Acinetobacter baumannii isolate BAL_212 from Vietnam produces the K57 capsular polysaccharide containing a rarely occurring amino sugar N-acetylviosamine

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Abstract

The structures of capsular polysaccharides (CPSs) produced by different Acinetobacter baumannii strains have proven to be invaluable in confirming the role of specific genes in the synthesis of rare sugars through the correlation of genetic content at the CPS biosynthesis locus with sugars found in corresponding CPS structures. A module of four genes (rmlA, rmlB, vioA and vioB) was identified in the KL57 capsule biosynthesis gene cluster of A. baumannii isolate BAL_212 from Vietnam. These genes were predicted to direct the synthesis of 4-acetamido-4,6-dideoxy-D-glucose (N-acetylviosamine, a-Qui4NAc) and the K57 CPS was found to contain this monosaccharide. The K57 structure was determined and, in addition to a-Qui4NAc, included three N-acetylglactosamine residues in the main chain, with a single glucose side branch. The KL57 gene cluster has not been found in any other A. baumannii genomes, but the rmlA-rmlB-vioA-vioB module is present in the KL119 gene cluster that would likely produce a a-Qui4NAc-containing CPS.

The capsular polysaccharide (CPS) is one of the principal virulence determinants of Acinetobacter baumannii, a bacterial pathogen that poses a significant threat to human health due to the global emergence of extensively and now pan-antibiotic resistant isolates [1]. However, the CPS has been shown to vary extensively in chemical structure between different strains due to differences in the gene content at the chromosomal capsule biosynthesis locus (KL) that directs its synthesis [2–4]. A number of sets of genes predicted to be responsible for the synthesis of specific sugars have been found within one or more forms of the gene cluster at the K locus, and, in several cases, their role in the synthesis of specific sugars has been confirmed by determination of the sugar content of the CPS. These sugars include 6-deoxy-L-talose [4] and 3-acetamido-3,6-dideoxy-D-galactose [5], as well as 5,7-diacetamido-3,5,7,9-tetraacetylglycerol-1-altro- and -d-glycerol-1-altro-non-2-ulosonic acid (di-N-acetylicini- netaminic acid and di-N-acetyl-8-epiacinetaminic acid, respectively), which have only recently been discovered and to date have only been found in A. baumannii [6, 7].

The novel capsule biosynthesis gene cluster has recently been discovered in A. baumannii BAL_212, a single carbapenem-resistant sequence type 52 (Institut Pasteur scheme) isolate recovered in 2010 at the Hospital for Tropical Diseases in Ho Chi Minh City, Vietnam [8]. In this work, the capsule biosynthesis gene cluster was retrieved from the draft BAL_212 genome sequence assembled from the available Illumina paired-end short-read sequences (European Nucleotide Archive project ERP001080, isolate accession number ERR190446), and the products of the genes present were analysed using BLAST [9] before being annotated according to the KL nomenclature system established for A. baumannii [2]. The BAL_212 gene cluster has an arrangement that is typical for the species, with capsule export genes on the left (as drawn in Fig. 1) and genes for the synthesis of simple sugars such as D-glucose on the right [2]. However, after comparison to the known KL gene clusters, the specific gene content and organization was found to be unique and, as gene clusters are numbered in order of identification [2], the novel gene cluster was named KL57.

Received 31 October 2017; Accepted 14 December 2017

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Keywords: Acinetobacter baumannii; capsule; K locus; K57; N-acetylviosamine.

Abbreviations: COSY, correlation spectroscopy; CPS, capsular polysaccharide; D-Qui4NAc, 4-acetamido-4,6-dideoxy-D-glucose (N-acetylviosamine); Gtr, glycosyltransferase; HMBC, heteronuclear multiple-bond correlation; HSGC, heteronuclear single-quantum coherence; ltr, initiating transferase; KL, K locus; NDE, nuclear Overhauser effect; ROESY, rotating-frame nuclear Overhauser effect spectroscopy; TOCSY, total correlation spectroscopy.

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The GenBank/EMBL/DDBJ/PDB accession number for the KL57 sequence is KY434631.1. One supplementary table is available with the online version of this article.
The sequence can be found in GenBank (accession number KY434631.1).

In the central variable segment, KL57 includes a group of genes, including rmlB, rmlA, and two open reading frames, which have no homologues previously described in A. baumannii. The first, adjacent to rmlA, predicts a protein (GenPept accession number ARR95883.1) that is 53% identical to the VioA transaminase from Escherichia coli O7 (GenPept accession number AAD44154.1), whereas the second (GenPept accession number ARR95884.1) is 27% identical (68% coverage) to the VioB acetyltransferase from the same bacterium (GenPept accession number AAD44155.1). 4-Acetamido-4,6-dideoxy-D-glucose (D-Quip4NAc, N-acetyl-viosamine) was found in the E. coli O7 polysaccharide [10], and RmlB, RmlA, VioA and VioB are all required for its synthesis [11] (Fig. 1). Hence, despite the low similarity for the VioB gene products, it seemed possible that D-Quip4NAc, a rarely occurring monosaccharide in nature, is present in the K57 CPS structure.

To determine the role of VioA and VioB in A. baumannii, the structure of the K57 CPS was determined essentially as described in [5]. Briefly, the 1H NMR and 13C NMR (Fig. 2) spectra of the CPS were assigned using two-dimensional 1H,1H (COSY, TOCSY) and 1H,13C HSQC experiments (Table S1, available with the online version of this article). Based on 3JH,1H coupling constants and characteristic 13C NMR chemical shifts, three residues of α-GalpNAC (units A-C) and one residue each of β-Quip4NAc (unit D) and α-Glcp (unit E) were identified.

Linkage and sequence analysis was performed by one-dimensional 1H,1H NOE and two-dimensional 1H,1H ROESY experiments, which showed the following correlations between anomic protons and protons at the linkage carbons: A H1/D H3, B H1/A H3, C H1/B H4 and D H1/C H3 at δ 5.08/3.72, 5.14/3.98, 4.94/4.08 and 4.58/4.12, respectively. There was no NOE correlation for H1 of the side branch 6-linked Glcp (E), and the site of its attachment was determined by a cross-peak between H1 of Glcp and C6 of 3-substituted GalpNAC (C) at δ 4.91/67.3 observed in the 1H,13C HMBC spectrum. The glycosylation pattern was confirmed by characteristic downfield displacements of the 13C NMR signals of the linkage carbons (Table S1). Based on these data, it was concluded that the K57 CPS has the structure shown in Fig. 3. It must be noted that serology for CPS is not available for Acinetobacter species, and the CPS and CPS repeat units are named after the corresponding gene cluster.

The KL57 gene cluster encodes an ItrA2 initiating transferase that is 96% identical to ItrA2 from KL30 in A. baumannii NIPH 190 (GenPept accession number ENV24273.1), and ItrA2 has previously been shown to be responsible for the linkage of D-GalpNAC to an undecaprenol phosphate lipid carrier to initiate K unit synthesis [12], making a D-GalpNAC residue the first sugar of the K57 unit.

Fig. 1. Genetic arrangement of the A. baumannii KL57 and KL119 capsule biosynthesis gene clusters. The figure is drawn to scale from GenBank accession numbers KY434631.1 and NGGP01000084.1 (coordinates 55,684 to 28,000), respectively. The arrows are open reading frames showing the direction of transcription, and the colours indicate the functional roles of the encoded proteins using the scheme shown below. Horizontal bars above denote the functional roles of proteins encoded by genes in modules. Dark grey shading between gene clusters indicates a sequence that is >90% identical, whereas light grey indicates a sequence that is 70–90% identical. The predicted synthesis pathway of dTDP-D-Quip4NAc in A. baumannii is also shown below. The pathway was originally predicted for E. coli O7 [10] and later confirmed biochemically [11]. Enzymes are shown in bold.
Immediately upstream of *itrA2* in KL57, there is a gene encoding a glycosyltransferase that is 85% identical to Gtr50 from KL30 in *A. baumannii* NIPH 190 (GenPept accession number ENV24274.1), which has been predicted to link another D-GalpNAc residue to the initial D-GalpNAc via an α-(1→3) linkage [12]. This feature is present in the K57 structure; therefore, α-D-GalpNAc-(1→3)-D-GalpNAc is drawn as the first linkage of the K57 unit (Fig. 3). Gtr117 (GenPept accession number ARR95889.1) is 57% identical to Gtr93 from *A. baumannii* KL45 (GenPept accession number ENW33213.1), which is predicted to form α-D-GalpNAc-(1→4)-D-GalpNAc [13]. Two further glycosyltransferase genes (*gtr115* and *gtr116*) are present in the K57 gene cluster. Gtr116 (GenPept accession number ARR95887.1) shares 52% identity with Gtr97 from *A. baumannii* KL48 (GenPept accession number ENW33213.1), which is predicted to form α-D-Glcp-(1→6)-D-GlcNAc [13], while Gtr116 was assigned to this linkage in K57 (Fig. 3). Gtr115 (GenPept accession number ARR95886.1) was assigned to the remaining β-D-Quip4NAc-(1→2)-D-GalpNAc linkage, and Wzy would therefore form the α-D-GalpNAc-(1→3)-D-Quip4NAc linkage between K57 units.

The *A. baumannii* K57 capsule structure is identical to that of a polysaccharide produced by *A. baumannii* strain 9 that was reported earlier [14]. Although the latter was described as an O-antigen polysaccharide, more recent studies have indicated that *A. baumannii* does not produce an O-antigen [2, 15, 16], and the major surface polysaccharide is CPS. A sequence for the strain 9 polysaccharide biosynthesis gene cluster is not available, and sequencing is not possible as the strain is no longer available.

To date, KL57 has only been reported in *A. baumannii* isolate BAL_212, and no strain carrying a homologous gene cluster was found in the NCBI non-redundant and Whole Genome Shotgun databases. However, the *rmlB*, *rmlA*, *vioA* and *vioB* genes were found in the genome of *A. baumannii* isolate ARLG1794 (Whole Genome Shotgun accession number NGGP01000084.1, coordinates 55 684 to 28 000) in a KL gene cluster, which we designated KL119. It differs from KL57 in the sequence for three glycosyltransferases and the Wzy polymerase required to link K units together. Therefore, it is predicted that the K119 CPS also includes D-Qui4NAc, although some of the linkages between the sugars in the K119 unit and the linkage of K119 units to each other may be different from those in the K57 CPS.

Although several CPS types appear to be prevalent in *A. baumannii* on a global scale, there have been sporadic...
The authors declare that there are no conflicts of interest.

Conflicts of interest

Acknowledgements

We thank Steven Baker from The Hospital for Tropical Diseases, Wellcome Trust Major Overseas Programme, Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam for providing isolate BAL_212.

Funding information

This work was supported by the Russian Foundation for Basic Research (project no. 17-04-01254) (Y. A. K.) and NHMRC (project no. 1026189) (R. M. H.).

Acknowledgements

We thank Steven Baker from The Hospital for Tropical Diseases, Wellcome Trust Major Overseas Programme, Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam for providing A. baumannii isolate BAL_212.

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Edited by: D. W. Hood and M. Whiteley

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