Biofilm-Based Nutraceuticals: A Better Solution to Diet-Based Interventions for Achieving Healthy Gut Microbiota

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Abstract

Microbial biofilms developed *in-vitro* and applied to agroecosystems are reported to restore their soil-plant-microbial relationships by supplying a mixture of diverse biochemicals that reinstate network interactions. Here we hypothesize that the same method can also be used to reinstate unhealthy human body ecosystem caused by altered gut microbiota due to modern lifestyle and dietary patterns. In the present study, we tested biochemicals exuded by a developed fungal-bacterial biofilm (BFEx) on the growth and development of five test gut microbes in a simulated gut environment with eight different dietary patterns. Live microbial cell concentrations of the cultures were analyzed after 24 and 48 hours of inoculation using a bacterial viability kit. In addition, BFEx was tested for cytotoxic activity using brine shrimp lethality assay. Results revealed that the live microbial cell concentrations of the mixed cultures increased while they decreased in the monocultures with the application of BFEx in all dietary patterns. The BFEx promoted the growth and possibly dormancy-breaking of the tested gut microbes. However, it seems that there is a need of an interaction of diverse microbes, if a beneficial outcome from the BFEx is to be achieved. Further, the BFEx showed no toxicity to brine shrimp nauplii, instead it supported for their survival for a while by supplying food sources. In conclusion, this biofilm-based method is a better solution than diet-based interventions for achieving healthy gut microbiota because the former is user-friendly and preserves food-choice-freedom of people, unlike the latter.

1. Introduction

Diversity of gut microbiota controls the host during homeostasis and illness [1, 2]. A healthy host–microorganisms balance is vital in maintaining the intestinal barrier and immune system functions and, hence, prevent disease-development. Moreover, recent advances in science have revealed the significance of gut microbiota in linking emotional and cognitive centers of the brain with peripheral intestinal functions (gut-brain axis) [3]. In fact, dietary components are chemically transformed by the microbiota, and gut-derived metabolites are distributed to all organs, including the brain. It is reported that the gut microbiota modulates neurotransmitter production in the gut and brain causing behavioral changes in mice [4]. However, gut microbial diversity in humans has decreased drastically with the adoption of modern lifestyle and dietary habits including use of antibiotics and processed food with low fiber content [5]. As such, it has turned out to be a growing health concern, since it is strongly associated with obesity and related metabolic diseases. In order to prevent diseases and maintain health, novel diet-based interventions are emerging. However, they restrict the food-choice-freedom of the people.

It has been found that the gut microbial diversity is a subset of soil microbial diversity [6]. Close linkage between the soil microbiome and the human gut microbiome has evolved during evolution and is still developing [7]. Application of microbial biofilms developed *in-vitro* has been reported to reinstate lost soil microbial diversity, because their exuded biochemicals break the dormancy of microbial seed bank formed under stress [8, 9, 10]. The strongest evidence to this has come from a study where soil application of a fungal-bacterial biofilm increased cyanobacterial diversity in an agroecosystem [11]. It is hypothesized that, the same method may be applied to reinstate unhealthy human body ecosystem

caused by altered gut microbiota due to modern lifestyle and processed foods [12]. Therefore, this study was designed to examine the effect of BFEx on human gut microbiota in a simulated gut environment with different dietary patterns.

2. Materials And Methods

2.1 Biofilm Formation

To develop a fungal-bacteria biofilm, *A. niger* and *S. maltophilia* were used to represent the fungus and the bacterium, respectively. The two microbes were selected based on the results of a previous study in which they produced the highest EPS-biochemical diversity compared to the other biofilms types tested (U.M.B. Premarathna, unpublished). From each, one loopful of microbes was inoculated to 250 mL CCM [13] broth to prepare monocultures. Hundred microliters of each monoculture was taken and inoculated in to 15 ml sterilized centrifuge tube containing 10 ml of CCM medium, and was incubated for seven days to develop the fungal-bacterial biofilm.

2.2 Extraction of the BFEx

The extraction of BFEx was performed by combining physical and chemical methods [14]. A NaCl solution was prepared by dissolving 5 g of NaCl in 100mL of sterilized distilled water. Ten microliters of the solution was poured to 15 ml centrifuge tube that contained the developed biofilm. Then, it was subjected to ultra-sonication for 10 minutes, followed by centrifugation at 5000rpm for 10 minutes. Finally, the supernatant in the centrifuge tube was taken for further experiments.

2.3 Potential of BFEx in breaking dormancy of gut microbes: in vitro gut simulation study with different dietary patterns

Five commonly found gut microbes [15, 16] viz. *Bacillus clausii, Lactobacillus sporogenes, Lactobacillus reuteri, Bacillus subtilis*, and *Aspergillus niger* were taken as test microbes (many of them are soil-based probiotics) that were isolated from fecal matter. They were grown as monocultures and mixed cultures in a simulated gut environment with eight different dietary patterns, i.e. low-carbohydrate, high-carbohydrate, low-protein, high-protein, low-lipid, high-lipid, low-fiber and high-fiber. Here, culture medium described by Parmanand et al. (2019) [17] and Macfarlane et al. (1998) [18] was used after modifying. The culture medium contained; casein 3 g/l, yeast extract 2 g/l, NaCl 0.1 g/l, K_2HPO_4 0.04 g/l, KH_2PO_4 0.04 g/l, $MgSO_4.7H_2O$ 0.01 g/l, $CaCl_2.6H_2O$ 0.01 g/l, $NaHCO_3$ 2 g/l, Tween-80 2 ml, glucose 10 g/l, vitamin K1 10 µl, cysteine HCl 0.5 g/l, bile salts 0.5 g/l, Starch 10 g/l, Pectin 2 g/l. The pH was controlled and maintained between 6.2–6.6. Temperature was set to 37 ^oC. Moreover, the culture medium was modified to have different levels of carbohydrate, protein, lipid, and fiber as diets. For that, starch, casein, tween 80, and pectin were selected to represent carbohydrate, protein, lipid, and fiber, respectively. Likewise, eight different media were prepared to represent relatively low and high concentrations (i.e. 50% and 150% of the above concentrations of each dietary source as the low and high concentrations, respectively) of the diets in the gut environment. Each concentration with or without BFEx formed two

treatments. Each treatment had three replicates in a completely randomized design. Live microbial cell concentrations were determined after 24 and 48 hours of inoculation using LIVE/DEAD™ BacLight™ bacterial viability kit (Invitrogen Molecular Probes, Eugene, OR, USA) [19, 20, 21].

2.4 Brine shrimp lethality assay for BFEx

Brine shrimp (*Artemia salina* L.) eggs were hatched in a half covered beaker (250 mL) containing artificial sea water and were allowed to stay for two days at room temperature (RT) [22]. During the hatching period, constant and continuous aeration was given through an oxygen pump and illumination was given with a 20 W bulb. After 48 hours, 2nd larval stage (nauplii) of brine shrimps was observed towards the illuminated side of the beaker. Test samples were prepared in artificial sea water having 0, 100 and 300-fold dilutions of the BFEx, triplicated for each concentration. The test samples of 2 mL each were poured in to a 24 well plate. Each concentration was added with 10 brine shrimp nauplii and allowed to stay for another 48 hours under illuminated conditions at RT. After 24, and 48 hours, observations were taken and the percentage lethality was calculated.

2.5 Statistical analysis

The data were analyzed using the statistical software Minitab version 17. ANOVA followed by Tukey's HSD test were done to compare the means. Probability < 0.05 was used as the threshold for significance.

3. Results And Discussion

3.1 Breaking dormancy of gut microbes using BFEx

The BFEx application produced higher live microbial cell concentrations than the treatment without BFEx application in all dietary patterns (Fig. 1). However, this was observed only in the mixed microbial cultures suggesting that there is a need of an interaction of diverse microbes to trigger the favorable mechanisms of BFEx (Figs. 1 and 2). In the soil, microbial biofilms developed *in vitro* are reported to reinstate lost microbial diversity in degraded agroecosystems as explained above [11], thus leading to restore network interactions for improved rice production in large scale cultivations [9, 23]. The same mechanism could have operated here to increase the live microbial cell concentrations of the gut microbes, because the gut microbial diversity is a subset of soil microbial diversity [6].

3.2 Brine shrimp lethality assay for BFEx

After 24 hours, 100% survival of brine shrimp nauplii was observed in all test samples of BFEx including the control. However, after 48 hours, all the brine shrimp nauplii died except 40% survival only in 100-fold diluted BFEx. In the control (without BFEx) and 300-fold diluted BFEx, all the brine shrimp nauplii died within 48 hours possibly due to starvation. Thus, the BFEx showed no toxicity on the brine shrimp nauplii, instead they supported for their survival for a while by supplying food sources like polysaccharides, proteins and fatty acids in the 100-fold BFEx dilution.

Generally, dietary diversity leads to food compound (biochemical) diversity which is transformed in to microbial metabolites like short chain fatty acids (SCFAs) that in turn increase the microbial diversity [24, 25]. However, it is reported that monotonous diets such as western style diets are not capable enough to be converted in to SCFAs via trimethylamine N-oxide (TMAO), which leads to increase TMAO levels causing many diseases and disorders [26, 27]. In this context, biofilm interventions like BFEx can facilitate the above conversion via biochemical and microbial diversities and their stability leading to human health [8, 11, 27, 28].

4. Conclusions

The BFEx promoted the growth and possibly the dormancy-breaking of the tested gut microbes, and hence they can be developed as biofilm-based interventions to reinstate gut microbiota for improved human health. To date, diet-based interventions are being researched to shape the gut microbiota. However, BFEx may be a better solution to do the same job because they are user-friendly as they preserve food-choice-freedom of the people, unlike the diet-based interventions. Therefore, it is concluded that the biofilm-based interventions derived from BFEx can be considered as the next generation medicines or rather nutraceuticals which hopefully will provide answers for various human health issues in the future.

Declarations

Acknowledgement All the members of Microbial Biotechnology Unit of the NIFS are acknowledged for their support during this study. We would also like to show our gratitude to all who collaborated by providing insights and expertise that greatly assisted the research. Compliance with Ethical Standards The authors declare that they do not have conflict of interest. The study was performed without the use of animals and without the involvement of human subjects.

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Figures

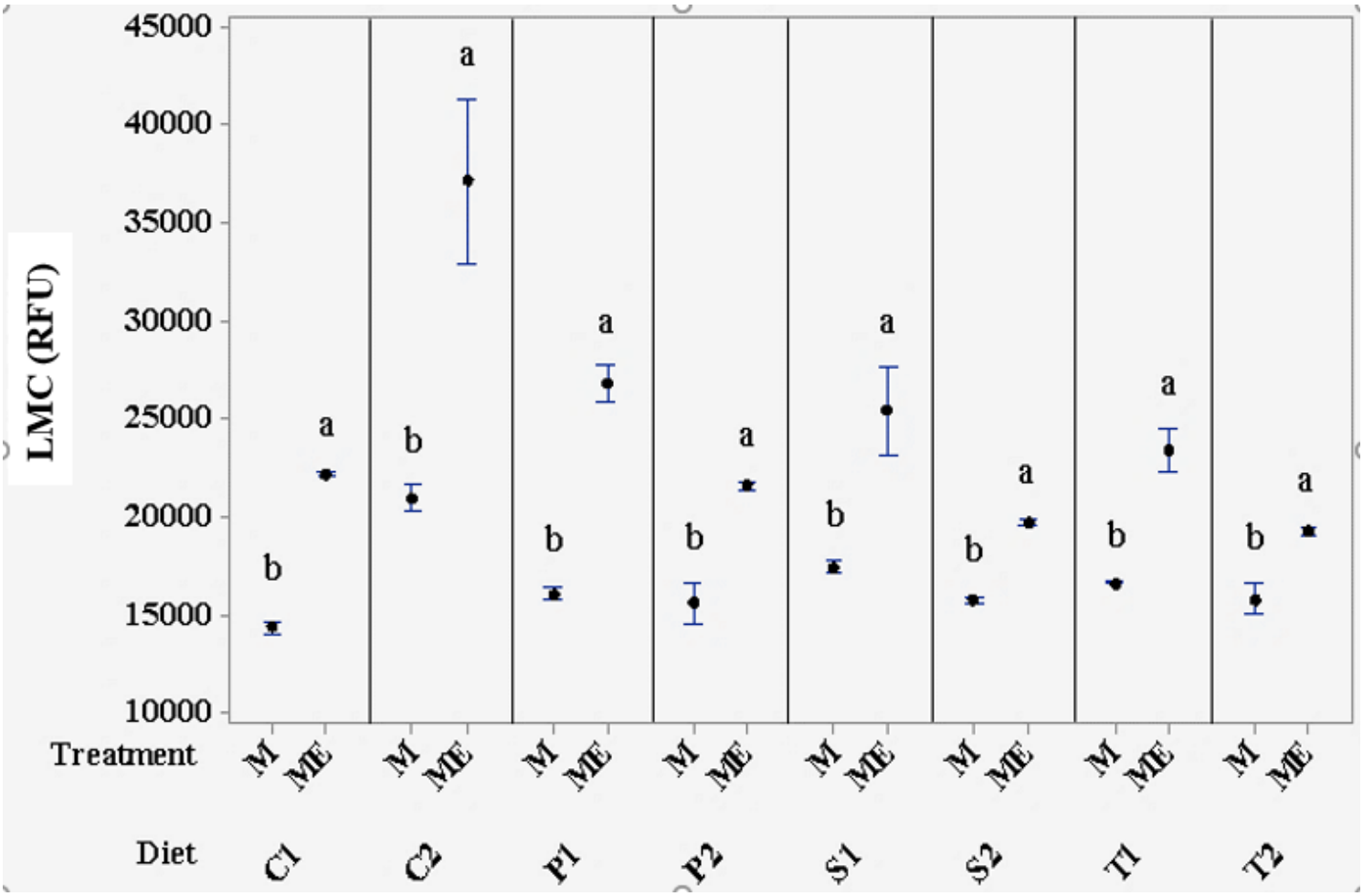

Figure 1

Figure 1. Live microbial cell concentrations (LMC) as evaluated from relative fluorescent units (RFU) of the microbial mixed culture with respect to the different dietary patterns after 24 hours. *M – without BFEx, ME – with BFEx, C1 – low protein, C2 – high protein, P1 – low fiber, P2 – high fiber, S1 – low carbohydrate, S2 – high carbohydrate, T1 – low lipid, T2 – high lipid.*

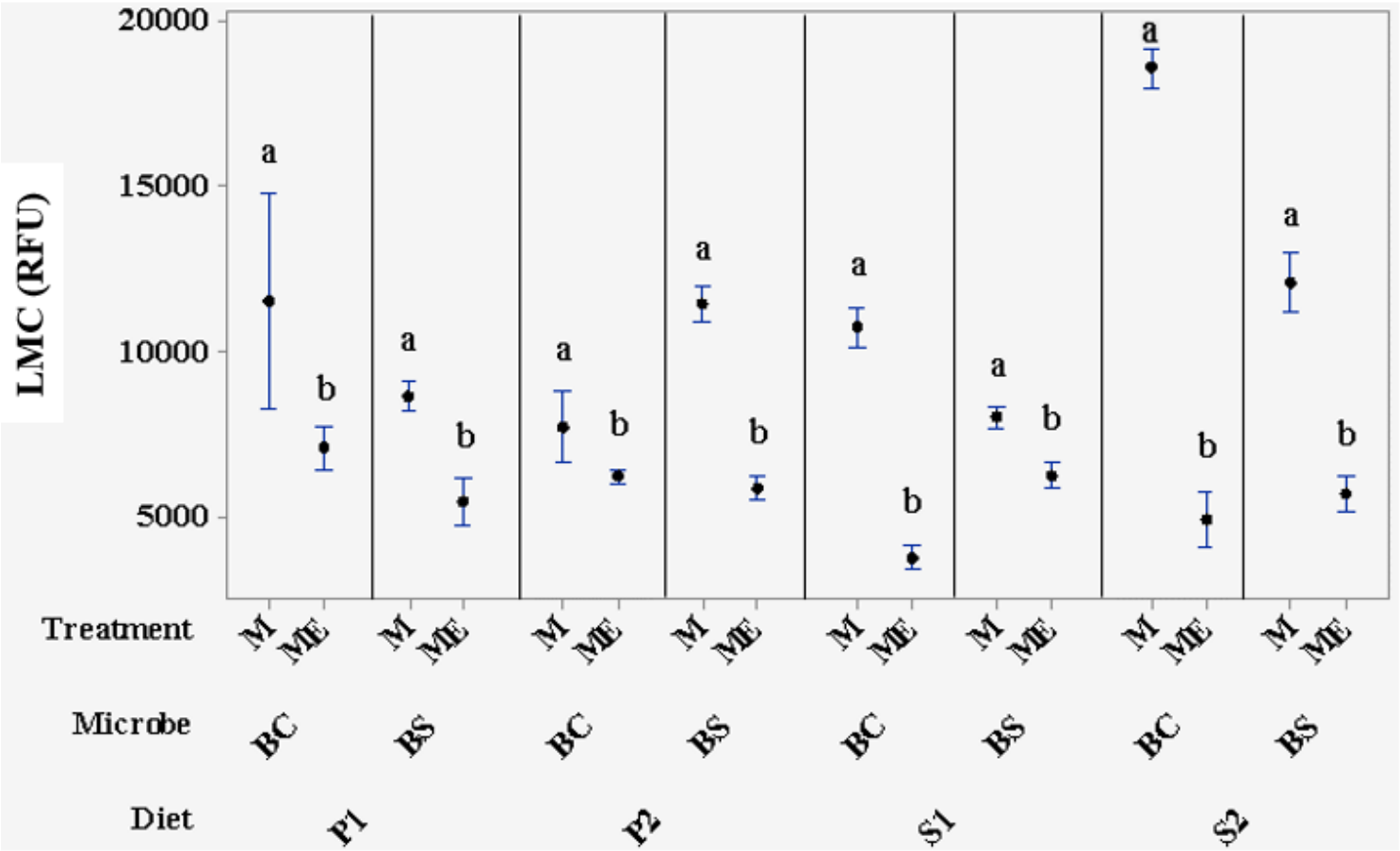

Figure 2

Figure 2. Live microbial cell concentrations (LMC) as evaluated from relative fluorescent units (RFU) of the microbial mono culture with respect to the different dietary patterns after 24 hours. *M – without BFEx, ME – with BFEx, P1 – low fiber, P2 – high fiber, S1 – low carbohydrate, S2 – high carbohydrate.*