

ENVIS Newsletter
on
Microorganisms and Environment Management

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EVENTS



Dear Readers,

Greetings!

Hydrosphere, the aquatic environment of the earth, supports numerous living things. Microbes are minute organisms that occur in all environments and are therefore omnipresent. Microorganisms in the aquatic environment affect man and other organisms either positively or negatively, directly or indirectly. In other words, these minute living things may be beneficial or harmful to man, his activities as well as other natural resources. For purposes of completeness, it is noted that the presence of some microorganisms in water may sometimes have no apparent or visible effect, being neutral to the ecosystem.

The newly evolved aquaculture system, called “Biofloc Technology (BFT)” is considered as an environmental friendly and efficient alternative system for aqua farming. Bioflocs are the mixer of detritus with associated microorganisms such as bacteria, algae, protozoa, rotifers, copepods, nematodes etc. This technology facilitates recycling of nutrients and converts nitrogenous waste into protein, thereby reduce water pollution, with the involvement of these microorganisms. The proteins produced in bioflocs by bacteria are called single cell proteins, similar to proteins originating from yeast and algae. Bioflocs are able to assimilate waste nutrients (or pollutants) from the water column, cleaning the water and producing new proteins.

In this issue, the importance of Biofloc Technology is highlighted, which help the students, researchers and industry to understand the basic aspects of such eco-friendly technology, aiming to encourage for further research. In addition, some interesting informations on fossil fuel, synthetic chromosome, role of microbes in chemical energy are also included.

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Prof. N. Munuswamy

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Microbial soup-Eco based approach for shrimp culture and management**A. Panigrahi*, M. Sundaram,
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email: apanigrahi2k@gmail.com; panigrahi@ciba.res.in**Abstract**

The concept of 'microbial soup' eventually led to development of "biofloc" technology in changing the facet of intensive aquaculture with scope to attain high productivity through a sustainable approach. By developing dense heterotrophic bacterial community, the system becomes bacterial dominated rather than algae dominated and takes care of the waste generated in the aquaculture system through *in-situ* bioremediation. Biofloc technology approach promises a healthy rearing system, which is increasingly identified as one possible solution for disease problems. Protein is utilized two times once as feed for the shrimp and second time as microbial floc when the heterotrophic microbes converts the nitrogenous waste into protein.

Central Institute of Brackishwater Aquaculture has successfully demonstrated the biofloc and periphyton based nursery and grow out technology for Pacific white shrimp *Litopenaeus vannamei*. This study deals with the evolution of the concept, principles and progress in research including dynamics of biofloc production through addition of different carbohydrate sources and inoculums *vis-a-vis* protein levels for better yield. Advantage in providing better growth performance, nutrition, the "natural probiotic" effect in BFT and protective response against pathogens with biofloc based rearing is elaborated.

Keywords: Microbial soup, Biofloc, Periphyton, Protein, BFT and, Eco-based approach, bacteria

Introduction

The concept of delivering high production with sustainability is gaining momentum in aquaculture practices and many technologies like Biofloc and Periphyton based farming system are being evolved nowadays. Biofloc

technology (BFT) is a promising technology which promotes the retention of waste and its conversion to biofloc as a natural food within the aquaculture system. Biofloc is the conglomeration of microorganisms (such as heterotrophic bacteria, algae (dinoflagellates & diatoms), fungi, ciliates, flagellates, rotifers, nematodes, metazoans & detritus). This is a relatively new biotechnological means to support high density, to maintain the water quality, reduce water exchange, maintaining biosecurity, reutilize the feed and reduce the production cost. In Asia, the shrimp-farming industry has been heavily affected by early mortality syndrome or, more descriptively, acute hepatopancreatic necrosis syndrome (AHPNS). Bio-floc technology approach promises a healthy rearing system and identified as one possible solution for these disease problems. Periphyton is a complex mixture of algae, cyanobacteria, heterotrophic microbes, and detritus attached to submerged surfaces in aquatic ecosystems. BFT in combination with Periphyton increases the natural production and in turn productivity.

Genesis and evolution

Going back to the genesis of this concept, Steve Surfing in 1976, put forward the 'microbial soup' concept that eventually led to development of "biofloc" based aquafarming. By developing dense heterotrophic bacterial community, this system becomes bacterial dominated. Accumulation of these bacteria, called soup or flocs, engulf up the nitrogenous wastes ten to hundred times more efficiently than algae, irrespective of the weather, and turn them into high-protein feed. The cell wall of the microbial constituent of this microbial soup (biofloc), such as bacterial lipopolysaccharide, peptidoglycan and β -1, 3-glucans, stimulate nonspecific immune activity of fish/shrimp (Panigrahi *et al.*, 2007).

BFT was first developed in early 1970s at Ifremer-COP (French Research Institute for Exploitation of the Sea, Oceanic Center of Pacific) with different penaeid species including *Penaeus monodon*, *Fenneropenaeus merguensis*, *Litopenaeus vannamei*. Later, Prof. Yoram from Israel, contributed immensely for the growth of this promising technology. Biofloc technology has recently gained attention as a sustainable method to maintain water quality with the added value of producing proteinaceous feed *in-situ* (Crab *et al.*, 2012). In addition to organic nitrogenous waste, ammonium

will be converted into bacterial biomass if C:N ratio is balanced at a ratio of 10-15:1 (Schneider *et al.*, 2005). The growth rate and microbial biomass yield per unit substrate of heterotrophs are higher than that of nitrifying bacteria, thus making many fold increase in heterotrophic bacteria (Hargreaves, 2006).

C: N Ratio and biofloc manipulation

The biofloc principle combines the removal of nutrients from the water with the production of microbial biomass, which can be used by the culture species, *in-situ* as additional food source. Application of immobile substrate and manipulation of carbon nitrogen ratio have drawn attention for their role in enhancement of aquaculture production through improved nutrients utilization and control of toxic nitrogen metabolites. Manipulation of C:N ratio of the feed affects the quantity and quality of periphyton production over the substrate. The optimum C:N ratio in an aquaculture system can be maintained (C:N ratio 12-15:1 is optimal for biofloc production) by adding different locally available cheap carbon sources and/or reducing protein percentage in feed. Under optimum C:N ratio, inorganic nitrogen is immobilized into bacterial cell, while organic substrates are metabolized. This technique was initially developed in Israel and subsequently spread to many other countries due to its several advantages. However, this is still in an initial stage and lot of research is necessary for its modification, standardization and implementation.

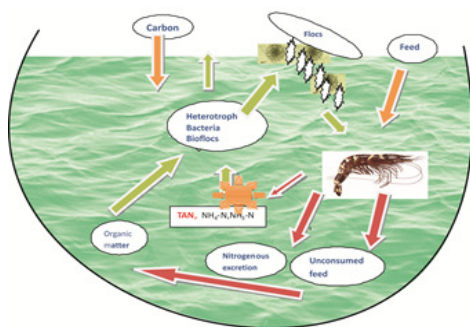


Fig 1: Schematic diagram showing the concept of biofloc in a grow – out system

Biofloc research in India

Central Institute of Brackishwater Aquaculture (CIBA), Chennai has initiated research for standardizing nursery and grow-out culture methods for *L. vannamei* on periphyton and biofloc based technology. A series of experiments on pilot scale was conducted at CIBA to standardise the bio-floc system and optimise the carbohydrate,

C:N ratio and inoculums, nutritional characterisation of different types of biofloc and protein requirement for maximum gain of their inclusion in feeds. There was substantial gain in the production as well as FCR in tiger shrimp *P. monodon* farming by following these eco based techniques (Anand *et al.*, 2013). In an outdoor growth trial, carbon nitrogen ratio (C:N ratio 10 and 20) was manipulated with shrimp feed (containing 32% crude protein and rice flour as carbohydrate source) resulted in 42% increase in final body weight. Ray *et al.* (2010) described this as a complex interaction between organic matter, physical substrate and large range of microorganisms. Bacteria and other microorganisms act as very efficient “biochemical systems” degrader and metabolize organic residues (Avnimelech,1999).

Biofloc constituents and characterization

The constituents of biofloc were characterised and found to be comprised of 70-80% of microorganisms such as phytoplankton, free and attached bacteria, aggregates of particulate organic matter and grazers, such as rotifers, ciliates and flagellates protozoa and copepods. Besides the biofloc volume and the physicochemical parameters, the level of protein, poly-β-hydroxybutyrate and fatty acids can be used to characterize microbial flocs.

CHO source and reduction in TAN and Nitrite

Carbohydrate (CHO) sources like molasses, wheat, tapioca or some other inexpensive carbon sources were added to make carbon/nitrogen ratio of 12:1, or higher, to promote heterotrophic bacteria (HB) as these bacteria use organic carbon as energy source and uptake of nitrogen to grow through the production of microbial proteins (Avnimelech, 1999). Simple sugars like sucrose and molasses found to induce the growth of the floc faster, however, it require frequent additions. In contrast, complex starches like corn, cassava, tapioca, wheat and cellulose most stable but slow to

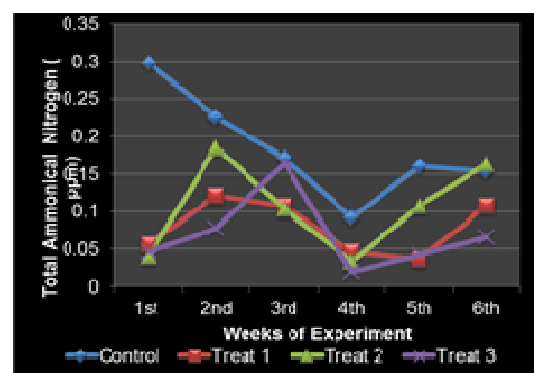


Fig 2: Reduced level of TAN in Biofloc treatments

react, can also act as bacterial substrates and contain suites of enzymes which facilitate digestion once ingested by shrimp. The impact of the carbon source type on biofloc characteristics and shrimp production economics is being investigated.

Biofloc technology for improving production efficiency in aquaculture

CIBA have successfully demonstrated the biofloc and periphyton based nursery technology for shrimp culture in tank system, where a very high survival of 98-99% was achieved compared to the conventional system (91-92%). Interestingly, it is found to reduce the risk of early mortality, due to any disease, at nursery phase, and ensuring better survival during grow-out phase.

Recently in another demonstration trial, a production up to 4 to 4.5 kg/ cu m (40-45 tonnes/ha) was achieved at the institute facility using different protein levels in feed through biofloc based farming system. The results also revealed a very high growth rate in the biofloc system compared to that of the conventional system and a very high standing crop could be maintained without any risk.

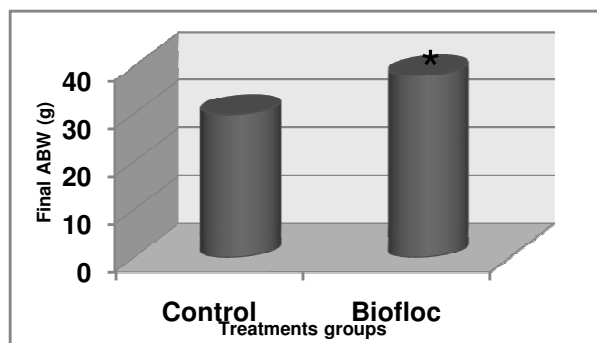


Fig 3: Gain in average body weight in the bio-floc group of shrimp during a grow-out experiment.

In our study, it was found that the protein content in pelletized feed can be reduced substantially without compromising growth and survival. It was observed that shrimp fed with minimum crude protein under biofloc conditions performed better, especially at higher density, compared to those fed with 40% protein under regular autotrophic intensive culture. This would help to reduce feed cost. It is important to reduce the protein content to achieve good C:N ratio and reduction of N-waste formation. At lower protein content, heterotrophic removal of total ammonium nitrogen (TAN) begins to dominate over autotrophic and the growth and survival was found to be better than higher protein feed in commercial closed pond systems due to enhanced nutrition on flocs and reduced N-wastes (Ballester *et al.*, 2010; Nunes *et al.*, 2010).

Bioflocs as a biocontrol measure, Immunomodulation and protective response

The “natural probiotic” effect in bio-floc could act internally and/or externally against, *Vibrio* sp. and ectoparasites. The regular addition of carbon in the water is known to select for polyhydroxy alkanooates (PHA) accumulating bacteria which produce several biodegradable polymer storage products, like poly-β-hydroxy butyrate (PHB), having antibacterial or biocontrol properties which provide immunity to the host (Sinha *et al.*, 2008). The number of total bacteria in biofloc group was significantly higher (10^6 to 10^8 cfu mL⁻¹) than in the conventional system. The cellwall components of these beneficial microbes have potential immunomodulatory properties. More than 50% of the bacteria in these systems were free living and the reminder was associated with detritus in the form of flocculated matter (Burford *et al.*, 2004). Our studies suggest that microbes associated with bioflocs may enhance expression of certain immune-related genes. Studies in the similar line also revealed an upregulation of immune genes like prophenoloxidase, prophenoloxidase activation enzyme and serine proteinase1 genes with exposure to biofloc thus implying immunomodulation in the shrimp. Probiotic interventions of probionts *L. rhamnossus* and *B. subtilis* were found to be advantageous in terms of better growth and survival rate (Panigrahi and Azad, 2007). Again, the expression of certain immune related genes were significantly up-regulated explaining the possible immunomodulations and in turn better protectionin to fish (Panigrahi *et al.*, 2007; 2011) which opens the scope of probiotics driven biofloc interventions.

Microbial biomass application as feed for aquaculture species

The macro aggregates (biofloc), in aquatic medium, is proteinlipid rich natural source available “*in-situ*” throughout the day (Avnimelech, 2007). It is possible to replace 1/3 of a conventional diet by low-protein biofloc meal without interfering survival and performance of the shrimp. Harvested bioflocs have been biochemically characterised as premium quality feed ingredients. This can be used instead of fishmeal in shrimp diets (Crab *et al.*, 2007) and is also cost effective (Kuhn *et al.*, 2009). This could also be incorporated into broodstock diet. Growth enhancement has been attributed to both bacterial and algal nutritional components, which is up to 30% of

conventional feeding ratio and can be lowered due to biofloc consumption in shrimp. Burford *et al.*, (2004) reported that more than 29% of daily food consumed by *L. vannamei* could be of biofloc. Shrimps could also be grown on a diet without any of the marine protein sources in a biofloc based rearing system without compromising growth.



Fig 4: (A-F) Biofloc technology commercial-scale at CIBA – harvest festival (A, B), harvested shrimp (C), pilot scale tanks (D), biofloc measuring cones (E) and commercial scale Hitide Sea farm (F).

Future Research

Immunomodulatory properties of the bioflocs for the host, molecular characterization of biofloc constituents, poly- β -hydroxybutyrate like constituents which influences the protective response, the potential antagonistic properties, and efficacy of bioflocs to influence viral and bacterial load are the other areas which need further investigation. Mechanism to control the unregulated growth of bio-floc in the existing pond systems and evaluating biofloc and/or periphyton based system for advantage also needs research attention.

Further fine-tuning and implementation of this technology will also attract more focused research and development. Researchers are challenged to refine this technique to farmers friendly to implement in their future aquaculture systems. Many research issues can be fashioned to optimise microbial floc production, maintaining the water quality, reducing the water exchange, risk of disease and reutilizing the nitrogenous metabolites and understanding its working mechanism with altering factors such as C:N ratio, temperature, dynamics of TAN and other metabolites.

Conclusion

Biofloc technology will enable aquaculture practices towards an environmental friendly approach. This technology as revealed through our studies brings an obvious advantage of minimizing water requirement, recycling *in-situ* nutrients and organic matter and in turn improving farm biosecurity by exclusion of pathogens, augmentation of natural food and improvement of FCR, zero/minimal water exchange system and *in situ* bioremediation to maintain water quality, protein requirements in feed could be brought down in these systems, providing stress-free environment, use of chemicals and other medicines are not required and negligible environmental impact. This ‘microbial soup’ based technology BFT have potential to revolutionise the aquaculture sector.

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RESEARCH REPORTS

New, fossil-fuel-free process makes biodiesel sustainable

A new fuel-cell concept, developed by an Michigan State University researcher, will allow biodiesel plants to eliminate the creation of hazardous wastes while removing their dependence on fossil fuel from their production process.

The platform, which uses microbes to glean ethanol from

glycerol and has the added benefit of cleaning up the wastewater, will allow producers to reincorporate the ethanol and the water into the fuel-making process, said Gemma Reguera, MSU microbiologist and one of the co-authors.

"With a saturated glycerol market, traditional approaches see producers pay hefty fees to have toxic wastewater hauled off to treatment plants," she said. "By cleaning the water with microbes on-site, we've come up with a way to allow producers to generate bioethanol, which replaces petrochemical methanol. At the same time, they are taking care of their hazardous waste problem."

The results, which appear in the journal *Environmental Science and Technology*, show that the key to Reguera's platform is her patented adaptive-engineered bacteria *Geobacter sulfurreducens*.

Geobacter are naturally occurring microbes that have proved promising in cleaning up nuclear waste as well in improving other biofuel processes. Much of Reguera's research with these bacteria focuses on engineering their conductive pili or nanowires. These hair-like appendages are the managers of electrical activity during a cleanup and biofuel production.

First, Reguera, along with lead authors and MSU graduate students Allison Speers and Jenna Young, evolved *Geobacter* to withstand increasing amounts of toxic glycerol. The next step, the team searched for partner bacteria that could ferment it into ethanol while generating byproducts that 'fed' the *Geobacter*.

"It took some tweaking, but we eventually developed a robust bacterium to pair with *Geobacter*," Reguera said. "We matched them up like dance partners, modifying each of

KNOW A SCIENTIST

Dr. Barry J. Marshall was born in Kalgoorlie, Australia, on September 30, 1951. While training in medicine, he joined pathologist Dr. J.



Robin Warren to investigate a stomach bacteria. They soon discovered that many gastritis and stomach ulcer patients had the spiral bacteria, which would eventually be named *Helicobacter pylori*.

Postulating that *H. pylori* was disease-causing, Marshall realized that **antibiotics could replace the current treatments for ulcers**. For this experiment, he experimented himself as a human subject at the age of 32. Marshall and Warren shared the **Nobel Prize in Physiology or Medicine** for their work in 2005.

them to work seamlessly together and eliminate all of the waste."

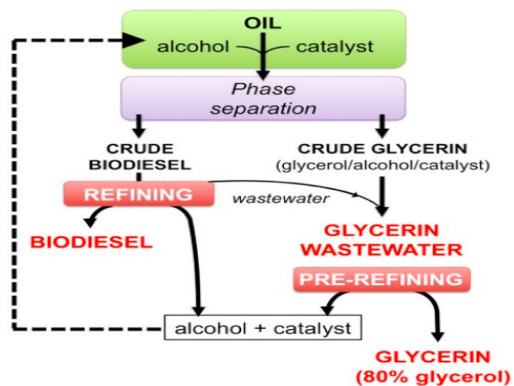
Together, the bacteria's appetite for the toxic byproducts is inexhaustible.

"They feast like they're at a Las Vegas buffet," she added. "One bacterium ferments the glycerol waste to produce bioethanol, which can be reused to make biodiesel from oil feedstocks. *Geobacter* removes any waste produced during glycerol fermentation to generate electricity. It is a win-win situation."

The hungry microbes are the featured component of Reguera's microbial electrolysis cells, or MECs. These fuel cells do not harvest electricity as an output. Rather, they use a small electrical input platform to generate hydrogen and increase the MEC's efficiency even more.

The promising process already has caught the eye of economic developers, who are helping scale up the effort. Through a Michigan Translational Research and Commercialization grant, Reguera and her team are developing prototypes that can handle larger volumes of waste.

Reguera also is in talks with MBI, the bio-based technology "de-risking" enterprise operated by the MSU Foundation, to develop industrial-sized units that could handle the capacities of a full-scale biodiesel plant. The next step will be field tests with a Michigan-based biodiesel manufacturer.



MSU is working to eliminate biodiesel producers' hazardous wastes and dependence on fossil fuels.

Image Credit: Courtesy of Gemma Reguera

Source: www.sciencedaily.com

How bacteria in the placenta could help shape human health

Even before a baby is born a microbial ecosystem takes up residence in the placenta, creating a microbiome that may help shape the newborn's immune system and perhaps exert influence over premature births. The revelation, based on the genetic profile of hundreds of placentas, provides the most definitive answer to date that the life-sustaining organ, which nourishes the

fetus and helps remove waste, is far from sterile.

Although the composition of human microbiota has become increasingly clear with genetic-sequencing technology, little is known about what shapes humans' early microbial communities and exactly when an infant is first exposed to and colonized by those microorganisms.

A new catalogue of placental critters is thrusting these microbes into the spotlight, fueling questions about how they may help pull the strings for human health before birth and where a mother's own microorganisms fit into the equation. Led by Kjersti Aagaard, Professor of Obstetrics and Gynecology at Baylor College of Medicine and Texas Children's Hospital, and published in *Science Translational Medicine*, her team's work analyzes the genetic sequences of hundreds of placental microbiomes from preterm and term infants and compares them with an existing inventory of microbiota from nonpregnant women's skin, airways, vaginas, guts and mouths.

The work provides initial insights into what microorganisms are present in placenta and where they come from. And the placental microbiome's apparent similarities to the smorgasbord of oral microorganisms also reignites the debate about links between the role of mother's gum disease and preterm births. For the placenta, this new work maps out "which organisms are present, what they are capable of doing and how the placental community is likely structured," the authors wrote. (This work does not, however, directly compare an infant's microbiota with that of the mother.)

Earlier studies already detailed the composition of the amniotic sac, the guts of newborns in the first week of life and infants' first stools, providing hints that microbiota may be at work before birth. Still, the placental microbiome including its existence remained relatively mysterious until the past couple years. "The beauty of this study is that they don't just do gram-negative stains of the placenta. They look at the types of microbes that are present by looking at their DNA signatures, which is novel," says Josef Neu, neonatologist at University of Florida, neonatologist whose earlier work found that even before babies have their first meals their stools host a diverse array of microorganisms, suggesting microbes are present and play a role before the baby is born. Neu was not an author on this new study.

Aagaard's work finds that placental samples of 320 infants actually most closely resembled the milieu of microbes found in the human mouth not that of the human gut, stool or vagina. So how would those microbes get there? The authors hypothesize that the microbiota from a mother-to-be's mouth travels by blood into the placenta, upending an earlier theory that suggests microbes in the placenta and amniotic cavity originate in the vagina. "There are lots of clues to biology and development to be unfolded from little nooks and crannies that we haven't looked at yet, and the placenta certainly has many of those," Aagaard says. "The biology of pregnancy is certainly important if we are going to understand the health of the next generation," she adds.

Although prior work has suggested a mother's periodontal disease may lead to health problems in infants, efforts to treat pregnant women's gum disease has not reduced premature births. Aagaard says her new research helps elucidate why such interventions did not work. "Even in the first or second trimester [the microbiome] is already well established," she says. When it comes to dental care for women before pregnancy, "You would really want to be thinking about primary prevention, not secondary treatment," she says, noting that oral health should be part of standard treatment for women before pregnancy.

In addition to drawing links to maternal oral microbes, this study also catalogued complex ecosystem differences between the placental microbiome of infants born slightly prematurely around 34 to 37 weeks and infants born at term. (Preterm births around this time are more common than early births at, say, 27 weeks). "We don't know if [the differences] trigger preterm births but we do know they are associated with, and are a good predictor of preterm birth," Aagaard says.

Differences included a higher prevalence of microbes that typically keep yeast in check among the preterm infants. Across the board, the work found that the single most prevalent microbe in the placentas was *Escherichia coli* a bacterium strongly associated with sepsis in premature infants. "The metagenomic survey alone can describe the microbial communities present, but clinical context and advanced inference analyses will be required to understand the factors that affect community composition," the authors noted.

Urinary tract infections early in pregnancy, too, may alter the placental microbiome, the study suggests. It found a difference in microbiome composition among women who had those infections versus those who did not but the study did not

answer if the infection itself or the antibiotics used to treat it may have caused the variance. Moreover, it remains unclear how that microbiome change may have impacted fetal health or preterm birth.

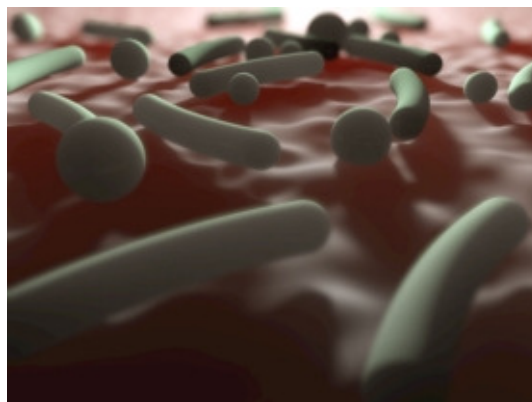


Image Credit: V. Altounian/*Science Translational Medicine*.

Another interesting finding is that regardless of whether infants were born via cesarean section or descended through the birth canal (and passed through the gauntlet of accompanying bacteria), the composition of the placental microbiome appeared to be roughly the same in this analysis,

Microbes Living in Deep Ocean Absorb Carbon Using Chemical Energy

They may not absorb enough carbon to curtail global warming, but the microbes that call the deep ocean home absorb enough of it to merit further study, according to a paper published in the *International Society of Microbial Ecology (ISME) Journal*.

Lead author of the paper Tim Mattes, at the University Iowa, explained that while most are familiar with the role of trees and grass in absorbing carbon from the air, organisms such as bacteria living in the dark recesses of the ocean hold between 300 million and 1.3 billion tons of carbon.

"A significant amount of carbon fixation occurs in the dark ocean," Mattes said. "What might make this surprising is that carbon fixation is typically linked to organisms using sunlight as the energy source." He continues, "In the dark ocean, carbon fixation can occur with reduced chemical energy sources such as sulfur, methane, and ferrous iron. The hotspots are hydrothermal vents that generate plumes rich in chemical energy sources that stimulate the growth of microorganisms forming the foundation for deep sea ecosystems."

Along with a team of researchers, Mattes explored the hydrothermal vents located in a volcanic

Using protein-based techniques, the researchers discovered sulfur-oxidizing microorganisms that were also converting carbon dioxide into biomass. Despite this discovery, according to Mattes "there is no evidence to suggest that they could play any role in mitigating global warming."

Rather, the primary value of the investigation, he says, is that of increasing understanding of how microorganisms living in the dark ocean function in addition to bettering the fundamental knowledge of global biogeochemical cycles.



While most people are familiar with microbes that occur above ground — such as this orange-colored colony surrounding Grand Prismatic Spring at Yellowstone National Park — microbes also occur around hydrothermal vents on the ocean floor, at depths where light cannot penetrate. There, at the ocean's floor, they trap carbon using chemical energy instead of sunlight. (Photo : Jim Peaco, National Park Service, via Wikimedia Commons)

Source: www.natureworldnews.com

indicating mode of delivery, at least for the placenta, does not alter its microbial community. The finding does not answer how that birth method impacts an actual infant, because a baby would typically swallow amniotic fluid and microbes along the birth canal during delivery.

With the new profile of the placental microbiome, “we do not know what is the cause and effect here. That will take a much longer ongoing longitudinal samples to answer,” Aagaard says. Next up, her team plans to delve further into answering questions including how cesarean section versus natural birth influences infants’ microbiomes.

Source: www.scientificamerican.com

ONLINE REPORTS ON MICROORGANISMS

First synthetic yeast chromosome revealed

It took geneticist Craig Venter 15 years and US\$40 million to synthesize the genome of a bacterial parasite. Today, an academic team made up mostly of undergraduate students reports the next leap in synthetic life: the redesign and production of a fully functional chromosome from the baker’s yeast *Saccharomyces cerevisiae*.

As a eukaryote, a category that includes humans and other animals, *S. cerevisiae* has a more complex genome than Venter's parasite. The synthetic yeast chromosome which has been stripped of some DNA sequences and other elements is 272,871 base pairs long, representing about 2.5% of the 12-million-base-pair *S. cerevisiae* genome. The researchers, who report their accomplishment in *Science*, have formed an international consortium to create a synthetic version of the full *S. cerevisiae* genome within 5 years.

“This is a pretty impressive demonstration of not just DNA synthesis, but redesign of an entire eukaryotic chromosome,” says Farren Isaacs, a bioengineer at Yale University in New Haven, Connecticut, who was not involved in the work. “You can see that they are systematically paving the way for a new era of biology based on the redesign of genomes.”

The project began a few years ago, when Jef Boeke, a yeast geneticist at New York University, set out to synthesize the baker’s yeast genome with much more drastic alterations than those demonstrated by Venter and his team in 2010.

The group at the J. Craig Venter Institute in Rockville, Maryland, had chemically synthesized short strands of

DNA and stitched them together to create a version of the 1.1-million-base-pair DNA genome of the bacterium *Mycoplasma mycoides*, which was then inserted into a recipient cell. Venter’s team wrote a few coded 'watermarks' into the genome sequence, which spelled out the names of the team members, as well as several famous quotes. But besides these tweaks and a few other changes, the synthetic *M. mycoides* genome was identical to its blueprint.

By contrast, Boeke and his team thought that by stripping the genome of certain features to test their importance, they could justify the enormous cost and effort of synthesizing whole yeast chromosomes.

“I wasn’t sceptical about whether it could be done,” Boeke says. The question, he explains, was: “How can we make this different from a normal chromosome and put something into it that’s really going to make it worthwhile?”

Source: www.nature.com

Lung microbes protect against asthma

Whether or not people develop asthma may be determined in the first few weeks after birth according to a study on mice funded by the Swiss National Science Foundation (SNSF). The study suggests that microbes in the lungs stimulate the newborn's immune system.

Our lungs were long considered to be germfree and sterile. It was only recently discovered that, like our intestines and skin, our respiratory organs are colonised by bacteria. Now, tests conducted on mice by researchers working with Benjamin Marsland at the University Hospital in Lausanne have shown that these lung microbes offer protection against allergic asthma.

The researchers exposed the mice to an extract obtained from house dust mites. Neonates had a much stronger allergic reaction to the extract than older mice. Why? The lungs in newborn mice have not yet been colonised by the microbes that alter the immune system and make its responses less prone to allergic reactions.

Two-week adaptation process

The researchers have discovered that the process of colonisation and adaptation takes place during the first two weeks of the mouse's life. Young mice which were kept

completely germ-free remained susceptible to asthma and had excessive immune responses to dust mite allergens even later in life. Marsland and his team have already started studying whether lung microbes ensure healthy airways in humans as well. Pilot studies involving newborn babies in Switzerland and New Zealand indicate that the situation may be similar for men and mice. Further studies are required, however, to identify the potential mechanisms in humans.

Focus on newborns

"There would appear to be a developmental window early in life that determines whether or not an individual will develop asthma later," Marsland says. Until now, scientists and doctors have focused on asthma essentially from the point of view of the course of the disease and possible direct triggers. "We should probably focus on a much earlier stage, that of newborns."

What Marsland wants to know now is how big the developmental window is for building up the immune system in childhood. He hopes that the new discovery will help prevent asthma. Perhaps by encouraging pregnant women to eat more fruits and vegetables quite recently Marsland showed that the dietary fibre contained in these foodstuffs also protects against allergic asthma by altering the microbial flora. That protection might be passed on to newborn babies.

Source: www.sciencedaily.com

NEWS

Marine bacteria are natural source of chemical fire retardants

Researchers at the University of California, San Diego School of Medicine have discovered a widely distributed group of marine bacteria that produce compounds nearly identical to toxic man-made fire retardants. Some marine bacteria produce potent persistent organic compounds that are nearly identical to flame retardant chemicals. Among the chemicals produced by the ocean-dwelling microbes, which have been found in habitats as diverse as sea grasses, marine sediments and corals, is a potent endocrine disruptor that mimics the human body's most active thyroid hormone. The study is published in the June 29 online issue of *Nature Chemical Biology*. "We find it very surprising and a tad alarming that flame retardant-like chemicals are biologically

synthesized by common bacteria in the marine environment," said senior author Bradley Moore, PhD, a professor at the UC San Diego Skaggs School of Pharmacy and Pharmaceutical Sciences and Scripps Institution of Oceanography.

The toxic compounds are known as polybrominated diphenyl ethers (PBDEs), a subgroup of brominated flame retardants that are combined into foam, textiles and electronics to raise the temperature at which the products will burn.

Certain formulations of PBDEs are no longer used in automobile and home products in the United States, but testing by the Environmental Protection Agency indicates that most Americans and Canadians carry traces of the chemicals. Indeed, levels exceed those of Europeans and others by a factor of ten or more. Californians, in particular, have higher than average "body burdens" of the compounds.

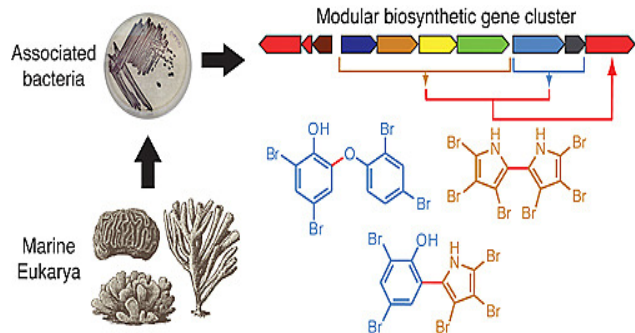
Although the presence, persistence and ability of PBDEs to accumulate in the fatty tissues of marine animals have long been recognized, researchers had previously believed the compounds were anthropogenic in origin and due to ocean pollution.

More recent examinations have shown a pervasiveness of PBDEs in prey and predatory species, suggesting a natural microbial source of the compounds as well as an anthropogenic one. This study is the first one to isolate and identify bacteria that synthesize these compounds and whose presence may help to explain the observed distribution pattern of PBDEs in the marine food chain.

In the study, the researchers identified a group of ten genes involved in the synthesis of more than 15 bromine-containing polyaromatic compounds, including some PBDEs. They have since conducted DNA sequencing analyses that will allow them to probe the ocean for other biological sources for these chemicals and to begin to assemble a complete picture of their human health risk.

"The next step is to look more broadly in the marine environment for the distribution of this gene signature and to document how these compounds are entering the food chain," said Vinayak Agarwal, PhD, a postdoctoral researcher with the Scripps Center for Oceans and Human Health at UC San Diego. Co-authors include Abraham El Gamal, Kazuya Yamanaka, Roland Kersten, Dennis Poth, Michelle Schorn, and Eric Allen, all at UCSD.

Funding for this study was provided, in part, by the National Science Foundation (grant OCE-1313747) and National Institute of Environmental Health Sciences (grant P01-ES021921) through its Oceans and Human Health program.



Some marine bacteria produce potent persistent organic compounds that are nearly identical to flame retardent chemicals.

(Source: www.ucsdnews.ucsd.edu, June 29, 2014.)

'Microbe sniffer' could point way to next-generation bio-refining

A new biosensor invented at the University of British Columbia could help optimize bio-refining processes that produce fuels, fine chemicals and advanced materials.

It works by sniffing out naturally occurring bacterial networks that are genetically wired to break down wood polymer.

"Nature has already invented microbial processes to degrade lignin--the tough polymer in wood and plant biomass that currently stymies industrial bio-refining," says UBC microbiologist Steven Hallam. "We needed to do the detective work, and develop the right toolkit, to isolate these processes in naturally occurring microbial communities from coal beds."

Developed by Hallam and his team, the biosensor screens DNA from environmental samples to isolate the lignin-busting genetic machinery encoded in the samples' resident microbes.

"We've found that bacteria harness adaptive genetic circuits to break down lignin and that these circuits can be mobilized in nature via horizontal gene transfer," says Hallam. "Our biosensor and screening enables us to uncover this genetic network, and then further optimize it in the laboratory."

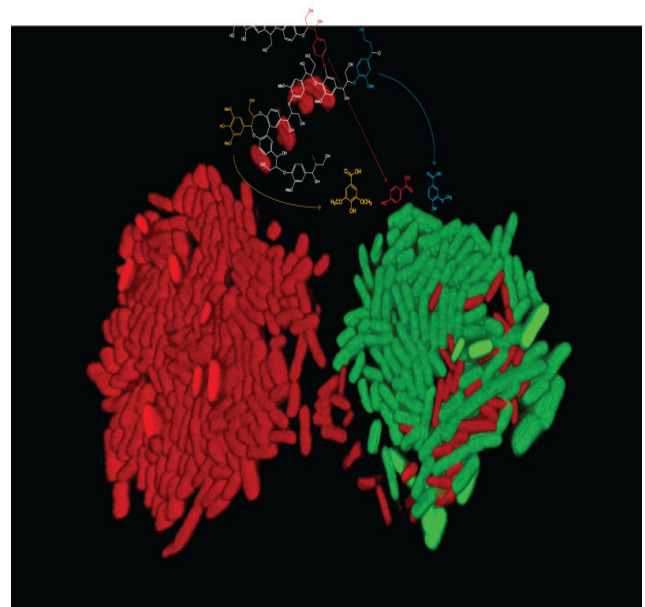
The improved understanding of adaptive, eco-engineered lignin transformation could also lead to more tunable industrial processes.

"We need to remain sensitive to the complexity of natural processes that act on lignin, but this project has unearthed some basic organizing principles that will also enable us to exploit microbial processes more quickly for any number of engineering applications," says UBC researcher Cameron Strachan. "It's a biological search function for biologists interested in harnessing naturally assembled genetic machinery."

The sensor, screening and adaptive genetic circuitry discovered with them have been licensed through the University Industry Liaison Office. A spin-off company, guided by the UBC program, is looking into ways to increase the scale of production of this technology.

Background

The findings validating the screening were published in the *Proceedings of the National Academy of Sciences*. This work was funded by Genome Canada, Genome BC and the Tula Foundation. Lignin, a promising and abundant feedstock, comprises up to 40% of plant biomass. However, lignin has so far resisted efficient decomposition into fuels, fine chemicals and advanced materials.



Red cells are carrying DNA that codes for lignin transformation. Green cells are biosensors detecting lignin transformation products.

(Image Credit: Cameron Strachan, University of British Columbia)

Most bio-refining agents are based on enzymes engineered from fungi. In this case, UBC researchers used the innovative screening approach to source and test genetic arrays from

bacteria inhabiting coal beds. The biosensor reacts to a set of small molecules that are the residue of lignin's natural degradation process. The researchers surmised that coal -- ancient wood and plant biomass deposited before the evolution of fungal lignin degradation pathways -- might contain bacterial pathways involved in the transformation process.

(Source: www.sciencedaily.com, June 30, 2014.)

Abstracts of Recent Publications

01. Aquacultural Engineering, 2014, **59**, Pages: 41- 47.

Efficiency of biofloc technology in suspended growth reactors treating aquacultural solid under intermittent aeration. Wenyan Liang, Guozhi Luo, Hongxin Tan, Niannian Ma, Nan Zhang, Li Li.

College of Fisheries and Life Science, Shanghai Ocean University, Shanghai 201306, China.

Aquacultural solid waste from a recirculation aquaculture system was used as a substrate to produce heterotrophic bacteria in suspended growth reactors. The efficiency of nitrogen recycling under intermittent aeration (IA, 0.5-h aeration/0.5-h non aeration) and continuous aeration (CA) strategy was investigated. The nitrogen dynamics, biochemical composition of biofloc and efficiency of nitrification/denitrification/ammonium assimilation of biofloc were determined. No significant differences were observed in the nitrogen recycling rate, crude protein and polysaccharides contents of biofloc between the IA and CA reactors. The energy used for intermittent aeration was almost one half of that for continuous aeration. IA strategy (0.5-h aeration/0.5-h non aeration) appears to be more effective to produce biofloc in aquaculture solid waste in reactors than CA strategy.

Keywords: Heterotrophic nitrogen assimilation; Intermittent aeration; Aquaculture solid waste; Biofloc technology

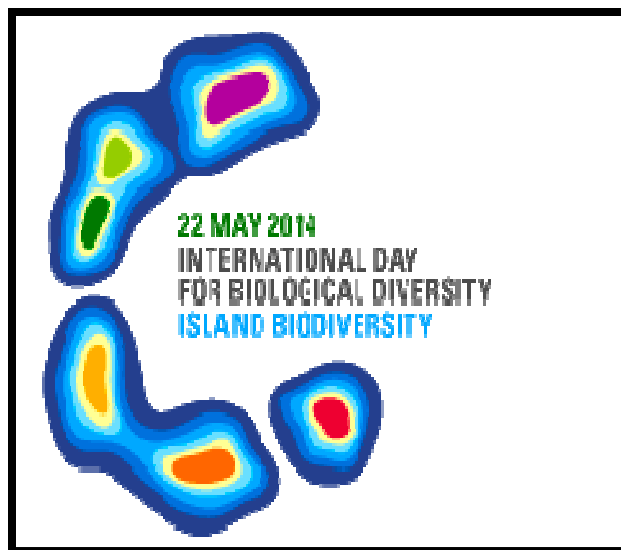
02. Water Science and Technology, 2014, **69**(4), Page: 694 – 702.

Bioflocculation behaviours of microbial communities in water treatment. Yanbin Zhu, Shan Li, Dengxin Li, Chunyan Liu and Fang Ma.

School of Environment Science and Engineering, Donghua University, Shanghai 201620, China.

We studied the flocculation behaviours of microbial communities in 21 soil, wastewater and activated sludge samples to clarify the effects of culture medium types on flocculation ability and screening efficiency, and to analyze diverse functions and microbial compositions. The bioflocculants produced by 33% of the microbial communities had flocculating efficiencies higher than 90%. Six out of the eight microbial communities with efficiencies over 94% were screened from the culture medium using dibutyl phthalate (DBP) as the carbon source. BF-BCT, which was derived from the Chinese cabbage soil sample, had the highest flocculating efficiency (99.6%), species diversity and uniformity. Nine highly efficient strains were separated and purified from seven different microbial communities, indicating that flocculating microorganisms are widely distributed in ecosystems. The 16S rRNA gene testing shows that the eight bacterial and the one fungal strains are common soil microorganisms. The flocculating abilities of BB11 (*Sphingobacterium multivorum*) and SE3 (*Galactomyces geotrichum*) have never been reported hitherto. Six strains, including the most flocculating-active TB13 and JB17, were screened from the culture medium using DBP as the sole carbon source. In particular, we compared the performance of culture media and analyzed analogous microbial communities with a Biolog automatic micro-analysis system for the first time.

Keywords: bioflocculation; dibutyl phthalate; microbial community; water treatment



E - Resources on Microorganisms

NATIONAL

Environmental Resources Management (ERM)
www.erm.com/

Bacterial identification
<http://www.tgw1916.net/>

Microbial Resource Research Infrastructure
<http://www.mirri.org/home.html>

Microbial Genome Resources
http://www.ncbi.nlm.nih.gov/genomes/MICROBES/microbial_taxtree.html

INTERNATIONAL

Microbial Genome Databases
<http://microbialgenomics.energy.gov/databases.shtml>

Microbial Ecology
<http://commtechlab.msu.edu/sites/DLC-ME/resources/index.html>

Microbial Antarctic Resources (mARS)
<http://mars.biodiversity.aq/>

BMRC Brazilian Microbial Resource Center
<http://www.bmrc.incc.br/>

EVENTS

Conferences / Seminars / Meetings 2014

Australian Society for Microbiology ASM, July 06 - 09, 2014. **Venue:** Melbourne, **Australia.**

Website: <http://asmmeeting.theasm.org.au/>

Microbial Toxins and Pathogenicity. July 19 - 20, 2014. **Venue:** Waterville Valley, NH, **USA.**

Website: http://www.grc.org/programs.aspx?year=2014&program=grs_mictox

International Union of Microbiological Societies 2014 Congresses. July 27 - August 01, 2014.

Venue: Montreal, **Canada.** **Website:** <http://www.montrealiums2014.org/>

15th International Symposium on Microbial Ecology. August 24 - 29, 2014. **Venue:** Seoul, **South Korea**

Website: <http://www.isme-microbes.org/isme15>

International Conference on Fisheries and Aquaculture (ICFA) – 2014. September 09 - 10, 2014.

Venue: Colombo, **Sri Lanka.** **Website:** <http://aquaconference.com/>

Ancient whodunit may be solved: Methane-producing microbes did it!



An outburst of methane produced effects similar to those predicted by current models of global climate change: a sudden, extreme rise in temperatures, combined with acidification of the oceans. In the case of the end-Permian extinction, virtually all shell-forming marine organisms were wiped out -- consistent with the observation that such shells cannot form in acidic waters.

Evidence left at the crime scene is abundant and global: Fossil remains show that sometime around 252 million years ago, about 90 percent of all species on Earth were suddenly wiped out -- by far the largest of this planet's five known mass extinctions. But pinpointing the culprit has been difficult, and controversial. Now, a team of MIT researchers may have found enough evidence to convict the guilty parties -- but you'll need a microscope to see the killers.

The perpetrators, this new work suggests, were not asteroids, volcanoes, or raging coal fires, all of which have been implicated previously. Rather, they were a form of microbes -- specifically, methane-producing archaea called *Methanosarcina* -- that suddenly bloomed explosively in the oceans, spewing prodigious amounts of methane into the atmosphere and dramatically changing the climate and the chemistry of the oceans.

Volcanoes are not entirely off the hook, according to this new scenario; they have simply been demoted to accessories to the crime. The reason for the sudden, explosive growth of the microbes, new evidence shows, may have been their novel ability to use a rich source of organic carbon, aided by a sudden influx of a nutrient required for their growth: the element nickel, emitted by massive volcanism at just that time.

The new solution to this mystery is published in the *Proceedings of the National Academy of Sciences* by MIT professor of geophysics Daniel Rothman, postdoc Gregory Fournier, and five other researchers at MIT and in China.

The researchers' case builds upon three independent sets of evidence. First, geochemical evidence shows an exponential (or even faster) increase of carbon dioxide in the oceans at the time of the so-called end-Permian extinction. Second, genetic evidence shows a change in *Methanosarcina* at that time, allowing it to become a major producer of methane from an accumulation of carbon dioxide in the water. Finally, sediments show a sudden increase in the amount of nickel deposited at exactly this time.

The carbon deposits show that something caused a significant uptick in the amount of carbon-containing gases -- carbon dioxide or methane -- produced at the time of the mass extinction. Some researchers have suggested that these gases might have been spewed out by the volcanic eruptions that produced the Siberian traps, a vast formation of volcanic rock produced by the most extensive eruptions in Earth's geological record. But calculations by the MIT team showed that these eruptions were not nearly sufficient to account for the carbon seen in the sediments. Even more significantly, the observed changes in the amount of carbon over time don't fit the volcanic model.

"A rapid initial injection of carbon dioxide from a volcano would be followed by a gradual decrease," Fournier says. "Instead, we see the opposite: a rapid, continuing increase." "That suggests a microbial expansion," he adds: "The growth of microbial populations is among the few phenomena capable of increasing carbon production exponentially, or even faster. But if living organisms belched out all that methane, what organisms were they, and why did they choose to do so at that time?"

That's where genomic analysis can help: It turns out that *Methanosarcina* had acquired a particularly fast means of making methane, through gene transfer from another microbe -- and the team's detailed mapping of the organism's history now shows that this transfer happened at about the time of the end-Permian extinction. (Previous studies had only placed this event sometime in the last 400 million years.) Given the right conditions, this genetic acquisition set the stage for the microbe to undergo a dramatic growth spurt, rapidly consuming a vast reserve of organic carbon in the ocean sediments.

But there is one final piece to the puzzle: Those organisms wouldn't have been able to proliferate so prodigiously if they didn't have enough of the right mineral nutrients to support them. For this particular microbe, the limiting nutrient is nickel -- which, new analysis of sediments in China showed, increased dramatically following the Siberian eruptions (which were already known to have produced some of the world's largest deposits of nickel). That provided the fuel for *Methanosarcina*'s explosive growth.

The resulting outburst of methane produced effects similar to those predicted by current models of global climate change: a sudden, extreme rise in temperatures, combined with acidification of the oceans. In the case of the end-Permian extinction, virtually all shell-forming marine organisms were wiped out -- consistent with the observation that such shells cannot form in acidic waters.

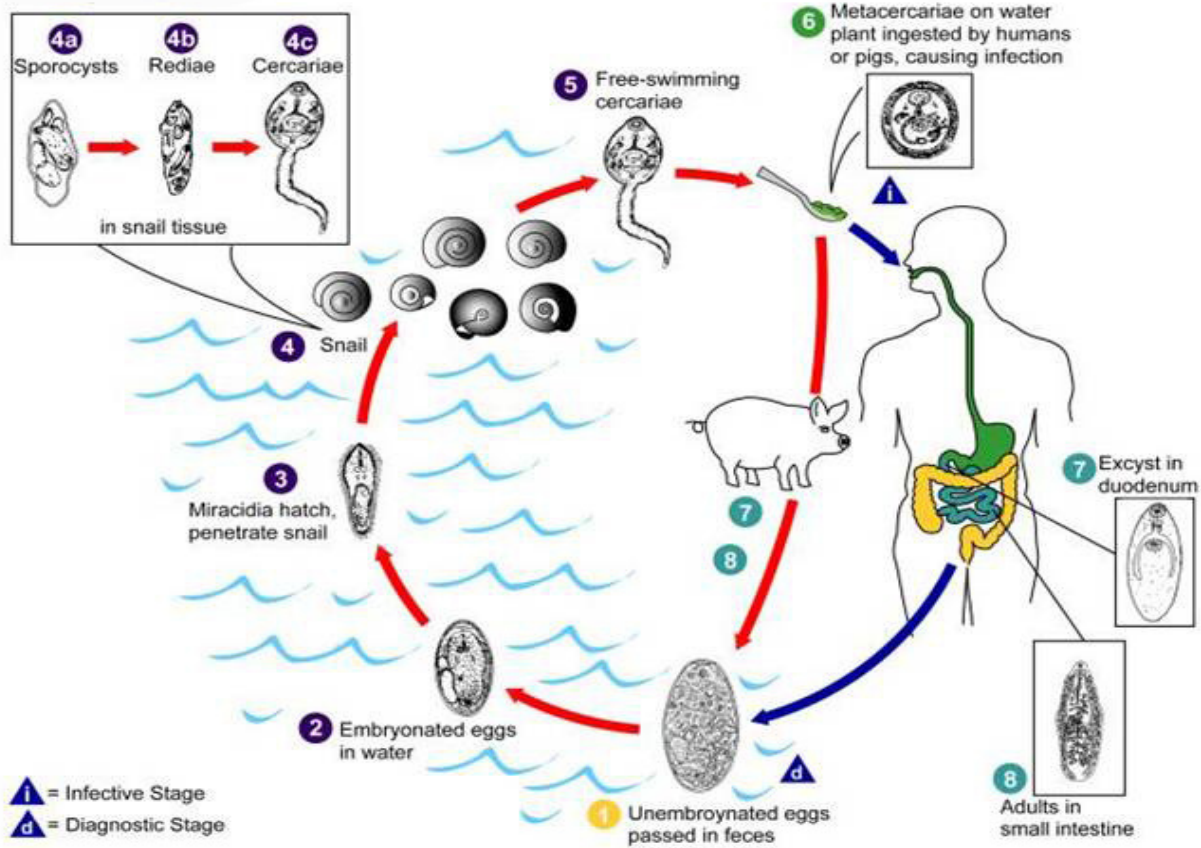
"A lot of this rests on the carbon isotope analysis," Rothman says, which is exceptionally strong and clear in this part of the geological record. "If it wasn't such an unusual signal, it would be harder to eliminate other possibilities."

While no single line of evidence can prove exactly what happened in this ancient die-off, says Rothman, who is also director of MIT's Lorenz Center, "the cumulative impact of all these things is much more powerful than any one individually." While it doesn't conclusively prove that the microbes did it, it does rule out some alternative theories, and makes a strong and consistent case, he says.

Source: www.sciencedaily.com

FASCIOLOPSIASIS

(*Fasciolopsis buski*)



UNIVERSITY OF MADRAS
ENVIRONMENTAL INFORMATION SYSTEM (ENVIS) CENTRE
(Funded by Ministry of Environment & Forests, Govt. of India)



DEPARTMENT OF ZOOLOGY

WORLD ENVIRONMENT DAY - JUNE 5, 2014



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5 June