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Characterization of a single-chain variable fragment specific to *Cronobacter* spp. from hybridoma based on outer membrane protein A

Qiming Chen [a], Tingting Tao [a], Xiaomei Bie [a], Fengxia Lu [a], Yuanhong Li [a,b], Zhaoxin Lu [a,*]

[a] College of Food Science and Technology, Nanjing Agricultural University, 1 Weigang, Nanjing 210095, People's Republic of China
[b] Department of Public Health, Xuzhou Medical College, 209 Tongshan Road, Xuzhou, Jiangsu 221004, People's Republic of China

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ABSTRACT

Monoclonal antibody and polyclonal antibody specific to *Cronobacter* spp. had been reported in previous studies. However, the preparation of single-chain variable fragment (scFv) was faster and convenient. Hence, the aim of this study was to construct a scFv using outer membrane protein A (OmpA) of *C. sakazakii* as antigen. The protein sequences of OmpA of *Cronobacter* spp. were analyzed first. The results showed protein OmpA with length of 347 amino acids was conserved in *Cronobacter* genus (94.83%–100% of protein identity) and was greater than that observed for the other genera tested (8.28–91.64% of protein identity). Then, purified protein OmpA expressed in *E. coli* was used to prepare hybridoma and to construct scFv further. The scFv was named scFvH81 and analyzed by bioinformatics. The model of scFvH81 built by homologous modeling had a good quality (residues in disallowed regions: 3%) and showed that scFvH81 had a standard pocket-like site. Purified scFvH81 was prepared by denaturation and renaturation of inclusion body and it showed a good specificity and its affinity of $Ka = 2.39 \times 10^6\ M^{-1}$. Therefore, it could be used in the detection and the pathogenesis study of *Cronobacter* spp.

1. Introduction

Cronobacter spp. is Gram-negative, rod-shaped, foodborne opportunistic pathogens of the family *Enterobacteriaceae* (Nazarowec-White and Farber, 1997). They can cause necrotizing enterocolitis, sepsis, and meningitis (Friedemann, 2009, Holy and Forsythe, 2014, Stoll et al., 2004), and the fatality rate of the infection can be 50–80% (Healy et al., 2010, Townsend et al., 2008). *Cronobacter* spp. had been isolated from a range of food sources (Friedemann, 2007, Joseph and Forsythe, 2012), and contaminated powdered infant formula (PIF) has been associated with many infant infection cases (Arseni et al., 1987).

Antibody-based methods have been widely used for the detection and pathogenesis studies of *Cronobacter* spp. (Blazkova et al., 2011, Chiang et al., 2012, Emami et al., 2012, Hu et al., 2013, Li et al., 2013, Park et al., 2012, Rodriguez-Emmenegger et al., 2011, Xu et al., 2014, Zhang et al., 2012a, 2012b). However, all of them were based on monoclonal and polyclonal antibody, and single-chain variable fragment (scFv) had not been used in the detection of *Cronobacter* spp. until now. ScFv is a small molecular antibody (25–30 kDa) that consists of two variable regions of the heavy and light chains (V_H and V_L) of IgG (~150 kDa) separated via a short peptide linker (Chen et al., 2014). There are a variety of ways to obtain scFv, such as cloning scFv genes from a hybridoma and isolating scFv genes from libraries by display technology (Lee et al., 2004, Molek et al., 2014, Rothe et al., 2007). ScFv has a simple and relatively small structure, hence, it is more easy to be modified to get extra features compared with monoclonal and polyclonal antibody (Duan et al., 2012, Le Gall et al., 1999, Liu et al., 2010). For the same reason, scFv can be largely produced in microorganisms such as *Escherichia coli*, *Saccharomyces cerevisiae* and *Pichia pastoris*. This makes the preparation more economical and fast. (Evans et al., 2010, Miller et al., 2005, Turki et al., 2014). ScFv is often used in the detection of micromolecules and proteins such as aflatoxin (Min et al., 2011, Moghaddam et al., 2001) and Cry1C toxin (Zhang et al., 2012a, 2012b), as well as diagnosis and treatment of cancer (Moricoli et al., 2014). However, there have been few reports concerning the use of scFv in detection of pathogen until now (Yuan et al., 2007), let alone *Cronobacter* spp. which was brought to the forefront recently.

Outer membrane protein A (OmpA) is a major outer membrane protein of gram-negative bacteria (Hellman and Warren, 2001). It is a two-domain protein composed of a eight-stranded-barrel transmembrane domain and a periplasmic domain (Maiti et al., 2011). OmpA plays an important role in the interface between the bacterial cell and the environment (Pore and Chakrabarti, 2013), as well as in the infection of *Cronobacter* spp. (Singamsetty et al., 2008). OmpA is widely distributed on the surface of *Cronobacter* and therefore it has the potential to contact with the host immune system directly (Chang et al., 2011, Hughes et al., 1992, Jeannin et al., 2000, Torres et al., 2006). All these make protein OmpA a good potential antigen for preparing antibody. As a matter of fact, a notion that is further supported by use of OmpA as a novel vaccine for bacterial infection (Chu et al., 2015, Maiti et al., 2011, Yan et al., 2010).

* Corresponding author.
E-mail address: fmb@njau.edu.cn (Z. Lu).

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Table 1
Identity of OmpA protein sequences of different bacteria.

Strains	Strain number	OmpA protein ID	Length	Protein identity (%)
Cronobacter sakazakii	ATCC 29544	ADB23432.1	347	100
Cronobacter malonaticus	DSM 18702	AGQ57144.1	347	99.71
Cronobacter universalis	NCTC 9529	AGQ57147.1	347	99.42
Cronobacter turicensis	DSM 18703	AGQ57145.1	347	99.42
Cronobacter muytjensii	ATCC 51329	AGQ57143.1	347	99.14
Cronobacter dublinensis	DSM 18705	AGQ57146.1	347	96.83
Cronobacter condimenti	LMG 26250	ALB64724.1	355	94.65
Escherichia coli	H474	AAT98590.1	346	89.05
Enterobacter cloacae	ECNIH4	AIX55461.1	359	91.64
Salmonella enterica	ATCC 35640	AHW19505.1	347	91.17
Klebsiella variicola	DX120E	AJA96499.1	358	87.19
Shigella flexneri	Shi06HN006	AIL39878.1	356	85.79
Vibrio fischeri	ES114	AAW88170.1	324	21.88
Campylobacter jejuni	NCTC 11168-rNRC	AHK58525.1	480	8.28
Shewanella baltica	OS195	ABX51262.1	202	10.63

In this study, OmpA protein sequences of *Cronobacter* species were analyzed and a recombinant from *C. sakazakii* was expressed in *E. coli* BL21 (DE3). The purified protein OmpA was used as antigen to prepare hybridoma that could secrete antibody specific to *Cronobacter* spp. ScFv genes were then cloned from the hybridoma and analyzed by bioinformatics. Finally, scFv expressed in *E. coli* was purified, the specificity and affinity of it were then tested by an indirect ELISA method developed in this study.

2. Materials and methods

2.1. Materials

Six-week-old BALB/c mice were bought from Yangzhou University (Jiangsu, China). Murine myeloma cell line SP2/0 was from our laboratory culture collection. The strains used in this study were purchased from the China Center of Industrial Culture Collection (CICC), the National Center for Medical Culture Collections (CMCC), the American Type Culture Collection (ATCC), the National Collection of Type Cultures (NCTC), and the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ).

All primers were synthesized by Sangon Biotech (Shanghai, China) and sequencing service was provided by Genscript (Nanjing, China). TRIZOL® Reagent was purchased from Invitrogen Inc. (Gaithersburg, MD, USA). SanPrep Column DNA Gel Extraction Kit and AMV First Strand cDNA Synthesis Kit were purchased from Sangon Biotech. Minitan ultrafiltration membrane with nominal molecular weight limit of 20,000 was purchased from Millipore Corp. (Bedford, MA, USA).

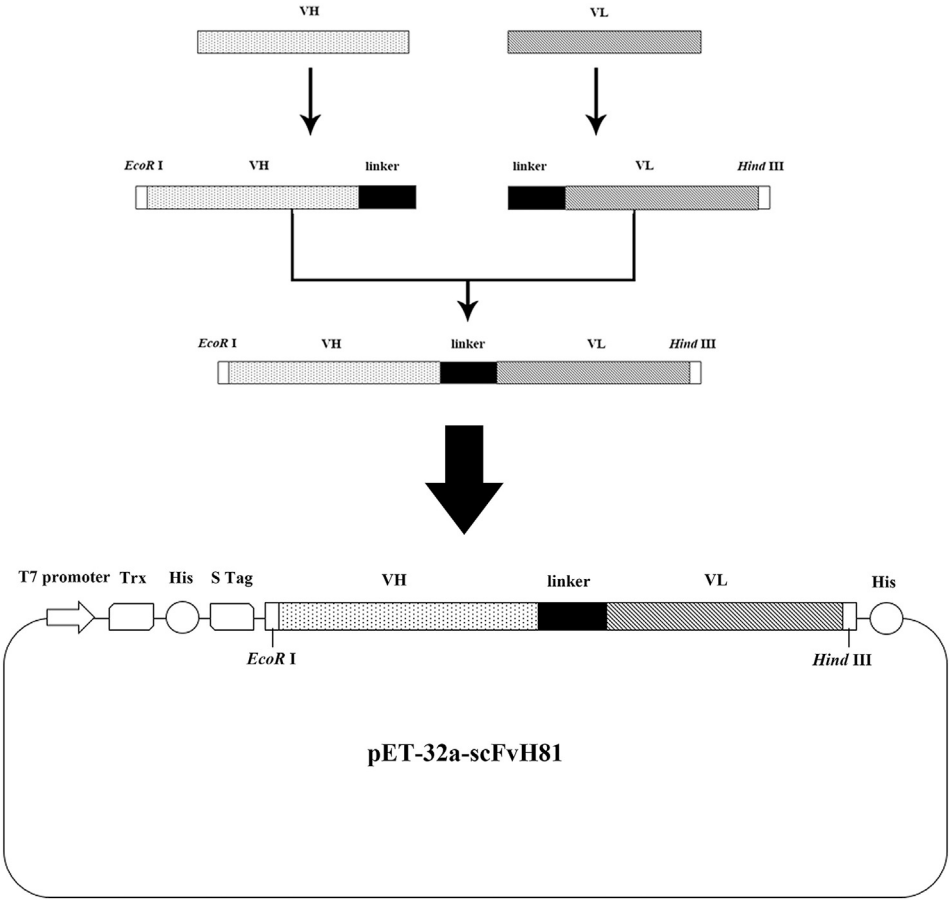

Fig. 1. Flow chart for the construction of recombinant plasmid pET-32a-scFvH81.