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Distribution and molecular characterization of *Porphyromonas gulae* carrying a new *fimA* genotype

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ABSTRACT

Porphyromonas gulae is a gram-negative black-pigmented anaerobe which is known to be a pathogen for periodontitis in dogs. Approximately 41 kDa filamentous appendages on the cell surface (FimA) encoded by the *fimA* gene are regarded as important factors associated with periodontitis. The *fimA* genotype was classified into two major types and strains in type B were shown to be more virulent than those in type A. In the present study, we characterized a strain with a novel *fimA* genotype and designated it as type C. The putative amino acid sequence was shown to be similar to the genotype IV *fimA* of *Porphyromonas gingivalis*, a major pathogen of human periodontitis. Analyses using an oral squamous cell carcinoma cell line derived from tongue primary lesions revealed that the type C strain inhibited proliferation and scratch closure more than genotype A and B strains. In addition, experiments using a mouse abscess model demonstrated that the type C strain could induce much higher systemic inflammation when compared with strains of the other genotypes. Furthermore, molecular analyses of oral swab specimens collected from dogs demonstrated that the detection frequencies of *P. gulae* and the genotype C in the periodontitis group were significantly higher than those in the periodontally healthy group. These results suggest that FimA of *P. gulae* is diverse with the virulence of genotype C strains the highest and that molecular identification of genotype C *P. gulae* could be a possible useful marker for identifying dogs at high risk of developing periodontitis.

1. Introduction

Periodontal diseases are initiated following gingivitis with localized inflammation without the destruction of the periodontal tissues (cementum, periodontal ligament and alveolar bone) (Pihlstrom et al., 2005). Without any intervention, this localized inflammation generally progresses to periodontitis, especially in elder subjects and

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those with systemic disorders. The symptoms of periodontitis are generally irreversible and include severe bleeding, pus discharge and mobility of the affected tooth and ultimately leads to tooth loss. The distribution of the periodontitis-related bacterial species has been investigated in the oral swab specimens taken from Japanese dogs and it was observed that *Porphyromonas gulae*, a gram-negative black-pigmented anaerobe, was one of the species frequently detected (Kato et al., 2011).

The cell surface 41 kDa fimbrillin (FimA), a subunit of fimbriae, was characterized in *P. gulae* and is known to be one of the major factors for periodontitis in dogs (Hamada et al., 2008). Furthermore, *P. gulae* invasion into gingival epithelial cells has also been demonstrated. In our recent study, FimA of *P. gulae* could be classified into two major genotypes, and analyses of the putative amino acid sequences of FimA in many clinical strains revealed that the type A FimA is specific for *P. gulae*, and the amino acid sequence of type B FimA is more closely related to that of genotype III FimA of *Porphyromonas gingivalis*, a major pathogen of human periodontitis (Nomura et al., 2012). On the other hand, there are several *P. gulae*-positive specimens which are negative for both types A and B, indicating the presence of *P. gulae* strains without FimA or those with additional *fimA* genotypes (Nomura et al., 2012).

In the present study, we successfully characterized a new genotype for *fimA* genes in *P. gulae* encoding a novel FimA and designated it as genotype C. The purpose of the present study was to compare the properties of each FimA genotype relative to its virulence potential in periodontitis. In addition, the distribution of the each group was analyzed focusing on the clinical conditions of the dogs sampled.

2. Materials and methods

2.1. Bacterial and cell culture conditions

Table 1 lists the *P. gulae* strains analyzed in the present study, among which all except for one strain (D049) were previously reported (Kato et al., 2011; Nomura et al., 2012). Strain D049 was isolated from an oral swab specimen from a dog and confirmed to be *P. gulae* by a molecular biological method described previously (Kato et al., 2011). In addition, 11 *P. gingivalis* strains listed in Table 1 were also used (Amano et al., 1999; Nakagawa et al., 2000, 2002). *P. gingivalis* and *P. gulae* strains were cultured in mhTS broth [Tripticase soy broth (Becton, Dickinson & Co, Franklin Lakes, NJ, USA) with hemin (50 mg/ml) and menadione (5 mg/ml)] under anaerobic conditions.

The SAS cells, an oral squamous cell carcinoma cell line, were obtained from Japanese Collection of Research Biosources (Tokyo, Japan). Cells were cultured in RPMI 1640 medium (Wako, Osaka, Japan) supplemented with 10% fetal bovine serum at 37 °C in 5% CO_2.

Table 1
P. gulae and *P. gingivalis* strains used in the present study.

Species	Name	*fimA* types	Length of *fimA* gene (bp)	Accession numbers of *fimA* gene	References
P. gulae	ATCC 51700[a]	A	1152	AB297918	Hamada et al. (2008)
	D024	A	1152	AB663087	Nomura et al. (2012)
	D025	A	1152	AB663088	Nomura et al. (2012)
	D028	A	1152	AB663089	Nomura et al. (2012)
	D034	A	1152	AB663090	Nomura et al. (2012)
	D035	A	1152	AB663091	Nomura et al. (2012)
	D036	A	1152	AB663092	Nomura et al. (2012)
	D042	A	1152	AB663093	Kato et al. (2011)
	D043	A	1152	AB663094	Kato et al. (2011)
	D060	A	1152	AB663095	Kato et al. (2011)
	D066	A	1152	AB663096	Kato et al. (2011)
	D067	A	1152	AB663097	Kato et al. (2011)
	D068	A	1152	AB663098	Kato et al. (2011)
	B43	B	972[b]	CS228034	Dreier et al. (2005)
	D040[a]	B	1161	AB663099	Kato et al. (2011)
	D044	B	1161	AB663100	Kato et al. (2011)
	D052	B	1161	AB663101	Kato et al. (2011)
	D053	B	1161	AB663102	Kato et al. (2011)
	D077	B	1161	AB663103	Nomura et al. (2012)
	D049[a]	C	1167	AB679295	This study
P. gingivalis	381	I	1044	D17794	Amano et al. (1999)
	ATCC 33277	I	1044	D17795	Amano et al. (1999)
	BH18/10	I	1044	D17796	Amano et al. (1999)
	HW24D1	II	1047	D17797	Amano et al. (1999)
	OMZ314[a]	II	1044	D17798	Amano et al. (1999)
	OMZ409	II	1047	D17799	Amano et al. (1999)
	ATCC 49417	II	1053	D17800	Amano et al. (1999)
	6/26	III	1062	D17801	Amano et al. (1999)
	HG564	IV	1083	D17802	Amano et al. (1999)
	HNA99	V	1104	AB027294	Nakagawa et al. (2000)
	HG1691	Ib	1044	AB058848	Nakagawa et al. (2002)

[a] Strains analyzed in the mouse abscess model.
[b] Only partial sequences corresponding to *fimA* of *P. gulae* are available.