Dear Reviewer B,

thank you for your comments and suggestions. Below are the answers to your questions.

Page 2, line 47: the sentence was rewritten.

Page 2, line 48: Regarding your questions “Can you explain why other groups start investigated glutamate and GABA? What was the first hypothesis and relation with MS? “ we can say that since GABA is a major inhibitory neurotransmitter, as well as immunosuppressive molecules the assumption is that it may be an important molecule in the treatment of Multiple Sclerosis. To our knowledge Demarkova et al. (2003) first measured the level of GABA and activity of the GAD enzyme in the blood serum of the MS patients and showed that they are reduced in MS patients in comparison to healthy controls. We added this in the Introduction, line 54-55.

Page 2, line 56, the requested reference was added into the manuscript.

Page 3, line 86, we have specified which bacterial strain was cultured in MRS broth.

Page 3, line 91 Regarding your questions „Why did you decide to use this strain for milk fermentation? Do you have some previous data about milk fermentation with this strain?„ we can answer that strain *Streptococcus thermophylus* BGKMJ1-36 is the strain from the laboratory collection with good technological characteristics suitable for fermentation (data not shown in this paper or previously published), which is traditionally used in our laboratory for making various fermented milk beverages.

Page 3. lines 96 and 97, we added requested information.

Page 5, line 138: Regarding first question „With or without MSG? Strain was grown in MRS without the addition of MSG, while regarding the question „What was the reason for 16h, and in other case with supernatant was 48h?“ we can explain that for the experiments in which we used live strain, 16 h old culture was used because the bacteria are in the early stationary phase of growth, alive and capable of colonizing the gastrointestinal tract. Regarding the question „Do you know what is conc of GABA in this moment? „ in the 16 h old BGZLS10-17 supernatant, when the strain is cultivated in MRS without the addition of MSG, there is no GABA production. In these experiments, the live strain was used with the assumption that it will adhere to cells in the gut and produce GABA *in situ*. Page 5, Line 140 regarding the question „Can you be absolutely sure that no GABA in this sample?“ yes we are, we measured the GABA concentrations on HPLC. Strain was cultivated 48 hours for the supernatant preparation because the GABA production was the highest at this time. The concentration of GABA after 48 h of cultivation of BGZLS10-17 in MRS with MSG was 6,4 mg/ml, as shown previously in our manuscript Sokovic Bajic et al. (2019).

Page 5 and 6, lines 168-180: The recommended suggestions were adopted.

Page 12, line 295, 296 The sentence was rewritten according to your suggestions. Line 302, The mistake has been corrected, we used pasteurized milk instead of skim milk.

Page 13, line 313. The recommended suggestion was adopted.

Dear Reviewer F,

thank you for your comments and suggestions. Below are the answers to your questions.

Regarding your comment „1. The title is misleading, since it suggests that the manuscript is focused on pre-clinical research in EAE animals treated with BGZLS10-17. Instead, the effects of BGZLS10-17 on clinical score of EAE DA rats are just described, and the immune-biological mechanisms involved are poorly investigated; on the contrary, the manuscript presents the GABA-mediated pH resistance, the proteolytic activity and the preparation of fermented milk beverage based on BGZLS10-17. Therefore, the title should refer also to this former part of the study, improving in this way the adherence of the manuscript to the journal topics – i.e biochemistry.

1. According to your suggestion we rephrased the title of the manuscript. Instead of „Administration of *Lactobacillus brevis* BGZLS10-17 alleviates symptoms of experimental autoimmune encephalomyelitis in DA rats“ we suggest „Characterization of GABA – mediated pH resistance and the proteolytic activity of *Lactobacillus brevis* BGZLS10-17 in preparation of fermented milk beverage and the effects on the symptoms of the experimental autoimmune encephalomyelitis“.

Regarding your question „2. Authors have previously observed that BGZLS10-17 treatment decreased IL8 production and increased the expression of TGFβ in vitro; did they analyze

these effects in EAE DA rats? Did they observe any modulation of immune system in vivo?“

1. In this stage of our investigation the focus was on the effect of the BGZLS10-17 strain only on the clinical symptoms of the EAE and the ability of preparing GABA-enriched fermented beverage with this strain. Future research will focus on the mechanisms underlying the therapeutic effect, including modulation of immune system *in vivo.*

Regarding your suggestion „3. In the introduction, a short paragraph on the gut-brain axis could be added to further support the importance of GABA-producing bacteria and their effects on the central nervous system.“

1. We added the short paragraph about the gut-brain axis in the Introduction (line 49-53).

Regarding your comment „4. Authors should specify the amount of BGZLS10-17 given to EAE rats in term of Colony Forming Unit (CFU) per dose, and not simply indicate the 'total volume' of 50ml given to the animals in the cages. It is quite difficult to evaluate if all animals have received the same amount of 'treatment'.“

1. Each animal received ~ 1x109 [CFU]/ml of the BGZLS10-17 strain which is sufficient dose of probiotic. Applying the treatments *per os* is the standard procedure in our laboratory practice Stanisavljevic et al. (2018) *Front. Immunol* (<https://doi.org/10.3389/fimmu.2018.00942> ) and Stanisavljevic et al. (2019) *Sci Rep* (<https://10.1038/s41598-018-37505-7> ), as well as in other groups Castillo et al. (2011) BMC Microbiology (<https://10.1186/1471-2180-11-177> ). *Per os* way of application was chosen over oral gavage (by which the precise amount of bacteria can be applied) to minimize the amount of stress in animals, which is significant in these type of diseases.

Regarding your comment „5. Authors should point out and discuss the fact that the administration of L. brevis significantly restrains EAE in the second peak of the disease (relapse) whereas the supernatant seems to just delay the disease onset.“

1. We accepted your suggestion and added several sentences into discussion (line 239-246: „Comparing the effects of live strain and its supernatants, it can be observed that treatment with both live BGZLS10-17 and its GABA-containing supernatant alleviate the development of the second peak of EAE (relapse), while application of the live strain shows more significant effect in alleviating EAE symptoms in this phase of the disease. At the same time only the treatments with supernatants statistically delay the onset of the disease and that could be the result of the time needed for the live strain to colonize the GIT, while bioactive molecules contained in the supernatants can achieve its effect immediately after implementation.“ )

Regarding your comment „6. In figure 3B, in the BGZLS10-17 spn without GABA group it is not represented the standard deviation.

1. Thank you for your observation. It was unintentional mistake. We corrected the figure 3.

Regarding your question „7. Which tests were used for the statistical analysis of clinical score?“

1. We used Student t-test to compare control with treated groups. We added this into the Experimental section (line 153-155).

Regarding your observation „8. In the text, line 255 and 257 the figure is the number 5 (not 4)“

1. We corrected the figure number in the text.