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# Cationic Composition of 22 Species of Bacteria Grown in Seawater Medium

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Twenty-two species of bacteria of marine, estuarine, and terrestrial origin were analyzed for cationic content by atomic absorption spectrophotometry after growth in a basal seawater medium. *Alcaligenes marinus* was analyzed from eight separate but replicate determinations yielding the following cationic concentrations: Na, 5,600 ± 2,260; Mg, 1,580 ± 740; K, 700 ± 360; Ca, 790 ± 390; Mn, 1.7 ± 0.5; Fe, 256 ± 57; Ni, 1.7 ± 0.7; Cu, 14 ± 4; Zn, 122 ± 27; Cd, 2.8 ± 0.7; and Pb, 10 ± 3 ppm/(dry weight). Washing *A. marinus* cells before analyses was necessary due to interstitial medium within the cell pellets after centrifugation and loose cationic retention by the cells. The principal source of error in the procedure was ascribed to variability due to washing cells with 0.5 M ammonium formate. The mean cationic concentrations for trace elements in the 22 bacterial cultures grown in the basal seawater medium to constant optical density and washed three times with 0.5 M ammonium formate were: Mn, 2.4 ± 3.8; Fe, 262 ± 112; Ni, 2.3 ± 1.8; Cu, 24 ± 17; Zn, 146 ± 72; Cd, 3.8 ± 2.5; and Pb, 17 ± 21 ppm (dry weight). Major ions were concentrated only occasionally by the cells after washing, whereas Mn, Fe, Ni, Cu, Zn, Cd, and Pb were concentrated from the medium by the following factors on the average: 180, 1,600, 140, 1,200, 750, 1,900, and 900, respectively.

The elemental composition of bacteria has not been analyzed extensively. Although increasing interest has developed in the role of mineral elements in the physiology of microbial cells (7, 17, 37, 43) and their transformations in nature (12, 15, 33, 35), data on the bioaccumulation of mineral cations by bacteria have remained sparse (2, 18, 20, 27). Washing procedures have been variable, if practiced at all. The conditions of growth such as pH (24, 32), elemental composition of the medium (7, 37), growth rate (32, 37), and the source of the electron donor in photosynthetic organisms (40) have influenced the elemental composition of bacteria.

The bioaccumulation of cations by marine bacteria has not been studied (14). Marine plants and animals reportedly concentrate trace elements from seawater by several orders of magnitude (8, 18, 23, 25, 26, 29, 30, 34, 39, 41, 42). Undersaturation of many trace elements in seawater has been attributed partially to assimilation by organisms (9).

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Since a sufficient biomass of specific marine bacteria free of contaminating particles cannot be obtained directly from natural seawater, 22 pure cultures of bacteria were grown in a basal seawater medium for cationic analyses. The elemental composition of bacteria from a single medium should provide data for analytical and generic variability.

## MATERIALS AND METHODS

**Microorganisms.** Twenty-two cultures of bacteria were distributed into three groups: (i) five previously identified marine bacteria, namely, *Alcaligenes marinus* (formerly *Arthrobacter marinus* ATCC 25374) (J. B. Rake and G. E. Jones, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, I33, p. 160; 3), *Alteromonas haloplanktis* (B16 strain of R. A. MacLeod; ATCC 19855 and NCMB 19) (22, 28), *Pseudomonas putida* Roche (formerly *P. cuprodurans* ATCC 29735) (F. J. Passman, Ph.D. thesis, University of New Hampshire, Durham, 1977; 14), *Pseudomonas* sp. (NCMB 130), and *Vibrio fischeri* (ATCC 15382 and NCMB 1143), the facultative psychrophilic strain (4, 5, 10); (ii) 12 different, unidentified pure cultures of marine bacteria freshly isolated from the Great Bay estuarine complex, Durham, N.H.; and (iii) five identified pure cultures of nonmarine bacteria, namely, *Bacillus megaterium* (laboratory strain), *Escherichia coli* (NCLB 8114), *Pseudomonas fluorescens* (NCLB 8858), *Pseudomonas ovalis* (NCLB 9229), and *Staphylococcus aureus* (NCLB 6571).

**Culture conditions.** The basal seawater medium contained 1 g of yeast extract (Difco Laboratories, Detroit, Mich.); 1 g of tryptone (Oxoid Ltd., London); and 1,000 ml of 75% synthetic seawater (14). The final pH of the medium was 7.5 to 7.8. The calculated cationic composition of the basal medium in parts per million was: Na, 7,459.255; Mg, 995.426; K, 297.863; Ca, 300.778; Mn, 0.013; Fe, 0.166; Ni, 0.016; Cu, 0.020; Zn 0.196; Cd 0.002; and Pb 0.019 (14).

The inoculum, growth conditions, and harvesting and drying of cells have been described (14).

**Cell analyses.** Ashing and flame and atomic spectrophotometric techniques have been described (14).

## RESULTS

The reproducibility of the elemental analyses for *Alcaligenes marinus* grown under identical conditions on eight separate occasions was examined initially. Cells were grown in 500 ml of basal seawater medium contained in 1-liter Erlenmeyer flasks inoculated with 0.2 ml of inoculum (optical density at 420 nm [$OD_{420}$] = 0.95 to 1.05) (14). The cells were harvested from three pooled flasks after 18 to 24 h ($OD_{420}$ = 0.96 to 1.03). Weights of washed cells were greater than 100 mg (dry weight) in all cases.

The coefficient of variation in single determinations of the major cation (Na, Mg, K, Ca) concentrations was high, 40 to 51% (Table 1). This was due to variability in washing techniques. The coefficient of variation in single determinations of the minor cations was lower, varying from 22% for Zn and Fe to 41% for Ni. The trace elements were retained more firmly than the major cations by the cells of *A. marinus*. Only those differences which lay outside the coefficient of variation of a single result as determined by this experiment were considered significant in subsequent experiments.

To determine the effect of different washing solutions on elemental composition, *A. marinus* was grown in basal seawater medium at 21 ± 1°C for 18 h in 1,400-ml replicate amounts to the same cell density. The highest dry weight was obtained from unwashed cells, 780.7 mg (Table 2). Since some of this dry material was interstitial medium constituents and adsorbed substances, washing was essential. Three washes with distilled water reduced the dry weight to 332.6 mg, a reduction of 57%. One wash of 0.5 M ammonium formate reduced the dry weight by 26%, and three washes reduced it by 48%. Three washes of 0.01 M phosphate buffer reduced the dry weight by 39%, and three washes of phosphate buffer plus $10^{-4}$ M ethylenediaminetetraacetic acid reduced it by 31%. Thus, washing was necessary to remove noncellular material. Some lysis may have occurred during the washing procedures.

The apparent removal of major ions by the washes was considerable when compared with the basal medium composition previously cited (Table 2). Thus, the unwashed cellular pellets retained appreciable amounts of these major ions by the negatively charged cells. Na was removed by all washing solutions to amounts less than in the medium. Mg was removed similarly by the washes except for distilled water and one wash of ammonium formate (Tables 1 and 2). K was only reduced drastically in the three washes of ammonium formate. Ca was retained by the cells or increased in most instances. Variability of results for the major ions was attributed to the type of washing solution,

TABLE 1. *Cation composition of A. marinus grown in basal seawater medium at 20 ± 3°C to late logarithmic grown in eight separate experiments*

| Expt | Cellular cation concn (ppm, dry wt) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Na | Mg | K | Ca | Mn | Fe | Ni | Cu | Zn | Cd | Pb |
| 1 | 4,600 | 1,850 | 600 | 950 | 1.5 | 328 | 2.7 | 16 | 84 | 3.9 | 13 |
| 2 | 2,000 | 1,390 | 300 | 530 | 2.2 | 227 | 1.7 | 22 | 119 | 2.6 | 12 |
| 3 | 8,000 | 1,890 | 1,100 | 820 | 2.2 | 292 | 1.5 | 14 | 131 | 3.4 | 14 |
| 4 | 4,700 | 1,090 | 200 | 230 | 1.1 | 176 | 0.8 | 14 | 94 | 1.9 | 7 |
| 5 | 3,700 | 700 | 300 | 370 | 1.3 | 257 | 1.2 | 15 | 153 | 1.9 | 11 |
| 6 | 7,600 | 2,800 | 900 | 1,350 | 2.1 | 272 | 2.0 | 9 | 146 | 3.3 | 5 |
| 7 | 8,300 | 2,180 | 900 | 1,170 | 2.0 | 313 | 2.8 | 11 | 150 | 2.2 | 10 |
| 8 | 6,200 | 700 | 1,000 | 900 | 1.4 | 184 | 1.0 | 14 | 97 | 3.0 | 7 |
| Mean | 5,600 | 1,580 | 700 | 790 | 1.7 | 256 | 1.7 | 14 | 122 | 2.8 | 10 |
| Standard deviation[a] | 2,260 | 740 | 360 | 390 | 0.5 | 57 | 0.7 | 4 | 27 | 0.7 | 3 |
| Coefficient of variation[b] | 40 | 47 | 51 | 49 | 29 | 22 | 41 | 29 | 22 | 25 | 30 |
| Standard error[c] | 800 | 260 | 130 | 140 | 0.2 | 20 | 0.3 | 1 | 10 | 0.3 | 1 |

[a] $\pm \sqrt{\frac{\sum y^2}{n-1}}$.

[b] (Standard deviation × 100)/mean.

[c] Standard deviation/$\sqrt{n}$.

TABLE 2. *Cation composition of A. marinus grown in basal seawater medium at 21 ± 1°C for 18 h, using different washing solutions*

| Washing treatment | Dry wt (mg) | % of wt retained during washing | Cellular cation concn (ppm, dry wt) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Na | Mg | K | Ca | Mn | Fe | Ni | Cu | Zn | Cd | Pb |
| None | 780.7 | 100 | 30,330 | 3,200 | 13,800 | 3,880 | 2.1 | 189 | 6.1 | 11 | 63 | 2.3 | 13 |
| 3 × 200 ml of distilled water | 332.6 | 43 | 3,100 | 3,550 | 3,000 | 3,090 | 1.2 | 280 | 3.9 | 24 | 89 | 2.4 | 17 |
| 1 × 200 ml of 0.5 M ammonium formate | 580.4 | 74 | 4,700 | 1,260 | 570 | 910 | 1.4 | 238 | 3.8 | 9 | 86 | 0.9 | 10 |
| 3 × 200 ml of 0.5 M ammonium formate | 410.3 | 52 | 2,000 | 390 | 80 | 530 | 2.2 | 296 | 5.3 | 22 | 119 | 2.6 | 27 |
| 3 × 200 ml of 0.01 M phosphate buffer | 477.3 | 61 | 4,000 | 510 | 4,350 | 620 | 1.7 | 249 | 5.2 | 39 | 82 | 3.3 | 45 |
| 3 × 200 ml of 0.01 M phosphate buffer × $10^{-4}$ M EDTA[a] | 534.9 | 69 | 4,100 | 500 | 6,210 | 260 | 1.5 | 217 | 4.7 | 18 | 60 | 3.2 | 18 |

[a] EDTA, Ethylenediaminetetraacetic acid.

inconsistencies during mechanical stirring of cells when washing, and maintenance of constant cold temperature.

Among the trace elements, firm retention by the cells during the various washing procedures was indicated. Mn, Fe, Ni, Cu, Zn, Cd, and Pb were retained by *A. marinus* cells within a half order of magnitude for each element regardless of washing solution. Where cells were only washed once with ammonium formate, the lower amount of the trace cations relative to three washings was ascribed to incomplete removal of other elements, resulting in a larger dry weight for the singly washed cells. The washing procedure was considered the greatest single source of variability in the elemental analyses of bacterial cells.

The cations retained by the identified marine and unidentified estuarine bacteria grown in basal seawater medium are presented in Table 3. Excluding widely anomalous amounts of Na, Mg, K, and Ca in a few samples, the concentration factors for these cations were 0.04, 0.6, 0.35, and 0.8, respectively, indicating less retention by the cells than the medium after three washes with chilled ammonium formate. The anomalous values were invariably higher than the mean; their inclusion in the calculations would tend to bring the mean closer to unity for major cations in cells and medium.

Other than the anomalous value of 16 ppm (dry weight) Mn in UNH 4, the mean concentration in marine bacteria was 0.9 ppm (dry weight). Mn concentration by the 15 other cultures indicated a concentration almost 70-fold higher than in the basal seawater medium.

Fe was concentrated on the average in the marine cells to 288 ppm (dry weight), representing a concentration 1,700-fold higher than in basal seawater medium. Ni was concentrated on the average in the cells to 2.5 ppm (dry weight), a concentration of 160-fold higher than in the basal seawater medium. Cu, Zn, Cd, and Pb content in the cells averaged 28, 157, 4.3, and 16 ppm (dry weight), representing average concentrations 1,400-, 800-, 2,150-, and 840-fold higher than in the basal seawater medium. Thus, Mn and Ni were concentrated by about two orders of magnitude by the marine bacterial cells from the medium, and Fe, Cu, Zn, Cd, and Pb were concentrated by three orders of magnitude. The coefficient of variation ranged from 36% for Fe to 81% for Ca, excluding several indicated anomalous amounts (Table 3).

Cationic concentrations of five identified nonmarine bacterial cultures grown in basal seawater medium indicated wide coefficients of variation for the major cations (Table 4). Since only five cultures were analyzed, anomalous amounts were more difficult to detect. *E. coli* retained the highest concentrations of Na, Mg, and K and the lowest concentration of Ca. Major seawater cations remained in the nonmarine bacterial cells to a greater extent on average than in the marine bacteria, but with larger coefficients of variation.

Among the major cations in the dry weight of the nonmarine bacteria, Fe (178 ppm), Cu (18 ppm), Zn (113 ppm), and Cd (2.3 ppm) were reduced compared with marine bacteria. Mn (4.2 ppm) and Pb (22 ppm) indicated some tendency to be concentrated in the nonmarine bacteria. Within standard deviation, there was similarity of the cationic concentration in bacterial cells

TABLE 3. *Cation composition of 16 marine bacteria grown in basal seawater medium at 22 ± 3°C to late logarithmic growth*

| Bacterium | Dry wt (mg) | Cellular cation concn (ppm, dry wt) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Na | Mg | K | Ca | Mn | Fe | Ni | Cu | Zn | Cd | Pb |
| Identified | | | | | | | | | | | | |
| *Alteromonas haloplanktis* (NCMB 19) | 281 | 299 | 420 | 36 | 267 | 0.4 | 262 | 1.5 | 22 | 159 | 3.9 | 3 |
| *Pseudomonas putida* (ATCC 29735) | 219 | 255 | 292 | 36 | 272 | 0.9 | 422 | 4.5 | 19 | 112 | 4.6 | 3 |
| *Pseudomonas* sp. (NCMB 130) | 147 | 327 | 156 | 55 | 286 | 0.5 | 365 | 1.5 | 49 | 180 | 8.8 | 2 |
| *Vibrio fischeri* (NCMB 1143) | 135 | 357 | 359 | 89 | 165 | 1.5 | 351 | 1.5 | 29 | 101 | 10.4 | 2 |
| Unidentified | | | | | | | | | | | | |
| UNH 1 | 641 | 11 | 440 | 47 | 80 | 2.0 | 343 | 3.4 | 24 | 168 | 2.0 | 15 |
| UNH 3 | 470 | 192 | 536 | 85 | 216 | 0.4 | 290 | 4.7 | 33 | 206 | 4.3 | 15 |
| UNH 4 | 287 | (25,800)[a] | (8,939) | (4,184) | —[b] | (16) | 137 | 1.5 | 19 | 44 | 4.9 | 3 |
| UNH 5 | 172 | (2,565) | 705 | (1,166) | 186 | 0.3 | 314 | 3.5 | 50 | 227 | 6.4 | 45 |
| UNH 7 | 105 | 531 | 698 | 123 | 266 | 1.0 | 462 | 8.5 | 84 | 308 | 6.6 | 96 |
| UNH 11 | 739 | 433 | 391 | 202 | 70 | 0.3 | 132 | 0.7 | 10 | 57 | 1.4 | 1 |
| UNH 14 | 70 | 315 | (4,822) | 143 | 830 | 1.4 | 436 | 2.9 | 24 | 302 | 1.7 | 11 |
| UNH 15 | 228 | 281 | 737 | 114 | 150 | 2.2 | 205 | 0.9 | 21 | 153 | 1.8 | 4 |
| UNH 16 | 86 | 232 | 672 | 46 | 104 | 1.2 | 243 | 1.7 | 16 | 118 | 3.5 | 27 |
| UNH 23 | 207 | 290 | 663 | 87 | 136 | 0.4 | 289 | 1.0 | 17 | 161 | 1.7 | 11 |
| UNH 25 | 316 | 392 | 1,362 | 57 | 596 | 0.5 | 147 | 1.5 | 8 | 53 | 2.6 | 4 |
| UNH 26 | 613 | 227 | 463 | 326 | 160 | 0.7 | 222 | 0.8 | 22 | 169 | 3.4 | 20 |
| Mean | | 296 | 564 | 103 | 252 | 0.9 | 288 | 2.5 | 28 | 157 | 4.3 | 16 |
| Standard deviation | | 121 | 289 | 79 | 204 | 0.6 | 104 | 2.0 | 19 | 78 | 2.6 | 24 |
| Coefficient of variation | | 40 | 51 | 76 | 81 | 66 | 36 | 80 | 67 | 50 | 60 | 66 |
| Concn factor | | 0.04 | 0.6 | 0.35 | 0.8 | 69 | 1,700 | 160 | 1,400 | 800 | 2,150 | 840 |

[a] Numbers in parentheses are anomalous values not calculated.
[b] —, Not determined.

TABLE 4. *Cation composition of five nonmarine bacteria grown in basal seawater medium at 20 ± 2°C to late logarithmic growth*

| Bacterium | Dry wt (mg) | Cellular cation concn (ppm, dry wt) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Na | Mg | K | Ca | Mn | Fe | Ni | Cu | Zn | Cd | Pb |
| *Bacillus megaterium* (laboratory strain) | 143 | 298 | 1,178 | 149 | 1,655 | 10.5 | 339 | 2.4 | 36 | 186 | 1.0 | 13 |
| *Escherichia coli* (NCB 8114) | 335 | 3,341 | 1,351 | 1,789 | 42 | 2.7 | 111 | 0.6 | 11 | 76 | 3.1 | 11 |
| *Pseudomonas fluorescens* (NCB 8858) | 454 | 365 | 544 | 99 | 48 | 3.1 | 99 | 2.6 | 19 | 93 | 2.9 | 39 |
| *P. ovalis* (NCB 9229) | 379 | 221 | 660 | 26 | 834 | 0.5 | 256 | 2.8 | 12 | 108 | 0.4 | 18 |
| *Staphylococcus aureus* (NCB 6571) | 482 | 66 | 776 | 207 | 112 | 4.4 | 86 | 0.6 | 14 | 104 | 4.3 | 30 |
| Mean | | 858 | 902 | 454 | 538 | 4.2 | 178 | 1.8 | 18 | 113 | 2.3 | 22 |
| Standard deviation | | 1,392 | 347 | 749 | 708 | 3.8 | 113 | 1.1 | 10 | 42 | 1.6 | 12 |
| Coefficient of variation | | 162 | 38 | 165 | 130 | 90 | 64 | 61 | 57 | 38 | 70 | 54 |
| Concn factor | | 0.1 | 0.9 | 1.5 | 1.7 | 320 | 1,100 | 110 | 400 | 600 | 1,150 | 1,200 |

grown in the same medium under similar cultural conditions.

## DISCUSSION

Chilled ammonium formate (0.5 M) was used in this study to wash the bacterial cell pellets since it was used by Riley and Roth (29) for washing phytoplankton, providing osmolarity and volatilizing during drying at 105 to 110°C. Although the necessity for washing *A. marinus* cells was established to remove loosely bound cations (Table 2), the efficacy of ammonium formate treatment must be questioned. The major cations of seawater were reduced drastically from the cells during this procedure. Other investigators (1, 6, 7, 16, 17, 21, 31, 32) using a variety of procedures indicated higher retention of these major ions by bacterial cells. Kung et al. (17) reported that *E. coli* B/r grown expo-

nentially contained 2,800 ppm of Mg, 12,000 ppm of K, 2,300 ppm of Ca, and 150 ppm of Zn when washed with 10 ml of warm 0.3 M glucose solution. Jasper and Silver (11) reported that total cellular Mg was generally in the range of 360 to 840 ppm (wet cells), although the growth medium may have varied 100,000-fold. Thus, our values for major ions in the ammonium formate-washed cells on a dry weight basis are low, indicating removal during the washing procedure. Table 2, subsequent work in this laboratory (Passman, Ph.D. thesis, 1977), and studies by others (6, 31) have indicated distilled water as a more effective wash which still reduces the concentrations of major cations in the cells.

Comparing our data for major cations with those of Takacs et al. (36) for *Alteromonas haloplanktis* indicates that only bound Na and K remain after washing with ammonium formate. Distilled water-washed cells of *B. megaterium* grown in a chemostat also generally indicated greater retention of major cations than those in Table 4 (32). An increase in the rate of growth resulted in increased content of Ca, Mg, and Fe, with no change in K (32). Although the present investigation indicates little to no concentration of major ions by the cells from the basal seawater medium due to removal by the ammonium formate wash, concentrations of an order of magnitude are not uncommon (37).

Minor ions were retained quite comparably to other studies (6, 31, 32) due to their strong retention by cellular components during washing. For Mn, marine bacteria averaged about 1 ppm and nonmarine bacteria averaged 4 ppm (Tables 3 and 4). These Mn concentrations are lower than those of Rouf (31) (20 ppm for *E. coli*) and Curran et al. (6) (40 ppm for *B. megaterium* vegetative cells). Mn increased from 40 to 250 ppm in cells of *Chromatium vinosum* grown photoheterotrophically versus photoautotrophically, suggesting its relative importance in autotrophic evolution (40). Freshwater cyanobacteria averaged almost 40-fold the Mn of marine heterotrophic bacteria (13). Bacteria from 18 strains of microorganisms isolated from the Mn biogeochemical province of the Georgian U.S.S.R. were reported to contain from 40 to 100,000 ppm of Mn (19).

*C. vinosum* grown photoautotrophically contained 210 ppm of Fe, whereas photoheterotrophically grown cells contained 590 ppm of Fe (40). Five freshwater cyanobacteria grown with light energy averaged a greater than 10-fold increase in Fe compared with heterotrophically grown bacteria (13). The Fe concentration in vegetative bacteria was quite consistent: 30 to 560 ppm (6); 140 to 280 ppm, excluding *Sphaerotilus natans* (31); 20 to 3,000 ppm in *B. megaterium*, depending on pH of the medium and rate of growth (32); and 86 to 462 ppm (Tables 3 and 4).

Ni concentration by bacterial cells was about two orders of magnitude higher in bacterial cells than in the medium (Tables 3 and 4). Only slightly higher concentrations of Ni in bacterial cells were reported by Rouf (31). $^{63}Ni$ appears to be transported by the system responsible for Mg accumulation by *E. coli* (11).

Cu uptake by *B. megaterium* was decreased from 22 to 3 ppm at pH 7 when the growth rate was increased in a chemostat from $D = \mu = 0.2$ $h^{-1}$ to $D = \mu = 0.7$ $h^{-1}$ (32). Cu concentration of the dry weight of vegetative bacterial cells by spectrographic analyses ranged only slightly higher than the concentrations in Tables 3 and 4 (6, 31).

The Zn content of bacterial cells ranged from 44 to 308 ppm (dry weight) (Tables 3 and 4). The mean concentrations in this study were slightly lower, about 100 ppm, than those of Rouf (31). Zn was shown to increase in the cells of *Alcaligenes marinus* as the concentration of Zn in the medium was elevated (14).

Although the Pb concentrations of bacterial cells reported here were only slightly higher than those of Rouf (31), *Micrococcus luteus* and *Azotobacter* sp. cells grown in a broth in contact with a dialysis membrane containing $PbBr_2$ immobilized 490,000 and 310,000 ppm Pb on a dry weight basis, respectively (38).

The elemental composition of bacterial cells still requires considerable investigation. The data presented indicate remarkably similar cation concentrations from 22 different bacterial species grown under the same conditions, suggesting a reasonable unity for their elemental composition.

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