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Comparison of Three Buffers Used in the Formulation of Buffered Charcoal Yeast Extract Medium

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Growth of *Legionella* spp. on buffered charcoal yeast extract medium supplemented with α-ketoglutarate and formulated with 3-(*n*-morpholino)propanesulfonic acid (MOPS), 3-(*n*-morpholino)-2-hydroxypropanesulfonic acid (MOPSO), or *n*-(2-acetamido)-2-aminoethanesulfonic acid (ACES) buffer was similar. With three exceptions, growth was no different in buffered yeast extract broth supplemented with α-ketoglutarate and formulated with MOPS or ACES buffer.

Pasculle and colleagues made a major advance in the cultivation of *Legionella pneumophila* by formulating buffered charcoal yeast extract (BCYE) culture medium containing activated charcoal and the organic buffer *n*-(2-acetamido)-2-aminoethanesulfonic acid (ACES) (5). ACES buffer provided an optimal pH for *L. pneumophila* growth, without causing the growth inhibition observed with some inorganic buffers. However, ACES buffer is costly ($265/kg) (6), and it decomposes unpredictably. We conducted experiments designed to find a less expensive and more stable organic buffer that produces equivalent growth of *L. pneumophila* on BCYE medium supplemented with 0.1% α-ketoglutarate (BCYEα medium) (1).

BCYEα medium was prepared as described previously, by using ACES buffer (BCYEα-A) (1, 2). In addition, the same medium was made by substituting either 3-(*n*-morpholino) propanesulfonic acid (MOPS) (BCYEα-MO) or 3-(*n*-morpholino)-2-hydroxypropanesulfonic acid (MOPSO) buffer for ACES buffer (Research Organics, Cleveland, Ohio). All buffers were used at a final concentration of 0.055 M. Two different lots of MOPS and MOPSO buffers were tested independently.

Media were tested by using frozen homogenized suspensions of lungs from guinea pigs with *L. pneumophila* serogroup 1 (strain F 889) pneumonia (4). Plating was done in quadruplicate for the study of the first lot of buffers and in quintuplicate for the study of the second lot of buffers. Because colony enumeration was destructive, one set of plates was incubated for 3 days and another set was incubated for 4 days; the plates read at 3 days and those read at 4 days postincubation were inoculated on different days from different vials of frozen bacteria. Thus, a total of four independent experiments was conducted (two different incubation times and inocula and two different buffer lots). Incubation of all plates was done at 35°C in humidified air. Diameters of the five largest and five smallest colonies on each plate were measured by using a calibrated dissecting microscope.

After completion of the studies described above, additional studies that compared BCYEα-A with BCYEα-MO medium were performed by plating a total of nine additional clinical isolates of *Legionella* species (Table 1). The bacteria, all of which were low-passage isolates that had been frozen at −70°C, were passaged once on BCYEα-A and then plated quantitatively on plates taken from one lot of BCYEα-A and from two different lots of BCYEα-MO medium. Also, the same strains plus *L. pneumophila* serogroup 1 strain F 1663 were inoculated (≈10^3 CFU/10 ml of broth) into buffered yeast extract broth supplemented with 0.1% α-ketoglutarate (BYEα) (2) and buffered with either ACES or MOPS. Growth in the broths was monitored by measuring optical density after 30 and 42 h of incubation at 35°C in a shaking ambient-air incubator.

Guinea pig alveolar macrophages were harvested, purified, and infected with *L. pneumophila* F 889 as described previously (3). The bacterium was grown on both BCYEα-A and BCYEα-MO. Bacterial growth in the macrophages was measured by plating culture supernates on BCYEα-A medium (3).

Standard descriptive statistics were calculated from the colony size and number measurements for each medium type, and the performances of different medium types were compared by using one-way analysis of variance (InStat version 2.01; GraphPad Software, San Diego, Calif.).

No significant differences in the yields of *L. pneumophila* F889 plated on BCYEα media made with the three different buffers were detected in the four independent experiments ($P > 0.2$) (Fig. 1). There were some statistically significant, but practically insignificant, colony size differences between the different media (Fig. 1).

No significant differences in colony numbers enumerated

TABLE 1. Growth of nine *Legionella* species strains on BCYEα medium made with either ACES or MOPS buffer

Strain[a]	Mean CFU/plate (SEM) on:		
	BCYEα-A	BCYEα-MO (lot 1)	BCYEα-MO (lot 2)
L. pneumophila F 1821	84 (5)	82 (5)	82 (5)
L. pneumophila F 1924	77 (4)	80 (12)	84 (3)
L. pneumophila F 1948	98 (4)	97 (5)	102 (3)
L. pneumophila F 2111	82 (3)	86 (3)	85 (3)
L. pneumophila F 2127	88 (6)	87 (4)	88 (5)
Legionella micdadei F 1438	136 (3)	153 (12)	140 (7)
L. longbeachae F 1548	72 (3)	68 (5)	68 (2)
Legionella dumoffii F 1717	77 (6)	80 (5)	67 (5)
L. bozemanii F 2115	59 (5)	68 (4)	68 (2)

[a] All isolates were in serogroup 1 of their respective species.

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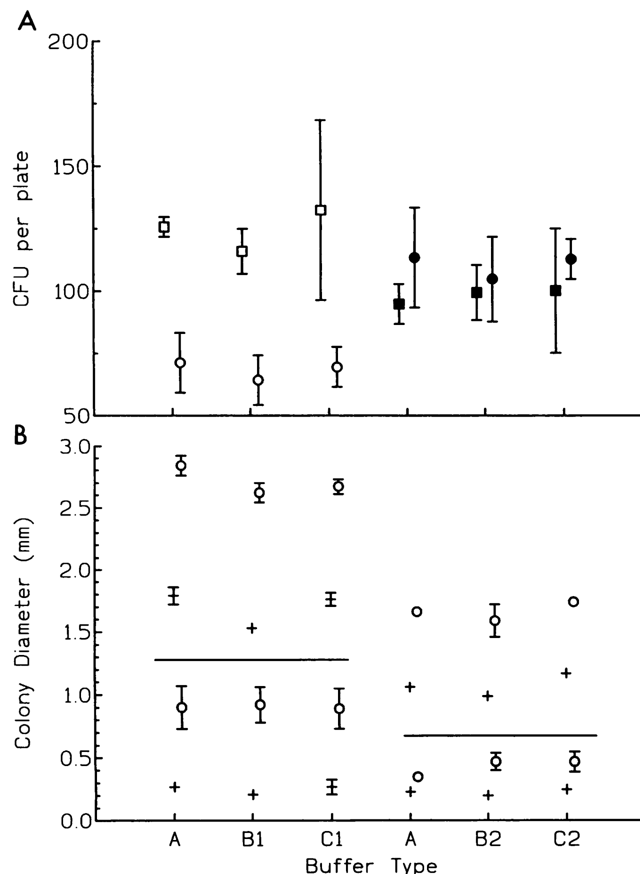

FIG. 1. Performance of BCYEα agar made with either ACES, MOPS, or MOPSO buffer. (A) Recovery of *L. pneumophila* F 889 plated on the different media after 3 days (□ and ■) and 4 days (○ and ●) of incubation; (B) colony diameters measured after 3 days (+) and 4 days (○) of incubation of the media (the two sets of values above the horizontal lines are for large colonies, and the bottom two sets of values are for small colonies). The mean values and 95% confidence intervals are shown for all points; in some cases the symbol size is larger than the confidence interval. Each set of datum points represents an independent experiment. Buffers used in the BCYEα media are shown on the abscissa: A, ACES; B1 and B2, MOPS lots 1 and 2; C1 and C2, MOPSO lots 1 and 2.

after 3 or 4 days of incubation were found for the nine other *Legionella* species strains plated on either BCYEα-A or BCYEα-MO medium (Table 1) ($P > 0.4$ for comparisons between medium types by one-way analysis of variance). No major differences in colonial morphology or size were observed for the two different medium types examined.

Eight of the ten strains inoculated into broth media grew to turbidity after 30 to 42 h of incubation. A significant difference ($P < 0.05$) in optical density between the two broth media was noted only for strain F 1717, which grew better in the broth made with ACES buffer. A single strain each of *Legionella longbeachae* and *Legionella bozemanii* failed to grow in either broth medium; retesting of these strains using a higher inoculum showed superior growth in the ACES-containing medium (data not shown).

Growth of *L. pneumophila* F 889 in guinea pig alveolar macrophages was nearly identical regardless of buffer type used in the BCYEα medium to grow the infecting bacterium ($P > 0.1$ after 2 and 3 days of incubation with the macrophages).

These results demonstrate no practically significant differences between the performances of BCYEα media made with buffers different from ACES. Because one of the test inocula (strain F 889) studied was *L. pneumophila*-infected lung tissue, these results are likely representative of the clinical performance of these media for *L. pneumophila*. In addition, other *Legionella* species and other *L. pneumophila* strains grew as well on BCYEα-MO as on BCYEα-A medium. We know that a change in buffers did not change the virulence of one strain of *L. pneumophila* for alveolar macrophages but do not know if this will apply to all *Legionella* bacteria.

We are less certain regarding the equivalency of MOPS and ACES buffers used in the formulation of BYEα broth, because growth of some *Legionella* species was more rapid in the ACES-containing broth. A universal broth or agar medium that grows all strains and species of *Legionella* equally well may not be possible, regardless of buffer type.

We feel that the use of MOPS buffer is to be preferred over that of MOPSO because of the slightly larger colonies obtained by using MOPS rather than MOPSO and because MOPSO is a little more expensive than MOPS ($154 versus $124/kg) (6). The use of MOPS buffer rather than ACES buffer in the formulation of BCYEα medium could result in substantial cost savings for large-volume medium manufacturers without any decrease in medium quality.

REFERENCES

1. **Edelstein, P. H.** 1981. Improved semiselective medium for isolation of *Legionella pneumophila* from contaminated clinical and environmental specimens. J. Clin. Microbiol. **14:**298–303.
2. **Edelstein, P. H.** 1985. Legionnaires' disease laboratory manual, 3rd ed. National Technical Information Service, Springfield, Va.
3. **Edelstein, P. H., K. B. Beer, and E. D. DeBoynton.** 1987. Influence of growth temperature on virulence of *Legionella pneumophila*. Infect. Immun. **55:**2701–2705.
4. **Edelstein, P. H., and M. A. C. Edelstein.** 1991. Comparison of different agars used in the formulation of buffered charcoal yeast extract medium. J. Clin. Microbiol. **29:**190–191.
5. **Pasculle, A. W., J. C. Feeley, R. J. Gibson, L. G. Cordes, R. L. Myerowitz, C. M. Patton, G. W. Gorman, C. L. Carmack, J. W. Ezzell, and J. N. Dowling.** 1980. Pittsburgh pneumonia agent: direct isolation from human lung tissue. J. Infect. Dis. **141:**727–732.
6. **Research Organics, Inc.** 1993. Research Organics catalog. Research Organics Inc., Cleveland.