Medium and Temperature Dependence of Decarboxylase Reactions in Aeromonas spp.

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Abstract. Decarboxylase/dihydrolase activities in Aeromonas spp. are important as diagnostic tools and indicators of enterotoxin production. We have analyzed the following media at 25°C, 29°C, and 37°C, respectively, for their ability to detect such activities: Möller's, Falkow's, and Fay and Barry's (F&B) containing ornithine, lysine, and arginine, respectively, as well as motility-indole-ornithine (MIO) medium and lysine decarboxylase broth with 0.1% agar (LDC). In order to retain ornithine negativity, but to get as much positivity as possible for arginine, optimal incubation conditions were 29°C for 96 h (Möller), 48 h (Falkow, MIO, and LDC), and 24 h (F&B). The F&B medium proved to be the most sensitive for the detection of lysine decarboxylase, a positive test being highly correlated with the two species A. hydrophila and A. sobria, and we suggest its use for routine detection of decarboxylase/dihydrolase activities.

Decarboxylase reactions in the genus Aeromonas are important as diagnostic tools and indicators of enterotoxin production. Ornithine decarboxylase should be negative in all members of this genus [4], whereas positivity in lysine decarboxylase has been correlated to the production of an enterotoxin that may act as a factor in determining virulence among this group of gram-negative bacteria [5]. Also, in our laboratory, we have encountered some problems in determining decarboxylase activity within this genus, i.e., ornithine decarboxylase was not consistently negative in all our strains, and lysine decarboxylase activity was dependent on the medium used. We have therefore analyzed 28 strains of Aeromonas spp. for decarboxylase/dihydrolase activity using ornithine, lysine, and arginine, respectively, as substrates to assess the reliability of different media at different temperatures.

Materials and Methods

Bacterial strains. A total of 28 Aeromonas strains (10 A. caviae, 9 A. hydrophila, and 9 A. sobria including 3 atypical strains) were identified with the API 20E system (API Systems SA, Montalieu-Vercieu, France) to the genus level and speciated on the basis of five biochemical markers (Table 1) at 29°C as described [1]. They included 18 clinical (feces, blood, wound) and six environmental isolates as well as four strains of unknown origin.

In memory of Dr. Sally Jo Rubin.

Five strains each of Providencia rettgeri, Hafnia alvei, Kluyvera ascorbata, and Enterobacter sakazakii were included as controls since they exhibit a very consistent behavior with respect to certain decarboxylase/dihydrolase activities [2]. The P. rettgeri strains were used as negative controls and H. alvei (100% lysine decarboxylase positive), K. ascorbata (100% ornithine decarboxylase positive), and E. sakazakii (99% arginine dihydrolase positive) were suitable as positive controls.

Media. Five different decarboxylase media (Table 2) were used throughout this study, incubated at 25°C, 29°C, and 37°C, respectively, and read every day for four days. With the exception of the medium of Fay and Barry [3] (F&B), all bases are commercially available (Difco, Detroit, Michigan). L-Arginine and L-ornithine (Fluka, Buchs, Switzerland), and L-lysine (Merck, Darmstadt, FRG) were added at a concentration of 10 g/liter.

Results

Controls. All five strains of P. rettgeri consistently showed no decarboxylase activity in all media tested and, thus, no false positive reactions were observed even after incubation for 4 days.

The five strains each of K. ascorbata, H. alvei, and E. sakazakii that were used as positive controls for ornithine, lysine, and arginine decarboxylase activity, respectively, were all positive after incubation for 48 h at 37°C. To a low extent, we observed some temperature dependence in that incubation at lower temperatures increased the incubation time necessary to give a positive result. From the results...
with the positive control strains (data not shown), it can be concluded that Møller, Falkow, and F&B media require incubation for 48 h, whereas for MIO and LDC medium incubation for only 24 h may be sufficient.

**Aeromonas strains.** As is shown in Table 3, considerable differences were observed between different media inoculated with *Aeromonas* strains. Ornithine decarboxylase activity was found in all media tested, but only one strain was positive in the Møller medium after incubation for 96 h at 25°C. In Falkow, F&B, and MIO media, the number of positive strains increased upon extended incubation independent of the temperature used.

For arginine dihydrolase activity, the F&B medium was significantly more sensitive than Møller and Falkow media, at least using short incubation times. These differences were even more prominent for lysine decarboxylase activity. In F&B medium, the maximal number of positive strains was recorded after incubation for only 24 h. Møller medium was undersensitive, and Falkow as well as LDC media needed incubation for at least 4 days (Table 3).

In order to minimize false positive reactions for ornithine decarboxylase activity and to be as sensitive as possible for arginine dihydrolase and lysine decarboxylase activities, we concluded that incubation at 29°C for 96 h (Møller), 48 h (Falkow, MIO, and LDC), and 24 h (F&B) seems appropriate.

Under incubation conditions as listed above, the lysine decarboxylase activity was compared in *A. hydrophila*, *A. caviae*, and *A. sobria* (Table 4). It is obvious that F&B medium is far superior to the other media tested, a total of 18 strains being positive in F&B compared to only one, six, and four in Møller, Falkow, and LDC, respectively. There is an excellent correlation between species and lysine decarboxylase activity (F&B medium): *A. hydrophila* (eight of nine) and *A. sobria* (nine of nine) are positive and *A. caviae* is usually negative (only one in ten positive).

**Discussion**

Decarboxylase activities are important diagnostic tools in identifying *Aeromonas* spp. from clinical and environmental samples. Popoff [4] states that ornithine decarboxylase is consistently negative and arginine dihydrolase consistently positive in all species of the genus *Aeromonas*. Incubation temperature used by this group was 30°C [6]. In contrast, Janda [7] found arginine dihydrolase to be positive in only 91% of the strains when tested at 37°C. We therefore decided to check, systematically, decarboxylase activities, media, and incubation temperatures, respectively, with 28 *Aeromonas* strains.

Essentially no differences with respect to medium and incubation temperature were observed with our control strains. As was expected, optimal incubation was at 37°C for 48 h. The *Aeromonas* strains behaved rather differently (Table 3): upon prolonged incubation, ornithine decarboxylase became positive in several strains, depending on medium as well as incubation temperature. Whether this result reflects true or false positive reactions remains uncertain since positive strains have been recorded in several laboratories (F. W. Hickman-Brenner, personal communication). For arginine dihydrolase, positivity was recorded in all strains at 29°C using F&B as well as Møller medium, but not