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Corrigendum

Corrigendum to ’Identification and structural determination of the capsular polysaccharides from two *Acinetobacter baumannii* clinical isolates, MG1 and SMAL’ Carbohydrate Research, 346 (2011) 973–8977.

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The authors regret about the structure of the capsule from *Acinetobacter baumannii* strain MG1. The N-acetyl-galactosaminuronic acid residue of this capsule has the D absolute configuration, and not L as reported in the original version of the manuscript. The authors would like to apologise for any inconvenience caused.

List of corrections

- Abstract: 8 lines from the top: “L” is changed in “D”
- Page 2, section 2.1, second line of second para: “L” is changed in “D”
- Page 3, Table 1: second line of first column: “L” is changed in “D”
- Page 4, Conclusion section, three lines before end of first para: the words “in the absolute configuration of the GalpNA residue and”are deleted and substituted with “for”
- Page 4, Figure 6a: “L” is changed with “D”
- Page 5, six lines from the beginning of section 4.2: “L-” is deleted.

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Identification and structural determination of the capsular polysaccharides from two *Acinetobacter baumannii* clinical isolates, MG1 and SMAL

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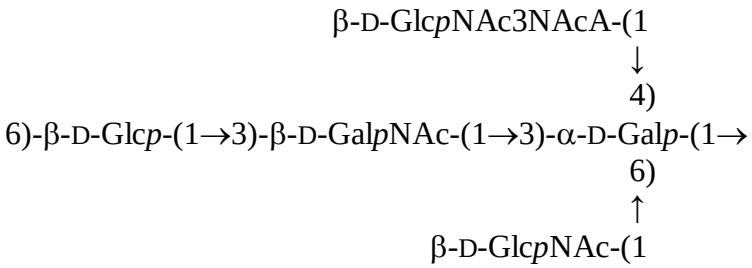
Abstract

The structures of the capsular polysaccharides (CPSs) of the two clinical isolates *Acinetobacter baumannii* SMAL and MG1 were elucidated. Hot phenol/water extractions of the dry biomasses, followed by enzymatic digestions and repeated ultracentrifugations led to the isolation of polysaccharides which were negative in Western blot analysis utilizing an anti-lipid A antibody, thus proving that they were not the LPS O-antigens but CPSs. Their structures were established on the basis of NMR spectroscopy and GC-MS analyses. The *A. baumannii* MG1 CPS consisted of a linear aminopolysaccharide with acyl substitution heterogeneity at the *N*-4 amino group of Qui*p*N4N:

4)-α-D-Glc*p*NAc-(1→4)-α-L-Gal*p*NAcA-(1→3)-β-D-Qui*p*NAc4NR-(1→

R= 3-hydroxybutyrryl or acetyl

The repeating unit of the CPS produced by strain SMAL is a pentasaccharide, already reported for the O-antigen moiety from *A. baumannii* strain ATCC 17961:



Keywords: *Acinetobacter baumannii*; Capsular polysaccharide; Western Blot; structural analysis

Abbreviations: AP: alkaline phosphatase; BCIP: 5-bromo-4-chloro-3-indolylphosphate toluidine salt; CPS: capsular polysaccharide; HB: (*S*)-3-hydroxybutyrryl; LOS: lipooligosaccharide; LPS: lipopolysaccharide; NBT: nitro-blue tetrazolium chloride; PCP: 90% phenol-chloroform-light petroleum; PVDF: polyvinylidene difluoride; D-QuiN4N: bacillosamine; UC: ultracentrifugation

Research highlights

Purification protocol

Western blot analysis

Bacterial Serotyping

1. Introduction

Acinetobacter baumannii is an opportunistic Gram-negative bacterium and considered the most common human pathogen species within its genus, followed by *A. lwoffii* and *A. haemolyticus.* This bacterium is widely distributed in nature and is extraordinarily adaptable to a variety of environmental conditions, e.g., it may colonize a variety of hospital surfaces such as surgical drains or aeration filters.[1] It is generally considered an opportunistic pathogen in immuno-compromised patients causing severe nosocomial, bloodstream, pneumonia, or urinary tract infections, and septicemia.[2]

The propensity of this organism to develop drug-resistance[3] (against carbapenem, beta-lactam, and tetracycline antibiotics) and the lack of development of new drugs to treat infections have resulted in a significant increase in *Acinetobacter*-related studies.[4] In this regard, the study of the main outer membrane components, lipopolysaccharides (LPSs) and capsular polysaccharides (CPSs) is of high importance, since these cell wall components may help to prevent bacterial killing and lyses.

The LPS is composed of the lipid A which is inserted in the bacterial outer membrane and substituted by the core region which in turn bears a third, optional region, termed the O-specific side chain (OPS, or O-antigen). Consequently, LPSs are classified as S- or R-form (also called lipooligosaccharide, LOS), depending on the presence or absence of the O-antigen, respectively. In addition to LPS, *Acinetobacter* bacteria can be surrounded by a thick polysaccharide layer, named capsule or K-antigen.

So far, the LPS structures from several *Acinetobacter* species have been described, and the occurrence of both, R-[5,6] and S-form LPSs was reported.[7,8] Additionally, more than 10 surface polysaccharides were isolated from different *A. baumannii* strains,[9] but in most cases it was not clear whether they originated from CPSs or OPSs. Thus, to date, only two CPSs from *Acinetobacter* species have been clearly defined, namely those of *A. calcoaceticus* BD4[10] and to *A. lwoffii* F78.[11]

In this article, the CPS of two clinical isolates of *A. baumannii*, SMAL and MG1, were investigated disclosing the occurrence of two different capsular polysaccharides. These data extend the basis for the development of an *Acinetobacter* serotyping scheme, analogous to that currently used for *Escherichia coli* isolates.

2. Results and Discussion

2.1 Isolation and chemical analysis of supernatants and sediments from *A. baumannii* MG1 and SMAL obtained by ultracentrifugation.

Freeze-dried cells of *A. baumannii* MG1 and SMAL were treated with aqueous 90% phenol-chloroform-light petroleum (PCP)[12] to extract R-form LPS, and the remaining pellets were successively extracted according to the hot phenol/water method.[13] The water phases were further purified, i.e. nucleic acid and protein contaminants were removed by enzymatic digestion and the resulting solutions were dialyzed and subjected to several cycles of ultracentrifugation (UC). Sugar analysis of the UC sediments gave the same compositions as identified for the LOSs from the two *A. baumannii* strains, namely D-Glc, D-GalN, D-GlcN, and 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo).

The monosaccharide composition of the UC supernatant from *A. baumannii* MG1 comprised L-GalNA, D-QuiN4N (bacillosamine), and D-GlcN. The absolute configuration of QuiN4N was assigned using the same sugar obtained from the CPS produced by *A. lwoffii* F78[11] as standard, and similarly the GalNA butylglycoside was compared to the analogue derivative obtained from the LPS of *Halomonas pantelleriensis*.[14] Furthermore, after weak methanolysis and acetylation, GC-MS analysis showed the presence of QuinN4N substituted at *N*-4 by (*S*)-3-hydroxybutyrryl group (HB). The substituted position was assigned on the basis of the retention time and mass spectrum analogies with the same residue identified in the CPS of *A. lwoffii* F78.[11] The sugar composition of the UC supernatant from *A. baumannii* SMAL revealed the presence of D-Gal, D-Glc, D-GlcN, D-GalN, and 2,3-diacetoamido-2,3-dideoxy-glucuronic acid (DAGA).

2.2 Electrophoretic and Western Blot analysis of UC supernatant and sediment from *A. baumannii* MG1 and SMAL

The sediments and supernatants from UC were analyzed by SDS-PAGE. The gel was first fixed with Alcian blue[15] then with silver nitrate,[16] and disclosed the occurrence of LOS molecules in the

sediment (figure 1), whereas the supernatants contained high molecular mass material which was sensitive to Alcian blue (figure 2, lines 1-4) and identified as CPS. Here, the presence of LOS was negligible and was detected only after having overloaded the gel (figure 2, lines 5 and 6).

In order to exclude that the isolated polysaccharides might originate from LPS, the CPS preparation was subjected to Western blot analysis developed after mild acid hydrolysis of the membrane with the monoclonal antibody (mAb) A6,[17] which specifically recognizes the free bisphosphorylated diglucosamine backbone of lipid A. This antibody had been used earlier for the identification of non-stainable S-form LPS in various *Acinetobacter* strains and LOS in *A. lwoffii* F78.[11] The CPS isolated from *A. baumannii* MG1 and SMAL did not bind mAb A6 (figure 3, lines 2 and 3). Only when applied in high amounts (figure 3, lines 4 and 5), a very faint smear appeared at the bottom of the membrane, with a migration similar to that observed for the purified LOS (figure 3, lines 6 and 7), confirming its occurrence in trace amounts. The lack of reaction of mAb A6 with the material in the upper part of the gel pointed at the presence of a CPS in both, *A. baumannii* MG1 and SMAL.

2.3 Spectroscopical analyses of the CPS from *A. baumanni* MG1

In order to establish the structure of the repeating unit of the CPS of *A. baumannii* MG1, the UC supernatant was analyzed by NMR spectroscopy. The ^{1}H NMR spectrum (figure 4) contained several diagnostic signals: three anomeric ones (5.30-4.50 ppm), and those of four *N*-acetyl methyl protons (approx. 2.00 ppm) and two methyl signals (approx. 1.2 ppm). Of the latter, the most intense signal originated from H-6 of the bacillosamine, and the other one was related to HB present in non-stoichiometric amounts. The sugar residues were labeled with a letter (**A-C)** in order of their decreasing anomeric chemical shifts, and **D** corresponded to HB. By analysis of the 2D NMR spectra of the polysaccharide all proton and carbon chemical shifts could be assigned (table 1). Residue **A** was classified as a *O*-4 substituted α-Gal*p*NAcA. The *galacto* stereochemistry was identified by the weak scalar correlations in both the COSY and TOCSY spectra of H-4 to its vicinal protons (H-3 and H-5), the α-configuration was established on the basis of the chemical

shift (5.21 ppm) and of the broad singlet shape of the H-1 signal. The chemical shifts of the ring carbons identified one nitrogen-bearing carbon, namely *C*-2 (50.4 ppm), which was *N*-acetylated as proven by the H-2 deshielded chemical shift and by the presence of *N*-acetyl signals in the proton spectrum. The *C*-4 signal appeared in the low field region of the HSQC spectrum (79.7 ppm) proving glycosylation at this position, and H-5 displayed a long-range correlation with a carbon at 175.1 ppm, confirming the uronic acid feature of this residue.

Similar considerations identified **B** as a *O*-4 substituted 2-acetamido-2-deoxy-α-glucopyranose.

With regard to unit **C** (bacillosamine), the multiplicity of the anomeric proton could not be assigned since it appeared as a very broad singlet at 4.60 ppm. Therefore, the β-configuration was suggested from both the ^{1}H and ^{13}C anomeric chemical shifts and confirmed by the NOE contacts (figure 5) of H-1 to both, H-3 and H-5, due to the axial orientation of these three protons. The examination of the carbon chemical shifts of this residue identified two nitrogen bearing carbons, i.e. *C*-2 and *C*-4 (both at 57.9 ppm), and the glycosylation site on *C*-3 (76.8 ppm).

Using the same approach, the HB residue was completely assigned and, based on the GC-MS data, found to substitute the amino group at *C*-4 of residue **C**. Information regarding the substitution degree at *N*-4 of **C** was deduced from the integration of two groups of signals in the high field region of the proton spectrum (figure 4), namely the broad peak at 2.33 ppm, which accounted for the two methylene protons of HB ($Area_1$), and the two methyl groups ($Area_2$) at 1.24 (H-4 protons of HB) and 1.17 ppm (H-6 protons of **C**). At this regard, methyl signal at 1.17 ppm comprehended the methyl protons from all the bacillosamine residues present in the polymer, regardless their acylation state on the nitrogen at *C*-4, that could be either acetylated or substituted with the HB unit. This methyl signal could not be integrated directly in the proton spectrum, but its contribute to $Area_2$ was deduced applying formula 1: both $Area_1$ and $Area_2$ were normalized with respect to the number of protons they represented (two and three, respectively), so that $(Area_2)/3$ represented the number of HB residues plus those of bacillosamine, while $(Area_1)/2$ reflected the total number of HB moieties. In this way, the bacillosamine amount was deduced subtracting $Area_1$ from $Area_2$ and

this value was then compared with that of the HB residue ($Area_1$) yielding an overall substitution degree of 19% (see formula 1).

The analysis of the NOESY spectrum (figure 5) showed a spatial proximity between H-1 of **A** and H-3 of **C**, H-1 of **B** and H-4 of **A**, and H-1 of **C** and H-4 of **B**, and thus, disclosed the sequence between the three residues as reported in figure 6a.

Finally, CPS ^{1}H NMR and HSQC spectra were recorded in 10 mM deuterated HCl (data not shown). Under this condition a large low field shift was found for H-5 resonance and to a lesser extent for H-1 signals of the Gal*p*NAcA residue, suggesting that the carboxylic group of this residue was in the free form and amidated, for instance.

2.4 Structural analysis of the CPS from *A. baumannii* clinical isolated SMAL

The proton NMR spectrum of the CPS produced by strain SMAL showed five anomeric signals and a crowded carbinolic area, together with the occurrence of four *N*-acetyl methyl signals. The analysis of the 2D NMR spectra was impaired by the high number of signals, many of which overlapped, and from their broad shape which resulted in poor quality spectra. These problems were circumvented by studying the deacetylated CPS and recording the sample under alkaline conditions causing the shift of the amino-geminal protons to high field region of the spectrum.

The five anomeric signals were labeled **A - E** in decreasing order of their chemical shifts, and the attribution of the 2D homo- and heteronuclear spectra (table 2) followed the strategy described above for the MG1 CPS and characterized the structure of the repeating unit (figure 7a).

On the basis of the above information and considering that the alkaline treatment removed the pre-existing acetyl groups, the structure of the repeating unit of the *O*-deacylated CPS produced from *A. baumannii* SMAL was as shown in figure 7b. This repeating unit was identical to that previously reported for *A. baumannii* strain ATCC 17961.[18]

3. Conclusions

In the course of this study, carbohydrate material produced from two *A. baumannii* clinical isolates, MG1 and SMAL, was studied with the purpose to establish their location in the cell envelope together with their structures.

Western blot analysis classified these materials as CPSs and the extensive use of NMR spectroscopy determined the carbohydrate sequence of each repeating unit. With regard to *A. baumannii* MG1, the repeating unit of the capsular structure is constituted by three different amino sugars (figure 6a) and it is quite similar to the O-antigen of the LPS from another strain of *A. baumannii* (figure 6b).[19] Indeed, both polymers possess a β-bacillosamine residue acylated non-stoichiometrically with HB at *N*-4, but they differ in the absolute configuration of the Gal*p*NA residue and the acetylation at *O*-6 of the Glc*p*N unit, which is absent in the MG1 CPS.

As far as *A. baumannii* SMAL is concerned, its CPS is constituted of a pentasaccharide repeating unit (figure 7b), rich in amino sugars, as was often found in CPS or O-antigens from this bacterial species. The structure of this polysaccharide is not new, and it was recently reported for the O-antigen of another *A. baumannii* strain, ATCC 17961.[18]

The structures of the CPSs from *A. baumannii* MG1 and SMAL may contribute to the establishment of a serotyping scheme for this bacterium.

4. Experimental

4.1 Bacteria growth and CPS isolation

A. baumannii strains SMAL and MG1 were cultivated in Luria Broth (LB) medium at 28°C and cells were collected by centrifugation (9 800 *g*, 20 min, 4°C), washed sequentially with distilled water, ethanol, acetone, and ethyl ether, then suspended in water and freeze-dried.

For each strain, the isolation of the LOS was performed on dry cells by PCP (2:8:5 v/v/v) extraction.[12] After removal of the light solvents under vacuum, the LOS was precipitated from the phenol with water and washed with aqueous 80% phenol and acetone. The precipitate was suspended in water and lyophilized (strain SMAL yield 0.8% g_{LOS}/g_{cells}; strain MG1 yield 0.7%

g_{LOS}/g_{cells}). The remaining cell pellet was extracted according to the hot phenol/water protocol,[13] and water and phenol phases were dialyzed and freeze dried. In both cases, preliminary chemical and electrophoretic analysis revealed the presence of polysaccharide material in the water phases (strain SMAL yield 22% g_{LOS}/g_{cells}, strain MG1 yield 20% g_{LOS}/g_{cells}). The phenol phases were not investigated.

The CPS were purified from nucleic acid, proteins and LOS by enzymatic treatment and repeated ultracentrifugations: for each strain, the crude water extract was solved in the digestion buffer (100 mM TRIS, 50 mM NaCl, 10 mM $MgCl_2$, buffer at pH 7.5) at a concentration of 5 mg/mL, and treated with DNAse (Roche, Germany 04716728001) at 37 °C overnight, successively Proteinase K (Roche, Germany 03115836001) was added and the solution left at 56 °C for 4 h. After dialysis and freeze-drying, enzyme-treated water phase (30 mg from each strain) was solved in distilled water and ultracentrifuged (105 000 *g*, 4°C for 12 h). The LOS-containing sediment was suspended in water and ultracentrifuged again at the same conditions. This sediment was lyophilized and used for further analysis (strain SMAL yield 9 mg, strain MG1 13 mg). The CPS-containing supernatants were combined and ultracentrifugated (500 000 *g*, 4°C, 48 h), the supernatant, namely the purified CPS, was freeze-dried and finally used for the chemical analyses (strain SMAL yield 18 mg, strain MG1 yield 15 mg).

4.2 Chemical Analyses

Monosaccharide compositional analysis (acetylated methyl glycosides) and absolute configuration analysis (octyl or butyl glycosides) were performed as reported elsewhere.[20]

Monosaccharide derivatives were recognized on the basis of their GC-MS spectrum fragmentation pattern and by comparison of their retention time with that of authentic standards. At this regards, L-Gal*p*NA, and D-bacillosamine reference compounds were obtained from the LPS of *Halomonas pantelleriensis*[14] and CPS of *A. lwoffii* F78, respectively.[11]

All samples were analysed with a Hewelett-Packard 5890 GC-MS instrument, equipped with a SPB-5 capillary column (Supelco, 30 m x 0.25 mm i.d., flow rate, 0.8 mL/min, He as carrier gas) and the temperature program: 150 °C for 5 min, 150→300 °C at 10 °C/min, 300 °C for 12 min; electronic impact mass spectra were recorded with an ionization energy of 70 eV and an ionizing current of 0.2 mA.

4.3 SDS-PAGE and immunodetection analysis

The sediments and supernatants obtained after UC were subjected to SDS-PAGE[21] and Western Blot using the anti-lipid A monoclonal antibody (mAb) A6.[17] Discontinuous SDS-PAGE was performed on a miniprotean gel system (Bio-Rad) with a 5% stacking gel. The separating gel composition was optimized for the type of molecule screened, i.e. 15% for LOS and 8% (top half) and 15% (bottom half) for CPS. Samples were run at constant voltage (150 V), and gels were silver stained,[16] applying Alcian blue first, as fixative.[15]

Western blot analysis was accomplished using a Biorad Trans Blot Cell System and a PVDF membrane (pore size 0.45 μm). The membrane blotting was performed in Trans-Buffer (Tris 2.4 g/L, glycin 11.5 g/L, 15% methanol) for 16 h, 10°C, 26 V. The membrane was incubated with 1% AcOH (100°C, 2h) to cleave the Kdo glycosidic linkage which was required because mAb A6 is specific for free lipid A.

For Western blot detection, the membrane was washed with Tris-Tween buffer (3 x 30 min; Tris 1.21 g/L, Tween 20 0.5 ml/L, pH 8.0) then blocked for 1 h in Tris-Tween buffer with evaporated defatted milk (5%) and incubated for 1 h with mAb A6 (diluition 1:200) in dilution buffer (Na_2HPO_4 1.78 g/L, NaCl 8.77 g/L, Tween 20 0.5 ml/L, pH 7.4) and 2% of evaporated defatted milk. The membrane was then washed with Tris-Tween buffer (3 x 15 min) and incubated for 1 h with the secondary antibody alkaline phosphatase (AP)-conjugated goat anti-mouse IgG (Jackson ImmunoResearch cod. 115-055-003, dilution 1:1000). Finally, the membrane was rinsed with Tris-Tween buffer (2x15 min) first and then with PBS, followed by washing with AP-buffer (10 min, 0.1

M $NaHCO_3$, 1 mM $MgCl_2 \cdot 6\ H_2O$, pH 9.0) and treatment with a solution containing NBT (Biomol 06428, 50 mg/mL in 70% DMF) and BCIP (Biomol 02291, 50 mg/mL in DMF), 2:1, v/v, in AP-buffer, until the color developed.

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4.5 Deacylation of CPS from *A. baumannii* SMAL

Deacylation of both CPS was performed treating the sample (10 mg) with 4 M KOH (1 mL) at 120°C for 16 h. After neutralization, the sample was desalted by SEC chromatography (Biogel P2, Biorad, 1.5 x 120 cm, eluent 50 mM NH_4HCO_3, flow rate 0.2 mL/min), from which it was recovered in the void volume of the column.

4.6 NMR spectroscopy

Homo-and heteronuclear spectra of the CPS from *A. baumannii* MG1 were recorded in deuterated water (D_2O) using Varian Inova 500 MHz spectrometer equipped with a inverse z-gradient probe (Consortium INCA, L488/92, Cluster 11) operating at 298 K. With regard to the CPS of *A. baumannii* SMAL, spectra were acquired on a Bruker 600 MHz equipped with a cryo probe; the fully deacetylated capsule (5 mg in 600 µl of 30 mM NaOD in D_2O) was measured at 291 K. Chemical shifts are expressed in ppm relative to internal acetone (^1H at 2.225 ppm, ^{13}C at 31.45 ppm). Two-dimensional spectra (DQ-COSY, TOCSY, NOESY, gHSQC and gHMBC) were measured using the standard Varian or Bruker software. For the homonuclear experiment, 512 FIDs of 2048 complex data points were collected, with 40 scans per FID, a mixing time of 120 or 200 ms was applied for the TOCSY and NOESY spectra, respectively. For the HSQC and HMBC spectra, 256 FIDS of 2048 complex points were acquired with 50 scans per FID. Processing of the Varian and Bruker data and analysis was performed with Bruker TopSpin 2.1 program.

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Conflict of interest: none declared

Figure Legends

Figure 1: SDS-PAGE (stacking gel 5%, separating gel 15 %), stained with Alcian blue and silver nitrate procedure) of the UC sediments; **1**: *A. b*. SMAL (2 μg); **2**: *A. b.* MG1 (2 μg); **3**: *E. coli* O16 LPS (2 μg)

Figure 2: SDS-PAGE (Stacking gel 5%, separating gel 8 and 15 %) of the UC supernatants; lines **1** and **2** are stained only with Alcian blue; lines **3**-**6** are silver stained applying Alcian blue as fixative first. **1**: *A. b*. SMAL (1 μg); **2**: *A. b*MG1 (1 μg); **3** and **5**: *A. b* SMAL (1 and 3 μg); **4** and **6**: *A.b* MG1 (1and 3 μg).

Figure 3: Western blot of the UC supernatants (lines **2**-**5**) and sediments (lines **6**-**7**) from *A. baumannii* SMAL and MG1, detected with mAb A6. **1**: LPS *E. coli* O16 (8 μg); **2** and **4**: UC supernatant of *A. b.* SMAL (5 and 25 μg); **3** and **5**: UC supernatant of *A. baumannii* MG1 (5 and 25 μg); **6** and **7**: UC sediments from *A. baumannii* SMAL and MG1, respectively (3 μg each).

Figure 4: (500 MHz, 298 K) ^{1}H NMR spectrum in D_2O of the capsular polysaccharide from *A. baumannii* MG1. Comparison of $Area_1$ and $Area_2$ according to formula 1, suggested a substitution degree with HB at *N*-4 of the bacillosamine residue of 19%.

Figure 5: The anomeric area of the NOESY spectrum (500 MHz, 298 K) of the CPS from *A. baumannii* MG1 in D_2O, the inset shows the NOE contacts identified for residue **C**. The broad anomeric signal of **C** induced the same pattern in the NOE densities, which appeared large and flattened on the baseline; these signals were recognized by amplifying the area of interest, as

indicated in the inset. The *intra*-residue NOE $C_{1,3}$ may be overestimated due to the coincidence of the signals from H-3 of **C** with H-3 of **B**.

Figure 6. a) structure of the repeating unit of the capsular polysaccharide from *A. baumannii* MG1. R is HB (19 %) or Acetyl; b) structure of the O-antigen produced from *A. baumannii* strain 24, R is HB or Acetyl.

Figure 7. Structure of the repeating unit of the capsular polysaccharide isolated from *A. baumannii* SMAL, in its not (a) or acetylated form (b); residue labels reflect those used in the NMR assignment.

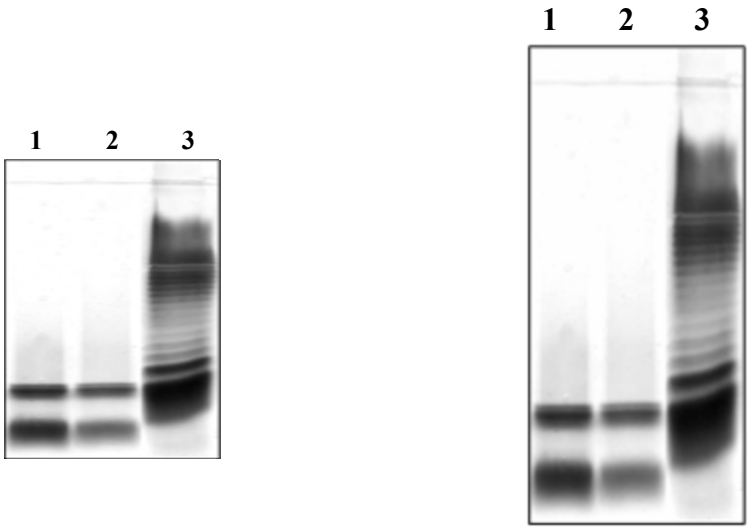


Figure 1: SDS-PAGE (stacking gel 5%, separating gel 15 %, stained with Alcian blue and silver nitrate procedure) of ultracentrifuge sediments; **1**: *A. b*. SMAL (2 µg); **2**: *A.b.* MG1 (2 µg); **3**: *E. coli* O16 LPS (2 µg)

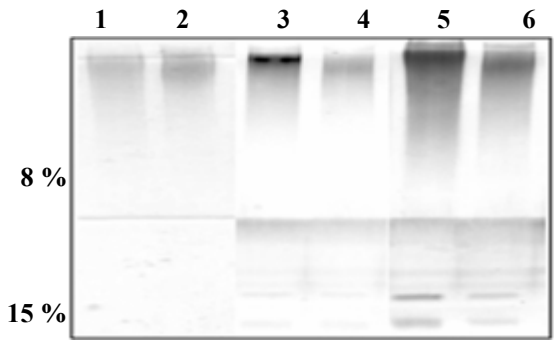


Figure 2: SDS-PAGE (Stacking gel 5%, separating gel 8 and 15 %) of ultracentrifuge supernatants; lines **1** and **2** are stained only with Alcian blue; lines **3**-**6** are silver stained applying Alcian blue as fixative first. **1**: *A.b*. SMAL (1 µg); **2**: *A.b* MG1 (1 µg); **3** and **5**: *A.b* SMAL (1 and 3 µg); **4** and **6**: *A.b* MG1 (1 and 3 µg).

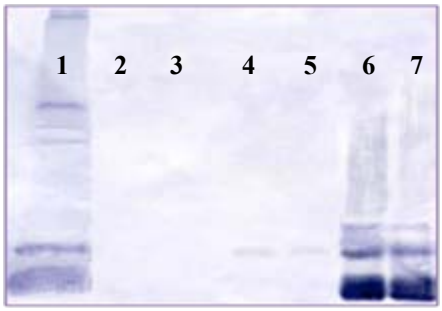


Figure 3: Western blot of ultracentrifuge (UC) supernatants (lines **2**-**5**) and sediments (lines **6**-**7**) from *A. baumannii* SMAL and MG1, detected with mAb A6. **1**: LPS *E. coli* O16 (8 µg); **2** and **4**: UC supernatant of *A. baumannii* SMAL (5 and 25 µg); **3** and **5**: UC supernatant of *A. baumannii* MG1 (5 and 25 µg); **6** and **7**: UC sediments from *A. baumannii* SMAL and MG1, respectively (3 µg each).

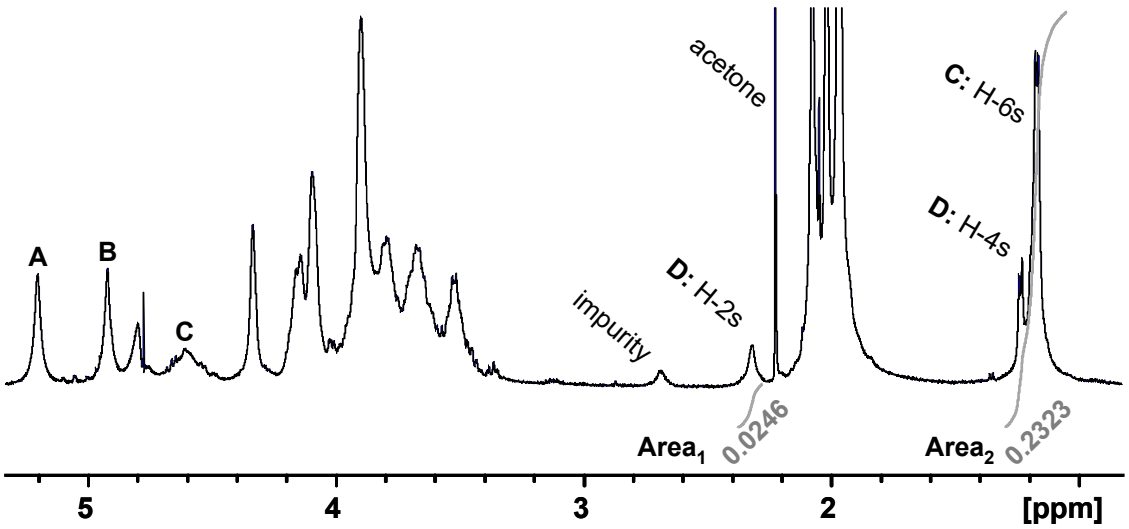
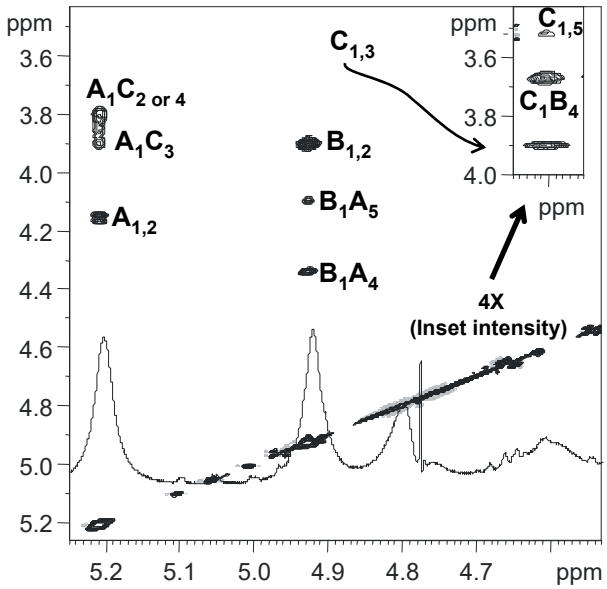


Figure 4: (500 MHz, 298 K) ^{1}H NMR spectrum of the capsular polysaccharide from *A. baumannii* MG1. $Area_1$ represents the integration of the methylene protons from HBA (residue **D**, $Area_1$); $Area_2$ accounts for all the methyl signals and comprehends H-4s from HBA (**D** residue) and H-6s from bacillosamine (**C** residue) regardless the type of *N*-substitution at *C*-4 of this last unit (HBA or acetyl). Comparison of $Area_1$ and $Area_2$ according to formula 1, suggested a substitution degree with HBA at *N*-4 of the bacillosamine residue of 19%.



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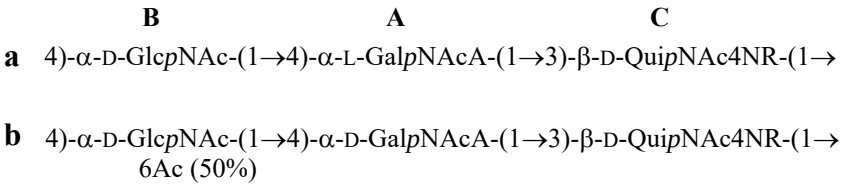

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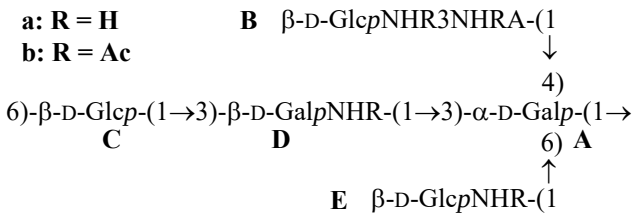

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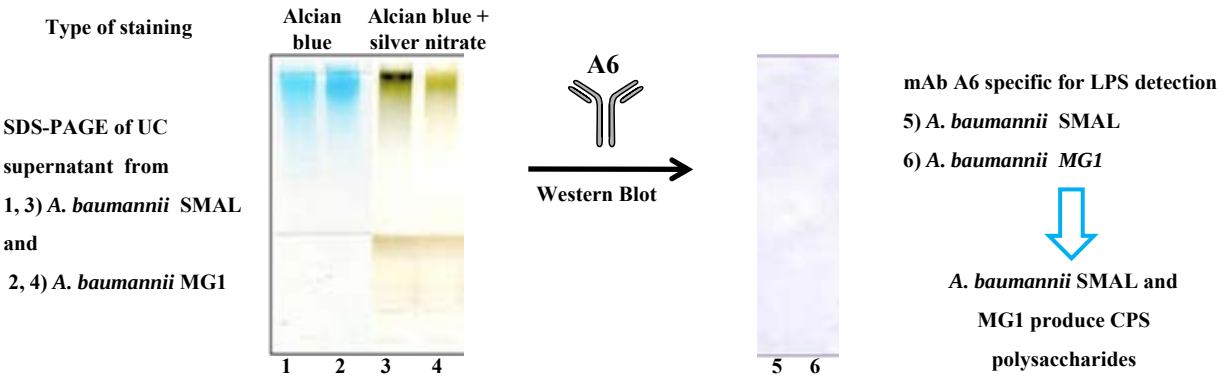

Capsule Polysaccharides from Acinetobacter baumannii strains

MG1 4)-α-D-Glc*p*NAc-(1→4)-α-L-Gal*p*NAcA-(1→3)-β-D-Qui*p*NAc4NR-(1→

SMAL

β-D-Glc*p*NHR3NHRA-(1
↓
4)
6)-β-D-Glc*p*-(1→3)-β-D-Gal*p*NHR-(1→3)-α-D-Gal*p*-(1→
6)
↑
β-D-Glc*p*NHR-(1