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Biological Properties and Structure of the Lipopolysaccharide of a Vaccine Strain of *Francisella tularensis* Generated by Inactivation of a Quorum Sensing System Gene *qseC*

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Abstract—A knockout mutant with a deletion in a quorum sensing system gene *qseC* was generated from the vaccine strain *Francisella tularensis* 15 by site-directed mutagenesis. The variant with the inactivated gene *qseC* differed from the parental strain in growth rate on solid nutrient medium but had the same growth dynamics in liquid nutrient medium. The mutation abolished almost completely the resistance of the vaccine strain to normal rabbit serum and its ability to survive in macrophages; in addition, the strain lost the residual virulence. A significant phenotypic alteration was observed in the lipopolysaccharide of *F. tularensis*. Particularly, the mutant strain synthesized no noticeable amount of the lipopolysaccharide with the high-molecular-mass O-polysaccharide, presumably as a result of impairing biosynthesis of the repeating unit, namely, a loss of the ability to incorporate a formyl group, an N-acyl substituent of 4-amino-4,6-dideoxy-D-glucose.

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Regulation of biosynthesis of proteins responsible for a variety of phenotype features of bacterial culture, particularly, structures of cell surface biopolymers including lipopolysaccharide (LPS), is one of the major mechanisms of adaptation of bacterial cells to environmental conditions. Many bacteria achieve this control through the quorum sensing (QS) system, sometimes referred to as autoinduction or cooperative sensitivity, a mechanism that allows determination of the population density of bacterial cells [1]. Various QS systems are widespread among Gram-negative and Gram-positive bacteria and known to play an important role in pathogenicity control [2]. Particularly, they are found in such emergency pathogens as the causative agents of Asian cholera *Vibrio cholerae* [3], melioidosis *Burkholderia pseudomallei* [4], glanders *Burkholderia mallei* [5], and plague *Yersinia pestis* [6]. Autoinducers have been demonstrated also in the causes of brucellosis *Brucella melitensis* [7] and anthrax *Bacillus anthracis* [8].

Despite that the tularemia microbe *Francisella tularensis* is one of the most virulent Gram-negative bac-

Abbreviations: CFU, colony-forming unit; dHexN, deoxyhexosamine; ESI FT ICR, electrospray ionization Fourier-transform ion-cyclotron resonance; GalNAcAN, 2-acetamido-2-deoxygalacturonamide; Hex, hexose; HexN, hexosamine; HexNAN, hexosaminuronamide; Kdo, 3-deoxy-D-*manno*-oct-2-ulosonic acid; LPS, lipopolysaccharide; M_r, molecular weight; QS, quorum sensing; QuiNAc, 2-acetamido-2,6-dideoxyglucose; Qui4N, 4-amino-4,6-dideoxyglucose; Qui4NFo, 4-formamido-4,6-dideoxyglucose; 3HO18:0, 3-hydroxyoctadecanoic acid; 16:0, hexadecanoic (palmitic) acid.

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teria and belongs to class A emergency pathogens – potential agents of bioterrorism – little is known about its QS system [9]. In this work, a *F. tularensis* vaccine strain knockout mutant with a deletion in a QS system gene *qseC* was constructed and studied in respect to its biological activities and structure of the LPS, the major component of the *F. tularensis* cell wall outer membrane, which plays an important role in the resistance of bacteria to the host immune system.

MATERIALS AND METHODS

Bacterial strains, plasmids, and primers. The bacterial strains and plasmids are listed in Table 1. Primers used for site-directed mutagenesis are listed in Table 2.

Bacterial growth. Strains of *E. coli* were grown at 37°C on Luria–Bertani agar and Luria–Bertani broth [10], when necessary supplemented with antibiotics: 100 μg/ml ampicillin or 20 μg/ml chloramphenicol.

Strains of *F. tularensis* were grown at 37°C on solid (FTA) and liquid (FTB) nutrient media. Composition of FTA: 3.8% erythritol-agar, 1% dried bovine blood, 1% glucose, 0.05% cysteine, 0.0025% thiamine chloride, pH 7.2. Composition of FTB: 2% casein enzymatic hydrolysate, 1% yeast extract, 1.2% KH_2PO_4, pH 7.2, 1% glucose, 0.001% cysteine, 0.001% $FeCl_2$. If necessary, the medium was supplemented with 100 μg/ml polymyxin B or 3 μg/ml chloramphenicol.

General DNA methods. All general DNA methods including extraction of DNA from *E. coli*, digestion with restriction endonucleases, ligation, electrophoresis in agarose gel, and transformation of plasmids in *E. coli* were performed using routine procedures [11]. Extraction of DNA from *F. tularensis* was performed as described previously [12].

Construction of the plasmid for *qseC* gene mutagenesis. Genome regions of 1513 bp 5′ (left flank) and 1546 bp 3′ (right flank) of the *qseC* gene including the first 102 and the last 130 nucleotides, respectively (Table 2), were PCR-amplified using the total DNA from *F. tularensis* 15 as a template. Primers for the left flank (qseCL15 and qseCR0) contained the restriction sites *Sal*I and *Bgl*II, respectively. Primers for the right flank (qseCL0 and qseCR15) contained the restriction sites *Bgl*II and *Xba*I, respectively.

The obtained fragments were digested with restrictases *Sal*I and *Bgl*II (left flank of *qseC*), *Xba*I and *Bgl*II (right flank of *qseC*), combined, mixed with the pPV plasmid DNA restricted at the *Xba*I and *Sal*I sites, and ligated. The ligate was transformed into *E. coli* DH5α cells. The transformants were selected by the Ap^RCm^R phenotype. The resulting clones were checked by PCR for the presence of the *qseC* deletion variant in the vector plasmid pPV. The plasmid DNA was extracted from the positive clones and transformed into *E. coli* S17-1. The transformants were selected by the Ap^RCm^R phenotype. Strain *E. coli* S17-1(ΔqseC) thus obtained contained the pPV plasmid with a 1185-bp deletion in the *qseC* gene flanked with the homologous sequences of ~1400 bp on each side. It was used for the mobilization transfer of the recombinant plasmid into *F. tularensis* 15 for the allelic replacement of the intact *qseC* gene with the mutant variant.

Construction of *F. tularensis* ΔqseC-mutant. The bacterial crossing was performed as follows: $1 \cdot 10^8$ cells of the donor strain *E. coli* S17-1(pPVΔqseC) were mixed with $3 \cdot 10^{10}$ cells of the recipient strain *F. tularensis* 15, inoculated on Luria–Bertani agar as a spot, and incubated at 25°C for 18 h. The biomass was washed away with buffered saline, inoculated from serial dilutions on FTA supplemented with 100 μg/ml polymyxin B for the counter-selection of the donor *E. coli* strain and 3 μg/ml chloramphenicol, and incubated at 37°C. The first transformants appeared after 96-120 h. The resultant clones were resuspended in buffered saline, inoculated on FTA supplemented with 100 μg/ml polymyxin B and 5% sucrose,

Table 1. Bacterial strains and plasmids

Strain or plasmid	Description	Source or reference
E. coli DH5α	*F⁻(φ80dlacZΔM15) recA1 endA1 gyrA96 thi-1 hsdR17(r_k^- m_k^+) supE44 relA1 deoR Δ(lacZYA-argF) U169*	[26]
E. coli S17-1	*thi thr leu tonA lacY supE recA*::RP4-2Tc::Mu, Kn::Tn7	[27]
F. tularensis 15	Pm^r, vaccine strain	collection of SRCAMB
E. coli S17-1/pPVΔqseC		this work
pPV	Amp^R, Cm^R, *sac B*, mob	[23]
pPVΔqseC	Amp^R, Cm^R, *sac B*, mob, 3.05-bp region of *F. tularensis* 15 genome with a deletion in the *qseC* gene	this work

Table 2. Oligonucleotide primers used for amplification of 5′- and 3′-flanking regions of the *qseC* gene

Amplified gene	Localization*, bp	Primer size, bp	Product size, bp	Primer	Sequence (from 5′ to 3′) and restriction site**
			Left flank		
qseC	1694424-1694452	29	1513	FtqseCL15	TAAGTCGACACTAAATGTGTATATTCAGG SalI
qseC	1695932-1695900	33		FtqseCR0	CCGAGATCTAACTAACTAAAAATTGATATAACC BglI
			Right flank		
qseC	1697110-1697135	26	1546	FtqseCL0	GTAAGATCTAGGTTTAGCAATCGTCG BglI
qseC	1698655-1698627	29		FtqseCR15	TTATCTAGACAATGAGCCAAGTACAGGAC XbaI

* Referred to the *F. tularensis* ssp. *holarctica* genome (Gen Bank NCBI, NC_007880).
** Recognition sites for restriction endonucleases are underlined and restriction endonucleases are indicated.

and incubated at 37°C. The isolated clones having the Cm^S phenotype were checked by PCR for selection of the *F. tularensis* 15(ΔqseC) mutant variants with the corresponding deletion in the *qseC* gene.

Resistance to normal rabbit serum. A 5-ml blood sample from the periphery vein of healthy non-immunized rabbits was drawn into a tube, taking care to avoid foam formation. The collected blood was incubated at 37°C for 1 h, and serum was gathered by pipette, centrifuged using a microfuge, and used for experiments. The remaining material was divided into aliquots and kept at −18°C.

A suspension of cells in buffered saline (pH 7.2) was prepared from the fresh agar culture by the turbidity standard. The concentration of cells was controlled by plating from serial dilutions on FTA supplemented with 100 μg/ml polymyxin B. The bacterial cells were introduced into undiluted serum to concentration of $2 \cdot 10^6$ CFU/ml (0.1 of the final volume of the cell suspension) and incubated at 37°C for 24 h. The concentration of the surviving cells was determined by plating from corresponding dilutions on FTA supplemented with 100 μg/ml polymyxin B.

Phagocytosis. The ability of *F. tularensis* strains to invade, and to survive in, macrophage cells was estimated in murine macrophage-like line J774.A cells. Infection of cells was determined by plating on agar and microscopy. The macrophages were cultivated at 37°C at concentration $(3\text{-}9) \cdot 10^5$ cells/ml concentration on Dulbecco's modified Eagle medium, containing 2 mM glutamine and 10% fetal calf serum in a CO_2-incubator in 5% CO_2. The cells were washed away by a trypsin and EDTA solution, centrifuged, and applied to the same cultural medium. The number of cells was counted in a Goryaev chamber, a suspension of $4 \cdot 10^5$ cells/ml was applied to a 24-well plate (0.5 ml per well) and incubated for 24 h at 37°C in 5% CO_2. The mutant and the parental *F. tularensis* strains were grown on FTA. The bacterial suspension in buffered saline (pH 7.2) was applied to a 24-well plate with a monolayer of J774.A cells in a 100 : 1 ratio (zero point) and the resulting mixture was kept at 37°C in 5% CO_2 for 2 h. The macrophages were washed with a phosphate buffer to remove non-phagocytized bacteria, and the plates were incubated in cultural medium containing 2 μg/ml gentamicin for 1 h. The medium was removed, the macrophages were lysed by the addition of 0.5 ml 0.05% sodium deoxycholate, and the samples were inoculated on plates with FTA (3 h after zero point). The same procedure was repeated after 21 h (24 h after zero point). The surviving cells count was performed after 72 h of incubation at 37°C.

Virulence. The virulence of *F. tularensis* strains was determined in an outbreed white mouse model. The mice were infected in a vivarium organized in accordance with the respective GAC (Good Animal Care) regulations and P03-20 protocol of the Bioethics Committee of the State Research Center for Applied Microbiology and Biotechnology. Five-to-eight-week-old mice of both genders (18-20 g, five animals in each group) were infected subcutaneously with 0.2 ml tenfold dilution of bacterial cells ($5 \cdot 10^1$ to $5 \cdot 10^2$ CFU per animal for the parental strain *F. tularensis* 15 and $5 \cdot 10^1$ to $1 \cdot 10^5$ CFU per animal for the mutant strain *F. tularensis* 15(ΔqseC)). Dead mice were autopsied and subjected to bacteriological studies. The surviving animals were monitored for 28 days.

After six days from the start of the experiment, some animals infected with 50 microbe cells were sacrificed, and their spleen was homogenized in 5 ml buffered saline. The resulting suspensions were titrated and inoculated

from serial dilutions on FTA supplemented with 100 μg/ml polymyxin B.

Isolation of lipopolysaccharides (LPS). LPS preparations were extracted from the parental and mutant strains by the phenol–water procedure [13]. The yields of the LPS were 0.8 and 3.1%, respectively. The LPS from the mutant strain was obtained also by extraction with a phenol–chloroform–light petroleum ether mixture as described by Galanos [14], which is usually employed for isolation of the R-form LPS. The LPS preparations obtained by dialysis against distilled water and lyophilization were studied without further purification.

PAGE and Western blotting. Electrophoresis of the LPS preparations was performed in 15% SDS-polyacrylamide gel as described [15, 16]. For LPS visualization, the gels were stained with a silver oxide ammonia solution after oxidation with periodic acid as described [17].

The isolated LPS preparations were transferred electrophoretically (250 mA, 40 min) from the gel onto a Hybond-C extra nitrocellulose membrane [18]. After blocking in 1% BSA in Tris-buffer (20 mM Tris-HCl, 500 mM NaCl, pH 7.5), the membrane was incubated for 1 h in a solution of murine monoclonal antibodies 11B6 against the LPS of *F. tularensis* 15 (primary antibodies). For visualization, the membrane was incubated for 1 h in a 1 : 5000 solution of secondary anti-mouse IgG antibodies conjugated with horseradish peroxidase (Sigma, USA) and stained with 3,3′-diaminobenzidine tetrachloride (Sigma) (5 mg of the reagent in 10 ml Tris-buffer, 50 μl 8% $NiCl_2$, 10 μl 33% H_2O_2).

Mild acid hydrolysis of the lipopolysaccharide. The LPS sample recovered from the aqueous layer after the phenol–water extraction of bacterial cells was subjected to mild acid hydrolysis (1% AcOH, 100°C, 1 h). The insoluble lipid A precipitate was removed by centrifugation (13,000*g*, 20 min), and the water-soluble supernatant was fractionated by gel-permeation chromatography on Sephadex G-50 Superfine (Amersham Biosciences, Sweden) in 0.05 M pyridinium acetate buffer (pH 4.5) using a differential refractometer (Knauer, Germany) for monitoring.

Mass spectrometry. ESI FT ICR mass spectra of negatively charged ions were recorded on a hybrid Apex Qe instrument (Bruker Daltonics, USA) equipped with an actively shielded 7 Tesla magnet and Apollo II Dual ESI/MALDI ion source using instrumental parameters as recommended by the manufacturer. Samples (~10 ng/ml) were dissolved in a 2-propanol–water–triethylamine mixture (50 : 50 : 0.001 v/v, pH ~ 8.5) and sprayed at a flow rate 2 μl/min. The Q-voltage was set to 5 V, and the drying gas temperature was set to 200°C. The mass scale for broadband spectra was externally calibrated using a compound with known structure. The spectra were charge-deconvoluted by DataAnalysis software version 3.4 (Bruker Daltonics), and the mass numbers given refer to monoisotopic molecular peaks.

RESULTS AND DISCUSSION

Generation of the *F. tularensis qseC* mutant. Analysis of nucleotide sequence in the genome of the holarctic race vaccine strain *F. tularensis* subsp. *holarctica* LVS enabled *in silico* identification of the *FTL1762* gene, which encodes a protein homologous to QseC in *E. coli*. The histidine-kinase QseC is a sensor protein that is autophosphorylated in response to QS system signals and then transfers the phosphate group to a regulatory protein QseB [19]. Both components of the QseBC QS system regulate the formation of flagella, motility, and colonization ability in such pathogens as enterohemorrhagic *Escherichia coli* (EHEC) 0157:H7 [20, 21] and *Salmonella enterica* serovar *typhimurium* [22]. A 1428-bp structural domain of the *FTL1762* (*qseC*) gene in *F. tularensis* LVS encodes a protein with the predicted molecular mass ~56 kDa. *Francisella tularensis* LVS was derived from the vaccine strain *F. tularensis* 15, and this allows extrapolation of the data of LVS to *F. tularensis* 15.

To study the effect of the *qseC* gene expression on biological properties and the structure of the LPS of the vaccine strain *F. tularensis* 15 (a derivative of the virulent wild strain *F. tularensis* ssp. *holarctica* 503), a knockout mutant *F. tularensis* 15(ΔqseC) with a deletion of 1185 bp in the *qseC* structural domain was generated. For this purpose, a pPVΔqseC plasmid carrying 1400-bp fragments of the *F. tularensis* chromosomal DNA that flank the mutant *qseC* gene was constructed based on the suicide vector pPV [23]. The deletion mutation was transferred by the allelic exchange from the plasmid into the *F. tularensis* 15 chromosome.

Biological properties of *F. tularensis* 15(ΔqseC). Colonies of the mutant and parental strains differed in the growth rate on a solid nutrient medium at 37°C: the colonies of *F. tularensis* 15(ΔqseC) appeared first after incubation for 36-48 h, whereas the colonies of *F. tularensis* 15 appeared only after 60-72 h. However, on a further incubation the colonies of the parental strain became larger than the mutant colonies. Marked differences were observed also in the appearance of the colonies: those of *F. tularensis* 15(ΔqseC) were distinguished by a deeper gray color, a more convex cone-like shape, and a denser consistence. Light microscopy revealed no difference in the morphology of the cells. The strains showed also the same growth dynamics in a liquid nutrient medium with the cell number doubling time of ~2 h.

The mutation in the *qseC* gene affected the serum resistance of the strain. Whereas the parental strain *F. tularensis* 15 survived in normal rabbit serum, the mutant strain *F. tularensis* 15(ΔqseC) completely lost the resistance. This difference indicated a cell-wall deficiency in the mutant strain.

Francisella tularensis 15(ΔqseC) was almost by one order of magnitude more efficiently associated with and

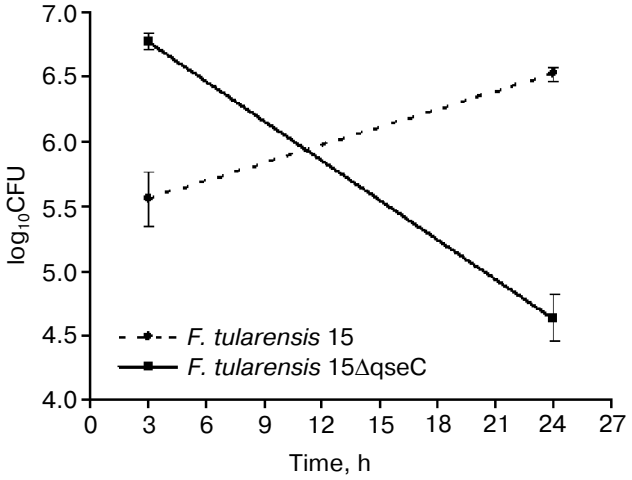

Fig. 1. Growth of *F. tularensis* in J774.1A cells. The data from a representative experiment are shown, and the results are expressed as the $\log_{10}$[CFU ± S.D. (ml^{-1})] of *F. tularensis* based on triplicate wells.

absorbed by macrophage-like cells of the J774.1A line than the parental strain *F. tularensis* 15. However, the mutant strain was unable to multiply in macrophages: within 24 h the number of the surviving mutant cells reduced 100 times, whereas the number of the surviving parental cells increased tenfold (Fig. 1).

LD_{50} of the *F. tularensis* 15 for white outbreed mice challenged subcutaneously was $1 \cdot 10^2$ CFU, whereas even $1 \cdot 10^5$ CFU of *F. tularensis* 15(ΔqseC) did not cause animals' death during the entire observation period (28 days). In mice infected with the parental strain, the degree of spleen colonization was ~$1 \cdot 10^5$ tularemia microbe cells per organ, while no surviving bacteria were found in the spleen of mice infected with the mutant strain.

Therefore, *F. tularensis* 15(ΔqseC) is almost avirulent for mice.

Structure of *F. tularensis* 15(ΔqseC) lipopolysaccharide. Like the LPS of most other Gram-negative bacteria, the LPS of *F. tularensis* consists of an O-polysaccharide (O-antigen), which is attached to lipid A via a core oligosaccharide (S-form LPS) [24, 25]. SDS-PAGE and Western blotting with monoclonal antibodies specific to the *F. tularensis* LPS revealed marked differences between the LPS phenotypes of the parental *F. tularensis* 15 and mutant *F. tularensis* 15(ΔqseC) strains (Fig. 2). Particularly, whether isolated by the Westphal [13] or Galanos [14] procedure, the ladder-like band pattern of the high-molecular-mass LPS that is typical of the parental strain was observed for the mutant strain only faintly and in a much narrower range of molecular mass distribution.

The structures of the O-polysaccharide and the core oligosaccharide of the *F. tularensis* 15 LPS were established earlier [24, 25] (Fig. 3, structures *1* and *2*, respectively).

The LPS was isolated from *F. tularensis* 15(ΔqseC) by the phenol-water extraction according to the Westphal procedure [13] and cleaved by mild acid hydrolysis to give two oligosaccharide fractions (OS1 and OS2), which were separated by Sephadex G-50 gel-permeation chromatography. No significant amount of a high-molecular-mass polysaccharide was detected.

The oligosaccharides were studied by high-resolution electrospray ionization Fourier-transform ion-cyclotron resonance (ESI FT ICR) mass spectrometry. The mass spectrum of the OS1 (Fig. 4a) showed the major peak for a compound with the molecular mass 1853.67 Da, which corresponds to a $Hex_4HexN_1Kdo_1Ac_1$ core hexasaccharide bearing one $dHexN_2HexNAN_2Ac_3$ O-polysaccharide repeating unit, where HexNAN is hexosaminuronamide (calculated molecular mass 1853.67 Da; Fig. 3, structure *3*). This oligosaccharide was evidently derived from the SR-form LPS of the mutant strain, in which the core oligosaccharide is substituted with one O-polysaccharide repeating unit rather than a high-molecular-mass O-polysaccharide. A distinctive feature of the OS1 is the lack of the formyl group, which is attached to Qui4N (4-amino-4,6-dideoxyglucose) in the O-polysaccharide of the parental *F. tularensis* 15 strain (Fig. 3, structure *1*). The spectrum contained also minor peaks for a sodium-

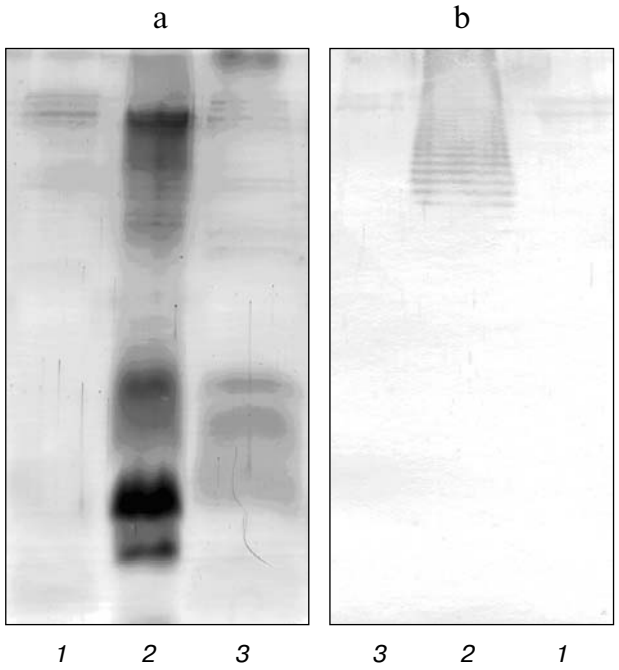

Fig. 2. SDS-PAGE in 15% gel (a) and Western blotting with monoclonal antibody 11B6 (b). *1*) LPS of *F. tularensis* 15(ΔqseC) isolated by the Galanos procedure; *2*) LPS of *F. tularensis* 15 isolated by the Westphal procedure; *3*) LPS of *F. tularensis* 15(ΔqseC) isolated by the Westphal procedure.

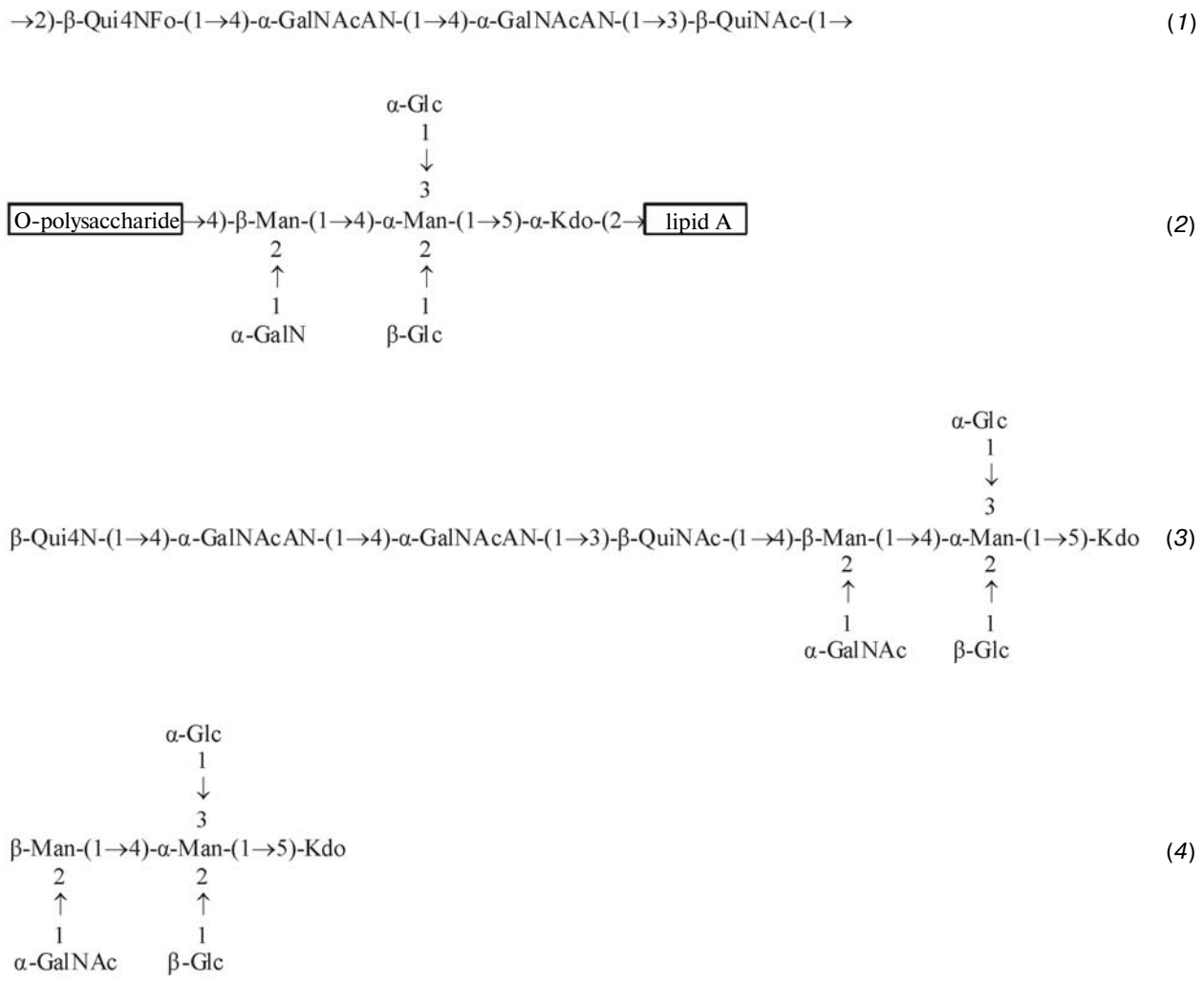

Fig. 3. Structures of the O-polysaccharide repeating unit of *F. tularensis* 15 [25] and ATCC 29684 [24] (*1*) and the core oligosaccharide of *F. tularensis* ATCC 29684 [25] (*2*) determined earlier, and structures of the major oligosaccharides in the OS1 (*3*) and OS2 (*4*) obtained after mild acid hydrolysis of the SR- and R-forms of the *F. tularensis* 15(ΔqseC) LPS.

adduct ion (ΔM 22 Da) and an oligosaccharide with Kdo residue in an anhydro form (ΔM 18 Da). In addition, there were peaks for incomplete oligosaccharides, which lack one of the hexose residues (M_r 1691.60 Da), non-acylated Qui4N residue (M_r 1708.59 Da), or both Qui4N and HexNAcAN residues (M_r 1474.51 Da).

The mass spectrum of the OS2 (Fig. 4b) showed a peak for the full $Hex_4HexN_1Kdo_1Ac_1$ core hexasaccharide (experimental and calculated molecular mass 1089.36 Da; Fig. 3, structure *4*). It was derived from the R-form LPS of the mutant strain, which contains no O-polysaccharide repeat. The most abundant peak in the spectrum belonged to a $Hex_3HexN_1Kdo_1Ac_1$ incomplete core, which lacks a hexose residue (M_r 927.31 Da). The spectrum contained also peaks for the core substituted with either complete or incomplete O-polysaccharide repeating unit (see above for the OS1).

The absence from the OS1 of any oligosaccharides containing a formyl group suggests that the SR-form LPS of the *F. tularensis* 15(ΔqseC) mutant strain is devoid of this component. In the LPS of the parental strain, the formyl group is the N-acyl substituent on the Qui4N residue (Fig. 3, structure *1*). A possibility that the formyl group could be cleaved under mild ionization conditions (ionization energy 5 V) used to run the mass spectra was ruled out by measuring the spectra of the O-polysaccharide of the parental strain under harsher conditions (ionization energy 20 V), which induced a cleavage of the glycosidic linkages in the polymer. The spectrum contained peaks for oligomers of the complete repeating unit bearing the formyl group, particularly, for a $dHexN_4HexNA_4Ac_6Fo_2$ dimer (experimental and calculated molecular mass 1584.60 and 1584.61 Da, respectively).

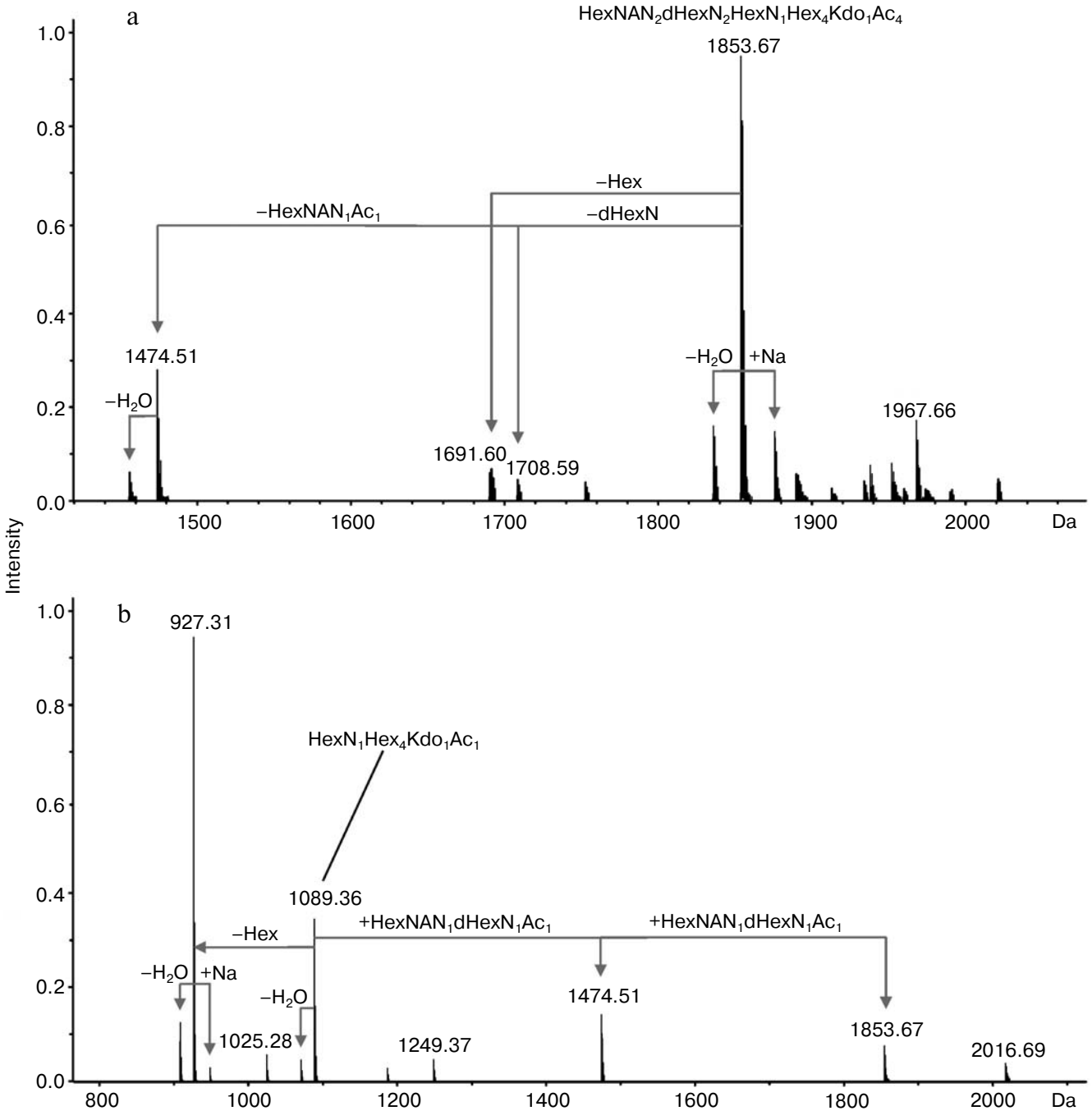

Fig. 4. ESI FT ICR mass spectra of OS1 (a) and OS2 (b). Spectra were charge-deconvoluted and the numbers refer to monoisotopic molecular peaks.

The presence in the spectrum of the OS1 (Fig. 4a) of a peak for an oligosaccharide with the molecular mass 1708.59 Da, corresponding to a Qui4N-lacking repeating unit, and the absence of any peak for an oligosaccharide that lacks HexNAcAN only showed that Qui4N is the terminal monosaccharide residue in the full SR-form LPS. This conclusion is in agreement with the structure of the biological repeating unit of the *F. tularensis* O-polysaccharide [25]. It could be suggested that the absence of the N-acyl substituent, i.e. the formyl group, on the terminal Qui4N residue prevents further elongation of the O-polysaccharide chain, which results in the inability of the mutant strain *F. tularensis* 15(ΔqseC) to produce a significant amount of the S-form LPS with a high-molecular-mass O-polysaccharide.

A comparison of the mass spectra of lipid A samples obtained by mild acid hydrolysis of the *F. tularensis* 15 and *F. tularensis* 15(ΔqseC) LPS revealed no difference

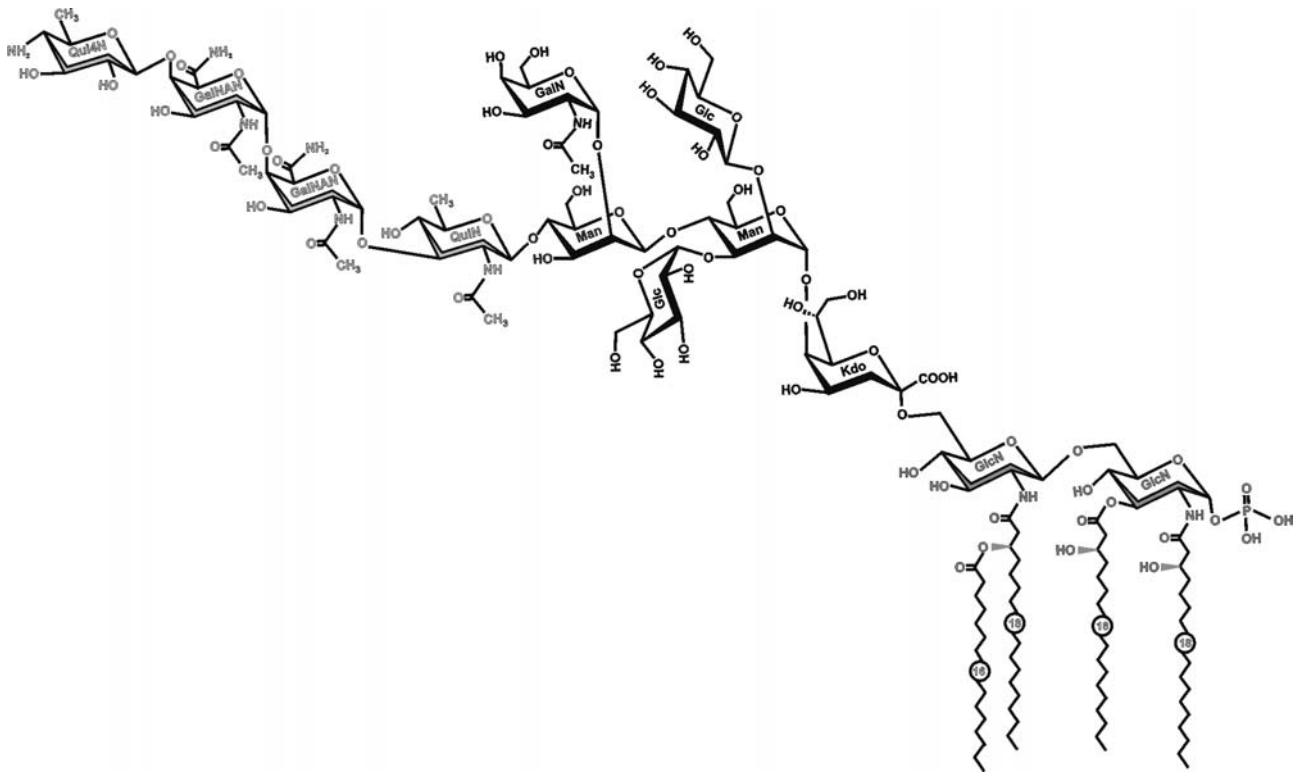

Fig. 5. Structure of the *F. tularensis* 15(ΔqseC) LPS. The figure shows the full carbohydrate chain of the SR-form LPS and the most abundant lipid A variant. The O-polysaccharide repeating unit (left) and lipid A (right) are shown in gray.

(spectra not shown). In both cases, the major peak in the spectrum belonged to a $HexN_2P_13HO18{:}0_316{:}0_1$ monophosphorylated tetraacylated lipid A variant (experimental and calculated molecular masses 1505.10 Da). Minor peaks in the spectra corresponded to a dephosphorylated lipid A (ΔM 80 Da) and/or to compounds with lower fatty acids (14:0 in place of 16:0 or 3HO16:0 in place of 3HO18:0; ΔM 28 Da). These data, together with the published data on the wild-type *F. tularensis* LPS structure [24, 25] enabled determination of the full LPS structure in the mutant strain *F. tularensis* 15(ΔqseC) (Fig. 5).

Therefore, the inactivation of *qseC* gene (or genes that might have been affected during the allelic exchange) had a dramatic effect on the phenotype of the *F. tularensis* LPS, which is an important pathogenicity factor of the tularemia microbe. The bacteria can no longer synthesize the LPS with a high-molecular-mass O-polysaccharide. Presumably, this is a result of impairing biosynthesis of the repeating unit, namely, a loss of the ability to incorporate the N-formyl group into the repeating unit, whose transfer seems to be controlled by the *qseC* gene.

The data show that the *qseC* gene plays an important role in the pathogenesis of tularemia. As a result of a deletion in the *qseC* gene, the *F. tularensis* 15 strain became avirulent for mice and lost the resistance to the bactericidal action of the normal serum. The strain demonstrated an increased capacity to associate with macrophages but a decreased ability to survive in macrophages.

Further studies are necessary to understand which changes in the biological properties of the mutant strain were directly related to its inability to produce the typical *F. tularensis* S-form LPS and which resulted from an altered expression of other genes under the control of the *qseC* gene and the QS system.

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