A REPORT FROM THE AMERICAN ACADEMY OF MICROBIOLOGY

RESEARCH OPPORTUNITIES IN FOOD & AGRICULTURE MICROBIOLOGY

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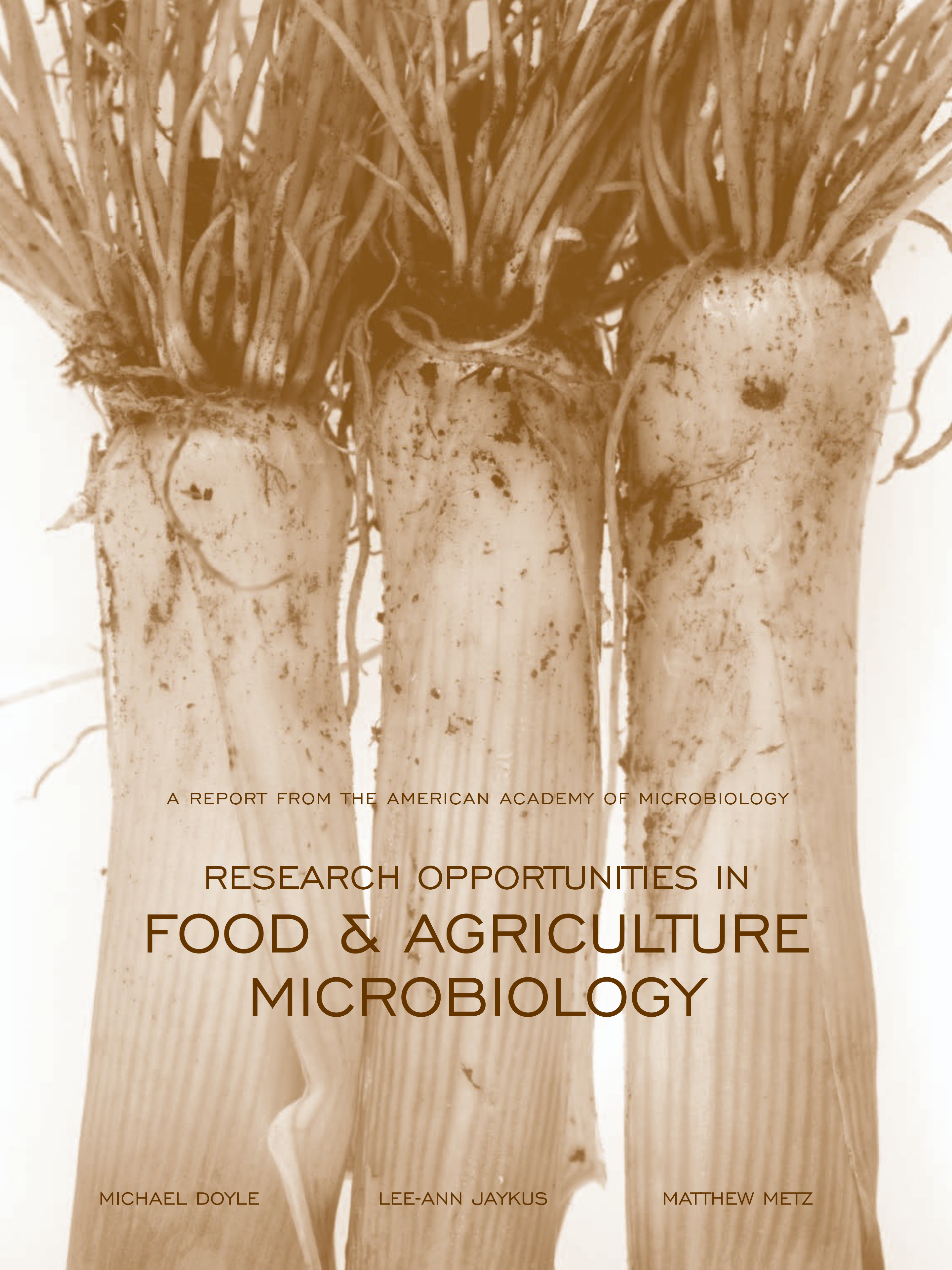

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EXECUTIVE SUMMARY

This report presents a wealth of research opportunities in food and agriculture microbiology. The backdrop for these research opportunities is a world of microorganisms teeming with threats and benefits to abundant, healthy food and associated environments. Threats come from microbial pathogens that perpetrate a wide range of plant and animal diseases, destroying agricultural productivity. The constant spread and evolution of agricultural pathogens provides a continually renewed source of challenges to productivity and food safety. Pathogens continue to cause harm once food has left the farm, causing spoilage, and in some cases poisoning and diseases of humans and animals. New vulnerabilities are generated for agriculture by global movement of agricultural products, trading policies, industrial agricultural practices, and the potential for malicious releases of pathogens by "bioterrorists." In addition to the threats, benefits also come from the many microorganisms associated with, or introduced into, our food supply where they serve important roles in bioprocessing, fermentation, or as probiotics.

Science and technology emerging from microbiology research can help meet these challenges to food and agriculture. Knowledge of microbial pathogens will lead to tools for surveillance and disease prevention. Beneficial microbes may find uses in protecting agriculture, preserving food, enhancing the value of food products and providing general benefits to health and well being. Complex interactions among microbes and agricultural systems must be better understood to facilitate the optimal use of beneficial microorganisms and maximal control of pathogens.

Opportunities in microbiology research are the gateway to sustaining and improving agriculture and food production, quality, and safety. Multidisciplinary research must be undertaken to capitalize on advances in different disciplines, such as genomics, nanotechnology, and computational biology. Research into the interactions of animal and plant hosts with pathogens and beneficial microbes is essential to preventing disease and encouraging mutualistic interactions. On a more holistic scale, interactions occurring among organisms within a microbial community require study so that a healthy balance between the highly managed ecosystems of industrial agriculture and the unmanaged ecosystems of the natural environment can be achieved. Finally, research is critical to determine why pathogens continue to emerge and where and how newly developed technologies should be put to use.

Barriers to seizing these research opportunities must be overcome. The lagging priority of food and agriculture research will be reversed as funding programs and research institutions recognize its importance and improve resources, infrastructure, and incentives accordingly. Endeavors, such as long-term research projects and the banking of diverse microbial specimens, require support so that a foundation of future innovation and discovery is established and sustained. A decline in the number of young scientists entering the fields of food and agriculture research will have to be reversed with funding and fellowship opportunities to provide a highly trained core that will carry out the research of the future. Regulatory hurdles impose stringent processes for research on certain organisms, but are viewed as out of step with actual hazards and must be revised consistent with scientific assessment of risk. Changes that are needed will have to be advocated by scientists, research institutions, professional societies, non-governmental institutions, and companies that are committed to food and agriculture.

This report offers recommendations for research priorities and identifies barriers to a strong food and agriculture research agenda.

BACKGROUND

Microbes permeate the entire food and agricultural process. While the most visible role of agriculture is probably that of producing and delivering food, microbiology is critical to other agricultural sectors as well, e.g., for production of energy and for bioremediation of agricultural wastes. Some microorganisms are a constant source of trouble for agricultural endeavors, while others are an integral part of successful food production. Microbial influences on food and agriculture have produced both advancements and disasters that have punctuated human history. Some examples of microbe-driven outcomes set the stage for describing how important it is to seize research opportunities in food and agriculture microbiology.

MICROBES ON THE FARM & IN OUR FOOD

In the fall of 1844 a horde of hungry microbes, whose name *Phytopthora infestans* was earned from the damage they were about to cause, lurked in the soil of Western Europe. This pathogen causes a disease known as Potato Late Blight, and over the next several years they spread to the fields of Ireland where subsistence farmers were completely reliant on growing potatoes. Devastation of the Irish potato crop led to a terrible famine where almost one million people died and more than twice that many fled their country in abject poverty. A new variant of this blight emerged in the United States in the 1980s, causing serious losses and even bankruptcy for modern potato growers.

The relationship of microbes to the human food supply also includes many examples of organisms that preserve rather than destroy. Early Mediterranean societies discovered that fermentation could be used to help create yogurt and cheese from dairy products. These products were flavorful, safe, and could be stored for extended periods of time. Different types of bacteria and fungi are now known to be involved in fermentation processes. For example, fermentation of sugars in extracts from grain or grape juice produces alcohol that serves as a preservative and provides its own added value. The ancient Egyptians are frequently credited with inventing beer.

Every category of microorganism has members that impact food and agriculture. These include bacteria, single-cell organisms without special compartments for storing their genes; fungi, which can be single- or multicellular, and like plants and animals store their more complex genomes in a compartment called a nucleus; and viruses, which are little more than an infectious set of genes that must operate inside a host cell to reproduce. Among all of these different organisms there are those that benefit agriculture and food, enhancing productivity or nutrition through their interactions with plants and animals. Some microorganisms provide benefit by virtue of their ability to harm other organisms that would cause damage or spoilage if not disrupted. Agriculture and food are prey to many microorganisms that, in the course of their life cycles, destroy crops, animals, and foodstuffs. Some of these microbial pathogens create toxins or are infectious enough to cause disease in humans exposed to the products they have tainted.

MICROBIOLOGY RESEARCH IN FOOD & AGRICULTURE

The wide-ranging impact that microbes have on agriculture and food has always been, and is expected to remain, a challenge. To eat and survive, humans have answered this challenge with ingenuity. Answers sometimes begin as empirical approaches to problems, like the early development of fermentation. Such solutions are subject to improvement and refinement through scientific study and discovery. The better the understanding of the living organisms involved in the agriculture and food chain, the better equipped people are to steer the course of these interactions in our favor.

Basic research is a critical driver for innovation in agriculture and food. For example, despite centuries of using fermentation to ward off spoilage, people still found their wine and beer spoiling over time. In the mid 1800s Louis Pasteur was embroiled in a scientific conflict, disputing the favored belief at the time that microorganisms could appear through “spontaneous generation” in nutrient broth. To disprove this contemporary view, Pasteur devised a method for heat sterilizing broth and keeping it sealed off from contamination. Lengthy demonstrations that the treatment prevented any growth of microorganisms, however, did not win his theory immediate acceptance in the intellectual community. The French navy, then struggling to deal with spoilage on its ships and eruptions of mutiny due to shortages of wine, was ready to perform a large scale test of the principle. “Pasteurization” proved effective, and a basic research problem led to a fundamental technological advancement.

REAPING BENEFITS FROM RESEARCH

Technological advancements do not always find immediate or the most opportune applications. In the case of pasteurization, the technology was shown to effectively rid milk of dangerous pathogens before the end of the 19th century. Despite promotion of the benefits of pasteurization, it was adopted very slowly due to reluctant producers and suspicious consumers. Milk remained responsible for one quarter of all food borne illness throughout the first third of the 20th century in the United States. Wide scale use of pasteurization now provides the invisible benefit of a much safer food supply. In another example, the new technology of genetic modification or engineering has led indirectly to decreases in mycotoxins produced by fungi in some growing crops. These toxins are highly detrimental to animals and humans, including being implicated in several cancers.

On the opposite end of the spectrum, some technologies experience rapid and extensive adoption before their impact is sufficiently understood. Extensive use of antibiotics in livestock and poultry production came into practice to protect large populations of closely quartered animals from infection, and for its poorly understood growth-promoting effect. The practice is associated with the appearance of some strains of antibiotic resistant microbial pathogens. Furthermore, this practice may provide a pool of resistance genes that can be transferred among organisms in both the gut of animals and the production environment.

The contribution of research towards providing a plentiful, healthy, and safe food supply reaches beyond the cycle of basic research and applied science. Research is also required after development of a technology to direct its prudent or appropriate use. For example, research predicted the selection of antibiotic resistant microbes in agriculture, but did not predict the potential consequences of changing the formulation and processing methods for feed used in British cattle production. Most scientists believe that the origin of bovine spongiform encephalopathy, the so-called "Mad Cow" disease, was the supplementation of cattle feed with animal protein derived from other ruminants. This resulted in a disease caused by a replicating protein that was propagated through British cattle herds and was subsequently epidemiologically linked to a deadly neurological condition in humans. Assurances of the safety of the meat supply were provided without scientific backing as an animal epidemic gained momentum. As of 2003, there have been over 180,000 confirmed diagnoses of BSE in British cattle and the most recent 2005 statistics cite over 100 confirmed deaths from variant Creutzfeldt-Jakob disease, the human form of the disease believed to be linked to BSE.

In an endeavor such as food production and distribution, tension is always present between technological advances and avoidance of unintended consequences. This tension can be heightened when disasters, such as the Mad Cow episode in Britain, are amplified by policies made without sufficient scientific understanding. Even with scientific understanding of benefits and risks, a technology may be scuttled by lingering mistrust or poor public understanding of complex issues. This is part of what caused the slow adoption of pasteurization, and still has the adoption of food irradiation mostly hamstrung in the United States. Irradiation, proven for decades to destroy pathogens in spices and food and protect against spoilage, lacks a confidence-inspiring

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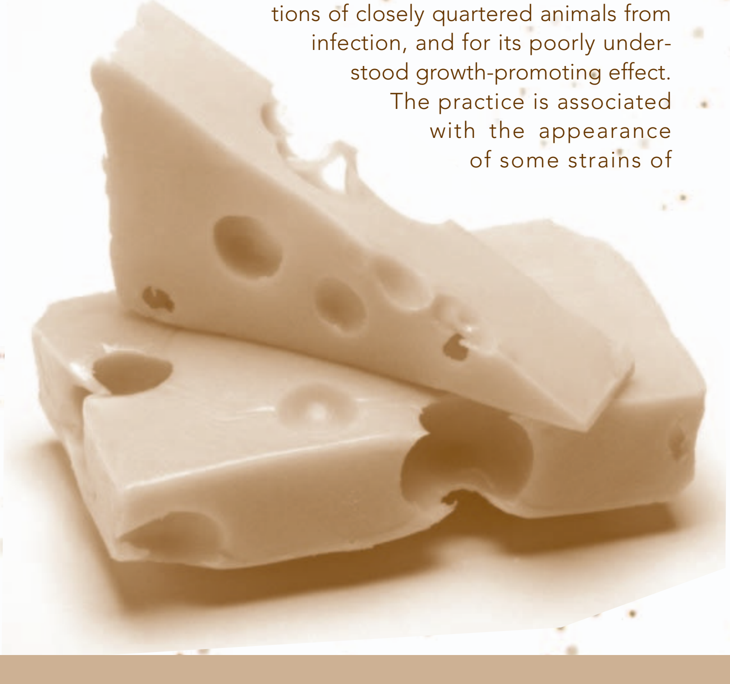

name and popular understanding. Similarly, the use of modern molecular biology to add genes into crops, animals, or microbes, creating so-called Genetically Modified Organisms (GMOs), has met with a cool reception in many parts of the globe. Despite their benefits in terms of production, reduced pesticide use, and now a record of safe use after one decade, there remains rancorous dispute about the potential risks of GMOs. Government-sponsored research and oversight, with reasonable transparency in some countries, has enabled commercialization of several crops. Use of rigorous science to study risks and weigh them against the benefits of any new technology has not yet been convincing enough to diffuse the tension that interrupts progress.

Nineteen scientists with expertise in areas ranging from plant pathology to food microbiology to microbial ecology were brought together for a two-and-a-half-day colloquium to examine the future of food and agriculture microbiology. Their deliberations and conclusions are captured in this report.

MICROBIOLOGICAL CHALLENGES TO FOOD & AGRICULTURE

An abundant and healthy food supply is expected from our agricultural systems. Fulfilling this demand is a complicated process involving plant cultivation, animal husbandry, soil and water management, harvesting, processing, storage, and transport. At each step the microbial world presents obstacles to success.

MICROBIAL DISEASE

Disease-causing microbes continually assault the animals and crops that humans raise for food. These unwelcome guests make their living off of our agriculture as well. Each animal or plant that we raise is host to an assortment of bacterial, viral, and fungal pathogens. One of the more famous examples of viral diseases in animals, Foot and Mouth Disease, infects cloven hoofed animals such as cattle and sheep. It is extremely contagious and persists in susceptible animals in the wild and in husbandry. The severe blisters and cankers caused by the virus are usually not deadly, but the lifespan and productivity of infected animals is severely reduced. The disease is dreaded globally as a problem for trade because it can be spread easily, not only by sick animals, but by meat products and even on clothing.

The most virulent diseases are crippling or deadly to agriculture, draining or even annihilating a crop harvest or animal population. Some of these diseases have consequences for humans reaching beyond their impact on the availability or expense of our food. Pathogens known as "zoonotic" are those that can be transmitted from animals to humans and include transmission via vectors (i.e., insects, rodents), food, or water that have become contaminated from these animal sources. The most notorious contemporary example is Anthrax. This soil dwelling bacterium can kill cattle and other herd animals that encounter it while grazing. It is also known to cause skin lesions, gastrointestinal infection, and serious systemic disease in people exposed to infected animals. This bacterium has some choice properties as a potential biological weapon, including deadly toxins it produces while growing in the warm tissues of an animal host. In the fall of 2001, illnesses and deaths resulted from letters containing special preparations of Anthrax spores, which infected individuals who had contact with the contaminated mail.

Plant diseases also impact humans. Most significantly, fungi leave behind toxins poisonous to people and animals that eat them, as well as to the host plant. As unlikely as it seems, there is some evidence that plant pathogens can also be infectious to people. The greatest number of documented cases so far are pathologies found in the immune compromised, but an increasing number are associated with apparently healthy humans. However, this is a neglected field of study, and it is not known how widespread or important such infections might be and with what types of syndromes these agents might be associated.

Fungal, bacterial, and viral pathogens are problems in any system where dense populations of the same kind of plants or animals are being cultivated for food. This principle extends beyond fields and pastures, to areas like ponds or net-cages, where aquaculture is performed, and caves, where mushrooms are grown. Diseases that assail agriculture can also be more complicated than an infection by a single pathogen; polymicrobial diseases result from the compound effects of multiple pathogens acting together. Diseases of this kind can be more difficult to predict, diagnose, and respond to than those caused by one organism alone.

> “FOOT AND MOUTH DISEASE OF LIVESTOCK COMES IN ABOUT 80 DIFFERENT “SEROTYPES” AROUND THE WORLD; EACH ONE IS SEROLOGICALLY DIFFERENT.”

MICROBIAL PATHOGENS MOVING & MORPHING

Pathogens have a variety of ways to invade agricultural plants, animals, and products, such as sliced meats and cheeses. Vector transmission, seed and aerial dispersion, environmental persistence, and living on alternate, often perennial hosts are some of the ways that pathogens can break into an agricultural setting. Vector transmission occurs when another organism, such as an insect, carries the pathogen from an infected host and inoculates a healthy host. For example, the glassy-winged sharpshooter can suck sap from a grape vine infected with Pierces’ Disease, and be able to transmit the disease-causing bacteria to other healthy vines for several days. Infected plants rapidly show complete loss of productivity. Vector transmission, seed and aerial dispersion, environmental persistence, and living on alternate, often perennial hosts are some of the ways that pathogens can break into an agricultural setting. The spores of the Anthrax bacterium mentioned above can remain viable in the soil for decades until one finds its way into the nutrient rich, warm setting of a skin scratch or the lung of a mammal. Some pathogens are not able to survive long without a host, but are able to linger on what are called “alternate hosts.” This allows the pathogen to last over a winter or for several years, with the alternate host providing a reservoir of infectious material upon reappearance of the susceptible agricultural host and the right conditions for infection.

Agricultural pathogens not only have diverse ways of infecting plants and animals, but also have ways to overcome host defenses directed against them. The sheer enormity of microbial populations provides them with an evolutionary advantage. In vast microbial populations which replicate very quickly, variations in genetic makeup become statistically more probable when compared to slower growing plant and animal populations. Genetic variation allows for the emergence of pathogens that are no longer recognizable to the immune system of a host, or that have improved mechanisms for inflicting disease. Foot and Mouth disease of livestock comes in about 80 different “serotypes” around the world; each one is serologically different. An animal resistant to one serotype through vaccination or exposure will still have an immune system that is unprepared for most of the other serotypes.

Evolution frequently produces pathogens resistant to pesticides and antibiotics through genetic routes more complex than simple mutation. For example, the problem of antibiotic resistant bacteria is driven by the swapping of genetic material between organisms. Genes can be transferred on mobile pieces of DNA, or shuttled from one cell to another by plasmids or bacteria-infecting viruses called bacteriophages. Not recognized by opponents of GMOs, microbial pests of agriculture and public health readily take advantage of “natural” gene transfer or genetic engineering for survival and spread.

Viruses are capable of even greater wholesale shuffling of genetic material. Virus genomes reproduce inside of a host cell. Co-infection of a cell with different viruses allows the opportunity for a broad array of hybrid genomes to result. These new "hybrid" variants are usually inactive, but occasional variants gain properties, such as increased virulence, enhanced infectivity, or altered host range or vector specificity. A frightening agricultural example of this phenomenon is Avian Influenza. In 1918, a pandemic flu emerged that decimated cities and countries around the world. Today, with high density poultry production and many people in close contact with flocks, zoonotic episodes of Avian Influenza are being reported. Experts are concerned that it is only a matter of time before another highly virulent pandemic strain of human influenza evolves, perhaps this time of avian origin.

MICROBES ROTTING & POISONING THE FOOD SUPPLY

Microbial interference with an abundant and healthy food supply continues once plant and animal products leave the farm. Since pathogens can be part of the normal gastrointestinal flora of many animals, they are difficult, if not impossible, to completely eradicate and may contaminate our food supply. Bacteria such as *Salmonella*, *Campylobacter*, and certain strains of *Escherichia coli*, as well as enteropathogenic viruses, can persist, and some bacteria will multiply. If not killed before the food is eaten, these microbes can cause serious illness. There are an estimated 76 million incidents of foodborne illness in the United States each year. This is despite all of the advancements in food handling and processing hygiene, such as pasteurization, already in practice. Pathogens can also reach the food supply through contaminated water, transmission between infected animals, using animal manure as fertilizer, and even from contaminated or infected food handlers. Some contaminated seafood acquires viruses, for example, from contaminated harvest waters.

Illness due to microbes is also caused by toxins left behind in food as a consequence of microbial growth. Fungi, such as those in the genus *Fusarium* and *Aspergillus*, grow well on grains and other crops. As they grow, they produce toxic substances called mycotoxins. These toxins remain in edible tissues, or can accumulate after harvest if the fungus continues growing, and are poisonous to humans and animals that eat them. The toxins cause a variety of damaging effects on the nervous, digestive, immune and vascular systems. Some are also highly carcinogenic, including one of the most potent cancer causing chemicals known, aflatoxin. The most potent neurotoxin known causes Botulism, and is a product of a bacterium that grows in food in the absence of oxygen.

Even without directly causing human disease, microbes can have a chilling effect on the efficiency and cost of food production. Most people are familiar with vegetables left too long in the refrigerator drawer which are transformed into black puddles of mush. This spoilage is caused by bacteria and/or fungi that eat, or rot, the food. The process of spoilage erodes the quality

and availability of our food supply at every step in the food production, processing, transportation, and marketing chain. Its costs are borne by producers, shippers, processors and consumers.

PRACTICES THAT INCREASE THE MICROBIAL THREAT TO FOOD & AGRICULTURE

A newly realized threat to agricultural production and food safety is the purposeful use of disease and damage-causing organisms. Whether thought of as "bioterrorism" or simply malicious sabotage, this is a real threat derived from the microbial world. The systems we have in place to detect and deal with the broad array of diseases and toxins discussed above now must take into account human-made versions of the threats as well.

Many elements of the modern world interact with the microbial world to create new problems. Regional and international shipment of agricultural products means that pathogens do not necessarily require natural dispersion. Although some pathogens, such as soybean rust, can be transported by wind, others that might not spread so easily by natural forces can arrive by plane or boat. Stiff trade restrictions are established to prevent the entry and spread of certain diseases, but these restrictions are only as good as the inspection and detection measures used. Foot and Mouth Disease is a notable example. Countries that are free of FMD prohibit import of any meat products from countries not considered free of the disease. However, because of limitations to current detection methods, countries that vaccinate against the disease are automatically treated as though they have infectious animals. Countries that are able to operate without using vaccine have privileged access to certain markets, but at the cost of having completely vulnerable herds if the disease were introduced. Many microbiological issues are thus serious international agricultural trade issues and often are a reason for blocking trade of certain commodities.

Modern, intensive agricultural practices provide advancements in efficiency and product uniformity, but also bear some elements contributing to their own demise. Overuse of antibiotics and pesticides can select for resistance in the microorganisms that they target. Many of these chemicals are also pollutants, contaminating the environment and perhaps reaching people or organisms that were never their intended targets. Heavy use of fertilizers feeds nutrients into waterways, fueling microbial growth that can kill fish and other wildlife. And animal manure, used as a fertilizer, can contaminate water sources used for animal and plant production, providing a source of foodborne pathogens if not applied using best management practices.

In the drive for uniformity, intensive agriculture, such as planting row upon row of genetically identical crops, or raising large herds or flocks with little genetic diversity in close quarters, may result in expansive populations of vulnerable hosts upon the emergence of pathogens that are newly resistant to pesticides, vaccines, or other critical barriers.

FOOT & MOUTH DISEASE & TRADE BARRIERS

Trade in cattle, sheep, goats, and pigs is controlled based on a variety of animal diseases, one of the most important being Foot and Mouth Disease (FMD). Countries that have the status "FMD-free where vaccination is not practiced" are, with few exceptions, the more economically advanced nations or those that have insignificant livestock operations. Countries with this status are entitled to exclude products from others, but at a perilous price. The unvaccinated herds are vulnerable to the highly infectious disease. An outbreak in 2001 in Great Britain led to the slaughter of over 6 million animals in order to eradicate the disease (without using vaccine) and return the country to its FMD-free status.

MEETING CHALLENGES WITH MICROBIOLOGICAL SCIENCE & TECHNOLOGY

The microbial world is not just a source of endless problems for food and agriculture. Some of the solutions to disease and spoilage lie in the application of microbiology. Solutions to non microbe-derived problems may also be provided through microbiology. An ever growing human population is demanding more food, and is also using more energy and creating more waste. Food and agriculture microbiology can present opportunities to address some of these problems. Furthermore, some of the solutions to agricultural problems, such as soil salinization and drought, may lie with microbes.

UNDERSTANDING MICROBIAL PATHOGENS & COMBATING THEM

Better understanding of the microorganisms that cause disease and spoilage in agriculture and food will lead to better ways of controlling them. Pesticides are needed that are more environmentally friendly and that have added barriers to the production of resistance. Improved vaccines and immunomodulators are needed to make immunization of herds and flocks against pathogens more effective. Advances in these areas are dependent on knowing more about the "enemy," i.e., microbes that cause the diseases.

It also pays to know about the enemies of one's enemies. Pathogens that attack crops and animals frequently have competitors and pathogens of their own. Harnessing the capabilities of these antagonists is an approach called biological control. Through biological control, relatively harmless microorganisms (or their metabolic products) that inhibit or kill a harmful organism are mass produced and applied to food or crops as a protective measure. Large scale production of biological control organisms is a difficult process, and their performance in a field setting is often unpredictable as a match with each local ecosytem's conditions is needed. Nevertheless, genetic engineering of biological control organisms is a possible way to overcome these shortcomings. As biological control using GMOs is more difficult to clear through regulatory and political hurdles due to the current climate, little work is in progress.

A strategy of containment and destruction is required in instances whereby plant and animal diseases cannot be controlled by selective breeding, pesticides, vaccines, drugs, or biological control. The approach of quarantining and then depopulating possibly infected plants and animals becomes more expensive and devastating with increased disease spread. This is illustrated by the expense of destroying millions of chickens during the 2003-04 outbreaks of Avian Influenza, and by the 2001 FMD outbreak in Great Britain. The appearance of citrus canker in Florida has required extensive cutting of citrus trees, even in urban areas, at the cost of millions of dollars. Reducing these losses relies upon the ability to detect and react quickly to the appearance of a pathogen.

SURVEILLANCE FOR MICROBIAL PATHOGENS

Improved surveillance of, and response to, disease outbreaks relies on a variety of technologies. Accurate models of pathogen spread are required to prevent costly overestimates of pathogen dispersion, or underestimates that render control measures incomplete or ineffective. Better coordination and networking between the different entities handling surveillance operations and the standardization of detection technologies will facilitate more rapid, thorough response to outbreaks.

Ideally, surveillance networks should also be capable of tracing the cause of an outbreak to its point of origin. Knowledge of how incidents were initiated is critical to instituting changes that will prevent future incidents. Under ideal circumstances, surveillance networks should be capable of distinguishing among incidents caused by natural, accidental, and purposeful release of pathogenic organisms. This would require a much greater knowledge of microbial communities than we presently have, as well as forensic capabilities. Robust systems for disease surveillance advance the capability of responding to microbiological threats, thereby reducing damage. This is the case in preemptive actions against some agricultural diseases. For example, planting of certain genotypes of wheat in North America is guided each year by a forecasting system that observes what wheat rust virulence types are appearing to the South, and then recommending what available resistance genotypes will fare best in the upcoming planting season. Surveillance is also applied to respond as early as possible to outbreaks such as Avian Influenza. Another form of surveillance is routinely applied when we monitor for contaminants in the food supply. For example, grains are screened for mycotoxin contamination, and raw meats are sometimes tested for the presence of enteropathogenic bacteria.

> "... SURVEILLANCE NETWORKS SHOULD BE CAPABLE OF DISTINGUISHING AMONG INCIDENTS CAUSED BY NATURE, ACCIDENTAL, AND PURPOSEFUL RELEASE OF PATHOGENIC ORGANISMS."

Successful surveillance depends on accurate, fast, and practical detection technologies. Most immediately, there is a need for diagnostics that can test for multiple organisms in a single test, a concept termed multiplexing. In addition, diagnostic tests must be robust enough to be applied to complex sample materials, such as soil, food, and fecal material. The complexity of these materials can cause so-called matrix effects, severely hindering the sensitivity and specificity of a diagnostic test that would otherwise perform perfectly when applied to a pristine sample matrix, such as a pure culture of the target organism.

Other needed improvements to diagnostics are ones that enable them to be more widely accessible for use and more widely relied upon. Improving the accuracy and versatility, as described above, will help accomplish this. Standardization of tests nationally and internationally will be a challenge. The technologies need to be more portable and more rapid, enabling field sampling and real time analysis. Diagnostics should be made less expensive so they can be used more frequently and by programs with restricted budgets. Finally, diagnostic technologies must contribute useable information to inform risk management. Wrong information in a practical sense can consist of more than a simple false-positive result; information can also be useless if the test correctly detects the presence of the target organism, but it is dead, or not present at levels of concern. To help resolve these issues, it is necessary for diagnostic tests to be made more quantitative. Along similar lines, test specificity needs to be refined so that we are detecting the presence of pathogenic variants of microorganisms. There are many cases where diagnostic specificity to the species level is insufficient to accurately reflect risk because different strains within a species can differ significantly in their pathogenic characteristics.

The versatility and ruggedness that is needed from diagnostic technologies is also dependent on improving methodologies for handling specimens before testing. Improved methods for the pre-analytical processing of specimens are required. Means of cultivating organisms that have previously been non-culturable will improve diagnostic capabilities, although knowledge of microbial genomics will enable identification of many non-culturable micro-organisms as well as viruses.

PRESERVING FOOD & ENHANCING ITS VALUE

A major point of inefficiency in food production will be improved with reductions in post-harvest spoilage. Finding ways to slow or even prevent microbial spoilage will provide one set of solutions to this problem. Inactivation of spoilage-causing microbes is only one way to preserve food. Better understanding the spoilage process itself will open opportunities to alternatives to spoilage control, such as the biocontrol option. A time honored example of this principle is in the production of yogurt. The bacteria that grow in milk to generate yogurt convert the nutrients into byproducts that make the food environment much less amenable to the growth of spoilage organisms, thereby extending the shelf life of the food.

Beneficial microbes cultivated in food can provide added value far beyond delay or prevention of spoilage. Many of these microbes have "probiotic" properties that can help exclude disease-causing organisms and prevent infections. Probiotic properties of beneficial microbes are thought to be derived in part from competitive exclusion of pathogenic microbial species. However, the phenomenon is complex and may include other elements, such as the release of compounds antagonistic to pathogens or stimulation of the host immune system. Deepening understanding of the nature of such probiotic effects and elucidated ways that these can be strengthened will allow scientists to capitalize further on the beneficial effects of these microbes.

The presence of beneficial microbes in agriculture and food holds the possibility of generating added value to products. Some microbes have properties that convey nutritional enhancement to food. For example, yeast is a source of B-complex vitamins. There is also speculation that interactions between plants and certain microbes can stimulate enhanced production of compounds with pharmaceutical properties. This possibility may provide opportunities to generate health-promoting foods with so-called nutraceutical properties.

A HELPING HAND IN AGRICULTURE FROM MICROBES

Other interactions with beneficial microbes can be of direct benefit to agricultural plants and animals. A classic example of mutualism in action is the partnership between legumes and bacteria called Rhizobia. The bacteria take up residence in plant roots, receiving nutrients. In exchange, they fix nitrogen from the air into a form that the plants can use, replacing a need for nitrogen-containing fertilizer. There are other cases of microbes helping a host organism scavenge essential nutrients, or fend off pathogens. In the intestinal tract of ruminants, a complex mixture of bacteria enables the animal to extract sufficient nutrients from a diet of grasses.

Even the best-recognized and most-studied forms of mutualism are not understood well enough to be effectively controlled or expanded to cover hosts previously unknown to benefit from a particular interaction. Scientists have been unsuccessful in getting Rhizobia to form a mutualistic relationship with wheat roots, for instance. For the few classic examples of mutualism in agricultural systems, there are likely to be many more interactions taking place in obscurity. Study of interactions between organisms that boost agricultural success is a field rich with opportunities. More knowledge of microbial ecology and mutualistic interactions will pave the way for advances that enhance agricultural organisms' nutrient use, pathogen resistance, and hardiness.

Microbial ecology will likely be found to have impacts on agricultural systems beyond those currently recognized. Complex interactions between plants and the consortia of microbes found in soil probably extend beyond resisting pathogens and scavenging nutrients. Properly tuned interactions could help improve drought resistance and salt tolerance of plants and have other growth-promoting activities. Understanding and managing microbial ecology will have major benefits for stressed agricultural systems.

The massive scale of human agricultural and food production enterprises brings with it an array of problems that microbiology can help address. Any technological advances that increase resistance to pathogens or nutrient scavenging will also contribute to reduced use of pesticides and fertilizers. This represents a corresponding reduction in pollutants. Other pollutants are a direct consequence of agricultural production itself, rather than production practices. Waste produced by animals, particularly when produced in high densities, frequently represents a serious environmental and health hazard. Animal manure is typically accumulated in bulk and some of the material is used as fertilizer on agricultural fields. Technology to harness microbes for digestion of animal waste could alleviate some of the environmental and health hazards generated by large-scale animal rearing operations. Microbes may also be harnessed for the remediation of agricultural chemicals or for mitigating greenhouse gases.

Microbial digestion, another form of fermentation, can be harnessed to produce alternative fuels. Fermentation of animal wastes can create flammable gases, such as methane. Devising bioreactors that efficiently convert animal waste on a large scale would help eliminate an environmental and health hazard, while also satisfying growing energy needs. Fermentative processes also produce fuels, such as ethanol from plant material. The inefficiency of this type of fermentation for fuel production has kept it from being widely adopted. Improvements in fuel generating technology would allow the gradual replacement of highly polluting fossil fuels with more environmentally friendly fuel sources. However, continued removal of plant waste from fields may have unintended effects, such as altering the composition and characteristics of the soil, affecting microbial populations and subsequent plant growth. Thus, this practice needs to be examined and followed over multiple years to monitor its effects.

ETHANOL FUEL FROM CELLULOSE

For many years, ethanol fuel has been available as a supplement to gasoline used for transportation. Ethanol can replace up to 85% of the gasoline volume and still be used to efficiently power vehicles. However, the fermentation of grain has been used for ethanol production until recently. Because the value of crop material as food far exceeds its value for ethanol production, this has not been a cost-effective use of grain. New fermentation technologies have been developed that produce ethanol from cellulose-based plant materials left over after food is harvested. Cellulose is broken down into sugars during a primary fermentation step, followed by a secondary fermentation to produce ethanol. The result is a clean burning fuel created from agricultural waste, putting otherwise discarded energy to use.

MICROBIOLOGY RESEARCH OPPORTUNITIES TO ADVANCE FOOD & AGRICULTURE

The previous lineup of microbiology-related problems confronting agriculture and food, followed by approaches to solving these problems, provides an empirical appreciation of the value of agriculture and food research. There have been attempts to measure this value. Estimates of the return on investment in agricultural research, based on a purely economic level, range from approximately 30-60%. This means that for every dollar invested in agricultural research, there is an annual net flow of return to society of 30 to 60 cents. However, many of the benefits of research in agriculture and food microbiology carry over into other important areas, such as public health and economic development. These returns have not yet been estimated.

PROGRESS THROUGH MULTIDISCIPLINARY RESEARCH

Food and agriculture microbiology research intersects with many other fields. This overlap is evident in the research opportunities associated with multidisciplinary research. For example, the need for improved detection technologies will be addressed by research that combines such diverse fields as microbiology, molecular biology, statistics, and engineering. Multidisciplinary research approaches will also lead the way in developing better tools to model the behavior of microbiological hazards, and successful application of massive amounts of biological information to the management of the living systems that comprise agriculture.

Assays and detectors used in surveillance for agricultural diseases and human pathogens in food have the potential to be improved as a result of several types of multidisciplinary research. One approach that is rapidly improving detection technology is the combination of microfluidic engineering and molecular biology. Devices arising from the marriage of these fields will be inexpensive to operate, portable, and rapid. The combination of genetically engineered microorganisms and optical devices is facilitating the creation of biosensors in which a living microbe is actually the interface that detects targeted microbes or toxins.

The ability to predict pathogen spread and persistence is being boosted by multidisciplinary research. Combining Global Information Systems (GIS) tools, mathematical modeling, and microbial physiology, it is now possible to simulate microbial behavior in the environment. This will enable integration of climate information and biology to predict or track microbial dispersion or viability upon release into the environment. Improving predictive capability is the key to predicting, recognizing, and containing outbreaks and enabling intervention efforts to be properly and efficiently applied in a timely manner.

Application of massive computing power to the handling and analysis of biological information has expanded our understanding of many biological systems and processes in ways not imagined previously. Computational analysis of genetic, protein, and metabolic data has spawned new approaches to studying complex, networked events that take place within living organisms to give rise to particular phenotypes. Genomics, proteomics, and metabolomics rely on microscopic handling of molecular samples (e.g., microarrays) to generate enormous data sets that measure the state of genes, proteins, or metabolic products in an organism. As computing advances allow processing of ever increasing amounts of data and nanotechnology improves the ability to precisely handle and detect molecular samples, genomic, proteomic, and metabolomic studies will become increasingly effective. These kinds of analyses will enable discoveries that can advance agricultural efficiency and food quality and safety. The power of these analyses is currently expanding to enable study of the interactions between diverse microbial species and the interactions between microbes and their environments.

MULTI-ORGANISM BIOLOGY — INTERACTIONS BETWEEN HOSTS, PATHOGENS & MUTUALISTS

An exciting and relevant area in food and agriculture microbiology is the study of disease and infection. Agricultural productivity and food safety can be improved by disruption of pathogen inflicted disease in plants and animals. Traditional studies of pathogen virulence and host range continue to contribute to our understanding of disease processes. Breeding of disease resistance into plants and animals, for example, remains an important effort to continue. Creation of improved vaccines is another area where sustaining the pursuit of previously successful approaches can continue to reap benefits. Many vaccines could be improved by increasing their breadth of immunogenic activity, facilitating the ability to differentiate vaccinated animals from disease carriers, and increasing the duration of immunity that is conferred.

Beyond traditional methods of combating pathogens, research into the disease process itself and the role of innate host resistance will open new insights into the complex set of interactions between hosts and pathogens associated with food and agricultural systems. Budding areas of research, such as the use of immunomodulators to fortify host innate immunity against pathogens, will contribute to this end. Research is revealing that, in many cases, a critical part of the disease process occurs when pathogens disrupt immune responses in the host. As multi-organism genomic, proteomic, and metabolomic studies reveal precisely how pathogens attack a host, subsequent studies can investigate immunomodulation as a way to interfere with the infection process of specific pathogens.

Organisms that are participating in complex biological interactions represent a hugely underexploited pool of interventions to prevent disease through efforts such as immunological fortification, production of antibiotics/probiotics, and other mechanisms. Current investigation and knowledge of probiotics scratches the surface of this area of research, and even in this area, specific knowledge is lacking about what interactions occur between microbes and the host and how those interactions can be capitalized upon to prevent disease. For example, beneficial organisms may contribute to the prevention of disease by producing substances that interfere with successful colonization or infection by a pathogen in a host, and/or the beneficial organisms may exclude pathogens by competing for resources while not damaging the host. Past methodological restraints have limited our ability to understand complex host-microbe interactions. However, functional genomics, proteomics, and metabolomic approaches can all be harnessed to answer basic science questions about these interactions. In so doing, probiotic approaches can be refined, providing public health benefits and enhancing the sustainability of agriculture. This is one area where agriculture may come face-to-face with human and animal clinical medicine.

Another related phenomenon where microorganisms protect against pathogens is biological control. Many microorganisms can actively antagonize or kill the organisms that damage or cause disease in our agricultural crops and animals. A famous example is the soil bacterium *Bacillus thuringiensis* (Bt) that produces insect-killing toxins. Microbiology research has extracted a wide range of toxin specificities from different strains of Bt and enabled these toxins to be expressed directly in genetically modified crops, providing the plants with their own protective compounds. Continued research will undoubtedly produce more discoveries from this biocontrol organism. Many other biological control options, such as fungi and viruses that are pathogenic to a wide variety of specific agricultural pests, are largely unexplored and may be exploited for protection of crops and animals. Genetic engineering of biocontrol organisms has promise for ensuring their effectiveness in targeted, large scale use against pests.

MICROBIAL ECOLOGY & HEALTHY AGRICULTURAL SYSTEMS

The role of beneficial organisms in promoting the health of agricultural plants and animals extends beyond combating pathogens. Research into how beneficial microorganisms can promote growth, improve stress tolerance, and aid in the uptake of nutrients are research areas ripe for discovery and innovation. Research into these complex and often delicate interactions between different organisms should ultimately pay off by revealing ways to assure that agriculture can become heartier and less environmentally taxing.

The same communities of microbes that benefit agricultural health and efficiency are likely to be disturbed by some of the practices of industrialized agriculture. One way to fortify agriculture against disease and stress is to supplement systems with probiotic and biocontrol organisms, but a complementary and sometimes alternative approach is to protect beneficial organisms that may already be present in the environment. Research in microbial ecology will help to determine how to preserve a balance in microbial communities that favors agriculture. Heavy pesticide and fertilizer use, in particular, are two practices that should be studied using a holistic or integrated approach to determine their impact on microbial ecology within the context of tradeoffs between risks and benefits. More knowledge in this area will help determine optimal tradeoffs such that the benefits of use outweigh the disruption caused by chemical inputs into our agricultural systems.

> "RESEARCH IN MICROBIAL ECOLOGY WILL HELP TO DETERMINE HOW TO PRESERVE A BALANCE IN MICROBIAL COMMUNITIES THAT FAVORS AGRICULTURE."

Microbial communities are both vulnerable to, and contribute to, removal of pollutants. Research into how multiple organisms work in partnership to degrade complex molecules is essential to increase options for dealing with the byproducts of industrialized agriculture. Understanding this aspect of microbial ecology may also lead to improvements in waste disposal and energy generation through fermentation, as well as bioremediation.

Organisms that cannot currently be cultivated in a laboratory setting are likely to be pivotal in advances in probiotics, biocontrol, and microbial ecology. Research efforts targeted on identification of these previously uncharacterized organisms, whether through new cultivation methods or by indirect detection approaches made possible by genomic knowledge, will strengthen our ability to use beneficial organisms effectively.

MAKING THE BEST USE OF AGRICULTURAL TECHNOLOGY

Research offers opportunities for understanding more completely the impacts of technologies that are already used in agriculture and food production. Questions remain concerning if genetically modified organisms interact differently in the environment as compared to their non-engineered counterparts. Whether or not there are negative impacts associated with the use of GMOs needs to be considered and balanced within the broader context of sustained use and potential benefits.

Increased attention to food hygiene, which has occurred in the developed world over the last century, has dramatically reduced incidence of foodborne infection. However the void of microbially-based immune stimulation has been proposed as a culprit in weakening immune systems in animals and people. Further research into this area will determine whether this is a real phenomenon and, if so, how to balance tradeoffs between a microbiologically safer food supply and maintaining a healthy immune system throughout life.

Food and agriculture microbiology research provides potential solutions to problems that cut across many fields. The scientific principles and the implications of food and agricultural research are increasingly linked to public health and the environment, topics that historically have received more public attention. For example, interventions against Avian Influenza, which has been devastating poultry production, may also prevent dissemination of another human influenza epidemic. Providing biologically-based alternatives for protection of crops and animals against pathogens will help reduce pesticide and antibiotic use, both of which have public health and environmental implications.

OVERCOMING

BARRIERS TO SUCCESSFUL AGRICULTURAL & FOOD MICROBIOLOGY RESEARCH

Despite the need for continued advances in agriculture and food microbiology, and the proven track record of agricultural research, research support for these fields over the last few decades has been lean and is, in fact, decreasing. Reversing the decline in funding and recognition of the value of agricultural research requires fundamental changes, in addition to an infusion of financial support. The major barriers to advancing agriculture and food research are institutional and perception based.

PUTTING FOOD & AGRICULTURE MICROBIOLOGY IN THE SPOTLIGHT

The profile and priority of agricultural research needs to be raised. Designated research centers of excellence, similar to those in the biomedical and defense arenas, would make strides in this respect. There are numerous institutions that provide a backbone of exceptional scientific work for U.S. agriculture, but their programs run in aging facilities and with limited financial resources. Within U.S. research institutions, agriculture is too often a subordinate priority. One way to reverse this would be to raise the institutional overhead rate that is currently allowed on USDA grants from its uniquely low level to a level on par with that provided by other funding agencies, such as the National Science Foundation (NSF) or the National Institutes of Health (NIH). With limited overhead capital, administrators and investigators are restricted in their efforts to build strong programs and recruit personnel to pursue state-of-the-art agricultural research. Increases in overall research expenditures for U.S. Department of Agriculture (USDA) programs would then be needed to maintain even the current level of direct funds for research.

A healthy agricultural research community depends on an influx of young scientific talent. Trouble recruiting and maintaining graduate students is impacting programs and will ultimately affect the field. Several measures can be taken to alleviate this problem. A program of prestigious and remunerative fellowships for graduate students and postdoctoral fellows would provide some needed recruiting leverage. Internships involving industry, non-governmental organizations (NGOs), and government agencies would have mutually beneficial value. Such internships would infuse awareness and technical knowledge of agricultural science to institutions and provide the visiting scientists with networking and training opportunities. The recent security-motivated tightening of immigration procedures has limited access of the U.S. scientific community to international talent. Making the U.S. more accessible to legitimate international students and scientists again would help all scientific endeavors, including invigorating the base for revival of agricultural science.

Funding for agriculture and food research is essential to fulfilling any of the potential benefits that have been proposed. Because of the shallow profit margin in agriculture and food, it is to be expected that industry/commodity funding for research in these areas will be minimal, and when it does occur, it is usually focused on short-term payoffs. Such sources of funding traditionally have provided no indirect costs, further perpetuating the declining research facility infrastructure. Therefore, basic research on high priority agriculture and food problems is deeply dependent on government sources to provide sufficient funding. Currently the National Research Initiative of the USDA explicitly favors "translational research," i.e., those projects with the promise of providing near-term, technological products or advances. By nature, many agriculture and food research endeavors are long-term, but competitive funding for long-term projects is more or less unavailable. An expansion of research priorities to emphasize basic and long-term research and dedicated funding to back this expansion will help revive agricultural research.

> "LONG-TERM AGRICULTURAL RESEARCH PROJECTS ARE THE ONLY WAY TO OBTAIN SCIENTIFIC ANSWERS."

RESEARCH PRIORITIES WITH REACH

Long-term agricultural research projects are the only way to obtain scientific answers to many questions in areas such as microbial ecology and epidemiology. Unfortunately, the formula-funded land grant university system once could support such long-term research, but funds have diminished to the point that this is no longer possible, even though the land facilities are still there under university ownership. One mechanism for overcoming barriers to long-term research in agriculture would be through projects like the Long-Term Ecological Research (LTER) stations. Sustained study of particular agriculture and food science problems is also hampered by certain institutionalized incentive structures. Tenure evaluation procedures draw heavily on publication records of individuals, but long-term projects may be incomplete and unpublished by the time of tenure or promotion review. It is also the case that research grants generally demand a structure that promises completion of a project within two or three, and at most, five years. Allowing for longer project duration, including sustained funding, and evaluation of productivity based on alternative measures, would make long-term research a more viable scientific pursuit.

Collections of microbial specimens are an essential asset to agriculture and food microbiology research. Availability of specimens for initiation and verification of research can be assured only if the resources and expertise are maintained to properly curate collections of microbes. In the same way that institutions and funding agencies determine the viability of long-term research, their commitment of resources and recognition to the maintenance of microbe collections will determine whether this asset is sustained or lost.

With multidisciplinary research at the center of many needed advances in food and agriculture research, some of the disincentives to this type of work should be lifted. Institutions frequently fail to recognize the effort required to coordinate across different research groups and disciplines to make a project run successfully. The many-author publications that result from this type of work are also not as highly regarded as ones where credit is less widely distributed or—in a view that is not necessarily justified—less diluted.

The research community is also responsible for some barriers to more successful food and agriculture science. Compartmentalization between fields of research and industrial practice prevent effective cross-fertilization and sharing of lessons learned. Divisions also stand in the way of greater multidisciplinary efforts. Research opportunities will expand as scientists become more aware and more engaged across fields dealing with microbiology, veterinary and human medicine, plant diseases (pathology), epidemiology, statistics and mathematical modeling, environmental sciences, and engineering, to name a few.

PAVING THE WAY FOR ESSENTIAL RESEARCH

Various regulations present obstacles to research because they are inappropriately stringent or lacking in discrimination. For example, listing certain pathogen species as "select agents" dramatically increases the expense and legal liability of working with these microorganisms. In many cases, agents that are on the select agent lists are already prevalent in the environment, and their study in a laboratory represents no extraordinary safety or security risk. For example, soybean rust recently reached and spread throughout the southeastern United States, creating the potential to cause havoc with growers in the current growing season. But because of the pathogen's select agent rating before its arrival into the country, there was a scarcity of research at a time when studies of the pathogen were urgently needed. Fortunately, the action by USDA to remove it from the select agent list will permit a greater number of researchers to work with the organism and monitor its progress across the soybean fields of the country. Regulations that are more science-based and flexible will allow essential scientific investigation to proceed. For the cases for which the select agent regulation remains sensible, it is important that funding programs allow investigators to budget for the biosafety and security measures that are legally required to work with these organisms. These requirements are becoming necessary for many non-select agent plant pathogens as well and must be met for basic research to continue.

Basic research is stifled by a number of forces in the research funding arena. The push for applied results in a short timescale as seen in the USDA's National Research Initiative is one such force. While understandable that agencies are under pressure to demonstrate an immediate payoff from research supported by taxpayers, this ultimately drains the scientific foundation of innovation. If every grant is charged with generating specific, applied solutions to problems that are most politically compelling at the time, there will not be time or resources for the occasional unexpected but remarkable discovery. This phenomenon is currently at its worst in the area of biodefense research. In fact, biological research funding programs are currently skewed in favor of supporting proposals with a biodefense spin, and the agricultural arena is no exception.

The product-oriented leaning of research funding programs also spills over to discourage proposals that are not stoked with preliminary data. This puts innovative but perhaps "high-risk" proposals at a funding disadvantage because they are seen as exploratory or speculative rather than hypothesis-driven. It also puts younger investigators at a disadvantage.

FOOD & AGRICULTURE ADVOCACY

Successful institution of the changes necessary to revitalize food and agriculture research must be influenced by various players. Consumers, commodity groups, and legislators need to be persuaded of the importance of food and agriculture microbiology research, particularly basic research. Organizations whose members are part of this research community include the American Society for Microbiology, the American Phytopathological Society, the Institute of Food Technologists, the Council on Agricultural Science and Technology, and the National Association of State Universities and Land-Grant Colleges.

More successful communication of the benefits of food and agricultural microbiological research is critical. Interested organizations and academic institutions can contribute by training some of their scientists in speaking to and writing for the lay public. They can further contribute by training educators of non-scientists to be able to convey basic scientific literacy. As scientific information can reach and be understood by the public, facts become more likely to be used for decision making. This will be of benefit to many arenas, including those that impact food quality and safety, public health, and environmental protection. It will also encourage the public to be a more informed participant in decisions regarding food and agriculture technology, rather than being either complacent or frenzied by rumor and conjecture.

Building awareness of the critical value of a broad, basic research agenda in food and agriculture microbiology is an important step to overcoming obstacles that have in fact hindered the progress of these disciplines.

RECOMMENDATIONS

RESEARCH AGENDA

- Study the impact of production and processing practices on microorganism evolution, persistence, and antibiotic/pesticide resistance as they affect agricultural animals, plants, and environments.

- Apply systems biology approaches to understanding communities of microorganisms within agricultural hosts, food matrices, and production/processing environments.

- Develop more sophisticated understanding of the nature, specificity and adaptation of microorganisms to food environments, hosts (human/animal/plant), and host responses to both pathogenic and beneficial microbes.

- Use a comparative pathobiology approach to understand the importance of pathogens that cross from animals or plants to humans and what characteristics enable their pathogenicity to multiple hosts.

- Develop microbial technologies that can be applied in agricultural contexts for reduction of inputs, bioremediation of pollution, conversion of biomass, and converting wastes to fuel.

- Pursue multidisciplinary strategies for developing knowledge and technologies to solve food and agriculture problems.

REBUILDING THE FOUNDATION FOR TECHNOLOGY USE & RESEARCH

- Coordinate development and standardize use of diagnostic tests across agricultural production, food processing, and public health systems to provide a foundation for integrated surveillance systems.

- Provide, through integrated educational initiatives, scientifically trained professionals who will serve the food and agricultural communities.

- Facilitate implementation of systems approaches, long-term projects, and multidisciplinary research in food and agricultural microbiology.

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