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Influence of medium composition on the growth and antigen expression of *Helicobacter pylori*

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E.J. WALSH AND A.P. MORAN. 1997. An evaluation of the ability of various solid and liquid media to support both growth and antigen expression, particularly lipopolysaccharide (LPS) expression, by *Helicobacter pylori* culture collection strains and clinical isolates was performed. Liquid-based basal media (brain heart infusion, Brucella broth, Mueller-Hinton broth and tryptone soya broth) supported the growth of strains, whereas solid basal media of the same formulation did not support growth. Optimal growth of all strains was obtained on solid and in liquid media containing blood. Supplemented solid media containing supplements other than blood supported growth but only to a small extent. In liquid media excluding blood, serum supplements enhanced growth and horse serum was found to be superior to fetal calf serum. In general, β-cyclodextrin did not increase growth. Mueller-Hinton broth or tryptone soya broth containing horse serum and a nitrogen source such as yeast extract or proteose peptone no. 3 were found to give optimal growth of *H. pylori* in a blood-free environment. Strains after cultivation in liquid media, irrespective of composition, maintained production of high-molecular weight (mol. wt) LPS with an O side chain independent of medium composition, whereas subculturing an solid media resulted in production of low-mol. wt LPS. Expression of proteins differed in liquid and an solid media, particularly proteins of 57 and 60 kDa, but qualitatively no differences were observed upon supplementation of basal media.

INTRODUCTION

Helicobacter pylori is a Gram-negative, micro-aerophilic, spiral or S-shaped bacterium which resides in the human stomach (Moran 1996). This bacterium is the causative agent of active chronic gastritis and is associated with the development of peptic and gastric ulcers and non-ulcer dyspepsia (Warren and Marshall 1983; Marshall and Warren 1984; Rauws and Tytgat 1990; Graham et al. 1992; Blaser and Parsonnet 1994). *Helicobacter pylori* is also a cofactor in the development of gastric cancer (Forman et al. 1991; Nomura et al. 1991; Wotherspoon et al. 1991; Blaser and Parsonnet 1994). Due to the fastidious nature of *H. pylori,* in vitro cultivation is difficult. Investigations have been performed an assessing the growth of *H. pylori* in various blood-based solid media (Goodwin et al. 1985; Buck and Smith 1987; Hazell et al. 1989; Oliveri el al. 1993; Hachem et al. 1995). Cultivation of the organism in small and large volumes of liquid media containing serum has also been reported (Buck and Smith 1987; Morgan et al. 1987; Secker et al. 1991; Shahamat et al. 1991; Xia et al. 1993a; Morshed et al. 1994; Coudron and Stratton 1995).

Like the cell envelope of other Gram-negative bacteria, *H. pylori* contains lipopolysaccharide (LPS) in its outer membrane (Moran 1995a). This molecule is important in the structure and function of the outer membrane (Nikaido and Vaara 1987), provides the basis for serological classifications and is a potential toxin of the bacterium (Brade et al. 1988; Rietschel et al. 1990). Chemically, LPS is composed of a polysaccharide moiety attached to a lipid component, lipid A, embedded in the bacterial outer membrane (Brade et al. 1988; Rietschel et al. 1990; Moran 1995b). High-molecular-weight smooth-form (S-) LPS consists of an O side chain, which is a polymer of repeating oligosaccharide units, a core oligosaccharide and lipid A, whereas low-molecular-weight rough-form (R-) LPS is devoid of the O side chain (Rietschel et al. 1990; Moran 1995b).

The LPS of various *H. pylori* strains can be visualized

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by silver staining the molecules in sodium dodecyl sulphatepolyacrylamide gel electrophoresis (SDS-PAGE) (Moran 1995a). The electrophoretic mobility of the molecules depends an their mass, with larger LPS molecules being retarded more in the gel than smaller ones. The relative mobilities of the LPS molecule thus depend an the structural variation and the degree of polymerization of the O side chain (Palva and Makela 1980). Previous studies with SDS-PAGE have shown that fresh clinical isolates of *H. pylori* produce O side chains of homogenous length (Moran et al. 1992). Upon serial subculturing of *H. pylori* isolates an solid media, loss of O side chains may occur and expression of LPS molecules shift to R-LPS (Moran et al. 1992). The number of subcultures an solid media necessary to induce the change from S- to R-LPS is dependent an the strain (Moran and Walsh 1993a,b). Thus, *in vitro* cultivation of *H. pylori* isolates on solid media may result in loss of the antigenic O side chain and potential loss of antigenic determinants. This phase shift can be reversed when the strains were grown an liquid media (Moran and Walsh 1993a) and upon numerous subcultures in liquid media clinical isolates have been shown to still produce O side chains (Moran and Walsh 1993a,b). On the other hand, SDS-PAGE analysis of LPS of *H. pylori* culture collection strains shows profiles characteristic of R-LPS (Moran et al. 1992; Moran 1995a). Nevertheless, culture collection strains revert from production of R-LPS to S-LPS when strains are grown in liquid media (Moran and Walsh 1993a).

An examination of the electrophoretic profiles of S-LPS of different *H. pylori* strains has indicated structural variation in the O side chain region (Moran and Walsh 1993a,b) and this has been confirmed by serological and chemical investigations (Mills et al. 1992; Aspinall and Monteiro 1996; Aspinall et al. 1996). Therefore, the antigenic variation in LPS particularly in the O side chain, may serve as the basis of a serotyping scheme for *H. pylori* once production of S-LPS is stabilized *in vitro* (Milk et al. 1992; Moran 1995a).

The aim of the present investigation was to evaluate the ability of different media to support the growth of *H. pylori* and, in addition, to investigate the ability of these to induce and maintain the production of S-form LPS by *H. pylori*.

MATERIALS AND METHODS

Bacteria and culture conditions

Helicobacter pylori NCTC 11637 and NCTC 11638 were obtained from the National Collection of Type Cultures (London, UK), other strains of *H. pylori* used in the present study were isolated from gastric biopsy specimens taken from patients referred for gastroscopy to the Gastroenterology Unit of University College Hospital, Galway, Ireland. All media were dissolved in distilled water according to manufacturers' instructions and sterilized at 121°C and 15lb in^{-2} for 15 min. Media containing glycerol were sterilized at 115°C and 10lb in^{-2} for 20 min. Horse blood, serum and other sterile constituents were added to the sterile media when its temperature reached 45°C. The biopsy samples were transported to the laboratory in 1ml of a hypotonic solution containing 20% (v/v) glycerol (BDH) and processed immediately. Samples were ground in the transport medium using a sterile mortar and pestle, and inoculated onto plates of enrichment medium consisting of Columbia agar base (Oxoid CM331), 7% (v/v) defibrinated horse blood (Oxoid SR50), 1% (v/v) Isovitalex enrichment (BBL) and *Helicobacter pylori* selective supplement (DENT; Oxoid SR147E). Inoculated enrichment medium was incubated at 37°C in a microaerobic atmosphere containing 6% O_2, 10% CO_2 and 84% N_2; the atmosphere was created using disposable gas generating kits (Oxoid BR60) with a palladium catalyst (Oxoid BR42) in an anaerobic jar (BBL). Stock cultures were prepared and maintained at --70°C by harvesting growth from plates of enrichment medium into a semisolid medium consisting of brain heart infusion (Oxoid CM225), 0-25% (w/v) yeast extract (Oxoid 1,21), 10% (v/v) horse serum (Oxoid SR35) and 20% (v/v) glycerol and thereafter were grown routinely an blood agar (Columbia agar base containing 7% (v/v) defibrinated horse blood) at 37°C for 72h in a microaerobic atmosphere. After incubation, the purity of cultures was examined by using the modified Gram stain of Ogg (1962) and by determining the catalase, oxidase and urease activity as described previously (Smibert and Krieg 1981).

Growth in liquid media

Liquid media used in the investigations were based an a series of basal media: brain heart infusion (BHIB), Brucella broth (BB; Difco), Mueller-Hinton broth (MHB; Oxoid CM405) or tryptone soya broth (TSB; Oxoid CM129). These basal media were supplemented with 5% (v/v) horse serum (HS), 5% (v/v) fetal calf serum (FCS; Gibco), 7% (v/v) defibrinated horse blood (BL), 1% (w/v) yeast extract (YE), 2% (w/v) proteose peptone no. 3 (PP3; Difco), 0.1% (w/v) β-cyclodextrin (βCD; Sigma) or 0.1% (w/v) corn starch (CS; Sigma) as appropriate.

An inoculum was prepared by harvesting 3 d blood agar cultures into sterile saline and adjusting the optical density at 620nm to 0.033 using a Milton Roy Spectronic 1001 plus spectrophotometer; this suspension corresponded to an inoculum of about 10^2 colony-forming units (cfu) ml^{-1}, which was determined by plate counts. Briefly, bacteria were harvested into sterile phosphate-buffered saline pH 7-3 (PBS; Oxoid BR14a), dilutions of this bacterial suspension were prepared and inoculated (100µl) onto blood agar,

inoculated blood agar plates were incubated at 37°C for 72 h and subsequently colonies counted. It was assumed that each colony arose from a single cell. Broths (9ml) were inoculated with 1ml of this suspension and transferred into 25cm^2 tissue culture flasks (Falcon). Duplicate flasks were inoculated and incubated, with loosely fitting screw caps, at 37°C for 48h under microaerobic conditions. Following incubation, the purity of cultures was examined by using the modified Gram stain and by determining the catalase, oxidase and urease activity as above. Also, samples (10µl) were removed from the cultures and duplicate sets of blood agar media were inoculated and incubated at 37°C for 24h under aerobic conditions, to rule out contamination by aerobic micro-organisms.

Estimation of bacterial concentration

Serial dilutions of broth cultures were made in sterile PBS. Samples (0.1ml) of each dilution were plated in duplicate onto blood agar and colonies counted after 72h incubation at 37°C in a microaerobic atmosphere. The remainder of the broth cultures were centrifuged at 5000g for 20 min, duplicate pellets were combined and stored at -20°C until analysis by SDS-PAGE.

Growth an solid media

Solid basal media, to which supplements were added were: brain heart infusion agar (BHIA; Oxoid CM375), Brucella agar (BA; Difco), tryptone soya agar (TSA; Oxoid CM131) or Mueller-Hinton agar (MHA; Oxoid CM337). An inoculum was prepared as described for liquid media and duplicate sets of media were inoculated with the bacterial suspension (0.1ml). Inoculated media were incubated for 37°C for 72h under microaerobic conditions. After incubation, 1ml of sterile saline was added to each plate and bacterial growth harvested, serial dilutions of this bacterial suspension were prepared and cfus determined as above. The remainder of the bacterial suspensions were centrifuged at 10000g for 15min and stored at -20°C until analysis by SDS-PAGE.

Analysis of antigen expression

Whole-cell (WC) and Proteinase K-treated whole-cell (PKWC) extracts of *H. pylori* strains were prepared as described by Hitchcock and Brown (1983). Samples were analysed by SDS-PAGE with the discontinuous buffer system described by Laemmli (1970). For the analysis of LPS in PKWC extracts, a 15% polyacrylamide separating gel containing urea (BDH), and a 5% polyacrylamide stacking gel were used. Electrophoretic analysis of proteins in WC extracts was performed with a separating gel containing 12% polyacrylamide and a 4% polyacrylamide stacking gel. Electrophoresis was conducted with a constant current of 35mA for 1h. After SDS-PAGE, gels were fixed and LPS was detected by silver staining as described by Tsai and Frasch (1982). For analysis of proteins, electrophoretic gels of WC extracts were stained in 0-2°16 (w/v) Coomassie brilliant blue (BioRad) for 30 min and destained overnight.

RESULTS

Evaluation of solid media

Unsupplemented solid basal media did not support growth of either *H. pylori* NCTC 11637 or 11638. Upon addition of blood to basal media, both strains yielded 10^6-10^7 cfu ml^{-1} after 72h incubation (Fig. 1). Optimal growth of NCTC 11637 was achieved an TSA containing blood, whereas MHA with blood supported optimal growth of NCTC 11638. Supplemented solid media containing HS, FCS, YE, PP3, βCD or CS supported growth to a small extent but this was too small to be quantified (less than 20 cfu per plate; data not shown).

Evaluation of liquid media

All the liquid basal media investigated including BB supported growth of *H. pylori* NCTC 11637 and NCTC

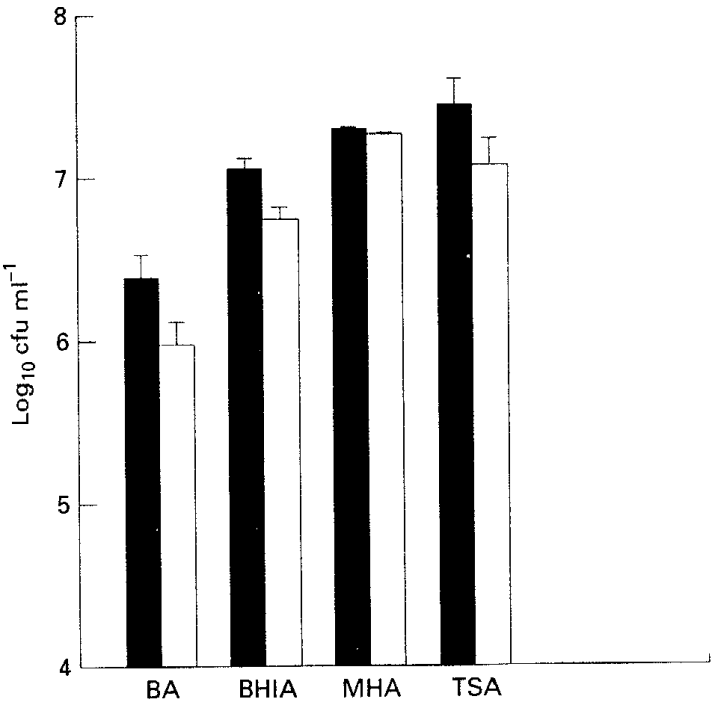

Fig. 1 Growth of *Helicobacter pylori* NCTC 11637 (■) and NCTC 11638 (□) on solid basal media supplemented with 7% (v/v) horse blood; BA, Brucella agar; BHIA, brain heart infusion agar; MHA, Mueller–Hinton agar; TSA, tryptone soya agar. Results are presented as mean values of two separate experiments; error bars represent standard deviation

11638 (Fig. 2). The best basal broth medium for growth of both strains was TSB; BB was the poorest. When blood was added as a single supplement (Fig. 3) or in combination with other supplements to basal broths (Table 1) optimal growth of both NCTC 11637 and NCTC 11638 was achieved. A medium of MHB with blood was the best liquid medium for growth of both NCTC 11637 and NCTC 11638 (Fig.3). Growth of each strain was optimal once the basal media contained blood and was independent of further supplementation. Thus, optimal growth of the *H. pylori* strains was not achieved in a blood-free environment (Table 1).

In general, addition of a single supplement other than blood to basal broths improved the growth of *H. pylori* NCTC 11637 and NCTC 11638 compared with basal media alone (Figs 2 and 3), but there was a difference in response of the strains to the different supplements. As shown in Table 1, addition of another supplement to a broth which contained HS increased growth of both NCTC 11637 and NCTC 11638. Similarly, addition of an extra supplement to a basal broth containing FCS increased growth. However, the growth of strains varied when another supplement was added to a basal broth containing two supplements, one of which was HS or FCS; in some cases an improvement in growth occurred whereas in others growth was diminished (Table 1). Furthermore, throughout the experiments supplementation with HS was, in general, found to be superior in supporting growth of both *H. pylori* strains than supplementation with FCS (Fig.3 and Table 1).

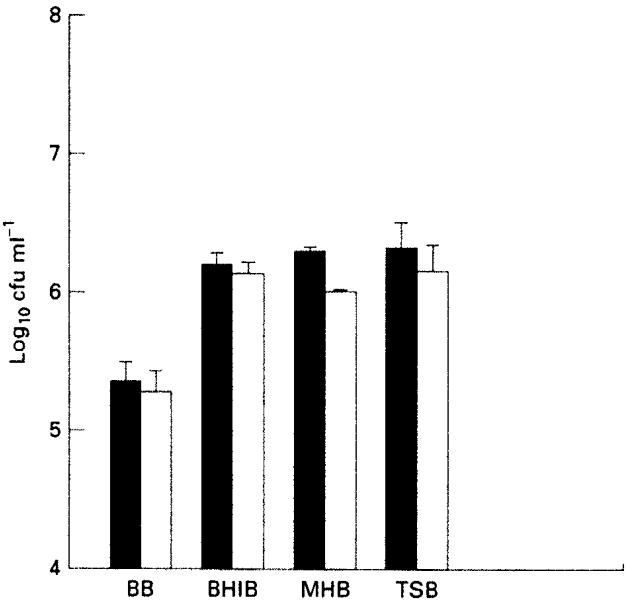

Fig. 2 Growth of *Helicobacter pylori* NCTC 11637 (■) and NCTC 11638 (□) in basal liquid media without supplementation; BB, Brucella broth; BHIB, brain heart infusion broth; MHB, Mueller–Hinton broth; TSB, tryptone soya broth. Results are presented as mean values of two separate experiments; error bars represent standard deviation

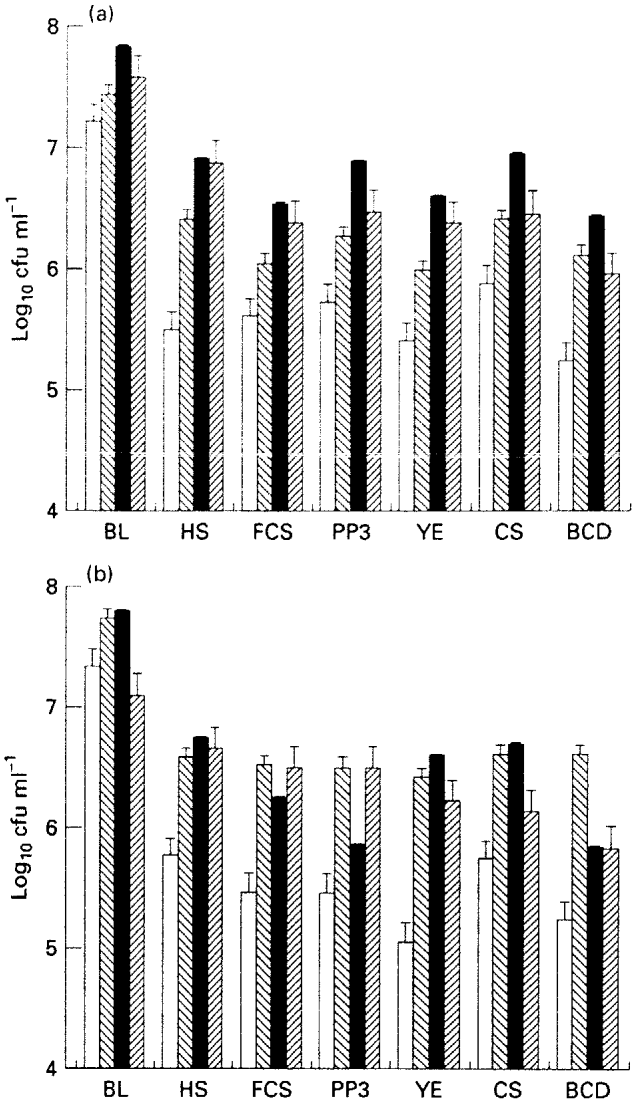

Fig. 3 Growth of (a) *Helicobacter pylori* NCTC 11637 and (b) NCTC 11638 in basal liquid media; □, Brucella broth; ▨, brain heart infusion broth; ■, Mueller–Hinton broth; ▨, tryptone soya broth. One supplement was added to each basal medium, either 7% horse blood (BL), 5% horse serum (HS), 5% fetal calf serum (FCS), 2% proteose peptone no. 3 (PP3), 1% yeast extract (YE), 0·1% corn starch and 0·1% β-cyclodextrin (βCD). Results are presented as mean values of two separate experiments; error bars represent standard deviation

Increased growth was observed when CS with one or two other supplements (Table 1). Similarly, addition of βCD with other supplements increased growth of NCTC 11637 and NCTC 11638 (Table 1), but when βCD was added as a single supplement to MHB or TSB, it inhibited growth of both strains (Figs. 2 and 3). MHB or TSB containing HS and, in addition, YE or PP3 yielded optimal growth of these H. pylori strains

Table 1: Ability of supplemented basal liquid media to support growth of *Helicobacter pylori*

	Colony count (log_{10}cfu ml^{-1}) of *H. pylori* strains after incubation in:							
Basal broths*	BB		BHI		MHB		TSB	
H.pylori strains†	37	38	37	38	37	38	37	38
Supplements added‡								
None§	5.4	5.3	6.2	6.1	6.3	6.1	6.3	6.0
BL+HS	7.4	7.3	7.5	7.7	7.9	7.6	7.1	6.9
BL+FCS	7.2	7.3	7.4	8.0	7.8	7.6	6.4	7.2
BL+βCD	7.3	7.4	7.4	7.9	7.8	7.6	7.1	6.8
BL+PP3	7.0	7.3	7.3	7.7	8.0	7.8	7.7	7.7
BL+YE	7.1	6.7	7.4	7.8	8.0	7.9	7.5	7.8
BL+CS	7.3	7.3	7.5	7.9	7.9	7.8	7.5	7.7
HS+βCD	5.8	6.3	6.5	6.7	7.0	6.7	6.8	6.8
HS+PP3 II	5.7	5.9	6.4	6.7	6.9	6.8	6.9	6.9
HS+YE II	5.6	6.2	6.4	6.8	7.0	6.8	6.9	6.8
HS+CS	6.0	6.1	6.4	6.6	7.1	6.9	6.8	6.8
FCS+βCD	5.7	5.6	6.3	6.6	6.6	6.3	6.4	6.4
FCS+PP3	5.7	5.9	6.1	6.8	6.4	6.3	6.7	6.7
FCS+YE	5.5	5.6	6.5	6.7	6.8	6.3	6.7	6.7
FCS+CS	6.0	6.0	6.5	6.8	7.0	6.6	6.9	7.0
HS+βCD+FCS	5.6	5.8	6.5	6.5	6.7	6.7	7.0	6.2
HS+βCD+PP3	5.7	5.7	6.4	6.4	6.9	6.8	6.8	6.8
HS+βCD+YE	5.6	5.6	6.4	6.4	6.8	6.8	7.3	6.8
HS+βCD+CS	6.1	6.2	6.9	6.9	7.1	7.2	7.3	7.4
HS+PP3+FCS	5.7	5.7	6.5	6.4	6.5	6.8	7.2	6.4
HS+PP3+YE	5.4	5.4	6.4	6.5	6.9	6.9	6.8	6.9
HS+PP3++CS	6.2	6.2	6.9	6.9	7.2	7.2	7.3	7.3
FCS+βCD+PP3	5.6	5.7	6.4	6.4	6.6	6.8	6.8	6.9
FCS+βCD+YE	5.6	5.7	6.4	6.4	6.5	6.4	6.5	6.4
FCS+βCD++CS	6.0	6.1	6.7	6.7	6.8	6.8	7.1	7.1
FCS+PP3+YE	5.7	5.7	6.4	6.5	6.7	6.6	6.6	6.7
FCS+PP3+CS	5.7	5.8	6.6	6.6	6.7	6.8	7.0	7.0
HS+FCS+YE	5.7	5.5	6.4	6.4	6.8	6.8	7.0	6.8
HS+FCS+CS	6.1	6.1	6.8	6.8	6.9	6.9	7.2	7.2
HS+CS+YE	6.2	6.2	6.9	6.9	7.2	7.2	7.3	7.3
FCS+CS+YE	5.8	5.8	6.6	6.6	6.7	6.7	7.0	7.0
PP3+CS+YE	6.2	6.2	6.9	6.9	7.1	7.1	7.3	7.3
βCD+CS+YE	6.1	6.1	6.9	6.9	7.1	7.1	7.3	7.3
βCD+PP3+YE	5.7	5.7	6.4	6.4	6.6	6.6	6.6	6.6
βCD+CS+PP3	6.2	6.1	6.9	6.9	7.2	7.1	7.4	7.3

* Basal broths: BB, Brucella broth; BHI, brain heart infusion broth; MHB, Mueller-Hinton broth; TSB, tryptone soya broth.

†Helicobacter pylori strains: 37, *H. pylori* NCTC 11637; 38, *H. pylori* NCTC 11638.

‡Supplements added: BL. 7% (v/v) horse blood; HS, 5% (v/v) horse serum; FCS, 5% (v/v) fetal calf serum; β-cyclodextrin; PP3, 2% (w/v) proteose peptone no. 3; YE, 1% (w/v) yeast extract; CS, 0,1% (w/v) corn starch

§ These values of growth of *H. pylori* in basal media without supplementation have been presented in Fig. 2, they are included here for comparison only.

II Simplest media for growth of *H. pylori* in a blood free environment.

in a blood-free environment. Comparable results were obtained with *H. pylori* clinical isolates grown an solid and in liquid media of the same composition although some variations in growth between the strains occurred (data not shown). Plates which were incubated aerobically did not yield growth of any micro-organisms.

Evaluation of antigen expression in solid and liquid media

SDS-PAGE was performed an WC and PKWC extracts of *H. pylori* strains grown in different media to examine the influence of medium composition an antigen expression in *H. pylori*. *Helicobacter pylori* NCTC 11637 and NCTC 11638 grown on solid media containing blood or other supplements expressed R-LPS and representative results are shown in Fig. 4. Previously it has been shown that fresh clinical isolates express S-LPS and upon numerous in vitro passages an solid media, R-LPS is produced (Moran et al. 1992). A similar R-LPS profile was seen with clinical isolates after numerous in vitro passages. Figure 5 is representative of that seen with the type strain and with clinical isolates after numerous in vitro passages; however, growth of *H. pylori* in liquid basal media was sufficient to obtain expression of the O side chain in LPS and various supplements added to the broths did not inhibit O side chain production (Fig.6). There was no change in protein expression when various single and combinations of supplements were added to basal liquid media (data not shown). However, comparison of the protein profiles of *H. pylori* NCTC 11637 when grown on solid and in liquid media demonstrated a change in expression of proteins, in particular, proteins of mol.wt 57 and 60 kDa. When *H. pylori* NCTC 11637 was grown on solid media, there was a higher expression of 57kDa protein than of 60kDa protein; the

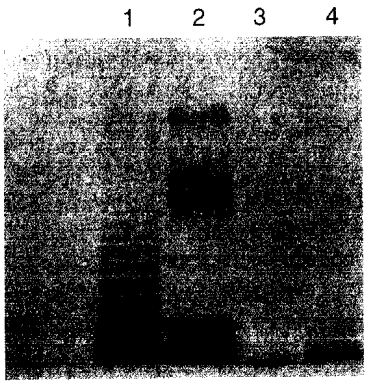

Fig. 4 Silver-stained SDS-PAGE gel of phenol–water-extracted lipopolysaccharide from *Salmonella enterica* serovar *typhimurium* SH2183 (lane 1), *Helicobacter pylori* NCTC 11637 grown in brain heart infusion broth containing 2% fetal calf serum (lane 2), *H. pylori* NCTC 11637 grown on blood agar (lane 3) and *Salm. enterica* serovar *typhimurium* SH9013 (lane 4)

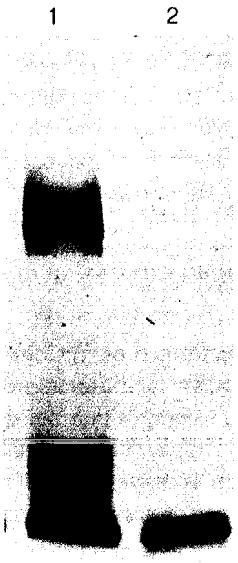

Fig. 5 Silver-stained SDS-PAGE gel of Proteinase K-treated whole-cell lysates of a *Helicobacter pylori* clinical isolate before (lane 1) and after (lane 2) numerous *in vitro* passages on blood agar

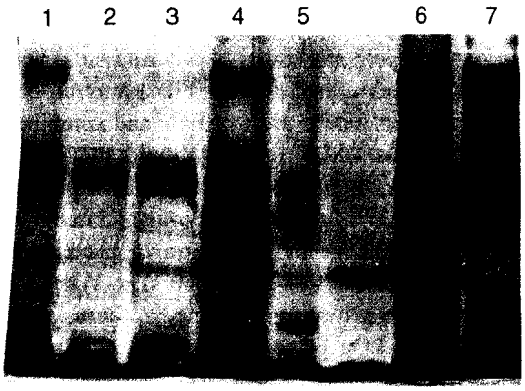

Fig. 6 Silver-stained SDS-PAGE gel of Proteinase K-treated whole-cell lysates of *Helicobacter pylori* NCTC 11637 grown in tryptone soya broth (TSB) with and without supplements added (lanes 1–6). Lanes: 1, TSB; 2, TSB + 0·1% β-cyclodextrin; 3, TSB + 2% proteose peptone no. 3; 4, TSB + 1% yeast extract; 5, TSB + 0·1% corn starch; 6, TSB + 5% fetal calf serum; 7, phenol–water-extracted lipopolysaccharide from *H. pylori* NCTC 11637 grown in brain heart infusion broth containing 2% fetal calf serum

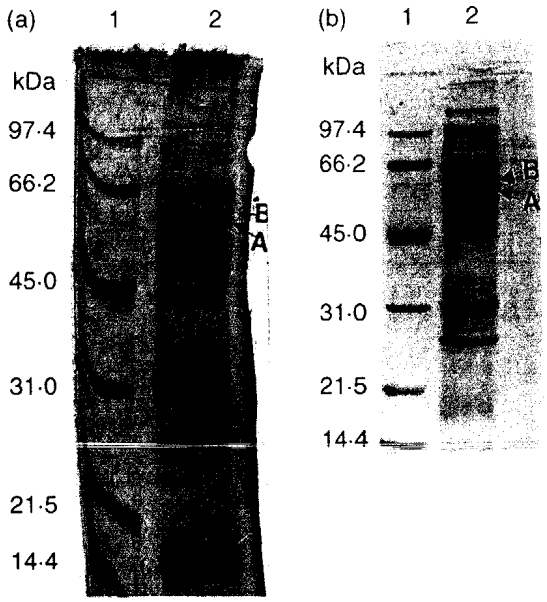

Fig. 7 Coomassie blue-stained SDS-PAGE gel of whole-cell extracts of *Helicobacter pylori* NCTC 11637. (a) Lanes: 1, low-molecular weight markers; 2, *H. pylori* grown on blood agar. (b) Lanes: 1, low-molecular weight markers; 2, *H. pylori* grown in tryptone soya broth containing 5% horse serum and 1% yeast extract. Arrows A and B indicate proteins of molecular weight 57 and 60 kDa, respectively

reverse occurred when *H. pylori* was grown in liquid media (Fig. 7).

DISCUSSION

Previous studies an the nutritional requirements of *H. pylori* have focused mainly an the growth of this bacterium in large and Small volumes of liquid media (Buck and Smith 1987; Morgan et al. 1987; Secker et al. 1991; Shahamat et al. 1991; Oliven et al. 1993; Xia et al. 1993x; Morshed et al. 1994) and on the influence of different solid media compositions on growth (Goodwin et al. 198; Buck and Smith 1987; Hazell et al. 1989; Cellini et al. 1992; Oliveri et al. 1993) or on the survival of *H. pylori* in different transport systems (Soltesz, et al. 1992; Xia et al. 19936, 1994). To date, no intensive study has examined the influence of media composition and supplementation on the production of LPS by *H. pylori*. Supplements selected and their quantities used were in general those previously described in the literature. *Helicobacter pylori* strains NCTC 11637 and NCTC: 11638 were chosen because they are well characterized strains. Using these strains, direct comparisons to previously published data could be made. However, it has been suggested that fresh clinical isolates are more fastidious than laboratory-adapted or culture collection strains (Hazell et al. 1989) and therefore the influence of culture conditions on fresh clinical isolates was examined also.

In basal liquid media growth of *H. pylori* NCTC 11637 and NCTC 11638 occurred in all four unsupplemented broths including BB. This is in contrast to reports which did not obtain growth in BB alone (Guck and Smith 1987; Morgan et al. 1987; Shahamat et al. 1991). However, this broth yielded the poorest growth of *H. pylori* in the present study and throughout the investigations BB and supplemented BB did not perform as well as the other media used.

Broths containing blood alone or containing blood with another supplement gave optimal growth of both *H. pylori* NCTC 11637 and NCTC 11638 (Fig.3 and Table l). Nevertheless, unsupplemented basal media supported the growth of NCTC 11637 and NCTC 11638, but solid basal media of similar composition to the liquid media did not. The failure of *H. pylori* to grow in unsupplemented solid basal media was reported by Buck and Smith (1987), Olivieri et al. (1993) and Coudron and Stratton (1995). However, Buck and Smith (1987) obtained slight growth an unsupplemented MHA. Thus, fluid conditions favoured the growth of the strains. When supplements were added to solid basal media, supplementation with blood as in broths gave optimal growth of *H. pylori*. In this study 7% horse blood was added to the basal media. Coudron and Stratton (1995) reported that the source of the blood was critical for growth of *H. pylori*, and in particular, that sheep blood, laked or whole blood inhibited the growth of the bacterium in liquid media. The results of the present study indicate that whole horse blood supports growth of *H. pylori* in both solid and liquid media; nevertheless, differences were observed in the ability of strains to grow in blood supplemented solid media compared to blood supplemented liquid media in agreement with previous reports (Goodwin et al. 1986; Queiroz el al. 1987; Ansorg et al. 1991).

In this investigation, a low inoculum of 102 cfu ml^{-1} was chosen resembling the low numbers of *H. pylori* present in gastric biopsies and the patchy distribution of the organism in the stomach (Goodwin et al. 1985). An incubation period of 48h, previously reported to achieve maximum growth of *H. pylori* strains with intermediate inoculum levels of 10^2-10^4cfu ml^{-1} (Morgan et al. 1987), was selected. It also avoided the generation of coccoid forms, a degenerate, non-viable form of *H. pylori* which begin to appear after 2-3 d incubation (Eaton et al. 1995). In this study an liquid media, 50ml of tissue culture flasks were used, which contained 10ml of broth with loose-fitting caps, thereby allowing diffusion of the microaerobic gaseous atmosphere into the culture medium and eliminating the need for gyration.

Solid and liquid media containing cyclodextrin have been reported to support the growth of *H. pylori* and may be used with selective supplements for isolating the bacterium (Olivieri et al. 1993). Another report from the same group described conditions for the optimized production of the vacuolation cytotoxin of *H. pylori* in such media (Marchini et al. 1994). In the latter studies BB was supplemented with (2,6-di-0-methyl)-β-cyclodextrin (dMeβCD) and not βCD as used in this investigation, and thus direct comparisons cannot be made. The results of the present investigation show an inhibition of growth of *H. pylori* in some supplemented broths containing βCD, as well as differences in the ability of *H. pylori* NCTC 11637 and NCTC 11638 to grow in βCD-supplemented media. Similarly, Morshed et al. (1994) reported differences in the ability of different strains of H. pylori to grow in BB supplemented with βCD alone. Nevertheless, the source and type of cyclodextrin may influence growth of *H. pylori* strains (Marchini et al. 1995).

Although addition of blood to solid and liquid media gave optimal growth of *H. pylori*, blood-containing broths are difficult to work with and cultivation in a blood-free environment would be desirable. Shahamat et al. (1991) found that either fetal calf serum or horse serum could be used to induce good growth of *H. pylori* strains. In these studies we found horse serum to be superior to fetal calf serum and supplementation with horse serum yielded growth secondary to that with blood (Fig. 3). Addition of an extra supplement to a basal broth containing horse serum improved growth further, but additions of a third supplement caused a decline in growth. In particular, TSB containing horse serum and PP3 yielded good growth of both NCTC 11637 and NCTC 11638 (Table 1). Also, NCTC 11637 grew well in MHB plus horse serum and yeast extract. Therefore, either TSB or MHB containing 5% horse serum and a nitrogen source such as PP3 or YE may provide optimal growth of *H. pylori* in a blood-free environment. Similar results were obtained with the clinical isolates studied.

In addition to examining the importance of the various media in supporting *H.* pylori growth, we also investigated the ability of the different media at inducing and maintaining S-LPS in *H. pylori*. Culture collection strains that were subcultured many times produced R-LPS when grown an solid media but when the same strains are grown under liquid conditions, S-LPS was produced (Fig.4) in agreement with our previous results (Moran et al. 1992; Moran and Walsh 1993x). When isolated from gastric biopsies, clinical isolates produced S-LPS (Moran el al. 1992) and maintained their O side chain when grown in liquid media. In the present investigation, however, when the isolates were grown an solid media, loss of O side chain occurred and R-LPS was produced (Fig. 5), consistent with our previous results (Moran et al. 1992; Moran and Walsh 1993a,b). This phase shift was reversed when the strains were grown in liquid media. Therefore, growing *H. pylori* under liquid conditions, mimicking *in vivo* conditions was sufficient for O side chain production and was found to be independent of media composition (Fig. 6). Protein antigen expression was also investigated and found not to be affected by supplementation.

These findings have major implications for the development of a serotyping system for *H. pylori* based on LPS. From these results it is apparent that clinical

isolates should be grown in liquid media to maintain O side chain production to be typeable in such a serotyping scheme. Furthermore, *H. pylori* LPS is an inducer of a range of immunological mediators (Moran 1995x, 1996) and is capable of inducing pepsinogen secretion which plays an important role in the pathogenesis of *H. pylori* (Moran 1996). The consequence of this study therefore allows optimal growth of the bacterium in vitro, as well as expression of intact LPS as *in vivo* to be maintained.

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