Pathogenicity of a Non-Hemagglutinating Mutant Strain of *Fusobacterium necrophorum* Biovar A in Mice

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ABSTRACT. A fimbriated and non-hemagglutinating (HA⁻) mutant strain was derived from a fimbriated, HA⁺ parent strain of *Fusobacterium necrophorum*, biovar A. An experiment was carried out to detect any difference in pathogenicity in mice between the two strains. After the intraperitoneal inoculation of the parent strain, 9 out of 10 mice died with hepatic abscess. Organisms with HA⁺ activity were recovered from the abscess, other parts of the livers, spleens, kidneys and lungs. In contrast, all the mice that received the HA⁻ mutant survived, and HA⁻ organisms were isolated only from the hepatic abscesses found in 6 out of 10 mice. The results indicate that the mere presence of the fimbriae is insufficient for *F. necrophorum* to cause pathogenicity in mice, or HA activity. These biological activities may be controlled by some qualitative character of the fimbriae or by some surface structures of the organism other than fimbriae.—**KEY WORDS:** *F. necrophorum*, fimbria, non-hemagglutinating mutant, pathogenicity.

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The fimbriae play an important role in bacterial adherence to host cells [2, 5, 6, 11, 17] and are regarded as one of the important virulence factors [7, 8, 9, 12, 16, 18]. *Fusobacterium necrophorum* biovar A is a major etiological agent of hepatic abscess in cattle. The organism agglutinates erythrocytes of many species of animals [4, 15] and possesses fimbriae [10]. Organisms belonging to biovar A differ from other biovars in that they cause fatal infection in mice [13, 14]. It is not clear whether there is a relationship between the presence of fimbriae and pathogenicity of this organism.

The purpose of the present investigation was to examine the relationship between the presence of fimbriae and the pathogenicity of *F. necrophorum*. A non-hemagglutinating mutant strain was isolated from a hemagglutinating strain of *F. necrophorum* biovar A. The presence of fimbriae was examined by electron microscope with the two strains. Their pathogenicity was compared between the mutant and parent strains in mice.

MATERIALS AND METHODS

Strains used: Two strains were used. One was the Fn 43 strain of *F. necrophorum* biovar A isolated from a case of bovine hepatic abscess. The other was the Fn 43 HA⁻ strain, a mutant of the former strain which had turned spontaneously to be non-hemagglutinating in the laboratory.

Pathogenicity: Male mice of the ICR strain were used at 5 weeks old. The strain were grown in BPPY medium [14] at 37°C for 24 hours. The viable bacterial number was counted for each inoculum. Two groups of 10 mice each were inoculated intraperitoneally with 0.3 ml of a culture of the parent (6.0×10^7 CFU/mouse) or mutant strain (6.4×10^8 CFU/mouse). Some mice died after inoculation, which were immediately examined at necropsy. Bacterial isolation was done in GAM agar medium (Nissui) with addition of bovine or equine blood to 5% from abscess in the liver, hepatic tissue free from abscess, spleen, kidney, and lung. The other surviv-

Table 1. Pathogenicity of the hemagglutinating or non-hemagglutinating strain of *F. necrophorum* after intraperitoneal injection into mice

Strain	Mouse No.	Survival or death	Days after inoculation	Body weight		Liver abscess formation	Recovery of <i>F. necrophorum</i> from		
				Initial	Final		Liver abscess	Liver	Spleen Kidney Lung
Parent strain	1	D	6	22	18	+	+	+	+
	2	D	6	22	17	+	+	+	+
	3	D	6	22	17	+	+	+	+
	4	D	7	22	17	+	+	+	+
	5	D	6	22	16	+	+	+	+
	6	D	8	22	18	+	+	+	+
	7	S	11	22	23	+	+	+	+
	8	D	8	23	16	+	+	+	+
	9	D	6	23	20	+	+	+	+
	10	D	6	23	20	+	+	+	+
HA ⁻ mutant strain	1	S	11	22	28	+	+	-	-
	2	S	11	22	28	-	-	-	-
	3	S	11	23	30	+	+	-	-
	4	S	11	23	29	+	+	-	-
	5	S	11	22	27	-	-	-	-
	6	S	11	22	26	+	+	-	-
	7	S	11	22	28	-	-	-	-
	8	S	11	23	31	+	+	-	-
	9	S	11	22	28	+	+	-	-
	10	S	11	22	28	-	-	-	-
Control	1	S	12	22	26	-	-	-	-
	2	S	12	23	28	-	-	-	-
	3	S	12	22	26	-	-	-	-
	4	S	12	22	26	-	-	-	-
	5	S	12	23	27	-	-	-	-

ing mice were sacrificed 11 or 12 days post inoculation by dislocation of the cervical vertebra and subjected to necropsy and isolation of the organism. Anaerobic culture was carried out by the CO₂-replacing steel-wool method [1]. Five mice were injected intraperitoneally with 0.3 ml of BPPY medium to serve as controls.

Observation of fimbriae: The method of Miyazato *et al.* [10] was used.

RESULTS

Pathogenicity in mice: All the mice inoculated with the mutant strain, Fn 43 HA⁻, survived for 11 days when the experiment

was terminated (Table 1). They weighed 22–23 g and 26–30 g on the first and last day, respectively. Hepatic abscess was found in six subjects and *F. necrophorum* was isolated as pure cultures. It was not recovered from the abscess-free hepatic tissues, spleens, kidneys, or lungs. The isolated organisms uniformly failed to agglutinate chicken erythrocytes.

All the mice, but one, that had been inoculated with the parent strain, Fn 43, died 6–8 days after the inoculation with some decrease in body weight (Table 1). The surviving one also exhibited a delay in body weight gain. Hepatic abscess was found in the surviving mouse, as well as in all the dead mice.

Fig. 1. Fimbriae of Fn 43 parent strain, shadowed. Bar=1 μ m.

Fig. 2. Fimbriae of Fn 43 mutant strain, shadowed. Bar=1 μ m.

Fusobacterium necrophorum was recovered from all the mice. The organism was also isolated from the hepatic tissues lacking abscess, spleens, kidneys, and lungs. All the isolated strains of *F. necrophorum* hemagglutinated chicken erythrocytes.

The five control mice, inoculated with the

medium alone, showed no lesions and no organism was isolated from their tissues.

Observation of fimbriae: The presence of fimbriae was noticed in organisms of both strains. There was no morphological difference between the two strains (Figs. 1 and 2).

DISCUSSION

It has been demonstrated with some bacterial species that fimbriated strains are higher in pathogenicity than non-fimbriated strains [3, 12, 18]. The fimbriae function as a tool to adhere to host cells as well as play an important role in the initial stage of multiplication and invasion [2, 5, 6, 11, 16]. *Fusobacterium necrophorum* biovar A is a major causative agent of hepatic abscess in cattle, and is able to form fimbriae and agglutinate erythrocytes of various animal species [14, 15]. The organism also causes necrobacillosis in domestic animals and highly pathogenic for mice [13, 14]. In the present investigation, an experiment was carried out to study the relationship between the pathogenicity of this organism and the presence of fimbriae.

The parent (Fn 43) strain was fimbriated and hemagglutinating. The mutant strain isolated from the present experiment, on the other hand, was non-hemagglutinating, but fimbriating. Hence, *F. necrophorum* biovar A may be hypothesized to possess two types of fimbriae; one participates in the adherence of organism to the cell and the other does not. The fimbriae of the parent strain seem to belong to the former and those of the mutant strain to the latter. When the mutant strain was examined for pathogenicity in mice, it was not fatal, showing a decrease in ability to cause hepatic abscess in comparison to the parent strain which caused fatality with the abscess.

It may be reasonable to assume that some qualitative change in fimbriae has caused the hemagglutinating ability to disappear and, at the same time, the pathogenicity to decrease. It can also be interpreted as follows; there is no relationship between the pathogenicity of the organism and the presence of fimbriae. The change in HA capacity and pathogenicity may have been associated with changes in lipopolysaccharide and/or outer membrane protein components. Both components are

related intimately with the pathogenicity of Gram-negative bacteria. It seems necessary to examine both the parent and mutant strains for their ability to adhere to cells other than chicken erythrocytes, to compare the biological and chemical properties of the purified fimbriae, and to compare lipopolysaccharide or outer membrane protein.

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要 約

Fusobacterium necrophorum 生物型 A の赤血球非凝集変異株のマウスに対する病原性：新城敏晴・清山絃子（宮崎大学農学部家畜微生物教室）——*Fusobacterium necrophorum* 生物型 A から赤血球非凝集性の変異株を分別し、線毛とマウスに対する病原性との関係について親株と比較した。親株を接種したマウスは10匹中9匹が死亡し、全例に肝膿瘍が形成された。接種菌は肝膿瘍、膿瘍非形成部の肝臓、脾臓、腎臓及び肺から回収され、すべての分離菌がニワトリ赤血球を凝集した。一方、変異株を接種したマウスは、10匹とも生残し、その中の6匹に肝膿瘍が認められた。接種菌は肝膿瘍部からのみ回収され、膿瘍非形成部の肝臓、脾臓、腎臓及び肺からは回収されなかった。肝膿瘍部から回収された菌株は、ニワトリ赤血球を凝集しなかった。線毛は両菌株に認められた。