Food Research International 64 (2014) 402–411

Contents lists available at ScienceDirect

Food Research International

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

Role of growth morphology in the formulation of NaCl-based selective media for injury detection of *Escherichia coli*, *Salmonella* Typhimurium and *Listeria innocua*

Estefanía Noriega, Eirini G. Velliou [1], Eva Van Derlinden, Laurence Mertens, Jan F.M. Van Impe *

CPMF² — Flemish Cluster Predictive Microbiology in Foods, http://www.cpmf2.be, Belgium
BioTeC — Chemical and Biochemical Process Technology and Control, Department of Chemical Engineering, KU Leuven, Leuven, Belgium

ARTICLE INFO

Article history:
Received 15 April 2014
Accepted 29 June 2014
Available online 4 July 2014

Keywords:
Sublethal injury
Selective media
Hyperosmotic NaCl stress
Colony growth
Xanthan gum
Escherichia coli
Salmonella Typhimurium
Listeria innocua

ABSTRACT

Sublethal injury (SI) poses major public health concerns since injured cells are responsible for serious limitations in food diagnostics and are susceptible to recovery, often developing adaptive stress responses. Detection of SI is based on the difference in plate counts between non-selective media, which represent the total cell population, and selective media, to which injured cells become sensitive. Selective media for detection of sublethal membrane damage are often based on NaCl supplement, although there is a lack of consensus in the literature about appropriate levels. Planktonic cells are generally used to investigate SI mechanisms, although they often exhibit different stress tolerance than cell colonies in/on solid food (model) systems. In this work, the effect of growth morphology, colony size and concentration of the gelling agent in the growth media, on the maximum non-inhibitory NaCl concentration in the plating medium was assessed for *Escherichia coli*, *Salmonella* Typhimurium and *Listeria innocua*. Stationary phase cultures of planktonic cells and large and small colonies grown in either 1.5% (w/v) xanthan gum-based system or 2.5% (w/v) xanthan gum-based system exhibited significantly different viable counts and osmotolerance. The effect of cell arrangement and xanthan gum percentage in the growth media depended on the microorganism under investigation. Additionally, differences in the maximum non-inhibitory concentration were evident, with 5.0% (w/v) NaCl for the Gram-negative bacteria and 6.5% (w/v), for *L. innocua*. Different extent of colony shrinkage and morphological damage was observed as NaCl concentration in the plating medium increased. This information will contribute to define NaCl-based selective media for accurate SI detection under realistic scenarios.

1. Introduction

Sublethal injury (SI) has been defined as "a consequence of exposure to a chemical or physical process that damages but does not kill a microorganism" (Hurst, 1977). More specifically, SI may affect cell wall or membrane permeability and also cause extensive damage to functional cell components (Brashears, Amezquita, & Stratton, 2001). SI is macroscopically detectable by the inability of injured survivors to form visible colonies on media containing selective agents, e.g. sodium chloride, to which healthy microbiota normally show resistance. Accordingly, the difference in plate counts between non-selective media, which support cell reparation before growth and represent both uninjured and injured cells, and the corresponding selective media, to which injured cells become sensitive, is a traditional means to quantify SI as a proportion or percentage of the entire population (Besse, 2002; Besse, Brissonnet, Lafarge, & Leclerc, 2000; Gilbert, 1984; Hurst, 1977; Jasson, Uyttendaele, Rajkovic, & Debevere, 2007; Kurbanoglu & Algur, 2006; Mackey, 2000; Osmanagaoglu, 2005; Semanchek & Golden, 1998).

Due to the food environment itself and hurdle technologies implemented throughout the food chain, an important microbial subpopulation in food products may undergo some degree of sublethal damage (Brashears et al., 2001; Jasson et al., 2007; Mossel & Van Netten, 1984; Wesche, Gurtler, Marks, & Ryser, 2009). SI is responsible for serious limitations in food diagnostics, such as underestimation of contamination levels and assumption of false negative results, due to the selective media used for detection and enumeration of specific target microorganisms (see Table 1), and the extended lag phase of injured survivors, which enter a physiological state that requires specific reparative

* Corresponding author at: BioTeC — Chemical and Biochemical Process Technology and Control, Department of Chemical Engineering, KU Leuven, W. de Croylaan 46, B-3001 Leuven, Belgium. Tel.: +32 16 32 14 66; fax: +32 16 32 29 91.
E-mail addresses: estefania.noriegafernandez@cit.kuleuven.be (E. Noriega), eirini.velliou@cit.kuleuven.be (E.G. Velliou), eva.vanderlinden@cit.kuleuven.be (E. Van Derlinden), laurence.mertens@cit.kuleuven.be (L. Mertens), jan.vanimpe@cit.kuleuven.be (J.F.M. Van Impe).
[1] Current address: Centre for Process Systems Engineering (CPSE) & Biological Systems Engineering Laboratory (BSEL), Department of Chemical Engineering, Imperial College London, South Kensington Campus, SW7 2AZ London, UK.

http://dx.doi.org/10.1016/j.foodres.2014.06.047
0963-9969/

Table 1
Selective media for injury detection of *Escherichia coli*, *Salmonella enterica* and *Listeria* spp.

Strain	Selective medium	Reference
Escherichia coli	Chromocult Coliform Agar (enhanced selectivity)	Gabriel and Nakano (2010)
	Hemorrhagic Coli Agar	Williams, Sumner, and Golden (2004)
	Sorbitol MacConkey Agar (SMA)	Kang and Siragusa (1999); Restaino et al. (2001); Uyttendaele et al. (2001);
	SMA + Cefixime Tellurite Supplement	Williams et al. (2004); Jasson et al. (2007); Ukuku, Jin, and Zhang (2008)
	MA + 0.3, 0.45, 0.6% (w/v) bile salts	
	Xylose lysine sodium tetradecyl sulfate	Ukuku et al. (2008)
	Levine's eosin methylene blue agar (LEMBA)	Restaino et al. (2001); Uyttendaele et al. (2001); Williams et al. (2004);
	Modified LEMBA	Yuste et al. (2004)
	TSA + 2, 3, 4 or 5% (w/v) NaCl	Semanchek and Golden (1998); Perni, Chalise, Shama, and Kong (2007); Ukuku et al. (2008); Walkling-Ribeiro, Noci, Cronin, Lyng, and Morgan (2008)
	Violet red-bile agar (VRB)	Ray and Speck (1973); Restaino et al. (2001)
	VRB with a double level of bile salts	
	VRB + 2% (w/v) NaCl	
	Deoxycholate-lactose agar	Ray and Speck (1973); Perrot et al. (1998)
	Nutrient agar + 4% (w/v) NaCl	García, Mañas, Gómez, Raso, and Pagán (2006)
	BHIA + 5% (w/v) NaCl	Noriega et al. (2013)
	TSAYE + 0.5, 1, 3, 4 or 5% (w/v) NaCl	Somolinos, García, Mañas, Condón, and Pagán (2008); Saldaña et al. (2009, 2010)
Salmonella enterica	TSA + 2, 3, 3.5, 4 or 5% (w/v) NaCl	Wuytack et al. (2003); Chambliss, Narang, Juneja, and Harrison (2006); Perni et al. (2007);
	TSA pH 5.5 or 6.5	Ukuku et al. (2008); Noriega et al. (2013)
	TSAYE + 3 or 4% (w/v) NaCl	Saldaña et al. (2009, 2010); Monfort, Gayán, Condón, Raso, and Álvarez (2011)
	Sorbitol MacConkey Agar	Ukuku et al. (2008)
	Double modified lysine agar	Chambliss et al. (2006)
	Xylose lysine sodium tetradecyl sulfate	Ukuku et al. (2008)
	Violet red bile glucose agar	Wuytack et al. (2003)
	Xylose lysine desoxycholate agar	Alexandrou, Blackburn, and Adams (1995); Kang and Siragusa (1999); Yuste et al. (2003); Yuste et al. (2004); Aljarallah and Adams (2007)
	Bismuth sulfite agar	Williams et al. (2004); Gabriel (2007)
	Nutrient agar + 4% (w/v) NaCl	Aljarallah and Adams (2007)
	Heart Infusion Agar + 2.5% (w/v) NaCl	Stephens et al. (1997)
	Xylose lysine tergitol 4 agar	Williams et al. (2004)
Listeria spp.	Agar *Listeria* Ottavani & Agosti	Jasson et al. (2007)
	TSAYE + 3, 4, 5, 6 or 6.5% (w/v) NaCl	McKellar, Butler, and Stanich (1997); Dykes and Moorhead (2001); Miller, Brandão, Teixeira, and Silva (2006); Jasson et al. (2007); Uyttendaele et al. (2008); Saldaña et al. (2009); Noriega et al. (2013)
	TSA + 4% (w/v) NaCl	Yu and Fung (1993); Novak and Juneja (2003)
	Listeria selective agar — Oxford formulation	Mackey, Boogard, Hayes, and Baranyi (1994); Bremer, Osborne, Kemp, and Smith (1998); Rowan and Anderson (1998); Hansen and Knøchel (2001); Pascual, Robinson, Ocio, Aboaba, and Mackey (2001);Yuste et al. (2004)
	Listeria selective agar — Palcam formulation	Miller et al. (2006)
	Tryptose phosphate agar + 4% (w/v) NaCl	Busch and Donnelly (1992); Chawla, Chen, and Donnelly (1996)
	BHIA + 5% (w/v) NaCl	Miller, Bayles, and Eblen (2000)

processes (Adams, 2005; Jasson et al., 2007; Restaino, Frampton, & Spitz, 2001; Van Houteghem et al., 2008). Moreover, injured cells are susceptible to recovery after exposure to appropriate (storage) conditions, often developing adaptive stress responses (Bozoglu, Alpas, & Kaletunç, 2004; Brashears et al., 2001; Skandamis, Yoon, Stopforth, Kendall, & Sofos, 2008; Vermeiren, Devlieghere, Vandekinderen, Rajtak, & Debevere, 2006). To avoid this major public health concern, resuscitation/repair steps on non-selective media, aimed at restoring the viability and culturability of injured cells, are incorporated in routine microbiological surveillance methods based on selective detection/enumeration. Several one-step recovery-enrichment methods have been developed using combinations of selective and non-selective media that allow the repair of injured cells and, at the same time, inhibit the proliferation of undesirable background microbiota (Kang & Siragusa, 1999; Yuste, Capellas, Fung, & Mor-Mur, 2004; Yuste et al., 2003).

The susceptibility of injured cells to selective media may be related, among others, to membrane damage (Camper & McFeters, 1979; Domek, Robbins, Anderson, & McFeters, 1987; Ray, 1979). Loss of tolerance to the presence of sodium chloride in plating media is attributed to damage to the functionality and/or integrity of the cytoplasmic membrane, thus preventing microbial growth on the corresponding NaCl-based selective media (Jay, 1992). Selective media to detect sublethal membrane impairment should contain the highest concentration of sodium chloride that does not inhibit the growth of non-stressed cells (Cebrián, Michiels, Mañas, & Condón, 2010; Noriega, Velliou, Van Derlinden, Mertens, & Van Impe, 2013; Saldaña, Puértolas, Condón, Álvarez, & Raso, 2010; Saldaña et al., 2009). Formulation of selective media with lower/higher NaCl concentrations would result in under/overestimation of sublethally injured cell populations. However, there is a lack of consensus in the literature on the appropriate levels of sodium chloride to formulate these selective media (see Table 1). The maximum non-inhibitory NaCl concentration in the plating medium depends not only on the microbial strain, but also on the growth phase and the physiological state of the NaCl-exposed cells (Hajmeer, Ceylan, Marsden, & Fung, 2006; Saldaña et al., 2009). In this sense, stationary phase cells often withstand stress scenarios (e.g. hyperosmotic NaCl stress) better than exponentially growing populations (Hurst, Hughes, & Collins-Thompson, 1974; Jenkins, Chaisson, & Matin, 1990; Knøchel & Gould, 1995; Smith, 1995), due to the de novo synthesis of stress proteins in the early stationary phase (De Angelis et al., 2004; Dodd & Aldsworth, 2002; Taylor-Robinson, Child, Pickup, Strike, & Edwards, 2003). Several common shock proteins have been reported to be synthesized after exposure to osmotic stress and during starvation at the stationary phase of growth (Mongkolsuk & Helmann, 2002). Whereas the stationary phase of growth is the most often encountered in a natural environment (Jiang & Chai, 1996; Rees, Dodd, Gibson, Booth, & Stewart, 1995), planktonic growth is not the natural state for bacteria in their normal habitats, for instance food products, which exhibit a solid structure that leads to surface/submerged colony growth (Brocklehurst, Mitchell, & Smith, 1997; Hodges, 2011; Wimpenny et al., 1995). Planktonic or freely-suspended cells are routinely used to investigate microbial inactivation/SI mechanisms, although, they often exhibit different susceptibility to inhibitory agents (e.g. NaCl) compared