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Bisulfite or Sulfite Inhibits Growth of *Helicobacter pylori*

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Bisulfite or sulfite was found to be inhibitory to *Helicobacter pylori* growth. A modified version of Brucella broth (BB), bisulfite-less BB (BLBB), supported rapid, robust, and consistent growth of *H. pylori*. We suggest that BLBB simply be called "Pylori broth" for distinction from Brucella broth.

Helicobacter pylori, a gram-negative, spiral bacterium, has been implicated as the etiological agent for gastritis and as a major contributing factor in the development of peptic gastroduodenal ulcers (2, 5, 8, 9, 12, 13). It has also been linked to an increased risk of gastric cancer (3). Since its first isolation in 1983 (9, 17), *H. pylori* has generally been described as an oxygen-susceptible, fastidious microaerophile (1, 10, 11, 14–16). Growth media and incubation conditions that provide an environment of reduced oxygen tension have commonly been used for culturing *H. pylori*. Brucella broth (BB) is one of the frequently used media (10, 11, 14–16). The medium consists of rich carbon and nitrogen sources, such as tryptone, peptamin, yeast extract, and glucose, and two salts, sodium chloride and sodium bisulfite. Bisulfite is thought to be useful in reducing oxygen tension in the medium. In our earlier experience with BB, growth of *H. pylori* was consistently more robust in older medium which had been stored in the refrigerator than in freshly prepared medium. Since bisulfite (HSO_3^{-1}) and sulfite (SO_3^{-2}) can gradually be oxidized to sulfate (SO_4^{-2}) on exposure to air, we suspected that the slow oxidation of bisulfite or sulfite to sulfate during medium storage might have been responsible for these differences, although this hypothesis did not seem to fit with the theory of oxygen susceptibility (i.e., preference or requirement of the microaerophile for a reduced oxygen environment provided, or encouraged, by the presence of bisulfite or sulfite). We report here that bisulfite or sulfite in the medium is not beneficial and actually inhibits the growth of *H. pylori*. A modified BB, bisulfite-less BB (BLBB), supported more rapid, robust, and consistent growth of *H. pylori*. Use of BLBB in place of the standard BB should facilitate the isolation of *H. pylori* from clinical specimens and the cultivation of large quantities of the organisms for research purpose.

MATERIALS AND METHODS

The following *H. pylori* strains were used in the study: UMAB41, a clinical isolate obtained from H. L. T. Mobley (7), and the type strain (ATCC 43504), NCTC 11637, obtained from the American Type Culture Collection (4). Stock cultures were prepared in BB with 10% serum and were stored at −80°C in 10% glycerol. The following media and medium components were used: BB (Difco), tryptone (Difco), peptamin (Difco), glucose (Sigma Cell Culture), sodium chloride (Sigma Cell Culture), sodium bisulfite (Baker), sodium sulfite (Baker), sodium sulfate (Baker), and fetal bovine serum (catalog no. A-1111-L; Hyclone).

BB was prepared by following the manufacturer's instructions and was sterilized either by autoclaving at 121°C for 30 min or by filtration through a 0.22-μm-pore-size nylon filter (150-ml filter system; Corning). In some experiments we elected to use the filter sterilization method in order to minimize the oxidation of bisulfite or sulfite, which could occur following autoclaving. BLBB was prepared by dissolving 10 g of tryptone, 10 g of peptamin, 1 g of glucose, 2 g of yeast extract, and 5 g of sodium chloride in 1 liter of deionized water. Sodium bisulfite, sulfite, and sulfate were prepared as 200× stock solutions and were added individually to BLBB to give a final concentration of 0.96 mM (BB normally contains 0.96 mM sodium bisulfite). Fetal bovine serum (10% [vol/vol]) was added to all media unless noted otherwise. An inoculum was prepared by using a 2-ml frozen stock culture to inoculate a 250-ml flask containing 50 ml of BB or BLBB. The inoculum was grown for 15 to 25 h to an optical density (OD) of 0.4 to 0.9 and was used to inoculate flasks with an inoculation ratio of

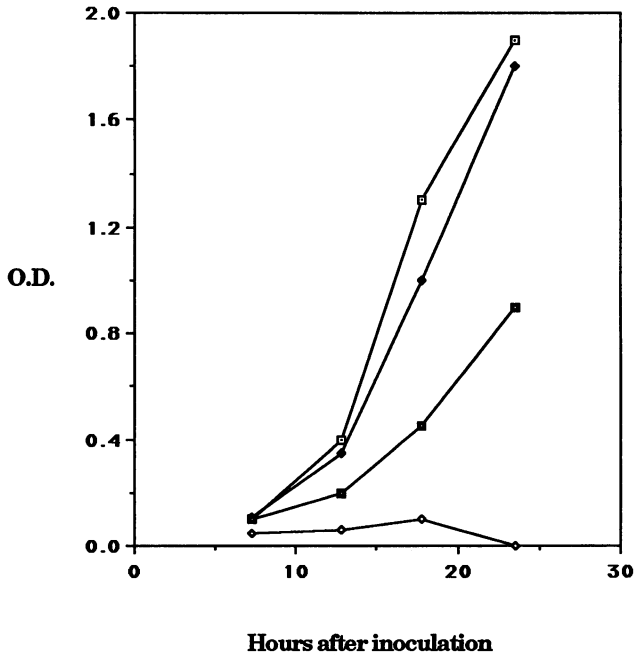

FIG. 1. Growth of *H. pylori* UMAB41 in filtered BLBB (⊡), autoclaved BLBB (◆), autoclaved BB (■), and filtered BB (◇).

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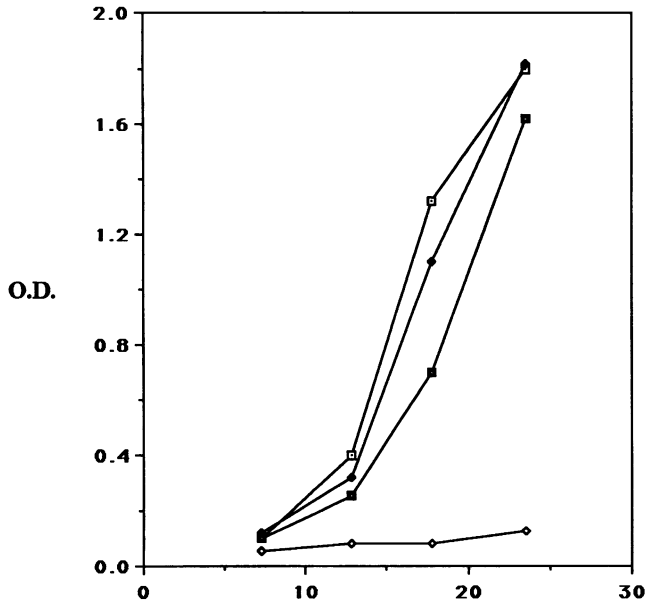

FIG. 2. Growth of *H. pylori* UMAB41 in filtered BLBB plus sulfate (⊡), autoclaved BLBB plus sulfate (◆), autoclaved BLBB plus sulfite (◘), and filtered BLBB plus sulfite (◇).

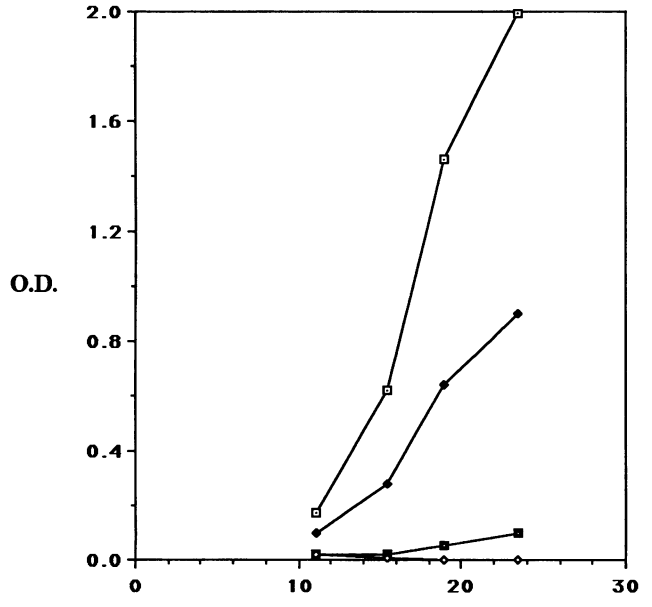

FIG. 3. Growth of *H. pylori* UMAB41 in BLBB plus serum (⊡), BLBB without serum (◆), BLBB plus sulfite plus serum (◘), and BLBB plus sulfite but without serum (◇). All media were filter sterilized.

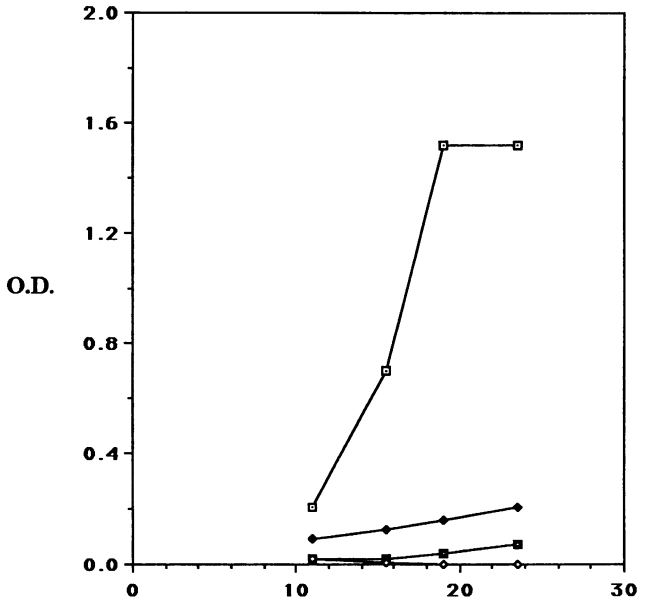

FIG. 4. Growth of *H. pylori* NCTC 11637 in BLBB plus serum (⊡), BLBB without serum (◆), BLBB plus sulfite (◘), and BLBB plus sulfite but without serum (◇). All media were filter sterilized.

1 to 2% (vol/vol) in order to achieve a calculated after-inoculation OD of about 0.01. Smooth flasks (250 ml; Corning) were used, and each flask contained 50 ml of medium. The flasks were incubated in a tissue culture incubator at 37°C with 10% carbon dioxide on a platform shaker (Innova 2100; New Brunswick Scientific) at a speed of 150 rpm (3/4-in. [1.9-cm] throw). Cell growth was monitored by measuring the OD at 550 nm.

RESULTS AND DISCUSSION

When growth of *H. pylori* UMAB41 in filtered BB was compared with that in either autoclaved or filtered BLBB (Fig. 1), it was apparent that bisulfite in BB was inhibitory to cell growth. Since there was some, although limited, growth in autoclaved BB, we believe that some of the bisulfite in BB must have been removed, probably oxidized to sulfate, after being autoclaved. To test this hypothesis further, we examined the growth of *H. pylori* in autoclaved or filter-sterilized BLBB with added sulfite or sulfate. The inhibitory effect of sulfite was apparent (Fig. 2), since cell growth was completely arrested in filter-sterilized BLBB to which sulfite was added. When the same medium was autoclaved, the inhibitory effect of sulfite lessened. Again, this effect was presumably due to the removal, such as through oxidation, of sulfite to sulfate following autoclaving. When sulfate was added to BLBB, it had no effect on growth whether the medium was autoclaved or filtered sterilized; cell growth in BLBB plus sulfate was essentially the same as that in BLBB.

Once the inhibitory effects of bisulfite and sulfite were established, we then examined the growth of *H. pylori* in filter-sterilized BLBB without the addition of serum. It was interesting that *H. pylori* grew relatively well in BLBB without

serum (Fig. 3). When sulfite was added to BLBB, there was, as expected, little or no growth whether or not serum was added. A similar inhibitory effect of sulfite was also demonstrated with the American Type Culture Collection type strain, NCTC 11637, as shown in Fig. 4.

It is not clear how bisulfite or sulfite inhibits the growth of *H. pylori*. It could affect the redox potential of the medium. It is also possible that bisulfite or sulfite might act indirectly by removing, through reduction, some essential growth factor(s), such as unsaturated fatty acids (6), from the medium.

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