Fungal Detection in Yogurt: New Rapid Methods

Introduction

Yogurt is a popular and nutritious dairy food that can be packaged easily to accommodate different serving sizes and flavors. Dairy producers are aware of potential spoilage issues that negatively affect the customer experience. Fungal contamination (yeasts and molds) can be introduced from a variety of sources, including added fresh fruit purees or airborne particles. Some packaged yogurts may also generate excess moisture under the foil cap that is a breeding ground for mold growth. While primarily a spoilage and shelf life issue, there have been recent occurrences of yeast and mold contamination causing illness.

Test Methods

The ISO method for enumeration of yeast and mold colonies in dairy products is ISO 6611:2004. In addition, ISO 21527-1: 2008 applies to foods with low water activity. Both methods are standard agar plate culture methods that rely on selective agars to display colonies. Often, methods such as ISO 21527 include the use of antibacterials such as chloramphenicol to suppress bacterial growth. The ISO and other regional methods usually take 5-7 days of incubation at 25C before the results are readable.

Drawbacks to Standard Methods

When using standard methods, the primary drawback is time-to-result. This is because yogurt products must be kept in the cold chain and typically have shelf lives of less than 2 weeks. The cell doubling times for yeasts and molds is much longer than for bacteria, hence the long incubation times. Molds usually demonstrate even longer doubling times than yeasts. Therefore, potential losses to food producers due to recall are high when test results take up to half the shelf life period to achieve.

Alternate methods sometimes rely on selective agar films to produce a colony forming unit (CFU) count, yet these suffer the drawback of not showing colony morphology that would allow the operator to see the difference between yeasts and molds.

For all plate incubation methods, the detection and enumeration sensitivity is suspect below about 20 CFU/mL. Since many samples exhibit low contamination, false negatives are a problem for QC operators.

The GreenLight® System

A novel alternative to these testing methods is GreenLight, a rapid microbial testing instrument and method that uses a fluorescence-based sensor to determine microbial growth inside a specialized sensor vial (the APCheck™ vial).

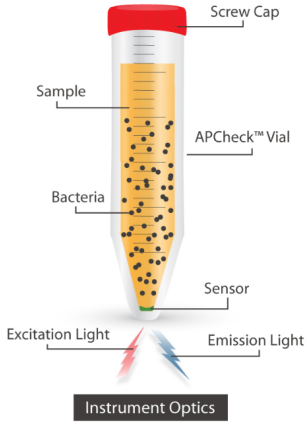

Figure 1. The APCheck vial.

Aerobic microbes consume oxygen as they grow inside the vial. When the sensor at the bottom of the vial is excited by light from the dedicated reader inside the instrument, it returns information about the oxygen content that can be directly related to the microbial load inside the vial. The time-to-result depends on the respiration rate and doubling time of the resident microflora as well as the growth

medium used, but generally the greater the initial microbial load, the faster the time-to-result.

No dilution of the test samples is necessary due to the sensor's sensitivity and wide measurement range. The sample and its growth media can be added directly to the APCheck vial. The size of the vials (either 2mL or 15mL) allows for the testing of a greater sample volume than the standard agar plate techniques. The time-to-result and CFU are calculated automatically, eliminating the need for manual counting and calculation as well as the operator-dependence that is present in the standard methods.

Laboratory Study

In a study conducted at STU in Bratislava, Slovakia, retail fruit yogurt samples were inoculated with commonly found yeast and mold organisms with the goal of achieving high correlation to standard plate count enumeration methods. In order to reject the effects of resident lactic-acid bacteria in the final colony count result, a selective broth was devised for use with the sample in the APCheck vial.

Materials and Methods

The selective media was composed of Sabouraud Dextrose Broth (SDB) with 0.01 g/L chloramphenicol (ref: Sigma-Aldrich, Part Number C0378).

Separate 1:10 dilution sample preparations of the yogurt samples were made by adding 10g into 90mL of the broth. An inoculum of two study organisms was made of *Candida tropicalis* (CCY 029007062) and *Geotrichum candidum* (raw milk cheese isolate G). These were inoculated into the yogurt samples at concentrations between 1 log CFU/mL and 7 log CFU/mL.

Results

- Results are for 24 data pairs in triplicate using the customized yeast/mold broth
- At 10 CFU/mL, in-matrix samples with *C. tropicalis* gave results in 18 hours versus 5 days for the ISO method
- Across a measurement range of 1 log CFU/mL to 7 log CFU/mL the GreenLight correlation to plate count was 0.95 (Pearson Coefficient)
- At 10 CFU/mL, in-matrix samples with *G. candidum* gave results in 22 hours versus 5 days for the ISO method
- The higher the microbial load, the faster the GreenLight result

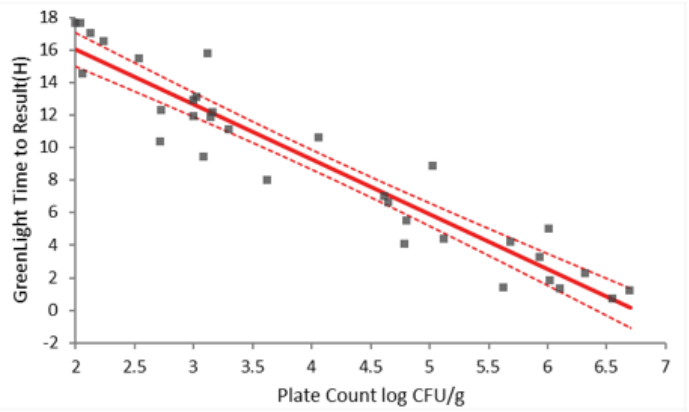

Figure 2: Time response of GreenLight vs. plate count for yogurt products inoculated with *C. tropicalis* and *G. candidum*. Equation y=-3.38x+22.78 (R^2=0.90).

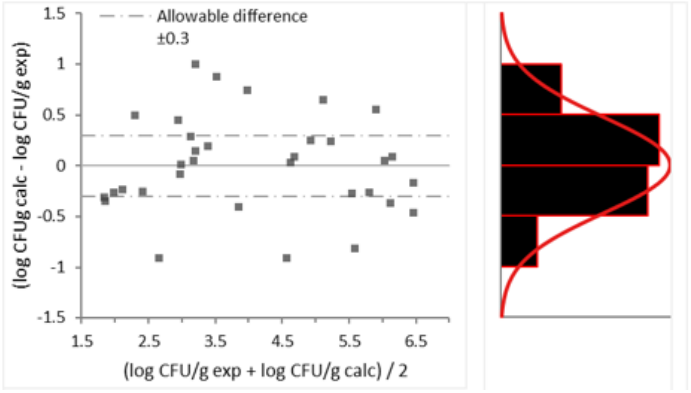

Figure 3: Distribution of differences between data calculated from Greenlight responses and plate counting in inoculated yogurt products.

Conclusion

The new GreenLight Rapid Test Method for Yeasts and Molds in yogurt products can improve sample time-to-result by 80% when compared to traditional methods while suppressing interference from lactic acid bacteria.

The preparation labor cost is lower. The results of testing are also less operator-dependent, allowing for greater precision than the standard test methods.

Acknowledgements

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