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FEATURE ARTICLE: Application of PCR Technique in Study of Genetic Relatedness of *E. coli*

DEANS' COLUMN: Brief News Column, Human Ecology in Home

RESEARCH NEWS: Starting Exotic, Exotic Smell, Exotic, Exotic in Park, Exotic, Exotic (IV), Exotic

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Application of PCR Technique (RAPD) in Studying Genetic Relatedness Among Strains of *Escherichia coli* Isolated From Raw Meat

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For many years, Malaysia obtained its supply of frozen beef from other countries. The consumption of the imported meat remains a concern in respect of the possible infectivity of the meat product with diverse microorganisms such as *E. coli* strains since the supplies are subjected to diverse surrounding during slaughtering, transportation and handling of the raw meat. It has been reported that imported beef retailed in this country serve as the increasing reservoir for this bacteria. This would have public health significance indicating the need for increased attention to microbiological safety implications associated with their consumption.

Several different methods for documenting genetic information are being used to identify genetic differences among *E. coli* isolates worldwide. Random Amplified Polymorphic DNA (RAPD) technique was found to be one of the relatively easy, highly stable and reproducible method of producing a biochemical fingerprint of a particular species in most researches using arbitrary primers. It generates genetic markers that are different in the sites at which DNA will be amplified by the PCR. Relationships between species may be determined by comparing their unique fingerprint information. Recognizing the advantage of this method, we decided to utilize this approach in studying the genetic relatedness among *E. coli* strains isolated from meat. The main objective of this study was to characterize and determine the genetic relatedness of a collection of *E. coli* strains isolated from various geographic origins and different animal host using RAPD technique. From this study, the RAPD fingerprinting profiles for the *E. coli* isolates can assist to resolve relationship among the *E. coli* isolates from animal sources.

In our study, we investigated a total of fifty *E. coli* strains isolated from animal sources. The isolates were obtained from samples of imported lamb from New Zealand (6 strains), imported beefs from Australia (12 strains) and India (19 strains), and local chicken meat (13 strains) sent to the Veterinary Department's Diagnostic Laboratory at Kota Samarahan, Sarawak for analysis. Twenty random primers were screened for their discriminatory abilities by visualizing the amplification products electrophoretically.



Two primers (OPC-06 and OPC-07) were selected and tested against all the isolates. RAPD analysis using the two primers in combination successfully produced 83 distinctive RAPD patterns. The RAPD profiles obtained contained 3 to 12 bands with the molecular size ranging from 260 bp to 5000 bp. The genetic relationship between the isolates was analyzed by RAPDistance package to produce a neighbour joining tree or dendrogram. Dendrograms of the neighbor joining tree (NJTREE) description file were achieved by means of the Tdraw clustering algorithm program (University of Texas, Houston). Clustering analysis allowed the differentiation of 4 major clonal clusters, each

[continued on page 4]

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Bird fact and myth—The White-rumped Shama (*Copsychus malabaricus*), is a medium size bird with glossy black back and white patch at its rump. It occurs throughout the Indian sub-continent, South China, SE Asia and the Greater Sunder Islands. It feeds mainly on insects and is well known for its melodious songs, which are often heard coming out from the undergrowth in the morning and late afternoon.

**FAUNA
FACTS**



Dean's Column

Professor Mohd Azib Salleh

Brave New Century: Human Cloning is Here!

The biggest news in science in 2001 was the announcement in March of a plan by a group of researchers led by an Italian doctor to start experiments towards human cloning. The proposed experiments, to start in November, gave hope to thousands of childless parents and 200 couples have purportedly volunteered to participate. The announcement drew immediate protests and condemnations from other scientists and the general public. Severe censures and threats of legal actions were also made by certain professional/government bodies. And guardians of science ethics had their field day criticising the new twist in modern biotechnology. The opponents say such plans are fraught with danger citing the case of Dolly the sheep — the world's first cloned mammal — which took 277 attempts before succeeding. The implications would be serious, and fearing bad socio-political repercussions some governments have already passed legislations that would impose a total ban on human cloning. But supporters among the medical fraternity think that a total ban would be counter-productive. There is a strong support, for instance in the UK, for the so-called therapeutic cloning which involves cloning of human embryos for stem cell research. Stem cell research have great potential benefits in medical sciences; it may lead to effective cures for such debilitating diseases as cancer, diabetes, hepatitis and Parkinsons disease.

The religious, ethical and medical aspects of human cloning have dominated debates about social impacts of biotechnology for a few years especially following the birth of Dolly in February 1977. Reproductive cloning would actually "dehumanize" human beings as it would do away with the need for sexual intercourse to unite male and female gametes to form an offspring. Cloning would also be against the known positive attributes of genetic variability. It is stretching the notion of genetic selfishness to its extreme. But the most feared "dark zone" that reproductive cloning is taking us into is the emotional dimension of future human civilizations. Will the future human race be still bound together by the sense of love, commitment and kinship that characterize the present traditional family units? Or will it be partly made up of highly individualistic biological "semi-robots" who owe allegiance only to their "creators" in some reproductive clinics? The beginning of the New

Millennium indeed marks a very critical moment in science and its impact on the human society.

FRST Research Highlights

Shrimp Farming: The Blessing and The Curse

Dr. Lee Nyanti

Aquatic Resource Laboratory

Shrimp farming has grown rapidly worldwide in the past three decades and it has become a large industry. As much of the wild fish stocks are fully or overexploited and landings from marine and inland captured fisheries have remained constant since a decade ago, shrimp farming is considered as one of the solutions to meet the demand for fish protein. Apart from meeting the human needs for food security, shrimp farming could also play an important economic role by creating new economic niches, generating employment, utilizing local resources in a more effective way, providing local sources of high-quality food and providing opportunities for productive investment in the local economy.

World shrimp production has increased from about 80,000 metric tons in 1982 to over 800,000 metric tons in 1999. The exceptional profitability of cultured shrimp is one of the driving forces that make this industry grows. It is not unusual that investments could be fully recovered from profits received from two crops of shrimp or in about a year in tropical countries.

However, if not properly planned, shrimp farming could have negative impacts to the environment such as the destruction of mangroves and wetlands, pollution of water, reduction of biodiversity, salinization of fresh water, and wastage of natural resources. A reduction in environmental quality of the adjacent water bodies can have negative feedback on shrimp pond operations due to the promotion of infectious diseases, noxious algae blooms, introduction of toxic substances, and increasing sediment loads. Poorly regulated shrimp industries have resulted in complete or partial collapse of shrimp industries in Taiwan, Thailand, China and Ecuador.

In order for shrimp farming to be sustainable, it must takes into account both the impact of shrimp ponds on the whole ecosystems as well as the ability of ponds to be used for a long period of time. Therefore, any reduction in the impact of shrimp pond effluent on the environment would be viewed as

contributing positively to sustainable shrimp farming. A number of techniques, including sedimentation pond have been used in an attempt to improve the quality of effluent from shrimp ponds.

A brief study carried out at an earthen commercial shrimp farm located in the Kuching area indicated that the amount of effluent discharged during harvest from a hectare of shrimp pond is about $1.1 \times 10^3 \text{ m}^3$. Therefore, waste from shrimp farms could have potentially deleterious effects to the environment if it is discharged into the adjacent water body untreated. However, effluent that was retained in sedimentation pond for 16 hours showed significant improvement in water quality. There was a decrease of 48% in the concentration of ammonia nitrogen, 92% in soluble reactive phosphorus, 79% in turbidity, 55% in total suspended solids and 45% in biochemical oxygen demand. Therefore, shrimp operators should seriously adopt the use of sedimentation pond to reduce the input of nutrients into the surrounding water bodies.



Figure: Pond effluent being discharged during harvesting.

First Incidence Of Paralytic Shellfish Poisoning On The East Coast Of Peninsula Malaysia

Lim Po Teen

Aquatic Resource Laboratory

The first incidence of paralytic shellfish poisoning (PSP) on the east coast of Peninsula Malaysia was reported in Tumpat, Kelantan. In September 2001, six persons were hospitalized and one fatality was reported following the consumption of contaminated toxic Lokan, *Polymesoda* sp. A plankton survey was conducted at the affected area a week after the incident. The area is a semi-enclosed lagoon formed by sand bars at the mouth of Sungai Golok. The lagoon is fringed by *Nipah* palms, with water depths ranged from 3 to 6 m and

[continued on page 3]

salinity ranged from 25 ‰ at the river mouth to 12 ‰ at the inner part of the lagoon. Plankton samples were collected using a 20µm mesh size plankton net from six locations. Lokan samples were also collected from the site where the contaminated shellfish were found.

Morphological observation of plankton samples showed that the dinoflagellates *Alexandrium minutum* and the yet unidentified *Alexandrium* species were most probably the progenitors of this incident. The results also showed that most of the *Alexandrium* cells were found at the inner part of the lagoon, whereas no *Alexandrium* cell was found at the other four seaward locations. Laboratory cultures of the dinoflagellates have been successfully established. Toxicity testing by mouse bioassays confirmed the presence of PSP toxins in extracts from the Lokan and the cultured *Alexandrium* cells.



**Research Report: Animal Resource
Biology Field Expedition To Pueh,
October-November 2001**

Assoc. Prof. Dr. Andrew Alek Tuen
Animal Resource Biology Core Group

Tropical highland has always fascinated scientists because they are well known as centres of endemism. The Pueh-Berumpit highland located along the Sarawak-Kalimantan border about



100 km to the west of Kuching has not been studied but montane species such as the mountain treeshrew *Tupaia montana* and Mountain Black-eye *Chlorocharis emiliae* have been recorded there (MacKinnon *et al.* 1996, Smythies 1999). In 2001, the Animal Resource Biology Core Group secured a research grant from Unimas to study the effect of

habitat disturbance on the diversity and abundance of butterflies, frogs, rodents, bats and birds of Gunung Pueh.

Our fieldwork was conducted in the forest at the lower slopes of Gunung Pueh (1,291m) above the Zuhrah Pelangi mulberry farm (1° 48.23' N and 109° 42.68'E) from 22nd October to 1st November 2001. Butterfly sampling was done using aerial net in daytime and restricted to the forest understory, open habitats in clearing and along the creek banks. In addition, nine traps baited with rotten pineapples and bananas were set up randomly in the forest and along the creeks. Frog surveys were conducted in freshwater streams and ponds in heavily disturbed areas which include the mulberry farm, pepper gardens and picnic sites as well as in the less disturbed forest located upstream from the farms. Sixty baited mousetraps were used to catch rodents and 20 mist nets were used for bats and birds. These were placed at random in highly disturbed areas represented by the secondary forest near the edge of the mulberry farm and in less disturbed habitat located about 400 m away from the edge of the farm. The animals and birds caught were identified, weighed, measured, tagged and then released. Some specimens of selected species were euthanised and preserved in alcohol for the Unimas museum.

The lowland forest of Pueh was found to support a great diversity of butterflies, particularly the family Pieridae, Nymphalidae and Satyridae. A total of 151 specimens of butterflies comprising 69 species from 7 families were collected. The species characteristics of the secondary vegetation such as Common Grass Yellow butterfly (*Eurema hecabe*), Blue Pansy (*Junonia orithya*) and *Ypthima*

pandocus were frequently encountered along the waysides and in the open areas.

Ninety-six frogs from 18 species were captured during seven nights of sampling. The dominant family was Ranidae with 65.6% of the total captures. The White-lipped Frog (*Rana chalconota*) was the dominant species

with 21.9% of the total captures followed by the Kuhl's Creek Frog (*Limnonectes kuhlii*) with 14.6%.



Trapping effort of 600 trap-nights yielded 13 rats, 9 squirrels, 21 treeshrews and one moonrat. The most abundant species was the large treeshrew, *Tupaia tana* with six individuals caught in highly disturbed habitat and eight individuals in less disturbed habitat. The most common species in the highly disturbed habitat comprising 6 individuals, was the Three-striped Ground Squirrel (*Lariscus insignis*) from the family Sciuridae. Plantain squirrel (*Callosciurus notatus*) and a moot rat (*Echinosorex gymnarus*) were caught in the more disturbed habitat only. In contrast, the common species in less disturbed habitat was the large treeshrew, *Tupaia tana* and the long-tailed giant rat, *Leopoldamys sabanus*.

A total of 173 bats representing 14 species from five families were captured in the mist nets; 90% of the bats were fruit bats. Eighty four percent of the bats were captured in more disturbed habitats while the rest were captured inside less disturbed habitats. Netting success was higher in the more disturbed habitat compared to less disturbed habitat (1.46 vs 0.27 bat/net-night). Short-nose fruit bat, *Cynopterus brachyotis*, was the most abundant species and make up 54% of the bats captured in the study. The orange tube-nosed bat, *Murina cyclotis*, caught in less disturbed habitat was the second record for Sarawak. According to Payne *et al.* (1985) the first record for this bat in Sarawak was in Mulu.

A total of 205 birds from 46 species were mist-netted during the expedition. The most abundant species was the Little Spiderhunter (*Arachnothera longirostra*) comprising 24.4% of the total capture followed by Grey-headed Babbler (*Stachyris pliocephala*), Yellow-bellied Bulbul (*Criniger phaeocephalus*) and Black-headed Bulbul (*Pycnonotus atriceps*). One hundred and twenty seven individuals (61.95 %) were caught in

[continued on page 4]

more disturbed area while 78 individuals (38.05%) were caught in less disturbed area. During an exploratory trip to the top of Gunung Berumpit (1,486 m) on 29-30 October 2001, a Temmincks babbler (*Pellorneum pyrrhogenys*) was caught. This bird is resident to montane areas.

The high capture rate of birds and bats in highly disturbed areas is probably related to the abundance of wild fruits there. Many bats and most birds caught were in breeding conditions suggesting that breeding probably coincided with the fruiting season in the expedition area.

Organotin(IV) Complexes With 2-Acetylpyridinebenzhydrazone Ligand, I: Reactivity Studies Of Organotin(IV) Chloride As A Lewis Acid Towards Crystal Structures Of [Me₂SnCl(C₅H₄N(Me)C=Nncoph)] II and [PhSnCl₂(C₅H₄N(Me)C=Nncoph)] III.

Dr. Md. Abu Affan

Inorganic Chemistry Laboratory

It is becoming increasingly apparent that the coordination chemistry of the various metal ions is of relevance in a wide variety of chemical, pharmacological activities and analytical applications. The chemistry of hydrazones have been intensively investigated in recent years, owing to their capability to coordinate to metal either in a keto or in an ionic (iminol) form via the loss of amide hydrogen. There has been a great deal of interest in organotin(IV) complexes of hydrazones because they displayed a variety of structural formulae and a lot of chemical applications such as new antitumour agents, pharmacological activities and bactericides.

Although the complexation reactions of hydrazones and nitrogen—oxygen donor ligands have been studied for many years, the derivatives of tin(IV) and organotin(IV) moieties with these ligands have aroused interest in view of the many salient observations in the organotin(IV) chemistry.

Recently, we have synthesized the 2-acetylpyridinebenzhydrazone ligand, I and studied its coordination characteristics towards two types of organotin(IV) chloride including the X-ray single crystal structures [Me₂SnCl(C₅H₄N(Me)C=Nncoph)], II

and [PhSnCl₂(C₅H₄N(Me)C=Nncoph)], III.

The research found that the ligand coordinated with dimethyltin(IV) through the azomethine nitrogen atom and deprotonated oxygen of the ethanolic group of 2-acetylpyridine benzhydrazone, I. The X-ray single crystal structure of II is shown in Figure 1, which revealed a distorted trigonal bipyramidal geometry. On the other hand, in the structure of III, the hydrazone ligand was found coordinated in a tridentate fashion to central tin atom which is in a distorted octahedral geometry.

The reactivity of benzhydrazone, I towards MeSnCl₂ and PhSnCl₃ acceptors depends on the electronic and steric features of the groups bonded to tin(IV). The acceptor strength of the tin derivatives R_nSnX_{4-n} decreases in the following order:



where X = NCS > F > Cl > Br and



From the above acceptor strength of this series, it is clear that tin atom in II has lower tendency to accept electron from I than III. It is argued that two methyl groups have electron donating tendency to tin atom in II but two chlorine atoms and one phenyl group have electron withdrawing tendency from tin atom in III, so tin atom easily coordinated to pyridine ring nitrogen in III. As a result, the complex III showed a distorted octahedral geometry. It is evident that PhSnCl₃ acts as a stronger Lewis acid than MeSnCl₂ towards benzhydrazone ligand I.



Figure 1: An ORTEP diagram for [Me₂SnCl(C₅H₄N(Me)C=Nncoph)], I

(from page 1)

comprising of 6 to 19 strains of *E. coli* which correspond to their animal host and geographical origins. Strains isolated from chicken meat were the most homogeneous whereas those isolated from beef were heterogenous.

Results from this study has revealed significant diversity among the *E. coli* strains even when they are isolated from a single source which is raw meat. It is likely that the organism, although isolated from an animal host of different country origins, may have undergone genetic changes with interaction in different environment conditions that increased the evolution of these bacteria strains. The different RAPD types revealed in this study can be considered variant genotypes of a major clonal group, irrespective of variations in their geographical origin or animal host, representing the result of recent evolutionary divergence. It is most likely a result of both multiple environmental conditions and selective pressure that the bacteria were subjected to their respective host. Among these strains, previously found to have identical antibiotic resistance or plasmid profiles do not display any similarity in RAPD patterns, indicating that the RAPD method is more sensitive and capable of evaluating the extent of genetic variation among similar strains of bacteria. The presence of single point mutation anywhere in the genome might be amplified by RAPD. Hence, it is notable that RAPD can differentiate between isolates that are clonally related, which represents an additional increase in sensitivity and flexibility, allowing detection of significance intraspecies variation that would otherwise have gone unnoticed.



Figure: RAPD profiles of *E. coli* strains isolated from imported beef using primer OPC-06.

In conclusion, RAPD analysis is an efficient technique in differentiating the *E. coli* isolates. This is important as the organism is potentially pathogenic and any new information can assist in future epidemiological investigation of diseases related to the organism.