→ **Comment 1:** The English language was improved, but there are still errors.

Response 1: As suggested by the reviewer, minor errors throughout the manuscript has been revised now.

→ **Comment 2:** The following comments were not addressed correctly:

Why was linkage analysis not performed? (I was referring to the chemical method by methylation.). Bacterial EPSs are normally composed of repeating units. Based on the 1H NMR spectrum, the purity and/or heterogeneity of the EPS is questioned. (I was referring to peak intensities that are not uniform.

The new spectra emphasize this even more: for example, I count at least 10 H1/H2 cross peaks of different intensities on the COSY.) The authors added a COSY spectrum but did not analyze it.

They deleted erroneous information about mannose residues, but came up with new conclusions about mannose identity and linkages based solely on chemical shift of anomeric proton/carbon.The information contained in the other 2D spectra was not exploited to provide structural information. Chemical shift similarity is not sufficient to identify monosaccharide and linkage in a polysaccharide repeating unit. The evidences for the partial structure given are not convincing. If the authors do not add methylation analysis and perform full NMR spectral analysis, the claims about structure determination should be removed from the manuscript.

Response 2: Dear reviewer, thanks for your valuable comments for improving the quality of this paper. We will answer in the following paragraphs because all comments were related to the same topic.

- We agree with some of the comments that you made. However, we would like to remind you that the main purpose of this manuscript is not the complete elucidation of the EPS chemical structure but also the optimization of the EPS production, and characterization of the EPS functional properties. At the same time, we provided different chemical properties like: sugar identification and its molar ratio, molecular weight, functional group analysis and thermal stability of the EPS, and this journal has recently published other papers with similar or less chemical information on the EPS, for instance:

Kuo, H.-C.; Liu, Y.-W.; Lum, C.-C.; Hsu, K.-D.; Lin, S.-P.; Hsieh, C.-W.; Lin, H.-W.; Lu, T.-Y.; Cheng, K.-C. Ganoderma formosanum Exopolysaccharides Inhibit Tumor Growth via Immunomodulation. *Int. J. Mol. Sci.* 2021, *22*, 11251. https://doi.org/10.3390/ijms222011251

Fetsiukh, A.; Conrad, J.; Bergquist, J.; Timmusk, S. Silica Particles Trigger the Exopolysaccharide Production of Harsh Environment Isolates of Growth-Promoting Rhizobacteria and Increase Their Ability to Enhance Wheat Biomass in Drought-Stressed Soils. *Int. J. Mol. Sci.* 2021, *22*, 6201. https://doi.org/10.3390/ijms22126201.

- On the other side, we performed the 1D and 2D - NMR analysis as complementary information to the other techniques in the present work. Such analysis was performed to try to replace the traditional acid hydrolysis, methylation/acetylation and GC-MS used in glycoside analysis. **On the opposite to NMR, we do not have access to this analysis, and we could not find it as a service.**

- The HPLC and NMR results from the present work confirmed the heterogeneity of the EPS (3 different sugar moieties with different molar ratios), which agrees with the other reported mannan polysaccharides. The 1H and 13C NMR chemical shifts are comparable with literature reports of other similar EPS or oligosaccharides, where complex structures are identified by NMR too (Casillo et al.,

2021; Chatterjee et al; 2018). Besides, **our 2D-NMR analysis supported EPS heterogeneity, identifying different types of monomer linkages.**

Casillo, A., Fabozzi, A., Russo Krauss, I., Parrilli, E., Biggs, C. I., Gibson, M. I., … Corsaro, M. M. (2021). Physicochemical Approach to Understanding the Structure, Conformation, and Activity of Mannan Polysaccharides. Biomacromolecules, 22(4), 1445–1457. doi:10.1021/acs.biomac.0c01659.

Chatterjee, S., Mukhopadhyay, S. K., Gauri, S. S., & Dey, S. (2018). Sphingobactan, a new α-mannan exopolysaccharide from Arctic Sphingobacterium sp. IITKGP-BTPF3 capable of biological response modification. International Immunopharmacology, 60, 84–95. doi:10.1016/j.intimp.2018.04.039

- Your mention of the non-uniform peak intensity confirming the three different sugar units found. **This is because the signal intensity of NMR peaks is related to an -H abundance. Different monomer ratios are responsible for this variation and support our findings.** Also, the 10 H1/H2 cross peaks observed in the COSY spectra supported our findings of EPS heterogeneity, given the following facts: 1) We found 3 different types of sugars by HPLC. 2) We identified by NMR at least 2 types of linkages α 1-2 Man-Man and alfa 1-4 Man-Man monosaccharides. 3) There are other 2 sugars for which the β-type of linkage was identified but not the exact sugar position. 4) Anomeric protons shifts from units at the reducing end of polysaccharide differs from the one in another position. In conclusion, there are different linkage possibilities (plus already identified) to different monosaccharides in different ring positions. This gives rise to a multiplicity of signals (not fully identified in this work), which could produce different H1/H2 correlations.

- About the COSY spectrum discussion, only a minor and partial discussion is presented that can be found below the Scheme in manuscript: “In the COSY spectrum, it was found its α-configuration by low-field H-1 signal at δ 5.34 and correlated with H-2 (δ 4.0), H-6 (δ 3.98 and 3.85), and H-5 (δ 4.12) (Speciale et al., 2022).”

- **Regarding the TOCSY and HMBC mentioned in the methods, they are now deleted from the manuscript, as suggested.**

- Finally, we kindly request the reviewer to consider that this is the first approach to studying a Chilean hot spring EPS isolation and its functional characterization. We also mention that further work to fully reveal this EPS structure will be necessary using higher resolution NMR spectra combined with chemical techniques. This work has given partial structural elucidation, which can be considered a previous background for other research on this topic.