

RAPID DETECTION OF ENTEROBACTERIACEAE IN DEHYDRATED FOODS USING IMPEDANCE MICROBIOLOGY: THE CRITICAL ROLE OF THE PRE-ENRICHMENT PHASE

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1 INTRODUCTION

The bacteriological examination of food in order to monitor sanitary practices, processing efficacy and post processing contamination encompasses screening for specific groups of bacteria or individual species. These organisms are commonly referred to as indicator organisms. Particular groups or genera may also be used to assess the potential risk of closely related pathogens being present in foods, a function for which the term index organism has been proposed^{1,2}.

The most popular group of indicator organisms has been the coliforms, although this group does not have great specificity. The genera detected by standard coliform tests are those members of the Enterobacteriaceae that are able to utilise lactose with acid and gas production. Thus, slow lactose-fermenting or lactose negative organisms would be overlooked. To improve this situation with respect to assessment of food safety and quality, it is now common practice to use a total Enterobacteriaceae count instead^{3,4}.

The present study examined the detection of Enterobacteriaceae in spray-dried baby foods produced from rice flour. This raw ingredient provides a natural source of Enterobacteriaceae, the levels of which are reduced by consecutive heating and drying stages in the manufacturing process. Detection of the surviving sub-lethally damaged organisms had conventionally been achieved using a two-stage enrichment/confirmation procedure. This provided adequate sensitivity for the target organisms but was labour-intensive and had a 48 hour test duration. Positive release of product on the basis of Enterobacteriaceae levels could potentially be accelerated by the use of a rapid detection assay. Work therefore commenced to develop an enhanced detection protocol for this group of organisms using the Rapid Automated Bacterial Impedance Technique (RABIT).

2 MATERIALS AND METHODS

2.1 Test Samples

Both savoury and dessert varieties of rice flour-based baby food were used to evaluate the detection methods used in this study. Each was naturally contaminated with Enterobacteriaceae at a level of approximately 0.5 cfu g^{-1} . These organisms had originated from untreated rice flour and had survived the normal manufacturing process.

2.2 Conventional Methodology

An initial tenfold dilution of each sample was prepared by rehydrating the required mass with Maximum Recovery Diluent (MRD) and processing in a Stomacher for 30 seconds. Rehydrated samples were allowed to stand at room temperature for 30 minutes and 100 x 10 ml aliquots were transferred to equal volumes of double-strength Enterobacteriaceae Enrichment Broth (EE Broth - Oxoid Limited; CM317). These were incubated at 30°C for 24 h to promote selective recovery of the target organisms. Each broth was then used to stab inoculate a 10 ml tube of Violet Red Bile Glucose Agar (VRBGA - Oxoid Limited; CM485) which was incubated at 30°C for a further 24 h. Tubes showing a colour change from maroon to bright purple in the lower parts of the agar were counted and were regarded as presumptive positive Enterobacteriaceae tests.

2.3 RABIT Methodology

2.3.1 Principle. Detection of microbial growth using the RABIT system is achieved by adding the prepared test sample to a suitable culture medium in reusable impedance cells (working volume 2 ml to 10 ml). Impedance cells are inserted in the RABIT incubator modules which maintain the selected incubation temperature while recording conductance measurements across the cell contents. Microbial utilisation of the culture medium during growth produces conductance changes which are presented graphically by the accompanying software. A positive result is recorded automatically when the rate of conductance change exceeds a threshold selected by the operator. In the present study, all RABIT protocols were tested in parallel with the conventional methodology using an equal number of replicates.

2.3.2 Single Stage RABIT Procedure. An initial tenfold dilution of each test sample was prepared in MRD as described above. After standing for 30 minutes, 100 x 5 ml aliquots were transferred to equal volumes of impedance medium 1 in sterile RABIT cells. The medium consisted of Whitley Impedance Broth (WIB - Don Whitley Scientific Limited; G50001) supplemented with 0.5 g sodium lauryl sulphate per litre and was prepared at double strength. RABIT cells were connected to the system and incubated at 30°C for 24 h, during which a positive test result was recorded if the rate of conductance change exceeded $10 \mu\text{S}$ in three successive 6 minute intervals. To provide confirmation of RABIT test results, the contents of each incubated impedance cell were used to stab inoculate a 10 ml tube of VRBGA, which was incubated and interpreted as described previously.

2.3.3 RABIT Procedures with Pre-enrichment. Three pre-enrichment protocols were evaluated in conjunction with impedance detection. Initial tenfold dilutions of each test sample were prepared in (a) Buffered Peptone Water (BPW) (b) BPW + 30 mg Brilliant Green per litre and (c) BPW + 20 mg novobiocin per litre. Each preparation was incubated at 30°C for 2 h and 30 x 5 ml aliquots were transferred to equal volumes of impedance medium 2 in sterile RABIT cells. The medium consisted of WIB + 0.5 g sodium deoxycholate per litre and was prepared at double strength. RABIT cells were incubated as described above. All BPW preparations were re-incubated at 30°C for a further 2 h and the RABIT tests were repeated. The results of all RABIT tests were again subjected to confirmation by subculture into VRBGA.

3 RESULTS

Using the single stage RABIT method a high rate of detection was reported by the system for both product types (Table 1a). However, the frequency of "false positive" detection (positive RABIT tests not confirmed by a VRBGA colour change) was up to 20% while the number of confirmed positive tests represented a lower rate of Enterobacteriaceae detection than that achieved using the conventional methodology.

A 4 h pre-enrichment phase in unmodified BPW increased the number of confirmed positive results in the subsequent RABIT tests (Table 1b). In this case the rate of Enterobacteriaceae detection was almost double that achieved conventionally, but the rate of "false positive" detection increased to 50% of replicate tests. A 4 h pre-enrichment phase in BPW + Brilliant Green produced the best correlation between conventional and RABIT methodologies (Table 1c). The impedance method produced a higher rate of confirmed positive results while "false positive" detection was observed with a mean frequency of only 1%. Pre-enrichment in BPW + novobiocin prior to RABIT testing produced distinct results for the two product types (Table 1d): in savoury product the detection rates of the two methodologies were very similar, while in dessert product the RABIT method exhibited a detection rate for the target organisms more than three times that of the conventional method.

The reduced pre-enrichment of 2 h duration prior to RABIT testing produced unsatisfactory results (data not shown). Using the optimal BPW + Brilliant Green protocol the confirmed positive detection rate was below that of the conventional methodology while the frequency of "false positive" detection was approximately 5%.

4 DISCUSSION

The efficacy of impedance instrumentation for detecting sub-lethally damaged bacteria has previously been demonstrated for a variety of sample types^{5,6,7}. It should be appreciated, however, that this technology is dependent on active bacterial metabolism, for which adequate resuscitation of the target organism is a prerequisite. In the initial

Table 1 Comparison of conventional and RABIT methodologies for detection of *Enterobacteriaceae* in spray-dried baby food*(a) Single stage RABIT test*

Product type	Savoury	Dessert
Number of replicates	100	100
Total positive RABIT tests	54	94
Confirmed positive RABIT tests	41	74
Confirmed positive - conventional method	73	68

(b) RABIT tests after BPW pre-enrichment

Product type	Dessert
Number of replicates	30
Total positive RABIT tests	28
Confirmed positive RABIT tests	13
Confirmed positive - conventional method	7

(c) RABIT tests after BPW + Brilliant Green pre-enrichment

Product type	Savoury	Dessert
Number of replicates	30	30
Total positive RABIT tests	9	9
Confirmed positive RABIT tests	7	9
Confirmed positive - conventional method	3	3

(d) RABIT tests after BPW + Novobiocin pre-enrichment

Product type	Savoury	Dessert
Number of replicates	30	30
Total positive RABIT tests	6	25
Confirmed positive RABIT tests	4	25
Confirmed positive - conventional method	3	

phase of the present study the Enterobacteriaceae within the test products did not regain sufficient metabolic vigour to allow detection within a 24 h RABIT test alone: competing organisms were thus detected at the expense of the target species. In products which have undergone heating, dehydration or a combination of these processes the predominant surviving bacteria are commonly gram positive sporeformers, the growth rate of which is likely to exceed that of sublethally damaged gram negative species upon entry into a high-moisture nutrient matrix.

In this scenario the pre-enrichment phase has a dual function: resuscitation of the target species must be accompanied by selective inhibition of the competing microflora. Prudent selection of the inhibitory agent ensures that the latter process occurs relatively quickly following rehydration of the test product, promoting multiplication of the target species in the absence of competitive inhibition. In the present study, brilliant green dye and novobiocin were selected as specific inhibitors of gram positive species. The design of the test protocol caused the concentration of each agent to be halved when the pre-enrichment broth was added to the impedance medium. This permitted the use of a relatively high inhibitor concentration in conjunction with a short pre-enrichment; when combined with RABIT detection the total test duration was 28 h. In practice, the majority of sample replicates were detected within 20 h of RABIT test initiation, thus halving the time required for reliable detection of the target organisms using conventional methodology.

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