APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 1990, p. 3223–3224
0099-2240/90/103223-02$02.00/0

Vol. 56, No. 10

Nutritional Features of *Syntrophomonas wolfei*

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Received 27 March 1990/Accepted 18 July 1990

***Syntrophomonas wolfei* subsp. *wolfei* grew poorly in a defined medium with crotonate as the energy source in the absence of rumen fluid. Thiamine, lipoic acid, biotin, cyanocobalamin, and *para*-aminobenzoic acid were required for growth comparable to that obtained with the rumen fluid-based medium. Iron and cobalt were also required for the growth of *S. wolfei* in the chemically defined medium.**

Propionate and longer-chain fatty acids are important intermediates in the conversion of organic matter to CH_4 and CO_2 (4). These compounds are degraded to methanogenic substrates (acetate, H_2, and formate) by syntrophic bacteria which require the presence of methanogens or other H_2-using bacteria to maintain low concentrations of H_2 for the degradation of fatty acids to be thermodynamically favorable (4). *Syntrophomonas wolfei* subsp. *wolfei* is an anaerobic bacterium that β-oxidizes short-chain saturated fatty acids, four to eight carbons in length, to acetate, propionate (from odd-numbered fatty acids), and H_2 only when grown in coculture with H_2-using bacteria such as methanogens (5, 6, 9). Other subspecies and species of the genus *Syntrophomonas* can also use long-chain fatty acids (3, 7). Study of the metabolism of these organisms has been difficult because of the very low growth rates and cell yields of these cocultures and the fact that these are two-member cultures (9).

Recently, *S. wolfei* was isolated in pure culture by using crotonate as the energy source (2). However, this medium contained rumen fluid, which is often difficult to obtain in quantity and causes foaming in fermentors used for the mass culture of *S. wolfei* for biochemical studies. Here, we describe the specific nutritional requirements of this organism and a defined medium which supports growth in the absence of rumen fluid.

The pure culture of *S. wolfei* subsp. *wolfei* isolated from the *S. wolfei-Methanospirillum hungatei* coculture (DSM 2245B) was grown anaerobically as described previously (2) by modifying the basal medium of McInerney et al. (6). The composition of the defined medium was that of the modified basal medium but with rumen fluid and the vitamin solution deleted. Each medium was anaerobically prepared by boiling under an 80% N_2–20% CO_2 gas phase. After the medium was cooled to room temperature, solid sodium bicarbonate was added. The medium was then dispensed into serum tubes which were sealed with black rubber bungs and aluminum crimp seals prior to autoclaving (121°C, 15 min). The cysteine-sulfide reducing solution (6) and the vitamin solutions were then added to each tube several hours before inoculation. All vitamin solutions were filter sterilized and made anoxic by aseptically evacuating and repressurizing the tube three times with O_2-free nitrogen.

All glassware used was chemically cleaned. Additions and inoculations to media were done with syringes and needles (1). A 1% (vol/vol) inoculum from a mid-exponential-phase culture was used. *S. wolfei* was transferred at least five times in medium lacking a particular compound before it was concluded that the compound was not required for the growth. Growth was monitored spectrophotometrically at 600 nm, and growth rates were calculated from changes in absorbance (6).

The pure culture of *S. wolfei* grew at a specific growth rate of 0.039/h in crotonate basal medium which contained rumen fluid. Decreasing the rumen fluid concentration of this medium from 5 to 0.5% (vol/vol) did not affect the rate of growth or the final absorbance (0.45 to 0.5) of the culture (data not shown). In the defined medium, the growth rate (0.023/h) was lower and final absorbance (0.08) was much less than those observed with rumen fluid-based media. The addition of a B-vitamin solution containing thiamine, lipoic acid, biotin (50 μg of each per liter), and cyanocobalamin (5 μg/liter) to the defined medium increased the growth rate and final absorbance (Fig. 1). These values were similar to the growth rate and final absorbance observed with rumen fluid-based media. Deletion of any one of these vitamins decreased the amount of growth of *S. wolfei* in the defined medium (Table 1). Increasing the concentration of thiamine to 100 μg/liter decreased the lag time but did not affect the growth rate or final change in absorbance. The addition of nicotinic acid, D,L-calcium pantothenate, pyridoxine, riboflavin, 1,4-naphthoquinone, hemin, and a volatile fatty acid mixture (8) did not affect the growth of *S. wolfei* (data not shown). After about five transfers in the defined medium

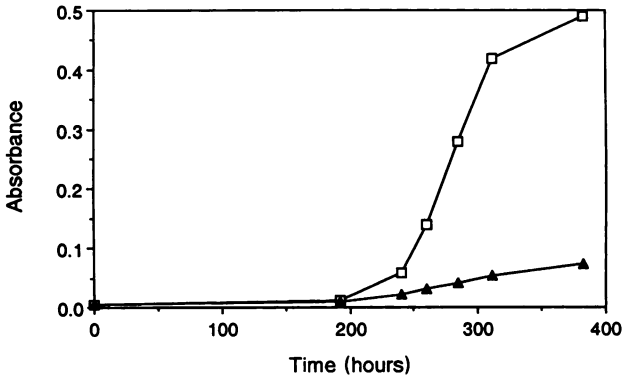

FIG. 1. Effect of B vitamins on growth of *S. wolfei* in defined medium with crotonate as the energy source. The B-vitamin solution contained lipoic acid, cyanocobalamin, biotin, and thiamine added at the concentrations indicated in the text. The inoculum was grown once in medium lacking the specific vitamin(s) in question. Symbols: □, all four vitamins present; ▲, all four vitamins absent.

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TABLE 1. Effect of vitamins on growth of *S. wolfei* in defined medium[a]

Component(s) deleted	Maximal absorbance[b]
None	0.67
p-Aminobenzoic acid	0.40
Biotin	0.10
Thiamine	0.09
Cyanocobalamin	0.06
Lipoic acid	0.03
All B vitamins	0.07

[a] *S. wolfei* was grown in the defined medium as described in the text. Values are means of three replicate tubes with standard deviations (not shown) less than 5% of the mean.

[b] Maximal absorbance was reached after 311 h of incubation. Little or no growth was observed within the first 192 h of incubation.

with the four B vitamins, the growth rate and final change in absorbance of *S. wolfei* decreased. The addition of 50 μg of *para*-aminobenzoic acid per liter to the defined medium with the four B vitamins stimulated the growth of *S. wolfei* (Table 1).

The deletion of $MnSO_4$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $AlK(SO_4)_2$, H_3BO_3, $NaMoO_4 \cdot 2H_2O$, $NaSeO_2$, and $NiCl_2 \cdot 6H_2O$ from the defined medium with the five B vitamins did not affect the growth of *S. wolfei* even after five transfers in medium without these minerals (data not shown). The deletion of $FeSO_4$ or $CoCl_2 \cdot 6\ H_2O$ from this medium decreased the final absorbance by 63 and 44%, respectively. No growth of *S. wolfei* occurred after two transfers in medium lacking iron and cobalt.

These data show that *S. wolfei* has simple nutritional requirements, with good growth occurring in a mineral medium with five B vitamins, cysteine-sulfide reducing solution, and crotonate as the energy source. The stimulation of the growth of *S. wolfei* by the addition of B vitamins is consistent with the observation of McInerney et al. (5) that the coculture of *S. wolfei-M. hungatei* grew faster in the butyrate basal medium which contained rumen fluid and a B vitamin solution than in a defined medium which lacked these two components. The fact that *S. wolfei* can be grown in a defined medium without rumen fluid will greatly facilitate the mass culturing of this organism for further biochemical studies.

We thank R. S. Tanner for helpful comments throughout the course of this work.

This work was supported by contracts DE-ASO583ER-13053 and DE-FG05-89ER14003 from the U.S. Department of Energy.

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