



Synthesis

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BioCarrier™:

A Novel Molecular Carrier

from Newcastle Disease Virus

Khatijah Yusoff, Tan Wen Siang, Kho Chiew Ling, Amir Rabu

Award Winner

Various kinds of carrier molecules have been used to deliver nucleic acids, drugs, vaccines and other important compounds. A molecular carrier for immunogenic peptides has been developed which can be used for the development of multicomponent vaccines, diagnostic reagents and the delivery of novel therapeutics or drugs (Malaysian Patent Pending: PI 20004837 and PI 20021709; US Patent Application No. 09/970,851).

It has been discovered that the nucleocapsid protein of a local Malaysian poultry virus (Newcastle disease virus) produced in *Escherichia coli* assembles into ring-like and herringbone-like particles. It is possible to manipulate the lengths of these particles by genetically engineering one end of this nucleocapsid protein. In

addition, these particles are able to be fused with foreign polypeptides. Some of the advantages of this carrier molecule are its ability to increase the solubility of proteins, deliver immunogenic peptides or proteins in animals, as well as aid the purification of specific protein molecules.

Several fusion proteins have been constructed. These include the antigenic regions of the Newcastle disease virus, Nipah virus, hepatitis B virus, enterovirus virus 71, chicken anemia virus, and infectious bronchitis virus. Chickens inoculated with these particles mounted an immune response against the foreign peptides, supporting the use of these particles as carriers for immunogens in the development of multicomponent vaccines and immunological reagents.

GOLD – I-Tex 2003

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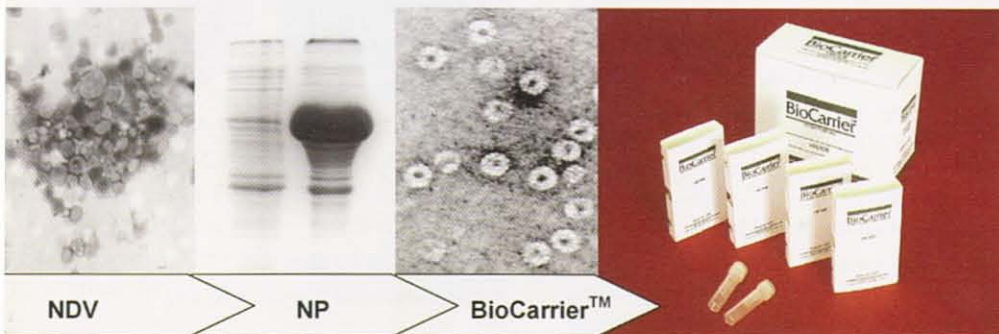
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Editorial

I have pleasure in presenting you with the first issue of this quarterly new research bulletin: R&D Digest of Universiti Putra Malaysia which is a continuation of "Berita Penyelidikan" that was published by the Universiti Press. The main focus of this bulletin is to keep you abreast of the latest developments in the multidisciplinary research being conducted at the university. This inaugural issue marks Research Management Centre's (RMC) entry into globalisation; and as you can visualise, we are doing it amidst a swirl of colours! Yes, the image of research at UPM is changing!

RMC believes that the interests of both science and development are best served by promoting and nurturing the research efforts of our scientists, particularly the young scientists, who are at the beginning of their research careers. RMC has also indeed put concerted effort to improve the image and voice of UPM's scientists to ensure that they continue to be at the forefront of its goals.

I truly hope that your challenges and expectations will be even more daunting. Let me say thank you to the UPM academia for their valuable contributions in reserach.

I hope that you will find this bulletin helpful and informative.

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Spotlight

Research is increasingly an activity that is not constrained by boundaries. Discipline boundaries are also increasingly irrelevant. The next big priority will be to promote interdisciplinary research in the University. The University Research Committee (URC) with Research Management Centre (RMC) acting as a secretariat has come up new initiatives to cater this aspect of research by grouping core areas of research into "research clusters". The formation of such clusters will allow the academic and research community at UPM to achieve its best by working closely across the various faculties, research institutes and centres of excellence at UPM. The initiative of restructuring our research into niche priority areas and grouping them by research clusters is to nurture integrative research towards the development of commercialised research output, further strengthening and enhancing R&D—thereby contributing significantly to the growth and development of the Malaysian economy.

The 8 clusters of research that have been launched at UPM are:

1. Agriculture, Food and Forestry (AFF);
2. Bioscience and Biotechnology (BAB);
3. Economics, Management and Accounts (EMA);
4. Health and Allied Sciences (HAS);
5. Information Technology and Mathematics (ITM);

6. Materials, Energy and Environment (MEE);
7. Science and Engineering (SAE); and
8. Social Science and Humanities (SSH).

Each research cluster is represented by a striking hallmark—an icon that clearly indicates the rationale behind the cluster.



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Probiotic for Poultry

Y. W. Ho, R. Kalavathy, L. Z. Jin,
N. Abdullah and
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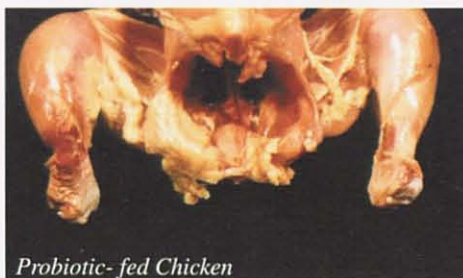
Award Winner

For more than 50 years, antibiotics have been used as growth promoters in livestock production. However, in recent years, there is a growing concern on the continuous feeding of sub-therapeutic levels of antibiotics to livestock which may result in the presence of antibiotic residues in animal products and the development of antibiotic-resistant bacteria that are dangerous and potentially lethal when transmitted to humans. Such negative potential motivated many developed countries, such as the US, the European Union and Japan to either severely restrict or totally ban the use of antibiotics in animal feeds as a growth promoter. This led to considerable interest to find other ways to achieve the same growth promotion without the use of antibiotics. Probiotics, which are viable friendly bacterial cultures that have a beneficial effect on the health of a host when taken as a feed supplement, have been considered as a substitute for the antibiotic growth promoters.

The product developed by us at the Institute of Bioscience, Universiti Putra Malaysia, is a probiotic for poultry which can be used as a natural substitute for the antibiotic growth promoters to increase performance in chickens (broilers and layers) and as a hypolipidaemic agent to reduce fat and cholesterol in broilers, and cholesterol in egg yolk. It consists of a mixture of 12 *Lactobacillus* strains isolated from the intestines of local chickens. The *Lactobacillus*-probiotic is in a powder form, made up of viable freeze-dried *Lactobacillus* strains mixed in cornstarch. Viability of the *Lactobacillus* strains in the mixture is maintained at a high level – at about 1×10^9 viable cells per gram. Only a low dosage of 0.1% of the probiotic is required to be incorporated into the feed daily.

Twelve feeding trials have been carried out to evaluate the effects of the *Lactobacillus*-probiotic, used in place of antibiotic growth promoters, to enhance the growth performance and feed efficiency of broiler chickens and laying hens. Results from the feeding trials consistently showed that probiotic-fed broiler chickens have better growth performance, feed efficiency and immune response, less mortality rate, less pathogenic bacteria and noxious bacterial enzymes in their intestinal tract, and less body fat and cholesterol than the control broilers fed without probiotic. When compared to antibiotic-fed broiler chickens, probiotic-

fed chickens also have better growth performance, feed efficiency and immune response, and less mortality rate. Similarly, supplementation of the probiotic to laying hens also improves the growth performance, feed efficiency, egg production, especially at the initial laying period, egg weight and egg size, and lowers the cholesterol level in egg yolk at the initial laying period. The egg weight is increased by 1.1 gram per egg, and there is a shift from small and medium to large and extra large eggs in the probiotic-fed hens.



Probiotic-fed Chicken

the chances of them surviving and proliferating in the intestinal environment of the chicken are even better.

The *Lactobacillus*-probiotic contributes not only economic benefits to the poultry industry, but also health benefits to society as a whole. Probiotic-fed broiler chickens, and eggs produced by probiotic-fed hens are considered safe and healthy food as they are antibiotic-free and have low fat and cholesterol. Cost of production is lowered since performance and feed efficiency of the chickens are enhanced. Additional profit can be obtained from premium price of antibiotic-free, low fat and cholesterol broiler chickens and eggs. Increase in egg production and egg size will further enhance profit.



Control Chicken

Large fat deposition in the control chicken compared to the reduced fat deposition in the probiotic-fed chicken

Although imported commercial probiotics are available in Malaysia and are used by some swine farmers, inconsistent results of using probiotics in poultry production have been a constraint to the promotion of their use. Variations in the foreign microorganisms and decreasing viability in local hot and humid conditions probably led to the inconsistent results in using imported probiotics in the poultry industry. However, the results from all our feeding trials consistently showed that our *Lactobacillus*-probiotic supplemented to chickens significantly improves their performance and feed efficiency, and can be used as a replacement for antibiotic growth promoters. The probiotic also has a hypolipidaemic (fat and cholesterol-reducing) effect on the chickens. The probiotic developed from our research, which consists of a combination of 12 *Lactobacillus* strains isolated from the intestines of local chickens (host-specific), has an advantage over other single-strain probiotic or strains isolated from other sources (non-host-specific). As the gastrointestinal tract is a complex and hostile environment, a multi-strain probiotic is more effective in influencing the microbial ecology of the host, particularly if it is intended to generate a beneficial impact on the intestinal microflora. If the probiotic strains are host-specific, as ours are,

The *Lactobacillus*-probiotic has great commercial potential as many countries have banned or severely restricted the use of antibiotics in animal feeds, and other countries that have not done so will eventually follow suit. The poultry industry, at present, is under pressure to seek a substitute for antibiotics as a growth promoter, and to produce chickens with less fat and cholesterol as consumers demand safer and healthier food. Commercial potential for global market is huge as the poultry industry in the world is a large and successful one. In Malaysia, the poultry industry produces 1 million broiler chickens and 40 million eggs a day.

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ZAPPA™ – the rice seeds germination enhancer and the control of "Padi Angin" infestation

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Ahmad Husni Mohd. Hanif.



Minister of Agriculture, LPP Chairman and Chief Director of LPP ZAPPA,
during the exhibition of PPK recently

The government aspiration to increase rice yield up to 10 t/ha may be hampered if infestation of "padi angin" or weedy rice cannot be controlled. Serious infestation of weedy rice had been reported to reduce rice yield up to 75 %. ZAPPA or "Zap Padi Angin" is specially formulated as paddy seed treatment to enhance rapid seed germination for direct seeding rice grown under aerobic and anaerobic systems.

ZAPPA treated paddy seeds have shown that the root and shoot growth of 3-day old rice seedling increased to about 120 % and 90 %, respectively. Paddy seeds treated with active oxygen in ZAPPA were able to grow vigorously under anaerobic direct seeding (about 5 cm water depth), thereby, delayed or

suffocated the untreated weedy rice seeds present in the soil. The local verification trials of ZAPPA conducted on two farmers plot each 1.2 hectares in Sg. Besar, Selangor, which were previously infected with weedy rice had shown a yield increment between 40 and 57 %. MARDI researchers in Tg. Karang and Bertam had also evaluated the effectiveness of ZAPPA for seed germination and the control of padi angin.

The result showed that seeds treated with ZAPPA were able to grow at 5 and 15 cm of water depth but tillers number was greatly reduced at 25 cm of water depth. Others benefits of using ZAPPA were i) reduce weeds problem, ii) reduce rat attack because of standing water, iii) conserved water

usage (water was not removed after plowing), iv) reduce seed borne diseases, and v) increase seed purity. Since March 2001, about 40,000 liters of ZAPPA were sold. Many farmers had accepted ZAPPA for both the aerobic and anaerobic direct seeding due to its effects on seedling vigor that helps to compete with the weeds growth.

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Enzyme-Aided Peeling of Local Citrus Fruit – An Alternative to Conventional Methods

A.Osman, H.M.Ghazali, R.Abdul Rahman, S.Yusof, F.Liu, H.Adnan and A.G. Aziz

Award Winner

Citrus is considered as one of the important fruit crops in the tropics and sub-tropical region. They are primarily evergreen species belonging to the order Geraniales and family Rutaceae. It has been reported that presently, there are about 1600 species of citrus in the world amongst which only a few of the more well known species are widely cultivated in Malaysia. These include the Chinese mandarin (*Citrus reticulata*), the local mandarin (*Citrus suhuiensis*) which is also known as "limau langkat", the pomelo (*Citrus grandis*), the musk lime (*Citrus microcarpa* Bunge or *Citrus mitis* Blanco) and the common lime (*Citrus aurantifolia*). In Malaysia, most citrus fruits are grown in Cameron Highlands in small farms. *Citrus reticulata* are grown in the cooler region while the species, *Citrus suhuiensis* or limau langkat is mainly grown in the warmer region. Limau langkat is a local loose-peel citrus species which has developed into many varieties and clones, the most prominent and popular being the Limau Madu (local mandarin).

Citrus fruit is one of the most widely marketed fruits in the fresh and processed form. Limau bali is the largest fruited citrus, round to flattened, or pear-shaped and the surface of the fruit is characterised by greenish dots which are oil glands. Fruit segments are very large and vary in colour from yellow to pink or pinkish red. The thick leathery skin that covers the juice vesicles is one of the important characteristics of the fruit. The pulp is light yellow or pink with a sour bittersweet taste. On the other hand, musk lime or locally known as limau kasturi is among the smallest citrus fruit, having a very thin skin that is strongly attached to the fruit segments and is difficult to peel normally. In the industry, problems usually arise in obtaining a high quality (non-bitter) musk lime juice. The extracted juice taste bitter due to the presence of the compound, naringin, which is found mainly in the flavedo (green portion) and albedo (white portion) of the peel and to a smaller extent in the seeds and the outer membrane of fruit segments. The present extraction of musk lime juice using the screw-press is impractical since the bitter compound is



Plate 1. Enzyme-peeled local mandarin

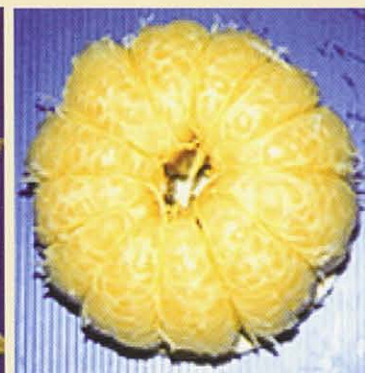


Plate 2. Enzyme-peeled pomelo

also extracted into the juice during processing, therefore, the peel and adhering albedo have to be removed before the extraction and this can be easily achieved by using enzyme to aid peeling.

In recent years, the demand for freshly prepared fruits and vegetables in supermarkets and restaurants has increased. The production of minimally processed, fresh produce is a new emerging industry in Asia, offering nutritious, convenient products with fresh-like qualities. These minimally processed fruits offer the advantages of fresh like quality without the inconvenience of waste of home peeling, slicing and trimming.

Peeling is one of the most important preparatory stages in the processing of fruits and vegetables. Proper peeling method employed is important, as the quality of the products depend, to a large extent, on the methods used. Peeled, cleaned, citrus segments were previously prepared traditionally by hand to remove excess albedo, strings and core material clinging to the segments causing undue bitterness or unsightly appearance. It also resulted in loss of juice from cutting through segment membranes during the trimming process. Other alternative methods are the steam and lye peeling. These two methods had some disadvantages such as the formation of brown ring (heat ring) below the surface of the fruit due to tissue damage by polyphenol enzyme activity during steam peeling. While the disadvantages for lye peeling are high peeling losses, loss of damaged fruits and pollution of large

volumes (2600 gal/ton fruit peeled) of water.

The peeling of fruit with enzyme is a new technology. This method uses vacuum infusion, whereby fruits were placed in an enzyme solution and put under vacuum to allow the solution to be drawn into the evacuated pores and intracellular spaces of the fruit. Enzymatic peeling has been defined as the application of exogenous enzyme specially to alter the characteristics of intact tissues. Pectic enzymes have been found to be able to selectively alter the albedo structure of citrus fruits and thus aid in the removal of citrus peel and the adhering albedo. In addition, this method is considered as "green" method as enzyme are proteins that are easily degradable and causes no harm to the environment. The advantage of this mode of peeling is that it incurs less wastage due to broken segments as compared to hand and chemical peeling. Furthermore, enzyme-peeled fruit segments are more appealing, as it is much shiny in appearance and has a more intense colour.

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Development of Malaysian isolate of *Spodoptera litura* nucleopolyhedrovirus (SpltnPV) biopesticide for controlling cruciferous vegetable pest, *Spodoptera litura* (Fabricius) (Lep., Noctuidae)

Ahmad Said Sajap, Mohamad. A. Bakir, Hussan. A. Kadir, Norani. A. Samad and Lau Wei Hong



Baculoviruses are pathogens that can cause lethal infections to many insect pests. In Malaysia, one of these baculoviruses is commonly associated with an armyworm, *Spodoptera litura* (Fabricius) (Lep., Noctuidae), a serious pest of many agricultural and forestry crops. Though the impact of the disease on *S. litura* population in the field has yet to be confirmed, SpltnPV has the potential to be used as a control agent to be incorporated in the management of the pest in Malaysia.

A study on the characteristics of SpltnPV is therefore warranted before it is recommended for field application. This study examines the infectivity of nucleopolyhedrovirus of *Spodoptera litura* (Fabricius) (Lep., Noctuidae) in different pH suspensions and exposure to sunlight prior to ingestion and effects of rearing temperature on the mortality of infected larvae.

The result shows that mortality significantly increased by 47% from 53% when the larvae were reared at 20°C to 100% at 30 and 35°C. There was a positive correlation between mortality and rearing temperature, indicating that the infectivity of the SpltnPV increased with the increase in larval rearing temperature as reflected by a significant reduction in LT50 values



from 24 to 6 days. All virus suspension used in the experiment induced mortality to more than 90% except in pH 11, where it was 64%. The LT50 increased to about four folds when the pH of suspension was increased from 7 to 11. Thus the virus infectivity was more adversely affected in alkaline than in acidic suspensions. Like many baculoviruses, sunlight also deleteriously affected the virus. A complete viral inactivation occurred after 12 h of direct sunlight. To offset this shortcoming, options in protecting the virus from detrimental effects of sunlight using optical brighteners was considered. Laboratory trial and field trials using SpltnPV formulated with UV-protectants show that Tinopal provided protection for SpltnPV against sunlight

inactivation and also enhanced its infectivity. The yield recorded from SpltnPV-treated *Brassica rapae* was comparable with that of the yield from chemically treated crop.

The results from this study prove that SpltnPV has the potential to be developed as a biopesticide for controlling *S. litura*.

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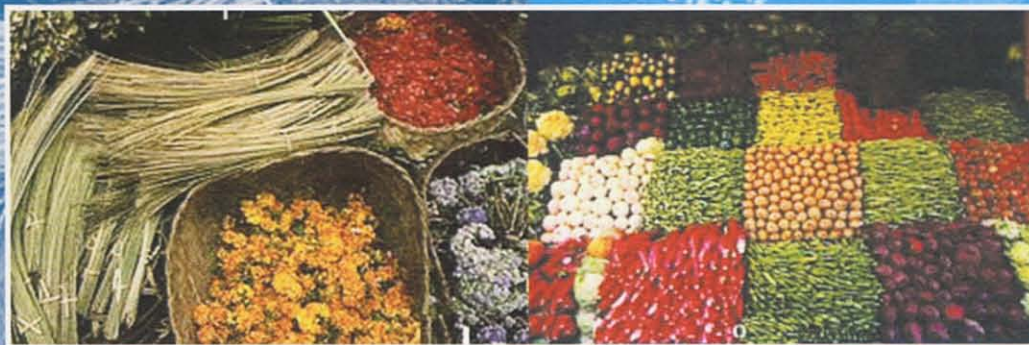
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Production of Natural Flavoring and Coloring Powders from Local Edible Plants by Using Different Drying Methods

Tan Chin Ping



Drying is an ancient process used to preserve foods. It is the most common food preservation process. With literally hundreds of variants actually used in drying of particulate solids, pastes, continuous sheets, slurries or solutions, it provides the most diversity among food engineering units operations. The quality of food powders is based on a variety of properties depending on the specific application. In general, the final moisture content, the insolubility index, the rheological properties and the bulk density are of primary importance.

In recent years, natural food ingredients have been recognized for their health-promoting qualities. Therefore, much interest has been devoted to preparing flavoring and coloring compounds from natural sources by extraction, purification, and

isolation. Continued interest in natural compounds by consumers showed that they are just as concerned about their health as they are about the quality of the foods they consume. In Malaysia, many plants, spices, and herbs contain natural flavoring and coloring compounds. In this study, several local plants such as pandan (*Pandanus amaryllifolius*), lemon grass (*Cymbopogon citratus*) and roselle (*Hibiscus sabdariffa* L.) will be selected.

Nowadays the main challenges in the production of powders are the development of specialities and the reduction of processing costs. For this the production capacity of available installations is maximized, the process conditions are directed towards minimal fouling of equipment, minimal product losses and reduction of energy

consumption, and on-line product quality control is implemented as far as possible. Therefore, it is important that standard/ optimum operating criteria and processing conditions be established that will ensure a prime quality feature of dried powder products during drying processes. The developed drying techniques can be applied to produce encapsulated flavor and color powder for use in various food products.

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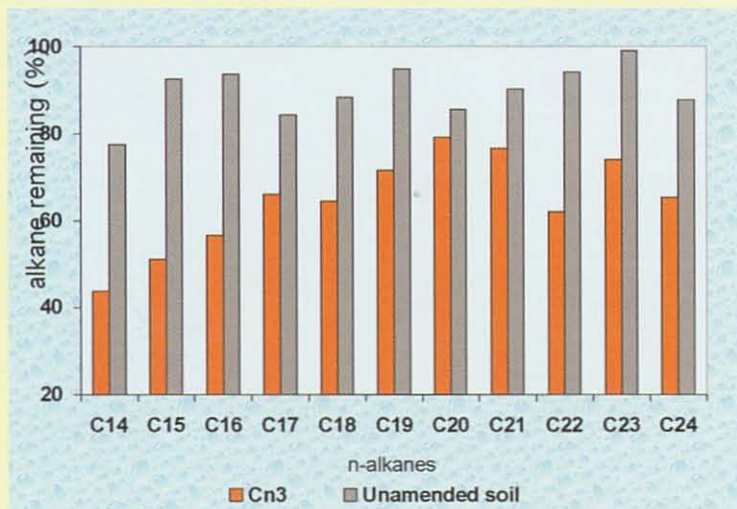
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Bugs at Work: The Oil-Busters

Abu Bakar Salleh, Farinazleen Mohamad Ghazali,
Raja Noor Zaliha Abd Rahman, & Mahiran Basri

Award Winner



Degradation of hydrocarbons in diesel-contaminated soil

This study investigated the biodegradation of hydrocarbons by hydrocarbon-degrading microorganisms isolated from contaminated Malaysian soils. Mixed cultures consisting of 3, 5 and 8 different isolates were constructed to test the effectiveness of the bacterial population in removing hydrocarbon contamination in our environment. Through preliminary studies, it was seen that that the microbial mixture consisting of 8 bacterial strains were more effective at biodegrading hydrocarbons compared to the mixtures containing 3 or 5 isolates. Under laboratory conditions in liquid medium, the consortium was found to be effective at degrading crude oil at temperatures between room and 40°C, at both seawater and freshwater salinity and at pH values between 6 and 9. Following the investigation in liquid medium, the consortium was applied to soil that were contaminated with diesel, used engine oil or crude oil. It was observed that the microbial mixture could effectively degrade hydrocarbons in the soil. Better remediation was seen in the soil that was contaminated with the engine oil. Short- and medium-length alkanes could not be detected in the soil thirty days after

the addition of the microbial mixture. These findings indicate the ability of the consortium to treat environmental pollution, offering a better alternative for the treatment of oily wastes.



Site of diesel spill

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BRONZE – I-TEX 2003

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About RMC

Research Management Centre (RMC) was officially established in May 2000 to replace the former University Research Unit that was formed in 1992. RMC is poised to play the role of the engine of growth by increasing and diversifying UPM's funding resources.

In brief, the role and functions of the RMC are:

- To enhance and strengthen the current research management system;
- To increase and diversify UPM's funding resources from the government or the private sectors;
- To provide world-class infrastructure for research;
- To increase collaborative R&D efforts between public R&D agencies and the private sector to facilitate commercialisation of research findings.

The Centre headed by a director of research and assisted by three deputy directors reports to the Deputy Vice Chancellor (Academic) and serves as the secretariat for the University Research Committee.



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