1. The Binding Efficiency and Interaction of *Lactobacillus casei* Shirota Toward Aflatoxin B1.

**Summary**
The use of probiotics as dietary approach to prevent exposure to food contaminant, aflatoxin B1 (AFB1) has greatly increased. Several studies found that AFB1 binding to the bacterial cell wall is strain-specific. Moreover, the interaction between AFB1 and bacterial cell wall is not well-understood, thus warrants further investigation. This research was conducted to assess the ability of *Lactobacillus casei* Shirota (LcS) to bind AFB1 at different concentrations and to determine AFB1 binding efficiency of different LcS cell components including live cell, heat-treated, and cell wall. In addition, the interaction between AFB1 and LcS was also evaluated via scanning electron microscopy (SEM) and through an animal study. The binding of AFB1 by all LcS cell components depends on the concentration of available AFB1. Among all LcS cell components, the live Lcs cells exhibited the highest binding efficiency (98%) toward AFB1. Besides, the SEM micrographs showed that AFB1 induced structural changes on the bacterial cell surface and morphology including rough and irregular surface along with a curve rod-shaped. In vivo experiment revealed that LcS is capable of neutralizing the toxicity of AFB1 on body weight and intestine through the binding process. The animal’s growth was stunted due to AFB1 exposure, however, such effect was significantly (p < 0.05) alleviated by LcS. This phenomenon can be explained by a significant (p < 0.05) decreased level of blood serum AFB1 by LcS (49.6 ± 8.05 ng/mL) compared to AFB1-exposed rats without treatment (88.12 ± 10.65 ng/mL). Taken together, this study highlights the potential use of LcS as a preventive agent against aflatoxicosis via its strong binding capability.
2. Oral Administration of Probiotic *Lactobacillus casei* Shirota Decreases Pneumonia and Increases Pulmonary Functions after Single Rib Fracture: A Randomized Double-Blind, Placebo-Controlled Clinical Trial.


**Summary**

Considerate proportion of elderly patients with a rib fracture are susceptible to pulmonary complications, especially pneumonia. This study aimed to assess the effect of oral administration of the probiotic *Lactobacillus casei* Shirota (LcS) on pneumonia and pulmonary functions among elderly patients with single rib fracture. The current study included 204 eligible elderly patients with a single rib fracture. Patients were randomly assigned to receive oral administration of skimmed milk containing either a commercial probiotic LcS or placebo daily for 1 month after the fracture. This was followed by pneumonia assessments, pulmonary function testing including forced expiratory volume (FEV), negative inspiratory pressure (NIP), and forced vital capacity (FVC), as well as evaluation of potential adverse effects including myocardial infarction, acute kidney injury, nonunion of fractured bone, or stroke. After 1-month consumption, patients in the LcS group exhibited decreased pneumonia and increased recovery of pulmonary functions, in terms of FEV, FVC, and NIP, compared to the placebo group. No difference was observed in incidence of adverse events between the 2 groups. In patients with a single rib fracture, oral administration of the probiotic LcS was associated with a lower incidence of pneumonia and increased pulmonary functions without causing severe adverse effects.


**Summary**

Suppression of immune function during long spaceflights is an issue that needs to be overcome. The probiotic *Lactobacillus casei* strain Shirota (LcS) could be a promising countermeasure, and a project was launched to investigate the efficacy of its use on the International Space Station (ISS). As a first step, a special probiotic product for space experiments,
containing freeze-dried LcS in capsule form (probiotics package) was developed. This was for tested its stability through 1 month of storage on the ISS. The temperature inside the ISS ranged from 20.0 to 24.5 °C. The absorbed dose rate of the flight sample was 0.26 mGy/day and the dose equivalent rate was 0.52 mSv/day. The number of live LcS was $1.05 \times 10^{11}$ colony-forming units/g powder (49.5% of the initial value) 6 months after the start of the study; this value was comparable to those in the two ground controls. Profiles of randomly amplified polymorphic DNA, sequence variant frequency, carbohydrate fermentation, reactivity to LcS-specific antibody, and the cytokine-inducing ability of LcS in the flight sample did not differ from those of the ground controls.

4. Redox Role of Lactobacillus casei Shirota Against the Cellular Damage Induced by 2,2'-Azobis (2-Amidinopropane) Dihydrochloride-Induced Oxidative and Inflammatory Stress in Enterocytes-Like Epithelial Cells.

Summary
In Western societies where most of the day is spent in the postprandial state, oxidative and inflammatory stress conditions makes postprandial stress a risk factor for the development of cardiovascular disease. A large body of evidence have been accumulated on the anti-inflammatory effects of probiotics, but no information is available on the mechanisms through which intestinal microbiota modulates redox unbalance associated with inflammatory stress. The study aimed to investigate the ability of Lactobacillus casei Shirota (LcS) to induce an antioxidant response to counteract oxidative and inflammatory stress in an in vitro model of enterocytes. The results show that pre-treatment of enterocytes with LcS prevents membrane barrier disruption and cellular reactive oxygen species (ROS) accumulation inside the cells. It also modulates the expression of the gastro-intestinal glutathione peroxidase (GPX2) antioxidant enzyme, and reduces p65 phosphorylation, supporting the involvement of the Nfr2 and nuclear factor kappa B pathways in the activation of antioxidant cellular defenses by probiotics. These results suggest, for the first time, a redox mechanism by LcS in protecting intestinal cells from AAPH-induced oxidative and inflammatory stress.
5. Are probiotic treatments useful on fibromyalgia syndrome or chronic fatigue syndrome patients? A systematic review.

Summary
Evidence suggests that the gut microbiota might play an important role in fibromyalgia syndrome (FMS) and chronic fatigue syndrome (CFS). The goal of the study was to systematically review the reported effect of probiotic treatments in patients diagnosed with FMS or CFS. A systematic review was carried out using 14 databases (PubMed, Cochrane Library, Scopus, PsycINFO, and others) in February 2016 to search for randomised controlled trials (RCTs) and pilot studies of CFS or FMS patient, published in the last ten years (from 2006 to 2016). The Jadad scale was used to asseverate the quality of the clinical trials considered. Two studies (n=83) met the inclusion criteria, which were performed in CFS patients and both studies were considered as a 'High range of quality score'. The administration of *Lactobacillus casei* strain *Shirota* in CFS patients, over the course of 8 weeks, reduced anxiety scores. Likewise, this probiotic changed the faecal composition following 8 weeks of treatment. Additionally, the treatment with *Bifidobacterium infantis* 35624 in CFS patients, during the same period, reduced inflammatory biomarkers. The evidence about the usefulness of probiotics in CFS and FMS patients remains limited. The studied strains of probiotics have demonstrated a significant effect on modulating the anxiety and inflammatory processes in CFS patients. However, more experimental research, focusing mainly on the symptoms of the pathologies studied, is needed.