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A highly selective, differential medium for the enumeration and isolation of *Klebsiella* spp. was developed. With pure cultures, 100% recovery of *Klebsiella* spp. was observed. Recovery of *Klebsiella* spp. on MacConkey-inositol-potassium tellurite (MCIC) agar was as good as or better than on MacConkey-inositol-carbenicillin agar either with pure cultures or environmental samples. Recovery and percent colony confirmation with MCIC agar were greater and easier to obtain than for other proposed *Klebsiella* selective media.

It is always desirable to have a selective, differential medium specific for the rapid detection and efficient recovery of an opportunistic pathogen such as *Klebsiella* spp. Much effort has been devoted in recent years to the isolation and enumeration of *Klebsiella* spp. in clinical, industrial, and natural environments (2, 6, 7, 9).

In 1970 Thom (13) developed a nutrient medium for the selective recovery of *Klebsiella* spp. from feces, MacConkey-inositol-carbenicillin (MCIC) agar. This medium owes its differential capacity to the fact that about 97 to 99% of *Klebsiella* spp. and only 0 to 1% of *Escherichia coli* strains are capable of fermenting inositol (4) and hence appear as red colonies. The selectivity of the medium is due to the presence of carbenicillin to which most *Escherichia coli* strains are also susceptible. Since about 10 to 15% of *Klebsiella* strains are susceptible to this concentration of carbenicillin (8), these strains will be missed when this medium is used.

In this study we devised and tested a new medium for the identification and selective recovery of *Klebsiella* spp. from environmental and clinical sources. The medium (MacConkey-inositol-potassium tellurite [MCIC]) was prepared by suspending 40 g of MacConkey agar base (Adsa, Barcelona, Spain) in 1 liter of distilled water. After the mixture was autoclaved at 121°C for 15 min and then cooled to 50°C, filter-sterilized myo-inositol (E. Merck AG, Darmstadt, Federal Republic of Germany) (final concentration, 10 mM) and potassium tellurite (final concentration, 3 μg/ml) were added. After mixing well, the medium was poured into sterile petri plates. The plates kept well at 4°C for at least 10 weeks.

We recently described potassium tellurite as a strong inhibitor of phosphate transport in *Escherichia coli* (J. M. Tomás and W. W. Kay, submitted for publication), and plasmid resistance to K₂TeO₃ in members of the family *Enterobacteriaceae* has been widely documented (11, 12; J. M. Tomás, D. E. Taylor, and W. W. Kay, unpublished data). When we tested the MIC of K₂TeO₃ (14) for enterobacteria we found that only *Klebsiella* spp. showed a high degree of resistance (10, 14), and this resistance was usually of chromosomal origin (data not shown). *Klebsiella* strains showed higher resistance to K₂TeO₃ than did other enterobacteria able to ferment inositol (Table 1), indicating that potassium tellurite is a good selective agent for *Klebsiella* identification.

Recovery was calculated as the number of viable cells on MCIC or MCIC (50 or 100 μg of carbenicillin per ml) agar divided by the number of viable cells on standard plate count agar. Recovery on MCIC agar with either 50 or 100 μg of carbenicillin per ml was lower (92 ± 3 and 75 ± 5%, respectively) than on MCIC agar (approximately 98 ± 2%) for the four *Klebsiella* species. Pure cultures of *Enterobacter aerogenes*, *Enterobacter agglomerans*, *Enterobacter cloacae*, *Serratia marcescens*, *Providencia stuartii*, *Providencia rettgeri*, *Proteus vulgaris*, *Citrobacter freundii*, *Yersinia enterocolitica*, and *Klebsiella oxytoca* were recovered with the new selective medium.

### Table 1. Bacterial strains used in this study and MIC of potassium tellurite

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Inositol fermentation</th>
<th>Source</th>
<th>MIC of K₂TeO₃ (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>+</td>
<td>ATCC 13048</td>
<td>1</td>
</tr>
<tr>
<td><em>Enterobacter agglomerans</em></td>
<td>+</td>
<td>ATCC 23216</td>
<td>2</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>+</td>
<td>ATCC 12666</td>
<td>3</td>
</tr>
<tr>
<td><em>Enterobacter agglomerans</em></td>
<td>−</td>
<td>Palma de Mallorca, Spain</td>
<td>2</td>
</tr>
<tr>
<td><em>Providencia stuartii</em></td>
<td>+</td>
<td>ATCC 29914</td>
<td>1</td>
</tr>
<tr>
<td><em>Providencia rettgeri</em></td>
<td>+</td>
<td>Barcelona, Spain</td>
<td>1</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em></td>
<td>+</td>
<td>ATCC 6750</td>
<td>1</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>+</td>
<td>Barcelona, Spain</td>
<td>1</td>
</tr>
<tr>
<td><em>Yersinia enterocolitica</em></td>
<td>+</td>
<td>ATCC 9610</td>
<td>1</td>
</tr>
<tr>
<td><em>Klebsiella oxytoca</em></td>
<td>+</td>
<td>Palma de Mallorca, Spain</td>
<td>200</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>+</td>
<td>Palma de Mallorca, Spain</td>
<td>100</td>
</tr>
<tr>
<td><em>Klebsiella ozonae</em></td>
<td>+</td>
<td>Palma de Mallorca, Spain</td>
<td>10</td>
</tr>
<tr>
<td><em>Klebsiella rhinoscleromatis</em></td>
<td>NT*</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella planticola</em></td>
<td>+</td>
<td>Palma de Mallorca, Spain</td>
<td>100</td>
</tr>
<tr>
<td><em>Klebsiella terrigena</em></td>
<td>+</td>
<td>This laboratory (5)</td>
<td>200</td>
</tr>
<tr>
<td><em>Escherichia coli</em> C600</td>
<td>−</td>
<td>This laboratory (1)</td>
<td>1</td>
</tr>
<tr>
<td><em>Escherichia coli</em> CSH57</td>
<td>−</td>
<td>CSH57</td>
<td>1</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>−</td>
<td>Victoria, Canada</td>
<td>1</td>
</tr>
<tr>
<td>LT2</td>
<td>−</td>
<td>Barcelona, Spain</td>
<td>1</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>−</td>
<td>Barcelona, Spain</td>
<td>1</td>
</tr>
</tbody>
</table>

*Corresponding author.

* + and −, Able or unable to ferment inositol, respectively.  
* NT, Not tested.
TABLE 2. Verification of colony type on MCIK medium

<table>
<thead>
<tr>
<th>Sampling location</th>
<th>Typical (pink-red)</th>
<th>Atypical</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. tested</td>
<td>Klebsiella spp.</td>
</tr>
<tr>
<td>El Prat</td>
<td>0</td>
<td>111</td>
</tr>
<tr>
<td>Llobregat River</td>
<td>129</td>
<td>100</td>
</tr>
<tr>
<td>Murtra</td>
<td>36</td>
<td>100</td>
</tr>
<tr>
<td>Castelldefels</td>
<td>19</td>
<td>100</td>
</tr>
<tr>
<td>Pineda</td>
<td>22</td>
<td>100</td>
</tr>
<tr>
<td>Sant Andriá</td>
<td>45</td>
<td>100</td>
</tr>
<tr>
<td>Besos River</td>
<td>146</td>
<td>100</td>
</tr>
</tbody>
</table>

* Values indicate percent verified as Klebsiella spp. by Simmons citrate, indole, motility, lysine and ornithine decarboxylase, and urease tests.

enterocolitica, Escherichia coli, and Salmonella typhimurium showed a 0% recovery after incubation at 37°C for 24 h. Klebsiella cultures appeared as pink to red colonies on the agar surface within 24 h of incubation at 37°C, indicating inositol fermentation.

Water and raw sewage samples from the Barcelona area were also tested for Klebsiella recovery on MCIK agar by using membrane filtration techniques. Any pink to red colonies, regardless of size, were considered as presumptive Klebsiella spp. In highly contaminated samples some nonenteric bacteria developed as grey microcolonies. The specificity of MCIK agar for Klebsiella detection was demonstrated by the fact that 100% of all presumptive colonies were verified as Klebsiella spp. (Table 2). Only 0.2% of the background-growth colonies were Klebsiella spp. Both indole-positive and indole-negative Klebsiella spp. were detected on MCIK agar.

Equal or greater numbers of Klebsiella spp. per 100 ml were detected in water environmental samples from the Barcelona area (Table 3) by using MCIK agar as compared with the numbers of Klebsiella spp. appearing as total coliforms on mENDO agar LES (Difco Laboratories, Detroit, Mich.). All (100%) of the typical colonies were confirmed as Klebsiella spp. Klebsiella spp. represented 0.05 to 25% of the total coliform count in water samples (3). The percentage of Klebsiella spp. obtained on MCIK agar was in agreement with these values (Table 3). When mixtures of Enterobacter aerogenes, Enterobacter agglomerans, Enterobacter cloacae, Serratia marcescens, Providencia stuartii, Providencia retgeri, Proteus vulgaris, Citrobacter freundii, Yersinia enterocolitica, Escherichia coli, and Salmonella typhimurium pure cultures and Klebsiella spp. were prepared, MCIK agar allowed the recovery of Klebsiella spp. constituting <10⁻⁶% of the total CFU.

MCIK was therefore found to be a highly specific medium for primary Klebsiella identification. The level of K₂TeO₃ used was sufficient to inhibit growth of the other enterobacteria able to ferment inositol in both pure culture and environmental samples. This medium has advantages over other proposed selective Klebsiella media in having higher or identical recovery, confirmation of typical colonies, and an incubation time faster than that of MCIC (in our hands MCIC needs an incubation time of more than 24 h). Another advantage is that K₂TeO₃ had a constant potency for 10 weeks at least, while carbenicillin potency decreased during storage. This makes it possible to store MCIC plates at 4°C at least for 10 weeks, as compared with MCIC medium (72 h at 4°C according to Bagley and Seidler [3]). Based on these results, MCIC medium could be used routinely for Klebsiella enumeration in both environmental and clinical samples.

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We thank F. Lucena for performing the total coliform counts.

LITERATURE CITED

5. Imperjal, J., R. Parés, and J. A. Cole. 1982. Defective nitrate assimilation by a derivative of Klebsiella pneumoniae strain C3 (formerly Citrobacter intermedius C3) which has lost the isoci-

TABLE 3. Comparative environmental detection of Klebsiella spp. on selective media

<table>
<thead>
<tr>
<th>Sampling location</th>
<th>No. of replications</th>
<th>No. of Klebsiella detected/100 ml on*:</th>
<th>Total coliforms/100 ml (mEndo agar LES)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MCIK</td>
<td>MCIC (50)²</td>
<td>MCIC (100)²</td>
</tr>
<tr>
<td>Sant Adriá</td>
<td>3</td>
<td>(3.0 ± 0.4) X 10⁴</td>
<td>(2.9 ± 0.4) X 10⁴</td>
</tr>
<tr>
<td></td>
<td>(100)¹</td>
<td>(100)</td>
<td>(100)</td>
</tr>
<tr>
<td>Marbella</td>
<td>3</td>
<td>(2.5 ± 0.3) X 10⁴</td>
<td>(2.3 ± 0.3) X 10³</td>
</tr>
<tr>
<td></td>
<td>(100)¹</td>
<td>(97)</td>
<td>(99)</td>
</tr>
<tr>
<td>Montgat</td>
<td>3</td>
<td>(3.2 ± 0.4) X 10⁴</td>
<td>(3.1 ± 0.4) X 10⁴</td>
</tr>
<tr>
<td></td>
<td>(100)¹</td>
<td>(99)</td>
<td>(100)</td>
</tr>
<tr>
<td>Barcelona</td>
<td>3</td>
<td>(2.2 ± 0.2) X 10⁴</td>
<td>(2.2 ± 0.2) X 10⁴</td>
</tr>
<tr>
<td></td>
<td>(100)¹</td>
<td>(100)</td>
<td>(100)</td>
</tr>
<tr>
<td>Badalona</td>
<td>4</td>
<td>(2.4 ± 0.2) X 10⁴</td>
<td>(2.0 ± 0.3) X 10³</td>
</tr>
<tr>
<td></td>
<td>(100)¹</td>
<td>(94)</td>
<td>(100)</td>
</tr>
<tr>
<td>Besos River</td>
<td>2</td>
<td>(3.1 ± 0.4) X 10⁴</td>
<td>(3.0 ± 0.3) X 10³</td>
</tr>
<tr>
<td></td>
<td>(100)¹</td>
<td>(96)</td>
<td>(100)</td>
</tr>
</tbody>
</table>

* The geometric mean recoveries for the different Klebsiella medium formulations were 5.8 X 10⁴ for MCIK, 5.4 X 10⁴ for MCIC (50), and 4.1 X 10⁴ for MCIC (100).
1 MCIC with 50 μg of carbenicillin per ml (3).
² MCIC with 100 μg of carbenicillin per ml (13).
3 Percent verification for each Klebsiella selective medium is reported in parentheses. Klebsiella verification was performed as indicated in Table 2.