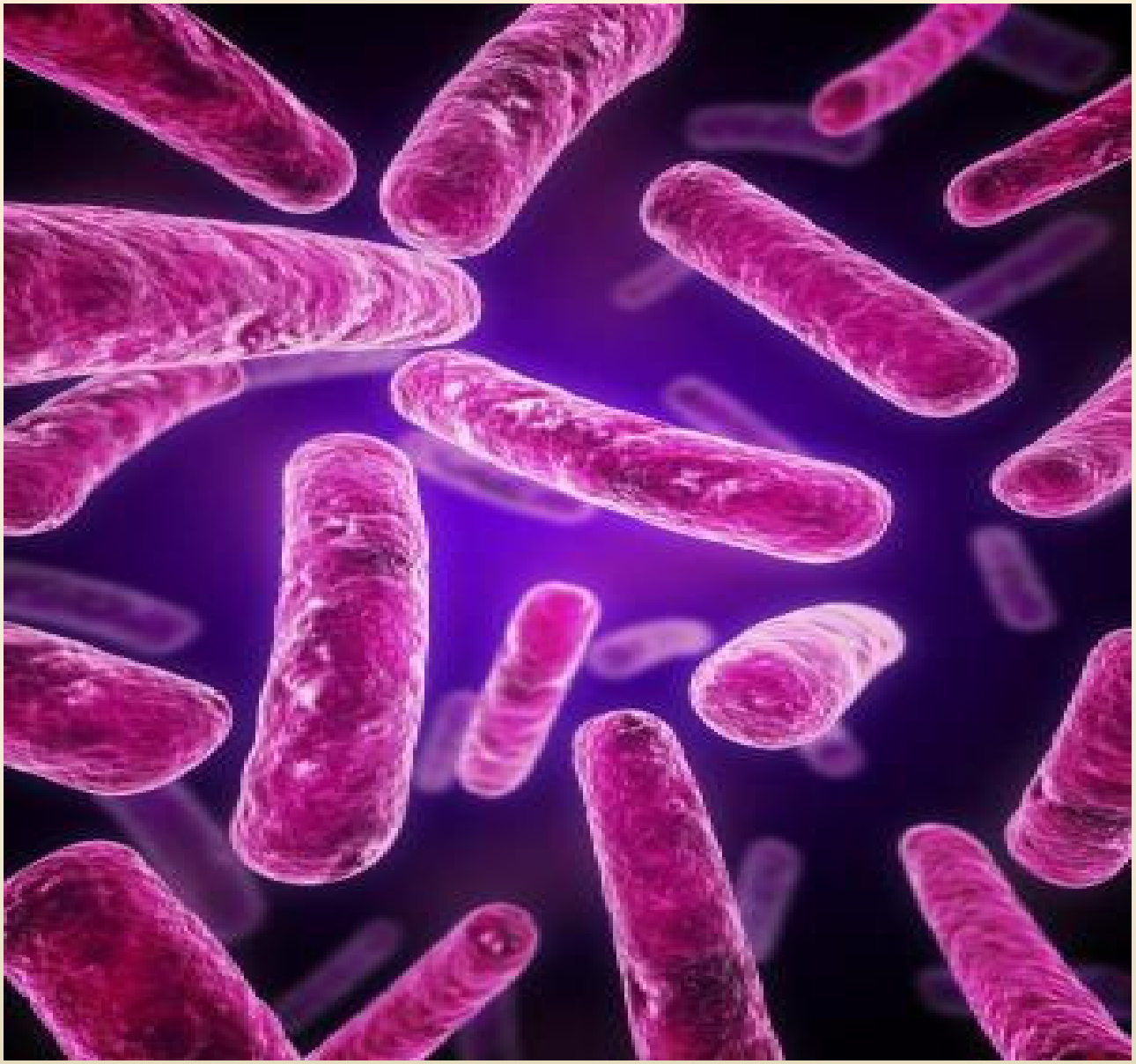
ISSN-0974-1550

ENVIS NEWSLETTER

MICROORGANISMS AND ENVIRONMENT MANAGEMENT

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

VOLUME 8 ISSUES 3 & 4 JUL & OCT - 2010



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ISSN - 0974 - 1550

Volume 8 | Issues 3 & 4 | Jul & Oct 2010

PUBLISHED BY

Environmental Information System (ENVIS)

Department of Zoology

University of Madras, Maraimalai Campus, Guindy,

Chennai - 600 025, Tamilnadu, India.

SPONSORED BY

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Xanthoria elegans (lichen)

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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.

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Cover page

Front : Artist's rendering of bacteria (Courtesy: iStockphoto/Sebastian Kaulitzki)

Back : A female *Daphnia magna* with eggs, species used in the study of bacterial hitchhikers (Courtesy: Adam Petrusek)

ENVIS Newsletter on Microorganisms and Environment Management

Contents

Dear Readers,

It has become mandatory on the part of each nation to cut the consumption of green house gases to save the universe from climate change. The existing UN pact obliges 40 developed nations to begin reducing their green house gas usage. This pact is under print carbon market and is meant to foster a vast shift in investment towards cleaner energies.

However, emerging economies say that the rich have burnt most fossil fuels since the industrial resolution and must continue to lead extending Kyoto. Carbon offset markets worth $20 billion last year depend on Kyoto emission caps to drive developed countries to pay for cuts in green house gases in developing nations.

The recently held Cancun meeting resolved many agreements such as establishing a **Green Climate fund!** This would protect tropical forests and provide a way to share clean emerging technologies and help developing nations adapt to climate change.

This issue includes articles on the importance of *Mycobacterium* in environmental clean - up, role of bacteria in extenuating nitrogenous waste management in aquaculture and production of protease from tannery solid waste.

We sincerely look forward to your suggestions and feedbacks. Please do mail us at www.envismadrasuniv.org/send_feedback.php.

Prof. N.Munuswamy

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International Mountain Day - 11th Dec, 2010.

Mycobacteria in environmental clean-up

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Introduction

Mycobacteria are a diverse group of rod-shaped acid-fast bacteria that include more than 70 different species. The obligate pathogens, *Mycobacterium tuberculosis* and *Mycobacterium leprae* cause tuberculosis and leprosy respectively. Most other mycobacteria live in the soil and water in both rural and urban settings throughout the world. There is no standard naming convention for this group of microorganisms. They may be referred to as nontuberculous mycobacteria (NTM), mycobacteria other than tuberculosis (MOTT), atypical mycobacteria and/or environmental mycobacteria. They can be found in aerosols, rivers and swamps, in treated city water, public swimming pools, hot spas, humidifiers, aquariums, garden soils, food, and many other places. Because they are protected by their waxy lipid-rich cell wall, mycobacteria are resistant to disinfectants and water treatment measures. Almost half of the NTM species identified are associated with opportunistic infections in animals and humans, and several have caused sporadic outbreaks. But, certain species of mycobacteria play an important role in environmental clean-up processes such as biodegradation of polycyclic aromatic hydrocarbons (PAHs), crude oils and chemical dyes and other recalcitrant molecules. This article will focus on the role of certain species of NTM in bioremediation of major environmental pollutant, PAHs.

PAHs

PAHs are a group of organic compounds of two or more fused benzene rings in linear, circular or cluster arrangements. Contamination by PAHs is widespread and has been detected in air, water, soil and sediment (Johnson *et al*., 2005)(Fig.1). Although PAHs are present in the terrestrial environment in low concentrations, pollution is mainly due to human activities. PAHs are produced from incomplete combustion of organic materials, fossil fuels, petroleum product spillage and various domestic and industrial activities.

Fig. 1. Oil (PAH) Contaminated site

Processes and sources that can produce or contain PAHs are given in table 1.

Table 1. Sources and processes of PAHs

Natural oil seeps	Combustion of fossil fuels
Refinery and oil storage waste	Tobacco and cigarette smoke
Accidental spills from oil tanks and other ships	Forest and prairie fires
Municipal and urban wastewater discharge runoff	Rural and urban sewage sludge
River-borne pollution	Refuse and waste incineration
Atmospheric fallout of fly ash particulates	Coal gasification and liquefaction processes
Petrochemical industrial effluents	Creosote and other wood preservative wastes
Coal tar and other coal wastes	Chronic input associated with boating activities
Automobile engine exhausts	

Source: Johnson *et al*. (2005)

PAHs, when discharged into the environment, pose serious health hazards owing to their carcinogenic, mutagenic and genotoxic properties. Based on their ecotoxicity, United States Environmental Protection Agency has prioritized 16 PAHs as environmental pollutants (Mohanty *et al*., 2010). Some important PAHs include naphthalene, fluorene, phenanthrene, anthracene, Benzo(a)fluorene, pyrene, chrysene and benzo(a)pyrene. Based on their molecular weight, PAHs are divided into high molecular weight PAHs (compounds with four or more benzene rings) and low molecular weight PAHs (compounds with two or three benzene rings)

(Law *et al*., 2002). High molecular weight PAHs are resistant to degradation and pose a concern to human health because of their carcinogenic potential. Low molecular weight PAHs do not pose a risk to human health as carcinogens but they are toxic to fish and other marine organisms. The persistence of these toxic pollutants in the environment can be attributed to their various physical and chemical properties (Tab.2). The solubility and volatility of these chemicals are decreased due to its hydrophobic nature and the presence of more number of benzene rings.

Table 2. Some physico-chemical properties of certain PAHs

PAH	Rings	Melting point (°C)	Boiling point (°C)	Solubility (mg/l)
Phenanthrene	3	101	340	1.29
Anthracene	3	216	340	0.07
Fluoranthene	4	111	250	0.26
Benz(a) anthracene	4	158	400	0.014
Pyrene	4	149	360	0.14
Chrysene	4	255	488	0.002
Benzo(a)pyrene	5	179	496	0.0038
Dibenz(a,h) anthracene	5	262	524	0.0005

Considering their chemical and physical properties, PAHs are not easily degradable and may persist in the environment. For example, the half life of tricyclic phenanthrene ranges from 16-126 days in soil, whereas for the five ringed high molecular weight PAH benzo(a)pyrene, the half life may range from 229 -1400 days (Mrozik *et al*., 2003).

Remediation of PAHs from the environment

The removal of PAHs has been demonstrated by various means including chemical oxidation, photo-oxidation, volatilization and bioremediation. Bioremediation seems to be the most promising method for the removal of these toxic pollutants using microbial populations. The microbial mediated approach is cost effective, exploits naturally existing diverse bacterial communities with high specificity for degradation and produces innocuous end products. But the success of bioremediation with microbes is limited to low molecular weight compounds. The high molecular weight compounds are resistance to bioremediation is due to various reasons such as their structural stability, low solubility and high tendency to interact with non-aqueous phases. In fact, the bioavailability of PAHs has been shown to decrease logarithmically in relation to the increasing molecular weight (Johnson *et al*., 2005). However, microbial degradation has been identified as a potential means of successfully removing PAHs from contaminated environments(Fig.2). Several researchers have reported the biodegradation kinetics of individual and mixture of PAHs by various microbial species.

Fig. 2. Remediation of oil contaminated site

Mycobacteria in PAHs remediation

A large number of PAH-degrading microorganisms have been isolated from PAH contaminated environmental communities and characterized. Almost all PAH-degrading isolates are aerobic and able to use PAHs as sole carbon and energy source. Members of the genera *Pseudomonas, Sphingomonas* and *Mycobacterium* are well known for their degradation potential towards PAHs and have acquired diverse capabilities to inhabit a wide range of environments.

Mycobacteria seem to be more specialized in the degradation of high molecular weight PAHs such as pyrene. They are also unique in using hydrophobic sorbed/organic dissolved PAHs while *Pseudomonas* and *Sphingomonas* strains prefer aqueous liquid systems. In *Sphingomonas* and *Pseudomonas* strains, PAH-catabolic genes are often located in conjugative plasmids while in *Mycobacterium* species they seem to be chromosomal. So far, all PAHs degrading mycobacterial isolates could be placed in the phylogenetic branch of fast growing *Mycobacterium* species. Most PAH-degrading mycobacteria are scotochromogenic and produce

smooth, round yellow colonies on solid media. The PAH-degrading mycobacterial isolates were, based on 16S rRNA gene sequence, often assigned to the species *M. frederiksbergense, M. gilvum, M. austroafricanum, M. vanbaalenii, M. holderi, M. flavescens, M. anthracenicum* and *M. chelonae.*

Mycobacterium vanbaalenii, a fast growing species of *Mycobacterium* present in soil, is an exceptional microbe in its ability to oxidatively degrade a great variety of low and high molecular weight PAHs in soil. Phenanthrene, a tricyclic, low molecular weight PAH, is considered as a prototype PAH and is often used to detect PAH contamination. The *M. vanbaalenii* can mineralise 90% of added phenanthrene in about 14 days. PAH degradation by *M. vabaalenni* is catalyzed by dioxygenases and monooxygenases, based on metabolites identified. Another study indicated the role of *M. vanbaalenii* in degradation of one of the most potent carcinogenic PAH, benzo(a)pyrene. Degradation of benzo(a)pyrene is confirmed to be a metabolic detoxification process based on the noncarcinogenic properties of the metabolites produced (Moody *et al.*, 2004). Mohanty *et al*. (2010) analysed the biodegradation of PAHs such as pyrene, anthracene and naphthalene by fast growing mycobacterium, *M. frederiksbergense.* The results showed that the PAH removals varied 54-81% when each PAH was at low concentrations in the mixture and 67-89% at higher concentrations. Herwijnen *et al*. (2003) had reported the degradation of anthracene by *Mycobacterium* sp. (strain LB501T) and concluded that the degradation proceeded via a novel pathway through O-pthalic acid. Cultures of *Mycobacterium* sp. (Strain PYR-1) were dosed with anthracene/phenanthrene and after 14 hours of incubation had degraded 92 and 90% of the added anthracene and phenanthrene, respectively (Moody *et al*., 2001). Boldrin *et al*. (1993) reported the degradation of phenanthrene, fluorine, fluoranthene and pyrene by a *Mycobacterium* sp. Strain BB1 isolated from a former coal gasification site. Lopez *et al.* (2008) reported the simultaneous biodegradation of creosote by a pyrene-degrading *Mycobacterium* sp. AP1. Miller *et al*. (2004) isolated three *Mycobacterium* strains from creosote wood preservative-contaminated soil and they were able to rapidly degrade phenanthrene. The phylogeny of the 16S rDNA analysis showed that they were distinct from other mycobacterial isolates with PAH degrading activities. Catalase and superoxide dismutase (SOD) isozyme profiles confirmed that each isolate was distinct from each other and from the PAH degrading mycobacterium, *M. vanbaalenii.* Doss-Ross and Cerniglia (1996) reported that *Mycobacterium flavescens* could degrade pyrene. Interestingly, mycobacteria have been repeatedly isolated as bacteria that are able to degrade high molecular weight PAHs, pyrene and benzo(a)pyrene. These bacteria are known for their comparatively slow growth. However, their growth on PAHs is faster than other bacteria: for example, the growth rate of *Mycobacterium* sp. BB1 was twice faster than *Rhodococcus* sp. UW1 (Heinkamp *et al*., 1988).

Certain ongoing experiments to improve biodegradation have proposed the use of surfactants or organic solvents to improve the bioavailability of PAHs. The use of chemical surfactants increases the concentration of hydrophobic compounds in the aqueous phase by the process of emulsification and hence, promotes bioavailability of substrates. It has also been hypothesized that mycolic acids, the major cell wall components of mycobacteria may act as a type of biosurfactant and play an important role in increasing the efficiency of degradation of PAHs by reducing the surface tension of the molecules (Johnson and Korlson, 2004). So it is conceivable that the versatility of mycobacteria in degradation of PAHs makes it a potential target for use in PAH remediation. However, more research is needed for its efficient application.

Conclusion

The diversity of fast growing *Mycobacterium* species in the environment is still greatly unknown but could be of major interest for bioremediation of PAH contaminated soils. Therefore, methods for community analysis and monitoring of indigenous and/or inoculated fast growing PAH-degrading *Mycobacterium* species in soil are needed.

References

Boldrin, B., Tiehn, A. and Fritzasche, C. (1993) Degradation of phenanthrene, fluorine, fluoranthene and pyrene by a *Mycobacterium* sp. Appl. Environ. Microbiol. **59**(6)**,** 1927 - 1930.

Doss-Ross, D. and Cerniglia, C. E. (1996) Degradation of pyrene of *Mycobacterium flavescens.* Appl. Environ. Microbiol. **46,** 307.

Heinkamp, M. A., Freeman, J. P., Miller, D.W. and Creniglia, C. E. (1998) Pyrene degradation by *Mycobacterium* sp.: identification of ring oxidation and ring fission products. Appl. Environ. Microbiol. **54,** 2556.

Herwijnen, R. V., Springael, D., Slot, P., Govers, H. A. J. and Parsons, J. R. (2003) Degradation of anthracene by *Mycobacterium* sp. Strain LB501T Proceeds via a novel pathway, through O-Phthalic acid. Appl. Environ. Microbiol. **69(1)** 186 - 190.

Johnson, A.R. and Korlson, U. (2004) Evaluation of bacterial strategies to promote the bioavailability of polycyclic aromatic hydrocarbons. Appl. Microbiol. Biotechnol. **63**(4)**,** 452 - 459.

Johnson, A. R., Wick, L.Y. and Harms, H. (2005) Principles of microbial PAH-degradation in soil. Environ. Poll. **133**(1)**,** 71 - 84.

Law, J.R., Kelly, C., Baker, K., Jones, J., Mclintosh, A. D. and Moffat, C. F. (2002) Toxic equivalency factors for PAH and their applicability in shellfish pollution monitoring studies. J. Environ. Monit. **4**(3)**,** 383 - 388.

Lopez, Z., Vila, J., Ortega-Calvo, J. J. and Grifoll, M. (2008) Simultaneous biodegradation of creosote-poolycyclic aromatic hydrocarbons by a pyrene-degrading *Mycobacterium* sp. Appl. Microbiol. Biotechnol. **78,** 165 - 172.

Miller, C.D., Hall, K., Nieman, Y. N., Sorenson, D., Issa, B., Anderson, A. J. and Sims, R. C. (2004) Isolation and characterization of Polycyclic Aromatic Hydrocarbon - degrading *Mycobacterium* isolates from soil. Microbial. Ecol. **48,** 230 - 238.

Mohanty, B., Pakshirajan, K. and Venkata Dasu, V. (2010) Batch biodegradation of PAHs in mixture by *Mycobacterium fredeiksbergense:* analysis of main and interaction effects. Clean Techn. Environ. Policy. **12,** 441 - 447.

Moody, J.D., Freeman, J. P., Fu, P. P. and Cerniglia, C. E. (2004) Degradation of benzo(a)pyrene by *Mycobacterium vanbaalenii* PYR -1. Appl. Environ. Microbiol. **70,** 340 - 345.

Moody, J.D., Freeman, J.P., Deorge, D.R. and Cerniglia, C.E. (2001) Degradation of phenanthrene and anthracene by cell suspensions of *Mycobacterium* sp. strain PYR – 1. Appl. Environ. Microbiol. **67,** 1476 - 1483.

Mrozik, A., Piotrowska-Seget, Z. and Labuzek, S. (2003) Bacterial degradation and bioremediation of Polycyclic Aromatic Hydrocarbons. Polish Journal of Environmental Studies. **21**(1)**,** 15 - 25.

Mitigating nitrogenous wastes in aquaculture

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Aquaculture globally has undergone tremendous growth during the last fifty years from a production of less than a million tonnes in the early 1950s to over 50 million tonnes in the year 2008 (FAO, 2009). Modern intensive and semi-intensive aquaculture practices involve use of supplementary feeds rich in protein (as much as 25-40 percent). In high intensity aquaculture, water quality becomes a limiting factor. Fish and shrimp accumulate about 20-25% of protein and the rest is released to the pond as ammonium and organic nitrogen (Boyd and Tucker, 1998). These proteinaceous wastes result in total ammonia nitrogen (TAN) and biochemical oxygen demand (BOD). It is estimated that in aquaculture ponds, for every kilogram of feed containing 35 percent protein, about 50.4g of ammonia nitrogen is generated (Ebeling *et al.*, 2006). Ammonia is also a major end product of protein catabolism, excreted by fish, crustaceans, and molluscs into the culture system. These nitrogenous organic wastes stimulate proliferation of heterotrophic microbes. Total ammonia-nitrogen (TAN) is composed of unionised (NH_3-N) and ionised forms (NH_4^+). The unionised ammonia is most toxic to aquatic organisms as it can readily diffuse through cell membranes and is highly lipid-soluble. Nitrite (NO_2) an intermediate product of nitrification is also one of the toxic forms of nitrogen that can be found in aquaculture ecosystems. These substances, despite being toxic to the cultured animals *per se,* increase their susceptibility to diseases, particularly shrimp, which are bottom dwelling organisms. Hence, it is extremely important that these organic pollutants generated during aquaculture have to be

treated using conventional better management practices(BMPs) and also by *in situ* or *ex situ* bioremediation approaches in order to achieve optimal aquaculture productivity. This article provides a brief overview of strategies of mitigating nitrogenous wastes in aquaculture.

Strategies for mitigating NH_3 and H_2S in Aquaculture

Remediation of aquaculture wastes needs to be addressed by developing appropriate strategies, keeping in view the specific requirements in the hatcheries and grow-out ponds. A number of approaches have been adopted for the removal of nitrogenous organic wastes in aquaculture ponds and hatcheries with varying degrees of success. In grow-out ponds, simple physical measures such as aeration, ozonation and replacement with freshwater on a regular basis have been practiced to provide good water quality to the cultured animal. The paddle wheel aerators, despite providing oxygenation, create a circular motion to water, facilitating concentration of wastes at the centre of the aquaculture ponds (Fig.1). Waste mitigation strategies in aquaculture include use of bioaugmentation probiotics (an example of *in situ* bioremediation) and use of biofilters or bioreactors (*Ex situ* bioremediation) for the management of water quality. The latter are used mainly in maturation systems and hatcheries. Certain innovative aquaculture practices utilizing microbial and algal biomass such as 'active suspension ponds' and 'partitioned aquaculture' for management of nitrogenous wastes for maintaining water quality in aquaculture ponds have been proposed.

Fig. 1. Shrimp farm with aerators being operated to provide dissolved oxygen in water and to concentrate wastes at the centre of the ponds

Bioaugmentation

The use of different metabolic pathways of microbes to stimulate autochthonous degradation processes to mitigate undesirable hazardous substances is the strategy of *in situ* bioremediation adopted in aquaculture. Bioaugmentation has been applied in aquaculture with exogenous microbes as "probiotics", the microbes with nitrification, denitrification and sulfur oxidation potential and ability to digest detritus for management of organic matter in aquatic ecosystems. The use of 'probiotics' in aquaculture has been extensively reviewed by Vershuere *et al.* (2000) and is defined as "A live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, by enhancing the host response towards disease, or by improving the quality of its ambient environment".

A wide range of microbes including nitrifiers, sulphur bacteria, *Bacillus* spp. and *Pseudomonas* spp. are commercially sold for *in situ* bioremediation in aquaculture for improving water quality by mineralization of organic matter to carbon dioxide and remove nitrogen from the pond ecosystem by nitrification and denitrification (Antony and Philip, 2006). The most common probiotic bacteria used in ponds or hatchery tanks are *Bacillus* spp. These bacteria being spore formers and resistant to relative extreme physicochemical conditions have the ability to occupy a wide range of niches and degrade a wide variety of substrates. The rationale is that the *Bacillus* spp. are generally more efficient in converting organic matter into CO_2 than the gram-negative bacteria, and convert a greater percentage of organic carbon to bacterial biomass or slime (Stanier *et al.*, 1963). By maintaining higher levels of *Bacillus* spp in the pond, it is believed that the buildup of dissolved and particulate organic carbon can be minimized during the culture cycle.

It appears that there is potential to employ beneficial microbes in aquaculture to improve water quality. But a number of issues such as the stochaic and deterministic factors that govern the microbial diversity and their population dynamics, the fate of exogenous microbe introduced into a new dynamic aquatic ecosystem and sustaining its activity, implications on the natural microbial ecology and impact on the environmental parameters have to be still understood. Boyd (1995) suggested that the bioaugmentation probiotic application must improve water quality, including increase in DO, prevention of off flavour, reduction of nitrogen and phosphorus levels, promoting organic mater decomposition, optimal algal boom, exclusion of pathogens from the production system, enhancement of animal's physical condition, indicators to improve the animals' overall health, appearance, better average size and weight of the

animal, and restoration of normal appetite and feed consumption.

Mitigating NH_3 and nitrite using recirculating aquaculture systems (RAS)

The primary objective of the recirculating aquaculture system is to save water, reduce health risks to aquatic animals caused by exchange of water that may contain contaminants and pathogens, and control pollution (Eding *et al*., 2006). In RAS, removal of total ammonia nitrogen (TAN), particularly the unionized form of ammonia is the main objective and the process relies mainly on microbial metabolism (See box). RAS have been identified as one of the main research areas by NOAA's Aquaculture Policy (http://swr.ucsd.edu/find/bill/aquapol.htm). RAS have been used successfully in aquaculture for the past 20 years, and are now increasingly used in shrimp maturation, hatcheries, nurseries, and ornamental fish breeding which require oligotrophic-grade water quality. Water is recycled through an external biofilter, where it gets purified (Gutierrez-Wing and Malone, 2006). These systems are well tested, proven efficient and are now commercially available. Basic elements of RAS design consider i) maximum biomass of fish / shrimp in the culture system, ii) maximum load of feed used and iii) waste production in the culture system on a diurnal basis. In addition, water quality characteristics required in the culture system are also taken into account in order to regulate flow rates. Components in RAS that perform four critical processes are: i) mechanical filtration to remove suspended solids, ii) foam fractionation for removal of small suspended particles and surfactant molecules, iii) degasification to remove excess carbon dioxide, iv) biofiltration for nitrification of ammonia, and v) aeration to replenish oxygen (Fig. 2).

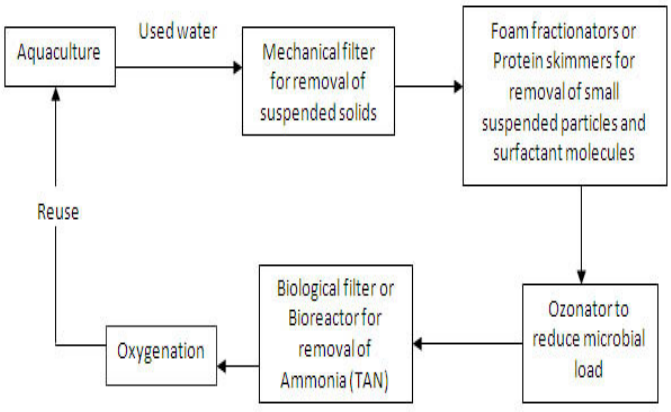

Fig. 2. Components of Recirculating Aquaculture Systems

Recent approaches for the mitigation of NH_3

With the discovery of anaerobic ammonia oxidizing (anammox) bacteria, new approaches of ammonia bioremediation have evolved during the past decade. Anammox are obligate anaerobic chemolithoautotrophs, belonging to planktomycetes group and are extremely slow growing in nature with 11 days of doubling time. The new approaches include processes such as combined SHARON-Anammox process (Single reactor system for High Ammonium oxidation Over Nitrite- anaerobic ammonia oxidation) and CANON (completely autotrophic nitrogen removal over nitrite) (Paredes *et al.*, 2007).

The combined SHARON-Anammox process is based on partial nitrification, followed by denitrification by anaerobic ammonia oxidizing (anammox) bacteria. In the SHARON process, oxidation of ammonium is regulated to proceed only up to nitrite production. The process is combined with anammox process wherein, the subsequent oxidation of nitrite takes place. The anammox process is the denitrification of nitrite with ammonium as electron donor. Hence the anammox process requires nitrite (produced through partial nitrification by nitrifying bacteria) as an electron acceptor. One of the problems with anammox process is the long start-up time because of the extremely slow growing nature of these bacteria. The SHARON-Anammox process has been patented and implemented for wastewater treatment in Rotterdam in The Netherlands.

The CANON process is also based on partial nitrification, wherein, oxidation of ammonium rich wastes is carried out sequentially by aerobic chemolithotrophs such as *Nitrosomonas* spp. and anaerobic anammox bacteria to dinitrogen gas (Paredes *et al.*, 2007). *Nitrosomonas* spp. are basically aerobic organisms, but can also survive under anaerobic conditions, whereas, the anammox bacteria are obligate anaerobes. Hence, these two groups of bacteria could be co-cultured under oxygen limiting conditions. The aerobic chemoautotrophs (*Nitrosomonas* spp., *Nitrosobacter* spp., and *Nitrosospira* spp.) oxidize ammonium to nitrite and consume oxygen. The anammox bacteria subsequently oxidize ammonium along with the nitrite produced by the aerobic chemoautotrophs to dinitrogen and nitrate.

Principles of ammonia removal

Ammonia occurs in two major forms, the unionised ammonia (NH_3-N) and ionised ammonia (NH_4-N), and the ammonia concentration generally includes both, in other words, total ammonia nitrogen (TAN).

Nitrification and denitrification processes play an important role in the removal of ammonia-nitrogen from wastewater. The nitrification process is aerobic and hence requires aeration and the denitrification process may require addition of an external carbon source.

Nitrification is the process of biological oxidation of ammonia with oxygen into nitrite followed by the oxidation of nitrite into nitrate. The oxidation of ammonia into nitrite is performed by two groups of chemoautotrophic ammonia oxidizing bacteria such as *Nitrosomonas* and *Nitrosococcus*, and ammonia oxidizing archaebacteria. The second step of oxidation of nitrite into nitrate is carried out by *Nitrobacter* and *Nitrococcus*.

$$NH_3 + O_2 \rightarrow NO_2^- + 3H^+ + 2e^-$$
$$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^-$$

Denitrification is a respiratory process, carried out by heterotrophic bacteria, in which nitrate and nitrite are converted into gaseous nitrogen intermediates nitric oxide (NO), nitrous oxide (N_2O) and finally nitrogen (N_2). The denitrification process takes place under oxygen limiting conditions, and the bacteria use nitrate as a terminal electron acceptor.

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \text{ gas}$$
$$2NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6H_2O$$

Anaerobic ammonium oxidation (**anammox)** is a process of denitrification of nitrite with ammonium as electron donor directly into dinitrogen gas under anoxic conditions. This process is known to contribute up to 50% of the dinitrogen gas produced in the ocean. The reaction is mediated by anaerobix bacteria of the group known as planktomycetes.

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$$

Conclusion

Predominant wastes generated during aquaculture include rapidly degradable organic nitrogenous wastes and to some extent reduced sulfur compounds, and these affect the shrimp and fish being cultured unless measures are employed to maintain their levels tolerable by the culture species. The perspective is essentially to provide a clean environment for the aquatic animal being cultured, in order to achieve optimal production from aquaculture. Simple management techniques such as aeration and ozonation have been practiced in semi-intensive and intensive aquaculture for providing adequate oxygen to the cultured animal, while oxidizing organic wastes generated during the culture process. However, these measures need to be supplemented through eco-friendly bioremediation tools in order to achieve freedom from organic loading. *In situ* bioremediation has been widely applied in aquaculture through bioaugmentation, using indigenous or exogenous microbes called 'probiotics' which ameliorate water quality (Wang *et al*., 2005). However, their efficacies are uncertain. Policy guidelines on the use of bioaugmentation probiotics in aquaculture do not exist. Application of biofilms and microbial mats independently for bioremediation of aquaculture wastes is still under research and development. However, these are the major microbial ecosystems in recirculating aquaculture systems that remove nitrogenous wastes. In hatcheries, in addition to bioaugmentation probiotics, importance of RAS has been recognized for management of nitrogenous wastes especially in maturation and larval rearing facilities. RAS rely on use of biological filters / bioreactors for the removal of toxic wastes such as ammonia and nitrite. New technologies such as SHARON-ANAMMOX and CANON are being recently explored for application in aquaculture for the mitigation of nitrogenous wastes (Tal and Schrier, 2008). However, because of the initial high installation costs with RAS and the newer technologies and falling prices of shrimp and fish, their use in grow-out systems is becoming prohibitive.

References

Antony, S. P. and Philip, R. (2006) Bioremediation in Shrimp Culture Systems NAGA, World Fish Center Quarterly. **29,** 62 – 66.

Boyd, C. E. (1995) Chemistry and efficacy of amendments used to treat water and soil quality imbalances in shrimp ponds. In: Swimming Through Troubled Water, Proceedings of the Special Session on Shrimp Farming (Ed. by C. L Browdy & J.S. Hopkins), pp. 183 - 99. Aquaculture '95, World Aquaculture Society, Baton Rouge, LA.

Boyd, C. E. and Tucker, C. S. (1998) Pond aquaculture water quality management. Kluwer Academic Publishers, Boston, USA.

Ebeling, J. M., Timmons, M. B. and Bisogni, J. J. (2006) Bioengineering analysis of the stoichiometry of photoautoterophic, autotrophic, and heterotrophic removal of ammonia nitrogen in aquaculture systems. Aquaculture. **257,** 346 - 358.

Eding, E. H., Kamstra, A., Verreth, J. A. J., Huisman, E. A. and Klapwijk, A. (2006) Design and operation of nitrifying trickling filters in recirculating aquaculture: A review. Aquacultural Engineering. **34,** 234 - 260.

FAO (2009). The state of world fisheries and aquaculture. ftp://ftp.fao.org/docrep/fao/011/i0250e/i0250e.pdf.

Gutierrez-Wing, M. T. and Malone, R. F. (2006) Biological filters in aquaculture: Trends and research directions for freshwater and marine applications. Aquacultural Engineering. **34,** 163 - 171.

Paredes, D., Kuschk, P., Mbwette, T. S. A., Stange, F., Müller, R. A. and Koser, H. (2007) New aspects of microbial nitrogen transformations in the context of wastewater treatment: A review. Engineering in Life Sciences. **7,** 13 – 25.

Stanier, R. Y. and Doudoroff, M. and Adelberg, E. A (1963) The Microbial World. 2nd ed. Prentice – Hall Engelwood Cliffs. NJ.

Tal, Y. and Schreier, H. J. (2008) Anaerobic ammonium oxidation for water treatment in recirculating aquaculture. US Patent No. 7314741 B2.

Vershuere, L., Rombaut, G., Sorgeloos, P. and Verstraete, W. (2000). Probiotic bacteria as biological control agents in Aquaculture. Microbiology and Molecular Biology Reviews. **64,** 655 – 671.

Wang, Y. B., Xu, Z. R. and Xia, M. S. (2005) The effectiveness of commercial probiotics in northern white shrimp *Penaeus vannamei* ponds. Fisheries Science. **71,** 1036 - 1041.

Environment-friendly plastics from corn

Making corn-derived plastics more heat-tolerant may broaden the range of applications for which corn-derived plastics could be used as an alternative to petroleum-based plastics and research is underway to that end.

Source: The Hindu, September, 09, 2010.

Production and characterization of thermotolerant alkaline serine protease from non pigmented *Serratia marcescens ANFLR2* isolated from the gut of *Labeo rohita*

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Introduction

A phenomenal increase has been observed in recent years in the use of alkaline protease as industrial catalysts for various biochemical processes. Microorganisms represent an attractive source for producing an abundant and uninterrupted supply of proteases through fermentation methods. Various organic substrates have been studied for the production of alkaline protease. This study deals with the hydrolysis and fermentation of animal fleshing (tannery solid waste) by the enzymatic activity of non pigmented strain of *Serratia marcescens ANFLR2* isolated from the gut of *Labeo rohita,* the fresh water fish. The digestion of food mainly depends on the enzymatic action of the gut microbial flora, which can change its profile enzymes according to the type of food the host feeds on. The focal theme of the present investigation was to adapt the fish gut symbiotic bacteria to animal fleshing and isolation of *Serratia marcescens* for hydrolyzing the same.

Materials and methods

Isolation of protease producing bacteria

Labeo rohita was acclimatized with animal fleshing as a feed for 30 days before sampling the fish. The acclimatized fish were sacrificed and immersed in 1% iodine solution for surface sterilization. The gastro-intestinal tracts were dissected under aseptic conditions using sterilized instruments. The gut contents were washed using sterile water. About one gram of the separated guts was immediately homogenized in a surface sterilized mortar and pestle with 10 ml of sterilized saline solution. The homogenized gut sample was serially diluted with saline solution and plated on skim milk agar medium and incubated for 24 to 48 hours at 37^0C. The colonies which

produced a zone of hydrolysis were picked up and streaked onto agar slants for further screening.

Screening of Animal fleshing hydrolyzing bacteria

The strains showing the highest proteolytic activity were inoculated in the liquid minimal media containing 1% ANFL. The protein content and protease activity were determined for every 24 hours for 4 days. The strain which reflected the maximum protein content and protease activity was selected and identified using standard biochemical assays.

Biochemical test and 16SrRNA analysis

The DNA was extracted from 24 hour old LB broth following the modified method of Hykin *et al.* 1994. The extracted DNA was amplified using two forward and two reverse primers in nested PCR. The purified DNA product, of approximately 1.5 kb, was sequenced using five forward and one reverse primer as described earlier. The deduced sequence was subjected to BLAST search tool, for the closest match in the database. Phylogenetic analysis was performed by subjecting the deduced sequence to the 16 S rRNA database to obtain the closely related sequences.

Fermentation of animal fleshing

The substrate ANFL was collected from a commercial tannery in Chennai and processed as described earlier (Ganesh kumar *et al.,* 2008). The fermentation was carried out in a 2000 mL fermentation chamber. The production medium consisted of NaCl (1.4g/L), NH_4Cl (0.005g/L), K_2HPO_4 (1.25g/L), KH_2PO_4 (0.9g/L) and 10g of animal fleshing. The medium was inoculated with *Serratia marcescens ANFLR2* of 10% concentration. The fermented medium was concentrated using ammonium sulfate precipitation, dialyzed and lyophilized for further use.

Characterization of the *Serratia marcescens ANFLR2* protease

Molecular weight determination of the purified protease

The purified protease was subjected to SDS PAGE analysis to determine the molecular weight of the purified protease using high molecular weight protein markers.

Effect of pH on Protease activity

The partially purified protease was dissolved in pure double distilled water. About 0.5 mL aliquots of enzyme were distributed in test tubes and incubated for 1 hour with various buffers of pH range 3 to 12. The protease activity of the aliquots were determined after one hour at different pH.

Effect of temperature on Protease activity

The partially purified proteases were dissolved in appropriate buffer and incubated at different temperatures (4, 20, 28, 37, 45, 55, 65 and 75^{0}C) for 60 minutes to determine the stability of the enzyme at these temperatures.

Effect of Ca^+ ions, metal ions, inhibitors and surfactants on protease activity

The enzyme was dissolved in appropriate buffers and incubated with different concentration (1mM, 5mM, and 10mM) of metal ions such as $MgCl_2$, $MnSO_4$, $CaCl_2$, $CuSO_4$, $ZnSO_4$ and $CaCl_2$, inhibitors (EDTA, Dithiothreitol and Polymethane Sulfonyl Fluoride (PMSF)) and surfactants (SDS, Tween 20, Tween 80 and Triton X 100) for 60 minutes to evaluate the effect of these components on the activity and stability of the proteases.

Results and Discussion

The isolates belonged to gram negative rod, non motile and non spore forming bacteria, exhibiting positive response to citrate, catalase and oxidase tests. The isolate had 98% similarity to *Serratia marcescens* strains and was assigned a Genbank accession number HM584905 and given a strain name as *Serratia marcescens ANFLR2*. The protease enzyme produced by the non pigmented *Serratia marcescens ANFLR2* have higher activity and stability in alkaline pH range of about 8 to 10 with maximum activity at 9 (Fig. 1).

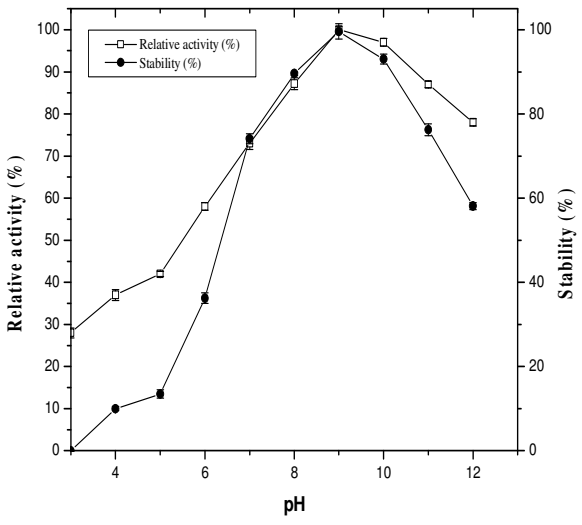


Fig. 1. The relative activity of *Serratia* protease as a function of pH

The enzyme was highly stable at high temperatures in the range 50^0 C to 65^0 C (Fig. 2).

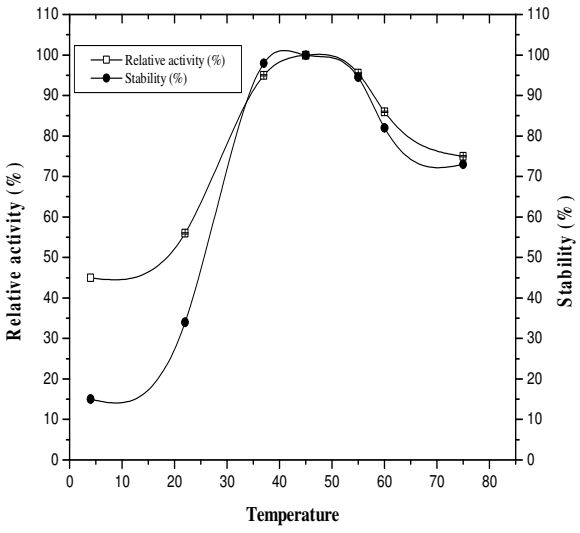


Fig. 2. The variation of relative activity and stability as a function of temperature

The protease activity produced by the fermentation process was 220 U at 37^0C and pH 8. On the addition of 1% triton X 100 to the medium, the activity was increased to 300 U. Further addition of $CaCl_2$ to the medium enhanced the protease activity to 370 U. The protease activity was inhibited by 10mM of PMSF having residual activity of 38% indicating that it could be classified under serine proteases. Ca^{2+} ions enhanced the stability of the protease to retain about 89% of relative activity after 60 minutes. The stability of the protease was also evaluated in the presence of 1% Triton X 100 which retained about 85% of the relative activity of the protease after 60 minutes. This may be interpreted that the protease produced should be effective enough to break all the disulfide and hydrogen bonds present in animal fleshing, because the bacteria was isolated from fish gut which were degrading high molecular proteins to the assimilating level to the fishes. The symbiotic association of the bacteria with that of the fish might have induced this capacity to degrade such high molecular mass animal proteins by producing high molecular weight protease of 64 Kda (Fig. 3).

The molecular weight reported by us is higher than the values reported in literature for *Serratia* sp. (40-58kda). Hence, the alkaline protease produced by the non pigmented *Serratia marcescens* is a novel enzyme having high molecular mass which is capable of cleaving the complex animal proteins.

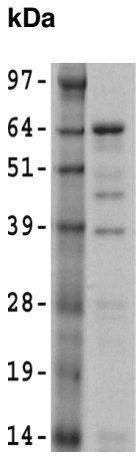


Fig. 3. Molecular Weight Profile of *Serratia* Protease

Conclusion

This study confirms the capacity of the non pigmented *Serratia marcescens* to produce a novel protease against animal fleshing to hydrolyze it. The protease produced by the *Serratia marcescens ANFLR2* was characterized as a thermo tolerant alkaline serine protease having a molecular mass of around 64 kda which has not been cited in literature so far described.

References

Ganesh Kumar, A., Nagesh, N., Prabhakar, T. G. and Sekaran, G. (2008) Purification of extracellular acid protease and analysis of fermentation metabolites by *Synergistes* sp. utilizing proteinaceous solid waste from tanneries. Biores. Technol. **99,** 2364 - 2372.

Hykin, P. G., Tobal, K. and McIntyre, G. (1994) The diagnosis of delayed post-operative endophthalmitis by polymerase chain reaction of bacterial DNA in vitreous samples. J Med. Microbiol, **40,** 408 - 15.

National Energy Conservation Day - Dec 14.

Hitchhiking bacteria can go against the flow

A new study co-authored by Professor Kam Tang of the Virginia Institute of Marine Science reveals that tiny aquatic organisms known as "water fleas" play an important role in carrying hitchhiking bacteria to otherwise inaccessible lake and ocean habitats.

The article, "Bacteria dispersal by hitchhiking on zooplankton," appeared in the Proceedings of the National Academy of Sciences. It was co-authored by scientists from the Leibniz-Institute of Freshwater Ecology and Inland Fisheries in Stechlin, Germany.

Bacteria and other microorganisms are key components of aquatic ecosystems, nurturing the base of the food web and recycling organic matter into carbon, nitrogen, and other elemental constituents of global biogeochemical cycles. Some, like *Vibrio*, can cause disease. *Vibrio* is responsible for cholera and other water- and shellfish-borne illnesses.

Aquatic microbes are also some of the most abundant, widespread, and diverse organisms on Earth. Scientists estimate that a single tablespoon of seawater holds 5 million marine bacteria, and that a liter can hold tens of thousands of microbial species. Aquatic microbes occur from the deep seafloor to polar lakes, and pretty much everywhere in between.

Yet despite their ubiquity throughout aquatic ecosystems, Tang says the manner by which microbes move from one habitat to another has been somewhat of a mystery. That's because for animals of this small size, water has the viscosity of honey, and the boundary between water masses of different temperature and salinity may as well be a brick wall.

Previous studies have shown that wind, birds, and land animals can help carry aquatic bacteria from lake to lake, and that bacteria can spread quickly through a particular water mass simply by dint of their extremely rapid growth rates. Earlier studies also show that bacteria can move downward with the larger, heavier particles of organic detritus that constantly rain upon the seabed.

Testing the conveyor-belt hypothesis

The study by Tang and his colleagues sought to test an alterative means of bacterial dispersal their "conveyor-belt" hypothesis that bacteria might also be able to move upward, against gravity and across stratified density boundaries, by hitching a ride with the many zooplankton species that migrate from the depths to the surface each day with the setting sun. Their idea requires that bacteria not only climb aboard the upwardly migrating zooplankton, but that they later disembark in surface waters. Another possibility is that zooplankton eat the bacteria, and later egest them unharmed.

Tang says that intermittent or permanent stratification due to differences in temperature and salinity is common in many water bodies, whether they be lakes, coastal bays, or the open ocean. Stratification occurs frequently in Chesapeake Bay and other estuaries when relatively fresh river water forms a layer that floats atop the saltier, denser seawater entering the estuary's mouth.

To test their hypothesis that bacteria might be moving between water masses by hitching a ride on "water fleas" like *Daphnia*, the researchers filled a series of graduated cylinders with fresh- and then salt-water to form a stably stratified water column, then added bacteria labeled with a green fluorescent protein to the lower layer and kept the top layer bacteria-free.

The researchers then added *Daphnia* to the cylinders and used a directed light source to manipulate their swimming direction (The species of *Daphnia* they used instinctively move toward light). By alternating the position of the light every 2 hours for 8 hours, the researchers were able to make the water flea swim up and down across the stratified water column several times, and followed the rate at which bacteria were transported upward. A cylinder with bacteria but without *Daphnia* served as the control. Afterward, the experiment was repeated by adding bacteria to the top layer of the cylinders and following the downward transport of the bacteria by migrating *Daphnia*.

The experiments were done with 3 different species of bacteria. At the end of their lab experiments, the team found labeled bacteria were transported in both upward and downward directions in the cylinders containing *Daphnia*, confirming their "conveyor-belt" hypothesis. Field studies of *Daphnia* and bacteria from a local lake further strengthened their laboratory findings.

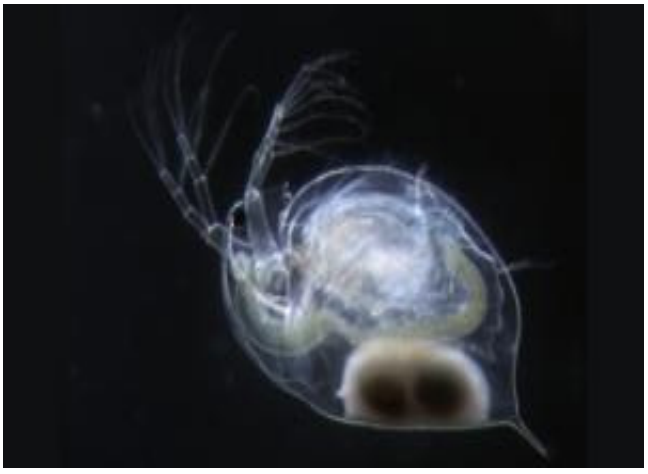
Aquatic hitchhikers

The team's study, says Tang, clearly shows that "bacteria, including pathogens, are able to travel and cross aquatic boundaries by hitchhiking on migrating organisms, thus facilitating exchanges between separate microbial communities and allowing access to otherwise inaccessible resources."

The authors note that "unlike slowly sinking aggregates and other detritus that mostly transport bacteria downward, mobile and migrating hosts can cover long distances rapidly and disperse bacteria in all directions repeatedly and effectively."

Tang cautions that in natural environments with diverse species of zooplankton, "dispersal of hitchhiking bacteria will not be uniform and can occur both vertically and horizontally, and on different time and space scales, from daily to seasonal migrations over millimeters to kilometers."

Although the team conducted their study in freshwater lakes and with freshwater organisms, Tang says their findings likely pertain to ocean ecosystems as well. "Many species of marine zooplankton migrate long distances vertically on daily or seasonal time scales, or during different stages of their life cycle," he says. "They may therefore transport and disperse bacteria over long distances, affecting the ecology and physiology of even deep-sea microbes."



A female *Daphnia magna* with eggs used in the study of bacterial hitchhikers.

Image Credit: Adam Petrusek

Source: www.sciencedaily.com

Some trees 'farm' bacteria to help supply nutrients

Some trees growing in nutrient-poor forest soil may get what they need by cultivating specific root microbes to create compounds they require. These microbes are exceptionally efficient at turning inorganic minerals into nutrients that the trees can use.

Researchers from France report their findings in the journal Applied and Environmental Microbiology.

"In acidic forest soils, availability of inorganic nutrients is a tree-growth-limiting factor. A hypothesis to explain sustainable forest development proposes that tree roots select soil microbes involved in central biogeochemical processes, such as mineral weathering, that may contribute to nutrient mobilization and tree nutrition," says Stéphane Uroz, an author on the study.

Certain microbes are efficient at breaking down inorganic minerals into nutrients. This process, called mineral weathering, is especially important in acidic forest soils where tree growth can be limited by access to these nutrients. Mineral-weathering bacteria can release necessary nutrients such as iron from soil minerals. This gives trees with increased concentrations of mineral-weathering microbes an advantage over other trees.

Distinct impacts of the tree species on the soil bacterial community structure have been previously reported, suggesting that the composition and activity of soil bacterial communities depend on tree physiology and notably on its impact on the soil physicochemical properties and nutrient cycling. However, no study has ever addressed the question of the impact of tree species on the structure of forest soil bacterial communities involved in mineral weathering.

"This question regarding the impact of tree species on the functional diversity of the bacterial communities remains a major issue in forestry, especially in the context of today's climate change, which will give rise to a shift in the spatial distribution of forest tree species," says Uroz.

The researchers took soil samples from the root areas of beech, oak and Norway spruce trees and cultured them to determine the bacterial populations. They observed heightened levels of mineral-weathering bacteria in the samples near the roots of oak and beech trees compared to surrounding soil samples. This difference was not seen in the Norway spruce samples.

"Our results suggest that certain tree species have developed indirect strategies for mineral weathering in nutrient-poor soils, which lie in the selection of bacterial communities with efficient mineral weathering potentials," says Uroz.

Oak trees that grow in nutrient-poor forest soil may get what they need by cultivating specific root microbes to create compounds they require

Image Credit: iStockphoto

Source: www.sciencedaily.com

Do our bodies bacteria play matchmaker !

Could the bacteria that we carry in our bodies decide who we marry. According to a new study from Tel Aviv University, the answer lies in the gut of a small fruit fly.

Prof. Eugene Rosenberg, Prof. Daniel Segel and doctoral student Gil Sharon of Tel Aviv University's Department of Molecular Microbiology and Biotechnology recently demonstrated that the symbiotic bacteria inside a fruit fly greatly influence its choice of mates.

The research was done in cooperation with Prof. John Ringo of the University of Maine, and was recently published in the Proceedings of the National Academy of Sciences (PNAS).

Love, marriage and fruit flies

Based on a theory developed by Prof. Rosenberg and Dr. Ilana Zilber-Rosenberg, the scientists propose that the basic unit of natural selection is not the individual living organism, plant or animal, but rather a larger biological milieu called a holobiont. This milieu can include plant or animal life as well as their symbiotic partners. In the case of animals, these partners tend to be microorganisms like intestinal bacteria.

"Up to now, it was assumed that the host organism undergoes evolution on its own, while its symbiotic bacteria undergo their own evolution," Prof. Rosenberg says. "The mechanism that we discovered enables evolution to occur more rapidly in response to environmental changes. Since a generation is shorter for bacteria than for multicellular organisms, they genetically adjust more quickly to changes in the holobiont," says Prof. Rosenberg.

Conducting their experiments on the rapidly-reproducing fruit fly, the scientists were able to test this new theory. The first experiment repeated a study carried out two decades ago by a Yale University researcher, in which a fly population was divided in half and fed different diets malt sugar versus starch. A year later, when the flies were re-integrated as one group, those who had been fed starch preferred starch-fed mates, while the sugar-fed flies preferred mates of a similar nutritional background. The repeat experiment carried out by the Tel Aviv University researchers shows that this dietary influence takes effect within just a generation or two rather than over an entire year.

In their second experiment, the Tel Aviv University team repeated the first, but with the addition of an antibiotic, which killed the bacteria and eliminated the specific mate preference. The mating process became random, with no dietary influence.

In subsequent experiments, the researchers successfully isolated the bacterial species responsible for reproductive isolation in flies with diet-related mating preferences, and found the bacteria *Lactobacillus plantarum* to be present in greater numbers in starch-fed fruit flies than in sugar-fed flies. When *L. plantarum* was

reintroduced into the antibiotic-treated flies, the preferential mating behavior resumed proving that this bacterial species is at least partly responsible for the mating preference.

Rewriting Darwin

Finally, in cooperation with Prof. Avraham Hefetz of Tel Aviv University's Department of Zoology, the team analyzed the sexual pheromones produced by the fruit flies. There turned out to be differences in pheromone levels between the two groups of flies differences that again disappeared after administering antibiotics.

"The finding indicates that pheromone alterations are a mechanism by which we can identify mating preferences. We therefore hypothesize that it is the bacteria that are driving this change," Prof. Rosenberg says. He adds that these discoveries have implications for our entire understanding of natural selection something which may even lead to the development of a new theory of evolution.

The symbiotic bacteria inside a fruit fly greatly influence its choice of mates.

Image Credit: iStockphoto/Tomasz Zachariasz
Source: www.sciencedaily.com

World's largest, most complex marine virus is major player in Ocean ecosystems

UBC (University of British Columbia) researchers have identified the world's largest marine virus an unusually complex 'mimi-like virus' that infects an ecologically important and widespread planktonic predator.

Cafeteria roenbergensis virus has a genome larger than those found in some cellular organisms, and boasts genetic complexity that blurs the distinction between "non-living" and "living" entities.

"Virus are classically thought of small, simple organisms in terms of the number of genes they carry," says UBC Professor Curtis Suttle, an expert in marine microbiology and environmental virology and lead author of the study.

"Much of the genetic machinery we found in this virus you would only expect to find in living, cellular organisms, including many genes required to produce DNA, RNA, proteins and sugars."

The findings are reported in the issue of the Proceedings of the National Academy of Sciences.

Viruses cannot replicate outside of living host cells and they depend on proteins provided by the cell, a boundary that is often used to delineate "non-living" from "living" organisms. Giant viruses challenge this definition, as they still need a cell to replicate, but encode in their own genome most of the proteins required for replication.

Discovered in Texas coastal waters in the early 1990s, Curtis and his team where able to determine that the pathogen's genome contains approximately 730,000 base pairs. That makes *Cafeteria roenbergensis* virus the largest known marine virus, and the second largest known virus, after the fresh water-borne *Acanthamoeba polyphaga* mimivirus, which weighs in at 1.2 million base pairs.

Cafeteria roenbergensis virus also infects a major marine zooplankton which occupies a key position in marine food webs.

"Even though predation by these marine plankton grazers is a major pathway of carbon transfer and nutrient recycling in marine and freshwater systems, we know next to nothing about the role viruses play in this system," notes Curtis, cross appointed to the departments of Earth and Ocean Sciences, Botany, and Microbiology and Immunology.

"There's little doubt that this virus is just one representative from a major group of largely unknown but ecologically important marine giant viruses."

Also on the research team were UBC graduate student Matthias Fischer, Michael Allen of the Plymouth Marine Laboratory, United Kingdom, and William Wilson of the Bigelow Laboratory for Ocean Sciences, United States.

Funding for the research was provided by the Natural Sciences and Engineering Research Council of Canada and the Tula Foundation through the UBC Centre for Microbial Diversity and Evolution.

Source: www.sciencedaily.com

Bacteria gauge cold with molecular measuring stick

Some bacteria react to the cold by subtly changing the chemistry of their outer wall so that it remains pliable as temperatures drop. Scientists identified a key protein in this response mechanism a few years ago, but the question of how bacteria sense cold in the first place remained a mystery. Based on a study by scientists at Rice University and Argentina's National University of Rosario, the answer is they use a measuring stick.

The study, published in Current Biology, involved a series of intricate experiments on the bacteria *Bacillus subtilis*. The researchers found a specialized protein that protrudes through the bacteria's outer cell wall acts as a measuring stick that's tuned to give a signal when temperatures outside the cell drop.

Scientists have long known that cells use specialized proteins called "transmembrane" proteins to sense and react to the outside world. Transmembrane proteins protrude through the cell's outer wall, or membrane.

"All living cells have the ability to respond to external stimuli, but in most cases that we are aware of, signal recognition the event that triggers the response occurs when a transmembrane protein binds physically to another chemical outside the cell," said study co-author Ariel Fernandez, research professor at Rice.

Fernandez said the *Bacillus subtilis* study is one of the first to determine how a transmembrane protein can respond indirectly to a physical stimulus outside the cell. The research was highlighted in review articles in both Current Biology and Nature Reviews Microbiology.

He and colleagues examined a transmembrane protein called DesK (pronounced des-KAY). In previous studies, scientists had found that DesK responded to cold temperatures by causing the cell to make a special compound that keeps the membrane pliable. Without the compound, the fatty acids inside the cell wall become more rigid as temperatures fall.

Fernandez and colleagues found that the part of the DesK protein that protrudes outside the cell contains a sensitized tip. As long as the tip remains in contact with water molecules outside the cell, DesK remains switched off. As temperatures fall and the cell membrane becomes more rigid, the membrane also becomes thicker. As it thickens, it engulfs the sensitized end of the temperature probe, cutting off contact with water molecules outside the cell. This, in turn, activates DesK and sends the signal to release the cold-protecting chemicals. This mechanism, which Fernandez named the buried buoy trigger, was proposed by Fernandez and probed experimentally by the Argentinean team.

The molecular biology and experimental probes were conducted in the laboratory of Diego de Mendoza at the National University of Rosario in Rosario, Argentina. To confirm the findings, the group constructed versions of DesK proteins of varying lengths. Using these as longer or shorter measuring sticks, the researchers confirmed that the signaling mechanism was triggered based upon whether the tip of the transmembrane sensor remained in contact with water molecules outside the membrane.

Co-authors of the research include Larisa Cybulski, Mariana Martin and Maria Mansilla, all of the National University of Rosario. The research was supported by the National Institutes of Health, Argentina's National Scientific and Technical Research Council, Argentina's National Agency for Science and Technology Promotion and the Howard Hughes Medical Institute.

Artist's rendering of bacteria.

Image Credit: iStockphoto/Sebastian Kaulitzki

Source: www.sciencedaily.com

Metal-mining bacteria are green chemists

Microbes could soon be used to convert metallic wastes into high-value catalysts for generating clean energy, say scientists writing in the issue of Microbiology.

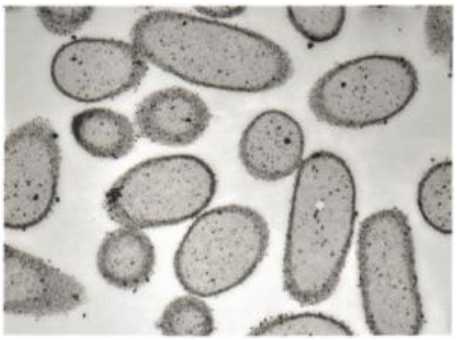
Researchers from the School of Biosciences at the University of Birmingham have discovered the mechanisms that allow the common soil bacterium *Desulfovibrio desulfuricans* to recover the precious metal palladium from industrial waste sources.

Palladium is one of the platinum group metals (PGMs) which are among the most precious resources on Earth. They possess a wide variety of applications, due to their exceptional chemical properties. PGMs are routinely used in many catalytic systems and are the active elements of autocatalytic converters that reduce greenhouse gas emissions.

Dr. Kevin Deplanche who led the study explained why new ways of recovering PGMs are needed. “These metals are a finite resource and this is reflected in their high market value,” he said. “Over the last 10 years, demand has consistently outstripped supply and so research into alternative ways of recovering palladium from secondary sources is paramount to ensuring future availability of this resource.”

Previous work in the team's lab showed that *Desulfovibrio desulfuricans* was able to reduce palladium in industrial wastes into metallic nanoparticles with biocatalytic activity. Now, the precise molecules involved in the reduction process have been identified. Hydrogenase enzymes located on the surface membrane of the bacterium carry out the reduction of palladium, which results in the accumulation of catalytic nanoparticles. The bacterial cells coated with palladium nanoparticles are known as “BioPd”.

The group believes that BioPd has great potential to be used for generating clean energy. “Research in our group has shown that BioPd is an excellent catalyst for the treatment of persistent pollutants, such as chromium, that is used in the paint industry. BioPd could even be used in a proton exchange fuel cell to make clean electricity from hydrogen,” said Dr. Deplanche. “Our ultimate aim is to develop a one-step technology that allows for the conversion of metallic wastes into high value catalysts for green chemistry and clean energy generation,” he said.



Escherichia coli cells are surrounded by nanoparticles of palladium and gold (black deposits).

Image Credit: Kevin Deplanche

Source: www.sciencedaily.com

Sewage water bacteria: ‘missing link’ in early evolution of life on Earth

A common group of bacteria found in acid bogs and sewage treatment plants has provided scientists with evidence of a 'missing link' in one of the most important steps in the evolution of life on Earth the emergence of cells with a nucleus containing DNA (eukaryotic cells).

For billions of years, bacteria (single celled organisms without a nucleus) were the only cellular life form on Earth. Then, about 1.6 to 2.1 billion years ago, eukaryotic cells emerged. These cells (with a nucleus) heralded the evolution of multi-cellular life on Earth including plants, insects, animals and humans.

Until now scientists have been unable to identify an 'ancestral cell' linking the early prokaryotes with the later eukaryotes, so fusion theory where two cells merge to form a new cell is often put forward to explain the appearance of these new cell types.

But new findings by scientists from University College Dublin, Ireland, and the European Molecular Biology Laboratory in Heidelberg, Germany, published in Science, have put paid to the fusion theory explanation, and suggest that an intermediate or 'missing link' cell did exist all those billions of years ago.

"Our discovery means that the appearance of eukaryotic cells on Earth can be explained by Darwinian evolution over billions of years rather than a 'big bang' fusion theory," says cell biologist Dr Emmanuel Reynaud from University College Dublin, one of the co-authors of the scientific paper.

"Our analysis shows that PVC [*Planctomycetes*, *Verrucomicrobiae, Chlamydiae*] bacteria, members of which are commonly found in today's sewage treatment plants or acid bogs, represent an intermediate type of cell structure. They are slightly bigger than other known bacteria, and they also divide more slowly."

"The structure of PVC suggests that it is an ancestor of a 'missing link' cell which connected prokaryotic to eukaryotic cells along an evolutionary path all those billions of years ago," says Dr Damien P Devos from the European Molecular Biology Laboratory, Heidelberg, Germany, who co-authored the scientific paper.

PVC (*Planctomycetes, Verrucomicrobiae, Chlamydiae*) bacteria members of which are commonly found in today's sewage treatment plants or acid bogs represent an intermediate type of cell structure.

Image Credit: iStockphoto/Viktor Balabanov
Source: www.sciencedaily.com

House-sharing with microbes

Household dust contains up to 1000 different species of microbes, with tens of millions of individual bacterial cells in each gram and these are just the ones that can be grown in the lab.

Dr. Helena Rintala, speaking at the Society for General Microbiology's autumn meeting in Nottingham describes how we share our living and working spaces with millions of microbes, not all of whom are bad news.

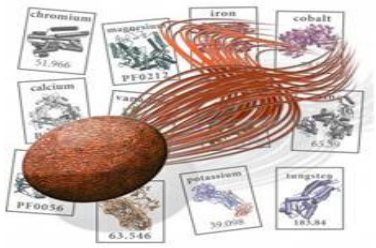
Microbes are a part of our normal environment and can be both beneficial and detrimental to our health. "Exposure to microbes in childhood can prevent the development of allergies. On the other hand, mould growth can increase the risk of asthma," said Dr Rintala from the National Institute for Health and Welfare in Finland.

In indoor environments microbes thrive on surfaces that are occasionally moist or wet, for example in the kitchen and bathroom. Prolonged damp anywhere in the house can lead to greater numbers of microbes. "These microbes, their spores and the molecules they secrete can be released into the air which can lead to health problems if they are breathed in," she said.

Dr. Rintala explains why it is important to study the microbes, both good and bad, that typically live in indoor environments. "When you consider that we spend more than 90% of our lifetime in indoor environments and breathe the indoor air with all its components, it is important to know that the environment is healthy and that the air is safe to breathe," she said. The Finnish group is working towards identifying microbial species that are important to our health both good and bad and developing rapid detection methods for them. "Culture methods are slow and selective but with the development of new DNA-based methods we can assess the indoor air quality of homes and workplaces more rapidly, enabling people to take faster action if there is a problem," said Dr. Rintala.

Source: www.sciencedaily.com

More surprises from bacteria



Scientists identified a new way of surveying microbes for metal - containing proteins found several unexpected metals in *Pyrococcus furiosus,* a bacteria such as lead, manganese and molybdenum. Their research will help for complete understanding of the far-reaching roles of microbial metals in biology and the earth's climate.

Source*:* www.sciencedaily.com

Microorganisms in the ground don't slack off in winter

It is known that soil microorganisms can maintain some activity during the cold winter months. Scientist at Swedish University of Agricultural Sciences (SLU) and Umeå University in Sweden have now shown that the microorganisms in frozen soils are much more viable than previously anticipated and also has large potential for growth.

In northern forest ecosystems, there is a great deal of carbon stored in the ground. The degradation of this carbon supply is a crucial component in computational models used to describe the effects of future climate changes.

In recent years it has been noticed that the winter half of the year can also have a great impact on the carbon balance of forests, as microorganisms (fungi and bacteria) continue to degrade organic carbon despite freezing temperatures and frozen ground. Just how microorganisms go about breaking down organic carbon under such adverse conditions has largely been unknown, which has rendered it difficult to carry out reliable calculations of a forest's carbon balance in wintertime.

"The results of previous studies have been interpreted as meaning that microorganisms in frozen ground cannot grow but merely give off a certain amount of carbon dioxide. A research team at SLU in Umeå and at Umeå University has now shown that this is not the case. Instead, the capacity of microorganisms to grow in frozen ground is astonishingly similar to that of the summer half of the year, although the growth rate is lower," says Mats Öquist from SLU, who directed the study.

These findings are being published in the journal PNAS(Proceedings of the National Academy of Sciences), published by the American Academy of Sciences.

The study was performed in close collaboration between Mats Öquist, Mats Nilsson, and Stina Harrysson Drotz at SLU, and Jürgen Schleucher and Tobias Sparrman (Umeå University).

In previous publications these scientists have established that the activity of microorganisms in frozen ground is mainly regulated by access to unfrozen water, and they have identified what characteristics in the ground govern the availability of water.

These studies have been possible thanks to a method for monitoring unfrozen water using nuclear magnetic resonance spectroscopy (NMR), a method that was developed by the team. In combination with the latest findings about the capacity of microorganisms to exploit organic materials and grow in frozen ground, this research makes it possible to develop more reliable computational models of the carbon balance of the northern hemisphere.

Source: www.sciencedaily.com

NEWS

Fungus turns ants into zombies

The oldest evidence of a fungus that turns ants into zombies and makes them stagger to their death has been uncovered by scientists. The gruesome hallmark of the fungus's handiwork was found on the leaves of plants that grew in Messel, near Darmstadt in Germany, 48 million years ago. According to the findings, parasitic fungi evolved the ability to control the creatures they infect in the distant past, even before the rise of the Himalayas.

The fungus, which is alive and well in forests today, latches on to carpenter ants as they cross the forest floor before returning to their nests high in the canopy.

It then grows inside the ants and releases chemicals that affect their behaviour. Some ants leave the colony and wander off to find fresh leaves on their own, while others fall from their tree-top havens on to leaves nearer the ground.

Infected ants move towards the underside of the leaf they are on and lock their mandibles in a "death grip" around the central vein, immobilising themselves and locking the fungus in position. One can find graveyards with 20 or 30 ants in a square metre.

Source: The Hindu, 19th August, 2010.

Green sink: molecular sponge to soak up CO_2

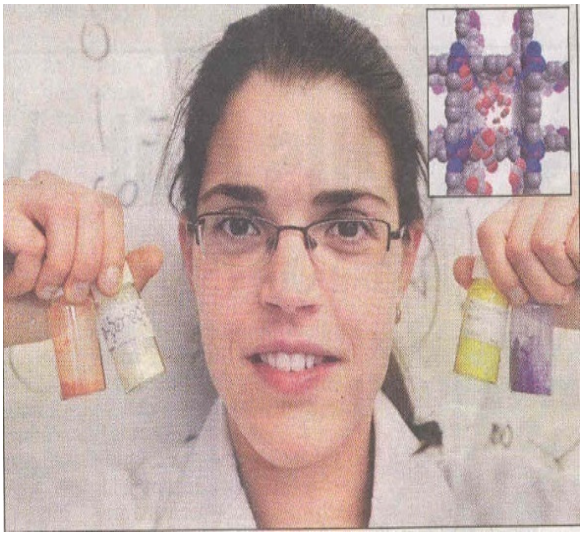
Crystals full of minute hole can retain gas

Australian scientists are working to develop "molecular sponges" that they hope will soak up carbon gases and help in the fight to contain greenhouse gases blamed for climate change.

Researchers at Sydney University have produced crystals full of minute holes which can retain gases such as carbon dioxide, and which they hope could be used in places where these gases are produced, such as power stations. "You could think of them a little bit like your kitchen sponge," lead researcher and postdoctoral fellow Deanna D'Alessandra told ABC(Australian broadcasting corporation) Radio.

The chemical frame works are full of so many tiny holes or pores that they have a far greater surface area than would be expected from their size, she said. "So if you thought of all of the area inside of the little pores of the sponge, then in fact it would add up to an incredible amount.

"So in fact if you took a tea spoon of one of the best materials we have at the moment, then it would actually have a surface area of about a rugby field, which is pretty amazing," she said. The process of soaking the "molecular sponges" with CO_2 could also be reversible, allowing the gas to be released under certain conditions. Thy are not yet ready for commercial adaptation.



Catching Carbon: Deanna D'Alessandro of the University of Sydney displays highly porous three dimensional solids that can filter and capture gases such CO_2 (Inset) 3D structure of the sponges

Source: The Times of India, September 14, 2010.

Atlantic sea turtles hit by fungal egg infection

Atlantic sea turtles are under threat from an infection which targets eggs.The fungus *Fusarium solani*, a strain representing over 45 phylogenetic and biological species, may be key to the 30-year decline in turtle numbers.

Source: The Hindu, Nov 04, 2010

Glue from bacteria can 'knit' cracks in concrete

British scientists have developed a genetically modified bacteria which they say can knit together cracks in concrete structures by producing a special glue.

The microbe, created by a team of researchers at the Newcastle University, has been programmed to swim down fine cracks in the concrete.

Once at the bottom, it produces a mixture of calcium carbonate and a bacterial glue which combine with the filamentous bacterial cells to "knit" the building back together, the researchers said.

Ultimately hardening to the same strength as the surrounding concrete, the "BacillaFilla" as it has been aptly named has been developed to prolong the life of structures which are environmentally costly to build, said the researchers who designed it as part of a science competition in the US.

Project instructor Jennifer Hallinan said: "Around 5% of carbon dioxide emissions are from the production of concrete. Finding a way of prolonging the lifespan of structures means we could reduce this environmental impact."

Source: The Times of India, November 22, 2010.

CO_2 emitted per person by eating

Confirming for the first time that human excrements contribute to water pollution, primarily with nitrogen and phosphorus, a study by the Universidad de Almería (UAL) says that every person emits the equivalent of approximately two tonnes of carbon dioxide a year from the time food is produced to when the human body excretes it, representing more than 20 per cent of total yearly emissions.

A team of researchers from UAL has estimated the environmental impact of the Spanish diet and role that human excrements play in the life cycle of food.

"Food in Spain produces emissions of around two tonnes of carbon dioxide per person and per year (more than 20 per cent of total emissions per person and per year) and consumes 20 gigajoules of primary energy," main author of the study and researcher at the UAL Iván Muñoz said.

The study, which was published recently in The International Journal of Life Cycle Assessment, analyses the relationship of the food production and consumption chain with global warming and the acidification and eutrophication (excess of nutrients) of the environment, taking what a person in Spain ate in 2005 (881 kilograms) as a reference.

Calculations included agricultural and animal production, industrial food processing, sale and distribution, preparation and cooking at home, solid waste treatment, as well as human excretion, according to a press release from the Spanish Foundation for Science and Technology.

As regards emissions, human excreta have a net null effect on global warming, as they are offset by carbon fixation in photosynthesis.

As a consequence of this, they do not contribute to increasing the concentration of Carbon dioxide in the atmosphere.

Source: The Hindu, November 04, 2010.

Urine: Waste product or future power source !

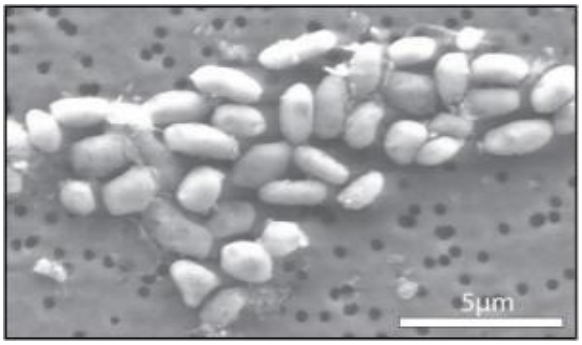
Researchers at Bristol Robotics Lab (BRL), are looking into the use of urine as the 'Fuel' for microbial fuel cells (MFCs). Urine is rich in nitrogen, urea, chloride, potassium and bilrubin that could be used by bacterial cultures to power the MFCs to generate energy. MFCs are a developing technology used to power autonomous robots.

Dr. Ioannis Ieropoulos holds a microbial fuel cell.

Image Credit: University of the West of England
Source: www.sciencedaily.com

First known bacteria able to thrive using Arsenic

Phosphorus is a central component of the energy-carrying molecule in all cells(ATP) and also a part of chemical backbone of DNA and RNA. Arsenic, which is chemically similar to phosphorus, is poisonous for most life on Earth. Researchers discovered new bacteria, the *Gammaproteobacteria* (strain GFAJ-1) from the harsh environment of Mono Lake in California able to thrive and reproduce using the chemical arsenic. It substitutes arsenic for phosphorous in its cell components.



Gammaproteobacteria (strain GFAJ-1) grown on arsenic

Image Credit: NASA
Source: www.sciencedaily.com

001. Brandon K. Swan, Christopher J. Ehrhardt, Kristen M. Reifel, Lilliana I. Moreno, and David L. Valentine. Department of Earth Science and Marine Science Institute, University of California, 1006 Webb Hall, Santa Barbara, CA 93106-9630. **Archaeal and Bacterial Communities Respond Differently to Environmental Gradients in Anoxic Sediments of a California Hypersaline Lake, the Salton Sea.** Applied and Environmental Microbiology, **76** (3), 2010, 757 – 768.

Sulfidic, anoxic sediments of the moderately hypersaline Salton Sea contain gradients in salinity and carbon that potentially structure the sedimentary microbial community. We investigated the abundance, community structure, and diversity of Bacteria and Archaea along these gradients to further distinguish the ecologies of these domains outside their established physiological range. Quantitative PCR was used to enumerate 16S rRNA gene abundances of Bacteria, Archaea, and Crenarchaeota. Community structure and diversity were evaluated by terminal restriction fragment length polymorphism (T-RFLP), quantitative analysis of gene (16S rRNA) frequencies of dominant microorganisms, and cloning and sequencing of 16S rRNA. Archaea were numerically dominant at all depths and exhibited a lesser response to environmental gradients than that of Bacteria. The relative abundance of Crenarchaeota was low (0.4 to 22%) at all depths but increased with decreased carbon content and increased salinity. Salinity structured the bacterial community but exerted no significant control on archaeal community structure, which was weakly correlated with total carbon. Partial sequencing of archaeal 16S rRNA genes retrieved from three sediment depths revealed diverse communities of Euryarchaeota and Crenarchaeota, many of which were affiliated with groups previously described from marine sediments. The abundance of these groups across all depths suggests that many putative marine archaeal groups can tolerate elevated salinity (5.0 to 11.8% [wt/vol]) and persist under the anaerobic conditions present in Salton Sea sediments. The differential response of archaeal and bacterial communities to salinity and carbon patterns is consistent with the hypothesis that adaptations to energy stress and availability distinguish the ecologies of these domains.

Keywords: Bacteria, Archaea, Crenarchaeota, Euryarchaeota, Crenarchaeota, 16S rRNA.

002. Stephen C. Nold, Joseph B. Pangborn, Heidi A. Zajack, Scott T. Kendall, Richard R. Rediske, and Bopaiah A. Biddanda. 410 10th Avenue East, Menomonie, WI 54751. **Benthic Bacterial Diversity in Submerged Sinkhole Ecosystems.** Applied and Environmental Microbiology, **76** (1), 2010, 347 - 351.

Physicochemical characterization, automated ribosomal intergenic spacer analysis (ARISA) community profiling, and 16S rRNA gene sequencing approaches were used to study bacterial communities inhabiting submerged Lake Huron sinkholes inundated with hypoxic, sulfate-rich groundwater. Photosynthetic cyanobacterial mats on the sediment surface were dominated by *Phormidium autumnale*, while deeper, organically rich sediments contained diverse and active bacterial communities.

Keywords: 16S rRNA gene, Photosynthetic cyanobacterial mats, *Phormidium autumnale, Bacteroidetes*, *Firmicutes, Chloroflexi.*

003. Fiona P. Brennan, Florence Abram, Fabio A. Chinalia, Karl G. Richards, and Vincent O'Flaherty. Department of Microbiology, School of Natural Sciences and Environmental Change Institute, National University of Ireland, Galway, Ireland. **Characterization of Environmentally Persistent *Escherichia coli* Isolates Leached from an Irish Soil.** Applied and Environmental Microbiology, **76** (7), 2010, 2175 - 2180.

Soils are typically considered to be suboptimal environments for enteric organisms, but there is increasing evidence that *Escherichia coli* populations can become resident in soil under favorable conditions. Previous work reported the growth of autochthonous *E. coli* in a maritime temperate Luvic Stagnosol soil, and this study aimed to characterize, by molecular and physiological means, the genetic diversity and physiology of environmentally

persistent *E. coli* isolates leached from the soil. Molecular analysis (16S rRNA sequencing, enterobacterial repetitive intergenic consensus PCR, pulsed-field gel electrophoresis, and a multiplex PCR method) established the genetic diversity of the isolates (n = 7), while physiological methods determined the metabolic capability and environmental fitness of the isolates, relative to those of laboratory strains, under the conditions tested. Genotypic analysis indicated that the leached isolates do not form a single genetic grouping but that multiple genotypic groups are capable of surviving and proliferating in this environment. In physiological studies, environmental isolates grew well across a broad range of temperatures and media, in comparison with the growth of laboratory strains. These findings suggest that certain *E. coli* strains may have the ability to colonize and adapt to soil conditions. The resulting lack of fecal specificity has implications for the use of *E.coli* as an indicator of fecal pollution in the environment.

Keywords: *Escherichia coli,* 16S rRNA sequencing, Luvic Stagnosol soil.

004. Anna M. Kielak, Johannes A. van Veen, and George A. Kowalchuk. Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), P.O. Box 40, 6666 ZG Heteren, Netherlands. **Comparative Analysis of Acidobacterial Genomic Fragments from Terrestrial and Aquatic Metagenomic Libraries, with Emphasis on *Acidobacteria* Subdivision 6.** Applied and Environmental Microbiology, **76** (20), 2010, 6769 - 6777.

The bacterial phylum *Acidobacteria* has a widespread distribution and is one of the most common and diverse phyla in soil habitats. However, members of this phylum have often been recalcitrant to cultivation methods, hampering the study of this presumably important bacterial group. In this study, we used a cultivation-independent metagenomic approach to recover genomic information from soilborne members of this phylum. A soil metagenomic fosmid library was screened by PCR targeting acidobacterial 16S rRNA genes, facilitating the recovery of 17 positive clones. Recovered inserts appeared to originate from a range of *Acidobacteria* subdivisions, with dominance of subdivision 6 (10 clones). Upon full-length insert sequencing, gene annotation identified a total of 350 open reading frames (ORFs), representing a broad range of functions. Remarkably, six inserts from subdivision 6 contained a region of gene synteny, containing genes involved in purine *de novo* biosynthesis and encoding tRNA synthetase and conserved hypothetical proteins. Similar genomic regions had previously been observed in several environmental clones recovered from soil and marine sediments, facilitating comparisons with respect to gene organization and evolution. Comparative analyses revealed a general dicho**to**my between marine and terrestrial genes in both phylogeny and G+C content. Although the significance of this homologous gene cluster across subdivision 6 members is not known, it appears to be a common feature within a large percentage of all acidobacterial genomic fragments recovered from both of these environments.

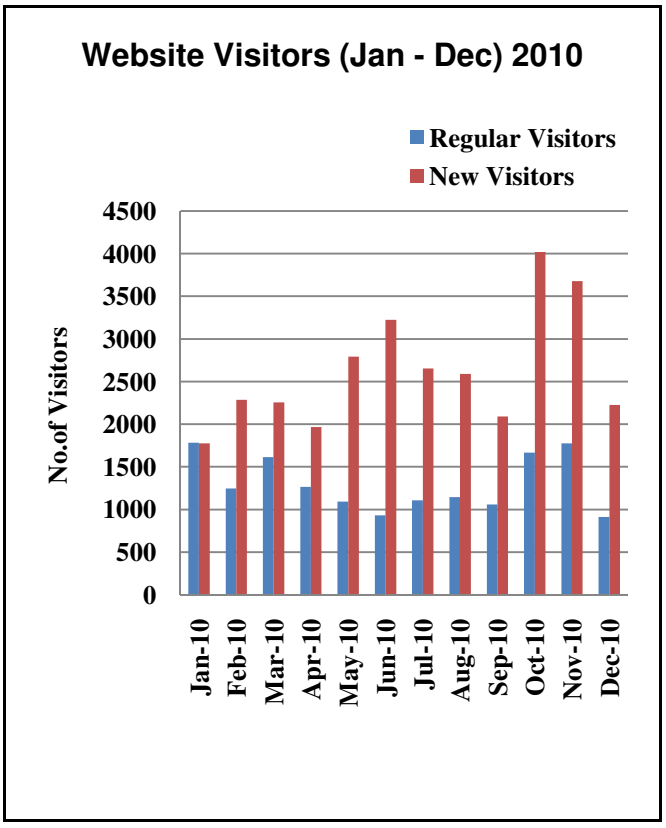
Keywords: phylum *Acidobacteria,* 16S rRNA genes, marine sediments.

005. Sara E. Blumer-Schuette, Derrick L. Lewis, and Robert M. Kelly. Department of Chemical and Biomolecular Engineering, North Carolina State University, Raleigh, NC 27695-7905. **Phylogenetic, microbiological and glycoside hydrolase diversities within the extremely thermophilic, plant biomass-degrading genus *Caldicellulosiruptor.*** Applied and Environmental Microbiology, **76**(24), 2010, 8084 – 8092.

Phylogenetic, microbiological and comparative genomic analysis was used to examine the diversity among members of the genus *Caldicellulosiruptor*, with an eye towards the capacity of these extremely thermophilic bacteria to degrade the complex carbohydrate content of plant biomass. Seven species from this genus (*C. saccharolyticus*, *C. bescii*, *C. hydrothermalis*, *C. owensensis*, *C. kronotskyensis*, *C. lactoaceticus*, and *C. kristjanssonii*) were compared on the basis of 16S rRNA phylogeny and cross-species DNA-DNA hybridization to a whole genome *C. saccharolyticus* oligonucleotide microarray, revealing that *C. saccharolyticus* was the most divergent within this group. Growth physiology of the seven *Caldicellulosiruptor* species on a range of carbohydrates showed that, while all could be cultivated on acid pretreated switchgrass, only

C. saccharolyticus, *C. besci*, *C. kronotskyensis*, and *C. lactoaceticus* were capable of hydrolyzing Whatman No. 1 filter paper. Two-dimensional gel electrophoresis of the secretomes from cells grown on microcrystalline cellulose revealed that the cellulolytic species also had diverse secretome fingerprints. The *C. saccharolyticus* secretome contained a prominent S-layer protein that appears in the cellulolytic *Caldicellulosiruptor* species, suggesting a possible role in cell-substrate interaction. Growth physiology also correlated with glycoside hydrolase (GH) and carbohydrate-binding module (CBM) inventories for the seven bacteria, deduced from draft genome sequence information. These inventories indicated that the absence of a single GH and CBM family was responsible for diminished cellulolytic capacity. Overall, the genus *Caldicellulosiruptor* appears to contain more genomic and physiological diversity than previously reported, and this argues for continued efforts to isolate new members from high temperature terrestrial biotopes.

Keywords: Phylogenetic, *Caldicellulosiruptor,* carbohydrate-binding module, glycoside hydrolase.



'Titanic' fast food for bacteria

In the deep: Bow railing of R.M.S. 'Titanic' showing rusticles.

The wreckage of the Titanic on the ocean floor will soon disappear as it is being fast eaten up by a newly discovered bacteria, according to Canadian researchers.The Titanic, which was the largest passenger ship at the time, sank on its maiden journey from England to New York April 14, 1912, after hitting an iceberg in mid-Atlantic. Of the 2223 passengers on board, only 706 survived.

Henrietta Mann, a Canadian civil engineering Professor at Dalhousie University, says the new bacterial species are eating away the wreckage so fast that soon the Titanic will be reduced to a "rust stain" on the ocean bottom.

"Perhaps if we get another 15 to 20 years out of it, we're doing good. Eventually there will be nothing left but a rust stain," she said."In 1995, I was predicting that Titanic had another 30 years, but I think it's deteriorating much faster than that now". Using DNA technology, Mann and Bhavleen Kaur from Dalhousie University and researchers from the University of Sevilla in Spain identified a new bacterial species collected from rusticles from the Titanic wreck, a statement by the researchers said.

Dark orange in colour, a rusticle is a formation of rust similar in shape to an icicle or stalactite. The wreck is covered with the knob-like mounds, formed as a 'consortium' of at least 27 strains of bacteria, including *Halomonas titanicae,* making a meal out of Titanic. But unlike icicles which are solid and hard, rusticles are porous and allow water to pass through. Indeed, they are rather delicate and will eventually disintegrate into fine powder.

"It's a natural process, recycling the iron and returning it to nature," said Mann who studies extreme environments.The Titanic's final resting was discovered by a joint American-French expedition in 1985, almost 73 years after its sinking. The wreck is located 3.8 kilometres below the ocean surface and some 530 kilometres southeast of Newfoundland (Canada).The discovery confirmed that the ship had split apart as the stern and the bow were located 600 metres apart from each other and are facing in opposite directions, the statement said. In the 25 years since the discovery of the wreck, Titanic has rapidly deteriorated, according to Mann.

Source: The Hindu, Dec 09, 2010.

E - Resources on Microorganisms

NATIONAL

The National Center for Biotechnology Information
www.ncbi.nlm.nih.gov/Taxonomy/

The Integrated Taxonomic Information System
www.itis.gov

Central Soil Salinity Research Institute (CSSRI)
www.cssri.org

Foundation for Research in Genetics and Endocrinology (FRIGE)
www.geneticcentre.org

Indian Bioresources Information Network (IBIN)
www.ibin.co.in

INTERNATIONAL

Biological Soil Crusts
www.soilcrust.org/

Few Aspects of Soil Microbiology and Biological Degradation of Pesticides
www.inapg.inra.fr/ens_rech/ager/ressources/supports/solpesticid/slide1.htm

Hazardous Waste Clean-Up Information
www.clu-in.org/

Japan Collection of Microorganisms (JCM)
www.jcm.riken.go.jp/

Belgian Co-ordinated Collections of Micro-Organisms
www.bccm.belspo.be/index.php

EVENTS
Conferences / Seminars / Meetings 2010 / 2011

80th Annual Session and National Symposium on "Climate Change - Research, Awareness and Capacity Building". Dec 2 - 4, 2010. **Venue:** Jaipur National University, Jaipur, **India.** **Website:** www.nasi.org.in

Mycobacteria: Physiology, Metabolism and Pathogenesis - Back to the Basics. Jan 15 - 20, 2011. **Venue:** Fairmont Hotel Vancouver, Vancouver, British Columbia, **Canada.**
Website: www.keystonesymposia.org/meetings/viewMeetings.cfm?MeetingID=1112

Antibiotics 2011 - Where Now?. Jan 20, 2011. **Venue:** Burlington House, London, **United Kingdom.**
Website:www.rsc.org/ConferencesAndEvents/RSCConferences/Antibiotics2011/index.asp

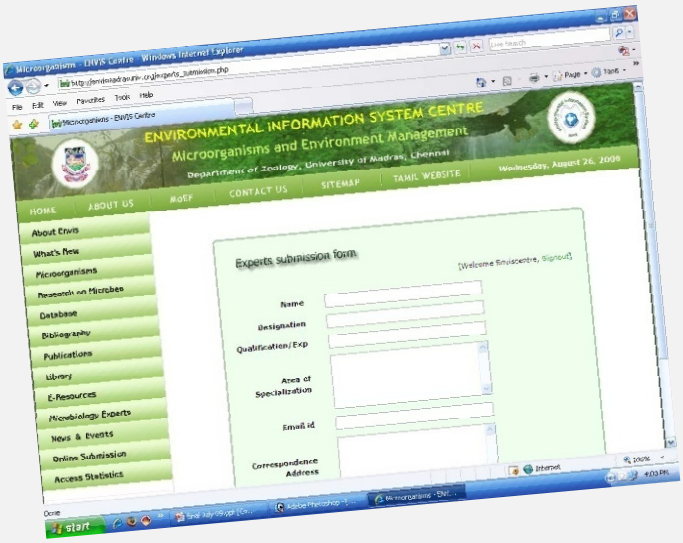
Microbes in Wastewater and Waste treatment, Bioremediation and Energy Production. Jan 24 - 27, 2011.
Venue: Birla Institute of Technology and Science - Pilani, Goa campus, NH 17B, Zuari Nagar, Goa, **India**.
Website: www.bits-goa.ac.in/mwt2011/

Biofilms in Nosocomial Fungal Infections. Jan 31- Feb 1, 2011. **Venue:** Pasteur Institute, Paris, **France**.
Website: www.escmid.org/profession_career/educational_activities/current_escmid_courses_and_ workshop

"Recent Biotechnological Perspectives in Diseases Diagnosis and Therapy" theme "Genes in Genomics Proteins in Proteomics". Feb 18 - 19, 2011. **Venue:** Mediclone Academy of Biosciences, Mediclone Research Center, 29, Biopavilion, Velichai, Kolathur post, Chennai, Tamil Nadu, **India.**
E-mail: drkrimmunotoxicol@yahoo.co.in

National Conference on "Emerging trends in Biological Research" (NCEBR' 11). Feb 21 - 22, 2011. **Venue:** Seminar Hall, Department of Zoology, Maraimalai Campus, University of Madras, Guindy, Chennai, Tamil Nadu, **India.**
Website: www.unom.ac.in/downloads/zoonatsem.pdf.

Microbial Communities as Drivers of Ecosystem Complexity. March 27 - April 1, 2011. **Venue:** Beaver Run Resort, Breckenridge, Colorado, **USA. Website:** www.keystonesymposia.org/meetings/viewMeetings.cfm?MeetingID=1059



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