Serum- and animal tissue-free medium for transport and growth of Helicobacter pylori

Cindy M. Dierikx1, Jaime Martodihardjo2, Ernst J. Kuipers1, Charles M.H. Hensgens2, Johannes G. Kusters1, Hidekazu Suzuki3, Nanda de Groot2 & Arnoud H.M. van Vliet1

Correspondence: Arnoud H.M. van Vliet, Department of Gastroenterology and Hepatology, Erasmus MC – University Medical Center Rotterdam, 's-Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands. Tel.: +31 10 463 5944; fax: +31 10 463 2793; e-mail: a.h.m.vanvliet@erasmusmc.nl

Received 13 September 2006; revised 8 November 2006; accepted 27 November 2006. First published online 12 February 2007.

DOI:10.1111/j.1574-695X.2007.00211.x

Editor: Alex van Belkum

Keywords
transport medium; growth medium; survival; Helicobacter.

Abstract

The important human gastric pathogen Helicobacter pylori is the subject of many studies, and as a consequence it is frequently being transported between national and international laboratories. Unfortunately, common bacterial growth and transport media contain serum- and animal tissue-derived materials, which carry the risk of spreading infectious diseases. We have therefore developed a growth and transport medium for H. pylori, designated ‘Serum- and Animal Tissue-Free Medium’ (SATFM), which does not contain serum- or animal tissue-derived components. SATFM supported growth of H. pylori isolates to similar levels as obtained with serum-supplemented Brucella medium, and SATFM with 0.5% agar supported transport and storage of H. pylori strains, as 4/4 reference strains and 11/11 clinical isolates survived for at least 3 days at room temperature in SATFM, with some strains (2/15) even surviving for up to 7 days. In conclusion, SATFM can be used both as transport and growth medium for H. pylori. The formulation of SATFM may allow its use in international transport of H. pylori, and may also allow certified use in immunization studies requiring growth of H. pylori and other bacterial pathogens.

Introduction

Animal tissue-derived materials have traditionally been used in the manufacturing of many biological products. Unfortunately their use is associated with the possibility of spreading and transferring infectious diseases, such as Bovine Spongiforme Encephalopathy (BSE) (Erstad, 2002). This is especially noteworthy with the use of bovine materials, as there might be links between consumption of contaminated bovine tissue, meat or bone meal and the development of new variant Creutzfeld Jacob Disease (vCJD) in humans (Novakofski et al., 2005). Therefore the use of alternatives for animal-derived materials should be considered, and may be subject to new and strict recommendations on their inclusion in products for human and animal use or consumption (http://www.fda.gov/cber/BSE/BSE.htm). This is an important issue, both when transporting bacterial isolates between (inter)national laboratories and when culturing bacterial isolates for production of antibodies or vaccines.

The important human pathogen Helicobacter pylori is a fastidious organism, which requires complex media and microaerobic conditions for survival and growth (Kusters et al., 2006). Therefore, peptic digest of animal tissue, beef extract, bovine serum albumine, blood or serum are commonly used in growth, storage and in transport media for H. pylori (Shahamat et al., 1991; Olivieri et al., 1993; Xia et al., 1994). Special care must be taken in using these materials in transport or growth media used for production of H. pylori lysates for immunization (Suzuki et al., 2004), as their use could potentially lead to the unintentional spread of infectious agents.

A consequence of the importance of H. pylori as a human pathogen is that H. pylori strains are being shipped between various laboratories at different geographical regions. Different protocols have been developed for the storage and transport of H. pylori strains (Wang et al., 1980; Owen et al., 1988; Soltesz et al., 1992; Xia et al., 1994). It is possible to ship the bacteria on dry ice, but this is expensive, and not always feasible. A different way of transporting H. pylori is using special transport media, in which the bacteria can maintain viability for several days at ambient temperatures (Wang et al., 1980). This medium has been used successfully for transporting H. pylori strains between laboratories.
(our unpublished results). A drawback of this medium, however, is that it contains many animal-derived components, and therefore its usefulness as transport medium is likely to become limited due to changing laws and regulations.

The aim of this study was the development of a transport and growth medium for \textit{H. pylori} that (1) does not contain serum or animal tissue-derived components associated with the transfer of infectious agents, and (2) in which sufficient cell densities can be obtained for growth experiments and production of lysates for immunization.

### Materials and methods

#### Media

SATFM was based on Brucella medium (Becton Dickinson, The Netherlands) supplemented with bovine serum, in which animal-derived components were replaced by non-animal derived components where possible (Table 1). Bacto-agar (Becton Dickinson) was used at a final concentration of 1.5% for solid media and 0.5% for transport media. SATFM was supplemented with $\beta$-cyclodextrins (Fluka, The Netherlands) to a final concentration of 0.2% (w/v). For growth experiments SATFM-broth was compared with Brucella media [Brucella broth supplemented with 3% (v/v) New born Calf Serum] (Life Technologies, Breda, The Netherlands; designated BBN).

SATFM was also compared to modified Wang transport medium, where 10% sheep blood was replaced by 10% defibrinated horse blood (Wang et al., 1980). The Wang transport medium furthermore consisted of Brucella broth and 0.5% bacto-agar. Dent Selective Supplement (Oxoid, The Netherlands) consisted of Columbia agar (Oxoid) supplemented with 7% saponin-lysed defibrinated horse blood, 0.004% triphenyltetrazolium chloride (Sigma, The Netherlands), and Dent Selective Supplement (Oxoid).

SATFM was also compared to modified Wang transport medium, where 10% sheep blood was replaced by 10% defibrinated horse blood (Wang et al., 1980). The Wang transport medium furthermore consisted of Brucella broth and 0.5% bacto-agar. Dent Selective Supplement (Oxoid, The Netherlands) consisted of Columbia agar (Oxoid) supplemented with 7% saponin-lysed defibrinated horse blood, 0.004% triphenyltetrazolium chloride (Sigma, The Netherlands), and Dent Selective Supplement (Oxoid).

SATFM was also compared to modified Wang transport medium, where 10% sheep blood was replaced by 10% defibrinated horse blood (Wang et al., 1980). The Wang transport medium furthermore consisted of Brucella broth and 0.5% bacto-agar. Dent Selective Supplement (Oxoid, The Netherlands) consisted of Columbia agar (Oxoid) supplemented with 7% saponin-lysed defibrinated horse blood, 0.004% triphenyltetrazolium chloride (Sigma, The Netherlands), and Dent Selective Supplement (Oxoid).

### Bacterial strains and growth conditions

The \textit{H. pylori} strains used in this study are listed in Table 2. \textit{Helicobacter pylori} was stored at $-80\,\text{°C}$ in BHI broth with 15% glycerol. Bacteria were routinely cultured on either SATFM or Dent-plates for 48–72 h at 37 °C in an atmosphere of 5% $\text{O}_2$, 10% $\text{CO}_2$, and 85% $\text{N}_2$. For broth cultures, \textit{H. pylori} was collected from either SATFM or Dent-plates and inoculated to an OD at 600 nm (OD$_{600}$) of 0.1. Inoculated media were incubated at 37 °C under microaerophilic conditions (see above) and shaken continuously at 40 r.p.m.

### Testing recovery from transport medium

\textit{Helicobacter pylori} was harvested from one quarter part of one SATFM or Dent plate (diameter 9 cm) with a cotton swab. The cotton swab with bacteria was then carefully inserted into 2 mL cryovials (Sarstedt, The Netherlands) containing 1 mL of, respectively, fresh-made SATFM or fresh-made Wang transport medium. The cryovials were closed after cutting of the tip of the swab with sterile scissors. Each plate was used to inoculate four vials, which were all held at room temperature and room atmosphere for up to 7 days. On days 0, 3, 5 and 7 one of the vials was opened and the swab was spread on one quarter part of a Dent-plate.

### Protein analysis

The effect of growth medium on protein profiles of \textit{H. pylori} strain 26695 was determined after growth in SATFM-broth or BBN for 18 h with moderate shaking to an OD at 600 nm (OD$_{600}$) of 0.3 to 0.4, centrifuged for 10 min at 4000 g at 4 °C and resuspended in ice-cold phosphate-buffered saline to a final OD$_{600}$ of 10. Eight microliters of this solution was size

### Table 1. Formulation of serum- and animal tissue-free medium for growth and transport of \textit{Helicobacter pylori} strains

<table>
<thead>
<tr>
<th>Components</th>
<th>SATFM g L$^{-1}$</th>
<th>Suppliers</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid hydrolysed casein</td>
<td>12.0</td>
<td>DMV International, Veghel – NL</td>
<td>Replaces pancreatic digest of casein</td>
</tr>
<tr>
<td>Papaine digest soya peptone</td>
<td>8.0</td>
<td>DMV International, Veghel – NL</td>
<td>Replaces both peptic digest of animal tissue &amp; beef extract</td>
</tr>
<tr>
<td>$\beta$-cyclodextrins</td>
<td>0.2</td>
<td>Fluka</td>
<td>Replaces new born calf serum</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0</td>
<td>ProLabo VWR</td>
<td></td>
</tr>
<tr>
<td>Corn starch*</td>
<td>1.0</td>
<td>Sigma</td>
<td></td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.0</td>
<td>Oxoid</td>
<td></td>
</tr>
<tr>
<td>Bacto-agar</td>
<td>5.0</td>
<td>Becton Dickinson</td>
<td>Used in SATFM plates</td>
</tr>
<tr>
<td></td>
<td>13.5</td>
<td></td>
<td>Used in SATFM transport medium</td>
</tr>
</tbody>
</table>

*May be replaced by $\alpha(+)$– glucose monohydrate at 1.0 g L$^{-1}$. 

© 2007 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved


Downloaded from https://academic.oup.com/femspd/article-abstract/50/2/239/682557 by guest on 25 December 2018
separated on a 10% Sodium Dodecyl Sulfate-polyacrylamide gel, followed by staining with Coomassie Brilliant Blue (Sambrook et al., 1989).

Results and discussion

SATFM supports plate and broth cultures of *H. pylori*

Animal tissue-derived components of Brucella broth were replaced by nonanimal tissue-derived alternatives as is shown in Table 1. Eleven out of 16 strains tested were able to grow in SATFM supplemented with 0.02% β-cyclodextrins, while all 16 tested strains were able to grow in SATFM supplemented with 0.2% β-cyclodextrins. Therefore, a β-cyclodextrin concentration of 0.2% was used in subsequent experiments. *Helicobacter pylori* reference strain 26695 was used to assess whether SATFM supported growth of *H. pylori* to similar densities as BBN (Fig. 1a). Although the final OD_{600} after 18 h of growth in SATFM was lower than that when strain 26695 was grown in BBN, this difference was relatively minor and not statistically significant (Fig. 1a). Comparison of protein profiles of BBN- and SATFM-grown *H. pylori* strain 26695 did not reveal any major changes in protein expression (Fig. 1b).

SATFM does not completely consist of non-animal derived materials. The caseins used in SATFM to replace pancreatic hydrolyzed caseins are derived from the milk of cows, but were hydrolyzed with acid instead of an animal-derived enzyme. Milk and its derivatives are considered to be unlikely to present any risk of transmissible spongiform encephalopathies (such as BSE) as long as no other rumen materials (with the exception of calf rennet) are used in the preparation of such derivatives, and the original milk is derived from healthy cows (European Medicines Agency, 2004). Therefore we do not expect problems with the use of acid-hydrolyzed caseins in the process of developing vaccines or other medicinal products. Some manufacturers have developed vegetable alternatives for animal derived components like vegetable peptone acid hydrolysate which may present an alternative for casein acid hydrolysate.

**Table 2. Helicobacter pylori strains used in this study**

<table>
<thead>
<tr>
<th>H. pylori strains</th>
<th>CagA status</th>
</tr>
</thead>
<tbody>
<tr>
<td>26695*, G27*, J99*</td>
<td>Positive</td>
</tr>
<tr>
<td>ATCC43504*, SS1*</td>
<td>Positive</td>
</tr>
<tr>
<td>KS76*, KS105*, KS107*</td>
<td>Positive</td>
</tr>
<tr>
<td>KS114*, KS125*</td>
<td>Positive</td>
</tr>
<tr>
<td>KS126*, LS1*</td>
<td>Positive</td>
</tr>
<tr>
<td>1061*, 2025*, 2047*, KS70*, KS91*, KS98*</td>
<td>Negative</td>
</tr>
</tbody>
</table>

*Source/reference: 26695 Tomb et al. (1997), G27 Covacci et al. (1993), J99 Alm et al. (1999); ATCC43504 (American Type Culture Collection); 1061 Goodwin et al. (1998), SS1 Lee et al. (1997); Crabtree et al. (2002), 2025 and 2047 Munzenmaier et al. (1997).

1Strains with KS annotation were obtained from Keio University, Japan.

1Strains with L designation were described in Loffeld et al. (2000).

SATFM can be used as transport medium for *H. pylori*

The survival of four reference strains and 11 clinical isolates in SATFM under simulated transport conditions was compared to that in Wang transport medium. *Helicobacter pylori*

![Fig. 1. Growth of *H. pylori* 26695 in SATFM and BBN results in similar OD\textsubscript{600} values and protein profiles. (a) Comparison of growth of *H. pylori* strain 26695 in SATFM-broth and BBN. Growth was monitored in inoculation. Black bars represent SATFM, white bars represent BBN. The error bars denote SDs. (NS, not significant; P = 0.07, Wilcoxon Signed Ranks test). (b) Protein profile of *H. pylori* 26695 grown in SATFM (S) and BBN (B). Relevant marker sizes are indicated on the left.](https://academic.oup.com/femspd/article-abstract/50/2/239/682557)
Conclusions

In the last decades, there has been increasing awareness of the risks of spreading infectious agents through the global transport of serum- and animal tissue-derived materials. The growing concern about the possible link between BSE in cattle and vCJD in humans (Novakofski et al., 2005) has resulted in creation of new legislation, and tightening of current rules (European Medicines Agency, 2004). Overall, the use of animal tissue-derived materials associated with a possible transfer of infectious agents are restricted by legislation (e.g., http://www.aphis.usda.gov/vs/ncie/biofacts.html for the USA). Next to affecting transport of pathogens in transport media, this has also significant impact on for example the manufacturing of vaccines (Anonymous, 2000) and pharmaceutical products (Erstad, 2002). When producing protein samples for vaccination or production of antibodies, the medium that was used to cultivate the bacteria should also be certified to allow national and international use. Standard growth media will not receive such a certification, while this should be possible with SATFM. Also, production of bacterial lysates for passive immunization studies is likely to be subject to restrictive legislation, and the use of SATFM may be an important step in allowing the continuation of these studies. It is important to note that the chances of H. pylori infection from transported strains is very low (Kusters et al., 2006), and that this infection can be treated with antibiotics (Gerrits et al., 2006), whereas prion-based diseases such as BSE and vCJD are difficult to diagnose early, are invariably lethal and are without a cure. Therefore, attention to protection against diseases such as BSE and vCJD is of prime importance (Novakofski et al., 2005).

In summary, the use of animal-derived materials in growth and transport media is expected to be constrained by new and existing regulations preventing the spread of infectious agents. This necessitates alternative transport media replacing animal-derived materials. The SATFM medium developed in this study may be used as alternative growth and transport medium for H. pylori, and possibly also for other microorganisms both related and unrelated to H. pylori.

Acknowledgements

This work was supported by DMV international (The Netherlands) and Ortho Corporation (Japan). Special thanks to DMV international for kindly supplying acid hydrolysed casein and papain digest soya peptone.

We thank Ruud Loffeld (De Heel Medisch Centrum, Zaandam, The Netherlands) for providing H. pylori clinical isolates used in this study.

References


