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Original Article

Lighting up my life: a LOV-based fluorescent reporter for *Campylobacter jejuni*

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Abstract

In this study, a LOV-based fluorescent reporter (light, oxygen, or voltage-sensing domains of phototropin), termed iLOV, was adapted for *Campylobacter jejuni* and used to investigate promoter activity via monitoring fluorescence intensity and to study the localisation of two chemotaxis proteins. The pC46 complementation vector contains coding sequence from *cj0046*, a *C. jejuni* NCTC11168 pseudo-gene and is used to integrate cloned genes onto the *C. jejuni* chromosome. The pC46 vector was used to construct plasmids containing iLOV, driven by three different *C. jejuni* constitutive promoters and plasmids containing transcriptional fusions of the iLOV reporter and two chemoreceptors, *tlp5* and *tlp8*. Expression from the *porA* promoter, p*porA*, produced the highest fluorescence signals compared to p*fdxA* (intermediate level) and p*metK* (lowest level). The cellular localisation pattern of transducer-like protein (Tlp) clusters, containing Tlp5 and Tlp8, was predominately polar, with Tlp5 positioned only at one and Tlp8 at both poles. Here, we demonstrate that a iLOV fluorescent reporter can be used as a promoter probe or as a gene fusion reporter in *Campylobacter* spp. This is a new system uniquely placed for studying *Campylobacter* spp., as it combines resistance to photobleaching and functionality under microaerobic conditions.

Keywords: *Campylobacter jejuni*; Chemotaxis; (FMN)-based fluorescent protein; Protein localisation

1. Introduction

LOV-based fluorescent protein (LOV-based FP) or flavin-based fluorescent protein (FbFPs) reporters have been used in vivo and in vitro to study promoter activity, protein expression and protein localisation [1,2]. LOV-based FPs belong to a highly conserved family of photoreceptors known as light, oxygen, or voltage-sensing proteins [1,3,4]. LOV-based FPs can be utilised under aerobic or anaerobic conditions in a wide variety of microbes, including *Bacteroides fragilis*, *Clostridium cellulolyticum*, *Candida albicans*, *Escherichia coli*, *Rhodobacter capsulatum* and *Saccharomyces cerevisiae* [3–6]. A promising reporter, previously developed from the LOV-flavoprotein domain of *Arabidopsis thaliana* phototropin [7,8], is structurally comprised of two regions, an N-terminal photosensory domain (LOV1 or LOV2), which acts by binding a flavin mononucleotide (FMN), and a C-terminal output serine/threonine kinase domain. Both LOV domains belong to a larger receptor family known as PAS (Per-ARNT-Sim) receptors, due to their association with a co-factor binding [1,9]. The mechanism of photocycle activation involves the FMN non-covalent dark state changing to the covalent state upon blue light irradiation. When the covalent bond is broken, the molecule is reverted to the dark state (photo-bleaching) and the reaction can be regarded as a reversible photocycle [10,11]. The iLOV polypeptide is an improved LOV2 domain with increased photostability [12]. The advantages of the iLOV reporter [1] compared to GFP include smaller size (11 kDa compared to ~25 kDa for GFP), thermal stability, stability over a wide pH range and spontaneous recovery from photobleaching by ultraviolet (UV) light exposure

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Table 1
List of bacterial strains used in this study.

Name of strain	Relevant genotype	Source
Campylobacter jejuni NCTC11168	Wild-type	National Collection of Type Culture, Colindale, London, UK
Escherichia coli DH5αe		Invitrogen (Paisley, UK).
C. jejuni M-iLOV46	P*metK*-iLOV::*cj0046* (Cm^R)	This study
C. jejuni F-iLOV46	P*fdxA*-iLOV::*cj0046* (Cm^R)	This study
C. jejuni P-iLOV46	P*porA*-iLOV::*cj0046* (Cm^R)	This study
C. jejuni F-Tlp8-iLOV	(P*fdxA-cj1110*::iLOV)::*cj0046* (Km^R)	This study
C. jejuni P-Tlp8-iLOV	(P*porA*-cj1110::iLOV)::*cj0046* (Km^R)	This study
C. jejuni F-Tlp5-iLOV	(P*fdxA-cj0246*::iLOV)::*cj0046* (Km^R)	This study
C. jejuni P-Tlp5-iLOV	(P*porA-cj0246*::iLOV)::*cj0046* (Km^R)	This study

Cm^R, chloramphenicol-resistant, Km^R, kanamycin-resistant.

[13,14]. Importantly, iLOV is an oxygen-independent fluorescent reporter, making it ideally suited to anaerobes and microaerobes [11,12], including *Campylobacter jejuni*. These attributes make this reporter easy to use for the expression of fluorescent-labelled proteins and to track protein distribution in living cells.

The components of the bacterial chemotaxis system include chemoreceptors that sense attractant and repellent signals and a phosphorelay-based signal transduction system, capable of sensory adaptation, that relays the signal response to the flagellar apparatus [15]. Chemoreceptors are inner membrane proteins that direct cellular movement in response to changing environmental conditions by responding to periplasmic ligands. However, some chemoreceptor proteins do not have periplasmic domains or any apparent membrane linkage, and may respond to cytoplasmic signals [15]. In most species, chemoreceptor proteins and associated signal transduction proteins (Che) localise to the poles of the cell [16–22]. Initial reports of Tlp1 (Transducer-like protein) localisation suggest that this is also likely to be the case in *C. jejuni* [23]. Here we describe the localisation of two *C. jejuni* cytoplasmic Tlps, Tlp5 and Tlp8, using a new iLOV reporter system. Our objective was to develop a system for expressing iLOV-labelled chemoreceptor proteins in *C. jejuni*. Such a system enables the tracking of Tlp distribution and determination of cellular localisation and will contribute to our understanding of the roles of different Tlp proteins in *C. jejuni* chemotactic responses.

2. Materials and methods

2.1. Bacterial strains and culture conditions

C. jejuni NCTC11168 (National Collection of Type Culture, Colindale, London, UK) was used in this study (Table 1) [24]. *C. jejuni* cells were grown at 42 °C microaerobically

Table 2
List of plasmids used in this study.

Plasmid	Description	Source
pKmetK46, pKfdxA46 and pKporA46	Vector with *metK*, *fdxA* or porA promoter and *aph3* (Km^R) flanked by *cj0046* sequence.	Dr. R. D. Haigh
pCmetK46, pCfdxA46 and pCporA46	Vector with *metK*, *fdxA* or porA promoter and *cat* (Cm^R) by *cj0046* sequence.	Dr. R. D. Haigh
pET28a-iLOV	pET28a containing fluorescent reporter iLOV	Dr. Anthony Buckley
pM-iLOV46	iLOV gene from pET28a-iLOV cloned into *Esp*3I site of pCmetK46 (Cm^R). iLOV is under the control of P*metK* and flanked by 5′ and 3′ sequences of *cj0046*.	This study
pF-iLOV46	iLOV gene from pET28a-iLOV cloned into *Esp*3I site of pCfdxA46 (Cm^R). iLOV is under the control of P*fdxA* and flanked by 5′ and 3′ sequences of *cj0046*	This study
pP-iLOV46	iLOV gene from pET28a-iLOV cloned into *Esp*3I site of pCporA46 (Cm^R). iLOV is under the control of P*PorA* and flanked by 5′ and 3′ sequences of *cj0046*	This study
pP-tlp5iLOV46	*cj0246c* (*tlp5*) with a 3′ fusion to iLOV gene cloned into pKporA46. *Tlp5*::iLOV is under the control of P*porA* and flanked by 5′ and 3′ sequences of *cj0046*. (Km^R)	This study
pF-tlp5iLOV46	*cj0246c* (*tlp5*) with a 3′ fusion to iLOV gene cloned into pKfdxA46. *Tlp5*::iLOV is under the control of P*fdxA* and flanked by 5′ and 3′ sequences of *cj0046*. (Km^R)	This study
pP-tlp8iLOV46	*cj1110c* (*tlp8*) with a 3′ fusion to iLOV gene cloned into pKporA46. *Tlp8*::iLOV is under the control of P*porA* and flanked by 5′ and 3′ sequences of *cj0046*. (Km^R)	This study
pF-tlp8iLOV46	*cj1110c (tlp8)* with a 3′ fusion to iLOV gene cloned into pKfdxA46. *Tlp8*::iLOV is under the control of P*fdxA* and flanked by 5′ and 3′ sequences of *cj0046*. (Km^R)	This study

Cm^R, chloramphenicol-resistant, Km^R, kanamycin-resistant.