International Journal of Food Microbiology 136 (2009) 169–178

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Review

Culture media for the isolation of *Cronobacter* spp

Patrick Druggan [a,*], Carol Iversen [b]

[a] Oxoid Ltd., Thermo Fisher Scientific, Basingstoke, Hampshire RG24 8PW, United Kingdom
[b] Centre for Food Safety, School of Agriculture, Food Science and Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland

ARTICLE INFO

Keywords:
Cronobacter
Enterobacter sakazakii
Microbiology media
Detection
Sensitivity
Specificity

ABSTRACT

Enterobacter sakazakii is a member of the Enterobacteriaceae and has been implicated in causing necrotising enterocolitis, as well as bacteraemia and meningitis in infants. In some cases, the infection has been linked to ingestion of infant formula milk (IFM) that has not been terminally sterilised. The nomenclature of *E. sakazakii* has been clarified and it has now been accepted as a group of six species comprising a novel genus, *Cronobacter*. Outbreaks in neonatal intensive care units resulting in relatively high case fatality rates and the recognition of IFM as a significant route of infection prompted the development of culture-based detection methods. Development of enrichment broths specific for *Cronobacter* spp., coupled to the use of fluorogenic and chromogenic substrates in culture media has significantly improved the sensitivity and specificity of methods. This review presents the history and rationale behind the currently available methods, and gives an overview of the principles involved in designing these microbiological media.

Contents

1. Introduction

Enterobacter sakazakii is a member of the Enterobacteriaceae that has been implicated in causing necrotising enterocolitis (van Acker et al. 2001), bacteraemia and meningitis (Anonymous 2002a; Biering et al. 1989; Bowen and Braden 2006; Lai 2001; Muytjens et al. 1983; Muytjens and Kollee 1982, 1990; van Acker et al. 2001) in infants. The nomenclature of this species has been clarified recently and it has now been accepted as a separate genus, *Cronobacter* spp. (Iversen et al. 2008b; Kuhnert et al. 2009-this issue).

1.1. The initial response to the need for an isolation method

An outbreak of *Cronobacter* spp. associated with feeding infant formula milk (IFM) to pre-term infants occurred in April 2001 in a neonatal intensive care unit in Tennessee (Anonymous 2002a; Himelright et al. 2002). This prompted the Food and Drug Administration (FDA) to introduce a method for the detection of *Cronobacter*

* Corresponding author. Tel.: +44 1256694415.
E-mail address: Patrick.Druggan@thermofisher.com (P. Druggan).

0168-1605/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.ijfoodmicro.2009.09.008

Nomenclature

Equation 1	The probability of detection of a pathogen as determined by its proportion of total positives on an isolation medium
D	Probability of detection
Q	Proportion of pathogen relative to all positives on an isolation medium, as a decimal fraction
n	Number of colonies picked from a plate.

spp. (Anonymous 2002b; Anonymous, 2009). Although this method has never been validated, and is not included in the FDA Bacteriological Analytical Manual (BAM), it has nevertheless been adopted worldwide. The method was based on the procedure used to enumerate Enterobacteriaceae in dried foods (Mossel and Ratto 1970) and in IFM (Muytjens et al. 1988) and is summarised in Fig. 1.

The method contains four of the five functional steps that are common to the isolation of Gram-negative pathogens (Andrews 1985). The pre-enrichment step is carried out in a non-selective broth that allows stressed cells to recover from any injury that has occurred during manufacturing or preparation that makes them sensitive to selective agents. The enrichment step is carried out in a broth that allows the pathogen to increase in number relative to the competitive microflora. The isolation step usually involves plating out samples from the enrichment broth on to a selective diagnostic medium that allows a pathogen to be differentiated from the competitive microflora that has survived the enrichment step. The identification step uses biochemical methods to give a presumptive identification of a pathogen. Methods for most pathogens also include a serological or molecular confirmation step, but this is missing in the FDA method for *Cronobacter* spp. as there was no serological or suitable molecular method available at the time of publication of the method in July 2002.

After the pre-enrichment step, each subsequent step in an isolation and enumeration protocol should improve the specificity of the method to reduce the number of batches that are falsely rejected, as this is a significant cost to the manufacturer. More significantly, from a public health perspective, poor specificity can lead to false negative results. This will be discussed later.

In the FDA method (Fig. 1) 333 g of IFM is tested and the number of cells present is determined by a most probable number (MPN) method (Anonymous 2002b). The samples are rehydrated in sterile distilled water and incubated at 36 °C overnight to resuscitate stressed cells. A 10 ml

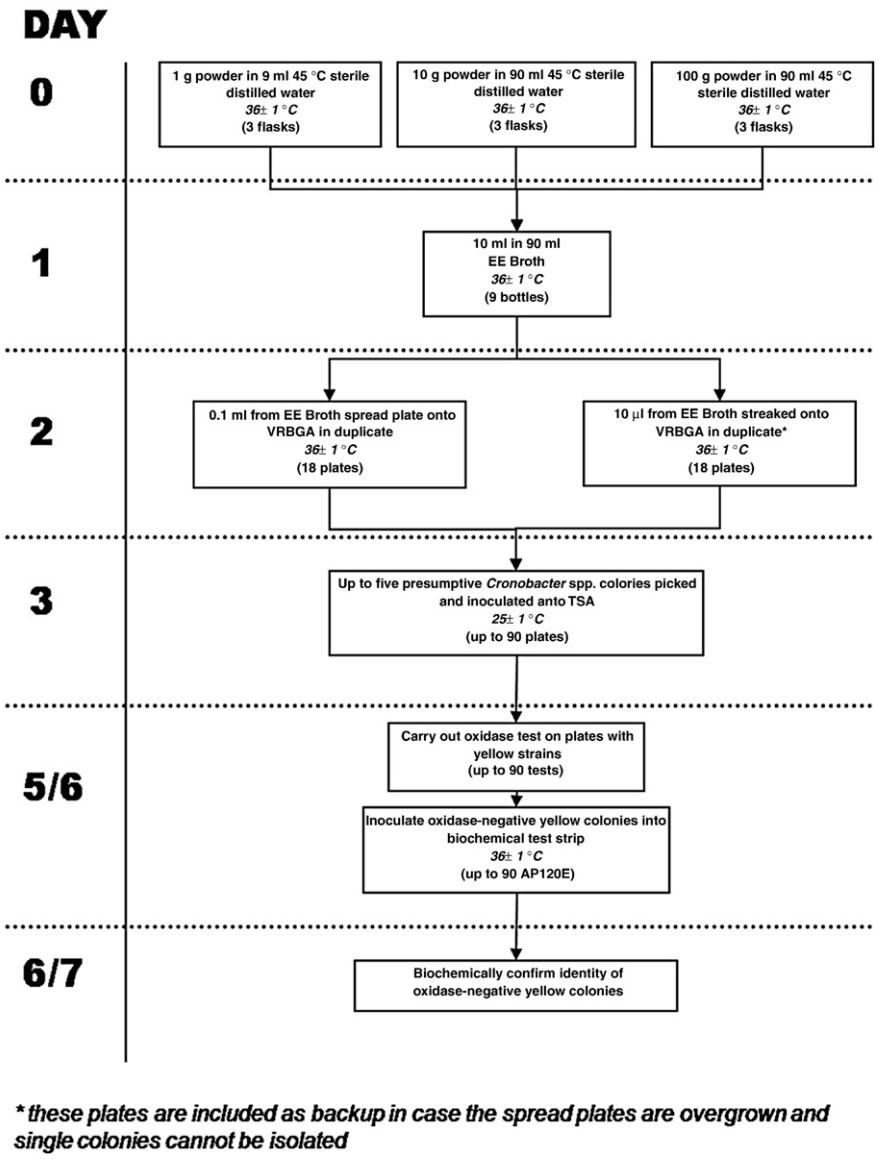

Fig. 1. Food and Drug Administration method for the detection of *Cronobacter* spp.