Note

A selective differential medium for *Lactobacillus plantarum*

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Received 3 October 2005; received in revised form 1 February 2006; accepted 9 February 2006
Available online 6 March 2006

Abstract

The quantification of exogenous lactobacilli in faecal samples is frequently required for the evaluation of the intestinal colonization by probiotic bacteria. In this study, a selective and differential medium, designated LPSM, was developed for the culture of exogenous *Lactobacillus plantarum*. In quantitative assays, LPSM showed a sensitivity similar to those of enriched and *Lactobacillus*-adapted media. The presence of ciprofloxacin made LPSM inhibitory to most intestinal bacteria, including endogenous acid lactic bacteria, whereas exogenous *L. plantarum* strains grew producing a yellow color caused by acid production from sorbitol in the presence of bromocresol purple. The results showed that LPSM is suitable for detection and enumeration of *L. plantarum* in faecal samples.

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Keywords: Ciprofloxacin; Intestinal microbiota; *Lactobacillus plantarum*; Probiotic bacteria; Selective medium

Probiotics are non-pathogenic microorganisms that, when administered in adequate amounts, exert health benefits on the host (Marteau et al., 2002; Reid et al., 2003). Lactobacilli are among the first bacteria to be described as probiotics. Strains of several *Lactobacillus* species have proven to exert a range of health promoting activities such as immunomodulation, enhancement of resistance against pathogens, reduction of blood cholesterol levels and others (Gill and Rutherfurd, 2001; Rosenfeldt et al., 2002; Shu and Gill, 2002; Jones et al., 2004).

The complexity of the intestinal microbiota, that includes members from the genus *Lactobacillus*, makes research on probiotic bacteria difficult (Savage, 1999; Moreau and Gaboriau-Routhiau, 2000). There are some selective media that support growth of lactobacilli and some non-*Lactobacillus* species, including MRS Agar that is the most commonly used. However, these media are not useful to distinguish between exogenous and endogenous lactobacilli in faecal samples from humans or animals fed probiotic lactobacilli. The interference of endogenous lactobacilli is specially serious in the case of assays in mouse models, since strains of *Lactobacillus* species are predominant in the mouse gastrointestinal tract (Moreau and Gaboriau-Routhiau, 2000). Advances in molecular genetics of bacteria have allowed more reliable methods for discrimination between *Lactobacillus* species (Satokari et al., 2003; Park and Itoh, 2003), but these methods require special instrumentation and appropriate oligonucleotide probes and do not discriminate between live and dead bacteria.

We hypothesized that, starting from the original formulation of MRS medium, it is possible to design modified media by adding selected antibiotics, an appropriate sugar instead of dextrose and a pH indicator, on which specific, exogenous lactobacilli can be selectively cultured and recognized on the basis of
antibiotic resistance and sugar fermentation. In this report, we describe a selective and differential medium for accurate detection and enumeration of exogenous Lactobacillus plantarum from mouse faecal samples.

Two catalase-negative, gram-positive strains were isolated from commercial fermented milk samples on Lactobacilli MRS agar (LMRS agar, Difco Laboratories, Francisco Soria Melguizo, Madrid, Spain) plates. Sugar fermentation pattern was determined using the API 50 CH system (BioMérieux, Lyon, France), according to the manufacturer’s instructions. Results were recorded after 48 h at 37 °C and a biochemical profile was obtained by the ApilabPlus software. Based on this profile strain C1 (from yogourth) was identified as Lactobacillus casei, and strain C4 (from kefir) as L. plantarum. A gram-positive coccus isolated on Trypti-case soy agar (TSA, Difco) from yogourth was identified as Streptococcus thermophilus by the ApilabPlus software. Strain C16 was isolated from mouse faeces on LMRS agar and identified by API 50 CH as Lactobacillus fermentum. Strain C17 was a gram-negative bacillus isolated from mouse faeces on MacConkey agar (Difco) and identified by API 20 E (BioMérieux) as Escherichia coli. Other bacterial strains used in this study are listed in Table 1 and were obtained from the Spanish Type Culture Collection (CECT).

The new selective differential medium designated LPSM (L. plantarum selective medium) was developed for isolating and enumerating L. plantarum from faecal samples. LPSM design was based on the resistance of the L. plantarum to ciprofloxacin and its ability to produce acid from sorbitol. The formulation per liter of medium was Bacto proteose peptone (Difco) (10 g), Bacto beef extract (Difco) (10 g), Bacto yeast extract (Difco) (5 g), d-sorbitol (Sigma) (20 g), ciprofloxacin (Sigma) (4 mg), sodium acetate (5 g), ammonium citrate (2 g), potassium phosphate (2 g), magnesium sulfate (0.1 g), manganesum sulfate (0.05 g), bromocresol purple (0.02 g) and Bacto agar (Difco) (15 g). The medium without ciprofloxacin was autoclaved for 15 min at 121 °C, and cooled at 50 °C. Ciprofloxacin was sterilized by filtration before being added to the cooled medium. The pH of the medium was 6.0±0.1. When solidified, LPSM was an purple color.

The sensitivity and specificity of LPSM were determined by comparison of four L. plantarum strains with other selected bacteria grown on LPSM, LMRS agar and the enriched medium TSA. All strains were grown on TSA tubes at 37 °C for 24 h. Bacteria were harvested, washed twice and resuspended in sterile phosphate-buffered saline (PBS), and 10-fold serial dilutions (10^{-1} to 10^{-8}) were prepared in PBS. 10-μl volumes of undiluted samples and dilutions were plated onto LPSM, LMRS agar and TSA plates and incubated at 37 °C for colony enumeration. The results are presented in Table 1. On LPSM, all four L. plantarum strains produced large (diameter >2 mm) yellow colonies surrounded by a yellow halo after 48 h of incubation. The yellow color change in the medium was due to acid production from sorbitol. Recovery rates of L. plantarum strains were similar in all three media. In contrast, strains of L. casei and L. fermentum produced similar colony counts on LMRS agar and TSA but they did not grow on LPSM above the detection level (10^2 bacteria/ml). Strains of S. thermophilus, E. coli and Salmonella enterica serovar typhimurium failed to form colonies on LPSM and LMRS agar but they did not grow on LPSM above the detection level (10^2 bacteria/ml). Strains of S. thermophilus, E. coli and S. enterica serovar typhimurium were streaked onto LPSM plates, supporting the selectivity of the medium (data not shown).

To evaluate the sensitivity of LPSM to detect L. plantarum in heavily contaminated samples, faeces from untreated mice were diluted in sterile PBS and blended in a Vortex mixer, and 10 μl of appropriate dilutions of C4 suspensions in saline were added to the diluted faeces (1-ml samples). Samples were homogenized in a Vortex mixer and 10-fold serial dilutions (10^{-1} to 10^{-8}) of the homogenates were plated onto LPSM plates and incubated at 37 °C for two days before colony enumeration. The original dilutions of C4 in

<table>
<thead>
<tr>
<th>Strain</th>
<th>Average of CFU on the following media</th>
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<tbody>
<tr>
<td></td>
<td>LPSM</td>
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<tr>
<td>L. plantarum C4</td>
<td>1.0 × 10^8</td>
</tr>
<tr>
<td>L. plantarum ATCC8014</td>
<td>4.0 × 10^7</td>
</tr>
<tr>
<td>L. plantarum ATCC10241</td>
<td>6.0 × 10^7</td>
</tr>
<tr>
<td>L. plantarum ATCC14431</td>
<td>5.9 × 10^7</td>
</tr>
<tr>
<td>L. casei C1</td>
<td>&lt;10^2</td>
</tr>
<tr>
<td>L. casei ATCC393</td>
<td>&lt;10^2</td>
</tr>
<tr>
<td>L. fermentum C16</td>
<td>&lt;10^2</td>
</tr>
<tr>
<td>S. thermophilus C5</td>
<td>&lt;10^2</td>
</tr>
<tr>
<td>E. coli C17</td>
<td>&lt;10^2</td>
</tr>
<tr>
<td>E. coli ATCC25922</td>
<td>&lt;10^2</td>
</tr>
<tr>
<td>S. enterica serovar typhimurium CECT4156</td>
<td>&lt;10^2</td>
</tr>
</tbody>
</table>

Each strain was suspended in saline and counted in triplicate on the corresponding media. The lowest detectable number of bacteria was 10^2 per ml of suspension.
Our results showed that LPSM is suitable for specific and sensitive tool to assess the intestinal colonization by *L. plantarum*, whose probiotic potential has been shown in several reports (Miettinen et al., 1996; Herias et al., 1999; Coeuret et al., 2004). In quantitative assays, LPSM has shown a sensitivity similar to those of the enriched medium TSA and the *Lactobacillus*-adapted medium MRS. A ciprofloxacin concentration of 4 μg/ml made LPSM inhibitory to most intestinal bacteria, including endogenous acid lactic bacteria that grow on MRS agar, but had no adverse effect on *L. plantarum* recovery. Some faecal bacteria that eventually were able to grow on LPSM plates formed pinpoint colorless colonies that were readily distinguished from those of *L. plantarum*. We propose that theoretical approach of LPSM design could be applied to the design of selective and differential media adapted to other *Lactobacillus* species with probiotic potential.

### Acknowledgements

This study was supported by Ministerio de Sanidad y Consumo and the European Regional Development Fund (Programa de Promoción de la Investigación Biomédica y en Ciencias de la Salud, Proyecto P1021774). C. Bujalance was supported by a predoctoral grant from Junta de Andalucía.

### References


