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RESEARCH ARTICLE

Gut Microbiota Modulation by Selenium and Zinc Enrichment Postbiotic on Dysbiosis Associated with Hypertension

Laura-Dorina Dinu[1,*], Florentina Gatea[2], Florentina Matei[4], Cristian Banciu[3] and Emanuel Vamanu[1,*]

[1]*Department of Industrial Biotechnology, Faculty of Biotechnology, University of Agronomic Sciences and Veterinary Medicine, Bucharest, Romania;* [2]*Department of Biotechnology, Centre of Bioanalysis, National Institute of Research and Development for Biological Sciences, Bucharest, Romania;* [3]*Department of Ecology, Institute of Biology of Romanian Academy, Bucharest, Romania*[4]*Faculty of Food Industry and Tourism, Transylvania University of Brasov, Brasov, Romania*

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Abstract: ***Background*:** Targeting gut dysbiosis to treat chronic diseases or to alleviate the symptoms is a new direction for medical adjuvant therapies. Recently, postbiotics have received considerable attention as they are non-viable probiotic preparations that confer various health benefits to the host without the safety problems associated with using live microbial cells.

***Objective*:** The aim of the study is to obtain selenium (Se) and zinc (Zn) enriched *Saccharomyces boulardii* postbiotic biomass and to analyze its modulation effect because these minerals play an important role in reducing gut dysbiosis linked to cardiovascular (CV) diseases.

***Method*:** The effect of the *S. boulardii* and Se/Zn enriched yeast postbiotics on CV microbial fingerprint was studied *in vitro* using the gastrointestinal system (GIS 1) and analyzed by microbiological, chemical, and qPCR methods.

***Result*:** There was a 2.2 log CFU/mL increase in the total bacterial load after SeZn postbiotic treatment and in the qPCR counts of Firmicutes phyla for both treatments. Beneficial taxa, *Bifidobacterium* spp. and *Lactobacillus* spp., as well as *Bacteroides* spp. were up to 1.5 log higher after mineral-enriched postbiotic application, while the acetic acid level increased.

***Conclusion*:** These preliminary studies highlight the therapeutic potential of using Se/Zn enriched yeast postbiotics as adjuvants for clinical treatments of CV diseases.

Keywords: *Saccharomyces boulardii*, postbiotic, selenium and zinc enriched biomass, *in vitro* gastrointestinal simulation, hypertension associated dysbiosis, gut microbiota modulation, cardiovascular disease treatment.

1. INTRODUCTION

Hypertension is the prevalent risk factor for cardiovascular (CV) diseases and is considered a major public health issue because it is estimated that 1.28 billion people worldwide are affected by this major cause of premature death [1]. The new mosaic theory about the pathophysiology of hypertension suggested that many genetic and environmental factors interact to raise blood pressure and cause end-organ damage, including the imbalance of the gut microbial community [2]. Gut dysbiosis in animal models and human patients with hypertension from childhood to adulthood has been reported, and recently, some reviews focusing on the relationship between gut microflora and CV diseases were published [3-8]. Numerous small-scale studies have provided data about the association between gut microbial dysbiosis and different groups of pre-hypertensive, systolic, and diastolic hypertensive patients [9-13]. Moreover, it has been suggested that mediocre results obtained after treatment of COVID-19 patients with hypertension are linked to their gut microbial imbalance [14]. Altogether, it appears that gut microbial dysbiosis promotes the development of hypertension, and the severity of the disease was found to be associated with increased Firmicutes/Bacteroidetes ratios, reduced diversity, a greater abundance of opportunistic pathogenic taxa, and a reduced population of acetate-butyrate-producing bacteria [4, 5, 7]. Microbiota metabolites production, especially short-chain fatty acids (SCFAs) such as acetate, propionate, butyrate, and other metabolites - lactate and trimethylamine (TMA), have been proven to lower blood pressure [6, 15, 16]. In hypertensive patients, changes in the gut environment are associated with changes in microbial composition and metabolic profile, as well as increased colonic permeability and inflammation [3, 7, 16]. Targeting gut dys-

* Address correspondence to these authors at the Department of Industrial Biotechnology, Faculty of Biotechnologies, University of Agricultural Sciences and Veterinary Medicine Bucharest, Bucharest, Romania;
Fax: +40.213182266; E-mails: laura.dinu@biotehnologii.usamv.ro, email@emanuelvamanu.ro

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biosis as an alternative treatment for hypertension or other chronic diseases closely related to hypertension is a possible therapeutic strategy to prevent the generation or alter the development of CV diseases [4, 16]. The antihypertensive probiotic widely reported is *Lactobacillus* spp., which can reduce blood pressure using multiple mechanisms, such as restoring the imbalance of microflora, changing the production of metabolites, reducing oxidative stress, and exerting anti-inflammatory effects [4, 16]. However, the benefits of relevant probiotics remain controversial as various strains showed inconsistent activities, and huge discrepancies exist between different studies or clinical trials [4]. A proposed therapeutical intervention to manipulate gut microbiota is related to postbiotics, non-viable probiotic preparations that confer various health benefits to the host without the safety problems associated with the use of live microbial cells [7, 17, 18]. The new approach in probiotic/postbiotic research, the minerals-enriched microbial biomass, is based on the synergistic effect of bioactive molecules released from postbiotic and minerals that can modulate the gut microbiota to prevent or treat dysbiosis [19]. Bacterial or fungal probiotic strains can accumulate minerals and transform them into organic compounds that are used by both intestinal microbiota and host [20]. Minerals, such as selenium (Se) and zinc (Zn), incorporated into probiotic biomass proved to be able to balance microbial flora and to increase the mineral bioavailability and subsequent absorption by the host [20-23]. Moreover, the active biomolecules from postbiotics and selenocompounds reduced the pathobionts' overgrowth [22, 24]. It is known that Zn and Se are powerful antioxidants that play a protective role against oxidative stress and defend the human and animal body from chronic conditions, such as heart and metabolic diseases and cancer [25, 26]. However, only a few studies have provided information about the interaction between gut microbiota and Se and/or Zn-enriched probiotics, mostly performed on murine models [27-33], chickens [34] and canine [24], while few trials have been conducted on humans or human cell cultures [35-37].

Therefore, the study aims to obtain enriched *Saccharomyces boulardii* postbiotic and to analyze the human gut microbiota modulation effect of Se/Zn postbiotic, comparing results with data obtained after *S. boulardii* postbiotic treatment. The changes in CV microbial fingerprint after treatment with postbiotic and Se/Zn enriched postbiotic were studied *in vitro* using the gastrointestinal system (GIS 1) [[38, 39]] and analyzed by microbiological and real-time PCR (qPCR) methods, while the metabolic profile was analyzed with a zonal electrophoretic method. These preliminary studies highlight the therapeutic potential of Se/Zn enriched yeast postbiotic as an adjuvant for clinical treatments that target CV gut dysbiosis.

2. MATERIALS AND METHODS

The probiotic strain used in the study is *Saccharomyces boulardii* S28, obtained from the University of Lille (France) and maintained at -80C. The (*Supplementary Materials)* file provides detailed information about the methods and materials used.

The strain was routinely maintained in yeast extract peptone dextrose (YEPD) agar (Acumedia, USA) medium and was grown aerobically at 30C for 48 h. The adaptation process of the yeast was started in the culture medium YEPD enriched with sodium selenite Na_2SeO_3 (Sigma-Aldrich, USA) 100 μg/mL and Zn sulfate $ZnSO_4x7H_2O$ (Sigma-Aldrich, USA) 30 μg/mL.

Postbiotic preparations from wild-type and Se/Zn enriched-strain (10^7 CFU/mL) were performed by thermal inactivation (80C for 30 min).

X-ray fluorescence system (XRF Rigaku ZSX100e, USA) was used to analyze the heavy metals from non-viable suspensions.

All tests were performed using the *in vitro* simulation system GIS1 (https://gissystems.ro/) and the microbiome of the target CV disease. The reconstitution process followed the protocol previously described and was performed with a mean interval of 7-10 days [40, 41]. The postbiotic *S. boulardii* S28 strain and Se/Zn enriched strain (1 ml) were added every day during 4-day experiments. The CV microbiota was grown microaerophile at constant temperature (37C) and pH 6 while their evolution was recorded during the simulation process. Luminal pH in the proximal small bowel ranges from 5.5 to 6.5, and the pH was kept constant with sterile 1 M NaOH solution. The samples (15 mL) were collected at regular intervals (0, 48, 96 h), then centrifuged (4000 rpm, 15 min), and the pellet was kept in sterile conditions at 4C, under glycerol.

Microbiological analysis and DNA isolation were performed within 24 h after collection using nutrient broth agar medium (Oxoid) and selective medium: MRS agar for *Lactobacillus* sp. (Oxoid), BSM agar for bifidobacteria (Sigma-Aldrich/Fluka) and MacConkey agar for Gram-negative enteric bacteria (Oxoid).

Similar to the previous studies, genomic DNA was extracted from all samples with a Quick DNA Miniprep Plus kit (ZymoResearch, USA). Quantitative PCR was performed using Rotor-Gene 6000 5plex HRM (Qiagen-Corbett Life Science, Australia), Maxima SYBR Green/ROX qPCR MasterMix (ThermoScientific, USA), and specific primers [42, 43, 15].

The microbial metabolic products (0, 96 h) were analyzed from the supernatant obtained after centrifugation. SCFA separation was performed using an Agilent G7100 capillary electrophoresis apparatus (Agilent Technologies, Germany) with a diode array detector (DAD) [44, 45].

The statistical analysis was calculated using the IBM SPSS Statistics 23 software package (IBM Corporation, USA). The significance level for the calculations was set as follows: significant, $p<0.05$; very significant, $p<0.01$; highly significant, $p<0.001$; and extremely significant, $p<0.0001$ using the letters from a to d.

Table 1. The tolerance test of *Saccharomyces boulardii* wild-type strain in YEPD media enriched with different concentrations of selenium and zinc.

Medium	Average Growth Rate (μ)	% From Initial μ
YEPD	0.273	100
YEPD-Se60	0.243	89.080
YEPD-Se120	0.209	76.890
YEPD-Zn30	0.286	104.869
YEPD-Zn60	0.265	90.020
YEPD-Se100Zn30	0.232	85.283

3. RESULTS

3.1. Preparation of Se/Zn-enriched Saccharomyces Boulardii Postbiotic

The first objective was to evaluate the ability of *S. boulardii* wild-type strain to grow in the presence of Se and Zn and select the metal concentrations that do not significantly influence the growth rate but promote metals bioaccumulation. The tolerance study was performed by growing the yeast cells in different media, YEPD, YEPD supplemented with Na_2SeO_3 (60 and 120 μg/mL), respectively YEPD-$ZnSO_4x7H_2O$ (30 and 60 μg/mL); then the exponential growth rate was calculated (Table **1**).

The growth rates of *S. boulardii* decreased in media supplemented with increased metal concentration, with one exception noted in the presence of 30 μg/mL zinc (YEPD-Zn 30), where the growth rate was higher (~104%) than for the control sample. Zinc is an essential element required by yeast growth and metabolism, and for *S. cerevisiae,* up to 2 mg/L appears to be required for optimal growth and glycolysis [46]. Se concentrations >7.9 μg/mL are considered to affect cell division negatively and yeast growth [47]. In our study, the *S. boulardii* growth rate was lower in YEPD-Se 60 medium than in the medium with YEPD-Zn 60, as it is known that selenium has stronger toxic effects. The *S. boulardii* growth rate gradually decreased, thus, in medium YEPD-Se, 120 μg/mL remained at 76.89% from the growth rate measured after cultivation in YEPD medium. Therefore, YEPD medium enriched with 100 μg/mL sodium selenite and Zn sulfate 30 μg/mL was selected for further adaptation experiments.

The probiotic strain *Saccharomyces boulardii* S28 was adapted to grow in a Se/Zn-enriched medium, and red colonies were selected. In the medium with a high Se concentration, microbial strains convert the inorganic Se into an element of Se and change the color to red [27]. The adapted yeast strain had a lower growth rate in selected minerals-enriched medium but maintained ~85% from the initial exponential growth rate of the wild-type (Table **1**).

The XRF analysis was used to assess the Se and Zn concentration in the *S. boulardii* postbiotic and the level of bioaccumulation in adapted Se/Zn-postbiotic suspension. The results are shown in Fig. (**1**). As expected, the adapted yeast strain contains 2.7 times more Se and an increased Zn (1.98x) concentration compared with the wild-type *S. boulardii* after growing on the YEPD medium. In medium enriched with Se (100 μg/mL) and Zn (30 μg/mL), the metal bioaccumulation capability of the adapted strain is higher, especially Se, which was detected at 7.68 times higher level than the wild-type, while Zn doubled (2.42x).

Wild-type and Se/Zn-enriched strains were used to obtain postbiotic preparations by thermal inactivation, and the loss of viability was checked by plating them onto a YEPD medium.

3.2. The Effect of the S. boulardii Postbiotic and Se/Zn-enriched Postbiotic on Microbial Load and Potential Taxa Involved in Blood Pressure Regulation

The cardiovascular gut microbiota used in the study has been previously analyzed, proving that it corresponds to the pathophysiology of hypertension and was used in different studies [38-40]. After restoration, CV microbiota was used for gastrointestinal simulation with the GIS 1 system. At the same time, *S. boulardii* postbiotic (WP treatment) or Se/Zn--postbiotic (SeZnP treatment) were added each day during the 4-day experiments.

At the beginning of the experiment, the total microbial load (aerobic and anaerobic counts onto non-selective media) was approximately 17.72 log CFU/mL. After the minerals-enriched treatment, the total bacterial population was ~2.19 log CFU/mL higher, increasing both aerobic and anaerobic populations. However, the administration of *S. boulardii* postbiotic changed the total bacterial population (Fig. **2**). Notably, the Firmicutes population detected by qPCR gradually increased up to 1.3 log after 96 h of both postbiotic treatments, especially after WP treatment (Table **2**).

Many works have identified thatpotential microbial taxa are involved in regulating blood pressure, species that can reduce hypertension, and some are potentially harmful. However, it is important to mention that the roles of some microbes in hypertension are debated, such as *Bacteroides* spp [3, 16]. At the same time, *Lactobacillus,* which belongs to Firmicutes, and *Bifidobacterium* from Actinobacteria are widely considered beneficial bacterial genus. Most studies proved the higher abundance of *Lactobacillus, Roseburia, Akkermansia, Coprococcus,* and *Bifidobacterium* in healthy or treated groups with lower blood pressure [5, 16]. Moreover, a significant depletion of *Bifidobacterium* was found in hypertensive rats and humans [9, 15].

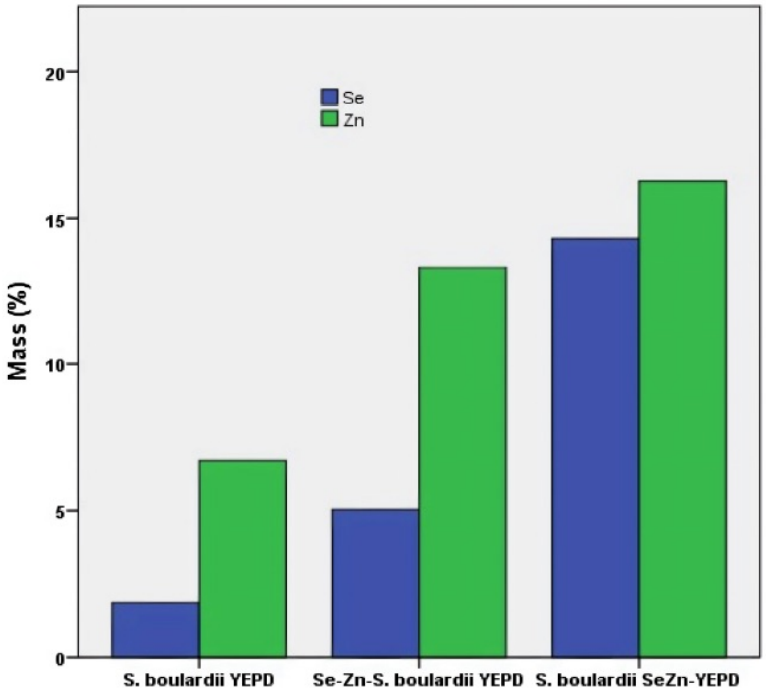


Fig. (1). Selenium and zinc bioaccumulation in *Saccharomyces boulardii* wild-type and SeZn-enriched postbiotic grown onto YEPD and Se100Zn60-YEPD media. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

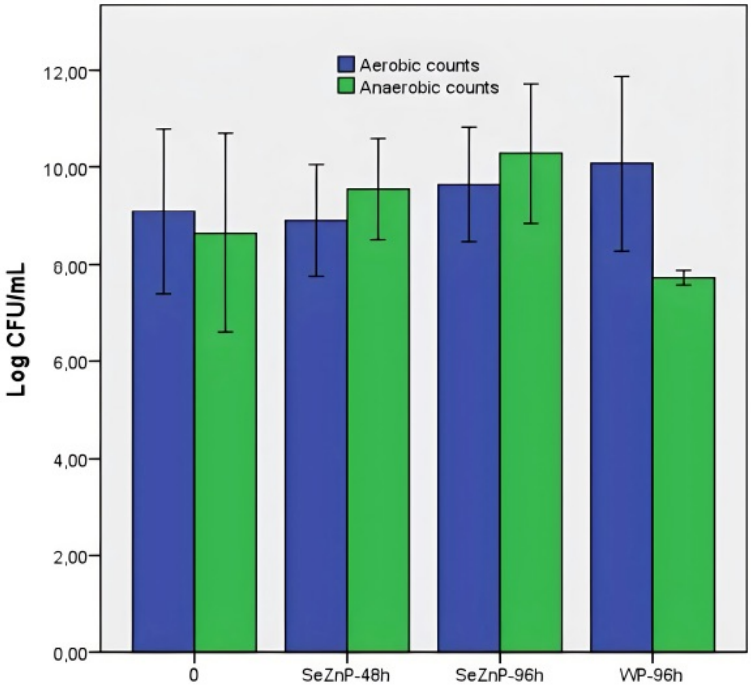


Fig. (2). The effect of the *S. boulardii* postbiotic (WP) and Se/Zn-enriched postbiotic (SeZnP) treatments on total microbial load, detected by microbiological methods on non-selective media before (0h) and after 48 and 96 hours of treatment. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 2. The effect of the *S. boulardii* postbiotic (WP) and Se/Zn-enriched postbiotic (SeZnP) treatments on Firmicutes phyla, detected by qPCR, before (0h) and after 48 and 96 h of treatment.

-	WP Treatment	SeZnP Treatment
0h	9.933 ± 0.057	
48h	10.633 ± 0.057	9.833 ± 0.208
96h	11.250 ± 0.050	10.850 ± 0.050

Our microbiological and qPCR analysis data showed that postbiotic and SeZn-enriched postbiotic administration increased the *Bifidobacterium* population. Therefore, counts on selective BSM media increased from 6.43 ± 0.24 log CFU/mL to 7.94 ± 0.23 log CFU/mL after 96 h of SeZnP treatment, while the *Bifidobacterium* population remained stable (6.36 ± 0.86 log CFU/mL) after WT treatment and similar results were obtained based on qPCR results (Fig. **3a**). Both postbiotics produce changes in the *Lactobacillus* counts detected by qPCR or in the amount of culturable lactobacilli onto MRS media after the treatment population increased up to 1 log/mL (Fig. **3b**). Based on qPCR results with specific primers *Bacteroides* spp. population is 1 log/mL higher after SeZn-enriched postbiotic administration than at the beginning of the experiment, but only 0.5 log/mL after using *S. boulardii* postbiotic (Fig. **3b**). However, in our study, the *Enterobacteriaceae* population detected on selective media remained constant (~5.5 log CFU/mL), while *E. coli* detected by qPCR significantly changed after postbiotic applications (Fig. **3ab**).

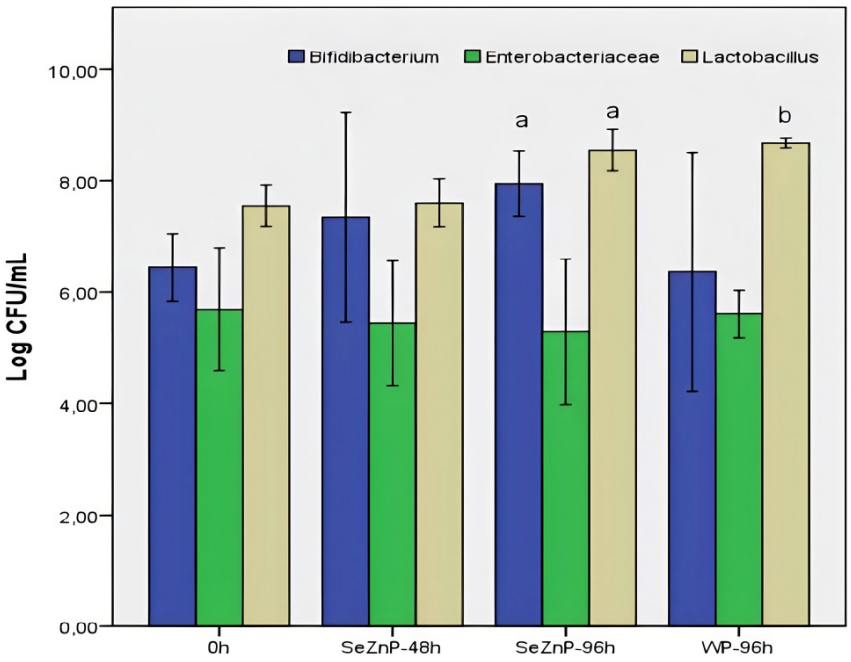
The major shift in the microbial composition after SeZn--postbiotic treatment, characterized by a higher acetate-producing *Bifidobacterium* population, was correlated with an increase in the acetic acid at the end of the experiment. Thus, the acetate level increased from 0.230 ± 0.024 mg/mL to 0.350 ± 0.027 mg/mL at the end of the experiment. Acetate is one of the major microbial metabolite SCFAs that helps regulate the acidity in the gut environment and promote the growth of beneficial bacteria [16, 48, 49]. The results obtained in this study showed that SeZnP treatment increased acetic acid concentration as well as the acetate-producing bacteria *Bifidobacterium* spp. and *Bacteroidetes* spp. However, other SCFAs (butyrate and propionate) did not change in samples treated for 96h with SeZnP, while lactate slowly decreased from 1.065 ± 0.076 mg/mL to 0.715 ± 0.046. Additionally, a small amount of oxalic acid was detected, which decreased from 0.042 ± 0.003 mg/mL to 0.029±0.001 mg/mL at the end of the experiment.

4. DISCUSSION

Furthermore, because cardiovascular diseases are the leading cause of death worldwide, an investigation of novel approaches that restore gut microbiome dysbiosis has great potential for managing CV diseases. To our knowledge, the study is the first attempt to evaluate *in vitro* the effect of mineral-enriched postbiotic preparations on human gut microbiota linked to hypertension. This approach is based on the health benefits of bioactive molecules from postbiotic preparations, their stability, and safety, as well as on the evidence that Se and Zn-enriched probiotic biomass modulated different gut dysbiosis. Using *S. boulardii* Se/Zn-enriched postbiotic possesses a few advantages, including the ability of yeast to accumulate high amounts of minerals and to transform inorganic into organic compounds with higher bioavailability for microbiota and human intestinal cells. At the same time, this is an eco-friendly and cost-effective approach.

In our study, the probiotic *S. boulardii* strain was tested for tolerance in media supplemented with sodium selenite (60 μg/mL, 120 μg/mL) and Zn sulfate (30 μg/mL, 60 μg/mL), then the concentrations that do not significantly influence the growth rate but promote metals bioaccumulation were selected. In the YEPD-Zn30 medium, the growth rate was slowly higher than for the control sample, and similar data were reported for an adapted *Saccharomyces cerevisiae* strain with the highest growth rate in the medium with 25 μg/mL Zn, promoting Zn uptake [50]. Using strain *S. cerevisiae* PTCC 5209 cultivated in medium with 30 mg/L ZnSO4, the total zinc accumulation and organically bound Zn fraction was maximum, while the Zn content in the biomass increased by 24-fold compared with the growth in basal medium [51]. At the same time, double Zn concentration (60 mg/L) decreased biomass yield without increasing the total metal accumulation [51]. Se has a more harmful effect than Zn, but it was shown for *S. boulardii* CCT4308 that Se accumulation is correlated with a metal concentration in a medium, up to 150 μg/mL Se. In this case, the best Se bioaccumulation was detected in a medium with 100 μg/mL Na2SeO3 (4.56 ± 0.44 mg Se/g biomass), while the biomass decreased by 4.79% [52]. In another study with *S. cerevisiae* NCYC 1026, 75 μg/mL Se concentration did not change the biomass production, and bioaccumulation increased in media with >50 μg/mL Se [53]. The adapted *S. boulardii* strain on YEPD-Se100Zn30 medium changed the color into red and maintained ~85% from the initial exponential growth rate of the wild-type yeast.

The adapted strain accumulated 7.68 and 2.42 times more amounts of Se and Zn, respectively, than the wild-type strain. Microbes can act as natural adsorbents for minerals, especially yeasts. In these cells, Se and Zn accumulation occurs in two steps: biosorption, or "passive capture," related to the binding of the microelement on the cell wall surface and bioaccumulation, or "active capture" that is a metabolism-dependent intracellular uptake [52, 54]. Firstly, metal ions accumulate in the yeast vacuole, and the cells can convert inorganic forms into organically bound Se and Zn [22, 51]. A higher bioaccumulation rate can be obtained in appropriate culture conditions with specially adapted Se/Zn-enriched yeast, but this was not the aim of this study.



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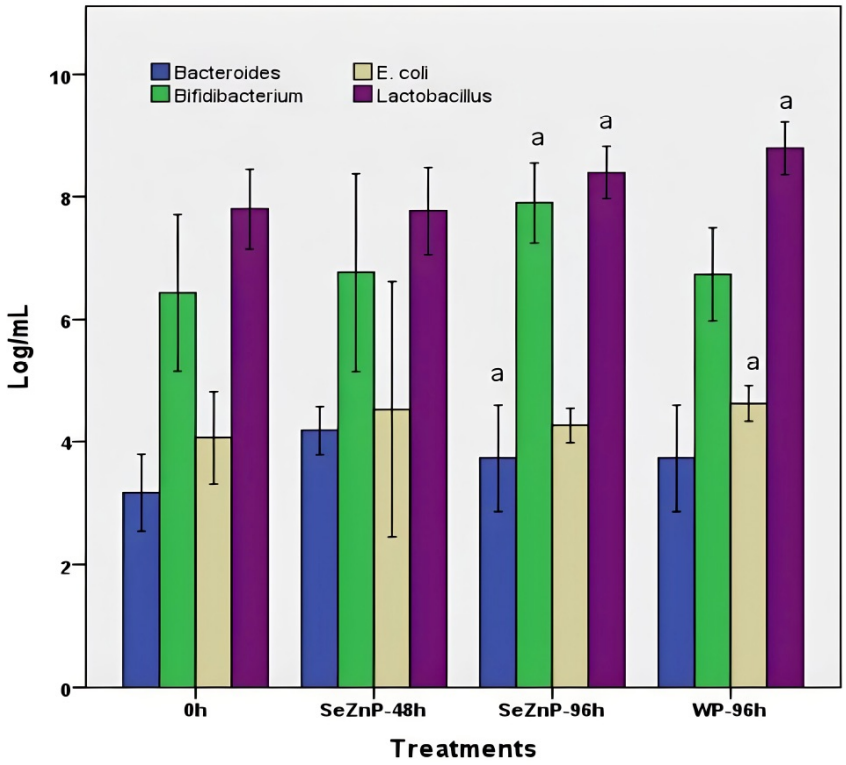

B

Fig. (3). The effect of the *S. boulardi*i postbiotic (WT) and Se/Zn-enriched postbiotic (SeZnP) treatments on potential taxa involved in the blood pressure regulation detected by microbiological (**A**) and qPCR methods (**B**). The significance level for the calculations was set as follows: significant, $p<0.05$; very significant, $p<0.01$; highly significant, $p<0.001$; and extremely significant $p<0.0001$ using the letters from a to d. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Se and Zn are essential trace elements that have various biological functions in humans. Selenium has antioxidant, anti-inflammatory, and immune functions, which is necessary for the conversion of thyroid hormones, and acts as a cofactor of several selenoproteins and selenoenzymes, including glutathione peroxidase (GPx1) [22, 25, 35]. Zn has important roles in growth, fertility, and immunity and is a key component of some important metalloenzymes, including antioxidant superoxide dismutase (SOD) [20, 30]. Zn and Se deficiency have been associated with a higher incidence of CV diseases, while increased blood Se level is associated with a lower risk for CV pathology evolution [20, 25]. However, environmental overexposure to Se increased blood pressure [25]. It is unclear how the development of CV pathologies is affected by Se and Zn deficiency, but some studies indicated that increased oxidative stress and its sequelae are involved [20, 25]. At the same time, it is proven that long-term Se and Zn deficiency significantly alters microbiota composition and function [20, 22, 55]. Therefore, the enrichment of Se and/or Zn by different probiotic bacterial strains (*Lactobacillus plantarum, Bifidobacterium longum, Bacillus subtilis*) and their effects on different dysbiosis has been extensively studied in the last years [24, 26-34, 36, 37]. The minerals-enriched probiotic biomass is considered a new type of dietary mineral source with less or no side effects and higher absorption efficiency for the human/animal body than other mineral supplements [26, 30, 36]. Moreover, a diet supplemented with Se/Zn probiotic (0.3 mg/L Se, 100 mg/L Zn) had an antioxidant effect and significantly improved serum enzyme activity of GPx1 and SOD, in rats model rising under heat stress [28].

The modulation effect on human CV dysbiosis of postbiotic preparations obtained by thermal inactivation of *S. boulardiic* wild-type and Se/Zn-enriched strains was investigated. Currently, limited information from animal studies has revealed the effect of Se and/or Zn-enriched bacterial probiotic treatments on different gut microflora, normal and pathological from irritable bowel syndrome or ulcerative colitis [26-34, 36, 37]. Probiotic and Se co-supplementation in a clinical trial with diabetic patients with coronary heart disease demonstrated the treatment's beneficial effect, and especially improved mental health indicators and CV metabolic profile [37].

In this study, the total bacterial load increased after the SeZnP application in both anaerobic and aerobic populations. However, the application of *S. boulardii* postbiotic preparation that contained non-viable cells and bioactive molecules did not induce changes in the total microbial counts. *Saccharomyces boulardii* is a probiotic yeast widely used for treating gastrointestinal disorders. Gu *et al*. indicated that the *S. boulardii* effect is related to the upregulation of serotonin transporter, which is responsible for disordered gastrointestinal sensation and motility [56, 57]. Mainly, dysbiosis associated with hypertension was related to a reduced microbial load and an imbalance of the gut microbial bacterial community [4, 16]. Two phyla, Firmicutes and Bacteroidetes, represent 90% of the gut microbiota [58]. The phylum Firmicutes consists of mostly Gram-positive bacteria, spore-forming, anaerobic or facultative aerobes, that are predominantly from the genera *Bacillus, Clostridium*, *Enterococcus*, *Lactobacillus*, and *Ruminicoccus*. The phylum Bacteroidetes includes different Gram-negative species, non-spore forming, aerobic, and anaerobic, predominantly from the genera *Bacteroides*, *Parabacteroides*, and *Prevotella*. *Bifidobacterium* spp. belongs to the Actinobacteria phylum, a relevant minority phylum important in the maintenance of gut homeostasis and is considered a beneficial bacteria that plays a vital role in the maturation and regulation of the immune system [15]. Both WP and Se/ZnP treatments for 96 h increased Firmicutes counts and *Lactobacillus* spp. population. Most studies have demonstrated that Firmicutes bacteria exhibit anti-inflammatory effects, which is important as inflammation promotes the CV risk [58]. In addition, a microbiota composition with abundant *Lactobacilli* and *L. plantarum* probiotic strains is known to lower blood pressure [6, 8]. A study on mice fed with chow supplemented with Se/Zn-enriched *Lactobacillus plantarum* confirmed abundant lactic acid bacteria DNA in fecal samples, genus *Lactococcus* and *Lactobacillus* [27]. Another study reported that Se- and Zn-enriched *Bifidobacterium longum* probiotic strains promoted the abundance of health-benefiting *Lactobacillus* in murine model [29, 30].

An imbalance in specific microbial taxa correlated with their metabolite production was reported in CV dysbiosis, especially depletion of *Bifidobacterium* spp [9, 15]. In the human model, the number of hypertension-associated taxa was strongly correlated with the severity of the disease, while microflora from pre-hypertensive and hypertensive groups were very similar [9, 16]. In our study, both beneficial bacteria *Lactobacillus* spp. and *Bifidobacterium* spp. increased after SeZnP treatment, as well as the acetic acid production. Similarly, preparations of selenium/zinc enriched probiotics (*Candida utilis*, *Lactobacillus* biomass) increased the amount of *Lactobacillus* and *Bifidobacterium* in the fecal samples of dogs fed with a diet supplemented with 2.0 g of Se/Zn-enriched probiotics [24]. Moreover, studies demonstrated that acetate supplementation or a diet rich in fiber that increases *Bifidobacterium* could reduce systolic and diastolic blood pressure. On the other hand, acetate supplementation could reduce systolic and diastolic blood pressure and increase the abundance of *Bacteroides acidifaciens* that belong to the Bacteroidetes phylum [5, 6, 59, 60]. We noticed a significant increase in *Bacteroides* spp. population after SeZnP treatments, probably as a result of acetate production. However, the role of this taxon in hypertension is disputed [3, 16]. Acetate can be produced by species from *Bifidobacterium* spp. and *Bacteroides* spp., and in the case of CV pathology, acetate, and propionate are suggested to lower blood pressure by decreasing systemic inflammation [6, 16, 48, 49, 61]. Moreover, a reduced number of acetate- and butyrate-producing bacteria was found in hypertensive rats and humans [9, 15]. However, after WP and SeZnP treatments, the butyrate level did not change. The reduced concentration of another microbial metabolite, lactate, after SeZnP treatment might also be beneficial, as it was suggested that a high serum lactate level increased blood pressure [62]. Simi-

lar data were reported for oral minocycline administration, an anti-inflammatory antibiotic that attenuated high blood pressure [15]. Minocycline restored gut microbiota by increasing Bacteroidetes in the hypertensive rat model. At the same time, the drug treatment did not reduce the bacterial load but expanded the acetate and butyrate-producing bacteria [15]. It has been reported that the abundance of lactate-producing bacteria (*Streptococcus* spp. and *Turicibacter*) in some CV dysbiosis is associated with high levels of serum lactate that increase blood pressure [5]. Therefore, the reduction in concentration of this metabolite after SeZnP application might be beneficial. However, more studies must be performed to clearly understand the mechanisms involved in SCFA effects on lowering blood pressure.

Dysbiotic patterns with abundant opportunistic pathogens (*e.g.*, *Escherichia coli*, *Klebsiella* spp., *Streptococcus* spp.) were reported in patients with hypertension [4, 7, 61]. Moreover, high blood pressure was associated with increased *Enterobacteriales*, producing system inflammation and affecting intestinal permeability [60]. *S. boulardii* supernatant reduced *Escherichia*, *Shigella*, and other taxa in mice, while Se/Zn enriched probiotics decreased fecal *E. coli, Staphylococcu*s, and *Enterococcus* in canine experiments [24, 56]. Acetate supplementation and probiotic *Bifidobacterium* spp. Treatment is suppose to lower the intestinal pH and thus pathobionts growth [59]. However, in our research, the *Enterobacteriaceae* population did not significantly decrease after applications of both WP and SeZnP preparations. During the gastrointestinal simulation in the GIS1 system, the pH value was checked and kept constant (pH~6) with a sterile 1 M NaOH solution. Therefore, the short period for *in vitro* simulation (96 h) and the pH control, could explain the constant population of *Enterobacteriaceae* detected after both postbiotics administration. However, in our study, *S. boulardii* postbiotic treatment increased *E. coli* counts based on qPCR detection.

The gut microbiota changes are intricately involved in the development and progression of various diseases, thus, understanding this relationship is critical for further targeted therapies to prevent and alleviate adverse events. In this context, mineral-enriched postbiotic research has great therapeutic potential as it combines the additive effects of bioactive molecules and essential minerals bioaccumulated by the postbiotic strains. This study strongly suggested the potential of Se/Zn-enriched postbiotics to be used in CV therapy. However, more research in a disease-induced animal model and clinical trials are needed to evaluate the functionality of the mineral-enriched preparations.

CONCLUSION

Increasing evidence suggests that gut microbiota dysbiosis is one of the modifiable risk factors for CV disease progression and development. Therefore, novel therapeutic strategies for preventing and treating CV dysbiosis that target intestinal microbial imbalance are most interesting. This *in vitro* study proved that Se- and Zn-enriched *S. boulardii* postbiotic modulate the composition and metabolic profile of human CV dysbiosis, increasing total bacterial load and beneficial taxa *Lactobacillus* spp. and *Bifidobacterium* spp., as well as the acetic acid level. At the same time, the potential of mineral-enriched postbiotics to be used as a novel therapeutic approach was revealed. More investigations are required to understand the mechanisms and functionality of this approach.

LIST OF ABBREVIATIONS

CV	=	Cardiovascular
SCFAs	=	Short-Chain Fatty Acids
Se	=	Selenium
Zn	=	Zinc

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data and supportive information are available within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material isavailable on the publisher's web site along with the published article.

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