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A latex agglutination assay for specific detection of Panton–Valentine leukocidin

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ABSTRACT

Panton–Valentine leukocidin (PVL) is produced by some isolates of *Staphylococcus aureus*, and has been associated with the high pathogenicpotential of these strains. To rapidly detect the toxin producer strains, we developed a reverse passive latex agglutination (RPLA) reaction assay specific for PVL. By testing 64 *S. aureus* strains, the assay could detect the 35 *pvl*-gene-positive strains with 100% specificity and sensitivity. Furthermore, the assay revealed an extensive variation in the amount of PVL produced by the *pvl*-positive strains.

1. Introduction

Community-acquired, methicillin-resistant *Staphylococcus aureus* is an emerging nosocomial pathogen. (Baba et al., 2002; Bohach and Foster, 2000; Hiramatsu et al., 2002; Torell et al., 2005). Recently, highly virulent community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has emerged and markedly increased worldwide (Hiramatsu et al., 2002; Naimi et al., 2001; Okuma et al., 2002; Vandenesch et al., 2005). Among these isolates, carriage of the genes encoding Panton–Valentine leukocidin, a toxin associated with increased (Diep et al., 2004; Etienne, 2005; Gillet et al., 2002; Ma et al., 2006; Torell et al., 2005). PVL toxin is likely to be involved in severe symptoms such as necrotic pneumonia and furunculosis (Gillet et al., 2002; Lina et al., 1999; Naimi et al., 2001; Panton and Valentine, 1932; Torell et al., 2005). On the other hands, there is a report that PVL is not critically involved in high virulence but small phenol soluble modulins (Wang et al., 2007). In order to clinch the controversy, methods to detect the toxins quantitatively and easily are urgently needed.

The PVL, having cytolitic activity against human and rabbit monocytes and polymorph nuclear cells, was first reported by Panton and Valentine in 1932 (Panton and Valentine, 1932), purified in 1991 from *S. aureus* strain V8 (ATCC49775) isolated from a patient with chronic furunculosis (Finck-Barbancon et al., 1991). The toxin consists of two polypeptides, S- (slow-eluted) and F- (fast-eluted) components, based on their elution profiles through cation-exchange chromatography (Kaneko and Kamio, 2004; Noda and Kato, 1988; Woodin, 1960). The gene for the toxin was then cloned and sequenced in 1995 by Prevostet al., and was renamed as PVL (LukS-PV+LukF-PV) to distinguish it from other homologous leukotoxins with hemolytic activities (Prevost et al., 1995). The presence or absence of the *PVL* gene in clinical strains can be detected by PCR (Deurenberg et al., 2004; Johnsson et al., 2004; Lina et al., 1999; McClure et al., 2006; McDonald et al., 2005; Nakagawa et al., 2005). However, carriage of the genes for PVL toxin does not necessarily mean this toxin is expressed in these isolates, which is a potential limitation of PCR-based approaches to circumvent this limitation of PCR-based *PVL* gene detection, we developed an agglutination-based immunoassay for the PVL toxin.

2. Results

2.1. Preparation of recombinant PVL toxins

Plasmid pGEX-4T3 (GE Healthcare Bio-Sciences KK) was employed to generate glutathione S-transferase (GST) fusion recombinant toxins. The fusion construction was done with PCR-amplified *lukS-PV*, *lukF-PV*, *lukE*, *lukD*, and the three members of gamma toxin, *hlgA*, *hlgC* and *hlgB* of a CA-MRSA strain MW2 (Baba et al., 2002). The *lukE*, *hlgA*, *hlgC* are the homologs for *lukS-PV*; and *lukD* and *hlgB* are the homologs of *lukF-PV*, respectively (Clyne et al., 1992; Cooney et al., 1988, 1993; Dalla Serra et al., 2005; Gravet et al., 1998). Since the amino acid homologies among the S and F class peptides are quite high (HlgA, HlgC, and LukE are 65–77% identical to LukS-PV, and HlgB

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and LukD are 71–82% identical to LukF-PV, respectively (Cooney et al., 1993; Gravet et al., 1998; Prevost et al., 1995)), they were cloned and expressed to produce recombinant toxins to be used for the absorption of anti-PVL antisera (see below).

The specific primers used for the amplification of the *lukS-PV* and *lukF-PV* genes were S2F (5′-AAGGGATCCGATAACAATATTGAGAA-TATTGGTG-3′) and S2R (5′-AAAGGCCGTCGACTCAATTATGTCCTTTCACTT-TAATT-3′), and F2F (5′-AAGGGATCCCAACATATCACACCTGTAAGTG-3′) and F2R (5′-AAAGGCCGTCGACTTAGCTCATAGGATTTTTTTCCT-3′). They contained *Bam*HI (S2F and F2F) or *Sal*I (S2R and F2R) restriction sites (underlined). The PCR products were treated with the restriction enzymes, and cloned into the corresponding sites of pGEX-4T3 allowing

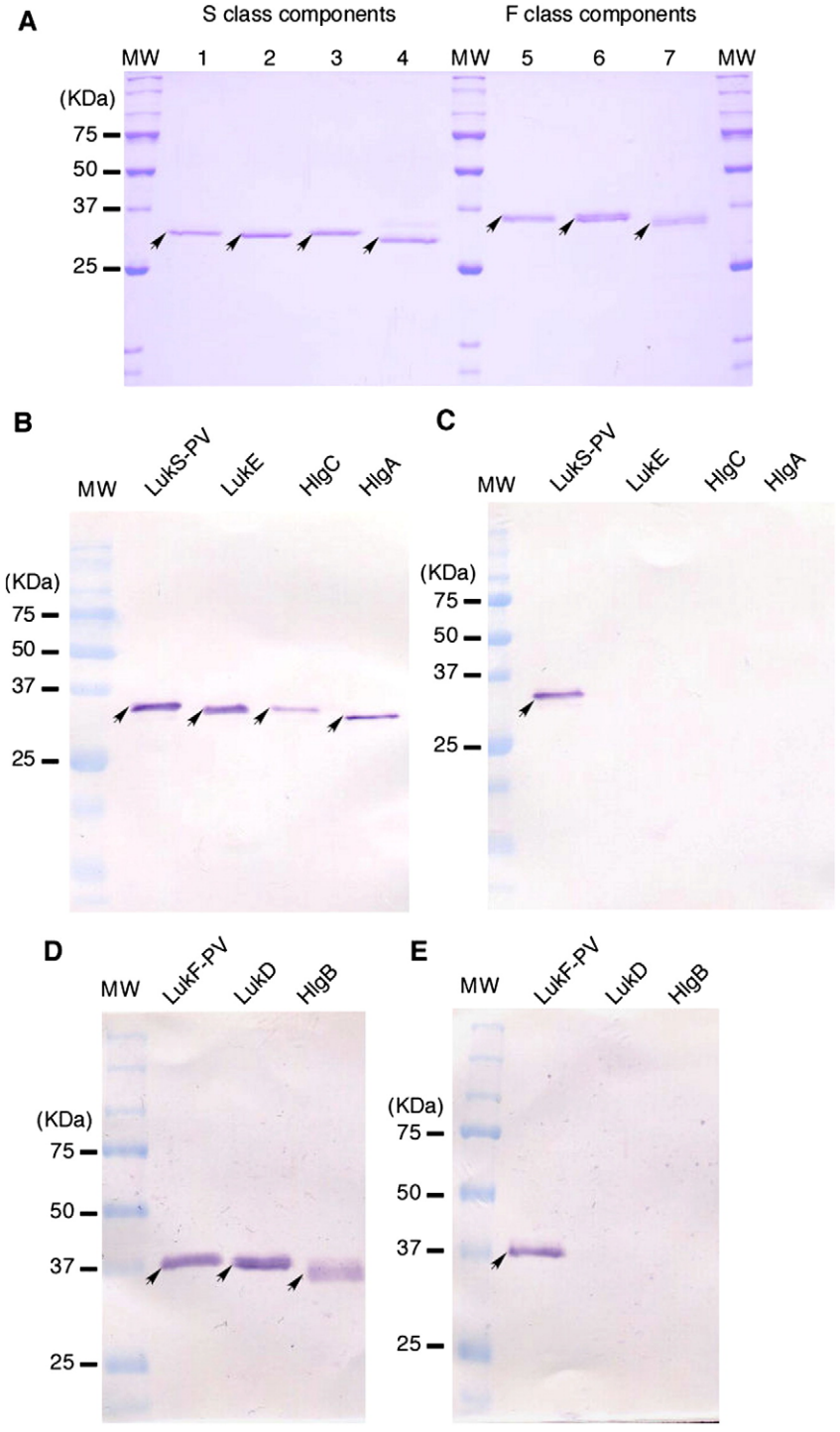

Fig. 1. A. Purified recombinant Panton–Valentine leukocidin and its homologous toxins. Purified toxin proteins were subjected to SDS-PAGE followed by Coomasie Brilliant Blue staining. Approximately 0.5 ng were applied to each lane. Lane 1, LukS-PV; lane 2, LukE; lane 3, HlgC; lane 4, HlgA; lane 5, LukF-PV; lane 6, LukD; lane 7, HlgB; MW, molecular weight markers. Note that lanes 1–4 are S-class components, whereas lanes 5–7 are F class ones. B. C. Specific detection of PVL with purified anti-LukS-PV antibody by immunoblotting. Purified PVL and homologous toxin components indicated in the panels were subjected to SDS-PAGE followed by electrotransfer to PVDF membrane. MW stands for molecular weight standards. Anti-LukS-PV rabbit polyclonal antibodies either before (B) or after affinity-purification (C) was used as detection antibodies. D. E. Specific detection of PVL with purified anti-LukF-PV antibody by immunoblotting. Experimental procedure was basically the same as panels B and C but indicated recombinant proteins were employed. Anti-LukF-PV rabbit polyclonal antibody (D) or affinity-purified anti-LukF-PV (E) was used.