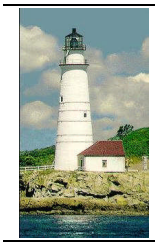

Northeast Branch Newsletter

Number 132 Winter 2009

2009 Programs in Review

Workshop - Clinically Relevant Anaerobic Microbiology

Our first program of 2009, a one-day intermediate workshop in anaerobic microbiology was held at Bristol Community College on January 14, 2009 and was co-sponsored by the Northeast Branch and Bristol Community College. The instructor was Gloria Petruzziello, MS, M,SM(ASCP), Supervisor, Clinical Microbiology & Section Head of the Anaerobe Laboratory, Brigham & Women's Hospital, Boston, MA. Twenty-six workshop attendees participated in lectures, discussion and demonstrations with hands-on training. Lectures covered the theoretical aspects of obligate anaerobiasis, infections involving anaerobic bacteria and organisms frequently encountered in infections. Subjects discussed included updated nomenclature of anaerobes, specimen collection, primary isolation, identification, susceptibility testing, GLC and MIDI analysis. Participants were also asked to identify fourteen unknown organisms using rapid, inexpensive and reliable procedures. The workshop was facilitated by Paulette Howarth, Professor of Microbiology at Bristol Community College, two of her students, Maria LePage and Edir Lopes, and Professor Frank Scarano, from the University of Massachusetts, Dartmouth.

Instructor Gloria Petruzziello (Center) and Students

Inside This Issue

NORTHEAST BRANCH -ASM OFFICERS and STANDING COMMITTEE CHAIRS
(Offices effective until June 30, 2010)

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NEB By-Laws Change

The recommendation by the NEB Council that the NEB voting procedure by paper ballot be updated to permit electronic voting was voted upon by the membership as required by the NEB Constitution. The majority of the membership voted in favor, with one vote opposed. Article VI, Item 6 will therefore now read: *Voting for all candidates for elected office shall be conducted by electronic and/or mail ballot not later than June of each year.*

Membership News

Dues reminders were sent in late 2009 by email and by postal service to members who did not provide an email address. Membership forms may be found on the NEB website or you may join the both the ASM and the Northeast Branch online through the ASM eStore. Please make the necessary corrections to your demographics and return to the Treasurer or email changes to: NEBranch-ASM@comcast.net. Please check mailing labels as they reflect existing information. Although membership in the national branch automatically makes you a member of the local branch in some organizations, this is NOT the case in the ASM. *To be both a National Member and a NEB member, you have to join each individually.* The Northeast Branch currently has 200 members.

Visit the NEB Web Site!!

The NEB has established a home page on the World Wide Web where all current events and the Newsletter are available. ASM has also established a Branch Meetings page. Visit us via the ASM Home Page or directly at:
http:/www.asm.org/branch/brNoE/ index.shtml

2009-10 Council Elections

Congratulations to the following NEB members whose terms began July 2009. James Kirby, MD was elected President (one year), Paulette Howarth was elected National Councilor, and Alfred DeMaria, Jr., was elected local Councilor (three-year term).

FUTURE PROGRAMS
Local Programs:

Announcements of Local Meetings are posted on our website at:
http:/www.asm.org/branch/brNoE/ index.shtml

February 23, 2010
Current Understanding and Status of the New Pandemic H1N1 Influenza Virus.
Speaker: Ed Balkovic, PhD., Quality Control Technical Services, Genzyme Corporation, Framingham, MA. Location Vinny Testa's. Dedham, MA

April 7, 2010
One Health Initiative/Emerging Infectious Diseases. ASM Branch Lectureships Program Speaker Stanley Maloy, PhD, Dean, College of Sciences, San Diego State University, CA. Location to be announced.

May, 2010
Dinner–Meeting at the Public Health Museum in Tewksbury, MA. Date and speaker to be announced.

National Meetings:

May 23-27, 2010
110th ASM General Meeting, San Diego, CA .

May 23-27, 2010
ASM Undergraduate Conference for Undergraduate Educators (ASMCUE) 2009
Town & Country Resort and Convention Center, Mission Valley area, San Diego.

For national ASM meetings contact: ASM Meetings,1752 N Street, NW, Washington, DC 20036. Tel: 202-942-9248. See www.asm.org

109th ASM General Meeting, Philadelphia, PA

Officers from the thirty-five ASM Branches are annually invited to attend a Branch Officers Forum which is held at the annual ASM General Meeting. This year, the Northeast Branch was among those invited to give a presentation on its activities.

James Kirby, President-Elect of the Northeast Branch, shared some success stories from his Branch, particularly highlighting how the Branch provided opportunities for students to learn about microbiology and related careers. For example, high school students were invited to the Woods Hole Marine Biology Laboratory to participate in the hands-on microbiology enrichment program. In addition, multiple Career Nights at local colleges and dinner lectures also attracted approximately 200 students in 2008.

The Branch was particularly proud of its involvement in the development of a Public Health Laboratory CareerPac. This multimedia product focused upon the kaleidoscope of careers available in public health laboratories. It is presently being distributed to middle and high school science departments across Massachusetts. Teachers are encouraged to adapt or customize their lesson plans with the CareerPac to meet the needs of their students.

Kirby also described the branch's diverse workshops, symposia, and conferences, showcasing the Northeast Branch's success in delivering interesting and provocative programming to its members.

From: Microbe/Volume 4/Number 7, 2009

The Northeast Branch currently supports three Student Chapters: the Boston Chapter, University of New Hampshire Chapter, and the Society for Microbiology at the University of Maine at Orono. Students are encouraged to attend ASM Annual Meetings. Maine Chapter students and advisor Anne Hanson attended the Philadelphia meeting (below).

The Origin of Virulence

The first dinner-meeting of the 2009 series was held on April 7, 2009 at Vinny Testa's of Boston in Dedham, MA. Arturo Casadevall, MD, PhD, from the Albert Einstein College of Medicine, Bronx, NY, spoke on *The Origin of Virulence in Pathogenic Microbes.* Dr. Casadevall is Professor of Microbiology and Immunology and Medicine, and Chair, Department of Microbiology and Immunology; he is also an ASM Branch Lectureships Program Speaker. In this talk he addressed the "damage-response" framework of microbial pathogenesis proposed by his laboratory (the concept that both host and microorganism affect the outcome of infection).

Dr. Casadevall first addressed whether pathogenic microbes are really different from nonpathogens. In the 1900's, it was thought they were different. One school of thought said yes: they had capsules and toxins (virulins and aggressins). However, there were exceptions, and an opposing school said there was no difference between pathogens and nonpathogens. Virulence is not a necessarily a stable trait, as in vitro or passage through an animal may negates it. He then surmised how the microbial property of virulence arises. Most organisms are acquired from the soil and have no need to be virulent in an animal host, while microbes acquired from another animal host would need to have survival mechanisms.

Many diseases from the 19th century have all but disappeared in the 20th century, the organisms are now nonpathogens. Some organisms need to be "tortured", as by giving them vancomycin, so that they become virulent again. In the 1950 and 1960's "commensals" gave rise to the concepts of "microbial opportunism". Now they were being called "opportunists" instead of "pathogens".

Dr. Casadevall's laboratory in 2003 published a theory called the "damage-response framework." *(Casadevall and Pirofski (2003) Nat Rev Microbiol 1:17-24).* This states that two entities are needed to interact, and that we really care about host damage not damage to the organism. It is the host's response that controls the amount of damage done. Therefore virulence is a microbial property that is expressed only in a susceptible host, and every microbe has the potential to be pathogenic, depending on the host.

ASM Branch Lectureships Program Speaker
Arturo Casadevall (R)

For example, the yeast *Saccharomyces cerevisiae* found in yeast packages in a grocery store can be viewed in a number of different ways; it can be used in food production (baking); a commensal organism (vaginal infections?), an opportunistic pathogen (AIDS) and as a primary pathogen (lung nodule). In all cases, the host must be taken into account.

Dr. Casadevall then spoke of host-acquired versus environmentally-acquired pathogenic microbes. Host-acquired microbes are communicable, have a limited host range, are not free-living, etc., while environmental microbes are not communicable, have an unlimited host range and are free living, etc. These appear to be exactly the opposite of organisms found in a host.

Dr. Casadevall's major research interests are in fungal *(Cryptococcus. neoformans)* pathogenesis and the mechanism of antibody action. He mentioned that there are about 1.5 million fungal species, but only about one hundred human pathogenic fungi; ten to fifteen of these are such common pathogens such as *Candida albicans, Pneumocystis* spp., and the dermatophytes. Of the pathogenic fungi, some are host-acquired such as *Candida albicans,* and *T. pedis*, while others such as *Aspergillus* are environmentally acquired. In the 1950s, *Histoplasma capsulatum* and *C. neoformans*

were isolated from the environment using mouse inoculation. Highly contaminated soil containing microbial spores was inoculated into mice, yielding the organisms. However virulence is often lost on subculture in the laboratory.

Focusing on *C. neoformans*, one study attempted to determine whether a low prevalence of disease in immunocompetent children in the Bronx was due to a lack of early exposure to the organism. Initial infection occurs in early childhood and it was shown that all children two-four years of age were infected; there was a high prevalence of infection but yet a low prevalence of disease. When this theory was published, newspaper headlines proclaimed "Pigeon Poop Poisoning 70% of our Children". Cryptococcus neoformans is a facultative intracellular pathogen Dr. Casadevall explained, and all soil organisms have a virulence strategy. He showed a slide of a phagosome "barfing" *C. neoformans*. The fungi have such a unique strategy that that allow themselves to be engulfed by the phagosomes and to multiply in them; they and their progeny are then expelled by the cells and the cells remain alive to be reinfected. The progeny hatch without wrecking their host. But why aren't they killed? The organism itself has a huge phagosome and avoids intracellular killing by macrophages.

C. neoformans has a very sophisticated virulence strategy. It produces disease in healthy hosts that are exposed to large numbers of the organisms (which is generally self-limited) or in those that are immunologically impaired (which is usually lethal). Looking at the habitat in which it lives, i.e. pigeon excreta and the soil, did it develop mechanisms to survive harsh and extreme environments, and ingestion and phagocytosis by amoebae, phages and other soil-dwelling microbes? Such mechanisms could also function as virulence factors in other environments, such as an animal host. Infection occurs when a host inhales airborne fungal spores; this may simply reflect airborne dissemination, which is also suitable for host pulmonary infection.

Dr. Casadevall then described melanins, which are dark high molecular weight biological pigments that are found just about everywhere in nature, i.e. in skin, hair, feathers, scales, eyes, etc. The various types of melanins vary in chemical structure, have great physical stability, and are linked to protection against environmental insults and resistance to host defense mechanisms in melanized microorganisms. Melanin synthesis occurs in *C. neoformans*, which protects it against oxidation, elevated temperature, amphotericin B, caspofungin, microbicidal peptides, enzymatic degradation, and macrophages in vitro. The pigment is formed as an end product during metabolism of the amino acid tyrosine. Melanins are conspicuous in dark skin moles of humans; in the black dermal melanocytes (pigment cells) of most dark-skinned peoples; and as brown, diffuse spots in the epidermis.

In radionucleotide-contaminated soil, such as at Chernobyl, the surrounding soil is black due to, melanins produced by dermatophytes. Inside the damaged reactor, where a lethal (to humans) dose of radiation exists, thirty-seven species of fungal species were found. The question then arose "Do the fungi eat radiation?" It was known that many fungi synthesize melanin, which protects again UV and solar radiation due to its absorption of UV and visible light of all wavelengths and its heat-dissipating properties. Studies in Dr. Casadevall's laboratory in 2007 showed that melanin functions as an energy transductor molecule, perhaps a type of "primitive photosynthesis". Following exposure to radiation, growth of melanized C. *neoformans* and *W. dermatidis* was preferentially enhanced. After this publication the newspaper headlines screamed: "Hungry Fungi Chomp on Radiation". Melanotic fungi also exist in other extreme environments such as high altitudes, and have contaminated the space station. They were found growing in grease and "eating" cables; the situation appears to worsen when solar flares occur.

All cellular structures in melanized *C. neoformans* can be solubilized by a chemical method, leaving only black particles. Electron microscopy analysis of the particles shows cell "ghosts" with electron-dense walls. *C. neoformans* strains can differ in melanin content. Therapeutic drugs given must pass through this melanin and thus are inactivated, adding to the organism's virulence. Melanized

cells are also less susceptible to microbial oxidants. However, from studying these "ghosts" we now have a new therapy for metastatic melanoma. Monoclonal antibodies can be produced to *C. neoformans* melanin peptides which will bind with tumor melanin, and could be used to deliver therapeutic radionuclides to melanoma tumors. Dr. Casadevall stressed that the funding of basic research is extremely important, discoveries such as this frequently occur accidentally.

Dr. Casadevall theorizes that virulence can be thought of as "game cards", or "cards of virulence". Organisms play games and play different hands; any given microbe has different cards, each card represents an attribute (virulence factor) that allows an organism to survive is a host, such as having phospholipase, melanin, or urease, or being able to grow at 37°C. Depending on the combination of attributes and hands an organism has, it can infect an insect, human, etc. If an organism has enough cards and enough different "hands" it can infect anything.

Dr. Casadevall lastly commented on the protozoa - fungal - animal timeline, i.e., what killed the dinosaurs. After the KT event catastrophe, dust in the atmosphere from asteroid impact or volcanic activity would have blocked sunlight and inhibited photosynthesis, causing a mass extinction of animal and plant species (a global compost). However, this favored the proliferation of organisms such as fungi (fungal bloom) that don't require photosynthesis and utilize nutrients from decaying organic matter. Most fungal species grow best at ambient temperatures and each degree centigrade increase in temperature excludes 6% of fungal diseases. He then added that most of our caloric intake is to maintain our body temperature - we eat three times a day, a seemingly wasteful lifestyle, to keep the fungi away. A mammalian temperature, he said, is really an 'extreme" temperature- and may have evolved to protect us against from most fungal diseases; we are well above the ambient temperature. Reptiles need to only eat once a day or less often. Dinosaurs were not endothermic 37°C creatures, nor are insects, plants, and amphibians, which are frequently attacked by fungi. Fungal diseases thus might have attributed to the extinction of dinosaurs and the proliferation of mammals. He added that mammals were a "failed experiment" and initially were probably food for small reptiles! Historically, a connection between infectious disease and extinction was not considered but chytridiomycosis today has definitely contributed to the extinction of many species of frogs worldwide.

The Current Status of Lyme Disease in Massachusetts

The second dinner-lecture of the year, *Danger is Lurking Everywhere-The Current Status of Lyme Disease in Massachusetts* was held on June 2, 2009 at Vinny Testa's of Boston in Dedham. Alfred DeMaria, Jr., MD, Medical Director, Bureau of Infectious Disease, Prevention and Services at the William A. Hinton State Laboratory Institute (SLI) in Jamaica Plain, MA, updated the audience on the problems associated with Lyme as well as some of the other diseases carried by the same ticks. Dr. DeMaria is also State Epidemiologist and believes this topic has not received the serious public attention it deserves.

NEB President-Elect James Kirby, PhD (L) and Alfred DeMaria, Jr., MD (R)

The lecture started with a time-lapse graph of Massachusetts (MA) showing the progression of Lyme disease in the state from the years 1988-

2007. The disease was first (predominantly) seen on the Cape and Islands, then gradually moved west, with most cases occurring during summer months. Dr. DeMaria pointed out that the incidence of Lyme disease is continuing to increase throughout MA. The spread of *Ixodes*, ticks that carry the disease, has been tracked across MA since the 1970's. People today have a good chance of encountering ticks, wherever they reside in the state. The MA Public Health Department is now keeping track of other tick-borne diseases.

The most common tickborne diseases in the Northeast are Lyme borreliosis (*Borrelia burgdorferi*), babesiosis (*Babesia microti*) and erlichiosis (HGE- *Anaplasa phagocytophylum*) carried by *Ixodes scapularis*. We also have tularemia (*Francisella tularensis*), Rocky Mountain spotted fever (*Rickettsia rickettsiae*) and Powassan virus (flavivirus encephalitis. Tickborne diseases elsewhere in the United States (US) include human monocytic erlichiosis (HME), *Erlichia chaffeensis*), southern tick-associated rash illness (*Borrelia lonestari*), *Babesia* sp., tickborne relapsing fever (*Borrelia* sp), Colorado tick-fever, tick paralysis (tick neurotoxin), West Nile virus infection, and possibly Q fever (*Coxiella burnetti*) and *Bartonella vinsonii*.

In the northeast, *Ixodes scapularis*, the black-legged deer-tick, is the prime vector, whereas the western black-legged tick *Ixodes pacificus* is the vector on the Pacific Coast. Dr. DeMaria showed photos of the various stages in the life cycle of the tick and described its anatomy and rather complicated two-year life cycle. An engorged female lays eggs in leaf litter etc. in forests (May-July); the larvae hatch and feed on deer mice (*Peromyscus*), small mammals and birds (August-September). At this point the larvae are infected with *Borrelia* and other pathogens from the disease reservoir of mice, chipmunks, etc. They molt to nymphs that overwinter (October-April), then awake to attach and voraciously feed (May-July) on small mammals and birds since they need a blood meal in order to molt to the adult stage. Nymphs can also be infected with pathogens at this point.

Adult ticks look for medium to large mammalian hosts, primarily deer, on which they mate, feed, and then drop off to lay eggs. Adult ticks are most active on warm days in the winter with a second peak of activity in the spring. Overwintering nymphs are also active at this time, looking for a blood meal. Nymphs, because of their small size, spread most of the disease because they are much harder to detect. Note that deer themselves do not carry the disease and that *Borrelia*, *Erlichia*, and *Babesia* are dormant in ticks and don't multiply in them. Therefore, a tick must be attached for about 24 hours before it can transmit disease; it takes this long for the dormant organisms to become active and reach the salivary glands of the tick where they can be transmitted. This latent period is important when trying to access the risk of disease when removing a tick.

An adult tick sits in higher grass or leaves, with its front legs extended; the tick is very sensitive to CO_2 emissions and movement from an animal. It will attach to any passing animal, imbedding itself into the flesh of humans, dogs, deer, or white footed deer mice, and try to find a warm place, such as the groin, axilla, behind the knees or ears or along the hairline. It then attaches, actually imbedding its mouth parts into the flesh and feeds, taking about three days to obtain a full meal of blood. Since tick saliva contains both an anticoagulant and an anesthetic there is no pain involved; also, there are no allergens as present in the mosquito saliva, so there is no itching.

Why does this problem occur? In the 18th century Massachusetts was mostly farm land; there were no communities, few trees, and very few deer wandering around. Now the entire state is urbanized, suburbs have expanded into wooded areas, there is a regrowth of trees and much open space to host diverse flora, food, brush and leaf litter for nymphs to hide in; we also have many white-footed mice. Due to these changing land use patterns, the suburbs, with no predators such as coyotes and mountain lions, provide an attractive environment for an overabundant deer population. The Islands and Essex County are currently over their maximum goal of deer, which are the definitive host for Lyme *Borrelia*, *Anaplasa*, and *Babesia*. A map of the US shows *Ixodes scapularis* well established in the northeast, southeast and great lakes region, with *Ixodes pacificus* on the West

coast. The lone star tick, *Amblyomma americanum*, found in southeastern US as far west as Texas, carries monocytic erlichiosis and tularemia, but the symptoms are not as severe as with Lyme. It is a very aggressive feeder and is also found in southern MA. Although tick bite can lead to paralysis, it is not associated with the same symptoms and long sequellae as with *Ixodes scapularis.* There is less Lyme disease in the southeast due to lizards eating the mice.

Lyme disease was recognized in Europe in 1909 and the first recognized cases in the U.S. occurred in the 1970s in Lyme, Connecticut. First seen in coastal areas in Nantucket, Lyme disease spread west and north into ME, VT, and NH. The same occurred with all other tick-borne diseases. Today it is the most common vector-borne disease in the U.S.

Graphs of Reported cases of Lyme disease in the US from 1992-2007 showed a steady increase in Lyme disease from 10,000 to over 25,000 cases; Lyme disease has been reportable in MA since 1987. Most cases in the U.S. are seen in men due to their outdoor activity and occupations (landscapers, hikers, etc.) There were about 300 confirmed cases of Lyme disease in MA in 1997; as of 5/26/09, 3938 cases were confirmed for 2008 and the number is expected to rise.

The lyme *Borrelia* is similar to the *Tremonema* spirochete, and invades any tissue in the body. Cases reported usually have the "typical" symptoms of lyme. Symptoms associated with physician diagnosed Lyme disease [in MA in 2005] were erythema migrans, arthritis, Bells palsy, meningitis, radiculoneuropathy, and heart conduction block. When looking at graphical representation of cases meeting CDC criteria, and those reported by physicians that did not meet CDC criteria, they mirror each other. Dr. DeMaria believes that for each case reported to the DPH, three to ten cases are not reported because they don't meet all of the CDC's case definition. Physician diagnosed cases included the following mixed bag of signs and symptoms: fatigue, joint/ muscle pain, night sweats, headache, other rash, neck pain, cognitive impairment, paresthesias, visual/auditory impairment, topic stations, arthritis, lymphocytic meningitis, and Bell's palsy. Criteria for lyme diagnosis should be specific enough so that everyone is using at the same criteria said Dr. DeMaria, which is currently not the case.

Incidence rates of Lyme disease show that the disease exists throughout MA, with the majority of cases occurring in the summer, peaking in July; however, you can be infected anytime, even on a warm November or February day. The disease is seen mostly in young children and males in mid-life which is associated with more exposure in tick-infested areas.

Dr. DeMaria then described Lyme disease and showed numerous slides of the clinical manifestations of Lyme borreliosis. He described the different stages of the disease, early localized disease, early disseminated disease, and late or chronic disease.

Symptoms of early localized Lyme disease may manifest a week to month after infection and can include erythema migrans, fatigue, malaise, lethargy, headache, stiff neck, myalgia, arthralgia and lymphadenopathy (regional or generalized). Dr. DeMaria showed slides depicting the numerous and varying appearances of the characteristic circular "bull's eye" pattern skin rash. The CDC case definition requires a 5 cm bull's eye – many cases don't meet this, in fact, 30% don't have the typical pattern (rash).

Symptoms in early disseminated Lyme disease include effects on the heart (carditis, rarely fatal conduction defects); skin (spreading rash, lymphocytoma); numerous neurologic symptoms (meningitis, Bell's palsy, encephalitis myelitis), musculoskeletal problems (fibro-myalgia, polyarthritis), eye problems (retinitis, conjunctivitis, iritis); lymphadenopathy (regional or generalized); hepatic problems (abnormal liver function tests, hepatitis); and renal problems (microhematuria, proteinuria).

Late stage Lyme disease includes the classic chronic arthritis, as well as other musculo-skeletal problems (migratory polyarthritis, the classical chronic arthritis, fibromyalgia); skin problems (acrodermatitis chronica atrophicans, morphea); and neurologic systems (encephal-opathy, encephalomyelitis, peripheral neuropathy, ataxia, dementia, sleep disorder). Such symptoms of Lyme disease are very real and everyone agrees that they do occur said Dr. DeMaria. Regarding women who are infected

during pregnancy, early treatment is critical to prevent such symptoms such as these. An association between transplacental transmission of *Borrelia burgdorferi* and adverse birth outcomes was not conclusively proven in the 1980s. Current literature shows that the effect of Lyme disease on fetus is benign. However, there is agreement that additional studies are needed.

Dr. DeMaria then described the differences between European and American Lyme disease. Eurasian Lyme disease is carried by *I. ricinis* and *I. persulcatus*, and the causative organisms are *Borrelia burgdorferi* ss, *B. afzelii*, and *B. garinii*. There is a more frequent memory of tick bite, long duration of rash, central clearing is more common, systemic symptoms are less common, arthritis is uncommon and lymphocytoma and acrodermatitis are well documented. In North America, *I. scapularis* or *I. pacificus* carry *Borrelia burgdorferi* ss, there is infrequent memory of tick bite, short duration of rash, central clearing is less common, and systemic symptoms are common. Arthritis is common in untreated patients, and there is rare lymphocytoma and acrodermatitis.

Lyme disease may be diagnosed clinically or by laboratory procedures. In the early stages, the diagnosis is fairly obvious if the characteristic “bulls-eye” rash is present. Other wise, Lyme is suggested by a variety of nonspecific clinical symptom, such as the physical symptoms already mentioned and a variety of time honored, but nonspecific tests as the presence of elevated ESR (erythrocyte sedimentation rate), ALT/AST (aspartate and alanine aminotransferases, enzyme indicators of organ damage), and cerebrospinal fluid (CSF) antibodies. Isolation of the spirochete using specialized medium (modified Barbour-Stoenner-Kelly medium) is rarely successful. Currently two-tiered approach is used. A positive or equivocal ELISA using *B. burgdorferi* antigen is done first (or IFA), followed by a confirmatory Western Blot for both IgM (which demonstrates a current or recent infection) and IgG (which demonstrates a previous infection). The immunoblot for IgG however, requires the presence of five of ten possible bands, and immunoblot for IgM requires the presence of two of three possible bands, thus making the test difficult to interpret and resulting in false-positives. The two-tier test should not be used until at least three weeks following a suspected infection (bite), as the test has a low sensitivity in the early stages of Lyme disease; people with a rash are frequently seronegative. The test can be used in cases with late-stage symptoms however. More recently, antigen detection tests such as OspA, OspB [outer surface proteins A and B], flagellin, etc. have been developed]. PCR (polymerase chain reaction) has been used to detect the spirochete in body fluids.

Early treatment is highly effective (87%) with nymphs and engorged ticks. A single dose of 200mg doxycycline is given within 72 hours of discovery of an attached tick (where there is endemic Lyme disease and an obviously engorged tick). Doxycycline is best and penetrates serum and tissue levels however, 30% of patients treated with doxycycline have some type of reaction. There are no recommendations regarding doxycycline for children and patients must always be counseled about erythema migrans and symptoms. Amoxicillin is also used for children and erythromycin for pregnant women. Dr. DeMaria reminded us that anyone can relapse.

Dr. DeMaria then spoke of other diseases in MA carried by ticks. There were 583 laboratory reports of Babesia in 2008 of which less than 100 cases were confirmed. Seven cases were transmitted by blood transfusions (in NY) where asymptomatic people had donated blood. Fourteen percent of people on Nantucket have Babesia antibodies and are asymptomatic. There have been ninety-two confirmed and probable tularemia cases in MA from 2000 to 2008. Most (sixty-seven) were associated with Martha's Vineyard, and related to ground work, while nine cases were not. Rocky Mountain spotted fever is rare in Massachusetts; one case is seen approximately every five years. Powassan is a North American flavivirus transmitted primarily by *Ixodes cookei* that causes a rare but severe illness in humans with resulting neurological sequelae and a 10 to 15% case fatality rate. There is a high seroprevalence in burrowing mammals in New England, and a related virus was isolated from *I. scapularis*.

Powassan gained recognition as a result the increased evaluation of encephalitis cases because of West Nile Virus. There have been no cases in MA to date but several were reported in Maine & Vermont in 2001.

Dr. DeMaria continued with a note on "Chronic Lyme Disease". Some clinicians say that if there are no symptoms there is no disease and others say there is no such thing. Numerous studies have been published supporting both sides of this controversy: in 1990 Alan Steere and colleagues described chronic manifestations of Lyme disease; in 1994, at the Brigham and Women's Hospital, Shadick et al. described long-term outcomes of Lyme Disease such as arthralgia, numbness of extremities, unusual fatigue, concentration difficulties and memory impairment; in 2001, Kalish et al. at Tufts described a variety of findings, but no full picture, and in 2001, a quality of life study showed no difference between patients having Lyme disease syndromes and being treated for Lyme or not treated at all.

Infectious Diseases Society of America (ISDA) Guidelines for Clinical Assessment, Treatment and Prevention of Lyme Disease, Human Granulocytic Anaplasmosis, and Babesiosis were published in 2006, while the International Lyme and Associated Diseases Society [Evidence-based guidelines for the management of Lyme disease] published their own guidelines. The ISDA this year will convene a review panel to examine if their 2006 guidelines should be revised or updated based on a rigorous review of the medical and scientific evidence of the diagnosis and treatment of Lyme disease. Dr. DeMaria remarked that treated people do get better.

How can Lyme disease be prevented? Dr. DeMaria presented a variety of ways you can protect yourself against infection from ticks. Among these are (1) Use insect repellants but read the labels carefully; use those containing no greater than 30% DEET on exposed clothing and skin and use those containing permethrin-that kills ticks on contact, only on clothing. (2) Use protective clothing sprayed with permethrin (that binds to the fabric) and the effect will last through many washes; (3) Do a "tick check" on people and pets after being outdoors, especially on the legs, groin, armpits, along the hairline and the ears, the beltline, between toes; (4) Wear long sleeved shirts, light-colored trousers and tuck them into your socks; (5) Change the environment around your property (ticks thrive in damp areas): create a tick-free buffer zone by placing a three foot gravel or paved path between wooded areas and the property, or use deer netting around your property; clean up areas around wood piles and rock walls; (6) Use Damminix Tick Tubes: these contain permethrin-soaked cotton that mice carry back to their nests, thus reducing the number of ticks and disease spread by mice; and (7) Use mouse traps. The use of deer stations is controversial.

Ticks, if discovered on the body, must be removed properly. Gently grasp the tick with fine point tweezers close to the skin and pull straight out. Don't twist or squeeze the tick; many times the body will come off but mouthparts will remain in the skin, causing a local infection. Don't crush the tick's body as its fluids may contain infectious organisms. Wear gloves if possible.

Dr. DeMaria lastly mentioned LYMErix recombinant vaccine, based on the outer surface protein A (OspA) of *B. burgdorferi* which was marketed in the late 1990s by GlaxoSmithKline. It was an effective vaccine but was never popular as most people don't contract Lyme disease. There were also some histocompatibility issues and lawsuits. The manufacturer withdrew the vaccine in 2002 because of low sales. Protection against ticks and other sources of infections are currently the only way to prevent contracting Lyme disease. There has been controversy over almost every aspect of this tick-borne disease, which is likely to continue into the future.

Microbial Awakenings

The third dinner-lecture of the year, *Microbial Awakenings,* was held on September 23, 2009 at Vinny Testa's of Boston in Dedham, MA. Slava S. Epstein, PhD, the evening speaker, received his PhD in microbial ecology at the Shirshov Institute of Oceanography in Moscow, several years later joined Northeastern University's Marine Science Center, and currently is Professor of Biology at Northeastern University. His interests lie in microbial discovery for the purpose of both basic and applied research and works with "uncultivable" organisms. In this talk, he described a hypothesis on how environmental microorganisms survive unfavorable conditions. Its principal element is a proposal that non-sporulating species could exit dormancy via scout cells that become active spontaneously and randomly, at low frequency, due to noise in gene networks. The talk presented experimental evidence for the existence of scouts in both environmental species and laboratory strains. He indicated that half of what he would speak on is taken from actual data, and the other half is theory. Dr. Epstein acknowledged the work of his laboratory staff and Dr. Kim Lewis in collaborative studies; also funding from the National Science Foundation, National Institutes of Health, Department of Energy and other sources.

Dr. Epstein carefully observed the microbial growth curve (with its rise, plateau and decline in the number of viable "culturable" organisms) and developed it into a model; he then tried to find data to support the model. He mentioned the work of Roberto Kolter at Harvard who showed that the "death phase" of the growth curve is a most interesting area to study. Here waves of competitive mutants arise that will outcompete all others on the curve. When organisms die, what becomes of their nutrients (the dead shells and cell walls etc.)? The explanation seems obvious; as cells die, others grow on nutrients made available and a drop in colony forming units (CFUs) is seen. Low numbers of organisms can be cultured months after the lowest point on the curve is reached, however, even after an experiment is extended

Slava Epstein, PhD

for months. The viable count remains low, but unchanged. But what actually happens at the end of the curve? Experiments many years ago have shown that low numbers of viable organisms can be cultured up to five years after the curve reaches its endpoint; there is little change in the numbers of organisms capable of growth throughout that time. But how do they survive, what keeps them alive? How can they maintain basic mechanisms of survival for years without nutrients and accumulated waste? Why do only some cells act in this manner and not all that were originally grown? Supposedly there are no additional nutrients on which to subsist as they have been used up during growth, hence organism death. A number of theories have been proposed to explain this, one of which being that mutants arise.

Dr. Epstein asked, "What if these cells aren't dead?" What if there are initially two populations - a large dormant population that remains invisible and doesn't grow, or grows at a low frequency and a second smaller population of dead cells. Within the dormant population is a small population of "scout" cells, that awake, then die if an unsuitable living environment is found. The number of dead cells now increases and the number of dormant cells decreases. Dr. Epstein theorizes that the dormant cells wake up continually, and a dynamic balance exists between waking and dying populations.

The next question is why the dormant cells wake up. Is it due to environmental stimuli, temperature, humidity, etc? What factors

"wake up" inactive cells; there is nothing in the medium that would do this. He then speculated on how this happens. If a clonal culture of *Bacillus subtilis* is induced to sporulate, some cells do, while a subpopulation does not. Why? This is not all auto regulation. It occurs in a series of steps, and only in some cells. There is no sporulation because there is no auto regulation, with others this does not happen, it is random.

Apparently identical cells act differently, this is microbial individuality. Cells in a single clone can be totally different. Division is spontaneous and random. What if the mechanism maintaining dormancy makes a mistake?

Dr. Epstein cited an experiment that showed that if organisms are treated with a high dose of drugs, most are killed, but the living cells are still sensitive to the drug. The CFU's drop to a minimum level and remain low, but organisms still retain sensitivity to the drug. The question is, if you have a few dormant cells, and they awake after treatment with drugs, why are they sensitive? The theory is that the silent majority of dormant cells never grow but are always present, and a few persist (grow) at one time and are there before, during, and after treatment with drugs. For example, if cells wake up a few at a time, and more are seen daily, in three days and in one week, more will be seen as additional cells wake, so that the number of cells surviving are more than at first count. This divides the population into two parts, one perhaps performing one function, the other no function at all; this occurs randomly. With *E. coli,* after two months one sees a greater and greater number of cells awakening and some behaving completely differently than expected.

Dr. Epstein then discussed the "Scout Model". In an environmental population, if you have a mix of both active and dormant cells growing under permissive conditions, if the conditions change to adverse, the cells become dormant. The dormant population produces, at a low frequency, consistently, a new "scout" that looks at the surroundings to see if they are good or bad. Scouts are sent out intermittently. If conditions are good, the organism grows into a small population. A scout under good conditions produces a signal to tell the rest of the dormant population to wake up. Other species, synergistic with these organisms can also send a wake-up signal. There are several layers to this complicated process, but in the simplest process, growth has been achieved.

He speculated as to whether dormancy leading to a wakening occurs in nature, i.e. where a few cells are able to be cultured first and more later on. In one experiment, for example, with 5000 marine cells placed onto a 384 well microtiter plate, turbidity occurred from well to well. The organisms were held for four months in an incubator and their ribosomal DNA was sequenced. He asked whether organisms that took 2-3 wks, 4-6 wks, etc. and up to 3 to 4 months to grow can be called slow growers? When these are put into a fresh dish, they will grow in 24 to 48 hours; therefore they can either grow slow or fast. The organisms were identified as Pseudomonas strain CH01; some cells grow at day 2, others at day 14, 18, etc., up to day 120, but on subculture all strains grew quickly. He speculated that this can happen only if you have a large number of dormant cells. It happens with other species, but not with all. Dormant organisms wake up one by one, each at its own time. Some bacilli grow at two days, others at 120 days.

Dr. Epstein then discussed whether there is evidence of microbial awakening via signaling. Organisms go from suspended animation to awakening he said. Dormant cells are analogous to bacterial spores, some wake up daily. The scout grows in good conditions, while its kin do not grow until the growing scout emits a signal to wake the others to grow.

It has long been known that most microbes from most environments are unculturable. Dr. Epstein showed a photograph of, and described a patented diffusion chamber for growing "uncultivable" organisms, which was designed in collaboration with Dr. Kim Lewis at Northeastern University. Organisms can be grown in the chamber that allows an exchange of chemicals with the environment via permeable membranes but restricts movement of the cells. Therefore a simulated natural environment is provided from which cells can be removed, studied, and returned to the environment. He then described an experiment using cultures of a marine environmental organism, MSC33, its 16S DNA was sequenced

to identify *Psychrobacter* sp. Most of these organisms don't grow on standard media, less than 1% will grow on Petri dishes, and others simply will not grow. One culture grew on any and all media; one organism grew in the diffusion chamber, inside the chamber; it also grew on laboratory media. By restricting cell movement inside the chamber, it was found that cells inside the chamber "talked" to cells outside the chamber via a peptide that now induced growth of the "noncultivable" organisms outside the chamber.

Populations of MSC33 normally don't grow in Petri dishes, however, if this peptide is added, it starts growing. Because of its effectiveness in low concentrations it is believed the peptide molecule "signals". The scout therefore is thought to provide a "signal" that wakes up the rest of the cells. For microbes this may serve as a good survival strategy, said Dr. Epstein. Consider a microcolony of 1000 cells. If 0.1% wake up daily, and if a scout lives for 10 days, then at any given time 1% of the cells are active. It then follows that the population will survive for over three years.

The implications of the scout model for microbial ecology and health sciences are many. Medically, Dr. Epstein referred to recurrent infections such as (latent) tuberculosis where organisms lie dormant in organs for decades. How do these cells wake up, duplicate? If they can all be awakened at the same time, the growing cells can be killed. Many questions still are unanswered such as whether there is a master regulator keeping dormancy, and what the role of co-cultures is, where some organisms grow on synthetic media only in the presence of other species that provide a "growth factor". It has not been possible to date, to cultivate many microorganisms implicated in human disease in the laboratory. The patented diffusion chamber developed to grow "uncultivable" environmental microorganisms is one major step toward isolating new species and perhaps answering some of these questions.

Biofilm Development

Immediate NEB Past-President Jeff Klinger, PhD (L) and ASM President Roberto Kolter (R)

The fourth NEB dinner-lecture of the year was held on November 2, 2009 at the Genzyme Center in Cambridge, MA. We were pleased to have Roberto Kolter, PhD, President of the American Society of Microbiology, and professor of Microbiology and Molecular Genetics Department at Harvard Medical School in Boston speak on *Biofilm Development*. He presented findings from his laboratory on how extracellular signaling controls cell fate determination during the process of biofilm formation by *Bacillus subtilis.* The audience consisted of nearly one hundred people, over half of which were students from the Boston and New Hampshire ASM Students Chapters and several Massachusetts Community Colleges. Prior to the lecture, Richard Mattila, Director of Environmental Affairs at the Genzyme Center gave the audience an overview of the Genzyme's world headquarters building, which meets the highest environmental standard set by the US Green Building Council and has about nine hundred employees.

Dr. Kolter began by explaining that cells in a biofilm grow differently than in culture. The cells encase themselves in an extracellular matrix of polysaccharide and other materials, forming unexpected spatial and temporal organizations, and behaving as structured communities, with cellular differentiation occurring. Populations of such surface-associated bacteria are commonly referred to as

biofilms, and there are only a few surfaces in the world that are not colonized by microbes. In the clinical environment, for example, dental plaques are body associated biofilms and contain dozens of different strains/species in a small area, much like agricultural films on grapes. Since it is difficult to analyze how biofilms occur in nature, we therefore study laboratory strains of organisms, such as *Bacillus subtilis*.

He gave us a little of the historical background of *B. subtilis*, which was first described in 1877 by botanist Ferdinand Cohn, in a botanical journal. In the journal, there was a series of articles entitled *Research on the Bacteria, Contributions to the Biology of the Bacilli*; Cohn's paper was the fourth in the series. He was the first to show in an earlier paper (1876) that *Bacillus* can change from a vegetative state to an endospore when subjected to an environment that was deleterious to its vegetative state. Fifth in that series, was an important paper by Robert Koch on *Bacillus anthracis*, which ultimately led to *Koch's Postulates*. Koch's figures appear on Cohn's plates, and are the first description of a pure culture; Koch drew pictures of bacilli (*anthracis*) in chains and spores in beautiful accurate drawings that remarkably resemble today's electron micrographs. Dr. Kolter emphasized that we don't pay enough homage to these men.

Dr. Kolter showed an electron micrograph of a *B. subtilis* biofilm. Sites where sporulation genes were being expressed were seen as protrusions all over the surface of colonies; Kolter addressed this spatial and temporal organization throughout his talk. He explained that it is not possible to get a sense of dimension from a photograph. Growth around the perimeter of a droplet of culture on an agar plate is seen in twelve hours, growth is more abundant in twenty four hours, colony growth without wrinkles appears in forty-eight hours, and in three days a fully mature biofilm is seen. The same beautiful architectural quality can also be observed in a liquid culture pellicle; the organisms make an extracellular matrix that holds the cells together in a biofilm. The wild strain NCIB 3610 has not been "domesticated" and forms this matrix while many specially selected isolates of *B. subtilis* used in laboratories today disperse easily but have lost the ability to make this matrix.

Dr. Kolter explained that the reason why *B. subtilis* is so exciting to us is because we know much about it already. Its features have been studied extensively for decades to understand how cells can differentiate. We know much about the cells types called the spore and its mother cell that eventually lyses to release the spore. A second type of cell called a "swimmer" can synthesize flagella. A third type of cell of interest to scientists is the "competent" cell. The focus of interest here is to learn how these cells can take up DNA from the environment and thus be transformed; elucidation of this may provide clues to understanding an evolutionary function. Dr. Kolter then spoke of the concept of the matrix builder; the cell that make the biofilm. The three previously mentioned cells are selfish cell types, i.e. they benefit by whatever they are doing. The matrix builder, while benefiting by protecting itself within the matrix is also putting outside a resource (the matrix) that all can share. This is interesting because it can be speculated that this may be an early example of possible cooperation between the cells-which leads to many interesting theories and debates.

Dr. Kolter went on to explain how individual cells theoretically follow different developmental pathways and differentiate within the biofilm; producing heterogeneous populations. There are four predominant types of cells: mother cell, spore, swimmer, and matrix builder. There are also the competent cells which contribute to genetic diversity, cannibal cells that kill cellmates but are immune to their own toxins, and miners that release hydrolytic enzymes to hydrolyze substrates that are needed but not generally available. It has been argued that cannibalism is important to delay the decision to sporulate. We now actually know that the cannibals and matrix builders actually arise from the same type of cell he said, and matrix builders use nutrients released by the cannibals. Regarding the nature of the shared resources, miner cells for example may put out a protease that produces amino acids from a protein for everybody to use.

We know which genes are required to make each of these cell types as well as the regulatory factors needed to turn these genes on. The fate of individual cells can thus be followed by incorporating within them a fluorescent transcriptional promoter protein which can be developed for cell type specific genes; then by following the fluorescence of the cell, we can follow its differentiation into a second type of cell. Dr. Kolter described these experiments, speaking of three cell types in particular. Swimmer cells and matrix builder are self exclusive; they are either one or the other. This happens mechanistically because there is a repressor called SinR that represses the matrix genes and indirectly activates the genes for swimmers. Sin R in turn is antagonized by a protein, called SinI, that binds it and prevents it from being a repressor. Therefore when Sin I is available matrix formation occurs. The condition under which this happens is when a transcriptional regulator SpoOA is phosphorylated (SpoOA-> SpoOA~P) because of some specific signal (hypothetical factor X) that transcribes SinI to antagonize SinR resulting in matrix and the inability to produce swimmers. When there is more signal or a different kind of signal that leads to higher concentrations of the regulator SpoOA~P, an interesting phenomenon occurs; Sin I actually becomes a repressor, matrix formation stops, and the pathway to phosphorylation is now activated. The important point is that there is a certain signal amount or a signal type that leads to a certain concentration of spoOA~P that will give you an expression of matrix genes, and later, if the concentration gets too high, the synthesis of spoOA~P is shut off and spores are made.

Dr. Kolter reiterated that for all cell types, there are gene specific promoters. Using those genes labeled with differently colored fluorescent proteins, we can observe at the individual cell level what each cell has become: for example, swimmers become blue cells, matrix builders become red cells, and sporulators become yellow cells. What we originally observed to be a homogeneous population gives rise to heterogeneous population types. The idea of heterogeneity in populations, as shown by such experiments, is something that we did not come to appreciate until recently. If you start off with a single cell that has a specific genotype and simply let it grow, if the genetic code states that only some of each population is a swimmer, matrix builder or sporulator, eventually you can observe coexisting types of populations.

Dr. Kolter then showed slides of the anatomy of these cells. If a colony is frozen, thin-sliced vertically, from the top down, and examined using both fluorescence and phase contrast microscopy, sporulation is seen at the top only. The bottom of the biofilm is blue but other cells are also present; various cells localize to distinct areas of the biofilm. At any given time, about one half of the population makes matrix and one half does not. We can actually watch the matrix builders proceed to sporulation, but swimmers don't become sporulators. First a cell becomes a swimmer, then a matrix maker, then a sporulator. This is all based on the amount of SpoOA~P present as described previously. The pathway actually has check points at each developmental point; it is almost as if each cell asks whether it has progressed through each sequential point before proceeding to the next. This can be shown by arresting matrix development; by making a mutant that won't build matrix, but still has the promoter to the matrix genes fused to a fluorescent protein. What occurs in that colony is that all the cells try to make matrix and don't proceed to sporulate. If you take that mutant and shake it in culture, it sporulates well; it is therefore completely dependent on the context of growing as a multicellular aggregate, as a biofilm.

Dr. Kolter again addressed the model he proposed: that a certain signal amount of a specific compound (hypothetical factor X previously mentioned) or a signal type will generate a certain amount of SpOA~P that will produce matrix builders. The *B. subtilis* cultures we previously saw were grown under optimal laboratory conditions where they would produce the maximum amount of matrix, but the organism can be grown in a medium where it produces no matrix at all. One interpretation of this is that what is missing is this factor X, and we should actually be able to elucidate what factor X is. But of more interest was whether there are any neighbors, or their metabolites, in

the soil that may sometimes be telling *B. subtilis* to start making matrix. *B. subtilis* may not like the neighboring cells and may make matrix for itself only, or finds it advantageous to make matrix that benefits both organisms. We don't know the answer to this yet, but reason that there may be natural compounds made by other soil organisms that will be the signal for building matrix.

Dr. Kolter's interest in natural antimicrobial activity arises from his fascination with the idea that while we use antibiotics to kill bacteria we don't know what antibiotics are doing in their specific ecological setting. His idea was to take known natural antimicrobials made by other soil microbes and add them to *B. subtilis* cells; a number of these compounds have little in common and are unrelated, but all cause the release of potassium ions that leads to *B. subtilis* matrix formation. In nature, the organism may respond to secondary metabolites to protect itself. Why this occurs is unknown; it may depend on the concentration. For example, nystatin, the antifungal made by *Streptomyces noursei* induces matrix building; so do gramicidin, valinomycin, and surprisingly, surfactin, produced by *B. subtilis* itself; all are cyclic compounds. As it happens, surfactin is a molecule made by *B. subtilis* that signals other cells to build a matrix. When grown in a specific broth, *B. subtilis* does not make surfactin or a matrix; surfactin is therefore is the missing signal (factor X) previously mentioned, that triggers the matrix genes and induces potassium leakage. And that is the answer; this is what might be called a quorum sensor, a molecule made by the organism itself that is acting upon itself. The end result is why only a certain percentage of the population becomes swimmers and signal their siblings to become matrix producers as part of this co-existing heterogeneous population. This is called "signaling" to distinguish it from quorum sensing (bacterial communication). In some way that we don't fully understand yet, that signal is transduced to activate a membrane protein kinase, *KinC*, probably because the signal is coming in and potassium is constantly leaving. This mechanism enables *B. subtilis* to respond to other bacteria; it is the concept that the organism, because of environmental conditions, makes surfactin, the signal that affects the same species. However, the organism also has the ability to respond in the exact same way to stimuli (signals) that are coming from other species. So one can speculate, said Dr. Kolter, that in nature these organisms might respond to the presence other organisms and their secondary metabolites by making matrix. We don't know if this might be a protective response or a cooperative response. Huge amounts of some of these compounds added in large amounts to some organisms will kill them; for example, gramicidin can act as a signal but we don't know what it's doing in the real world. Julian Davies has espoused for a long time that antibiotics in nature could be both signals (gene modulators) and antibiotics.

Dr. Kolter then spoke of the cyclic polyene antifungal nystatin, which induces matrix building. He digressed to say that interestingly, biology teaches us that one of the differences between eukaryotic cells and bacterial cell is the absence of steroids (with exceptions of course!). There are fascinating compounds called the hopanoids, which contain polycyclic terpenoids that regulate plasma membrane permeability in bacteria, much as cholesterol does in eukaryotes. These molecules, precursors of steroids and sterols in animals, are present in everything living and are so recalcitrant to degradation that they form one of the major components of sediments on the planet; sterols are also recalcitrant. What do molecules like ergosterol, found in fungal cell membranes, primarily yeast, and chemically related cholesterol found in mammalian cells, do? Nystatin can be used as an antifungal agent because it interacts with ergosterol and creates a pore that causes potassium leakage, eventually leading to cell death. So how can it be active on *B. subtilis*? As we saw earlier, it causes the release of potassium ions that leads to *B. subtilis* matrix formation. Dr. Kolter now showed how ergosterol can easily be synthesized from the organic compound squalene, which is important in sterol synthesis. The biochemical pathway includes the intermediate isopentenyl pyrophosphate; squalene synthase then combines two molecules of farnesyl pyrophosphate into squalene, which doesn't

look much like ergosterol or cholesterol if you just look at shapes of molecules. But these structures are flexible and all that is needed no to produce ergosterol is a cyclase and a number of additional steps. No one had previously looked for a compound like squalene synthase in *B. subtilis*. When investigating this, it was discovered that a natural product, zaragozic acid, when added to *B. subtilis*, indeed inhibited matrix production. The compound, which inhibits squalene synthase, is made by *Streptomyces intermedia.*

Returning to the question as to what molecules like cholesterol and ergosterol do in cells, he explained that eukaryotic cell membranes contain microdomains called lipid rafts-which contain large amounts of cholesterol; carotenoids and other lipids also converge in microdomains. These are associated with cell processes such as signaling and protein secretion. Upon investigating whether bacteria had microdomains (lipid rafts), microdomains in the membrane were indeed found and then sequenced. They contained enzymes for signal transduction and molecules such as *KinC* which signals for *B. subtilis* matrix production, *Opp*, a quorum sensing molecule, squalene synthase and other compounds. Dr. Kolter also added that wild type *B. subtilis* had different proteins in its membrane than laboratory strains. Any of these can be disturbed by molecules which disrupt their function and thus affect the bacterial cells. The interactions of all the pathways Dr. Kolter mentioned in his talk are actually much more complex than he described in this lecture.

Can some of these ideas have medical applicability in humans? Compounds such as cholesterol lowering drugs might be used to disrupt/inhibit biofilms that cause chronic disease. Biofilms are composed of many bacterial species that are difficult to eradicate and can be found in diabetic ulcers on the foot, lung disease, cystic fibrosis, nose, throat, and intestinal diseases, and human implanted devices for example. The use of natural antimicrobial compounds to coat IVs or human implanted devices should be considered (such as the use of zaragozic acid to coat catheters). The current cost in industry to reduce industrial surface contamination is high; the protection of boat surfaces for example is only one concern. There are natural compounds, non-toxic for humans, such as genes for anti-virulence and anti-biofilms to which organisms cannot develop resistance that should be investigated.

Dr. Kolter concluded by saying that we can only speculate what happens in the environment with *B. subtilis* and other organisms, or what happens in the human microbiome. We know very little about the complex interactions between organisms and how they respond to changing surroundings. As we begin to explore other organisms we may find others that differentiate like *B. subtilis*. Much needs to be learned!

NEB Education Chair Gregory Reppucci (3rd from right) and North Shore Community College students at the Genzyme Center lecture

The New Chief of "Staph", the "Difficile" Bacillus and Other Pending Disasters

The fifth and final NEB dinner-lecture of the year was held on November 12, 2009 at Woodland Commons, UMass Dartmouth, North Dartmouth, MA, and was sponsored by the Northeast Branch and University of Massachusetts Dartmouth Department of Medical Laboratory Science Student Association. Stephen M. Brecher, PhD, Director of Microbiology, VA Boston Health Care System, West Roxbury, MA, spoke on *The New*

Chief of "Staph" the "Difficile" Bacillus, and Other Pending Disasters. He mentioned that the opinions in his presentation are his own and do not necessarily represent the views of the Veterans Affairs Health-Care System.

Dr. Brecher began his lecture with two case presentations of *Clostridium difficile* infections (CDI). One elderly female was hospitalized and treated with levofloxacin for community acquired pneumonia; in six days she developed seven 7-14 loose bowel movements and a rising WBC. Stool for *C. difficile* EIA toxins A and B were negative; this was later resolved as the test was negative because the stool was not refrigerated. Her physician thought the test should be positive and should have treated her because she had the hallmarks of severe disease.

An elderly male was hospitalized community acquired pneumonia, treated with levofloxacin, discharged, hospitalized elsewhere seven days later with abdominal distension and hypertension. He was afebrile, without diarrhea, and was given ciprofloxacin to complete the treatment for pneumonia. He was discharged three days later, returned again in three days with fever, a high WBC and admitted with *C. difficile* as a possible diagnosis but not treated. Complications occurred; on day four he had toxic megacolon and he died on day five.

Dr. Brecher then described the symptoms of CDI which include asymptomatic colonization, mild to severe diarrhea, abdominal pain, distention, fever, pseudomembraneous colitis, toxic megacolon and perforated colon leading to sepsis and death. He also listed the markers of severe disease among which are leukocytosis, more than ten bowel movements daily, hypertension, pseudo-membranous colitis, toxic megacolon, severe distention and abdominal pain. He mentioned that these markers are more important than laboratory testing for the organism and remarked that both patients in the preceding case studies should have been treated aggressively for CDI. The female patient's specimen was left at room temperature for 24 hours, and the male patient should have been treated with oral vancomycin much sooner.

Historically, in the 1960's, when patients on antibiotics developed diarrhea it was called staphylococcal colitis and thought to be caused by *Staphylococcus aureus*; stools were cultured routinely for *S. aureus* only and treatment consisted of oral bacitracin. A new explanation was found in the early 1970's; severe diarrhea, pseudo-membranous colitis, and occasional deaths occurred in patients on clindamycin and *C. difficile* was isolated from these. The organism received its name in 1935 when it was cultured from healthy neonatal, with difficulty, and was called *Bacillus difficilus* (now *C. difficile*); therefore it took a long time to associate the organism with the disease. Antibiotic treatment in general disrupts normal intestinal flora which plays a major role in food digestion and may cause diarrhea. In recent years an epidemic strain of *C. difficile* was found which was highly resistant to fluoroquinolones, had binary toxin genes and produced large quantities of toxins A and B; it had a tcdC gene deletion. Although numerous case reports have been published on epidemic strains describing them as extremely virulent, there are also reports to the contrary. Dr. Brecher described a third case study of a 31-year-old pregnant female with twins who was seen in a local emergency room with a history of three weeks of intermittent diarrhea, and three days of cramping and watery diarrhea. Her stools were positive for *C. difficile* toxin; she was admitted, treated with metronidazole and discharged. She was readmitted the next day with severe colitis, treated for 18 days with metronidazole, oral vancomycin, cholestyramine and discharged. She was readmitted four days later with diarrhea, hypertension, and spontaneously aborted her fetuses; in spite of aggressive therapy she died on the third day. Post-mortem showed toxic megacolon with pseudomembranous colitis. Dr. Brecher mentioned that we really know nothing about community-based CDI and testing for *C. difficile* is now done both on inpatient and outpatient basis; risk factors other than antibiotic use must be considered. CDI is not a reportable disease therefore the exact number of cases and deaths remain unknown but we do know that CDI cases have tripled over the last five years. The exact causes of fatal outcomes are not really known.

A number of tests are available for the laboratory diagnosis of *C. difficile* infection, including stool culture, molecular tests (PCR or Loop-Mediated Isothermal Amplification [LAMP]), toxigenic culture (culture and cell culture neutralization essay [CCNA]), glutamate dehydrogenase (GDH), enzyme immunoassay (EIA), and the cell culture neutralization essay. When determining which tests to use, accuracy, time to detection, the prevalence in your population, cost, and ease-of-use should be considered. Dr. Brecher emphasized that at this time there is no perfect test for the diagnosis of CDI. As specimen quality influences test results, he suggested some guidelines to follow when testing for *C. difficile*. First is to accept only liquid cultures or soft stools as there are no formed stools in *C. difficile* disease, and then to limit repeat testing once the patient is positive because it is not clinically relevant. The organism can be present in asymptomatic individuals and carriage can continue. A fresh specimen tested within two hours of collection is best; but it can be refrigerated at 4°C up to three days or frozen at -70°C if testing is delayed.

The "Gold Standard" for testing appears to be the cell culture neutralization assay but this has been questioned recently. Conflicting results have been obtained with EIA, the advantages being that it is rapid, inexpensive, and relatively easy, does not require costly equipment and can be run in batches or a single test format. Disadvantages are great variations in published sensitivity and specificity, technologist error and contamination. There is a question as to whether EIA is accurate enough to be used as a screening or confirmatory test. Glutamate dehydrogenase (GDH) can be used as a screening test to detect nearly all true and false positives; and works best in a low prevalence population. Molecular-based assays may be the test of choice in the near future. Considerations here should be ease-of-use, capital equipment, can it be used as a single test, accuracy, whether multiple targets unnecessary, and cost. There are three assays based on the polymerase chain reaction; Prodesse and BD have FDA approved tests and Cepheid has a test proved only for one gene (Toxin B). There is also the Loop-Mediated Isothermal Amplification (LAMP) assay. Dr. Brecher then discussed results attained using EIA versus Clinical Judgment, and PCR versus Clinical Judgment. He found that EIA has a high number of false positives in the low positive range and by changing the positive cutoff value from 0.100 to 0.130, 11/18 specimens were reclassified as true negatives. The Meridian assay correlated nearly 100% for values below 0.100 and above 0.200. PCR performed well but had four false positives. Dr. Brecher based his decision as to what testing would be done in his laboratory on the fact that PCR is costly but highly sensitive and specific and EIA works most of the time. He created a two-step algorithm and now the laboratory tests low EIA positives by PCR. They have a very strict stool rejection policy regarding solid and semisolid specimens in order to cut down on colonization positives by PCR. To date the two-step algorithm is working well. PCR costs significantly more but results are available within 60 minutes and the test is easy to perform. He stressed that the cost of testing is only a small part of the cost of treating a patient for CDI in an intensive care unit.

Dr. Brecher now focused his attention on the staphylococci. He mentioned that keeping up with the changing break points for staphylococci is quite challenging. Case report 4 was a 44-year-old male admitted from the emergency room with a diagnosis of possible right-sided bacterial endocarditis and a history of drug abuse. Blood cultures were positive for Gram positive cocci in clusters eighteen hours after admission and within one hour the laboratory was able to report methicillin-resistant *S. aureus* (MRSA). Primers released in 2009 for three gene sequences (spa gene, mecA gene and SCCmec gene) now allow the testing of Gram positive cocci directly from blood cultures by rapid PCR, in 60 minutes.

The test cost is $65-$75, however equipment costs over 100K, but clinically this is invaluable because you can optimize antibiotic therapy very quickly based on the results. Dr. Brecher then reviewed staphylococcal resistance standards according to CLSI M100-S19, 2009 Table 2C. He reviewed cefoxitin and oxacillin disk tests for mec-A-mediated resistance, cefoxitin and oxacillin MIC breakpoints for mec-A-mediated resistance, cefoxitin disc

diffusion for mec-A-mediated resistance in coagulation negative staphylococci, and oxacillin MIC tests for for mec-A-mediated resistance collective waste negative staphylococci. He mentioned that the cefoxitin disc diffusion test works better than the oxacillin disk in detecting isolates with mecA because mecA is expressed at higher levels in the presence of cefoxitin as compared to oxacillin. In summary the cefoxitin disc diffusion test can be used to predict the presence or absence of mecA in staphylococci especially in the central nervous system, and cefoxitin and oxacillin MIC methods both reliably detect mecA-mediated resistance in *S. aureus.*

Dr. Brecher now turned his attention to vancomycin and staphylococci and reviewed the new CLSI vancomycin breakpoints. Heteroresistant vancomycin-intermediate *S. aureus* (hMRSA) represent subpopulations of lesser susceptible organisms within a population of susceptible organisms that are not detected or inconsistently detected by standard MIC tests. It is difficult to differentiate strains with MIC's between 2-4. The CLSI guidelines for detecting vancomycin resistance in staphylococci state that MIC tests should be performed although they are not always reliable. Isolates with an MIC greater than or equal to four should be sent to a reference laboratory. The CLSI deleted the vancomycin disk tests in 2009 because they do not differentiate vancomycin susceptible strains from intermediate strains, do not differentiate among S, I, and R in CNS, and the test detects isolates containing vanA. Dr. Brecher then spoke of method specific differences using E-Test, MicroScan, Phoenix, Vitek, agar dilution and Sensititre.

S. aureus fully resistant to vancomycin (VRSA) was reported in MMWR in July, 2002; the MIC was >1024. This was the first isolate to naturally acquire the vanA gene from *Enterococcus faecalis*. There have been an additional nine cases as of November, 2009; 7/9 were in Michigan and do not appear to be clonally related. This has not yet spread widely, perhaps because *S. aureus* has an enzyme system that protects it from foreign DNA, and for gene transfer from enterococci to occur staphylococcal strains must have mutations that allow foreign DNA to integrate. Whether to screen or not screen for MRSA remains a controversial subject.

Case study number five with the 64-year-old male who is admitted to a local emergency room with a lesion on his leg. He said he was chopping wood outdoors when he was bitten by a spider however he admitted he did not actually see a spider bite him although there were many around. The question was whether he should be treated for a spider bite. Dr. Brecher then showed a number of slides depicting community acquired MRSA (CA-MRSA) skin and soft tissue infections which are increasingly being seen in the US; this is currently the most common pathogenic organism seen in the Emergency Room, in epidemic proportion.

CA-MRSA causes a variety of clinical syndromes, several of which include skin and soft tissue infections (spider bite abscesses), necrotizing fasciitis, septic thrombophlebitis and rapidly progressive necrotizing pneumonia. Outbreaks have occurred in prisons, among high school and college football and wrestling teams, military recruits, injection drug users, in pediatrics, and even globally. Many victims are young healthy people going about daily activities. There were four deaths and two hundred documented cases of MRSA among children ages 1-13 in Minnesota and North Dakota. In the fatal cases, the initial isolates were resistant to oxacillin and other beta-lactam antibiotics. Dr. Brecher pointed out that the organism differs from hospital acquired MRSA in that nasal colonization may not be a factor in its spread; the major source of infection may be skin to skin contact and fomites (whirlpools, razors, towels, etc). Many strains carry Panton-Valantine leukocidin (PVL) genes that encode for a cytotoxin associated with tissue necrosis, leukocyte destruction, necrotic lesions and abscess formation. Reinfection and transmission to family members commonly occurs. Although several strains of *S. aureus* cause CA-MRSA infections in the US, strain USA300 appears to be more virulent and easily spread. Treatment of these infections consists of first performing susceptibility studies. The organisms are usually susceptible to rifampin and trimethoprim sulfamethoxazole and most are susceptible to clindamycin and tetracycline; about 60% are susceptible to fluoroquinolones.

An important factor in treating skin and soft tissue infections is incision and drainage, thus controlling the source of the infection.
The lecture ended with Dr. Brecher showing a slide of the Cocci Market in Italy, then proceeding to make us all laugh with the final slide about male genetics. The audience was simultaneously entertained and educated; Dr. Brecher's mix of humor and fact was very well received.

Career Night

(L to R) Harvey George, Linda Foote, Marcia Walsh, Trish Dawson, Vincent Mucci and Gregory Reppucci

The final Northeast Branch program of the year was *Microbiology Careers Night*, which was held on Monday, November 16, 2009 at the Sakowich Students Center of Merrimack College in North Andover, MA. Approximately 35 people from Merrimack College, North Shore Community College, Salem State College, and Middlesex Community College attended this interesting evening at which four professionals described different career paths in Microbiology. Career Night was organized by Gregory Reppucci from North Shore Community College and Marcia Walsh, from Merrimack College, with financial support from the American Society of Microbiology.

The first speaker was Trish Dawson, Food Microbiologist, from Stoneyfield Yogurt in Londonderry, NH. She received her degree in general microbiology and graduate degrees in applied science of food microbiology. After graduation she started working in chemistry but found it too repetitive, and so went on to more adventurous undertakings. She began working in food and wine microbiology and found that she had a passion for food microbiology, recognizing that after all is said and done, people must eat! She spent the next ten years in this area. She became interested in food safety and spoilage, and the organisms responsible for fermented food products such as beer, wine, cheese, bread, pickles, salami, and sauerkraut. In dairy microbiology fermented milk is used to produce cottage cheese, yogurt and cheese. She explained that sophisticated technical support is needed to develop and maintain the organisms critical in the production of about 800 types of cheese. This experience led to her employment at Stoneyfield Yogurt. She pointed out that in order to repeatedly make any specific type of yogurt, or to improve the product, one must have tight control of the pH, a pure seed culture of the specific organism, and the precise culture time, 4 to 5 hours. Ms Dawson is currently the Department Head and has 13 people in the laboratory; four of which are microbiologists. They address problems such as food safety and spoilage, equipment sanitization, and Hazard Analysis of Critical Control Points (HACCP), i.e. using risk based assessment to identify potential sources of contamination or any other hazards. Agencies such as the FDA are also involved in regulating consumer food products and regularly inspect the facility. The laboratory role is to assure consistency, safety and nutrition of the products for public consumption. They do this by carefully monitoring cultures used to produce the products, and check products for flavor, pH, etc. Stoneyfield purchases its raw materials, such as milk and starter cultures from outside suppliers. Dr. Dawson summarized her talk by emphasizing the large number of opportunities in the food industry, including microbiology, quality control, teaching and sales.

Vincent A. Mucci, Senior QC Manager, Biopharmaceutical Analysis Microbiology, Pfizer Corporation, Andover, MA was the next speaker. (The Corporation was formerly Wyeth and was acquired by Pfizer a month ago). He has a BS in biology and MS from the University

of Massachusetts in Amherst. Starting in the laboratory as a laboratory technician, he has worked for 30 years in the areas of industrial quality assurance and quality control. His experience includes environmental monitoring to ensure products distributed to the public remain bioburden free. He has been both a supervisor and laboratory manager, and provided examples of a *good, bad and ugly day* in industry. On a good day, there are no testing failures and your products are approved by the FDA. On a bad day there may be a failure in sterility. On an "ugly" day, you may detect mycoplasma which is difficult to remove from pharmaceuticals, and your product may not be approved after millions of dollars are spent to develop it! He described a career in industry which may start with laboratory rotation and work in microbiology, then progressing to quality assurance that encompasses compliance and validation, and from there to research process development in the manufacturing area, or even to regulatory affairs. Positions available in quality control start at a technician level with a BS and may lead to a scientific or managerial ladder. In order to progress, you need to learn how to multitask and wear many hats, and to problem solve. Good writing skills and good communication skills are essential, and you must learn how to "think outside the box" in order to do good investigative work. You can learn how to operate laboratory equipment. i.e. in entry-level quality control positions, associates do routine assays such as microbial identification or gene sequencing. He concluded by saying that you must be passionate in your work, and must always remember that whatever you are doing, however inconsequential you think it is, your work *always* affects patients.

Linda C. Foote, Ph.D., Program Manager, Harvard Vanguard Infection Control Program, Harvard Vanguard Medical Associates spoke of her career. She received an MT degree after three years of undergraduate lecture/laboratory-based studies with a twelve month internship in an affiliated hospital. She then went to the Boston Veteran's Administration Hospital to work as a generalist in medical technology, now seeing how things learned in school actually worked in the laboratory. After working as a clinical laboratory technologist, she served as both an adjunct and tenured professor at Merrimack College. She did graduate work at Harvard University (MS) and PhD and Boston Medical School. After teaching at Merrimack College for ten years, she started a new career at Harvard Vanguard in Infection Control, a group consisting of about 300 physicians at twenty different sites. The infection control program consists of a team of four people who perform clinically based work that is used to make diagnostic treatment decisions.

During Dr. Foote's training and working at major Boston Teaching hospitals, she had the opportunity to become proficient in chemistry, hematology, microbiology, blood banking and quality control. She also presented research findings at American Society for Microbiology Annual Meetings. Her microbiology experience gave her first hand exposure to both local and international patients. This broad patient contact enabled her to become experience with a variety of exotic and non-routine disease, including cytomegalovirus and lung infections which the medical community was not aware was the beginning of the HIV epidemic. She also worked as supervisor at Hunt Memorial, a small community hospital, for four years.

In her current position in infection control at Harvard Vanguard, her work includes monitoring when only drug resistance patterns and not the identification of an organism is known, providing staff and program members with health screening, and providing vaccines and vaccinations for both seasonal flu and influenza H1N1. Her department also runs a prevention campaign that emphasizes handwashing and educating people on droplet transmission and precautions. They also operate a Travel Clinic, and provide advice and immunizations for people preparing to travel abroad. Dr. Foote emphasized that in all of the work experience and areas she has worked in, there are ample opportunities for employment.

Harvey George, PhD spoke of his four careers encompassing various scientific disciplines. The three preceding speakers were microbiologists but he received a BA from Cornell University and an MS and PhD in chemistry from The University of Tennessee. Dr. George described a distinctly non-typical

career, having worked in research (biochemistry, microbiology and organic chemistry), education (taught seminar courses at Boston Medical School and Northeastern University), clinical laboratory science (Director of Clinical Laboratories at Lahey Medical Center), Public Health (Director of the Clinical Laboratory for the Massachusetts State Laboratory Institute) and currently in forensic toxicology (Director, Calloway Laboratories). He made the point that although he is not a microbiologist in the classical sense, by virtue of continuing education and keeping up with the literature, he has been able to make significant contributions to all of these fields. He pointed out that laboratory science is a very broad area, involving many disciplines, all of which require a fundamental education in the physical sciences, for example chemistry, biochemistry, microbiology, mathematics, etc. There are numerous opportunities for employment in research and clinical practice. In fact, many institutions are currently paying finders fees of up to $5000 for qualified medical technologists.

Additional Northeast Branch Activities – 2009

The NEB annually donates an award of $100 to each of five MA regional fairs and the VT science fair, and $200 to the MA Science Fair. This year we also supported the Darwin Festival held annually in February at Salem State College, and co-sponsored the 61st Annual Meeting of the Clinical Laboratory Science Society of Central New England (ASCLS/CNE). The Branch likewise presented a seminar entitled *Influenza from a Human, Animal and Laboratory Testing Perspective* at the 44th Region I Meeting hosted by the Connecticut Valley Branch in Cromwell, CT in October. Sandra Smole from the MA Dept of Public Health convened the session. Speakers were (1) Ed Balkovic, Genzyme Corp. on C*urrent Understanding and Status of the Influenza A (H1N1) Virus Pandemic* (2) Catherine Brown, MA Dept. of Public Health, on *Influenza A Viruses in Non- Human Animals* and (3) Sandra Smole, MA Dept. of Public Health, who spoke on *Initial PHL Response to the Pandemic H1N1 (2009) Virus: Diagnostics and Surveillance*

ASM Website Updated

Please visit the newly upgraded ASM website, http://www. asm.org. One prominent feature is a large blue button, “ASM Community", where you can find the newest technological tools in social networking made available to members and branches through the ASM Community website, http://community.asm.org.

ASCP and NCA Unite

The American Society for Clinical Pathology (ASCP) Board of Registry (BOR) and the National Credentialing Agency for Laboratory Personnel (NCA) on July 21, 2009, signed an agreement forming a single certification agency for medical laboratory professionals. The agency will be called the ASCP Board of Certification (BOC). The agreement is effective on Friday, October 23, 2009. At that time, the NCA will be dissolved as a Corporation.

ASCP provides answers to questions regarding the transition on their website, http://www.ascp.org.

American Medical Technologists (AMT), through December 31, 2009, is extending a hand to lab professionals holding the certification credential NCA. From their website you can download and complete an application for MT or MLT, and with documentation of having passed and NCA generalist exam for CLS or CLT, AMT will extend MT(AMT) or MLT(AMT) certification and membership free of charge.

From: Advance for Medical Laboratory Professionals, July 27, 2009 and MLO. September 2009

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Has your membership expired?