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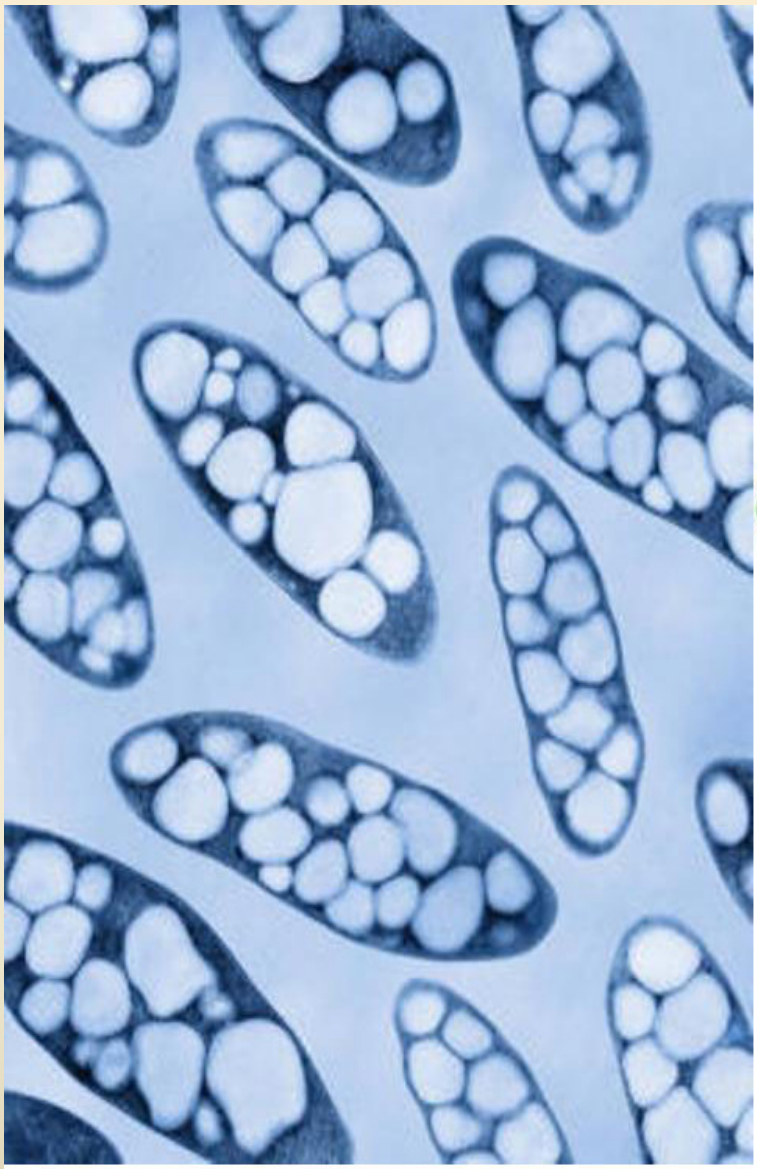

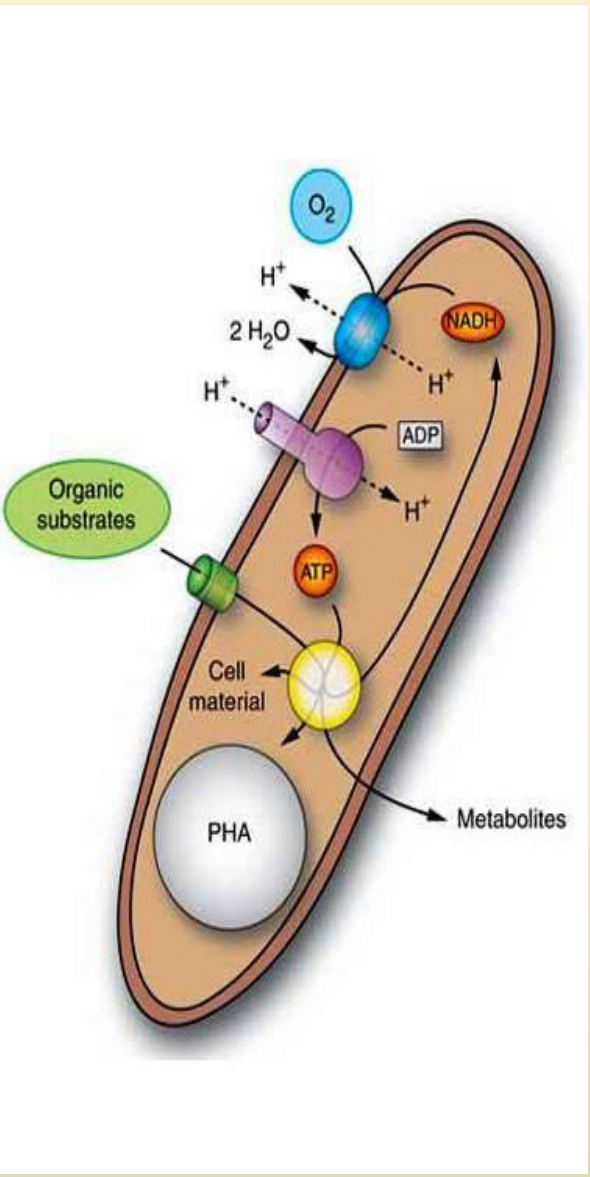

ENVIS CENTRE

Department of Zoology

University of Madras, Maraimalai Campus, Guindy, Chennai - 600 025

Telefax: 91-44-22300899; E-mail: dzum@envis.nic.in; enviscoordinator@gmail.com

Websites: www.envismadrasuniv.org; www.dzumenvis.nic.in; www.envismicrobes.in (Tamil website)

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EDITORS

Prof. N. Munuswamy

(ENVIS Co-ordinator)

Dr. V. Balasubramanian

(Scientist - D)

ENVIS TEAM

Prof. N. Munuswamy (Co-ordinator)

Dr. V. Balasubramanian (Scientist - D)

Mr. T. Tamilvanan (Programme Officer)

Mr. D. Siva Arun (Programme Asstt.)

Mr. R. Ramesh (Data Entry Operator)

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Department of Zoology

University of Madras, Maraimalai Campus,

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

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

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The Co-ordinator

ENVIS Centre

Department of Zoology

University of Madras

Maraimalai Campus, Guindy,

Chennai - 600 025.

Tamil Nadu, INDIA

(OR)

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enviscoordinator@gmail.com

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Cover page : Genetically engineered bacteria that consume corn sugar and produce PHA that can be used to make biodegradable plastics, including the types used in shopping bags. Schematic representation illustrating the key aspects of heterotrophic metabolism of PHA producing bacteria.

(Source: www.technologyreview.com & www.nature.com/nbt/journal/v24/n10/fig_tab/nbt1244_F1.html

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on
Microorganisms and Environment Management

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Dear Readers,

In our present lifestyle plastics have become an integral part. Most of our routine usage materials are made up of plastics. Due to increase usage, the plastic wastes dumped in land affects the ground water system and pollute the environment. Similarly, burning the plastics releases toxic gases like dioxin and carbon dioxide causing respiratory diseases to living beings. Further, recycling of plastic wastes reduces the quality of the plastics besides involving high expenditure. As they are non-biodegradable we have to find alternative way to reduce the pollution caused by plastics.

Moreover, microbes can produce many compounds as secondary metabolites which are important to human welfare. Scientific communities describe the compounds which mimic the plastic characteristics as polyhydroxyalkanoates (PHAs). Instead of using plastics we can use PHAs which are biodegradable and eco-friendly. Therefore, in recent years, there is an upsurge in the production of PHA by selected microbes which may probably reduce the usage of synthetic plastics.

In this context, this issue includes an article on Exploration of polyhydroxyalkanoates production from rhizosphere soil bacteria. Other regular features included are research reports, online reports, abstracts, e-resources and events on microorganisms.

It gives us great pleasure to share our views with you. We sincerely look forward to your suggestions and feedbacks. Please do mail us at:

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Prof. N. Munuswamy

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World Fisheries Day, November 21st 2012

SCIENTIFIC ARTICLE

Exploration of polyhydroxyalkanoates production from rhizosphere soil bacteria

Vijaya Abinaya. R, Balasubramanian. V, Ramesh. N, Natrajan. K and Rajeshkannan. V

Rhizosphere Biology Laboratory,
Department of Microbiology, Bharathidasan University,
Tiruchirappalli - 620024, Tamil Nadu, India

Abstract

Polyhydroxyalkanoates (PHA) accumulating bacteria were isolated from Rubber plant growing areas of Kerala. They were isolated by PHA production medium with sucrose as a sole carbon source. PHA producing bacteria were screened by staining with Sudan black B and PHA from the same bacteria was extracted by sodium hypochlorite. An efficient PHA producing isolate (PHA7) was identified and subjected for its morphological, biochemical properties, and molecular characterization. The identified *Bacillus cereus* yielded PHA of 0.436 g/l, amounting to 13.77% (w/w) of dry cell weight. The extracted polymer was analysed for oil absorbing, oil retention and biodegradability of PHA. Further, characterization of this polymer using Gas chromatography-Mass spectrometry (GC-MS) confirmed it as polyhydroxyhexonate.

Introduction

Global dependence on petroleum derived plastics has increased considerably over a decade (Philip *et al.,* 2007). Plastics have versatile qualities of strength, lightness, durability and resistance to biodegradation (Khanna and Srivastava, 2005). They are produced from non-renewable resources such as petro-chemicals and are not compatible with natural carbon cycle, because of their non-degradable characteristics. They pose serious threat to environment and wild life due to their persistence (Chua *et al.,* 2003). Accumulation of recalcitrant plastics in the environment has become a worldwide problem. Recycling of plastics can be done but it is a tedious time consuming process. In such cases, biodegradable plastics offer the best solution to the environmental hazard posed by conventional plastics as they are 'eco-friendly' in nature. Biodegradable plastics can be divided into four categories, such as polyhydroxyalkanoates (PHAs), polylactides, aliphatic polyesters and polysaccharides and in those, PHAs are the only 100% biodegradable polymer.

PHAs are optically active biopolyoxoesters and composed of (R) 3-hydroxy fatty acids, which represent a complex class of storage polyesters. They are synthesized by some Archaea and wide range of gram-positive and gram-negative bacteria in aerobic and anaerobic environments. These biopolymers are accumulated as inclusions (PHA granules) in the bacterial cytoplasm in response to organic nutrient limitation, generally, when the microbes are cultured in the presence of an excess carbon source. At present, PHAs are classified in two major classes: short-chain-length PHAs (scl-PHAs) with C4-C5 monomers and medium-chain-length PHAs (mcl-PHAs) with C6-C14 monomers. Mcl-PHAs are mainly produced by *Pseudomonas* sp. Because of structural differences, the physical properties of mcl-PHAs are generally quite different from the archetypal polyhydroxybutyrate (PHB) and other scl-PHAs (Matrinez *et al.,* 2011).

In this study, an attempt has been made to explore the polyhydroxyalkanoates production from rhizosphere soil bacteria of rubber plant. The extracted PHA was subjected to structural characterization by GC-MS analysis and the rate of biodegradation of the PHA film was done by open windrow composting method.

Materials and Methods

Sample collection

Isolates were obtained from rhizosphere soil of Rubber plantation areas located at different places in the state of Kerala such as Guruvayur, Trivandrum, and Sabarimala. Collected soil samples were kept in plastic bags and stored at 4ºC for further use. Each sample was homogenized by sieving (2.0 mm pores); dry weight equivalents were established by treating three samples having 20 g of fresh soil (Moreno *et al.,* 2007).

Isolation of bacteria

One gram of rhizosphere soil was serially diluted in sterile distilled water and plated on nutrient agar plates and incubated at 30ºC for 24 hours. Various colonies of different morphologies including branched, circular and rhizoidal forms were individually picked and subcultured 3-4 times on nutrient agar plates to obtain pure culture (Aarthi and Ramana, 2011). The bacterial isolates were maintained as pure culture on nutrient agar slants and stored at 4ºC for further use.

Screening of PHA producing bacteria

Sudan Black B staining was used for detecting the presence of PHA in cytoplasm of bacterial cells by Moreno *et al.* (2007). However, before screening, the isolates were induced to accumulate PHA by growing in PHA production liquid medium; a nitrogen-limiting medium containing 2% (w/v) glucose for 24 hours. Smear was made on a clean glass slide. After drying, 0.3% of Sudan black was added and kept for 10 minutes. Later, the slide was washed gently with distilled water and allowed to air dry. The dried slides were then immersed in xylene for few seconds and allowed to dry. Then 0.5% of safranin was added to the slides for 30 sec, the slide were washed gently, allowed to air dry and observed under light microscope.

Those PHA producing bacteria screened by Sudan Black B staining were subjected to sodium hypochlorite (Moreno *et al.,* 2007). The screened bacterial isolate was grown in conical flask containing PHA production medium on a shaker at 30ºC for 72 h with an agitation rate of 125 rpm. After 72 h, the bacterial culture was centrifuged at 6500×g, the supernatant was discarded and the pellet was transferred into pre-weighed petriplates by dissolving it in distilled water. The plates were dried by keeping it in hotplate at 80ºC. The dried weight of the pellet was taken in order to know the biomass weight. Sodium hypochlorite solution was added to the dried pellet and transferred to the centrifuge tubes and kept in shaker for 30 minutes at 37ºC. After incubation, the samples were centrifuged at 6500×g for 15 minutes. The supernatant was discarded and the pellet was washed with the distilled water and acetone was added to remove the hypochlorite solution by centrifugation. 10 ml of chloroform was added to the pellet and filtered into the pre-weighed petriplates. The chloroform gets evaporated which leaves the PHA film in the petriplates. The weight of the PHA film was observed. The PHA production was calculated by following formula, % of PHA production = (Weight of PHA/ Weight of biomass) × 100.

Identification of efficient PHA producing bacteria by 16S rRNA sequencing

The efficient PHA accumulating bacterium PHA7 was characterized by 16S rRNA gene sequencing. The genomic DNA from the PHA7 was isolated as per the standard protocol described by Sambrook *et al.* (1989). Amplification of bacterial 16S rRNA gene from the extracted genomic DNA was performed using the universal 16S rRNA gene primers: 16S rRNA forward primer 5' AgAgTTTgA TCM TGG CTC Ag3' (27f) and the reverse primer 5' TAC ggY TAC CTT gTTACg ACT T3' (1492r). The amplification of 16S rRNA gene was confirmed by 1.5% agarose gel electrophoresis in 1X Tris-acetate-EDTA buffer. The amplified product was further resolved and amplicon size corresponding to 1400bp was purified from agarose gels using QIA gel extraction kit following the manufacturer's protocol. The amplified 16S rRNA gene after gel elution was sequenced using forward and reverse, about 1400bp were carried out in Applied Biosystems™ by Life Technologies™ ABI Prism® 3730, Ocimum Bio solutions, Hyderabad, India.

Determination of oil-absorbing capacity

PHA film was weighed prior to immersion into mineral oil. After 1 min, the film was removed and excess oil was allowed to drip and PHA film weight was measured. The oil-absorption capacity is expressed as mass of oil per unit mass of initial film (Sudesh *et al.,* 2007) according to the formula: Oil absorbing property (g/g) = [(Film weight after dipping in oil – Initial film weight) / Initial film weight)].

Determination of oil-retention capacity

After determining the oil-absorption capacity, the PHA film was placed under a Petriplate wrapped with aluminium foil. Weight was placed on the plate for 1 min and then the film was weighed again (Sudesh *et al.,* 2007). The percentage oil retention was calculated as : Oil retention (%) = {[1-[(Film weight after dipping in oil - Film weight after pressing) / Film weight after dipping in oil] × 100]}.

GC- MS analysis

Sample preparation

Five milligrams of the sample was taken and added to dry test tubes. One milliliter of chloroform, 850 µl of methanol, and 150 µl of sulphuric acid were added and mixed well. The tubes were sealed and allowed for methanolysis. For methanolysis, the sample containing test tubes were kept in a beaker containing glycerine and boiled up to 100ºC. During methanolysis, the sample gets converted into methyl esters (HB/HV) and dissolved in chloroform. The sample was then heated to 2 h and 15 min. After cooling, the seal was broken and 1 ml of distilled water was added and shaken well. Bilayer formation occurred. These tubes were centrifuged at 9000×g

for 5 min. The bottom layer, which contains chloroform with methyl esters was taken and transferred to Eppendorf tubes. The tubes were kept at - 40ºC (Chia *et al.,* 2010).

Conditions of GC-MS

The capillary column was used with the following conditions: Capillary Column Elite - 5MS (5% Phenyl, 95% dimethlpolysiloxane); Dimensions (L × OD): 30 m × 0.25 mm; Carrier gas: He at flow rate 1ml/minute; Injector temperature: 290ºC; Column temperature: 200ºC; Detector temperature: 250ºC; and Temperature programming:60ºC (1min) at 6ºC/min 220ºC (3min) @ 8ºC/min 290ºC (5 min).

Biodegradability of PHA in soil

The PHA film produced from the fermented broth of PHA7 isolate was subjected to biodegradability test. The PHA film was buried into the soil in plastic tray to check its biodegradation rate and was maintained for 54 days in open windrow composting method. The film was visually inspected for changes in their morphology at different time interval (1, 14, 28, and 54th days).

Results and Discussion

Isolation and screening of PHA producing bacteria

Morphologically different types of bacteria were isolated from rhizosphere soil of rubber plantation collected from three different places of Kerala. The total number of 152×10^6 CFU/g of rhizosphere soil in Trivandrum area, 148×10^6 CFU from Sabarimala area, and 83×10^6 CFU for Guruvayur were recorded. The bacterial population varied significantly among all these three areas; probably due to the temperature, pH, and fertility of the soil.

The bacteria were initially screened by Sudan black- B staining method, in order to find out the PHA granules present in the cytoplasm of bacteria. Among all the isolates, 4 isolates from Trivandrum, 3 from Sabarimala, 1 from Guruvayur were screened as PHA producers. On microscopic observation, the 24 hours grown culture had showed the bluish black granules of PHA, which were ovoid or spherical in shape (Fig.1). The PHA producing isolates were designated as PHA1, PHA5, PHA7, PHA9, and PHA10.

In order to find out the efficient PHA producer from 8 isolates the sodium hypochlorite extraction method was performed. Based on the results, PHA7 isolates were found as more efficient than other PHA producing isolates. The film produced by the PHA 7 was found to be 0.028 g/l.

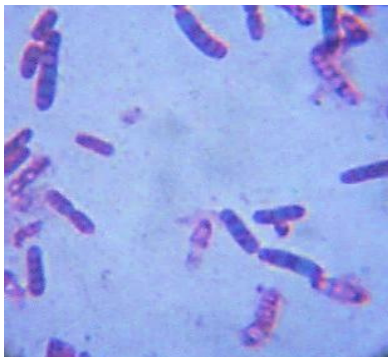

Fig. 1: Photomicrograph showing the PHA granules present in *B. cereus* (400X)

The film produced by PHA10 was found to be low, hence it was considered as a least producer of PHA. PHA5 was efficient PHA producer which produced PHA up to 21.90% but the recovery of the PHA was very low (0.02 g/l), whereas, PHA7 has produced PHA 13.77% of bacteria cell biomass and the recovery of PHA was high up to 0.028 g/l (Table 1). Hence, PHA7 was considered as the efficient strain and selected for further studies.

Table 1: Screening of efficient PHA producing bacteria

S. No	Bacterial isolate	Biomass (g/l)	PHA film weight (g/l)	PHA (%)
1	PHA1	0.211	0.017	7.65
2	PHA5	0.254	0.02	21.90
3	PHA7	0.465	0.028	13.77
4	PHA10	0.154	0.006	2.08

Identification of efficient bacterial isolate (PHA7)

Amplification of 16S rRNA gene was confirmed by 1.5% agarose gel electrophoresis in 1X Tris - acetate - EDTA buffer. The amplified product was further resolved and amplicon size corresponding to 1400bp was purified from agarose gels using QIA gel extraction kit following the manufacture's protocol. The PCR product was sequenced. The PHA7 was identified as *Bacillus cereus*.

Determination of oil - absorption and retention capacity of PHA

PHA films have the good capacity to absorb oil. The amount of oil absorbed and retained depends upon the efficiency of the organisms which produce PHA. Pre-weighed PHA film extracted form *B. cereus* was immersed in oil and

reweighed for oil absorption (Fig. 2). PHAs with good commercial applications are often compared with PP (polypropylene) because of their similar characters in some respects. So, that PHA cast from the solution has an extraordinary ability to absorb oil. The oil rapidly absorbed into the film and caused obvious change to the film appearance, which was indicated by greater transparency.

The initial film weight of PHA film without oil was 0.036 g, after immersing in oil the weight of the PHA drastically increased up to 0.103 g. The difference after and before immersing in oil was found to be nearly up to 0.79 g (Fig. 3). Applying the respective values in the formula, the results were found to be 1.861 g. The value obtained was high when compared to the PHBHx oil retention capacity which was found to be around 1. The plant - fiber based facial blots absorb around 1.4 (Sudesh *et al.,* 2007). The results showed that the PHA film produced by *B. cereus* had the ability to absorb oil.

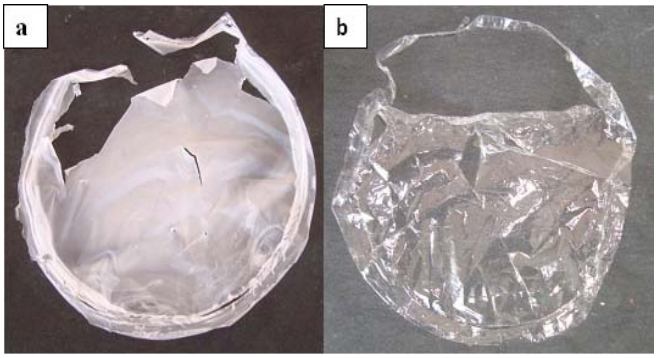

Fig. 2: Appearance of PHA film produced by *B. cereus*
(a) before and (b) after immersed in mineral oil

After absorbing, the oil in the film was then pressed in between the petriplates in order to find out the oil retention capacity. The PHA film weight after pressing was found to be 0.066 g, the weight of PHA film decreased. On by applying the respective values in the formula the result obtained was found to be 34.92% (Fig. 2). The oil retention capacity of PHA film was found to be 80% (Sudesh *et al.,* 2007), which was higher than the PHA produced by *B. cereus*.

Characterization of PHA film

B. cereus was grown on production media amended with glucose as carbon source was used for direct chloroform extraction procedure to obtain PHA product, and was subjected to GC-MS analysis. 3HHx (3-hydroxyhexonate) was found at 28.66 RT and the molecular weight of the compound was 270 Da (Fig. 3) by MS library search. The compound was referred with MS library and identified as hexonate. GC–MS revealed that the *B. cereus* was mainly composed of 3HD (3- hydroxyl hexonate).

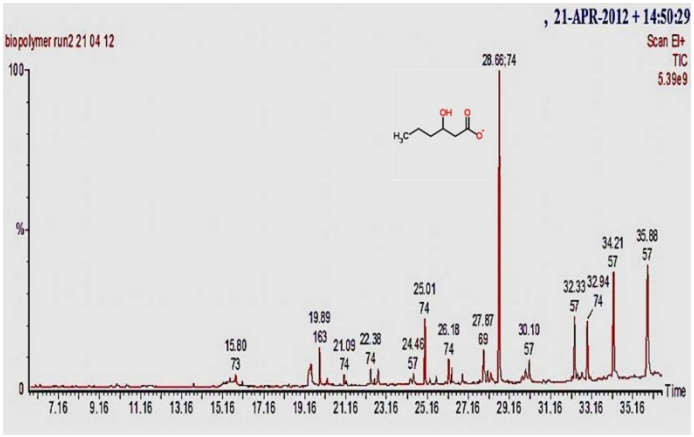

Fig. 3: GC–MS analysis of PHA produced by *B. cereus*

Biodegradability of PHA in soil

Degradability is the main difference between the synthetic plastics and bioplastics. Hence, the degradability of the PHA films produced by the *B. cereus* was tested in soil. The PHA film of *B. cereus* had degraded within 54 days. Biodegradation of PHA film on soil surface using open windrow composting method was examined (Fig. 4). The PHA film has the greatest affinity towards the depolymerase enzyme. The polymer film P (3HB-co-1 mol% 3 HV-co-3 mol% 3H4MV-co-18 mol% 3HHx) has been degraded on 54th day. P(3HB-co-5mol% 3HHx) did not get degraded even after 60 days (Chia *et al.,* 2010). This result showed that PHBHHx degrades faster than the PHBV.

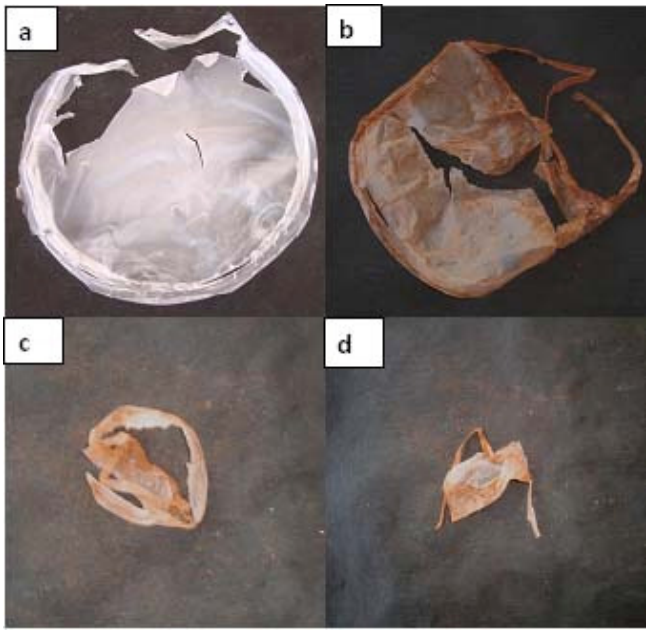

Fig. 4: Biodegradation of PHA film produced from *B. cereus*.
(a) 1st day; (b) 14th day; (c) 28th day; (d) 54th day of incubation

Conclusion

PHAs are found to be promising biodegradable plastics which show materials similar to that of synthetic plastics. The produced PHA was characterized as 3-hydroxyl hexonate by GC-MS. It showed oil absorption and retention capacity. Due to these properties, it may have commercial application value in oil absorbing films in the cosmetics and skin care industries. Further studies on the increased production of PHAs are required for commercial application

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RESEARCH REPORTS

Plants recognize pathogenic and beneficial microorganisms

In collaboration with national and international experts, researchers from Aarhus University have revealed new fundamental features of biomolecular interactions that enable plants to identify and respond appropriately to microorganisms. The new results provide a better understanding of the mechanisms governing the ability of plants to interact with beneficial microorganisms while being resistant to pathogenic bacteria and fungi. This could have implications for future sustainable agriculture, where useful microbes are increasingly sought to replace pesticides.

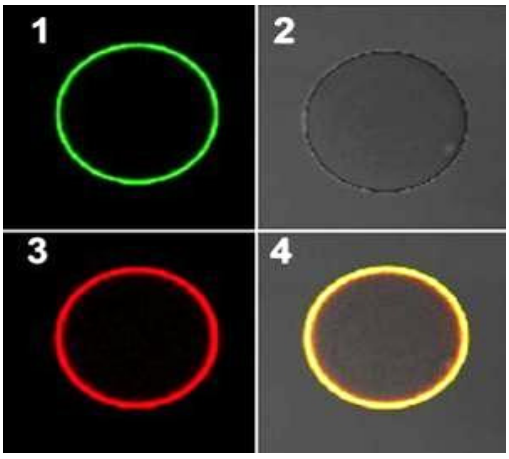
Plant roots are surrounded by thousands of bacteria and fungi living in the soil and on the root surfaces. To survive in this diverse environment, plants employ sophisticated detection systems to distinguish pathogenic microorganisms from beneficial microbes. Here the so-called chitin molecules from microorganisms, along with modified versions, play an important role as they are detected by the plant surveillance system. Legumes, for example, build a defence against pathogenic microorganisms in response to simple chitin molecules. However, when the plant detects a specific modified chitin molecule (called a Nod factor) that is secreted from the *Rhizobium* soil bacteria, formation of new organs in the form of "root nodules" occurs. *Rhizobium* bacteria are allowed to enter and colonise in these symbiotic organs, and they ultimately produce nitrogen for the plant.

The plant's detection of ligands such as chitin and modified Nod factors takes place through protein receptors that are localised on the surface of cells. Research at the Centre for Carbohydrate Recognition and Signalling (CARS) has now shown that ligand recognition through direct Nod factor binding is a key step in the receptor-mediated signal transduction that leads to root nodule development in legumes. High-affinity binding was observed in the nano-molar range, comparable to the biologically relevant concentrations where Nod factor has *in vivo* activity. In contrast to this, simple chitin molecules bind to the receptors with low affinity.

Structure-dependent ligand specificity and ligands binding affinities at different receptors may therefore determine which response mechanism is activated in plants exposed to different microbes or microbial communities in the environment.

Interdisciplinary approaches combining advanced biochemistry, chemoselective chemistry and microbial genetics made it possible to investigate the molecular mechanisms involved in distinguishing between Nod factor molecules secreted from rhizobia and chitin secreted by pathogenic microorganisms. The challenging task of purifying plant receptor proteins, which are present in very low amounts in roots of the model legume *Lotus japonicus*, was successfully accomplished by expressing the receptors in heterologeous plant-based systems and purifying them from membrane fractions.

Another challenge was the establishment of binding assays with the carbohydrate ligands. Nod factor labelling and Nod factor immobilisation facilitated this, following application of chemoselective chemistry. The researchers behind the results that have just been published in the international journal *PNAS* are affiliated with the Danish National Research Foundation's Centre for Carbohydrate Recognition and Signalling at the Department of Molecular Biology and Genetics, Aarhus University (Denmark), Department of Chemistry, University of Copenhagen (Denmark) and Department of Microbiology and Immunology, University of Otago (New Zealand).



Nod factors were isolated from the supernatant of a rhizobia culture, purified by HPLC and identified by MS. A fluorescent label (Alexa546) was attached to the purified Nod factor by chemoselective chemistry.

(**Image Credit:** Angelique Broghammer)

Source: www.sciencedaily.com

Bacteria talk to each other and our cells in the same way, via molecules

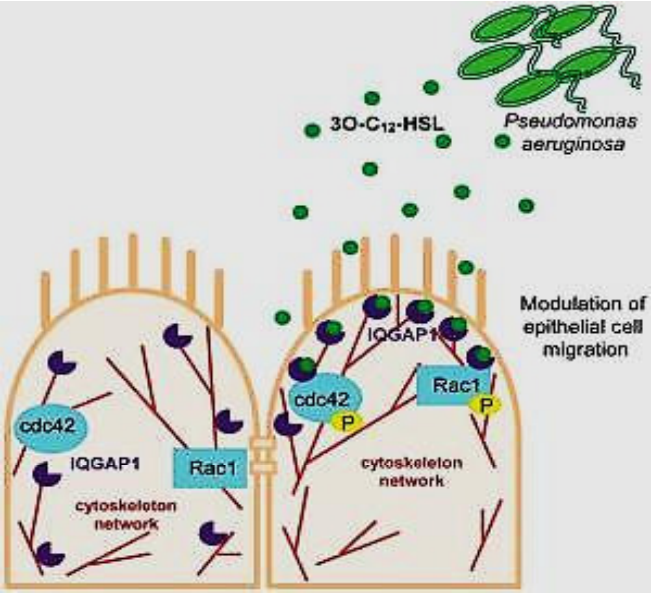
Bacteria can talk to each other via molecules they themselves produce. The phenomenon is called quorum sensing, and is important when an infection propagates. Now, researchers at Linköping University in Sweden are showing how bacteria control processes in human cells the same way. The results are being published in *PLoS Pathogens* with Elena Vikström, researcher in medical microbiology, as the main author.

Bacteria 'talk'

When the announcement goes out, more and more bacteria gather at the site of the attack, a wound, for example. When there are enough of them, they start acting like multicellular organisms. They can form biofilms, dense structures with powers of resistance against both antibiotics and the body's immune defense system. At the same time, they become more aggressive and increase their mobility. All these changes are triggered when the communication molecules short fatty acids with the designation AHL fasten to receptors inside the bacterial cells; as a consequence various genes are turned on and off. AHL can wander freely through the cell membrane, not just in bacterial cells but also our own cells, which can be influenced to change their functions. In low concentrations white blood cells, for example, can be more flexible and effective, but in high concentrations the opposite occurs, which weakens our immune defenses and opens the door for progressive infections and inflammations.

A team at Linköping University is the first research group to show how AHL can influence their host cells. Using biochemical methods, the researchers have identified a protein designated IQGAP, which they single out as the recipient of the bacteria's message, and something of a double agent. "The protein can both listen in on the bacteria's communication and change the functions in its host cells", Vikström says. Their laboratory studies were carried out on human epithelial cells from the intestines, which were mixed with AHL of the same type produced by *Pseudomonas aeruginosa*, a tough bacterium that causes illnesses in places like the lungs, intestines, and eyes. With the help of mass spectrometry, they have been

able to see which proteins bind AHL. "We have proof that physical contact between bacteria and epithelial cells is not always required; the influence can happen at a distance", Vikström says. The team's discovery can open the door to new strategies for treatment where antibiotics cannot help. One possibility is designing molecules that bind to the receptor and block the signal path for the bacteria something like putting a stick in a lock so the key won't go in. It's a strategy that could work with cystic fibrosis, for example, an illness where sticky mucus made of bacterial biofilm and large amounts of white blood cells is formed in the airways.



Model of the communication between *P. aeruginosa* 3O-C12-HSL and human epithelial Caco-2 cells. *P. aeruginosa* 3O-C12-HSL interacts and co-localizes with IQGAP1. The targeting of IQGAP1 by 3O-C12-HSL initiates early event of communication between Caco-2 cells and bacteria via 3O-C12-HSL and can further trigger the essential changes in the cytoskeleton network of epithelial cells. Also, 3O-C12-HSL modulates Caco-2 cell migration in a dose- and time-dependent manner. It also alters the phosphorylation status of Rac1 and Cdc42, and cellular distribution and localization of IQGAP1 from the basolateral to apical side of epithelial cells.

Image Credit: Karlsson *et al.,* The *P. aeruginosa* N-Acylhomoserine Lactone Quorum Sensing Molecules Target IQGAP1 and Modulate Epithelial Cell Migration.

Source: PLoS Pathogens, 2012; 8 (10): e1002953 DOI: 10.1371/journal.ppat.1002953

ONLINE REPORTS ON MICROORGANISMS

Ancient microbes found living beneath the icy surface of Antarctic lake

This week a pioneering study published in the *Proceedings of the National Academy of Sciences (PNAS)* and co-authored by Dr. Alison Murray and Dr. Christian Fritsen of Nevada's Desert Research Institute (DRI) reveals, for the first time, a viable community of bacteria that survives and ekes out a living in a dark, salty and subfreezing environment beneath nearly 20 meters of ice in one of Antarctica's most isolated lakes. Lake Vida, the largest of several unique lakes found in the McMurdo Dry Valleys, contains no oxygen, is mostly frozen and possesses the highest nitrous oxide levels of any natural water body on Earth. A briny liquid that is approximately six times saltier than seawater percolates throughout the icy environment that has an average temperature of minus 13.5 degrees centigrade (or 8 degrees Fahrenheit).

"This study provides a window into one of the most unique ecosystems on Earth", said Murray, the report's lead author, and molecular microbial ecologist and polar researcher for the past 17 years, who has participated in 14 expeditions to the Southern Ocean and Antarctic continent. "Our knowledge of geochemical and microbial processes in lightless icy environments, especially at subzero temperatures, has been mostly unknown up until now. This work expands our understanding of the types of life that can survive in these isolated, cryoecosystems and how different strategies may be used to exist in such challenging environments". Despite the very cold, dark and isolated nature of the habitat, the report finds that the brine harbors a surprisingly diverse and abundant assemblage of bacteria that survive without a present-day source of energy from the sun. Previous studies of Lake Vida dating back to 1996 indicate that the brine and it's inhabitants have been isolated from outside influences for more than 3,000 years. Murray and her co-authors and collaborators, including the project's principal investigator Dr. Peter Doran of the University of Illinois at Chicago, developed stringent protocols and specialized equipment for their 2005 and 2010 field campaigns to sample the lake brine while avoiding contaminating the pristine ecosystem.

To sample the unique environment researchers worked under secure, sterile tents on the lake's surface to keep the site and equipment clean as they drilled ice cores, collected samples of the salty brine residing in the lake ice and then assessed the chemical qualities of the water and its potential for harboring and sustaining life, in addition to describing the diversity of the organisms detected. Geochemical analyses suggest that chemical reactions between the brine and the underlying iron-rich sediments generate nitrous oxide and molecular hydrogen. The latter, in part, may provide the energy needed to support the brine's diverse microbial life. "It's plausible that a life-supporting energy source exists solely from the chemical reaction between anoxic salt water and the rock", explained Fritsen, a systems microbial ecologist and Research Professor in DRI's Division of Earth and Ecosystem Sciences. "If that's the case", echoed Murray. "This gives us an entirely new framework for thinking of how life can be supported in cryoecosystems on earth and in other icy worlds of the universe". Murray added further research is currently under way to analyze the abiotic, chemical interactions between the Lake Vida brine and the sediment, in addition to investigating the microbial community by using different genome sequencing approaches. The results could help explain the potential for life in other salty, cryogenic environments beyond Earth. The Lake Vida brine also represents a cryoecosystem that is a suitable and accessible analog for the soils, sediments, wetlands, and lakes underlying the Antarctic ice sheet that other polar researchers are just now beginning to explore.

While Antarctica's Lake Vida will never be a vacation destination, it is home to some newly discovered hearty microbes.

(**Image Credit:** Courtesy of the Desert Research Institute.)

Source: www.sciencedaily.com

Remote sensing, microbiology used to trace food-borne pathogens

In 2011, an outbreak of *Listeria monocytogenes* in cantaloupe led to almost 150 illnesses and 30 deaths. With a spate of recent outbreaks of such food-borne pathogens as *Salmonella*, Shiga toxin-producing *E. coli* and *L. monocytogenes*, the ability to predict where and how these deadly microbes enter the food supply chain could save lives and prevent disease. Cornell researchers have created a method that uses geospatial algorithms, food-borne pathogen ecology and Geographic Information System (GIS) tools to predict hot spots where these pathogens may be present and spread on farms prior to harvest. Many of the recent outbreaks of food-borne pathogens have been linked to contamination on the farm.

The method, which can be applied to any farm, uses classification tree tools with remotely sensed data, such as topography, soil type, weather trends, proximity to various sources (water, forests) and more, to predict areas where pathogens are likely to be present. "We wanted to see if we could identify factors that gave us a higher or lower prevalence of finding these pathogens", said Laura Strawn, a graduate student in the field of food science and lead author of a study published online Nov. 9, 2012 in the journal *Applied and Environmental Microbiology*. "We can look at a farm and use this data analysis tool to tell the farmer where these hotspots may be for food-borne pathogens", she said.

"These tools are likely to provide a completely new science-based approach for guidance on how to reduce the likelihood of contamination with these bacteria", said co-author Martin Wiedmann, a food science professor and study co-author. By knowing where the hot spots are, farmers may then implement such preventive practices as draining standing water, adjusting where livestock graze, or planting crops that should be consumed cooked rather than raw, for example, Strawn added. The researchers collected 588 samples soil, water, feces and drag swabs (gauze attached to a string and dragged over a field) from four produce fields on five farms each. Samples were collected four times a year, during each season, from 2009 to 2011. The prevalence of *L. monocytogenes*, *Salmonella* and

E. coli were 15.0, 4.6 and 2.7 percent respectively across all the samples. *L. monocytogenes* and *Salmonella* were detected more frequently in water samples from irrigation sources or nearby streams, while *E. coli* was found in equal distributions across all the sample types.

L. monocytogenes and *Salmonella* were found in higher frequencies in areas with moist soils. For *Salmonella*, "if you had more precipitation before a sample was collected, you were more likely to find that pathogen", said Strawn. Also, well-drained fields had lower *Salmonella* prevalence. Knowledge of such factors would help predict whether an area of a farm may be at higher risk. For *Listeria*, proximity to water, pastures, livestock and grazing cattle, wildlife habitation and nearby impervious surfaces, roads and ditches all predicted a higher prevalence of the pathogen. Once such factors have been identified, the GIS platform may be used to filter out specific areas based upon those factors (such as filtering areas that have moist soils and close proximity to water) to create a color-coded map of any farm area with predicted prevalence for a pathogen. "This work advances our understanding of the environmental microbiology of food-borne pathogens and permits tailored solutions to predict contamination of produce commodities during cultivation", said Peter Bergholz, a research associate in food science and the study's corresponding author.

The research was funded by the U.S. Department of Agriculture.

Esther Fortes, a member of the Cornell Produce Sampling Team, collects a water sample from a stream located adjacent to one of the produce fields used in the study.

(**Image Credit:** Laura Strawn)

Source: www.sciencedaily.com

NEWS

A bacterium that turns toxin into gold

WASHINGTON: Scientists have discovered bacteria that have the ability to withstand incredible amounts of toxicity and can be the key to creating 24-carat gold. Researchers from the Michigan State University have found that the metal-tolerant bacteria *Cupriavidus metallidurans* can grow on massive concentrations of gold chloride or liquid gold, a toxic chemical compound found in nature. The researchers fed the bacteria unprecedented amounts of gold chloride, mimicking the process they believe happens in nature. In about a week, the bacteria transformed the toxins and produced a gold nugget. "Microbial alchemy is what we're doing transforming gold from something that has no value into a solid, precious metal that's valuable", said KazemKashefi, assistant professor of microbiology and molecular genetics in a statement.

In their art installation, "The Great Work of the Metal Lover", researchers used a combination of biotechnology, art and alchemy to turn liquid gold into 24-carat gold. The artwork contained a portable laboratory made of 24-carat gold-plated hardware, a glass bioreactor and the bacteria, a combination that produces gold. The work has been put on display at the cyber art competition, Prix Ars Electronica, in Austria. Don't get up hopes that the procedure could make gold mines out of toxic waste dumps, though. The process would be cost prohibitive on a larger scale, said Adam Brown, Associate professor of electronic art and intermedia at MSU. However, Brown added, the experiment's success does raise questions about greed, the economy, environmental impacts and ethics as related to science and the engineering of nature.

Source: The Times of India, October 4, 2012.

Chloroquine returns to take on malaria again

NEW DELHI: Chloroquine has made a comeback to combat malaria. Studies have found that 70% of the malaria parasites in Senegal are reacting once again to chloroquine. A similar trend is being seen in Tanzania, Mozambique and Malawi as well. Scientists say choice

of drugs against malaria is limited and related, so when the malaria parasite once again reacts to a substance, its welcome news. Interestingly, chloroquine was highly efficacious for over 50 years before the vector started becoming resistant to it. The latest finding can greatly help malaria treatment.

Chloroquine costs only 25 cents for a four-day cure, while the current and corresponding artemisinin combination therapy cost $ 2. Antimalarial drug resistance is a major public health problem which hinders the control of malaria. In India, resistance of *Plasmodium falciparum* to chloroquine the cheapest and the most used drug was first reported in 1973 from Diphu in Assam's Karbi-Anglong district. Scientists say, "If healthcare personnel in developing countries can begin using chloroquine again, it will be possible to protect the currently used medicine and delay the reappearance of resistance, and it will also give a large group of patients access to cheaper treatment". Every year, 250 million cases of malaria infections are reported around the world, causing nearly one million deaths. According to the recent World Malaria report, 2011, over 70% of India's population, or 100.41 crore face the risk of malaria infection. Around 31 crore, however, face the "highest risk" of getting infected by the vector-borne disease.

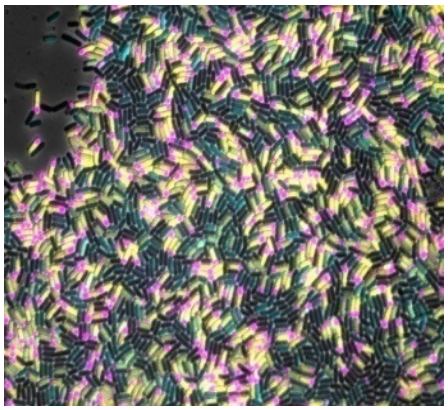
WHO said India has over 10 crore suspected malaria cases, but only 15.9 lakh could be confirmed last year. Scientists and healthcare personnel across the world fear that the malaria parasite will develop resistance to the current frontline treatment against malaria, Artemisinin-based Combination Therapies (ACTs). The resistance monitoring at the University of Copenhagen shows that in several African countries, malaria parasites are succumbing to chloroquine. The results have recently been published in the American Journal of Tropical Medicine and Hygiene. The first case of chloroquine resistance was found along the Thai-Combodian border in the late 1950s.

By 1973, chloroquine had to be replaced by the combination of sulphadoxine and pyrimethamine (SP) as first line drug for the treatment of uncomplicated malaria in Thailand, and in more than 10 African countries. Chloroquine-resistant falciparum strains had spread in all endemic areas of South America by 1970 and almost all in Asia and Oceania by 1989. In India, chloroquine resistance was first detected in 1973 in Assam, which gradually spread towards the west and south, covering almost the entire country.

Source: The Times of India, October 4, 2012.

Deciphering Bacterial Doomsday Decisions

As a homeowner preparing for a hurricane, the bacterium *Bacillus subtilis* uses a long checklist to prepare for survival in hard times. In a new study, scientists at Rice University and the University of Houston uncovered an elaborate mechanism that allows *B. subtilis* to begin preparing for survival, even as it delays the ultimate decision of whether to "hunker down" and withdraw into a hardened spore. The new study by computational biologists at Rice and experimental biologists at the University of Houston was published online on November 19, 2012 in PNAS. "The gene-expression program that *B. subtilis* uses to form spores involves hundreds of genes", said Dr. Oleg Igoshin, lead scientist on the study and professor of bioengineering at Rice. "Many of these genes are known and have been studied for decades, but the exact mechanism that *B. subtilis* uses to make the decision to form a spore has remained a mystery". *B. subtilis* is a common soil bacterium that forms a spore when food runs short. Spore formation involves dramatic changes. The cell first asymmetrically divides within its outer wall, forming one large chamber and one small one. As spore formation progresses, one chamber envelopes the other, which becomes a vault for the organism's DNA and a small set of proteins that can "reboot" the organism when it senses that outside conditions have improved. *B. subtilis* is harmless to humans, but some dangerous bacteria like anthrax also form spores. Scientists are keen to better understand the process, both to protect public health and to explore the evolution of complex genetic processes. During spore formation, scientists know that a bacterium channels its energy into producing proteins that help prepare the cell to become a spore.



Source: www.phys.org

Abstracts

001. Runa Antony, K.P. Krishnan, C.M. Laluraj, MelothThamban, P.K. Dhakephalkar, Anupama S. Engineer, S. Shivaji. National Centre for Antarctic and Ocean Research, Headland Sada, Vasco-da-Gama, Goa 403 804, India. **Diversity and physiology of culturable bacteria associated with a coastal Antarctic ice core.** Microbiological Research, 2012, **167** (6), 372 - 380.

Microbiological studies of polar ice at different depths may provide important comparisons, as they preserve records of microbial cells and past climate. In this study, we examined bacterial abundance, diversity and glaciochemical composition from three depths of an ice core from coastal Dronning Maud Land, East Antarctica. Higher bacterial abundance corresponded with high *in situ* sea-salt Na^+ and dust concentration, suggesting that bacteria might have been transported and deposited into ice along with dust particles and marine aerosols. Fourteen bacterial isolates belonging to the genera *Methylobacterium*, *Brevundimonas*, *Paenibacillus*, *Bacillus* and *Micrococcus* were retrieved. Frequent isolation of similar bacterial genera from different cold environments suggests that they possess features that enable survival and metabolism for extended periods of time at sub-zero temperatures. The highest number and diversity of recoverable bacteria was obtained from 49 m depth corresponding to 1926 AD and consisted of bacteria from 4 different genera whereas at 11 m (1989 AD) and 33 m (1953 AD) samples only species belonging to the genera *Bacillus* was recovered. Among the *Bacillus* species, *Bacillus aryabhattai* which has been reported only from the upper stratosphere, was isolated and is the first record from the Earth's surface. *Methylobacterium* was the most dominant genera at 49 m depth and its prevalence is attributable to a combination of high *in situ* methanesulfonate concentration, specialized metabolism and environmental hardiness of *Methylobacterium*. Some of the isolated bacteria were found to respire and grow using methanesulfonate, suggesting that they may utilize this substrate to sustain growth in ice. In addition, NO_3^- (2.93-3.69 μM), NH_4^+ (1.45-3.90 μM) and PO_4^{3-} (0.01-0.75 μM) present in the ice could be potential sources fueling bacterial metabolism in this environment. It could be deduced from the study that variation in bacterial abundance and diversity was probably associated with the prevailing *in-situ* conditions in ice.

Keywords: Bacteria, Diversity, Ecology, Adaptation, Ice, Antarctica.

002. Jan-HendrikHehemann, Amelia G. Kelly, Nicholas A. Pudlo, Eric C. Martens, and Alisdair B. Boraston. **Bacteria of the human gut microbiome catabolize red seaweed glycans with carbohydrate-active enzyme updates from extrinsic microbes.** Proceedings of the National Academy of Sciences, 2012, **109** (48), 19786 -19791.

Humans host an intestinal population of microbes-collectively referred to as the gut microbiome-which encode the carbohydrate active enzymes, or CAZymes, that are absent from the human genome. These CAZymes help to extract energy from recalcitrant polysaccharides. The question then arises as to if and how the microbiome adapts to new carbohydrate sources when modern humans change eating habits. Recent metagenome analysis of microbiomes from healthy American, Japanese, and Spanish populations identified putative CAZymes obtained by horizontal gene transfer from marine bacteria, which suggested that human gut bacteria evolved to degrade algal carbohydrates-for example, consumed in form of sushi. We approached this hypothesis by studying such a polysaccharide utilization locus (PUL) obtained by horizontal gene transfer by the gut bacterium *Bacteroides plebeius*. Transcriptomic and growth experiments revealed that the PUL responds to the polysaccharide porphyran from red algae, enabling growth on this carbohydrate but not related substrates like agarose and carrageenan. The X-ray crystallographic and biochemical analysis of two proteins encoded by this PUL, *BACPLE_01689* and *BACPLE_01693*, showed that they are β-porphyranases belonging to glycoside hydrolase families 16 and 86, respectively. The product complex of the GH86 at 1.3 Å resolution highlights the molecular details of porphyran hydrolysis by this new porphyranase. Combined, these data establish experimental support for the argument that CAZymes and associated genes obtained from extrinsic microbes add new catabolic functions to the human gut microbiome.

Keywords: *Bacteroides plebeius,* human gut microbiome, degrade algal carbohydrates, polysaccharides.

E - Resources on Microorganisms

NATIONAL

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

The Energy and Resource Institute
http://www.teriin.org/index.php?option=com_ongoing&task=about_project&pcode=1993BM61

Analyzing software database for Bioinformatics
http://www.imtech.res.in/raghava/

National Bureau of Agriculturally Important Microorganisms
http://www.nbaim.org.in/Page.aspx?Page=Rch_cc

International Crops Research Institute for the Semi-Arid Tropics
http://www.icrisat.org/bt-pathology-viral.htm

INTERNATIONAL

Biocatalysis, Biodegradation Database, University of Minnesota
http://umbbd.ethz.ch/resources.html

Regnum Prokaryote
http://www.tgw1916.net

The Fungal Records Database of Britain and Ireland
http://www.fieldmycology.net/FRDBI/FRDBI.asp

Fungi Images on the Net
http://fungi.fvlmedia.dk/

Fungi
http://www.in2.dk/fungi/imageframe1.htm

EVENTS
Conferences / Seminars / Meetings - 2013

Immunology of Fungal Infections. January 12 - 13, 2013. **Venue:** Galveston, TX, **USA.**
Website: http://www.grc.org/programs.aspx?year=2013&program=grs_immuno

Geobiology: Microbe-Mineral Interactions, Biomineralization, and the Rock Record. January 27 - February 1, 2013. **Venue:** Ventura, CA, **USA.**
Website: http://www.grc.org/programs.aspx?year=2013&program=geobiology

Genomics and Epidemiological Surveillance of Bacterial Pathogens. February 03 - 08, 2013. **Venue:** San Jose, **Costa Rica.**
Website: http://www.wellcome.ac.uk/Education-resources/Courses-and-conferences/Advanced-Courses-and-Scientific-Conferences/Advanced-Courses/index.htm

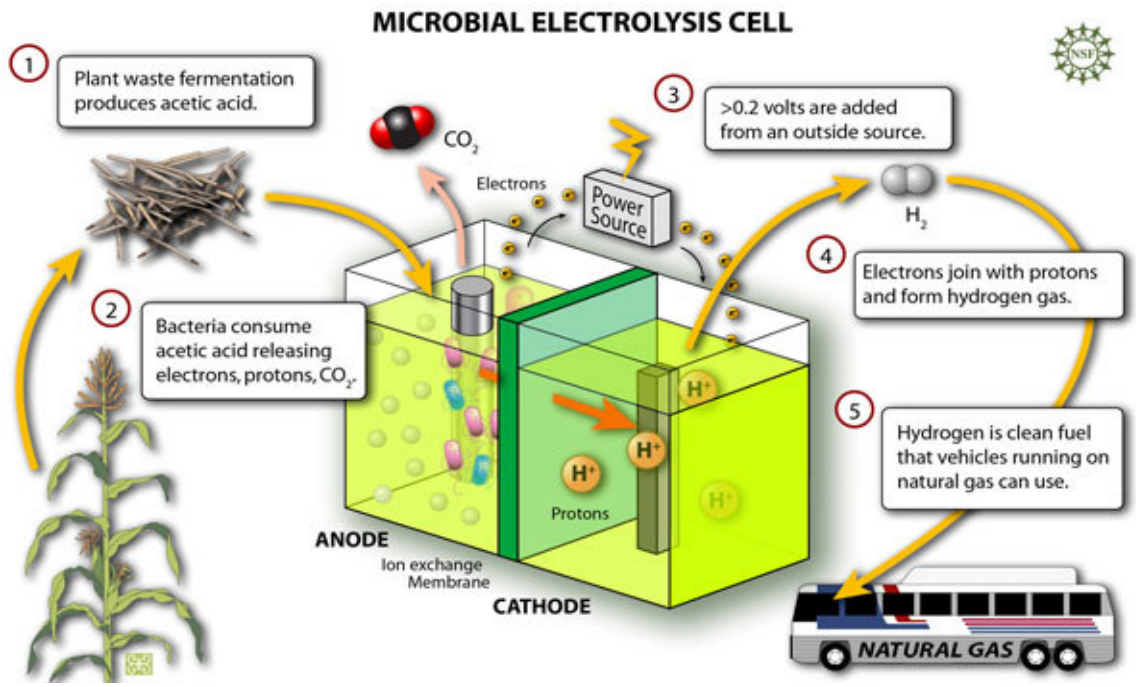
2nd Biotechnology World Congress. February 18th - 21st, 2013. **Venue:** Dubai, **UAE.**
Website: http://www.biotechworldcongress.com/index.php

Cyanobacteria can survive in extreme habitats is remarkable, such as the hot springs of Yellowstone National Park.

Ancient genetic building block discovered in cyanobacteria

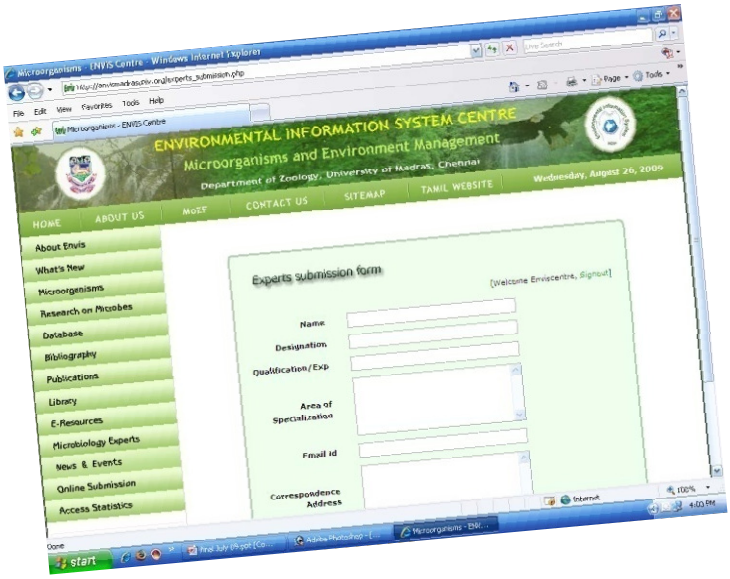
Scientists believe that prior to the advent of DNA as the earth's primary genetic material, early forms of life used RNA to encode genetic instructions. What sort of genetic molecules did life rely on before RNA? The answer may be AEG, a small molecule that when linked into chains form a hypothetical backbone for peptide nucleic acids (PNA), which have been hypothesized as the first genetic molecules. Synthetic AEG has been studied by the pharmaceutical industry as a possible gene silencer to stop or slow certain genetic diseases. The only problem with the theory is that up to now, AEG has been unknown in nature. A team of scientists from the USA and Sweden announced that they have discovered AEG within cyanobacteria which are believed to be some of the most primitive organisms on earth. "While we were writing our manuscript", Dr. Cox says, "we learned that our colleagues at the Stockholm University Department of Analytical Chemistry had made a similar discovery, so we asked them to join us on the paper". To determine how widespread AEG production is among cyanobacteria, the scientists analyzed pristine cyanobacterial cultures from the Pasteur Culture Collection of Paris, France.

(**Image Credit:** © GalynaAndrushko / Fotolia)
Source: www.bioquicknews.com



Penn State researchers have developed a microbial electrolysis cell, which they call BEAMR, to produce hydrogen. The process uses bacteria to break down organic material, such as acetic acid and cellulose. A small external burst of voltage aids in boosting hydrogen production.

Image Credit: Zina Deretsky, National Science Foundation



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