Sensitive, Rapid Detection of Bacterial Spores

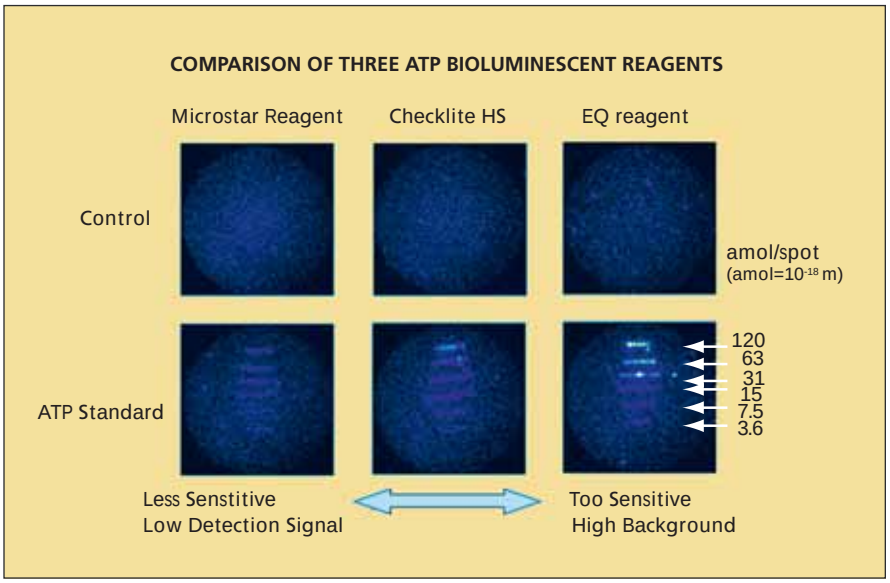
This capability is beneficial for medicine, public health, and biowarfare defense.

NASA's Jet Propulsion Laboratory, Pasadena, California

A method of sensitive detection of bacterial spores within delays of no more than a few hours has been developed to provide an alternative to a prior three-day NASA standard culture-based assay. A capability for relatively rapid detection of bacterial spores would be beneficial for many endeavors, a few examples being agriculture, medicine, public health, defense against biowarfare, water supply, sanitation, hygiene, and the food-packaging and medical-equipment industries.

The method involves the use of a commercial rapid microbial detection system (RMDS) that utilizes a combination of membrane filtration, adenosine triphosphate (ATP) bioluminescence chemistry, and analysis of luminescence images detected by a charge-coupled-device camera. This RMDS has been demonstrated to be highly sensitive in enumerating microbes (it can detect as little as one colony-forming unit per sample) and has been found to yield data in excellent correlation with those of culture-based methods. What makes the present method necessary is that the specific RMDS and the original protocols for its use are not designed for discriminating between bacterial spores and other microbes.

In this method, a heat-shock procedure is added prior to an incubation procedure that is specified in the original RMDS protocols. In this heat-shock procedure (which was also described in a prior *NASA Tech Briefs* article on enumerating spore-forming bacteria), a sample is exposed to a temperature of 80 °C for 15 minutes. Spores can survive the heat shock, but non-spore-forming bacteria and spore-forming bacteria that are not in spore form cannot survive. Therefore, any colonies that grow during incubation after the heat shock are deemed to have originated as spores.



These **Luminescence Images** were obtained in tests of three bioluminescence reagents with successively diluted samples of an ATP solution. The tests led to the selection of one of the reagents (Checklite HS) as offering the best compromise between requirements for high sensitivity and low background.

This method also provides for reduction of the incubation time from the typical range (18 to 24 hours) required by the original RMDS protocols. This reduction was effected by evaluation of three commercial bioluminescence reagents (see figure), leading to the selection of one of them that makes it possible to effect detection after an incubation time of only ≈5 hours. The sensitivity and rapidity afforded by this method were demonstrated in tests in which seven species of *Bacillus* that had been repeatedly isolated from clean rooms were detected after incubation times of about 5 hours.

This work was done by Roger G. Kern, Kasthuri Venkateswaran, and Fei Chen of Caltech; Molly Pickett of Millipore Corp.; and Asahi Matsuyama of Kikkoman Corp. for NASA's Jet Propulsion Laboratory. For more information, contact iaoffice@jpl.nasa.gov. NPO-40976

Adenosine Monophosphate-Based Detection of Bacterial Spores

AMP is released by means of heat shock, then detected via bioluminescence.

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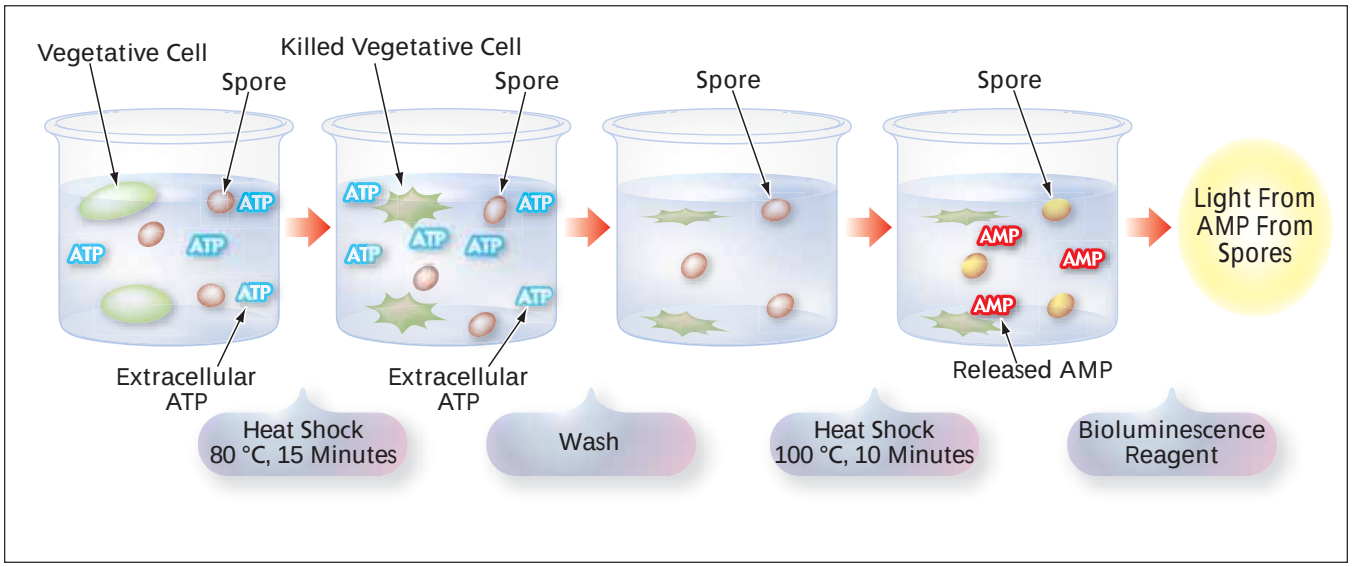
A method of rapid detection of bacterial spores is based on the discovery that a heat shock consisting of exposure to a temperature of 100 °C for 10 minutes causes the complete release of adenosine monophosphate (AMP) from the spores. This method could be an alternative to the method described in the immediately preceding article. Unlike that method and related prior methods, the present method does not involve germination and cultivation; this feature is an important advantage because in cases in which the spores are those of pathogens, delays involved in germina-



This **Flow Chart** summarizes the laboratory procedure for rapid detection of bacterial spores.

tion and cultivation could increase risks of infection. Also, in comparison with other prior methods that do not involve germination, the present method affords greater sensitivity.

At present, the method is embodied in a laboratory procedure, though it would be desirable to implement the method by means of a miniaturized apparatus in order to make it convenient and economical enough to encourage widespread use. The main steps of the laboratory procedure (see figure) are the following:

1. A sample suspected of containing spores is suspended in an aqueous solution.
2. In the first of two heat shocks, the suspension is heated to a temperature of 80 °C for 15 minutes to kill non-spore-forming bacteria and vegetative cells.
3. The suspension is subjected to a standard low-acceleration centrifugation wash.
4. The cells from the centrifuged suspension are resuspended in a solution that contains adenosine phosphate deaminase, which eliminates extracellular adenosine triphosphate (ATP) and AMP.
5. The new suspension is subjected to the aforementioned standard low-acceleration centrifugation wash.
6. The cells from the centrifuged suspension are resuspended in distilled water.
7. In the second heat shock, the suspension is heated to 100 °C for 10 minutes, causing the release of AMP from any spores that may be present.
8. A fraction of the heat-shocked suspension is treated using a commercially available bioluminescence agent with pyruvate, orthophosphate dikinase, causing luminescence in proportion to the concentration(s) of ATP that are converted from AMP by oxidative phosphorylation. The luminescence is measured by use of a standard laboratory luminometer.
9. To further ensure that the bioluminescence is of spore (AMP) origin and to discriminate against any residual ATP in the suspension, a remaining untreated fraction of the suspension from step 6 is similarly tested for luminescence that responds to ATP but not AMP.

In experiments on eight strains of *Bacillus*, this method was found to enable detection of spores in suspension down to sub-femtomolar levels of AMP. These levels correspond to sensitivity of the order of 100 or fewer spores per sample.

This work was done by Roger G. Kern, Fei Chen, and Kasthuri Venkateswaran of Caltech and Nori Hattori and Shigeya Suzuki of Kikkoman Corp. for NASA's Jet Propulsion Laboratory. For more information, contact iaoffice@jpl.nasa.gov.
NPO-40938