TESTING METHODS IN FOOD MICROBIOLOGY

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Summary

Microbial safety and quality of foods are determined by the kinds and number of microorganisms occurring in them. Food-borne microorganisms may cause spoilage of foods, or after ingestion may cause disease of the consumer by infection or intoxication. The primary aim of food microbiology is to use testing methods suitable to detect, enumerate and identify microorganisms in a food product. Enumeration of viable cells can be achieved by taking a sample of food, bringing it into a homogenous suspension and inoculating solid or liquid growth media to obtain colony counts or most probable number of cells. Detection is made with the use of specific and differential growth media, in the case of suspected pathogens, generally followed with the identification of species and typing of subspecific strains. Conventional culturing methods are slow and material- and labour-intensive. Modified versions facilitate obtaining results rapidly. Non-traditional testing methods relying on physical, chemical, immunological or molecular principles have been introduced to supplement or replace conventional testing methods. Rapid techniques are particularly useful in modern procedures of quality management and control systems, such as HACCP, to ensure the microbial quality and safety of foods in a preventive way that cannot be attained by end-product testing.

1. Introduction

Microbiological assessment for quality and safety of foods traditionally relies upon the enumeration and specific detection of pathogenic and spoilage microorganisms. Conventional testing methods make use of growth media and cultivation to enumerate and isolate cells of microorganisms from food samples brought into suspension with diluents. Isolated colonies of microbes are then subjected to a series of biochemical, physiological and serological tests in order to find species identity and subtypes of the microorganisms in question. These methods can provide both quantitative and qualitative information on the importance of microorganisms present in foods, and have been verified over the years. However, testing methods relying on cultivation are generally slow and give results after a period of several days; moreover they use a lot of material and labour. Modern quality management and control systems, such as good manufacturing practice (GMP) and hazard analysis and critical control point (HACCP) systems require methods and techniques that can be used on-line and give results in real-time. Hence, food microbiologists seek more rapid, sensitive and specific methods to get adequate information in due time to monitor the safety and quality of products. In recent decades a number of improved conventional and alternative non-traditional methods and techniques have been developed that appear suitable for early detection and characterization of microorganisms significant in foods. This chapter will briefly summarize the very broad and growing field of microbiological testing methods.

2. Basic Microbiological Techniques

For the majority of microorganisms, the size of individual cells is very small, in the range of micrometers; hence examination of individual cells is limited; for the most part microbiological studies deal with **populations**, containing millions of individuals. Such populations are obtained by growing microorganisms in an artificial environment under well defined conditions. This is called **cultivation**, and the result is a microbial **culture.**

A culture obtained from a natural sample or foods usually contains several kinds of microorganisms—it is a mixed culture. However, to study the properties of a single organism (all the cells in a population), a **pure culture** must be established, containing only one kind (species) of microorganism to be characterized. Accordingly, in addition to cultivation, another basic operation is **isolation—**the separation of a particular species in pure culture from the mixed populations that exist in nature. Furthermore, both cultivation and isolation are to be exercised under conditions to avoid **contamination** from external sources other than the material under study (equipment, containers, air, etc). The procedures used in the prevention of contamination (and infection, for that matter) during handling and manipulations of cultures are collectively called **aseptic techniques** that should be followed meticulously in a microbiological laboratory. Some specific details of the basic principles are given below.

Microorganisms are ubiquitous—they are everywhere. Because of their small size, they are easily carried by air, and settle on all laboratory surfaces, means and tools of cultivation. Our body, in particular the hands and clothes, are always contaminated by microorganisms. Therefore, the equipment and containers commonly used to cultivate and maintain microorganisms must be **sterilized** before use; this is usually done by heat. The rooms of the laboratory should be cleaned and **disinfected**, and our hands carefully washed and **sanitized**.

Equally important are the precautions during handling of microorganisms. Containers (test tubes, flasks, petri dishes, etc.) must be rendered initially sterile, and when opened, must be protected from air contamination; their cups or plugs should never put on the table. Tools used for transfer of cultures (pipettes, metal loops, needles) must be sterilized before and after use or disposal.

One of the most important procedures to be carried out under aseptic conditions is **sampling** and sample preparation. For reliable microbiological analysis of foods, proper sampling procedures are essential. The sample should represent the whole microbial community from which it is taken. To this end, statistical sampling plans are recommended for the microbiological qualification of various foods (see 6.4). Aseptic techniques must be followed during collecting and transportation of samples.

In the laboratory, subsamples are removed and prepared for analysis. Food samples could be solid or liquid, and from the food environment (factory, store, kitchen) surface and air samples are also delivered. A common requirement for all samples is to bring them into suspension aseptically. Solid samples need to be **homogenized** with a sterile blender or in a sterile plastic bag when using a stomacher. In most cases, the initial suspension of food samples is prepared in a 1:10 **dilution**, that can be further diluted when required. Repeated steps of dilution are not only a source of error but also a source of contamination if aseptic conditions are not met.

2.2. Cultivation

A **culture medium** for growing microorganisms under lab conditions should provide all the nutrients required by them. Obviously, no universal growth medium exists, and no single medium will support the growth of all different types of organisms that occur in

foods. **Complex media** are useful for the cultivation of a wide range of microorganisms; these contain certain natural ingredients (such as peptone, meat extract, yeast extract and others) rich in nutrients and provide growth factors. A medium prepared entirely of chemically defined components is termed a **synthetic medium**. A medium may include some compound to inhibit the growth of most microorganisms while selectively permit some others. **Selective media** can be used to favor the development of one particular organism over others and to obtain an increased population of a particular type by **enrichment culture**. Also, certain substances or reagents can be incorporated in a medium in order to develop different and characteristic colonies; **differential media** can reveal differences among the microorganisms grown on it.

Media differ in consistency, and can be liquid or solid. Media are solidified most frequently by agar, or some other gelifying agent. Agar media when molten are used for the preparation of **pour plates**, whereas when already in gel state, colonies can develop on the surface of the plate by **streaking** or **spreading**.

Preparation of media requires skill and knowledge. Manuals describe the precise composition of a large variety of media; the prescribed amount of components, the way of adding ingredients, the adjustment of pH, the distribution into flaks or tubes, and the temperature regime of sterilization to be followed accordingly. Otherwise, commercially prepared and dehydrated media can be purchased from specialized suppliers with instructions for preparation. Also, specific guidelines refer to the storage of media and reagents; prepared media may require refrigeration. All media must be labeled with the date received, prepared or opened; media and reagents with an expired shelf life must be discarded.

Media are not the only provisions to cultivate microorganisms under artificial conditions—other requirements for growth are also to be met. Most important are the suitable temperature, humidity, and atmosphere composition. A medium after **inoculation** is placed into a cabinet at a constant temperature, and kept for a period until growth and development has occurred. For **incubation** of anaerobic organisms air (oxygen) should be removed or replaced with inert gas such as nitrogen or carbon dioxide.

2.3. Pure Culture Technique

Food samples and other materials commonly contain mixed populations of different microorganisms. The preparation of a pure culture aims at the **isolation** of a given microorganism from a mixed population. The isolate can be maintained in pure culture for further study. Pure cultures can be simply obtained from discrete colonies formed on agar media, and streaking on plates is generally the most useful method for isolating pure cultures from liquids as well.

The streak plate method is a rapid and simple technique for obtaining well-isolated colonies on the surface of the plate, each arising hopefully from a single cell, so that pure cultures from a mixture can be established. If the number of cells in the suspension is believed to be small, a loopful is transferred to the plate and the streaking is done

from the edge of the Petri dish toward the center. Otherwise, with a dense population, only a quarter of the plate area is streaked. After this, the loop is flamed and cooled again before continuing streaking the next part by touching the loop to the far end of the primary streak. The three or four-phase streaking pattern should give satisfactory results for obtaining isolated colonies. The pour plate and spread plate methods (see below), though used primarily for enumeration of microorganisms, often result in well separated, discrete colonies which are available for the isolation of pure cultures with repeated plating or streaking. The procedure should be repeated at least three times until the uniform appearence of colonies becomes obvious.

Quantitative microbiology is concerned with determining the concentration of microbial cells, i.e. the number of living cells per ml or per gram of the sample. Enumeration is often made by direct counting of colonies developed on plates. It is assumed that each colony arises from a single cell originally inoculated into the medium. However, this is not always the case. Even after thorough homogenization, chains or clumps of cells may remain attached, and produce a single colony. Hence, instead of cell counts, the term **colony forming units (CFUs)** per g or ml is commonly used in food microbiology.

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Biographical Sketch

Tibor Deák, Ph.D., D.Sc., is Professor Emeritus at the Department of Microbiology and Biotechnology, Faculty of Food Science of the University of Economics, Budapest, Hungary.Dr. Deák received his B.Sc. degree in biology and chemistry from the University of Szeged in 1957, and his M.Sc. degree in microbiology from the Eotvos University of Budapest in 1963. He received his Ph.D. and D.Sc. degrees in biological sciences in 1970 and 1989, respectively, from the Biology Section, Hungarian Academy of Sciences, Budapest. After gaining experiences at the Budapest Canning Co. and the Research Institute for Canning Industry, he was appointed an Assistant Professor in Microbiology at the University of Horticulture and Food Science in 1967. He became an Associate Professor in 1970 and full Professor in 1980. He was the Head of the Department from 1970 to 1996, and served two terms as Dean of the Faculty of Food Science and Technology (1986-1991) and one term as the Rector of the University (1993-96).Dr. Deak served as the President of the Hungarian Scientific Society for Food Industry, and currently is Vice-President of the International Committee for Food Microbiology and Hygiene, board member of the International Committee for Yeast, International Committee for Food Mycology, Hungarian Society of Microbiology, and member of the World Federation of Culture Collection, European Culture Collection Organization, American Society of Microbiology. He has been granted post-doctoral research fellowships from the Hungarian Academy of Sciences, British Council, FAO-UNO, and has twice been a Senior Fulbright Scholar. Among other awards he has been the recipient of the Sigmund Award for Food Science, the Manninger Award for Food Microbiology, and received twice the Magister Optimus teaching award from the student association. He is a Fellow of the American Academy of Microbiology, a Distinguished Fellow of the Kansas State University, Manhattan, and appointed External Examiner of the Free State University, Bloemfontein, South Africa.Dr. Deak is author or co-author of more than 230 research papers and eleven books, 15 textbooks and manuals. His current research interests include the microbial ecology of foods, biodiversity of yeasts in agro-ecosystems, yeasts as spoilage agents in foods and their detection and identification.