Osmoregulation in Symbiosis-Independent Mutants of *Bdellovibrio bacteriovorus*

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*Bdellovibrios* capable of axenic growth grow in a cell-free medium at a rate considerably lower than that attainable in a two-membered culture with *Escherichia coli*. The axenic growth rate may be improved either by adjustment of the osmoticity of the medium or by the addition of low concentrations of spermine.

*Bdellovibrios* grow within the periplasmic space of other bacteria on precursors obtained from the inhabited cells. They have, therefore, been described as bacterial parasites, predators, or symbionts. However, the intraperiplasmic space, in addition to nutrients, may also provide a special protective environment, rarely available extracellularly. Symbiosis-independent (*S*<sup>in</sup>) mutants (7) isolated from symbiosis-dependent *bdellovibrios* can obtain all nutrients from organic cell-free media and grow axenically. However, they achieve their optimal growth rate only in a two-membered culture. This phenomenon has been observed by us for several *bdellovibrio* strains such as 109J, GB, and 6-5-S and is hereby described for several mutants of *Bdellovibrio bacteriovorus* 109J. In an attempt to improve the growth rate of *S*<sup>in</sup> *bdellovibrios* in axenic culture, we examined the effect of increased osmoticity on growth rates of several strains.

An osmoticity of 0.03 improved the growth of *Bdellovibrio* 109J *S*<sup>in</sup> mutant G-4715; sodium chloride and sucrose decreased the mass doubling time from 20-25 h to 8-10 h (Fig. 1; Table 1). The same effect was obtained by addition of the chloride salts of potassium, calcium, or magnesium (osmoticity, 0.03), and the amino acids glutamic acid and proline (0.1 M). Higher concentrations of both inorganic salts (NaCl or KCl) at an osmoticity of 0.05) or amino acids (1 M) were inhibitory. Another *S*<sup>in</sup> mutant of *Bdellovibrio* 109J, mutant C-3915, was similarly affected by NaCl and sucrose (Table 1).

By repeated passage of the two *S*<sup>in</sup> mutants, G4715 and C3915, in axenic culture, more rapidly growing derivatives were obtained. Apparently, these were "fitter" cells predominating the culture due to their faster growth rate (7). The latter, designated *S*<sup>in</sup> *comp*<sup>-</sup> mutants, differed from the symbiosis-dependent, wild-type parent not only in symbiosis independence, but also in symbiosis incompetence, having lost the ability to symbiotically associate with other bacteria (7). The growth rate of these *S*<sup>in</sup> *comp*<sup>-</sup> strains was not markedly affected by the addition of either NaCl or sucrose (Table 1).

Another *S*<sup>in</sup> *comp*<sup>-</sup> mutant, 204, derived from

**Fig. 1. Growth of *S*<sup>in</sup> mutant G4715 under various conditions:** □, in a two-membered culture with *Escherichia coli* B (10<sup>5</sup> cells per ml of TM buffer) as the sole substrate; ○, in unsupplemented PY medium; △, in PY medium supplemented with NaCl at a 0.03 osmoticity; ●, in PY medium supplemented with sucrose at a 0.03 osmoticity; ▼, in PY medium supplemented with 10<sup>-4</sup> M spermine. TM buffer was composed of Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O(200 mg/liter), Fe(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>·6H<sub>2</sub>O (10 mg/liter), and MnSO<sub>4</sub>·H<sub>2</sub>O (10 mg/liter) dissolved in 0.002 M tris(hydroxymethyl)aminomethane at pH 7.8. PY medium contained Difco peptone (10 gliter) and Difco yeast extract (3 gliter) at pH 7.0. All cultures were incubated in 100-ml flasks in a New Brunswick gyratory shaker operating at 30°C and 250 rpm.
Bdellovibrio 109D was also examined; its mass doubling time in unsupplemented PY medium (for composition, see legend of Fig. 1) was 6 to 7 h; sodium chloride or sucrose at an osmosy of 0.02 to 0.03 had no effect.

Several investigators have noted that osmotically fragile bacteria such as Neisseria perflava and Pasteurella tularensis grow well in media of low toxicity provided that minute amounts of polyamines such as spermine or putrescine are added (3, 4). The growth-promoting effect of the polyamines apparently stems from their capacity to prevent osmotic lysis. Polyamines have been shown to bind to the cytoplasmic membrane of bacteria with defective cell envelopes, thereby stabilizing them (1, 2, 6). We, therefore, examined the effect of spermine on the above four mutants of Bdellovibrio 109J. The growth rate of mutants G4715 and C3915 was enhanced, although the fast-growing comp− mutants were unaffected (Fig. 1; Table 1). The growth rate of mutant G4715 in the presence of both sodium chloride (10−2 M) and spermine (10−4 M) was equal to that observed in the presence of sodium chloride alone.

We suggest that the intraperiplasmically growing wild-type bdellovibrios are adapted to their special, protective environment. Their membrane is either suited to a milieu of relatively high osmosy or, if the periplasmic environment is not of high osmosy, is protected by osmotic stabilizer present. A mutation to symbiosis independence need not affect the membrane, and therefore the S^n mutants grow very slowly in a medium of lower osmosy. The fact that spermine at low concentrations can substitute for salts or sucrose is consistent with this hypothesis. The osmotically fragile bacteria, at a selective disadvantage in the absence of membrane stabilizers, are, under such conditions, soon outnumbered by mutants with decreased osmotic sensitivity and, perhaps, modified membranes. The fact that all three fast-growing mutants are also comp− might be coincidental. However, the possibility that modification of the membrane could abolish the capacity for intraperiplasmic growth cannot be ruled out.

The fact that increased osmosy of the medium or the addition of spermine, although increasing the growth rate of the S^n mutants twofold, does not enable them to attain the intraperiplasmic rate indicates that changed osmotic pressure is but one of the as yet undefined differences between the periplasmic milieu and the medium.

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