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Construction of an inducible expression shuttle vector for *Laribacter hongkongensis*, a novel bacterium associated with gastroenteritis

Patrick C.Y. Woo [a,b], Shirley S.L. Ma [a], Jade L.L. Teng [a], Maria W.S. Li [a], Richard Y.T. Kao [a,b], Susanna K.P. Lau [a,b], Kwok-yung Yuen [a,b,*]

[a] *Department of Microbiology, Faculty of Medicine, The University of Hong Kong, Queen Mary Hospital Compound, Pokfulam Road, Hong Kong*
[b] *Research Centre of Infection and Immunology, Faculty of Medicine, The University of Hong Kong, Hong Kong*

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Abstract

An *Escherichia coli–Laribacter hongkongensis* shuttle vector (pPW380) was constructed by ligating the 4701-bp *Eco*RI digested fragment of pHLHK8 to *Eco*RI digested pBK-CMV. An *E. coli–L. hongkongensis* inducible expression shuttle vector was further constructed by ligating a 2105-bp fragment that contains the tetracycline repressor and tetracycline-inducible promoter region of pALC2084 to the 8897-bp fragment of pPW380, deletion of the green fluorescent protein gene, and insertion of a multiple cloning site. This inducible expression system was able to express two commonly used reporter genes, the green fluorescent protein gene and the glutathione *S*-transferase gene, efficiently in *E. coli* and *L. hongkongensis*.

Keywords: *Laribacter hongkongensis*; Inducible; Expression; Shuttle vector

1. Introduction

Laribacter hongkongensis, a novel genus and species, was first discovered in Hong Kong in 2001 from the blood and empyema pus of a 54-year old Chinese man with alcoholic cirrhosis and bacteremic empyema thoracis [1]. Phenotypically, it is a facultative anaerobic, motile, non-sporulating, urease-positive, Gram-negative, S-shaped bacillus. Genotypically, by phylogenetic analysis using 16S rRNA gene sequences, *L. hongkongensis* belongs to the Neisseriaceae family of the β-subclass of Proteobacteria. Since the patient's underlying liver cirrhosis and ascites suggested that the gastrointestinal tract might be a possible primary site of infection, *L. hongkongensis* was intensively sought in fecal specimens of patients with gastroenteritis. During a period of two months, *L. hongkongensis* was discovered, on charcoal cefoperazone deoxycholate agar, in three of our patients with community-acquired gastroenteritis [2]. A similar finding was also observed in three other patients in Switzerland [2]. In a recent multi-centered prospective study using cefoperazone MacConkey agar as the selective medium [3], we confirmed that *L. hongkongensis* is associated with community-acquired gastroenteritis and traveler's diarrhea [4]. Furthermore, freshwater fish was also confirmed to be a reservoir of *L. hongkongensis* [4,5]. By comparing the pulsed-field gel electrophoresis patterns of fish and patient isolates, it was observed that most patient isolates were clustered

* Corresponding author. Tel.: +852 2855 4892; fax: +852 2855 1241.
E-mail address: hkumicro@hkucc.hku.hk (K.-y. Yuen).

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together, suggesting that some clones could be more virulent than others [5]. The isolation of *L. hongkongensis* from patients who resided in or have recent travel histories to Asia, Europe, America, and Africa implied that the bacterium is likely to be of global importance. Recently, a class C β-lactamase gene of *L. hongkongenis*, responsible for its resistance to multiple β-lactam antibiotics, was cloned and characterized [6].

Development of genetic manipulation systems is of paramount importance in the study of pathogenesis and virulence factors in *L. hongkongensis*. In this study, a small plasmid in one of the isolates was completely sequenced and characterized. Using this plasmid, an *Escherichia coli–L. hongkongensis* inducible expression shuttle vector was constructed.

2. Materials and methods

2.1. Strains and plasmids

The bacterial strains and plasmids used in this study are shown in Table 1. All *L. hongkongensis* strains used in this study were clinical isolates from patients in Hong Kong [1,2,4]. pCL52.2, a temperature sensitive *E. coli–Staphylococcus aureus* shuttle vector and pALC2084, an *E. coli–S. aureus* shuttle vector with a tetracycline-inducible green fluorescent protein (GFP) gene, were gifts from Cheung [7,8].

2.2. Plasmid extraction and sequencing and in silico analysis of pHLHK8

Extraction of plasmids of <20 kb in *L. hongkongensis* strains was performed using the High Pure Plasmid Isolation kit (Roche Applied Science) according to manufacturer's instructions. The extracted plasmids were electrophoresed in 1.0% (w/v) agarose gel, with molecular size markers, Lambda DNA *Eco*471 Digest Marker (Roche Applied Science) and Lambda DNA *Hin*dIII Digest Marker (Roche Applied Science), in parallel. Electrophoresis in Tris-borate-EDTA (TBE) buffer was performed at 100 V for 45 min. The gel was stained with ethidium bromide (0.5 μg ml^{-1}) for 15 min, rinsed and photographed under ultraviolet light illumination.

pHLHK8, the 8266-bp plasmid of *L. hongkongensis* strain HLHK8, was digested with *Cla*I and *Eco*RI and the respective fragments were cloned into pBSKII(−). Both strands of the fragments were sequenced twice with an ABI 3700 DNA analyzer according to manufacturers' instructions (Applied Biosystems), using primers T7 and T3 and additional primers designed from the first and second rounds of the sequencing reactions. The nucleotide and deduced amino acid sequences of the open reading frames of pHLHK8 were compared with sequences in the GenBank. Direct and inverted repeats were determined using dotmatcher (EMBOSS-GUI).

Table 1
Bacterial strains and plasmids

Organism/plasmid	Feature	Source/reference
Strains		
Laribacter hongkongensis HKU1	Type strain	[1]
Laribacter hongkongensis HLHK8	Human strain isolated from patients with community-acquired gastroenteritis in Hong Kong. Origin of pHLHK8	[4]
Laribacter hongkongensis HLHK9	Human strain isolated from patients with community-acquired gastroenteritis in Hong Kong. Used in GFP and GST expression experiments	[4]
Laribacter hongkongensis HLHK2-4, 10–24	Human strains isolated from patients with community-acquired gastroenteritis in Hong Kong	[2,4]
Escherichia coli DH5α	F^-, Φ80d *lac*ZΔM15, Δ(*lac*ZYA-argF)U169, *end*A1, *rec*A1, *hsd*R17(rK-mK+) *deo*R, *thi*-1, *sup*E44, λ^-, *gyr*A96(Nal^r), *rel*A1	Invitrogen
Plasmids		
pHLHK8	Plasmid isolated from HLHK8	This study
pBSKII(−), Ap^r	Cloning vector	Stratagene
pBK-CMV, Km^r	Cloning vector	Stratagene
pCL52.2, Ap^r	Temperature sensitive *E. coli–S. aureus* shuttle vector	[8]
pBR322, Ap^r Tc^r	Cloning vector	Promega
pUC19, Ap^r	Cloning vector	Invitrogen
pALC2084, Ap^r	*E. coli–S. aureus* shuttle vector with a tetracycline-inducible GFP gene	[7]
pPW380, Km^r	pHLHK8 replicon + pBK-CMV	This study
pPW632, Km^r	pALC2084 + *Sph*I–*Aat*II adaptor	This study
pPW576, Km^r	*Aat*II fragment of pPW632 + pPW380	This study
pPW633, Km^r	pPW576–GFP	This study
pPW578, Km^r	pPW633+ multiple cloning site	This study
pGEX-2T, Ap^r	GST gene fusion vector	Amersham Bioscience
pPW585, Km^r	pPW578 + GST	This study