

AMP Based Sensitive and Rapid Spore Detection Assay

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Abstract

Guidelines for space missions to other planetary bodies, as well as for extraterrestrial sample return, stress the importance of avoiding contamination of hardware components with terrestrial organisms, their remains, and organic matter in general. Spore-forming organisms are of particular concern. The traditional spore detection method is laborious and requires a long cultivation time. A firefly luciferase ATP bioluminescence assay has been developed to determine the microbial cleanliness of spacecraft and associated-environments. This ATP assay is rapid and simple. However, the level of ATP is very low in spores, and the ATP assay is not very sensitive for spore detection. As the AMP level is much higher than the ATP level in spores, AMP may be a better biomarker for spore detection. An AMP bioluminescence assay has been developed by Kikkoman Corp. In this method, AMP is converted to ATP using pyruvate orthophosphate dikinase (PPDK), and ATP is subsequently detected by luciferase. In this present study, we measured the AMP content of several spores of *Bacillus* species to develop an AMP based bioluminescence assay for rapid spore detection. Spores of several *Bacillus* type strains as well as spacecraft-associated environmental isolates of the same species were purified and compared for AMP content. Spore samples were heat shocked at 80°C for 15 minutes to kill vegetative cells and extracellular ATP was removed by washing. AMP was released from the spores by heat shock at 100°C for 10 minutes. A suitable aliquot of sample was mixed with equal volume of bioluminescence reagent, and light emission was measured using a Kikkoman P100 lumitester. AMP content in a sample was determined from a standard curve that was established with purified chemical AMP. For the *Bacillus* strains tested, the mean AMP content of a bacteria spore is 10^{-18} moles per colony-forming units (CFU). The limit of this method for spore detection is ~100 CFU. In summary, this AMP based spore assay is rapid and sensitive. It may have wide applications in homeland security, defense against bio-warfare agents, environmental monitoring, as well as in the food and pharmaceutical industries.

Key words: AMP, PPDK, Spore detection.