ISSN-0974-1550

ENVIS NEWSLETTER

MICROORGANISMS AND ENVIRONMENT MANAGEMENT

(Sponsored by Ministry of Environment and Forests, Government of India)

VOLUME 9 ISSUES 2&3 Apr & Jul 2011

ENVIS CENTRE
Department of Zoology
University of Madras, Maraimalai Campus, Guindy, Chennai - 600 025
Telefax: 91-44-22300899; E-mail: dzum@envis.nic.in; enviscoordinator@gmail.com
Websites: www.envismadrasuniv.org; www.dzumenvis.nic.in; www.envismicrobes.in (Tamil website)

ISSN - 0974 - 1550

Volume 9 | Issues 2&3 | Apr & Jul 2011

EDITORS

Prof. N. Munuswamy

(ENVIS Co-ordinator)

Dr. T. Sathish Kumar

(Scientist - D)

ENVIS TEAM

Prof. N. Munuswamy (Co-ordinator)

Dr. T. Sathish Kumar (Scientist - D)

Mr. T. Tamilvanan (Programme Officer)

Mr. D. Siva Arun (Programme Asstt.)

Mr. M. Manirajan (Data Entry Operator)

PUBLISHED BY

Environmental Information System (ENVIS)

Department of Zoology

University of Madras, Maraimalai Campus, Guindy

Chennai - 600 025, Tamilnadu, India.

SPONSORED BY

Ministry of Environment and Forests

Government of India

New Delhi.

International Biodiversity Day - 22nd May 2011

INSTRUCTIONS TO CONTRIBUTORS

ENVIS Newsletter on Microorganisms and Environment Management, a quarterly publication, publishes original research articles, reviews, reports, research highlights, news-scan etc., related to the thematic area of the ENVIS Centre. In order to disseminate the cutting-edge research to user community, ENVIS Centre on Microorganisms and Environment Management invites original research and review articles, notes, research and meeting reports. Details of forthcoming conferences / seminars / symposia / trainings / workshops also will be considered for publication in the newsletter.

The articles and other information should be typed in double space with maximum of 8 - 10 typed pages. Photographs/line drawings and graphs need to be of good quality with clarity for reproduction in the newsletter. For references and other details, the standard format used in referred journals may be followed.

Articles should be sent to:

The Co-ordinator

ENVIS Centre

Department of Zoology

University of Madras

Maraimalai Campus, Guindy, Chennai – 600 025.

Tamil Nadu, INDIA

(OR)

Send your articles by e-mail:

enviscoordinator@gmail.com

dzum@envis.nic.in

Cover page : A leaf infected by a pathogen called an oomycete which releases the next generation of infectious spores (the white tree-like structures emerging from the leaf surface).

Courtesy: Petra Epple, Dangl Lab, UNC-Chapel Hill.

ENVIS Newsletter
on
Microorganisms and Environment Management

Contents

Dear Readers,

This year, the United Nations Environment Programme (UNEP) has selected India as host of World Environment Day, 2011. **"Forest: Nature at your service"** is the Environment Day slogan. Forest cover one third of the Earth's land mass and play a key role in our battle against climate change and releasing oxygen into the atmosphere while storing carbon dioxide.

Natural products from the environment play an important role throughout the world in treating human diseases. Medicines from natural products have come from various source materials including plants and microorganisms. The plant and algal group comprise of many species of medicinal value, which are yet to be explored. The development of resistance of pathogens against antibiotics has become a difficult issue, hence the demand for new and effective antimicrobial agents from natural sources are increasing day by day.

In recent years, more research is being carried out on the environmental wastage. Researchers are constantly looking for solutions to this ever-growing problem. The fine solution is the introduction of biodegradable products. The new class of bio-based and biodegradable plastics are on the rise in all levels of society and industry. This issue includes articles on anti-hepatitis activity of marine brown alga and current trends and future prospects of bio-plastics. Other interesting reports on microorganisms are also available.

We sincerely look forward to your suggestions and feedbacks. Please do mail us at.

www.envismadrasuniv.org/send_feedback.php.

Prof. N.Munuswamy

For further details, visit our website

www.envismadrasuniv.org

Strong anti hepatitis B virus activity of *Padina tetrastromatica,* a marine brown alga

S. Dinesh, M. Lavanya, M. Jeevan, V. Mythily [1], and M. Elanchezhiyan *
Department of Microbiology, Dr. ALM. PG Institute of Basic Medical Sciences,
University of Madras, Taramani, Chennai - 600 113, India.
[1]Blood Bank, Voluntary Health Services, Adyar, Chennai - 600 025, India.
e-mail: emanickan@yahoo.com*

Hepatitis B virus (HBV) causes significant morbidity and mortality worldwide. HBV infection can be acute which can be cured completely or chronic during which the virus persists in the liver for a long time and occasionally for their life. Despite the universal vaccination of neonates and infants during the last years and the subsequent reduction in the incidence of new infections with hepatitis B virus (HBV), chronic HBV infection remains a significant public health problem worldwide (Maddrey, 2000). It is estimated that there are approximately 40 billions people with chronic HBV infection and that more than 500 000 people die every year due to complications of HBV related chronic liver disease (Maddrey, 2000). Although considerable improvements in the evaluation and treatment of patients with chronic HBV infection have occurred during the last decade, several issues regarding the optimal management of such patients still challenging. All patients with chronic HBV infection are at increased risk for hepatocellular carcinoma (HCC) compared with the general population and the risk increases substantially in patients with prolonged high viremia and cirrhosis (Chen *et al.,* 2006). Recent data suggest that patients with chronic HBV infection and HBV DNA above 10^4 copies/mL (approximately 2000 IU/mL) are at increased risk for cirrhosis and HCC regardless of alanine aminotransferase (ALT) activity and are therefore possible candidates for treatment (Chen *et al.,* 2006)

Lamivudine treatment is widely employed to curtail chronic HBV infection (Alberti and Caporaso, 2011). Treatment with Interferon α is also an approved method of treatment for chronic HBV infections (Dusheiko, 1995). However, interferon α therapy is associated with several side effects and often unsatisfactory response rates. Among the reasons that have been cited for explaining the poor response rate to interferon are: 1) the immune tolerance to HBV after infection at birth or during early childhood (Lai *et al.*, 1998) and 2) persistence of the viral covalently closed circular (ccc) DNA in the liver (Lin *et al.*, 1998). Thus the optimal first-line anti-HBV therapy with the best long-term cost/benefit ratio is yet to be developed.

Now there is a need for the clinical development of more efficacious yet safe and non-toxic cytoprotective agents for the adequate management of hepatitis. In this context antiviral and hepatoprotective drugs development from alternative natural sources play important role. Several medicinal herbs has been reported in Ayurvedha, Sidda and Unani for their medicinal properties. In recent times, many attempts to identify the active anti-HBV substances in *Phyllanthus* extracts is being investigated (Thyagarajan *et al*., 1988; Yoon *et al.,* 2000). Though *Phyllanthus amarus* (*P. amarus*) extracts shown to possess bioactivities to clear the carrier status of HBV infections its efficacy during chronic HBV infections is reported to be equivocal (Venkateswaran *et al.,* 1987). This necessitates the search for novel anti HBV drugs from marine sources. Drug from the sea program is a major initiative by the Government of India to develop novel drugs against infectious diseases. Significant interest on marine organisms has developed recently due to their possession of pharmacologically bioactive substances that had been used against bacteria, viruses and tumors (Smit, 2004). Despite the increasing number of new findings about seaweed metabolites possessing biological activity on the last three decades few products having actual potential have been identified or developed (Smit, 2004). Antiviral activities of extracts derived from various marine algae have been documented (De Almeida *et al.,* 2011). The present study documents the possible anti HBV activity of *Padina tetrastromatica* by HBsAg binding inhibition assay.

The marine alga, *Padina tetrastromatica* (Fig. 1) was collected from Rameshwaram coast, India. The impurities were removed by rinsing in sterile distilled water and authenticated at the Department of Botany, University of Madras, Chennai. The algae was shade dried, powdered and stored at room temperature until use. Methanol extract of *P. tetrastromatica* was prepared by adding 50 gms of algal

powder in 500 ml of methanol and then filtered using Whatman filter paper (No.1). The filtrate was allowed to evaporate for about 2-3 days. The dried filtrate was collected, weighed and stored at 4°C until use.

Fig. 1. *Padina tetrastromatica* - simple, freely branched filaments to highly differentiated forms and distinguished into blades, stipes and holdfast.

Equal volume of pre-titrated HBV and varying concentration of methanolic extract was mixed and incubated at 37° C for 5 days. The mixture was assayed on day 5 for the presence of bound/unbound HBsAg using ELISA kit (Hepanostika HBsAg kit). Controls included in the experiment were drug positive control (Elan-PA001) and drug negative control (Nonoxynol-9). Other controls included the kit positive and negative controls. ELISA was performed as per the manufacturer protocol. Briefly, to the anti HBsAg antibody precoated plates extract treated HBV virus was added and incubated for 1 hour at 37°C. Then the plates were washed and secondary antibody-HRP conjugate was added and further incubated for 1 hour at 37°C. Then the plates were washed and TMB substrate was added and incubated at room temperature for 30 minutes. To this stop solution was added and the plates were read at 450 nm in ELISA reader (BioTek). Experiments were conducted thrice and one representative experiment was described. Results are represented as ELISA optical density (OD) and percentage (%) inhibition.

Percentage inhibition = OD of Test-OD of the control/OD of the control × 100.

Anti HBV property of *P. tetrastromatica* was evaluated by studying the inhibition of HBsAg binding by the extracts. In this evaluatory study first varying concentration of *P. tetrastromica* (dose response) were tested for its anti HBV activity. Secondly two different doses of virus were tested for the drug efficacy. Table 1 represents the dose response profile of *P. tetrastromatica,* percent inhibition above 90% was considered significant. As shown in the table above 90% inhibition (virus concentration 1.5pg/ml) of HBV was noticed at a drug concentration of 5 mg/ml and above. Up to 10 mg/ml concentration of *P. tetrastromatica,* the algal extract did not show any drug toxicities as described by MTT assay (data not shown). An in-house preparation, Elan-PA001 served as drug positive control which completely inhibited HBV. A potent anti HIV drug Nonoxonol-9 was used as negative HBV drug which showed <1.0% activity. As anticipated the group received sterile distilled did not show any anti HBV activity.

Table: 1. Anti-HBV activity of methanolic extract of *Padina tetrastromatica*

Groups	Dosage	OD	% of inhibition
Untreated	1.5 pg/ml	OF	<1.0
Padina extract treated	10 mg/ml	0.083	96.67
	5 mg/ml	0.054	98.64
	2.5 mg/ml	2.258	43.46
	1.25 mg/ml	OF	<1.0
	0.625 mg/ml	OF	<1.0
	0.312 mg/ml	OF	<1.0
Nonoxynol-9 (Drug negative control)	100 mg/ml	OF	<1.0
Elan-PA001 (Drug positive control)	5mg/ml	0.021	99.31

Various concentrations of *Padina tetrastromatica* were tested for its anti HBsAg inhibition activity on day 5 by ELISA. Virus concentration = 1.5 pg/ml; Untreated = group that received sterile distilled water; OF = OD over 4.0.

In order to assess whether the anti HBV activity of *P. tetrastromatica* was virus dose dependent, varying concentrations of HBV such as 3 pg/ml, 1.5 pg/ml and 0.75pg/ml were tested against 5 mg/ml concentration of the *P. tetrastromatica* extract. The results revealed that *P. tetrastromatica* extracts completely inhibited the 1.5 pg/ml of HBV as shown in the above experiment (Table 2). A similar inhibitory activity was noticed with the lower virus dose of 0.75 pg/ml also. When the virus dose was increased to 3 pg/ml

this inhibitory activity was abrogated suggesting the 1.5 pg/ml may be the optimum concentration that could be nullified by the *P. tetrastromatica* (data not shown).

Table 2. Anti HBV activity against two different doses of HBV

Groups	Virus concentration			
	1.5 pg/ml		0.75 pg/ml	
	OD	% inhibition	OD	% inhibition
Untreated	OF	<1.0	OF	<1.0
Padina tetrastromatica (5 mg/ml)	0.034	98.9	0.029	99
Nonoxynol-9 (Drug negative control)	3.995	<1.0	OF	<1.0
Elan-PA001 (Drug positive control)	0.016	99.49	0.019	99.40

Stock virus was diluted to 1.5 pg/ml and 0.75 pg/ml and tested for the drug's anti HBV activity by ELISA

Exploring novel drugs to combat HBV infections especially the chronic HBV hepatitis is needed very desperately. Marine sources serve as a potential treasure hunt platforms for unique drug development that could be used to treat viral hepatitis. In this evaluatory study varying concentration of *P. tetrastromatica* were tested for the anti HBV activity and it was found that 5 mg/ml concentrations of *P. tetrastromatica* completely neutralized the HBV suggesting its medicinal scope to treat viral hepatitis. *P. tetrastromatica* extracts inhibited 1.5 pg/ml of HBV suggesting the magnitude of inhibition. Bioactivities of several marine algae have been reported but medicinal value of *P. tetrastromatica* has not been studied so far suggesting the pioneering nature of our study.

Chronic HBV infections can lead to liver cirrhosis and hepatocellular carcinoma. Considering the severity of clinical outcome proper treatment modalities must be in place to fight against human HBV infection. One of the very important proteins of HBV is the surface antigen (HBsAg) which helps the virus in adherence to the target tissue (Neurath *et al.,* 1990). Importance of HBsAg is multifold and it is highly immunogenic (Carman *et al.,* 1993). HBsAg is also known as "australia antigen" and found in 4 phenotypes namely adw, ayw, adr and ayr and each phenotype is epidimiologically important (Tiollais *et al.,* 1985). Presence of HBsAg in a patient is an indication that it is a recent infection and antibodies to HBs (anti HBs antibody) are efficient in clearing the HBV (Halliday *et al.,* 1992). Besides that there are two other important antigens namely HBcAg and HBeAg are important for the complete clearance of the virus during chronic infections. (Liaw *et al.,* 1984). In chronic HBV infection both HBsAg and antibodies to HBs (anti HBs) are found in the patients and presence of HBsAg helps in the new infection of hepatocytes.

HBV infects hepatocytes and causes viral hepatitis. Receptors for HBV is not fully known and it is speculated that preS domain of surface protein of the virus bind to carboxypeptidase D molecules found on hepatocytes. Thus the surface antigen (HBsAg) plays an important role in virus attachment to the hepatocytes and any methodology that would interfere with this initial binding can prevent the virus attachment to the host tissue. In this context the current investigation is very important as the study clearly showed that *P. tetrastromatica* extract inhibits HBsAg binding to its receptor, anti HBs antibody. This study also clearly demonstrated that 5 mg/ml of the extract inhibited the virus binding and this inhibition was noticed upto 1.5 pg/ml concentration of the virus. This report is of first of its kind in augmenting the efficacy of *P. tetrastromatica* extract in inhibiting HBV binding to its receptor. This study open up new vistas on the molecular mechanism of HBV viral entry inhibition. Therefore cataloging marine algae and their medicinal property have more scope in combating infectious diseases.

References:

Alberti, A. and Caporaso, N. (2011) HBV therapy: guidelines and open issues. Dig Liver Dis. **43** suppl 1, S57 - 63.

Carman, W., Thomas, H. and Domingo, E. (1993) Viral genetic variation: hepatitis B virus as a clinical example. Lancet. **341**, 349 - 353.

Chen, C. J., Yang, H. I., Su, J., Jen, C. L., You, S. L., Lu, S. N., Huang, G. T. and Iloeje, U. H. (2006) Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA. **295**, 65 - 73.

De Almeida, C. L., Falcao, Hde. S., Lima, G. R., Montenegro, Cde. A., Lira, N. S., De Athayde - Filho, P. F., Rodrigues, L. C., De Souza, Mde. F., Barbosa-Filho, J. M. and Batista, L. M. (2011) Bioactivities from marine algae of the genus *gracilaria*. Int. J. Mol. Sci. **12**(7), 4550 - 73.

Dusheiko, G. M. (1995) Treatment and prevention of chronic viral hepatitis. Pharmacol Ther. **65**, 47 - 73.

Halliday, M. L., Kang, L.- y., Rankin, J. G., Coates, R. A., Corey, P. N. J., Hu, Z. H., Zhou, T. K., Yuan, G. J. and Yao, E. L. (1992) An efficacy trial of a mammalian cell-derived recombinant DNA hepatitis B vaccine in infants born to mothers positive for HBsAg, in Shanghai, China. lnt. j. Epidemiol. **21**, 564 - 573.

Lai, C. L., Chien, R. N. and Leung, N. W. Y. (1998) A 1-year trial of lamivudine for chronic hepatitis B. N Engl J Med. **339**, 61 - 68.

Liaw, Y. F., Chu, C. M., Huang, M. J., Sheen, I. S., Yang, C. Y. and Lin, D. Y. (1984) Determinants for hepatitis B e antigen clearance in chronic type B hepatitis. Liver. **4**, 301 - 306.

Lin, E., Luscombe, C., Colledge, D., Wang, Y. Y. and Locarnini, S. (1998) Long-term therapy with the guanine nucleoside analog penciclovir controls chronic duck hepatitis B virus infection *in vivo*. Antimicrob Agents Chemother. **42**, 2132 - 2137.

Maddrey, W. C. (2000) Hepatitis B: an important public health issue. J. Med. Virol**. 61**, 362 - 366.

Neurath, A. R., Strick, N., Sproul, P., Ralph, H. E. and Valinsky, J. (1990) Detection of receptors for hepatitis B virus on cells of extrahepatic origin. Virology, **176**, 448 - 457.

Smit, A. J. (2004) Medicinal and Pharmaceutical uses of seaweed natural products: a review. Journal of Applied Phycology. **16**, 245 - 262.

Thyagarajan, S. P., Subramanian, S., Thirunalasundari, T., Venkateswaran, P. S. and Blumberg, B. S. (1988) Effect of *Phyllanthus amarus* on chronic carriers of Hepatitis B virus. The Lancet. **1**, 764 - 766.

Tiollais, P., Pourcel, E. and Dejean, A. (1985) The hepatitis B virus. Nature. **317**, 489 - 495.

Venkateswaran, P. S., Millman, I. and Blumberg, B. S. (1987) Effects of an extract from *Phyllanthus niruri* on hepatitis B and woodchuck hepatitis viruses: *In vitro* and *in vivo* studies. Proc. Natl. Acad. Sci. USA, **84**, 274 - 278.

Yoon, S., Chung, T. H. and Hung, C. C. (2000) Pharmaceutical composition for the treatment of hepatitis B comprising corilagin or brevifolin. Patent wo/2000/027413.

Bioplastics : Current trends and future prospects

S. Priyanka and R. Rajam *
School of Bio Sciences and Technology
VIT University, Vellore - 632 014, India.
e-mail: rajendran.n@vit.ac.in*

Introduction

Biomaterials are natural products synthesized and catabolised by different organisms and have broad biotechnological applications. They can be assimilated by many species and do therefore possess biocompatibility with the host. In this way, they confer upon them a considerable advantage with respect to other conventional synthetic products. Bioplastics are biomaterials that are polyesters produced by a range of microbial sources, and plants under different nutrient and environmental conditions. They are derived from renewable biomass sources, such as vegetable oil, corn starch, and pea starch. Table 1 summarizes the different types of bioplastics based on the chemical nature. The production and use of bioplastics is generally regarded ecofriendly as compared to plastic production from petroleum. The reason for this is former relies less on fossil fuel as a carbon source and also hazardous waste released is lesser or even negligible when compared to that of oil-derived plastics. In Europe, bioplastics account for 60% of the biodegradable materials market. The most common end use market is for packaging materials. Japan has also been a pioneer in bioplastics, incorporating them into electronics and automobiles.

Table 1 : Types and properties of bioplastics

S.No.	Types	Properties
1	**Starch based plastics**	The thermoplastic starch, such as plastarch material, currently represents the most important and widely used bioplastic.
2	**Aliphatic polyesters**	The aliphatic biopolyesters are mainly polyhydroxyalkanoates (PHAs) like the poly-3-hydroxybutyrate (PHB), polyhydroxyvalerate (PHV) and polyhydroxyhexanoate PHH.
3	**Polylactic acid (PLA) plastics**	PLA is a transparent plastic produced from cane sugar or glucose.
4	**Poly-3-hydroxybutyrate (PHB)**	The biopolymer poly-3-hydroxybutyrate (PHB) is a polyester produced by certain bacteria processing glucose or starch.
5.	**Polyamide 11/ PA 11**	PA 11 is a biopolymer derived from natural oil. PA 11 belongs to the technical polymers family and is not biodegradable. It is used in high-performance applications like automotive fuel lines, pneumatic airbrake tubing, electrical cable etc
6.	**Bio-derived polyethylene**	The monomer of polyethylene is ethylene. This is produced by fermentation of agricultural feedstocks such as sugarcane or corn.

Biological Sources

Plastics manufactured by conventional methods come from non-renewable hydrocarbon resources. They cannot be broken down easily by microorganisms as they consist of long polymer molecules tightly bound to one another. Biodegradable plastics can be made using polymers from bacteria, algae and plants as they consist of shorter, more easily degraded polymers and hence seem to be a fascinating option today. Following is a brief account of how each group of organisms can be utilized for the purpose.

1. Algae

Algae serve as an excellent feedstock for plastic production owing to its many advantages such as high yield and the ability to grow in a wide range of environments. Algae bioplastics mainly evolved as a byproduct of algae biofuel production. Algae based plastics have been a recent trend in the era of bioplastics compared to traditional methods of utilizing feedstocks of corn and potatoes as plastics. While algae-based plastics are in their infancy, once they are into commercialization they are likely to find applications in a wide range of industries. However, before commercialization is realized, many technical problems have to be negated. Cereplast the company which makes 'Cereplast Algae Plastics' produce the plastic that contains only 50% algae. Plastics that comprise material derived 100% from algae are still not a reality and require innovative developments. The use of biotechnological techniques can play a key role in conducting the feasibility and sustainability studies in algae bioplastics.

2. Fungi

The contribution of this group of organisms towards bioplastic production is still not perceptible.

3. Plants

Crop plants are capable of producing large amounts of a number of useful chemicals at a low cost compared to that of bacteria or yeast. Commercialization of plant derived bioplastics, particularly PHAs will require the creation of transgenic crop plants that in addition to high product yields have normal plant phenotypes and transgenes that are stable over several generations. In contrast to bacteria, plant cells are highly compartmentalized hence the desired genes for example *phb* must be targeted to the compartment of the plant cells where the concentration of the precursor molecule is high. Many oil crops such as rapeseed, sunflower and soybean could be potentially engineered for the production of PHA. The other plants currently in use for PHA production are *Gossypium hirsutum* and *Zea mays*. The advantage looks more with the starch-producing crops than oil crops in terms of yield (kg/hectare) but the diversion of precursor molecules towards PHB synthesis is likely to be more complex in starch crops since the flux of carbon is primarily directed towards sucrose.

4. Bacteria

Bacteria are so far the most widely studied organisms with regard to production of bioplastics. Particularly, PHAs are synthesized by many gram-positive and gram-negative bacteria from at least 75 different genera. PHAs extracted from bacterial cells show material properties that are similar to polypropylene (Braunegg *et al.* 1998). These polymers are accumulated intracellularly (Fig. 1) to levels as high as 90% of the cell dry weight under conditions of nutrient stress and act as a carbon and energy reserve (Madison and Huisman, 1999). The occurrence of PHAs in bacteria has been known since 1920s, when Lemoigne reported the formation of poly 3-hydroxybutyrate (PHB) inside bacteria (Lemoigne, 1926). Non-storage PHA that are of low molecular weight, have been detected in the cytoplasmic membrane and cytoplasm of *Escherichia coli*. The following are some of the most important bacterial species utilized for the production of PHAs. They are *Alcaligenes eutrophus*, *Bacillus megaterium* QMB1551, *Klebsiella aerogenes* recombinants, *Methylobacterium rhodesianum* MB 1267, *Pseudomonas aeruginosa*, *P. denitrificans*, *P. putida*, *P. oleovorans* and *Sphaerotilus natans* (Reddy *et al.*, 2003). Additional information on this aspect can be obtained from Verlinden *et al.* (2006).

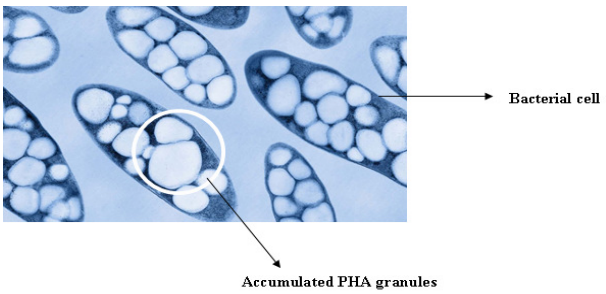


Fig. 1: Intracellular accumulation of PHAs in bacterial cells (**Source:** www.technewsdaily.com)

The main candidates for the large-scale production of PHAs are plants and bacteria. However, plant cells give very low yields [<10% w/w dry weight]. High levels [10 - 40% w/w dry weight] have been shown to have a negative effect on growth and development of plant. In bacteria they can be accumulated up to 90% of the cell dry weight. Accumulating PHAs is a natural way for bacteria to store carbon and energy, when nutrient supplies are imbalanced. These polyesters are accumulated when bacterial growth is limited by depletion of nitrogen, phosphorous (Shang *et al.,* 2003) or oxygen and an excess amount of a carbon source is still present. While the most common limitation is nitrogen, for some bacteria, such as *Azotobacter* sp., the most effective limitation is oxygen (Dawes, 1990). The first PHA to be discovered and therefore the most studied is PHB. In their metabolism, bacteria produce acetyl-coenzyme-A (acetyl-CoA), which is converted into PHB by three biosynthetic enzymes(Fig. 2)

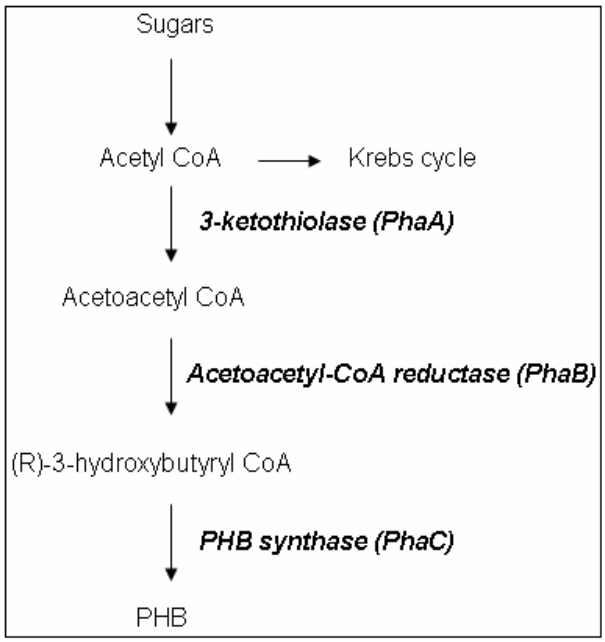


Fig. 2. Metabolic pathway for PHB

Petroplastics vs Bioplastics : Pros and Cons

Although a lot of expectations have been pinned on bioplastics, but many aspects have to be dealt to make the future of these commercially viable. Most important of all is the cost feasibility of bioplastics, apart from this, there is a concern about genetically modified organisms, sustainably grown biomass, there is an urgent need to develop composting programs and infrastructure, also there is a lack of adequate labeling and concern over contamination of recycling systems. Inspite of all these points, bioplastics have many merits over the petroplastics as depicted in figure 3.

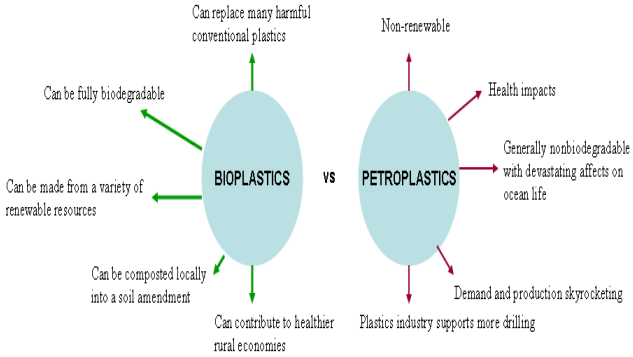


Fig. 3. Bioplastics vs Petroplastics

Applications

Apart from the general applications like manufacture of polythene bags, trays, containers and bottles for soft drinks and dairy products, blister foils for fruit, vegetables and medicines are manufactured from bioplastics. However, now this trend is expected to change with the innovation and upgradation in technology and these biomaterials will intervene in the manufacture of products such as mobile phones, cameras, medical devices, electronics, as well as automotive parts. PHAs vary in toughness and flexibility, depending on their formulation. As such they can be used either in pure form or as additives to oil derived plastics such as polyethylene. However, these bioplastics are currently far more expensive than petrochemically based plastics and are therefore used mostly in applications that conventional plastics cannot perform, such as medical applications. Most interesting application to come up is the application of these bioplastics in tissue engineering. PHAs are immunologically inert and are only slowly degraded in human tissue, which means they can be used as devices inside the body. Scientists have envisaged the use of these for making artificial bones, pacemakers, shunts etc. Additionally, they have the potential to be used as drug delivery agents for constant and uniform release of drug over a period of time in human body. Studies related to these aspects are still very preliminary and a lot of research has to be done in this area.

References

Braunegg, G., Lefebvre, G. and Genser, K. F. (1998) Polyhydroxyalkanoates, biopolyesters from renewable resources: Physiological and engineering aspects. Journal of Biotechnology **65**, 127 - 161.

Dawes, E. (1990) Novel Biodegradable Microbial Polymers. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Lemoigne, M. (1926) Produits de dehydration et de polymerisation de l'acide ß- oxobutyrique. Bulletin de la Société de Chimie Biologique. **8**, 770 - 782.

Madison, L. L. and Huisman, G. W. (1999) Metabolic engineering of poly (3-hydroalkanoates): from DNA to plastic. Microbiology and Molecular Biology Reviews. **63**, 21 - 53.

Reddy, C. S. K., Ghai, R., Rashmi and Kalia, V. C. (2003) Polyhydroxyalkanoates: an overview. Bioresource Technology. **87**, 137 - 146.

Shang, L., Jiang, M. and Chang, H. N. (2003) Poly (3-hydroxybutyrate) synthesis in fed-batch culture of *Ralstonia eutropha* with phosphate limitation under different glucose concentrations. Biotechnology Letters. **25**, 1415 - 1419.

Verlinden, R. A. J., Hill, D. J., Kenward, M. A., Williams, C. D. and Radecka, I. (2007) Bacterial synthesis of biodegradable Polyhydroxyalkanoates. Journal of Applied Microbiology **102**, 1437 – 1449.

Research Reports

E. coli engineered to produce record - setting amounts of alternative fuel

Researchers at UCLA's (University of California, Los Angeles), Henry Samueli School of Engineering and Applied Science have developed a way to produce normal butanol often proposed as a "greener" fuel alternative to diesel and gasoline from bacteria at rates significantly higher than those achieved using current production methods. The findings, reported online in the journal Applied and Environmental Microbiology, mark an important advance in the production of normal butanol, or n-butanol, a four-carbon chain alcohol that has been shown to work well with existing energy infrastructure, including in vehicles designed for gasoline, without modifications that would be required with other biofuels.

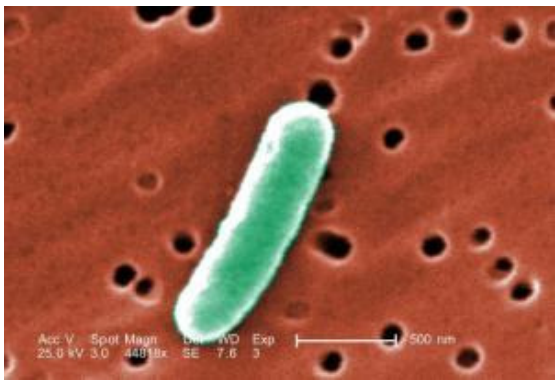
The UCLA team, led by James C. Liao, UCLA's Chancellor's Professor of Chemical and Biomolecular Engineering, demonstrated success in producing 15 to 30 grams of n-butanol per liter of culture medium using genetically engineered *Escherichia coli* a record-setting increase over the typical one to four grams produced per liter in the past.

For the study, Liao and his team initially constructed an n-butanol biochemical pathway in *E. coli*, a microbe that doesn't naturally produce n-butanol, but found that production levels were limited. However, after adding metabolic driving forces to the pathway, the researchers witnessed a tenfold increase in the production of n-butanol. The metabolic driving forces pushed the carbon flux to n-butanol.

"Like human beings, microbes need an incentive to work," said Liao, the study's senior author. "We created driving forces by genetically engineering the metabolism," said Claire R. Shen, a UCLA Engineering graduate student and lead author of the study.

While certain microbes, including species of the bacteria *Clostridium*, naturally produce n-butanol, Liao's team used *E. coli* because it is easier to manipulate and has been used industrially in producing various chemicals. "By using *E. coli*, we can make it produce only the compound with no other byproducts," Liao said. "With native producing organisms like *Clostridium*, which naturally produces n-butanol, there are other byproducts that would add cost to the separation process."

The next step in the research, the researchers say, will be to transfer the study to industry for the development of a more robust industrial process. The study was funded by the KAITEKI Institute Inc. of Japan, a strategic arm of Mitsubishi Chemical Holdings Corp., Japan's largest chemical company.



***E. coli* at an extremely high magnification of 44, 818X.**

(**Credit:** Janice Haney Carr).

Source: www.sciencedaily.com

Novel microorganism '*Nitrososphaera viennensis*' isolated

Microorganisms play an important role in global nutrient cycles. A research team led by Christa Schleper, Head of the Department of Genetics in Ecology at the University of Vienna, has isolated the first ammonium oxidizing archaeon from a soil in Vienna and thus proved its activity. The researchers present their results on *Nitrososphaera viennensis* in the newest edition of the Proceedings of the National Academy of Sciences.

Life on Earth would be impossible, without the metabolic capacities of the smallest of all living forms, the bacteria and the archaea. These microorganisms play a central role in global nutrient cycles, because they degrade organic matter to the smallest compounds, thus bringing them back to the atmosphere or recycling them for the synthesis of novel cells.

"However, the great diversity and high numbers of bacteria and archaea in soils have only been detected relatively recently, with the help of molecular biological methods," says Christa Schleper. Already six years ago co-workers who are now working at the department have predicted the high abundance of archaea in soil with the help of such molecular techniques. Since then it was hypothesized that these archaea contribute significantly to the nitrogen cycle, based on their capability to oxidize ammonia to nitrite.

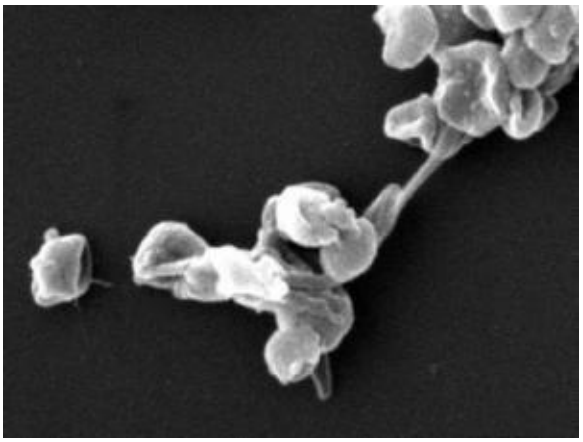
Co-workers of Christa Schleper, have now succeeded to obtain the first ammonia oxidizing archaeon from soil in pure culture and to directly demonstrate its physiological activity. It stems from the garden of the University Center at Althanstrasse in Vienna's 9th district and carries the name *Nitrososphaera viennensis* (the spherical ammonia oxidizer from soil). A single cells has a diameter of only 0.8 micrometers.

Most of the archaea live in extreme environments, such as e.g. volcanic hot springs and are therefore often regarded as evolutionary relicts. "*Nitrososphaera viennensis* could also have evolutionary old traits, because different from its bacterial counterparts who like well-fertilized agricultural soils, it grows preferably under low nutrient conditions that are more reminiscient of pristine soils," says Schleper.

Different from bacterial ammonia oxidiziers "*Nitrososphaera viennensis"* needs low amounts of organic material for growth beside ammonia and carbon dioxide, as demonstrated with the help of a NanoSIMS. This highly modern secondary ion mass spectrometer which works at nano-scale resolution has only recently been installed through the Department of Microbial Ecology and with support of different faculties of the University. It is used by researchers of the faculty of Life Sciences, the faculties of Geology, Geography and Astronomy, and the faculty of Chemistry as well as the Max F. Perutz Laboratories.

Relevance for agriculture

Nitrososphaera viennensis is the first cultivated representative of archaeal ammonia oxidizers, and therefore a model organisms of this ecologically relevant group of microorganisms. The study of this species will be of relevance in agriculture, because ammonia oxidation has a great influence on the availability of nitrogen for plants and on the accumulation of nitrate in groundwater says Schleper. She sees a wide field of upcoming research, e.g. to test *Nitrososphaera viennensis* for its capability to produce N_2O (nitrous oxide). This gas which is produced in considerable amounts by the bacterial counterparts, contributes to the depletion of ozon and thus plays a role in global warming. "Since relatives of *Nitrososphaera viennensis* are broadly distributed and account for up to 10 million cells per gram of soil it will be of relevance to measure their contribution to such pocesses."



A single *Nitrososphaera viennensis* cell has a diameter of just 0.8 micrometers.

(**Credit:** University of Vienna, Department of Genetics in Ecology)

Source: www.sciencedaily.com

Jellyfish blooms shunt food energy from fish to bacteria

A new study by researchers at the Virginia Institute of Marine Science (VIMS) shows that jellyfish are more than a nuisance to bathers and boaters, drastically altering marine food webs by shunting food energy from fish toward bacteria. An apparent increase in the size and frequency of jellyfish blooms in coastal and estuarine waters around the world during the last few decades means that jellie's impact on marine food webs is likely to increase into the future.

The results of the study, led by recent VIMS (Virgina Institute of Marine Science) Ph.D. graduate Rob Condon appear in the latest issue of the Proceedings of the National Academy of Sciences. Condon conducted his field studies by sampling jellyfish blooms in the York River, a tributary of lower Chesapeake Bay. The team's experimental work took place in laboratories at VIMS, and in Canada and France. The researchers tracked the flow of food energy in the lab by measuring the amount of carbon taken up and released by jellyfish and bacteria within closed containers during "incubation" experiments of varying length. Carbon is the "currency" of energy exchange in living systems.

"Jellyfish are voracious predators," says Condon. "They impact food webs by capturing plankton that would otherwise be eaten by fish and converting that food energy into gelatinous biomass. This restricts the transfer of energy up the food chain, because jellyfish are not readily consumed by other predators."

Jellyfish and marine bacteria

Jellyfish also shunt food energy away from fish and shellfish that humans like to eat through their affects on the bacterial community. "Marine bacteria typically play a key role in recycling carbon, nitrogen, phosphorus, and other byproducts of organic decay back into the food web," says Condon. "But in our study, we found that when bacteria consumed dissolved organic matter from jellyfish they shunted it toward respiration rather than growth."

The upshot of this "jelly carbon shunt" is that bacteria in jelly-laden waters end up converting carbon back to carbon dioxide, rather than using it to grow larger or reproduce. This means the carbon is lost as a direct source of organic energy for transfer up the food web.

The researchers think the shift toward bacterial respiration happens because jellyfish produce organic matter that is extra rich in carbon. They do so through excretion and the sloughing of mucus. "The mucus is the slime you feel when you pick up a jelly," says Steinberg (co - author). The jellyfish in Condon's experiments released large quantities of carbon-rich organic matter with 25 to 30 times more carbon than nitrogen. That compares to a ratio of 6 parts carbon to 1 part nitrogen for the organic matter found dissolved in typical marine waters. "The bacteria metabolized this carbon-rich material two to six times faster than they did with dissolved organic matter from water without jellyfish," says Condon. "This rapid metabolism shunted carbon toward respiration rather than production, reducing their potential to assimilate this material by 10% to 15%."

The microbial community

A final significant finding from the team's research is that an influx of dissolved organic matter from jellyfish blooms changes the make-up of the local microbial community. "Dissolved organic matter from jellyfish favored the rapid growth and dominance of specific bacterial groups that were otherwise rare in the York River," says Condon. "This implies that jelly-DOM was channeled through a small component of the local microbial assemblage and thus induced large changes in community composition." Overall, says Condon, the team's findings "suggest major shifts in microbial structure and function associated with jellyfish blooms, and a large detour of energy toward bacteria and away from higher trophic levels." He adds that a host of factors, including climate change, over-harvesting of fish, fertilizer runoff, and habitat modifications could help to fuel jellyfish blooms into the future. "Indeed," he says, "we have seen this already in Chesapeake Bay. If these swarms continue to emerge, we could see a substantial biogeochemical impact on our ecosystems."

"Simply knowing how carbon is processed by phytoplankton, zooplankton, microbes or other trophic levels in space and time can lead to estimates of how much carbon energy is available for fish to consume," he said. "The more we know, the better we can manage ecosystem resources."

Dense bloom of jellyfish (*Aurelia aurita*) in the York River, a tributary of Chesapeake Bay.

(**Credit:** Photo by Scott Kupiec)
Source: www.sciencedaily.com

Hunting for deadly bacteria

You can't see them, or smell them or taste them. They can be in our water and in our food, multiplying so rapidly that conventional testing methods for detecting pathogens such as *E.coli*, *Salmonella* and *Listeria* come too late for the tens of thousands of Canadians who suffer the ill effects of these deadly bacteria. Biochemist Yingfu Li and his research team have developed a simple test that can swiftly and accurately identify specific pathogens using a system that will 'hunt' for bacteria, identifying their harmful presence before they have a chance to contaminate our food and water.

Like any living thing, bacteria have their own spoor, leaving behind DNA trails of bacterial 'droppings'. Li tracks these metabolic by-products with molecular beacons little lighthouses on a molecular scale that actually light up when they detect the DNA sequence left behind. Li created a DNAzyme sensor that will be able to identify any bacteria, utilizing a method that doesn't require the steps and specialized equipment typically used to identify whether or not harmful bacteria are present.

"Current methods of foodborne bacterial detection take time. The five days it takes to detect listeria, for example, can translate into an outbreak that costs lives. We have developed a universal test that uses less complex procedures but still generates precise and accurate results," says Li, a Canada Research Chair in Directed Evolution of Nucleic Acids.

Li's fluorescent test system was highlighted in Angewandte Chemie International Edition. Li's paper, co-authored with lab members Monsur Ali, Sergio Aguirre and Hadeer Lazim, was designated a 'hot paper' by Angewandte's editors for its "importance in a rapidly evolving field of current interest."

"McMaster researchers are known for their ability to provide solutions to problems that impact the public's well-being. The test that Professor Li has developed will help safeguard the health of Canadians, and supply industry with a reliable means to bring safe food products to consumers and reduce their time to market," said Mo Elbestawi, vice-president, research and international affairs. Li's research was funded by the Natural Sciences and Engineering Research Council (NSERC) and the Sentinel Bioactive Paper Network.

Source: www.sciencedaily.com

News

New antibiotic fished out of sea

For years, scientists at the National Institute for Research in Tuberculosis (NIRT) (formerly Tuberculosis Research Centre) here in collaboration with Periyar University, Salem have been digging in deserts and under the sea hoping to unearth chemical compounds that had the potential to be developed into drugs to fight tuberculosis. Recently they announced that they had hit pay dirt at a coral reef off Rameswaram. From a soil sample obtained from under the sea there, the researchers isolated a novel *Streptomyces* sp. which produced transitmycin, a novel antibiotic that could fight TB and HIV together. Scientists hunt for new drugs in extreme environments, such as deserts and under the sea where there's little human presence. They believe that life forms in these regions could battle disease causing organisms in humans better than those in contact with humans.

"We were thrilled when we saw the compound we isolated turn yellow in petridish - a sure sign of a potent antibiotic," said principal investigator Dr. Vanaja Kumar, Head of the Department of Bacteriology, NIRT. While NIRT scientists are celebrating, they say there's much work to be done before a drug can be made available for patients. The project has so far cost Rs 25 lakh and may take another Rs 300 crore to develop the drug. The team has prepared a 64-page report and has urged the Indian Council of Medical Research (ICMR) to fund animal and human trials. "It would take another 10 years for the drug to hit the market, but we are hopeful that this would be our holy grail," said Dr. Vanaja Kumar.

Source: The Times of India, June, 16, 2011.

Engineered fungus to fight malaria

Scientists have genetically engineered a fungus to be a potent, specific and eco-friendly tool against malaria. Transgenic fungal approach is a very flexible one that allows design and delivery of gene products targeted to almost any disease-carrying arthropod. Researchers from the University of Westminster, London, created their transgenic anti-malarial fungus by starting with *Metarhizium anisopliae*. They inserted *anisopliae*, a fungus that naturally attacks mosquitoes, into genes for a human antibody or a scorpion toxin. Both the antibody and the toxin specifically target the malaria-causing parasite *Plasmodium falciparum*. The team then compared three groups of mosquitoes all heavily infected with the malaria parasite. In the first group were mosquitoes sprayed with the transgenic fungus, in the second were those sprayed with an unaltered or natural strain of the fungus, and in the third group were mosquitoes not sprayed with any fungus. The research team found that compared to the other treatments, spraying mosquitoes with the transgenic fungus significantly reduced parasite development. Even in the 25 per cent of mosquitoes that still had parasites after being sprayed with the transgenic fungi, parasite numbers were reduced by over 95 per cent compared to the mosquitoes sprayed with the wild-type fungus.

Source: The Hindu, March 03, 2011.

'Harmless' soil bacteria can destroy tumours

London: Scientists are harnessing a harmless soil bug to kill tumours making it a drug delivery vehicle. The therapy uses *Clostridia sporogenes* a bug found abundantly in soil. Its spores are injected into patients and only grow in solid tumours, where a specific bacterial enzyme is produced. An anti-cancer drug is injected separately into the patient. After reaching the tumour site, the bacterial enzyme activates the drug, allowing it to destroy only the tumour cells.

University of Nottimgham's Nigel Minton, who led the research, said, "*Clostridia* are an ancient group of bacteria that evolved on the planet before it had an oxygen-rich atmosphere and so they thrive in low-oxygen conditions." When *Clostridia* spores are injected into a cancer patient, they will only grow in oxygen-deficit environments, i.e. the centre of solid tumours, according to a Nottingham statement.

Researchers have introduced a gene for an improved version of the enzyme into the *Clostridia sporogenes* DNA. It can now be produced in far greater quantities in the tumour than previous versions, and is more efficient at converting the pro-drug into its active form.

Source: The Times of India, September, 03, 2011.

Abstract

001. Xiaoying Lu, Tong Zhang, Herbert Han-Ping Fang, Kenneth M.Y. Leung, Gan Zhang. Environmental Biotechnology Laboratory, Department of Civil Engineering, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, China. **Biodegradation of naphthalene by enriched marine denitrifying bacteria.** International Biodeterioration & Biodegradation, **65**, 2011, 204 - 211.

Numerous studies have been investigated on the PAHs biodegradation in aerobic and anaerobic environments; however, the biodegradation of PAHs under anoxic conditions, especially denitrifying conditions, has drawn less attention. In this study, four series of batch experiments were conducted to investigate the effect of temperature, pH, naphthalene concentration and nitrate concentration on the naphthalene degradation under denitrification condition. Our results showed that the degradation of naphthalene was most favorable at pH 7 and 25°C. Results also indicated that 30 mg/l naphthalene inhibited the biodegradation and the removal efficiencywas only 20.2%. Significant degradation (91.7% and 96.3%) of naphthalene occurred when nitrate concentrations were 1.0 and 5.0 mM. Moreover, the maximum degradation rates were 0.13 and 0.18 mg-NAP/(lh) depending on the concentration of nitrate. Based on 16S rDNA analysis, the denitrifying enriched culture was mainly composed of *g-Proteobacteria* (19 clones out of a total of 23 clones) and *Actinobacteria* (4 clones). Using a primer set specific for naphthalene degrading functional gene nahAc, two operational taxonomy units were obtained in the clone library of nahAc. Both of them were closely related to nahAc genes of known species of *Pseudomonas*. Quantitative polymerase chain reaction (qPCR) was employed to quantify the change of naphthalene degrading population during the degradation of naphthalene using nahAc gene as the biomarker. The maximum degradation rate and removal efficiency were strongly correlated with nahAc gene copy number, with R2 of 0.69 and 0.79, respectively.

Keywords: Polycyclic aromatic hydrocarbons, 16S rDNA analysis, nahAc genes, *Pseudomonas, Mycobacterium, Rhodococcus, Neptunomonas* and *Stenotrophomonas*.

E - Resources on Microorganisms

NATIONAL

Indian Institute of Ecology and Environment
www.ecology.edu/iiee/iiee.htm

Microbial Type Culture Collection and Gene Bank
www.mtcc.imtech.res.in

National Fungal Culture Collection of India
www.aripune.org/NFCCI.html

NII Microbial Culture Collection
www.niist.res.in

Visva-Bharati Culture Collection of Algae
www.visva-bharati.ac.in

INTERNATIONAL

Agricultural Research Service Culture Collection of the USDA
www.nrrl.ncaur.usda.gov

Culture Collection, University of Göteborg
www.ccug.se

British Mycological Society
www.britmycolsoc.org.uk

Society for General Microbiology
www.sgm.ac.uk

International organization for Mycoplasmology
www.the-iom.org

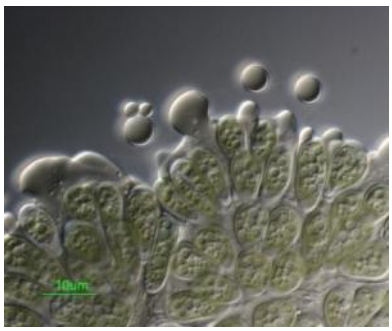
EVENTS
Conferences / Seminars / Meetings 2011 - 2012

National Level Training Workshop on Monitoring and Evaluation of Recalcitrant Chemicals in the Environment. October 28 - 31, 2011. **Venue:** Department of Environmental Biotechnology, School of Environmental Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu, **India. Website:** www.bdu.ac.in

MicroBiotec11. December 12 - 16, 2011. **Venue:** School of Health Sciences of the University of Minho, in the Gualtar Campus, **Portugal. Website:** www.microbiotec2011.org/

International Conference on Molecular Ecology. February 4 - 7, 2012. **Venue:** Lecture Hall A, University of Veterinary Medicine, Veterinärplatz 1, 1210 Vienna, **Austria. Website:** www.vipca.at/MOLECOL/index.html.

Bio-informatics and Computational Biology. March 12 - 13, 2012. **Venue:** Chatrium Hotel Riverside, Bangkok, **Thailand. Website:** www.bioinfoconf.org/index.html.

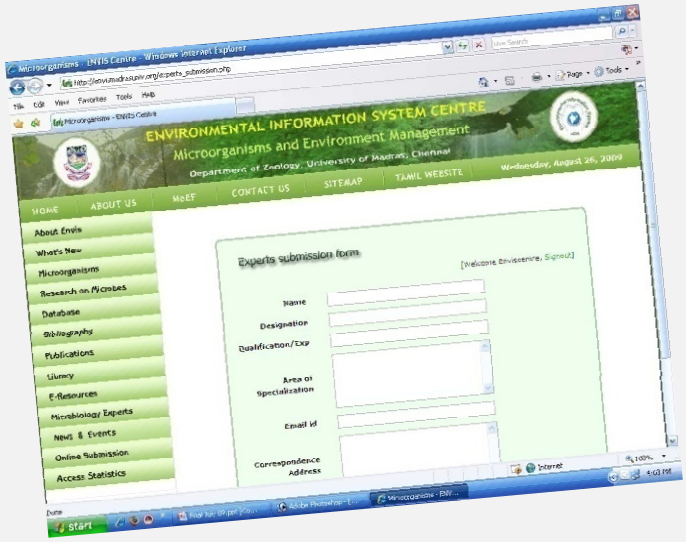


Oil oozing from the green alga

Ancient algae: a path to new energy sources

Scientists previously established that oil and coal have their roots in the organisms that lived on the planet over 500 million years ago. *Botryococcus braunii,* Race B, is an ancient, colony-forming unique green alga contributed to these natural resources. It has attracted interest because it accumulates large amounts of high-value, petrochemical replacement oils. The oil oozing from the algal colony is evident in this picture. It has been the target of studies from the large chemical and petrochemical industries.

Source: www.sciencedaily.com



Join with us in "Experts Directory Database"
Send your details through:
www.envismadrasuniv.org/experts_submission.php

ENVIS CENTRE, Department of Zoology, University of Madras, Maraimalai Campus, Guindy, Chennai - 600 025, Tamilnadu, INDIA. Telefax: 91 - 44 - 2230 0899.