短　報

Pulsed Field Gel Electrophoresis Analysis Conditions for Molecular Epidemiological Group B *Streptococcus* Identification

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Introduction : In studying PFGE process conditions, using 7 noninfected Group B *Streptococcus* (GBS) detected in 3 mother-neonate pairs[1)], some type-III serotype GBS, which produce a large amount of pathogenic neuraminidase[2)], were not detected when we applied PFGE to strains detected in neonates developing meningitis, so we investigated PFGE process conditions under which the GBS type-III serotype could be detected.

Methods : Subjects were 26 strains of meningitis causing GBS and 22 noninfected strains. Different lysis processes and restriction enzymes were studied under PFGE process conditions, and the mixture of lysozyme (5mg/mL [120－02674 ; Wako]) and mutanolysin (100U/mL [M4782 ; Sigma-Aldrich]) was examined in the lysis process. Lysozyme increased from 50 to 4,000μL, and mutanolysin from 20 to 300μL. We prepared and heated several plugs at 90℃ for 10 minutes. After restriction enzyme digestion, we applied electrophoresis to the plugs for 19.8 hours (initial ramp time, 5.3 ; final ramp time, 34.9) with the running buffer temperature maintained at 14℃ and at an angle of 120° with a CHEF-DRIII system (Bio-Rad, USA).

Results : Strains detected using the PFGE analysis[1)] (lysozyme 50μL and mutanolysin 20μL for bacteriolysis, restriction enzyme *Apa* I) were 17 of 22 noninfected strains—type III, 7 strains ; type NT6, 3 strains ; type Ib, JM 9, 2 strains ; and type Ia, II, V, one strain—and 11 of 26 meningitis-causing strains—type Ib, 4 strains ; type III, 3 strains ; type Ia, 2 strains ; and type NT6, 2 strains (Fig. 1, Lane 1－11)—. Strains not detected—5 non-infected, and 15 infected (Fig. 1, Lane 12－26) — were all type-III serotype. We detected these 20 strains by lyzing －using 50μL of lysozyme and 20μL of mutanolysin, and heating at 90℃ for 10 minutes—and by using restriction enzymes －*Sma* I and *Sal* I－ (Fig. 2, 3).

Discussion : Strains not detected by PFGE analysis[1)]—5 noninfected, and 15 infected－ were all type-III serotype GBS. We detected these 20 strains by improving detection conditions involving heating temperature and restriction enzymes *Sma* I and *Sal* I. Further work is need, however, to determine the relationship between results of PFGE analysis here and pathogenic agents of strains examined. PFGE analysis detected 11 of 26 meningitis-causing strains, 3 of which were type-III serotype and the remaining 15 strains all type-III serotype.

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Fig. 1 PFGE of meningitis causing GBS with the *Apa* I restriction enzyme
Lane M, λ-size marker; serotype Ia, Lane 8, 11; serotype Ib, Lane 1,3,5,6; serotype II, Lane 4; serotype III, Lane 9, 10; serotype NT6, Lane 2, 7.
※ Lane12 ～ 26 serotype are serotype III.

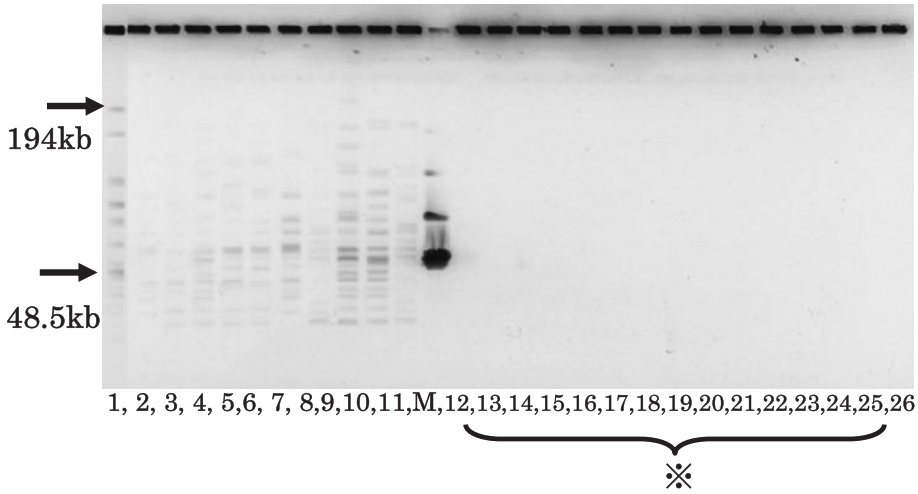


Fig. 2 PFGE of meningitis causing GBS with the *Sma* I restriction enzyme
Lane M, λ-size marker; Lane12 ～ 26 serotype are serotype III

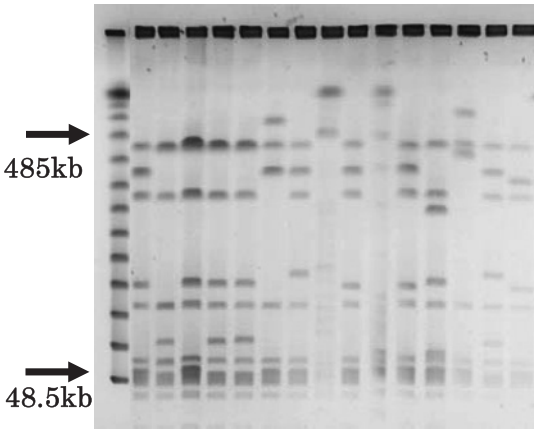
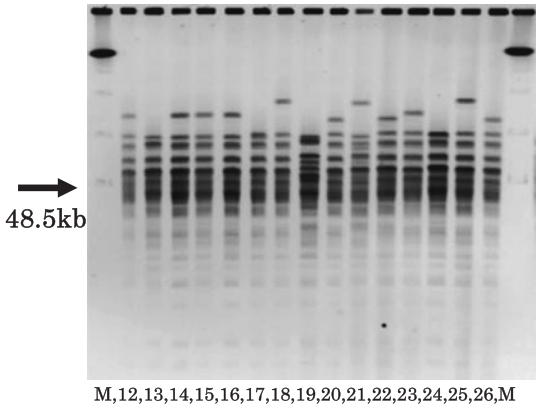


Fig. 3 PFGE of meningitis causing GBS with the *Sal* I restriction enzyme
Lane M, λ-size marker; Lane12 ～ 26 serotype are serotype III



This means some but not all− type-III serotype strains can be detected by PFGE analysis[1]. After changing restriction enzyme *Apa* I to *Sma* I and Sal I, we could detect all 15 type-III serotype strains. Changing restriction enzymes is reported to effectively detect strains for other bacteria such as Group A *Streptococcus*[3] and *Clostridium perfringens*[4], so we thus attempted to use different restriction enzymes. We found that PFGE analysis showed different results when applied to the same type of serotype. It follows that PFGE is effective in determining infection routes.

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References

1) Wakimoto H, Yano H, Baba S, Okuzumi K, Okamoto N, Wakimoto Y, *et al.* : Pulsed field gel electrophoresis Analysis Conditions for Molecular Epidemiological Identification of Vertical Group B *Streptococcus* infection. The

Journal of the Japanese Association for Infection Disease 2005；79：561―3.
2）Terakubo S, Ichihata Y, Takemura H, Yamamoto H, Shimada J, Nakashima H：Serotype and Antibody levels of Group B Streptococci in Pregnant Women. The Journal of the Japanese Association for Infection Disease 2003；77：121―6.
3）Yokoyama E, Kishida K, Koiwai K：The significance of DNA fingerprinting for determination of food poisoning. Bulletin of the Chiba Prefectural Institute of Public Health 1999；23：23―5.
4）Koiwai K：Foodborn Epidemic of Group A Streptococci. Bulletin of the Chiba Prefectural Institute of Public Health 2002；25：16―9.

Group B *Streptococcus* における Pulsed field gel electrophoresis 工程条件の検討

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