

Identification of a Functional Type VI Secretion System in *Campylobacter jejuni* Conferring Capsule Polysaccharide Sensitive Cytotoxicity

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Abstract

The pathogen *Campylobacter jejuni* is the principal cause of bacterial food-borne infections. The mechanism(s) that contribute to bacterial survival and disease are still poorly understood. In other bacterial species, type VI secretion systems (T6SS) are increasingly recognized to contribute to bacterial pathogenesis by toxic effects on host cells or competing bacterial species. Here we report the presence of a functional Type VI secretion system in *C. jejuni*. Proteome and genetic analyses revealed that *C. jejuni* strain 108 contains a 17-kb T6SS gene cluster consisting of 13 T6SS-conserved genes, including the T6SS hallmark genes *hcp* and *vgrG*. The cluster lacks an ortholog of the ClpV ATPase considered important for T6SS function. The sequence and organization of the *C. jejuni* T6SS genes resemble those of the T6SS located on the HHG1 pathogenicity island of *Helicobacter hepaticus*. The *C. jejuni* T6SS is integrated into the earlier acquired *Campylobacter* integrated element CJIE3 and is present in about 10% of *C. jejuni* isolates including several isolates derived from patients with the rare clinical feature of *C. jejuni* bacteremia. Targeted mutagenesis of *C. jejuni* T6SS genes revealed T6SS-dependent secretion of the Hcp needle protein into the culture supernatant. Infection assays provided evidence that the *C. jejuni* T6SS confers contact-dependent cytotoxicity towards red blood cells but not macrophages. This trait was observed only in a capsule-deficient bacterial phenotype. The unique *C. jejuni* T6SS phenotype of capsule-sensitive contact-mediated hemolysis represents a novel evolutionary pathway of T6SS in bacteria and expands the repertoire of virulence properties associated with T6SS.

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Introduction

Gram-negative bacteria have evolved at least six types of protein secretion systems (type I–VI) to export proteins to the periplasmic space or the environment [1]. Several secretion systems are composed of needle-like structures that span the bacterial cell wall and protrude from the cell surface. These nanomachines include the classical type III and type IV secretion apparatus involved in the injection of bacterial proteins into eukaryotic cells. One more recently discovered bacterial needle structure is the type VI secretion system (T6SS) as originally described for *Vibrio cholerae* and *Pseudomonas aeruginosa* [2,3]. Today whole genome analyses have predicted T6SS gene clusters to be present in more than 100 Gram-negative bacterial species. These gene clusters often have of a variable composition but typically contain at least 13 core genes that encode the basic elements of the injection apparatus [4–6].

Structurally the T6SS consists of a membrane-associated assembly platform and a needle structure that transports effector molecules into neighboring bacteria or eukaryotic cells. A number of the T6SS core proteins show similarity to elements of tailed

bacteriophages. Examples are the baseplate gp-25-like protein VCA109, the tail sheath-like proteins TssB and TssC (VipA/VipB), the tail subunit-like hemolysin co-regulated protein (Hcp) that polymerizes into the T6SS needle structure, and the valine-glycine repeat protein (VgrG) that forms the spike of the nanotube [4–6]. The structural similarity with bacteriophage proteins has led to the hypothesis that T6SS resemble an inverted bacteriophage tail structure that is exposed at the surface of the bacterial cell wall [7,8]. Recently, contraction and extension of the VipA/B tubular sheath of the T6SS of *V. cholerae* have been visualized *in vivo*, supporting the model that the T6SS sheath is a dynamic contractile structure that projects the T6SS spike into the target cell analogous to bacteriophage entry [9,10]. Disassembly of the contracted sheath requires the T6SS ClpV ATPase [9,11,12]. Another group of T6SS building blocks (TssM-L) seems related to proteins of the type IV secretion system (i.e. IcmF and IcmH/DotU) [13,14]. These proteins may be involved in the recruitment of Hcp to the T6SS inner membrane assembly platform [15].

The hallmark of a functional T6SS is the presence of Hcp and VgrG in the culture supernatant [3,16–18]. Both proteins may