

ABSTRACT

A collaborative effort between Morinda, Inc. and Advanced Analytical Technologies, Inc. was established to develop a rapid test for yeast detection in nutraceutical products. Currently, Morinda uses a 7-day test for yeast in their products, and the RBD 3000 was investigated as a prescreening tool to ensure the absence of yeast in Morinda's products. However, in the case of a contamination event, a Most Probable Number (MPN) test was also developed to determine possible contamination levels within 24 hours. Two product types were tested: TAHITIAN NONI® Juice, a dietary supplement (TNJ) and Moëa™ Body Butter. Each product was diluted 1:10 in Tryptic Soy Broth + Soy Lecithin + Tween 20 (TSBST) in duplicate. One tube for each product was spiked with ~100 *Candida albicans* (ATCC 10231)/g product, while the other tube was spiked with ~10 *C. albicans*/g product. These spike levels were chosen to represent a yeast specification of 100cfu/g product. Serial 10-fold dilutions of the spiked samples were prepared in TSBST to final product concentrations of 1:100, 1:1,000 and 1:10,000 (10mL/tube). All tubes were enriched at 30°C for 23 hours. Following enrichment, samples were gravity filtered (40µm), diluted in phosphate buffer and analyzed on the RBD 3000 using Advanced Analytical's *FASTest* Total Viable Organism detection kit. All samples were plated in parallel on 3M Petrifilm™. Detection of yeast spiked into the TNJ and body butter was achieved in 24 hours using the RBD 3000. Test samples spiked with ~100 yeast/g product had positive results at the 1:100 and 1:1,000 dilutions on the RBD 3000, and negative results at the 1:10,000 dilutions. Samples spiked with ~10 yeast/g product had positive results at the 1:100 dilution and negative results at the 1:1,000 and 1:10,000 dilutions on the RBD 3000. Since a 1:1,000 dilution of the products was the determinative dilution of interest, the results indicated a successful MPN test. Non-spiked samples of the TNJ and body butter were analyzed on the RBD 3000 and 3M Petrifilm™ at the same product concentrations. All RBD 3000 results for the non-spiked TNJ and body butter samples were negative. 3M Petrifilm™ results correlated with all RBD 3000 results in all cases.

MATERIALS

Bacterial Cultures: *Candida albicans* #10231 (ATCC, Manassas, VA). Yeast Extract and Malt Extract (YM) broth (Difco, Sparks, MD) was used for culturing, Tryptic Soy Agar (TSA, Difco) and Yeast and Mold (YM) Petrifilm™ (3M, St. Paul, MN) were used for plating. Tryptic Soy Broth + Soy Lecithin + Tween 20 (TSBST) was used as the enrichment media. **Products:** TAHITIAN NONI® Juice, a dietary supplement (TNJ) and Moëa™ Body Butter (Morinda, Inc., Orem, UT). **Detection:** *FASTest* Total Viable Organism (TVO) kit and fully automated RBD 3000 (Advanced Analytical Technologies, Inc. Ames, IA).

METHODS

Enrichment: Each product was diluted 1:10 in TSBST in duplicate and neutralized for 30 minutes. One tube for each product was spiked with ~100cfu *Candida albicans*/g product, while the other tube was spiked with ~10cfu *C. albicans*/g product. To verify spike levels, these *C. albicans* dilutions were spread-plated onto TSA, and one mL of each 1:10 dilution was plated on YM Petrifilm™. (Actual 100cfu spike levels were confirmed at 110-350cfu on TSA and 60-160cfu on YM Petrifilm™. Actual inocula for the 10cfu spikes ranged from 10-35cfu on TSA and <10-30cfu on YM Petrifilm™.) Serial 10-fold dilutions of the spiked samples were prepared in TSBST to final product concentrations of 1:100, 1:1,000 and 1:10,000 (10mL/tube), n=6 for each dilution at each spike level. Non-spiked samples (negative controls) were prepared in triplicate at the above product concentrations. All tubes were enriched at 30°C with rocking for 23 hours. **Sample Processing:** Following enrichment, samples were 40µm filtered and diluted in 10mM phosphate buffer. Samples were placed on the RBD 3000 and analyzed for viable organisms using the *FASTest* TVO kit. All samples were plated in parallel on YM Petrifilm™. Enriched samples were considered positive for microbial growth on the RBD 3000 if the counts were ≥5 times the negative control counts.

RESULTS

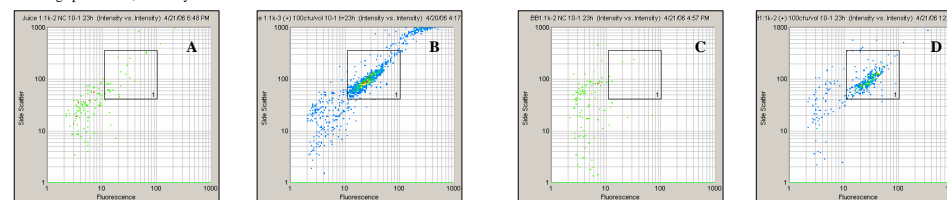
Table 1. Detection of *C. albicans* @ ~100cfu/g in enriched TAHITIAN NONI® Juice and Moëa™ Body Butter

Sample Dilution	Theoretical Result	Detection by RBD 3000	Growth on YM Petrifilm™
1:100	+	+	+
1:1,000	+	+	+
1:10,000	-	-	-

Table 2. Detection of *C. albicans* @ ~10cfu/g in enriched TAHITIAN NONI® Juice and Moëa™ Body Butter

Sample Dilution	Theoretical Result	Detection by RBD 3000	Growth on YM Petrifilm™
1:100	+	+	+
1:1,000	-	-	-
1:10,000	-	-	-

Figure 1. Intensity plots of enriched (A) Negative Control 1:1,000 TNJ, (B) 100cfu/g spike in 1:1,000 TNJ, (C) Negative Control 1:1,000 Body Butter, and (D) 100cfu/g spike in 1:1,000 Body Butter



DISCUSSION/CONCLUSIONS

- The RBD 3000 in conjunction with the *FASTest* TVO kit provide a rapid and sensitive detection method for yeast cells in these Morinda nutraceutical products.
- Spiked TAHITIAN NONI® Juice and Moëa™ Body Butter yielded positive results within 24 hours on the RBD 3000 vs. 7 days on YM Petrifilm™.
- For both product types tested, spiked samples were considered positive if the RBD 3000 counts were ≥5 times the negative control sample counts. Pass/Fail acceptance criteria was established using the RBD 3000 software, allowing for objective interpretation of results.
- All negative control (non-spiked) samples were negative by the RBD 3000 and YM Petrifilm™.
- Since the 1:1,000 product dilution was the determinative dilution of interest for the MPN test on the RBD 3000, analysis of the other dilution levels would not be necessary.
- When the RBD 3000 is used as a pre-screening method for the absence of yeast, the 1:10 product dilution would be the only dilution required for analysis (data not shown).