Purification of K88 and K99 Pili from Porcine Enterotoxigenic *Escherichia coli* by Affinity Chromatography

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Enterotoxigenic *Escherichia coli* (ETEC) is an important causative agent of diarrhea in neonatal animals and humans. ETEC induces diarrhea in two steps: first, by colonizing the mucosal surface of small intestine, and then producing heat-labile or heat-stable enterotoxin or both [8]. In recent years, it has been reported that K88, K99, 987P and F41 pili play a significant role in the colonization of porcine ETEC [2, 8].

Various purification methods have been used for isolation of immunologically pure pili from ETEC [1, 2, 4, 5, 7]. However, these methods are time consuming and require several steps wherein large amount of pili may be lost. The present paper describes the purification of K88 and K99 pili from porcine ETEC by affinity chromatography.

ETEC strains 19304 (O157:K88ac:NM, LT⁺) and 431 (O101:K30:K99:NM, ST⁺) were obtained from Salsbury Laboratories Inc. (Charles City, Iowa, USA). Each strain was streaked onto tryptic soy agar (Difco Laboratories, Detroit, Michigan, USA) supplemented with 5% horse blood and incubated at 37°C for 18 hr. A colony of pilated organisms, identified by agglutination in pilus-specific antiserum (Salsbury Laboratories Inc.), was inoculated into 10 ml of Minca broth [3]. After incubation for 18 hr at 37°C with shaking (200 rpm), 0.1 ml of the medium was added to 500 ml of the fresh Minca broth in 1-liter bottle, and the culture was done in the same manner. The cells from 10 liters of the medium were harvested by centrifugation and suspended in 200 ml of 0.1 M Tris-HCl buffer (pH 7.2). The pili were detached from the cells by using a homogenizer (Kinematica, Luzern, Switzerland) for 30 min in ice bath at half speed. After centrifugation at 10,000 g for 30 min at 4°C, the supernatant was saved as crude extract.

To obtain the ligands for affinity chromatography, anti-pilus antibodies were prepared. The pili

were first purified from the crude extracts by a combination of gel filtration and ion exchange chromatography [4, 7]. Each rabbit was then subcutaneously received the purified pili with Freund's complete adjuvant (Difco Laboratories). The specificity of the antisera was determined by Ouchterlony's immunodiffusion test [9]. Both antisera gave single precipitin lines against the crude extracts of corresponding bacteria (Fig. 1).

Gamma-globulin fractions of the antisera were obtained by precipitation with ammonium sulfate, and 60 mg of the fraction was linked to 3 g of Formyl-Cellulofine (Seikagaku Kogyo Co., Ltd., Tokyo) in accordance with the manual. The antibody-linked gels were crammed into the column (1 by 10 cm) and washed with 0.1 M Tris-HCl buffer (pH 7.2).

The crude extract was concentrated to approximately 20 ml by ultrafiltration (UK-10 filter, TOYO ROSHI, Tokyo), and 5 ml of the sample was applied to the column. The column was washed and eluted with 0.2 M glycine-HCl buffer (pH 2.25). The elution patterns are shown in Fig. 2. Both pili were eluted with single peaks. The purity was analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) in 10% acrylamide gel [6] and electron microscopy. As shown in Fig. 2, each preparation gave a single band, and the molecular weights of K88 and K99 pili were 28 and 19 kilodaltons, respectively. In electron microscopy, no contaminating membrane vesicle was observed (Fig. 3). The average yields of K88 and K99 pili from the crude extracts (total protein, 100 mg) were 15 mg and 10 mg, respectively. These yields were two to five times as much as those of the combination of gel filtration and ion exchange chromatography (Table 1).

Thus, the affinity chromatography procedure was proved to have substantial advantage over the combination of gel filtration and ion exchange chromatography for the purification of pili. This method also allows the purification of

Fig. 1. Ouchterlony's immunodiffusion test of the anti-pilus sera. Wells: 1, purified pili; 2, crude extract; 3, antiserum against purified pili; 4, antiserum against crude extract.

Fig. 2. Purification of K88 and K99 pili by affinity chromatography. Each arrow indicates the application of 0.2 M glycine-HCl buffer (pH 2.25). The insets show the analysis of the purified preparations by SDS-PAGE.

Fig. 3. Electron micrographs of purified K88 (1) and K99 pili (2), negatively stained. Magnification, $\times 20,000$.

Table 1. Average yields of pili from the crude extracts (total protein, 100 mg)

purification method	K88	K99
gel filtration and ion exchange chromatography	3 mg	5 mg
affinity chromatography	15 mg	10 mg

pili on a large scale.

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要 約

アフィニティークロマトグラフィーによる豚毒素原性大腸菌線毛の精製(短報): 葛谷光隆・横山英明・児玉義勝(株)ゲン・コーポレーション, 岐阜ラボトリー)——豚毒素原性大腸菌線毛(K88 および K99)の簡便な精製法について検討した。その結果, 両線毛は抗線毛抗体をリガンドとして用いたアフィニティークラムにより, 従来の方法に比較して効率的に精製された。