

ABSTRACT

The concept of “enteric dialysis” as an alternative strategy to both slow progression of chronic kidney disease (CKD) and treat Stage 5 CKD avoiding the need for dialytic therapy is being explored. Probiotic bacteria, living strains of purportedly beneficial bacteria have been formulated into a patented proprietary product termed “Kibow Biotics®”. Administered in the form of enteric coated gel capsules, marketed as Azodyl™, in the United States and Canada, broad acceptance has been gained by veterinarians managing cats and dogs afflicted with moderate to severe kidney failure.

A constraint on extending clinical trials is the requirement that live probiotic bacteria within enteric coated gel capsules (Kibow Biotics®) must be refrigerated (4°C) to maintain stability and viability. To stabilize the current gel capsule formulation for transit and storage at room temperature, we explored improved delivery strategies, such as compressed coated caplets that may retain bacterial viability at room temperature avoiding a requirement for refrigeration that may prove difficult to meet in developing countries. We investigated the efficacy of enteric-coated caplets containing three different probiotic bacteria (*Streptococcus thermophilus*, *Lactobacillus* spp. and *Bifidobacterium* spp.) testing their viability under simulated gastrointestinal conditions and storage under refrigeration (4°C) or room temperature (24°C). Selective enumeration of microbes was determined by plating on appropriate media. We found a 1-log decrease in total count of probiotic strains in enteric coated caplets (2.0×10^{10} to 5.0×10^9 CFU/caplet) compared to the number of organisms in their original growth flasks after three hour incubation in simulated gastric conditions (pH 2.5). No significant loss in viability was observed in enteric coated caplets after 6 h incubation in simulated bile conditions. There was no significant reduction in the viability of probiotic strains after 6-month storage under refrigeration or room temperature. Overall, enteric-coated compressed caplets were found effective in protecting viability of probiotic bacteria in simulated gastrointestinal conditions and also during storage shelf life at room temperature.

MATERIALS AND METHODS

I. Evaluation of Neutraceutix Bacterial Strains:

Enteric coated caplets consisting of Neutraceutix bacterial strains *Streptococcus thermophilus*, *Lactobacillus* species, and *Bifidobacterium* species were manufactured by Neutraceutix Inc. of Redmond, Washington. Initial count on the bacterial species was performed by aseptically crushing two random caplets in a sterile bag and placing the crushed caplet into 100 ml of physiological saline (PPS). Serial dilution was performed and the bacterial strains were enumerated on the following media in triplicates: ST agar (10 g sucrose, 2 g dipotassium phosphate, 5 g yeast extract, 10 g pancreatic digest of casein, and 20 g agar per liter) for *S. thermophilus*, MRS agar for *Lactobacillus species* (obtained from Acumedia Manufacturer Inc, Lansing, MI), and Reinforced Clostridium Agar (10 g beef extract, 10 g pancreatic digest of casein, 5 g NaCl, 5 g dextrose, 3 g yeast extract, 3 g sodium acetate, 0.5 g L-cysteine-HCl, 1 g soluble starch, and 20 g agar per liter) for the enumeration of *Bifidobacterium spp.* The selected plates were incubated at 37°C for 48 hours in Napco 5100 incubator. The caplets were then stored at room temperature (24°C) for a period of 6 months. The 6 month count of *S. thermophilus*, *Bifidobacterium* species and *Lactobacillus* species was performed by the same method as the initial count (described above).

II. Evaluation of Kibow’s Bacterial Strains:

Freeze dried bacterial strains *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium longum*, provided by Kibow Biotech Inc. were manufactured into caplets by Neutraceutix in order to assess the stability of Kibow’s probiotic strains. The caplets were stored in the same conditions as the Neutraceutix bacterial strains for a period of 6 months. The initial count as well as the count after 6 months was performed by the same method that was used to assess the viability of Neutraceutix strains (described above).

RESULTS

I. The initial count for *S. thermophilus* was 1×10^8 colony forming units (cfu) per caplet. After a period of 6 months storage at room temperature, *S. thermophilus* decreased by 57.5% (4.25×10^7 cfu/ caplet). The *Lactobacillus species* also resulted in a loss when stored at room temperature, a loss of 41.5% in viability. For *Bifidobacterium species*, there was a 60% loss when stored at room temperature for the same time period.

II. The initial count for Kibow’s bacterial strain *S. thermophilus* was 18.2×10^9 cfu per caplet and 3.5×10^9 cfu for *L. acidophilus*. The initial count for *B. longum* resulted in the viability of 5×10^4 cfu per caplet. Upon completion of the 6 month storage period at room temperature resulted in a 99.89% loss for *S. thermophilus*, 99.30% loss for *L. acidophilus*, and 100% loss in viability for *B. longum*.

CONCLUSIONS

In summary, the viability of bacterial strains provided by Neutraceutix showed to be less affected by the room temperature storage for 6 months, as compared to Kibow’s bacterial strains, in the form of caplets. Therefore, it is not feasible to manufacture Kibow’s proprietary formulation in the form of caplets specifically formulated for uremic toxin removal for pre-CKD as well as CKD patients worldwide.

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