Biosafety, biodiversity and significance of Microbial Resource Centers (MRCs) in microbiology education

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Summary

Interest in the intrinsic behavior of microorganisms has led to the establishment of centers of expertise, known as Microbial Resource Centers (MRCs) built around specialized collections of cultures of microorganisms. Their main function is to accession, preserve and distribute authenticated, viable samples of microorganisms in consultation with the international scientific community for study and application. Microorganisms, which are exceptionally diverse, are found almost everywhere and affect human society in countless ways. Thus, modern microbiology has a great impact on medicine, agriculture, food science, ecology, genetics, biochemistry and many other fields. MRCs appear to be equally important to industry, to academia, and from the elementary to high school science teachers who can order specific educational strains. The basic microbiological knowledge of human society possesses the potential to revolutionize many aspects of human life. MRCs are a key resource to underpin these developments.

Introduction

Microorganisms are not only of value for the production of useful substances; they also play unique roles in the biogeochemical cycles involving plants and animals. To a great degree, humans depend on individual microorganisms in biotechnology and diverse ecosystems on the earth. For example, microorganisms have key roles in the processes of mineralization, organic matter production, geochemistry and nitrogen availability. Some microbes cause disease but others are completely harmless. Since microbes are of such bioeconomic and social significance, it is important that microbiology becomes a part of everyone's education.

Microbiology is a discipline with a rich history of interweaving research and teaching, providing a model for other branches of science to strengthen education through the influence of research. An early forwardlooking statement about the importance of education to the field of microbiology was made in 1916 with the launching of the *Journal of Bacteriology*. The first article in this journal, "The Pedagogics of Bacteriology" (Bergey 1916) contained a strong appeal for expanding the offerings in bacteriology to a broader group of science and health majors in universities as well as introducing bacteriology to general education students to enhance their understanding of the world in which they live. This article summarizes the issues concerning the safety in teaching with microorganisms in schools, importance and the functions of MRCs as key repositories of biodiversity, their continued role in microbiology education and focuses on the emergence, as well as the prospects of MRCs and microbiological education in Turkey.

Teaching with microorganisms in schools

Microorganisms are an ideal teaching tool for science; they are ubiquitous, cost-effective, and infinitely interesting organisms that can bring biology to life in any classroom (Handelsman 2002). Although students' preconceptions of microorganisms general regard them as pathogens (Williams & Gillen 1991), the fundamental rules of personal, public and domestic hygiene rely on an understanding of the characteristics of these organisms. Microorganisms possess many indispensable features that make them ideal subjects for safe and practical exercises in schools. Staff in schools and colleges should be in no doubt of the considerable educational value of thoughtful, practical microbiological work and of the need for every pupil to possess a basic knowledge of the biology of microorganisms (DfEE 1996).

Microbiological studies in schools are also a part of the European Initiative for Biotechnology Education (EIBE) that was financed by the European Commission. The aim of EIBE is to inform the public and in particular school students, about the benefits of Biotechnology (Grainger 1996). Microorganisms are indispensable for biotechnology because the biotechnological processes make use of the metabolic activities of microorganisms or develop microorganisms for specific biotechnological use.

As we know elementary and middle schools students' preconceptions of microorganisms in general and on bacteria in particular have had a negative influence on the acquisition of scientific concepts (Williams & Gillen 1991). Therefore it is necessary to find out students' preconceptions on microorganisms and integrate them in teaching programmes dealing with microorganisms and biotechnology (Harms 2002). Microbiology is often a difficult subject to introduce to students, but the study of microorganisms should begin in the elementary grades (AAAS 1993).

Work in microbiology and biotechnology in schools may be categorized into four biosafety Levels (BSLs) which are described in outline below. These levels are also similar to the "levels of containment" used by professional microbiologists. Risk groups are another way of assessing certain health and safety related characteristics of microorganisms. U.S., and many European countries, as well as the World Health Organization, use risk groups in addition to biosafety levels.

Biosafety

Four BSLs are described which consist of combinations of laboratory practices and techniques, safety equipment, and laboratory facilities. Each combination is specifically appropriate for the operations performed with the documented or suspected routes of transmission of the infectious agents, and for the laboratory function or activity. The recommended biosafety level(s) for the organisms represent those conditions under which the agent can ordinarily be safely handled. The laboratory teacher is specifically and primarily responsible for assessing risks and for properly applying the recommended biosafety levels. In most cases, work with known biological agents should be conducted at the biosafety level recommended. When specific information is available to suggest that virulence, pathogenicity, antibiotic resistance patterns, vaccine and treatment availability, or other factors are significantly altered; appropriate stringent practices may be specified.

BSL1. This represents a basic level of containment that relies on standard microbiological practices which can be carried out by teachers with no specialist training. Practices are appropriate for undergraduate and secondary educational training and teaching laboratories, and for other facilities in which work is done with defined and characterized strains of viable Microorganisms not known to cause disease in healthy adult humans. Yoghurt bacteria (*Lactobacillus* spp.), yeast such as *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* for the production of bread or beer and some unicellular algae (*Chlorella* sp. or *Spirulina* sp.), asporogenic *Bacillus subtilis* or *Bacillus licheniformis, Escherichia coli* K12 (cloning strains) are representative of those microorganisms that are not associated with disease in healthy adult humans, are of minimal potential hazard to laboratory personnel, and of minimal potential hazard to the environment. Viruses are rarely used in schools and colleges but Baculoviruses and Tobacco Mosaic Virus might be considered (ASE 2001; HSC 2001).

Many bioagents that are normal flora of skin and mucosal membranes are not ordinarily associated with disease processes in humans. Their presence is not considered clinically significant. Although, organisms in this category such as *Escherichia, Proteus, Klebsiella, Citrobacter, Enterobacter* and *Serratia* species are normal flora or commensal flora, cultures should be viewed in the unopened containers in which they were grown for preventing splashes or aerosols. They may cause infection in the young, the aged, and immunodeficient or immunosuppressed individuals.

BSL2. Work is done with the broad spectrum of indigenous moderate-risk agents present in the community and associated with human disease of varying severity. Representative organisms assigned to BL2 are the salmonellae, Staphylococcus, Streptococcus, and Toxoplasma spp. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low. Organisms may be cultured from the classroom or laboratory environment but not from environments which are likely to contain harmful organisms, for example feces or wastewaters other than hair, fingers or hands. Primary barriers such as splash shields face protection, gowns, and gloves should be used. Secondary barriers such as hand washing, waste decontamination and disposal facilities must be available to reduce potential environmental contamination.

BSL2 work may be carried out with pupils between the ages of 11 and 16 years and by science teachers who require training and some supervision, which can be provided through a short in-service course or in school by an experienced and knowledgeable biology teacher. Non-specialist teachers should not carry out or supervise such works. Before work with microbes is started, students should wash their hands with soap and water and cover any cuts with waterproof plasters. Hands should also be washed after working with microbes. There must be no hand-to-mouth operations such as chewing, sucking, licking labels or mouth pipetting. Teachers should use laboratory coats or aprons for BSL2 work.

Teachers wishing to study or to use organisms at the BSL2 level, must be aware of potential hazards, and must be trained and proficient in the practices and standard microbiological techniques required for handling such material safely and should consult an appropriate advisory body such as national, local or university microbial culture collections or teaching laboratories. Cultures should be examined by students in containers (such as Petri dishes, screw-topped tubes, bottles) which have been taped closed. Cultures and contaminated material must be heated to kill after use for safe disposal. Autoclaving is the preferred method of sterilization for culture media, aqueous solutions and discarded cultures. The process uses high pressure steam, usually at 121.6 °C at 15 lb/in² pressure, 15– 30 min. Autoclaving can be done in school laboratories with a domestic pressure cooker or a purpose-built autoclave.

BSL3. Here, practices, safety equipment, and facilities are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. *Mycobacterium tuberculosis*, St. Louis encephalitis virus, and *Coxiella burnetii* are representative of microorganisms assigned to this level. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols.

BSL4. In this case practices, safety equipment, and facilities are applicable for work with dangerous and exotic agents that pose a high individual risk of lifethreatening disease, which may be transmitted via the aerosol route, and for which there is no available vaccine or therapy. Viruses such as Marburg or Congo-Crimean hemorrhagic fever are manipulated at Biosafety Level 4. The primary hazards to personnel working with Biosafety Level 4 agents are respiratory exposure to infectious aerosols, mucous membrane exposure to infectious droplets, and autoinoculation. All manipulations of potentially infectious diagnostic materials, isolates, and naturally or experimentally infected animals pose a high risk of exposure and infection to laboratory personnel, the community, and the environment. BSL4 facilities are generally in a separate building or completely isolated zone with complex, specialized ventilation and waste management systems to prevent release of viable agents to the environment.

BSL 3 and BSL4 practices and facilities are only applicable for university level clinical, diagnostic teaching hospitals or pharmaceutical research laboratories etc. and not suitable for investigations in secondary schools and colleges. These works are normally confined to students over the age of 16 and institutions where facilities are appropriate. Main requirements are safety equipment including biological safety cabinets, enclosed containers, and other engineering controls designed to eliminate or minimize exposures to hazardous biological materials. Laboratory managers, supervisors, technicians and others who provide supervisory roles in BSL 3 and BSL4 practices are responsible for providing or arranging for appropriate training of personnel and overseeing the safety in laboratories and reporting any problems, accidents, and spills to the appropriate faculty

member. Students working in or using the laboratory facilities in the course of their employment or studies are responsible for knowing and following all safety procedures and also responsible to and must notify to the supervisors or teachers in case of accidents, chemical spills, and hazardous waste disposal.

Role of Microbial Resource Centers (MRCs)

Culture collections, herbaria, museums and libraries are critical components of the scientific infrastructure and MRCs necessary for advancing the missions of education, health, agriculture etc. It is therefore vitally important that MRCs continue to provide the authenticated reference strains necessary in conformity with new guidelines that ensure biosecurity and biosafety so that future generations have access to the standards on which to base the work. Microorganisms are also significant gene pools, and these gene pools must not be lost. From this point of view, microorganisms can be regarded as a cultural heritage and a cultural property, and they must be transferred to the next generation in a normal and healthy condition (Komagata 1998).

The International Committee on Systematics of Prokaryotes (ICSP) of International Union of Microbiological Societies (IUMS) approved the bacterial species of which a culture of the type strain should be deposited in two publicly accessible recognized culture collections in two different countries, from which it would remain readily available and published the names of bacteria in the "Approved lists of bacterial names". The Approved Lists of Bacterial Names contain 2212 names of genera, species or subspecies, and 124 names of higher taxa (Skerman et al. 1980). All new names have from January 2000 been published only in the International Journal of Systematic Bacteriology (IJSB) renamed in 2000 International Journal of Systematic and Evolutionary Microbiology (IJSEM), either as an original article or in the "Validation Lists" regularly appearing in that journal. The Validation Lists constitute valid publication of new names and new combinations that were previously effectively published outside the IJSB/IJSEM.

Culture collections have been developed to provide one or more general functions: (1) To establish repositories of interesting, rare or useful organisms, (2) to provide the research community with taxonomic type strains, or control/reference strains for experimental use and standardized testing, or specialized genetic or clinical strains, and (3) to establish a repository of organisms necessary to permit enablement of patented inventions (ASM-PSAB 1995).

The mainstay of school culture collections has been located in conjunction with the teaching laboratory. The microbial culture collections for teaching purpose, usually maintained by the teacher or graduate students in training, in order to provided reliable and well-characterized cultures for laboratory courses or experimental works. This is the case because teaching laboratories expanded due to the cost pressures of cultures and curricular decisions. Most often, it is not clear who owns the microbial cultures or is responsible for their preservation. These small individualized collections are at great risk as fund sources end or as investigators relocate, retire or change research direction. It can be claimed that these culture collections must be reduced or eliminated basing on the facts that even the well-organized microbiological culture collections, which provide a limited number of frequently used microorganisms at minimum cost, are known as "strains for educational purposes". Educational strains are less pathogenic strains of microorganisms, specifically for teaching, considerably less expensive than the regular strains and, therefore, more suitable for school budgets.

According to the data of World Data Center for Microorganisms (WDCM) 483 culture collections are exist in 65 countries in the world, and there are in total 1,118,351 microorganisms (bacteria, fungi, virus, cell lines etc.) in the registered culture collections. However, major public culture collections, which hold a wide variety of microorganisms and distribute them to the public, are estimated to be about 30 collections. In addition, bacterial cultures account for 445,112 (39.8%), filamentous fungi for 375,052 (33.5%), viruses for 10,214 (0, 9%), cell lines for 10,520 (0.9%), and other kinds of microbial (algae, protozoa etc.) for 277,453 (24%) by the data of WDCM (WDCM 2004). Essentially MRCs provide authenticated strains for reference in biosystematics, and to support bioscience research, development and education. However, the expertise that drives MRCs is under threat, to combat the current trend of operating and financing, collections are driven toward becoming more commercially-oriented and more competitive (Smith & Ryan 2001).

Biodiversity

It is important that students realize that all life on this planet ultimately depends on the activities of microorganisms. Found nearly everywhere, microorganisms are the most widely distributed group of organisms on earth. According to the action statement (Colwell & Hawksworth 1992) of "*Microbial diversity 21*" by IUMS and International Union of Biological Societies (IUBS), less than 5% of microorganisms on the earth are recognized, while the remaining 95% are awaiting our exploitation. It is generally thought that 1% of the microorganisms found in the nature have been cultivated. For this reason, even people who are not scientists should have some familiarity with the properties and activities of microorganisms.

Recently, the term of "biological diversity" or "biodiversity" has become well known to the public. Biological diversity means variability among living organisms from all sources including soil, water, air environments and involves the great number of biological species on the earth. Article 13 (Public Education and Awareness) of the Convention on biological diversity (COD), Rio de Janeiro, 1992 is one of the important cornerstones of the Convention (Glowka 1996). Because microorganisms cannot be readily seen, the difficulties of raising standards of public education and awareness are enormous. The key roles that microbes play in all aspects of human life should be emphasized. Providing information about microorganisms living in bizarre environments such as hydrothermal vents may provide an ideal opportunity to educate and to make people concerned about microbial diversity. Plants, animals, and microorganisms are consistent elements of ecosystems, and play important roles in biogeochemical cycles and other functions to sustain an active and clean earth. However, biological diversity has been reduced significantly by varied human activities (e.g., air and water pollution, clearing of tropical moist forests for agricultural purposes, global climate change).

In the near future, uncountable numbers of microbial strains will be isolated through the study of microbial diversity, and the attributes of a large number of strains will be improved. One way to protect the genetic diversity of the biosphere is the "*ex-situ*"- conservation of organisms in culture collections (Hawksworth & Colwell 1992). Therefore, reliable and well-organized culture collections are needed as the depository and for the promotion of research and application of the strains. In fact, culture collections play a depository role of the type strains in bacteriology, and as a consequence the study of bacterial systematics will not be complete without culture collections.

Overall condition of MRCs, microbiology education and related problems and prospects in Turkey

MRCs in Turkey are relatively recent phenomenon. Currently, in Turkey, three governmental and three university-based, in total six MRCs are registered in WDCM. Of these, the Center for Research and Application of Culture Collections of Microorganisms (KUKENS, WDCM101) is university based and also the oldest one in the related field in Turkey. Refik Saydam National Type Culture Collection (RSKK, WDCM828) is the Turkish national and official type collection, established in 1954. These two Turkish MRCs are also members of the European Culture Collections' Organisation (ECCO). Concerning the microbiology-related organizations and events, the Turkish Microbiological Society, established in 1931, a full member of the Federation of European Microbiological Societies (FEMS), organizes many national and international microbiology related events are held in Turkey on a regular basis. The society is active in organizing symposia and conferences. However a symposium concerning the problems and development of microbiology education or MRCs in Turkey should have been organized.

The Turkish scientific establishment is very much aware of the importance of MRCs and improvement of

teaching microbiology in Turkish schools has not been identified as one of the priority research areas. Today, there are 76 (53 public and 23 private) universities in Turkey. Out of 76, 43 (one private and 42 public) universities have faculties of education, most of which offer dual (both regular and evening) programs. The numbers of universities, in Turkey that offer microbiology as a component of other undergraduate degree programs are increasing. However, it is still low compared to other European countries. Amongst Turkish universities, some biology departments offer microbiology as one of the option packages to final year undergraduate students but do not have any specific microbiology undergraduate degree programmes and options, although offering microbiology related courses.

To stimulate student interest and to simplify the study of microorganisms, there is a need to define an educational policy on microbiology education at all levels, with a special emphasis on raising public/community awareness of issues related to biosafety and biodiversity. There is a variety of mechanisms that can be used for this purpose, including involving non-governmental organizations (NGOs) and local community-based organizations in both formal and informal awareness and education programs as well as linking with local, national, and international electronic and mass media, scientific societies, nature clubs, local schools. The Turkish scientific establishment, other related governmental and NGOs should evaluate the how the quality of teaching microbiology in Turkish schools can be improved and the status of support for MRCs, their significance in biosafety, biodiversity and microbiology education. Enhanced funding of current MRCs and greater emphasis on microbiology education and research in microbial diversity and systematics will be essential for the continued evolution of these activities. New funds should be used to strengthen certain types of MRCs, either by governmental funds alone or in conjunction with other national or international agencies. Developing an effective biosafety system may well require substantial training, (e.g., to raise awareness of policymakers), introduce ethical and safety issues to researchers.

Conclusion

The laboratory teacher or director is specifically and primarily responsible for the safe operation of the laboratory. His/her knowledge and judgment are critical in assessing risks and appropriately applying these recommendations. The recommended biosafety level represents those conditions under which the agent can ordinarily be safely handled. Special characteristics of the agents used, the training and experience of personnel, and the nature or function of the laboratories are the issues that need to be taken into consideration by the director in the application of these recommendations. Society's need for culture collections are increasing year-by-year. MRCs are the pivotal information sources by selecting, acquiring, organizing, preserving, maintaining, and providing access to the microbial resources that address the interests and needs of the members of a diverse and complex scientific community. They are also an educational institution concerned with the diversity and relationships among microorganisms. Their effective and smooth management is required to provide the services required. For educational purposes care in the choice of suitable microbiological cultures must be obtaining from recognized specialist biological suppliers which would include MRCs. Effective teaching needs adequate and reliable sources of properly preserved cultures. Strain information given in current strain databases is not enough to satisfy the demands of science teachers. Additional documentation is needed for strains deposited in the major culture collections, such as ATCC (American Type Culture Collection), DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen), CBS (Centraalbureau voor Schimmelcultures), NCIMB (National Collection of Industrial and Marine Bacteria, National Collections of Industrial, Food and Marine Bacteria) or BCCM (Belgian Co-ordinated Collections of Micro-organisms); fortunately, these and other culture collections generally provide growth conditions, geographical origin, some biological properties, other collection numbers etc. that guide depositors through the documentation process. Ideally, the catalogue of culture collections should provide a guide to find organisms with desired educational properties. They not only provide the organisms but also detailed information including experimental protocols.

With regard to issues related to biosafety, biodiversity programmes implemented at the primary and secondary school levels could reach many young people between 8 and 16 years that are seen as most receptive age in secondary schools if care is taken to develop close ties with educators, create appropriate curricula and evaluate curricula implemented. Informal education may be useful in supplementing more formal educational experiences. MRCs should also explore developing educational partnership with zoos, botanic gardens, aquaria, natural history museums as well as national parks, other protected areas and environment related non-governmental organizations.

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