Isolation and structure elucidation of cell surface polysaccharides from *Oenococcus oeni*

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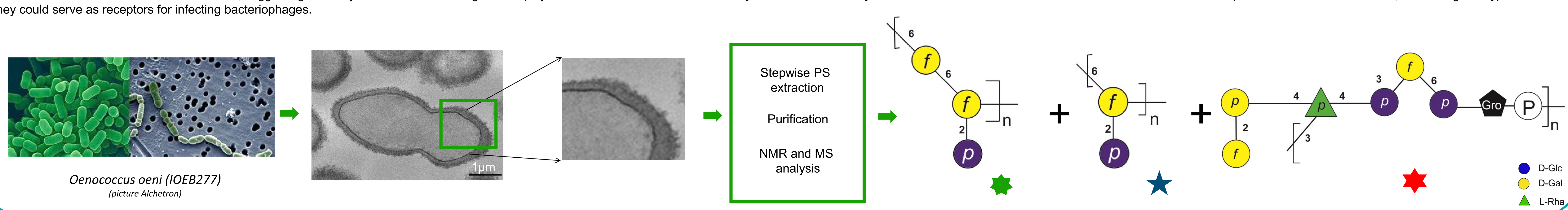
INTRODUCTION

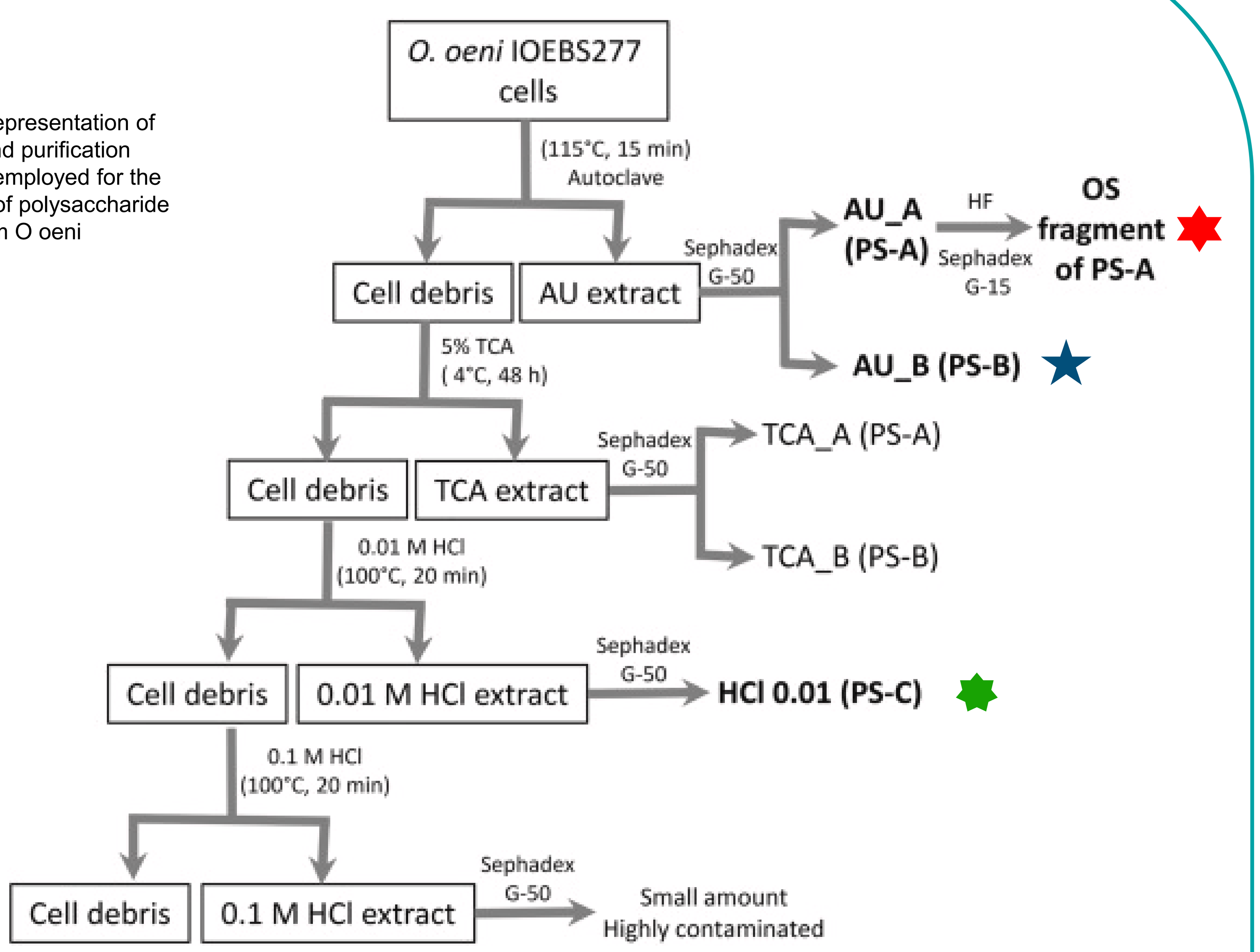
The Gram-positive bacterium *Oenococcus oeni* is a major player in wine malolactic fermentation. In *O. oeni*, cell wall polysaccharides are considered putative receptors for bacteriophages, virus predators that lead to fermentation failures. In this study, we have developed an efficient stepwise extraction protocol to extract polysaccharides from the cell wall of *O. oeni* IOEBS277, which were analyzed by methylation, 1D-, 2D-NMR spectroscopy, and MALDI-QIT-TOF mass spectrometry. The chemical structures of the two major purified polysaccharides were elucidated. The first one (PS-A) is a heteropolysaccharide with repeating units consisting of a branched hexasaccharide and one glycerol residue, linked by phosphodiester bonds. The second one (PS-B) consists of a linear galactofuranan chain regularly substituted with glucose. In addition, a possible linker between PS-A and PS-B was identified, suggesting that they could constitute a large heteropolysaccharide in *O. oeni* cell wall. Finally, HR-MAS NMR analysis of intact *O. oeni* cells indicated that both PS-A and PS-B are exposed to the bacterial surface, reinforcing the hypothesis that they could serve as receptors for infecting bacteriophages.

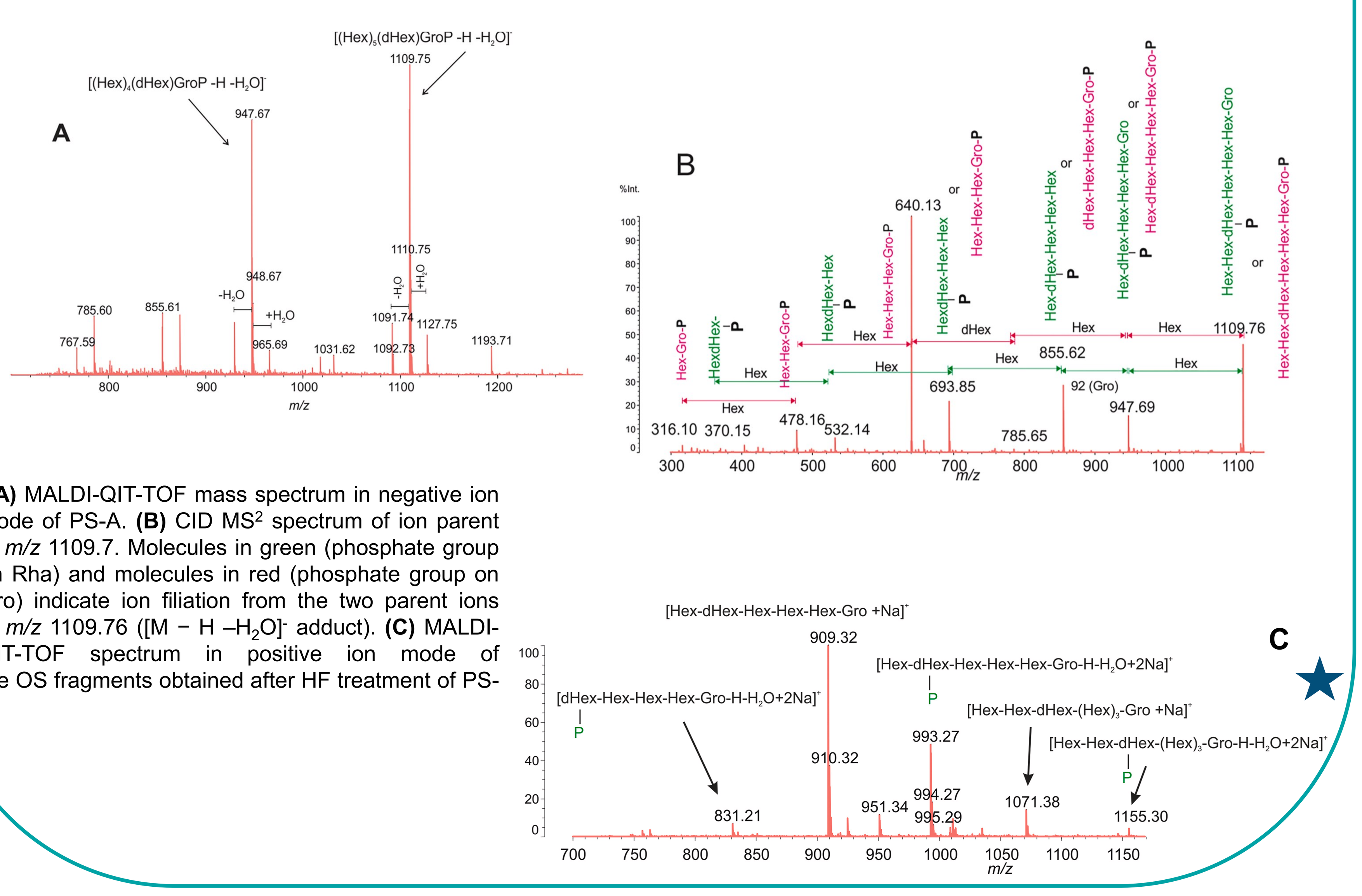


Oenococcus oeni (IOEB277)
(picture Alchetron)

Purification procedure

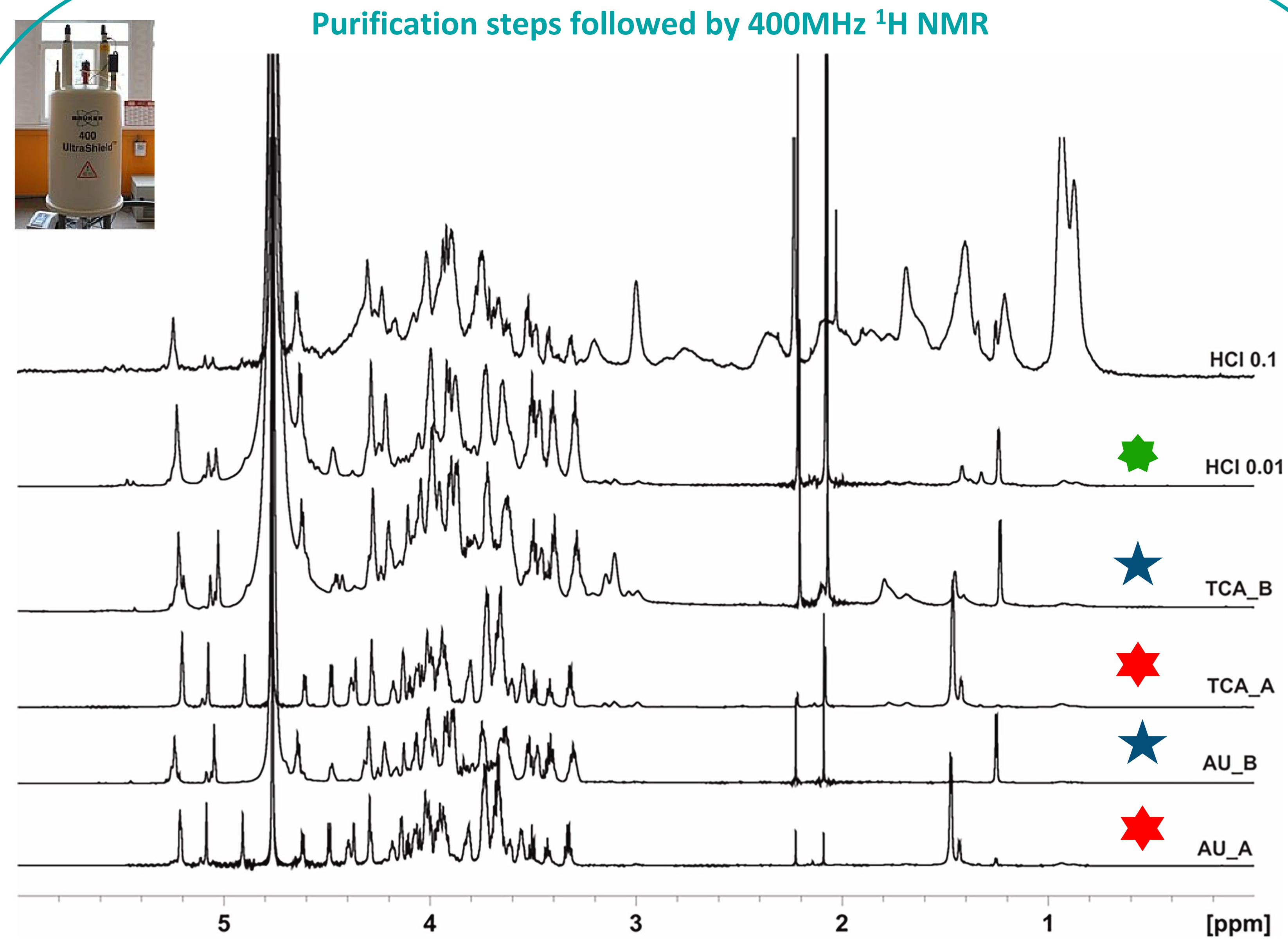
Schematic representation of extraction and purification procedures employed for the preparation of polysaccharide samples from O oeni IOEBS277.



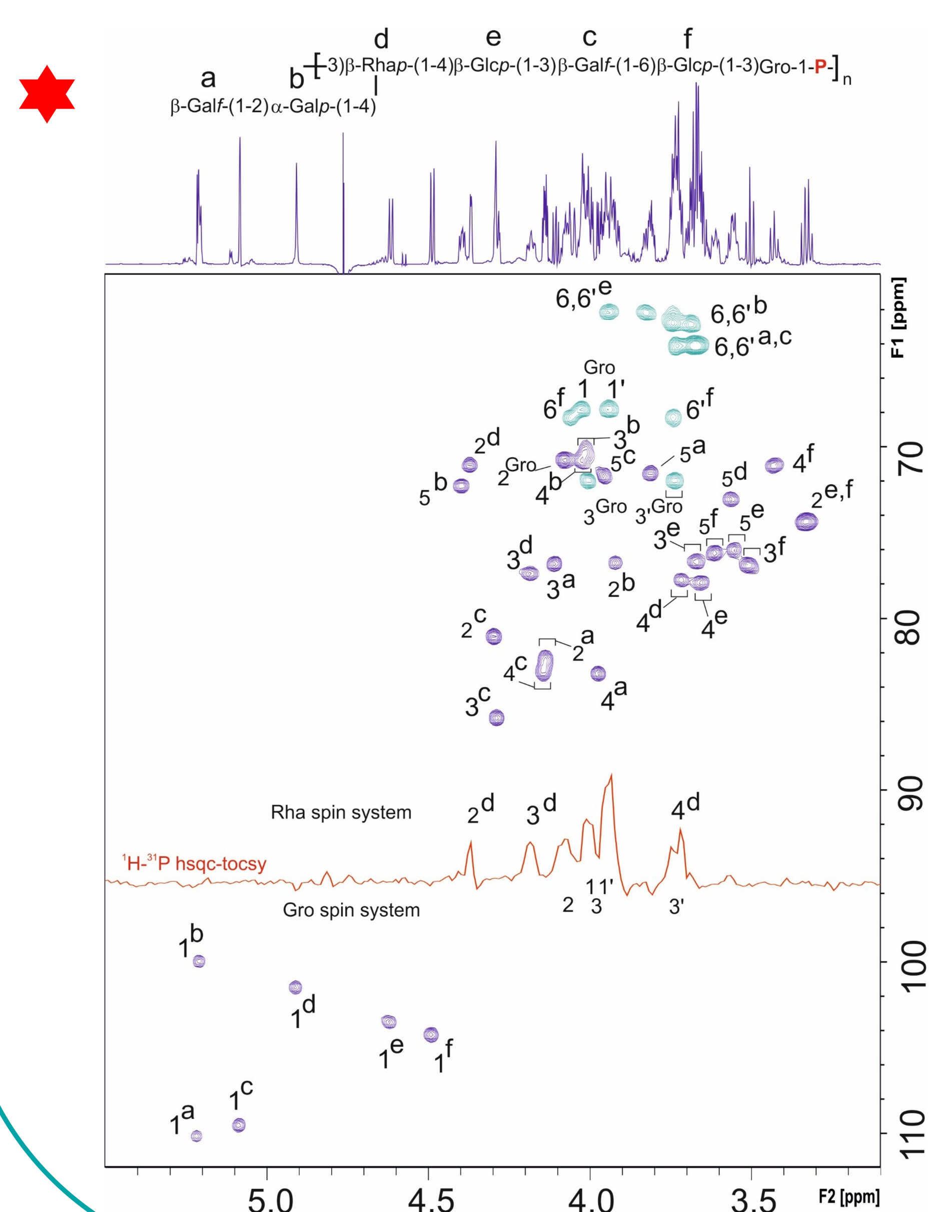


(A) MALDI-QIT-TOF mass spectrum in negative ion mode of PS-A. **(B)** CID MS^2 spectrum of ion parent at *m/z* 1109.7. Molecules in green (phosphate group on Rha) and molecules in red (phosphate group on Gro) indicate ion filiation from the two parent ions at *m/z* 1109.76 ([M − H −H_2O]$^-$ adduct). **(C)** MALDI-QIT-TOF spectrum in positive ion mode of the OS fragments obtained after HF treatment of PS-A.

Purification steps followed by 400MHz ^{1}H NMR



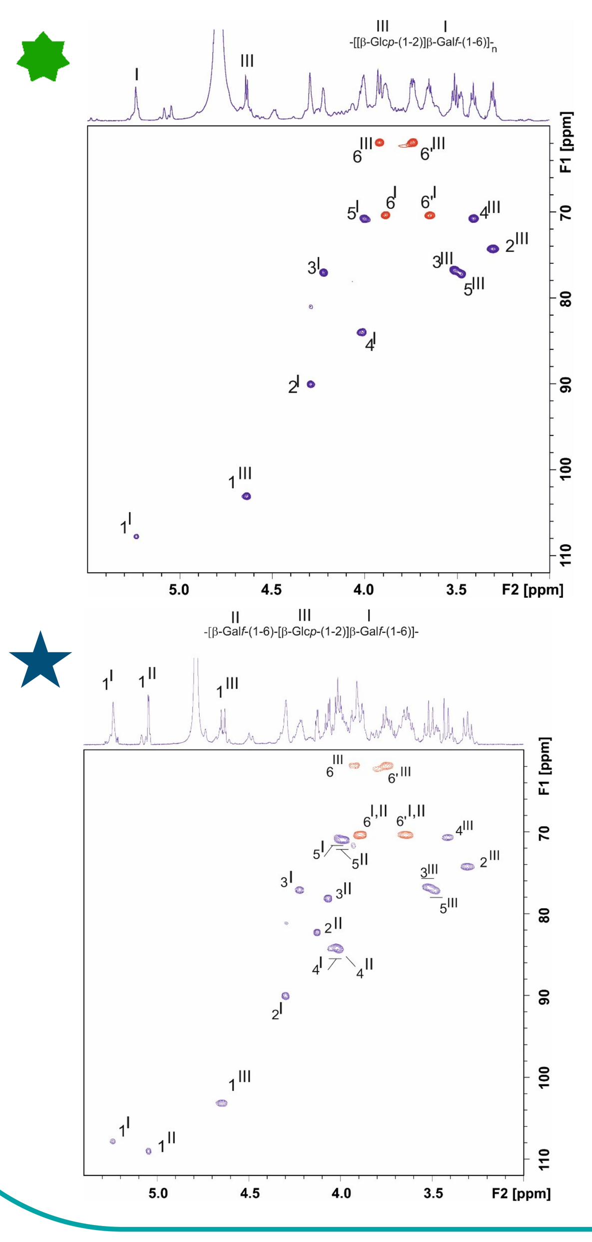
NMR analysis of PS-A with 800MHz



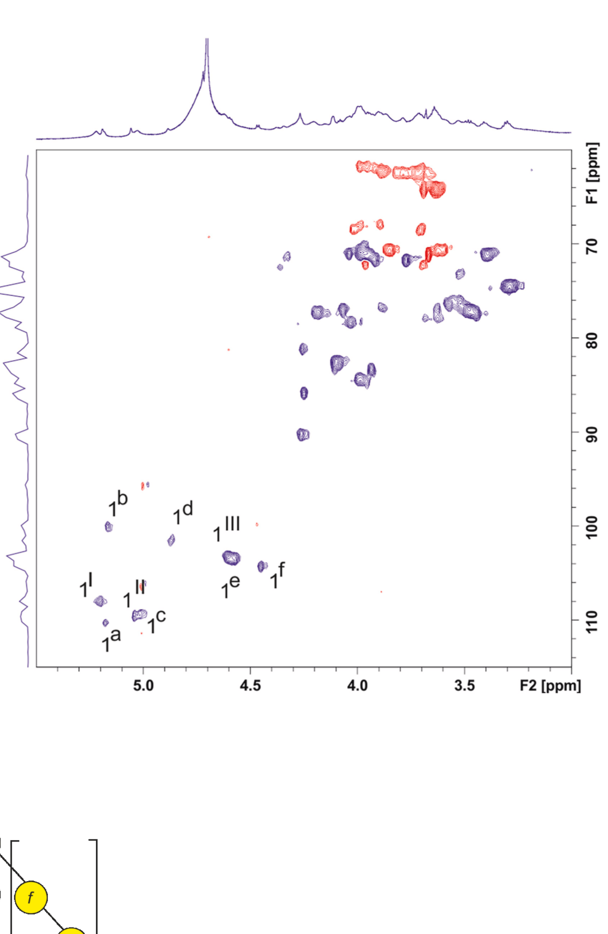
Magnet of 18.8 T from Chevreul Institute (Villeneuve d'Ascq)
^{1}H, ^{13}C and ^{31}P resonate at 800,13, 201,2 and 323,9 MHz respectively

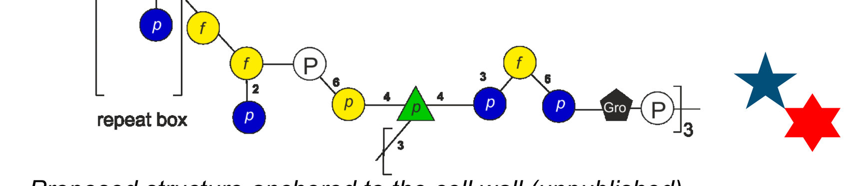
^{1}H–^{13}C edited-HSQC spectrum of purified PS-A from *O. oeni* IOEBS277. In red, superimposed 1D ^{1}H–^{31}P HSQC-TOCSY spectrum indicating the phosphodiester linkage between C1 of Gro and C3 of Rha (d unit). HSQC is recorded with an inept sequence giving –CH– and –CH_3 positive signals in purple and –CH_2- negative signals in green

NMR analysis of PS-CB and PS-C with 800MHz



HR-MAS NMR analysis of whole bacteria with 800MHz





Proposed structure anchored to the cell wall (unpublished)

Discussion

CWPS play a crucial role in bacterial cell wall homeostasis and mediate the interactions of bacteria with their biotic and abiotic environment [1,2]. We have elucidated the chemical structures of the main CWPS of *O. oeni* IOEBS277 wine LAB, which will enable us to study their functional properties in greater detail.

The first polysaccharide (PS-A) is a heteropolysaccharide made of repeating units consisting of a branched hexasaccharide and one glycerol residue, linked by phosphodiester bonds. Thus, PS-A is an anionic CW glycopolymer that could play roles analogous to teichoic acids [1] in *O. oeni*. The two other polysaccharide chains (PS–B and PS-C) are both (1 → 6)-linked β-D-galactofuranans with a different degree of side chain β-D-Glc*p* substituents in position O-2. They could be part of the same polymer comprising two regions with different degrees of Glc substitution or, alternatively, they could be variant chains with different locations inside the cell wall. Of note, two genetic loci named *eps1* and *eps2* were previously annotated in the genome of *O. oeni* strains [3], which could encode the biosynthesis of PS-A and PS-B/C.

The occurrence of the hexofuranose Gal*f* in microbial glycopolymers and their absence in mammalian glycoconjugates was previously highlighted [4]. Galactofuranan made of an alternance of β-D-Gal*f* (1 → 5) and β-D-Gal*f*(1 → 6) is a well-known constituent of the arabinogalactan complex polysaccharide of *Mycobacterium tuberculosis* cell wall [4]. In *O. oeni*, we have found (1 → 6) linked galactofuranan with β-D-Glc*p*(1 → 2) substituents. Similar (1 → 6) linked galactofuranans with various side chain substituents were previously characterized in the CW of several bacterial species including several phytopathogenic Clavibacter species [5], and in the soil bacterium Arthrobacter sp. [6].

The characterized oenococcal CWPS were extracted with harsh treatments of bacterial cells, high temperature (autoclaving), or strong acid treatment, which strongly suggests that they are covalently attached to the cell wall. Moreover, they were detected by HR-MAS NMR analysis, indicating that they are exposed to the bacterial surface. These data reinforce the hypothesis that they can be recognized by phages to adsorb onto *O. oeni* cells and infect them.

Elucidation of the CWPS chemical structure is the first step toward identifying phage receptors in *O. oeni* IOEBS277. Since *O. oeni* is not currently amenable to genetic modification, a preferred strategy will be to isolate spontaneous phage-insensitive mutants (BIMs). Whole genome sequencing of BIMs combined with structural characterization of mutant CWPS will enable us to map the saccharidic structural motifs involved in the adsorption of the different families of *O. oeni* phages.

References

[1] H. Guérin, S. Kulakauskas, M.P. Chapot-Chartier, *J. Biol. Chem.*, 298 (10) (2022), **[2]** C. Whitfield, S.S. Wear, C. Sande *Annu. Rev. Microbiol.*, 74 (2020), pp. 521-543, **[3]** M. Dimopoulou, M. Vuillemin, H. Campbell-Sills, P.M. Lucas, P. Ballestra, C. Miot-Sertier, M. Favier, J. Coulon, V. Moine, T. Doco, M. Roques, P. Williams, M. Petrel, E. Gontier, C. Moulis, M. Remaud-Simeon, M. Dols-Lafargue, *PLoS One*, 9 (6) (2014) **[4]** M. Senicar, P. Lafite, S.V. Eliseeva, S. Petoud, L. Landemarre, R. Daniellou *Int. J. Mol. Sci.*, 21 (10) (2020), **[5]** A.V. Perepelov, D. Kim, E.M. Tul'skaya, N.V. Potekhina, A.S. Dmitrenok, S.N. Senchenkova, L.V. Dorofeeva, L.I. Evtushenko, A.S. Shashkov, *Carbohydr. Res.*, 525 (2023), **[6]** K. Yamazaki, K. Inukai, M. Suzuki, H. Kuga, H. Korenaga, *Carbohydr. Res.*, 305 (2) (1997), pp. 253-260

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