RAPID MICROBIOLOGICAL METHODS IN MEAT AND MEAT PRODUCTS

ANDRÉS OTERO, MARÍA-LUISA GARCÍA-LÓPEZ & BENITO MORENO
Department of Food Hygiene and Food Technology. Veterinary Faculty, University of León. E-24071-León (Spain)
Correspondence address: Andrés Otero. Department of Food Hygiene and Food Technology. Veterinary Faculty, University of León. E-24071-León (Spain). Phone: +34/987/291286. Fax: +34/987/291284. E-mail: dhtaoc@unileon.es

ABSTRACT

Modern analytical techniques have to provide accurate results in time to permit forward approaches for controlling the microbiological quality of foods. Although the classical microbiological procedures of counting and identification of microorganisms have been automated, several techniques with quite different principles are now used.

The quantification of the microbial load of meat samples can be done in 4-24 hours with methods involving the growth of microorganisms in liquid media (impedance and turbidimetric methods) and in less than 2 hours with methods counting stained microbial cells or detecting components of microbial cells.

Rapid detection of undesirable (pathogens or spoilers) microorganisms in meat samples can be done with immunological and DNA-based methods.

The main techniques which can be used for the microbiological quantification and identification in the meat industries, as well as their applications are reviewed. Possibilities for rapid typing of bacterial isolates are also presented. Finally, some future trends in the microbiological analysis of foods are discussed.

INTRODUCTION

Microbiological analysis in the meat industry is mainly targeted to count total microorganisms or a specific part of the microflora (mesophiles, psychrotrophs, indicators, etc.) or to detect the presence of selected genera or species of microorganisms (pathogens such as *Salmonella, Listeria monocytogenes, Escherichia coli* O157:H7, *Yersinia enterocolitica, Campylobacter,* etc., potential spoilers such as *Pseudomonas, Brochothrix thermosphacta,* etc.). Microbiological based techniques are also used in order to evaluate the activity of starter cultures or in compositional studies (determination of vitamins, detection of antibiotics or mutagenic compounds, etc.).

Since the end of the last century, microbial numbers have been determined by so-called colony counts on solid media. The detection of bacteria belonging to genera or species commonly involved in food-borne outbreaks of infections and intoxications is done (as a general scheme) by a laborious procedure involving one or more of the following steps: preenrichment, selective enrichment, isolation in selective plating media, and confirmation of the identity of isolates by microscopic, biochemical and immunological characteristics.

This traditional approach to microbiological analyses in the meat industry is the basis of the retrospective use of the results in order to improve the microbiological quality of future lots of meat products. However, modern approaches of food quality assurance (HACCP and similar systems) demand procedures by which the microbiological status of raw materials or end products may be rapidly assessed so as to allow active measures before marketing meat products. The HACCP-based systems require information not only on the genera or species of bacteria present in the different raw materials and end products, but also takes account of the "infection chain" and the transfer of particular strains by the use of modern typing methods.

Also, the quality and reliability of results of microbial analysis have to be assured (accreditation of laboratories). Automation of techniques as well as internal controls, are some of the ways of assuring the quality of analytical results.

Techniques now used in the detection of pathogenic bacteria from foods are commonly adapted from methods previously developed for clinical applications, although the gap between the application of a concrete clinical technique to the analysis of foods is decreasing (Fung, 1995). Also, the application to the food analysis of a technique with great success when it is used with clinical samples has to overcome the complexity of the food matrix. Different physical, chemical and biological methods of separation of microorganisms from substances of the foods interfering the analytical technique are now available.

In order to respond to the above cited analytical demands, several technological developments have taken place in the last years. The majority of these developments are using a quite different basis for the microbiological quantification or identification. The complexity of current techniques is growing as some of them are combining several technological developments, which makes it difficult to classify the microbiological techniques available to the meat industry microbiologist.

A classification of rapid and automated methods which can be used in the meat industry is presented in Table 1. A great number of such methods can easily be performed as specific instruments and/or media or reactives/kits are now marketed. The equivalence of results obtained with such commercial systems and those obtained with classical procedures has been commonly proved (see Patel and Williams -1994- for a review).

Table 1. Classification of rapid and automated methods commonly employed for the microbiological analysis in the meat industry

1. Methods used for quantification
   1.1. Automation in colony counts methods:
      Media preparators/pourers and dispensers
      Gravimetric diluters, liquid diluters
      Spiral plating systems
      Automated counting procedures
      Ready-to-used commercialised systems (pre-poured plates, dip slides; Redigel™; Petrifilm™)
      Membrane filtration based methods: Hydrophobic Grid Membrane Filter (HGMF) system.
   1.2. Methods involving growing microorganisms in liquid media and detection of electrical or optical changes. Quantification is done in hours (4-24, depending on the initial flora and the searched group of microorganisms).
      Methods based on impedance determinations
      Turbidimetric methods
   1.3. Very rapid methods (results in less than 2 h).
      Methods for counting microbial cells:
         Fluorescence microscopy methods (Direct Epifluorescent Filter Technique, DEFT)
         Flow or solid-phase cytometry-based methods
      Methods for detecting components of microbial cells:
         Aminopeptidase test, Catalase test
         Limulus lysate test
         ATP-bioluminescence methods

2. Rapid methods for the detection of selected genera or species of bacteria and/or toxins of bacterial origin
   2.1. New media with chromogenic or fluorogenic substrates
   2.2. Automated systems for the identification of isolates based on phenotypic features
   2.3. Immunological techniques
   2.4. DNA-based methods

3. Rapid methods for typing of bacteria isolated from meat and meat products.
   3.1. Phenotypic typing.
   3.2. Genotypic typing.

1. METHODS FOR MICROBIAL QUANTIFICATION

1.1. Innovations in colony counts methods.

The traditional procedure for counting microorganisms from meat samples involves: (i) homogenisation, (ii) dilution, (iii) plating onto appropriate media, (iv) incubation at selected temperatures for more than 24 hours, and (v) counting of specific colonies.

Several technological advances enable the automation of this classic procedure. Stomaching is the reference method of homogenisation. Gravimetric diluters considerably reduce errors from manual weighing and dispensing (García-Armesto et al., 1993). Not only different automated systems for preparing agar filled Petri dishes are available, but also previously prepared and certified plates with different types of culture media are marketed. Plating of homogenates and certain dilution can be done with the Spiral plater, which has been tested for different meat samples (von Gerats and Snijders, 1978). The sensitivity of the instrument is 10^3-10^6 cfu/ml or g. A Spanish patent (IUL S.A.) satisfactorily solves problems of cross-contamination between samples. Automated colony counters (some of them adapted to specific plating procedures, such as the laser colony counter for spiral plates) solves, through software analysis of images, interferences from food particles and those derived from the different optical properties of the diverse types of colonies.

Several ready-to-use marketed systems can be used for reducing materials (agar, space in incubation ovens, etc.): Redigel,

Petrifilm™ (García-Armesto et al., 1993). Petrifilm™ plates can be used for counting mesophiles, psychrotrophs, coliforms, *Escherichia coli,* and yeasts and moulds. Sampling of surfaces for estimating hygienic status is easily done with contact slides coated with an appropriate agar medium, some of which are commercially available; however, recovery of bacteria from surfaces is scarce (McGoldrick et al., 1986). A rapid method, combining direct plating on a Petrifilm plate and a direct blot enzyme immunoassay, was described for the detection of Escherichia coli O157:H7 on beef carcasses (Calicchia et al., 1997).

In the Hydrophobic Grid Membrane Filter (HGMF) procedure, a special filter (with a pre-printed hydrophobic grid), a most probable number-like system of enumeration of microorganisms (after counting squares with microbial growth) and specific media (depending on the group of microorganisms) are combined (Sharpe and Michaud, 1974). HGMF-based techniques are available for counting viable organisms, coliforms, faecal coliforms, *Escherichia coli,* enterococci, yeasts and moulds, lactic acid bacteria, *Salmonella, Staphylococcus aureus, Yersinia enterocolitica, Pseudomonas aeruginosa, Clostridium perfringens,* as well as several spoilage bacteria (García-Armesto et al., 1993; Greer and Dilts, 1997).

1.2. Methods based on the inoculation of liquid media and the quantification of microbial numbers after modifications in optical or electrical properties of the media.

Changes in impedance, conductance or capacitance during the growth of microorganisms in liquid media can be easily and frequently (every 5-10 minutes) measured, recorded and analysed with appropriate equipments (see Bolton & Gibson, 1994, for a description of the main marketed instruments). Specific media (with a predifinite electrical stability and susceptible to electrical changes after chemical modifications caused by the growth of microorganisms) have to be used. After standardisation (including the construction of a calibration curve between the initial numbers of microorganisms and the time of incubation necessary for the development of a sharp change in the measured electric property -detection time-), such systems can be used for routinely determinations of total viable flora, *Enterobacteriaceae,* coliforms, *Escherichia coli,* enterococci, and yeasts and moulds.

With appropriate media (specifically designed for preventing the growth of background flora, as well as for detecting electrical changes related mainly to a specific biochemical property of the searched microorganisms) and, in some cases, combining the electrical measures with other microbial techniques (preenrichment in culture media, immunomagnetic separation, etc.), protocols have been designed for the detection of selected food-borne pathogens *(Salmonella, Listeria, Campylobacter, Staphylococcus aureus, Yersinia enterocolitica, Clostridium botulinum;* Bolton & Gibson, 1994) or some of the genera or species of bacteria involved in meat spoilage (Russell, 1997; Salvat et al., 1997).

Commercially availabe instruments have different potentialities, but all of them are including computer interface, different temperature combinations (in the range 4-60 °C, depending on the instrument), and the possibility of carrying out simultaneously a great number of tests (until 1200).

Results of the microbial analysis are produced in a very short period of time compared to classical methods. For example, in the determination of *Enterobacteriaceae,* counts can be done in 7.3-10.3 h when the initial contamination is 10^2 cfu/g and 1.8-3.8 h with 10^6 cfu/g (Bolton & Gibson, 1994).

Electrical instruments can also be used for sterility tests, and for shelf-life predictions. The activity of starters may be quickly checked with this kind of equipment. An automated approach to typing of bacterial isolates using conductance measurements has recently been proposed (Jason & Jason, 1997). Profiles obtained by plotting the first derivate of the growth of a particular organism (the growth rate) as a function of time are suggested to be used as fingerprintings for typing and identification of bacterial isolates.

Changes in turbidimetric measurements during the incubation of specific liquid media have been used for counting total viable microorganisms, lactic acid bacteria, *Brochothrix thermosphacta, Pseudomonas, Enterobacteriaceae, Micrococcaceae* in several meat samples (Schulz et al., 1988; Jakob et al., 1989). A calibration curve between the initial counts and the time necessary to reach a definite change in optical density is used for establishing bacterial counts. Time for obtaining results is considerably reduced. Lactic acid bacteria in the range 10^3 to 10^9 cfu/g can be detected within 48 h. Counts exceeding 10^7 cfu/g can be detected within 6 h. Two automated instruments have been designed and marketed (Mattila, 1987; Schulz et al., 1988). Both have the possibility of analysing a great number of samples per run, temperature control, as well as computer recording and analysis of data. One of them includes a centrifugation step prior to optical readings.

Although their use in quality control is possible, the main usefulness of turbidimetric-based instruments is probably related to the development of the high number of experiments which are necessary to design mathematical models predicting the behaviour of selec-

ted pathogenic bacteria in different internal environments (meat products with different levels of antimicrobials) (Begot et al., 1997).

Specific liquid media with redox or pH indicators are used in another optical instrument designed for the microbiological analysis of foods. Aliquots of homogenate are inoculated and changes in optical properties of the media are periodically recorded along incubation. Detection times (when a sharp variation in optical density occurs) are related to initial counts. In less than 10 hours, the aerobic flora of hamburger meat can be assessed if it is in the range 10^5-10^7 cfu/g (Shelef & Eden, 1996). Specific protocols for the detection of *Listeria monocytogenes, Salmonella* and *Escherichia coli* can be done (Shelef & Eden, 1996). Probably, other dye indicators can be used (Shelef & Eden, 1996).

1.3. Very rapid methods.

Although electrical and optical methods provide results about the microbiological quality of samples in time to take measures before marketing of meat products, some techniques are able to inform about the microbiological quality of perishable raw materials before the application of a determined technological process, that is, microbial results can contribute to design the parameters of the process.

In the Direct Epifluorescent Filter Technique (DEFT), a part of the homogenate is filtered, the retained microorganisms stained with fluorescent dyes (usually acridine orange) and then counted with an epifluorescence microscope or an automated system based on image analysis. A completely automated instrument for doing the process from filtration to counting is available (Pettipher et al., 1992). Applications of the DEFT have been revised (García-Armesto et al., 1993). Although both viable and non-viable bacterial cells are stained, there is a good agreement, in meat and fish samples, between mesophilic plate counts and DEFT-counts in the range 10^4-10^7 cfu/g (Pettipher et al., 1992). On the other hand, results are available in less than 1 hour. The sensitivity of the method, when it is applied to the analysis of raw and cooked beef, can be improved with a previous 1-h separation and concentration procedure, which includes an enzyme treatment and differential centrifugation (Rodrigues-Szulc et al., 1996).

Flow cytometry (FCM) is an optically-based technique which can provide cell-by-cell analysis. A recent review presents the principles of FCM and the main applications in microbial analysis (Davey and Kell, 1996). Cell suspensions are treated with appropriate fluorescent stains, passed through a laser beam and light scattering and fluorescence parameters are determined. Mathematical analysis of the different signals is done with appropriate software. Different kinds of fluorochromes can be used, including fluorescent-labelled antibodies or oligonucleotides. Flow cytometric sorting can also be used as an automated and selective enrichment procedure (Davey and Kell, 1996).

Modern solid phase cytometers including automated systems for doing the several steps from the preparation of samples to the computerised analysis of data are now marketed. Such equipments scan liquid samples after filtering and labelling. Specific applications useful in the analysis of meat samples have been carried out. So, viable counts (after staining with viability label) can be done in meat samples with more than $10^{4\text{-}5}$ cfu/g within 45 minutes. A previous 6-hours enrichment step and fluorescent antibodies can be used for the determination of *Salmonella,* although the sensitivity is 1 cell/ml of liquid media (McClelland & Pinder, 1994).

Some very rapid methods for the estimation of microbial contamination of food samples are based on the detection of components of the microbial cells. A spectrophotometric method for the quantification of the aminopeptidase activity in meat samples has been described (Alvarado et al., 1992). Aminopeptidase activity is related with the cell walls of most of psychrotrophic Gram-negative bacteria. In less than 3 hours, it is possible to have an indication of the levels of psychrotrophs, ranging between 10^4 and 10^8 cfu/cm^2 of meat surfaces (Alvarado et al., 1992).

Bacterial catalase can be determined on meat samples with a simple test based on the disc-flotation method (Wang & Fung, 1986). A good correlation was observed between transformed log counts of mesophiles or psychrotrophs and log of the flotation time (indicating the catalase activity) for swab-samples from cold-stored chicken (Wang & Fung, 1986). Results are available in less than 1 hour.

Lipopolysaccharide (endotoxin) from cells of Gram negative bacteria is able to clot a lysate of *Limulus* amoebocytes. Endotoxin titers increase with viable counts of Gram-negative bacteria in beef samples (Jay, 1992). The method, which provides results in less than 2 hours, has been used for estimating the microbial quality of raw fish and cooked turkey rolls (Jay, 1992). Several modifications in the original clotting method have been developed, including colorimetric and turbidimetric systems for reading of tests (González et al., 1994).

Three kinds of analytical methods for microbial examination of foods are based on luminescence detection: (i) ATP-bioluminescence methods, (ii) bacterial luminescence-based systems, and (iii) methods employing chemiluminescent labels or chemiluminescent substrates (Kyriakides & Patel, 1994).

Although bioluminiscence methods for the detection of ATP from a selected group of microorganisms (by using specific ATP extractants) have been published (Tuncan & Martin, 1987; Griffiths, 1996), the bioluminescence assays are mainly employed for the assessment of cleanliness of surfaces and hygienic practices in food-processing plants, through the estimation of total ATP, both from bacterial and organic residues origin (Kyriakides and Patel, 1994).

Several types of systems for the ATP detection are now marketed, the majority of them being portable and equipped with all reactives and utensils necessary for the evaluation of surfaces in contact with foods (Hawronskyj & Holah, 1997). A specific relationship between the readings of relative light units (RLU) and the hygienic status is necessary for each application and each luminometer. Results are available in minutes, but the provided information is of the semaphore type. Selection of an adequate luminometer (Anon., 1997) and the influence of the different cleansers and sanitizers used in the food-processing plants on the results of the ATP-based methods (Velazquez & Feritag, 1997) are two of the key elements when it is considered to implement an ATP-bioluminescent based procedure for the evaluation of cleanliness of food-processing plants.

Genes responsible for bacterial bioluminescence (*lux* genes) have been used for the development of specific assays for the detection of pathogens and indicator microorganisms. In general, *lux* genes are inserted into bacteriophages specific for the searched organism *(Salmonella, Listeria)* or group of organisms (coliforms, *Enterobacteriaceae)*. The modified viruses are employed in a very sensitive systems for the detection of pathogens or indicator groups, but it is usually necessary to resuscitate and to increase numbers of the target microorganisms before using the *luminescent* bacteriophages.

2. RAPID METHODS FOR THE DETECTION OF SELECTED GENERA OR SPECIES OF BACTERIA AND/OR TOXINS OF BACTERIAL ORIGIN

Some of the methods previously described are also applied to the detection of pathogenic or selected spoilage microorganisms, however, a considerable number of methods with quite a different analytical approach have been developed.

2.1. New media with chromogenic or fluorogenic substrates.

The inclusion of fluorogenic or chromogenic substrates for the detection of specific enzymatic activities of microbial origin in culture media permits a quick detection and identification of microorganisms, bypassing the need for time-consuming isolation procedures prior to identification. Media including such substrates for rapid reading have been designed for the detection of *Enterobacteriaceae, Escherichia coli,* coliforms, *Salmonella* and others (Manafi, 1996).

2.2. Automated systems of identification of isolates based on phenotypic features.

Conventional identification (by considering mainly cultural, biochemical, morphological, and serological features) is cumbersome and is not appropriate for routinely analysis in food industries. Thus, several automated systems for microbial identification have been designed and marketed. The majority have been developed for the identification of isolates from clinical samples, although some companies are now expanding their microbial databases and are including data from microbial species of food origin.

Apart from the different miniaturised biochemical kits for the identification of selected groups of microorganisms (API strips and others), several instruments for an automated identification of isolates are now available (Stager and Davis, 1992).

The AutoMicrobic System of bioMérieux uses a computer-driven optical reader and specific cards for generating a biochemical profile. The identification is done by comparing each profile with those included in a database. Cards for Gram-negative bacteria, Gram-positive bacteria and yeasts are available.

The Biolog system uses 96-microwell plates in which a basal medium with different carbon sources (94 carbon sources and two additional control wells) is included, as well as a redox dye, for rapid detection of the microbial activity in each well. After 4-24h of incubation, the resulting pattern of active wells yields a "metabolic fingerprinting" of the tested microorganism, which is later identified by comparison with patterns of reference database. The Biolog GN microplate (for the identification of Gram-negative bacteria), the Biolog GP microplate (for the identification of Gram-positive bacteria), and the Biolog yeast microplate are marketed. The whole automated Biolog system includes a pipettor, a turbidimeter, a computer-linked MicroPlate Reader, and the appropriate software for the microbial identification. The usefulness of this system for the identification of food and beverage yeasts has been recently evaluated (Praphailong et al., 1997).

Gas chromatographic profiles of whole-cell fatty acids has been used with taxonomic purposes. A fully automated, compute-

rised system is marketed (the Microbial Identification System, MIS or MIDI). This system has been employed for microbial species identification, and has been checked for typing of bacterial isolates (Steele et al., 1997).

2.3. Immunological techniques.

Specific antibodies are used in different formats for the detection of selected food-borne pathogenic or spoiler microorganisms and for the detection and quantification of microbial toxins (Table 2). Several commercial kits based on an immunological approach are now marketed (Patel & Williams, 1994). Both monoclonal and polyclonal antibodies are being used. Different agglutination tests are available for the rapid identification of bacterial isolates *(Staphylococcus aureus, Salmonella)*. However, the majority of commercial kits are employing labelled reactives. The main labels used in immunoassays are enzymes (peroxidase, alakaline phosphatase, etc.) and compounds participating in a luminescent reaction (acridinium ester, isoluminol derivatives, etc.).

Table 2. Main applications of enzyme immunoassays in the microbial analysis of foods

Detection of pathogens
Salmonella
Listeria spp
Listeria monocytogenes
Escherichia coli
Escherichia coli O157:H7
Vibrio spp.
Yersinia enterocolitica
Campylobacter jejuni
Detection of microbial toxins
Clostridium botulinum neurotoxins
Staphylococcus aureus enterotoxins
Bacillus cereus diarrhoeal type enterotoxin
Clostridium perfringens enterotoxin
Escherichia coli enterotoxins
Mycotoxins (aflatoxins, ochratoxin)
Detection of microorganisms involved in the food spoilage

Several types of solid-phase supports and surfaces are being used in the different diagnostic products. In general, a concentration of 10^4-10^5 of targeted cells per ml is necessary to be detected in an immunological assay. In order to increase the sensitivity of techniques, amplification systems (avidin-biotin, digoxigenin-antidigoxigenin, etc.), are frequently introduced in some of the immunoassays. Depending on the type of reactives employed, the system in which the antibody-antigen reaction is developed and the ways for reading the result, six groups of immunoassays have been defined (Deshpande, 1994).

Fully automated ELISAs for the analysis of foodborne pathogens are marketed (Patel & Williams, 1994). Immunological tests for the detection of the most common microorganisms involved in fresh meat spoilage have been described (Gutiérrez et al., 1997; García-López et al., 1998).

2.4. DNA-based methods

Specific probes and several polymerase chain reaction (PCR) assays have been described for the detection of food-borne bacterial pathogens or selected food spoilers (Olsen et al., 1995; Venkitanarayanan et al., 1997).

Several DNA-hybridization kits are commercially available. Probes are now labelled mainly with enzymes, biotin or digoxigenin. Both solid phase hybridization (filter hybridization or colony hybridization) and solution or liquid hybridization formats are used. Probes used in the hybridization assays have been selected from randomly cloned chromosomal fragments or from well-known genes (related to specific enzymatic activity or with specific virulence mechanism, etc.). Commercial hybridization probe assays are commonly used after one or more steps of microbial cultivation (enrichment, isolation).

Numerous methods based on the amplification of genetic material have been described for the detection of selected microorganisms from foods. The most frequently used are based on the polymerase chain reaction (PCR, nested-PCR, inverse-PCR, anchored-PCR, etc.), although other amplification systems are also employed (Ligase amplification reaction -LCR-, Transcription-based amplification systems -TAS, 3SR, NASBA-,..). Selected primers are used for the amplification of the searched genetic material,

and the amplicons are detected by gel electrophoresis or employing one of the modern signal amplification and detection methods (chemiluminescent label, enzymatic label, antibody capture, hybridization capture, magnetic separation, etc.).

Amplification methods for the detection of food-borne microbial pathogens have been recently reviewed (Hill and Olsvik, 1994, Olsen et al., 1995). Systems targeted to a genus or species of microorganisms can be designed. Also, protocols for the simultaneous detection of several species of food-borne pathogens have been described (Wang et al., 1997).

Although some amplification methods can be applied directly to food homogenates, in this format, their sensitivity is very low. Food matrix is frequently interfering with the amplification reactions (Wilson, 1997). Several methods for the preparation of samples before the amplification of genetic material can be used. Apart from cultural methods, physical methods, DNA-extraction methods and adsorption methods have been described (Lantz et al., 1994). Internal standards for the detection of false-negative PCR results can be used (Lambertz et al., 1998). Sensitivity of amplification methods is varying with the target microorganism and the food matrix, but, with adequate preparation of samples, *Salmonella* can be detected at levels over 0.1 cfu/g of meat in 30 hours (enrichment, immunomagnetic separation and PCR; Fluit et al., 1993).

Some PCR-based and automated assays for the detection of the main meat-borne bacterial pathogens *(Salmonella, Escherichia coli* O157:H7, *Campylobacter, Listeria)* are marketed or close to the marketing stage (Batt, 1997).

3. RAPID METHODS FOR TYPING OF BACTERIA ISOLATED FROM MEAT AND MEAT PRODUCTS

Bacterial typing is done with phenotypic or genotypic methods, recently reviewed by Farber (1996). Although some phenotypic methods give reasonable results and are easy to perform, in general, genotypic methods have higher discriminatory power.

New modifications of molecular typing methods are frequently described, but the most commonly used methods are: chromosomal DNA restriction endonuclease analysis (REA, RFLP), ribotyping, pulse-field gel electrophoresis (PFGE), and PCR-based methods (randomly amplified polymorphic DNA -RAPD-, PCR ribotyping, PCR-RFLP, rep-PCR). A commercial system for automated ribotyping is now available (Farber, 1996).

4. FUTURE TRENDS

Fung (1995) selected ten attributes of an ideal rapid and/or automated foodborne microbial assay system: accuracy of results, speed, initial and current costs, acceptability for the scientific community, simplicity to operate, training needs, availability and stability of reagents and supplies, company reputation, technical service and space requirements.

The ideal techniques of microbial analysis to be applied in quality assurance units of meat industries will probably be selected from those with a great versatility (determination of multiple microbial parameters), with a high level of automation, including enough internal controls for assuring results, and providing results directly transferred to an integrated food process monitoring system. Miniaturisation of biological detection systems and their integration in microchips (biosensors) is being announced from several years ago as one of the "magic" analytical solutions.

ACKNOWLEDGEMENTS

We thank the Spanish Comisión Interministerial de Ciencia y Tecnología (Project No. CICYT ALI95-0132) for financial support.

REFERENCES

Alvarado, R., Rodríguez-Yunta, M.A., Hoz, L., García-de-Fernando, G.D., & Ordóñez, J.A. (1988) *J. Food Sci.* 57 (6), 1330.

Anon. (1997) *Food Quality March,* 20.

Batt, C.A. (1997) *J. Dairy Sci.* 80 (1), 220.

Begot, C., Lebert, I., & Lebert, A. (1997) *Food Microbiol.* 14 (5), 403.

Bolton, F.J. & Gibson, D.M. (1994) in *Rapid analysis techniques in food microbiology* (Patel, P., Ed.) pp 131-169, Blackie Academic & Professional, London.

Calicchia, M.L., Parker, E.L., Gambrel-Lenarz, S., & Matner, R.R. (1997) *J. Food Protect.* 60, 870.

Davey, H.M. & Kell, D.B. (1996) *Microbiol. Rev.* 60, 641.

Deshpande, S.S. (1994) *Food Technol.* 48 (6), 136.

Farber, J.M. (1996) *J. Food Protect.* 59 (10), 1091.

Fluit, A.C., Widjojoatmodjo, M.N., Box, A.T.A., Torensma, R., & Verhoef, J. (1993) *Appl. Environ. Microbiol.* 59 (5), 1342.
Fung, D.Y.C. (1995) *Food Technol.* 49 (6), 64.
García-Armesto, M.R., Prieto, M., García-López, M.L., Otero, A., & Moreno, B. (1993) *Microbiol.* SEM 9, 1.
García-López, M.L., Prieto, M., & Otero, A. (1998) in *The microbiology of meat and poultry* (Davies, A. & Board, R., Eds.) pp 1-34, Blackie Academic and Professional, London.
González, I., Martín, R., García, T., Morales, P., Sanz, B., & Hernández, P.E. (1994) *Alimentación, Equipos y Tecnología* 13 (4), 97.
Greer, G.G. & Dilts, B.D. (1997) *J. Food Protect.* 60 (11), 1388.
Griffiths, M.W. (1996) *Food Technol.* 50 (6), 62.
Gutiérrez, R., García, T., González, I., Sanz, B., Hernández, P.E., & Martín, R. (1997) *J. Food Protect.* 60 (8), 908.
Hawronskyj, J.-M. & Holah, J. (1997) *Trends Food Sci. Technol.* 8 (3), 79.
Hill, W.E. & Olsvik, O. (1994) in *Rapid analysis techniques in food microbiology* (Patel, P., Ed.) pp 268-290, Blackie Academic & Professional, London.
Jakob, R., Lippert, S., & Baumgart, J. (1989) *Zeitschrift für Lebensmittel-Untersuchung und-Forschung* 189 (2), 147.
Jason, D. & Jason, A.C. (1997) *Lett. Appl. Microbiol.* 25 (6), 431.
Jay, J.M. (1992) in *Modern food microbiology,* Van Nostrand Reinhold, New York.
Jorgensen, H.L. & Schulz, E. (1985) *Int. J. Food Microbiol.* 2, 177.
Kyriakides, A.L. & Patel, P.D. (1994) in *Rapid analysis techniques in food microbiology* (Patel, P., Ed.) pp 196-231, Blackie Academic & Professional, London.
Lambertz, S.T., Ballagi-Pordány, A., & Lindqvist, R. (1998) *Lett. Appl. Microbiol.* 26 (1), 9.
Lantz, P.G. & Hahn-Hägerdal, B.R. (1994) *Trends Food Sci. Technol. 5 (12),* 384.
Manafi, M. (1996) *Int. J. Food Microbiol.* 31 (1-3), 45.
Mattila, T. (1987) *J. Food Protect.* 50 (8), 640.
McClelland, R.G. & Pinder, A.C. (1994) *J. Appl. Bacteriol.* 77 (4), 440.
McGoldrick, K.F., Fox, T.L., & McAllister, J.S. (1986) *Food Technol.* 40 (4), 77.
Olsen, J.E., Aabo, S., Hill, W., Notermans, S., Wernars, K., Granum, P.E., Popovic, T., Rasmussen, H.N., & Olsvik, O. (1995) *Int. J. Food Microbiol.* 28 (1), 1.
Patel, P.D. & Williams, D.W. (1994) in *Rapid analysis techniques in food microbiology* (Patel, P., Ed.) pp 61-103, Blackie Academic & Professional, London.
Pettipher, G.L., Watts, Y.B., Langford, S.A., & Kroll, R.G. (1992) *Lett. Appl. Microbiol.* 14, 206.
Praphailong, W., Gestel, M.V., Fleet, G.H., & Heard, G.M. (1997) *Lett. Appl. Microbiol.* 24, 455.
Rodrigues-Szulc, U.M., Ventoura, G., Mackey, B.M., & Payne, M.J. (1996) *J. Appl. Bacteriol.* 80 (6), 673.
Russell, S.M. (1997) *J. Food Protect.* 60, 385.
Salvat, G., Rudelle, S., Humbert, F., Colin, P., & Lahellec, C. (1997) *J. Appl. Microbiol.* 83 (4), 456.
Schulz, E., Jensen, B., & Celerynova, E. (1988) *Int. J. Food Microbiol.* 6, 219.
Sharpe, A.N. & Michaud, G.L. (1974) *Appl. Microbiol.* 28, 223.
Shelef, L.A. & Eden, G. (1996) *Food Technol.* 50 (1), 82.
Stager, C.E. & Davis, J.R. (1992) *Clin. Microbiol. Rev.* 5 (3), 302.
Steele, M., McNab, W.B., Raad, S., Harris, L., & Lammerding, A.M. (1997) *Appl. Environ. Microbiol.* 63, 757.
Tuncan, E.U. & Martin, S.E. (1987) *Appl. Environ. Microbiol.* 53 (1), 88.
Velazquez, M. & Feirtag, J.A. (1997) *J. Food Protect.* 60, 799.
Venkitanarayanan, K.S., Faustman, C., Crivello, J.F., Khan, M.I., Hoagland, T.A., & Berry, B.W. (1997) *J. Appl. Microbiol.* 82, 359.
Von Gerats, G.E. & Snijders, J.M.A. (1978) *Arch. Lebensmittelhyg.* 29, 57.
Wallner, G., Fuchs, B., Spring, S., Beisker, W., & Amann, R. (1997) *Appl. Environ. Microbiol.* 63 (11), 4223.
Wang, R.-F., Cao, W.-W., & Cerniglia, C.E. (1997) *J. Appl. Microbiol.* 83 (6), 727.
Wang, G.I.J. & Fung, D.Y.C. (1986) *J. Food Sci.* 51, 1442.
Wilson, I.G. (1997) *Appl. Environ. Microbiol.* 63 (10), 3741.