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Plasmid in *Fusobacterium necrophorum*

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ABSTRACT. Nine strains of *Fusobacterium necrophorum* biovars A, B, and C were examined for the presence of plasmid by agarose gel electrophoresis. Although none of biovars A and B possessed extrachromosomal deoxyribonucleic acids, all the strains of biovar C examined so far contained a single plasmid with a molecular weight of 118 megadaltons. This suggests the presence of plasmid may link to the biological variation in the *F. necrophorum* species.—**KEY WORDS:** *Fusobacterium necrophorum*, plasmid.

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Fusobacterium necrophorum, a causative agent of the hepatic abscess in cattle, has been divided into three biovars A, B, and C by Fiévez *et al.* [3]. The strains of biovars A and B are β -hemolytic and pathogenic for mice, but those of biovar C are not. It is therefore of interest to examine whether this variation may attribute to the presence of plasmid. Although plasmids are commonly found in a variety of prokaryotes, there has been no report on plasmid in the genus *Fusobacterium* so far. In the present study we have examined for the presence of plasmid in *F. necrophorum* by agarose gel electrophoresis.

Bacterial strains examined are given in Table 1. Two *Escherichia coli* strains, JP990 (R621a) and JP990 (TP116), containing drug-resistant plasmids of 65 megadaltons (Mdal) and 143 Mdal in size, respectively, were obtained from A. J. Pittard, University of Melbourne, Australia, and used as molecular mass references [2]. Nine strains of *F. necrophorum* were cultured on GAM plates (Nissui Seiyaku Co.) at 37°C for three days in an anaerobic jar filled with carbon dioxide, and the two strains of *E. coli* were grown on nutrient agar plates (Eiken Chemical Co.) at

37°C overnight. Plasmid deoxyribonucleic acid was extracted by the method of Birnboim and Doly [1] with minor modifications. The colonies were picked up directly from agar plates with sterile toothpicks and suspended in 20 μ l of lysozyme solution at 0°C for 10 min. After adding 40 μ l of 1% sodium dodecyl sulfate (SDS) in 0.2 N sodium hydroxide solution, the lysate was mixed gently by inverting the tubes about ten times at 0°C for 5 min, followed by extraction with an equal volume of phenol-chloroform mixture (1:1, by volume) in a 1.5 ml tube. After centrifugation in an Eppendorf model 5414 microcentrifuge for 3 min, 20 μ l of the aqueous phase was mixed with 4 μ l of tracking dye solution consisting of 0.07% bromophenol blue, 7% SDS and 33% glycerol in water. The resulting solution was subjected to 0.8% agarose slab gel electrophoresis by using Tris-borate buffer, pH 8.3, as described by Meyers *et al.* [4]. After pre-run at 50 volts for 30 min, electrophoresis was done at 100 volts for 3.5 hr. The gels were stained in a 0.4 μ g/ml solution of ethidium bromide, visualized with shortwave ultraviolet light on a Chromato-Vue transilluminator model C-61 (Ultra-Violet Products, San Gabriel, CA) and

Table 1. *F. necrophorum* strains examined for the presence of plasmid

Biovar	Strain	β -hemolysis	Pathogenicity for mouse	Hemagglutination (Chicken erythrocyte)	Antibiotic resistance			Source	
					Tc	Ap	Cm Rif	Sm Km Na [±]	
A	VPI 2891 ^{a)}		lethal						bovine liver abscess
	VPI 6054-A ^{b)}	+	(Diffuse abscess in the liver)	+	—			+	foot rot of sheep
	Fn 43 ^{c)}								bovine liver abscess
B	VPI 6161 ^{d)}		nonlethal						unknown
	SPH 6 ^{e)}	+	(Focal abscess in the liver)	—	—			+	bovine stomatitis
	Fn 45 ^{c)}								bovine liver abscess
C	410 ¹⁾								human
	118 ¹⁾	—	—	—	—			+	human
	130 ¹⁾								human

a) From W. E. C. Moore, Virginia Polytechnic Institute and State University, Va., U.S.A.
b) From T. D. Wilkins, Virginia Polytechnic Institute and State University, Va., U.S.A.
c) From our collection.
d) From M. Kanoe, Yamaguchi University, Yamaguchi, Japan.
e) From B. Aalback, Royal Veterinary and Agricultural University, Copenhagen, Denmark.
f) From H. Beerens, Institut Pasteur de Lille, Lille, France.
g) Tc: tetracycline, Ap: ampicillin, Cm: chloramphenicol, Rif: rifampicin, Sm: streptomycin, Km: kanamycin, Na: nalidixic acid.

Fig. 1. Agarose gel electrophoresis of plasmid DNA from *F. necrophorum* and *E. coli*. (A) *E. coli* JP990 (R621a), (B) *E. coli* JP990 (TP116), (C) *F. necrophorum* 410, (D) *F. necrophorum* 118, (E) *F. necrophorum* 130.

photographed with Polaroid type 55 films, using a Polaroid MP4 land camera with a Kodak 23A Wratten gelatin filter (Eastman Kodak Co., Rochester, NY).

Electrophoretic patterns are shown in Fig. 1. All the strains of biovar C examined in this study possessed a single plasmid with a same molecular weight of approximately 118 Mdal, but all the strains of biovars A and B were negative. This is the first evidence of plasmid in the genus *Fusobacterium*. Vandenberg *et al.* [5] have reported an unsuccessful attempt to detect plasmid in *F. nucleatum*. We are currently investigating the presence of plasmid in domestic isolates of *F. necrophorum*.

The plasmid in *F. necrophorum* did not associate with the pathogenicity for mice, production of β -hemolysin, and antibiotic resistance (Table 1). In our additional examination, bacteriocin production was not linked to this plasmid. Temperate phages may persist in the cell in extrachromosomal state, like plasmids, but we failed to detect phages after ultraviolet irradiation in all the plasmid-containing strains. Although the phenotypic function of this plasmid is currently un-

known, its presence may associate with the biological variation of *F. necrophorum* under the biovar level. We have tried curing of the plasmids in these strains, but only to fail. The strains of biovar C are not susceptible to a bacteriophage isolated from a strain of *F. necrophorum* (Tamada *et al.*, submitted for publication). This probably is due to the presence of plasmid, but any definitive elucidation must wait until its function is demonstrated explicitly.

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要 約

Fusobacterium necrophorum のプラスミド (短報): 中村華月, 原沢 亮, 新城敏晴 (宮崎大学農学部) —— *Fusobacterium necrophorum* biovar A, B, C に属する 9 株について, アガロース・ゲル電気泳動によりプラスミドを検索した。Biovar C に属する株からのみ, それぞれ同一の分子量 (118.Mdal) を持つ単一のプラスミドが検出されたことから, このプラスミドは biovar の分類基準に関連している可能性が示唆される。