

# Analysis of Glycans from *Vibrio crassostreae* isolated during an episode of mortality of the Mediterranean Sea urchin *Paracentrotus lividus*



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Disease in aquaculture systems has been steadily rising over the past decade, in many cases threatening the long-term survival of commercial and natural stocks (Le Roux. A well-documented example is the successive mass mortalities of juvenile oysters (*Crassostrea gigas*), in which bacteria of the genus *Vibrio* have been implicated (Petton et al., 2015). A previous study demonstrated that the onset of disease in oysters coincides with progressive replacement of diverse benign colonisers by members of a phylogenetically coherent virulent population (Lemire et al., 2015) that was assigned to the previously characterised species *Vibrio crassostreae*. *Vibrio crassostreae* is a Gram-negative bacterium known to be pathogenic to shellfish, mostly oysters and reported in other marine species during episodes of morbidity and mortality, causing significant losses in the aquaculture industry. Here we reported the investigation of *V. crassostreae* polysaccharide isolated during an episode of sea urchin mortality (*Paracentrotus lividus*) in Procida Island. The study aims to determine whether *Vibrio crassostreae* may also be pathogenic to sea urchins, specifically investigating its potential contribution to "bald sea urchin disease," a condition resulting in spines.

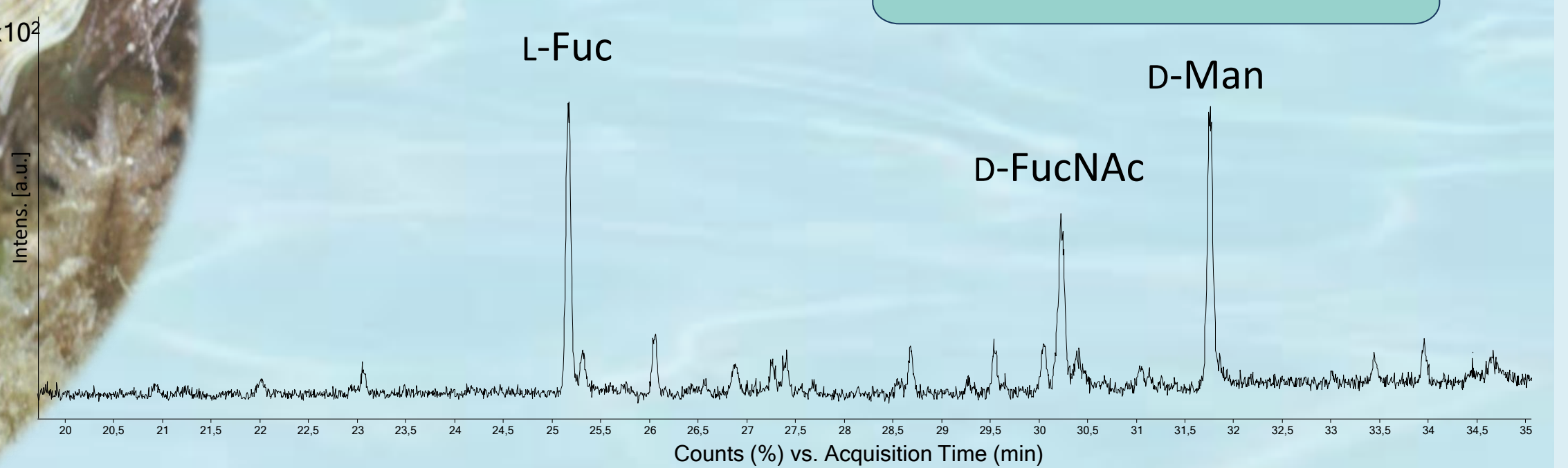
*Vibrio* spp., was isolated from soft tissues (oesophagus/intestines) and spines from *P. lividus*, inoculated into 10 ml of Marine Broth (MB) for 48 h at 30°C. Serial dilutions were performed for each sample and 100 µl of 10<sup>-2</sup> and 10<sup>-3</sup> of diluted samples were plated on the selective medium Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) Agar. Plates were incubated for 24-48 hours at 30°C to allow the growth of *Vibrio* spp.

**1**  
ESTRAZIONE  
PCP e P/W

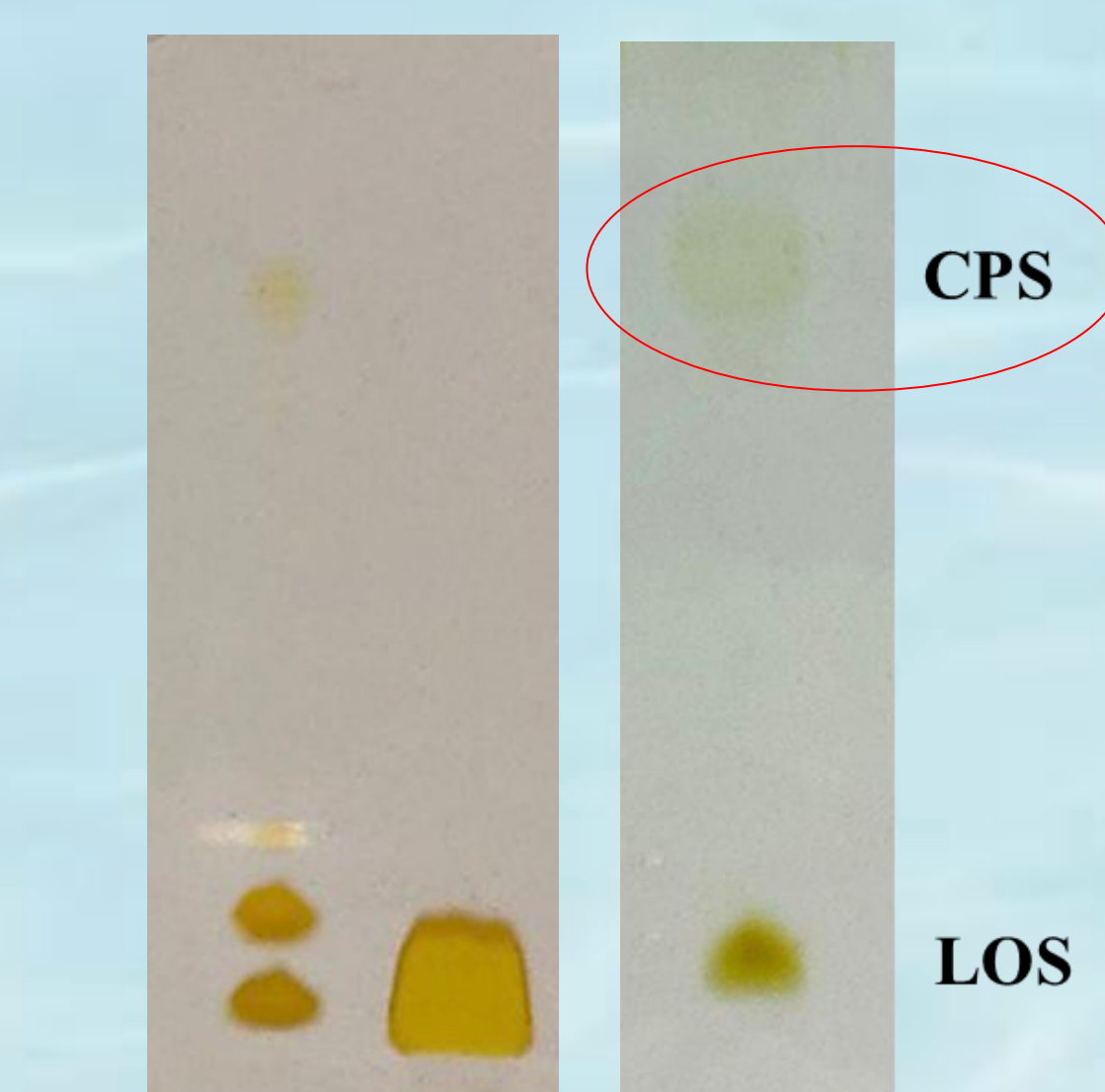
*V. crassostreae* cells (1,4 g) were extracted by (PCP) phenol/chloroform/petroleum ether (2:5:8) followed by (P/W) phenol/water extraction. The obtained extracts were dialysed against water and dried.



**5**  
MONOSACCHARIDE  
COMPOSITION



GC-MS chromatogram of acetylated octyl glycosides of CPS from *V. crassostreae*



**2**  
DOC-PAGE

DOC-PAGE visualised after silver staining suggested the presence of a rough-LPS. Furthermore, the presence of a capsular polysaccharide (CPS) was revealed after staining with Alcian blue and silver nitrate.

Aqueous extract of P/W was subjected to enzymatic digestion with DNase, RNase and protease.

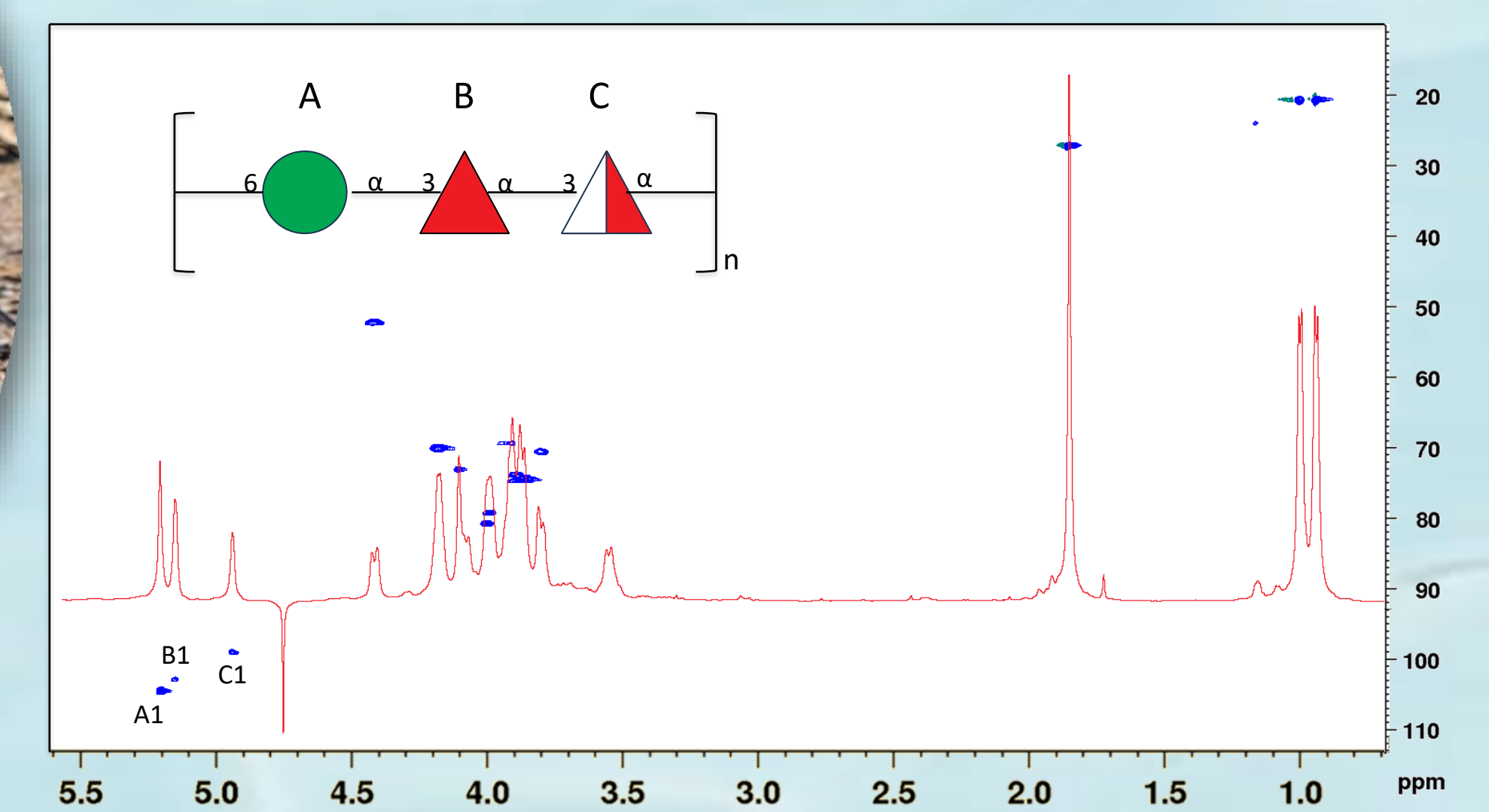
**3**  
ENZYMATIC  
HYDROLYSIS

CPS was purified from LOS by mild acid hydrolysis followed by gel filtration chromatography, eluted with 50mM AMBIC

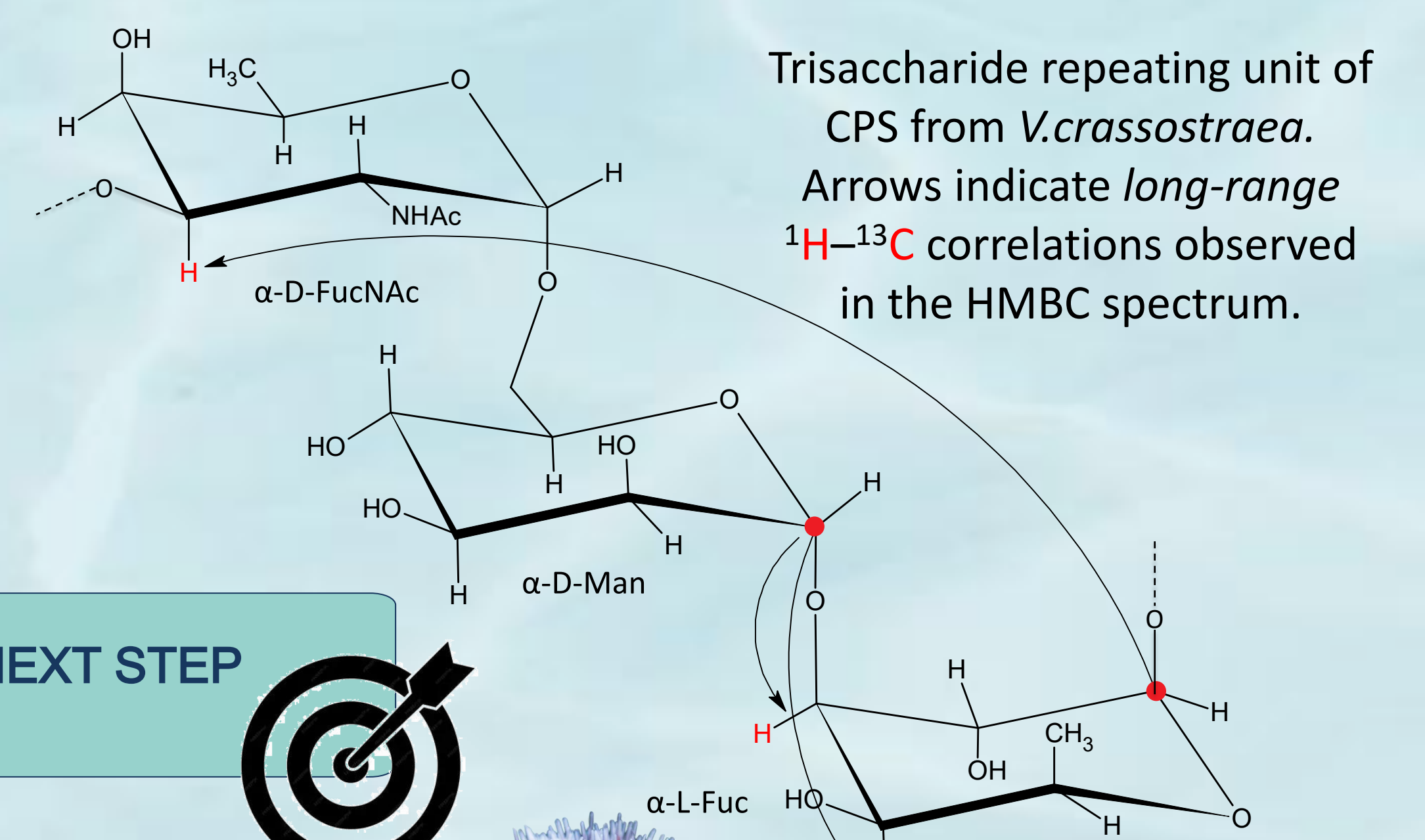


**6**  
NMR

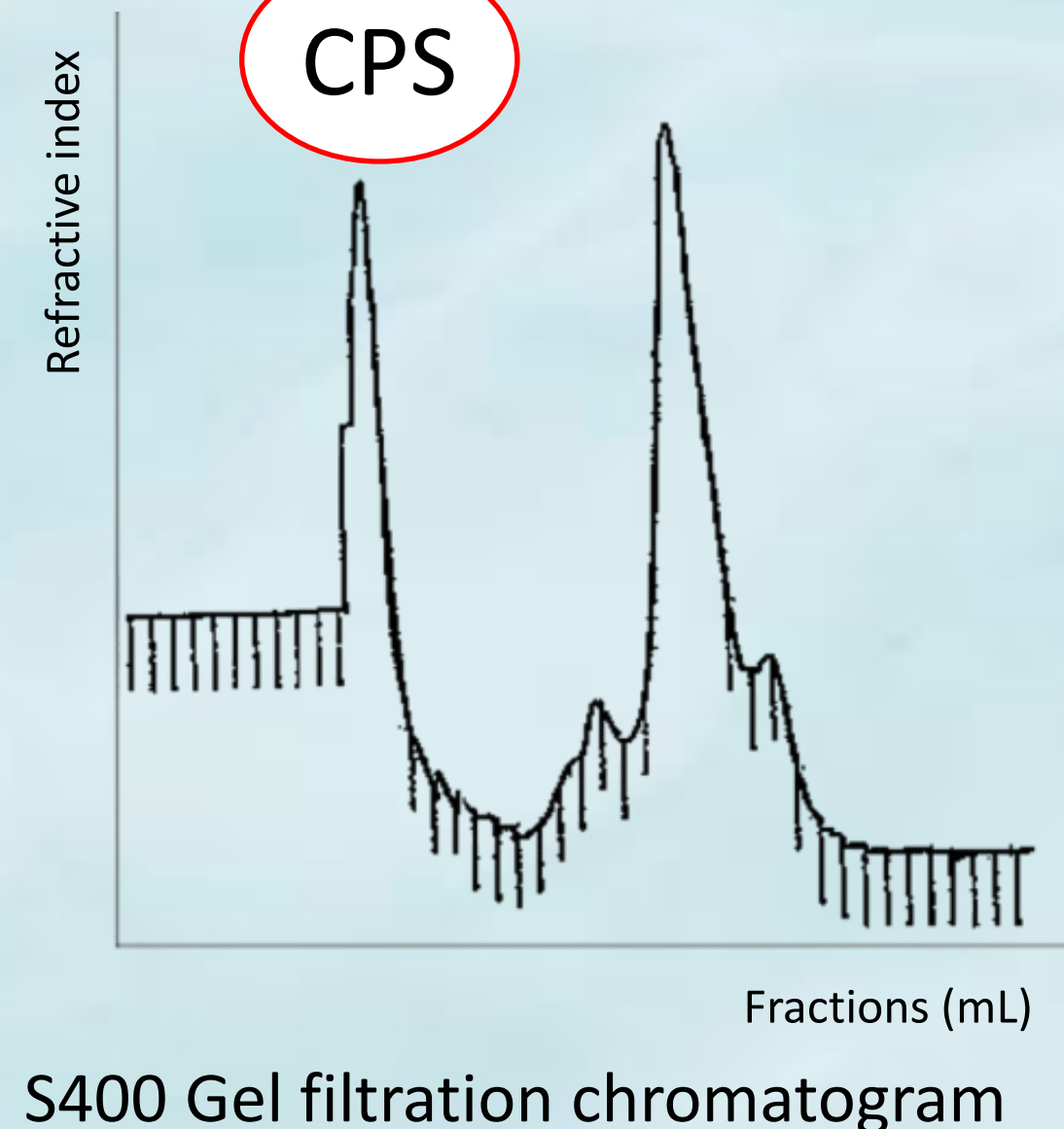
Purified CPS was analysed by homo- and heteronuclear 2D NMR spectroscopy. NMR spectra were acquired in D<sub>2</sub>O at 307K, at 600MHz.



Selected region of <sup>1</sup>H, <sup>13</sup>C DEPT-HSQC spectrum of the polysaccharide structure along with the proton profile.



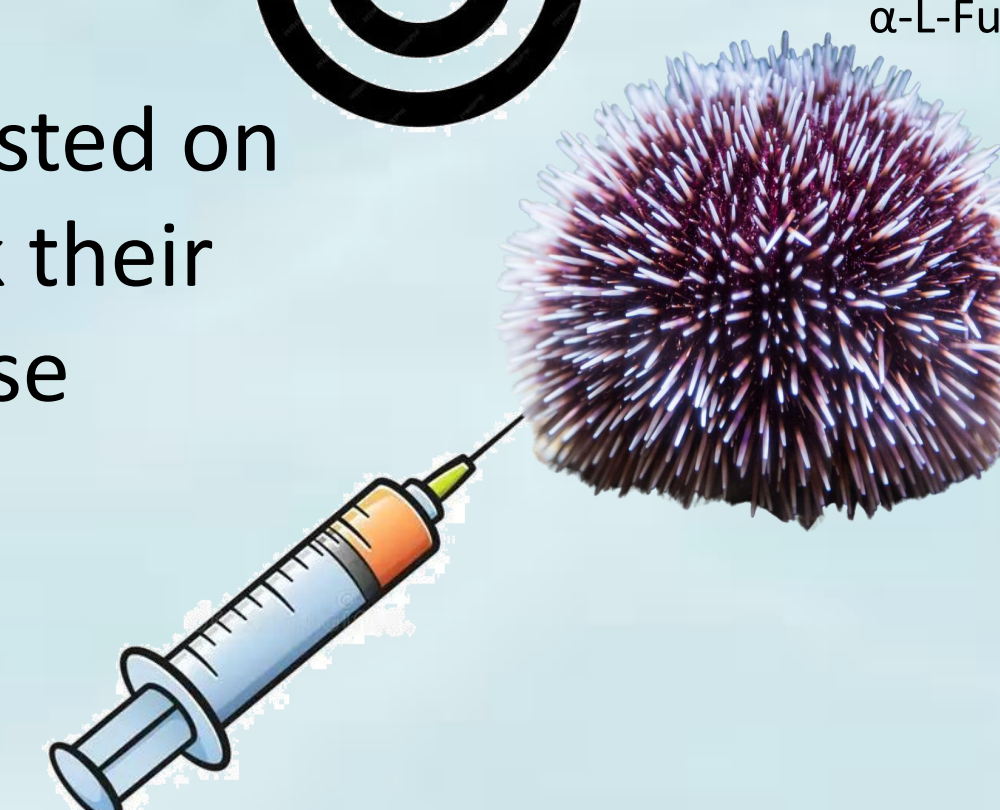
**4**  
MILD ACID  
HYDROLYSIS AND  
GEL FILTRATION  
CHROMATOGRAPHY



S400 Gel filtration chromatogram

**NEXT STEP**

LPS and purified CPS will be tested on healthy sea urchins to check their involvement in the disease



Funding



Lemire, A., et al. (2015). Populations, not clones, are the unit of vibrio pathogenesis in naturally infected oysters. *The ISME journal*, 9(7), 1523-1531.  
Petton, B., et al. (2015). *Crassostrea gigas* mortality in France: the usual suspect, a herpes virus, may not be the killer in this polymicrobial opportunistic disease. *Frontiers in microbiology*, 6, 686.