

Improved Growth Media for *Vibrio succinogenes*

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Five new culture media for *Vibrio succinogenes* are described. Of these, a medium composed of 0.4% yeast extract, 100 mM ammonium formate, 120 mM sodium fumarate, and 0.05% sodium thioglycolate, pH 7.3, supports the best growth.

Vibrio succinogenes is an unusual anaerobe originally isolated from the bovine rumen (7). The organism possesses a cytochrome-linked electron transport system that utilizes either hydrogen or formate as electron donors and either fumarate or nitrate as electron acceptors (2, 3, 7). L-Malate, L-aspartate, or L-asparagine can substitute for fumarate in media by virtue of their intracellular conversion to fumarate (5, 7; B. Maloney and M. Wolin, Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 143, P47, 1972). An unusual nutritional requirement for succinate has been demonstrated (5), as has the ability of the organism to produce large quantities of L-asparaginase (4). *V. succinogenes* has also been used as a hydrogen sink in a model anaerobic ecosystem (1).

One of the difficulties encountered in the study of this organism is the inability of published media to support good growth. A further difficulty is the high salts content of the media since harvested cells are heavily contaminated with precipitated salts. This note described a variety of media formulations that overcome both of these difficulties.

The media developed in this study contain 0.4% yeast extract supplemented with appropriate concentrations of electron donor and acceptor (Table 1). The pH of the media is adjusted to 7.3 before autoclaving, and sterile sodium thioglycolate is added after autoclaving to a final concentration of 0.05%. Incubations are in air at 37 C. The doubling time in VSF is about 70 min, and the cell yield is 2.0 to 2.2 g (wet weight)/liter. Typical cell yields obtained with FF medium (5, 7) are 0.5 to 0.7 (wet weight)/liter, including precipitated salts. When sodium formate is substituted for ammonium formate in VSF, significantly less growth is obtained (mean optical density [OD] of 10 cultures was 1.25) and is not improved by the addition of 10 mM NH₄Cl. Sodium formate is, however, more effective when other electron acceptors are pro-

vided. When L-malate was tested with ammonium formate, only one of 15 cultures grew within 24 h, five grew within 48 h, and nine showed no growth at 72 h. With sodium formate, 15 L-malate cultures reached maximal growth within 24 h. Results with L-aspartate and L-asparagine were less clear cut, but growth rates and cell yields were more consistent with sodium formate.

Titration of the formate and fumarate concentrations in VSF indicate that the concentrations in Table 1 are potentially growth limiting. However, when higher concentrations of either or both compounds were tested, complete inhibition of growth was frequently encountered. Thus, the values in Table 1 represent a compromise between cell yield and reliability.

The final pH of the cultures listed in Table 1 is 8.3 to 8.5. The addition of 40 mM *N*-2-hydroxyethyl-piperazine-*N'*-2'-ethanesulfonic acid results in a final pH of 7.5 to 7.6 but does not improve growth. Tris(hydroxymethyl)aminomethane and triethanolamine are also effective buffers but are inhibitory at concentrations above 25 mM.

Attempts to improve growth in nitrate media have not been successful. A medium (VSN) composed of 0.4% yeast extract, 100 mM sodium formate, 100 mM potassium nitrate, 25 mM potassium phosphate, and 10 mM sodium succinate, adjusted to pH 7.3 and reduced with 0.05% sodium thioglycolate, supports somewhat better growth than FF-NO₃ medium (5). The mean OD at 48 h with VSN is 0.80 compared with a mean OD of 0.45 with FF-NO₃. VSN is the simplest of about a dozen formulations that support essentially the same growth. If the phosphate is omitted from VSN, the pH rises to 9.3 to 9.5 and growth terminates at OD 0.1 to 0.2. With phosphate, the final pH is 8.6 to 8.7. The addition to VSN of 20 mM *N*-2-hydroxyethyl-piperazine-*N'*-2'-ethanesulfonic acid results in a final pH of 8.3 to 8.4, but does not

TABLE 1. Concentrations of electron donors and acceptors in *V. succinogenes* media

Medium	Electron donor	Electron acceptor	Growth ^a
FF (ref. 5, 7)	Sodium formate (44 mM)	Sodium fumarate (26 mM)	0.34
VSF	Ammonium formate (100 mM)	Sodium fumarate (120 mM)	1.50
VSM	Sodium formate (100 mM)	Sodium L-malate (120 mM)	1.40
VSASP	Sodium formate (100 mM)	L-Aspartate (120 mM)	1.00
VSASN	Sodium formate (100 mM)	L-Asparagine (120 mM)	0.95

^a Growth is expressed as OD at 550 nm measured in 10-mm cuvettes with a Hitachi-Coleman model 101 spectrophotometer. Screw-capped tubes (16 by 125 mm) containing 10 ml of media were autoclaved for 10 min at 121 C, cooled to approximately 37 C, reduced with sodium thioglycolate, and inoculated (0.1%, vol/vol). FF-grown cells were used as inocula for the first transfer; second and subsequent cultures were inoculated by repetitive transfer in the experimental media. Growth was usually complete in 15 to 18 h; OD was measured at 24 h. The OD values are the means of at least 10 cultures and were determined by diluting the cultures threefold with 0.4% yeast extract solution (pH 7.3), which was also used as the medium blank. The standard deviation of the VSF cultures was 0.09. The other cultures had similar standard deviations.

improve growth. Ammonium formate never supported growth in nitrate media (45 attempts). Wolin et al. (7) showed that resting cells of *V. succinogenes* provided with nitrate show a hydrogen uptake stoichiometry consistent with complete reduction of nitrate to ammonia. Growing cells, however, were found to produce nitrite. In the present study, qualitative testing with Trommsdorf's reagent (6) showed that nitrite was produced in all nitrate test media. This observation, coupled with the failure of some 500 different media formulations containing nitrate to support good growth, suggests that growth is ultimately limited by the toxicity of accumulated nitrite.

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