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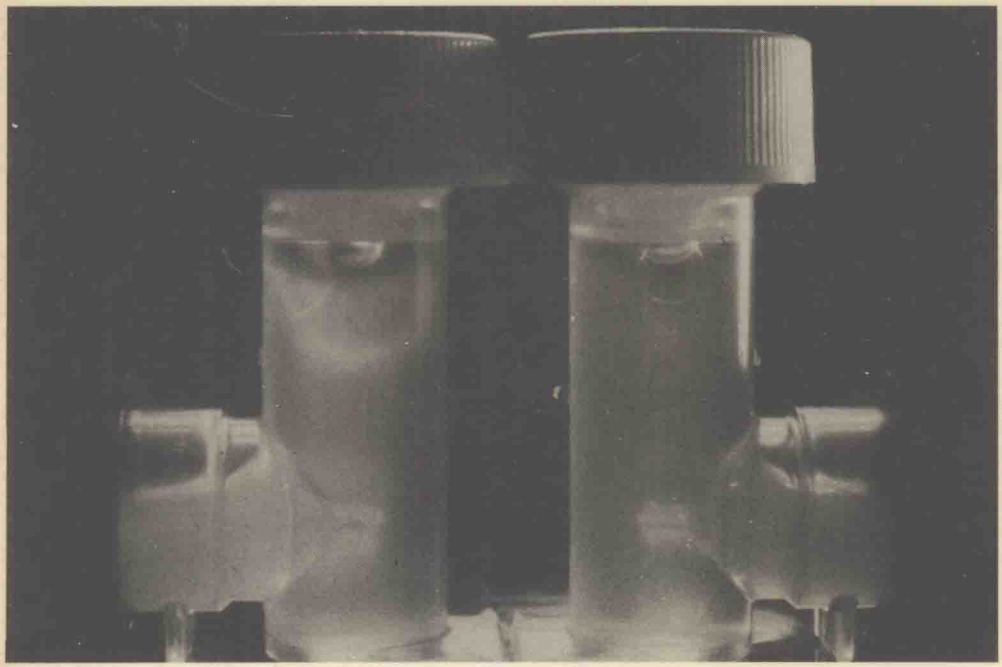
25

RAPID MICROBIOLOGICAL METHODS FOR FOODS, BEVERAGES AND PHARMACEUTICALS

Edited by
C.J. Stannard, S.B. Petitt and F.A. Skinner



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Rapid Microbiological Methods for Foods, Beverages and Pharmaceuticals

Edited by

C. J. STANNARD
Applied Research Department,
Pedigree Petfoods,
Melton Mowbray,
Leicestershire LE13 7BB

S. B. PETITT
U.B. (Ross Youngs) Ltd,
Ross House, Wickham Road, Grimsby,
South Humberside DN31 3SW

F. A. SKINNER
Harpenden,
Herts

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Contributors

M. ALLEN, *Bioelectronics Division, Biotechnology Centre, Cranfield Institute of Technology, Cranfield, Bedfordshire MK43 0AL, UK*

M. L. ARNOTT, *Cadbury Schweppes PLC, Group Research, The Lord Zuckerman Research Centre, The University of Reading, Reading RG6 2LA, UK*

J. W. AVIS, *Research and Development Laboratory, Allied Breweries Ltd, 107 Station Road, Burton-on-Trent, Derbyshire DE14 1BZ, UK*

R. P. BETTS, *Campden Food & Drink Research Association, Chipping Campden, Gloucestershire GL55 6LD, UK*

ANIA BIALKOWSKA, *Leatherhead Food R.A., Randalls Road, Leatherhead, Surrey KT22 7RY, UK*

JULIE A. BIRD, *Microbiology Department, Unilever Research and Engineering, Colworth Laboratory, Colworth House, Sharnbrook, Bedfordshire MK44 1LQ, UK*

C. DE W. BLACKBURN, *Applied Microbiology Section, Leatherhead Food R.A., Randalls Road, Leatherhead, Surrey KT22 7RY, UK*

B. J. BROOKS, *Beecham Pharmaceuticals Research Division, Chemotherapeutic Research Centre, Brockham Park, Betchworth, Surrey RH3 7AJ, UK*

K. COLEMAN, *Beecham Pharmaceuticals Research Division, Chemotherapeutic Research Centre, Brockham Park, Betchworth, Surrey RH3 7AJ, UK*

S. P. DENYER, *Department of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, UK*

M. C. EASTER, *Express Foods Group Ltd, 430 Victoria Road, South Ruislip, Middlesex HA4 0HF, UK*

L. J. FARR, *Foss Electric (UK) Ltd, The Chantry, Bishopsthorpe, York Y02 1QF, UK*

KATE FORDE, *Unigate Foods Ltd, Station Road, Wincanton, Somerset BA9 9ED, UK*

SHEILA M. FRYER, *St Ivel Technical Centre, Abbey House, Church Street, Bradford-on-Avon, Wiltshire BA15 1DH, UK*

D. M. GIBSON, *Ministry of Agriculture, Fisheries and Food, Torry Research Station, 135 Abbey Road, Aberdeen AB9 8DG, Scotland, UK*

M. W. GRIFFITHS, *Hannah Research Institute, Ayr KA6 5HL, Scotland, UK*

S. GAYE HADFIELD, *Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, UK*

B. W. T. HEPBURN, *Wessex Water, Regional Scientific Centre, Mead Lane, Saltford, Bristol BS18 3ER, UK*

T. E. IRVING, *Water Research Centre, Henley Road, Medmenham, PO Box 16, Marlow, Buckinghamshire SL7 2HD, UK*

D. I. JERVIS, *St Ivel Technical Centre, Abbey House, Church Street, Bradford-on-Avon, Wiltshire BA15 1DH, UK*

R. G. KROLL, *Department of Microbiology, AFRC Institute of Food Research, Reading Laboratory, Shinfield, Reading RG2 9AT, UK*

A. L. KYRIAKIDES, *Grand Metropolitan Brewing Ltd, Stag Brewery, 91 Brick lane, London E1 6QN, UK*

STEPHANIE E. LEAROYD, *Department of Microbiology, King's College, University of London, Campden Hill Road, London W8 7AH, UK*

R. A. P. LYNN, *Department of Pharmaceutical Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, UK*

E. MAY, *School of Biological Sciences, Portsmouth Polytechnic, King Henry I Street, Portsmouth, Hampshire PO1 2DY, UK*

H. MERRETT, *Virology Unit, Welsh Water PLC, Engineering and Environment Ltd, Bridgend Office and Laboratory, Tremains House, Tremains Court, Bridgend, Mid Glamorgan CF31 2AR, Wales, UK*

P. NEAVES, *Technical Division, Milk Marketing Board, Thames Ditton, Surrey KT7 0EL, UK*

R. A. PATCHETT, *Department of Microbiology, AFRC Institute of Food Research, Reading Laboratory, Shinfield, Reading RG2 9AT, UK*

N. P. PATEL, *Campden Food & Drink Research Association, Chipping Campden, Gloucestershire G155 6LD, UK*

S. B. PETITT, *U.B. (Ross Youngs) Ltd, Ross House, Wickham Road, Grimsby, South Humberside DN31 3SW, UK*

G. L. PETTIPHER, *Cadbury Schweppes PLC, Group Research, The Lord Zuckerman Research Centre, The University of Reading, Reading RG6 2LA, UK*

J. D. PHILLIPS, *Hannah Research Institute, Ayr KA6 5HL, Scotland, UK*

P. S. POVER, *Analytical Measuring Systems, London Road, Pampisford, Cambridge, CB2 4EF, UK*

G. A. PRENTICE, *Milk Marketing Board, Thames Ditton, Surrey KT7 0EL, UK*

S. J. PUGH, *Cadbury Ltd, Technical Laboratories, Bournville, Birmingham B30 2LU, UK*

SALLY A. ROSE, *Campden Food & Drink Research Association, Chipping Campden, Gloucestershire GL55 6LD, UK*

B. H. SCHNEIDER, *Bioelectronics Division, Biotechnology Centre, Cranfield Institute of Technology, Cranfield, Bedfordshire MK43 0AL, UK*

A. O. SCOTT, *Campden Food & Drink Research Association, Chipping Campden, Gloucestershire GL55 6LD, UK*

B. G. SHAW, *AFRC Institute of Food Research Bristol Laboratory, Langford, Bristol BS18 7DY, UK*

P. SMITH, *Research and Development Laboratory, Allied Breweries Ltd, 107 Station Street, Burton-on-Trent, Derbyshire DE14 1BZ, UK*

C. E. STACKHOUSE, *Virology Unit, Welsh Water PLC, Engineering and Environment Ltd, Bridgend Office and Laboratory, Tremains House, Tremains Court, Bridgend, Mid Glamorgan CF31 2AR, Wales, UK*

G. STANFIELD, *Water Research Centre, Henley Road, Medmenham, PO Box 16, Marlow, Buckinghamshire SL7 2HD, UK*

C. J. STANNARD, *Applied Research Department, Pedigree Petfoods, Melton Mowbray, Leicestershire LE13 7BB, UK*

M. F. STRINGER, *Department of Microbiology, Campden Food & Drink Research Association, Chipping Campden, Gloucestershire GL55 6LD, UK*

A. SWAIN, *Bioelectronics Division, Biotechnology Centre, Cranfield Institute of Technology, Cranfield, Bedfordshire MK43 0AL, UK*

F. TAYLOR, *Bioelectronics Division, Biotechnology Centre, Cranfield Institute of Technology, Cranfield, Bedfordshire MK43 0AL, UK*

C. F. THURSTON, *Department of Microbiology, King's College, University of London, Camden Hill Road, London SW14 7ET, UK*

P. A. THURSTON, *Grand Metropolitan Brewing Ltd, Mortlake, London SW14 7ET, UK*

A. P. F. TURNER, *Bioelectronics Division, Biotechnology Centre, Cranfield Institute of Technology, Cranfield, Bedfordshire MK43 0AL, UK*

M. J. WADDELL, *Technical Division, Milk Marketing Board, Thames Ditton, Surrey KT7 0EL, UK*

A. P. WILLIAMS, *Leatherhead Food R.A., Randalls Road, Leatherhead, Surrey KT22 7RY, UK*

Preface

This book is the 25th in the Technical Series of the Society for Applied Bacteriology. Each chapter is the written version of a practical contribution given at the Demonstration Meeting of the Society, held at the University of Bath on 30 September, 1987.

For many years, more rapid and labour-saving methods have been sought as alternatives to conventional microbiological techniques. Many of the pioneering studies have taken place in clinical laboratories. For foods, beverages and pharmaceuticals, the materials tested and the organisms sought are more varied. The contributions to this book illustrate the wide variety of approaches that workers in these industries have taken in order to solve the particular problems associated with their own products.

The methods described in this book include electrometric techniques, ATP assay, and immunological methods for a wide range of organisms from salmonellas to viruses. We feel that it is apparent that the choice of a rapid method for industry depends upon the equipment available and the accuracy required.

This book should be useful to those in the food, beverage and pharmaceutical industries, or in research or teaching, who require a practical guide to the use of rapid microbiological methods. We should like to thank the contributors for all their hard work in preparing the demonstrations and contributions for the book, and Dr Ron Board and his staff at the University of Bath for the organization of the meeting.

Catherine J. Stannard
S. B. Petitt
F. A. Skinner

Contents

The Use of ATP Bioluminescence for the Analysis of Beer in Polyethylene Terephthalate (PET) Bottles and Associated Plant

J. W. AVIS* AND P. SMITH
Research and Development Laboratory, Allied Breweries Ltd, 107 Station Street, Burton-on-Trent, Derbyshire DE14 1BZ, UK

The growing demand for products packaged in polyethylene terephthalate (PET) bottles (which cannot be pasteurized) has led to increased emphasis on plant hygiene and end-product quality assurance. Rapid methods of detecting microbial contamination are especially useful for these products and this work concentrates on the rapid membrane filtration of products directly from PET bottles and the analysis of membrane filters and production plant swabs by adenosine triphosphate (ATP) bioluminescence.

Many changes are currently taking place in microbiology. Micro-organisms are being studied increasingly as part of their natural environment rather than in isolation; automation has allowed large numbers of 'routine' samples to be examined and there is increased use and development of rapid methods of detection. In the brewing industry rapid methods would be particularly helpful to detect process failure, assess the quality of pitching yeast and the microbiological status of packaged products (Hope & Tubb 1985).

Two electrical methods which have shown promise are impedence measurement as a rapid forcing test for beer (Evans 1982) and conductance measurement for the rapid detection of both lactobacilli in beer (Evans 1985) and *Obesumbacterium proteus* in pitching yeast (Kilgour & Day 1983). The sensitivity of the method is low, however, because cells are detected only when they reach a level of 10^5-10^6/ml and incubation times can be in excess of 48 h for some organisms.

* Present address: Group Quality Control Laboratory, Bass Brewing Ltd, 137 High Street, Burton-on-Trent, Derbyshire DE14 1JZ, UK.

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The Direct Epifluorescent Filtration Technique (DEFT) has been used with success for milk (Pettipher *et al.* 1980) and to a more limited extent for beer (Kilgour & Day 1983) where the method for heat-treated samples was improved by counterstaining with methylene blue. Counterstaining with Janus Green B was found to give more consistent results than methylene blue (Rodrigues & Kroll 1986) but DEFT preparations on heat-treated beverages were still found to be unreliable in differentiating between viable and non-viable yeast cells.

Adenosine triphosphate bioluminescence has been used to detect microbial contamination in carbonated beverages (Littel & La Rocco 1986), wine (Lonvaud-Funel & Joyeux 1982) and beer (Hysert *et al.* 1976; Kilgour & Day 1983; Dick *et al.* 1986). The methods detect only viable organisms at levels of the order of 100 yeast cells or 1000 bacterial cells/ml suspension.

In the work to be described ATP bioluminescence was used specifically for the problems of PET bottled beer. The PET bottle is a popular package in the UK for many bottled drinks and sales are continuing to increase. The package cannot be pasteurized or heat-treated, however, so plant hygiene is particularly important. The ATP method has a role in assuring the quality of both packaging plant and finished product. In developing the method we have concentrated on the detection of yeasts rather than bacteria, following our findings that yeasts were the cause of over 95% of contamination problems examined over an 8-month period (Avis 1988).

Materials and Methods

ATP analysis

The work was carried out using the Lumac Biocounter 2010 (Lumac BV, Schaesberg, The Netherlands). Samples are contained in disposable plastic cuvettes which are inserted into a light-tight chamber for reading by a sensitive photomultiplier tube. The amount of light emitted is displayed as a digital readout and is expressed in Relative Light Units (RLUs). The absolute amount of ATP contained in a sample may be found by injecting an ATP standard into the sample and taking a second reading. To be accurate the RLU value of the standard should be two to five times that of the sample so a range of standards are made up from a stock solution and the RLU values of these are checked before samples are analysed.

The reagents and standards required for the analyses were supplied by Lumac, and were: 'Somase' (a non-microbial ATP-ase), 'F-NRS' (used here as a buffer), 'NRB' (a nucleotide-releasing agent for the extraction of ATP from microbial cells) and 'Lumit-PM' (luciferin–luciferase reagent). ATP standards were made up from a stock solution of 1.65×10^{-6} mol/l ATP. All

powdered reagents were stored between 0° and 2°C and, when reconstituted, Lumit-PM, Somase and ATP standard stock solution were split into 1- or 2-ml aliquots which were stored for a maximum of 4 weeks at −18°C. Temperature-sensitive reagents were kept on ice during analysis.

PET bottle filtration device

Filtration methods currently used for PET bottled products suffer from two disadvantages. Firstly, the majority of commercial membrane filtration units have only a 250-ml reservoir for beer, which is inconvenient if beer has to be continually poured into the unit, particularly if a laminar flow cabinet is not available. Secondly, there exists the possibility of contamination of the beer from the outside of the neck of the bottle as the beer is poured into the filter funnel. This has led to a piercing technique in which a portion of the bottle is swabbed with 70% methylated spirit and then pierced with a hot needle; the beer is then poured into the filter. This technique is also inconvenient. The device which will be described here was developed to avoid these problems by allowing the entire contents of the bottle to be taken directly from an upright bottle without the necessity of pouring or piercing.

The device is shown in Fig. 1 and is in two parts: a quick-release clamp and the gas inlet and beer outlet tube assembly. The ¼-inch National Pipe Thread fitting on the beer outlet tube accepts a conventional 47-mm membrane filter holder (e.g. Swinnex) and the whole device can be autoclaved with the membrane in place, after which it can be attached very quickly to the bottle to be sampled. All the bottle contents are then passed through the membrane by applying a top pressure of gas which is filtered in-line. The membrane is then removed for plating on agar or ATP analysis.

Filters

Conventional cellulose acetate (0.45 μm) and Ultipore (1.2, 0.8, 0.65 μm) nylon membrane filters (Pall Process Filtration Ltd, Portsmouth) were used. The latter type are electrostatically charged, the magnitude and polarity of the zeta potential being dependent on the pH. At pH 4 (approximately the pH of beer) the filter has a positive charge.

Swabs

Plain, cotton wool sterile swabs (Northern Media Supply Ltd, Hessle) were used to assess the cleanliness of plant associated with PET bottling. Charcoal-impregnated swabs must not be used as these affect the results of ATP analysis.