TABLE 5. Effects of ion deletion on the growth yields and rates of Bacteroides species

Ion deleted	Organism								
	GA33ª		2044		J1		7CM		
	Yield*	Rate	Yield	Rate	Yield	Rate	Yield	Rate	
None	2.2 (0.06)	5.3 (0.84)	4.5 (0.03)	2.5 (0.22)	1.3 (0.01)	5.5 (0.28)	1.8 (0.04)	3.3 (0.04)	
Na+	0		1.7 (0.27)	7.0 (2.5)	0		1.7 (0.04)	3.9 (0.74)	
K+	0		0.9 (0.02)	18.7 (6.5)	0		0.6 (0.03)	21.1 (2.1)	
PO48-	0		0.6 (0.01)	5.1 (1.8)	0		0.04 (0.05)	8.8 (3.1)	
SO <sub>4</sub> 2-	2.2 (0.03)	6.3 (0.88)	4.3 (0.06)	3.5 (0.10)	1.3 (0.01)	6.3 (0.03)	2.0 (0.02)	11.6 (0.11)	
Ca <sup>2+</sup>	2.0 (0.15)	4.9 (0.62)	2.2 (0.26)	4.4 (0.34)	1.3 (0.05)	8.9 (0.55)	1.0 (0.10)	3.1 (0.11)	
Mg <sup>2+</sup>	0.5 (0.04)	21.3 (1.3)	2.4 (0.10)	3.8 (0.51)	0.5 (0.02)	180.4 (39.7)	0.5 (0.01)	5.9 (1.6)	
Co²+	2.4 (0.03)	5.7 (1.46)	4.1 (0.17)	5.4 (0.97)	1.2 (0.08)	4.2 (0.26)	1.8 (0.04)	5.6 (0.06)	
Mn²+	2.3 (0.02)	6.6 (1.7)	4.7	4.1 (0.04)	1.4 (0.07)	5.4 (0.33)	1.8 (0.02)	5.6 (0.25)	

<sup>a</sup> The numbers denote B. ruminicola subsp. brevis GA33, B. fragilis subsp. fragilis 2044, and B. oralis strains J1 and 7CM. Results qualitatively similar to those shown for strain GA33 were also obtained for strains 23, B<sub>1</sub>18, 118B, B<sub>1</sub>4, and 8503, except that growth of strain 8503 was not completely eliminated when K<sup>+</sup> was deleted as a major medium ingredient. Results qualitatively similar to those shown for strain 2044 were also obtained for strains H, 9344, 8560, 2393, 8483, and 8482, except that the growth yield reductions accompanying Ca<sup>2+</sup> were less severe for strains 8482 and 8483 than was the case with the other strains. Results similar to those shown for strain 7CM were also obtained with strains 2366 and 25560, except that a Ca<sup>2+</sup> effect on growth could not be detected for strains B. hypermegas. Variability was observed among strains within species and also between species in the ability to detect growth rate effects as a function of divalent ion deletion.

## Table 4 footnote 'c':

<sup>&</sup>lt;sup>b</sup> See Table 4, footnote c.

<sup>&</sup>lt;sup>c</sup> The medium denoted by "None" was the medium described in Table 1 devoid of mineral-containing vitamins and contained all required cations at growth-supporting concentrations. The remaining media are identical to the cation-sufficient medium, except for the deletion of the ions indicated. The concentrations of Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Co<sup>2+</sup>, and Mn<sup>2+</sup> in inoculated media from which they were deleted as major ingredients were 43, 1.8, 28, 10, 1.8, and 0.5  $\mu$ M respectively.

<sup>&</sup>lt;sup>c</sup> Figures in columns denoted "Yield" are the growth yields in billions of cells per milliliter. Figures denoted "Rate" are the growth rates as measured by generation times in hours. Each value is the average of duplicate cultures from two or more experiments. Values of 0 indicate no growth detected by turbidity within 200 h of incubation at 37 C.

TABLE 1. Defined medium used to study the inorganic and metal-organic growth requirements of Bacteroides species

Component	Quantity	
Maltose	5.0	
Resazurin	0.001	
Methionine	0.45	
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.90	
Menadione	0.001	
Hemin <sup>b</sup>	0.001	
Cysteine hydrochloride water	0.5	
Volatile fatty acid solution <sup>c</sup>	10.0	
Vitamin solution <sup>d</sup>	10.0	
Mineral solution	50.0	
Buffer		
Distilled-demineralized water		
100% carbon dioxide gaseous phase		

<sup>a</sup> The quantities of maltose, resazurin, methionine, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, menadione, hemin, and cysteine hydrochloride monohydrate are expressed in grams per liter. The quantities of volatile fatty acids, vitamins, and minerals are expressed as milliliters of aqueous stock solution per liter of medium.

<sup>b</sup> Hemin was incorporated into media in a 47.5% (vol/vol) ethyl alcohol-0.1 M KOH solution when factors other than K<sup>+</sup> were studied. When K<sup>+</sup> was studied, it was isomolarly replaced in the stock solution with NaOH.

<sup>c</sup> The volatile fatty acids used and their final medium concentrations were the following: acetic, 2.8  $\times$  10<sup>-2</sup> M; propionic, 9.9  $\times$  10<sup>-3</sup> M; *n*-butyric, 4.5  $\times$  10<sup>-3</sup> M; isobutyric, *n*-valeric, isovaleric, and DL-2-methylbutyric, 9.0  $\times$  10<sup>-4</sup> M each.

<sup>d</sup> The vitamins used and their final medium concentrations were the following: thiamine hydrochloride,  $5.9 \times 10^{-6}$  M; nicotinamide,  $1.6 \times 10^{-6}$  M; riboflavine,  $5.6 \times 10^{-6}$  M; p-aminobenzoic acid,  $7.3 \times 10^{-7}$  M; biotin,  $2.0 \times 10^{-7}$  M; folic acid,  $1.1 \times 10^{-7}$  M; lipoic acid,  $2.4 \times 10^{-7}$  M; cyanocobalamin,  $3.7 \times 10^{-9}$  M.

The minerals used and their final medium concentrations were the following: KH<sub>2</sub>PO<sub>4</sub>,  $6.5 \times 10^{-3}$  M; NaCl,  $1.5 \times 10^{-2}$  M; CaCl<sub>2</sub>,  $1.8 \times 10^{-4}$  M; MgCl<sub>2</sub>·6H<sub>2</sub>O,  $9.0 \times 10^{-5}$  M; MnCl<sub>2</sub>·4H<sub>2</sub>O,  $5.0 \times 10^{-5}$  M; CoCl<sub>2</sub>·6H<sub>2</sub>O,  $4.2 \times 10^{-6}$  M; FeSO<sub>4</sub>·7H<sub>2</sub>O,  $3.6 \times 10^{-5}$  M. When the effects of ion concentration were studied, ion concentrations were isoosmotically altered as described by Caldwell et al. (5).

'For all of the studies except those involving Na<sup>+</sup>,  $CO_2$ -equilibrated  $7.4 \times 10^{-2}$  M NaHCO<sub>2</sub> buffer (pH 6.7 to 6.9) was used, and media were neutralized with 2.5 M NaOH. When Na<sup>+</sup> was studied,  $CO_2$ -equilibrated  $7.4 \times 10^{-2}$  M KHCO<sub>2</sub> replaced NaHCO<sub>3</sub>, and media were neutralized with 2.5 M KOH. Neither of these procedures affected growth of any of the organisms in media containing adequate Na<sup>+</sup> and K<sup>+</sup>.