

Isolation and characterization of protective anti-LPS nanobody against *V. cholerae* O1 recognizing Inaba and Ogawa serotypes

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Abstract *Vibrio cholerae* is considered one of the major health threats in developing countries. Lack of efficient vaccine, short incubating time of the disease, and bacterium ability to survive in aquatic environment have made cholera one of the most epidemic diseases yet known. The lipopolysaccharide is one of the bacterium key antigens used to classify *V. cholerae* into 206 serogroups. *V. cholerae* serogroup O1 is a causative agent of all cholera pandemics. Research has shown that anti-lipopolysaccharide (LPS) antibodies could provide protective immunity in cholera cases. In this research, we used N-terminal fragments of the camel's heavy-chain antibodies called VHH or nanobodies and produced a phagemid library. The obtained library was panned against *V. cholerae* O1 LPS, and four monoclonal nanobodies were isolated. Isolated nanobodies were tested in LPS ELISA and bacterial ELISA. The nanobody with the highest affinity toward the bacterium was used in an in vivo challenge and successfully neutralized the bacterium infection. The isolated nanobody showed high thermostability and proteolytic resistance in characterization tests.

Keywords *Vibrio cholerae* O1 · Lipopolysaccharide · VHH · Phage display

Introduction

Vibrio cholerae is a gram-negative bacterium with polar flagella mainly found in marine environments (Brooks et al. 2004) and still considered one of the major health threats across the world especially in developing countries (Qadri et al. 2005; Igbinsola and Okoh 2008). Cholera disease is characterized by watery diarrhea which if left untreated could lead to severe dehydration and death (Siddique et al. 2010; Ogunbanjo and Durrheim 2011). The ability of *V. cholerae* to survive in aquatic ecosystems, inappropriate sewage disposal, and contamination of water supplies in addition of short incubation period of the disease play a major part in cholera epidemics (Bhunia et al. 2009; Krickeberg et al. 2012; Alam et al. 2007). About 75 % of *V. cholerae* infections are asymptomatic, and only 2–5 % of infections will cause severe diarrhea (Bronze and Greenfield 2005). Symptoms could start as soon as 2 h or after 5 days and in severe cases could lead to loss of up to 2 l of water per hour that could result in death in less than 24 h (Musekiwa and Volmink 2011; Sánchez and Holmgren 2011). Cholera is still endemic in many countries, and outbreaks occur at regular intervals (Mishra et al. 2004; Alam et al. 2006; Taneja et al. 2003). Since 1817 *V. cholerae* has caused seven pandemics (Mandal 2011). Although *V. cholerae* O139 has been recently causing outbreaks across the globe, all the cholera pandemics were caused by O1 serotype (Faruque et al. 2003).

Serological classification of *V. cholerae* is based on difference in the carbohydrate structure of the heat-stable O antigen or lipopolysaccharide (LPS) (Weil et al. 2009). Based on distinctive serological response of different LPS compositions,

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