POSTER COMMUNICATIONS

**Human microbiota modulation by functional food consumption**

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The main activities of PhD thesis project deal with the relationship between microbiota and functional food, in particular dietary supplements with probiotic and prebiotic addition. First of all, the relationship between synbiotic administration to nephropathic (Chronic Kidney Disease, CKD) subjects and variation of gut microbiota composition was evaluated. Secondly, the effects of probiotic yeast (*Saccharomyces bayanus* var. *uvarum*) assumption on composition and metabolism of microbiota in healthy subjects were defined. These effects were evaluated to consider their addition to food matrices and assess the effect of a functional food on the microbiota.

Modulazione del microbiota umano mediante l’assunzione di alimenti funzionali

Le attività principali del progetto di tesi di dottorato riguardano la relazione tra microbiota e alimenti funzionali, in particolare integratori alimentari con aggiunta di probiotici e prebiotici. In primo luogo, è stata valutata la relazione tra la somministrazione di un simbiotico a soggetti nefropatici (malattia renale cronica, MRC) e la variazione della composizione del microbiota intestinale. In secondo luogo, sono stati definiti gli effetti dell'assunzione di lievito probiotico (*Saccharomyces bayanus* var. *uvarum*) sulla composizione e sul metabolismo del microbiota. Questi sono stati valutati per considerare la loro aggiunta a matrici alimentari e valutare l’effetto di un alimento funzionale sul microbiota.

**Key words**: gut microbiota, functional food, synbiotic, health-promoting bacteria, probiotic yeasts.

# **1. Introduction**

In accordance with the PhD thesis project previously described, this poster reports the main results of the first two activities concerning:

(A1) the restoration of the dysbiotic gut microbiota by synbiotic NatuRENG® administration in CKD subjects;

(A2) the variation of gut microbiota composition and metabolites by the consumption of probiotic yeast SERIUS.

# **2. Materials and Methods**

**2.1** **Restoration of gut microbiota by synbiotic NatuREN G® in CKD subjects**

A placebo-controlled, randomized, single-blind study in healthy and CKD subjects was planned. Patients were randomized to receive NatuREN G® (*Bifidobacterium* *animalis* BLC1, *Lacticaseibacillus* *casei* LC4P1, fructo-oligosaccharides, inulin, quercetin, resveratrol, and proanthocyanidins) or placebo. The two groups were named, respectively, CDK-S and CDK-P. Faecal samples were collected at the beginning of the study (T0), after two months of treatment (T60) and after one month of wash-out (T90). DNA extraction from feces and quantitative PCR were performed to evaluate an increment in *Lactobacillus* and *Bifidobacterium* genera.

**2.2 Evaluation of probiotic potential of *Saccharomyces bayanus* var. *uvarum***

The yeast probiotic properties were evaluated by a placebo-controlled, randomized cross-over study in 50 healthy subject who received SERIUS (*Saccharomyces bayanus* var. *uvarum* IRIS-SERIUS) or placebo (similar package containing xanthan gum). Faecal samples were collected at the beginning of the study (T0), at the end of first treatment (T60), at the end of wash-out period (T75) and at the end of second treatment (T135). DNA extraction, quantitative PCR and metabolome analysis (volatile organic compound, VOC) were performed to evaluate variation of composition of gut microbiota and variation of metabolites.

# **3. Results and Discussion**

**3.1 Restoration of gut microbiota by synbiotic NatuREN G® in CKD subjects**

At the beginning of the study (T0), no significant changes were found among all subjects and bacterial targets. After 60 days of treatment (T60) and after 30 days wash-out (T90), there was no significant increase in the CKD-S group, while in CKD-P there was a decrease of *Lactobacillus* genus. Otherwise, for *Bifidobacterium* genus, there was a significant decrease in its abundance in the CKD-P group at the end of the trial (T90) compared with the beginning of the study and after 60 days of placebo treatment (T0 and T60). In the CKD-S group, *Bifidobacterium* abundance increased in every check point of the study. However, 60 days of treatment with NatuREN G® was not sufficient to reach significance, the values collected at T90 were significantly different from those assessed at T0. The symbiotic NatuREN G® exerted selective efficacy in patients with stage IIIb-IV CKD. It was able to modulate the gut microbiota profile and related metabolism by increasing the ratio of Firmicutes to Bacteroidetes. A decrease of this ratio was previously noticed as a signature of chronic relapsing inflammation affecting the intestinal mucosa (Carding 2015). Although species belonging to Firmicutes and Actinobacteria contain relatively few fiber-metabolizing enzymes *per* organism, these phyla are the main responders to plant-derived nutrients (Deehan 2017). Both Firmicutes and Actinobacteria generally exert specialized roles, such as the initiation of complex substrate degradation (Martínez 2010). Therefore, the increased intake of fiber assessed by the dietary recall after the treatment with NatuREN G® may have sustained the abundance of Firmicutes in this pilot study. Therefore, the present work paves the way for further studies based on nutrition and adjuvant therapies based on the administration of probiotics and prebiotics, while having as interest the treatment of diseases without gastrointestinal background, such as nephropathy.

**3.2 Evaluation of probiotic potential of *Saccharomyces bayanus* var. *uvarum***

Faecal volatile organic compounds (VOCs) from healthy subjects following treatment with the probiotic (S. bayanus SERIUS/IRIS) or placebo (maltodextrins/xanthan gum/erythrol) were analysed. Untargeted analysis of faecal metabolites (GC-MS) identified a total of 134 VOCs, grouped into the following chemical classes: alcohols (16), aldehydes (10), esters and methyl esters (24), hydrocarbons (7), indoles (3), ketones (10), organic acids (18), phenols (4), sulfuric compounds (4), terpenes (24), and others (14). An initial analysis comparing the percentage of each of the chemical classes examined was conducted by comparing them in the two treatment groups at the beginning and end of administration. Phenols, indoles and organic acids were the most represented classes in all groups examined. The highest percentage of organic acids (29.73%) was presented in samples from healthy subjects after treatment with probiotic (*S. bayanus* SERIUS/IRIS). The compounds that increased in the probiotic-treated group (3-methyl-Indole; 2-Pentadecanone; 2-Undecanol; 2,6-Dimethylphenyl isocyanate and others).

3-Methylindole is the product of intestinal metabolism of tryptophan. Gut bacterial species convert tryptophan to tryptamine and indole-3-pyruvic acid, as well as convert it to indole, indole-3-acetaldehyde and indole-lactic acid. Some species belonging to the phylum Firmicutes (*Lactobacillus johnsonii*, *Limosilactobacillus reuteri*, *Ligilactobacillus murinus*, and *Lactobacillus acidophillus*) convert indole-3-acetaldehyde to indole-3-acetic acid, which by decarboxylation generates 3-methylindole. Intestinal tryptophan metabolites participated in the host-gut microbiota cross-talk and acted as aryl hydrocarbon receptor (AhR) ligands and agonists (Agus 2018; Dong & Perdew 2020; Hubbard 2015; Lamas 2018; Nicholson 2012). The AhR played a key role in regulating the immune system, as well as regulating the production of enzymes involved in metabolic processes. In particular, AhR can stimulate the immune response at the level of the intestinal mucosal barrier by modulating intraepithelial lymphocytes, T cells, and the production of interleukin-17 (IL-17) and IL-22, also contribute the maintenance of gut eubiosis (Lamas 2020). Finally, a targeted analysis of only short-chain fatty acids (SCFAs) (acetic, propionic, isobutyric, butanoic, and isovaleric) showed a statistically significant increase in acetic acid in healthy subjects following probiotic treatment. Short-chain fatty acids are final products of gut fermentation with trophic effect on the intestinal epithelium. In addition, they are involved in regulating the immune response and improving the barrier function of the intestine (LeBlanc 2017; Parada Venegas 2019; Ríos-Covián 2016). The statistically highlighted significant increase in acetic acid in faecal samples following treatment with probiotic (S. bayanus SERIUS/IRIS). This suggests an effect of counteracting gut dysbiosis and stimulating immune response.

Preliminary qPCR results showed an increase in the genus *Bifidobacterium*, *Bifidobacterium adolescentis*, and *Limosilactobacillus fermentum* after placebo intake, and an increase in *Bifidobacterium bifidum* after probiotic intake, as well as an increase in *Saccharomyces bayanus*. These evidences have been reported in Log (Copy Number). A standard curve for each primer was constructed with sequential dilutions of DNA extracted from type strain culture. The Copy Number (CN) and the Logarithms were calculated based on DNA concentration and amplicon length. The standard curve was constructed by interpolating the Log (Copy Number) and Cycle threshold (CT) obtained from qPCR analysis.

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