Performance of a new chromogenic plating medium for the isolation of *Listeria monocytogenes* from marine environments

A. El Marrakchi¹, N. Boum’handi² and A. Hamama¹

¹Institut Agronomique et Vétérinaire Hassan II, Rabat-Instituts, Rabat, and ²Centre Régional de l’Institut de Recherche Halieutique, Agadir, Morocco

2004/0372: received 1 April 2004, revised 12 May 2004 and accepted 13 May 2004

ABSTRACT


Aims: This study investigated the performance of a new chromogenic plating medium for the detection of *Listeria monocytogenes* from naturally contaminated samples obtained from marine environments in Morocco in comparison with the conventional plating media PALCAM and Oxford.

Methods: A total of 479 marine samples (sea water, sediment and mussels) were collected from 16 littoral sites in the region of Agadir (western centre of Morocco). They were examined for the presence of *L. monocytogenes* using a slight modification of the standardized French method (AFNOR V 08-055) for the detection of *L. monocytogenes* from food and three different isolation media: PALCAM, Oxford and a new chromogenic plating medium.

Results and Significance of the Study: The Oxford and the new chromogenic plating media were found relatively more efficient than the PALCAM medium for the isolation of *L. monocytogenes* (chi-square test, \( P < 0.05 \)) from marine samples. However, the new chromogenic plating medium was significantly more selective for *L. monocytogenes* (\( P < 0.005 \)) than the two other isolation media as 87.5% of the suspect colonies on this medium were indeed confirmed through identification of the isolates vs 12.7% for Oxford and only 3.8% for the PALCAM medium.

Keywords: Chromogenic medium, isolation, *Listeria monocytogenes*, marine environments, performance, selective plating media.

INTRODUCTION

Since 1981 when *Listeria monocytogenes* has been recognized as an agent of food-borne infection (Rocourt et al. 2000), this pathogen has been associated with a numerous outbreaks of food-borne listeriosis; foods involved include vegetables, dairy products, meat products and seafoods (Farber and Peterkin 1991). The presence of this pathogen in foods is a major concern to the food industry and public health regulators (Cassiday et al. 1989). Detection and enumeration of *L. monocytogenes* from environments such as food, which can be heavily contaminated with other organisms are often difficult (Golden et al. 1988). One of the major challenges of the food sanitarians is to identify and apply reliable and rapid techniques for the recovery of this pathogen from foods (Foret and Dorey 1997).

In general, the current conventional procedures for the detection of *L. monocytogenes* from foods suffer from a certain lack of performance particularly in foods where this pathogen is expected to occur in low numbers and/or in debilitated condition. Moreover, the presence of non-listeriae bacteria or even the very competitive *L. innocua* may interfere with the recovery of *L. monocytogenes*.

The plating media PALCAM and Oxford have been developed for the selective isolation of listeriae on the basis of the hydrolysis of esculin, which contribute to the differentiation of the listeriae from the other bacteria (Mc
Lauchlin 1987). Although these media have been found efficient for the isolation of listeriae from food products with injured listeriae cells and/or rich in competitive microflora (Ben Embarek 1994), they are not able to allow distinctive visualization of L. monocytogenes colonies. Therefore, their use as plating media does not permit a rapid detection of this pathogen from foods (Scotter et al. 2001). The need for isolation media capable of clearly differentiating L. monocytogenes colonies from the other listeriae colonies and particularly from those of L. innocua has been expressed by Poyksi et al. (1993). Thus, different chromogenic media such as ALOA chromogenic medium have been developed to respond to this need (Carles et al. 1997; Vlaemynck et al. 2000; Smith et al. 2001).

The present work aims to assess the performance of a new chromogenic plating medium CHROMagar™ Listeria (CHROMagar Microbiology, Paris, France) in comparison with the conventional plating media PALCAM and Oxford for the selective isolation of L. monocytogenes. This comparative study was based on the tentative recovery of this pathogen from samples obtained from naturally contaminated marine products using the three considered isolation media.

MATERIALS AND METHODS

Sampling procedure

Within the public health monitoring programme of the Moroccan southern Atlantic coast between the towns of Essaouira and Tan-Tan, a total of 479 marine samples were collected during a 2-year period (2001–02) from 16 sites located on the shores of the Atlantic coast of the region of Agadir (western centre of Morocco). These included 161 samples of sea water, 160 samples of marine sediment and 158 samples of mussels (Mytilus galloprovincialis).

All the samples were collected at low tide. Samples of sea water were taken at a depth of 20 cm under the sea surface and those of marine sediment at the surface of sea ground. These two types of samples were collected in separate sterile flasks while those of mussels were introduced into plastic sterile bags. All the samples were transported at the laboratory in ice boxes and then stored at 6–8°C before being analysed within 16 h of sampling.

Detection of L. monocytogenes

The standardized French method (AFNOR NF V 08-055, 1993) for the detection of L. monocytogenes in food was used with a slight modification. Portions of 25 ml of sea water or 25 g of marine sediment were introduced aseptically into sterile 500-ml flasks containing 225 ml of half strength Fraser broth (E. Merck, Darmstadt, Germany) and incubated for 24 h at 30°C. However, mussels (from three to 10 according to their size) were scrubbed, rinsed with tap water and then flamed with ethylic alcohol before being opened aseptically. The flesh with the intervalar liquor (25 g) were added to 225 ml of half strength Fraser broth (E. Merck) and homogenized for 40–60 s at 1400 g using a Waring blender and then incubated at 30°C for 24 h. A 0.1 ml subsample of each of the primary enriched cultures was added to 10 ml of full strength Fraser broth (E. Merck) and kept for 24 h at 37°C. A loopfull of the secondary enrichment culture was streaked onto each of the following isolation plating media: PALCAM (E. Merck), Oxford (E. Merck) and CHROMagar™ Listeria (CHROMagar Microbiology). The latter medium is composed of a base and a supplement. The base contains the followings ingredients: 23 g l⁻¹ peptones and meat extract; 5 g l⁻¹ sodium chloride; 15 g l⁻¹ agar and 17-5 g l⁻¹ special chromogenic mix. The inoculated plates were incubated for 24–48 h at 37°C. A number of one to three suspect colonies showing the typical morphology of listeriae on these three isolation media (blackish colonies on PALCAM, dark grey/green colonies with depressed black centre on Oxford, blue colonies with white halo on CHROMagar™ Listeria) were selected at random from the plates, checked for purity on Columbia agar and kept on slants of this medium for 24 h at 37°C before their final identification. This latter was based on reactions to confirm the genus affiliation: Gram stain, catalase test, motility at 25°C, urease activity, indole production, esculin hydrolysis and methyl red and Voges Proskauer tests. Then, the listeriae-confirmed isolates were speciated using the API-Listeria strips (Bio-Mérieux, Marcy l’Etoile, France) and the β-haemolysis test on sheep blood agar.

Statistical analysis

The chi-square test was used to compare the results of recovery rate of L. monocytogenes from marine samples with respect to the type of the isolation plating media used.

RESULTS AND DISCUSSION

Occurrence of listeriae in the marine samples

In general, 31 (6.5%) and seven (1.5%) of the overall samples were found to be contaminated with Listeria spp. and L. monocytogenes respectively (Table 1). Proportions of 8.1, 6.5 and 3.1% of sea water, 6.9, 6.2 and 0.6% of sediments and 4.5, 3.4 and 0.6% of mussels contained organisms of Listeria spp., L. innocua and L. monocytogenes respectively. L. ivanovii was detected only once and was from a single sediment sample (Table 1). These results are somewhat surprising as bivalve molluscs like mussels are expected to contain more listeriae than sea water and
resistant in high salt concentrations (Shahamat et al. 2000) because of differences in growth rates of L. monocytogenes-positive samples of 22 Listeria presumptive (9-1)% when using the PALCAM medium. In addition, as expected, none of the presumptively L. monocytogenes-positive samples on CHROMagar<sup>TM</sup> Listeria medium contained L. innocua while this latter made up the major parts of presumptive Listeria samples on both PALCAM (20/22; 90-9)% and Oxford (26/30; 86-6%). As already mentioned by several authors (Petran and Swanson 1993; Mc Donald and Sutherland 1994; Carles et al. 1997), L. innocua (a nonpathogen species for humans) can mask the presence of L. monocytogenes on both Oxford and PALCAM agars because of differences in growth rates of Listeria spp. in selective broths. Thus, species identification from a definite number of colonies per plate can be biased (Scotter et al. 2001). The antagonistic effect of L. innocua over L. monocytogenes has been attributed by some authors (Yokohama et al. 1998; Kalmokoff et al. 1999) to the production of phages as well as bacteriocin-like substances by this organism. Recently developed chromogenic isolation media such as CHROMagar<sup>TM</sup> Listeria are then capable of improving greatly the visual differentiation of these two Listeria species.

### Table 2 Recovery rate of *Listeria monocytogenes* from marine samples according to type of the plating isolation medium used

<table>
<thead>
<tr>
<th>Isolation medium</th>
<th>No. of samples</th>
<th>Listeria spp.</th>
<th>L. monocytogenes</th>
<th>L. innocua</th>
<th>L. ivanovii</th>
<th>L.m./L. innocua</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxford</td>
<td>479</td>
<td>30 (6-3)</td>
<td>7 (1-5)</td>
<td>22 (5-4)</td>
<td>None</td>
<td>7/30 (23-3)</td>
</tr>
<tr>
<td>PALCAM</td>
<td>479</td>
<td>22 (4-6)</td>
<td>2 (0-4)</td>
<td>20 (4-2)</td>
<td>None</td>
<td>2/22 (9-1)</td>
</tr>
<tr>
<td>CHROMagar</td>
<td>479</td>
<td>ND</td>
<td>7</td>
<td>None</td>
<td>1</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values are presented as n (%) unless indicated.

L.m., *Listeria monocytogenes*; ND, not determined.

Figures in Table 3 are relative to the performance of each of the examined plating media with regard to the isolation of \( L. \) monocytogenes from the total suspicious colonies isolated from that medium. It is important to indicate that in samples found to be positives for \( L. \) monocytogenes, the number of colonies showing the phenotypic characteristics of \( L. \) monocytogenes on the CHROMagar\textsuperscript{TM} Listeria medium was very small (mostly one colony per sample). This explains the low number of \( L. \) monocytogenes suspect colonies (eight colonies) observed on CHROMagar\textsuperscript{TM} Listeria medium (Table 3). However, seven of them were identified as \( L. \) monocytogenes (87.5\%) confirming the high selectivity of this medium to this micro-organism. Data from Table 3 show that although PALCAM was able to detect more listeriae among the \( L. \) monocytogenes \( L. \) innocua \( L. \) ivanovii \( L. \) monocytogenes from the total suspicious colonies isolated from each medium as Oxford or PALCAM. Although CHROMagar\textsuperscript{TM} Listeria cannot visually differentiate colonies of \( L. \) monocytogenes from those of \( L. \) ivanovii, this latter species is fortunately less frequently encountered than \( L. \) innocua in the environment (Rocourt and Seeliger 1985; Lovett and Twedt 1988). Therefore, the probability of a \( L. \) monocytogenes suspect colony on CHROMagar\textsuperscript{TM} Listeria to belong indeed to this species is very high as shown in this present study. Because of its high level of selectivity, the CHROMagar\textsuperscript{TM} Listeria plating medium, has also the advantage of exempting the microbiological laboratories from performing the haemolysis test, which is critical in the routine procedure for the differentiation of \( L. \) monocytogenes from \( L. \) innocua. This test has caused numerous problems to laboratories because of difficulties in reading and interpreting the results of the haemolysis reaction (Scotter et al. 2001).

In conclusion, this study has demonstrated that isolation plating media able to target specifically the detection of \( L. \) monocytogenes such as CHROMagar\textsuperscript{TM} Listeria are highly recommendable as they allow a relatively rapid presumptive response and an economy on the analysis cost.

**ACKNOWLEDGEMENTS**

This work has been conducted with the financial support of the PRAD projects 20/98 and 02/08. At the time of study, the medium CHROMagar\textsuperscript{TM} Listeria was not yet available in the market, the authors are grateful to Dr A. Rambach (CHROMagar, Paris, France) for providing samples of this new medium.

**REFERENCES**


