NOTE *Bacteriology*

Variation of Genes Encoding GGPLs Syntheses among *Mycoplasma fermentans* Strains

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ABSTRACT. The information of the biosynthesis pathways of *Mycoplasma fermentans* specific major lipid-antigen, named glycoglycerophospholipids (GGPLs), is expected to be some of help to understand the virulence of *M. fermentans*. We examined primary structure of cholinephosphotransferase (*mf1*) and glucosyltransferase (*mf3*) genes, which engage GGPL-I and GGPL-III synthesis, in 20 strains, and found four types of variations in the *mf1* gene but the *mf3* gene in two strains was not detected by PCR. These results may have important implications in virulence factor of *M. fermentans*.
KEY WORDS: cholinephosphotransferase, glucosyltransferase, *Mycoplasma fermentans*.

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Mycoplasma fermentans was first isolated from the urogenital tract of patients with ulcerative balanitis several decades ago [19] and then detected from respiratory tract of children with community-acquired pneumonia [4], adults with acute respiratory distress syndrome [10], the joints of patients with rheumatoid and other inflammatory arthritic disorders [5, 6, 8, 21] and so on. Although it was previously assumed that humans were the only natural hosts, *M. fermentans* has been isolated from genital lesions in sheep [15], suggesting a zoonotic aspect of this particular pathogen.

Interest in this organism has recently increased because of its possible role in the pathogenesis of rheumatoid arthritis and reports indicating that this organism may function as a cofactor accelerating the progression of human immunodeficiency virus infection [17]. Although *M. fermentans* is a typical extracellular microorganism able to adhere to human epithelial cells, ultrastructural studies performed with engulfed *M. fermentans* revealed mycoplasmas within membrane-bound vesicles [24, 25].

In mycoplasmas, adherence is the major virulence factor, and adherence-deficient mutants are avirulent [2, 18]. It seems that *M. fermentans* utilizes at least two surface components for adhesion to HeLa cells, a protease-sensitive surface protein, apparently the lipoprotein recently described [23], and a phosphocholine-containing glycolipid. Phosphocholine-containing lipids were detected in all *M. fermentans* strains tested by Ben-Menachem *et al.* [3].

Matsuda *et al.* have identified several alkali labile glycophospholipids designated as glycoglycerophospholipids (GGPLs) [13]. Of them, GGPL-I and GGPL-III are expressed in *M. fermentans* specifically, and these lipid-antigens are the major lipid-antigens of *M. fermentans* [14]. The structures of GGPL-I and GGPL-III were identified as 6'-O-phosphocholine-α-glucopyranosyl-(1'-3)-1,2-diacylglycerol and 1'-phosphocholine-2'-amino dihydroxypropane-3'-phospho-6'-α-glucopyranosyl-(1'3)-1,2-diacylglycerol, respectively [12, 13], and GGPLs have been chemically synthesized by Nishida *et al.* [16].

Based on unique structures and bioactivities, GGPLs have been considered as a hypothetical factor in the pathogenesis of *M. fermentans* [11]. Because GGPLs have strong immunogenicity, they may play roles as immunodisturbing agents in cell functions such as inflammation and cell differentiation [22]. GGPL-III antigens were detected in synovial tissues from RA patients and significantly induced TNF-α and IL-6 production from peripheral blood mononuclear cells, and also proliferation of synovial fibroblasts [9].

The information of the biosynthesis pathways of relative compounds of GGPLs is expected to contribute to identification of those of GGPLs, and Ishida *et al.* determined one of the putative GGPL-I biosynthetic genes, according to whole genome analysis of *M. fermentans* PG18 [7]. In the present study, we examined the presence of cholinephosphotransferase (*mf1*) gene and glucosyltransferase (*mf3*) gene, which engage GGPL-I and GGPL-III synthesis, in human mycoplasma species as well as in 20 strains of *M. fermentans*.

M. fermentans strains used in this study are listed in Table 1. Other human mycoplasmas examined include *M. genitalium* G37, *M. pneumoniae* Mac, *M. pneumoniae* FH, *M. penetrans* GTU, *M. orale* CH19299, *M. buccale* CH20247, *M. primatum* HRC92, and *M. hominis* PG21. *M. fermentans* strains were obtained from Dr. Tsuguo Sasaki of the National Institute for Infectious Disease, Tokyo, Japan and

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Table 1. Origin of *M. fermentans* strains and *mf1*, *mf3*, *MCGp* sequence type and PCR type based on the major part of the IS*1550* element

Origin	Strain	Year of isolation	*mf1* sequence	*mf3* sequence	IS*1550* PCR type (25)
Genital ulcer	PG18^T	1955	A	+	B
Rheumatoid	KL4	1990	D	+	A
Arthritis	KL8	1990	D	+	B
Joint fluid	GIM	1995	D	+	A
Leukemic bone	E10	1960–1969	D	+	A
marrow	K7	1960–1969	D	+	A
	Z62	1960–1969	D	+	A
Urethral isolate AIDS patients	BRO	1990	D	+	A
Urine	#5	1995	C	+	B
Urine	#29	1995	D	+	B
Blood	AOU	1990	D	+	A
Respiratory	M39	1990–1995	A	+	B
tract	M51	1990–1995	D	+	B
	M52	1990–1995	B	+	B
	M64	1990–1995	D	–	B
	M70	1990–1995	D	+	B
	M73	1990–1995	D	–	B
Cell culture	A6	1982–1992	D	+	B
	C5	1982–1992	D	+	B
	2059	1982–1992	D	+	A
	28AC	1982–1992	D	+	B

they include strains KL4, KL8 (from rheumatoid arthritis), GIM (from human joint), E10, K7, Z62 (from bone marrow of leukaemic patients), BRO (from human urethral), #5, #29 (from urine deposits from AIDS patients), AOU (from blood of an AIDS patient), M39, M51, M52 M64, M70, M73 (from human respiratory tract), 2059, 28AC, A6 and C5 (from cell culture). The *mf1* and *mf3* were PCR-amplified from *M. fermentans* genome. Briefly 1 μl of broth cultures (approximately 10^6 cfu/m*l*) were diluted in 1 m*l* of water and heated at 95°C for 3 min for lysis of mycoplasma cells. Mycoplasma lysate (5 μl) was directly added into 45 μl of the PCR master mixture consisting of 1 unit of KOD plus DNA polymerase (TOYOBO, Osaka, Japan), 5 μl of 10 × PCR buffer, 5 μl of 2 mM deoxynucleoside triphosphates, 75 nmol of $MgSO_4$, and 10 pmol of each primer, combination of mf1F (5'-ATAATAAAAACTATGAATGA-3') and mf1R (5'-CTATTTGTCATTTTTCTT-3'), or mf3F (5'-ATGATATGAAAGTTTTTTGTAAAAAAGAAAGG-3') and mf3R (5'-TTATTTTTTATAATGTTCAATAATTT-TTTTGTATTT-3'). Amplification was done under the following conditions; 30 cycles of 94°C for 40 sec, 50°C for 90 sec, and 68°C for 2 min after 94°C for 2 min. *mf1* and *mf3* amplified products (777 and 1,221 bp respectively) were sent to a reference laboratory (TaKaRa Custom Services, Shiga, Japan) for DNA sequencing.

In our study, *mf1* and *mf3* are thought to be *M. fermentans* specific genes because they were not amplified in case of other human mycoplasmas (*M. genitalium* G37, *M. pneumoniae* Mac, *M. pneumoniae* FH, *M. penetrans*, *M. orale, M. buccale* CH20247, *M. primatum* HRC92, *M. hominis* PG21).

The sequence data of *mf1* gene were deposited to the international DNA databases under the accession number AB480306 ~ AB480325. Although the *mf1* gene was shown to be conservative in 20 strains of *M. fermentans* by PCR, *mf3* gene was not amplified in two strains, M64 and M73, among these 20 strains examined. Besides, the *mf1* and *mf3* genes were not evident in other human *Mycoplasma* species by specific PCR, suggesting that these two genes are unique to the *M. fermentans* species. Nucleotide sequences of *mf3* gene in the 20 *M. fermentns* strains were identical, but those of *mf1* gene showed a minor variation causing some amino acid substitutions, and categorize A, B, C, and D types for descriptive purposes (Fig. 1). These amino acid changes may be responsible for enzymatic activity of cholinephosphotransferase in *M. fermentans*. In addition, although the *mf3* gene was not amplified from strains M64 and M73, the reason was currently unknown since defection of glucosyltransferase in these strains was not examined in the present study. These diversities in enzymes may engage specific major lipid-antigen syntheses, and also influence immunogenic potential and RA pathogenesis. No significant homology to the *mf3* gene was apparent in other prokayotes in databases, supporting that the GGPLs are unique to *M. fermentans*. Currently recombinant enzymes, based on the nucleotide sequences of the enzyme genes from PG18 strain of *M. fermentans*, have been successfully expressed in *Escherichia coli* [7]. PG18, a type strain of *M. fermentans*, has been shown particularly unique among *M. fermentanss* strains [20]. The difference of *mf1* posttranslational amino-acid sequence may have influenced the activity of choline phosphotransferase, and concern GGPL-I

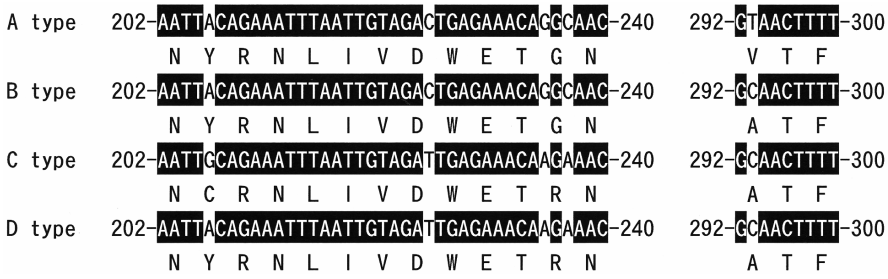

Fig. 1. The part of *mf1* DNA (top) and posttranslational amino-acid sequence data (bottom) of *M. fermentans* PG18 reference strain. The 20 strains tested were differentiate into four types A including PG18 and M39, B including M52, C including #5 and D including the other strains.

antigenicity, so studies on not only PG18 strain but also on other strains will become increasingly important. In addition, all strains presenting A type in IS*1550* PCR pattern were D type in *mf1* and detected *mf3*, however, there seems to be poor correlation among IS*1550*, *mf1* and *mf3*.

In conclusion, our genetic analysis on the 20 strains of *M. fermentans* showed four variations in *mf1* posttranslational amino-acid sequence but undetected *mf3* gene in two strains. Our results may have important implications for the virulence of *M. fermentans* especially in RA pathogenesis.

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