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SUPPLEMENTARY MATERIAL

Partial characterization and biological properties of the novel exopolysaccharide produced by a probiotic strain *L. reuteri B2*

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2.1 Bacteria and culture conditions

2.1.1 Strain identification.

Using the universal primers (UNI16Sfw and UNI16Srev) [1], the representative isolates were identified by 16S rDNA sequencing. Amplification was carried out in a thermal cycler (Applied Biosystems, ThermoFisher Scientific) and DNA fragments were amplified as follows: initial denaturation at 94 °C for 5 min, followed by 30 cycles consisting of denaturation at 94 °C for 1 min, annealing at 55°C for 1 min, and polymerization at 72°C for 1 min, and a final extension at 72°C for 7 min. The expected length was 1549 bp. Aliquots (5 µl) of the amplified products were subjected to electrophoresis in 1% agarose gel (ThermoFisher Scientific) in TAE buffer (40 mM Tris acetate, 1 mM EDTA, pH 8.2). Gels were stained with ethidium bromide (500 ng/mL) and visualized under UV light (BioDoc Analyze). All amplicons were eluted and purified using GeneJet PCR Purification Kit (ThermoScientific) by following the manufacturer`s protocol. The PCR products that we obtained were sequenced by the Macrogen Sequencing Service (Macrogen, Amsterdam, The Netherlands) and analyzed by using BLAST algorithm (http://www.ncbi.nlm.nih.gov/index.html). Selected isolates were identified as follows: isolate B2 - *Lacotbacillus reuteri,* isolate H10 - *Lactobacillus murinus, and isolate* J7 - *Klebsiella oxytoca* [2]. The most numerous colonies belong to isolate B2, hence it was chosen for further characterization as a potential source for exopolysaccharide (EPS) production.

3.1.2 Characterization of EPS isolated from L. reuteri B2

^{1}H NMR spectra of new EPS from *L. reuteri* B2

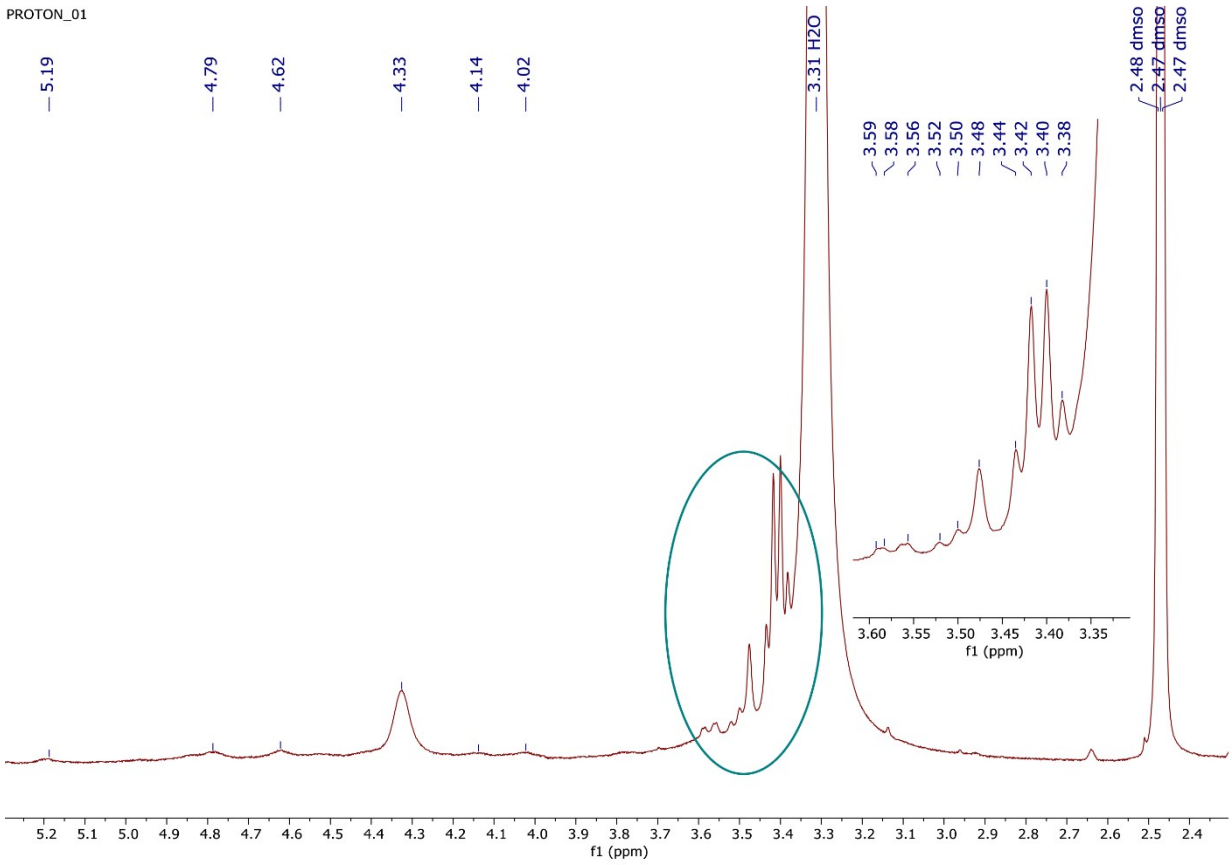


Fig S1.^{1}HNMR spectra of EPS from *L. reuteri* B2 recorded on a Bruker Avance III 500 spectrometer in DMSO-d6

3.2.4 Determination of point zero charges (PZC) of EPS from L. reuteri B2

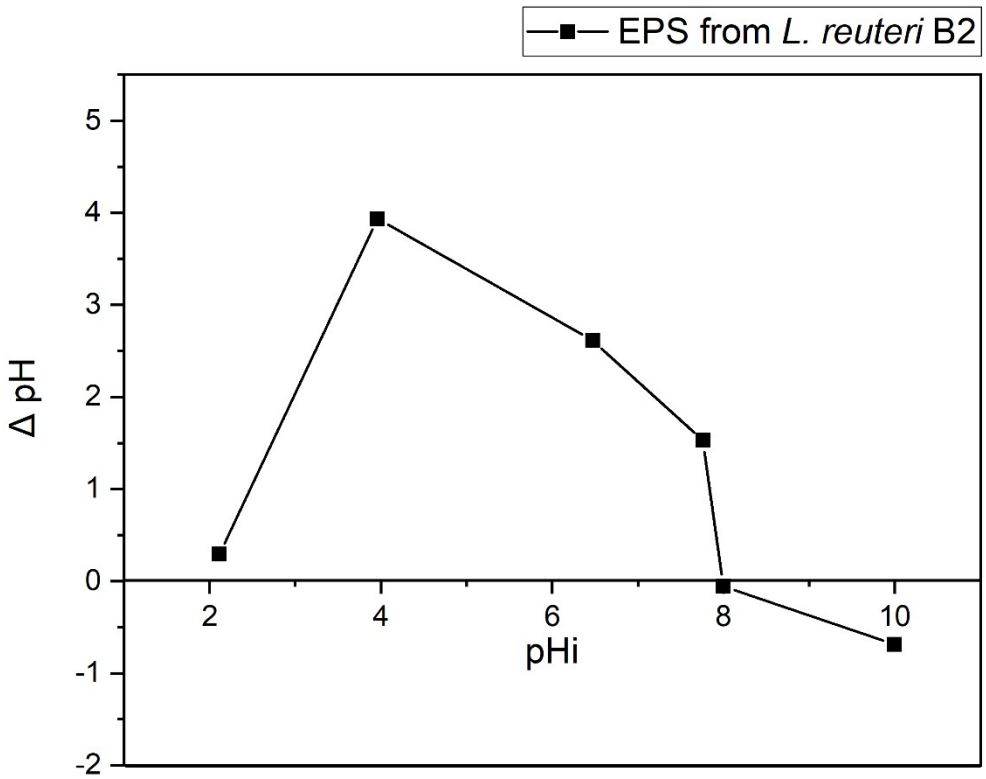
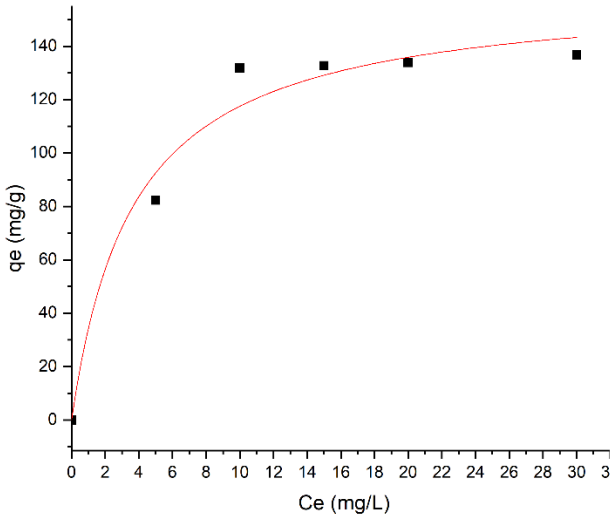


Fig. S2. Point of zero charge of EPS from *L. reuteri* B2

3.4.1 Isotherm study

a)



b)

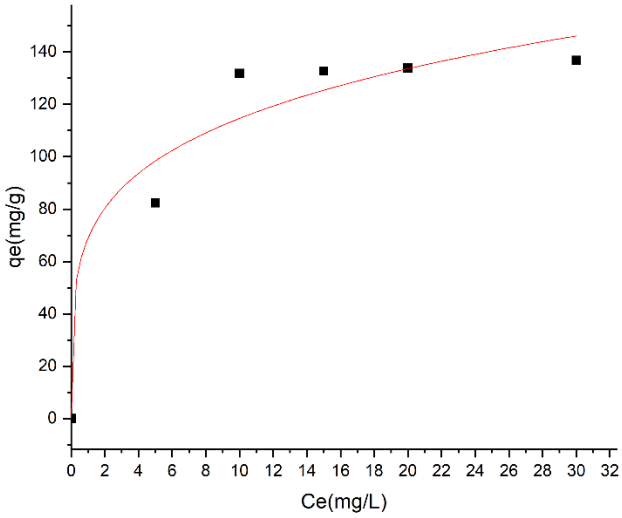


Fig. S3. Isotherm study, Langmuir a), and Freundlich b) isotherms for adsorption of Ni^{2+} ions on EPS isolated from *L. reuteri* B2, equilibrium experiments were performed for different Ni^{2+}concentrations (0-35 mg/L) at 25 ˚C, for 60 min at pH 8.0.

3.4.2 Thermodynamic study

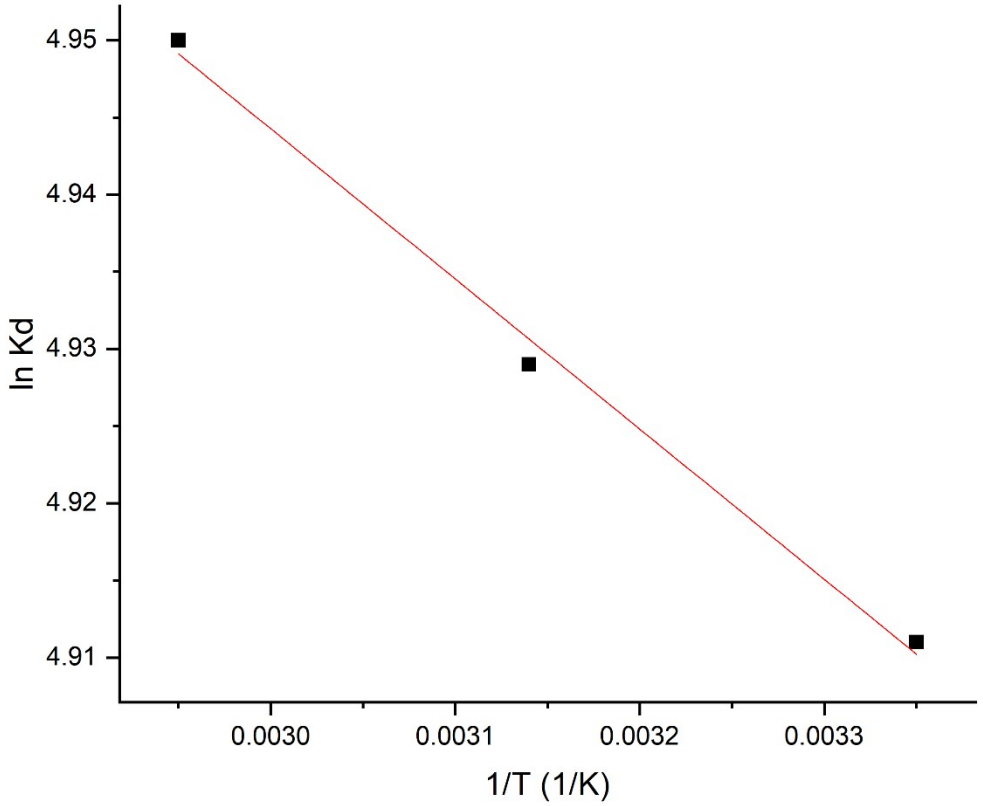


Fig. S4. Thermodynamic study of the adsorption for Ni^{2+} ions on EPS isolated from *L. reuteri* B2, equilibrium experiments were performed for Ni^{2+}concentrations 10 mg/L at different temperatures (298,318 and 338 K) for 60 min at pH 8.0.

References

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2. Popović M, Stojanović M, Veličković Z, et al (2021) Characterization of potential probiotic strain, L. reuteri B2, and its microencapsulation using alginate-based biopolymers. Int J Biol Macromol 183:423–434. https://doi.org/10.1016/j.ijbiomac.2021.04.177