Figure S1. 300 nm-thick plastic section of *A. longum* spores. Traditional EM preparation methods result in variability of poly-P storage granule preservation. Left: storage granules (black arrows) appear dense and are well preserved; Right: the dense poly-P material is lost leaving an empty hole. A thin layer of dense material (white arrows) surrounds in some empty storage granules. The spores represented here were picked from the same plastic section.
Figure S2. Spores of other species lack storage granules. Tomographic slices through mature spores from A) *Clostridium sporogenes* and B) *Bacillus subtilis* that lack the genes associated with poly-P formation. C) *B. cereus* and D) *B. thuringiensis* have the genes for poly-P formation. All imaged Gram-positive organisms lack dense SGs in their mature spores. Scale bar 200 nm.
Figure S3. Elemental composition of pure spores using NanoSIMS. A and B) Elemental analysis of three *A. longum* spores. Distribution of $^{14}$N$^{12}$C$^{-}$ and $^{31}$P$^{-}$ ions are shown in A and B respectively. Cts indicates counts of ions detected. The region of interest applied for the generation of ratio images in panel C is contoured in white. C) Ratio image of $^{31}$P$^{-}$/14N$^{12}$C$^{-}$ showing areas of elevated phosphorus ions in the spores. A smoothing factor of 9 was applied to images A-C. Due to the lower sensitivity of NanoSIMS for phosphorus, the $^{31}$P$^{-}$ signal for DNA and RNA from the core of mature spores is not readily detected. D) Representative even distribution of $^{12}$C$^{-}$, $^{19}$F$^{-}$, $^{31}$P$^{-}$ and $^{35}$Cl$^{-}$ in a single *B. thuringiensis* spore. Lighter colors represent higher counts. Panel D reprinted from Ghosal et al 2010.
Figure S4. Storage granule identification. A) Presence (black squares) or absence (empty squares) of genes encoding granule formation in *A. longum* and six other control organisms. Abbreviations: *glgBI* and *glgBII* – glycogen-branching enzyme 1 and 2; *ppk* – polyphosphate kinase 1 or 2; *ppx* – exopolyphosphatase; *sgpA* and *sgpB* – sulfur globule protein A and B; *phaP* – phasin; *phaC* – poly(3-hydroxybutyrate) polymerase; *phaZ1* and *phaZ2* – poly(3-hydroxybutyrate) depolymerase. *A. longum* has the genes required for glycogen and polyphosphate granule synthesis. B) Appearance of storage granules in representative organisms. From top to bottom: Negative-stained glycogen storage granules in *E. coli* appear as large numbers of small granules. Reprinted from Leduc et al 1989. A tomographic slice through a *C. crescentus* cell showing phosphorus-rich bodies with diameters ranging from 30 to 180 nm. Reprinted from Comolli et al 2006. Negative-stained *A. vinosum* cells showing empty sulfur globules enclosed in a protein envelope. Reprinted from Prange et al 2004. A tomographic slice though PHB granules in *R. eutropha*. The granules appear dense, uniform in texture and circular. Reprinted from Beeby et al 2012.