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QUANTITATIVE ELEMENTAL ANALYSIS OF BACTERIAL POLYPHOSPHATE BODIES USING SCANNING TRANSMISSION ELECTRON MICROSCOPY AND ENERGY-DISPERSIVE X-RAY SPECTROSCOPY

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The elemental composition of polyphosphate bodies (PPB's) has been determined qualitatively previously using energy dispersive x-ray spectrometers (EDX) and in bodies isolated from cells. In this present study we determine in several bacteria their quantitative elemental composition using EDX in conjunction with a STEM.

Bacteria were grown as previously described or were collected from natural sources.[1,2] Portions of each sample were air dried on formvar coated grids. Separate samples were fixed and embedded in Epon. For x-ray analysis cells of interest were first located using the TEM mode. The microscope was then switched to the STEM mode. Analysis of cell components was carried out in the spot mode (75 kV) of the STEM. The spot diameter at 100,000x is approximately 20nm. The objective lens current was adjusted so a total x-ray count of 600 to 1,000 cps was attained for the 100 sec. count time. Spectra were collected on a PGT IMIX (EDX). The data was analyzed using a bulk sample analysis program (ZAF method) in standardless mode (w/w). Latex spheres of known density (1.05 g per cu cent) and volume were analyzed to obtain a standard (mass/weight).[3,4]

Approximately 40 bodies in each sample were analyzed and averages of the elements present were calculated in various laboratory grown stock cultures of photoautotrophs and heterotrophs as well as isolates from Lake Arthur in Black Rock Forest, NY. The quantitative analysis of laboratory grown organisms revealed (bulk analysis) that a typical invivo PPB contains on average O (65%), C (19%), P (10%) and 6% of the minor elements. Minor elements include Mg (2%) and Ca (1%) and frequently K (1%), Fe (0.9%), S (0.8%) and Al (1%). When this data is used in the equations provided by Heldal[3,4] it translates to O (4.3×10^{-8}ug), C (1.2×10^{-8}ug), P (6.7×10^{-9}ug), Mg (1.3×10^{-9}ug), Ca (6.7×10^{-10}ug), K (6.7×10^{-10}ug), Fe (6.0×10^{-10}ug), S (5.4×10^{-10}ug) and Al (5.9×10^{-10}ug). PPB's in thin sectioned material had essentially the same composition as those from laboratory cultured air dried preparations.

Quantitative x-ray analysis of samples from nature indicate that there are more minor elements present than in the cultured cells particularly Zn. The PPB's in the natural samples had O (66%), C (19%), P (7%). On average they were composed of 8% minor elements with Mg (4%), Ca (1%), Zn (0.5%), S (1%), K (1%) and Fe and Al together (0.5%). When these mass percentages are converted to actual amounts with the Heldal equations[3,4] they are O (1.63×10^{-8}ug), C (4.75×10^{-9}ug), P (2.50×10^{-9}ug), Mg (5.0×10^{-10}ug), Ca (2.50×10^{-10}ug), K (2.50×10^{-10}ug), Fe (2.25×10^{-10}ug) and S (2.0×10^{-10}ug).

The presence of C at 19% of the bodies and the excess O in relation to the ratio P to O in PO_4 units indicates an organic component. In a study of isolated bodies from *Micrococcus lysodeikticus* they found 24% protein and 30% lipid in addition to the other elements. These and our results indicate

that the PPB's may have other unknown functions in addition to essential element storage and acting as a detoxification method by sequestering heavy metals. [1,2]

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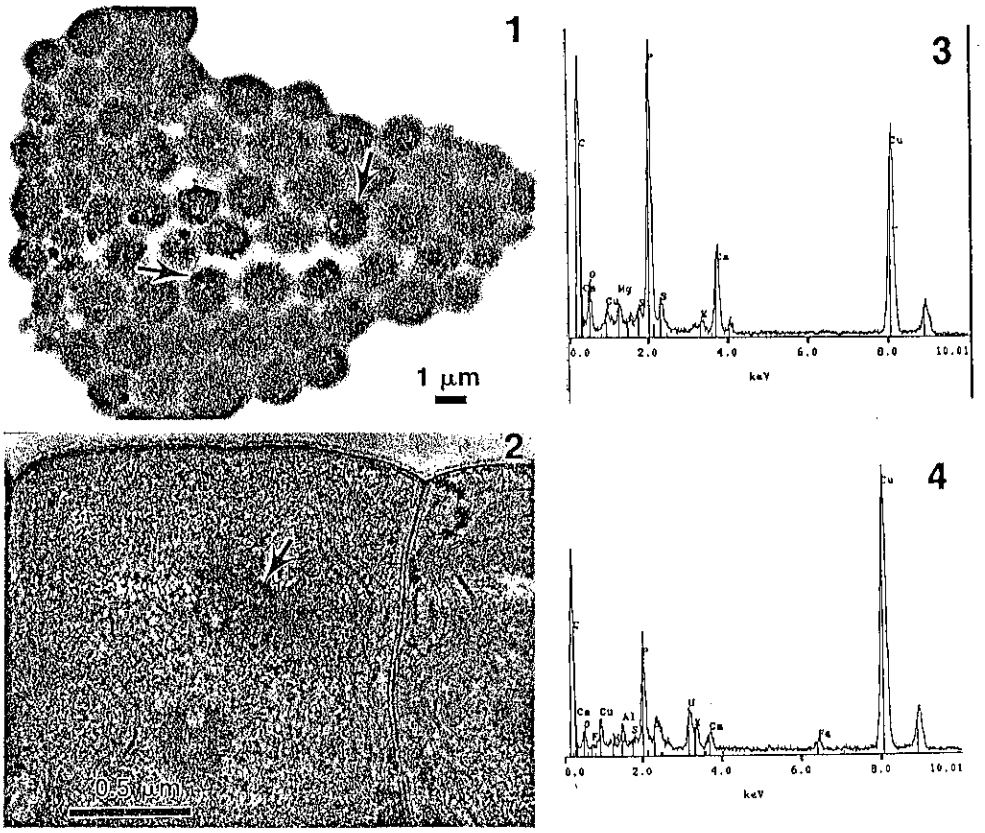

Fig. 1 Whole cells air dried of *Staphlococcus aureus*. Dense bodies at arrows are PPB's.
Fig. 2 Thin section of *Plectonema boryanum* . Dense body at arrow is a PPB.
Fig. 3 Spectrum of a PPb
Fig. 4 Spectrum of a PPB with a significant Fe peak.