Purification and characterization of P fimbriae from an Escherichia coli strain isolated from a septicemic turkey

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Abstract: A pap + Escherichia coli isolate from a turkey with colisepticemia expressed P fimbriae with a major subunit of an apparent molecular mass of 18 kDa which reacted with anti-F11 serum. This fimbriae was purified and polyclonal antiserum was produced in rabbits. The N-terminal amino acid sequence of the major fimbrial subunit of the avian P fimbriae was identical to that of F11. On immunoblotting, the antiserum against the avian P fimbriae strongly reacted with the major subunit of the homologous fimbriae, with F11, and with F165, fimbriae. Some antigenic determinants on the major subunits of F13, F71, and F72 fimbriae, with a stronger reaction against F13 fimbriae, were also recognized. The F11 antiserum reacted similarly to the antiserum against avian P fimbriae although cross-reactions against F13, F71, and F72 fimbriae were equivalent. In a competitive enzyme-linked immunosorbent assay, serological differences were observed between the purified avian P fimbriae and F11. Thus, the avian P fimbriae is closely related but not identical to F11 fimbriae which are associated with E. coli isolated from human urinary tract infection.

Key words: Escherichia coli; Turkey; P fimbriae; Purification; Characterization

Introduction

Escherichia coli septicemia or colibacillosis, one of the principal causes of morbidity and mortality in poultry, is characterized by airsacculitis, pericarditis, perihepatitis, and occasionally salpingitis, and causes significant economic losses to the poultry industry [1]. Bacterial colonization is the initial step in most E. coli infections and is generally mediated by fimbrial adhesins present on the bacterial surface [2]. Pathogenic avian E. coli strains have been shown to possess mainly 17 kDa mannose-sensitive hemagglutinating (MSHA) F1A (Type 1) fimbriae, in the case of strains from serogroup O78, and MSHA fimbriae which have been classified as F1A-like, in the case of strains from serogroups O1 and O2 [3]. In addition, mannose-resistant hemagglutinating (MRHA) P or P-related fimbriae may be expressed by some E. coli of avian origin [4,5]. In one study, up to

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78% of strains isolated from chickens with colibacillosis expressed F11 fimbriae with a major subunit molecular mass of 18 kDa [6]. Only 41% of E. coli strains isolated in our laboratory from chickens and turkeys with coliseptisemia possessed pap sequences which usually code for P fimbriae [7]. However, only a small proportion of these isolates, mostly from turkeys, expressed P fimbriae in vitro as determined by MRHA. These fimbriae reacted most strongly with anti-F11 serum when tested by immunofluorescence, immunodot, and immunoblotting (unpublished results). Thus, the objectives of the present study were to purify and characterize the P fimbriae produced by one of these isolates from turkeys with colibacillosis.

Materials and Methods

Bacterial strains and culture conditions

A pap + E. coli isolate (O1:K1) from the heart of a turkey with colisepticemia [7] was used in this study. Prior to phenotypic and immunological characterization, this wild-type isolate was subjected to three consecutive passages of 18 h at 37°C on tryptic soy agar (TSA) (Difco Laboratories, Detroit, MI) for maximal production of P fimbriae and minimal production of type 1 fimbriae.

All cloned strains were cultivated on Luria agar containing either 50 μg of ampicillin or 100 μg of chloramphenicol per ml, depending on the cloning vector used. The characteristics of the recombinant strains encoding for the serologically different P fimbriae and parental wild-type strains are presented in Table 1.

### Purification of fimbriae

Purification of fimbriae was carried out essentially as described by Korhonen et al. [13]. Briefly, fimbriae were precipitated in 20% crystalline ammonium sulfate, treated with 0.25% sodium deoxycholate and subjected to 10–60% sucrose gradient centrifugation at 72,000 rcf (g) for about 20 h. The final preparation was first dialyzed extensively against Tris buffer (10 mM Tris-HCl) and then against water. The fimbrial solution was concentrated by ultracentrifugation and examined by SDS-PAGE [14], and Western blotting [15]. Protein was estimated by the method of Lowry et al. [16]. For N-terminal sequence analysis, protein samples were electroblotted onto Immobilon-P transfer membrane using the CAPS buffer (pH 11.0). Automated Edman degradation was performed using the general protocol of Hewick et al. [17].

**Antisera**

Polyclonal antisera against the fimbriae purified from the avian isolate 12 (O1:K1) or F11 fimbriae purified from reference strain C1976 (O1:K1:H7:F11) were prepared in New Zealand White rabbits by immunization with purified fimbriae according to standard procedures. Antisera were absorbed with the homologous strains grown for 2 days at 16°C on TSA to remove non-specific reactions as described [18].

**Competitive ELISA**

The relationship between the purified avian P fimbriae and F11 fimbriae was examined by an adaption of the enzyme-linked immunosorbent assay (ELISA) as described previously [19]. A 100 μl volume of purified avian P fimbriae (2.5 μg

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<table>
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<tr>
<th>Cloned strain</th>
<th>Fimbrial clone</th>
<th>Parental strain</th>
<th>Parental serotype</th>
<th>Reference</th>
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<tr>
<td>AM1727(pPIL110-70)</td>
<td>F7</td>
<td>AD110</td>
<td>O6:K2:H1</td>
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<tr>
<td>AM1727(pPIL110-37)</td>
<td>F7</td>
<td>AD110</td>
<td>O6:K2:H1</td>
<td>[9]</td>
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ml⁻¹), or purified human F11 fimbriae diluted in 0.1 carbonate buffer (pH 9.6), was coated overnight at 4°C onto the wells of polystyrene microtitre plates (Dynatech Laboratories Inc., Chantilly, VA). A 1:200 dilution of the absorbed antiserum against human F11 or purified avian P fimbriae was preincubated at 56°C for 2 h with one of the following antigens: purified avian P fimbriae, F11, F165₁, F13, F7₁, or F7₂ fimbriae, or with PBS (pH 7.4) as a negative control. The fimbrial preparations were denatured with HCl (pH 2.0) to prevent fimbrial aggregation. These preparations were serially diluted two-fold in carbonate buffer prior to the addition of antiserum. The ELISA optical density at 415 nm was measured 10 min after addition of 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) (Sigma), using a model 3550 microplate reader (Bio-Rad).

**Results and Discussion**

Examination of the purified fimbriae by SDS-PAGE demonstrated a major fimbrial subunit of approximately 18 kDa. This band was also observed on Western blot using the anti-F11 serum (Fig. 1C). Three other O1 E. coli isolates from turkeys with colisepticemia also demonstrated a major fimbrial subunit of 18 kDa which reacted equally strongly to that of strain 12 on Western blot using the anti-F11 serum. F7₁, F7₂, F11, F13, and F165₁ fimbriae from recombinant strains were also purified as described in Materials and Methods. These fimbriae demonstrated major fimbrial subunits of 20 kDa, 18 kDa, 18 kDa, 19 kDa, and 18 kDa respectively (Fig. 1A). The N-terminal

*Fig. 1. SDS-polyacrylamide gel electrophoresis and Western blots of different purified fimbriae. (A) SDS-PAGE of different purified fimbriae with staining by Coomassie blue. (B) Western blotting of the same purified fimbriae with antiserum against the fimbriae of avian strain 12. (C) Western blotting of the same purified fimbriae with antiserum against F11. All samples contained equal concentrations (100 µg ml⁻¹) of protein. Lanes: 1, purified fimbriae from avian strain 12; 2, F11; 3, F165₁; 4, F13; 5, F7₁; 6, F7₂ fimbriae. The apparent molecular masses of the marker proteins (× 10³) are indicated on the left.*
amino acids of the purified 18-kDa major fimbriae subunit from the avian strain 12 were identical to those of the F11 major fimbriae subunit as published by Van Die et al. [20]. Thus, we provide evidence that this fimbriae on avian E. coli strains is closely related to F11 fimbriae. Similarly, van den Bosch et al. [6] found that fimbriae produced by pathogenic avian E. coli were identical to F11 as demonstrated by Western blots, ELISA, and N-terminal amino acid analysis.

On Western blots, the 18-kDa major fimbrial subunits of the avian P fimbriae, F11, and F1651 reacted equally strongly with the absorbed serum against both the purified avian P fimbriae and F11 (Fig. 1B and C). The major fimbrial subunits of F71, F72, and F13 reacted less strongly with both antisera, although the antiserum against the P fimbriae from the avian strain reacted more strongly with the major fimbrial subunit of F13 than did the anti-F11 serum (Fig. 1B and C). Our results contrast with those of van den Bosch et al. [6] who demonstrated that there was no reaction or only a very weak reaction between absorbed antiserum against avian F11 fimbriae and F71, F72, or F13 cloned fimbriae in an ELISA with

![Figure 2](image-url)

**Fig. 2.** Competitive ELISA. A 100 μl volume of purified avian fimbriae (A), or purified human F11 fimbriae (B) diluted in carbonate buffer (pH 9.6), was coated overnight at 4°C onto the wells of microtitre plates. Antiserum against fimbriae of the avian strain 12 was incubated for 2 h at 56°C with the purified fimbriae of the same strain (F11 A), F11 fimbriae (F11 H), F1651, F13, F72, or F71 fimbriae, or with PBS (A). Anti-F11 serum was incubated with the same samples as above (B). All tests were done in triplicate, and the mean absorbance and standard deviation were calculated. Standard deviations were too small to be visible in the figure.
whole bacteria. P fimbriae show extensive cross-reactivity which might be due to the presence of common antigenic determinants in the major subunit [21]. On comparison of the deduced amino acid sequences of the major fimbrial subunits, F11 has highest identity with F13 (up to 79%) and to a lesser extent with F7, and F7 (up to 57% and 58% respectively) [22].

When preparations of either avian P fimbriae or F11 fimbriae, coated on the microtiter plates, were incubated with their respective homologous antisera, pretreatment of antisera by either the homologous or heterologous antigen preparation at a high concentration (50 μg ml−1) resulted in complete inhibition of ELISA reactivity (Fig. 2A and B). However, for both antisera, pretreatment with the antigen preparations at lower concentrations resulted in less effective inhibition of ELISA reactivity for the heterologous than for the homologous antigen. Pretreatment of either antiserum with concentrations as high as 50 μg ml−1 of F165 fimbrial preparation did not result in complete inhibition of ELISA reactivity. At lower concentrations of the F165 fimbrial preparation, inhibition of ELISA reactivity was similar to that for the heterologous F11 or avian P fimbrial preparation at the same concentration. Pretreatment of either antiserum with various concentrations of the F7, F7, or F13 fimbrial preparations resulted in much less effective inhibition of the ELISA reactivity than for either the avian P fimbrial antigen or for F11. Thus, we have demonstrated by a more specific competitive ELISA that the fimbriae produced by avian strain 12 and F11, although highly similar, are not identical. As the major fimbrial subunit as well as the minor fimbrial subunits, including the G adhesin, and their homologous antibodies would have taken part in the competitive ELISA reaction, serological differences between the fimbriae of avian strain 12 and F11 could have been due to differences in any one or more of these components. It is interesting to note that the F165 antigen was unable to completely inhibit either the avian P fimbriae or F11 antibody-antigen homologous systems. This phenomenon could be due to fact that F165 consists of a mosaic of a major fimbrial subunit identical to that of F11 except for 4 amino acids and minor fimbrial subunits very similar to those of the Prs fimbriae [22].

Thus, P fimbriae serologically related to F11 have been found on E. coli strains associated with extra-intestinal diseases in humans (F11), swine (F165), and poultry (F11-related) [6,10,22], although receptor binding specificity of these fimbriae appears to differ depending on the host origin of the isolates, suggesting that homologous fimbrial subunits can carry different adhesin molecules which may be responsible for species-specific adherence. Similarly, other fimbrial types have adapted to specific host species. Enterotoxigenic E. coli expressing K88 or 987P fimbriae are predominantly found in pigs whereas those expressing CFA I fimbriae are found in humans [2,23].

The structure of fimbrial systems, consisting of a major fimbrial subunit and at least three minor components, indicates that their serological reactions are very complex and that minor proteins may participate in determining the antigenicity of the fimbriae [24,25]. Our competitive ELISA results demonstrated different levels of inhibition by F11 and avian P fimbriae of each of their respective homologous and heterologous antisera. These results, together with differences in crossreaction of antisera against F11 and the avian P fimbriae with F13, F7, and F7 in Western blots suggest that there may be some minor differences in the composition of major or minor subunit proteins of these two fimbriae. Further analysis of the genes coding for the major and minor fimbrial proteins is needed to elucidate the difference between the avian P and F11 fimbriae.

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