Spot the difference - chemotyping of Gram-negative bacteria by HR-MAS NMR

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The standard NMR "in solution" approach is not applicable for bacterial samples. Semi-solid samples of bacteria and suspensions of lipopolysaccharides yield poor quality NMR spectra. The High Resolution Magic Angle Spinning (HR-MAS) NMR technique allows to acquire spectra of intact bacteria. Such spectra are typically complex and contain resonances from different types of surface molecules and metabolites. The NMR spectra of Gram-negative bacterial cells include signals from the main surface component - lipopolysaccharide (LPS) [1]. The structurally diverse O-specific polysaccharide part (PS, O-antigen) of LPS is characteristic for the microbial strains and provides unique NMR profiles. These NMR profiles constitute "fingerprints" of bacteria and can be used for chemotyping.

We have performed the initial screening of *Plesiomonas shigelloides* and *Klebsiella pneumoniae* bacterial cells and LPSs by ^{1}H HR-MAS NMR. The spectra of bacteria were complex whereas those from LPSs represented a typical pattern of signals corresponding to the O-antigen carbohydrate structures. We have observed similarities between the NMR profiles of *P. shigelloides* strain CNCTC 78/89 and *K. pneumoniae* Kp20 O-antigens (*Scheme*) [2]. We hypothesized that the shared structural elements of the O-antigens contribute to the observed similarities of the NMR profiles. In-depth studies of *P. shigelloides* LPS and the isolated PS by ^{1}H and ^{13}C NMR spectroscopy and complementary mass spectrometry and chemical methods confirmed that the PS is composed of a disaccharide →3)-α-D-Gal*p*-(1→3)-β-D-Gal*f*2OAc-(1- repeating unit. The O-acetylation is incomplete and only 32% of β-D-Gal*f* molecules are modified. The NMR profile of the non-O-acetylated glycoform of *P. shigelloides* 78/89 O-antigen was identical to this of *K. pneumoniae* Kp20 D-galactan I. A serological cross-reactivity of the both LPSs confirmed the presence of the shared structural motif. The corresponding signals in the ^{1}H,^{13}C HSQC-DEPT HR-MAS NMR spectra of *P. shigelloides* bacterial cells and LPS confirmed the presence of all the O-antigen resonances directly on bacteria (*Figure*), including signal of the structure reporter groups (e.g. O-acetyl). This findings indicate that: (1) the HR-MAS NMR profile of bacteria can provide a structural fingerprint and (2) that it can be directly used for chemotyping of O-antigens.

The fast screening of O-antigens by HR-MAS NMR allows to reveal the similarities among even non-closely related bacterial strains.

The HR-MAS NMR spectra of Gram-negative bacteria include signals corresponding to the O-antigenic carbohydrate segments. The HR-MAS NMR technique demonstrates a potential application for screening of the bacterial O-antigen structures *in situ*, providing robust information on the chemotype of the O-antigens.

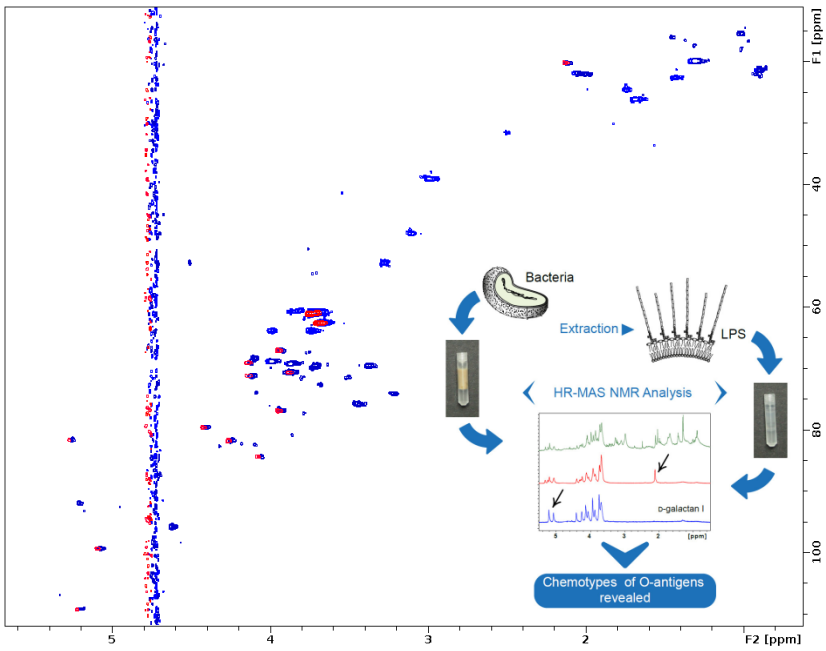

Figure. HR-MAS HSQC-DEPT NMR spectra of the O-antigen of *P. shigelloides* 78/89 acquired directly on bacteria (blue signals) and on the isolated LPS (overlay spectrum, red signals). The comparison demonstrates that the superimposed signals correspond to LPS molecules. *Scheme* represents the procedure of preparing the bacterial and LPS samples for O-antigen chemotyping [2]. The complex ^{1}H NMR profile of *P. shigelloides* 78/89 bacteria include LPS-derived signals of the structure reporter groups.

References:
1. Jachymek W., Niedziela T., Petersson C., Lugowski C., Czaja J., Kenne L. (1999) *Biochemistry* 38: 11788–11795.
2. Ucieklak K., Koj S., Pawełczyk D., Niedziela T. (2017) *Int. J. Mol Sci*. 18: 1-14.