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## RESEARCH ARTICLE

### IN VITRO STUDY ON THE INFLUENCE OF SELECT ASIAN FOOD SUPPLEMENTS ON PROBIOTIC CULTURES

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#### ABSTRACT

Probiotics, due to their beneficial gut effects, are widely sold in combination with a variety of food products such as infant feeds and health beverages. An *in-vitro* study was carried out to test three commonly used Asian food supplements - brown rice (BR), white rice (WR), American ginseng (AG) and casein hydrolysate (CH) for their influence on the growth on three probiotic bacteria *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The viable cell counts of the probiotics grown in the presence of 1.5% concentrations of the food supplements were plotted against time for 20 hours and were compared with that of a known prebiotic Inulin (PI) and a growth control (GC). No significant variation in growth data was observed on *L.acidophilus* when grown in the presence of BR, WR, G and CH. An inhibitory effect on *S.thermophilus* could be observed only with BR and PI. Supplement AG had an inhibitory effect on *L.bulgaricus*, whereas, PI, CH and BR improved its growth performance. This study reflects the need to study the survival rate of probiotic strains in the presence of combination ingredients in health food, as some of them might reduce or help to enhance the probiotic potential of the final marketed product.

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## INTRODUCTION

The therapeutic role for the manipulation of gut microflora in the maintenance of human health and treatment of various mucosal disorders cannot be undermined. In the past years, probiotics were limited to yoghurt and fermented drinks. Of recent, a vast majority of pre-term infant, toddler and child milk formulae (Athalye-Jape *et al.*, 2014); body building whey powders, and food bars are supplemented with probiotics, to enhance both the nutritive and therapeutic potential of the products. The three often discussed and used probiotics include, *Lactobacillus acidophilus* (*L.acidophilus*), *Lactobacillus bulgaricus* (*L. bulgaricus*) and *Streptococcus thermophilus* (*S.thermophilus*). The intent of this study was, to test the growth and viability of the probiotic microorganisms, in the presence of brown and white rice powder, casein hydrolysate and American ginseng. These ingredients are traditionally used especially in East Asian health foods.

## MATERIALS AND METHODS

Cultures used in this study were isolated from commercial dairy products and were confirmed by colony morphology, Gram stain and API 50 CH strip (bioMérieux® SA) identification (Ozgun & Vural, 2011).

Culture media used for *L. acidophilus* and *L.bulgaricus* included MRS broth and M17 broth for *S. thermophilus*. The supplements tested included brown rice (BR) powder, white rice (WR) powder, American ginseng (AG) powder (Hock Hua chinese medicine) and Casein hydrolysate (CH) (Acumedia). A commercially used prebiotic was included in the study –Inulin (PI) (Sigma Aldrich). Growth controls (GC) which comprised broth cultures, without any supplement was used for comparison. All the supplements were prepared in the broth medium as 1.5% concentrations. The media was inoculated with 4% inoculum of overnight probiotic culture (Su *et al.*, 2007) with a turbidity equivalent to 4 McFarland.

The cultures were incubated at 37°C for the lactobacilli and at 42°C for *S. thermophilus*. Growth of probiotic microorganisms was monitored by serial dilution followed by direct spread plate method, every two hours, for 20 hours. Growth curves were plotted using viable cells (log CFU/ml) against incubation time (hours), for each of the test supplements and compared with the effect of prebiotic inulin (PI) and the GC.

## RESULTS AND DISCUSSION

While *L.acidophilus*, *L.bulgaricus* and *S.thermophilus* might be a popular blend of probiotics in several formulations, their viability and growth might be affected by the co-ingredients.

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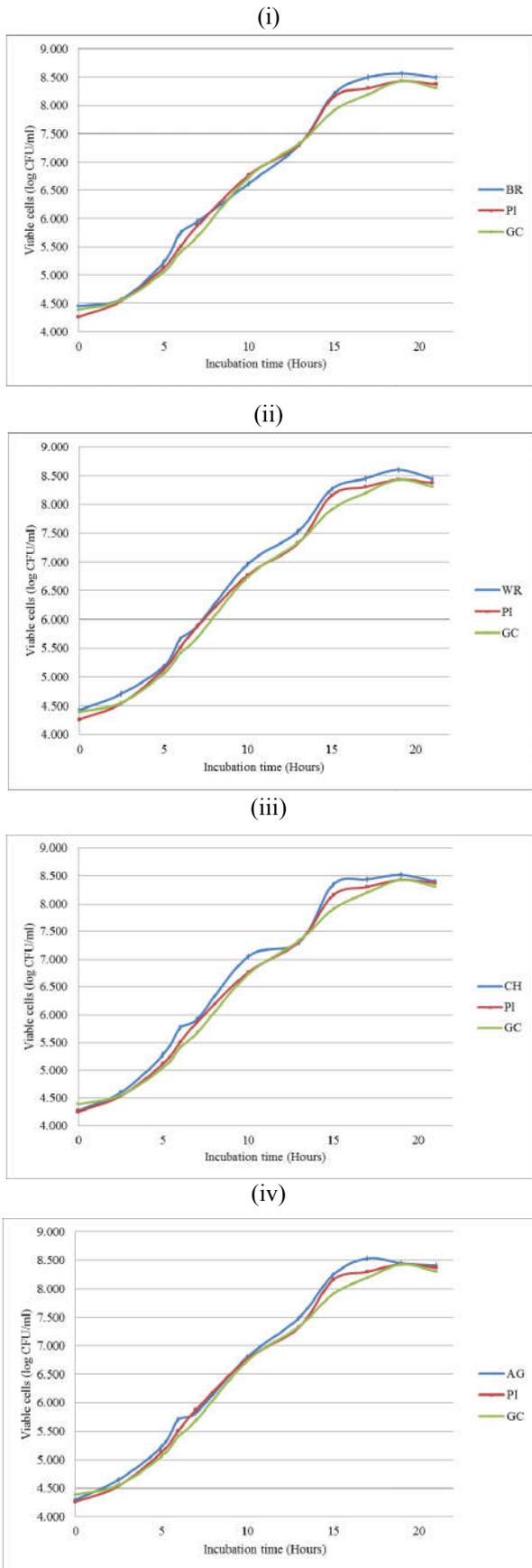


Figure 1. Growth curve for *L. acidophilus* in MRS broth supplemented with (i) 1.5% BR (ii) 1.5% WR (iii) 1.5% CH (iv) 1.5% AG. All comparisons were done against PI and GC

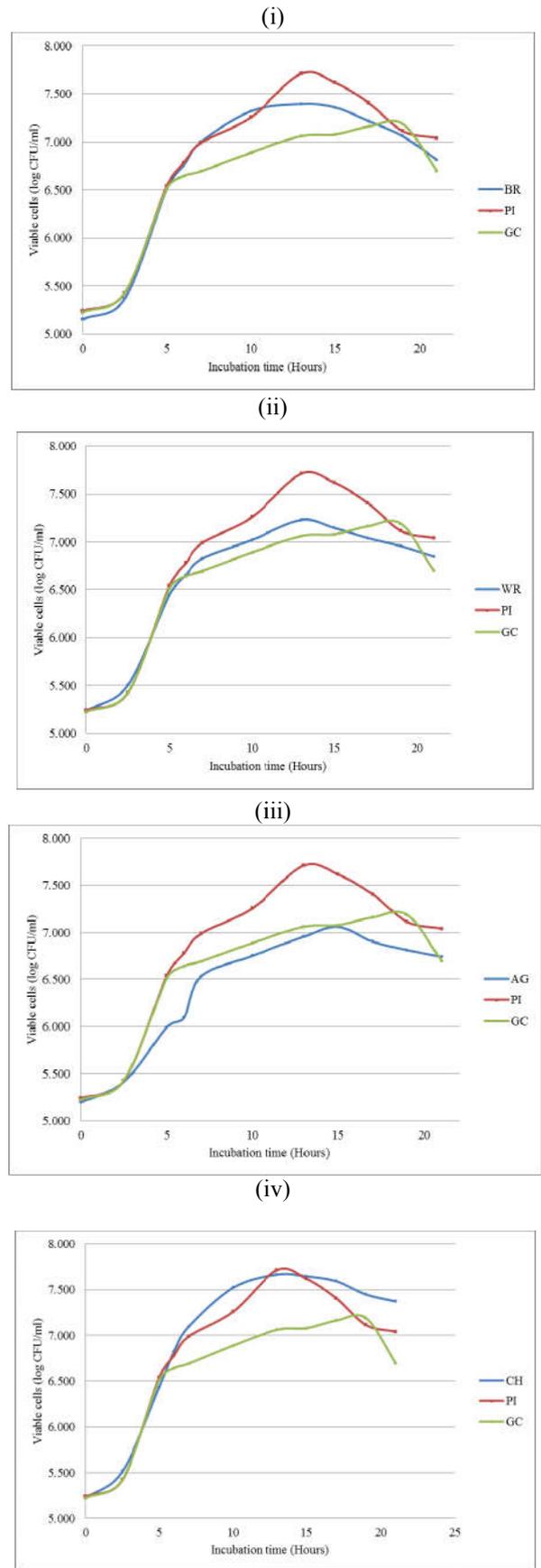
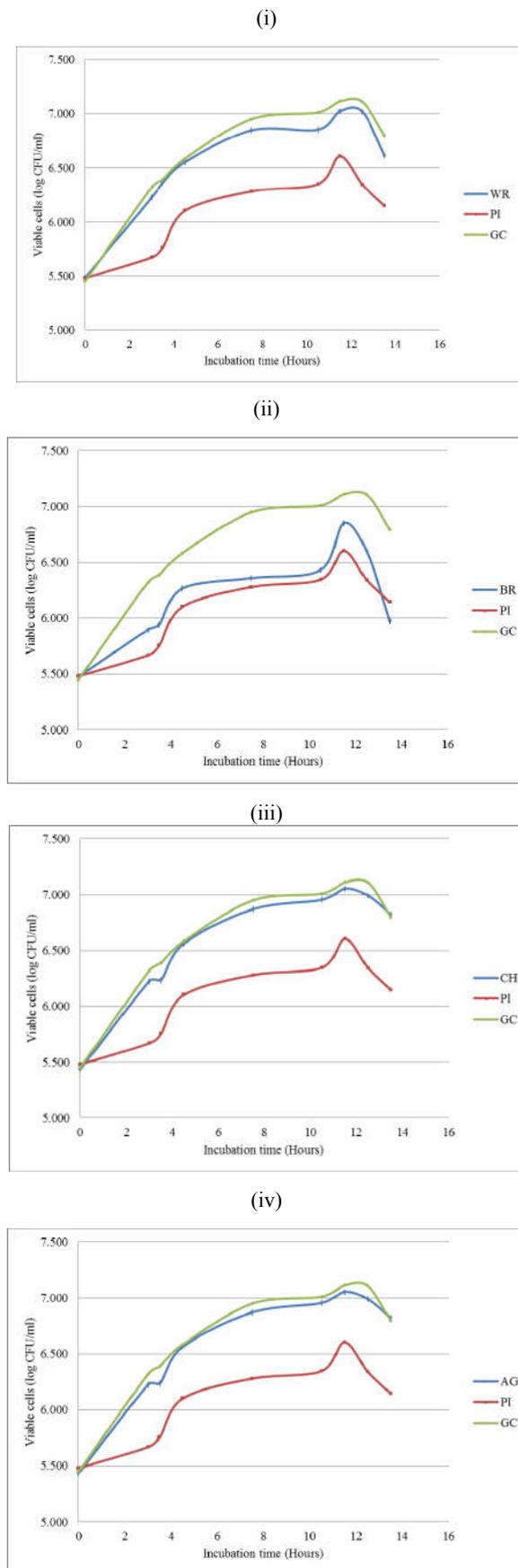


Figure 2. Growth curve for *L. bulgaricus* in acidified MRS broth supplemented with (i) 1.5% BR (ii) 1.5% WR (iii) 1.5% CH (iv) 1.5% AG. All comparisons were done against PI and GC



**Figure 3. Growth curve for *S.thermophilus* in M17 broth supplemented with (i) 1.5% BR (ii) 1.5% WR (iii) 1.5% CH (iv) 1.5% AG. All comparisons were done against PI and GC**

Results in our study show that growth of *L. acidophilus* didnot significantly vary in the presenceof BR, WR, CH, AG and PI (Fig 1).

Growth of *L. bulgaricus* was increased by PI (0.4 log CFU/ml) from 6-17h, when compared to the GC. Growth promoting effects (0.4 log CFU/ml) after 5 hours with BR and CH were also observed. However, a significant inhibitory effect could be seen after 2 hours in the test culture grown with AG (Fig. 2). In case of *S. thermophilus*, both the BR and PI were inhibitory; whereas, growth with CH, WR and AG showed no significant variation (Fig. 3). The study shows that *L. bulgaricus* and *S. thermophilus* growth profiles were altered in the presence of the test supplements. Manufacturers would need to test probiotic organisms with supplements for survival capability, before including them in the currently exploding range of probiotic shelf products. *L.acidophilus*, on the other hand, seemed to be more hardy over 20 hours, and thus could be preferred as a probiotic supplement in formulations.

While dietary fibers are reported to be good prebiotics, due to their indigestibility and probiotic promoting activity (Tuohy *et al.*, 2005); our study showed that WR was a relatively neutral supplement and BR had variable results. Rice bran has been reported to have protective efficacy against rotaviral diarrhoea (Yang *et al.*, 2015), its supplementation with probiotics may enhance its use in treating gut infections, while being more palatablethan pure probiotic formulations. Hydrolyzed casein is used in infant formulae to prevent lactose allergy and is usually supplemented with *L.rhamnosus* (Guest *et al.*, 2015). It is also used to increase cell count, slow down acidification and improve adhesiveness and sensory acceptability (Zhao *et al.*, 2006). Our study supports the synergistic effect of CH with probiotic microorganisms. While scientific reports on BR are few, its growth supporting ability on both lactobacilli isolates in our study provides a purpose for its use, along with its anti-inflammatory applications in colon cancer (Phutthaphadoong *et al.*, 2010).

Ginseng, a popular Chinese medicine and flavour enhancer in Asian nutritive and therapeutic foods was inhibitory on *L. bulgaricus* but had no significant effects on *L.acidophilus* and *S.thermophilus*. Ginseng is marketed under various names from Korea, America and China. While reports support some of the varieties as pro-probiotic (Bang *et al.*, 2014), it would be necessary to experimentally prove the growth-promoting effects of specific varieties before using them in therapy. On the other hand, PI, a known prebiotic, increased growth of *L. bulgaricus* by 0.6 log CFU/ml, but showed no significant effect on the other probiotics.

## Conclusion

While the beneficial effects of probiotics have been promoted in several reports, leading to their wide-spread application in several over-the-counter confectionary, food, dietary, therapeutic and prophylactic formulations; their survival along with other ingredients would need to be tested.

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