Comparative Analysis of the Viability and Functional Performance of Mono- and Multi-Species Probiotic Cultures in a Non-Dairy Food Matrix

By

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STATEMENT OF ORIGINALITY

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Signed……………………… Date ………………..

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<tbody>
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<td>CFU</td>
<td>Colony forming unit</td>
</tr>
<tr>
<td>DC</td>
<td>Dendritic cell</td>
</tr>
<tr>
<td>EIA</td>
<td>Enzyme immuno-assay</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immuno sorbent assay</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and agriculture organisation</td>
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<tr>
<td>GIT</td>
<td>Gastro-intestinal tract</td>
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Abstract

Probiotics are increasingly being included into food products in order to develop functional foods with health promoting effects, but have to date been exploited mainly in dairy products. Development of non-dairy probiotic foods such as fruit juices may provide consumers with greater choice and be attractive to those who can not eat dairy foods. Orange juice presents as an ideal vehicle for probiotic delivery as it is the most popular fruit beverage worldwide, and like other fruit juices has a short gastro-intestinal transit time which reduces exposure of probiotics to harsh environment of the stomach.

Since probiotic organisms vary in the type and level of their health promoting effects, it is likely that probiotic combinations would offer the consumer more benefit than single strains. Effective design of functional foods containing probiotic combinations, must take into consideration the likely occurrence and impact of potential interactions between individual species within a proposed combination, and between the probiotic and the carrier matrix. The main objectives of the current study were 1) to identify the effect of combining probiotics on their viability and adhesion to intestinal cells and 2) to examine the combined effect of exposure of probiotics to orange juice and low temperatures during refrigerated storage, on their viability and functional properties.

The initial study of long-term (14 days) growth interactions of several lactobacilli and Bifidobacterium animalis subsp lactis Bb12 (Bb), both alone and in co-culture with Propionibacterium jensenii 702 (PJ), revealed that growth patterns of Lactobacillus strains were not adversely affected by the presence of PJ, whereas lactobacilli strongly inhibited growth of PJ. In the co-culture of Bb and PJ, a significant enhancement of the growth of both bacteria was observed. The effect of combining probiotics on their adhesion to human intestinal epithelial Caco-2 cells was only evident in the case of Lb. casei 01 and Lb. rhamnosus GG (LG) which exhibited a decrease in adhesion rate in the presence of PJ.

The viability of LG, Lb. reuteri ATCC 55730 (LR), Bb and PJ, both individually and as 2- or 3- multispecies combinations, were then monitored in orange juice (OJ) (with and without 20% pulp) as well as bottled drinking water (BW) over 8 weeks of refrigerated
(4°C) and non-refrigerated storage (only for BW). Lactobacilli remained viable in higher numbers in OJ relative to that observed in BW under refrigeration. In contrast, a better outcome was observed for Bb and PJ in BW. Combining of probiotic species was observed to affect individual strain viability. Presence of pulp did not affect the viability of probiotics in OJ, while storage of BW at room temperature had an adverse effect on viability of all probiotics except of PJ, relative to storage under refrigeration.

Influence of combined exposure to OJ and refrigerated storage of the same probiotic preparations on their in vitro gastro-intestinal tolerance, adhesion to intestinal epithelial cells and immunomodulatory effects was then investigated at 10-day intervals during one month of storage. Suspension in OJ did not adversely affect the tolerance of any of the strains examined to simulated gastric juice (SGJ), with the tolerance of LG and PJ considerably enhanced relative to that observed in PBS, but did appear to impair the tolerance of lactobacilli and PJ to simulated intestinal juice (SIJ) at the baseline. High tolerance to SGJ was maintained throughout the storage period. The tolerance of both Bb and PJ to SIJ remained relatively constant during storage. Combining with both Bb and PJ enhanced the tolerance of the lactobacilli to SIJ with little impact on Bb, but adversely affected PJ in all combinations.

The adhesion rate of LG remained relatively constant in all preparations along with the viability during storage. In contrast with LG, adhesion rates and viabilities of other probiotics exhibited variation in relation to strain, presence of other microorganisms, and storage duration. In terms of both viability and adhesion rate, the preparations that provided the best outcomes for all constituents were LG and LR-PJ.

With the exception of LG, all probiotic preparations significantly enhanced non-stimulated interleukin-8 (IL-8) but not interleukin-6 (IL-6) or tumor necrosis factor-α (TNF-α) secretion by Caco-2 cells. Probiotic preparations enhanced Escherichia coli lipopolysaccharide (LPS) induced IL-8 release at baseline however this effect was not evident in all preparations at day 10. With the exception of LG, all probiotic preparations enhanced TNF-α induced IL-8 secretion towards day 20 after which it returned to the
control level. In contrast, probiotic preparations significantly reduced IL-1β induced IL-8 secretion at baseline, with no further effect evident during storage. The relative probiotic effect on IL-1β and TNF-α induced IL-8 secretion showed an upward and downward trend respectively over the storage period. Probiotic preparations did not affect LPS or IL-1β induced secretion of IL-6 up to 10 days of storage, while thereafter some of them exhibited variable effects on IL-1β induced IL-6 secretion. Compared to baseline (day 0), the effect of all four probiotic strains on IL-1β induced TNF-α production was found to decrease significantly by day10 of the storage period.

In conclusion, the results provided evidence of variation between individual strains in terms of their viability and intestinal adhesion capacity, and for the same strain when combined with different probiotics. When included in bottled drinking water and orange juice, the viabilities and functional properties of the probiotic preparations were further affected by the duration of their exposure to the carrier matrix and refrigerated storage. Such effects should be considered when formulating probiotic products, and further research is recommended to confirm the observed in vitro functional effects in vivo.