Microbial Detection at Low Levels by [125]I Radiolabeling. D. P. Summers[1] and H. K. Kagawa[2], [1]Carl Sagan Center, SETI Institute (c/o NASA Ames Research Center, MS 239-4, Moffett Field, CA, David.P.Summers@nasa.gov), 2Carl Sagan Center, SETI Institute (c/o NASA Ames Research Center, MS 239-15, Moffett Field, CA).

Introduction: It has long been acknowledged that culturing is an inadequate method for the detection of microorganisms. Modern methods that detect the presence of life typically rely on detection of the presence of a type of molecule present in life. Two common modern methods are the LAL (Limulus Amebocyte Lysate) test, which relies on endotoxins present in certain types of bacteria, the ATP (Adenosine Triphosphate) assay, and PCR (polymerase chain reaction), which involves the detection and amplification of DNA. We are working on a method that has potential advantages over these, the detection of microbial contamination by the detection of the organism's proteins. This is accomplished by the labeling of these proteins with a radioactive label, ^{125}I.

Detection by ^{125}I Labeling: Detection of proteins as signatures for the presence of organisms only requires the most general of assumption, that the organism uses proteins. All known organisms, across all three domains of life, use proteins. Another advantage of using proteins as a signature for life is that the large number of proteins contained in a cell makes detection easier, allowing the detection of very small numbers of organisms. An average *E. coli* is over 50% protein by dry weight and contains millions of protein molecules [1]. (50% by dry weight or 15% by volume is a good rule of thumb for most cells). The large size of proteins means that they will have a number of tyrosine residues (the easiest residue to label), allowing for multiple labels to be attached to each molecule, further multiplying sensitivity.

What are our expected detection limits for protein radiolabeling analysis? For ordinary detection of ^{125}I, 30 counts per second is fairly typical and 10 counts per second is reasonably achievable. This also results in measurement times (to achieve acceptable statistics) of seconds, which is more than fast enough for convenient analysis. If one assumes 30 cps and a detector efficiency of 75%, that corresponds to 40 disintegrations per second. A half life of 60 days correspond to a first order rate constant of 1.3×10^{-7} sec^{-1}. One would thus need 3×10^{8} labels to give 30 cps. An average *E. coli* bacteria has 2.3×10^{6} protein molecules per cell [1]. Assuming that one is able to place only one label per protein, one would be able to detect on the order of 130 cells. This is a fairly conservative estimate since most proteins will have multiple tyrosine residues and one could easily get below 30 cps. A typical protein might have 10-20 tyrosine residues, allowing the detection limits to potentially be cut by and order of magnitude. Multiple labeling and a well shielded counter (with a 10 cps or lower background) could detect less than 10 cells.

Radiolabeling techniques are inherently sensitive and ^{125}I benefits from a 60 day half-life, providing greater activity and signal per unit number of labels. Additional sensitivity can be obtained by use of a Multiphoton Detection (MPD). By taking advantage of selected isotopes, that decay by the emission of multiple photons, MPD can use coincidence methods to screen out many background events and detect radioisotopes, such as ^{125}I, at below background levels. This can enable thousand-fold improvements in sensitivity. That would make single cell detection easy to achieve. Even spores will have ample number of proteins for single cell detection.

The detection of live cells is accomplished by detecting the proteins that are released upon cell lysis by…

1) Separation of cells/spores from background proteins.

2) Lysis of cells.

3) Labeling of released proteins & separation from unreacted label.

4) Detection of radiolabeled proteins.

Comparison to Other Methods: This method has advantages in both generality and sensitivity. The LAL test will only detect organisms that produce endotoxins [2, 3]. The detection limits of organisms in the LAL test is about 10^{4} cells/ml [2].

The ATP test is a fairly general test, all known organisms use ATP [4-6]. However, the amount of ATP is more variable between organisms (ATP is only present in trace amounts in spores). In any case, the number of proteins in a cell is much higher than the number of ATP molecules. The ATP test can detect 10^{3} cells, or 10^{4} cells/ml, of "average" bacteria [6]. (For cells with very low levels of ATP, such as spores, sensitivities will be correspondingly worse.)

PCR techniques certainly have the potential for high sensitivity, though they require much more time and technique than the other methods. Significant issues revolve around the requirement for primers, as even general or "universal" primer sets most likely can detect only ~80% of known organisms. It is unlikely

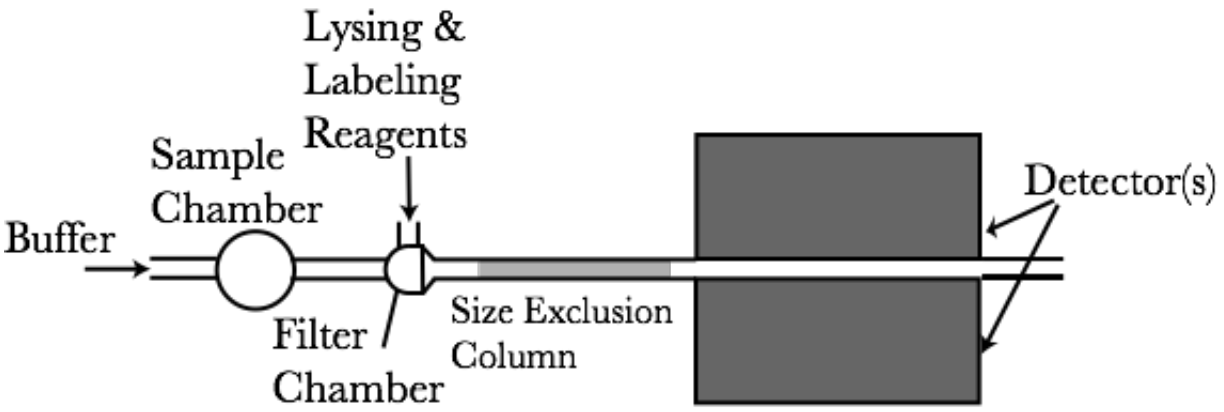

Figure 1. Schematic of flow system for analysis.

to be able to detect unknown organisms in extraterrestrial samples.

Current work: We are currently working on develop of this method through a grant from NASA's planetary protection program. There we seek to detect contamination of Earth organisms on spacecraft. Recent attention has been toward methods of universally lysing organisms in ways that are compatible development of a fast and automated analysis method. The best results were obtained by the use of XS (potassium ethyl xanthogenate) buffer, used for opening Halococcus (1% XS, 20 mM EDTA, 1% SDS, 800 mM ammonium acetate, 100 mM Tris-HCl, pH 7.5, incubated at 60 °C for 2 hr, Vortex for 10 sec, on ice for 10 min.)

This seems to allow good lysis while not requiring extensive handling steps, allowing it to be applied easily between cell separation and protein labeling.

While it is being developed for detection of Earth organisms on spacecraft, this method is very suitable for use with extraterrestrial samples. For samples of extraterrestrial origin, with unknown organisms, a method of high generality is needed. This method makes only the most general assumptions (that organisms use proteins). This makes it very applicable to the analysis of extraterrestrial samples. The durability of proteins (especially since there is no requirement to maintain enzymatic function) means that no significant restrictions are imposed upon sample handling and storage.

References:

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