

Improving the enrichment procedure for *Enterobacteriaceae* detection

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Abstract

The current ISO standard method for detection of *Enterobacteriaceae* (21528-1:2004) includes enrichment in EE broth which has been shown to be inhibitory to some members of this family, notably *Cronobacter* spp. A shortened procedure omitting the EE broth has been proposed, however competition from Gram-positive flora may be detrimental to the effective recovery of low levels of target organisms in some sample matrices. In this study we investigated novel cost effective modifications, designed to improve ISO 21528-1:2004 for the detection of *Enterobacteriaceae*. Initial experiments used a worse-case scenario involving stressed *Enterobacteriaceae* strains known to grow poorly in laboratory media as well as representative background competitors from powdered milk. The interaction between the *Enterobacteriaceae* and their competitors was characterised and additives to enhance the growth of target strains over non-target strains were investigated.

Supplementation of BPW with 40 μM 8-hydroxyquinoline, 0.5 g L^{-1} ammonium iron(III) citrate, 0.1 g L^{-1} sodium deoxycholate and 0.1 g L^{-1} sodium pyruvate (BPW-S) improved the recovery of *Enterobacteriaceae* from artificially and naturally contaminated samples. This improvement of the pre-enrichment broth may also be of interest for methods

designed to detect specific foodborne pathogens belonging to the *Enterobacteriaceae* (e.g. *Salmonella* spp., *Cronobacter* spp.) that require a pre-enrichment step in BPW.

Keywords: *Enterobacteriaceae*; Detection; Enrichment

Article Outline

1. [Introduction](#)
2. [Methods](#)
 - 2.1. [Bacterial isolates used in this study](#)
 - 2.2. [Isolation and identification of Gram-positive background flora from powdered milk products](#)
 - 2.3. [Inhibitory interactions between Gram-positive strains and *Enterobacteriaceae*](#)
 - 2.4. [Comparing growth rate in skimmed milk and infant formula](#)
 - 2.5. [Heat stability of vancomycin hydrochloride](#)
 - 2.6. [Optimization of pre-enrichment medium: inhibition of Gram-positive organisms](#)
 - 2.7. [Optimization of pre-enrichment medium: addition of ammonium iron\(III\) citrate and pyruvate](#)
 - 2.8. [Optimization of pre-enrichment medium: efficacy of 8-hydroxyquinoline in milk with and without ammonium iron\(III\) citrate](#)
 - 2.9. [Optimization of pre-enrichment medium: recovery of desiccated cells](#)
 - 2.10. [Comparison of methods for recovery of *Enterobacteriaceae* from artificially inoculated samples](#)
 - 2.11. [Comparison of methods for recovery of *Enterobacteriaceae* from naturally contaminated samples](#)
3. [Results](#)
 - 3.1. [The influence of Gram-positive background flora on the growth of *Enterobacteriaceae*](#)
 - 3.2. [Optimization of pre-enrichment medium: inhibition of Gram-positive organisms](#)
 - 3.3. [Optimization of pre-enrichment medium: addition of ammonium iron\(III\) citrate](#)
 - 3.4. [Optimization of pre-enrichment medium: recovery of desiccated cells](#)
 - 3.5. [Comparison of methods for recovery of *Enterobacteriaceae* from artificially inoculated and naturally contaminated samples](#)
4. [Discussion](#)
- [Acknowledgements](#)
- [References](#)

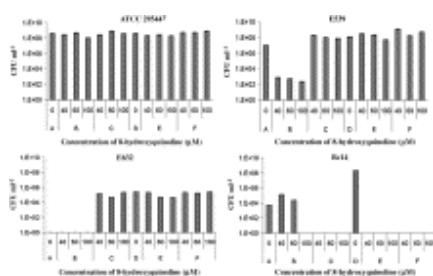


Fig. 1. The combined effect of 8-hydroxyquinoline and ammonium iron(III) citrate in milk. A, skimmed milk; B, skimmed milk plus 8-hydroxyquinoline; C, skimmed milk plus 8-hydroxyquinoline and 0.5 g L⁻¹ ammonium iron(III) citrate; D, infant formula; E, infant formula plus 8-hydroxyquinoline; F, infant formula plus 8-hydroxyquinoline and 0.5 g L⁻¹ ammonium iron(III) citrate. Strains ATCC 29544^T, E539 and E 632 are *Cronobacter sakazakii*; strain Bc14 is *Bacillus velezensis*.

Table 1.

Strains used in this study.



^a Primary isolates from powdered milk and formula.

^b From the culture collection at the Institute for Food Hygiene and Safety, University of Zurich, Switzerland.

^c From the culture collection at the Centre for Food Safety, University College Dublin, Ireland.

^d From the culture collection at Oxoid Ltd., Thermo Fisher Scientific, Basingstoke, UK; all other isolates were obtained from the culture collection at Nestlé Research Centre, Lausanne, Switzerland.

Table 2.

Recovery of lyophilised *Enterobacteriaceae* from artificially contaminated milk powder.



^a ISO 21528 without EE broth.

^b BPW plus 10 mg L⁻¹ vancomycin.

^c BPW plus 40 µM 8-hydroxyquinoline plus 0.5 g L⁻¹ ammonium iron(III) citrate plus 0.1 g L⁻¹ sodium deoxycholate plus 0.1 g L⁻¹ sodium pyruvate.


Table 3.

Recovery of endogenous *Enterobacteriaceae* strains from real samples.



^a ISO 21528 without EE broth.

^b BPW plus 40 µM 8-hydroxyquinoline plus 0.5 g L⁻¹ ammonium iron(III) citrate plus 0.1 g L⁻¹ sodium deoxycholate plus 0.1 g L⁻¹ sodium pyruvate.

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