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# SPECTROCHEMICAL ANALYSIS OF INORGANIC ELEMENTS IN BACTERIA

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ABSTRACT

ROUF, M. A. (Washington State University, Pullman). Spectrochemical analysis of inorganic elements in bacteria. J. Bacteriol. 88:1545-1549. 1964.—Quantitative spectrochemical analyses of inorganic elements in the vegetative cells of *Escherichia coli*, *Sphaerotilus natans*, *Micrococcus roseus*, *Bacillus cereus*, and the spores of *B. cereus* were made. The following elements were found to be present in the ash samples: B, Na, Mg, Al, Si, P, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Sr, S, Ag, Sn, Ba, Pb, V, and Mo. These could be divided into major, minor, and trace elements, depending on the relative amounts in the cells. Mg, P, K, and S were considered as the major elements; Ca, Fe, Zn, and, perhaps, Cu and Mn as the minor elements, and the rest as trace elements. Mg concentrations were higher in the cells of the gram-positive *M. roseus* and *B. cereus* than in the gram-negative *E. coli* and *S. natans*. The latter organism contained 2.6% $Fe_2O_3$ (dry weight basis). The vegetative cells of *B. cereus* were higher in Mg, P, K, Na, Ag, and lower in Si, Ca, Zn, Mn, and Cu than were its spores.

Numerous biochemical and physiological investigations have demonstrated the importance of inorganic elements in biological systems. The present knowledge about elements in bacteria is derived mainly from gross chemical analyses. The early investigators, Guillemin and Larson (1922), analyzed large quantities of *Escherichia coli* ash by chemical methods and found such common elements as Cl, Fe, Ca, P, Mg, S, Na, and K. More recently, Curran, Brunstetter, and Myers (1943) investigated some of the common inorganic elements of *Bacillus macerans*, *B. megaterium*, *B. cereus*, *B. albolactis*, *B. cohaerens*, *B. subtilis*, *Clostridium sporogenes*, *C. bifermentans*, and four other unidentified thermophiles by spectrographic method. However, the available data, especially for the less abundant elements, are few and not reliable (Luria, 1960). Knowledge of the elemental composition of bacteria is important for devising optimal nutrient media. Because of the accepted variation in elemental composition of bacteria, it is also important that a body of data on the elements of various organisms be developed.

The physiological activity of numerous trace elements has suggested their possible existence in protoplasm, although most of these elements have not been detected by analytical procedures. It was the purpose of the present investigation to obtain quantitative data on the inorganic elements, especially the less abundant ones, in bacteria by spectrochemical analyses. *Escherichia coli*, *Sphaerotilus natans*, *Micrococcus roseus*, and the vegetative cells and spores of *B. cereus* were analyzed quantitatively by spectrochemical methods for all of the detectable elements. Each organism was grown in a medium which supported good growth, and the medium varied with each organism.

## MATERIALS AND METHODS

*Bacteriological organisms and cultural conditions.* For *E. coli*, a medium composed of 1.25% $K_2HPO_4$, 0.25% $KH_2PO_4$, 0.02% $MgSO_4 \cdot 7H_2O$, 0.10% $(NH_4)_2SO_4$, and 1.0% glucose was used.

*S. natans* was cultivated in a medium described by Rouf and Stokes (1962). The medium contained 0.2% each of peptone (Difco) and glucose, 0.02% $MgSO_4 \cdot 7H_2O$, 0.005% $CaCl_2$, 0.001% $FeCl_3 \cdot 6H_2O$, and 0.01 M phosphate buffer (pH 7.0).

*B. cereus* was cultivated by the active culture technique of Nakata and Halvorson (1960) in a medium described by Nakata (1963). This medium contained 0.00005% $FeSO_4 \cdot 7H_2O$, 0.0005% $CuSO_4 \cdot 5H_2O$, 0.0005% $ZnSO_4 \cdot 7H_2O$, 0.005% $MnSO_4 \cdot 7H_2O$, 0.02% $MgSO_4$, 0.2% $(NH_4)_2SO_4$, 0.05% $K_2HPO_4$, 0.008% $CaCl_2$, 0.2% glucose, and 0.4% yeast extract.

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*M. roseus* was grown in a 3% Brain Heart Infusion (Difco) broth.

All media were prepared with distilled water, adjusted to pH 7.1, and distributed in 200-ml amounts into 2-liter flasks. Phosphates, wherever used, were sterilized separately.

Inocula were prepared from actively growing cultures. Cultures were grown on a New Brunswick rotary shaker at about 270 oscillations per min at 30 C. The vegetative cells were harvested by centrifugation late in the exponential phase of growth. *E. coli* was harvested after 20 hr of incubation, *S. natans* after 48 hr, and *M. roseus* after 72 hr. The inoculum for *B. cereus* was taken from an actively growing slant culture, transferred to the liquid medium (to slight turbidity), and incubated in the shaker at 30 C for 4 hr. A 10% (v/v) inoculum was then transferred to a second flask and incubated for 2.5 hr, and a third flask was inoculated in turn with the same size inoculum and incubated for 3.5 hr before harvesting. Spore stains at this stage showed no spores present. Incubation of the third flask for an additional 10 hr resulted in nearly 100% sporulation.

*Preparation of samples.* The distilled water used in the washing and preparation of samples was prepared by redistilling ordinary distilled water in an all-Pyrex glass apparatus. Extreme care was taken in all phases of the work to avoid metallic contamination from any possible source. Spatulas made of plastic were used. The harvested samples were washed four times in centrifuge bottles made of polypropylene, and the residue after final centrifugation was resuspended in a known small volume of water; samples of this were removed for dry weight determinations.

Ordinary methods of ashing may introduce errors in spectrochemical analyses. When a sample is dried and ashed in any container, the ash is difficult to remove without scraping the container. In doing so, one might contaminate the sample with materials from the container. Therefore, for ashing of the cells, Whatman filter paper no. 42 was sprayed with Krylon spray and air-dried. The sprayed and dried filter paper was folded in the shape of a cup and was placed in a porcelain cup. The filter papers thus prepared were leakproof and permitted easy removal of the ashed samples from the crucibles. Samples in 20-ml volume were placed on these cup-shaped filter papers and dried. The dried samples were ashed in a muffle furnace for 10 to 15 min at 700 C. The ash weight and analysis of the filter paper were made previously to correct for quantitative estimation of the bacterial samples.

*Spectrographic equipment and conditions (as reported by the Spectrographic Analyst).* Spectrograph: ARL 2-meter grating type, 5A/mm dispersion, wavelength range photographed (35-mm film) 2,280 to 4,950 A. Source conditions: direct current arc, 10 amperes. Film analysis: ARL projection-comparator densitometer. Other accessories: all standard equipment. Electrodes: Ultra Carbon Corp. (Bay City, Mich.) preformed electrodes. Type 101L was used for samples yielding 10 mg or more of ash. Types UCP 118 (5 mg), 117 (2 mg), 116 (1.0 mg), and 115 (0.5 mg) were used for smaller weights of ash. In each case, the largest size electrodes were burned to completion, with the burn time and exposure varied according to the electrode selected.

*Method.* The samples were ashed to constant weight in a muffle furnace at 700 C. A 10-mg portion or less, depending on the total weight of the residue, was weighed on a suitable balance and an equal weight of high purity powdered graphite (Grade SP No. 2) was added to, and mixed with, the sample. The mixture was tamped into an electrode of suitable size. A similar procedure was used for the standards. In analyzing the films, the concentrations of the various elements in the samples were determined by densitometric comparison of spectrum lines in standards and samples (Harvey, 1950).

*Preliminary survey.* A preliminary survey was made of those samples with more than sufficient amounts of residue, to determine the range of concentration of various elements that would have to be accommodated in the standards.

*Preparation of standards.* On the basis of the preliminary survey, a series of seven basic standard mixtures were prepared from the highest purity dry reagents available, avoiding salts that were hygroscopic or those containing water of crystallization. These seven samples contained only the major constituents (Table 1), with the widest concentration ranges occurring in calcium, potassium, and silicon. Two series of mixtures of oxides of those elements present in minor or trace amounts were prepared. One contained, as oxides, Al, Ti, Mn, Fe, Zn, Sr, Sn, and Pb. The other contained, as oxides, V, Cr, Ni, Cu, Mo, Ag, and Ba. These were added in varying amounts to the basic standards, to provide a range of concentra-

TABLE 1. *Composition of standard solutions*

| Salt used | Oxide form | Percentage as oxide in standard | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| $Ca_3(PO_4)_2$ | CaO | 40.6 | 24.4 | 10.8 | 5.4 | 4.2 | 2.16 | 1.1 |
| $K_2HPO_4$* | $K_2O$ | 5.4 | 13.7 | 24.4 | 40.5 | 16.5 | 10.8 | 5.4 |
| Total | $P_2O_5$ | 38.5 | 20.6 | 27.5 | 35.3 | 16.0 | 10.0 | 5.0 |
| MgO | MgO | 1.0 | 5.0 | 10.0 | 2.0 | 10.0 | 5.0 | 2.0 |
| $SiO_2$ | $SiO_2$ | 5.0 | 20.0 | 10.0 | 2.0 | 40.0 | 60.0 | 80.0 |
| $Na_2CO_3$ | $Na_2O$ | 0.59 | 2.92 | 5.85 | 1.17 | 2.92 | 1.17 | 0.59 |
| $B_2O_3$ | $B_2O_3$ | 2.0 | 1.0 | 0.50 | 0.20 | — | — | — |
| Inclusive | $CO_2$ | 0.42 | 8.5 | 4.2 | 0.83 | 2.08 | 0.83 | 0.42 |
| Inclusive | Water | 0.52 | — | 2.34 | 3.9 | 1.56 | 1.04 | 0.52 |
| Total | | 94.0 | 96.0 | 95.5 | 91.2 | 93.0 | 91.0 | 95.0 |
| Total minor elements to be added | | 6.0 | 4.0 | 4.5 | 8.8 | 7.0 | 9.0 | 5.0 |

* $K_2CO_3$ used in standard 2.

tions and to restore the total oxides to 100% (Table 1).

After trace and minor elements had been added to the seven basic standards, a further variation in composition was made. A portion of each standard was mixed with an equal part of $Na_2CO_3$, $K_2CO_3$, and $CaCO_3$ successively, thus effectively halving the concentration of all of the other elements in each standard and making each series largely a sodium, potassium, or calcium system. This was done to determine the effect of matrix changes on the percentage of the remaining elements.

## RESULTS AND DISCUSSION

The quantitative estimation of some elements is influenced by the amount of washing which the samples receive prior to their analysis. At the same time, it is important to wash the samples properly before analyses to eliminate extraneous materials. Curran et al. (1943) found that, of the various elements, only potassium was lost in an appreciable amount because of washing. Also, since sodium is present in the free salt form (Guillemin and Larson, 1922), it, also, is expected to be lost to a considerable extent by washing. Other elements occur to a much greater degree in bound form. In the present investigation, the various samples were washed four times, to eliminate possible impurities which may have adhered to the cells.

The results of the quantitative analyses of the various samples are presented in Tables 2 and 3. For convenience, the data in Table 2 are presented as per cent dry weight, and the data in Table 3 as parts per million. The calculations of the various elements present in the dry weight of the bacterial samples were made in the common oxide form. The data from the spectrochemical films were first calculated as per cent of oxide in the ash and then converted to the dry weight of the bacterial samples. The per cent of ash on a dry weight basis for *E. coli* was 6.2; for *S. natans*, 10; for *M. roseus*, 7.1; for the vegetative cells of *B. cereus*, 6.7; and for the spores of *B. cereus*, 9.6. Sulfate, although not detected by spectrochemical analysis, was calculated by the addition of all oxides in the ash and then restoring the total to 100%. Thus, if all of the oxides in the ash came to 84%, then sulfate was considered to be 16%.

It is apparent from Tables 2 and 3 that the inorganic elements present in bacterial samples can be classed into three groups: major, minor, and trace elements. Mg, P, K, and S may be considered major elements, since they are present in relatively high concentrations. Ca, Fe, Zn, and, perhaps, Cu and Mn may be considered minor, and the rest, trace elements. Curran et al. (1943) also found that K, P, Mg, and Ca are present in relatively high concentrations in *Bacillus* and *Clostridium* species.

Major elements in some organisms are minor or trace in other organisms. For example, Fe, which is a minor element in most of the bacteria,

TABLE 2. *Inorganic elements in vegetative cells and spores of bacteria*

| Organism | Per cent dry weight of cells and spores | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | MgO | $P_2O_5$ | $K_2O$ | $SO_4$ | $Na_2O$ | $SiO_2$ | CaO | $Fe_2O_3$ | ZnO |
| *Escherichia coli* | 1.04 | 9.83 | 1.42 | 2.58 | 0.01 | 0.09 | 0.02 | 0.03 | 0.01 |
| *Micrococcus roseus* | 1.99 | 7.46 | 1.11 | 2.39 | 0.85 | 0.14 | 0.10 | 0.02 | 0.03 |
| *Sphaerotilus natans* | 0.95 | 4.89 | 0.20 | 1.72 | 0.10 | 0.05 | 0.23 | 2.60 | 0.03 |
| *Bacillus cereus* | 1.72 | 7.42 | 5.52 | 2.09 | 0.75 | 0.06 | 0.04 | 0.03 | 0.03 |
| *B. cereus** | 0.95 | 2.81 | 2.19 | 2.11 | 0.05 | 1.05 | 0.80 | 0.04 | 0.32 |

* This entry refers to spores. The rest are vegetative cells.

TABLE 3. *Inorganic elements in vegetative cells and spores of bacteria*

| Organism | Parts per million of dry wt of cells and spores | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $B_2O_3$ | $Al_2O_3$ | $TiO_2$ | $Cr_2O_3$ | MnO | NiO | CuO | SrO | $Ag_2O$ | $SnO_2$ | BaO | PbO | $V_2O_5$ | $MoO_3$ | Co |
| *Escherichia coli* | 40 | 44 | 2 | 3 | 27 | 6 | 42 | 4 | 0.6 | 5 | 5 | 12 | —* | 4 | — |
| *Micrococcus roseus* | 25 | 64 | 7 | 2 | 9 | 7 | 18 | 5 | 0.7 | 14 | 5 | 4 | — | 7 | — |
| *Sphaerotilus natans* | — | 36 | 8 | 1 | 53 | 9 | 31 | 8 | 1.0 | 30 | 5 | 15 | 11 | — | — |
| *Bacillus cereus* | 8 | 31 | 3 | 0.5 | 620 | 3 | 43 | 4 | 2.0 | 5 | 2 | 8 | — | — | — |
| *B. cereus*† | — | 29 | 3 | 3 | 1,560 | 4 | 937 | 10 | 0.6 | 6 | 5 | 4 | 5 | — | — |
| Minimal detectable amount | 3.0 | | 0.5 | 0.1 | 0.7 | 0.4 | | 0.1 | 0.1 | 0.1 | 0.6 | 0.1 | 0.5 | 0.2 | 0.5 |

* None detectable.

† This entry refers to spores. The rest are vegetative cells.

is a major one, however, in *S. natans*. The high concentration of Fe in this case is understandable, since this organism is known to accumulate iron. Likewise, Mn, which is a trace element in most of the bacteria, is a minor one in *B. cereus*. The reason for the high Mn concentration in *B. cereus* is not clear. These variations, however, may merely reflect variations in the amounts of the different elements present in the growth media.

It is necessary to mention here that, although the organisms in the present investigation were grown in different media, the results offer some interesting differences and similarities in mineral composition. The primary objective of this study was to obtain reliable information about all elements that are present in the bacterial cells when the cells are grown in media which support good growth. No attempt was made in this investigation to determine what influence, if any, various concentrations of an element in the medium had on the concentration found in the cells.

The concentration of sodium and potassium is difficult to estimate quantitatively, as both of the elements can be lost to a considerable extent by washing. Sodium ions are taken up in considerable amounts by microorganisms to equalize the osmotic pressure caused by various ions present in the media. It is also interesting to note that the Mg concentration is higher in gram-positive *B. cereus* and *M. roseus* than in gram-negative *E. coli* and *S. natans*. Vanadium was detected in the cells of *S. natans* and the spores of *B. cereus*. Molybdenum was detected in *E. coli* and *M. roseus* cells. Molybdenum, replaceable by vanadium, is known to be present in *Azotobacter*, as well as in other organisms (Mulder, 1948). The presence of either V or Mo, but not both, in the cells suggests that these two elements are perhaps interchangeable in microorganisms. Cobalt is known to be a part of the vitamin $B_{12}$ molecule which is synthesized by many bacteria, but it is present in quantities too small to be detected by spectrochemical analysis of the ash (minimal detection level is 5 ppm in ash).

The uninoculated media were also analyzed (Table 4) to determine the initial abundance of the elements. Some elements, such as Ti, Cr, Mn,

TABLE 4. *Mineral composition of the initial media and distilled water (as oxides in per cent of ash)*

| Oxide form | Medium for | | | | Distilled water* |
|---|---|---|---|---|---|
| | *Escherichia coli* | *Micrococcus roseus* | *Sphaerotilus natans* | *Bacillus cereus* | |
| $B_2O_3$ | —† | — | — | 0.009 | 2.3 |
| $Na_2O$ | 0.056 | 50.0 | 1.3 | 6.0 | 1.8 |
| MgO | 8.4 | 0.10 | 2.3 | 1.9 | 0.74 |
| $Al_2O_3$ | 0.0057 | 0.0024 | 0.009 | 0.003 | 0.50 |
| $SiO_2$ | 0.016 | 0.15 | 0.022 | 0.24 | 49.0 |
| $P_2O_5$ | 17.0 | 7.6 | 16.2 | 5.4 | 2.9 |
| $K_2O$ | 37.0 | 3.9 | 54.0 | 22.0 | 0.10 |
| CaO | 0.015 | 0.10 | 0.27 | 0.55 | 7.2 |
| $TiO_2$ | — | — | — | — | 0.048 |
| $Cr_2O_3$ | — | 0.0008 | — | — | 0.006 |
| MnO | — | — | — | 1.3 | 0.055 |
| $Fe_2O_3$ | 0.055 | 0.10 | 0.11 | 0.06 | 2.7 |
| NiO | — | — | — | 0.007 | 0.017 |
| CuO | 0.0002 | 0.0002 | 0.001 | 0.13 | 0.068 |
| ZnO | — | — | — | 0.90 | 0.26 |
| SrO | — | 0.0009 | 0.03 | 0.022 | 0.011 |
| $Ag_2O$ | 0.0005 | — | 0.0004 | 0.0004 | 0.034 |
| $SnO_2$ | — | — | — | — | 0.15 |
| BaO | 0.0016 | — | — | 0.001 | 0.012 |
| PbO | — | — | — | 0.002 | 0.04 |
| $V_2O_5$ | — | 0.004 | — | 0.0015 | — |
| $MoO_3$ | — | — | — | 0.0009 | — |
| Total‡ | 62.5500 | 61.9583 | 74.2424 | 38.5268 | 67.9410 |

* Distilled water (1 liter) yielded 0.18 mg of ash.

† None detectable.

‡ The remaining amount to bring the total to 100% is considered to be sulfates.

Ni, Zn, Sn, Pb, V, and Mo were, in most cases, below the detectable level. These elements were present, however, in the bacterial cells. They must have been concentrated by the organisms from the undetectable level in the media to the detectable level in the cells. The elements can be detected in the distilled water used in the preparation of the media, as shown in Table 4, if the ash from large amounts of water is analyzed.

The differences in composition of the bacterial cells were not large, in most instances. Most organisms have a similar general pattern of mineral composition. There are, however, some interesting differences in mineral composition, which may be of significance, between the vegetative cells and the spores of *B. cereus*. The cells were higher in Mg, P, K, Na, and Ag and lower in Si, Ca, Zn, Mn, and Cu than were the spores. These results agree with those of Curran et al. (1943), who found the vegetative cells high in K and low in Ca and the spores low in K and high in Ca, Cu, and Mn. The significance of the high content of Si, Zn, Mn, and Cu in spores is not understood. Ca is perhaps associated with high tolerance of spores to heat.

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