

## NOTES

### Survival of Anaerobic Bacteria in Common Laboratory Diluents

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The survival of six species of anaerobic bacteria was studied in simple or commercially available diluents. *Bacteroides fragilis* and *Fusobacterium nucleatum* showed excellent survival in all diluents including distilled water. *Fusobacterium mortiferum* survived well in all diluents except water and water supplemented with 0.1% gelatin. *Clostridium perfringens* survived best in phosphate-buffered saline with gelatin. *Peptococcus asaccharolyticus* required gelatin added to the basic diluent, and *Streptococcus intermedius* showed excellent survival only in minimal essential medium with gelatin. These diluents could provide effective and economical alternatives to more complex and costly diluents often used in work with anaerobic bacteria.

Investigations involving anaerobic bacteria frequently require the use of diluents (1). Several different types of diluents for anaerobes are available, such as the complex mixture of salt solutions, buffers, carbohydrates, yeast extract, liver digest, and protein prepared in a prerduced manner which is described in the *Wadsworth Anaerobic Bacteriology Manual* (4). The Virginia Polytechnic Institute *Anaerobe Laboratory Manual* describes a simpler prerduced diluent consisting of a gelatin-salt solution (3). The Center for Disease Control's *Laboratory Methods in Anaerobic Bacteriology* also describes a simple buffered gelatin diluent which is prepared aerobically (2). However, information comparing the survival of anaerobes in any kind of diluent is scarce. We undertook the current study to determine the survival of several anaerobic bacteria in a number of readily available laboratory diluents. The diluents studied were relatively inexpensive, easy to prepare (or commercially available), and not dependent on a special apparatus for preparing them in a reduced state.

Single strains of *Bacteroides fragilis* Wadsworth Anaerobe Laboratory (WAL) 1361, *Clostridium perfringens* WAL 2164, *Fusobacterium mortiferum* WAL 538, *Fusobacterium nucleatum* WAL 38, *Streptococcus intermedius* WAL 1534, and *Peptococcus asaccharolyticus* WAL 2191 were studied. Frozen stocks were cultured

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anaerobically, washed, and suspended to a standard optical density. Dilutions of the suspension provided starting concentrations of  $5 \times 10^4$  to  $1 \times 10^6$  organisms per ml for all bacteria.

Six different diluents were investigated: bottled distilled water, phosphate-buffered normal saline (PBS), minimal essential medium with Eagle salts without glutamine (MEM; Grand Island Biological Co., Grand Island, N.Y.), and each of these three diluents supplemented with 0.1% gelatin (denoted by the suffix "-gel"). Bacterial survival in the diluents at room temperature (21°C) and 37°C was assayed by the pour plate method in room air as previously described (1). The tested diluent was incorporated into the assay at the time of harvesting the organisms from the cultures and was used throughout the assay, including the pour plate dilution tubes. Each study was done in duplicate, and the mean percentage of the original number of colony-forming units surviving was determined. Table 1 summarizes the survival of the six anaerobes in each of the diluents at 1, 2, and 4 h of incubation at room temperature.

*B. fragilis* survived very well in all diluents. The poorest survival was 20% at 4 h in water. No difference in survival at room temperature or 37°C was noted in any of the diluents except distilled water, in which case survival was moderately reduced after 2 h of incubation at 37°C. In related studies, we confirmed these observations in our laboratories for *B. vulgatus*, *B.*

TABLE 1. Survival of anaerobic bacteria in diluents at room temperature<sup>a</sup>

Diluent	<i>B. fragilis</i>			<i>C. perfringens</i>			<i>F. nucleatum</i>			<i>F. mortiferum</i>			<i>S. intermedius</i>			<i>P. asaccharolyticus</i>		
	1 h	2 h	4 h	1 h	2 h	4 h	1 h	2 h	4 h	1 h	2 h	4 h	1 h	2 h	4 h	1 h	2 h	4 h
Water	A	A	D	C	D	E	A	C	E	D	D	E	D	E	E	B	E	E
Water-gel	A	A	A	B	C	D	A	A	B	C	C	E	C	D	E	A	A	B
PBS	A	A	B	A	B	E	A	B	E	A	A	C	C	E	E	B	C	E
PBS-gel	A	A	A	A	A	C	A	A	A	A	A	A	B	C	C	A	A	C
MEM	A	A	A	C	D	D	A	A	A	A	A	A	B	B	C	C	C	D
MEM-gel	A	A	A	B	C	C	A	A	A	A	A	A	A	A	B	A	A	A

<sup>a</sup> Three periods of incubation are shown for each assay (1, 2, and 4 h). Survival of bacteria is symbolized by: A, excellent (75 to 100%); B, very good (50 to 74%); C, adequate (25 to 49%); D, fair (10 to 24%); E, poor (<10%).

*distasonis*, and *B. thetaiotaomicron* as well (D. Casciato, R. Bluestone, J. Rosenblatt, L. Goldberg, and S. Finegold, Clin Res. 22:438a, 1974). Thus, the simplest of diluents would be suitable for studies performed with the *B. fragilis* group of organisms.

*C. perfringens* had good to excellent survival for 1 h in all diluents except plain water and MEM. At 4 h survival fell to less than 35% in all diluents. Survival was best in PBS and PBS-gel, with more than half of the organisms alive after 2 h of incubation (range, 55 to 82%). Survival was somewhat poorer in most diluents at 37°C when compared to room temperature results.

Both species of *Fusobacterium* showed excellent survival in MEM, MEM-gel, and PBS-gel and very good (>71%) survival in PBS for at least 2 h. *F. nucleatum* also survived well in water-gel (59% at 4 h), but poorly in plain distilled water (<1% at 4 h). In contrast, *F. mortiferum* survived poorly in both water and water-gel (<35% at 1 h). For both organisms survival was poorest in distilled water and was further decreased at 37°C for water, water-gel, and PBS compared to room temperature incubations.

Both anaerobic cocci survived best in MEM-gel. Results with the other diluents varied. *S. intermedius* survival was reduced but "adequate" in PBS-gel and MEM (35 to 40% at 4 h), fair to poor in water, water-gel, and PBS, and further decreased when incubated at 37°C in nearly all diluents. *P. asaccharolyticus* survival was very good in water-gel and PBS-gel (>45% at 4 h), decreasing somewhat in 37°C incubations, but fair to poor in the diluents not supplemented with gelatin.

Eh and pH values were measured using a combination reference pH electrode and a combination platinum reference Eh electrode (Beckman 39186). Readings were not different in inoculated (*B. fragilis*) compared to uninoculated diluents. Eh readings were all very high, the lowest being +219 mV. None of the Eh values was low enough to have influenced anaerobe

survival in that diluent. However, considerable differences were noted in the pH values obtained, ranging from 6.9 to 8.4 units. It is doubtful that these pH differences per se affected survival here since bacteria in diluents with different pH values had similar poor survival rates (i.e., water and PBS) and bacteria in diluents with similar pH values had different survival rates (e.g., PBS and PBS-gel). It is more likely that the solution's composition provided ionic, osmotic, and perhaps minimal nutritional requirements to the bacterial cell (especially the cell wall) which determined the ability of the organism to survive in that diluent.

All of the anaerobes studied survived well enough for qualitative and semiquantitative assays in all of the diluents tested at 1 h of incubation. In each case, survival at room temperature was equal to or greater than survival at 37°C, an observation which is compatible with the use of these and related diluents for work on the laboratory bench. Although some quantitative work with mixtures of anaerobes, including the most fastidious (such as bowel flora studies), might require more complex, prerduced diluents, our work indicates that some strains of commonly studied anaerobes survive very well in inexpensive and readily available diluents.

#### LITERATURE CITED

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