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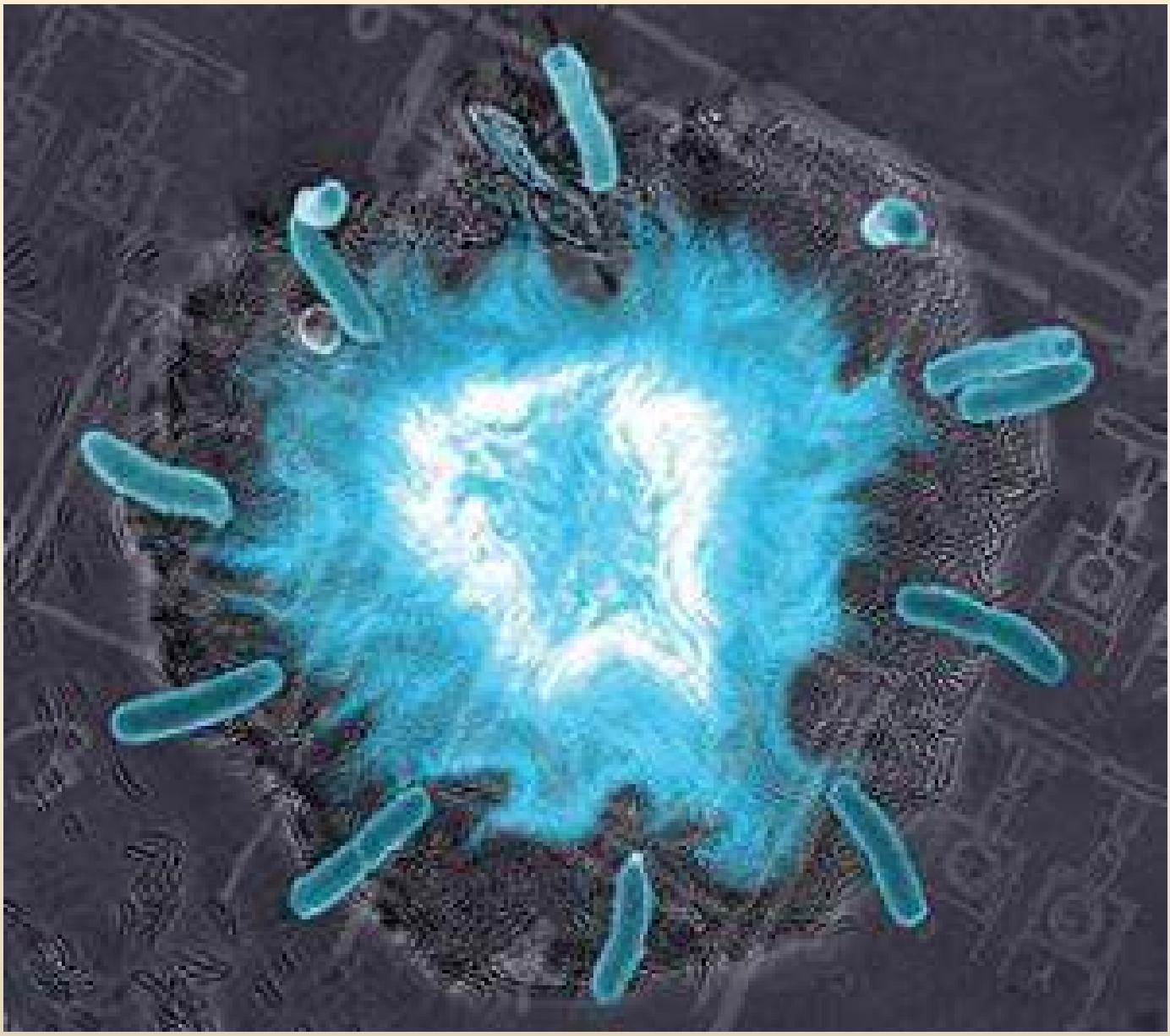

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A soft toy of *Shigella* bacterium, which causes dysentery

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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.

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

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Cover page : *Escherichia coli* bacteria that flash in fluorescent light and keep time like clock

Courtesy: University of California , San Diego (UCSD)

ENVIS Newsletter
on
Microorganisms and Environment Management

Contents

SCIENTIFIC ARTICLES Page No

Production of a novel lipase from slaughterhouse waste using *Pseudomonas gessardii* 2

K. Ramani and G. Sekaran

Prevalence of airborne fungi in the suburban of North Chennai, India - A case study 4

T. Sathish Kumar and M. Ramesh Babu

RESEARCH REPORTS

Microbes produce fuels directly from biomass 7

Researchers synchronize blinking 'Genetic Clocks' using bacteria 8

ONLINE REPORTS ON MICROORGANISMS

How ocean bacterium turns carbon into fuel 10

NEWS

Bio-pesticides to enrich the soil and enhance crop productivity 11

Discovery of millions of new microbes opens 'huge frontier' 11

ABSTRACTS OF RECENT PUBLICATIONS 12

E-RESOURCES ON MICROORGANISMS 13

EVENTS 13

Dear Readers,

Happy New Year 2010!

We are celebrating 'International year of Biodiversity 2010' and our country is one of the twelfth mega biodiversity region in the world. Let us conserve our rich biodiversity by safeguarding the pristine and fragile ecosystems.

Air pollution has become a major public concern since the past three decades all over the world. Air borne particles of industrial origin attract growing attention as potential contaminants and inducers of many respiratory diseases.

Use of waste products from any source is not only a partial solution to environmental and ecological issues, but it also solves the problems encountered in its disposal.

This issue also carries articles on the use of slaughter-house waste for the production of lipase, and the prevalence of airborne fungal spores in industrial region and its impact on human health.

Do write to us expressing your views on this and any other matter concerning Microorganisms and Environment Management.

We are happy to share our views with you and look forward to your suggestions and feedback (www.envismadrasuniv.org/send_feedback.php) on the articles published in this Newsletter.

Prof. N.Munuswamy

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World Water Day - 22nd March

Production of a novel lipase from slaughterhouse waste using *Pseudomonas gessardii*

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Introduction

Current industrial practices have led to an enormous generation of various crude fatty materials as waste that are difficult to treat and valorise (Fickers *et al*., 2005). Among these, the important ones are lipid waste from slaughterhouses. Slaughterhouse lipid waste, tallow, is a fat-rich material and has been used as raw material for the production of low value products like soap and detergents. Recovery of high value - added products from the above solid wastes has been largely a neglected field due to the survival of microorganisms in such highly hydrophobic substrates as a result of which the bio - transformations into value added products becomes difficult. It has been reported that fermentation of lipids under acidic conditions favour the surface attachment of the micro organisms onto substrate. Hence, there has been a constant search for the screening of extremophiles for lipid hydrolysis with the emphasis to produce high value added products such as highly active and stable lipases. In the present study, the lipolytic strain, *Pseudomonas gessardii* with high yield coefficient and high lipase yield was isolated from the tallow acclimatized soil. There are no reports available in literature on the utilization of slaughterhouse lipid waste for the production of lipase. Thus, the present study was focused on the production of lipase from *Pseudomonas gessardii* using slaughterhouse lipid waste as a substrate.

Substrate

Slaughterhouse waste, namely goat tallow, was used as a substrate in the present study. It is a whitish solid substance rich in fat with a mild odour which was obtained from a slaughterhouse in Chennai.

Isolation and identification of *P. gessardii*

Lipase producing microbial cultures was isolated from the tallow acclimatized soil. 10 g of tallow was buried in black soil for a period of 3 weeks. During this period, the soil microbes utilize the tallow as a nutrient (carbon) source. The decomposed tallow along with contaminated soil were dispersed in nutrient broth (NB) of composition peptone (5.0 g/l); yeast extract (1.5 g/l); beef extract (1.5 g/l); NaCl (0.5 g/l) and was incubated for 48 h at 37 °C. The cultured broth was plated on tributyrin agar and incubated at 37 °C for 24 h. The colonies showing clear zones were picked out from the plate and inoculated into the basal medium containing the goat tallow for the maximum yield of lipase. Among the fifteen isolated strains, one strain exhibited a better lipolytic activity than the others and thus it was selected for the production of lipase in our study. The strain was identified by 16S ribosomal DNA (16S rDNA) sequencing and phylogenetical analysis. Based on nucleotides homology (16S rDNA sequencing) and phylogenetical analysis, the organism was identified as *Pseudomonas gessardii.* The 1466 bp sequence was submitted to GenBank (NCBI) and the accession number "FJ943496" obtained. The BLAST algorithm was used to search for homologous sequences in GenBank.

Production, purification and characterization of acidic lipase from goat tallow using *P. gessardii*

P. gessardii was grown in the basal medium containing KH_2PO_4 (1.0 g/L); $NH_4(SO_4)_2$ (0.5 g/L); $CaCl_2$ (1.0 g/L); 0.31 % (w/v) goat tallow and 1% (v/v) gum arabic at pH 3.5. The maximum lipase production (52 U/ml) was found at the beginning of the stationary phase in the presence of goat tallow as a substrate at pH 3.5 and temperature 37°C. The glycerol and fatty acid formation during the hydrolysis of goat tallow is presented in Fig. 1.

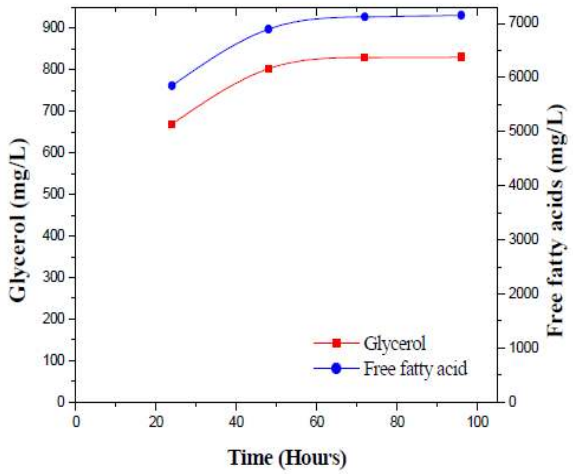

Fig. 1. Effect of time on the formation of glycerol and fatty acids from the goat tallow

This shows the maximum concentration of 830 mg/L and 7152 mg/L of glycerol and fatty acid respectively, which was produced from the 12444 mg/L of lipid substrate. Such high concentration of fatty acids might be formed due to the affinity of lipase towards the polar group of lipid substrate, goat tallow, because lipase attaches onto glyceride bond leading to the formation of 3 fatty acid molecules for every one molecule of glycerol. This higher yield of free fatty acid is possible only with high polar lipases which has high selectivity in solvating the glycerol moiety. The lipase was characterised by high polar/apolar amino acid ratio of 10.02. This is the highest ratio compared to other lipases in the literature. The maximum lipase production was observed at a pH of 3.5, thus the lipase production may be regarded as extremely acidic lipase. The purified *P. gessardii* acidic lipase was able to tolerate a broad range of pH from 1.0 to 6.0 with maximum lipase activity at pH 3.5. The enzyme remained stable with in the pH range of 1.0 to 5.5 (Fig. 2).

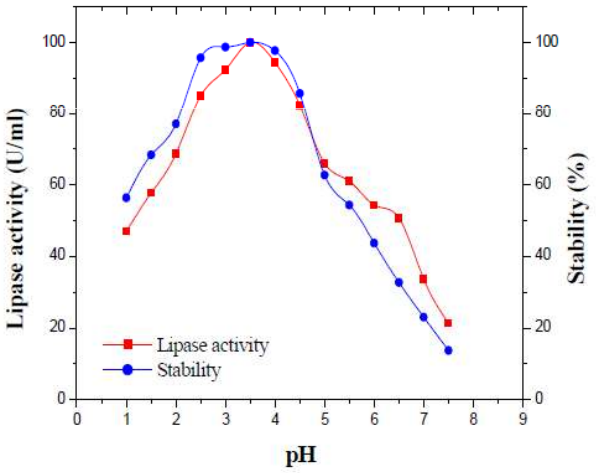

Fig. 2. Relative lipase activity and stability of purified acidic lipase at different pH

On the other hand many *Pseudomonas* sp. reported in the literature were known for alkaline lipases, which were stable in the pH range of 8.5 to 10 (Lin *et al*., 1996 ; Ogino *et al*., 2000; Rahman *et al*., 2005). There is no report on the production of lipase from bacterial strain, *P. gessardii* using goat tallow and other lipid substrates. The acidic lipases (less than pH 4.0) have wide potential for application in medicinal field as a substitute for pancreatic lipases in enzyme therapy (Sani, 2006).

The crude acidic lipase was purified and the specific activity of the purified acidic lipase was 1473 U/mg protein.

The molecular weight of purified acidic lipase was 94 kDa corresponding to a well defined single band which is the characteristic feature of monomeric enzyme (Fig. 3). The purified acidic lipase from fungal source (*Aspergillus niger*) was reported to have lower molecular weight of 32.2 kDa (Mhetras *et al*., 2009). The high molecular weight acidic lipase produced by *P. gessardii* is due to the fact that the substrate goat tallow used in the present study was of high molecular weight consisting of 18 oleic acid molecules arranged in a chain and it is embedded in palmitic acid residue. It is known that the essential characteristic of enzymes to cleave or solvate the high molecular weight substrates must possess more number of active sites i.e., with large number of peptide linkages. Thus, enzymes with larger number of peptide bonds are characterised by high molecular weight. This is reflected by high protein (116 mg) and total amino acid content (102 mg) of *P. gessardii* compared to other lipases.

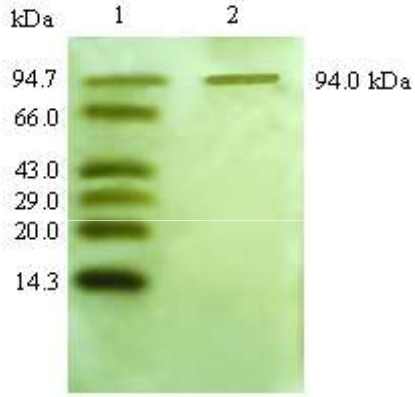

Fig. 3. SDS-PAGE of purified acidic lipase from *P. gessardii.*

Lane 1: Molecular mass standards (14.3 kDa to 94.7 kDa).

Lane 2: Lipase (10 µg), purified by ammonium sulfate precipitation in combination with ion exchange chromatography and gel filtration chromatography.

Cancer killer found in the Ocean

Marine biotechnologists treat cancer with mud-loving Ocean bacteria. Scientists identified and sequenced the genes of a bacteria called *Salinispora tropica.* It produces anti-cancer compounds and can be found in ocean sediments off the Bahamas. A product called salinosporamide A has shown promise treating a bone marrow cancer called multiple myeloma, as well as solid tumors.

Source: www.naturalnews.com

Conclusion

The extremely acidic lipase producing strain, *Pseudomonas gessardii* was isolated from tallow acclimatized black soil and identified by 16S rDNA sequencing. The presented lipase is a novel, extremely acid tolerant, biocatalyst from *P. gessardii* using goat tallow as a substrate, with wide industrial applications potential. Slaughterhouse waste, goat tallow, can be considered as a potential lipid substrate to produce extremely acidic lipase with high lipase activity. The maximum lipase production was observed at a pH of 3.5, for 48 h with the temperature of 37ºC. The purified lipase was highly stable under extremely acidic conditions (pH 1.0 to 5.5). The acidic lipase was characterised by high polar/apolar amino acid ratio than the other *Pseudomonas* lipases reported in the literature. The purified lipase from *P. gessardii* has a high molecular weight enzyme. The acidic lipase can be exploited in the hydrolysis of lipid wastes from slaughterhouses, households and oleochemical industries.

References:

Fickers, P., Benetti, P. H., Wache, Y., Marty, A., Mauersberger, S., Smit, M. S. and Nicaud, J. M. (2005) Hydrophobic substrate utilisation by the yeast *Yarrowia lipolytica*, and its potential applications. FEMS Yeast Research. **5**, 527 - 543.

Lin, S. F., Chiou, C. M., Yeh, C. M. and Tsai, Y. C. (1996) Purification and partial characterization of an alkaline lipase from *Pseudomonas pseudoalcaligenes* F-111. Appl. Environ. Microbiol. **62**, 1093 - 1095.

Mhetras, N. C., Bastawde, K. B. and Gokhale, D. V. (2009) Purification and characterization of acidic lipase from *Aspergillus niger* NCIM 1207. Biores. Technol. **100**, 1486 - 1490.

Ogino, H., Nakagawa, S., Shinya, K., Muto, T., Fujimura, N., Yasudo, M. and Ishikawa, H. (2000) Purification and characterization of organic solvent tolerant lipase from organic solvent tolerant *Pseudomonas aeruginosa* LST-03. J. Biosci. Bioeng. **89**, 451 - 457.

Rahman, R. N. Z. R. A., Baharum, S. N., Basri, M. and Salleh, A. B. (2005) High-yield purification of an organic solvent-tolerant lipase from *Pseudomonas* sp. strain S5. Analytical Biochemistry. **341**, 267 - 274.

Sani, D. G. (2006) Step up: Bacterial lipase to substitute pancreatic lipase for enzyme therapy. Available from: http://microbepundit.blogspot.com/2006/10/step-up-bacterial-lipase-to- substitute.html.

Prevalence of airborne fungi in the suburban of North Chennai, India - A case study

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Abstract

Exposure to airborne fungi is being proposed as a cause of adverse health effects. The study was undertaken to examine the concentrations of airborne fungal spores in eight suburban areas which are strategically located near industries in North Chennai, India. N - 6 Andersen single stage sampler was used to collect the air with Potato Dextrose Agar (PDA) at a flow rate of 25 l/min. A total of 25 species belonging to 13 genera of fungi were recorded. Total concentration of airborne fungi in each area ranged from 266.00 – 441.76 CFU/m^3 of air. Major prevalent airborne fungi belong to *Aspergillus* and *Penicillium.* This study provides a useful index for relative risk exposure of airborne fungi in the suburban areas of North Chennai.

Keywords: Industry, Airborne fungi; Bioaerosol; Fungal spores.

Introduction

Bioaerosols are natural or artificial particles of microbial, plant or animal origin suspended in air and may be called as organic dust. It includes live or dead bacteria, fungi, viruses, allergens, bacterial endotoxins (compounds of cell membranes of Gram - negative bacteria), antigens (molecules that can induce an immune response), toxins (toxins produced by microorganisms), mycotoxins (toxins produced by fungi), glucans (components of cell walls of many molds), pollen, plant fibers, etc. Microorganisms are frequently absorbed onto dust particles and transported along with the dust. Many bioaerosols are known to cause symptoms and/or illness, including a wide range of adverse health effects and infection. Individuals may become increasingly sensitized to some bioaerosols through repeated exposure.

Several epidemiological studies in several countries have indicated an association between human exposure to fungal spores in indoor air and adverse respiratory symptoms. More than 80 genus of fungi have been associated with

symptoms of respiratory tract allergies (Horner *et al*., 1995). *Cladosporium, Alternaria*, *Aspergillus* and *Fusarium* are amongst the most common allergenic genera. Airborne fungi are among the most common organisms in nature. They were considered to be correlated with air pollution and were proposed as a cause of adverse health effects on humans, animals and plants (Shelton *et al*., 2002). It was reported that the dominant fungi were *Cladosporium, Alternaria, Penicillium*, and *Aspergillus* in the atmosphere (Adhikari *et al*., 2004).

Chennai, one of the major metropolitan cities in India, was chosen for the present study due to its dense population and industrialization. Emission of air pollutants and dust particles (including organic dust) from industries are continuous, and seems to affect the normal life style of the residents of nearby areas. However, no studies on airborne fungal molds in heavily industrialized part of Chennai city were performed so far. Therefore the objectives of this study were to reveal the distribution characteristics and nature of airborne fungal spores in such areas.

Materials and methods

Sampling site

Eight suburban areas in North Chennai viz; Thiruvottiyur, Manali, Andarkuppam, Manali New Town (NT) , Ernavur, Kathivakkam, Ennore, and Mathur were selected which are immediate surroundings of industries. The study was carried out from the period of January 2008 to July 2008. Almost 5 different sampling locations were selected randomly in each area.

Sampling method and Mold identification

N - 6 Andersen single stage sampler was used to isolate fungal molds from the open environment at a suction rate of 25 l/ min. Samples were collected for 3 minutes between 10.00 am to 12.00 pm. For each sampling the sampler was loaded with 9.0 cm petri dishes containing Sterile Potato Dextrose Agar (PDA) with streptomycin to inhibit bacterial growth. Exposed petri dishes were incubated for 72 h at 27 ± 2°C. Few colonies did not generate spores even after incubation upto 7 days and were classified as "non-sporing colonies" group. After incubation, fungal colonies growing on each dish were counted and sub cultured for species identification. The number of colonies recorded is expressed as colony forming unit per cubic meter of air [CFU/m^3] and calculated as follow.

$$CFU/m^3 = \frac{\text{No. of Colonies obtained}}{\text{Sampling time} \times \text{Suction rate of the sampler}} \times 1000$$

Isolation frequency of a fungi is denoted by the number of sampling in which a fungi is recorded against the total number of samplings. On the basis of percent isolation frequency the molds are grouped into Most common (>80 %), Common (60 - 80 %), Frequent (40 - 60 %), Occasional (20 - 40 %) and Sporadic (< 20 %).

Results

Considering all sampling sites, concentration (Mean CFU/m^3 of five locations in each area) of airborne fungi in 8 suburban areas are represented in Table 1. Higher fungal concentrations of 441.76 and 441.56 CFU/m^3 were found in Thiruvottiyur and Mathur respectively. Minimal level of 266 CFU/m^3 was recorded in Manali New town. The concentration of fungi in other areas ranges from 316 CFU/m^3 to 423 CFU/m^3. Concerning non-spore formers, the highest concentration of 29.26 CFU/m^3 was observed in Thiruvottiyur, while in other areas it ranges from 2.66 CFU/m^3 to 23.94 CFU/m^3 except Mathur.

Fungal diversity

Thirteen genera, including 25 species of culturable fungi, were identified. The genus of *Aspergillus* with 9 species occupied more than 32 % of the total number of isolated fungal species. The genus of *Penicillium* only had five species and all other genera were represented by single species. A maximum number of eleven genera and 19 species were identified in the area of Mathur and a minimum of six genera and 12 species were identified in Manali New Town. The fungal species isolated from all the suburban areas and its isolation frequency are given in Table 1.

The most dominant group was found to be *Aspergillus* and the maximum frequency of isolation varied from 10 to 95 % followed by *Penicillium* with a range from 5 to 47.5 %. Within the group of *Aspergillus, Aspergillus niger* had the highest isolation frequency (95%). Other fungal groups varied from 2.5 % to 35 %. The Isolation frequency of non spore

Table 1. Prevalence of airborne fungi (Mean CFU/m^3) in eight suburban areas of North Chennai

S. No.	Species	Thiruvottiyur	Manali	Andarkuppam	Manali NT	Ernavur	Kathivakkam	Ennore	Mathur	Isolation Frequency
1	*Rhizopus stolonifer*	-	-	5.32	15.96	-	-	-	-	S
2	*Syncephalastrum racemosum*	-	5.32	-	-	2.66	2.66	-	2.66	S
3	*Aspergillus flavipes*	-	5.32	-	2.66	-	-	-	2.66	S
4	*Aspergillus flavus*	42.56	18.62	39.9	45.22	90.44	26.6	61.18	37.24	C
5	*Aspergillus fumigatus*	10.64	21.28	5.32	-	37.24	7.98	2.66	2.66	O
6	*Aspergillus japonicus*	101.08	26.6	29.26	26.6	45.22	77.14	53.2	82.46	C
7	*Aspergillus nidulans*	29.26	10.64	13.3	10.64	18.62	34.58	15.96	29.26	F
8	*Aspergillus niger*	117.24	135.66	125.02	114.39	82.46	82.46	77.14	103.74	MC
9	*Aspergillus ochraceus*	2.66	2.66	10.64	5.32	15.96	5.32	-	-	S
10	*Aspergillus tamarii*	21.28	7.98	13.3	2.66	13.3	-	18.62	21.28	O
11	*Aspergillus terreus*	7.98	18.62	5.32	-	-	-	-	2.66	S
12	*Aureobasidium pullulans*	-	-	-	-	-	-	2.66	-	S
13	*Chrysoporium pannorum*	7.98	7.98	-	-	-	5.32	-	2.66	S
14	*Cladosporium cladosporioides*	5.32	-	5.32	-	-	18.62	2.66	5.32	S
15	*Curuvalaria lunata*	-	5.32	5.32	-	-	-	-	15.96	S
16	*Drechslera australiensis*	5.32	-	2.66	7.98	29.26	2.66	13.3	7.98	O
17	*Fusarium oxysporum*	-	-	-	-	-	13.3	-	2.66	S
18	*Monilia sitophila*	7.98	2.66	18.62	2.66	23.94	7.98	23.94	15.96	O
19	*Paecilomyces variotii*	-	2.66	5.32	2.66	-	-	7.98	5.32	S
20	*Penicillium citrinum*	-	21.28	-	-	-	2.66	-	-	S
21	*Penicillium frequentans*	18.62	-	-	-	-	37.24	21.28	-	O
22	*Penicillium funiculosum*	-	-	-	-	-	-	2.66	5.32	S
23	*Penicillium oxalicum*	15.96	15.96	18.62	21.28	34.58	15.96	71.82	90.44	F
24	*Penicillium restrictum*	-	7.98	-	-	18.62	-	-	-	S
25	*Trichoderma viride*	18.62	-	2.66	-	2.66	-	5.32	5.32	S
	Non spore formers	29.26	23.94	10.64	7.98	7.98	5.32	2.66	-	O
	Total	441.76	340.48	316.54	266.00	422.94	351.12	383.04	441.56	

S - Sporadic; C - Common; F - Frequent; O - Occasional; MC - Most Common

formers was 35%. *Aspergillus niger* was recorded as Most Common (MC) in its occurrence. *Aspergillus flavus* and *Aspergillus japonicus* were recorded as Common (C) in their occurrence. Of the remaining, 2 species were recorded as Frequent (F), 5 as Occasional (O) and 15 as Sporadic (S). Non-spore formers were recorded as Occasional (O) in their occurrence.

Conclusion

The present study has clearly demonstrated the prevalence of airborne molds especially *Aspergillus* and *Penicillium*. Among the several species of *Aspergillus* isolated, the level of *Aspergillus niger, A. flavus*, and *A. japonicus* was relatively high, posing greater risk to population residing in these industry oriented suburban areas. The presence of *Penicillium* species like *Penicillium oxalicum, P. restrictum*, and *P. citrinum* are of great concern as they are considered to be potential toxin producers.

Many organic materials expelled from different industries are found to be dispersed in the environment. This is due to the excessive production of wastewater, dumping of waste and emission of air pollutants in the atmosphere. This may favor the growth and survival of many fungal spores within the environment. The difference in the nature of organic materials expelled may be the cause of abundance of certain species in certain areas. Many reports are also available for the prevalence of fungal spores in outdoor environments very close to industries. Although, the study was carried over in few areas, it clearly revealed the concentration and multiple fungal species present in the environment. Hence, few recommendations have to be carried out to minimize /control the airborne spore levels in these areas, otherwise they may pose a serious potential health risk to nearby populations.

References

Adhikari, A., Sen, M. M., Gupta - Bhattacharya, S. and Chanda, S. (2004) Airborne viable, non-viable and allergenic fungi in a rural agricultural area of India: a 2- year study at five outdoor sampling stations. Sci. Total Environ. **326**, 123 - 141.

Horner, W. E., Helbling, A., Salvaggio, J. E. and Lehrer, S. B. (1995) Fungal allergens . Clin. Microbiol. Rev. **8**, 161 - 179.

Shelton, B. G., Kirkland, K. H., Flanders, W. D. and Morris, G. K. (2002) Profiles of airborne fungi in buildings and outdoor environments in the United States. Appl. Environ. Microbiol. **68**(4), 1743 - 1753.

Microbes produce fuels directly from biomass

A collaboration led by researchers with the U.S. Department of Energy's Joint BioEnergy Institute (JBEI) has developed a microbe that can produce an advanced biofuel directly from biomass. Deploying the tools of synthetic biology, the JBEI researchers engineered a strain of *Escherichia coli* bacteria to produce biodiesel fuel and other important chemicals derived from fatty acids.

The fact that our microbes can produce a diesel fuel directly from biomass with no additional chemical modifications is exciting and important, says Jay Keasling, the Chief Executive Officer for JBEI, and a leading scientific authority on synthetic biology. “Given that the costs of recovering biodiesel are nowhere near the costs required to distill ethanol, we believe our results can significantly contribute to the ultimate goal of producing scalable and cost effective advanced biofuels and renewable chemicals”.

Keasling led the collaboration, which was made up of a team from JBEI's Fuels Synthesis Division that included Eric Steen, Yisheng Kang and Gregory Bokinsky, and a team from LS9, a privately-held industrial biotechnology firm based in South San Francisco. The LS9 team was headed by Stephen del Cardayre and included Zhihao Hu, Andreas Schirmer and Amy McClure. The collaboration has published the results of their research in the journal Nature. The paper is titled, “Microbial Production of Fatty Acid-Derived Fuels and Chemicals from Plant Biomass”.

A combination of ever increasing energy costs and global warming concerns has created an international imperative for new transportation fuels that are renewable and can be produced in a sustainable fashion. Scientific studies have consistently shown that liquid fuels derived

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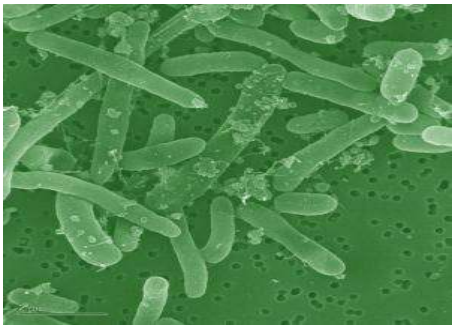
from plant biomass are one of the best alternatives if a cost-effective means of commercial production can be found. Major research efforts to this end are focused on fatty acids, the energy rich molecules in living cells that have been dubbed nature's petroleum.

Fuels and chemicals have been produced from the fatty acids in plant and animal oils for more than a century. These oils now serve as raw materials not only for biodiesel fuel, but also for a wide range of important chemical products including surfactants, solvents and lubricants. The increased demand and limited supply of these oils has resulted in competition with food, higher prices, questionable land use practices and environmental concerns associated with their production, Keasling says. "A more scalable, controllable, and economic alternative route to these fuels and chemicals would be through the microbial conversion of renewable feedstocks, such as biomass-derived carbohydrates".



Electron micrograph shows rod-shaped *E. coli* secreting oil droplets containing biodiesel fuel, along with fatty acids and alcohol.

(**Image Credit:** Eric Steen, Joint BioEnergy Institute (JBEI))

E. coli is a well-studied microorganism whose natural ability to synthesize fatty acids and exceptional amenability to genetic manipulation make it an ideal target for biofuels research. The combination of *E. coli* with new biochemical reactions realized through synthetic biology, enabled Keasling, Steen and their colleagues to produce structurally tailored fatty esters (biodiesel), alcohols and waxes directly from simple sugars. Biosynthesis of microbial fatty acids produces fatty acids bound to a carrier protein, the accumulation of which inhibits the making of additional fatty acids, Steen says. "Normally *E. coli* doesn't waste energy making excess fat, but by cleaving fatty acids from their carrier proteins, we're able to unlock the natural regulation and make an abundance of fatty acids that can be converted into a number of valuable products. Further, we engineered our *E. coli* to no longer eat fatty acids or use them for energy". After successfully diverting fatty acid metabolism toward the production of fuels and other chemicals from glucose, the JBEI researchers engineered their new strain of *E. coli* to produce hemicellulase enzymes that are able to ferment hemicellulose, the complex sugars that are a major constituent of cellulosic biomass and a prime repository for the energy locked within plant cell walls.

Engineering *E. coli* to produce hemicellulases enables the microbes to produce fuels directly from the biomass of plants that are not used as food for humans or feed for animals, Steen says. "Currently, biochemical processing of cellulosic biomass requires costly enzymes for sugar liberation. By giving the *E. coli* the capacity to ferment both cellulose and hemicellulose without the addition of expensive enzymes, we can improve the economics of cellulosic biofuels". The JBEI team is now working on maximizing the efficiency and the speed by which their engineered strain of *E. coli* can directly convert biomass into biodiesel. They are also looking into ways of maximizing the total amount of biodiesel that can be produced from a single fermentation.

Productivity, titer and efficient conversion of feedstock into fuel are the three most important factors for engineering microbes that can produce biofuels on an industrial scale, Steen says. "There is still much more research to do before this process becomes commercially feasible". This research was supported by funds from LS9, Inc. and the UC Discovery Grant program. LS9 is using synthetic biology techniques to develop patent-pending UltraClean™ fuels and sustainable chemicals. The UC Discovery Grant program is a three-way partnership between the University of California, private industry and the state of California that is aimed at strengthening and expanding California's economy through targeted fields of research.

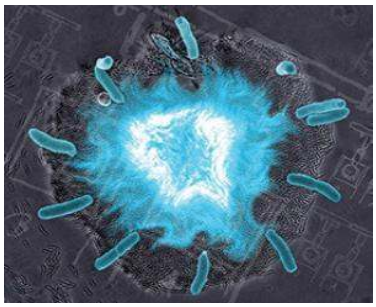
Source: www.sciencedaily.com

Researchers synchronize blinking 'Genetic Clocks' using bacteria

Researchers at University of California, San Diego (UCSD) who last year genetically engineered bacteria to keep track of time by turning on and off fluorescent proteins within their cells, have taken another step toward the construction of a programmable genetic sensor. The scientists recently synchronized these bacterial

"genetic clocks" to blink in unison and engineered the bacterial genes to alter their blinking rates when environmental conditions change. Their latest achievement, detailed in a paper published in the journal Nature, is a crucial step in creating genetic sensors that might one day provide humans with advance information about temperature, poisons and other potential hazards in the environment by monitoring changes in the bacterium's blinking rates. Programming living cells is one defining goal of the new field of synthetic biology, said Jeff Hasty, associate professor of biology and bioengineering at UCSD who headed the research team with Lev Tsimring, Associate Director of UCSD's BioCircuits Institute.

Dr. Hasty and colleagues have used powerful genetic tools, backed by decades of detailed knowledge of bacterial processes, to create a system that delivers on the promise of synthetic biology to engineer living organisms to meet pressing societal needs, said James Anderson, who oversees computational biology grants at the NIH's National Institute of General Medical Sciences. "The oscillating system they engineered sets the stage for the development of highly sensitive sensors that could have multiple applications in basic research, biotechnology and medicine". Synchronization of clocks and oscillators in general has been a fascinating subject for physicists and applied mathematicians for centuries, said Tsimring. "This began with the Dutch mathematician and astronomer Christiaan Huygens, who is credited with its serendipitous discovery in 1665 when he suspended a pair of nearly identical pendulum clocks (which he invented and patented some 8 years earlier) on the same wooden beam".



A supernova burst in a colony of coupled genetic clocks show them flashing in synchrony.

(**Image Credit:** University of California, San Diego (UCSD))

Synchronization plays a crucial role in physics and biology as a way of self-organization of highly regular behavior with less than perfect components. This phenomenon has a myriad of applications in modern technology, from communication networks to GPS. Our study demonstrates how inherently noisy gene oscillators can operate together with beautiful synchronicity and regularity once coupled together in a specific way. Over the past decade, researchers have gone from wiring genetic toggle switches and oscillators in living cells to building living circuits capable of pattern generation, noise shaping, edge detection and event counting. In their latest development, the UCSD researchers took advantage of a type of bacterial communication in which bacteria exchange small molecules. Many bacteria species are known to communicate by a mechanism known as quorum sensing, that is, relaying between them small molecules to trigger various behaviors, said Hasty. Other bacteria are known to disrupt this communication mechanism by degrading these relay molecules. Taking these communication elements from different organisms, Hasty and his team of researchers who included UCSD bioengineering graduate students Tal Danino and Octavio Mondragon designed and constructed a network in the genetic model bacterium *E. coli* with positive and negative feedback components to produce a colony of synchronized clocks.

Hasty said the architecture of the circuit is similar to his team's previous genetic clock, but with the quorum sensing components allowing the phase information that is, the oscillations between the bacterial cells to be relayed. The researchers constructed devices to precisely control the sizes of the bacterial colonies between two different scales: a micron, or a millionth of a meter, and a millimeter, or one-thousandth of a meter. At the micron scales, Hasty said the cells in the colonies oscillate synchronously from 50 to 90 minutes, a period that can be tuned externally. But at the longer, or millimeter scales, he noted, the time for diffusion of the signal becomes more important, allowing the researchers to actually observe the propagation of the signal through the colony. The use of quorum sensing is a promising approach to increase the sensitivity and robustness of the dynamic response to external signals, said Hasty. In nature, synchronization typically helps stabilize a desired behavior arising from a network of intrinsically noisy and unreliable elements. We think the synchronized genetic clock sets the stage for the use of microbes as a macroscopic biosensor with oscillatory output, or

applications of using a synchronized periodic signal in drug delivery.

The scientists received funding for their development from the National Institute of General Medical Sciences, which is part of the National Institutes of Health.

Source: www.sciencedaily.com

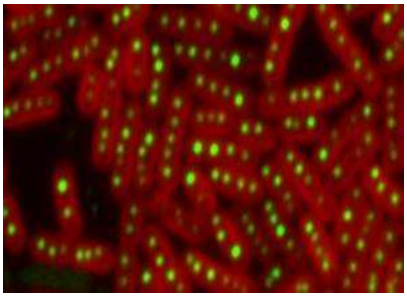
ONLINE REPORTS ON MICROORGANISMS

How ocean bacterium turns carbon into fuel

Reduce. Reuse. Recycle. We hear this mantra time and again. When it comes to carbon, the "Most Wanted" element in terms of climate change, nature has got reuse and recycle covered. However, it's up to us to reduce. Scientists at Harvard Medical School (HMS) are trying to meet this challenge by learning more about the carbon cycle, that is, the process by which carbon moves from the atmosphere into plants, oceans, soils, the earth's crust, and back into the atmosphere again. One of the biggest movers and shakers is the lowly cyanobacteria, an ocean-dwelling, one-celled organism. Pamela Silver, HMS Professor of systems biology, and colleagues have uncovered details about how this bacteria fixes or digests carbon. These bacteria build miniature factories inside themselves that turn carbon into fuel. Silver and her colleagues report that the bacteria organize these factories spatially, revealing a structural sophistication not often seen in single-celled organisms. This regular and predictable spacing improves the efficiency of carbon processing. In the future, an understanding of the mechanisms that govern this spatial organization may help improve the efficiency of designer bacteria engineered to produce carbon-neutral fuels such as biodiesel and hydrogen. These findings will be published online in the journal Science.

The rod-shaped *cyanobacteria* are among the most abundant organisms on earth. Forty percent of the carbon in the carbon cycle is reused and recycled through these tiny creatures. To process carbon, *cyanobacteria* build soccer-ball-shaped structures inside themselves called carboxysomes. These tiny factories absorb carbon dioxide and convert it into sugar, which the bacteria then use to produce energy. The ocean is just packed with these bacteria. By studying them, we're understanding more about how the earth works, said Silver, who is also on the faculty of the Wyss Institute for Biologically Inspired Engineering at HMS. “I'm blown away by what's happening in the ocean and what we don't understand about it. There are a lot of things in the ocean that are going to be useful to us”. The research team, led by co-first authors, research fellows David Savage and Bruno Afonso, attached a fluorescent tag to proteins involved in building the carboxysome, then grew the tagged bacteria under a microscope. The resulting images revealed that, instead of being randomly numbered and haphazardly placed, *cyanobacteria* build carboxysomes in numbers that scale with their size, and they space the factories evenly along their length.

The finding adds evidence for new ways to think about bacteria. “We had this idea of bacteria as a bag of enzymes, but that has been completely shattered,” said Afonso. A single protein, called parA, acts as a kind of inner-bacterium stage manager, arranging the carboxysomes in a neat, single-file row, the researchers found. When they disabled the bacteria's ability to make the protein, the carboxysomes were distributed far more randomly.



Fluorescent labeling of proteins inside the carboxysome show that C*yanobacteria* create carboxysomes in numbers proportional to length and space them evenly along their longest axis.

(**Image Credit:** Harvard Medical School)

The *cyanobacteria* lacking parA were also less “fit” for survival, said Savage. While wild-type bacteria cells have a consistent number of carboyxsomes, which in turn optimizes carbon processing and fitness, the knockout bacterium created daughter cells whose numbers of carboxysomes ranged from none to an excess. The daughter cells with few or no carboxysomes divide more slowly and also process fifty percent less carbon than daughter cells at the other end of the spectrum. By tagging parA in wild-type bacteria, they discovered interesting dynamics in the protein. Thousands of parA proteins repeatedly cluster together and shoot quickly from one end of the bacterium to the other. It's amazing that you can

generate this regularity and symmetry potentially from a single protein, said Savage. It's amazing that it is somehow tuned by the dynamics of the protein. The researchers have not yet identified the exact mechanism parA uses to govern the spacing.

Many other bacteria also have the parA protein, which is known for separating chromosomes during cell division. "This work highlights how bacteria cobble together spare parts to achieve similar goals such as organization and segregation," said David Rudner, HMS Assistant Professor of Microbiology and Molecular Genetics, who was not involved in the study. These findings may help synthetic biologists one day create designer bacteria. Knowledge about how cells create and deploy specialized factories like the carboxysome opens the way to creating other kinds of mini factories that could perform useful functions, said Richard Losick, Harvard University Professor of Molecular and Cellular biology, who was not involved in the study. Silver's lab is looking into whether the carboxysome might be useful for optimizing the production of hydrogen by engineered bacteria. One challenge in designing hydrogen-producing bacteria is that the enzymes that produce hydrogen are sensitive to oxygen. The carboxysome may help solve this problem because its outer shell blocks out oxygen, protecting the enzymes inside from its toxic effects.

Source: www.sciencedaily.com

NEWS

Bio-pesticides to enrich the soil and enhance crop productivity

Efforts of the budding scientists and researchers of India, in the field of microbiology are set to help in serving mankind in the near future. The formulation of bio-pesticides, which is in the form of a microbial product, has been developed by a senior scientist at the Chatrapati Sahu Ji Maharaj (CSJM) university. This formulation, when put in the field, would not only enrich the soil but also enhance the crop productivity. Reader and Head of the Institute of Bio-Sciences and Bio Technology CSJM University Naveen Arora, who has been working on the formulation of the bio-pesticides since the past four years, has claimed that his work has yielded successful results. Presently, the research is in its advanced stage and requires implementation on ground, after a first round of successful trials, will soon be patented.

It is to be mentioned that pesticides sprayed on the crops, make it poisonous for human and animal consumption. Once such crops are consumed, there are high chances for human beings falling prey to diseases like cancer, early aging, nervous disorders, liver and kidney failure and others. They also ruin the soil content and quality. All the factors led to the need for biological alternatives and serious efforts in this direction are going on.

Arora while talking to Times of India said "The existing bio-products (pesticides) used widely in the world are not very successful as they do not behave uniformly everywhere due to the differences in the soil and environmental conditions. We are using a bio-formulation which is in the form of a microbial product." Explaining about the new microbial product, he said it will work on any type of soil in the world and that too at diverse climatic conditions. "We are now using it on a wide variety of crops including plants like sunflower, groundnuts to measure its strength. And so far the results have been wonderful," he added excitedly. The field trials clearly indicate that the pesticides (bio-products) are multi-faceted organisms (extracted from the soil for developing biological alternatives for eco-friendly agriculture). Arora said "Due to the indiscriminate use of chemicals like pesticides, we are losing these important bacteria". He then stated the microbes as our national heritage and apprised that they are being conserved at the national and international collection centre and should be protected and saved from extinction.

Source: Times of India, dated January 08, 2010.

Discovery of millions of new microbes opens 'huge frontier'

Discovery of millions of new microbes has opened a 'huge frontier' in science. Scientists have discovered millions of tiny microbes, hitherto unknown to science, at the bottom of the sea. These organisms include microbes of bacteria, worms and ocean insects less than 1mm long. Scientists made the path - breaking discovery using technology such as DNA sequencing, that allows researchers to differentiate between different species, and submarines that can be operated thousands of feet under the sea. A survey was conducted as part of a 10 year international project to find out more about the oceans, the Census of marine life. For one study, ocean samples were gathered from over 1,200 sites around the world to find out

more about microbial life. It discovered microbes with 18 million different DNA sequences, suggesting the presence of millions of yet unknown species. Another project found 7,000 new genera of bacteria in the Western English Channel alone. Nearly 3,000 types of bacteria were found in a sponge from Australia's Great Barrier Reef. The findings of the survey have led scientists to believe that there could be a billion microbial cells in every litre of seawater. A separate study of holozooplankton, that look like tiny transparent insects, increased the number of known species to 14,000 from 7,000. A study into roundworms found 500,0000 in a single square metre of ocean floor, while currently there are 16,000 known species of seaworms. John Baross of the University of Washington, a contributor to the census, said the findings would open up new doors in science that could help understand changes in the food chain, weather patterns and carbon cycles. Marine animals alone may account for hundreds of millions of microbial species. This is a huge frontier for next decade.

Source: The Hindu, dated April 22, 2010.

Abstracts

001. Dean O. Cliver. University of California, VM: PHR, One Shields Avenue, Davis, CA 95616 USA Dean O. Cliver, Phone: +1-530-7549120, docliver@ucdavis.edu. **Early Days of Food and Environmental Virology.** Food Environ Virol, Vol. 2(1) 2010, 1–23.

In July 1962, the author joined the Food Research Institute (FRI), then at the University of Chicago, to become its food virologist. There was a limited record of water - borne viral disease outbreaks at the time; recorded data on food - borne outbreaks were fewer still. Laboratory environmental (water and wastewater) virology was in its infancy, and food virology was in gestation. Detection of viruses was most often attempted by inoculation of primary primate cell cultures, with observation for plaque formation or cytopathic effects. Focus was initially on enteroviruses and reoviruses. Environmental and food samples had to be liquefied if not already in liquid form; clarified to remove solids, bacteria, and fungi; and concentrated to a volume that could be tested in cell culture. Cytotoxicity was also a concern. Studies at the FRI and some other laboratories addressed all of these challenges. The FRI group was the World Health Organization's Collaborating Center for Food Virology for many years. Other topics studied were virus inactivation as functions of temperature, time, matrix, disinfectants, and microbial action; peroral and ex-vivo infectivity; and the suitability of various virus surrogates for environmental monitoring and inactivation experiments. Detection of noroviruses and hepatitis A virus required molecular methods, most often RT-PCR. When it was found that inactivated virus often gave the same RT-PCR signal as that of infectious virus, sample treatments were sought, which would prevent false-positive test results. Many laboratories around the world have taken up food and environmental virology since 1962, with the result that a dedicated journal has been launched.

002. Li F, Wang S, Liu W, Chen G. State Key Laboratory of Microbial Technology, University of Shandong, Jinan 250100. **Progress on biodegradation of polylactic acid - a review.** Wei Sheng Wu Xue Bao. [Article in Chinese], 48(2), 2008, 262-268.

Polylactic acid is high molecular-weight polyester made from renewable resources such as corn or starch. It is a promising biodegradable plastic due to its mechanical properties, biocompatibility and biodegradability. To achieve natural recycling of polylactic acid, relative microorganisms and the underlying mechanisms in the biodegradation has become an important issue in biodegradable materials. As on date, most isolated microbes capable of degrading polylactic acid belong to actinomycetes. Proteases secreted by these microorganisms are responsible for the degradation. However, subtle differences exist between these polylactic acid degrading enzymes and typical proteases with respect to substrate binding and catalysis. Amino acids relative to catalysis are postulated to be highly plastic allowing their catalytic hydrolysis of polylactic acid. In this paper we reviewed current studies on biodegradation of polylactic acid concerning its microbial, enzymatic reactions and the possible mechanisms. We also discussed the probability of biologically recycling PLA by applying highly efficient strains and enzymes.

E - Resources on Microorganisms

NATIONAL

The Integrated Taxonomic Information System
www.itis.gov

Institute of Microbial Technology
www.imtech.res.in

Institute of Human Genetics
www.geneticcentre.org

Sugarcane Breeding Institute (SBI)
www.sugarcane-breeding.tn.nic.in/organisation.htm

National Centre for Biological Sciences
www.ncbs.res.in

INTERNATIONAL

Bioremediation - Environmental Inquiry
www.ei.cornell.edu/biodeg/bioremed

Microbial Ecology Group - University of Vienna
www.microbial-ecology.net

The Center for Microbial Ecology
www.cme.msu.edu

The Culture Collection of Algae at University of Texas (UTEX)
www.web.biosci.utexas.edu/utex

Microbial Genome Databases
www.microbialgenomics.energy.gov/databases.shtml

EVENTS
Conferences / Seminars / Meetings 2010

Viruses of Microbes, June 21 - 25 2010. **Venue:** Paris, **France**. **Website:** www.pasteur.fr/infosci/conf/sb/virusmicrobes.

Marine Microbes - From Genes to Global Cycles, July 4 - 9, 2010. **Venue:** Tilton School **Tilton**, NH. **Website:** www.grc.org.

Waste Management 2010, Fifth International Conference on Waste Management and the Environment, July 12 - 14 2010. **Venue:** Tallinn, **Estonia**. **Website:** www.wessex.ac.uk/10-conferences/waste-management-2010/page-7.html.

9th International Mycological Congress (IMC9: The Biology of Fungi), 1 - 6 Aug, 2010. **Venue:** Usher Hall and Edinburgh International Conference Centre, Edingburgh, **United Kingdom**. **Website:** http://www.imc9.info.

Microbial Biofuels Symposium, Sep 1 - 3, 2010. **Venue:** Edinburgh Napier University, Edinburgh, **United Kingdom**. email: m.tangney@napier.ac.uk.

Bacteria with a built-in Thermometer

Researchers demonstrate for the first time that bacteria of the *Yersinia* genus possess a unique protein thermometer – the protein RovA. Depending on the environment of the bacteria, this protein reads the temperature for it. RovA is a multi-functional sensor which measures both the temperature of its host as well as the host's metabolic activity and nutrients.

Source: www.sciencedaily.com

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