

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# Flagellar Glycosylation: Current Advances

---

Jumpei Hayakawa and Morio Ishizuka

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/48352>

---

## 1. Introduction

In this chapter, we present the current advances in flagellar glycosylation. Glycosylation is well-known as one of the most frequent posttranslational protein modification. Glycosylation is well studied in eukaryotes as the superficial and secretory proteins are mostly glycosylated in the eukaryotic cell. Protein glycosylation was considered to be a eukaryotic organism specific modification for many years. However, reports of bacterial glycosylation have increased since the discovery of surface layer glycosylation on the cell envelope in archaea and hyperthermophiles in the mid-1970's (Mescher & Strominger, 1976; Sleytr, 1975; Sleytr & Thorne, 1976).

### 1.1. Protein glycosylation

Protein glycosylation is largely classified as N-linked or O-linked while C-mannosylation is rarely identified (Furmanek & Hofsteenge, 2000). Glycan structures are enzymatically transferred to amino acid residues where they can covalently conjugate via the amino group of asparagine residues (N-glycosylation) or the hydroxyl group of serine or threonine residues (O-glycosylation). Both linkage types are distributed in eukaryotes and prokaryotes. The N-linkage glycosylation pathway is characterized in all three domains of life (eukarya, archaea, and bacteria) (Calo et al., 2010; Haeuptle & Hennet, 2009; Szymanski & Wren, 2005; Weerapana & Imperiali, 2006). The carbohydrate chain is synthesized at the membrane (endoplasmic reticulum (ER) in eukarya, or on the cytoplasmic side of the plasma membrane in archaea and bacteria) via a specific glycosyltransferase which transfers a nucleotide-activated sugar precursor onto the lipid carrier (dolichol-phosphate in eukarya and archaea, or undecaprenyl-phosphate in bacteria). The synthesized carbohydrate chain (oligosaccharide) is flipped across the membrane using a specific flippase, and is transferred to the asparagine residue in the nascent protein en bloc by an oligosaccharyltransferase (OST), which is composed of nine subunits in eukarya. Archaeal and bacterial OST are encoded by the *aglB* (Abu-Qarn et al., 2007) or *pglB* (Wacker et al., 2002) gene to yield a

single protein, respectively. In eukarya and archaea, the asparagine residues of the N-linkage glycosylation site are conserved in a sequon, the N-X-S/T motif (where X is any amino acid except proline). Recently, the bacterial N-linkage sequon was characterized from *Campylobacter jejuni* (Kowarik et al., 2006). The two N terminal extended residues, D/E-X1-N-X2-S/T (where X1 and X2 are any amino acid except proline), were required for the bacterial OST recognition of the glycosylation site.

In eukaryote, glycoproteins typically possess a pentasaccharide core carbohydrate structures which consist of (Man)<sub>2</sub>-Man-GlcNAc-GlcNAc with one or more glycan chain (N-glycosylation) and di-, or trisaccharide core structure based on GalNAc attached to serine or threonine (O-glycosylation). However, recent reports on *C. jejuni*, *Haemophilus influenzae*, and *Desulfovibrio desulfuricans* N-glycosylations indicate that there is no conserved core structure in bacteria (Gross et al., 2008; Ielmini & Feldman, 2011; Young et al., 2002).

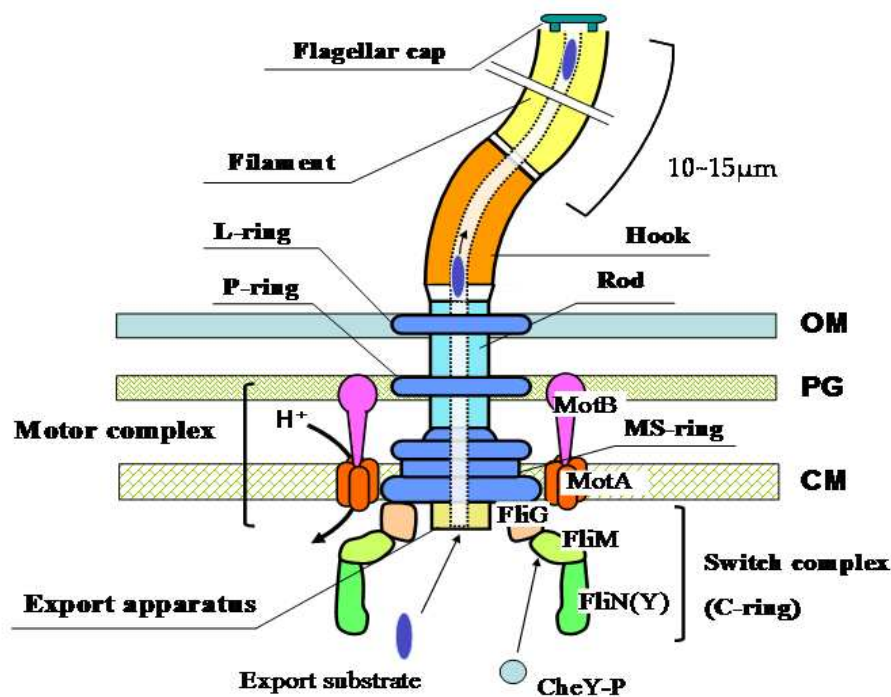
Thus, in prokaryotes many different glycoprotein structures have been observed that display much more variation than those observed in eukaryotes.

Glycoproteins have many biological functions: one example is recognition and adhesion among cells (Varki, 1993). The interactions between cells are mediated by the glycan structures on the cell surfaces. Therefore, the different glycan moieties on the cell surfaces serve as markers for cell recognition events, and modifications of the glycan structures can render several biological functions to the protein in eukarya. In recent years, accumulating studies for glycosylated bacterial proteins indicate that glycan structures mainly participate in the virulence of the mucosal pathogen (Szymanski & Wren, 2005). Most bacterial glycoproteins appear to be associated with the surface of the organism as in pili or flagella. Flagellin is one of the extensively studied glycosylated bacterial proteins and it is suggested that the flagellin glycosylation is responsible for their virulence, adherence, filament assembly, and filament stability (Arora et al., 2005; Goon et al., 2002; Szymanski et al., 2002; Taguchi et al., 2008).

## 1.2. Flagellar structures

Most bacterial species swim by means of rotating flagella that are powered by the monovalent cation (H<sup>+</sup> or Na<sup>+</sup>) influx. Many bacteria have extracellular flagellar structures and the pattern of flagellar arrangement is an identification tool in bacteria. A variety of flagella structures and swimming patterns have been discussed in previous works (Armitage & Macna, 1987; Bardy et al., 2003; Charon & Goldstein, 2002; Macnab, 1977, McCarter, 2001, 2004; Shigematsu et al., 2005). There are classified in-to four flagella arrangements as follows: multi-flagella are randomly distributed on the overall cell surface (peritrichous; e.g. *Escherichia coli*, *Salmonella typhimurium*, *Bacillus subtilis*), several flagella are present at one end of the cell (lophotrichous; e.g. *Pseudomonas fluorescens*), a single polar flagella is projected from the cell end (monotrichous; e.g. *Vibrio cholerae*, *Rhodobacter sphaeroides*), and a single flagellum is present at each pole of the bacterium (amphitrichous; e.g. *Spirillum serpens*). Although bacteria possess different flagella arrangement, a basic flagella structure is common among many bacterial species. The study of bacterial flagella

has been intensively investigated in *Escherichia coli* and *Salmonella typhimurium* by using genetic and biochemical approaches. A typical flagella structure is shown in Figure 1. About 50 genes, which are related to the bacterial flagellar assembly, have been identified. More than 20 distinct proteins make up the flagella structure and it consists of three main parts: a basal body, a hook, and a filament. The basal body consists of four rings (L, P, MS, and C-ring) and rod which is located just above the MS-ring and connected to the proximal region of the hook. However, the L and P ring are not observed in gram-positive bacterial species, because of the difference in the cell wall architecture (DePamphilis & Adler, 1971; Francis et al., 1995). The C-ring which is a part of flagella rotor consists of three proteins, FliG, FliM, and FliN (in gram-positive bacteria FliY correspond to FliN). In particular, FliG is the most directly involved in the rotation of the flagella motor among the C-ring proteins, as it interacts with the motor complex (MotA/B or PomA/B) component of the force-generating unit in the flagellar motor. The motor complex acts as the stator and the rotation energy is generated by the monovalent cation influx from the periplasmic space across the inner membrane. The C-ring is also called the “switch complex” as it can switch the direction of flagellar motor (Irikura et al., 1993; Kihara et al., 1996; Sockett et al., 1992). The switching event is caused by the binding of phosphorylated CheY (chemotaxis related gene) to the FliM of switch complex, and clockwise (CW)/counterclockwise (CCW) switching enable the bacterial cell to change swimming direction (Mathews et al., 1998; Sockett et al., 1992).



**Figure 1.** The model structure of the flagellar motor in gram-negative bacteria.

The motor consists of the Mot complex (MotA/B) and rotor (MS- and C-ring). The L- and P-ring do not exist in the gram-positive bacterial flagellar structure. The Mot complex is supposed to function as the force-generating unit via proton conduction while the C-ring functions as the switch. The phosphorylated form of CheY (CheY-P) interacts with FliM and

promotes CW rotation. When CheY-P is not bound to it, the motor rotates CCW. Flagellin subunits are transported from the cytoplasm and are delivered into a central channel in the basal body–hook filament structure (the diameter of the central channel is only 2 nm). OM, Outer membrane; PG, peptidoglycan layer; CM, cytoplasmic membrane (Irikura et al., 1993; Mathews et al., 1998).

The most impressive structure of the bacterial flagella motor is an extracellular long helical filament. In general, the flagella filament is composed of 20,000 ~ 30,000 subunits of a single protein called flagellin, and it reaches to more than 10  $\mu\text{m}$  in length (Namba & Vonderviszt, 1997). The flagellar specific export apparatus is located on the inside of the C-ring, and most of the flagellar components proteins are translocated across the cytoplasmic membrane by this apparatus, and then the proteins are diffused in the narrow nascent lumen structure and self-assemble at the distal end of the flagellar structure (Aizawa, 1996).

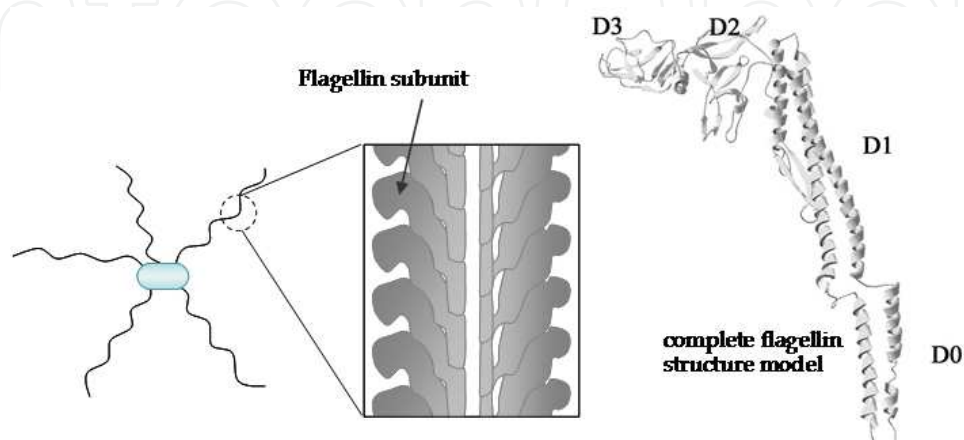
Flagellar-based motility is also common to archaea, but its structural features are quite distinct from bacteria. Archaeal flagella are closely related to bacterial type IV pili in their structure and assembly and the origin of bacterial flagella is considered to be a type III secretion system (Bardy et al., 2004). In bacteria, the flagellar filament is composed of a single flagellin subunit, in contrast, two or more distinct flagellin subunits are required for production of the flagellar filament. The other notable differences include that archaeal flagella rotation is powered by ATP, the flagellin subunit has a signal peptide which is cleaved by a specific peptidase for the secret matured flagellin subunit from the cell, and the flagella is grown from the proximal end of the cell surface by the addition of subunits to the base.

### 1.3. Flagellin glycosylations

Although flagella structures from both eubacteria and archaea are different, flagellin glycosylation is reported in both organisms. Eubacterial flagellin glycosylations are classified as either N- or O-linkage in a single subunit, and to date, most reports are about O-linkages. The O-linked glycan positions of bacterial flagellin proteins appear to be limited to the central region of the primary flagellin structure. The amino acid sequence alignment indicates that flagellin proteins are well conserved in the N- and C-terminal regions, while the central region is highly variable (Beatson et al., 2006). Although the intensively studied peritrichous flagella from *Salmonella typhimurium* do not have a glycosylated flagellin, a complete atomic model of its flagellin protein was resolved by excellent X-ray crystallography analysis and electron cryomicroscopic observation of the intact flagella filament (Samatey et al., 2001; Yonekura et al., 2003, 2005). These structural data revealed that the *S. typhimurium* flagellin protein consists of four major domains, D0, D1, D2, and D3 (Figure 2). The N- and C-terminal regions of flagellin correspond to D0 and D1, which are composed mainly of  $\alpha$ -helices, and they form the core part of a flagellar filament. D0 has two  $\alpha$ -helices (ND0 and CD0), whereas D1 has two long  $\alpha$ -helices (ND1a and CD1), one short  $\alpha$ -helix (ND1b) and one short  $\beta$ -sheet. These domains are positioned in the filament core. Gugolya et al. suggested that the alpha-helical terminal regions that correspond to the D0 domains are important for the coiled-coil model of flagellar filament formation (Gugolya et al., 2003). The central variable region of flagellin forms the outside surface-exposed

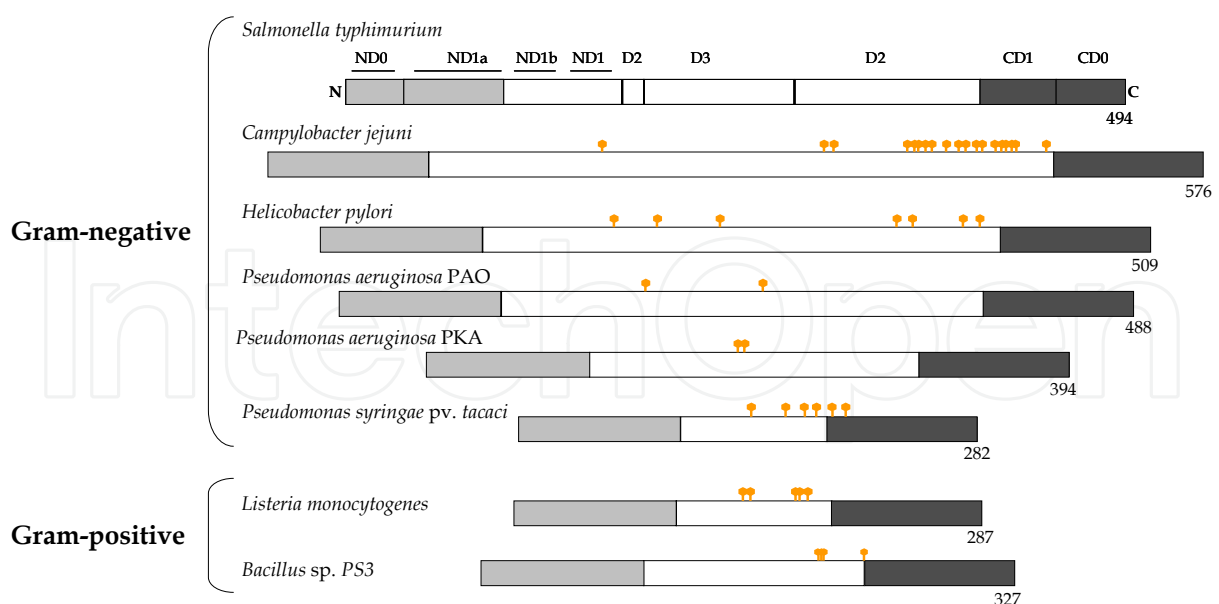


domain (D2 and D3) in the assembled filament. Studies of the variable region have focused on its role in H antigenicity, the effect of deletions on filament formation and motility, and the insertion of foreign peptides for extracellular display on bacterial flagella (Malapaka et al., 2007; Reid et al., 1999; Westerlund-Wikström, 2000; Woodset al., 2007; Yoshioka et al., 1995). Thus, flagellin glycan structures are restricted in the D2 and D3 domains (a few glycans were located at the D2 proximal end of the D1 domain), and it is considered that these glycan moieties are exposed to environmental conditions (Figure 3).



**Figure 2.** Bacterial flagellar filament.

Flagellar filament structure and complete flagellin 3D model of *Salmonella typhimurium* (Samatey et al., 2001; Yonekura et al., 2003, 2005). A flagellar filament consists of substantial amount of flagellin subunit and takes on a tubular structure.



Glycosylated flagellins are schematically aligned with the *S. typhimurium* flagellin domain. Light and dark grey boxes indicate 100 highly conserved terminal amino acid residues which constitute the alpha-helical structures. The corresponding amino acid size of the flagellin proteins is indicated at the C-terminal. Positions of glycosylation on each flagellin are indicated (●).

**Figure 3.** O-linked glycosylation sites of bacterial flagellin.

In contrast with eubacteria, three archaeal flagellin glycosylations are reported as N-linkage (Chaban et al., 2007; Voisin et al., 2005; Wieland & Sumper, 1985). Work on the most extensively studied flagellin from *Methanococcus voltae* are demonstrated that the glycan attached positions are not limited to anywhere specific in the flagellin primary structure and the N-linked asparagine residues seem to follow the classic eukaryotic type consensus sequon (N-X-S/T) rather than the recently identified bacterial N-linkage sequence (D/E-X<sub>1</sub>-N-X<sub>2</sub>-S/T).

2. Flagellar glycosylation

There have been many reports on flagella glycosylation since it was discovered about 20 years ago. Flagellin glycosylation is mainly found in gram-negative pathogenic bacterial species, and has been identified in about 30 microorganism strains including the archaea and gram-positive species (Logan, 2006). The distribution of flagellin glycosylation among several species is shown in Table 1, and a gene cluster which is potentially involved in the posttranslational modification of flagellin glycosylation is shown in Figure 4.

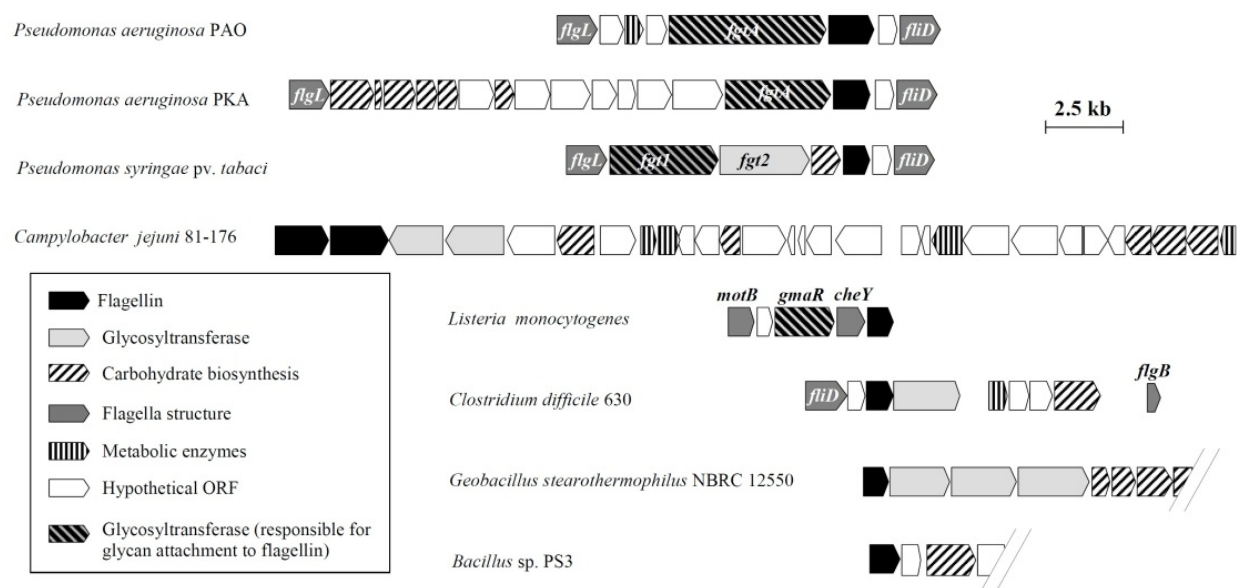


Figure 4. Organization of the glycosylation island located around the flagellin gene.

The location of glycosylation islands was not restricted to directly upstream or downstream of the flagellin gene, and the component genes were highly-diverse. A glycosyltransferase, which is responsible for the glycan attachment to a flagellin protein is usually included in the proximal glycosylation island of a flagellin gene, whereas, it was not identified to date in this region in *C. jejuni*.

Organism	linkage type	Glycan characterization	Function	GTase <sup>*5</sup> (OST)	Reference
<b>Gram-negative</b>					
<i>Aeromonas caviae</i> Sch3	O	Pse5Ac7Ac	Motility	n.d.	Tabei et al., (2009)
<i>Aeromonas caviae</i> UU 51	O	Pse5Ac7Ac8Ac	n.d.	n.d.	Schirm et al., (2005)
<i>Aeromonas hydrophila</i> AH3	n.d.*1	PAS <sup>*2</sup>	n.d.	n.d.	Rabaan et al., (2001)
<i>Agrobacterium tumefaciens</i> C58C1	n.d.	PAS	n.d.	n.d.	Deakin wt al., (1999)
<i>Azospirillum brasilense</i> Sp7 <sup>*2</sup>	O	$\beta$ -elimination <sup>*3</sup>	Adsorption	n.d.	Moens et al., (1995)
<i>Campylobacter jejuni</i> 81-176	O	Pse5Ac7Ac, PseAm, Pse8OAc PseAmGlnAc	Assembly	n.d.	Thibault et al., (2001) Schirm et al., (2005)
<i>Campylobacter jejuni</i> 11168	O	Leg5Am7Ac, Leg5AmNMe7Ac	n.d.	n.d.	Zampronio et al., (2011)
<i>Campylobacter coli</i> VC167	O	Pse5Ac7Ac, PseAm, Pse/PseAm-deoxypentose, Leg5Ac7Ac, Leg5Am7Ac, Leg5AmNMe7Ac	Assembly	n.d.	Logan et al., (2002)
<i>Caulobacter crescentus</i> CB15	n.d.	SDS-PAGE <sup>*4</sup>	Adherence Stability	n.d.	Faulds et al., (2011) Johnson et al., (1983)
<i>Helicobacter pylori</i>	O	Pse5Ac7Ac	Assembly	n.d.	Schirm et al., (2003)
<i>Helicobacter felis</i> CS1	n.d.	SDS-PAGE	n.d.	n.d.	Josenhans et al., (1999)
<i>Pseudomonas aeruginosa</i> PAK	O	Rha-(2-7 variable oligosaccharide chain)- deoxyhexosamine (dhexN)- deoxyhexose (dHex)	Virulence	FgtA	Schirm et al., (2004a)
<i>Pseudomonas aeruginosa</i> PAO1	O	dHex(PO <sub>4</sub> )-192Da	Virulence	FgtA	Verma et al., (2006)
<i>Pseudomonas aeruginosa</i> JJ692	O	Rhamnose	n.d.	n.d.	Schirm et al., (2004a)
<i>Pseudomonas syringae</i> pv. <i>tabaci</i>	O	$\beta$ -D-Quip4N(3-hydroxy-1-oxobutyl) 2Me-(1-3)- $\alpha$ -L-Rhap-(1-2)- $\alpha$ -L-Rhap	Virulence Stability	Fgt1	Takeuchi et al., (2007)
<i>Burkholderia pseudomallei</i>	O	291 Da carbohydrate	Motility	n.d.	Scott et al., (2011)
<i>Burkholderia thailandensis</i>	O	acetylated hexuronic acid	n.d.	n.d.	Scott et al., (2011)
<i>Acidovorax avenae</i>	O	n.d.	epitope recognition in rice	Fgt	Che et al., (2000) Hirai et al., (2011)
<i>Borrelia burgdorferi</i>	N	SNA, GNA lectin binding	n.d.	n.d.	Ge et al., (1998)
<i>Serpulina hyodysenteriae</i>	N	SDS-PAGE	n.d.	n.d.	Li et al., (1993)
<i>Spirochaeta aurantia</i>	n.d.	Lectin binding	n.d.	n.d.	Brahamsa & Greenberg, (1988)
<i>Treponema pallidum</i>	n.d.	PAS	n.d.	n.d.	Wyss, (1998)
<i>Shewanella oneidensis</i>	n.d.	n.d.	Motility	n.d.	Wu et al., (2011)
Marine magnetotactic ovoid bacterium MO-1	n.d.	n.d.	filament lubricant?	n.d.	Zhang et al., (2012)
<i>Xanthomonas oryzae</i> pv. <i>Oryzae</i>	O	n.d.	Virulence	<i>rbfC</i> <sub>Xoo</sub>	Sun et al., (2009)



Organism	linkage type	Glycan characterization	Function	GTase* <sup>5</sup> (OST)	Reference
<i>Thermus thermophilus</i> HB8* <sup>4</sup>	N	N-glycosydase F sensitive	n.d.	n.d.	Papaneophytou et al., (2012)
<i>Clostridium tyrobutyricum</i>	n.d.	β-elimination	n.d.	n.d.	Bedouet et al., (1998)
<i>Clostridium acetobutylicum</i>	O	PAS	n.d.	n.d.	Lyristis et al., (2000)
<i>Clostridium difficile</i>	O	HexNAc	Assembly	n.d.	Twine et al., (2009)
<i>Clostridium botulinum</i>	O	αLeg5GluNMe7Ac	n.d.	n.d.	Twine et al., (2008)
<i>Butyrivibrio fibrisolvens</i>	n.d.	PAS	n.d.	n.d.	Kalmokoff et al., (2000)
<i>Listeria monocytogenes</i>	O	N-acetylglucosamine (GlcNAc)	n.d.	GmaR	Shen et al., (2006)
<i>Geobacillus stearothermophilus</i>	O	PAS, β-elimination	Assembly	n.d.	Hayakawa et al., (2009a)
<i>Bacillus</i> sp. PS3	O	PAS, β-elimination	Assembly	n.d.	Hayakawa et al., (2009a)
<b>Archaea</b>					
<i>Halobacterium salinarum</i>	N	Glc(4-1)GlcASO4(4-1)GlcASO4 (4-1)GlcASO4	Stability	n.d.	Wieland et al., (1985)
<i>Methanococcus voltae</i>	N	β-ManpNAcA6Thr-(1-4)-β-GlcpNAc3NAcA-(1-3)-β-GlcpNAc	Assembly	AgIA (OST)	Voisin et al., (2005)
<i>Methanococcus maripaludis</i>	N	Sug-4-β-ManNAc3NAmA6Thr-4-β-GlcNAc3NAcA-3-β-GalNAc	Motility	AgIB (OST)	VanDyke et al., (2009)

\*<sup>1</sup> not determined; \*<sup>2</sup> Periodic acid stain; Carbohydrate specific staining method; \*<sup>3</sup> Removal of Ser/Thr binding carbohydrate structure; \*<sup>4</sup> Protein anomalous migration on SDS-PAGE; \*<sup>5</sup> Responsible for glycan attachment to flagellin.

**Table 1.** Flagellin glycosylations

## 2.1. Gram-negative

### 2.1.1. *Pseudomonas* spp.

*Pseudomonas* spp. are ubiquitous in nature and frequently isolated as opportunistic pathogen of both plant and animal. *P. aeruginosa* has a single polar flagellum which is classified by its type of flagella filament (type-a and type-b) by flagellin subunit size, amino acid sequence, and antigenicity (Allison et al., 1985; Lanyi, 1970). Both types of *P. aeruginosa* flagellin are known to contain O-linked glycosylated proteins. *P. aeruginosa* PAK and *P. aeruginosa* JJ692 produce glycosylated type-a flagellin protein, which is modified with a rhamnose (Rha) residue based on the glycan attached at two sites of each flagellin monomer (Schirm et al., 2004a). The glycan structure of PAK flagellin is a complex oligosaccharide which is composed of Rha-(2-7 variable oligosaccharide chain)-deoxyhexosamine (dhexN)-deoxyhexose (dHex), whereas JJ692 flagellin has only a single Rha glycosylation on both glycosylation site. The glycan form of PAO type-b flagellin is simpler than that of the PAK type-a flagellin, as it has a dHex linked sugar containing a

phosphate moiety at two sites of the flagellin monomer (Verma et al., 2006). With regards to the *Pseudomonas* spp. of plant pathogens, flagellin glycosylation from *Pseudomonas syringae* pv. *glycinea*, *Pseudomonas syringae* pv. *tomato*, and *Pseudomonas syringae* pv. *tabaci* 6605 have been identified (Taguchi et al., 2003, and Takeuchi et al., 2003). The structural characterization of the flagellin protein from *P. syringae* pv. *tabaci* 6605 revealed that six sites of the flagellin were modified with a novel trisaccharide, which was composed of two rhamnosyl (Rha) residues and one modified 4-amino-4,6-dideoxyglucosyl (Qui4N; trivial name, viosamine; Vio) residue,  $\beta$ -D-Quip4N(3-hydroxy-1-oxobutyl)2Me-(1-3)- $\alpha$ -L-Rhap-(1-2)- $\alpha$ -L-Rhap (Takeuchi et al., 2007). The flagella glycosylation island of these *Pseudomonas* spp. have been identified and located in the upstream region of their flagellin gene (Arora et al., 2001; Taguchi et al., 2006) (Figure 4). The PAK glycosylation island is composed of 14 ORFs (~16 kb) containing putative carbohydrate synthesis related genes and glycosyltransferase (*orfN*). In contrast, both the PAO and *syringae* pv. *tabaci* glycosylation island are more simple (only 4 and 3 genes, respectively), and a putative glycosyltransferase is encoded for each. Functional analysis of these glycosyltransferases demonstrated that they are essential for the addition of glycan structure to flagellin protein (Schirm et al., 2004a; Verma et al., 2006; Taguchi et al., 2006). Recently, a flagellin glycan biosynthesis gene cluster was newly identified from *P. syringae* pv. *tabaci* (Nguyen et al., 2009). The gene cluster is related to viosamine biosynthesis (viosamine island) and these genes are homologous to a part of the PAK glycosylation island (*orfA-E* and *orfG*) (Chiku et al., 2011). Mutagenesis analysis of glycosyltransferases and flagellin subunits demonstrated that flagellin glycosylation of PAK and PAO was not required for flagella biosynthesis and motility, but a remarkable reduction of virulence was observed upon mutation (Montie et al., 1982; Arora et al., 2005). Whereas, in *P. syringae* pv. *tabaci*, loss of flagellin glycosylation reduce not only virulence but also motility, in addition, mutations which resulted in a loss of glycosylation showed differences in the bundle formation of flagella, i.e. flagella bundles on the wild-type cell were loose, and in contrast mutant filaments seemed to be tightly interacting with each other. These results indicated that glycosylation stabilizes the filament structure and lubricates the rotation of the bundle (Taguchi et al., 2008, 2010). A similar conclusion was drawn for the glycan function of flagellin in the marine magnetotactic ovoid bacterium MO-1. Flagella bundles of MO-1 were enclosed with in sheaths structure and its glycosylation was required for smooth swimming (Lefèvre et al., 2010). Flagellin proteins were also glycosylated and each flagella bundle consisted of seven individual flagella filament, which were organized in a hexagon with a seventh in the middle. Considering the compact arrangement of the seven flagella filaments in the bundle, flagellin glycosylation might function as a lubricant (Zhang et al., 2012). Recently, an *fgt2* inactivation mutant from the biosurfactant producing species *P. syringae* pv. *syringae* B728a demonstrate upregulation of the late-stage flagellar genes (class IV), and increase surfactant production (Burch et al., 2012; Xu et al., 2012). The authors suggested that over-production of the biosurfactant helps smooth cell migration and minimize flagella breakage on sticky surfaces, such as a leaf surface.

### 2.1.2. *Campylobacter* spp.

*Campylobacter jejuni* have polar flagellum at one or both ends of the cell. The flagellin proteins are extensively O-glycosylated with structural analogues of the nine-carbon sugar pseudaminic acid (Pse), legionaminic acid (Leg), and their derivatives. Flagellin glycosylation is well characterized in three species of *Campylobacter*, i.e. *Campylobacter jejuni* 81-176, *C. jejuni* NCTC 11168, and *C. coli* VC167. Flagellin modification of *Campylobacter* species were identified at 19 serine or threonine residues, 16 sites, and at least 4 sites, in *C. jejuni* 81-176, *C. coli* VC167, and *C. jejuni* NCTC 11168, respectively (Thibault et al., 2001; Logan et al., 2002; Zampronio et al., 2011). The flagellin molecular weight from *C. jejuni* 81-176 is predicted by its amino-acid sequence to be 59.5 kDa, however flagellin from this strain is actually approximately 65 kDa. The additional 10% mass is attributed to attachment of substantial glycan structure (Thibault et al., 2001). In strain 81-176, the probable flagellin glycosylation related genes are largely involved in pseudaminic acid and acetamidino pseudaminic acid biosynthesis (Pse family), and lie downstream of the flagellin gene (about 27 kb) (Guerry et al., 2006). Similarly, Pse family glycosylation islands were identified in both *C. coli* VC167 and *C. jejuni* NCTC 11168, and in addition, in *C. coli* VC167 other flagellin modification genes which are involved in the synthesis of legionaminic acid and its derivatives (ptm family) were identified (McNally et al., 2007). However, the glycosyltransferase which catalyzes the addition of sugar residues to the protein backbone has not been identified. A mutation in the first step Pse biosynthesis gene leads to intercellular accumulation of unglycosylated flagellin protein (Goon et al., 2003). The biological roles of *Campylobacter* flagellin glycosylation have been mentioned in many reports. In 2007, an excellent review of the functions of flagellin glycosylation from *Campylobacter* species was published (Guerry, 2007).

### 2.1.3. *Helicobacter pylori*

*Helicobacter pylori* is a human gastric pathogen associated with gastric and duodenal ulcers as well as gastric cancer. Flagella and motility are important for colonization onto the mucosal of the human stomach. Two distinct flagellin subunits (FlaA and FlaB) were identified as glycosylated, and their glycan structures were characterized in a similar manner to that of *C. jejuni*, with Pse5Ac7Ac found at seven sites on FlaA and ten sites on FlaB, in addition flagellin glycosylation is required for functional filament assembly (Schirm et al., 2003, 2005; Josenhans et al., 2002). The mutagenesis analysis of four genes (HP0178, HP0326A, HP0326B, and HP0114) previously reported to be involved in flagellar glycosylation and polysaccharide biosynthesis demonstrated a non-motile phenotype with no structural flagella filament and only minor amounts of flagellin protein (Schirm et al., 2003). In contrast, inactivation of HP0518 resulted in altered motility and an increased level of flagellin glycosylation (Asakura et al., 2010). Complementation of a *H. pylori* HP0518 mutant and a recombinant HP0518 protein assay demonstrated the decreased glycosylation level of *H. pylori* flagellin *in vivo* and *in vitro* suggesting that HP0518 functions in the deglycosylation of flagellin. The *H. pylori* HP0518 mutant showed an increased colonization capability for the gastric tissues of mice. These results indicate that HP0518 is involved in the deglycosylation of flagellin, thereby regulating pathogen motility.

#### 2.1.4. *Burkholderia* spp.

*Burkholderia pseudomallei* is also known as *Pseudomonas pseudomallei* and is important as a human and animal pathogen. In contrast, *Burkholderia thailandensis* is closely related to *B. pseudomallei* but is a nonpathogenic bacterium. Top-down and bottom-up mass spectrometry (MS) analyses of both flagellin proteins identified that there were posttranslationally modified with novel glycans (Scott et al., 2011). MS analysis of the flagellin carbohydrate moiety suggested that *B. pseudomallei* flagellin was modified with a glycan with a mass of 291 Da, while *B. thailandensis* flagellin protein was modified with related glycans with a mass of 300 or 342 Da which included an acetylated hexuronic acid. A mutagenesis analysis of the lipopolysaccharide (LPS) O-antigen biosynthetic cluster demonstrated that it was important for flagellin glycosylation and motility in *B. pseudomallei*.

## 2.2. Gram-positive

### 2.2.1. *Clostridium*

*Clostridium* spp., a gram-positive spore-forming anaerobic bacterium, is an emerging opportunistic pathogen towards humans and plants, and includes *Clostridium botulinum*, *Clostridium difficile*, and *Clostridium glumae*. The genus *Clostridium* provides the most examples of gram-positive bacterium flagellin glycosylation which has been known since the discovery of *Clostridium tyrobutyricum* (Bédouet et al., 1998, Arnold et al., 1998). Structural characterization of the carbohydrate moiety from *C. botulinum* flagellin has been achieved, and it was shown to be composed of the Leg derivative, 7-acetamido-5-(N-methylglutam-4-yl)-amino-3,5,7,9-tetradeoxy-d-glycero- $\alpha$ -d-galacto-nonulosonic acid ( $\alpha$ Leg5GluNMe7Ac) (Twine et al., 2008). For the *C. botulinum* strain Langeland, a bioinformatic analysis of the flagella glycosylation island was completed between *flgB* and *fliD* as a large gene cluster (~48 kb), many of which appeared to be involved in carbohydrate biosynthesis (Sebahia et al., 2007). This glycosylation island could be divided into two regions, a variable region which was located immediately downstream of the flagellin gene, and a subsequent conserved region. The carbohydrate biosynthesis genes, which are significantly related to the legionaminic acid biosynthesis genes (ptm family) in *Campylobacter coli*, were encoded in the variable region, whereas the conserved region also encoded the carbohydrate biosynthesis genes (McNally et al., 2007). The *C. botulinum* strain Langeland was found to have homologous proteins to the capsular biosynthetic proteins from *Streptococcus agalactia*, including those derived from a second set of the sialic acid biosynthetic genes, *neuA* and *neuB*.

### 2.2.2. *Listeria monocytogenes*

*Listeria monocytogenes* is a gram-positive bacterium responsible for listeriosis, and *Listeria* species are found throughout the food-processing environment. Flagellin subunits are covalently modified by monomeric  $\beta$ -O-linked N-acetylglucosamine (GlcNAc) residues at three to six sites per subunit (Schirm et al., 2004b). The functional consequence of flagellin



glycosylation in *L. monocytogenes* was investigated by modification of the O-GlcNAc transferase (Lmo0688 renamed to GmaR), which is located just upstream of the flagellin gene (Shen et al., 2006). An in-frame deletion mutant of lmo0688 ( $\Delta 688$ ) resulted in a nonmotile bacteria similar to what was observed for *Campylobacter* species and *Helicobacter pylori* (Josenhans et al., 2002), but this phenotype differed from that reported for gram-negative species, as it was caused by a loss of flagellin expression. The point mutation analysis of the functional residues involved in the glycosyltransferase activity demonstrated full flagellin expression (without glycosylation) and motility. The authors concluded that GmaR is a bifunctional glycosyltransferase. However, glycosylation of flagellin is not required for any flagella functions and it remains to be determined what role glycosylation of the flagellin protein plays in *Listeria monocytogenes*.

### 2.2.3. Thermophilic *Bacillus* spp.

Thermophilic *Bacillus* species have been isolated from deepest sea mud, hot springs, and soil, and produce multiple peritrichous flagella. These thermophiles belong to genus *Geobacillus* and are not considered to be pathogens regardless of their flagellin glycosylation. In recent years, O-linked flagellin glycosylation was reported in two thermophilic *Bacillus* species, *Geobacillus stearothermophilus* NBRC 12550 and *Bacillus* sp. PS3 (Hayakawa et al., 2009a). These flagellin glycosylations were confirmed by PAS staining and beta-elimination. The analysis of the modification sites indicated that glycan structures were attached to at least 4 sites of the flagellin monomer in *Bacillus* sp. PS3, but the structural detail of the carbohydrate chains and total number of the modification sites is currently unknown. Although it was a partial sequence, the probable glycosylation islands from both thermophilic bacterial species were confirmed downstream of these flagellin genes (J. Hayakawa and M. Ishizuka, unpublished data). In *G. stearothermophilus*, a dTDP-L-rhamnose biosynthesis gene cluster (rml operon) was also identified immediately after GTases, which is highly homologous to the glycan biosynthesis genes of the S-layer glycoprotein from a closely related *G. stearothermophilus* strain (*G. stearothermophilus* NRS2004/3a) (Novotny et al., 2004 and Steine et al., 2007). The heterologous gene expression of these flagellin in a *Bacillus subtilis* flagellin deficient mutant demonstrated that unglycosylated flagellin proteins were intracellularly accumulated and phenotypically paralyzed (Hayakawa et al., 2009a), however amino acid substitutions could restore functional filament assembly and motility (Hayakawa et al., 2009b. described below). These results supported the proposal that flagellin glycosylation is important for filament assembly. However, the carbohydrate structure and more detail of the biological functions remain to be elucidated.

## 2.3. Archaea

Archaeal flagellin glycosylation was first identified in *Halobacterium salinarum* (Wieland et al., 1985). Its flagellin subunit was glycosylated with sulfated glucuronic acid which is the same type as the cell surface S-layer glycoprotein. The detailed structural characterization of flagellin attached carbohydrate was accomplished for *Methanococcus voltae* (Voisin et al.,



2005). *M. voltae* flagellin proteins were modified with a novel trisaccharide,  $\beta$ -ManpNAcA6Thr-(1-4)- $\beta$ -GlcNAc3NAcA-(1-3)- $\beta$ -GlcNAc, N-linked to Asn. In addition, the peptide containing the N-linked sequence motif of the flagellin protein was Asn-X-Ser/Thr, which is identical to that observed for S-layer protein glycosylation. Recently, a tetrasaccharide glycan which was N-linked to the flagellin subunits in *M. maripaludis* was also characterized, with a reported structure of Sug-4- $\beta$ -ManNAc3NAcA6Thr-4- $\beta$ -GlcNAc3NAcA-3- $\beta$ -GalNAc, where Sug is a (5S)-2-acetamido-2,4-dideoxy-5-O-methyl- $\alpha$ -l-erythro-hexos-5-ulo-1,5-pyranose, representing the first example of a naturally occurring diglycoside of an aldulose (Kelly et al., 2009, Jones et al., 2012). A deletion mutant analysis of three glycosyltransferases and an oligosaccharyltransferase (Stt3p homologue) from *M. maripaludis* revealed that these genes were responsible for flagellin glycosylation supported by the fact that glycan reduced flagellins were not assembled into the flagella filament (VanDyke et al., 2009). The structural and genetic analysis of archaeal flagellin glycosylation is frequently linked with S-layer protein glycosylation, and the reader is referred to a recent detailed review (Jarrell et al., 2010).

### 3. Glycosylation pathway

The complete pathway of bacterial flagellin glycosylation is still not clarified. There are two reviews which provide an overview of the O-linked flagellin glycosylation pathway (Logan et al., 2006; Nothaft & Szymanski, 2010). Bacterial flagella assembly occurs at the distal end of the basal body. The nascent flagellin protein is transported across the cytoplasmic membrane by a type three secretion system, and then proceeds through the narrow central channel of the flagella structure. Finally, the flagellin subunit associates with the tip of the filament structure which is elongated and reaches a length of about ten micrometers. In contrast to the archaeal flagellin export pathway, bacterial flagellin protein is not exposed outside of the inner membrane containing the periplasmic space until assembled into the filament. In other words, if flagellin glycosylation occurred extracellularly, it must be achieved far away from the cell. Therefore, it is reasonable to assume that the flagellin glycosylation machinery is located in the vicinity of the flagella basal body. Recently, the *C. jejuni* O-linked flagellin glycosylation machinery was localized at the pole of the cell along with the flagella (Ewing et al., 2009). Three genes involved in pseudaminic acid biosynthesis (PseC, which is the enzyme involved in the second step of PseAc synthesis, PseE, the putative PseAc transferase, and PseD, the putative PseAm transferase) were labeled with GFP fusion and expressed in *C. jejuni* 81-176. The fluorescent microscopic observation demonstrated that some, but not all, of the enzymatic glycosylation machinery was localized at the poles of the cells, consistent with a possible association with the flagellar basal body/export apparatus. Further study indicated that O-linked glycan biosynthesis could be reconstructed *in vitro* (Schoenhofen et al., 2009). The flagellin monomers from *Campylobacter* species are predominantly glycosylated with pseudaminic acid (Pse) and legionaminic acid (Leg). The precursors of these glycans are utilized in the form of CMP-activated sugars (CMP-Pse, CMP-Leg, and their derivatives), and they are added to the serine or threonine residues of flagellin by a specific glycosyltransferase (Note that the glycosyltransferases

responsible for O-glycan attachment to flagellin have yet to be identified). The eleven candidates of glycan biosynthetic enzymes (PtmF, PtmA, PgmL, PtmE, GlmU, LegB, LegC, LegH, LegG, LegI, and LegF) from *Campylobacter jejuni* have been individually purified and characterized. It was confirmed that Leg and its CMP-activated form were synthesized from fructose-6-phosphate. The authors also suggested that O-linked glycan biosynthesis was involved in the synthesis of the N-linked glycan.

## 4. Amino acid substitutions of flagellin protein

Many attempts have been carried out to obtain insight into the significance of flagellin glycosylation. One of the most visible experiments is the disruption of glycosyltransferase activity which allows the evaluation of the flagella assembly, filament morphology, motility, and virulence (see above). In this section, we focus on the effects of amino acid substitution in glycosylated flagellin proteins.

### 4.1. Influence of loss of glycosylation to the motility and virulence

#### 4.1.1. *Campylobacter jejuni* 81-176

The major flagellin of *Campylobacter jejuni* 81-176, FlaA, has been shown to be glycosylated at 19 serine or threonine residues, and this glycosylation is required for flagellar filament formation (Thibault et al., 2001; Goon et al., 2003). Mutants were constructed in which each of the 19 serine or threonines that are glycosylated in FlaA was converted to an alanine. Eleven of the 19 mutants displayed no observable phenotype, but the remaining 8 mutants had two distinct phenotypes. Five mutants (mutations S417A, S436A, S440A, S457A, and T481A) were fully motile but defective in autoagglutination. Three other mutants (mutations S425A, S454A, and S460A) were reduced in motility and synthesized truncated flagellar filaments (Ewing et al., 2009).

#### 4.1.2. *Pseudomonas syringae* pv. *tabaci*

Flagellin glycosylation of *Pseudomonas syringae* pv. *tabaci* 6605 has been reported at six serine residues, positioned at amino acids 143, 164, 176, 183, 193 and 201 (Taguchi et al., 2006). Mutants where 6 serine residues were converted to alanine individually were compared with the mutant containing the flagellin specific glycosyltransferase, *fgt1*. All mutants displayed reduced swarming ability, swimming speed, filament stability, and virulence (Taguchi et al., 2006; 2008; Takeuchi et al., 2008). In addition, reduction of the molecular weight of each mutant flagellin protein corresponded to the loss of a single carbohydrate chain moiety, and the degree of reduced biological functions were smaller than that of an all glycosylation-serine-replacement mutant (6 S/A).

### 4.2. Restoration of filament formation without glycosylation

Flagellin glycosylation of a thermophilic bacillus species was recently reported for *Bacillus* sp. PS3. Although there was low coverage of the flagellin sequence, at least four serine and

threonine residues were identified as glycosylation sites (Hayakawa et al., 2009a). This potentially glycosylated flagellin protein was expressed in *B. subtilis*  $\Delta hag$  (flagellin deficient mutant strain) for complementation. The resulting transformant was non-motile, and the produced flagellin protein derived from *Bacillus* sp. PS3 was not glycosylated and accumulated intercellularly. However, spontaneously isolated flagellin mutants partially restored the motility and produced a truncated flagella filament without glycosylation (Hayakawa et al., 2009b and J. Hayakawa and M. Ishizuka, unpublished data). All characterized suppressing mutations contained single or double point mutations and about 30 residue intragenic duplications in the flagellin in the highly variable region (D2 and D3 domain) and the end of the  $\alpha$ -helical structure (D1 domain). The positions of these mutations were in good accordance with the previously reported flagellin glycosylation sites from many other bacterial species. To our knowledge, this is the first report of a gain-of-function mutant of flagellin glycosylation.

## 5. Conclusions

Glycosylation is no longer a rare event regardless of whether bacteria or eukaryote are considered. Complete genomic information for several bacteria is now available and bioinformatic analyses demonstrated that bacterial flagellin glycosylation is widely spread over several genera. Many speculative functions of flagella glycosylation have been demonstrated, for example filament assembly (including flagellin export), filament stability, motility, virulence, gene regulation and mimicry with host-cell surface glycan structure. These glycosylation functions are similar regardless of the variety of eukaryote. In addition, the bacterial glycosylation pathway is becoming better defined; many genes which participate in flagellin glycosylation have been identified, but their number and loci are diverse in each bacterial species. Rapid increases in the knowledge of glycosyltransferases and glycan biosynthesis gene clusters will undoubtedly be achieved through glycoengineering with an aim to design a bacterial flagella motor for the development of a novel vaccine or drug-delivery-system.

## Author details

Jumpei Hayakawa and Morio Ishizuka\*

*Department of Applied Chemistry, Faculty of Science and Engineering, Chuo University, Tokyo, Japan*

## Acknowledgement

The authors acknowledge In-Tech-Open Access Publisher for the kind invitation to contribute this chapter. This work was supported in part by Chuo University Grant for Special Research to M. I.

---

\* Corresponding Author

## 6. References

- Abu-Qarn M., Yurist-Doutsch, S., Giordano, A., Trauner, A., Morris, H. R., Hitchen, P., Medalia, O., Dell, A., & Eichler, J. J. (2007). *Haloferax volcanii* AglB and AglD are involved in N-glycosylation of the S-layer glycoprotein and proper assembly of the surface layer. *Journal of Molecular Biology*, Vol. 374, No. 5, (December 2007), pp. 1224-1236, ISSN 0022-2836
- Aizawa S-I. (1996). Flagellar assembly in *Salmonella typhimurium*. *Molecular Microbiology*, Vol. 19, No. 1, (January 1996), pp. 1-5, ISSN 0950-382X
- Allison, J. S., Dawson, M., Drake, D., & Montie, T. C. (1985). Electrophoretic separation and molecular weight characterization of *Pseudomonas aeruginosa* H-antigen flagellins. *Infection and immunity*, Vol. 49, No. 3, (September 1985), pp. 770-774, ISSN 0019-9567
- Armitage, J. P. & Macnab, R. M. (1987). Unidirectional, intermittent rotation of the flagellum of *Rhodobacter sphaeroides*. *Journal of Bacteriology*, Vol. 169, No. 2, (February 1987), pp. 514-518, ISSN 0021-9193
- Arnold, F., Bédouet, L., Batina, P., Robreau, G., Talbot, F., Lécher, P., & Malcoste, R. (1998). Biochemical and immunological analyses of the flagellin of *Clostridium tyrobutyricum* ATCC 25755. *Microbiology and immunology*, Vol. 42, No. 1, pp. 23-31, ISSN 0385-5600
- Arora, S. K., Bangera, M., Lory, S., & Ramphal, R. (2001). A genomic island in *Pseudomonas aeruginosa* carries the determinants of flagellin glycosylation. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 98, No. 16, (July 2001), pp. 9342-9347, ISSN 0027-8424
- Arora, S. K., Neely, A. N., Blair, B., Lory, S., & Ramphal, R. (2005). Role of motility and flagellin glycosylation in the pathogenesis of *Pseudomonas aeruginosa* burn wound infections. *Infection and immunity*, Vol. 73, No. 7, (July 2005), pp. 4395-4398, ISSN 0019-9567
- Asakura, H., Churin, Y., Bauer, B., Boettcher, J. P., Bartfeld, S., Hashii, N., Kawasaki, N., Mollenkopf, H. J., Jungblut, P. R., Brinkmann, V., & Meyer, T. F. (2010). *Helicobacter pylori* HP0518 affects flagellin glycosylation to alter bacterial motility. *Molecular microbiology*, Vol. 78, No. 5, (December 2010), pp. 1130-1144, ISSN 0950-382X
- Bardy, S. L., Ng, S. Y., & Jarrell, K. F. (2003). Prokaryotic motility structures. *Microbiology*, Vol. 149, No. 2, (February 2003), pp. 295-304, ISSN 1350-0872
- Bardy, S. L., Ng, S. Y., & Jarrell, K. F. (2004). Recent advances in the structure and assembly of the archaeal flagellum. *Journal of Molecular Microbiology and biotechnology*, Vol. 7, No. 1-2, pp. 41-51, ISSN 1464-1801
- Beatson, S. A., Minamino, T., & Pallen, M. J. (2006). Variation in bacterial flagellins: from sequence to structure. *Trends in Microbiology*, Vol. 14, No. 4, (April 2006), pp. 151-155, ISSN 0966-842X
- Bédouet, L., Arnold, F., Robreau, G., Batina, P., Talbot, F., & Binet, A. (1998). Evidence for an heterogeneous glycosylation of the *Clostridium tyrobutyricum* ATCC 25755 flagellin. *Microbios*, Vol. 94, No. 379, pp. 183-192, ISSN 0026-2633

- Brahamsha, B. & Greenberg, E. P. (1988). Biochemical and cytological analysis of the complex periplasmic flagella from *Spirochaeta aurantia*. *Journal of Bacteriology*, Vol. 170, No. 9, (September 1988), pp. 4023–4032, ISSN 0021-9193
- Burch, A. Y., Shimada, B. K., Mullin, S. W., Dunlap, C. A., Bowman, M. J., & Lindow, S. E. (2012). *Pseudomonas syringae* coordinates production of a motility-enabling surfactant with flagellar assembly. *Journal of Bacteriology*, Vol. 194, No. 6, (March 2012), pp. 1287–1298, ISSN 0021-9193
- Calo, D., Kaminski, L. & Eichler, J. (2010). Protein glycosylation in Archaea: sweet and extreme. *Glycobiology*, Vol. 20, No. 9, (September 2010), pp. 1065–1076, ISSN 0959-6658
- Chaban, B., Ng, S. Y., Kanbe, M., Saltzman, I., Nimmo, G., Aizawa, S., & Jarrell, K. F. (2007). Systematic deletion analyses of the fla genes in the flagella operon identify several genes essential for proper assembly and function of flagella in the archaeon, *Methanococcus maripaludis*. *Molecular Microbiology*, Vol. 66, No. 3, (November 2007), pp. 596–609, ISSN 0950-382X
- Charon, N. W. & Goldstein, S. F. (2002). Genetics of motility and chemotaxis of a fascinating group of bacteria: the *spirochetes*. *Annual Review of Genetics*, Vol. 36, (December 2002), pp. 47–73, ISSN 0066-4197
- Che, F. S., Nakajima, Y., Tanaka, N., Iwano, M., Yoshida, T., Takayama, S., Kadota, I., & Isogai, A. (2000). Flagellin from an incompatible strain of *Pseudomonas avenae* induces a resistance response in cultured rice cells. *The Journal of biological chemistry*, Vol. 275, No. 41, (October 2000), pp. 32347–32356, ISSN 0021-9258
- Chiku, K., Ishii, T., Ono, H., Yoshida, M., & Ichinose, Y. (2011). Identification of genes involved in the glycosylation of modified viosamine of flagellins in *Pseudomonas syringae* by mass spectrometry. *Genes*, Vol. 2, No. 4, pp. 788–803, ISSN 2073-4425
- Deakin, W. J., Parker, V. E., Wright, E. L., Ashcroft, K. J., Loake, G. J., & Shaw, C. H. (1999). *Agrobacterium tumefaciens* possesses a fourth flagellin gene located in a large gene cluster concerned with flagellar structure, assembly and motility. *Microbiology*, Vol. 145, No. Pt 6, (June 1999), pp. 1397–1407, ISSN 1350-0872
- DePamphilis, M. L. & Adler, J. (1971). Fine Structure and Isolation of the Hook-Basal Body Complex of Flagella from *Escherichia coli* and *Bacillus subtilis*. *Journal of Bacteriology*, Vol. 105, No. 1, (January 1971), pp. 384–395, ISSN 0021-9193
- Ewing, C. P., Andreishcheva, E., & Guerry, P. (2009). Functional characterization of flagellin glycosylation in *Campylobacter jejuni* 81-176. *Journal of Bacteriology*, Vol. 191, No. 22, (November 2009), pp. 7086–7093, ISSN 0021-9193
- Faulds-Pain, A., Birchall, C., Aldridge, C., Smith, W. D., Grimaldi, G., Nakamura, S., Miyata, T., Gray, J., Li, G., Tang, J. X., Namba, K., Minamino, T., & Aldridge, P. D. (2011). Flagellin redundancy in *Caulobacter crescentus* and its implications for flagellar filament assembly. *Journal of Bacteriology*, Vol. 193, No. 11, (June 2011), pp. 2695–2707, ISSN 0021-9193
- Francis, N. R., Sosinsky, G. E., Thomas, D., & DeRosier, D. J. (1994). Isolation, characterization and structure of bacterial flagellar motors containing the switch



- complex. *Journal of Molecular Biology*, Vol. 235, No. 2, (January 1994), pp. 1261-1270, ISSN 0022-2836
- Furmanek, A. & Hofsteenge, J. (2000). Protein C-mannosylation: facts and questions. *Acta biochimica Polonica*, Vol. 47, No. 3, pp. 781-789, ISSN 0001-527X
- Ge, Y., Li, C., Corum, L., Slaughter, C. A., & Charon, N. W. (1998). Structure and expression of the FlaA periplasmic flagellar protein of *Borrelia burgdorferi*. *Journal of Bacteriology*, Vol. 180, No. 9, (May 1998), pp. 2418-2425, ISSN 0021-9193
- Goon, S., Kelly, J. F., Logan, S. M., Ewing, C. P., & Guerry, P. (2003). Pseudaminic acid, the major modification on *Campylobacter* flagellin, is synthesized via the Cj1293 gene. *Molecular Microbiology*, Vol. 50, No. 2, (October 2003), pp. 659-671, ISSN 0950-382X
- Gross, J., Grass, S., Davis, A. E., Gilmore-Erdmann, P., Townsend, R. R., & St. Geme, J. W. 3rd. (2008). The *Haemophilus influenzae* HMW1 adhesin is a glycoprotein with an unusual N-linked carbohydrate modification. *The Journal of biological chemistry*, Vol. 283, No. 38, (September 2008), pp. 26010-26015, ISSN 0021-9258
- Guerry, P. (2007). *Campylobacter* flagella: not just for motility. *Trends in Microbiology*, Vol. 15, No. 10, (October 2007), pp. 456-461, ISSN 0966-842X
- Guerry, P., Ewing, C. P., Schirm, M., Lorenzo, M., Kelly, J., Pattarini, D., Majam, G., Thibault, P., & Logan, S. (2006). Changes in flagellin glycosylation affect *Campylobacter* autoagglutination and virulence. *Molecular Microbiology*, Vol. 60, No. 2, (April 2006), pp. 299-311, ISSN 0950-382X
- Gugolya, Z., Muskotál, A., Sebestyén, A., Diószeghy, Z. & Vonderviszt, F. (2003). Interaction of FliS flagellar chaperone with flagellin. *FEBS letters*, Vol. 535, No 1-3, (January 2003), pp. 66-70, ISSN 0014-5793
- Haeuptle, M. A. & Hennet, T. (2009). Congenital disorders of glycosylation: an update on defects affecting the biosynthesis of dolichol-linked oligosaccharides. *Human mutation*, Vol. 30, No. 12, (December 2009), pp. 1628-1641, ISSN 1059-7794
- Hayakawa, J., Kambe, T., & Ishizuka, M. (2009b). Amino acid substitutions and intragenic duplications of *Bacillus* sp. PS3 flagellin cause complementation of the *Bacillus subtilis* flagellin deletion mutant. *Bioscience, biotechnology, and biochemistry*, Vol. 73, No. 10, (October 2009), pp. 2348-5231, ISSN 0916-8451
- Hayakawa, J., Kondoh, Y., & Ishizuka, M. (2009a). Cloning and characterization of flagellin genes and identification of flagellin glycosylation from thermophilic *Bacillus* species. *Bioscience, biotechnology, and biochemistry*, Vol. 73, No. 6, (June 2009), pp. 1450-1452, ISSN 0916-8451
- Hirai, H., Takai, R., Iwano, M., Nakai, M., Kondo, M., Takayama, S., Isogai, A., & Che, F. S. (2011). Glycosylation regulates specific induction of rice immune responses by *Acidovorax avenae* flagellin. *The Journal of biological chemistry*, Vol. 286, No. 29, (July 2011), pp. 25519-25530, ISSN 0021-9258
- Ielmini, M. V. & Feldman, M. F. (2011). *Desulfovibrio desulfuricans* PglB homolog possesses oligosaccharyltransferase activity with relaxed glycan specificity and distinct protein

- acceptor sequence requirements. *Glycobiology*, Vol. 21, No. 6, (June 2011), pp. 734-742, ISSN 0959-6658
- Irikura, V. M., Kihara, M., Yamaguchi, S., Sockett, H., & Macnab, R. M. (1993). *Salmonella typhimurium* *fliG* and *fliN* mutations causing defects in assembly, rotation, and switching of the flagellar motor. *Journal of Bacteriology*, Vol. 175, No. 3, (February 1993), pp. 802-810, ISSN 0021-9193
- Jarrell, K. F., Jones, G. M., Kandiba, L., Nair, D. B., & Eichler, J. (2010). S-layer glycoproteins and flagellins: reporters of archaeal posttranslational modifications. *Archaea*, Vol. 2010, (July 2010), ISSN 1472-3646
- Johnson, R. C., Ferber, D. M., & Ely, B. (1983). Synthesis and assembly of flagellar components by *Caulobacter crescentus* motility mutants. *Journal of Bacteriology*, Vol. 154, No. 3, (June 1983), pp. 1137-1144, ISSN 0021-9193
- Jones, G. M., Wu, J., Ding, Y., Uchida, K., Aizawa, S. I., Robotham, A., Logan, S. M., Kelly, J., & Jarrell, K. F. (2012). Identification of Genes Involved in the Acetamidino Group Modification of the Flagellin N-linked Glycan of *Methanococcus maripaludis*. *Journal of Bacteriology*, published ahead of print 9 March 2012, ISSN 0021-9193
- Josenhans, C., Ferrero, R. L., Labigne, A., & Suerbaum, S. (1999). Cloning and allelic exchange mutagenesis of two flagellin genes of *Helicobacter felis*. *Molecular microbiology*, Vol. 33, No. 2, (July 1999), pp. 350-362, ISSN 0950-382X
- Josenhans, C., Vossebein, L., Friedrich, S., & Suerbaum, S. (2002). The *neuA/flmD* gene cluster of *Helicobacter pylori* is involved in flagellar biosynthesis and flagellin glycosylation. *FEMS Microbiology Letters*, Vol. 210, No. 2, (May 2002), pp. 165-172, ISSN 0378-1097
- Kalmokoff, M. L., Allard, S., Austin, J. W., Whitford, M. F., Hefford, M. A., & Teather, R. M. (2000). Biochemical and genetic characterization of the flagellar filaments from the rumen anaerobe *Butyrivibrio fibrisolvens* OR77. *Anaerobe*, Vol. 6, pp. 93-109, ISSN 1075-9964
- Kelly, J., Logan, S. M., Jarrell, K. F., VanDyke, D. J., & Vinogradov, E. (2009). A novel N-linked flagellar glycan from *Methanococcus maripaludis*. *Carbohydrate research*, Vol. 344, No. 5, (March 2009), pp. 648-653, ISSN 0008-6215
- Kihara, M., Francis, N. R., DeRosier, D. J., & Macnab, R. M. Analysis of a *FliM-FliN* flagellar switch fusion mutant of *Salmonella typhimurium*. *Journal of Bacteriology*, Vol. 178, No. 15, (August 1996), pp. 4582-4589, ISSN 0021-9193
- Kowarik, M., Young, N. M., Numao, S., Schulz, B. L., Hug, I., Callewaert, N., Mills, D. C., Watson, D. C., Hernandez, M., Kelly, J. F., Wacker, M., & Aebi, M. (2006). Definition of the bacterial N-glycosylation site consensus sequence. *The EMBO journal*, Vol. 25, No. 9, (May 2006), pp. 1957-1966, ISSN 0261-4189
- Lányi, B. (1970). Serological properties of *Pseudomonas aeruginosa*. II. Type-specific thermolabile (flagellar) antigens. *Acta microbiologica Academiae Scientiarum Hungaricae*, Vol. 17, No. 1, pp. 35-48, ISSN 0001-6187

- Lefèvre, C. T., Santini, C. L., Bernadac, A., Zhang, W. J., Li, Y., & Wu, L. F. (2010). Calcium ion-mediated assembly and function of glycosylated flagellar sheath of marine magnetotactic bacterium. *Molecular microbiology*, Vol. 78, No. 5, (December 2010), pp. 1304-1312, ISSN 0950-382X
- Li, Z., Dumas, F., Dubreuil, D., & Jacques, M. (1993). A species-specific periplasmic flagellar protein of *Serpulina* (*Treponema*) *hyodysenteriae*. *Journal of Bacteriology*, Vol. 175, No. (December 1993), pp. 8000-8007, ISSN 0021-9193
- Logan, S. M. (2006). Flagellar glycosylation - a new component of the motility repertoire? *Microbiology*, Vol. 152, No. 5, (May 2006), pp. 1249-1262, ISSN 1350-0872
- Logan, S. M., Kelly, J. F., Thibault, P., Ewing, C. P., & Guerry, P. (2002). Structural heterogeneity of carbohydrate modifications affects serospecificity of *Campylobacter* flagellins. *Molecular Microbiology*, Vol. 46, No. 2, (October 2002), pp. 587-597, ISSN 0950-382X
- Lyrstis, M., Boynton, Z. L., Petersen, D., Kan, Z., Bennett, G. N., & Rudolph, F. B. (2000). Cloning, sequencing and characterization of the gene encoding flagellin, *flaC* and the posttranslational modification of flagellin, FlaC from *Clostridium acetobutylicum* ATCC824. *Anaerobe*, Vol. 6, pp. 69-79, ISSN 1075-9964
- Macnab, R. M. (1977). Bacterial flagella rotating in bundles: a study in helical geometry. *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 74, No. 1, (January 1977), pp. 221-225, ISSN 0027-8424
- Malapaka, R. R., Adebayo, L. O., & Tripp, B. C. (2007). A deletion variant study of the functional role of the *Salmonella* flagellin hypervariable domain region in motility. *Journal of Molecular Biology*, Vol. 365, No. 4, (January 2007), pp. 1102-1116, ISSN 0022-2836
- Mathews, M. A., Tang, H. L., & Blair, D. F. (1998). Domain Analysis of the FliM Protein of *Escherichia coli*. *Journal of Bacteriology*, Vol. 180, No. 21, (November 1998), pp. 5580-5590, ISSN 0021-9193
- McCarter, L. L. (2001). Polar flagellar motility of the *Vibrionaceae*. *Microbiology and Molecular Biology Reviews*, Vol. 65, No. 3, (September 2001), pp. 445-462, ISSN 1092-2172
- McCarter, L. L. (2004). Dual flagellar systems enable motility under different circumstances. *Journal of Molecular Microbiology and Biotechnology*, Vol. 7, No. 1-2, (May 2004), pp. 18-29, ISSN 1464-1801
- McNally, D. J., Aubry, A. J., Hui, J. P., Khieu, N. H., Whitfield, D., Ewing, C. P., Guerry, P., Brisson, J. R., Logan, S. M., & Soo, E. C. (2007). Targeted metabolomics analysis of *Campylobacter coli* VC167 reveals legionaminic acid derivatives as novel flagellar glycans. *The Journal of biological chemistry*, Vol. 282, No. 19, (May 2007), pp. 14463-14475, ISSN 0021-9258
- Mescher, M. F. & Strominger, J. L. (1976). Purification and characterization of a prokaryotic glucoprotein from the cell envelope of *Halobacterium salinarium*. *The Journal of biological chemistry*, Vol. 251, No. 7, (April 1976). pp. 2005-2014, ISSN 0021-9258

- Moens, S., Michiels, K., Keijers, V., Van-Leuven, F., & Vanderleyden, J. (1995). Cloning, sequencing, and phenotypic analysis of *laf1*, encoding the flagellin of the lateral flagella of *Azospirillum brasilense* Sp7. *Journal of Bacteriology*, Vol. 177, No. 19, (October 1995), pp. 5419-5426, ISSN 0021-9193
- Montie, T. C., Doyle-Huntzinger, D., Craven, R. C., & Holder, I. A. (1982). Loss of virulence associated with absence of flagellum in an isogenic mutant of *Pseudomonas aeruginosa* in the burned-mouse model. *Infection and immunity*, Vol. 38, No. 3, (December 1982), pp. 1296-1298, ISSN 0019-9567
- Namba, K. & Vonderviszt, F. (1997). Molecular architecture of bacterial flagellum. *Quarterly Reviews of Biophysics*, Vol. 30, No. 1, (February 1997) pp. 1-65, ISSN 0033-5835
- Nguyen, L. C., Yamamoto, M., Ohnishi-Kameyama, M., Andi, S., Taguchi, F., Iwaki, M., Yoshida, M., Ishii, T., Konishi, T., Tsunemi, K., & Ichinose, Y. (2009). Genetic analysis of genes involved in synthesis of modified 4-amino-4,6-dideoxyglucose in flagellin of *Pseudomonas syringae* pv. *tabaci*. *Molecular genetics and genomics*, Vol. 282, No. 6, (December 2009), pp. 595-605, ISSN 1617-4615
- Nothaft, H., & Szymanski, C. M. (2010). Protein glycosylation in bacteria: sweeter than ever. *Nature reviews. Microbiology*, Vol. 8, No. 11, (November 2010), pp. 765-778, ISSN 1740-1526
- Novotny, R., Schäffer, C., Strauss, J., & Messner, P. (2004). S-layer glycan-specific loci on the chromosome of *Geobacillus stearothermophilus* NRS 2004/3a and dTDP-L-rhamnose biosynthesis potential of *G. stearothermophilus* strains. *Microbiology*, Vol. 150, No. Pt 4, (April 2004), pp. 953-965, ISSN 1350-0872
- Papaneophytou, C. P., Papi, R. M., Pantazaki, A. A., & Kyriakidis, D. A. (2012). Flagellin gene (*fliC*) of *Thermus thermophilus* HB8: characterization of its product and involvement to flagella assembly and microbial motility. *Applied Microbiology and biotechnology*, (February 2012), ISSN 0175-7598
- Rabaan, A. A., Gryllos, I., Tomás, J. M., & Shaw, J. G. (2001). Motility and the polar flagellum are required for *Aeromonas caviae* adherence to HEp-2 cells. *Infection and immunity*, Vol. 69, No. 7, (July 2001), pp. 4257-4267, ISSN 0019-9567
- Reid, S. D., Selander, R. K., & Whittam, T. S. (1999). Sequence diversity of flagellin (*fliC*) alleles in pathogenic *Escherichia coli*. *Journal of Bacteriology*, Vol. 181, No. 1, (January 1999), pp. 153-160, ISSN 0021-9193
- Samatey, F. A., Imada, K., Nagashima, S., Vonderviszt, F., Kumasaka, T., Yamamoto, M., & Namba, K. (2001). Structure of the bacterial flagellar protofilament and implications for a switch for supercoiling. *Nature*, Vol. 410, No. 6826, (March 2001), pp. 331-337, ISSN 0028-0836
- Schirm, M., Arora, S. K., Verma, A., Vinogradov, E., Thibault, P., Ramphal, R., & Logan, S. M. (2004a). Structural and genetic characterization of glycosylation of type a flagellin in *Pseudomonas aeruginosa*. *Journal of Bacteriology*, Vol. 186, No. 9, (May 2004), pp. 2523-2531, ISSN 0021-9193



- Schirm, M., Kalmokoff, M., Aubry, A., Thibault, P., Sandoz, M., & Logan, S. M. (2004b). Flagellin from *Listeria monocytogenes* is glycosylated with beta-O-linked N-acetylglucosamine. *Journal of Bacteriology*, Vol. 186, No. 20, (October 2004), pp. 6721-6727, ISSN 0021-9193
- Schirm, M., Schoenhofen, I. C., Logan, S. M., Waldron, K. C., & Thibault, P. (2005). Identification of unusual bacterial glycosylation by tandem mass spectrometry analyses of intact proteins. *Analytical chemistry*, Vol. 77, No. 23, (December 2005), pp. 7774-7782, ISSN 0003-2700
- Schirm, M., Soo, E. C., Aubry, A. J., Austin, J., Thibault, P., & Logan, S. M. (2003). Structural, genetic and functional characterization of the flagellin glycosylation process in *Helicobacter pylori*. *Molecular Microbiology*, Vol. 48, No. 6, (June 2003), pp. 1579-1592, ISSN 0950-382X
- Schoenhofen, I. C., Vinogradov, E., Whitfield, D. M., Brisson, J. R., & Logan, S. M. (2009). The CMP-legionaminic acid pathway in *Campylobacter*: biosynthesis involving novel GDP-linked precursors. *Glycobiology*, Vol. 19, No. 7, (July 2009), pp. 715-725, ISSN 0959-6658
- Scott, A. E., Twine, S. M., Fulton, K. M., Titball, R. W., Essex-Lopresti, A. E., Atkins, T. P., & Prior, J. L. (2011). Flagellar glycosylation in *Burkholderia pseudomallei* and *Burkholderia thailandensis*. *Journal of Bacteriology*, Vol. 193, No. 14, (July 2011), pp. 3577-3587, ISSN 0021-9193
- Sebaihia, M., Peck, M. W., Minton, N. P., Thomson, N. R., Holden, M. T., Mitchell, W. J., Carter, A. T., Bentley, S. D., Mason, D. R., Crossman, L., Paul, C. J., Ivens, A., Wells-Bennik, M. H., Davis, I. J., Cerdeño-Tárraga, A. M., Churcher, C., Quail, M. A., Chillingworth, T., Feltwell, T., Fraser, A., Goodhead, I., Hance, Z., Jagels, K., Larke, N., Maddison, M., Moule, S., Mungall, K., Norbertczak, H., Rabinowitsch, E., Sanders, M., Simmonds, M., White, B., Whithead, S., & Parkhill, J. (2007). Genome sequence of a proteolytic (Group I) *Clostridium botulinum* strain Hall A and comparative analysis of the clostridial genomes. *Genome research*, Vol. 17, No. 7, (July 2007), pp. 1082-1092, ISSN 088-9051
- Shen, A., Kamp, H. D., Gründling, A., & Higgins, D. E. (2006). A bifunctional O-GlcNAc transferase governs flagellar motility through anti-repression. *Genes and development*, Vol. 20, No. 23, (December 2006), pp. 3283-3295, ISSN 0890-9369
- Shigematsu, M., Meno, Y., Misumi, H., & Amako, K. (1995). The measurement of swimming velocity of *Vibrio cholerae* and *Pseudomonas aeruginosa* using the video tracking methods. *Microbiology and immunology*, Vol. 39, No. 10, pp. 741-744, ISSN 0385-5600
- Sleytr, U. B. & Thorne, K. J. (1976). Chemical characterization of the regularly arranged surface layers of *Clostridium thermosaccharolyticum* and *Clostridium thermohydrosulfuricum*. *Journal of Bacteriology*, Vol. 126, No. 1, (April 1976), pp. 377-383, ISSN 0021-9193



- Sleytr, U. B. (1975). Heterologous reattachment of regular arrays of glycoproteins on bacterial surfaces. *Nature*, Vol. 257, No. 5525, (October 1975), pp. 400–402, ISSN 0028-0836
- Sockett, H., Yamaguchi, S., Kihara, M., Irikura, V. M. & Macnab, R. M. (1992). Molecular analysis of the flagellar switch protein FliM of *Salmonella typhimurium*. *Journal of Bacteriology*, Vol. 174, No. 3, (February 1992), pp. 793–806, ISSN 0021-9193
- Steine, K., Novotny, R., Patel, K., Vinogradov, E., Whitfield, C., Valvano, M. A., Messner, P., & Schäffer, C. (2007). Functional characterization of the initiation enzyme of S-layer glycoprotein glycan biosynthesis in *Geobacillus stearothermophilus* NRS 2004/3a. *Journal of Bacteriology*, Vol. 189, No. 7, (April 2007), pp. 2590–2598, ISSN 0021-9193
- Sun, Y., Wen, J., Wu, M., Chen, H., & He, C. (2009). Deletion mutation of *rbfC<sub>xoo</sub>*, encoding a putative glycosyltransferase in *Xanthomonas oryzae* pv. *oryzae* leads to enhanced virulence expression. *Acta microbiologica Sinica*, Vol. 49, No. 6, (June 2009), pp. 740–745, ISSN 0001-6209
- Szymanski, C. M. & Wren, B. W. (2005). Protein glycosylation in bacterial mucosal pathogens. *Nature reviews. Microbiology*, Vol. 3, No. 3, (March 2005), pp. 225–237, ISSN 1740-1526
- Szymanski, C. M., Burr, D. H., & Guerry, P. (2002). *Campylobacter* protein glycosylation affects host cell interactions. *Infection and immunity*, Vol. 70, No. 4, (April 2002), pp. 2242–2244, ISSN 0019-9567
- Tabei, S. M., Hitchen, P. G., Day-Williams, M. J., Merino, S., Vart, R., Pang, P. C., Horsburgh, G. J., Viches, S., Wilhelms, M., Tomás, J. M., Dell, A., & Shaw, J. G. (2009). An *Aeromonas caviae* genomic island is required for both O-antigen lipopolysaccharide biosynthesis and flagellin glycosylation. *Journal of Bacteriology*, Vol. 191, No. 8, (April 2009), pp. 2851–2863, ISSN 0021-9193
- Taguchi, F., Shibata, S., Suzuki, T., Ogawa, Y., Aizawa, S., Takeuchi, K., & Ichinose, Y. (2008). Effects of glycosylation on swimming ability and flagellar polymorphic transformation in *Pseudomonas syringae* pv. *tabaci* 6605. *Journal of Bacteriology*, Vol. 190, No. 2, (January 2008), pp. 764–768, ISSN 0021-9193
- Taguchi, F., Shimizu, R., Inagaki, Y., Toyoda, K., Shiraishi, T., & Ichinose, Y. (2003). Post-translational modification of flagellin determines the specificity of HR induction. *Plant and cell physiology*, Vol. 44, No. 3, (March 2003), pp. 342–349, ISSN 0032-0781
- Taguchi, F., Takeuchi, K., Katoh, E., Murata, K., Suzuki, T., Marutani, M., Kawasaki, T., Eguchi, M., Katoh, S., Kaku, H., Yasuda, C., Inagaki, Y., Toyoda, K., Shiraishi, T., & Ichinose, Y. (2006). Identification of glycosylation genes and glycosylated amino acids of flagellin in *Pseudomonas syringae* pv. *tabaci*. *Cellular Microbiology*, Vol. 8, No. 6, (June 2006), pp. 923–938, ISSN 1462-5814
- Taguchi, F., Yamamoto, M., Ohnishi-Kameyama, M., Iwaki, M., Yoshida, M., Ishii, T., Konishi, T., & Ichinose, Y. (2010). Defects in flagellin glycosylation affect the virulence of *Pseudomonas syringae* pv. *tabaci* 6605. *Microbiology*, Vol. 156, No. Pt 1, (January 2010), pp. 72–80, ISSN 1350-0872

- Takeuchi, K., Ono, H., Yoshida, M., Ishii, T., Katoh, E., Taguchi, F., Miki, R., Murata, K., Kaku, H., & Ichinose, Y. (2007). Flagellin glycans from two pathovars of *Pseudomonas syringae* contain rhamnose in D and L configurations in different ratios and modified 4-amino-4,6-dideoxyglucose. *Journal of Bacteriology*, Vol. 189, No. 19, (October 2007), pp. 6945-6956, ISSN 0021-9193
- Takeuchi, K., Taguchi, F., Inagaki, Y., Toyoda, K., Shiraishi, T., & Ichinose, Y. (2003). Flagellin glycosylation island in *Pseudomonas syringae* pv. *glycinea* and its role in host specificity. *Journal of Bacteriology*, Vol. 185, No. 22, (November 2003), pp. 6658-6665, ISSN 0021-9193
- Thibault, P., Logan, S. M., Kelly, J. F., Brisson, J. R., Ewing, C. P., Trust, T. J., & Guerry, P. (2001). Identification of the carbohydrate moieties and glycosylation motifs in *Campylobacter jejuni* flagellin. *The Journal of biological chemistry*, Vol. 276, No. 37, (September 2001), pp. 34862-34870, ISSN 0021-9258
- Twine, S. M., Reid, C. W., Aubry, A., McMullin, D. R., Fulton, K. M., Austin, J., & Logan, S. M. (2009). Motility and flagellar glycosylation in *Clostridium difficile*. *Journal of Bacteriology*, Vol. 191, No. 22, (November 2009), pp. 7050-7062, ISSN 0021-9193
- Twine, S. M., Paul, C. J., Vinogradov, E., McNally, D. J., Brisson, J. R., Mullen, J. A., McMullin, D. R., Jarrell, H. C., Austin, J. W., Kelly, J. F., & Logan, S. M. (2008). Flagellar glycosylation in *Clostridium botulinum*. *The FEBS journal*, Vol. 275, No. 17, (September 2008), pp. 4428-4444, ISSN 742-464X
- VanDyke, D. J., Wu, J., Logan, S. M., Kelly, J. F., Mizuno, S., Aizawa, S., & Jarrell, K. F. (2009). Identification of genes involved in the assembly and attachment of a novel flagellin N-linked tetrasaccharide important for motility in the archaeon *Methanococcus maripaludis*. *Molecular Microbiology*, Vol. 72, No. 3, (May 2009), pp. 633-644, ISSN 0950-382X
- Varki, A. (1993). Biological roles of oligosaccharides: all of the theories are correct. *Glycobiology*, Vol. 3, No. 2, (April 1993), pp. 97-130, ISSN 0959-6658
- Verma, A., Schirm, M., Arora, S. K., Thibault, P., Logan, S. M., & Ramphal, R. (2006). Glycosylation of b-Type flagellin of *Pseudomonas aeruginosa*: structural and genetic basis. *Journal of Bacteriology*, Vol. 188, No. 12, (June 2006), pp. 4395-4403, ISSN 0021-9193
- Voisin, S., Houlston, R. S., Kelly, J., Brisson, J. R., Watson, D., Bardy, S. L., Jarrell, K. F., & Logan, S. M. (2005). Identification and characterization of the unique N-linked glycan common to the flagellins and S-layer glycoprotein of *Methanococcus voltae*. *The Journal of biological chemistry*, Vol. 280, No. 17, (April 2005), pp. 16586-16593, ISSN 0021-9258
- Wacker, M., Linton, D., Hitchen, P. G., Nita-Lazar, M., Haslam, S. M., North, S. J., Panico, M., Morris, H. R., Dell, A., Wren, B. W., & Aebi, M. (2002). N-linked glycosylation in *Campylobacter jejuni* and its functional transfer into *E. coli*. *Science*, Vol. 298, No. 5599, (November 2002), pp. 1790-1793, ISSN 0036-8075

- Weerapana, E. & Imperiali, B. (2006). Asparagine-linked protein glycosylation: from eukaryotic to prokaryotic systems. *Glycobiology*, Vol. 16, No. 9, (June 2006), pp. 91R-101R, ISSN 0959-6658
- Westerlund-Wikström, B. (2000). Peptide display on bacterial flagella: principles and applications. *International journal of medical Microbiology*, Vol. 290, No. 3, (July 2000), pp. 223-230, ISSN 1438-4221
- Wieland, F., Paul, G., & Sumper, M. (1985). *Halobacterial* flagellins are sulfated glycoproteins. *The Journal of biological chemistry*, Vol. 260, No. 28, (December 1985), pp. 15180-15185, ISSN 0021-9258
- Woods, R. D., Takahashi, N., Aslam, A., Pleass, R. J., Aizawa, S.-I., & Sockett, R. E. (2007). Bifunctional nanotube scaffolds for diverse ligands are purified simply from *Escherichia coli* strains coexpressing two functionalized flagellar genes. *Nano letters*, Vol. 7, No. 6, (June 2007), pp. 1809-1816, ISSN 1530-6984
- Wu, L., Wang, J., Tang, P., Chen, H., & Gao, H. (2011). Genetic and molecular characterization of flagellar assembly in *Shewanella oneidensis*. *PloS one*, Vol. 6, No. 6, pp. e21479, ISSN 1932-6203
- Wyss, C. (1998). Flagellins, but not endoflagellar sheath proteins, of *Treponema pallidum* and of pathogen-related oral spirochetes are glycosylated. *Infection and immunity*, Vol. 66, No. 12, (December 1998), pp. 5751-5754, ISSN 0019-9567
- Xu, J., Platt, T. G., & Fuqua, C. (2012). Regulatory linkages between flagella and surfactant during swarming behavior: lubricating the flagellar propeller? *Journal of Bacteriology*, Vol. 194, No. 6, (March 2012), pp. 1283-1286, ISSN 0021-9193
- Yonekura, K., Maki-Yonekura, S., & Namba, K. (2005). Building the atomic model for the bacterial flagellar filament by electron cryomicroscopy and image analysis. *Structure*, Vol. 13, No. 3, (March 2005), pp. 407-412, ISSN 0969-2126
- Yonekura, K., Maki-Yonekura, S., & Namba, K. (2003). Complete atomic model of the bacterial flagellar filament by electron cryomicroscopy. *Nature*, Vol. 424, No. 6949, (August 2003), pp. 643-650, ISSN 0028-0836
- Yoshioka, K., Aizawa, S.-I., & Yamaguchi, S. (1995). Flagellar filament structure and cell motility of *Salmonella typhimurium* mutants lacking part of the outer domain of flagellin. *Journal of Bacteriology*, Vol. 177, No. 4, (February 1995), pp. 1090-1093, ISSN 0021-9193
- Young, N. M., Brisson, J. R., Kelly, J., Watson, D. C., Tessier, L., Lanthier, P. H., Jarrell, H. C., Cadotte, N., St Michael, F., Aberg, E., & Szymanski, C. M. (2002). Structure of the N-linked glycan present on multiple glycoproteins in the Gram-negative bacterium, *Campylobacter jejuni*. *The Journal of biological chemistry*. Vol. 277, No. 45, (November 2002), pp. 42530-43549, ISSN 0021-9258
- Zampronio, C. G., Blackwell, G., Penn, C. W., & Cooper, H. J. (2011). Novel glycosylation sites localized in *Campylobacter jejuni* flagellin FlaA by liquid chromatography electron capture dissociation tandem mass spectrometry. *Journal of proteome research*, Vol. 10, No. 3, (March 2011), pp. 1238-1245, ISSN 1535-3893

Zhang, W. J., Santini, C. L., Bernadac, A., Ruan, J., Zhang, S. D., Kato, T., Li, Y., Namba, K., & Wu, L. F. (2012). Complex spatial organization and flagellin composition of flagellar propeller from marine magnetotactic ovoid strain MO-1. *Journal of molecular biology*, Vol. 416, No. 4, (March 2012), pp. 558-570, ISSN 0022-2836

IntechOpen

IntechOpen