JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1989, p. 2118–2119
0095-1137/89/092118-02$02.00/0

Vol. 27, No. 9

Rapid Method for the Detection of DNase of Campylobacters

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Received 29 March 1989/Accepted 5 June 1989

A rapid agar diffusion method for the detection of DNase production of *Campylobacter jejuni*, *C. coli*, and *C. pylori* was developed. A strong pink zone indicating DNA hydrolysis was seen around the wells after 20 to 24 h of aerobic incubation at 37°C. Pretreatment of cells with polymyxin B, which releases the cell-associated DNase, both shortened the time needed to read positive results to 8 h and increased the zone size.

One of the tests used for the biotyping of *Campylobacter jejuni*, *C. coli*, and *C. laridis* is the production of DNA hydrolase. In the biotyping systems for thermophilic campylobacters developed by Hébert et al. (2), Lior (6), and Roop et al. (9), the production of DNase is included. Roop et al. (9) used brucella agar with FPB ($FeSO_4 \cdot 7H_2O$, sodium pyruvate, sodium metabisulfite) supplement and methyl green as an indicator for DNA hydrolysis. Hébert et al. (2) used DNase test agar (Difco Laboratories, Detroit, Mich.) supplemented with 0.005% methyl green, while Lior (6) used methyl green at 0.007%. These studies produced some inconclusive results. Recently, Lior and Patel (7) described a toluidine blue-DNA agar used to detect staphylococcal thermonuclease (4) for the detection of DNA hydrolysis of thermophilic campylobacters and *C. pylori*. In these studies, Lior (6) showed that methyl green is toxic for at least some *Campylobacter* strains. Toluidine blue-DNA agar gave a greater number of positive results and required a shorter incubation time than did the medium containing methyl green. It is also known that methyl green methods require a highly purified DNA substrate (3).

In the present study, production of DNase was measured by using DNase agar (Difco) with 0.01% toluidine blue 0 (E. Merck AG, Darmstadt, Federal Republic of Germany) and by the well agar diffusion method developed by Lachica et al. (4). The sensitivity of the well agar diffusion method was improved by incubating *Campylobacter* cells with polymyxin B (Sigma Chemical Co., St. Louis, Mo.) before inoculating the cell suspension into a well in the DNase test plate. Polymyxin B, a polycationic antibiotic, has been used to release the cell-associated secretory proteins, such as enterotoxins (1), protease, alkaline phosphatase, and DNase of *Vibrio cholerae* (10). DNase production by the well agar diffusion method was tested at pH 7.3 and 9.0. The pH of the media was adjusted with 0.05 M Tris buffer (Sigma). The unautoclaved medium was poured into petri dishes (10 ml per dish), and wells of about 0.5 mm in diameter were cut. These plates could be stored for several weeks in a refrigerated place if protected from desiccation.

In the DNase production studies, 81 *C. jejuni* and *C. coli* strains and 7 *C. pylori* strains were used. A turbid suspension of cells grown either on brucella blood agar plates (*C. jejuni* and *C. coli*) or on BHI (brain heart infusion agar; Difco) blood agar plates (*C. pylori*) was made in Tris buffer (pH 7.3 and 9.0). Two wells in each medium at pH 7.3 or 9.0 were filled with the bacterial suspension of each strain. To encourage the leakage of the cell-bound DNase, 50 μl of polymyxin B (2 mg/ml) was added to 0.5 ml of the bacterial suspension, which was kept at 4°C for 15 min. After this time, another two wells on each plate were filled with the polymyxin B-treated cells. The control wells contained Tris buffer with polymyxin B. Plates were incubated aerobically at 37°C and examined after 4, 8, 20, and 40 h of incubation. A strong pink zone with distinctive edges around the wells was considered a positive result, and colorless or lightly pinkish, narrow zones with irregular edges were considered negative. In the conventional DNase production testing method, a circular area about 1 cm in diameter was inoculated heavily with organisms. The plates were incubated microaerophilically at 37°C for 3 days. A pink zone around the growth was considered a positive result.

DNase was produced by 32 of the *C. jejuni* and *C. coli* strains and by all of the *C. pylori* strains. There was 100% agreement between the conventional and well agar diffusion methods. However, the well agar diffusion method was more rapid than the conventional DNase test, and no specific incubation conditions were needed. Initial positive results with the well agar diffusion method were visible after approximately 20 h of incubation, while the conventional cultivation method needed 48 to 72 h. The agar diffusion method offered a more reliable method for DNase detection in *C. pylori* strains, which did not grow well in the DNase test medium. In the well agar diffusion method, pretreatment of *C. jejuni* and *C. coli* cells with polymyxin B shortened the detection time for visible results to 8 h. These results were confirmed after 20 to 24 h of incubation. After 20 h of incubation, the pink zones around the wells with polymyxin B-treated cells were larger than those around wells with untreated cells. Polymyxin B pretreatment did not increase the width of the pink zones in the plates with *C. pylori*. These preliminary results suggested the presence of a cell-associated DNase in addition to the extracellular DNase in *C. jejuni* and *C. coli*. On the other hand, DNase of *C. pylori* was found only in the extracellular space, because polymyxin B pretreatment did not increase the width of the pink zone around the well. The DNase detection of *C. jejuni* and *C. coli* was better at pH 7.3 than at pH 9.0, but there were no differences for *C. pylori*.

The present well agar diffusion method showed that DNase of *Campylobacter* species can be detected without cultivating the cells. The effect of the growth phase on the production of DNase in a broth culture was also studied. DNase production was determined during the growth of five DNase-producing strains and one DNase-nonproducing *C. jejuni* strain in a biphasic medium which contained brucella agar with FBP supplement and brucella broth with FBP supplement (8). The growth medium was inoculated with approximately 10^5 cells per 50 ml of broth and incubated

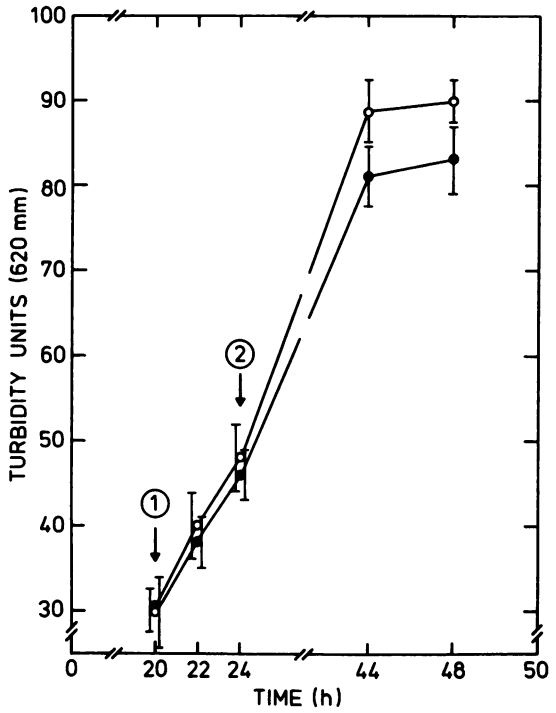

FIG. 1. Turbidity and DNase production of *C. jejuni* KH (○) and L69 (●) in the biphasic medium containing brucella agar with FBP and brucella broth with FBP. The arrows indicate the time when DNase was first detected in cells treated with polymyxin B (1) or in cells without polymyxin B treatment (2).

with agitation at 37°C. The turbidity (620 nm; Klett-Summerson; Klett Mfg. Co., New York, N.Y.), DNase excreted in the broth, and DNase associated with the cells were assessed during the growth. Cells from 8 ml of broth were collected by centrifugation and suspended in 0.4 ml of 0.05 M Tris buffer (pH 7.3). DNase production was determined from cells with and without polymyxin B as described above. The growth and DNase production of two *C. jejuni* strains are shown in Fig. 1. The DNase production was first detected in the cells incubated for about 20 h. The supernatant was never positive in the DNase test during the 48-h incubation period. This probably was due to the insensitivity of the method used to detect small amounts of DNase excreted by *Campylobacter* strains into broth culture. After 48 h of incubation, *Campylobacter* counts were 10^8 to 10^9 cells per ml. Lachica et al. (5) noted that at least 10^6 *Staphylococcus aureus* cells per ml were needed for the detection of thermonuclease. It was evident from the present study that *Campylobacter* species are poor producers of DNase. Thus, a heavy suspension of bacteria was needed. During the development of this method, *S. aureus*, *Serratia marcescens*, and *Aeromonas hydrophila*, which were used as positive controls, produced much wider pink zones around the wells than *Campylobacter* species.

In the present study, a rapid DNase detection method for *Campylobacter* species was developed. This method detected both extracellular and cell-associated DNase. For the testing of unknown strains of *C. jejuni* and *C. coli*, the well agar diffusion method at pH 7.3 is recommended. A heavy bacterial suspension with and without polymyxin B was inoculated into the wells. A known DNase-producing *Campylobacter* strain and a DNase-negative *Campylobacter* strain should be included in the test. The results can be read first after 8 h and confirmed after 20 to 24 h of incubation.

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