Pseudomonas Species Detection in Water: New Rapid Methods

Introduction

The *Pseudomonas* genus of aerobic bacteria is prevalent in plant material and water. There are several strains of pathogenic *Pseudomonas* including *P. aeruginosa* (Pa) that can affect immuno-compromised humans and others. In industrial applications, Pa is typically detected and enumerated by an accumulation technique (e.g. ISO 16266:2006[1]) due to the low expected overall bacterial loads of *Pseudomonas* species. For example, a method employing a sample of 100mL or more filtered through a suitable membrane is typical. Loaded filters are then plated on selective agars.

Test methods can be applied to process water that is used in manufacturing for industries such as the paper industry and the food and beverage industry. Process water may be used in the rinsing of pipes or in-process products or for the cleaning of work surface or even as a component of the finished product. In addition, as the demand for bottled water increases, there is potential for increased surveillance of water sources or final product.

Study Goals

A study[2] was conducted by MOCON® in collaboration with Transia GmbH and the University of Hannover, Germany. Its goal was to assess the relative performance of commercially available selective broths when used to detect *Pseudomonas* species in the MOCON GreenLight® rapid microbial detection system. In addition, the study compared GreenLight system performance to plate count reference method ISO 16266.

GreenLight Technology

GreenLight relies on a novel sensor developed by Luxcel Biosciences (Cork, Ireland). The GreenLight sensor is embedded in a sampling vial and provides an optical fluorescent signal based on the oxygen level of the sample (Figure 1). Aerobic microbes consume oxygen as they grow and respire, and this oxygen consumption can be directly related to the bacterial load in the sample. GreenLight offers significant advantages to food producers and other industrial users in terms of ease of use, sensitivity and reduced lab sample preparation.

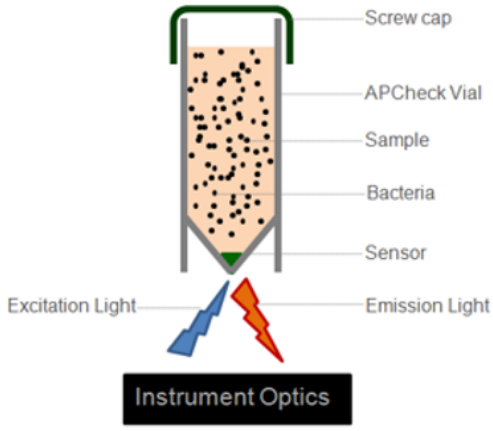
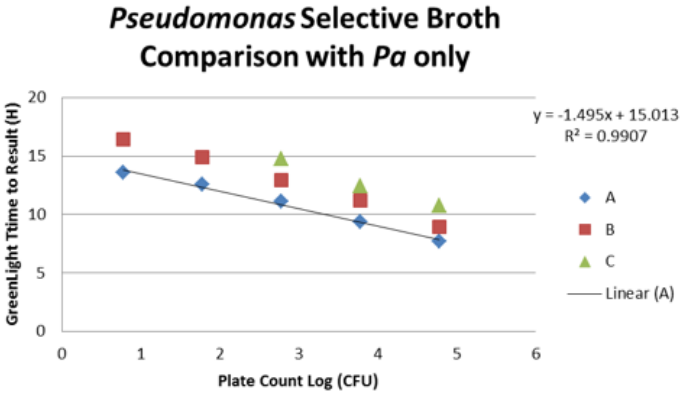


Figure 1. The APCheck Vial.

Test Methods and Results

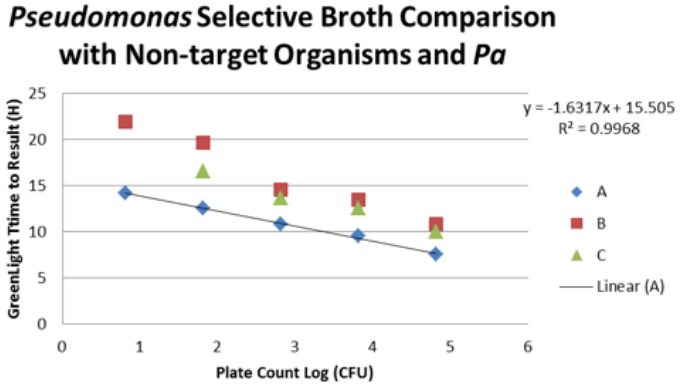
A trial was conducted to select the best broth for *Pseudomonas* detection in the GreenLight system. In the first stage, each candidate media was spiked with a serial dilution series of *Pseudomonas Aeruginosa*[1]. The data showed that broth A was the fastest for all dilutions over the range 1 to 5 Log (CFU) (Graph 1).



Graph 1: Shows the plate count Log (CFU) on the X axis and the GreenLight Time to Result (hours) on the Y axis. All three broths are presented and a linear regression is shown for broth A.

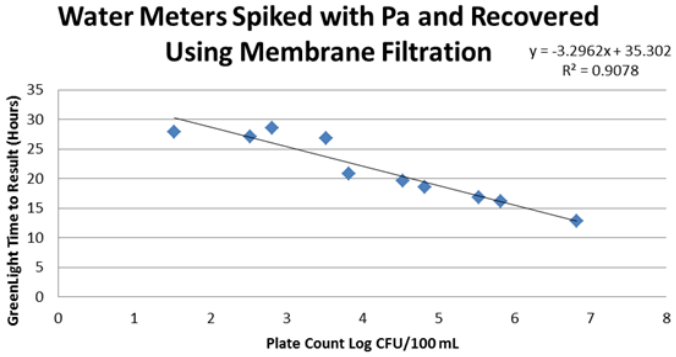
In the second stage, the broths were tested for non-target organisms. The broths were all spiked with a serial dilution series of *Escherichia coli* (DSM 682), *Enterococcus hirae* (DSM 3320), and *Salmonella enterica* (DSM 17420) from 1 to 5 Log (CFU). There was no reaction or growth for any of the tests, demonstrating that the broths were sufficiently selective.

In the third stage of testing, the first two stages were combined to prove that, in the presence of non-target organisms, the broth would still be able to differentiate dilutions of Pa. Every test was inoculated with a 5 Log (CFU) spike of a mixture of *Escherichia coli*, *Enterococcus hirae*, and *Salmonella enterica*. Each test was then spiked with successive dilutions of Pa (Graph 2). As before, broth A was the fastest time-to-result, and it also had the best correlation between time-to-result and plate count.



Graph 2: Shows the plate count Log (CFU) on the X axis and the GreenLight Time to Result (hours) on the Y axis. All three broths are presented and a linear regression is shown for broth A.

In the fourth stage, industrial water metering products were spiked with the same Pa inoculant strain. The products were rinsed internally and the collected rinsate was filtered using a 0.45 nitrocellulose membrane filter. The entire filter was aseptically inserted into a GreenLight APCheck vial and 10mL of broth "A" was added to it (Graph 3).



Graph 3: Shows the plate count Log (CFU) on the X axis and the GreenLight Time to Result (hours) on the Y axis. The linear line of regression and equation can be used for enumeration with GreenLight in logCFU/100mL.

Conclusion

Trial results confirm that GreenLight supports several commercially available selective broths for *Pseudomonas* species. One broth clearly demonstrated the best growth of the target species while selecting against other bacteria. A real world application was demonstrated using water meters with simulated *Pseudomonas* contamination.

This method shows the capability to return results in approximately 32 hours for bacterial loads of 10 CFU/100mL, compared to the ISO reference method of 48 hours. Higher loads would be much faster.

MOCON has released a GreenLight protocol for the detection of *Pseudomonas* species in water samples. Further studies will confirm its performance as a *P. aeruginosa* assay.

References

1. ISO 16266:2006
2. Werlein, Hans-Dieter and Raha Vatanparast (2015, September). *Pseudomonas aeruginosa* - Determination of Contamination in Water Flow Meter using GreenLight® System. Poster session presented at 67th Annual Meeting of the German Society for Hygiene and Microbiology eV 09.27.2015 - 30.09.2015 - Exhibition and Congress Center, Münster

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