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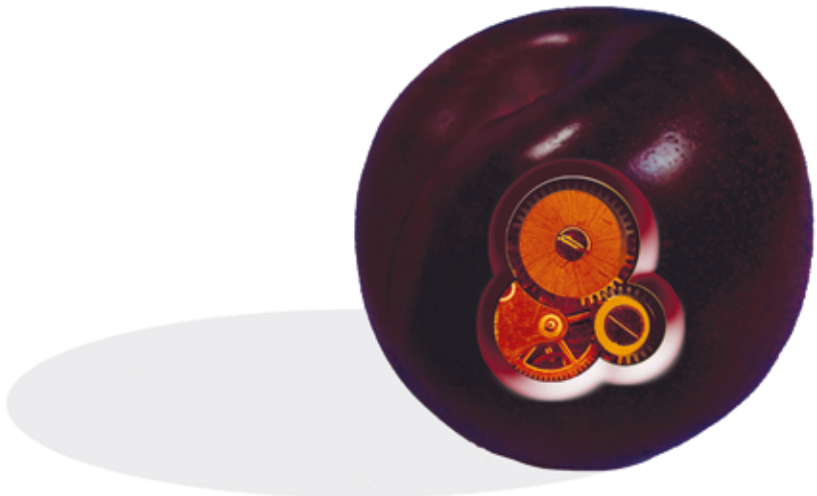

ST. ANTHONY'S COLLEGE
Shillong, Meghalaya, India
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Editorial

The second issue of Spectrum: Science and Technology has eight articles of which seven are from the life sciences and one from mathematics. The article on mathematics focuses onthe fear of mathematics among students of undergraduate classes and suggests innovative methods to attract students towards this important pillar of the basic sciences.

There has been a renewed effort to rejuvenate the fisheries sector in Meghalaya and as a reflection of that activity three articles in the current issue of the journal deal with that sector. One of these articles presents an analysis of the structure of fish markets in Meghalaya and describes the various risks that fish farmers face in selling their produce. Two articles present the findings on the distribution and on the reproductive biology of an endangered fish of Meghalaya.

Meghalaya is home to a number of medicinal plants. Research results on the one such medicinal plant has been presented in this issue. In another article researchers have presented their findings on the antibacterial activity of disinfectants and antiseptics. Some secondary metabolites of bacteria have applications as pharmaceuticals and food preservatives.One research articles details the isolation and partial purification of a food preservative from bacteria.The objective of the sole review article is to raise awareness on bacteria that reside inside plant tissue spaces and influence the physiology of the host.

This science and technology journal of St. Anthony's College, Shillong presents a platform to authors to make public their findings and express opinion in their respective domain areas and authors of eight articles of the current issue did a laudable job in utilising that facility. I thank the reviewers for their contribution in improving the quality ofmanuscripts. I am also grateful to members of the editorial board for their valuable inputs. A special thanks to Prof. Thy Answer Challam for designing the cover page and preparing the layout of the present issue. He was assisted efficiently by the co-opted members particularly, Prof. Jeremy N. Syiem.

I on behalf of the editorial board of Spectrum: Science and Technology and on my behalf thank Rev. Br. Albert L. Dkhar, Principal; Rev. Fr. Joby Joseph, Vice-Principal and Rev. Fr. Saji Stephen, Rector for their support and encouragement at all stages of the publication of the journal.

Dr. M.A. Laskar

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STUDY OF REPRODUCTIVE BIOLOGY OF CHOCOLATE MAHSEER, *Neolissochilus hexagonolepis* (MCCLELLAND, 1839) AND PHYSICO-CHEMICAL PARAMETERS OF DIFFERENT WATER BODIES AT MID ALTITUDINAL REGION OF MEGHALAYA, INDIA

Lydia B. Jyrwa and R. N. Bhuyan*

ABSTRACT

Meghalaya, the important hill state of North-Eastern India lies between 25°1'N and 26°5'N latitude and 85°49'E and 92° 52'E longitude and its capital city Shillong is situated at an altitude of 1496 meters MSL. Chocolate Mahseer (*Neolissochilus hexagonolepis*) is an important food and game fish in India, particularly the high lands of Meghalaya, recorded a sharp decline in recent years due to various natural and anthropogenic factors. This fish is considered as a threatened species. The male fish attains the gonadal maturity within the first year of life, while the female fish attains maturity within four years of its life span. The fecundity has been recorded to be 3,000 to 5,000 eggs/kg of body weight. The natural breeding season stretchesup to September with the peak during June and July (GSI of male was 2.65-2.49; GSI of female was 4.25-4.43). Studies also showed the desirable water quality parameters (temperature ranges from 10-25°C) and dissolved oxygen (6-9mg/L) of different rivers for survival and reproduction of the fish in terms of physico-chemical characteristics.

***Keywords*:** *Neolissochilus hexagonolepis*, Conservation, Physico-Chemical parameter

Introduction

Chocolate Mahseer (*Neolissochilus hexagonolepis*) locally known as "Khasaw" is an important food as well game fish in Meghalaya. But due to various natural and anthropogenic factors, the species recorded a sharp decline in recent years. This fish is considered as one of the endangered species, but has tremendous scope for culture, tourism and aquarium trade(Menon, 1999). Although the distribution of Chocolate Mahseer is restricted to the North Eastern Himalayan region, this fish is a native species to the state of Meghalaya (Dasgupta, 1982).

Steps are to be taken for culture, breeding and conservation of this important food and sport fish. This fish can adopt and grow very well under the agro climatic conditions of the region.The aim of the present work was to assess the water quality parameters of the natural habitat of this fish and to study a few biological parameters like gonado-somatic index, fecundity and size at first maturity.

In Meghalaya, the rivers have been known to be the natural habitat for Mahseer. But in recent years, due to various anthropogenic activities, the population of the fish has declined

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sharply (Sarma and Bhuyan, 2007). It is therefore important to understand more about the water quality parameters and their management which have influence on the growth and survival of aquatic organisms (Keramah *et al.,*2014).

Materials and Methods

Experimental protocol:

The fishes were collected by netting from the natural habitat of The Khri river (25°47¹N 91°25¹E) of West Khasi Hills, Umrynjah river (25°42¹N 9°58¹E) of Ri-Bhoi district, Amlayee river (25°31¹N 92°14¹E) of West Jaintia Hills, Umngi river (25°16¹N 91°25¹E) and Janiaw river (25°14¹N 91°35¹E) of East Khasi Hills during the year 2012-2014. The fishes were then brought to the hatchery complex of the Department of Fishery Science, St. Anthony's

For assessing the gonadal development, random samplings of the fishes were done in four different seasons. Visual and microscopic examination of reproductive organs was done to determine the periodic changes in gonadal morphology (Biswas, 1993).

The gonado-somatic index (GSI) was calculated following the method by Nikolsky, 1963. The fecundity was determinedfollowing the method by Bagenal, 1978.

Water quality analysis:

In total five different water bodies of Meghalaya were chosen for assessment of water quality from May, 2013 to May, 2014. Within this period, water samples were collected from each river using BOD bottles and plastic bottles of 1 litre capacity. The sampling bottles were labelled with dates and collection sites. Water temperature was measured at the sampling sites using a mercury in-glass thermometer graduated at °Celsius (0-100°C). The pH of the water was measured using Waterproof pH Tester 20 instrument. The Dissolved Oxygen was determined using Winkler's method (Welch, 1948). Other parameters (Free Carbon-dioxide, Total Alkalinity and Total Hardness) were analysed by APHA (2005) method.

Results

Gonado-Somatic Index:

The monthly gonado-somatic index (GSI) for both testis and ovary were calculated. The GSI of testis ranged from 0.53 to 2.65, with the maximum index during May - June (2.51-2.65) and minimum during January (0.53) (Fig.-I). The GSI of Ovary ranged from 1.79 to 4.43 (Fig.-II) and the maximum index was recorded during June-July (4.25 - 4.43) and minimum was during the month of January (1.79).

Gonadal maturity:

Corresponding to the Gonado-Somatic Index, both testis and ovary were examined for study of gonadal maturity. The photographs of gonads (Testis and Ovary) were taken during climatologically classified four different seasons of a year (pre-monsoon, monsoon, retreating monsoon and winter (Plate- I& II) for morphological studies and were placed in maturity scale (Table-I &II) based on Khanna (1993).

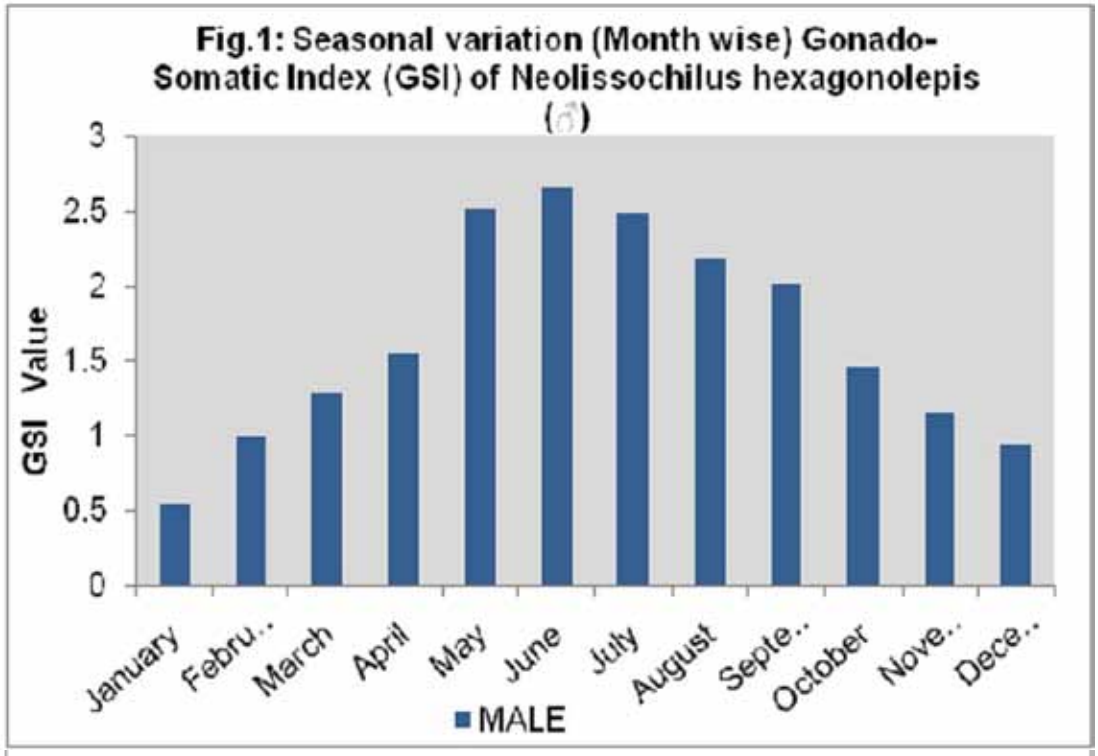


Fig.1: Seasonal variation (Month wise) Gonado-Somatic Index (GSI) of Neolissochilus hexagonolepis (♂)
GSI Value
3
2.5
2
1.5
1
0.5
0
January
Febru..
March
April
May
June
July
August
Septe..
October
Nove..
Dece..
MALE

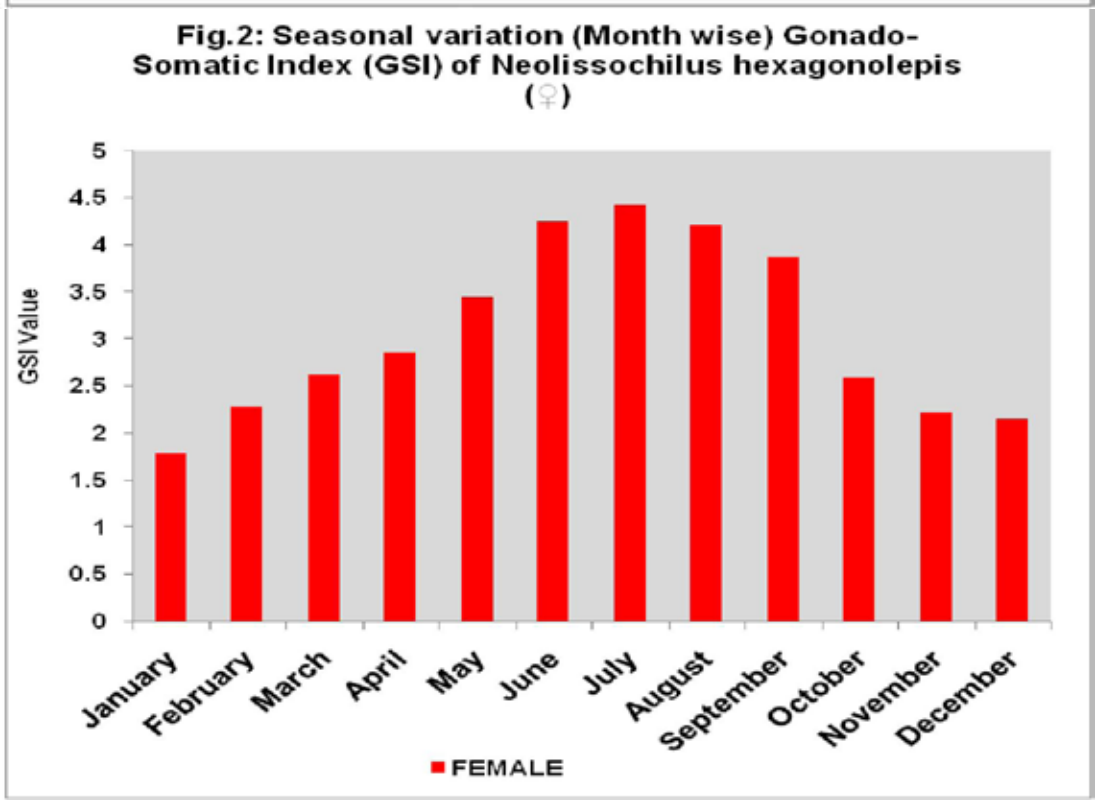
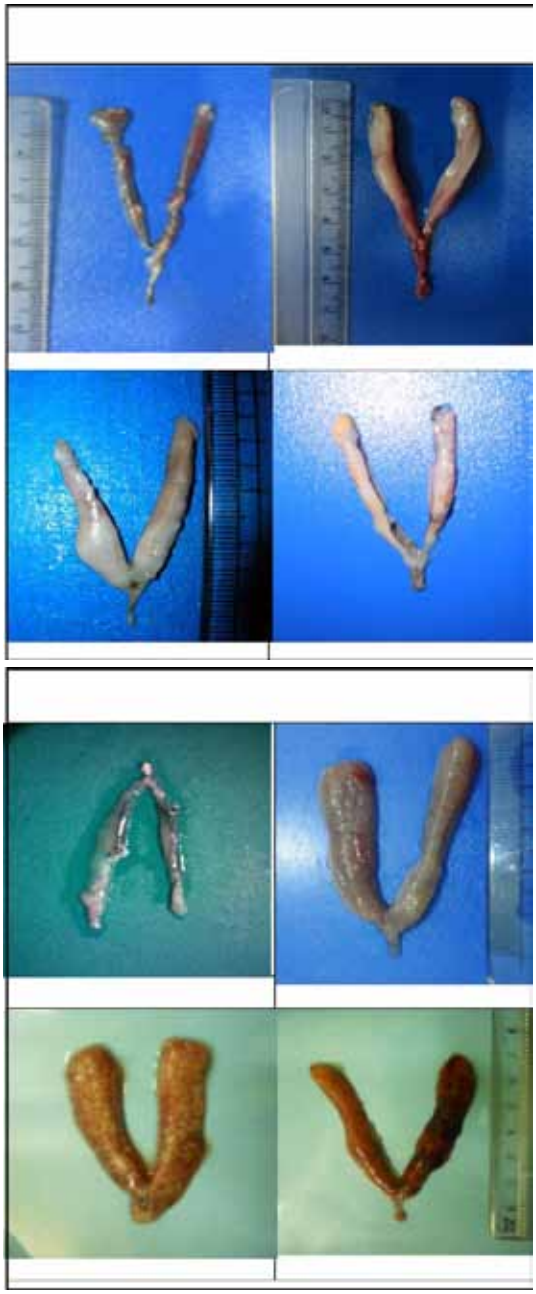


Fig.2: Seasonal variation (Month wise) Gonado-Somatic Index (GSI) of Neolissochilus hexagonolepis (♀)
GSI Value
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
January
February
March
April
May
June
July
August
September
October
November
December
FEMALE



Fecundity

The fecundity of *Neolissochilus hexagonolepis* has been recorded and it ranged from 3,000-5,000 /kg body weight of fishes in the specimens measuring from 200-250 gm of body weight.

Physico-Chemical parameters:

The result of the study is represented in Table.3. A total of six different physiochemical parameters were analyzed. The analysis was based on the samples taken from each site. The analysis showed that the highest water temperature and pH was from Umngi River. The result also showedthe value of Dissolved Oxygen, Free Carbon dioxide, Total Alkalinity and Total Hardness of the rivers. The Free Carbon dioxide was absent in all the rivers.

The classified stages are as follows (table I and II); Stage I (Immature): though winter season starts from December but its effects extends from January to February. During this period the gonads mostly remain at resting stage or immature stage. Stage II (Maturing): The Pre-monsoon season initiates from March and continues up to May. During this period, environmental temperature gradually increases and the gonadal development becomes very fast producing maturing sperms and ova. Stage III (Mature): Monsoon season extends from June to September. During the monsoon period matured eggs and sperms are produced and natural breeding of fish starts. Stage IV (Spent): During retreating monsoon the eggs and sperms are released and gonads become flaccid. This classified stages of gonads in *Neolissochilus hexagonolepis* has been confirmed with the calculated Gonado-Somatic Index of the fish (fig.1 and 2).

Table-1: Different maturity stages and morphological appearance of testis of *Neolissochilus hexagonolepis* at mid-altitudinal region of Meghalaya.			
Maturity stage	**Season**	**Morphological characters of testis**	**GSI**
Stage I (Immature)	Winter (January –February)	Very thin and translucent	0.53 0.99
Stage II (Maturing)	Pre-monsoon (March –May)	Testis thickened and became creamy white in colour	1.28 1.54 2.51
Stage III (Mature)	Monsoon (June- August)	Testes were enlarged at this stage, became opaque and pure whitish in colour	2.65 2.49 2.18
Stage IV (Spent)	Retreating-monsoon (September-December)	Testes were flaccid and became translucent	2.01 1.46 1.15 0.93

Table-2: Different maturity stages and morphological appearance ovary of *Neolissochilus hexagonolepis* at mid-altitudinal region of Meghalaya.			
Maturity stage	**Season**	**Morphological characters of ovary**	**G.S.I.**
Stage I (Immature)	Winter January -February	The ovaries were thin; The ova were not visible to the naked eye.	1.79 2.28
Stage II (Maturing)	Pre-monsoon March -May	Ovaries became larger, thicker. Vascular supply increased and blood capillaries became conspicuous.	2.61 2.86 3.45
Stage III (Mature)	Monsoon June- August	Ovaries were much enlarged, occupying the entire body cavity. Ova were found extruded on gentle pressure on the abdomen.	4.25 4.43 4.21
Stage IV (Spent)	Retreating-monsoon September-December	Ovaries were flaccid and shrunken, reduced in volume, dull in colour.	3.87 2.59 2.22 2.15

Table.3: Physico-Chemical Parameters of Different Rivers of Meghalaya						
Parameters	**Umrynjah river**	**Khri river**	**Amlayee river**	**Umngi river**	**Janiaw river**	**Desirable range**
Temperature (°C)	21.8	28.2	24.4	29.1	16.3	20-26
pH	7.28	7.46	7.50	7.88	7.02	6.5-8.5
Dissolved O_2 (mg/lt)	10.0	14.0	13.5	12.4	14.0	6-9
Free CO_2 (mg/lt)	0	0	0	0	0	<5
Total Alkalinity (mg/lt)	60	90	68	55	57	50-180
Total Hardness (mg/lt)	70	110	80	68	70	40-180

Discussion

Water is the home of aquatic animals and its quality means the component of water which must be present for optimum growth of the fish (Ehiagbonare & Ogundiran, 2010). Water quality is made up of various physico-chemical factors (including Temperature, pH, Dissolved Oxygen, Free Carbon-dioxide, Total alkalinity and Total Hardness) which influence the productivity of the fish (Huct, 1996).

It has been observed that the morphological appearance of gonads, both testes and ovary, could be correlated with the climatologically classified season of the year of North East India in general and Meghalaya in particular (Barthakur, 1986). The analysis of Gonado-Somatic Index confirmed that the chocolate Mahseerbecomes fully gravid during the month of Juneto August with peak maturity in the month of July. Thus the natural peak breeding season of the fish would be during July and August. In this context, it could be noted thatthere are reports of natural breeding of chocolate Mahseer during rainy season in Assam (Langer *et al*., 2001).

However, the gradual change of morphological appearance of gonads, both testes and ovary and corresponding Gonado-Somatic Index confirms that the important sport fish of the state has a longer spawning period in comparison to other carps, extending from June to September under the agro climatic conditions of Meghalaya particularly Khasi and Jaintia Hills Districts. This was in total agreement with the reports on the prolonged spawning season of Mahseer from Pagladia River of Assam and Simsang River of Garo hills (Dasgupta, 1982). It has also been observed that the size at maturity differs in male and female sexes. The size difference between the two sexes is basically due to reproductive requirements as the female carrying the bulky eggs is larger than the male (Bond, 1979). The fecundity of the chocolate Mahseer was found to be low. The finding of the present study confirms the earlier observations (Dasgupta, 1982). However, there was slight difference in results which could be attributed to the change in the seasonal rhythm of environmental factors specific to the area. The environmental factors like water temperature, photoperiod, rainfall, etc. are known to play a significant role in gonadotrophic activity of pituitary gland which in turn playsa major role in gonadal development (Lin and Peter, 1996).

It has been also observed that the male fish reaches sexual maturity in the first year of its life. However, the female takes almost four years to become sexually mature under the captive rearing condition.This indicates that the ecological transition from riverine to captive condition has not much impact on the gonadal maturation of the fish. But the difference in age for sexual maturation has great impact on development of protocol for artificial propagation of the fish.

The physical and chemical characteristics of water bodies affect species composition, abundance, productivity and physiological conditions of aquatic organisms (Bagenal, 1978). Natural water bodies may exhibit seasonal and diurnal variation and is closely related with change in atmospheric temperature (Kundanagar *et al*., 1996). The water temperature of all the rivers ranged from 16.3 to 29.1 °C. Temperature in the range 20 to 32 °C is ideal for majority of freshwater fishes (Boyd, 1990).

The pH value ranged from all the rivers was approximately 7.0. Water having pH below 5.0 and above 9.5 is not suitable for aquatic life (APHA, 2005). The pH is considered as a measure of environmental suitability and a range of 7.0 to 8.5 is considered to support a rich biota and fish (Bell, 1971).

The value of Dissolved Oxygen ranged from 10.0 mg/lt to 14.0 mg/lt. Dissolved Oxygen above 5.0 mg/lt is considered favourable for growth and activity of most aquatic organisms. Dissolved Oxygen < 3.0 mg/lt is stressful to most aquatic organisms while Dissolved Oxygen > 2.0 mg/lt does not support fish life (USEPA, 2000).

Free Carbon dioxide in water is the by-product of metabolism and is toxic to aquatic life. The value of Free Carbon dioxide was nil in all the rivers.

Alkalinity is a measure of the total concentration of bases in water and the ability of water to resist change in pH. Total alkalinity of 50 to 400 mg/lt is necessary for good water productivity. The value obtained in this study was appreciable and fall within desirable range (Boyd, 1990).

Water Hardness is a measure of the alkaline earth metal such as Calcium and Magnesium concentration on water samples (Ehiagbionare & Ogundiran, 2010). Calcium and Magnesium are essential to fish for metabolic reactions in bone and scale formation. A total hardness of < 50 mg/lt is referred to as soft water while a total hardness of 50 to 400 mg/lt is known as hard water (Boyd, 1990).

From the analysis of water quality parameters of five different rivers of Meghalaya revealed that the water of Khri River in West Khasi hills district is desirable for natural habitat of Mahseer in terms of growth, generaland reproduction. The condition of the river Amlayee and river Janiaw are also good for growth of the fish. However, results of water quality of the river Umngi shows some kind of anthropogenic interference.

The Physico-chemical parameters studied are within the normal range as shown in Table.1 which is suitable for fish culture. Besides, the fish *Neolissochilushexagonolepis* showed sexual maturation at captive condition. Therefore, this could be used for artificial propagation as better productivity andgrowth of the fishes can be obtained leading to natural conservation.

Acknowledgement:
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EXPLORATION OF DIFFERENT WATER BODIES OF MEGHALAYA, INDIA, FOR *Neolissochilus* POPULATION AND THEIR IDENTIFICATION: A PRELIMINARY REPORT.

Raffealla Nongrum and R. N. Bhuyan*

ABSTRACT

Nine different rivers across Khasi and Jaintia hills region of Meghalaya were selected for exploration of *Neolissochilus* species and its availabilty. The rivers were selected based on the preliminary survey conducted earlier. Highest population of Chocolate Mahseer was found in Khri River, West Khasi Hills district followed by Leshka in West Jaintia Hills and Umran, Ri Bhoi District. The least population was reported from Umrynjah and Umiam, Ri-Bhoi District and Umngi River in East Khasi Hills District. Based on the difference in morphometric measurements and meristic counts, two different species of genus *Neolissochilus* viz. *N hexagonolepis* and *N hexastichus* were identified. It was observed that *Neolissochilus hexagonolepis* is widespread across the state but *Neolissochilus hexastichus* is localized in certain pockets at the mid-altitude.

Key words: *Neolissochilus hexagonolepis*, *Neolissochilus hexastichus*, meristic counts, mid- altitude, preliminary survey.

Introduction

Meghalaya lies between $25^0$12 N and 26^0 52 N latitude and 85^0 492 E and 92^0 522 E longitudes. Due to the elevation which ranges from 72- 1580 meter MSL the state is considered to be in mid-altitudinal region with the capital city Shillong situated at an attitude of 1496 meters MSL. The state is rich in rivers which are natural habitat of different indigenous fish species including Mahseer species. Cyprinid fishes of the three genera *Naziritor*, *Tor* and *Neolissochilus* are often referred to as Mahseer (Nguyen *et al*., 2008). So far 46 Mahseer species have been reported (Mani *et al*., 2012). Mahseers are available in almost all the rivers, streams and reservoirs of Meghalaya. But recently its population has drastically reduced and in some of the rivers and streams where the population of Mahseer particularly that of Chocolate Mahseer is nil. The systematic approach for exploration of Chocolate Mahseer in the different rivers, streams and reservoirs of Meghalaya is of high priority to conserve the fishes from extinction. This fish can adapt and grow very well under the agro-climatic conditions of the region. Hence, there is an urgent need to study and conserve its natural habitat and also to rear the hatchery produced larvae of Chocolate Mahseer under captive rearing conditions to increase the population of this fish in the natural water bodies of Meghalaya, which is an important component of biodiversity. In the state of Meghalaya reports on Mahseer are scanty although there are Meghalaya has got tremendous scope for Mahseer fisheries. It is over-exploited in most of its range with populations continuing to

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decline due to various anthropogenic activities such as extensive fishing and industrialization. The species is assessed as near threatened in the IUCN Red List (2015) of Threatened species on the basis of inferred population decline.

Chocolate Mahseer is locally known as "Khasaw" in the East Khasi Hills, West Khasi hills and Ri-Bhoi district and it is known as "Khapnar" in Jaintia hills district. These fish species play an important role in day to day life of fishers of the state. The presence of *Neolissochilus hexagonolepis* in rivers of Meghalaya were reported by Mandal *et al*., (2012) from War Jaintia Hills and also by Yazdani (1975) but reports on *Neolissochilus hexastichus* are scanty and there are no reports on *Neolissochilus stracheyi* in rivers of Meghalaya. The fish, Chocolate Mahseer have high demand in the state and has afuture in angling tourism pursuit (Nguyen, 2008) and the species is important from the eco-tourism point of view. The market price for these fishes is one of the highest because of its taste though not much is known about its nutritional value. These species are large scales barbels and usually live in upstream, clear and running waters (Shrestha, 1990). It is an indigenous and endemic species of Meghalaya (Mandal *et al*., 2012). In North East India, Laskar *et al*., (2013) reported the presence of *Neolissochilus hexastichus* in the river Diyung in the Dima Hasao district of Assam. He also added that no other reports on the presence of the species from anywhere else in North East India. Mahapatra *et al*., (2011) reported the reduced in the abundance of *Neolissochilus hexagonolepis* in the state of Meghalaya.

In the present study, an attempt has been made to explore different rivers for presence of different species of *Neolissochilus* and identify the fish species *Neolissochilus hexagonolepis* and *Neolissochilus hexastichus* by analysis of morphometric measurements and meristic counts (Das Gupta, 1989). The fish specimens were also sent to ZSI, Shillong who also confirmed the identified specimens as *Neolissochilus hexagonolepis.*

Materials and Methods

Study area

The work was carried out during 2012-2014 in selected water bodies of Meghlaya. For exploration, nine rivers were selected. The rivers were selected based on the preliminary survey conducted earlier. These rivers are River Umran (Ri Bhoi District), Khri (West Khasi Hills District), Umiam (East Khasi Hills District), Janiaw (East Khasi Hills District), Rtiang (Ri Bhoi District), Umngi (West Khasi Hills District), Leshka (West Jaintia Hills) Lakroh (West Jaintia Hills District), Umraleng (East Khasi Hills District) and Amlayee (West Jaintia Hills District).

Materials and Methods

In total 300 specimens of chocolate Mahseer were collected. For collection of specimens angling as well as netting was employed with the help of local fishers. Live specimens of Mahseer were collected and transported using closed system with oxygen to the hatchery complex of Department of Fishery Science, St. Anthony's College, Shillong and reared in different cemented tanks. Maximum numbers of the live fish specimen were collected from River Khri, West Khasi Hills. The least number were collected from River Umngi. Some of the explored river systems are shown below (Plate .1).

Plate.1 Some of the collection site of the explored rivers

Lakroh River, Jaintia Hills.

Khri River, West Khasi Hills.

Umraleng River, East Khasi Hills District

Umngi River, West Khasi Hills

Janiaw River, East Khasi Hills

Amlayee River, West Jaintia Hills

Biometric Studies

For meristic counts Sex of the fishes was determined by examination of external morphology and secondary sexual character of the fish species. After preliminary investigation, fish specimens were send to ZSI (Zoological Survey of India), Shillong for species confirmation.

Results

Locations of places where samples were collected and the number of collected fishes were recorded in Table 1. During the morphometric measurements and meristic counts it has been found that there are difference in the meristic counts (Table. 2).

Table. 1: Name and location and number fish collected of the rivers explored				
Sl. No.	**River**	**District (latitude & longitude)**	**Altitude**	**No. of fishes collected**
1.	Umran	Ri-Bhoi District (25^0 46′ N and 91^0 52′ E)	813 m MSL	50
2.	Khri	South West Khasi Hills (25^0 47′ N and 91^0 25E)	1366mMSL	62
3.	Umiam	Ri-Bhoi District (25^0 40′ N and 91^0 55′ E)	930 m MSL	7
4.	Amlayee	West Jaintia Hills	609 m MSL	50
5.	Lakroh	West Jaintia Hills (25^0 10′ N and 92^0 09' E)	597 m MSL	20
6.	Leishka	West Jaintia Hills	N/A	50
7.	Umngi	North West Khasi Hills ($25^0$16′N and 91^0 25′ E	N/A	6
8.	Janiaw	East Khasi Hills (25^0 14′ N and 91^0 35' E)	914 m MSL	35
9.	Umrynjah	Ri-Bhoi (25^0 42′ N and 91^0 58′ E)	893m MSL	5
10.	Umraleng	East Khasi Hills (25^0 40′ N and 91^0 55′ E)	940m MSL	15

Table.2: Meristic counts of the n=200 species of *Neolissochilus*		
Meristic Parameters	*Neolissochilus hexagonolepis*	*Neolissochilus hexastichus*
Lateral line scale	28-32	24
Scales above lateral line	6	5
Scales below lateral line	4	4
Dorsal fin	9+i	8+i
Pectoral fin	12+i	12+i
Pelvic fin	8+i	8+i
Anal fin	6+i	6+i
Caudal fin	17+ii	16+ii

Based on the difference in meristic counts the fish were identified as *Neolissochilus hexagonolepis* and *Neolissochilus hexastichus* (Plate. 2). The findings were confirmed by ZSI, Shillong to whom sample specimens were sent. This result is of preliminary in nature and further confirmation is necessary by using advanced tools like DNA barcoding etc.

This could be correlated with the size and nature of the river, where river formed pool type structure with less water current and sandy bottom that could be considered ideal as the breeding ground of the chocolate Mahseer. Collection of the fish during the night was easier as compared to the day. This can be considered indicative of the nocturnal nature of the fish.

Plate.2: Two different species of chocolate mahseer reported from Meghalaya.

Neolissochilus hexagonolepis: With single lateral line: available in most of the river explored.

Neolissochilus hexastichus: Six prominent lateral line: available in Wah Janiaw, Mawsynram,

Discussion

During the preliminary explorations which focus on the availability of the species, its presence was observed in all the rivers of Meghalaya. The availability of chocolate mahseer in Leshka River, West Jaintia Hills district was found to be highest followed by Khri River in West Khasi hills district. The least number was collected from Umrynjah River and Umiam River, Ri-Bhoi district and Umngi river in North West Khasi hills district. The presence of chocolate Mahseer in Umran River was comparatively higher than that in any other river in the district.

The population of *Neolissochilus hexagonolepis* was found to be more than that of *Neolissochilus hexastichus*. Moreover it has been observed that the *Neolissochilus hexastichus* population is restricted only to a few rivers i.e., in our survey they were confirmed only from Umran and Umngi Rivers in Ri Bhoi district and West Khasi Hills respectively. In specific pockets of the state where water is not polluted and least human interference is present. The onset of sexual maturation and spawning of fish at a particular environmental condition is of great significance, as it ensures the favorable condition, for the development of embryo and

the young fishes (Schwassmann, 1971). The findings of the present study indicated that the prevalent environmental conditions of the individual rivers and food availability were the key for development of the presence those rivers.

The Umiam River and Umrynjah River appeared to be highly eutrophicated due to presence of higher percentage of nutrients coming from the different urban area directly as waste disposal. This was also in agreement with the results of other observations (Rajurkar *et al.*, 2003). Pollution followed by the destruction of the natural habitat could be identified as one of the important factors for rapid declination of this species in some rivers. It has been observed that *Neolissochilus* species are usually found in river waters of the state but in less disturbed areas. *Neolissochilus* is locally called 'KhaSaw' or "KhaPnar" and also as 'Kha Smet', *Tor* species is locally referred to as 'KhaLad'. The taxonomy of Mahseer is confusing due to the morphological variations they exhibit. Therefore there is a need to resolve taxonomic ambiguities for developing strategies for aquaculture and its artificial propagation (Mohindra *et al*., 2007). Hence, it is important to clear the impasse of the presence of different species of *Neolissochilus* as well as *Tor* species in the area. During the present exploration of different rivers for Mahseer population, it has been observed that two different species of Chocolate Mahseer are present in Meghalaya. These are *Neolissochilus hexagonolepis* (single lateral line with scale count: 28) and *Neolissochilus hexastichus* (presence of six line; lateral line scale count: 24) Table.2; Plate. 1. This was confirmed by the report of ZSI, Shillong, where sample specimen was sent for species verification.

However, identification of different species of *Neolissochilus* in the rivers of Meghalaya, relying only on morphological characters of the species found in this region is not enough to clarify the authenticity of these groups of Mahseer. Analysis of meristic counts gives better understanding of the species identification. Historically, with the work of Hamilton- Buchanan (Hamilton, 1822) various distinguished naturalists have been able to proposed new descriptions of different species of Mahseer from Indian waters (Laskar *et al*., 2013). But using advance technique like molecular markers, in particular the use of microsatellite markers for chocolate Mahseer diversity in the state of Meghalaya would help in understanding its population structure and in conservation of this species.

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ISOLATION AND IDENTIFICATION OF ENDOPHYTIC BACTERIA

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ABSTRACT

Plants are associated with different kinds of microorganisms that attribute different properties to the host plant. So knowing and understanding these microbes associated in different plants, known as plant microbiome, is a major thrust in modern microbiology research. In this review we are giving insight into isolation, identification and genetic approach to study endophytic bacteria.

Keywords- *Citrus plant, endophyte, bacterial identification 16S rRNA gene, sequencing.*

Introduction

Plant associated bacteria can be grouped under two categories: ectophytes define to bacteria that remain associated to plant surface and colonize there; and endophytes define to bacteria that remain associated to the internal part of plants and colonize there. Unlike ectophytes, endophytes have to overcome the plant defence responses, have to develop elaborate regulatory system to adapt, colonize and grow inside the plant without evoking the plant defence response. Nitrogen fixing bacteria are well-known plant inhabiting bacteria. Different plant pathogenic bacteria also inhabit plants. Here neither pathogenic bacteria nor nitrogen fixing bacteria are included under endophytes. We are including bacteria under endophytes that are more dynamics in nature that is these bacteria live in plants like their habitat and may not contribute to plant health. Relating to endophytes there are many interesting questions as follows:

- How do these bacteria overcome the plant defence responses?
- Is there a common feature all endophytes have?
- Are endophytes between annual and perennial plants different?
- How does the population of endophyte vary in relation to age of a plant?

Therefore endophytes have been a major thrust in plant-microbe interactions. Here we are giving a brief account of our study of endophytes in citrus plant.

Studying endophytes from citrus plant

Association of endophytic bacteria Bacterial identification and characterization based on biochemical and physiologic features have been greatly emphasized during the early twentieth

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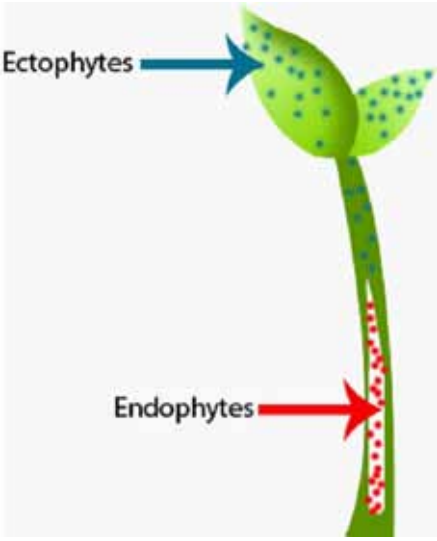

Figure 1. Ectophytic and Endophytic bacterial colonies on a plant

century. *Bergey's Manual of Determinative Bacteriology* is based on these features along with the Morphological traits as observed under light microscopy. Bacteria are small in size which makes their identification more difficult as they display limited morphological diversity (J.E. Clarridge III, 2004).

Later in twentieth century, a new standard method for identification was developed which could determine phylogenetic relationship of bacteria and other life-forms (D. Emerson *et al.* 2015). This method analyses a conserved gene sequence and in case of bacteria 5s, 16S and 23s rRNA coding genes are considered as candidate genes. 16S rRNA encoding gene is highly conserved and moreover, the size of this gene (1500 nucleotides) is ideal for generating adequate sequence information (Woo, P.C, 2008). The size of 5s rRNA gene is too short *i.e* 120 nucleotides and the size of 23s rRNA gene is too long *i.e* 2900 nucleotides and 16S rRNA gene, being the intermediate, is most commonly used.16S rRNA gene is also called as 16S rDNA. All Archaea bacteria and bacteria can be compared using 16S rRNA gene whereas in case of eukaryotes 18s rRNA gene is compared (J.E. Clarridge III, 2004).

There are a variety of definitions put forward to define the term endophyte. The bacteria that reside in the tissues internal to the epidermis are called endophytes (W.L. Arau´jo *et. al.*, 2000, 2002). This definition is modified as, the bacteria that can colonize the interior of the plants including either active or in-active pathogens can be termed as endophytes (Lodewyckx, C., 2010).However, the colonizing bacteria which are harmless to the plants and are only securing residency in the plants has to be taken into account. Moreover, the bacteria which exists as endosymbionts i.e. the plant obtains ecological benefits from such bacteria, has to be also considered (W.L. Arau´jo *et. al.* 2002).The bacteria which are present at the cortex of the roots are also defined as endophytes. Scientists have put forward the criteria based on which a "true" endophytic bacteria can be recognized (C. M. Press *et.al,* 1997). One, the bacteria have to be isolated from surface dis-infected or surface sterilized tissues of the plant. Two, the bacteria have to be "tagged" in order to visualize it microscopically inside the respective plant tissues. The bacteria which do not fulfil the above mentioned second criterion are termed as putative endophytes. Endophytic bacteria can also possess the ability to re-infect the dis-infected plant tissues.

The aim of this review is to describe one of the methods used for isolating endophytic bacteria from *Citrus sp.* and mechanism of 16S rDNA sequencing and its contribution to identify and characterize novel bacteria. Moreover, the identification and classification of bacteria at the correct time is centre to areas viz. anti-microbial therapy, food-safety, diagnosis of diseases and

environmental monitoring.

Isolation of Endophytic Bacteria

The branches of *Citrus reticulata* were chosen as the source of shoot ex-plant for endophyte isolation. Some of the endophytic bacteria associated with *Citrus sp.* plants are mentioned in Table 1.The branches are first surface-sterilized by the following method (Arau´jo, W.L. *et.al.* 2002)-

1.The branches are washed under running tap water and the branches showing superficial damage or symptoms of disease are excluded.

2.The branches are then washed with 70% ethanol for five minutes followed by treatment with 2% sodium hypochlorite solution for five minutes.

3.In this step the, branches are again treated with 70% ethanol for 30 seconds followed by two rinses in sterile distilled water.

4. In order to confirm the dis-infection method, the branches are pressed on Tryptic Soy Agar medium plates and the aliquot of the last rinse was poured on to the same medium. The plates were incubated for one to three days at 28^0 C.

Once the dis-infection process is successful, we proceeded to the isolation step-

1. The barks of the surface-sterilized branches are peeled off with a sterilized blade and the branches are plated on Tryptic Soy Agar amended with 100 units of nystatin, which inhibits yeast contamination and 150 units of fluconazole, which inhibits *Candida albicans* contamination (un-published report).

2.The plates were incubated at 28^0 C for 1-12 days or until growth is observed (un-published report).

n an another method, the surface dis-infected branches are homogenized with 5 ml of sterile phosphate buffered saline (containing NaCl at 8 g/liter, KCl at 0.2 g/liter, Na_2HPO_4 at 1.4 g/liter, and KH_2PO_4 at 0.24 g/liter) with a blender and the serial dilutions were plated on Tryptic Soy Agar followed by the incubation of the plates at 28^0 C for 1-20 days or until growth is observed (M. Rosenblueth *et.al.,* 2006).

The bacteria growing on the Tryptic Soy Agar plates were grouped on the basis of their phenotypic characteristics viz. colony colour, colony morphology, motility, shape and Gram reaction. Some isolates are chosen or further identification by 16S rDNA sequencing method (un-published report).

Mechanism of 16S rDNA sequencing

The 16S rRNA gene behaves as a molecular chronometer (D. Emerson *et. al.,* 2015, J.T. Staley, 2006) as it is universally distributed, functionally homologous and is a molecule of identical function. The peculiarity of this gene, which codes for small sub-unit of ribosome, lies in the fact that it has highly conserved stretches of DNA sequence interspersed among semi-conserved and non-conserved sequences (U. Edwards *et.al,*1989).The highly conserved sequences are exploited as primer binding sites and the amplified DNA sequence is analysed. Generally, universal primers are designed complementary to the highly conserved sequences at the start of the sequence or at 540 base pair region or at the end of the entire sequence (J.E. Clarridge III, 2004) .The variable sequences in between are used for taxonomical comparisons (D. Emerson *et. al.,* 2015).

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The following is the protocol for identifying bacteria using 16S rDNA sequencing method –

1. Pure cultures of unknown bacteria are isolated from any plate.

2. Colony Lysis - The pure colonies are picked from the plates in PCR tubes and lysed by adding lysis medium (95 µl sterilized distilled water and 5µl of 200 mM sodium hydroxide).

3. Complete colony lysis reaction is done at 95°C for 15 minutes.

4. Colony PCR is performed using a set of primers which are complementary to the highly conserved region of the 16S rDNA gene.

5. PCR reaction products are run on agarose gel along with the DNA ladder and visualized for amplification.

6. The targeted amplified bands are extracted from the agarose gel and purified.

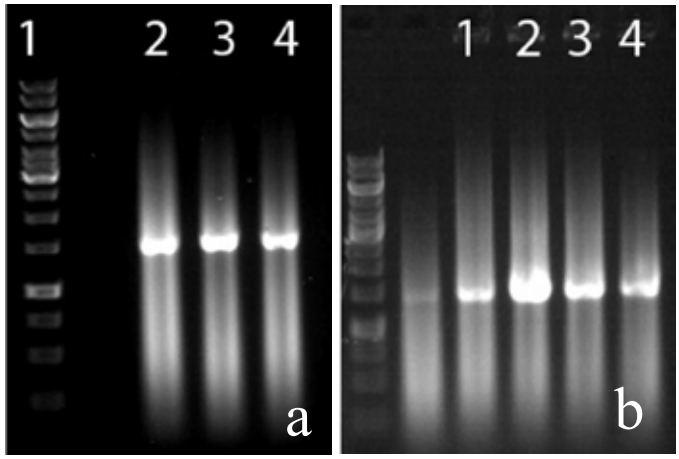
7. Sequencing of the 16S rDNA gene is done using the purified PCR product.

8. The sequences which are generated are edited using software and less than 5 minutes is required to edit the sequences if the sequencing run is good.

9. Sequence alignment was performed by BLAST tool. In case of novel organisms different databases are selected and search for alignment. Moreover, comparisons are also done on the basis of phenotypic characteristics of the organisms.

Table 1- Reported endophytes present in *Citrus sp.* (M. Rosenblueth *et.al,* 2006)

Endophytes	Plant species	Reference
Methylobacterium mesophilicum	Citrus plants	Araujo *et al.* 2002
Enterobacter cloacae	Citrus plants	Araujo e*t al.* 2002; Hinton et al. 1995
Pantoea agglomerans	Citrus plants	Araujo *et al.* 2001, 2002; Asis and Adachi 2003
Bacillus spp.	Citrus plants	Araujo *et al.* 2001, 2002
Curtobacterium flaccumfaciens	Citrus plants	Araujo *et al.* 2002



Primer sequences:
Forward Primer- 27R:
AGAGTTTGATCMTGGCTCAG

ReversePrimer-1492R
CGGTTACCTTGTTACGACTT

Figure 2.

A, Lane 1- DNA ladder, Lane 3, 4, 5- 1500bp PCR product. B, Lane 1, 2, 3, 4- 1500bp PCR products excised and extracted for sequencing. (Unpublished data).

Conclusion

16S-rDNA is important to for initial identification bacteria. Because of its conservation, it also helps in studying previously uncharacterized and uncultured bacteria. This has given rise to the idea of metagenomics. Moreover, the amount of time and labour required in 16S rDNA sequencing is less as compared to phenotypic and morphological characterization.

It is to be also understood that there are now more than 2000 strains of archaea and bacteria have been sequenced (Emerson .D. *et.al,* 2008). Therefore, many closely related bacteria likely to exhibit very much similarity in their 16S rDNA sequence. So based on 16S rDNA sequence, the bacterial identification cannot be conclusive. Multi-locus sequence typing (MLST) is important for future identification and characterization of bacteria. In this process sequence analysis of conserved coding sequences such as *gyrA* (codes for gyrase in bacteria), *rpoB* (codes for RNA polymerase â subunit of RNA polymerase in bacteria), *tufA* (codes for elongation factor TU in bacteria) remains helpful. Considering the economy of genome sequence these days, the ultimate aim any laboratory these days is to sequence the whole genome of any bacterial strain.

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SOME INNOVATIONS IN TEACHING OF MATHEMATICS (AT UNDER GRADUATE LEVEL)

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ABSTRACT

Presently, science educational approaches have resulted in a mismatch between what is taught to the students and what a student really needs. As such, many institutions are moving towards problem-based learning as a solution to produce students who are creative, can think critically, analytically and are able to solve problems. As Mathematics is one of the pillars of the basic sciences, one of the solutions is to remove the mathematics phobia that has been plaguing the minds of the students. In this paper, we focus on the problems, objectives, needs and on the innovative methods of teaching and attracting students to this subject. Some pedagogic tools with which a teacher should be equipped have been mentioned. A brief survey of the number of students, of some colleges in the state, opting for this subject has been done and highlighted in this paper.

Keywords: *Innovations, Mathematics, Undergraduate, Syllabus enrichment, Oral Presentation*

Introduction

Mathematics, being an important subject and occupying a central position since the ancient period till date, has not been of interest to many students. The reason is mainly because there is aspiration but it is hard to achieve. Being highly abstract, it is concerned with ideas, which are interrelated, and with the manipulation of symbols. Teaching of mathematics is not only concerned with the computational knowhow of the subject but is also concerned with the selection of the mathematical content and communication leading to its understanding and application. So while teaching mathematics one should use the teaching methods, strategies and pedagogic resources that are much more fruitful in gaining adequate responses from the students. Teaching and learning mathematics involves complexities which can be overcome if certain rules are followed. The nature and quality of instructional material, the presentation of content, the pedagogic skills of the teacher, the learning environment, the motivation of the students are all important and must be kept in view in any effort to ensure quality in teaching-learning of mathematics. Mathematics has a role to play in many different fields: innovations in medicine, digital encryption, communication technology, modeling real life phenomena, predicting disasters, organization of enterprises, business and transport to name a few.

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At the heart of mathematics education lies undergraduate mathematics education. It would be impossible to tackle any of the problems associated with mathematics education, at any level without intervention at the undergraduate level. After all, the harbingers of change, if there are to be any, will be the teachers, policy makers, the creators and imparters of curriculum and pedagogy. And each one of them will have been shaped by their undergraduate (mathematics) education. Hence it is necessary that we examine the doctrines that govern undergraduate mathematics education in India.

Objectives

In this paper, efforts to discuss innovations and innovative practices in teaching mathematics at the undergraduate level, under teaching methods, strategies and pedagogic resources have been made. The process of innovation is generally described as consisting of three essential steps, starting with the conception of an idea, which is then proposed and is finally adopted. Though many ideas have been conceived to bring about change in the teaching of Mathematics, it is yet to be proposed and adopted. So, the innovations discussed may not be new in terms of the idea but is new in terms of practice.

Innovations in Teaching Mathematics

This can be diversified in terms of methods and Pedagogic resources used in teaching-learning process.

Methods

Method is a style of presentation of content in classroom. The following are the innovative methods that can be used to make teaching-learning process of Mathematics effective. which are beyond the understanding of the students. Formulas, theorems,

1. Inducto-Deductive Method

Inductive method is to move from specific examples to generalization and deductive method is to move from generalization to specific examples. In classrooms, usually instructions start with the abstract concepts, examples, results are derived, proved and used. But teacher needs to start with specific examples and concrete things and then move to generalizations and abstract things. Then teacher again needs to show how generalization can be derived and it holds true through specific examples. This method helps students for better understanding; they don't have to cram the things and will have long lasting effect.

Examples:

(i) Pythagoras Theorem - In a right-angled D ABC, right angled at B,

$$\mathbf{AB^2+BC^2=AC^2}$$

(Considering right angle triangles of different measurements leading to generalization and then establishing it through the theoretical proof)

(ii) The sum of two sides of a triangle is greater than the third side.

(Ask the student to take any triangle, measure the sides, add any two of them, the result will always be greater than the third. The teacher can then proceed with the general proof.)

2. Analytico-Synthetic Method

Analytic is breaking down and moving from unknown to known and Synthetic is putting together known bits of information and moving from known to unknown. These methods are basically used in proving the results and solving problems. In textbooks, mostly synthetic method

Analytico-Synthetic Method

Synthetic method	Analytic method
$\int \tan x dx = \int \frac{\tan x \sec x}{\sec x} dx$ $= \log\lvert\sec x\rvert + c$	$\frac{d(\log\lvert\sec x\rvert + c)}{dx} = \frac{1}{\sec x} \sec x \tan x$ $= \tan x$

is used, to prove something unknown we start with a certain known thing, but that leaves doubts in minds of students as to why we have started with that step and using this particular known thing. So a teacher has to combine both in order to explain and relate each step logically.

3. Play-Way Method

This method includes play and fun activities that are related to numbers.

Examples:

(i) checking of divisibility of one number by another number,

123456712345688 is divisible by 4

(ii) generation of Pythagorean triplets (3,4,5), (6,8,10), (5,12,13)

(iii) formation of Pascal's triangle in solving $(1+x)^n$

Students don't realize that they are learning but in a way they are gaining knowledge by participating in these activities.

4. Laboratory Method

With the advent of computers, many of the collegesare well equipped with computer laboratories. The availability of computing softwares can be utilized in complementing classroom mathematical teaching to promote students´ active engaging and learning; to exchange long and difficult numerical and algebraic manipulations by communication of supporting reasoning when answering mathematical questions; to make experimental activities easier to handle; to develop problem resolution skills dealing with more interesting and difficult problems in so far as numerical, algebraic graphical and programming resources are available; to encourage discussion of different solutions or strategies as one works with multiple representations of the same mathematical object or process; to motivate the development of paired notions like discrete/continuous and finite/infinite. The pedagogical work needed to construct and implement learning situations to actualize these potentialities constitutes a major challenge to teachers.Some mathematical problems can be solved through Computer programs such as Maple, Mathematica, Matlab, Group algorithm program (GAP), which are powerful software programs used to solve general-purpose mathematical problems. Problems in the areas of mathematics, science and engineering (and many more) can be investigated using in-built commands of these programs or by utilizing these programming languages to create one's own

personalized programs. They can be used for solving problems in Calculus, Algebra, Solution of Differential Equations, Linear Programming, Statistics, plotting of points in two and three dimensions and also to create a three dimensional view of an object and many more.

Hence, introduction of laboratory component, in mathematics teaching, at the under graduate level, may enhance a better understanding of the subject for all papers for which there is feasibility of working in a laboratory environment.

5. Oral Presentation in Mathematics Learning

Anne B D'Arcy-Warmington(2008) mentioned that " it is important to consider the merits of oral presentations in mathematics service units as students' educational needs are diverse. Reaching parts of the brain that usual educational methods don't reach may be the answer to those poor students who do not have a 'mathematical brain'. The theory of multiple intelligences and brain-based learning may be the tool that will aid these students to be more confident about their mathematical ability. Oral presentations provide all students with a chance to display their knowledge in fun and creative ways. The interest aroused when researching the topic may give rise to a new curiosity about mathematics. With the declining numbers of students wishing to study mathematics perhaps, an injection of creativity in service units may spark an interest in mathematics in these and other students".

A study done by Lianghuo and Shu Mei (2007) showed that both teachers and students overall developed positive views about the benefits and usefulness of using oral presentation tasks into their daily mathematics teaching and learning. Oral presentation is an activity of sharing ideas and clarifying understanding verbally. Firstly, this method is regarded as an alternative mode of assessment for teachers to gather information about their students' learning of mathematics and hence make relevant instructional decisions. Secondly, it is also viewed as a tool for developing students' communication skills. One general purpose of oral presentation is to allow teacher to hear what students think about mathematics, and how they express it and their understanding of mathematics in their own words. Furthermore, teachers using oral presentation tasks must provide opportunity for students to think through questions and problems; express their ideas; demonstrate and explain what they have learnt; justify their own opinion; and reflect on their own understanding and on the ideas of others. *Thus, in the existing syllabus, changes can be made so as to include oral presentation as a process of mathematics learning by allocating some grades/marks to every paper. This incorporation may induce a better understanding of the subject.*

6. Syllabus enrichment

Generally the Mathematics courses of both BSc and BA programmes (with Honours/ Major in Mathematics) are the same as indicated in the NEHU syllabus curriculum to be followed at the undergraduate level in all affiliating colleges in Meghalaya; the two programmes differ in the nature of the stream, a student chooses from, in addition to mathematics, that is, whether from science or social sciences stream.

The BA/ BSc (Honours/ Major in Mathematics) curriculum of most of the universities include the following as compulsory courses:

Algebra (Classical and Linear Algebra) and Trigonometry

Calculus (Differential and Integral Calculus, Advanced Calculus)

Differential Equations (*Ordinary Differential Equations and Partial Differential equations)*

Vector Analysis

Analytic Geometry of two and three dimensions

Analysis (Real and Complex analysis, Metric Spaces)

Modern/Abstract Algebra

Mechanics

List of Optional papers

Principles of Computer Science-Theory and Practical

Differential geometryDiscrete Mathematics
Mathematical Modeling

Applications of Mathematics in Finance and Insurance

Special theory of Relativity

Combinatorial Number Theory

Computational Mathematics Laboratory

Numerical Analysis

Operational Research

Astronomy

Complex function Theory and Real Analysis

With new ways of improvement in the teaching-learning process, the Syllabus may also be modified keeping pace with the all round development of the society. Some of the above mentioned optional papers in many of the colleges are options made by the teachers and college authorities and not by the students themselves and as a result the purpose of an optional paper at undergraduate gets defeated in many cases. Thus in order to eliminate such practices, some of the vital optional papers mentioned above can be included into core courses. The courses that can be incorporated into the core courses, to name a few, are topics from Computer Science (Data storage, Data Manipulations, Operating system and network, algorithm, Programming languages, Software Engineering, Data Structures),

Discrete Mathematics (Propositional logic, Relations, Lattices, Boolean algebra, Graphs, Combinatorics)

Mathematical Statistics (Probability theory, Descriptive Statistics , Statistical Methods (Sampling, Statistical Tests), Distributions, Sampling theory, Correlation and Regression and multivariate analysis)

Differential Geometry (Curves, Surfaces, Manifolds, tensor Analysis)

Cryptography (Classical and Modern techniques, Elliptic curves Cryptography)

The inclusion of the above topics to the present syllabus can prove beneficial for the students in enhancing their employability.

This is an exploratory research paper and the above recommended syllabus enrichment is not a technique, but rather a suggestive approach

which when followed may prove beneficial for the students studying mathematics in enhancing their employability.

Pedagogic Resources

These are resources that a teacher may integrate in a method for the transaction of a particular content and draw upon to advance the students' learning.

7. Programmed Learning Material (PLM)

As internet usage by the students is increasing day by day, colleges can provide soft copies of important textbooks/learning materials and make them available to students through the colleges/ institutions websites.

An interactive environment by the use of web 2.0 can also be created by every department of a college/Institution so as to encourage students-teachers interaction as a PLM through which a learner can proceed his self study at his own pace.

Activities here include works wherein students play active roles, interact with different resources and generate knowledge. Some activities are listed below.

It has the characteristics of all sequential steps, learner's response, self-pacing, immediate feedback, reinforcement and self-evaluation. It is helpful in acquisition of concepts like fractions, number systems, etc. and can be used as a remedy for slow learners for a specific content.

Tablet

This is essentially an interactive whiteboard (IWB) or EWB that enables the lecturer to write with a special pen on the screen of a tablet that is connected to a data projector. Any work done on the tablet is then simultaneously (real time) broadcast to the whole class. The tablet enables the lecturer to, inter alia, annotate notes, make comments and use colour schemes to highlight important points in a lecture.

Activities

Explorative Study

A brief explorative study is done in connection with mathematics performances of the students in the

Activity	**Situations related to Activity**
Quiz competition	Mathematical rules, results, formulae, Properties of numbers
Projects	Contribution by Mathematicians
Seminars	Applications of Mathematics, talks on Ancient Mathematics etc.
Discussion	Concept of Pi , Golden ratio, Presence of Mathematics in real world viz, nature and music
Mathematics Clubs	Preparing models , Paper folding
Assignments	Solving problems, proving of theorems
Field trips	Visit to banks, Insurance companies
Self study	Library, internet, resource centers
Scholarship exams	Mathematics Olympiads, Mathematics Training and Talent Search (MTTS), Advanced Training in Mathematics etc, all funded by NBHM (National Board for Higher Mathematics)

state by using the data obtained from Meghalaya Board of School Education and North Eastern Hill University, Shillong.

One can draw a clear picture (Table 1) that the percentage of students failing in Mathematics in class X is increasing.

Data from Table 2 reveals a marginal decline on the percentage of students failing in Mathematics for class XII in the year 2011. However, it may not be authentic to draw a definite conclusion about this percentage as it again shows an increase in the following year. But an overall study reveals that more than 50% of the students are not able to stand to the subject and as a result few of them may continue with higher studies related to the subject

The figures from Table 3 are inconsistent and do not show an increase or a decrease in the trend .The previous data revealed that approximately 3000 students appeared for the class XII exams in Meghalaya. However, in spite of this, only a handful of students opt for Mathematics as an Honours paper.

Mathematics is believed to be the key for all other subjects but it is surprising that most students fail and yet pass in other subjects. Some of the reasons may be because there is a negative attitude towards mathematics, fear due to pressure from friends that the subject is tough, limited or even lack of learning materials or lack of enough practice by the students.

The present exploratory example is simply an attempt to quantify crudely the success level of students at different level of education in the subject mathematics, and this failure rate may not have a connection to the methods of teaching and curriculum of the subject. However this study is simply an attempt to bring out new ideas for making the subject more interesting and appealing to the learners, which in the process can also benefit the students in enhancing employability by the choices of optional papers listed above.

Conclusion:

At present, we are in the growing needs of our society and the needs of the discipline itself, unless we take strong ameliorative steps, the rate at which we are improving is just not going to be enough. If we take a closer look we can see many gaps and lacunae that require immediate healing. There is a requirement to both work out long-term strategies and at the same time to also have good achievable short-term goals.

To sum up, the curriculum in most of the high weightage undergraduate mathematics Programmes seem to be focused on fast-tracking young men and women to be research Mathematicians. On average, however, much less than a fourth of undergraduate Mathematics students actually decide to pursue an academic career in mathematics. Further the pedagogy and assessment patterns followed actually do not do much to foster or enhance the ability to think originally or to critically analyze and solve unseen questions. Thus on average the undergraduate programmes in mathematics fail in at least two important ways: firstly, they are not really equipping and training the minority that plan to take up a career in mathematics in the manner they should; secondly, the majority are neither gaining any understanding of the role of mathematics in society nor are they learning the skills required by all in terms of communication, presentation, or the use of modern computer technology.

Table 1: Number and percentage of students who failed in Mathematics at the Secondary School Leaving Certificate (Std X) Examination conducted by Meghalaya Board of School Education (MBOSE).

Year	Number Appeared	Number Failed in Mathematics	Percentage Failed
2010	36153	14027	38.79
2011	36122	17874	49.48
2012	38942	38942	54.93

Source: MBOSE

Table 2: Number and percentage of students who failed in Mathematics at the Higher Secondary School Leaving Certificate (std XII), Science Examination conducted by the Meghalaya Board of School Education (MBOSE).

Year	Number Appeared	Number Failed in Mathematics	Percentage Failed
2010	2946	1941	65.89
2011	3058	1708	55.8
2012	3072	1803	58.69

Source: MBOSE

Table 3: Number of students who appeared as Mathematics Major Students at the First year Bachelor of Science Examination conducted by North Eastern Hill University(NEHU).

Year	2003	2004	2005	2006	2007	2008	2009	2010	2011
Number Appeared	65	57	99	72	40	59	45	51	66

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ANTIBACTERIAL PROPERTIES OF AQUEOUS EXTRACTS OF FRUITS OF *Prunus nepalensis* L.

Angkana Kalita [a], Laishram Indira Singha [a] and Ivee Boiss [b]

ABSTRACT

Prunus nepalensis L. is a member of the family Rosaceae. Locally known as Sohiong, it is a common plant found in Meghalaya. In this study, the aqueous extract of the ripe fruits was studied for possible anti-bacterial activities by using six strains of pathogenic bacteria. Significant anti-bacterial activities were observed in all the six cases.

Keywords: *Prunus nepalensis* L., anti-bacterial, well diffusion method.

Introduction

Plant extracts have been used since time immemorial to treat a number of diseases for their therapeutic potential (Raturi *et al.,* 2011). Plants have proved to be the source of novel compounds that are used to design new drugs for well-being of human kind. (El Astal *et al.*, 2005).

The family Rosaceae consists of a large number of plants represented by fruits such as plums, cherries, quinces, pears, pears, apricots, loquats, strawberries etc. (Jorge & Markman, 1993). Many members of Rosaceae have shown a wide array of anti-bacterial activities (Richards, Durham & Liu, 1994). They contain compounds such as peptides,alkaloidal constituents, long chain aldehydes, essential oils etc. that are soluble in different types of solvents including water(Alma *et al.,* 2003).

Prunus nepalensis L. is a medium to large evergreen tree, 15-20 m high, with dark brown The leaves are lanceolate and crenate serrate. Flowers are white, arranged in terminal racemes or axillary. Fruits are fleshy, dark purple drupes, globose, subacid, edible and ready for harvest from August to October (Dr. A.G. Gaikwad, NBPGR 2013). The fruits have a high content of TSS, beta-carotene, anthocyanins, fiber and a number of minerals like sulphur, zinc, manganese, phosphorus, iron and copper(Rymbai *et al,* 2005). This study attempts to study the anti-bacterial activity of aqueous extract of *P.nepalensis L.* fruits.

Materials and methods

Plant material:

The ripe fruits of *P. nepalensis* L. were collected from local vendors from Shillong, Meghalaya.

Extract preparation:

The ripe fruits were cleaned and peeled. The flesh was removed and used for extract preparation.

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The seeds were discarded. For extract preparation, 10g of flesh was weighed and ground into a paste using sterile mortar and pestle. The ground flesh was suspended in 200ml double distilled water and kept in a magnetic stirrer for 24 hours. After 24 hours, the suspension was filtered using sterile muslin cloth. The resultant filtrate was then evaporated in a rotary vacuum evaporator (Equitron Roteva rotary flash evaporator) for about 2 hours at 65°C until a dried extract was obtained at the bottom of the flask. This was scrapped and stored for further use.

· **Preparation of microbial cultures: -** The lyophilized bacterial cultures were obtained from Institute of Microbial Technology (IMTECH), Chandigarh and inoculated in 50ml sterile nutrient broth (HIMedia). The bacteria chosen were-*Mycobacterium smegmatis (*MTCC 943*), Shigella flexneri (*MTCC1457*), Bacillus cereus (*MTCC 430*), Pseudomonas aeruginosa (*MTCC424*), Escherichia coli (*MTCC1678*)* and *Staphylococcusaureus (*MTCC 9886*).*

· **Anti-bacterial test: -**The anti-bacterial test was carriedout using well diffusion method (Tagg *et al*, 1976). On sterile Petri dishes, a layer of sterile Nutrient agar (HiMedia) was poured and allowed to solidify. Actively growing cultures of the chosen bacteria were mixed with 0.7% agar (HiMedia) and plated on top of the nutrient agar layer. After the agar solidified, wells were carefully punched on the agar. The dried extract obtained was dissolved in distilled water in three different concentrations to allow a comparative study. The wells were loaded with 20µl of the extract in concentrations 20mg/ml, 40mg/ml and 80mg/ml. As positive control 20µl of Ampicillin (2mg/ml) (HiMedia) was loaded and distilled water was used as negative control. The test was carried out in triplicates for each bacterium. The Petri dishes were then incubated for 24 hours at 37°C. After 24 hours, the zones of inhibition around the wells were measured. An average of the three readings was taken and tabulated.

Results and discussion:

The results of the anti-bacterial test are given in Table 1 and Fig 1(a)-1(f):-

The results of the anti-bacterial test given in Table 1 and Fig. 1(a)-(f) indicate antibacterial action of the aqueous extract of the fruits of *P. nepalensis* L. The extract has been found to have bactericidal effect on all the six strains of bacteria (*S.aureus, E. coli, P. aeruginosa, S. flexneri, M. smegmatis* and *B. cereus*) tested. The extract has been found to be effective at concentrations 80mg/ml and 40mg/ml against all said bacteria. The diameter of the inhibition zone increases with the increase in the concentration of the aqueous extract.The extract (40mg/ml) showed similar antibacterial activity against all the six tested pathogenic bacteria. The fruit extracts showed highest activity against *Bacillus cereus*, the causative agent of skin infections and many food borne illnesses and *Staphylococcus aureus,* the causative agent of nosocomial infections.While the zones of inhibition produced by the extract were smaller than the positive control ampicillin for most bacteria tested in this study, the inhibition against *S. aureus* is found to be close to that of ampicillin. The other four bacterial strains are also the causative agent of many diseases. *S. flexneri* is the causative agent of diarrhea and dysentery, *M. smegmatis* causes skin and soft tissue infections, *P. aeruginosa* causes many nosocomial infections like bacteremia and pneumonia and *E. coli* is the major causative agent of cholecystis, urinary tract infections, cholangitis and neonatal meningitis. Since the studied extract showed promising activity

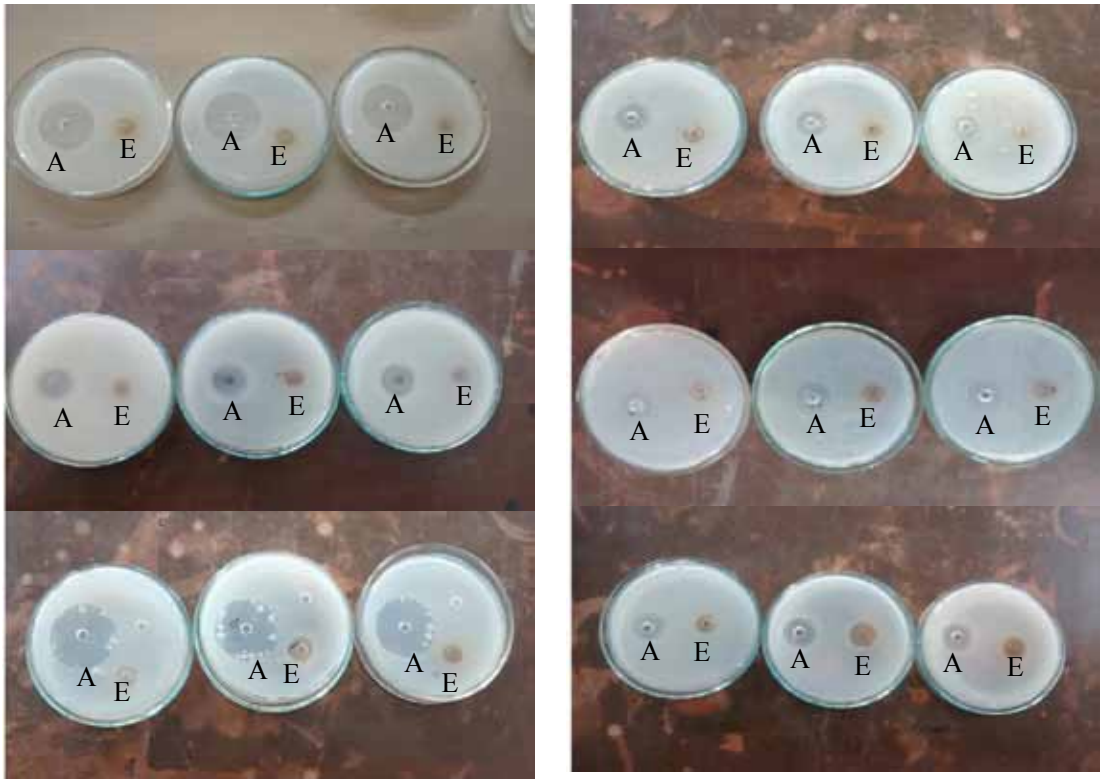

Fig. 1(a)-1(f):- Nutrient agar plates with different bacteria and wells loaded with extract (80mg/ml) and Ampicillin (2mg/ml) showing zones of inhibition. (A-ampicillin, E-extract)

against all the six tested pathogenic bacteria which are the causative agents of a wide range of diseases, it is can be said that the studied extract has the potential to be developed into anti-bacterial drugs to cure the diseases caused by these studied bacteria.

Conclusion:

All the tested bacteria were found to be susceptible to the aqueous extract at 40mg/ml and 80mg/ml.The results reported here indicate the presence of antibacterial compound(s) in the aqueous extract of *P. nepalensis* L. It can be concluded that the fruits of *P. nepalensis* L. is not only nutritious but also represents a rich source of valuable medicinal compounds. Thus the fruits of *P. nepalensis* L.contain high antibacterial property whichis further beingexplored for the isolation and identification of its bioactive compounds in our lab. These compounds have potential for development of anti-bacterial drugs against various pathogenic bacteria.

Table 1:- Anti-bacterial effect of aqueous extract of fruit of *Prunus nepalensis* L. on various bacteria

Bacteria	Zones of inhibition (in millimeters) (-) *indicates no zone of inhibition*				
	Aqueous extract(20mg/ml)	**Aqueous extract(40mg/ml)**	**Aqueous extract(80mg/ml)**	**Ampicillin (2mg/ml)**	**water**
Mycobacterium smegmatis	-	*10*	*11*	*32*	-
Shigella flexneri	-	*10*	*11*	*21*	-
Bacillus cereus	-	*11*	*14*	*38*	-
Pseudomonas aeruginosa	-	*10*	*12*	*21*	-
Escherichia coli	-	*10*	*12*	*20*	-
Staphylococcus aureus	-	*11*	*17*	*18*	-

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FISH MARKETING SYSTEM IN MEGHALAYA: A STUDY

Rupak Nath [a]

ABSTRACT

The present study reveals that fish marketing in Meghalaya is almost exclusively maintained by the private sector. Three distinct tires viz. primary, secondary and consumer market of marketing systems were observed in the process of distribution of fishes in the state. Approximately 99% of total fishes produced by the local fish farmer of the state are sold in the primary market located at rural areas and only 1% reaches to secondary market or whole sale market. Fish marketing in the state is mainly depending on imported fishes. Fishes arrived in the market from farm gate and distributed to consumers through a number of intermediaries: Auctioneer cum wholesaler, retailer and vendor. Intermediaries perform an array of important marketing functions. Fish traders in Meghalaya are generally confronted with two risks in the fish market viz., physical risks and market risk. Physical risk associated with improper handling of fishes and market risk is mostly linked with price risk and uncertainty of supply of fishes. Physical risk can be controlled by traders but market risk is not easy to manage because it is associated with supply of imported fishes.

Key words: *Fish market, Fish marketing functions, channels, and market risks*

Introduction

Fishery today is an important mode of earning a livelihood for rural people of India. In general fishery comprises the fishing sector, processing sector and marketing sector and these constituents are interlinked with each other. Marketing is the key sector which is being capable of influencing the efficiency and performance of the others. Fishing produces a "commodity" i.e. fish which is highly perishable. The fish catch, consisting of 'dead fish", has to be sold to the consumers within a short time, or iced, frozen, salted, dried before decomposition sets in (Kohis R L and J N Uhl, 1980). Fish marketing is the commercial aspect of the fishery sector encompassing all the business activities in the chain of flow of the produce from producers to consumers. It includes various activities like pooling of catches from producers or fishers, handing and initial processing, grading, storing and packing, transport and sale, institutional fee such as market fee, financing and risk bearing. These activities also known as marketing function. The domestic fish marketing system in India is neither efficient nor modern and is mainly carried out by private traders with a large number of intermediaries between producer and consumer, thereby reducing the fisherman's share in consumer's rupee. Physical facilities and infrastructure in all types of fish markets are far from satisfactory (FAO, 2001). Meghalaya is one of the North eastern states in the country where majority of the population is non vegetarian where fish comprise

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an important part of the diet of local indigenous people. Fish marketing in Meghalaya; encompass marketing of fresh water fishes produced by fish farmers, wild fishes caught by fishermen, fishes imported from several other states and also processed fishes. Fish production in the state is insufficient to satisfy the present demand of fish. In recent time, fish production in the state was recorded 5.89 thousand tonnes against the demand estimated 30.97 thousand tonnes per annum. A huge quantity of fish is imported from other states like Assam, Andhra Pradesh, and West Bengal to meet the demand of fish in the state. In this paper, an attempt is made to classify fish markets of Meghalaya, identifying various marketing functions, channels and risk involved in the fish marketing.

Materials and Methods

In the study both primary and secondary data were collected over one year period from March 2012 to April 2013. Primary data were gathered through interview and direct observation methods from minor landing centre of river Umngot at Dawki, Umiam reservoir site; wholesale, retail markets of the state and government officials. Information was collected on nature of the fish market, marketing functions, intermediaries, marketing channels and distribution system and market risk. Secondary data were collected from government publications of department of Fisheries. Suitable photographs, tables and figures were incorporated for the presentation of results.

Results

Classification of fish market:

Fish markets of Meghalaya can be classified based on their location and volume of transactions. On the basis of location or place of operation fish markets may be classified as primary market, secondary market and final consumer market. Primary fish markets are located within the rural market or the fishing areas. Fishers land their catch from the boat and sell fishes in the vicinity of landing centre. Fishes produced by the farmers in the state are sold in their rural market. Involvement of middlemen is nil in the primary market. Secondary markets are located in district head quarters are also known as wholesale market. Secondary markets are operated by numbers of intermediaries namely auctioneer, wholesaler, retailer (Nath *et al*, 2009). There is only one secondary market in Meghalaya located at Shillong. Final consuming markets are located in rural, sub-urban and urban areas of the state. On the basis of volumes of transactions at a time, fish markets may also be classified as wholesale market and retail market. Wholesale fish market is one in which fishes are bought and sold in large lots or in bulk. There is one wholesale market in Shillong located in Iewduh and is known as "Bara Dohkha". This wholesale fish market occupies an extremely important link in the marketing chain of all retail markets and is meeting the entire demand of fishes for both Khasi and Jaintia hills. Fish catches landed in the wholesale market basically consist of 3 kinds of fish: small fish varieties from local areas, medium varieties from Assam and large uniform sized import varieties from Andhra Pradesh. Fishes imported from outside the states namely Andhra Pradesh, Assam and West Bengal are sold through auction and retail traders participate in auctions. The key intermediaries in fish marketing process found are auctioneer cum wholesaler, retailer and the vendor. After the transactions, retail traders carry the fish to various retail markets by taxi and buses. A retail market is one in which transactions take place between retailers and consumers. Retail markets are located in different parts of a big city or town and even in rural areas.

Marketing Functions:

A marketing function can be defined as a "a major specialized activity performed in accomplishing the marketing process (Rao, 1995). The important functions performed by various agents in fish marketing process in Meghalaya are (i) fish harvesting and handling, (ii) fish transportation and (iii) fish selling. Fish harvesting and handling functions are carried out by farmers or fishers but fish selling function is performed by traders. Transportation function is performed by both fishers and traders. Fish is often transported long distances to the whole sale market by trucks or taxi. Fish arrives to the wholesale market from the local fish farm and outside the states are scattered all over the rural, sub urban and town areas located far and wide from the whole sale market by various routes. Fish moves through several intermediaries to the consumer. Intermediaries carry fish from landing centres or fish farms to fish market and ultimately to fish consumers. The intermediaries are involved in providing services of grading, icing, packing and transporting and these activities result in cost addition at every stage of marketing. Several other intermediaries like local fish collectors and fishermen. The key intermediaries observed in the fish market of Meghalaya are: Auctioneer cum wholesaler, retailer and the vendor.

Auctioneer cum wholesaler: Auctioneer cum wholesalers is the first intermediary in fish marketing channel in Meghalaya. They generally perform two different types of functions: (i) Facilitating the operation and (ii) Preservation of fish. The wholesalers buy fish in bulk and sell it to retailers. Wholesaler makes all necessary arrangement like sorting, grading, cleaning, icing before fish sale to the retailers and vendors.

Retailer and Vendors: Retailer and Vendors purchase fish from whole saler at the wholesale market and take it to different retail market places and sell it. But Vendors are mostly carry fish directly to the consumer households.

Marketing channels:

Fish marketing channel is the path by which the fishes are moved from producer to ultimate consumer. Fish marketing channels in Meghalaya follow multilevel marketing process. There are five marketing channels observed in Meghalaya through which the fishes are moved from one agency to another.

Channel I consist of only two agents. Fishers or fish farmer catch or harvested fishes from rivers, streams, wetlands, ponds and sale in the local rural market. Fishes does not reach to whole sale market. Quantity sold by fishers through this chain is not large. Large quantities of fishes are distributed in the state through Channel III which consists of four agents. Fish producers or fishers bring fishes to auctioneer cum commission agents who auctions fishes to traders at whole sale market. Huge quantity of fishes moves from outside Meghalaya through channel III. Through channel IV and V small quantity of fishes are being sold by fishery department particularly during festival and by street vendor. Processed fish products are very popular delicacies among tribal communities in the Meghalaya in general. Marketing of processed fish in the state is not organized like fresh fish. Except dry fish other processed products like smoked, semi fermented fish products are mainly consumed at domestic level. A huge quantity of dry fish is imported from other states which are routed through Assam (Jagiroad) to Meghalaya. Now days many Self Help Groups (SHGs) have started marketing of processed fish products and value added products in a small scale.

Table 1: Prevalent fish marketing channels in Meghalaya

Channel Number	Marketing channel
Channel I	Fisher/fish farmer → Consumer
Channel II	Producer → Retailer → Consumer
Channel III	Producer (outside state) → Whole seller → Retailer → Consumer
Channel IV	Producer → Fishery department → Consume
Channel V	Producer → Whole seller → Street trader → Consumer

Risk in the fish market:

Fishers and traders in Meghalaya are confronted with two major risks in the fish market. One is of physical risks which are associated with improper handling of fishes by fishers during the time of harvesting and by traders during the time of loading/ unloading fish and at the time of transportation to retail market. Buyers do not like to buy poor quality fish. Another risk associated with fish marketing is market risk. Market risk is mostly linked with price risk and uncertainty of supply of fishes. Fishes arrived from other states unloaded in the whole sale market and routed to different retail markets. Both whole sale and retail fish market does not have proper drainage facilities. Preservation is of great importance in fish marketing because fish is highly perishable product. Icing of fish is the only technique of preservation of fish in the fresh form. Though icing is carried out to preserve fishes but method put into practice to crash ice blocks may lead to contaminating fishes with dirt and microbes. Similar condition was observed in retail market also.

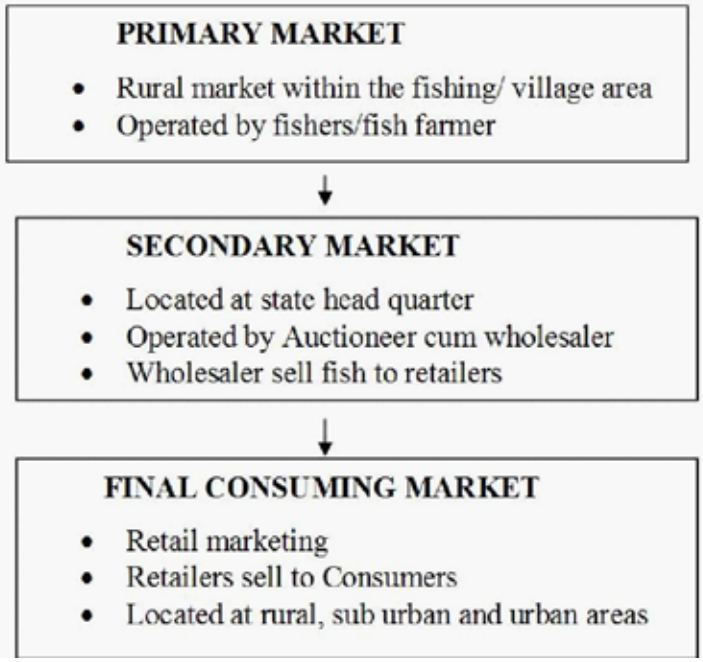
Discussion

Fish marketing in Meghalaya is mainly depending on imported fish and marketing is almost exclusively maintained by the private sector.

There is only one wholesale market in Meghalaya located at Shillong. According to market survey, the daily supply of fish in Shillong wholesale market varies depending on variations of seasons. Demand of fish more during the summer (March to August) than winter seasons i.e December to February. Most of the fish nearly 98% is imported from outside (Andhra Pradesh, Assam and West Bengal) and 2% is locally supplied. Mainly trucks are used for transportion of fish to wholesalers from outside. The wholesale market does not have facilities for cold storage and ice plants. Retail fish markets are characterized by the size of the markets in terms of quantity of fish, number of traders and proximity to the consumers (Kumar *et al,*2008).Most retailers in the state were found in selling fish in separate retail fish market but some retailers were also found selling fish by the roadside without fish dressing platforms. Hygienic condition of both whole sale and retail fish market of Meghalaya is poor. There is no drainage system observed and hygiene receives a low priority at all stages of marketing. In the wholesale market mostly block ice is used with manual methods being used to change the form of blocks to crushed ice. With regard to distribution of fishes it was observed that local fishes produced do not reach to secondary

market i.e. wholesale market and are sold in the primary market itself. 99% of total fish produced by local fish farmers were being sold in the primary market and only 1% reaches to whole sale market. Fish producers or fishers of the state do not confronted much with physical and market risk. Most of the local fishers and fish farmer sale in their fishes in the village market and are aware of current price of fishes and hence do not confronted physical and market risk. Mainly fish traders confronted both the risks. Traders take maximum possible care of his fish load to reduce physical risk because quality damages to fish affect his reputation. Collection of market intelligence is one way to reduce market risk. If proper market information is collected from various sources traders can minimise market risk. Domestic fish marketing holds a huge potential in Meghalaya. An efficient fish marketing system is vital requisite to increase productivity of fish farmers of the state. The improvement in fish marketing system is not only increasing the farmers profit share but also contribute economic security and nutritional security to fish traders and consumers.respectively.

Fig 1: Fish Market in Meghalaya



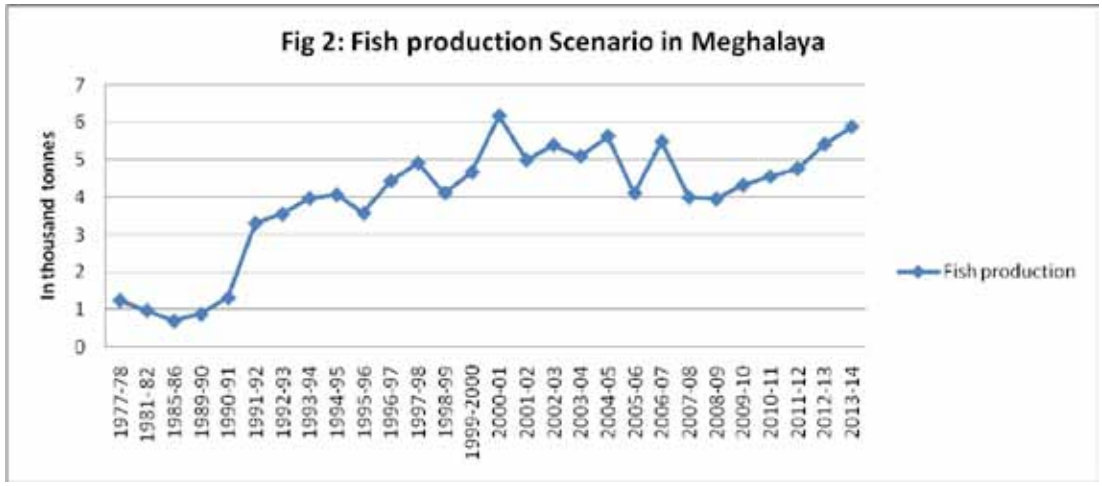


Figure 3&4. Icing a common fish preservation technique in the fish market of Meghalaya

Figure 5 Fish trader in Shillong whole sale fish market

Figure 6. Local dry fish market in Shillong

Table 2: Fish production and Demand in Meghalaya

Census	Population	Fish production ('000 t)	Demand ('000 t)	Deficit
1981	13,35819	0.98	13.96	93%
1991	17,74778	3.31	18.55	82%
2001	23,18,822	6.18	23.00	73%
2011	29,64007	4.77	30.97	86%

Demand estimation is based on 95% of population fish eater & 11 kg per capita/year

Table 3: District wise population in Meghalaya (2011 census) and requirement of fish

District	Population	Demand/annum (In tonnes)
East Khasi Hills	824059	8611
West Khasi Hills	385601	4029
Ri-Bhoi	258380	2700
Jaintia hills	392852	4105
West Garo hills	642923	6718
East Garo hills	317618	3319
South Garo hills	142574	1489
Total	**2964007**	**30,971**

On the basis of 95% fish eating population in the state & 11kg per capita/annum requirement

Table 4: Approximate quantity of fish arrived to Khasi, Jaintia and Garo hills (2013)

Region	Quantity of fish imported/annum	Per capita availability
Khasi and Jaintia hills	11,400 t	-
Garo hills (South, East Garo, West Garo)	2160 t	-
Quantity of imported fish	13,560 t	-
Quantity of local production	5420 t	1.92 kg
Total fish supply to consumers (local + imported)	18,980 t	6.741 kg

Table 5: Particulars of Bara Bazaar wholesale fish market, Shillong

Particular	Details
Location	Shillong
Coverage	Regional (East Khasi Hills, West Khasi Hills, South West Kha hills, East and West Jaintia hills).
Year of establishment	More than 70 years
Time span	Long period market
Volume of transaction	Whole sale and also retail sale
Nature of transaction	Cash mainly
No. Of commodities	All fresh water and some marine
Scale of operation	Approximately 30 tonnes/day
Main species handle	*Rohu, Catla, Cirrhinus mrigala,* murrels, *Pangasius, Walla attu, Sperata aor, Tenualosa ilisha*, *Labeo bata Mastacembelus*, Fresh water Prawns, exotic carps, *Puntius* sp *Notopterus notopterus, Chitala chitala*, Pomfret (marine), a small local varieties.
Weighing process	Physical balance
Preservation technique	Icing
Cold storage facilities	None
Maintenance of sanitation	None

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DETERMINATION AND COMPARISON OF ANTIBACTERIAL PROPERTIES OF COMMONLY USED DISINFECTANTS AND ANTISEPTICS

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and Jeremy N. Syiem [a]

ABSTRACT

Disinfectants and antiseptics are regularly used to reduce the risk of infection by bacteria that we may encounter every day. In this study, five common brands of disinfectants and antiseptics which included an antiseptic liquid, a mouth wash, a contact lens cleaning liquid, a floor cleaner and a toilet bowl cleaner were experimented to determine and compare their antibacterial properties against *Escherichia coli*. The methods used in this study were the "Disc Diffusion test" and the "Disc volatilization test". The results of the disc diffusion test confirmed the presence of antibacterial properties in the test samples with the most sensitivity shown towards the toilet bowl cleaner and the least sensitivity shown towards the contact lens cleaning liquid. The disc volatilization test however yielded no result, showing the absence of any volatile antibacterial agent in the test samples. This study proved the efficacy of common disinfectants and antiseptics in combating microbes.

Keywords: Disinfectants, Antiseptics, Antibacterial properties, Disc Diffusion, Disc Volatilization, *Escherichia coli*.

Introduction

Microorganisms, as the name suggests, are microscopic life forms, usually too small to be seen by the naked eyes and are composed entirely of a single cell. They include bacteria, fungi, protozoa and algae and their vast diversity can be seen not only in the differences between their morphology, physiology and genetics but also in their habitats and their interactions with their environment (Madigan, Martinko, Stahl & Clark, 2012). They also form an important part of our ecosystem by playing important roles in the various biogeochemical cycles and by forming the base of several food chains (Prescott, Harley & Klein 2005).

Mankind's interaction with microorganisms like bacteria has been known since the discovery of their existence. Some of these interactions are beneficial for humans like the use of microbes in the production of bread, wine, medicine, enzymes and other important products. Most interactions between humans and microbes, however has not been so delightful. In these interactions, usually, the microbes cause diseases in humans that may be mild to life threatening.

To combat the threat of infection by disease causing microorganisms, various precautionary measures have been devised and

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adopted by humans especially in their day- to-day activities. One of the measures that is adopted by members of the human population is the hygienic practice of washing or bathing. However washing or bathing only removes the microbes present on the skin surface. Microbes present on inanimate surfaces that humans may come in contact with, also have to be removed to prevent infection. Removal of microbes present on living tissue is called antisepsis, while the removal of microbes from inanimate objects is called disinfection. The chemical agents used for antisepsis and disinfection are therefore called antiseptic and disinfectant respectively (Ryan & Ray 2004; Singleton & Sainsbury 2006; Tortora, Funke & Case 2004). Even though the two chemicals are called differently, their purpose is the same. The only difference is the type of material on which they are used.

Some of the commonly used hygienic products that are said to have a disinfecting activity are mouth wash liquids, floor cleaners, toilet bowl cleaners, contact lens cleaning liquids and antiseptic liquids. The active antibacterial ingredients of these products are Chloroxylenol present in antiseptic liquids, Chlorhexidine gluconate present in mouth wash, Polyhexamethylene biguanide found in contact lens cleaning liquid, Benzalkonium chloride present in floor cleaners and Hydrochloric acid found in toilet bowl cleaners (Aboh, Oladosu & Ibrahim 2013; Masri et al. 2013; Okore et al. 2014; Saha, Haque, Karmaker, & Mohanta, 2009).

In this study, five products belonging to each of the earlier mentioned types of disinfectants and antiseptics were used to determine and compare their antibacterial properties against *Escherichia coli*. These products were designated as DA-1, DA-2, DA-3, DA-4 and DA-5 for antiseptic liquid, mouth wash, contact lens cleaning liquid, floor cleaner and toilet bowl cleaner respectively.

Materials and Methods

Preparation of Medium and Inoculation

100 ml of sterilized Nutrient Broth was aseptically inoculated with lyophilized culture of *Escherichia coli* (MTCC no. 40.) and incubated for 24 hours at 37 °C in an Incubator shaker. 0.5 ml of the 24 hour revived *E. coli* culture was then spread uniformly onto petri plates containing sterilized Nutrient Agar.

Disc Diffusion Test

Sterilized paper discs made from Whatman's filter paper no. 1 with the help of a paper puncher were then fully dipped into the respective disinfectant and antiseptic by using sterilized forceps. The discs were then placed on the inoculated agar plates. The plates were then incubated at 37°C for 24 hours (Masri et al. 2013; Ortez 2005). Control plates were also incubated along with discs containing sterilized distilled water.

Disc Volatilization Test

This method was used to test for the presence of any volatile substances in the disinfectants and antiseptics that may have antimicrobial properties. The disinfectant or antiseptic containing discs were placed on the lids of some of the inoculated agar plates that were not used in the disc volatilization test. The plates were thereafter incubated at 37°C for 24 hours. Discs impregnated with sterile distilled water were used as control.

Results

The results obtained after carrying out the two tests showed the presence of varying antibacterial properties in the experimented disinfectants and antiseptics. This was observed by the presence of a zone of inhibition around the discs, where, the larger the

Table 1. Disc Diffusion test

Designation of Disinfectant / Antiseptic	Name of active compound present	Diameter of Zone of inhibition (mm)
DA-1	Chloroxylenol	20
DA-2	Chlorhexidine gluconate	25
DA-3	Polyhexamethylene biguanide	15
DA-4	Benzalkonium chloride	20
DA-5	Hydrochloric acid	30

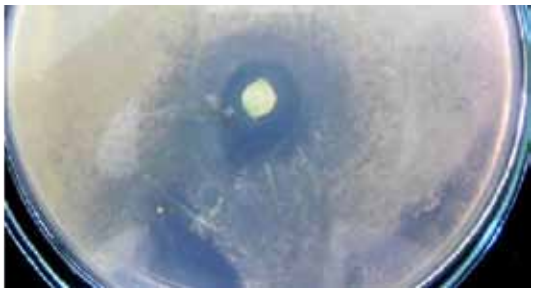

Figure 1. Zone of inhibition produced by DA-1

Figure 1. Zone of inhibition produced by DA-2

Figure 3. Zone of inhibition produced by DA-3

Figure 4. Zone of inhibition produced by DA-4

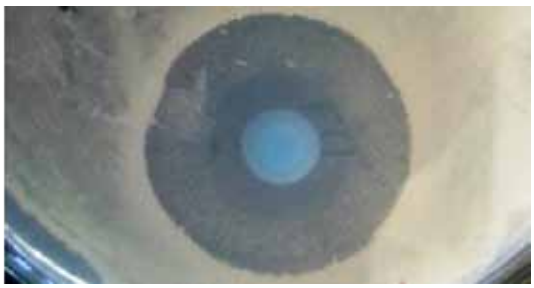

Figure 5. Zone of inhibition produced by DA-5

diameter of the zone of inhibition, the greater will be the antibacterial property of the tested product.

The Disc Volatilization test did not yield any result and all the cultured plates tested had full bacterial lawns on them.

Discussion

This study showed the presence of antibacterial activities of commonly used disinfectants and antiseptics. The most effective antibacterial property was shown by DA-5 (a toilet bowl cleaner) with an inhibition zone of diameter

30 mm. The least effective disinfectant was DA-3 (Contact lens cleaning liquid) with an inhibition zone of only 15 mm in diameter. The antibacterial properties of DA-1 (antiseptic liquid), DA-2 (mouth wash), DA-4 (floor cleaner) and DA-5 (toilet bowl cleaner) had earlier been reported (Aboh *et al.* 2013; Masri *et al.* 2013; Okore *et al*. 2014; Saha *et al.* 2009) and this has been confirmed again in this study. DA-3 (contact lens cleaning liquid) also showed antibacterial activity in the present study. The absence of any zone of inhibition in the disc volatilization tests ruled out the possibility of any volatile substances in the disinfectants and antiseptics having antibacterial effects. Based on the results, the antibacterial effects of the experimented disinfectants and antiseptics can be attributed to the active ingredients present in them. Other ingredients present in the disinfectants and antiseptics may also play a role in inhibiting the growth of bacteria (Masri *et al.* 2013).

Conclusion

The use of disinfectants and antiseptics has become part of our everyday life. Their effectiveness in inhibiting the growth of microbes as can be seen in this study, has played a vital role in preventing the spread of infections not only at home but also in other public places like hospitals, work places, airports, railway stations and schools. It is therefore necessary to encourage the use of disinfectants and antiseptics so as to reduce the chances of infection.

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ISOLATION OF BACTERIOCIN-PRODUCING LACTIC ACID BACTERIA FROM MILK AND MILK PRODUCTS AND PARTIAL PURIFICATION OF THE BACTERIOCINS PRODUCED.

Biju Bharali [a], Herbert. G. Lyngdoh [a] and Thyanswer Challam [a]

ABSTRACT

Lactic acid bacteria (LAB) are growing in importance due to the attention that they have gained as potential candidates for use in food preservation. LAB have been conferred Generally Recognised As Safe (GRAS, Grade One) status making them particularly well suited to meet the modern day demand for safe and healthy food. Among the substances produced by the LAB, bacteriocins are potentially among the most promising substances for future the food industry. These substances can be applied in the food industry as natural preservatives without much processing. This study was carried out to screen for potential bacteriocin producers from milk and milk products. Milk, yogurt and cottage cheese (paneer) samples were collected from shops in and around Shillong, Meghalaya, India. These were then grown in production medium and the bacteriocins produced were partially purified using Gel filtration and ion-exchange chromatography.

Keywords: *lactic acid bacteria, bacteriocins, potential preservatives*

Introduction

Bacteriocins are protein or protein complexes produced by bacteria and have antimicrobial activity against closely related species and various Gram positive and Gram negative bacteria including food spoilage bacteria and pathogens (Gaeng *et al*., 2000). However, most bacteriocins have a bactericidal or bacteriostatic mode of action only against closely related species. Occasionally, the inhibitory spectrum of some bacteriocins may also include bacteria which cause food spoilage and/or food-borne pathogenic microorganisms such as *Listeria* as proven by Cardinal *et al*., (1997). That, bacteriocins are, in fact, proteins and that they are ribosomally synthesized is demonstrable by their susceptibility to protease degradation (Montville and Kaiser, 2014). Agents produced that do not display this characteristic are not considered bacteriocins. Bacteriocins produced by bacteria form a rather heterogenous group differing in the species producing them, mode of action and antimicrobial spectrum, physical and chemical characteristics and molecular size (Vuyst & Vandamme, 1994). Bacteriocins that are important include Nisin, Diplococcin, Acidophilin, Bulgarican, Helveticins, Lactacins and Plantaricins (Nettles and Barefoot, 1993). Heng *et al*., (2007: 45) proposed that the bacteriocins produced by Gram-positive bacteria can be classified into four broad groups.

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The four groups are

(1) lantibiotics, (2) small non-modified peptides (3) large proteins, and (4) cyclic peptides.

Lactic acid bacteria (LAB) inhabit carbohydrate-rich environments and are especially at home in milk and its products. LAB have been classified as Generally Recognised as Safe (GRAS) organisms. Over the past few years there has been a lot of interest in the role that these bacteria would play in the food and food processing industry due to their ability to produce bacteriocins (Rodríguez, 2003). Nisin, a bacteriocin produced by *Lactococcus lactis* is widely used the world over in the food processing industry as a food preservative due to its efficacy, broad spectrum antimicrobial activity and proven safety (Hansen & Sandine, 1994).

This study was carried out to screen for potential bacteriocin producers from milk and milk products. Milk, yogurt and cottage cheese (paneer) samples were collected from shops in and around Shillong, Meghalaya, India. The study was aimed at isolating bacteriocins with activity against both *Staphylococcus aureus* which is a proven agent in some food-poisoning cases involving the consumption of milk or milk products (Asao et al., 2003; Kérouanton et al., 2007) and *Lactobacillus fermentum* which has been implicated with some cases of Cholecystitis (Chery et al., 2013).

Materials and Methods

Isolation and Identification of the LAB isolates

LAB strains were isolated from milk and milk products. Samples were plated directly on Man Rogosa Sharpe (MRS) and M16 agar at 37°C for 24 – 48 hours at pH 6.5 under both aerobiotic and anaerobiotic conditions. Samples were then purified using standard microbiological techniques. The cultures were then stored at -20°C supplemented with 30% (v/v) sterile glycerol. Working cultures were sub-cultured thrice (2% inoculum, 24 hours, 37°C) prior to use. The pure isolates selected as potential bacteriocin - producers were identified on the basis of its cultural, morphological, physiological and biochemical characteristics. Selected LAB isolates were characterized by Gram stain, absence of spores and catalase test. Gram positive, catalase and spores negative strains were maintained at -20°C until needed for antimicrobial activity testing.

Bacterial Strain used as indicator strains

Staphylococcus aureus MTCC 3160 (grown in Tryptic Soy Broth) and *Lactobacillus fermentum* MCC 2759 (grown in MRS Broth) were selected as indicator organisms to demonstrate and measure bacteriocin activity of the isolated LAB.

Selection of LAB strains

Of the 78 LAB isolates obtained from milk and milk product samples, 21 isolates showed promise as producers of bacteriocins which were shown to effectively inhibit either *Staphylococcus aureus* or *Lactobacillus fermentum*.

Preparation of Cell Free Extract (CFE)

The method outlined by Daba *et al*., (1991) was followed. Isolated cultures were grown overnight in MRS broth at 37°C in a rotary shaker at 150 rpm speed. Cells were centrifuged at 10,000 x g for 20 min at 4°C. The culture supernatant was concentrated by ammonium sulfate precipitation. 50 ml aliquots of culture

supernatant were made up to 60% saturation by addition of ammonium sulphate (HiMedia Labs, India) and kept overnight at 4°C. After centrifugation at 10,000 x g, for 20 min at 4°C the sedimented pellet was recovered and suspended in 5 ml of 0.1 M Potassium Phosphate buffer at pH 6.5. Dialysis using a dialysis membrane (HiMedia Labs, India) with a cut off between 12000-14000 was used to dialyse the suspended pellet in 0.1 M Potassium Phosphate buffer over a period of 48 hours with four changes of buffer. After dialysis, the solution in the dialysis bag was sterilized by filtration through a 0.22 um- PVDF hydrophilic membrane syringe- driven filter (HiMedia Labs, India). This is the cell-free extract (CFE) which was tested for bacteriocin activity against indicator bacteria by using agar well diffusion assay outlined below.

Agar Well Diffusion test

Isolates were selected based on apparent potency using Agar well diffusion assay by the method of Benkerroum *et al*. (1993). Petri plates were overlaid with 6 ml of soft MRS agar (0.7% agar) cooled to 45°C mixed with 400 ìl of 24-hour cultures of the indicator bacteria. The soft agar was then poured into a petri dish which contained previously solidified 1.5% agar. After the soft agar had solidified, 3 holes (wells) were bored on each plate using the broad-end of a 200 ìl micropipette tip. The agar 'buttons' that resulted were then removed and the well bottoms were sealed with a drop of soft agar (Daba *et al.*, 1991). The first well was then filled with 100 µl CFE of bacteriocin solution. The second well was filled with 10 µl 0.1M NaOH (HiMedia Labs, India) solution and 100 ìl of the CFE to rule out the possible inhibition due to organic acid production. The third well was filled with 10 µl 0.1 M NaOH solution and 10 µl of 1mg/ml catalase (Sisco Research Laboratories, India) to eliminate the possible inhibitory action of H_2O_2. If inhibition zone is observed in the third well, it is due to bacteriocin or bacteriocin-like inhibitory substances (BLIS).The plates were incubated for 24 hours at 37°C. At the end of the incubation period, the diameters of the inhibition zones were measured.

Ion exchange chromatography

10 ml of the CFEs obtained from the selected strains were loaded on to a DEAE- Cellulose 52 (HiMedia Labs, India), A-50 column of length 60mm. The gel was equilibrated with 20mM PBS buffer (pH 7.0). The prepared column was washed with three volumes of PBS buffer. The CFE of bacteriocin was then applied to the gel. The extract was eluted by increasing ionic strength and decreasing pH of Citrate Phosphate buffer. The flow rate maintained was 24ml per hour and 1ml fractions were collected and the absorbance recorded at 280 nm. The antimicrobial activity of the collected fractions showing high absorbance at 280nm was determined according to the methods outlined above.

SDS-PAGE analysis

Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) of the CFE samples purified by Ion Exchange chromatography was performed. The partially purified samples obtained by Ion Exchange chromatography were then mixed in a 1:1 ratio with sample buffer (4.6% SDS, 10% b-mercaptoethanol, 20% glycerol, 1.5% Tris base, 1% bromophenol blue) and heated at 100°C for 3 min. 20 ml samples were loaded on to the wells of the gel (4% stacking gel and 15% resolving gel) using low range (3-40KDa) molecular weight standard marker (Sisco Research Laboratories, India) as molecular weight markers. After 3 hours of electrophoresis at 100V, the gel was stained with

Coomassie Blue R-250 for 2 hours and destained overnight.

Characterization of the bacteriocins produced

Cell-Free Extracts (CFE) of the selected strains were subjected to heat treatment (100°C for 30 min) and protease treatment (100 ìl of alkaline protease (Sisco Research Laboratories, India) and Trypsin (Sisco Research Laboratories, India) at 2 mg/ml added to 100 ìl of bacteriocin solution and incubated at 37°C for2 hours. Remnant bacteriocin activity was assayed using agar well diffusion method against the indicator organisms as outlined above. Untreated CFE was used as the control. After 24 hours of incubation at 37°C, the inhibition zones were measured.

Temperature Optimization of Bacteriocin Production

Selected isolates was subjected to different culture conditions to derive the optimum conditions for bacteriocin production in MRS broth (Todorov and Dicks, 2004). Growth and bacteriocin production were estimated at temperatures 15°C, 30°C and 37°C. Bacteriocin assay was performed by agar well diffusion as outlined previously.

Results & Discussion

Selection and characteristics of the isolates

A total of 78 strains of bacterial isolates were collected from the different yogurt and milk samples. Of these 78 isolated strains, 21 showed some promise as bacteriocin-producers. This number was then narrowed down to 4 isolates (viz., YKPMRS-1, YKPM16-2, YKPM16-3, MKPM16-5) based on the size of the zone ofnhibition of the indicator organism. All four were found to be have inhibitory action against *Staphylococcus aureus* but not against *Lactobacillus fermentum*. All the strains were Gram positive, ovoid shape associated in pairs and/or short chains, negative catalase reaction. All the isolated strains could grow at 15°C, 30°C and 37°C but not at 4°C and 45°C. All isolated strains could grow between pH range of 4.8 to 7.8, no growth was seen above or below this range. Strains could grow between 2% to 6% NaCl. Most of the strains were able to grow at 4% NaCl concentration however only some of them resisted the concentration of 6% of NaCl. Concerning the carbohydrate fermentation profile, all the isolates used glucose and lactose. The morphological, biochemical and physiological characteristics of the 4 selected strains of the bacteria isolated strains are shown in Table 1.

Nature of the antibacterial substance

Of the four selected strains, only YKPM16-2 showed no loss of bacteriocin-activity upon trypsin treatment, however, the activity of all four was neutralised by treatment with alkaline protease. All four substances were heat-resistant and showed very little loss of activity after heating at 100 °C for 30 minutes, indicating that all four substances are heat-stable protein.

Production of Bacteriocins

The four isolates showed inhibition diameters between 8mm and 16mm (**Figure 2. & Table 2**). A reduction of bacteriocin production was noticed at 15°C and 30 °C or 96 hours as compared to their control incubated at 37 °C for 96 hours (**Figure 1.**). This is probably due to the problems of growth related issues at temperatures below 37°C. The extract from each production broth was then used in an agar diffusion test which proved that cultures grown at 37°C showed maximum zone of inhibition.

Table 1. -Morphological, biochemical and physiological characteristics of the 23 strains of the bacteria isolated strains

Isolate	Gram Stain	Shape	Size(μm)	Catalase	Oxidative Fermentative (O/F)	Fermentation Tests	Motile	Oxidase	Spore Stain	Acid Fast	Organism identified
YKP MRS-1	+	Rod	2.0x2.5	-	F	HETERO	-	-	-	-	Lactobacillus sp.
YKP M16-2	+	Coccus	1x1	-	F	HETERO	-	-	-	-	Lactococcus sp.
YKP M16-3	+	Coccus	1.5x1.5	+	F	HETERO	-	-	-	-	Lactococcus sp.
MKP M16-5	+	Rod	2.0x2.5	-	F	HETERO	-	-	-	-	Lactobacillus sp.

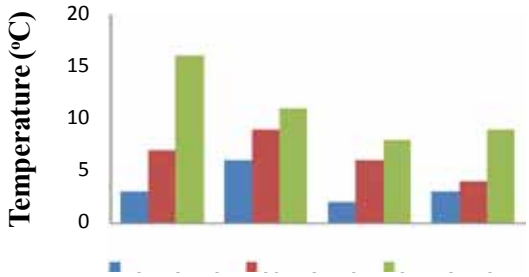

Figure 1. Production of Bacteriocin at varying temperatures

Table 2: Agar Diffusion Assay of the four CFEs

Isolate	Zone of Inhibition Size (mm)		
	Well 1	Well 2	Well 3
YKPMRS-1	8	9	10
YKPM16-2	16	14	13
YKPM16-3	11	13	12
MKP M16-5	14	14	13

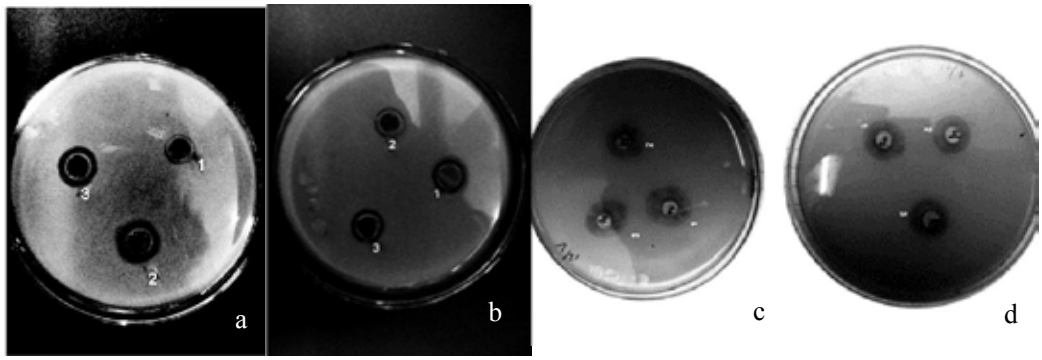

Figure 2. (a, b, c, d) Agar diffusion assay with observed inhibition zones against the indicator organism ***Staphylococcus aureus.***

*(*Well 1-CFE; Well 2-CFE+ pH 7+ NaOH; Well 3-CFE+ pH 7+ NaOH+ Catalase)*

Purification of bacteriocins

The substances produced by strains YKPM16-2, YKPM16-3, YKPMRS-1 and MKPM16-5 and were selected for production and purification. These strains had shown the largest zone of inhibition.

Ion Exchange Chromatography

Elution conditions for DEAE - Cellulose were chosen with a decreasing pH and increasing ionic concentration. **Figures 3(a-d)** are the elution profiles of the bacteriocins isolate from the 4 selected strains.

SDS-PAGE

SDS-PAGE results reveal that the bacteriocins produced by YKPM 16-3. YKPMRS-1 and MKPM16-5 were single proteins in the range of ~20 kDa (17.8 KDa) revealed by the plot of

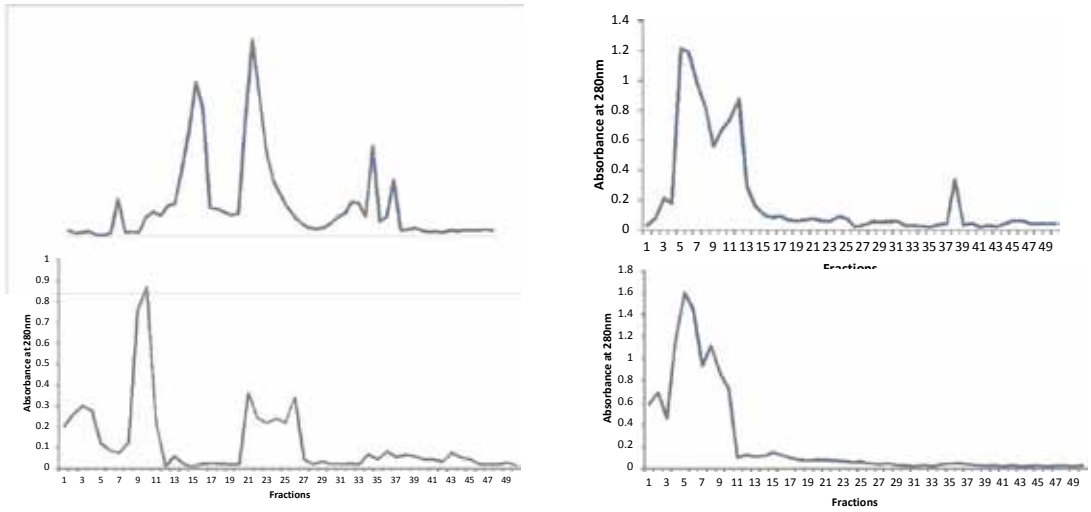

Figure 3(a.) YKP M16-2 DEAE-Cellulose Elution Profile (Fraction 25-34); **(b.)** YKP M16-3 DEAE-Cellulose Elution Profile (Fraction 5-9) **;(c.)** YKP MRS-1 DEAE-Cellulose Elution Profile (Fraction 9-11); **(d.)**. MKP M16-5 DEAE-Cellulose Elution Profile (Fraction 4-11)

Table 3: Table depicting the Rf values of the molecular markers

Size	Log value	Mobility (cm)	Rf	Solvent Front (cm)
43000	4.642465	1.4	0.15	9.2
29000	4.462398	2.1	0.23	9.2
20100	4.303196	2.7	0.29	9.2
14300	4.155336	3.8	0.41	9.2
6500	3.812913	5	0.54	9.2
3000	3.477121	5.9	0.64	9.2

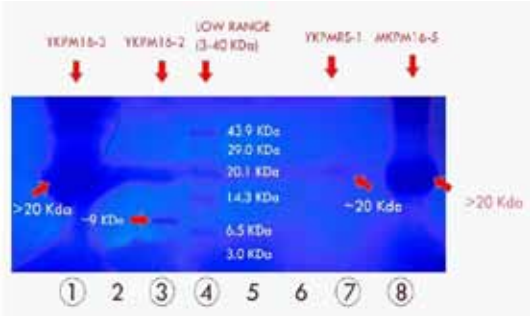

Figure 4. SDS PAGE of the obtained samples after purification

log mol mass versus Rf (**Figures 4 & 5**). The bacteriocin produced by YKPM16-2 however is ~9kDA (8.7 KDa) (**Figures 4 & 5**). **Table 4** describes the molecular masses of the 4 isolated bacteriocins.

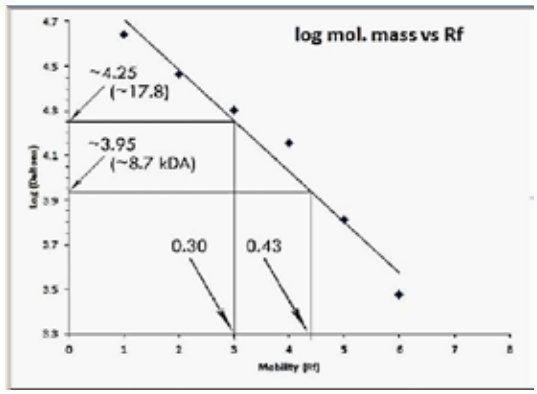

Figure 5. Log molecular mass of the markers vs. Rf

Table 4: Analyzed molecular weight of the samples

Sample	*Molecular weight obtained*
YKPM16-3	~17.8KDa
YKPM16-2	~9KDa
YKPMRS-1	~20KDa
MKPM16-5	~17.8KDa

Concusion and Discussion

Based on the results of SDS PAGE, stability at different pH values, it can be said that the bacteriocins produced by strains YKP M16-3, YKP M16-2, YKP MRS-1, YKP M16-5

belong to class II. Further studies such as the use of adsorption/desorption techniques instead of ammonium sulphate precipitation could probably yield higher purification and greater recovery rates as shown by some authors. Further work could involve the inclusion of more indicator species testsagainst the bacteriocins produced in this study.

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spectrum
SCIENCE AND TECHNOLOGY

St. Anthony's College, Shillong, established in 1934, became the first institute of higher education of the Don Bosco Society world-wide. The college was accredited by NAAC with the rank of Five Stars in 2000. It was also awarded the status of College with Potential for excelence by the UGC in 2006, 2011 and again in 2015. The college was reaccredited with Grade 'A' (3.14/4.00 CGPA) by NAAC in 2014. With more than 75 years of experience and expertise, St. Anthony's College, Shillong excels as one of the top colleges in the country.

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*The front cover page features a photomanipulated image of *Prunus nepalensis* locally called 'sohiong'.