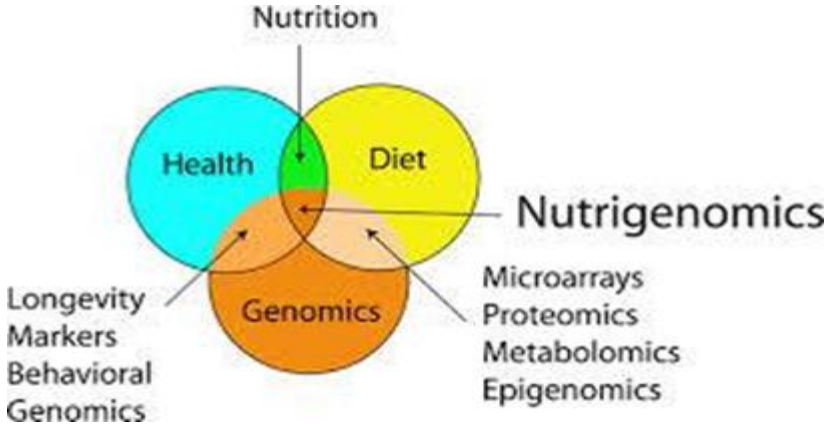

Figure 1: Image Courtesy- www.collegenaturalmedicine.com

According to WHO (2002) genomics is the study of genes and their function, and related techniques. Genomics takes into consideration the entire set of genes and their inter relationship to assess their combined influence on growth and development of an organism. Nutrigenomics includes known interactions between food and inherited genes (Neeha & Kinth; 2012). Nutrigenomics has ushered a new era in nutrition science that involves high throughput technology to study gene expression under the influence of a particular nutrient.

The primary principles of nutrigenomics are**:**

1. Diet is a serious risk factor for a number of diseases.
2. Nutrients present in diet can affect the human genome by altering gene activity or gene structure.
3. The extent to which a person gets affected (good or bad) by the food they take depends on their genetic makeup.
4. Diets regulate some genes and are likely to play a role in the onset and progression of chronic diseases. It also impacts the severity of the disease.
5. Using a personalized approach to a person's diet, based on their nutritional requirements, nutritional status and their genotypes can prevent, mitigate or even cure chronic disease (Yasothai, 2016).

Nutrigenomics is the study of interaction of nutrient and gene interactions and the variations in genes which cause individuals to react uniquely to different nutrients. Figure 2 shows the impact of nutrients on metabolism, cell signalling and gene expression.

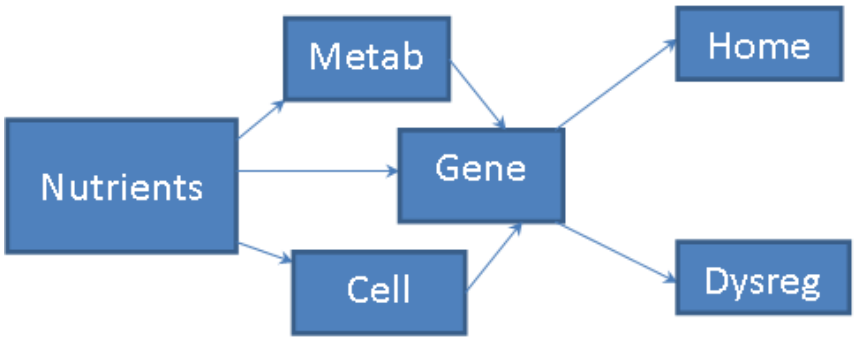

Figure 2: Represents the impact of nutrients on major life processes (recreated from Muller & Kersten, 2003)

Nutrients present in diet can alter gene expression by binding to transcription factor receptors. They also act as signalling molecules and can be metabolized by primary or secondary metabolic pathways, thereby altering concentration of substrates or intermediates (Yasothai, 2016). This review highlights the different aspects of nutrients interaction with genome, transcriptome, proteome and metabolome.

Diet and its impact on gene expression

The human genome project has revealed that humans have 99.9% identity between their genomes. Single nucleotide polymorphisms or (SNPs) are the reasons for genetic variation and they can change the protein encoded (Dauncey, 2012). Several studies have reported that some genes and their variants can be regulated by components from food and that these molecular variations have beneficial impact on the health of individuals (Cozzolino and Cominetti, 2013). A very common example of this can be seen in case of milk digestibility. Generally the digestion capacity of milk is much reduced in adults however, many northern European, northern Africans and Arabs maintain their ability to digest lactose because they have the dominant *LAC P* gene which controls adult lactose digestion. Either one or two alleles confer high digestive capacity (Lucock, 2014). Diet –gene interaction and evolution is very evident in the above example. A lot of data has emerged from recent research which shows the impact of nutrients on gene expression (Munshi and Duvvuri, 2008). It has been observed that a set of oxidative stress associated genes and the genes of thioredoxin carrier system are affected by selenium (Dawson, 2006). Another essential micronutrient metal, Zinc, interacts with DNA binding proteins forming motifs known as Zinc fingers which act like molecular switches affecting gene expression by blocking transcription (Cousins, 1994). Macronutrients such as dietary fibres have been shown to affect the colonic gene expression (Coursins, 1996).

Trancriptomics, Proteomics and Metabolomics:

Several bioactive components bind to transcription factors (Table 1) which act as sensors regulating and modulating transcription of cells according to need of the body (Fialho, 2008). Members of nuclear receptor super family are gaining much attention in this context.

Table 1: Enlists the nutrients and the transcription factors they regulate (Courtesy: Muller and Kersten, 2003)

Table 1 | **Transcription-factor pathways mediating nutrient–gene interactions**

Nutrient	Compound	Transcription factor
Macronutrients		
Fats	Fatty acids Cholesterol	PPARs, SREBPs, LXR, HNF4, ChREBP SREBPs, LXRs, FXR
Carbohydrates	Glucose	USFs, SREBPs, ChREBP
Proteins	Amino acids	C/EBPs
Micronutrients		
Vitamins	Vitamin A Vitamin D Vitamin E	RAR, RXR VDR PXR
Minerals	Calcium Iron Zinc	Calcineurin/NF-ATs IRP1, IRP2 MTF1
Other food components		
	Flavonoids Xenobiotics	ER, NFκB, AP1 CAR, PXR

AP1, activating protein1; CAR, constitutively active receptor; C/EBP, CAAT/enhancer binding protein; ChREBP, carbohydrate responsive element binding protein; ER, oestrogen receptor; FXR, farnesoid X receptor; HNF, hepatocyte nuclear factor; IRP, iron regulatory protein; LXR, liver X receptor; MTF1, metal-responsive transcription factors; NFκB, nuclear factor κB; NF-AT, nuclear factor of activated T cells; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; RAR, retinoic acid receptor; RXR, retinoid X receptor; SREBP, sterol-responsive-element binding protein; USF, upstream stimulatory factor; VDR, vitamin D receptor.

These transcription factors are stimulated by physiological signals triggered by bioactive food compounds or metabolites resulting from them (Fialho, 2008). In nutrition research, transcriptomics helps in identifying genes, proteins, or metabolites that are changed in prediseased and diseased conditions. It also helps in recognizing and characterising pathways and mechanisms regulated by nutrients (Liu and Qian, 2011).

Proteins form an important part of our diet and they play a variety of roles in the body such as structural, biochemical, transport and cell signalling and storage (Cozzolino & Cominetti, 2013). The number of proteins in the body far outnumbers the number of genes. Proteomics is the study of structure, expression, biochemical level of proteins, protein-protein interaction etc. It can be of great importance in nutritional research. Determining nutritional status by protein derived biomarker may be a possibility soon (Afman and Muller, 2006). As of now very few studies, deciphering the relationship of diet to protein, are available. A study by

Roos *et al.* (2005) discusses the potential of proteomics to study associations between dietary lipoproteins, fatty acids and metabolic disorders.

Metabolomics is that area of functional genomics which studies the changes in metabolites, their isolation and characterization (Cozzolino & Cominetti, 2013). In nutrition, metabolomics allows a better understanding of how the excess or lack of some nutrients can affect the human body (Sales *et al.,* 2014). Studies have shown that dietary patterns have a powerful effect on metabolic profiles (Solanky *et al.,* 2003). Aggregating individuals by difference in metabolic profiles provides a strategy for evaluating the effects of a particular diet (Ferguson *et al.,* 2016).

Nutrients and nutrigenomic studies are now down for an array on non-communicable diseases such as Obesity, type-II Diabetes, CVD etc. However, these topics are not detailed here as they are outside the purview of the current review.

Conclusions:

Nutrigenomics is a fast developing science however there is still a lot of work to be done before it can be applied in regular treatment of diseases and customized diets. Facilites to provide genome, proteome or metabolome profiles are still rare especially in countries like India. Also rules need to be framed and ethical issues need to be addressed before giving a go head in nutrigenomics studies. Public awareness is also required for better understanding of genomics in modern health and nutritional sciences by the common man. Extensive research and development of better tools in nutrigenomics can definitely assist in a better quality of life and a healthy diet for human populations.

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References:

1. Afman L & Muller M (2006). Nutrigenomics from molecular nutrition to prevention of disease, *J Am Diet Assoc*, 106 569-576.
2. Cousins R J (1994). Metals elements and gene expression. Annu Rev Nutr, 14 449-469.
3. Cousins R J (1996). Nutritional regulation of gene expression, Am J Med, 106(1A) 20S-151S
4. Cozzolino S.M.F., Cominetti C. (2013). Biochemical and Physiological Bases of Nutrition in Different Stages of Life in Health and Disease, Monole, Sao Paulo, Brazil, 1st edition.
5. Dawson K A (2006) Nutrigenomics: Feeding the genes for improved fertility, Anim Reprod Sci , 96 312-322.

6. Dauncey, M. J. 2012. Recent advances in nutrition, genes and brain health. The Proceedings of the Nutrition Society 71: 581-591.
7. de Roos B, Duivenvoorden I, Rucklidge G, Reid M, Ross K *et al* (2005). Response of apolipoprotein E*3-Leiden transgenic mice to dietary fatty acids: Combining liver proteomics with physiological data, *FASEB J*, 19 813-815.
8. Fialho E., Moreno F. S., and Ong T. P. P. (2008). Nutrition in the post-genomics: fundamentals and applications of omics tools. *Journal of Nutrition*, vol. 21, no. 6, pp. 757–766.
9. Ferguson J. F. et al. (2016). Nutrigenomics, Microbiome and the Gene-Environment Interactions: New Directions in Cardiovascular Disease Research, Prevention, and Treatment. AHA Scientific Statement. Circ Cardiovasc Genet Vol. 9, 291-313.
10. Genomics and World Health: Report of Advisory committee on health research, Geneva, WHO (2002).
11. Neeha,V.S. and Kinth P. (2013). Nutrigenomics research: a review. J Food Sci Technol 50 (3): 415-428.
12. Liu B. and Qian S. B.(2011). Translational regulation in nutrigenomics. *American Society for Nutrition*, vol. 2, pp. 511–519.
13. Lucock M. (2014) Molecular Nutrition and Genomics Nutrition and Ascent of Humankind (Wiley Publications).
14. Muller M. and Kersten S. (2003). Nutrigenomics goals and Strategies. Nature Reviews Genetics Vol. 4, 315-322.
15. Munshi A, Duvvuri VS (2008) Nutrigenomics: looking to DNA for nutrition advice. Indian J Biotechnol 7:32–40
16. Sales N.M.R., Pelegrini P.B., Goersch M.C. (2014). Nutrigenomics: Defination and Advances of this New Science. Journal of Nutrition and Metabolism Vol. 2014, Article ID 202759
17. Solanky K S, Bailey N J, Beckwith-Hall B M, Davis A, Bingham S *et al* (2003). Application of biofluid 1H nuclear magnetic resonance-based metabonomic techniques for the analysis of the biochemical effects of dietary isoflavones on human plasma profile, *Anal Biochem*, 323 197-204.
18. Yasothai R. (2016) Development and Principles of Nutrigenomics. International Journal of Science, Environment and Technology. Vol.5 , 2765-2768.

PHYTOESTROGEN: A POTENTIAL PHYTONUTRIENT

Rukshana Irani
Department of Food & Nutrition, Raidighi College
Corresponding author E-mail: rukshana9@gmail.com

Abstract:

Phytoestrogens are phenolic, non-steroidal plant derived compounds which exert oestrogenic activity on Central Nervous System, induce estrus and stimulate growth of female genital tract. Phytoestrogens are also called dietary estrogens and can mainly be categorised into three main classes i.e., isoflavones, coumestans and lignans with several subclasses. Dietary sources of phytoestrogen include soyabean, legumes, flax seeds, sesame seeds, ginseng, alfalfa, liquorice etc. Phytoestrogen imparts its biological activity by mimicking the action of endogenous estrogen. They possess diverse physiological effects like stimulating uterine growth, improving the level of antioxidant enzymes and depressing the risk of atherosclerosis, cancer, osteoporosis, cognitive decline. It's a blessing for women as it relieves the symptoms of menopause and is preferred more than Estrogen Replacement Therapy by post-menopausal women. Several adverse effects of phytoestrogens are also reported and hence should be consumed cautiously.

Keywords: Phytoestrogen, menopause, cancer, estrogen, antioxidant.

Introduction:

Phytoestrogens are potential plant derived compounds that are structurally or functionally similar to mammalian estrogen (17β-estradiol) and thus mimic its action and shows numerous benefits for human health [1, 2]. The biological activity of phytoestrogens is due to their ability to act estrogenically as estrogen agonists and antiestrogenically as antagonists. As estrogen agonists, phytoestrogens mimic endogenous estrogens and cause estrogenic effects. As estrogen antagonists, they may block or alter estrogen receptors (ER) and prevent estrogenic activity, causing antiestrogenic effects [3].

Mode of Action:

The phytoestrogenic effects on human health have been proposed by both genomic and non-genomic mechanisms [4].

a.) Genomic Mechanism:

Phytoestrogens are able to interact with enzymes and receptors, and because of their stable structure and low molecular weight they can pass through cell membranes **[5].**

These interactions allow them to bind to ERs (Estrogen Receptors), induce specific estrogen-responsive gene products, stimulate ER-positive breast cancer cell growth **[**6], and interfere with steroid hormone metabolism or action [5].

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Alters ER structure and affects transcription [7].

Figure 1: Genomic mechanism of phytoestrogens

b.) Non-genomic Mechanism:

It involves diverse mechanisms.

i. Induction of Cancer Cell Differentiation
ii. Inhibition of Tyrosine Kinase
iii. Inhibition of DNA topoisomerase activities
iv. Suppression of angiogenesis
v. Antioxidant properties of phytoestrogens [6].

c.) Other Effects:

Changes occurs in the cellular and molecular level and potentially influence the biosynthesis and metabolism of steroids and fatty acids, the serum steroid carrier proteins (sex steroid binding proteins and α- fetoprotein), and the intracellular and transmembrane transfer of hormones to a membrane and to nuclear receptors [8].

Classification:

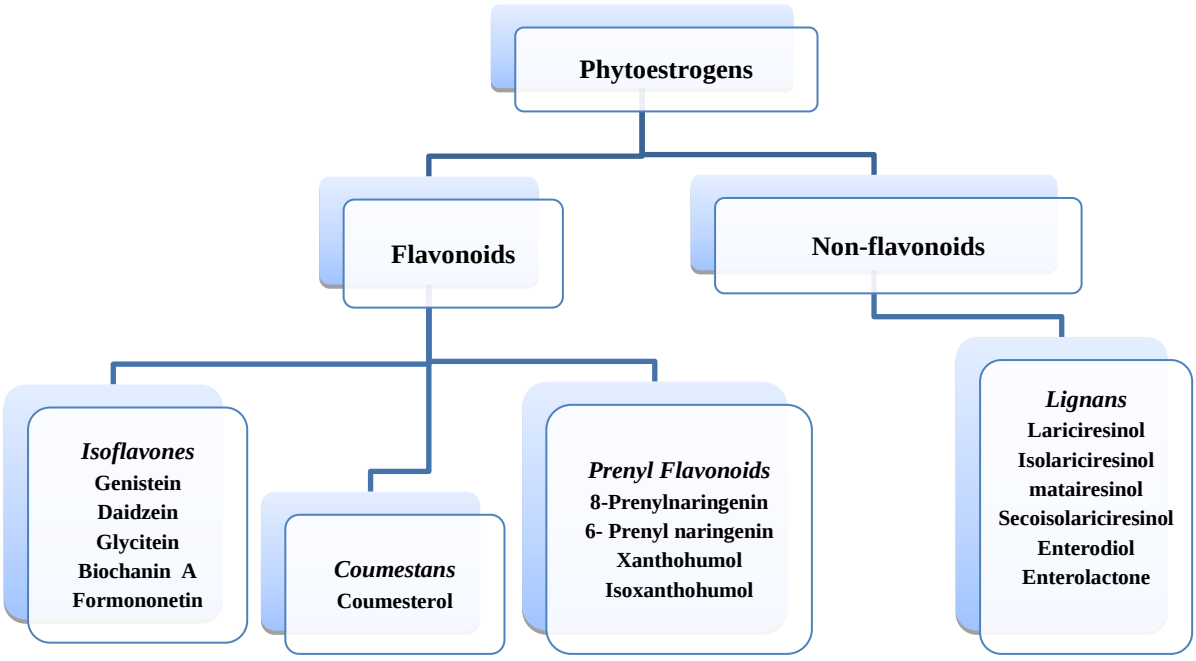

Figure 2: Different types of Phytoestrogens [9]

Dietary Sources:

Plant compounds can be limited to specific botanical families that may assist researchers in identifying other species that contain the same compounds. This concept is referred to as chemotaxonomy. For example, there is a higher concentration of phytoestrogens in legume plants even though they are also found in grains, vegetables and fruits distributed across the plant kingdom [10].

Other Sources of Phytoestrogens

Table 1: Sources of Phytoestrogens [11 - 14]

Common Name	Scientific Name
Alfalfa	*Medicago sativa*
Mung bean	*Vigna radiate*
Kudzu root	*Pueraria lobata*
Psoralea	*Psoralea corylifolia*
Red clover	*Trifolium pratense*
Soybean	*Glycine max*
Flax seed	*Linum usitatissimum*
Sesame seed [11]	*Sesamum indicum*
Ginseng	*Panax*
Liquorice [12]	*Glycyrrhiza glabra*
Black cohosh	*Actaea racemosa*
Hops [13]	*Humulus lupulus*
Broccoli	*Brassica oleracea*
Green Tea [14]	*Camellia sinensis*

Physiological benefits of phytoestrogens:

Phytoestrogens lower the incidence of cardiovascular diseases, osteoporotic fractures, rates of breast cancer and hot flushes in Asian population consuming a diet rich in soy [13]. Several studies have discussed the potential effects of phytoestrogens in treating endometrial cancer, liver diseases and prostate cancer. Phytoestrogens are potent antioxidants which can reduce the progression of atherosclerosis and have positive effects on hot flushes, vaginal symptoms, cognitive function/ dementia in postmenopausal women [15].

It exerts wide spectrum of effects on the following diseases:

I. Cancer:

Epidemiological studies have shown that the incidence and mortality rates of breast cancer in the western world are much higher in comparison to Asian Countries. When Asian people emigrated to the USA, it was observed that the first generation female migrants had a lower risk of breast cancer, but the protection was lost in the second generation with an increasingly western diet. It suggested that certain phytochemicals present in Asian Diets can affect cancer incidence. Nowadays, there are an increasing number of human (and animal) studies demonstrating that a high soy intake during childhood is associated with a reduced breast cancer risk [16, 17].

Protective effects of isoflavones including soy are measured by tumor numbers, incidence, metastasis, and latency in animal models with experimentally induced breast cancer. Prepubertal genistein-treated rats developed fewer mammary gland terminal-end buds, with significantly less cells in the S-phase of the cell cycle, and more lobules than controls at 50-days-old [18].

In vitro studies using human prostate cancer cells have shown the inhibition of cell growth with high concentrations of phytoestrogens [19]. Rats consuming soy and rye bran had delayed growth of implanted prostate tumors [20].

The population-based studies show that the mortality due to breast, ovarian, prostate, and colon cancer has a negative correlation with the phytoestrogens and cereal intake in the diet [21].

II. Cardiovascular diseases:

Phytoestrogens possess significant potential in reducing coronary artery diseases via favourable effects on the lipid profiles [22]. The serum total Cholesterol, LDL, and VLDL cholesterol levels have been shown to be lowered in both male and female rhesus monkeys fed on phytoestrogen-rich diet [23]. Several mechanisms of action are reported to explain the Cardio protective role.

a. Increased bile acid secretion, which aids removal of low density lipoprotein (LDL).
b. Affected hepatic metabolism coupled with increased removal of LDL by hepatocytes.
c. Enhanced Thyroid Function.
d. Inhibition of Platelet aggregation [1] [24].

Reduced oestrogen levels during menopause may influence the development of obesity, fat distribution, the lipid profile in plasma, and rheological properties of plasma and platelet function [25]. These observations suggest that oestrogen deficiency may promote cardiovascular disease in women and trigger the idea that phytoestrogens may reduce the risk [26] [27].

III. Diabetes mellitus:

An improvement in glucose metabolism and a significant reduction in insulin levels and insulin resistance in menopausal women, by especially genistein were reported. Also, a meta-analysis concluded that soy isoflavone supplementation could improve glucose metabolism and insulin control in non-Asian postmenopausal women.

Nutritional intervention studies in both animals and humans suggested that an intake of soy protein with isoflavones and flaxseed improved glucose control and insulin resistance [28].

IV. Osteoporosis:

In postmenopausal women, estrogen deficiency is a major risk factor for osteoporosis **[**29]. The incidence of hip fracture increases and may lead to immediate disability. It has been observed that osteoporosis and risk of hip fracture is low in postmenopausal Japanese women than their Western counterparts [30] [31].

A diet rich in phytoestrogens has been shown to be accompanied by an increase in bone mineral density (BMD) [32]. Recent study carried out among Chinese pre- and post-menopausal women demonstrated that high isoflavone diet is associated with high BMD (at both spine and hip region) in post- but not in pre-menopausal women [33].

V. Effects of phytoestrogens on menopausal Symptoms:

Several meta-analyses were conducted to investigate the effects of phytoestrogens or soy isoflavone extracts or supplements on menopausal symptoms. These studies reported a reduction in the frequency and severity of hot flushes and varying effects on spine bone mineral density and no effects on femoral neck, hip total and trochanter bone mineral density [28].

VI. Cognitive function:

Cognition and memory functioning have been reported to decrease around menopause, and therefore studies have investigated the association of Estrogen Replacement Therapy and cognition. A study with female rats proposed that soy phytoestrogens function as estrogen agonists because they increased choline acetyltransferase and nerve growth factor messenger RNA in the frontal cortex and hippocampus. Other human studies have also suggested improved memory with dietary phytoestrogens [13].

VII. Phytoestrogen and skin health:

Isoflavones may have a protective effect on skin health. It improves skin elasticity by increasing local blood flow. In addition, the carcinogenic effect of exogenous noxious agents such as ionizing radiation and chemical compounds may be alleviated by the antioxidant and anti-inflammatory properties of phytoestrogens [34].

Red clover protects from inflammation and immune suppression induced by ultraviolet radiation [35].

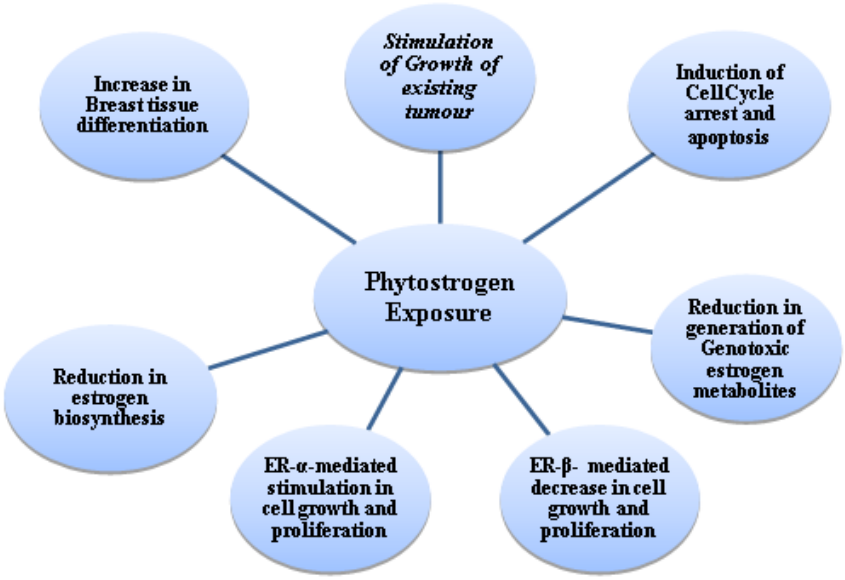

Figure 3: Summary of potential actions of Phytoestrogens [36]

Adverse effects of phytoestrogens:

The mechanism and potential of Phytoestrogens are not completely clarified and hence should be taken cautiously. The genetic toxicity potential of phytoestrogens has recently been reviewed. As potential endocrine disrupters, phytoestrogens may act as antiestrogens and harm the reproductive health of males. High doses of genistein are shown to alter pituitary responsiveness and basal luteinising hormone secretion in castrated post-pubertal rats. Coumesterol consumption by rat pups suppressed testicular testosterone concentrations and resulted in abnormal sexual behaviour in adulthood. Studies in cultured human lymphoblastoid cells reported that coumesterol was mutagenic and clastogenic. Concern has been associated with thymic weight and effects on the immune system. Ovariectomised adult mice injected with genistein produced dose responsive decrease in thymic weight of upto 80% [13].

Conclusion:

Research associated with Phytoestrogen and its impact on health has increased dramatically. There are several types of phytoestrogen with variable structural and functional differences, so further investigation is needed to evaluate the safety of Phytoestrogen on Human system. These polyphenolic compounds derived from plants are very common constituents of human and animal diet and can have significant consequences on reproductive health. They are recognised as therapeutic compounds claiming diverse health benefits which include hypolipidemic, hypoglycaemic, anticarcinogenic properties. But they may act as endocrine

disruptors indicating a potential cause of adverse health effects such as infertility and suppression of immune system.

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References:

1. Price KR, Fenwick GR. (1985). Naturally occurring oestrogens in foods- a review. Food Addit Cotam. 2: 73-106.
2. Knight DC, Eden JA. (1996). A review of the clinical effects of phytoestrogens. Obstet Gynecol 87: 897-904.
3. Brzezinski A, Debi A. (1999). Phytoestrogens: the 'natural' selective estrogen receptor modulators. Eur J Obstet Gynecol Reprod Biol. 85: 47-51.
4. Anderson JJB, Anthony M, Messina M, Garner SC. (1999). Effects of phyto-oestrogens on tissues. Nutr Res Rev 12: 75-116.
5. Adlercreutz H. (1998b). Evolution, nutrition, intestinal microflora, and prevention of cancer: a hypothesis. Proc Soc Exp Biol Med 217: 241-246.
6. Kurzer MS, Xu X. (1997). Dietary phytoestrogens. Annu Rev Nutr 17: 353-381
7. Santti R, Makela S, Strauss L, Korkman J, Kostian M-L. (1998). Phytoestrogens: potential endocrine disruptors in males. Toxicol Ind Health 14:223-237.
8. Benassayag C, Perrot-Applanat M, Ferre F. (2002). Phytoestrogens as modulators of steroid action in target cells. J Chomatogr B Analyst Technol Biomed Life Sci 777:233-248.
9. Sreedhar Reddy Puluputturi, Jhansi Rani Dayapulae. (2011). Phytoestrogens: Risks and Benefits. International Journal of Pharmaceutical Sciences Review and Research, Volume 10, Issue 1,122-126.
10. Dewick PM. (1993). Isoflavonoids. In The Flavonoids: Advances in Research Since 1986. Harborne JB (ed.). Chapman & Hall : London 117-238.
11. Ivana Lj. Nikolić, Ivana M. Savić-Gajić, Ana D. Tačić, Ivan M. Savić.(2017). Classification and Biological Activity of Phytoestrogen- a review. Advanced technologies. 6(2) 96-106.
12. Elena M. Umland, Jacintha S. Cauffield, Julienne K. Kirk,Tracy E. Thomason.(2000). Pharmacotherapy; 20(8)981–990).
13. Andreana L. Ososki, Edward J. Kennelly. (2003). Phytoestrogens: a Review of the Present State of Research. Phytother. Res. 17, 845-869.
14. Heather B. Patisaul* and Wendy Jefferson. (2010).The pros and cons of phytoestrogens. Front Neuroendocrinol; 31(4): 400–419.

15. IzabelaWocBawek-Potocka, Chiara Mannelli, Dorota Boruszewska,Ilona Kowalczyk Zieba,Tomasz WaVniewski and Dariusz J. SkarHyNski1. (2013). Diverse Effects of Phytoestrogens on the Reproductive Performance: Cow as a Model. International Journal of Endocrinology. pp 1-15
16. Messina M, PerskyV, Setchell KDR, Barnes S. (1994). Soy intake and cancer risk: a review of the in vitro and in vivo data. Nutr Cancer; 21: 113-31.
17. Stark A, Madar Z. (2002). Phytoestrogens: a review of recent findings. J Pediatr Endocr Met. 15; 561-72.
18. Murrill WB, Brown NM, Zhang JX, Manzolillo PA, Barnes S, Lamartiniere CA. (1996) Prepubertal genistein exposure suppresses mammary cancer and enhances gland differentiation in rats. Carcinogenesis. 17:1451–1457.
19. Adlercreutz H, Mazur W, Bartels P et al.(2000a). Phytoestrogens and prostate disease. J Nutr, 130, 658S–659S.
20. Landstom M, Zhang JX, Hallmans G, et al. (1998) Inhibitory effects of soy and rye diets on the development of Dunning R3327 prostate adenocarcinoma in rats Prostate, 36, 151–161.
21. Rose DP, Boyar AP, Wynder EL.(1986). International comparisons of mortality rates for cancer of the breast, ovary, prostate and colon, and per capita food consumption.Cancer; 58:2363- 71.
22.Ariyo AA, Villablanca AC.(2002).Estrogens and lipids Can HRT designer estrogens, and phytoestrogens reduce cardiovascular risk markers after menopause. Postgrad Med, 111, 23-30.
23. Anthony MS, Clarkson TB, Hughes CL, Moregan TM, Burke, GL. (1996).Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. J Nutr,126, 43-50.
24. Wroblewski, Lissin L, Cooke JP. (2000). Phytoestrogens and cardiovascular health. J Am Coll Cardiol, 35, 1403–1410.
25. Gorodeski GI (1994). Impact of the menopause on the epidemiology and risk factors of coronary artery heart disease in women. Exp Gerontol 29: 357–375.
26. Nagata C (2000). Ecological study of the association between soy product intake and mortality from cancer and heart disease in Japan. Int J Epidemiol 29: 832–836.
27. Zhang XL, Shu XO, Gao YT, Yang G, Li Q, Li HL et al. (2003). Soy food consumption is associated with lower risk of coronary heart disease in Chinese women. J Nutr 133: 2874–2878.
28. Ivonne M C M Rietjens, Jochem Louisse and Karsten Beekmann. 2017. The potential health effects of dietary Phytoestrogens. British Journal of Pharmacology. 174, 1263–1280 1263

29. Dempster DW, Lindsay R.(1996). Pathogenesis of osteoporosis. Lancet;341:797-801.
30. Cooper C, Campion G, Melton LJ III. (1992) Hip fractures in the elderly: A worldwide projection. Osteoporos Int;2: 285-9.
31. WHO Study Group Report.(1994).Assessment of fracture risk and an application to screening for postmenopausal osteoporosis. WHO Tech Rep Ser 1994.843:11-3.
32. Arena S, Rappa C, Del Frate E, Cenci S, Villani C. (2002). A natural alternative to menopausal hormone replacement therapy. Phytoestrogens. Minerva Ginecol. 54:53-7.
33. Mei J, Yeung SS, Kung AW.(2001). High dietary phytoestrogen intake is associated with higher bone mineral density in postmenopausal but not premenopausal women. J Clin Endocrinol Metab. 86:5217-21.
34. Widyarini S, Husband AJ, Reeve VE. (2005) Protective effect of the isoflavonoid equol against hairless mouse skin carcinogenesis induced by UV radiation alone or with a chemical co-carcinogen. Photochem Photobiol. 81:32–7.
35. Widyarini S, Spinks N, Husband AJ, Reeve VE.(2001).Isoflavonoid compounds from red clover (Trifolium pratense) protect from inflammation and immune suppression induced by UV radiation. Photochem Photobiol, 2001; 74:465–70.
36. Sarah M. Mense, Tom K. Hei, Ramesh K Ganju, Hari K. Bhat. (2008). Environmental Health Perspectives. Vol.116, no. 4. pp 426-433.

PROBIOTIC SPECTRA OF FERMENTED FOODS

Shakuntala Ghorai

Department of Microbiology, Raidighi College

Corresponding author E-mail: shakuntalaghorai@gmail.com

Abstract:

Fermented foods are part of human diet long before the days of Christ. At that time, they were made solely for the purpose of long term preservation. With ever increasing demands from food lovers and taste enthusiasts, fermented foods have come a long way. Varieties of dairy products, vegetables, fruits and grains are fermented and they are consumed heavily in almost all cuisine. Some fermented foods are confined in their known territories only whereas majority of them have global appeal. Their huge popularity in every civilization through the ages can be attributed to several factors. These foods not only act as great flavour enhancers but also have several health benefits as well. Making the food more digestible, increasing the nutritive value, curbing the sugar craving and reducing the toxic components etc are some of them. But, one healthy dose of probiotics is the icing on the cake. Those probiotics in turn boost the immune system of the consumer by competing with the pathogenic microorganisms present in the gut while reducing inflammatory response. Recent studies involving fermented foods show reduction in risk of cardiovascular disease and type 2 diabetes in human. Also, evidences of improved glucose metabolism, reduction of hyperlipidemia, hypertension, osteoporosis, muscle soreness and even depression are well supported with experimental data. Interestingly, there is an indication that consumption of fermented food can alter mood and brain activity, paving the way for a nascent field of research as 'microbiota-gut-brain axis'.

Introduction:

According to Campbell-Platt, "fermented foods are animal or plant tissues subjected to the action of microorganisms and/or enzymes to give desirable biochemical changes and significant modification of food quality" (1). Fermented foods have several health promoting benefits over regular food stuffs as these foods are enriched with nutritive factors while having protective, therapeutic and immunological properties. Moreover, they boost bioavailability of minerals and provide a healthy dose of antioxidants and omega-3-poly unsaturated fatty acids etc (2, 3). There are certain instances that vitamins, amino acids and other bioactive compounds are generated during fermentation. Presence of *Rhizopus oligosporus* in the foods have resulted in increased levels of niacin, nicotinamide, riboflavin, and pyridoxine, whereas non-pathogenic strains of *Klebsiella pneumoniae* and *Citrobacter freundii* have promoted synthesis of cyanocobalamin or vitamin B12 during fermentation (4, 5).

Several microorganisms also produce enzymes which assist in breakdown of complex substrates to simple ones. For example, *Bacillus subtilis* produces proteinase, amylase, mannase, cellulase, and catalase during *natto* and *kinema* fermentation (6, 7). Conversion of lactose to more digestible lactate and proteins to free amino acids by lactic acid bacteria made fermented milk the most popular fermented food throughout the world (1). *Leuconostoc sp*, *Lactobacillus sp* and *Streptococcus sp* are capable of detoxifying the cyanogenic glycoside linamarin from the bitter varieties of cassava (8). According to Tamang (9), fermentation also improves taste of the food by imparting typical flavour and texture to it. Due to low pH or high acid content shelf life or storage life of the food is also increased.

Every fermented food is associated with one or more functional microorganisms. These probiotic cultures are well known for their beneficial effect on gastrointestinal tract (10). Yakult (commercial probiotic milk product from Japan) has several reported health promoting benefits like modulation of the immune system, maintenance of gut flora, regulation of bowel habits, alleviation of constipation, and curing of gastrointestinal infections (11).

Dairy products:

Among all, this is the most popular category of fermented food available to us. Every section of human population is acquainted with one or other fermented dairy product like yogurt, sour cream/dips and natural or flavoured cheese etc. The milk source can be cow, buffalo, goat, sheep or yak. Frequently used Lactic acid bacteria for preparing Lassi(India) /Yogurt(Bulgaria) /Amasi(Africa) /Kefir (Eastern Russia)/Ymer (Denmark) /Filmjölk (Sweden) /Kumis (Central Asia) are *Lactobacillus delbrueckii* subsp. *bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, Lactococcus lactis* subsp. *lactis, and Lactococcus lactis* subsp. *Cremoris* etc (12).*Saccharomyces unisporous, Saccharomyces turicensis, Saccharomyces cerevisiae, Saccharomyces exiguous, Bacillus coagulans* are also found in various products.

During preparation of cheese, *Lactococcus lactis* subsp. *lactis/cremoris*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii* subsp. *bulgaricus,* and *Streptococcus thermophilus* are provided for acid and distinct flavor development while *Propionibacterium shermanii* produces propionate which acts as a natural shelf life extender (13). In addition to bacteria, edible molds are responsible for typical colour and flavour of various well-known cheese products like blue, Roquefort, stilton, Camembert, Brie and Gorgonzola etc. *Leuconostoc spp* creates typical flavour of sour cream. Acetaldehyde is considered as a typical flavour compound in yogurt whereas diacetyl is a distinctive flavour compound in buttermilk and sour cream (14, 15).

Cereals

Wheat, rice, maize, pearl millet, sorghum, barley, oats and rye etc are served as staple foods because they are rich in carbohydrate and fibre. However, cereals and other plant foods

may contain significant amounts of toxic or antinutritional substances. Legumes in particular are rich in protease inhibitors, amylase inhibitors, metal chelators, flatus factors, hemagglutinins, saponins, cyanogens, lathyrogens, tannins, allergens, acetylenic furan and isoflavonoid phytoalexins (16). Most cereals contain phytates, enzyme inhibitors which severely affect bioavailability of minerals, solubility, functionality and digestibility of proteins and carbohydrates (17). During fermentation, phytate is converted to inorganic orthophosphate and a series of myoinositols by action of phytases. Microorganisms are reportedly potent producers of 3-phytase whereas 6-phytase is available from cereal grains and other plant seeds (18). Several reports have shown that trypsin inhibitor, chymotrypsin inhibitor and amylase inhibitor levels are reduced during fermentation (19, 18).

Leuconostoc mesenteroids, Streptococcus faecalis are regular probiotics of idli (India, Sri Lanka) and puto (Philippines). Kichuddok (Korea) is enriched with *Saccharomyces cerevisiae.*Starch noodles from Thiland, Korea and Japan are prepared using *Lactobacillus cellobiosus* and *Lb. fermenti* as starter cultures. Mungbean (China) contains *Leuconostoc mesenteroids* (20).

Vegetables

Mainly lactic acid bacteria (LAB) are involved in fermentation of vegetables like cabbage, cauliflower, carrot, garlic, tomato, soybean, olive, cucumber, onion, turnip, radish, cauliflower, chillies, ginger and pepper etc. Depending on salt concentration and other factors, sometimes yeast and/ or other microorganisms may be present in the process. Presence of salt controls the nature and growth of microbes in the fermentation mixture. *Leuconostoc mesenteroids, Lactobacillus brevis, Lactobacillus plantarum, Pediococcus cerevisiae etc* are essential components of fermented vegetables (20). At the start, *Leuconostoc mesenteriodes* grows and produces lactic acid, acetic acid and carbon dioxide. Then *Lactobacillus brevis* grows, producing more acid. Finally, *Lactobacillus plantarum* grows, producing still more lactic acid and lowering the pH to below 4.0 (21). At this pH and under anaerobic conditions, the cabbage and other vegetables can be preserved for a long period of time.

Applying genome probing DNA chip (GPM) method to identify and monitor microbial behaviour during fermentation, more than 100 species of microorganisms were identified in kimchi fermentation (22). Among these, *Weisella confuse, Leuconostoc citreum, Lactobacillus curvatus, Lactobacillus sakai,* and *Lactobacillus fermentum* were identified as the important microorganisms.

Fish and meat

Fermented fish (both freshwater and sea fish) products are consumed as a staple food in many parts of the Scandinavian region of Europe, Africa, Middle East, South Asia and Southeast Asia. Although these foods are very popular, unfortunately very few studies have been done

regarding the microbial content of the fermented products. Mainly Southeast Asian foods are explored for probiotics. *Ngari*, one fermentented fish product from Manipur, India contains mainly lactic acid bacteria. These are *L. fructosus, L. amylophilus, L. coryniformis* spp. *torquens, L. plantarum, Lactococcus plantarum, L. lactis* spp. *cremoris*, and *Enterococcus faecium*. Sometimes *B. subtilis* and *B. pumilus, Micrococcus, Candida*, and *Saccharomycopsis* are also found. (23). *L. mesenteroides, Lb. plantarum* are associated with sikhae (Korea) and narezushi (Japan). *Lb. brevis, Streptococcus sp.* are key LAB of burong-isda (Philippines) whereas *Pediococcus cerevisiae*is found in balao-balao (Philippines) and kungchao (Thailand).

Generally, pork and beef meats are fermented on a regular basis and *Lactobacillus* are the dominant variety of microorganisms in them. *Lb. sake, Lb. curvatus,* and *Lb. plantarum* (24) are common with a relatively low number of fungi and yeast. Fungi and yeast decrease the pH of the medium and add typical flavour. *Penicillium, Aspergillus, Mucor, Eurotium* and *Cladosporium* are flavour inducer species of different sausages.

Risk factor

Several unwanted microorganisms can contaminate the fermentation mixture like *Escherichia coli, Salmonella paratyphi, Listeria monocytogenes, Brucella melitensis* and *Clostridium botulinum*. They constitute potential hazard as they release bacteriotoxin which can poison the food (25). Certain yeasts like *Candida parapsilosis, Rhodotorula mucilaginosa* and *Debaromyces hansenii* are reported to spoil fermented products(26). *Staphylococcus spp*., *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli* O157:H7, *Bacillus* and *Clostridium* spores are found in raw/ fermented meat and in dry sausages (27, 28). The presence of those contaminants may be attributed to insufficient acid production during fermentation, anaerobic fermentation condition and other improper steps employed during fermentation procedure.

End note

With a bit of precaution, fermented foods can pamper our taste buds while maintaining nutrition quotient. If we follow requisite steps during fermentation and storage, these foods can be a great treat to all age groups.

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References:

1. Campbell-Platt, G. (1994) Fermented foods- a world perspective. Food Res. Int. 27, 253-257

2. Tamang, J.P. (2007) Fermented foods for human life. In *Microbes for Human Life*, eds. Chauhan, A.K., Verma, A., andKharakwal, H. pp. 73-87, I.K. International Publishing House Pvt. Limited, New Delhi, India
3. Liong, M.T. (2008) Safety of probiotics: Translocation and infection. Nutr. Rev.66,192-202
4. Liem, I.T.H., Steinkraus, K.H., and Cronk, T.C. (1977) Production of vitamin B12 in tempeh, a fermented soybean food. Appl. Environ. Microbiol.34, 773-776
5. Keuth, S. and Bisping, B. (1994) Vitamin B12 production by *Citrobacter freundii* or *Klebsiella pneumoniae* during tempeh fermentation a proof of enterotoxin absence by PCR. Appl. Environ. Microbiol.60, 1495-1499
6. Ueda, S. (1989)*Bacillus subtilis. Molecular Biology and Industrial Applications*, eds. B. Maruo and Yoshikawa, H. pp. 143-161, Elsevier and Kodansha Ltd, Tokyo, Japan
7. Tamang, J.P. and Nikkuni, S. (1996) Selection of starter culture for production of kinema, fermented soybean food of the Himalaya. World J. Microbiol.Biotechnol.12, 629-635
8. Westby, A. and Twiddy, D.R. (1991) Role of microorganisms in the reduction of cyanide during traditional processing of African cassava products. Proceeding of Workshop on Traditional African Foods—Quality and Nutrition, eds. Westby, A. andReilly, P.J.A. pp. 127-131, International Foundation for Science, Stockholm, Sweden
9. Tamang, J.P. (2001) Kinema. Food Culture.3, 11-14
10. Lee, Y.K. and Salminen, S. (2009)Handbook of Probiotics and Prebiotics, 2nd Ed. Wiley, Hoboken, NJ
11. Kiwaki, M. and Nomoto, K. (2009) *Lactobacillus casei*Shirota.Handbook of Probiotics and Prebiotics, 2nd Ed, eds. Lee, Y.K.andSalminen, S.pp.449-457, Hoboken, Wiley, NJ
12. Chandan RC (2011) Nutritive and health attributes of dairy ingredients. Dairy Ingredients for Food Processing,eds. Chandan, R.C., Kilara A., Ames, IA, pp. 387-419, Wiley-Blackwell Publishing
13. Fox PF (2003a) Cheese: overview. Encyclopedia of Dairy Sciences, eds.Roginski H, Fuquay JW, Fox PF, pp. 252–261, Academic Press, London
14. Vedamuthu ER (2013a) Starter cultures for yogurt and fermented milks. Manufacturing Yogurt and Fermented Milks, eds. Chandan, R.C., Kilara, A. pp. 115-147, John Wiley Chichester, West Sussex
15. Vedamuthu ER (2013b) Other fermented and culture containing milks. Manufacturing Yogurt and Fermented Milks,eds. Chandan, R.C., and Kilara, A. pp. 393-410, John Wiley, Chichester, West Sussex
16. Pariza, M. W., (1996) Toxic substances. Food Chemistry, pp. 825-840, Marcel Dekker,New York

17. Reddy, N. R., Pierson, M. D., Sathe, S. K. and Salunkhe, D. K. (1989) Phytates in Cereals and Legumes. CRC Press, Boca Raton, FL
18. Reddy, N. R. and Pierson, M. D. 27 (1994) Reduction in antinutritional and toxic components in plant foods by fermentation. Food Res. Int. 27, 281-290
19. Chaven, J. K. and Kadam, S. S. (1989) Nutritional improvement of cereals by fermentation. Crit. Rev. Food Sci. Nutr. 28, 349-400
20. Rhee, S. J., Lee, J-E. and Lee, C-H (2011) Importance of lactic acid bacteria in Asian fermented foods. Microb. Cell Fact. doi:10.1186/1475-2859-10-S1-S5
21. Fleming, H.P., McFeeters, R.F., and Daeschel, M.A. (1992) Fermented and acidified vegetables in Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington, D.C.
22. Park HY, Bae JW, Lee IS, Kim HI, Lee JS, Ryu BH, Chang YH, Yoon JH, Nam YD, Kang KH (2005) Kimchi starter culture and molecular monitoring technology for the production of new functional Kimchi.Proceedings of Korean Society of Microbiology and Biotechnology Symposium Seoul, pp. 49-53. Korean Kimchi and Fermentation Technology
23. Thapa, N., Pal, J., and Tamang, J. P. (2004) Microbial diversity in ngari, hentak, and tungkap fermented fish products of North-East India. World J. Microbiol. Biotechnol.20, 599-607
24. Leroy, F., Verluyten, J., and De Vuyst, L. (2006) Functional meat starter cultures for improved sausage fermentation. Int. J. Food Microbiol.106, 270-275
25. Ramsaran H, Chen J, Brunke B, Hill A, Griffiths MW (1998) Survival of bioluminescent Listeria monocytogenes and E. coli O157:H7 in soft cheese. J. Dairy Sci. 81, 1810-1817
26. Adebayo, C.O., Aderiye, B.I., and Akpor, O.B. (2014) Assessment of bacterial and fungal spoilage of some Nigerian fermented and unfermented foods. African J. Food Sci. 8, 140-147
27. Erkkila, S. (2001) Bioprotective and probiotic meat starter cultures for the fermentation of dry sausages. Academic dissertation, p. 1-64, Department of Food Technology, University of Helsinki
28. Farber, J. M., and Peterkin, P.I. (1991) Listeria monocytogenes, a food-borne pathogen. Microbiol. Rev. 55, 476-511

AUGMENTATION OF DIRECT ADVENTITIOUS *IN VITRO* SHOOT REGENERATION OF AN IMPERILED & MEDICINAL *DENDROBIUM NOBILE* LINDL. ORCHID BY POLYAMINES

Madhumita Majumder

Department of Botany, Raidighi College,

P.O. + P.S. - Raidighi, 24 Parganas (S), Pin- 743383, West Bengal, India

Corresponding author E-mail: madhumita2363@rediffmail.com

Abstract:

An efficient method of mass propagation of *Dendrobium nobile* Lindl, was developed using shoot-tip culture system. Thin shoot-tip explants was excised from aseptically raised 3-month's old seedlings and cultured on modified Knudson's C nutrient solution containing 4µM BAP and polyamines. Polyamines like Putrescine, Sprmine and Spermidine were tested. After 3 months of culture direct adventitious shoots as well as direct protocorm-like –body (PLB) regeneration from the basal cut ends of explants were observed. Among various polyamines tested in *D. nobile* it was observed that all the polyamines were effective in increasing direct adventitious shoot regeneration with several proliferating branches. The optimal concentration for maximum shoot regeneration (70%) was observed in 0.4 µM spermidine. Avery low frequency of callusing occurred only in presence of spermine. But a very small percentage (10-17) % of explants was responsible for the formation of direct PLBs. By repeated subculturing on the primary inductive media, high frequency multiplication rate was established. Proliferating shoots produced when cultured on basal medium. The well rooted shoots were transferred to clay pots containing a mixture of dried coconut husk, small pieces of brick and leaf mould on 1:1:1 ratio. Potted plantlets were acclimatized to greenhouse condition and a preliminary planting trial of the plantlets in the natural habitat resulted in 70% survival rate.

Keywords: Shoot-tip culture, adventitious shoots, protocorm-like body, subculture, plantlets.

Introduction:

Orchidaceae forms one of the world's largest families of flowering plants of angiosperm. Because of its diverse shapes, forms and colors orchids are outstanding in many ways. They are marketed as potted plants and cut flowers leading to their production over years [21]. To save the diverse orchid species from extinction, in vitro culture techniques have been utilized to propagate them [5].

Dendrobium nobile is one of the commercially and ethno-botanically important taxa. This species is a major stake holder in the worldwide cut flower market [11] due to its violet colored

attractive flowers. It has a great traditional use in various drug preparations [22]. The presence of various active compounds like dendrobine, nobiline and dendrophenolin the stems and leaves of *D. nobile* has greatly increased its medicinal importance [12, 21]. These compounds also have strong antimutagenic properties and it has been found to be anticarcinogenic against lung carcinoma, ovary adenocarcinoma and promyelocytic leukemia [10]. Polyamines are organic compounds having 2 or more primary amino groups and found in plants both in free and bounded form. They appear to be important in cell division, plant growth and senescence as well as stress responses [1]. Putrescine for instance has been proven to increase the proliferation of *Dendrobium* Sonia PLBs. [16]. [20] reported that the application of spermidine and putrescine increased the endogenous polyamine level and resulted in the increased frequency of conversion of PLBs to shoots in *D. huoshanense*. In 2006, [7] also proved that adding putrescine into media increased the formation of multiple shoots of *Gossypium hirsutum* L. plantlets.

The present study also describes the role of exogenous polyamines in the induction of embryogenic callus formation of adventitious shoots and regeneration of PLBs from the shoot-tip explants and their subsequent plantlet development in *D. nobile.*

Material and Method:

The experiment was performed with shoot-tips of about 4-6mm length, isolated aseptically raised 5-months old seedlings. The basic culture media was based on modified nutrient solution of Knudson's C [8] and the original iron source was replaced by the iron-EDTA as described by Murashige & Skoog [13]. The medium was also supplemented with 2% (w/v) sucrose and 0.1% (w/v) peptone. The polyamine treatments consisted of five different concentrations at 0.2-1µM. The pH was adjusted with 5.2 prior to autoclaving. After adding 0.9% agar (w/v) the media were autoclaved at 1.02kg/cm^2 for 20 min. Various morphogenetic responses of the shoot-tips were recorded periodically and the final data for analysis were gathered after 3 months of culture.

Results:

- **Survival of shoot-tips:**

Shoot tip survival was dependent on the type and concentration of polyamines present in the medium. In polyamines free control medium explant survived at very low percentage. While spermine (0.2-1µM) was inhibitory for explants survival. Putrescine an spermidine exerted a marked stimulation in explant survival.

- **Growth responses:**

The surviving populations of *D. nobile* exhibited two types of growth responses in presence of polyamines. In the first type, they propagated through the formation of adventitious shoots and PLBs directly from the base of the shoot-tip (Fig.1).

But in the second type, low frequency of explants produced callus in some treatments.

a) **Formation of adventitious shoots:**

The explants followed the natural ontogenetic route for axial growth. Multiple adventitious shoots along with several branches were initiated from the base of the shoot-tip after 21-28 days. Among the different treatments tested, consistent shoot formation occurred at all the PAS treatments. Putrescine at 0.4 µM gave optimum response in shoot regeneration (4 shoots/explants) after 4-5 weeks of culture.

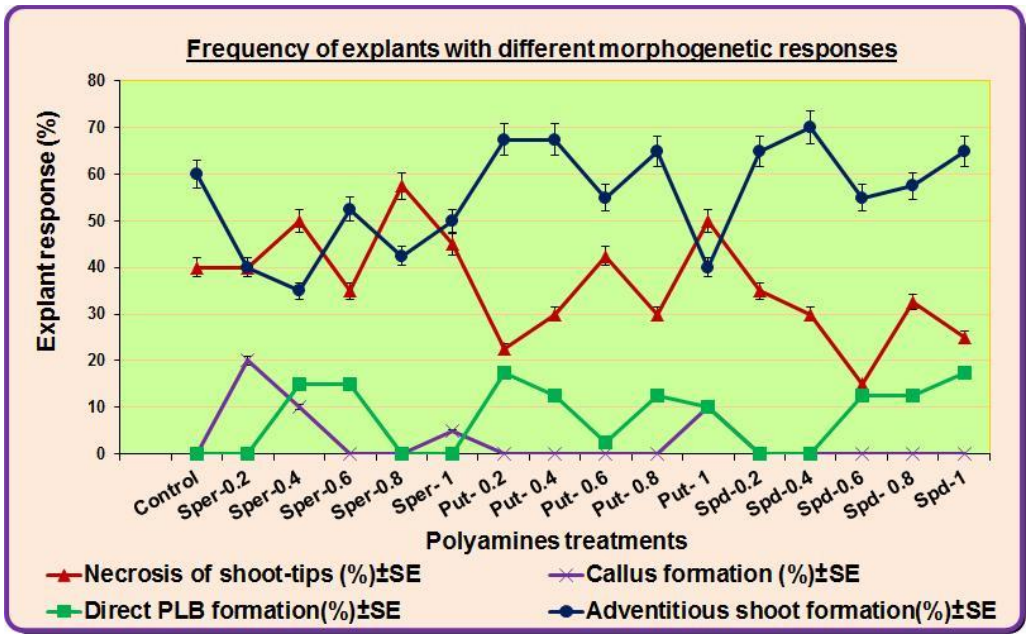


Figure 1: Graphical representation showing the different morphogenetic responses of *D. nobile* in presence of polyamines

b) Formation of direct PLBs:

Basal medium supplemented with 4 µM BAP was not suitable for the regeneration of PLBs. The efficacy of explnts for producing PLBs observed with the application of PAs in the medium, though the frequency was very low. Incorporation of putrescine at 0.2 µM, spermidine at 0.6-1 µM and spermine 0.4-0.6 µM induced PLB regeneration (Fig.2). Among the different polyamine treatments the best result was achieved in the medium containing 0.2 µM putrescine and 1 µM spermidine (both17.50%). Putrescine at 0.6 µM caused a steady decline (2.50%) in the production of PLBs. Mean number D-PLBs per explant was optimum in the medium

supplemented with 0.2 µM putrescine (mean number 1.89), followed by 0.6 µM sprmine (1.85per explant).

c) Formation of callus and callus mediated PLBs:

Apart from the formation of either adventitious shoots or D-PLBs, some of the explants got partly disorganized and propagated via callus mediated pathway (Fig2.). Such callusing occurred only in four PA concentrations. Spermidine failed to produce any such callus. The highest frequency of callusing (20%) was obtained at 0.2 µM spermine. Calli also exhibited low frequency of PLB regeneration and these PLBs have been designated as callus-mediated PLBs. (C-PLBs).

d) Acclimatization of plantlets:

For rooting, in vitro raised plantlets from different treatments were transferred to KC medium containing 1-2 µM NAA or 0.1% peptone. After 4-5 weeks they were placed in the greenhouse where they have acclimatized and the survival percentage was 75-80%.

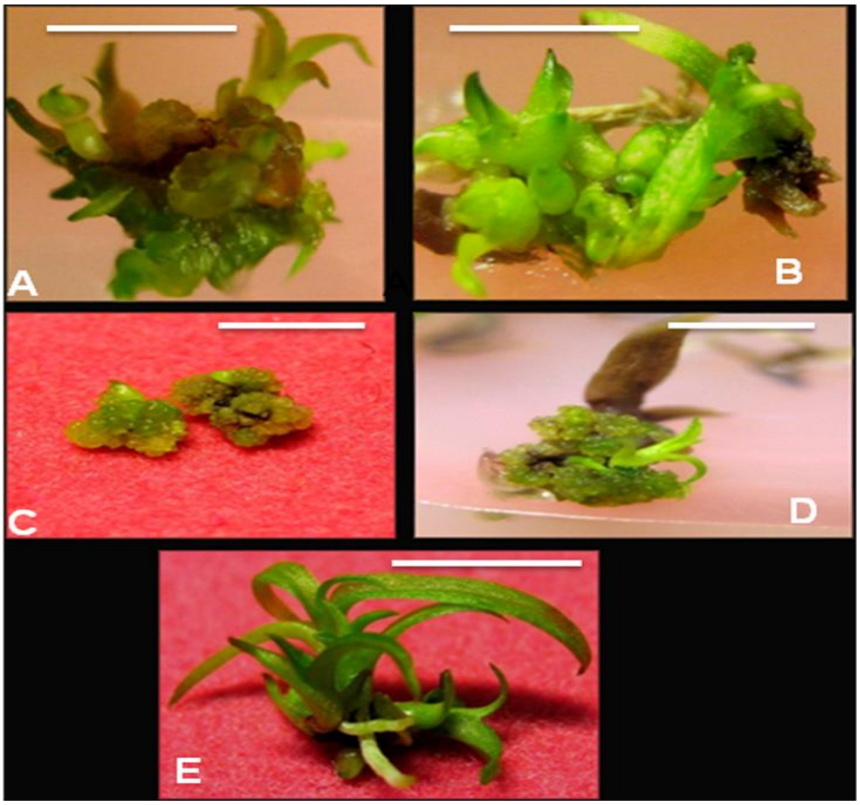


Figure 2: Effect of different polyamines on different morphogenetic responses of *D. nobile* (Bar = 7.5mm) A. PLBs at different stages directly regenerated from shoot-tips. B. PLB mediated plantlets. C. & D. Embryogenic callus and callus mediated PLBs and plantlet E. Multiple adventitious shoots with roots

Discussion:

The results of the present investigation have proved that application of exogenous polyamines is most effective for the tridirectional micropropagation pathway of *D. nobile.* Polyamines played a positive role in several tissue culture system, such as *Helianthus tuberosus* [14], micropropagation of *Asparagus* [7], and somatic embryogenesis in *Heveabrasiliensis* [4]. According to Kumar & Palni [9] the accumulation of spermine and spermidine at high concentration in the culture of *Gladiolus* hybridus enhanced the production percentage. Callus induction responses differ notably from earlier observation made on orchid. Among the polyamine treatment, only spermine had little inducing effects on its own. The inhibitory effects of spermidine and putrescine over callus production in *D. nobile* also supports the previous reports made in Coffee [2]. In *Sideritis anguslifolia* a dose-dependent inhibitory effect of exogenously supplied spermidine over calli.

Putrescine is a diamine, while spermine is tetramine and spermidine is triamine. The methylene group of the polyamines will give a hydrophobic interaction for this compound. Takao [17] showed that exogenous putrescine might not be absorbed by the PLBs than polyamines and it might accumulate in the media and at certain concentration it may be toxic. Such studies also incorporate with our studies. According to Dutra *et al.* [3], incorporation of spermidine and spermine enhanced the changes of cellular structure in proembryogenic masses by producing more embryogenic cell in *Araucaria angustifolia.*

So, it appears that when spermine and spermidine were added into the media the plant cells absorb and use these polyamines without any processing because these PAs were already synthesized in spermine and spermidine form. The interplay between hormones and polyamines and their combined effects are probably complex sensitivity of intact plant tissues to growth substances is an important factor in understanding growth regulation by polyamines and hormones.

References:

1. Alcázar R, Marco F, Cuevas JC, Patron M, Ferrando A, Carrasco P, Tiburcio AF, Altabella T: (2006) Involvement of polyamines in plant response to abiotic stress;Biotecnol. Letter; 28:1867-1876.
2. Calheiros MBP, Fuentes SRL, Veira LGE, Manettifilho J: (1994) Uso de fitoreguladores durante a fase de seleção de explantes de café. In: Congresso Brasileiro de pesquisas cafeei-ras 18: 89-90.
3. Dutra NT, Silveria V, De Azevedo IG, Gomes-Neto LR, Facanha AR, Steiner N et al.: (2013) Polyamines affect the cellular growth and structure of pro-embryogenic masses in

Araucaria angustifolia embryogenic cultures through the modulation of proton pump activities and endogeneous levels of polyamines; Physiol. Plant;148:121-132.

4. El- Hadrami I, Carron MP, Dauzac J: (1989) Variabilité clonale du potential embryogéne chez *Hevea brasiliensis*: relation avec les polyamines et les peroxidases des cals; Comptes Rendus – Acad. des Sci Paris; 308: 299-305.
5. Fay M: (1992) Conservation of rare and endangered plants using in vitro methods; In vitro Cell. Dev. Biol. - Plant; 28: 1-4.
6. Fiala V, Querou Y, Dore C: (1991) Polyamine changes during *in vitro* morphogenesis of *Asparagus* cloning; J of Plant Physiol; 138: 172-175.
7. Ganesan M, Jayabalan N: (2006) Isolation of disease-tolerant cotton (Gossypium hirsutum L. cv. SVPR 2) plants by screening somatic embryos with fungal culture filtrate; Plant Cell Tiss & Org Cult; 87(3): 273-284.
8. Knudson L: (1946) A new nutrient solution for the germination of orchid seeds; Amer. Orch. Soc.Bull; 14: 214-217.
9. Kumar A, Palni LMS: (2013) Changes in endogenous polyamines during in vitro cormlet formation in Gladiolus hybrids Hort; Sci Hort;162: 513-521.
10. Lee S, Park S, Kim T, Paek K, Lee SS, Park SS, Kim TJ, Paek KY: (1997) Effect of orchid habitat soil on growth of tissue cultured *Cymbidium kanran* and *C. goeringii* and root infection by orchid mycorrhizal fungus. J. Kor. Soc. Hort. Sci. 38: 176-182.
11. Martin KP, Madassery J: (2006) Rapid in vitro propagation of *Dendrobium* hybrids through direct shoot formationfrom foliar explants and protocorm-like bodies; Sci. Hort;108: 95-99.
12. Miyazawa M, Shimamura H, Nakamura S, Kameoka H: (1997) Antimutagenic activity of Gigantol from *Dendrobium nobile*; J. Agric. Food Chem; 45(8): 2849-2853.
13. Murashige T, Skoog F: (1962) A revised medium for rapid growth and bio assays with Tobacco tissue cultures; Physiol. Plant; 15: 473-497.
14. Phillips R, Press MC, Eason A: (1987) Polyamines in relation to cell division and xylogenesis in cultures explants of *Helianthus tuberosus*: lack of evidence for growth-regulatory action; J of Exp. Bot; 38: 164-172.
15. Sanchez-Gras MC, Segura J: (1988) Morphogenesis *in vitro* of *Sideritis angustifolia*: effects of auxins, benzyladenine and spermidine; Plant Sci; 57: 151-158.
16. Saiprasad GVS, Raghuveer P, Khetarpal S, Chandra R: (2004) Effect of various polyamines on production of protocorm-like bodies in orchid- *Dendrobium* 'Sonia'; Sci. Hortic; 100:161-168.

17. Takao K, Rickhag M, Hegardt C, Oredsson S, Persson L: (2006) Induction of apoptotic cell death by putrescine. Int J Biochem Cell Biol; 38:621-628.
18. Tokuhara K, Mii M: (2003) Highly efficient somatic embryogenesis from cell suspension cultures of *Phalaenopsis* orchids by adjusting carbohydrates sources; In vitro Cell. Dev. Biol.- Plant; 39(6): 635-639
19. Ye Q, Quin G, Zhao W: (2002) Immunomodulatory sesquiterpene glycosides from *Dendrobium nobile*; Phytochem; 61(8): 885-890.
20. Ying W, Luo JP, Wu HQ, Hong J: (2009) Conversion of protocorm-like bodies of *Dendrobium huoshanense* to shoots: the role of polyamines in relation to the ratio of total cytokinins and indole-3-acetic acidindole -3; J. Plant Physiol; 166(18): 2013-2022.
21. Zhao W, Ye Q, Tan X, Jiang H, Li X, Chen K, Douglas KA: (2001) Three new sesquiterpene glycosides from *Dendrobium nobile* with immunomodulatory activity; J. Natural Prod; 64(9): 1196-1200.

BIOACTIVE COMPOUNDS FROM CYANOBACTERIA - A REVIEW

Prasenjit Das* and Sahana Ghosh
Department of Microbiology,
Vijaygarh Jyotish Ray College, Kolkata- 700032
*Corresponding author E-mail: pdmicrobio@gmail.com

Abstract:

Beside producing cyanotoxins like the hepatotoxic microcystins and nodularins or the neurotoxic anatoxins and saxitoxins, cyanobacteria, have the ability to synthesize structurally distinct compounds such as proteins, fatty acids, vitamins or pigments, and various secondary metabolites with different bioactivities that can be used as food and feed, fuel, dyes, sunscreen agents and as therapeutic drugs. Most of the cyanobacterial metabolites are accumulated in the cyanobacterial biomass while some cyanobacteria excrete various organic compounds into their environment. So far, several biologically active compounds were identified among these exometabolites, e.g. some antibacterial diterpenoids in *Nostoc commune* or antifungal peptides in *Tolypotrixbyssoidea*. Cyanobacterial bioactive secondary metabolites are synthesized mainly by polyketide and nonribosomal biosynthetic pathways. The majority of isolated bioactive compounds are polyketides, amides, alkaloids and peptides. A large number of cyanopeptides have been identified with cytotoxic, antifungal, antibacterial and antiviral activities. Further, cyanobacterial metabolites show immunosuppressant, anticancer, anti-HIV, anticoagulant, anti-inflammatory and antiprotozoal activities. Some of these compounds have successfully reached Phase II and Phase III clinical trials.

Keywords: Cyanobacteria, primary metabolites, secondary metabolites, cyanopeptides

Introduction:

Cyanobacteria are prokaryotic, photoautotroph belonging to the phylum of eubacteria (1). Due to the presence of two pigments, chlorophyll a(green) and phycocyanin (blue), appearance of these bacteria is blue-green. Cyanobacteria can survive in wide variety of aquatic environments. Some species of cyanobacteria are mixotrophic and heterotrophic and can survive as consortia with other microorganisms (2). Cyanobacteria have abilities to synthesize peptide with non-ribosomal system (Polyketide Synthases) and have various types of methylases, oxidases and modification enzymes and are capable of producing many natural products containing linear peptides (3), cyclic peptides (4), linear lipopeptides (5), depsipeptides (6), cyclic depsipeptides (7), fatty acid amides(8), swinholides(9), glycomacrolides (10) or macrolactones(11).Cyanobacteria also possess cytochrome P450 system, a superfamily of heme

containing oxygenases that provide them the capacity for synthesizing and transforming bioactive molecules such as steroids, terpenes and alkaloids, and biodegrading xenobiotics and contaminants including pesticides, dyes and hydrocarbons (12).

Cyanobacteria are a rich resource of novel categories of biologically active carbohydrates, lipids, peptides, alkaloids, polyketides and terpenoids which perform activities such as antibiotic, anticancer, UV-protecting, vitamin or even toxin. Cyanobacteria from orders Oscillatoriales (49 %), Nostocales (26 %), Chroococcales (16 %), Pleurocapsales (6 %) and Stigonematales (4 %) are rich source of secondary metabolites (13). Species such as *Anabaena*, *Nostoc*, *Microcystis*, *Lyngbya*, *Oscillatoria*, *Phormidium* and *Spirulina* are reported to be the most promising ones for producing variety of bioactive compounds like carotenoids, fatty acids, lipopeptides, polysaccharides etc. (14). An overview of the cyanobacterial bioactive molecules with their potential uses is presented here.

Potential uses of Cyanobacterial bioactive compounds

Cyanotoxins

Several cyanobacteria produce toxins of various types including hepatotoxins, neurotoxins, dermatotoxins and cytotoxins (15). Hepatotoxins like microcystins, nodularins (protein phosphatase inhibitor) and cylindrospermopsin (protein synthesis blocker) (16), are obtained from cyanobacteria such as *Microcystis, Anabaena*, *Nostoc*, *Planktothrix* and *Nodularia spumigena* (17). Among the ten variants identified until now, Nodularin-R is the most abundant (18). Alkaloid neurotoxins like Anatoxins A, homoanatoxin A and saxitoxins are found in cyanobacteria species like *Anabaena*, *Aphanizomenon*, *Cylindrospermum* and *Microcystis* (19-21). Cyanotoxins have beneficial uses as algaecides, herbicides and insecticides (22) with significant biodegradability. Investigations on therapeutic effects of cyanotoxins as anticancer agents are in progress.

Antibiotics

Cyanobacteria can be a prospective source of innovative antimicrobial agents to tackle the serious problem of widespread occurrence of antibiotic resistance and rapid emergence of new infectious agents (23, 24). Major cyanobacterial sources of novel antibacterial compounds are *Fischerella ambigua* (25,26), *Hapalosiphon fontinalis* (27), *Tolypothrix nodosa*, *Nostoc* spp. (28,29), *Scytonema mirabile*, *Anabaena* spp., *Microcystis aeruginosa*, *Phormidium* sp., *Microcoleous lacustris*.

Antivirals

Some cyanobacterial molecules with antiviral activity can block absorption or penetration of viruses into cells or inhibit replication in viruses (30). For example, cyanovirin-N (CVN) is a cyanobacterial lectin isolated from *Nostoc ellipsosporum* (31) showing affinity towards mannose

rich oligosaccharides on virus surface and prevents fusion of enveloped viruses with animal cells. CVN is found to be active against enveloped viruses like HIV, influenza, Ebola, hepatitis, and herpes virus. Other antiviral compounds such as Spirulan (32), Nostoflan (33), Scytovirin N (34), Sulfoglycolipid (35) are derived from *Spirulina* sp., *Nostoc flagilliforme*, *Scytonema varium*, *Scytonema* sp., respectively.

Food and vitamins

Traditionally, cyanobacteria such as *Spirulina*, *Nostoc* and *Anabaena* species has been used as human food in several countries of South America, South-East Asia and Africa. *Spirulina* is an edible cyanobacterium, containing mainly high quality protein (60 to 71 % of its dry weight). *Synechococcus* sp. consists of 63% protein, 15% carbohydrate and 11% lipid (36). These cyanobacteria, therefore can be used as single-cell protein source. *Spirulina* is also a rich source of β-carotene, thiamine, riboflavin, absorbable iron, and other minerals, phenolic compounds, anti-inflammatory, antioxidants and essential fatty acids (37, 38). Strains of *Nostoc* have high content of fibers and proteins to be considered as healthy food (39). *Crocosphaera watsonii,* a marine cyanobacterium, can be used as a rich source of vitamin B_{12} (40). Growth rate of fishes was increased when nourished with various marine cyanobacteria like *Phormidium valderianum* in indoor and outdoor cultures (39).

Anticancer Agents

Some toxins and other secondary metabolites from cyanobacteria have activity against malignant cells (41, 42). Cyanotoxins such as microcystins and nodularins are inhibitors of protein phosphatases and downregulate signaling pathways in the cell and disintegrate cytoskeletal arrangement, thereby inducing cell death (apoptosis) in the tumor. Curacin A from *Lyngbya majuscula* and dolastatin 10 from *Symploca* sp. specifically affect tubulin or actin filaments in animal cells and are reported for their antimitotic and antiproliferative activity in clinical trials for potential use in neoplasias (43). Dolastatin derivatives such as soblidotin and tasidotinare in clinical trial for use as oncolytic drugs (44,45). Cryptophycins, obtained from *Nostoc* spp, is a type of depsipeptides with cytotoxic activity even on malignant cell lines with multiple drug resistance (MDR) (46). However, clinical trial on cryptophycin-52 in phase II was discontinued because of lack of efficacy *in vivo* and dose-limiting adverse effects; but investigation for development of analogues of cryptophycin is currently undergoing and already two patents on cryptophycin conjugates with immunoglobulins have been filed (47).

Pigments and sunscreens

Cyanobacteria produce high amounts of chlorophyll *a*, carotenoids and phycobiliproteins which are frequently utilized as natural colorants or antioxidants in food, cosmetic and

pharmaceutical industries (48). They also show other pharmacological activities including anti-proliferative, anti-lipid and neuroprotective (49,50). Phycocyanin, a phycobiliprotein derived from *Spirulina* and utilized in cosmetics, is a free radical scavenger and inhibitor of cytochrome oxidase; therefore it can be used in anti-inflammatory and hepatoprotective medicines (51). Moreover, this pigment has fluorescent activities for use in fluorescence-based diagnostic tests (52). Sun protection metabolite like scytonemin, a radical-scavenging pigment produced by *Nostoc punctiforme* (53), *Lyngbya aestuarii*(54) etc.is produced under UV exposure. Another pigment shinorine (a specific mycosporine-like amino acid), produced by *Anabaena variabilis* ATCC 29413 (55), also can be used to manufacture sunscreen.

References:

1. Castenholz, R. W. *et al* (2001) Bergey'sManualR of Systematic Bacteriology. Phylum BX. Cyanobacteria; (pp. 473–599): Springer; New York, NY, USA.
2. Subash chandra bose, S. R. *et al* (2013) Mixotrophic cyanobacteria and microalgae as distinctive biological agents for organic pollutant degradation. Environ. Int. 51, 59–72.
3. Simmons, T. L. *et al* (2006) Belamide A, a new antimitotic tetrapeptide from a Panamanian marine cyanobacterium. Tetrahedron Lett. 47(20), 3387–3390.
4. Sisay, M. T. *et al* (2009) Inhibition of human leukocyte elastase by brunsvicamides A-C: cyanobacterial cyclic peptides. Chem. Med. Chem. 4(9), 1425–1429.
5. Nagle, D. G., & Gerwick, W. H. (1995) Nakienones AC and nakitriol, new cytotoxic cyclicC11 metabolites from an Okinawan cyanobacterial (Synechocystis sp.) overgrowth of coral. Tetrahedron Lett. 36(6), 849–852.
6. Han, B. *et al* (2005) The wewakpeptins, cyclic depsipeptides from a Papua New Guinea collection of the marine cyanobacterium *Lyngbyasemiplena*. J. Org. Chem. 70(8), 3133–3139.
7. Soria-Mercado, I. E. *et al* (2009) Alotamide A, a novel neuropharmacological agent from the marine cyanobacterium *Lyngbyabouillonii*. Org. Lett. 11(20), 4704–4707.
8. Chang, T. T. *et al* (2011) Isomalyngamide A, A-1 and their analogs suppress cancer cell migration in vitro. Eur. J. Med. Chem. 46(9), 3810–3819.
9. Andrianasolo, E. H. *et al* (2005) Isolation of swinholide A and related glycosylated derivatives from two field collections of marine cyanobacteria. Org. Lett. 7(7), 1375–1378
10. Teruya, T. *et al* (2009) Biselyngbyaside, a macrolide glycoside from the marine cyanobacterium *Lyngbya sp*. Org. Lett. 11(11), 2421–2424.
11. Salvador, L. A., Paul, V. J., &Luesch, H. (2010) Caylobolide B, a macrolactone from symplostatin 1-producing marine cyanobacteria *Phormidium spp*. from Florida. J. Nat. Prod. 73(9), 1606–1609.

12. Alder, A. *et al* (2009) In vitro characterization of Synechocystis CYP120A1 revealed the first non animal retinoic acid hydroxylase. FEBS J. 276(19), 5416–5431.
13. Gerwick, W. H., *et al* (2008) Giant marine cyanobacteria produce exciting potential pharmaceuticals. Microbe. 3(6), 277–284.
14. Abed, R., Dobretsov, S., & Sudesh, K. (2009) Applications of cyanobacteria in biotechnology. J. Appl. Microbiol. 106(1), 1–12.
15. Codd, G. (1995) Cyanobacterial toxins: occurrence, properties and biological significance. Water Sci. Technol. 32(4), 149–156.
16. Froscio, S. M. *et al* (2001) Cell-free protein synthesis inhibition assay for the cyanobacterial toxin cylindrospermopsin. Environ. Toxicol. 16(5), 408–412.
17. Rantala, A. *et al* (2004) Phylogenetic evidence for the early evolution of microcystin synthesis. Proc. Natl. Acad. Sci. 101(2), 568–573.
18. Sivonen, K. *et al* (1990) Toxic cyanobacteria (blue-green algae) in Finnish fresh and coastal waters. Hydrobiologia. 190(3), 267–275.
19. Rapala, J. *et al* (1993) Anatoxin-a concentration in Anabaena and Aphanizomenon under different environmental conditions and comparison of growth by toxic and non-toxic Anabaena strains– a laboratory study. J. Appl. Phycol. 5(6), 581–591.
20. Codd, G. A. (2000) Cyanobacterial toxins, the perception of water quality, and the prioritization of eutrophication control. Ecologic. Eng. 16(1), 51–60.
21. Lyra, C. *et al* (2001) Molecular characterization of planktic cyanobacteria of Anabaena, Aphanizomenon, Microcystis and Planktothrix genera. Int. J. Sys. Evol. Microbiol. 51(2), 513–526.
22. Berry, J. P. *et al* (2008) Cyanobacterial toxins as allelochemicals with potential applications as algaecides, herbicides and insecticides. Marine Drugs. 6(2), 117–146.
23. Schmidt-Dannert, C. (2015) NextGen microbial natural products discovery. Microbial Biotechnol. 8 (1), 26–28.
24. Pandey, V. (2015) Cyanobacterial natural products as antimicrobial agents. Int. J. Curr. Microbiol. Appl. Sci. 4(1), 310–317.
25. Raveh, A., &Carmeli, S. (2007) Antimicrobial ambiguines from the cyanobacterium *Fischerella sp.* collected in Israel. J. Nat Prod. 70(2), 196–201.
26. Mo, S. *et al* (2009) Antimicrobial ambiguine isonitriles from the cyanobacterium *Fischerella ambigua.* J. Nat. Prod. 72(5), 894–899.
27. Moore, R. E. *et al* (1987) Hapalindoles, antibacterial and antimycotic alkaloids from the cyanophyte *Hapalosiphon fontinalis*. J. Org. Chem. 52(6), 1036–1043.

28. Jaki, B., Orjala, J., & Sticher, O. (1999) A novel extracellular diterpenoid with antibacterial activity from the cyanobacterium *Nostoc commune*. J. Nat. Prod. 62(3), 502–503.
29. Bui, H. T. *et al* (2007) Carbamidocyclophanes A-E, chlorinated paracyclophanes with cytotoxic and antibiotic activity from the Vietnamese cyanobacterium *Nostoc sp*. J. Nat. Prod. 70(4), 499–503.
30. Boyd, M. R. *et al* (1997) Discovery of cyanovirin-N, a novel human immunodeficiency virus-inactivating protein that binds viral surface envelope glycoprotein gp120: potential applications to microbicide development. Antimicrobial Agents & Chemotherapy. 41(7), 1521–1530.
31. Hayashi, T. *et al* (1996) Calcium spirulan, an inhibitor of enveloped virus replication, from a blue-green alga *Spirulina platensis*. J. Nat. Prod., 59(1), 83–87.
32. Kanekiyo, K. *et al* (2005) Isolation of an antiviral polysaccharide, nostoflan, from a terrestrial cyanobacterium, *Nostoc flagelliforme*. J. Nat. Prod. 68(7), 1037–1041.
33. Bokesch, H. R. *et al* (2003) A potent novel anti-HIV protein from the cultured cyanobacterium *Scytonema varium*. Biochem. 42(9), 2578–2584.
34. Loya, S. *et al* (1998) The inhibition of the reverse transcriptase of HIV-1 by the natural sulfoglycolipids from cyanobacteria: contribution of different moieties to their high potency. J. Nat. Prod. 61(7), 891–895.
35. Christaki, E., Florou-Paneri, P., &Bonos, E. (2011) Microalgae: a novel ingredient in nutrition. Int. J. Food Sci. &Nutr. 62(8), 794–799.
36. Annapurna, V., Deosthale, Y., & Bamji, M. S. (1991) Spirulina as a source of vitamin A. Plant Foods for Human Nutrition. 41(2), 125–134.
37. Sanchez, M. *et al* (2003) Spirulina (Arthrospira): An edible microorganism: A review. Universitas Scientiarum, 8(1), 7–24.
38. Deng, R., & Chow, T. J. (2010) Hypolipidemic, antioxidant, and antiinflammatory activities of microalgae Spirulina. Cardiovascular Therapeutics, 28(4), 33–45.
39. Abed, R., Dobretsov, S., & Sudesh, K. (2009) Applications of cyanobacteria in biotechnology. J. Appl. Microbiol. 106(1), 1–12.
40. Bonnet, S. *et al* (2010) Vitamin B12 excretion by cultures of the marine cyanobacteria *Crocosphaera* and *Synechococcus.* Limnology & Oceanography. 55(5), 1959–1964.
41. Tan, L. T. (2010) Filamentous tropical marine cyanobacteria: a rich source of natural products for anticancer drug discovery. J. Appl. Phycol. 22(5), 659–676.
42. Costa, M. *et al* (2012) Marine cyanobacteria compounds with anticancer properties: A review on the implication of apoptosis. Marine Drugs, 10(10), 2181–2207.

43. Newman, D. J., & Cragg, G. M. (2004) Advanced preclinical and clinical trials of natural products and related compounds from marine sources. Curr. Med. Chem. 11(13), 1693–1713.
44. Wipf, P. *et al* (2002) Synthesis and biological evaluation of structurally highly modified analogues of the antimitotic natural product curacin A. J. Med. Chem. 45(9), 1901–1917.
45. Garg, V. *et al* (2007) Preclinical analysis of tasidotin HCl in Ewing's sarcoma, rhabdomyosarcoma, synovial sarcoma, and osteosarcoma. Clin. Can. Res. 13(18), 5446–5454.
46. Butler, M. S. (2008) Natural products to drugs: natural product-derived compounds in clinical trials. Nat. Prod. Rep. 25(3), 475–516.
47. Trimurtulu, G. *et al* (1994) Total structures of cryptophycins, potent antitumor depsipeptides from the blue-green alga *Nostoc sp*. strain GSV 224. J. Am. Chem. Soc. 116(11), 4729–4737.
48. Bouchard, H. *et al* (2012) Novel conjugates, preparation thereof, and therapeutic use thereof. US20120225089.
49. Sidler, W. A. (2004) Phycobilisome and phycobiliprotein structures. The molecular biology of cyanobacteria. (pp. 139–216): Springer.
50. Gonzalez, R. *et al* (1999) Anti-inflammatory activity of phycocyanin extract in acetic acid-induced colitis in rats. Pharmacol. Res. 39(1), 55–59.
51. Romay, C. *et al* (2003) C-phycocyanin: a biliprotein with antioxidant, anti-inflammatory and neuroprotective effects. Curr. Prot. & Pep. Sci. 4(3), 207–216.
52. Reddy, C. M. *et al* (2000) Selective inhibition of cyclooxygenase-2 by C-phycocyanin, a biliprotein from *Spirulina platensis*. Biochem. Biophysic. Res. Comm. 277(3), 599–603.
53. Soule, T. *et al* (2007) Molecular genetics and genomic analysis of scytonemin biosynthesis in *Nostoc punctiforme* ATCC 29133. J. Bact. 189(12), 4465–4472.
54. Rath, J., Mandal, S., & Adhikary, S. P. (2012) Salinity induced synthesis of UV-screening compound scytonemin in the cyanobacterium *Lyngbya aestuarii*. J. Photochem. Photobiol B: Biol. 115, 5–8.
55. Balskus, E. P., & Walsh, C. T. (2010) The genetic and molecular basis for sunscreen biosynthesisin cyanobacteria. Sci. 329(5999), 1653–1656.

BIOPESTICIDES: RECENT RESEARCH AND APPLICATIONS

Anamika Ghatak*, Harsha Sharma and Neha Kumari Prasad
Department of Microbiology,
Tara Devi Harakhchand Kankaria Jain College, Cossipore, Kolkata
*Corresponding author E-mail: anughatak@gmail.com

Abstract:

Agricultural economy depends on crop quality, yield and disease management in developing countries. Application of Green Revolution technology (GR) increases the variety of crops per hectare thereby enhancing food supply to 12-13%. Fertilizers and pesticides have huge impact on GR in agro-market. Weeds and pests are major matters of concern in agricultural field as they cause considerable reduction in crop yield. In past decades, this crisis was resolved to some extent with the advent of chemical pesticides. But large-scale exposure of these chemicals has worsened ecological conditions. Bio-pesticides are developed gradually as an alternative of chemical pesticides, encompassing a diverse array of microorganisms, plants, animals to control pests in an eco-friendly manner. Categorically, these are of three types: Biofungicides (*Trichoderma*), bioherbicides (*Phytopthora*) and bioinsecticides (*Bacillus thuringiensis*). The worldwide production of biopesticides is over 3000 tons/year. In India, development of new, non-target specific biopesticides is the thrust area of research as both the purpose and process of implementation in field need knowledge of growers. This review highlights the types, applications and ethical constraints of various biopesticides available commercially.

Keywords: Agriculture, pest, biopesticides, applications, constraints.

Introduction:

The agricultural industry is undergoing rapid changes. In developing countries, organo-chemical pesticides exert a good impact on yield and quality of crops from decades. Sustainable development in the agricultural economy has also imposed advanced ideas of implementing various safety measures on fields. Success in global agro-business results in production of invasive, non-native pest species. Destructive activities of pests like fungi, weeds and insects reduce the total yield of crops. It was already estimated that the overall cost of the damage to both the environment and social economy is about $ 8.1 billion a year (1,2). Long-term use of chemical pesticides without recommended safety norms has resulted in serious health risks of humans and other warm-blooded animals. Chemicals when released into the environment can increase contamination of both the nature and the commodities utilized. Pest resurgence, resistance properties and lethal effects on non-target organisms are some inevitable consequences of using chemical pesticides (3, 4, 5). Therefore enhancement of product quality is

always in demand. In this regard, public awareness about the adverse effects of chemical pesticides is increasing gradually. Development and commercialization of eco-friendly, biodegradable alternatives are challenging areas of research. The conventional pesticide industry and market has undergone major changes adopting new practices of Integrated Pest Management (IPM) programmes. So, biopesticides reach the goal because of its sustainability, crop-quality and harvest flexibility benefits. These are considered as environmentally compatible tools for growers for the following advantageous aspects (6) --

- These are designed to affect only one specific pest or a few target organisms.
- Low residue and high performance.
- High impact when used in Integrated Pest Management (IPM) programmes.
- They exert non-toxic mechanism and inherently less harmful and less environmental load.
- Effective in very small quantities and often decompose quickly, thereby resulting in minimized nature pollution.

Some registered biopesticides under insecticides act, 1968 (7)

1. *Bacillus thuringiensis var. israelensis,*
2. *Bacillus thuringiensis var. kurstaki*
3. *Bacillus thuringiensis var. galleriae,*
4. *Bacillus sphaericus,*
5. *Trichoderma viride*
6. *Pseudomonas fluorescens,*
7. *Beauveria bassiana,*
8. *NPV of Helicoverpa armigera,*
9. *NPV of Spodoptera litura,*
10. Neem based pesticides,
11. Cymbopogan,
12. *Trichoderma harzianum*

Types of biopesticides and their use

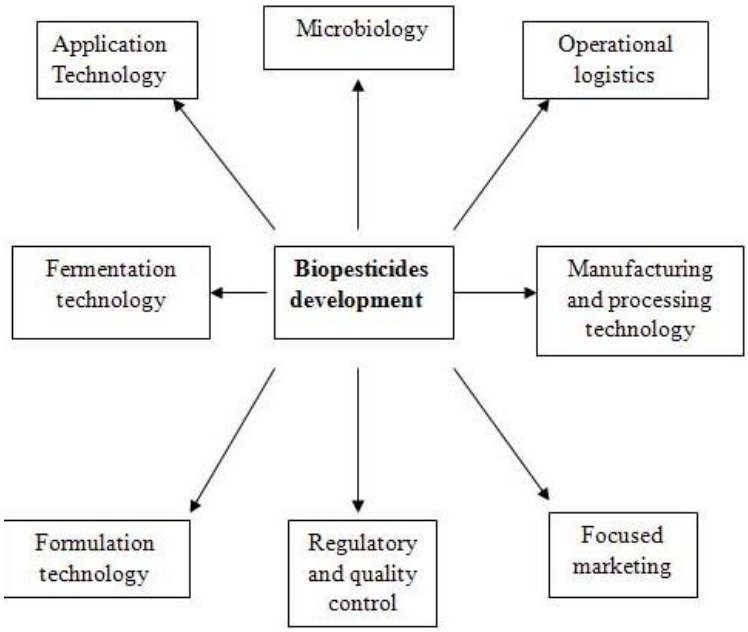
The U.S. Environmental Protection Agency (EPA) categorizes biopesticides into three classes: Microbial, Biochemical and Plant-Incorporated Protectants (PIPs). These include- Biofungicide (*Trichoderma),* Bioherbicides (*Phytophthora)* and Bioinsecticides (*Bacillus thuringiensis).*

Table 1: Major Active ingredients used in Biopesticides sector are as follows

Sub-sector	Types	Description	Some common term and Uses
Microbial pesticides [Insecticides and Fungicides]	Microorganisms – bacteria, fungi, virus, protozoa	Living or dead bacteria, fungi and other microorganisms, e.g. protozoa, virus, yeasts of specific strains	**1.** The commercial mycoinsecticide 'Boverin' based on *Beauveria bassiana* with reduced doses of trichlorophon have been used to suppress the second-generation outbreaks of *Cydia pomonella*. **2.** Commercial formulations of *Metarhizium anisopliae* and *Isaria fumosorosea* and *abamectin* were found to reduce plant damage and zebra chip symptoms. **3**. The genus *Bacillus* is often considered as microbial factories for the production of myriad array of toxins (coded by *cry* genes) for fungal growth. (8,9,10,11).
Biochemical pesticides	Biostimulants, growth stimulants, plant-growth promoters (PGPs) and sex pheromones.	Microbes, plant products	Azadirachtin, a tetranortritarpinoid, is a major Active ingredient isolated from neem, which is known to disrupt the metamorphosis of insects. (12).
PIPs (Plant Incorporated Protectants)	Pesticidal substances that plant produce from genetic material that has been added to the plant.	Plant products	The production of transgenic plants that express insecticidal δ-endotoxins derived from the *Bacillus thuringiensis* (*Bt* plants) were first commercialized in the US in 1996. The expression of these toxins confers protection against insect crop destruction (11).

Semiochemicals	Pheromones used as insecticides	Complex chemicals transmitting signals within and between species	Current research has found that herbivore-induced plant volatiles obtained from arthropod herbivores interaction used in slow release dispensers to attract predators and parasitoids (13,14).
Peptidomimetics	Small protein like chain designed to mimic a peptide.	Modification of an existing peptide or by designing similar systems that mimic peptide, such as β-peptide.	Insect control agent based on Backbone cyclic neuropeptide-antagonist (BBC-NBA), applied to the insect pyrokinin / pheromone biosynthesis (11).
Nematicides	Entomopathogenic nematodes	Microscopic or worm-like organisms	Most of the nematode-based products currently available are formulations of various strains of *Steinernema carpocapsae* such as ORTHO BioSafe, BioVector (15)
Extracts	Plant derived chemical extracts, biochemicals	Pure biochemicals or crude extracts, maybe salts e.g. soaps of fatty acids or esters.	Limonene and Linalool can be used against Fleas, aphids and mites, also kill fire ants, several types of flies, paper wasps (14)
Viricides	Nucleopolyhedrosisvirus (NPVs) and Granulovirus (GVs)	Viral component of bacteria	Elcar™ was a preparation of *Heliothis zea* NPV which is broad range baculovirus and infects many species belonging to genera *Helicoverpa* and *Heliothis* (11).

For all crop types, bacterial biopesticides claim about 74% of the market; fungal biopesticides, about 10%; viral biopesticides, 5%; predator biopesticides, 8%; and “other” biopesticides, 3% (16).



Global biopesticide research

The field attracts attention due to its superior application prospects, which in turn confer socio-economical benefits. Various biotechnological tools such as nanotechniques are now available to facilitate production of biopesticides in a safe and sustainable manner.

In 2005, the total demand for all kinds of biopesticides in China reached 145,000 tons, while the total sales were valued at about 0.8 to 1 billion Yuan. There were almost 122 biochemical pesticide registered with the Environmental Protection Agency (EPA), which include 18 floral attractants, 20 plant growth regulators, six insect growth regulators, 19 repellents and 36 pheromones. In 2006, Sichuan Academy of Agricultural Science succeeded in developing nano-pesticides (<100 nm) from a plant source, with advantages of environmental protection, high efficiency and low toxicity. There are more than 30 research institutions and 200 biopesticide (about 2000 pesticide manufacturing enterprises) in China with annual production of approximately 100,000 tons (17).

Canada, between 1972 and 2008, the Pest Management Regulatory Agency approved registration of 24 microbial substances with 83 formulations. The majority of the registrations occurred since 2000.In 2006, the global leading species of biopesticides were as follows: B.t. Cry F1, NRRL21882 (*Aspergillus flavus*), *Bacillus licheniformis* strain SBB3086 etc (18).At present, the world market for microbial pesticides is in excess of US $125 million per annum which is still less than 1 percent of the total global market for agrochemical crop protection of $20-25 billion.According to USEPA, over 1 billion tons of pesticides are used in the USA every year, and this is 22 % of the estimated 5.2 billion pounds of pesticides used worldwide (USEPA 2011)

(19).According to EPA data in the USA, 102 microbials, 52 biochemicals, and 48 semiochemicals are being used as biopesticides (USEPA 2011).

The largest individual European biopesticide market is Spain, followed by Italy and France. The first Bt-based product (Thuricide) was approved in Europe in 1964, whereas first registration for an entomopathogenic fungus *L. longisporum* was given in 1981 to Tate and Lyle in the UK. Europe also belongs to continents where the MRL (Maximum Residue Level) regulations in food products have been strictly decided (Regulation EC 396/2005). The largest increases since 2005 were seen in non-Bt bacteria, notably *B. subtilis,* and in fungal-based products, including *C. minitans* and *Trichoderma.* In Latin America, a proportion of Bt-based products cover about 40 % of the market. In Argentina, Bt products were first used in 1950 against *Colias lesbia* in alfalfa. The first virus-based product was registered in 2000 by Agro Roca, and the fungal product based on *B. bassiana* for controlling *Triatoma infestans* and *Musca domestica* was also registered. Approximately 40 commercial mycoinsecticides available in the Brazilian market are registered by 19 for-profit companies. More than 20 laboratories operated by sugar/ethanol mills produce *M. anisopliae* for their own use to control cercopids in cane fields (20, 21). According to estimation, the annual biocontrol sales for the whole of Africa in 2003 were approximately $23 million, including $5 million for bacterial products (22). Currently, there are 25 microbial insecticides on the market constituting slightly less than 2% of all insecticides used in Japan (23). In India, by 2006 only 12 biopesticides (such as Bt., *Trichoderma, Pseudomonas* and *Beauveria* species) had been registered, but 194 substances were listed as chemical pesticides (24). Some success stories about successful utilization of agents in Indian agriculture include (25) -

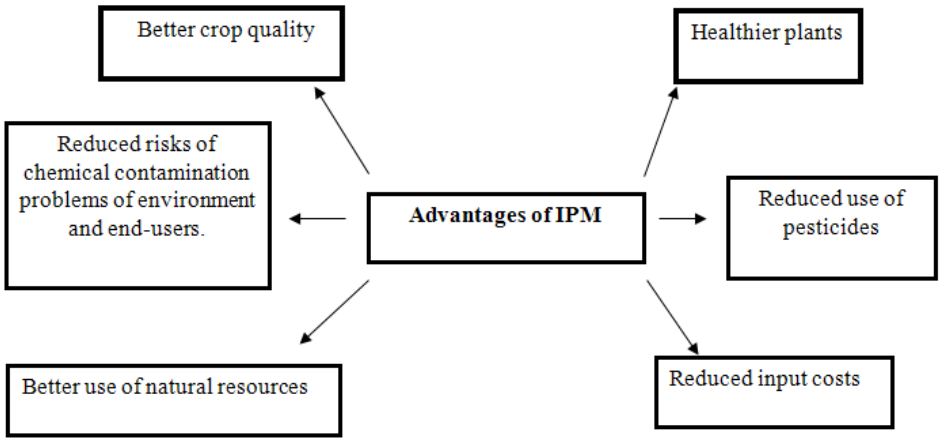
1. Control of *Helicoverpa* on cotton, pigeon pea, and tomato by *Bacillus thuringiensis*,
2. Control of mango hoppers and mealy bugs and coffee pod borer by *Beauveria*,
3. Control of diamondback moths by *Bacillus thuringiensis*,
4. Control of white fly on cotton by neem products,
5. Control of *Helicoverpa* by N.P.V.
6. Control of sugarcane borers by *Trichogramma*
7. Control of rots and wilts in various crops by *Trichoderma*-based products.

There are several constraints also (26)--

- Already established strong market of chemical pesticides.
- Lack of awareness about the formulations of biopesticides and confidence in growers.
- Unreliable supply and inconsistent field performance which often results in the rapid decline in the size of populations of active cells.

- Biopesticides available in the market are often found to be contaminated and have a low count of microorganisms.
- Implementation of Hi-tech instrumentation and not getting acceptance owing to its economic unfeasibility.
- Prolonged use renders carcinogenicity to end-users.
- Registration is expensive and time consuming as these live forms are treated like pathogens by the agencies. Import and export of biopesticides raise other serious issues.
- Development of tissue culture laboratories often needs ethical clearances from the government.

Scopes of IPM



Conclusion:

Biopesticide industry requires strategic, comprehensive schemes for establishment as the position and situation of biopesticides still remains in dilemma. The exploitation of "Live forms" could be done by developing strong governmental policies for betterment of industry and business. The use of bio-pesticides has emerged as promising alternative to chemical pesticides. Biopesticides have a precious role to play in the future of the Integrated Pest Management (IPM) strategies.

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References:

1. Pengfei, L., Zhiming, Z., Guangtang, P. and Maojun, Z. (2011) Applications and development trends in biopesticides. African J. of Biotechnol. 10, 86, 19864-19873.
2. Shen, Y.C., Zhang, Y.B. (2000). Biopesticides. Chemical Industry Press, Beijing.
3. Mazid, S., Kalita, J.C. and Rajkhowa, R. C. (2011) A review on the use of biopesticides in insect pest management.Int. J. Sci. and Advanced Technol. 1, 7, 169-177.
4. Kumar, S., Chandra, A. and Pandey, K.C. (2008) *Bacillus thuringiensis* (*Bt*) transgenic crop: an environmentally friendly insect-pest management strategy. J. Environ. Biol. 29, 641-653.
5. Abudulai, M., Shepard, B.M. Mitchell, P.L. (2001) Parasitism and predation on eggs of *Leptoglossus phyllopus* (L) (*Hemiptera*: Coreidae) in Cowpea: Impact of Endosulfan Sprays. J. Agric. Urban Entomol. 18,105-115.
6. EPA. Biopesticides. Available online: www.epa.gov/pesticides/biopesticides (accessed on 9 September 2017).
7. Gupta, S. and Dikshit, A. K. (2010) Biopesticides: An ecofriendly approach for pest control. J. of Biopesticides. 3, 186 – 188.
8. Husain, M.M. and Khatun, M. (2005) Response of Bishkatali (Polygonumhydropiper Linn.) and Malathion on *Tribolium castanium* Herbst. Bangladesh J. Sci. Ind. Res. 40, 1-2, 57-62.
9. Mensink, B.J.W.G. and Scheepmaker, J.W.A. (2007). How to evaluate the environmental safety of microbial plant protection products: A proposal. Biocontrol Sci. and Technol.17, 3-20.
10. USEPA, United States Environmental Protection Agency (2008) Pesticide fact sheets. Washtington, DC: US Environmental Protection Agency.
11. Sharma, S and Malik, P. (2012) Biopestcides: Types and Applications. International Journal of Advances in Pharmacy, Biology and Chem.1, 4, 508-515.
12. Thakore, Y. (2006) The biopesticide market for global agricultural use. Ind. Biotechnol. 2, 194-208.
13. Asogwa, E. U., Ndubuaku, T. C. N., Ugwu, J. A. and Awe, O. O. (2010) Prospects of botanical pesticides from neem, Azadirachta indica for routine protection of cocoa farms against the brown cocoa mirid – *Sahlbergella singularis*. Nigeria. J. of Med. Plants Res. 4, 1, 1-6.

14. Reddy, G. V. P., Cruz, Z. T. and Guerrero, A. (2009). Development of an efficient pheromone-based trapping method for the banana root borer Cosmopolites sordidus. J. Chem. Ecol. 35, 111–117. Doi: 10.1007/s10886-008- 9580-6.
15. Nawaz, M., Mabubu, J.I. and Hua, H.(2016)Current status and advancement of biopesticides: Microbial and botanical pesticides. J. of Ent. and Zoo. Studies. 4, 2, 241-246
16. Vashisth, S., Chandel, Y.S. and Sharma, P. K. (2013) Entomopathogenic nematodes-review. Agri. Reviews.34, 3,163-175.
17. Thakore, Y. (2006) The biopesticide market for global agricultural use. Ind. Biotechnol. 2,194-208.
18. Zhu, C.H.X., Sun, D.Y. and Jiang, X.L. (2004). Suggestion for standards and counter measures of microbiological pesticides industrialization in China. Mod. Chem. Ind. 24, 6-10.
19. Bailey, K.L., Boyetchko, S.M., Langle, T. (2010) Social and economic drivers shaping the future of biological control: a Canadian perspective on the factors affecting the development and use of microbial biopesticides. Biol. Control. 52, 221-229.
20. Arora, N.K. (2015) Plant Microbes Symbiosis: Applied Facets, 37-75. DOI 10.1007/978-81-322-2068-8_2, Springer India.
21. Moazami, N. Biopesticide production. Iranian Research Organization for Science and Technology.
22. Damico, T. (2017) Biopesticides Are in High Demand in Today's Pest Management Programs. Special to the Adviser. Certis. USA.
23. Ivase et al. (2017) Current status, challenges, and prospects of biopesticide utilization in Nigeria. Agriculture and Environment, 9, 95-106.
24. Kunimi, Y. (2007) Current status and prospects on microbial control in Japan. J. Invertebrate Pathol. 95, 181-186.
25. Kandpal, V. (2014) Biopesticides. International Journal of Environmental Research and Development.4, 2,191-196.
26. Kalra, A and Khanuja, S.P.S. (2007) Research and Development priorities for biopesticide and biofertiliser products for sustainable agriculture in India, In. Business Potential for Agricultural Biotechnology (Teng, P. S. ed.), Asian Productivity Organisation. 96-102.
27. Chandler *et al.* (2011) The development, regulation and use of biopesticides for integrated pest management. Phil. Trans. R. Soc. B. 366, 1987–1998. Doi: 10.1098/rstb.2010.0390.

MODE OF ACTION OF HOMEOTIC GENE

Ria Rakshit
Department of Botany,
Baruipur College, Baruipur, South 24 Parganas, W.B., INDIA
Corresponding author E-mail: daisyriya.408@gmail.com

Abstract:

Homeotic gene determines the developmental fate of a cell. The gene contains MAD box and Homeo box. Conserved nucleotide sequences present in these boxes and regulate the activity of the gene. Alternation in the gene develops homeotic mutants that develop organ at wrong places. Genetic influences have been identified to play an active role in homeotic mutation. Regulation of homeotic gene expression play a central role in the evolution of flower in *Arabidopsis* and *Antirrhinum*. On the other hand, mutation of the gene develops MONSTERS in *Drosophila melanogaster*. The gene that controls the developmental fate of a cell type and mutation of the gene that causes one cell type to follow the developmental pathway of other cell type regarded as HOMEOTIC GENE. The gene constitutes an intricate regulatory network in which one gene may activate or repress other genes. They are situated at the particular segment of the chromosome which interacts in complicated interlocking pattern. One example is hox gene which is important for segmentation, another is MAD box containing gene in ABC model of flower development. Alteration in the genes causes the formation of homeotic mutants that develops organs at wrong places due to incapability to recognize the positional information property and as a result the organ themselves are normal but the positions at which they develop are improper. The homeotic gene changes genetic make up of an organism and play a significant role in evolution.

Keywords: Homeotic Gene, MAD Box, Hox genes.

Introduction:

Among the most fascinating kinds of abnormalities in animals are those in which one body part is replaced by another. In 1894 William Bateson [1] catalogued several oddities of this nature coining the term "HOMEOTIC" to describe them. Calvin Bridges(1915) [2]isolated a spontaneous mutant of DROSOPHILA in which a part of halter was transformed into wing tissue and the mutant is called BITHORAX.

The gene that controls the developmental fate of a cell type and mutation of the gene that cause one cell type to follow the developmental pathway of other cell type is regarded as homeotic gene. The genes constitute an intricate regulatory network in which one gene may

activate or repress other gene. They are situated at the particular segment of the chromosome which interacts in complicated interlocking pattern.

Components of homeotic gene:

Homeotic gene that have been cloned so far belong to the class related to sequences known as MADBOX genes in case of plant homeotic genes, whereas animal homeotic gene sequences are known as HOMEOBOXES [Fig. 1].

MADBOX gene includes DEFICIENTS gene of snap dragon, AGAMOUS, PISTILATA-1, APETALATA-3 gene of ARABIDOPSIS. The MADBOX gene shares a characteristic nucleotide sequence which encodes a protein structure known as MAD DOMAIN. But not all genes containing MADBOX DOMAIN are homeotic gene i.e. AGL-20 is a MADBOX genes but not homeotic because it function as meristem identity gene.

HOMEOBOX genes encode HOMEODOMAIN protein that act as transcription factors. HOMEODOMAIN proteins belong to KNOTTED-1(KN1) class involve in maintaining the shoot apical meristem, it was found in Maize is a gain of function mutation develops the abnormal expression of gene. Because they contain a homeobox, homeotic genes of this class are sometimes called *Hox genes* for short.

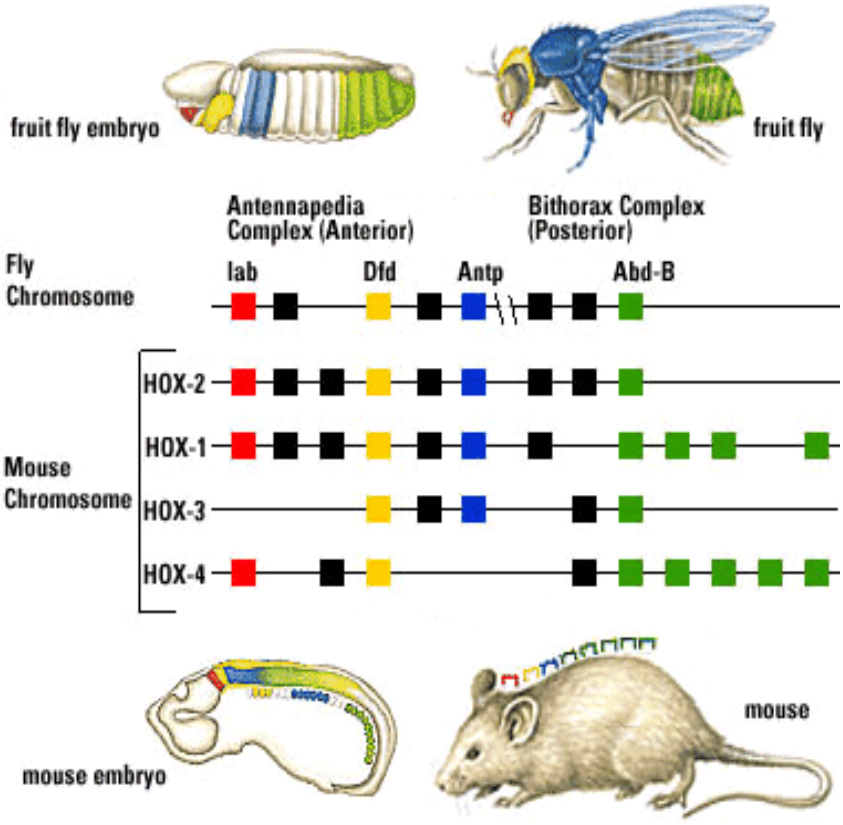

Figure 1: The homeotic genes of fruit flies and mice are arranged according to the colinearity principle

Phenotypic expression of homeotic mutation:

Homeotic mutations change genetic makeup of an organism and alter their differentiation pattern, producing integrated structures but in unusual locations that produce differences in body shape[3]. This means that homeotic mutation can be effective for a evolutionary change.

1. Homeotic mutation in *Drosophila*

The structural complexity of homeotic gene and their interaction makes it necessary to combine classical genetic studies with molecular analysis.

a) Antennapedia complex [Fig. 2 and Fig. 3]

Numerous studies have been devoted to the genetic analysis of Antennapedia complex. The distal region spans 260 kb of DNA. The Antennapedia (ANTC), composed of two promoters, eight exons spanning more than 100 kb and two termination processing region open reading frame has been analyzed in detailed. The M repeats is present in some 200 copies per genome. ANTC determines the anterior identity of the fly, namely the head and thoracic segment T1 and T2. ANTC contains five gene labial(lab), proboscipedia(pb), deformedL (Dfd), sexcombs reduced (Scr) and Antennapedia (Antp). Most ANTC mutations are lethal. One group of non-lethal mutant allele results in leg parts instead of an antenna growing cut of the cells near the eye during the development of eye disc. Note that the leg has a normal structure, but it is obviously positioned in an abnormal position.

Development of hopeful monster involving antenapedia complex:

Adult fly heads wild type, antennapedia and a double mutant prboscipedia. It has been noted that a clean transformation of one head structure does not much affect the adjacent structure due to homeotic mutation. The fly is put together like a construction toy with interchangeable parts.

b) Bithorax complex [Fig.4]

The pioneering studies of Lews were on a cluster of homeotic gene called Bithoraxcomplex (BX-C). BX-C determines the posrerior identity of the fly, namely thoracic segment T-3 and abdominal segments A1-A8. BX-C contains three genes called ultrabithoraxcomplex(ubx), abdominal-A(abd-A) and Abdominal-B(abd-B). Each of which constitutes one protein coding transcription unit. Mutations in these homeotic genes often are lethal and fly does not survive past embryogenesis. Some non-lethal alleishave been characterized, however that allow an adult fly to develop. Another possibly related gene is cad gene is specifically expressed in the last abdominal segment. Since the deletion of the entire BX-C does not transfer the most posterior abdominal segment, cad gene may specify the identity of these segments.

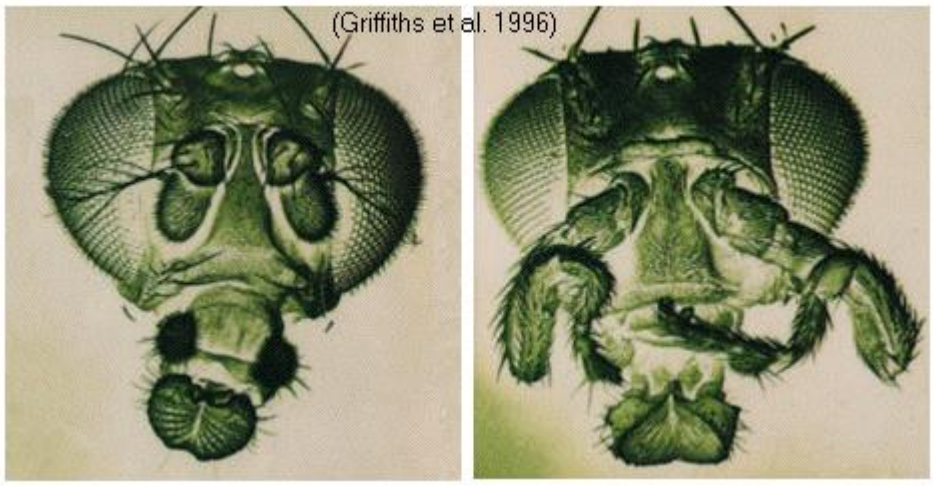

Figure 2: Antennapedia complexes of *Drosophila melanogaster*

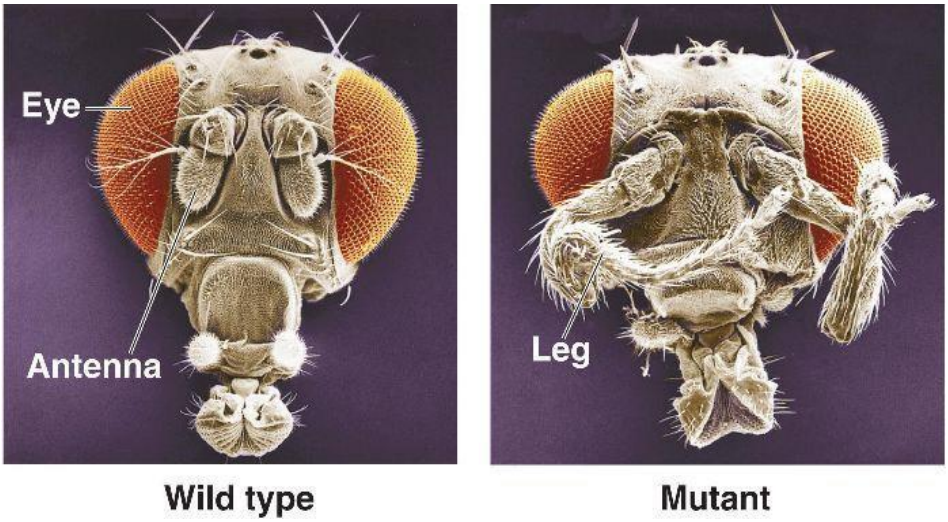

Figure 3: Images of *Drosophila melanogaster* showing WILD and MUTANT type

Figure 4: Brithorax *Drosophila* mutant with four wings

2. Homeotic mutation in humans [fig. 4]

Homeotic mutation causes several human diseases. Lymphomas, white blood cell detour on to the wrong lineages, and in Digeorge syndrome, there is missing of thymus, parathyroid, abnormalities in eyes, nose. A human homeotic transformation resulting from mutations in $PLCB_4$ and $GNAI_3$ causes auriculocondyler syndrome. AuriCulocondyler syndrome is a rare, autosomal dominant malformation syndrome characterized by question mark ear malformation. A careful phenotypic characterization suggested the presence of mandibular patterning defect resulting the maxillary type.

Homeotic mutation causes aniridia syndrome [Fig. 5]

If the homeotic gene PAX-6 is mutated as a result a child born with complete loss of iris. This condition known as aniridia, if there is no iris in the eye it is impossible to control the inlet of light. The pupil stands wide open, the only treatment of is as yet the use of coloured eye lenses in order to reduce the eye inlet. Aniridia is a congenital malformation of eye chiefly characterized by iris hypoplasia causes blindness.

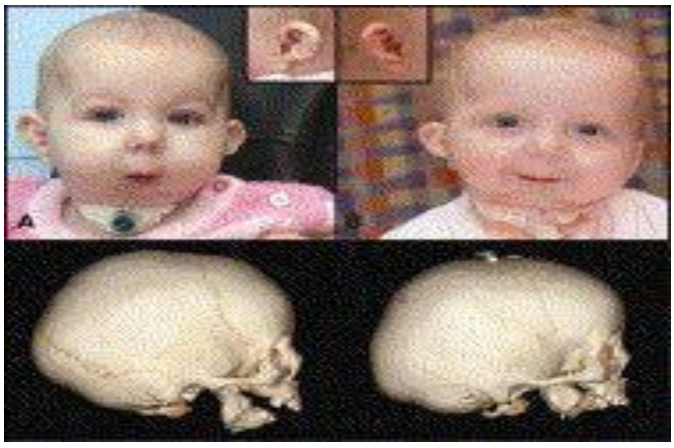

Figure 4: Homeotic mutation in human

Figure 5: Aniridia syndrome in a child

3. Homeotic gene controlling flower development in *Antirrhinum*

Forinacula, a homeotic gene is required for flower development in *Antirrhinum majus* [Fig. 6 and Fig.7]. In order to study these genes, we have to carry out an extensive transposon-mutagenesis experiment in Majus [4]. At least 3 categories of genes will be defined. The first

gene includes florinacula(flo), a primary gene required for the initiation of the flower developmental pathway. In the absence of the wild type flower product, proliferating inflorescence meristems arise in a place of flowers. The second category includes genes that effect the identity and also sometimes the number of whorls of organs in the flower. Genes of the third category control differences between organs in the same whorl and hence the overall symmetry of the flower. So the basic plan of the flower and inflorescence may arise through interactions of three categories of genes.

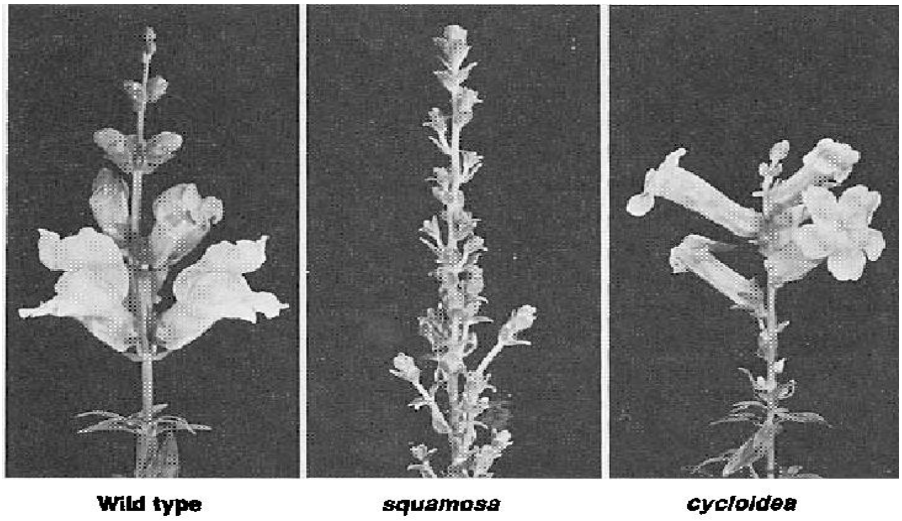

Figure 6: Three categories of genes controlling flower development in *Antirrhinum majus*

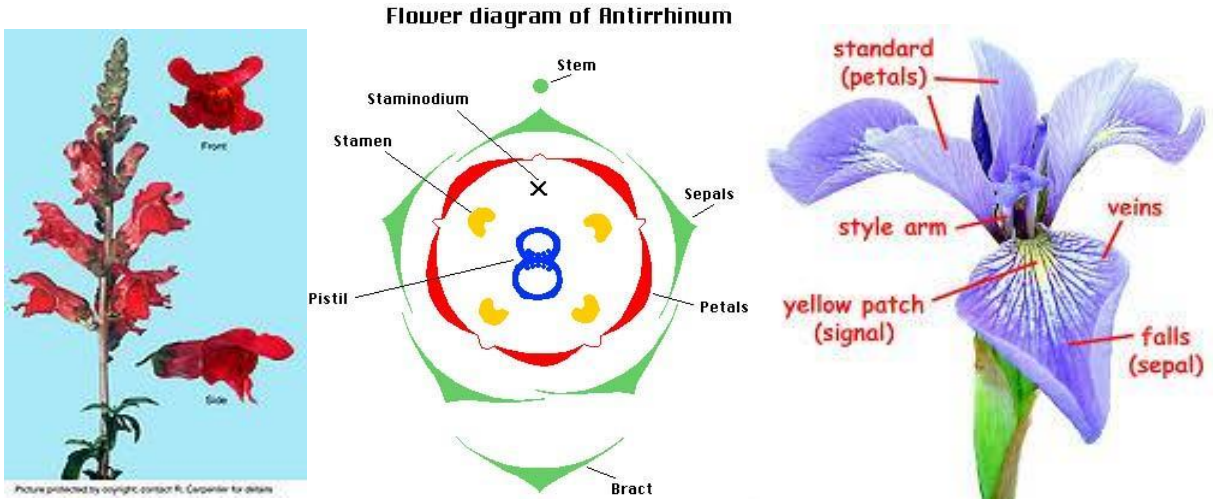

Figure 7: Wild-type Antirrhinum showing (a)schematic illustration of plant where lateral shoots arising in the axils of leaves are omitted for clarity, (b) Floral diagram and (c) Drawing of flower in side view (left) and face view (right). The flower in side view is shown slightly open to illustrate the hinge more clearly. Adapted from Weberling (1989) [5].

4. Determination of floral organ in arabidopsis mediated by homeotic mutants

Mutation in the PISTILLATA gene of *Arabidopsis thaliana* cause homeotic conversion of plants to sepals and stamens to carples. Surprisingly the initial pattern of PI and AP_3 expressions are different. By positive regulatory interaction between PI and AP_3, latter expression patterns are coincident. The pattern of PI expression also depends on the activity of the floral developmrntgenes $APETALA_2$. These PI and $APETALA_3$ proteins specifically associate together and regulating other genes.

Three classes of mutations were identified in Arabidopsis.

Class A: flowers with these mutations have (unfused) carpels instead of sepals in whorl 1, and stamens instead of petals in whorl 2. The pattern of organs (from outside to inside) is carpel, stamen, stamen, carpel. The genes containing these mutations were named APETALA1 (AP1) and APETALA2 (AP2). Bellow is an image[fig.8] of an apetala2 mutant flower (right) next to a wild type flower (left).

Figure 8: Images showing AP_2 and PI mutants

Class B: flowers with these mutations have sepals in whorl 2 instead of petals, and (unfused) carpels in whorl 3 instead of stamens. The pattern of organs (from outside to inside) is sepal, sepal, carpel, carpel. The genes containing this mutation were named APETALA3 (AP3) and PISTILATA (PI)

Class C: flowers with this mutation have petals in whorl 3 instead of stamens and sepals in whorl 4 instead of carpels. In addition the floral meristem is not determinate – flowers continue to form within the flowers, so the pattern of organs (from outside to inside) is: sepal, petal; sepal, petal; sepal, petal, petal, etc. The gene containing this mutation was called AGAMOUS (AG). Bellow is an image[Fig.9 and Fig 10] of an agamous mutant flower (right) next to a wild type flower (left) lata mutant flower (right) next to a wild type flower (left).

Figure 9: Agamous

Figure 10: Wild type

The ABC model [Fig. 11 and Fig. 12]

Each class of genes is required in two adjacent whorls. Class A genes are required in whorls 1 and 2, class B genes are required in whorls 2 and 3, and class C genes are required in whorls 3 and 4. Both class A and class B genes are required in whorl 2, and both class B and class C genes are required in whorl 3. The ABC model [6] that summarizes these results is shown bellow.

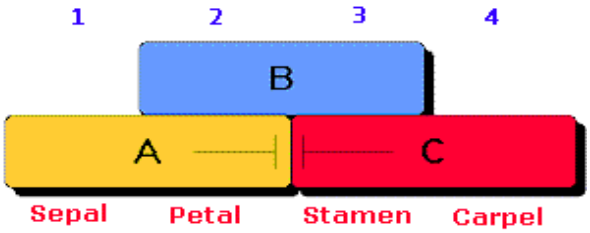

Figure 11: The ABC model

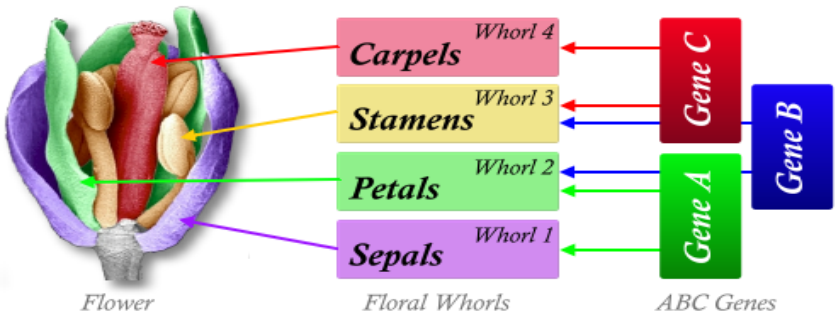

Figure 12: The ABC model

The A, B and C genes are transcription factors. Different transcription factors are needed together to turn on a developmental gene program –such as A and B needed to initiate the program for petals [Fig. 12].

Expression of homeotic genes:

1. Ectotopic expression of floral homeotic gene AGAMOUS in transgenic Arabidopsis plant alters floral organ identity. In the Arabidopsis and Alpiniaoblongifolia(zingiberaceae) ,

floral homeotic gene is required for leads to conversion of these organ in AG mutants. The loss of AG function leads to conversion of these organs to perianth organs. In contrast, mutation in another homeotic gene APETALA-2 result in the replacement of perianth organ by reproductive organ. On the basis of observation it has been proposed that AG and AP-2 act in antagonistic function.

2. Homeotic gene play a vital role in Drossophila melanogaster. Homeotic mutants develop in Antennapedia and bithorax complex. The Antpproten contain of polyglutamin is encoded by repeat called M repeat. Bithorax complex contains three genes which develop homeotic mutants.
3. Expression of the cdx1 and cdx2 homeotic gene lead to reduced cancer. Overexpression of both genes showed strongly decreased Bcl-2 expression and must be expressed to reduced tumreogenic potential.

Regulation of homeotic gene:

There is some evidence suggesting that a common mechanism exists for controlling the expression of homeotic gene. First, there is a set of genes that appears to affect the expression of many homeotic gene as judged from mutant phemotype. Secondly, the similarities exist between the patterns of expression of different homeotic genes during development. T-rans regulatory and Cis-acting sequences control regulation of homeotic gene [7].

Homeotic gene evolution is conserved through evolution:

The extent to which gene sequences and organization are conserved between organisms, give us clues about the amount of evolutionary time that has passed since the two-organism diverted from one another [Fig.13]. The presence of homeotic gene sequences in animals as different as insects and mammals suggests that this type of gene has a circular function.

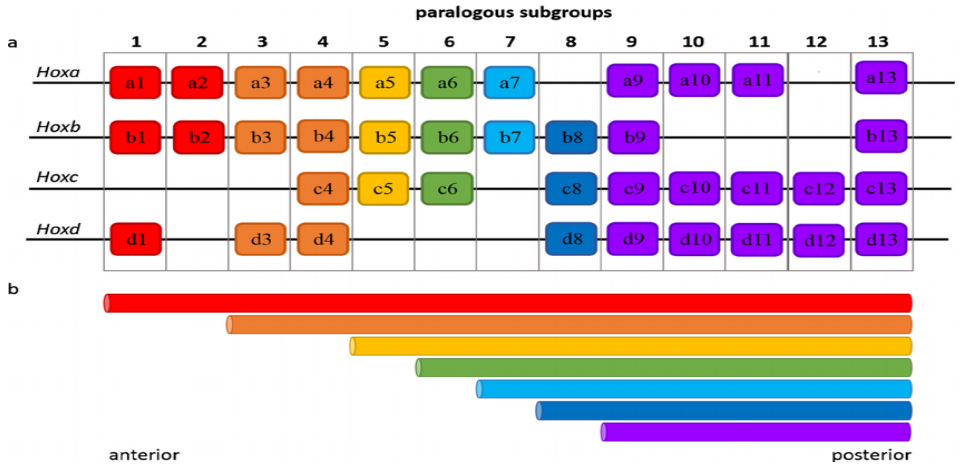

Figure 13: *Hox* genes have been duplicated over evolutionary history and now exist as four similar gene clusters labeled A through D

Conclusion:

1. Research into the gene that cause erroneous developments in the fruitflies have led to one of the most exciting discoveries in the field of developmental biology: the same type of gene controls early embryonic development in Drosophila, also controls early embrogenesis of the organisms including human. A small monster -fruitfly with an extra pair of wings- helps in the discovery of genes that control embryonic development.
2. Homeotic gene controls the diversity of segment development, but the domains of action of homeotic gene not obiviously correspond with major morphological subdivision of insect body. In one or more para segments, each homeotic gene is expressed metamerically, that is expressed from blastoderm stages. Elsewhere, the same homeotic gene may be deployed adventitiously, only in subsets of cell and latter stages of development.
3. The development of multicellular animals requires that all cells have two types of information where they relative to their neighbors (positional information) and what structure they should generate (identity information). In recent years our work has focused on homeotic sector which links two types of information their large regulatory region interprets positional information and their protein product control cellular identities by regulating transcription of downstream target genes.
4. Floral homeotic genes are targets of gibberelin signaling in flower development in Arabidopsis [8]. The GA-deficient ga-1-3 mutants shows retarded growth of all floral organs.
5. Homeotic gene play a crucial role in commercial production of transgenic crop by the process of genetic engineering for desirable character such as pest resistance capability, pathogen defence mechanism [9].
6. Empigrnetic inactivation of HOXA gene cluster is coupled to human cancer [10]. The disruption of epigenetic process can lead to altered gene function and malignancy occur.
7. 7)Floral homeotic genes are targets of gibberelin signaling flower development in Arabidopsis [8]. The GA-deficient ga-1-3 mutants shows retarded growth of all floral organs. Anthocyanin pigment synthesis also occur in plants through MAD BOX gene family.
8. The initiation of flowering is carried out by genetic pathway like Vernalization [11]. The process is regulated by the formation of Homeotic genes. The integrated signal of floral induction is transmitted to floral meristem identity genes LFY and API-1 and floral morphogenesis is performed.

References:

1. Bateson, W. (1894). Materials for the Study of Variation. Macmillan and Co., London.
2. C. B. (1915). A linkage variation in Drosophila, Journal of Experimental Zoology, 19(1), 1-21
3. Angenent GC, Busscher M, Franken J, Mol JN, van Tunen AJ.(1992). Differential expression of two MADS box genes in wild-type and mutant petunia flowers, Plant Cell,4(8),983-993.
4. Carpenter R, Coen ES. (1990). Floral homeotic mutations produced by transposon-mutagenesis in Antirrhinum majus. Genes Dev, 4(9),1483–1493
5. Weberling F. (1989). Morphology of flowers and inflorescences. Nordic Journal of Botany,11(5),496
6. Weigel D., Meyerowitz E. M. (1994). The ABCs of floral homeotic genes. Cell, 78, 203-209
7. Drews GN, Bowman JL, Meyerowitz EM 9(1991). Negative regulation of the Arabidopsis homeotic gene AGAMOUS by the APETALA2 product, Cell, 65(6),991–1002
8. Hao Y, Toshiro I, Yuanxiang Z, Jinrong P, Prakash K, and Elliot M. M. (2004). Floral homeotic genes are targets of gibberellin signaling in flower development, Proc Natl Acad Sci U S A, 101(20): 7827–7832.
9. Peria L and Seguin (2002). A Recent advances in genetic transformation of trees, Trends in Biotechnology,19(12),500-506
10. Sharma S, Kelly TK, Jones PA(2010) Epigenetics in cancer, Carcinogenesis, 31(1),27-36.
11. Shahryar Megan N. H Sandra N. O *et al.* (2009). The influence of vernalization and daylength on expression of flowering-time genes in the shoot apex and leaves of barley (*Hordeum vulgare*), Journal of Experimental Botany,60(7), 2169–2178.

CURRENT RESEARCH TRENDS IN WEST BENGAL COLLEGES VOLUME I

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About Editors

Dr. Arunima Biswas did her M.Sc. from Department of Biophysics, Molecular Biology and Genetics, University of Calcutta. She qualified CSIR-NET and was awarded Ph. D. degree from University of Calcutta, in 2006, on her thesis entitled 'Studies on interaction of tRNA and Unfolded Protein with Ribosome'. She then worked for some time as DBT-Post-doctoral Fellow in Department of Life Science and Biotechnology, Jadavpur University, on the final steps of protein synthesis. She joined Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, New York, USA, as Post-doctoral Research Associate and worked on Ribosome Biogenesis from 2006-2008. She then worked as a Research Associate in the Department of Medicine of the same institute and worked till 2010 on PXR, a crucial orphan nuclear receptor, with role in xenobiotic metabolism, lipogenesis and colon cancer progression. After returning to India, she worked as CSIR Pool Officer (SRA) from 2011-2014 at Jadavpur University on how the Eukaryotic Translation Initiation Factor 6 connects Ribosome Biogenesis with Calcium Signaling and Ribosomopathy. In 2014, she joined Department of Microbiology, Raidighi College, Raidighi, South 24 Parganas, West Bengal, as Assistant Professor. The college is NAAC accredited, UGC recognised and affiliated to the University of Calcutta. Dr. Biswas has published several articles in international and national peer-reviewed research journals with high impact factor. She has also published several book chapters under various international and national publishing houses. Her current research focus is on the anti-oxidant and anti-microbial potential of Sundarban honey and its role in nano-medicine. She is a life member and one of the state coordinators of Microbiologists Society of India. She is also a member of New York Academy of Sciences, USA and of RNA group, India.

Dr. Madhumita Majumder is presently an Assistant Professor, Head of Department of Botany, Raidighi College, Raidighi, South 24 Parganas, West Bengal. The college is NAAC accredited, UGC recognised and affiliated to the University of Calcutta. Dr. Majumder completed her Master's degree in the field of Botany with first class 2nd from the Darjeeling Govt. College. Her area of specialization is Cytogenetics and Plant Tissue Culture. She obtained her Ph. D degree in Botany from Visva-Bharati University and her field of research is Orchid Biotechnology. She has been serving as Assistant Professor in Raidighi College since 2016. Dr. Majumder has published more than 10 research papers in various peer reviewed Journals of National and International repute. She has also published 7 book chapters from several National and International publishing house. Her area of research interest is focused on plant tissue culture, Mangrove Vegetation and Eco-restoration, Biodiversity and Conservation of Medicinal plant, Organic farming etc. She is a life member of Botanical Society of Bengal and Prabir Chatterjee Research Foundation.

Dr. Shakuntala Ghorai did her M.Sc. in Biochemistry from University of Calcutta in 2002. Afterwards, she received Ph. D. from Jadavpur University in 2010. Her research involved isolation, purification, characterization and cloning of cellobiase enzyme from filamentous fungus Termitomyces clypeatus. She has published more than 20 research articles in international and national journals. She has also published 4 book chapters through international publishing house. Currently she is working as an Assistant Professor of Microbiology in Raidighi College, South 24 Parganas, West Bengal. The college is NAAC accredited, UGC recognised and is affiliated to University of Calcutta. Dr. Ghorai's research interest includes fungal enzyme technology, bioremediation and traditional knowledge of edible mushrooms. Processes and products related to fermented foods also fascinate her.

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