Growth of Aeromonas spp. on Cefsulodin-Irgasan-Novobiocin Agar
Selective for Yersinia enterocolitica

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Received 10 April 1985/Accepted 8 July 1985

Twenty-eight strains of Aeromonas spp. were analyzed for their ability to grow on two different kinds of cefsulodin-Irgasan (triclosan; Ciba-Geigy AG, Basel, Switzerland)-novobiocin (CIN) agar containing 15 or 4 mg of cefsulodin per ml and on inositol-bile salts-brilliant green (IBB) agar. Relative to blood agar, 68% of the strains were inhibited by more than 2 logs (i.e., less than 1% growth) at 37°C (39% at 25°C) on CIN I (high cefsulodin concentration), 7% were inhibited at either temperature on CIN II (low cefsulodin concentration), 4% were inhibited on IBB agar at 37°C, and none were inhibited on IBB agar at 25°C. These results reflect the MICs of cefsulodin on CIN Base: the MIC for 50% of the strains was 8 mg/liter at 37 and 25°C, and the MICs for 90% of the strains were 16 mg/liter at 37°C and 64 mg/liter at 25°C. The MICs of Irgasan and novobiocin were far beyond the concentrations used in CIN media. We argue that CIN agar containing 4 mg of cefsulodin per ml (CIN II) can be used for the simultaneous detection of Aeromonas spp. and Yersinia spp.

Yersinia spp. and Aeromonas spp. are accepted as major and possible enteric pathogens, respectively (8, 13). For the detection of Yersinia spp. in stools, our laboratory uses Yersinia Selective Agar Base (CIN Base; Oxoid Ltd., London, England) plus the appropriate supplement (also Oxoid), which contains cefsulodin (15 mg/liter), Irgasan (triclosan; Ciba-Geigy AG, Basel, Switzerland) (4 mg/liter), and novobiocin (2.5 mg/liter). This CIN agar (9) is also the subculture medium for two different enrichments (phosphate-buffered saline and Rappaport broth [1]).

By chance we isolated three fecal strains of Aeromonas spp. on this medium (hereafter called CIN I), as have others (2, 10; C. Seymour, K. T. Longo, and S. J. Rubin, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, C112, p. 318). The fact that the MICs of cefsulodin for 50% of the Aeromonas strains (12.5 mg/liter [3]) were close to those of CIN I but that those of Irgasan and novobiocin (10 and 10 to 50 mg/liter, respectively [5, 12]) were much higher than those of CIN I suggested a possible use for this medium in the selection of Aeromonas spp. from feces. The purpose of the present study was to determine whether Aeromonas strains would regularly grow on CIN I as well as on a commercial CIN agar preparation with a cefsulodin content of only 4 mg/liter but unchanged Irgasan and novobiocin contents (Yersinia Selective Agar; Difco Laboratories, Detroit, Mich.; hereafter called CIN II).


MATERIALS AND METHODS

Bacterial strains. Twenty-eight strains of Aeromonas spp. (11 Aeromonas caviae, 4 atypical A. caviae, 4 A. hydrophila, 4 atypical A. hydrophila, and 8 atypical Aeromonas sp. strains) were used in this study. Of these, 25 were from our stock culture collection (all isolated from human specimens); the remaining 3 were isolated from human feces by chance on CIN I. Biochemical identification was as described below.

Media. CIN I and CIN II were used in addition to inositol-bile salts-brilliant green (IBB) agar and blood agar (Tryptic soy agar [BBL Microbiology Systems, Cockeysville, Md.] with 5% human blood).

Biochemical identification. Identification of the strains was done with the API 20E system (API Systems SA, Montalieu-Vercieu, France) to the genus level (all motile aeromonads are designated Aeromonas hydrophila in this system). Conventional methods (esculin hydrolysis, salicin, L-arabinose, production of acetoin from glucose, and production of gas from glucose) provided species identification (7). Incubation was carried out at 25 and 37°C for all strains taken from our stock culture collection and at 29°C for all fresh isolates. If incubated at two different temperatures, a strain was considered positive for the respective marker if positive at either or both temperatures. If all reactions, based on the above-described parameters, were like those of one of the three motile species (7), we considered an isolate to be a typical strain of that species. Isolates with one atypical reaction were considered to be atypical strains of the respective species (e.g., atypical A. hydrophila). All other isolates were classified as atypical strains.

Determination of MICs. MICs of cefsulodin (Ciba-Geigy AG), Irgasan, and novobiocin (Merck Sharp & Dohme, Haarlem, The Netherlands) were determined by using the agar dilution test (6) on CIN Base (no antimicrobial agents) (Oxoid). Incubation was carried out at 25 and 37°C.

Evaluation of selective media. All strains were suspended in physiological saline at a density corresponding to a 0.5 McFarland standard. Samples (0.1 ml) of this suspension, diluted 1:10,000, were plated on CIN I and CIN II. For comparative purposes, blood agar and IBB agar (10), a medium known to be sensitive and specific in the detection of Aeromonas spp., were used. The plates were incubated aerobically for 48 h at 25 and 37°C. CFU were determined on all agars.

Fecal specimens. From 15 August to 14 November 1985, 1,282 stool specimens were plated on CIN I, incubated for 48 h at 25°C, and assayed for the presence of Yersinia spp. and

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Aeromonas spp. by standard biochemical procedures; enrichments were done with Rappaport broth (24 h, 29°C) and phosphate-buffered saline (14 days, 4°C), respectively (1), and subcultured onto CIN I.

RESULTS

Biochemical identification. Identification to the species level with tubed media revealed the temperature dependence of many strains for salicin, esculin hydrolysis, production of gas from glucose, and L-arabinose; i.e., these strains were usually positive at 25°C and negative at 37°C. Therefore, identification of fresh isolates was done at 29°C.

Growth on selective media. On blood agar, 0.1-ml samples of the bacterial suspensions diluted 1:10,000 contained 50 to 400 CFU for all 28 strains. The number of CFU was lower on CIN I, CIN II, and IBB agar than on blood agar (Table 1). This was true for incubation temperatures of 37° and 25°C. In addition, growth on CIN I seemed to be temperature dependent: 10 strains exhibited a difference in CFU of more than 10% (range, 11 to 50%) between 25 and 37°C. Three of these strains grew better at 25°C, six grew only at 25°C and not at 37°C, and one strain grew better at 37°C. In contrast, five strains exhibited a growth difference of more than 10% (range, 11 to 50%) between the two temperatures on CIN II, but of these, four strains grew better at 37°C. Finally, of five strains with a growth difference on IBB agar of more than 10% (range, 11 to 26%), four grew better at 25°C. There was no correlation evident between the species and the extent of growth on CIN agar.

MICs. Determinations of the MICs on CIN Base indicated that all 28 strains tested were resistant to Irgasan at the concentration used in CIN media at both temperatures. Only one strain was susceptible to novobiocin at 37°C. It is evident from Table 2 that the MICs of cefsulodin were in the concentration range of this agent in CIN media. Again, a temperature dependence could be recognized for several strains. At 37°C the MIC for 4 strains was 4 mg/liter, and for 21 strains it was 8 mg/liter; at 25°C these 4 strains were susceptible to 4 mg/liter, but only 13 strains were susceptible to 8 mg/liter. Correspondingly, the MICs of cefsulodin for 90% of the strains were 16 mg/liter at 37°C and 64 mg/liter at 25°C. The temperature dependence for Irgasan and novobiocin was less pronounced than that for cefsulodin, except for one strain, which was inhibited by 4 mg of novobiocin per liter at 37°C but not 25°C.

Fecal specimens. During 3 months of the summer of 1984 we isolated 6 strains of Yersinia spp. and 31 strains of Aeromonas spp. from 1,282 stool specimens. Of the Aeromonas strains, 18 were recovered on primary CIN I plates, whereas 2 and 11, respectively, were found only after enrichment with Rappaport broth and phosphate-buffered saline. The strains isolated represented 21 A. caviae, 1 A. sobria, 4 atypical A. sobria, 1 A. hydrophila, and 5 atypical Aeromonas sp. isolates.

DISCUSSION

In the present study we compared the relative growth of 28 strains of Aeromonas spp. on two different commercially available CIN media (differing mainly in their cefsulodin content) and on IBB and blood agars. Our results (Table 1) demonstrated that the growth of Aeromonas spp. is somewhat better on IBB agar than on CIN II and significantly better on IBB agar than on CIN I. Furthermore, there were growth differences for some strains on CIN I at the two temperatures tested. On CIN II (which contains less cefsulodin than CIN I), no temperature dependence was detected. We believe that this phenomenon is cefsulodin related. Although susceptibility to Irgasan and novobiocin is independent of the incubation temperature at concentrations relevant for the isolation of Aeromonas spp., the MICs of cefsulodin clearly indicated a lower susceptibility at 25°C than at 37°C. The fact that our MICs differed from those previously reported (3, 12) is most probably due to the use of CIN Base in our experiments instead of blood agar.

The temperature dependence exhibited by many strains of our stock culture collection when tested for biochemical markers raises the question of whether identification should be done at 37°C or at lower temperatures (or both). For species identification of our stock culture strains, we considered strains to be positive if they were positive at either or both temperatures and decided to use incubation at 29°C for our fresh isolates. In view of the uncertain taxonomy of the genus Aeromonas, this temperature dependence requires further clarification.

The question arises of whether CIN media, in particular the less inhibitory CIN II, can be used as selective media for Aeromonas spp. We do not yet have figures for the recovery rates of Aeromonas spp. in artificially inoculated stools as compared with IBB agar. Such figures would depend not only on the sensitivity and specificity of the medium but also on its selectivity. Semiquantitative data from our laboratory show that CIN II is much more inhibitory to normal stool flora than IBB agar (data not shown). Obviously, the spectrum of its inhibitory agents is greater than that of brilliant green and bile salts, the inhibitory ingredients of IBB agar (2, 10). This is also illustrated by the fact that of seven media selective for Aeromonas spp., IBB was 100% specific and sensitive at Aeromonas/clostridial ratios of up to 1:10 but...
only 22% sensitive at a ratio of 1:>10^3, performing significantly worse than ampicillin-containing media at this ratio (14). If Aeromonas spp. as a causative agent of diarrhea behave like enteric salmonellae, i.e., usually showing at least 10^4 organisms per g of stool but often 10^5 or more (4, 11), the inhibition of Aeromonas spp. by CIN media should not make much difference in view of the fact that few other species grow on these media (2, 10). An incubation temperature of 25°C was thought to be useful for the isolation of both Aeromonas spp. and Yersinia spp.

During 3 months of the summer of 1984, an isolation frequency for Aeromonas spp. of 2.4% was recorded on CIN I. This is above the 1.6% reported by von Graevenitz and Bucher (14), who used IBB agar and alkaline peptone water as the enrichment. Taken together with the fact that we used CIN I for culturing stool specimens (the medium that proved to be less suitable than CIN II in the experiments done simultaneously; see above), this is good evidence that CIN media (especially CIN II) will be very useful for the isolation of Aeromonas spp. from fecal specimens.

ACKNOWLEDGMENT
The help extended for this project by Ciba-Geigy AG is gratefully acknowledged.

LITERATURE CITED