Discovery of quinovosan : a unique dHex-rich polysaccharide structuring the spore crust of *B. subtilis*

Élodie Évangélaire[1], Christelle Lemy[1], Nao Yamakawa[2], Frederic Krzewinski[3], Emmanuel Maes[2], Thomas Dubois[1]

[1] Univ. Lille, CNRS, INRAE, Centrale Lille, UMR 8207, UMET, Unité Matériaux et Transformations, Lille, France .

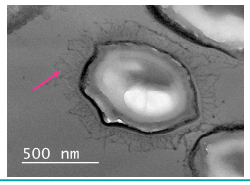
[2] Univ. Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, US 41 – UMS 2014 – PLBS, Lille, France

[3] Univ. Lille, CNRS, UMR 8576, UGSF, Unité de Glycobiologie Structurale et Fonctionnelle, Villeneuve d'Ascq, France

INTRODUCTION

Approximately 30% of foodstuffs produced are lost or wasted [1]. A significant portion of these losses is attributed to microbial spoilage, which is estimated to account for around 25% of production losses [2]. *Bacillus subtilis* spores pose a major challenge to the food industry due to their persistence in processing environments and their ability to cause recurrent contamination of finished products. The outermost layer of the spores, known as the crust, plays a key role in their adhesion to surfaces [3]. The crust is composed of proteins and glycans; however, it remains incompletely characterized, particularly with regard to its glycan components. Currently, only the presence of **Glc**, **Gal**, **Rha**, **Qui**, **MurNAc**, **GlcNH$_2$**, and **Leg** has been identified [3,4]. The objective is therefore to purify the glycans from the spore crust, characterize their structure, and investigate their physiological function.

MET of *B. subtilis* (PY79) spore section after ruthenium red staining

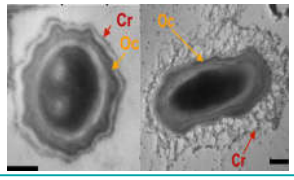


***B. subtilis* crust :**

☛ Determines **surface and adhesion properties** of spores [3]

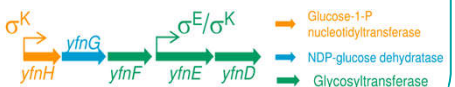
☛ Provides **hydrophilic** character to the spore [3]

PY79 (WT) [5]

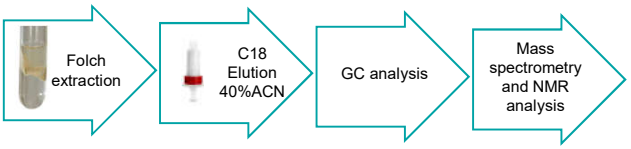


ΔyfnH ☛ Web-like structure

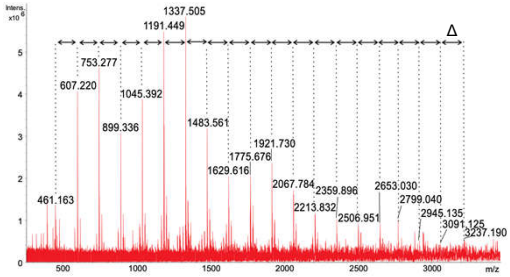
Opéron *yfnHGF-yfnED* :



Structural characterization of quinovosan



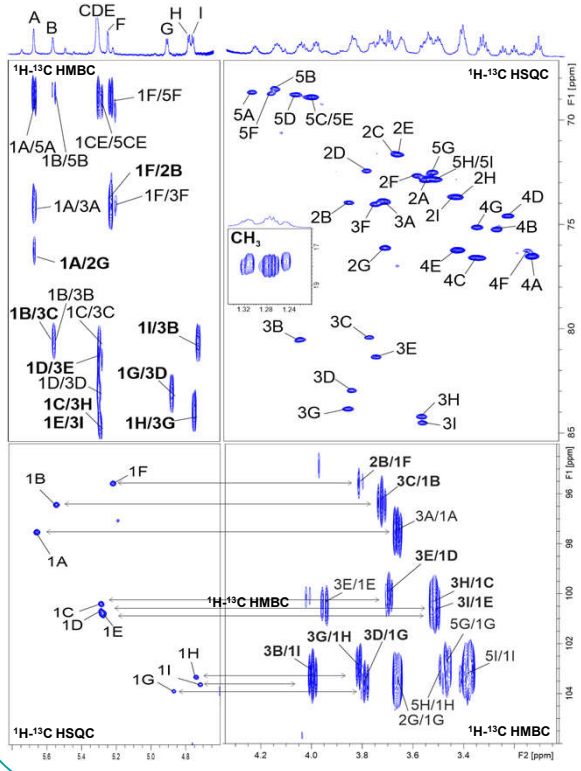
MALDI-FT-ICR

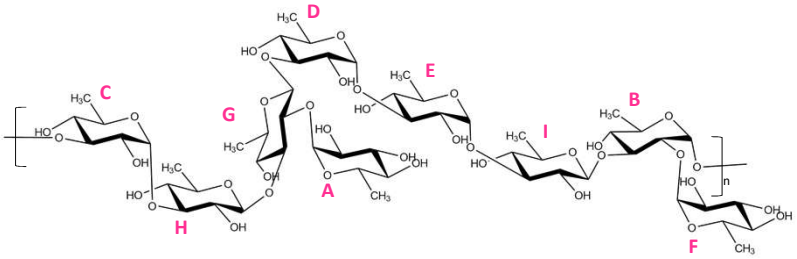


Δ : *m/z* 146 → dHex

→ Homopolymer of Qui

NMR 900 MHz

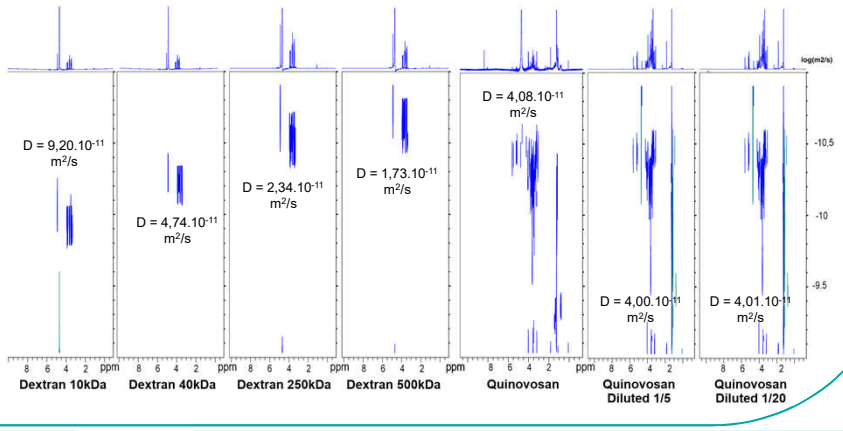




→ Primary structure of a *D*-Qui polysaccharide named : **Quinovosan**

DOSY-NMR 900 MHz

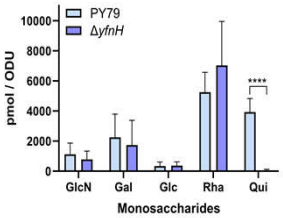
→ Quinovosan: estimated molecular weight of 65 kDa

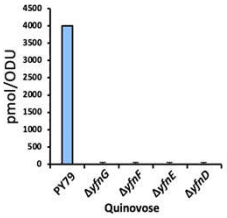


The *yfn* genes are involved in Qui synthesis and transfer

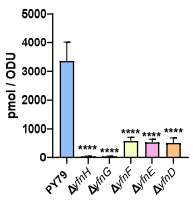
RP-HPLC-FL, anthranilic acid derivatization

Monosaccharide composition of crust





Qui - Dosage intracellulaire T10 - yfn



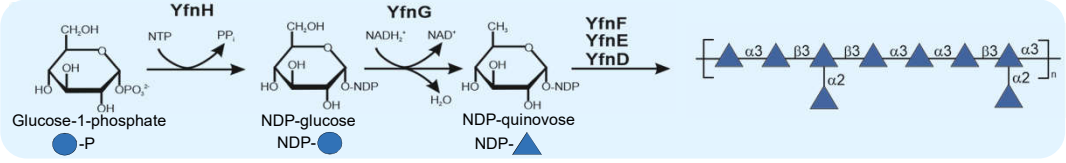
☛ *yfnH,G,F,E,D* genes involved in the synthesis and/or transfer of quinovose to the spore surface

CONCLUSION

This study is the first to report the identification of a homopolymer composed of quinovose residues in a bacterium.

- We determined the primary structure of this polysaccharide composed of quinovose. Extended NMR analysis identified a repeating unit of 9 quinovosyl units composed of 2/3 *α-D*-Qui residues and 1/3 *β-D*-Qui residues. 2 are in terminal non-reducing position, 2 of these are di-substituted in *O*-2 and *O*-3, and the other 5 in *O*-3. Interestingly, this polymer shares similarities with other dHex-rich polysaccharides, such as rhamnan, which mainly contains rhamnosyl residues arranged through α-1,3 and α-1,2 linkages. Due to the structural similarity to rhamnan, we designated this quinovose-based polymer as **quinovosan.**
- We also identified that the *yfnH-D* genes are involved in the synthesis and/or transfer of Qui. Qui biosynthetic pathway is functionally and genetically distinct from previously described pathways, although it shares mechanistic similarities with Rha biosynthesis pathways.
- This study also shed light on the role played by quinovosan at the spore surface. Quinovosan have a minor effect to the hydrophilic nature of spores. Taken together, our results support the conclusion that quinovosan plays only a minor role in surface hydrophilicity, but a prominent structural role by maintaining the integrity and architecture of the crust.

Quinovose biosynthesis pathway in *B. subtilis*



RÉFÉRENCES [1] Snyder, A.B. *et al.*, 2024. Nature Reviews Microbiology. [2] Bondi *et al.*, 2014. BioMed Research International. [3] Faille, C *et al.*, 2014. Biofouling, 30(7). [4] Dubois, T *et al.*, 2020. mBio [5] Shuster, B *et al.*, 2019. Journal of Bacteriology. [6] Nakaya, Y *et al.*, 2023. Molecular Microbiology [7] Zhang, J *et al.*, 1993. Journal of Bacteriology. [8] McKenney, P.T *et al.*, 2010. Current Biology. [9] Imamura, D *et al.*, 2010. Journal of Bacteriology.