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Validation of highly effective clean label antimicrobial, Verdad® Opti Powder N510 to control resistant lab and *listeria monocytogenes* in a high moisture uncured turkey (#603)

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Introduction

Meat products are spoiled by organic acid resistant lactic acid bacteria (LABs). High pH, moisture and low salt combined with ineffective antimicrobials can pose a food safety risk due to *Listeria monocytogenes*. Application of antimicrobials in concert with good manufacturing practices can control *Listeria* in ready-to-eat (RTE) meat products (Mellefont et al. 2007). However, our global studies have shown that regularly used clean label antimicrobials like vinegar is ineffective in controlling resistant LAB's. Vinegar is also very ineffective in pH of 6.4 of higher values in deli meat to control *Listeria monocytogenes*. The objectives of this study were (1) to screen several LAB isolates for resistance to common antimicrobials by analysis of lag phase and growth rate, then combine the most resistant strains into a cocktail; (2) analyze a new antimicrobial (Verdad® Opti Powder N510 – cultured cane sugar and vinegar (CSV)) for control of resistant LABs; and (3) analyze the control against *L. monocytogenes* in very susceptible (high pH, low salt, high aw, high moisture, long shelf life like 150 days) formulations.

Methods

Resistant LAB cultures, obtained from a commercial poultry and maintained frozen were activated in De Man, Rogosa, and Sharpe (MRS) broth (18 h at 30°C). Solutions of MRS with and without various concentrations of acetic and lactic acid (range 0-5%) were created, filtered, and transferred (200 µL) to sterile bioscreen plates. LAB cultures (independent and in a cocktail) were diluted and inoculated (3 µL) into the bioscreen plates. Plates were incubated (3 d at 30°C) in a bioscreen, and optical density was measured every 15 min at 420-580 nm. Growth curves were fitted to the exponential and Gompertz models to determine the growth rate. Next, restructured uncured turkey rolls were manufactured using a formula with 2% salt and varying levels of antimicrobial to achieve 20% extension. In one experiment, turkey was inoculated with LABs (ca. 9 log CFU/g) prior to cooking, and a portion of samples were enumerated upon initial inoculation, 0 h after heating, and 24 h heating to demonstrate lethality and any synergistic impact of cultured sugar and vinegar (CSV) solution. In a second experiment, turkey was stuffed in high barrier casings, then cooked to an internal temperature of 73°C. Samples were sliced (25 g) and inoculated with ca. 3 log CFU/g resistant LAB cocktail, vacuum packaged, and stored at 4.4°C to determine shelf life. In both experiments, enumeration was performed on MRS agar (30°C for 48 h). Finally, samples were prepared as previously described for shelf life analysis, but were instead inoculated with *L. monocytogenes* following cooking by applying ca. 3 log CFU/g cocktail to each turkey slice prior to vacuum packaging. Samples were stored at 4.4°C until enumeration on modified Oxford agar (MOX; 35°C for 48 h).

Results

The effect of lactic and acetic acid on relative growth rate (γ; Fig. 1) and impact of lag time (h) were determined via bioscreen. Fig. 1 demonstrates that, while γ decreases with increasing acid concentration as expected, there are differences among strains that would impact cocktail performance. Similar differences were observed for lag time.

Figure 1

The novel CSV solution was challenged by a cocktail containing all 7 strains of resistant LABs since the previous experiment showed that the resistant LAB cocktail has an average resistance as compared the individual strains. Application of CSV at varying concentrations demonstrates immediate destruction of LABs following heating and 24 h thereafter (Fig. 2A). Specifically, noticeable differences are observed at concentrations of 2%, 2.5%, and 3% antimicrobial compared to control (no antimicrobial) and a market reference (2.2% dry vinegar powder). Control of resistant LABs by the CSV solution throughout shelf life was also demonstrated (Fig. 2B) compared to control and a 2.2% dry vinegar powder market reference. Specifically, while control treatment and market reference visually spoiled (>6.5 log CFU/sample LAB) within 21 days, no growth of resistant LABs were observed throughout the 120 d study period.

Figure 2

Finally, application of Verdad® Opti Powder N510 also controlled *L. monocytogenes* throughout a 70 d shelf life period. While control samples failed (> 2 log outgrowth of *L. monocytogenes*) within 7 d, Verdad® Opti Powder N510 prevented *L. monocytogenes* growth throughout the analyzed shelf life at all concentrations tested (range 2.5% to 3.5%).

Figure 3

Conclusion

Lactic acid bacteria, particularly those resistant to antimicrobial interventions, are a challenge for deli meat producers, shortening shelf life and resulting in undesirable organoleptic changes. Furthermore, evolving factors such as high pH of processed meat, high moisture, long shelf life needs, low salt driver (resulting in high aw) make the control of *Listeria monocytogenes*

Notes

very difficult with standard antimicrobial classes such as vinegar etc. and more effective and potent clean label solutions such as novel CSV are critical to ensure food safety. The studies presented here elucidated the wide spectrum antimicrobial performance of Verdad® Opti Powder N510 to control both resistant LABs and *L. monocytogenes*.

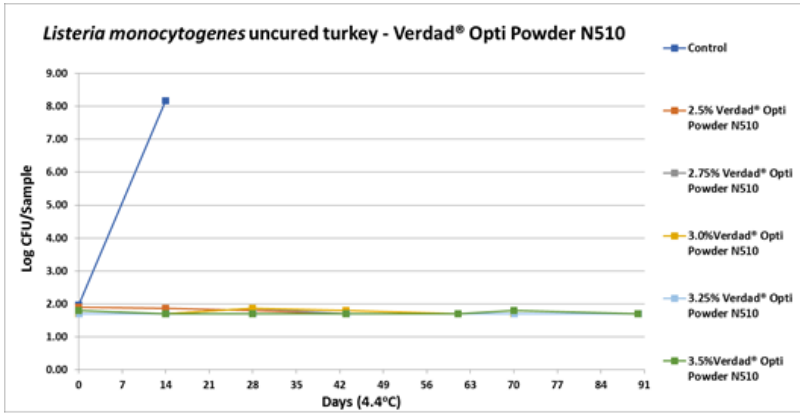

Figure 3.
Control of L. monocytogenes by Verdad® Opti Powder N510 in uncured turkey.

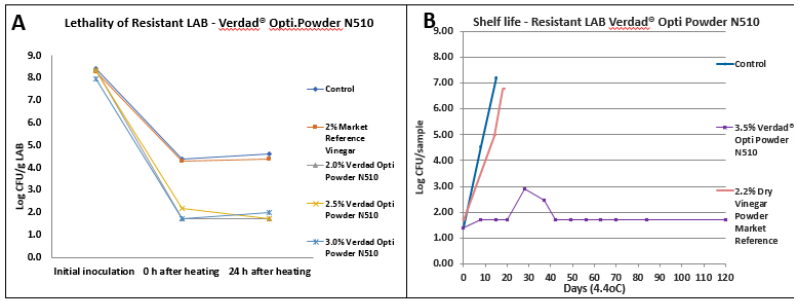

Figure 2.
Resistant LABs kinetics with Verdad® Opti Powder N510 in un-cured turkey. (A) demonstrates

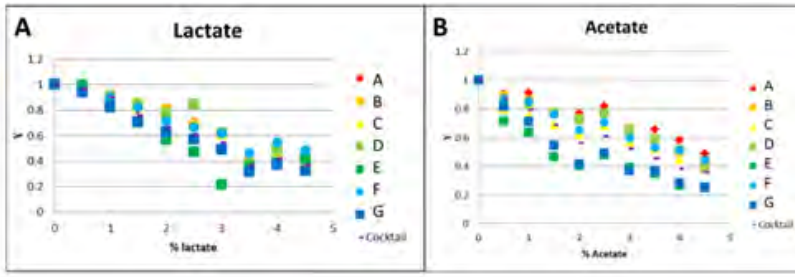

Figure 1.
Relative growth rate of seven (A-G)resistant LAB strains at varying concentrations of lact

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