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Purification and structural characterization of bacterial exopolysaccharides from microorganisms of biotechnological interest

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In recent years, prebiotics have gained popularity as tools to support gut health, as they provide selective nourishment to beneficial gut bacteria. [1] In this context, exopolysaccharides (EPS), usually employed in fermented products for providing viscosity and stability, have also shown several beneficial effects on human health.[2] These extracellular carbohydrate polymers are synthesized by several lactic acid bacteria (LAB) and bifidobacteria during microbial growth and contribute to microbial adaptation. Moreover, several studies have shown that EPS can interact with the intestinal microbiota and influence host–microbe interactions, as well as exerting different antioxidant, anti-inflammatory and immunomodulatory activities within the gastrointestinal tract. [1-3-4]

From a structural point of view, EPS can be classified into homopolysaccharides, formed by one type of monosaccharide, and heteropolysaccharides, composed of different monosaccharides arranged in more complex structures. [5]. Within the vast diversity and structural complexity of EPS molecules, the characterization of their chemical composition is crucial to fully understand how EPS exert its beneficial effects. Here, we describe the purification and characterization of EPS molecules produced by *Leuconostoc mesenteroides 404* strain; starting from the biofilm material, EPS molecules were extracted through precipitation with pure ethanol, followed by extensive dialysis. 1D and 2D Nuclear Magnetic Resonance (NMR) spectra allow the elucidation of the chemical structure resulting in (1→6) glucan, branched with glycosidic bonds (1→3). In the future, the same protocol will be applied also to the EPS produced by a *Bifidobacterium longum* subsp. *longum*, which was isolated from geriatric fecal samples and has been linked to healthy aging. [6] Moreover, to better understand their biological activities, the obtained EPS molecules will also be characterized for their immunomodulatory properties and protective effects on intestinal barrier and for their resistance to human digestion through INFOGEST 2.0 protocol [7].

References

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INTRODUCTION

Prebiotics have gained popularity as tools to support gut health, as they provide selective nourishment to beneficial gut bacteria.[1] In this context, exopolysaccharides (EPS), usually employed in fermented products for providing viscosity and stability, have also shown several beneficial effects on human health.[2] Moreover, several studies have shown that these extracellular carbohydrate can interact with the intestinal microbiota and influence host–microbe interactions, as well as exerting different antioxidant, anti-inflammatory and immunomodulatory activities within the gastrointestinal tract.[1,3,4] Here, we purified and structurally characterized the EPS produced by *Leuconostoc mesenteroides* 404 (EPS 404), a heterofermentative lactic bacterium already widely exploited in food biotechnology for its ability to synthesize high molecular weight EPS, such as dextran and levan, which improve texture viscosity and stability of fermented foods.[5]

ISOLATION AND PURIFICATION

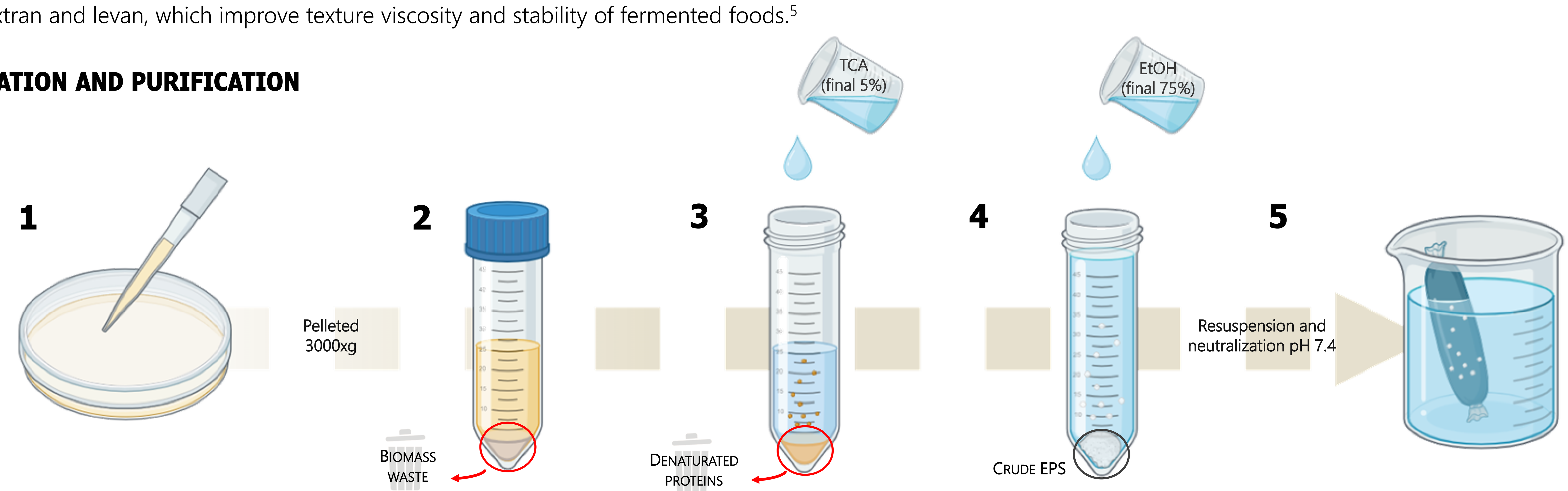


Fig. 1: EPS purification process. **1)** Crude EPS harvesting from petri dish. **2)** Dilution and centrifugation to remove cells. **3)** Deproteinization with trichloroacetic acid (TCA), final concentration 5%. **4)** EPS precipitation with 3V EtOH. **5)** Dialysis of precipitated, resuspended and neutralized EPS.

CHARACTERIZATION

Purified EPS 404 was characterized with High Resolution Magic Angle Spinning (HR-MAS) Nuclear Magnetic Resonance (NMR) spectroscopy. 1D and 2D spectra were acquired at 600 MHz, in D_2O, 323 K, pH 7.4.

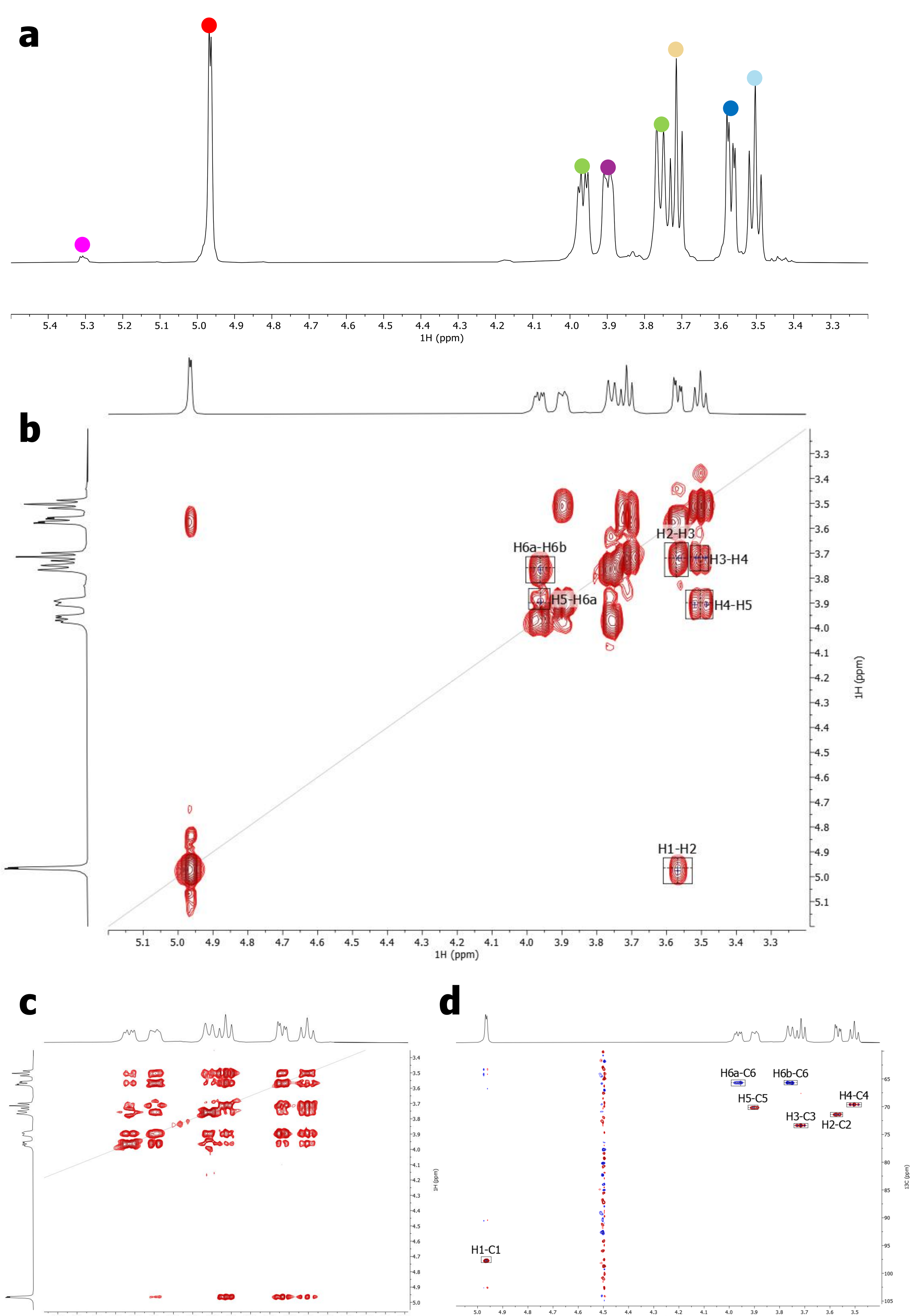
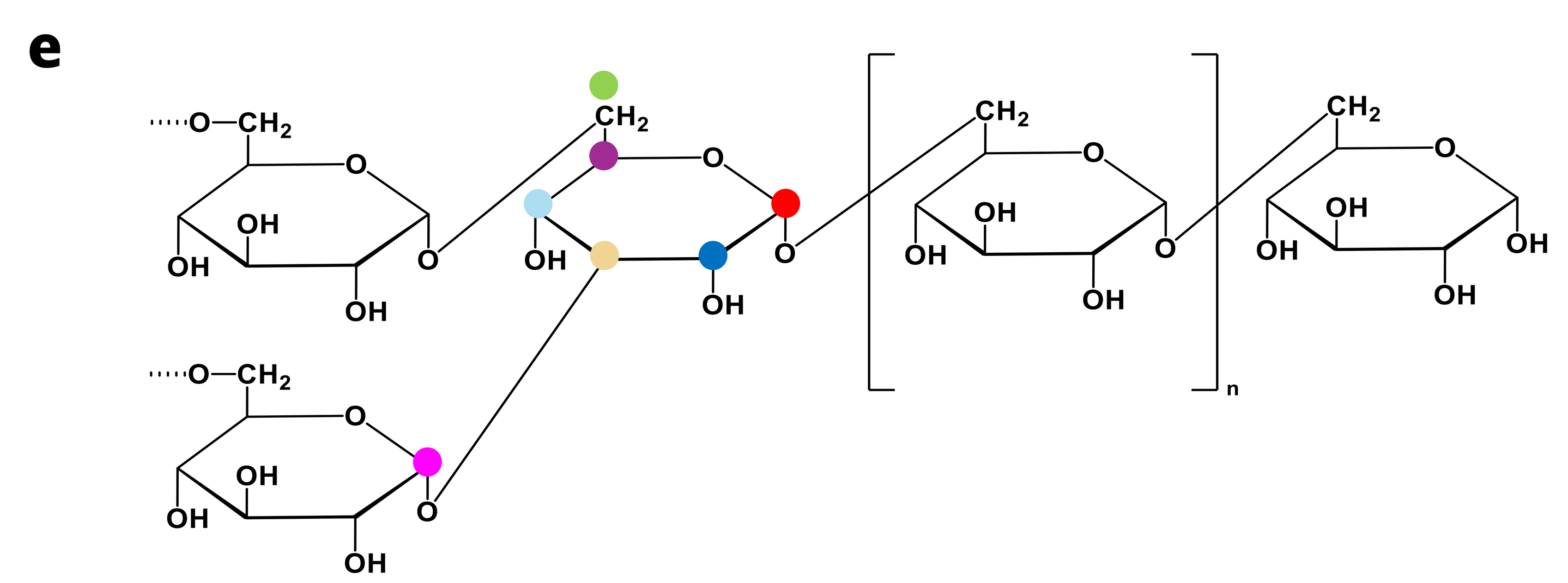


Fig. 2: EPS 404 NMR spectra and chemical structure **a)** ^1H spectrum 30 mg/mL in D_2O pH 7.4; **b)** ^1H-^1H COSY spectrum 30 mg/mL in D_2O pH 7.4; **c)** ^1H-^1H TOCSY spectrum 30 mg/mL in D_2O pH 7.4; **d)** ^1H-^{13}C HSQC spectrum 30 mg/mL in D_2O pH 7.4; **e)** EPS 404 chemical structure; **f)** summary table of EPS 404 ^1H and HSQC spectra. Results were in line with other studies which characterized the same molecule[8,9].



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Assignment	δ (ppm)	Multiplets	δ correlated ^{13}C HSQC (ppm)
H1 α(1→6)	4.97	d (J=3.66 Hz)	97.66
H1′ α(1→3)	5.30	m	99.30
H2	3.57	dd (J=3.61, 9.82 Hz)	71.40
H3	3.71	t (J=9.42 Hz)	73.40
H4	3.50	t (J=9.56 Hz)	69.64
H5	3.90	m	70.17
H6a	3.96	dd (J=4.90, 11.25 Hz)	65.66
H6b	3.76	d (J=11.03 Hz)	65.66

CONCLUSIONS

EPS 404 is an α(1→6) glucan with few ramifications α(1→3), which is commonly produced by *Leuconostoc* species[5]. By calculating the ratio between the H1 α(1→6) and H1′ α(1→3) integral signals, we can estimate that 95% of the glycosidic bonds are α(1→6), with the remaining 5% being α(1→3).

FUTURE PERSPECTIVES

In the future, the same protocol will be applied also to the EPS produced by *Bifidobacterium longum* subsp. *longum*, which was isolated from geriatric fecal samples and has been linked to healthy aging[6]. Moreover, to better understand their biological activities, the obtained EPS molecules will also be characterized for their immunomodulatory properties and protective effects on intestinal barrier and for their resistance to human digestion through INFOGEST 2.0 protocol[7].

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