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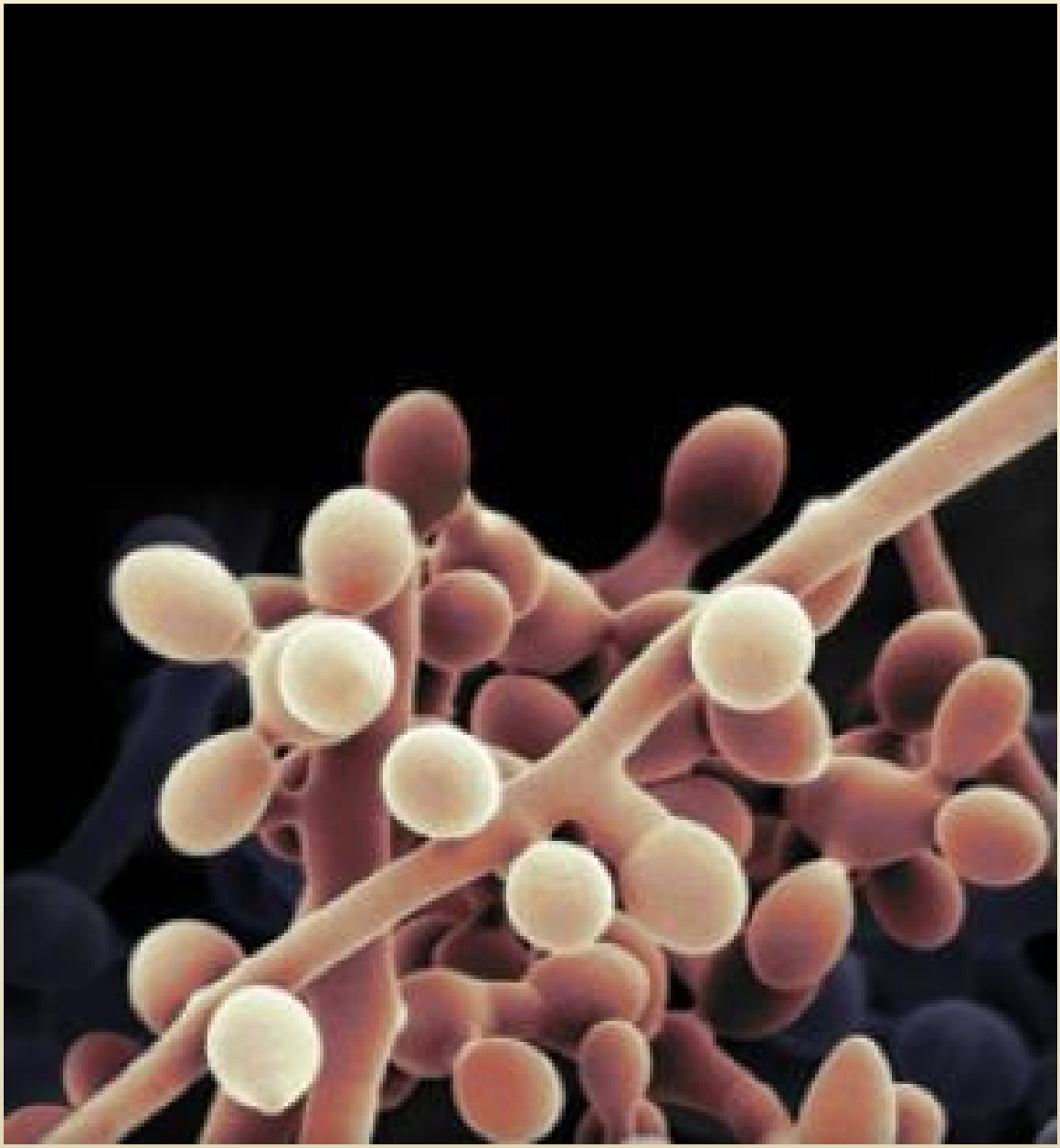

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Cover page : *Myceliophthora thermophila*, fungus, a source of clean energy

(Credit : Concordia University)

ENVIS Newsletter
on
Microorganisms and Environment Management

Contents

Dear Readers,

The theme for **World Environment Day 2012** is **'Green Economy: Does it include you?** A Green Economy is "one that results in improved human well-being and social equity, while significantly reducing environmental risks and ecological scarcities". In its simplest expression, a green economy can be thought of as one which is low carbon, resource efficient and socially inclusive. A Green Economy thus reduces carbon emissions and pollution, enhances energy and resource efficiency, and prevents the loss of biodiversity.

The importance of microbes on green economy is becoming more prominent. Their biosynthetic capabilities have been valuable in finding solutions for several problems to mankind have encountered in maintaining the quality of the environment. Researchers have been hard at work developing green energy from bacteria, fungi and algae. Green technologies such as bio-fuels, bio-plastics, bio-gas, electricity generation and waste treatment harnessing the capabilities of microbial systems for clean, alternative energy resources with considerable success.

Many different species of microorganisms not only play a key role in promoting bio-diversity and ecosystem stability, but also been used as bio-sensors for environmental monitoring. In this issue, article on probiotics and microbial insecticides review a few important applications of bacteria and fungi to human and animal health. Other interesting reports on microorganisms are also available.

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Gut microbes of laboratory reared zebrafish as potential probiotics

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Introduction

World aquaculture has grown tremendously over the last fifty years and became the fastest growing food producing sector in the world. Global intensification and commercialization of aquaculture invariably resulted in disease problems. For decades, outbreaks of infectious diseases caused by a variety of bacteria such as ***Flavobacterium, Vibrio*** **and** ***Mycobacterium*** are focused as primary constraints to the culture of many aquatic species, impeding both economic and social development. Hence, making aquaculture products disease-free and more acceptable to consumers has become the primary challenge.

The limitations associated with the use of antibiotics and vaccinations point to the need for the development of a more user-friendly methodology, which became the focus of attention of international aqua culturists. Probiotics, "live micro-organisms that confer a health benefit on the host" were suggested to be the safe alternative to control proliferation of pathogenic bacteria (Decamp and Moriaty, 2005). The diverse range of bacteria belonging to *Bacillus, Lactobacillus*, *Enterococcus, Pediococcus, Bifidobacteria* and the yeast**,** *Saccharomyces* (Watson *et al.*, 2008) have shown potential as probiotic organisms for aquaculture application. Several studies emphasized the role of gut colonizing probiotics in reducing mortality of the host, alleviating harmful organisms and producing polyamines and digestive enzymes. However, more attention has to be paid on the selection of a suitable probiotic in order to achieve the desired benefit in the host species. Isolation and identification of putative probiotic from the host, in which the probiotic is intended for effective use, has already been claimed as an elegant logic. Thus, identification of gut inhabitant bacteria with probiotic nature to design species-specific feed formulations with the inclusion of indigenous probiotic strain attains priority for successful disease management in aquaculture.

Bacteria are generally identified by sequencing specific sections of the genomic DNA and searching for a close match in the database to find out their taxonomy/genus/species. Among molecular tools,16s rRNA gene is generally accepted as the potential target for identification and phylogenetic analysis of bacteria (Amann *et al.*, 1995) and this technique is a valuable tool for rapid and fairly reliable identification of bacteria. For long, ***Bacillus*** **spp**. are known to be responsible for the exclusion of pathogenic strains due to their ability to produce antagonistic antibiotics, amino acids and enzymes. Therefore the present study documents ***Bacillus*** species inhabiting the gut of Zebrafish as probiotics against common pathogenic bacteria of freshwater fishes.

Materials and methods

Test species

Zebrafish, *Danio rerio* Hamilton obtained from Southern Aquafarms, Chennai, India were maintained in concrete tanks of 50 L capacity at a stocking density of 100 / tank. They were acclimatized with optimal pH (8.0 ± 0.2) and temperature (26 ± 0.5^{0}C).

Isolation and propagation of *Bacillus*

Fish starved for over 12 h were used for experimentation. Gut was dissected out and homogenized aseptically using sterile phosphate buffer (pH 7.0) and centrifuged at 6000 rpm for 10 minutes. Supernatant obtained was serially diluted using sterile phosphate buffer and the suspect colonies were isolated and purified using nutrient agar. The purified discrete colonies were further inoculated on *Bacillus* selective medium, incubated at 37ºC for 18 hours. The colonies from the selective medium were subjected to standard microbial screening techniques such as differential staining, motility test, methyl red test, spore formation and catalase test (Pacarynuk *et al.,* 2004). Pure culture was stored for further antagonistic and DNA studies.

Assessment of antagonistic activity

Antimicrobial activity of probiotic strain was assayed separately using agar diffusion method (Benkerroum *et al.*, 1993). Protein was extracted from probiotic bacteria (10^{5}CFU/ml) and partially purified by sonication followed by dialysis at 4^{0}C. Sterile luria agar medium was prepared and

100µl of about 10^5 CFU/ml of each pathogenic bacteria were evenly spread on the surface of the medium. Discs (0.5 mm) prepared with 100% (49.6µg/ml), 75% (36.8µg/ml), 50% (24.7µg/ml), and 25% (12.1µg/ml) of protein extracts were placed in each of the plates containing pathogenic strains (*Escherichia coli / Pseudomonas fluorescens / Klebsiella aerogenes / Xanthomonas maltophilia).* Another set of four discs prepared by dipping in 5ug/50uL of Gentamicin / Chloramphenicol / Ofloxacin / Erythromycin were placed separately in each of the plates containing the same pathogens and the set is considered as the positive control. All the plates were incubated for 24 hours at 37ºC and the zone of inhibition was observed against respective controls.

DNA extraction and 16s rRNA gene amplification

Broth culture (16h) was used for DNA isolation using phenol:chloroform extraction followed by ethanol precipitation. From the isolated DNA, 16S rRNA gene fragment was amplified using universal primers 27F (5'AGAGTTTGACCTGGCTCAG 3') and 1492R (5' GGTTACCTTGTTACGACTT 3') (Weisburg *et al*., 1991). Amplification was carried out using gradient thermal cycler (Eppendorf, Germany) up to 35 cycles with initial denaturation at 94°C for 3 minutes, denaturation at 94°C for 1 minute, annealing at 58° C for 1 minute, extension at 72°C for 1 minute and final extension at 72°C for 5 minutes. 10 µl of PCR amplified 16S rRNA gene product was separated through 2% agarose gel electrophoresis using 1% TAE buffer (for 1h at 80V). The band was visualized and documented using a gel documentation system.

Analysis of PCR product

Sequencing of PCR product of 16S rRNA gene was carried out in a commercial automated DNA Sequencer (Applied Biosystems Inc, Hyderabad). The sequence information of the bacterial isolate was compared with the similar sequences that are available in the GenBank of National Centre for Biotechnological Information (NCBI) (www.ncbi.nlm.nih.gov) using BLAST.

Results and discussion

Biochemical identification of bacteria isolated from the gut of Zebrafish revealed to be *Bacillus* spp. (Fig.1). Occurrence of *Bacillus* spp. in the gastro-intestinal tract of all fresh water Indian Major Carps and *Labeo rohita* have been demonstrated earlier.

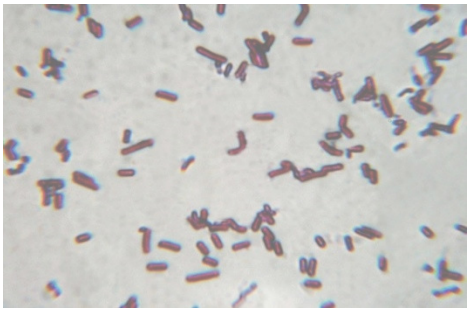

Fig.1 *Bacillus* strain isolated from the gut of Zebra fish

Antagonistic activity test carried out in the present study using 100%, 75%, 50%, and 25% of proteins extracted from *Bacillus* spp**.** showed inhibitory effect of the genus against *Escherichia coli, Pseudomonas fluorescens*, *Klebsiella aerogenes* and *Xanthomonas maltophilia* (Fig. 2). The impact of protein extracts on zone of inhibition against all the pathogenic strains was examined. The zone of inhibition of 0.2 ± 0.03 cm at 25 % showed gradual increase to 0.6 ± 0.01 at 100 % against *E. coli*. Likewise the inhibition zone of 0.1 ± 0.03 cm at 25 % increased to 0.3 ± 0.03 cm at 100% against *P. fluorescens***.** Similar concentration dependent inhibition was observed against *K. aerogenes* and *X. maltophilia* with increase in the zone of inhibition from 0.2 ± 0.03 and 0.1 ± 0.03 at 25 % to 0.4 ± 0.03 and 0.2 ± 0.03 cm at 100% respectively (Fig. 3). Thus the inhibitory effect of *Bacillus* spp. was found to be significantly higher at all the concentrations tested against *E. coli* compared to the rest of pathogenic bacteria. Antagonistic studies carried out earlier by Ghosh *et al*. (2007) considering *B. subtilis* demonstrated effective inhibition of *P. fluorescens* and *Aeromonas hydrophila* species in *L. rohita*. Chantharasophon *et al*. (2011) also demonstrated inhibitory role of *Bacillus* against growth of *A. hydrophila* in *Nile tilapia.*

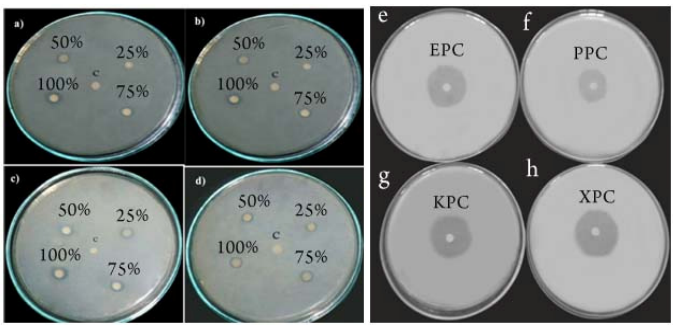

Fig. 2 Antagonistic activity of different concentrations (100, 75, 50, 25 %; C= LB medium) of protein extracts from zebrafish gut inhabiting *Bacillus* spp. *against a) E. coli , b) P. fluorescens, c) K. aerogenes* and *d) X. maltophilia.*
e) EPC, f) PPC, g) KPC & h) XPC represent respective positive controls.

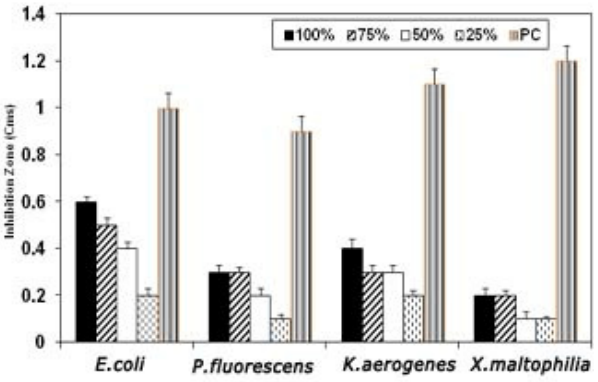

Fig. 3 Inhibition zone of different concentrations of proteins extracted from *Bacillus* species and antibiotics (PC = Positive Control) against *E.coli, P. fluorescens, K. aerogenes* and *X. maltophilia.*
(Values are Mean ± SD (n=5) of 5 individual observations)

Isolated DNA of ***Bacillus*** run through agarose gel against 500 bp marker exhibited a discrete band with molecular weight of > 5000 bp. 16s rRNA gene amplification carried out through PCR and run through 2 % agarose gel showed a clear band of 1600 bp (Fig.4 lane2). Partial sequence of 16s rRNA gene product obtained using forward primer amplification provided the sequence of 689 bases (Fig. 5). Further, the homology search made using BLAST revealed 98% similarity of the product with that of *Bacillus thuringiensis* (www.ncbi.nlm.nih.gov/blast/) (Gene Bank accession number JQ894337). Furthermore, *Bacillus* spp. showing 99 % homology with *B. substilis* (Geethanjali and Anitha Subash, 2011) and 99.9% homology with *B. brevis* (Chantharasophon *et al*., 2011) were more prevalent in the gut of laboratory reared *L. rohita* and *Nile tilapia* respectively.

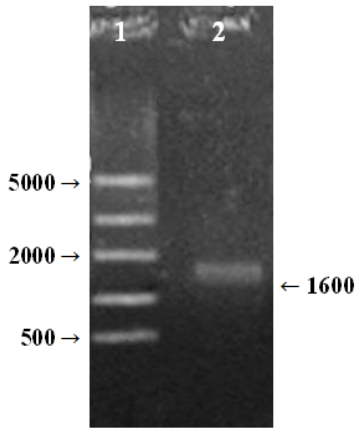

Fig. 4 PCR product of DNA isolated from Zebrafish gut inhabitant *Bacillus* spp. amplified using 16S rRNA primers. (Lane 1 - 5000 bp Marker; Lane 2 - amplified gene product)

TAGGCGGCTGGCTCCAAACGGTTACCCGACCGACTTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAG

GCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGATTACAAGCGATTCCGGCTTCATGTAGGCAAGTTGCAGCCTAC

AATCCGAACTGAGAACGGTTTTATGAAATTAGCTCCACCTCGCGGTCTTGCAGCTCTTTGTACCGTCCATTGTAGCACGT

GTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCACCTTAG

AGTGCCCAACTAAATGATGGCAACTAAAATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGC

TGACGACAACCATGCACCACCTGTCACTCTGCTCCCGAAGGAGAAGCCCTATCTCTAGGGTTGTCAAAGGATGTCAAGAC

CTGTAAGGTTCTTCGCGTTGCTTCAAATTAAACCACATGCTCCACCGCTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTC

AGCCTTGCGGCCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAACTTCAGCACTAAGGGGCGGAAACCCTCTAACCACTT

AGCAACTCATCGTTTACCGCCGTGGACTACCAGGTTATCTAATCCTGTT

Fig. 5 Partial sequence of PCR product of 16S rRNA gene of *Bacillus* spp. isolated from the gut of laboratory reared Zebrafish (www.ncbi.nlm.nih.gov)

Conclusion

Non-pathogenic nature and probiotic role of ***Bacillus*** species demands its use as an antagonistic agent against fish pathogens. The present study for the first time document, the occurrence of ***Bacillus*** in the gastrointestinal tract of laboratory reared Zebrafish. The strain showed high homology to ***Bacillus thuringiensis*** and significantly inhibited the growth of ***Escherichia coli / Pseudomonas fluorescens / Klebsiella aerogenes / Xanthomonas maltophilia,*** and thus provide strong evidence as a feed probiont for disease management in fresh water fish. Further studies on complete sequencing of 16S rRNA gene and phylogenetic status of the newly isolated ***Bacillus*** are in progress.

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Microbial insecticides in eco-friendly insect pest management

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Introduction

Insect pest control is almost an unavoidable element of agricultural practice throughout the world. The most important problem in insect pest control is the development of resistance by the pests to chemical insecticides. Farmers are under enormous pressure to reduce the use of chemical insecticides without sacrificing yield/crop quality, but at the same time the control of pests is becoming increasingly difficult due to insecticide resistance and the decreasing availability of products. The immediate need is the use of alternate pest control methods. Many farmers are now familiar with the use of insect predators and parasitoids for biological control of pests, but it is also possible to use specific micro-organisms that kill pests of insects. These include insect pathogens (most popularly called as entomopathogens or microbial insecticides) such as bacteria, viruses and fungi. These are all widespread in the natural environment and cause infections in many insect pest species. Entomopathogens contribute to the natural regulation of many insect pest populations. Many can be mass produced, formulated and applied to insect pest populations in a manner similar to chemical insecticides. Microbial insecticides are thus especially valuable because their toxicity to non-target animals and humans is extremely low. They are safe for both the user and consumers of treated crops when compared to other commonly used insecticides.

Types of Entomopathogens or Microbial Insecticides

1. Bacterial insecticides

More than ninety species of naturally occurring insect-specific (entomopathogenic) bacteria have been identified but only a few have been studied most intensively. Much interest has been given to *Bacillus thuringiensis* (*Bt*), a species that has been developed as a commercial microbial insecticide.

Some commercially known *Bt* varieties and target insect pests

Bacillus thuringiensis

var. *tenebrionis* - Colorado potato beetle and elm leaf beetle larvae

var. *kurstaki* - caterpillars

var. *israelensis* - mosquito, black fly and fungus gnat larvae

var. *aizawai* - wax moth larvae and various caterpillars, especially the diamondback moth caterpillar

Bacillus thuringiensis (*Bt*) occurs naturally in the soil and on plants. Different varieties of this bacterium produce a crystal protein that is toxic to specific groups of insects. The toxic crystal *Bt* protein in commercial formulations is only effective when consumed orally by insects with a specific gut pH (usually alkaline) and the specific gut membrane structures are required to bind the toxin. It is not only the insect must have the correct physiology and be at a susceptible stage of development, but the bacterium must be consumed in sufficient quantity. When ingested by a susceptible insect, the protein toxin damages the gut lining, leading to gut paralysis. Affected insects stop feeding and die from the combined effects of starvation and tissue damage. *Bt* spores do not usually spread to other insects or cause disease outbreaks as seen with many pathogens. *Bt* genes have been transferred into other microorganisms to produce more active formulations, some of which are commercially available. Additionally, researchers

have genetically engineered varieties of several plant species to express the *Bt* toxin as part of the plant's normal development. This has led to the production of "insect-resistant" *Bt*-transformed varieties of tobacco, cotton, corn, tomatoes, potatoes and others.

Other bacterial insecticides

Insecticides available in market in the generic name "milky spore disease" contain the bacterial species such as *Bacillus popillae* and *Bacillus lentimorbus.* It is very difficult to culture these bacteria in fermentation tanks but, they are obtained from laboratory-reared infected insect larvae. Insecticidal products containing *B. popillae* and *B. lentimorbus* can be applied to the soil for the control of larval / grub stage of several beetles. When a susceptible grub consumes spores of these bacteria, they proliferate within it, and the grub's internal organs are liquefied and turned milky white (most commonly called as milky spore disease). These symptoms develop slowly, often over a period of three to four weeks after initial infection. *B. popillae* and *B. lentimorbus*, unlike *Bt*, undergo proliferation in the environment if a substantial grub population is present at the time of application. Once grubs are killed by these bacteria, a new batch of spores is released into the soil. These spores can survive in the undisturbed soil for a period of 15 to 20 years.

2. Viral insecticides

The larvae of many insect species are vulnerable to devastating epidemics of viral diseases. The viruses that cause these outbreaks are very specific, usually acting against only a single insect genus or even a single species. Most of the viruses that are nuclear polyhedrosis viruses (NPVs), in which numerous virus particles are "packaged" together in a crystalline envelope within insect cell nuclei. Some granulosis viruses (GVs), in which one or two virus particles are surrounded by a granular or capsule-like protein crystal found in the host cell nucleus. These groups of viruses infect caterpillars and the larval stages of many dipteran insects.

Viruses, like bacteria, must be ingested to infect insect hosts. In sawfly larvae, virus infections are limited to the gut, and disease symptoms are not as obvious as they are in caterpillars. In caterpillars, virus particles pass through the insect's gut wall and infect other body tissues. As an infection progresses, the internal organs of caterpillars are liquefied, and its cuticle become discolors and eventually ruptures. Caterpillars killed by viral infection appear limp and soggy (Fig. 1). They often remain attached to foliage or twigs of plants for several days, release viral particles that may be consumed by other larvae. The pathogen can be spread throughout an insect population in this way and by virus-contaminated eggs of infected adult females. Dissemination of viral pathogens is deterred by exposure to direct sunlight, because direct ultraviolet radiation destroys virus particles. Although naturally occurring epidemics do control certain pests, these epidemics rarely occur before pest populations have reached out peak levels.

Fig. 1 Nuclear Polyhedrosis Viruses (NPVs) infected larvae of silkworm, *Bombyx mori*

The development and use of virus-based insecticides have been limited. Unlike *Bt*, insect viruses must be produced in live host insects. Production is therefore both expensive and time-consuming. As these viruses are genus or species specific, each viral insecticide has a limited market value. Nonetheless, though they are not well known or widely available, several insect viruses have been developed and registered for use as insecticides. Most are specific to a single species or a small group of related pests; they are not commercially available but are produced and used. Forest pests are especially good targets for viral pathogens because the permanence of the forest environment contributes to cycling of the pathogen (transmission from one generation to the next). The forest canopy also helps to protect viral particles from destruction by ultraviolet radiation.

Other insect viruses investigated for use as insecticides include those that infect the loopers, armyworms and imported cabbageworm. Although some of these viruses have been commercially formulated and applied in field tests, none has been registered or sold in the market. Most viruses are host-specific and effective only against immature stages of the target insect species.

The users must make sure to match the viral pathogen and the target pest correctly. Virus particles are killed by ultraviolet radiation and treating in the evening or on cloudy days should increase their effectiveness.

3. Fungal Insecticides

Fungi, like viruses, often act as important natural insect control agents that limit pest populations. Most of the species that cause insect diseases spread by means of asexual spores called conidia. Although conidia of different fungi vary greatly in ability to survive adverse environmental conditions, desiccation and ultraviolet radiation are important causes of mortality in many species. The viable conidia reach a susceptible insect pest host, free water or very high humidity is usually required for their germination. Unlike bacterial spores or virus particles, fungal conidia can germinate on the insect cuticle and produce specialized structures that allow the fungus to penetrate the cuticle and enter the insect's body (Fig. 2). Fungi do not have to be ingested to cause infections. In most instances, as fungal infections progress, infected insects are killed by fungal toxins, not by the chronic effects of parasitism.

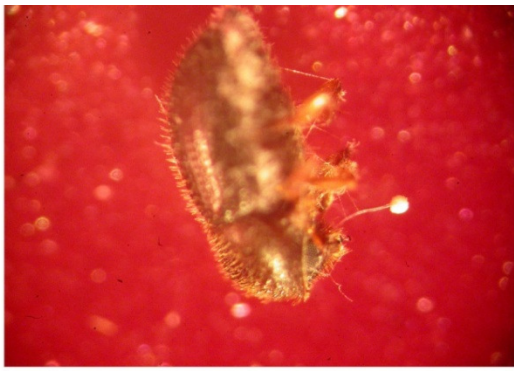

Fig. 2 Dead *Hypothenemus hampei* (adult Coleopteran beetle) on the application of fungus *Beauveria bassiana*

Many important fungal pathogens attack eggs, immature stages and adults of a variety of insect pest species. Others are more specific to immature stages or to a narrow range of insect species. Although fungal pathogens can be produced on artificial media, large-scale production of most pathogens has not yet been accomplished. Precise production and storage conditions must be established and maintained to ensure that infective spores are produced and stored without loss of viability before they are applied. Once applied, pathogenic fungi often are effective only if environmental conditions are favorable; high humidity or rainfall is usually important. In the case of fungal pathogens incorporated in soil to control soil pests, the adverse effects of ultraviolet radiation and desiccation are minimized, but other microorganisms that act as competitors or antagonists often alter pathogen effectiveness.

Fungi used as insecticides include the following:

Beauveria bassiana: This common soil fungus has a broad host range that includes many beetles. It infects both larvae and adults of many species. Understanding the interactions between *B.bassiana* and other soil microorganisms may be the key to successful use of this fungus.

Nomuraea rileyi: Naturally occurring epidemic infections of *Nomuraea rileyi* cause dramatic reductions in populations of foliage-feeding caterpillars in soybeans.

Vericillium lecanii: This fungus has been used to control aphids and whiteflies.

Lagenidium giganteum: This aquatic fungus is highly infectious to larvae of several mosquito genera. It propagates effectively in the aquatic environment, even when mosquito density is low. Its effectiveness is limited by high temperatures.

Hirsutella thompsonii: It is a pathogen of the citrus rust mite. Although preparations of this pathogen were once registered and marketed, it is no longer available commercially.

Conclusion

Microbial insecticides provide effective alternatives in an eco-friendly way for the control of many insect pests. Their greatest strength is safety, as they are importantly non-toxic and non-pathogenic to animals and humans. Although not every pest species can be controlled by the use of a microbial insecticide, their products can be used successfully in place of more toxic synthetic insecticides to control several important insect pests. Most microbial insecticides are effective against only a narrow range of pests because these insecticides are vulnerable to quick inactivation in the environment. Hence farmers must properly identify target insect pests and plan the most effective type of microbial insecticides. Moreover, they can be used without undue risks of human injury and environmental damage. Consequently, microbial insecticides are likely to become increasingly important tools in insect pest management.

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Research Reports

From compost to sustainable fuels: heat-loving fungi sequenced

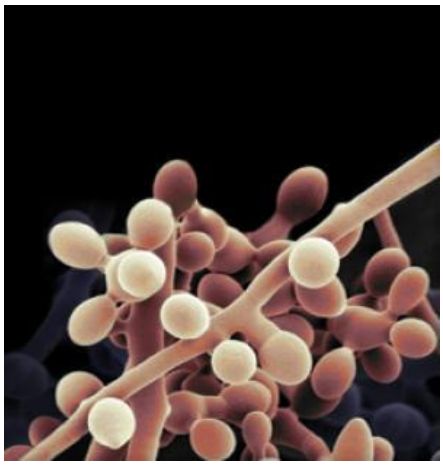
Two heat-loving fungi, often found in composts that self-ignite without flame or spark, could soon have new vocations. The complete genetic makeup of *Myceliophthora thermophila* and *Thielavia terrestris* has been decoded by an international group of scientists. The findings, published in *Nature Biotechnology*, may lead to the faster and greener development of biomass-based fuels, chemicals and other industrial materials.

"Organisms that thrive at high temperatures are rare. Fewer than 40 heat-loving fungi have been identified and they hold great promise in the production of many chemicals and biomass-based fuels," says senior author Adrian Tsang, a Biology Professor at Concordia University and Director of its Centre for Structural and Functional Genomics. "We have cracked the genetic blueprint of two such fungi. To our knowledge these are the only organisms, aside from a few bacteria, whose genomes have been fully sequenced from end-to end."

In sequencing *Myceliophthora thermophila* and *Thielavia terrestris*, the research team also discovered that both fungi could accelerate the breakdown of fibrous materials from plants at temperatures ranging from 40 to 70 degrees Celsius. This temperature range is too hot for many of the typical enzymes, which form an important component of some industrial processes used to degrade biomass into a range of chemicals and products. But where others fail, these fungi thrive. "Our next goal is to figure out how these organisms flourish at high temperatures and what makes them so efficient in breaking down plant materials," says Tsang.

These discoveries will further stimulate the search for better ways to transform green waste stalks, twigs, agricultural straws and leaves into renewable chemicals and fuels. Enzymes produced by these fungi could also be tweaked to replace the use of environmentally harmful chemicals in the manufacture of plant-based commodities such as pulp and paper. Having a multi-sectorial research team, composed of scientists from academia, government and industry, is essential to making these new advances.

"We could not have made these findings separately, since this type of research benefits tremendously from the intellectual input of researchers from different sectors," Tsang says. "This is an important discovery as we position ourselves from a fossil-fuel economy to one that uses biomass materials." This study was supported by the U.S. Department of Energy, the Cellulosic Biofuel Network of Agriculture and Agri-Food Canada, Genome Canada and Génome Québec.



The complete genetic makeup of *Myceliophthora thermophila,* a fungus, has been decoded for clean energy sources.

(Credit: Image courtesy of Concordia University)

Source: www.sciencedaily.com

Report seeks to integrate microbes into climate models

The models used to understand how Earth's climate works include thousands of different variables from many scientific including atmospherics, oceanography, seismology, geology, physics and chemistry, but few take into consideration the vast effect that microbes have on climate. Now, a new report from the American Academy of Microbiology, "Incorporating Microbial Processes into Climate Models," offers a plan for integrating the latest understanding of the science of microbiology into climate models. "Climate scientists and microbiologists usually work in isolation from each other, and yet their work is intimately connected. Microbes are critical players in every geochemical cycle relevant to climate.

The sum total of microbial activity is enormous, but the net effect of microbes on climate-relevant gases is currently not known," says Edward DeLong of the Massachusetts Institute of Technology, who co-chaired the report with Caroline Harwood of the University of Washington. The past two decades have witnessed an explosion in scientific recognition of the diversity of the microbial world. New DNA-sequencing technologies spurred by the Human Genome Project have made it technically and economically possible to sequence the collective DNA from whole microbial communities. This approach, called metagenomics, has revealed a previously undreamed-of degree of diversity in the microbial world. These microbial community analyses many "omics" approaches, such as proteomics and metabolomics, that together provide a detailed picture of community function, potential and change over time.

The report is based on a colloquium convened by the Academy in 2011. Experts in diverse disciplines in microbiology as well as computational and climate modeling participated in the meeting designed to identify specific efforts and activities that will lead to improved integration of microbial biology, biogeochemistry, and climate modeling. "While the gap between these disciplines is daunting, the need to bridge it is urgent and the science and technology needed to begin to do so is within reach," says Harwood. The report suggests a multipronged approach, breaking the challenge into manageable parts. The first recommendation is to choose a few specific biogeochemical cycles that are important, microbially driven and tractable to serve as demonstration projects. Specifically, the report identifies methane, carbon storage and nitrous oxide.

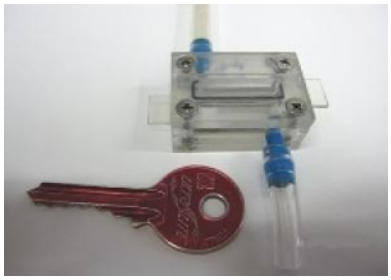
Other recommendations include:

- Assess current data collection methodologies and develop a monitoring/data collection strategy
- Implement validation processes to integrate data collection, modeling and experimentation
- Facilitate and provide incentives for collaborations and interdisciplinary training
- Address technology needs

"There is clear evidence that microbes can have an enormous impact on climate. In light of the increasingly urgent need to understand and find ways to mitigate climate change, the centrality of microbes in global biogeochemical cycles, can no longer be ignored," says DeLong.

Source: www.sciencedaily.com

Dip chip technology tests toxicity on the go



A biosensor device called the "Dip Chip"

Biosensors have long been used to safeguard against exposure to toxic chemicals. Scientists have combined biology and engineering to produce a biosensor device called the "Dip Chip," which detects toxicity quickly and accurately, generating low false positive and false negative readings.

The device, which looks like a dip stick, immobilizes these specially-produced microbes next to the sensing electrodes. Once the microbes come into contact with a questionable substance they produce a chemical signal that is converted to an electrical current by a device that can interpret the signals, producing a binary "toxic" or "not toxic" diagnosis. In the future, scientists hopes that smaller versions of the Dip Chips might be plugged into existing mobile electronic devices, such as cell phones or tablets, to give the user a toxicity reading. This would make it an economically feasible and easy-to-use technology for people such as campers or for military purposes.

(**Credit:** Image courtesy of American Friends of Tel Aviv University)

Source: www.sciencedaily.com

Hot new manufacturing tool: A temperature-controlled microbe

Many manufacturing processes rely on microorganisms to perform tricky chemical transformations or make substances from simple starting materials. The authors of a study appearing in mBio, the online open-access journal of the American Society for Microbiology, have found a way to control a heat-loving microbe with a temperature switch: it makes a product at low temperatures but not at high temperatures. The innovation could make it easier to use microorganisms as miniature factories for the production of needed materials like biofuels. This is the first time a targeted modification of a hyperthermophile (heat-loving microorganism) has been accomplished, say the authors, providing a new perspective on engineering microorganisms for bioproduct and biofuel formation.

Originally isolated from hot marine sediments, the hyperthermophile *Pyrococcus furiosus* grows best at temperatures around 100ºC (212ºF). *P. furiosus* is an archaeon, single-celled organisms that bear a resemblance to bacteria, but they excel at carrying out many processes that bacteria cannot accomplish. Like other hyperthermophiles, *P. furiosus'* enzymes are stable at the high temperatures that facilitate many industrial processes, making it a well-used tool in biotechnology and manufacturing. But not all products can be made at high heat. Some enzymes will only work at lower temperatures.

In the study in mBio, the authors inserted a gene from another organism into *P. furiosus* and coaxed it to use that gene to make a new product by simply lowering the temperature. The donor organism, *Caldicellulosiruptor bescii*, prefers to grow at a relatively cool 78ºC, so the protein product of its gene, lactate dehydrogenase, is most stable at that comparatively low temperature.The authors of the study inserted the lactate dehyrogenase gene into a strategic spot, right next to a cold shock promoter that "turns on" the genes around it when *P. furiosus* is out in the cold at 72ºC. This essentially gives scientists a switch for controlling lactate production: put the organism at 72ºC to turn on lactate production, restore it to 100ºC to turn it off, thus preventing the need for chemical inducers. What's more, since *P. furiosus* is mostly shut down at these lower temperatures, making the new product doesn't interfere with its metabolism, or vice-versa.

The lead author on the study, Michael Adams of the Department of Biochemistry & Molecular Biology at the University of Georgia, explains that this is the key benefit of this system: although *P. furiosus* now makes the enzyme that carries out the process, at these lower temperatures the organism's other metabolic processes don't get in the way. "The hyperthermophile is essentially the bioreactor that contains the foreign enzymes," says Adams. *P. furiosus* just supplies cofactors and a cytoplasmic environment for the highly active foreign enzymes, according to Adams. This makes for a cleaner, more controllable reaction.

Source: www.sciencedaily.com

Killer silk: making silk fibers that kill anthrax and other microbes in minutes

A simple, inexpensive dip-and-dry treatment can convert ordinary silk into a fabric that kills disease-causing bacteria even the armor-coated spores of microbes like anthrax in minutes, scientists are reporting in the journal ACS Applied Materials & Interfaces. They describe a range of potential uses for this new killer silk, including make-shift curtains and other protective coatings that protect homes and other buildings in the event of a terrorist attack with anthrax.

Rajesh R. Naik and colleagues explain that in adverse conditions, bacteria of the *Bacillus* species, which includes anthrax, become dormant spores, enclosing themselves in a tough coating. These spores can survive heat, radiation, antibiotics and harsh environmental conditions, and some have sprung back to life after 250 million years. Certain chemicals most popular among which are oxidizing agents, including some chlorine compounds can destroy bacterial spores, and they have been applied to fabrics like cotton, polyester, nylon and Kevlar. These treated fabrics are effective against many bacteria, but less so against spores. The researchers tried a similar coating on silk to see if it could perform better against these hardy microbes.

They developed a chlorinated form of silk, which involves soaking silk in a solution that includes a substance similar to household bleach and letting it dry. Silk treated for just an hour killed essentially all of the *E. coli* bacteria

tested on it within 10 minutes and did similarly well against spores of a close anthrax relative used as a stand-in. “Given the potent bactericidal and sporicidal activity of the chlorinated silk fabrics prepared in this study, silk-Cl materials may find use in a variety of applications,” the authors say. Other applications, they add, include purifying water in humanitarian relief efforts and in filters or to mitigate the effects of toxic substances. The authors acknowledge funding from the Defense Threat Reduction Agency and the Air Force Office of Scientific Research.

Source: www.sciencedaily.com

News

Did those bacteria really dine on lethal arsenic?

It was research that appeared set to turn the biological world on its head. A paper published online by the journal Science in December 2010 described a strain of bacteria that not only thrived in high levels of arsenic but appeared to incorporate it in its biomolecules, including DNA, displacing phosphorus that all other known forms of life utilise.

The U.S. space agency, the National Aeronautics and Space Administration (NASA), which had funded the research, loudly trumpeted the discovery. “The definition of life has just expanded,” exulted a senior agency official in a press release. But the paper by Felisa Wolfe-Simon and others failed to convince their peers in the scientific community. Instead, what followed was an outcry from scientists about flaws in the research. There was good reason, they said, to doubt that the bacterium was using arsenic in its DNA.

Science, according to its Editor-in-Chief, Bruce Alberts, received “a wide range of correspondence that raised specific concerns” about the paper’s research methods and interpretation of results. In May 2011, the journal took the unusual step of publishing online eight technical comments that raised a number of issues. But the question remained shouldn't someone else try to replicate the experiment using methods that avoided the pitfalls of the earlier work? It was not an alluring prospect, considering that the most likely outcome would be to merely corroborate the flaws that had already been pointed out.

Rosemary Redfield, a microbiologist at University of British Columbia in Canada, decided to take on the task. In a post on her blog ‘RRResearch’, which received a good deal of attention, she had criticised the Science paper as “lots of flim-flam, but very little reliable information.” “I’ve been saying that researchers shouldn’t invest the time and resources needed to test Wolfe-Simon *et al*’s claims because of the vanishingly small probability that they are correct,” she remarked in a blog post in May last year. “But I’m having second thoughts because the most important claims can, I think, be very easily tested.”

Having got the bacterial strain from the original group of researchers, Dr. Redfield set about planning and carrying out experiments in a remarkably different fashion. The experiments she wanted to do, the problems that cropped up and the results she got were all written up on her blog. “One of the thing that had always been unusual about my blog was that I was writing openly about the experiments that I was doing before they were published,” she said on a recent episode of the podcast ‘This Week in Microbiology’. It was important that the process of science be made much more open. It was also a useful way to clarify her thinking.

By January this year, Dr. Redfield and her collaborators at Princeton University in the U.S. had finished the lab work and prepared a paper. The paper was submitted to Science. But she also did something that is common enough in physics but rare in biology. The full manuscript was posted on arXiv.org, the preprint server that is freely accessible.

“The advantage of arXiv is that the physicists all use it,” she remarked on the podcast. So Science would have had to deal with physicists posting papers there before or after they submitted them for publication. Indeed, the editor at Science handling their paper said that the journal had no problem with the manuscript being put on arXiv.

Science later responded with a provisional acceptance and comments from three reviewers. The manuscript was revised in the light of those comments and sent back to the journal.

But Dr. Redfield has also posted the full reviewers comments on a web site and the revised manuscript was made available on arXiv.

Asked on the podcast whether the reviewers' comments could be released publicly, she responded, "I don't see why not." There was nothing to indicate that those comments were to be kept in confidence. As for their finding, the manuscript declares that there was no sign that the bacterium was able to grow by using arsenic or that the element had been incorporated in its DNA. "On April 13, we submitted the revised version [of the manuscript to Science], and we're waiting with fingers crossed for final acceptance," said Dr. Redfield on her blog.

Felisa Wolfe-Simon processing mud from Mono Lake to inoculate media to grow microbes on arsenic.

Source: The Times of India, May 10, 2012.

Abstracts

001. Bao MT, Wang LN, Sun PY, Cao LX, Zou J, Li YM. Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, Ocean University of China, Shandong, Qingdao 266100, China. **Biodegradation of crude oil using an efficient microbial consortium in a simulated marine environment.** Marine Pollution Bulletin, 2012, **64** (6), 1177 – 85.

Ochrobactrum sp. N1, *Brevibacillus parabrevis* N2, *B. parabrevis* N3 and *B. parabrevis* N4 were selected when preparing a mixed bacterial consortium based on the efficiency of crude oil utilization. A crude oil degradation rate of the N-series microbial consortium reached upwards of 79% at a temperature of 25°C in a 3.0% NaCl solution in the shake flask trial. In the mesocosm experiment, a specially designed device was used to simulate the marine environment. The internal tank size was 1.5m (L) ×0.8m (W) ×0.7m (H). The microbial growth conditions, nutrient utilization and environmental factors were thoroughly investigated. Over 51.1% of the crude oil was effectively removed from the simulated water body. The escalation process (from flask trials to the mesocosm experiment), which sought to represent removal under conditions more similar to the field, proved the high efficiency of using N-series bacteria in crude oil degradation.

Keywords: *Ochrobactrum* sp. N1, *Brevibacillus parabrevis* N2, *B. parabrevis* N3 and *B. parabrevis* N4, crude oil degradation.

002. Chrzanowski L, Dziadas M, Lawniczak L, Cyplik P, Bialas W, Szulc A, Lisiecki P, Jelen H. Institute of Chemical Technology and Engineering, Poznan University of Technology, Pl. M. Sklodowskiej-Curie 2, 60-965 Poznan, Poland. **Biodegradation of rhamnolipids in liquid cultures: Effects of biosurfactant dissipation on diesel fuel/B20 blend biodegradation efficiency and bacterial community composition.** Bioresource Technology, 2012, 328 – 35.

Bacterial utilization of rhamnolipids during biosurfactant-supplemented biodegradation of diesel and B20 (20% biodiesel and 80% diesel v/v) fuels was evaluated under conditions with full aeration or with nitrate and nitrite as electron acceptors. Rhamnolipid-induced changes in community dynamics were assessed by employing real-time PCR and the ddCt method for relative quantification. The experiments with rhamnolipids at 150 mg/l, approx. double critical micelle concentration (CMC) and diesel oil confirmed that rhamnolipids were readily degraded by a soil-isolated consortium of hydrocarbon degraders in all samples, under both aerobic and nitrate-reducing conditions. The presence of rhamnolipids increased the dissipation rates for B20 constituents under aerobic conditions, but did not influence the biodegradation rate of pure diesel. No effect was observed under nitrate-reducing conditions. The biodegradation of rhamnolipids did not favor the growth of any specific consortium member, which proved that the employed biosurfactant did not interfere with the microbial equilibrium during diesel/biodiesel biodegradation.

Keywords: Biosurfactant, Biodegradation of diesel, Rhamnolipids.

E - Resources on Microorganisms

NATIONAL

North Maharashtra Microbial Culture Collection Centre
http://www.wfcc.info/ccinfo/index.php/collection/by_id/972

Goa University Fungus Culture Collection and Research Unit
http://www.wfcc.info/ccinfo/index.php/collection/by_id/946

The Energy and Resources Institute
http://www.teriin.org/index.php?option=com_division&task=view_area&id=47

Culture Collection, Microbiology and Cell Biology Laboratory, Indian Institute of Science
http://mcbl.iisc.ernet.in/

Culture collection, Department of Microbiology, Bose Institute
http://bic.boseinst.ernet.in/micro/

INTERNATIONAL

Bacteria, Fungi, Algae
http://www.atcc.org/

World Federation of Culture Collections
http://www.wfcc.info/

Australian Collection of Microorganisms
http://www.uq.edu.au/departments/unit.html?unit=405

National Collection of Plant Pathogenic Bacteria
http://www.ncppb.com/

National Collections of Industrial, Food and Marine Bacteria
http://www.ncimb.com/

EVENTS
Conferences / Seminars / Meetings 2012

Molecular Basis of Microbial One-Carbon Metabolism, August 5 - 10, 2012. **Venue:** Lewiston, ME, **USA.**
Website: http://www.grc.org/programs.aspx?year=2012&program=molbasis

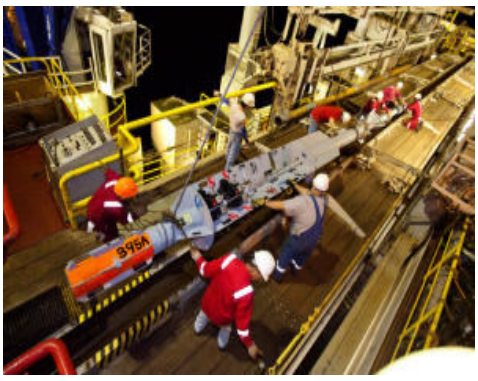
14th International Symposium on Microbial Ecology, ISME14, August 19 - 24, 2012. **Venue:** Copenhagen, **Denmark.**
Website: http://www.isme-microbes.org/isme14

Bacteria, Archaea and Phages, August 21 - 25, 2012. **Venue:** Cold Spring Harbor, NY, **USA.**
Website: http://meetings.cshl.edu/meetings.html

Medical Biofilm Techniques 2012, August 27 - 30, 2012. **Venue:** Copenhagen, **Denmark. Website:**
http://www.escmid.org/profession_career/educational_activities/current_escmid_courses_and_workshops/biofilms/

3rd Food and Environmental Virology Conference, October 7 - 10, 2012. **Venue:** Lisbon, **Portugal.**
Website: http://fevcongress.ist.utl.pt/

Scientists look to microbes to unlock earth's deep secrets



JOIDES Resolution crew members prepare a CORK for installation beneath the seafloor.

Of all the habitable parts of our planet, one ecosystem still remains largely unexplored and unknown to science: the igneous ocean crust. This rocky realm of hard volcanic lava exists beneath ocean sediments that lie at the bottom of much of the world's oceans. An international team of scientists sailing onboard the research vessel JOIDES Resolution recently returned from installing observatories beneath the seafloor in "North Pond" - a remote area in the middle of the Atlantic Ocean. They hope that data collected from these sub seafloor observatories (known as **CORKs, or Circulation Obviation Retrofit Kits**), along with studies of rock and sediment samples collected during the expedition, will help to shed light on the role tiny subsea floor microbes play in shaping Earth's oceans and crust.

(**Credit:** IODP/USIO)
Source: www.sciencedaily.com

Awareness Program

World Environment Day Celebration - 05 June, 2012

World Environment Day was celebrated by the **ENVIS Centre** by conducting various awareness programs. Banners were placed at different locations in University campus to explore the theme of the event.

EARTH DAY

April 22, 2012

This year's Earth Day theme is **"Mobilize the Earth"**. This theme is designed to provide people of all nationalities will voice their appreciation for the planet and demand its protection. Together we will stand united for a sustainable future and call upon individuals, organizations and governments to do their part.

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Send your details through:
www.envismadrasuniv.org/experts_submission.php

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