JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2002, p. 3035–3037 Vol. 40, No. 8
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.8.3035–3037.2002

Isolation from a Healthy Carrier and Characterization of a *Neisseria meningitidis* Strain That Is Deficient in γ-Glutamyl Aminopeptidase Activity

Hideyuki Takahashi,[1] Hiroshi Tanaka,[2] Hiroo Inouye,[2] Toshiro Kuroki,[3] Yuko Watanabe,[3] Shiro Yamai,[3] and Haruo Watanabe[1]*

Department of Bacteriology, National Institute of Infectious Diseases, Tokyo,[1] Department of Bacteriology, Ehime Prefectural Institute of Public Health and Environmental Science, Matsuyama,[2] and Department of Bacteriology and Pathology, Kanagawa Prefectural Public Health Laboratory, Yokohama,[3] Japan

Received 27 December 2001/Returned for modification 22 April 2002/Accepted 1 May 2002

γ-Glutamyl aminopeptidase (GGT) activity is used as a specific marker for the identification of *Neisseria meningitidis*. We isolated from a healthy carrier and characterized an *N. meningitidis* isolate which lacked the activity due to the insertional mutation of the *ggt* gene, suggesting that naturally occurring *N. meningitidis* isolates do not always possess GGT activity.

Neisseria meningitidis is a gram-negative diplococcus pathogen that colonizes the nasopharynx. Selective media such as modified Thayer-Martin medium and New York City medium are usually used for the isolation of pathogenic neisseriae from swabs of the nasopharynx, where *N. meningitidis*, *Neisseria gonorrhoeae, Neisseria lactamica*, and sometimes *Branhamella catarrhalis* would exist. Further identification is performed on the basis of the biochemical properties listed in Table 1. Of the biochemical properties used for classification, γ-glutamyl aminopeptidase (EC 2.3.2.2; GGT) is considered a specific marker for *N. meningitidis* because its activity is positive in *N. meningitidis* but not in the other three species mentioned above (2). In fact, the detection of GGT activity is used by the Gonochek II enzymatic identification system (E-Y Laboratories Inc., San Mateo, Calif.) in the identification of *N. meningitidis* (1, 5, 6, 11). Here, we report the isolation and characterization of a natural isolate of *N. meningitidis* that is defective in GGT activity.

During an investigation of healthy carriers of *N. meningitidis* in Japan, *N. meningitidis* strain NIID113 was isolated from a 19-year-old healthy male by selection on modified Thayer-Martin medium using swabs of his nasopharynx. After confirming the oxidase-positive and gram-negative phenotypes, we examined the profile of biochemical properties of NIID113, such as sugar fermentation, which identified this strain as *N. meningitidis* (Table 1). The serogroup could not be specified because of the absence of agglutination with any kind of antisera (Difco), but it was suspected to be serogroup B based on a PCR method (8). In contrast to the results of the biochemical property assessment, the strain was identified as *B. catarrhalis* when it was assayed by the Gonochek II system (Table 1). The sole difference in the results of the Gonochek II assay from the criteria of biochemical properties for *N. meningitidis* was the absence of GGT activity. Consequently, we further studied the *ggt* gene.

At first, to characterize a whole coding region of *ggt*, we tried to amplify a *ggt* allele by PCR with primers ggt-1 (ATGCCC TTGTATGGATCA) and ggt-2 (CTAATCACCCATCACTC GACCT) and ExTaq DNA polymerase (Takara Shuzo) and using purified chromosomal DNA as a template in GeneAmp PCR system 2400 (Applied Biosystems). Although a 1.8-kb fragment was amplified from H44/76, a typical *N. meningitidis* strain belonging to the electrophoretic type 5 complex isolated in Norway in the 1970s (kindly provided by L. O. Frøholm), a 2.9-kb fragment was amplified from NIID113 (Fig. 1). Amplified PCR products of the same size (1.8 kb) were observed

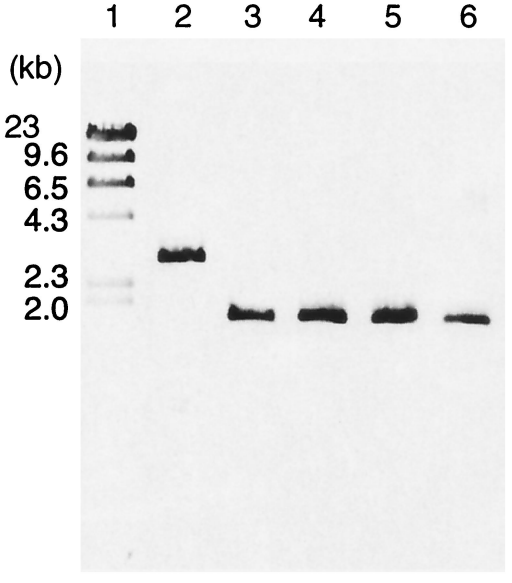

FIG. 1. PCR amplification of the *ggt* gene from NIID113 (lane 2), H44/76 (lane 3), and other *N. meningitidis* clinical isolates, namely, NIID57 (lane 4), NIID99 (lane 5), and NIID100 (lane 6). Molecular mass markers are shown in lane 1.

* Corresponding author. Mailing address: Department of Bacteriology, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo, 162-8640, Japan. Phone: 81-3-5285-1111(-2201). Fax: 81-3-5285-1171. E-mail: haruwata@nih.go.jp.

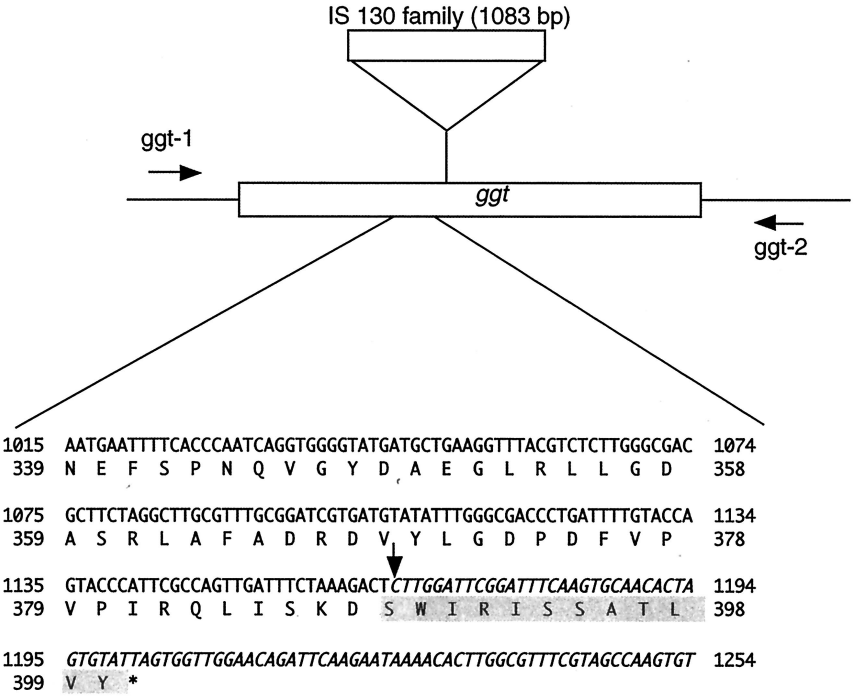

FIG. 2. Insertional site of IS in *ggt*. The vertical arrow behind the T residue (position 1165) indicates the insertional site of the IS. The IS sequence is shown in italics. Amino acid residues shaded in gray indicate the additional amino acid residues derived from the insertional sequence. An asterisk indicates the stop codon.

among other GGT-positive clinical isolates of *N. meningitidis* (Fig. 1). This result suggests that the *ggt* gene of NIID113 was inactivated by an insertional mutation. To further analyze the *ggt* alleles, we performed sequencing with a BigDye Terminator Cycle Sequencing Ready Reaction kit (version 2.0; PE Biosystems) according to the manufacturer's protocol and using the PCR products as templates and an ABI PRISM 310 genetic analyzer (PE Biosystems). From the sequence data (DDBJ accession number AB084259), it was found that a 1,083-bp fragment of insertion sequence (IS) (DDBJ accession number AB076582) was inserted 1,165 bp downstream from the start codon of *ggt*, resulting in the loss of 206 C-terminal amino acid residues of GGT and the addition of 12 foreign amino acid residues (Fig. 2). The IS was identical to one that is widely distributed in the *N. meningitidis* genome (10). The *ggt* gene product, GGT, was not expressed in NIID113; this finding was confirmed by Western blotting with anti-GGT rabbit serum (data not shown). Furthermore, GGT activity was recovered by the introduction of pHT198, which carries a wild-type *ggt* gene on the pHT128 vector (9), into NIID113 (Table 1), suggesting that the defect in GGT activity was due to the insertional mutation of the *ggt* gene.

TABLE 1. Biochemical properties of *Neisseria* strains

Property	Result for strain[a]	
	NIID113	NIID113/pHT198[b]
Oxidase	+	+
Catalase	+	+
Fermentation of sugar		
Glucose	+	+
Maltose	+	+
Sucrose	−	−
Levulose	−	−
Lactose	−	−
Nitrate reductase	−	−
Nitrite reductase	−	−
Production of polysaccharide	−	−
Gonochek II		
Prolyl imonopeptidase	−	−
γ-Glutamyl aminopeptidase	−	+
β-Galactosidase	−	−

[a] +, reaction positive; −, reaction negative.
[b] pHT198 is pHT128(9) carrying a wild-type *ggt* gene amplified with primers ggt-3 (GACTGCTGATGACATTAGCGG) and ggt-4 (GATTACTCACAATT TCCCCCTA) and using H44/76 chromosomal DNA as a template.

This is the first reported characterization of an *N. meningitidis* isolate from a healthy carrier with a deficiency in GGT activity resulting from an insertional mutation of the *ggt* gene. Since strains with carbohydrate degradation profiles that are inconsistent with the identification of *Neisseria* spp. have been occasionally found among *Neisseria* isolates (3, 4, 7), the detection of GGT activity would be a powerful and helpful method for identifying and differentiating *Neisseria* spp. However, researchers should carefully interpret a finding of GGT activity when the identification of *N. meningitidis* depends mainly on this characteristic, since the *ggt* gene in *N. meningitidis* strains can be inactivated by an insertional mutation in the strain's natural habitat, as shown by this study.

Nucleotide sequence accession numbers. The nucleotide sequences of *ggt* allele and IS in NIID113 have been deposited in the DDBJ/EMBL/Genbank nucleotide sequence data-

base under accession numbers AB084259 and AB076582, respectively.

This work was supported by grants from the Ministry of Health, Welfare and Labor.

REFERENCES

1. **Brown, J. D., and K. R. Thomas.** 1985. Rapid enzyme system for the identification of pathogenic *Neisseria* spp. J. Clin. Microbiol. **21:**857–858.
2. **D'Amato, R. F., L. A. Eriquez, K. M. Tomfohrde, and E. Singerman.** 1978. Rapid identification of *Neisseria gonorrhoeae* and *Neisseria meningitidis* by using enzymatic profiles. J. Clin. Microbiol. **7:**77–81.
3. **Granato, P. A., R. Howard, B. Wilkinson, and J. Laser.** 1980. Meningitis caused by maltose-negative variant of *Neisseria meningitidis*. J. Clin. Microbiol. **11:**270–273.
4. **Hoke, C., and N. A. Vedros.** 1982. Characterization of atypical aerobic gram-negative cocci isolated from humans. J. Clin. Microbiol. **15:**906–914.
5. **Janda, W. M., M. G. Ulanday, M. Bohnhoff, and L. J. LeBeau.** 1985. Evaluation of the RIM-N, Gonochek II, and Phadebact systems for the identification of pathogenic *Neisseria* spp. and *Branhamella catarrhalis*. J. Clin. Microbiol. **21:**734–737.
6. **Philip, A., and G. C. Garton.** 1985. Comparative evaluation of five commercial systems for the rapid identification of pathogenic *Neisseria* species. J. Clin. Microbiol. **22:**101–104.
7. **Saez-Nieto, J. A., A. Fenoll, J. Vazquez, and J. Casal.** 1982. Prevalence of maltose-negative *Neisseria meningitidis* variants during an epidemic period in Spain. J. Clin. Microbiol. **15:**78–81.
8. **Taha, M. K.** 2000. Simultaneous approach for nonculture PCR-based identification and serogroup prediction of *Neisseria meningitidis*. J. Clin. Microbiol. **38:**855–857.
9. **Takahashi, H., and H. Watanabe.** 2002. Broad-host-range vector of incompatibility group Q can work as a plasmid vector in *Neisseria meningitidis*: a new finding as a genetical tool. Microbiology **148:**229–236.
10. **Tettelin, H., N. J. Saunders, J. Heidelberg, A. C. Jeffries, K. E. Nelson, J. A. Eisen, K. A. Ketchum, D. W. Hood, J. F. Peden, R. J. Dodson, W. C. Nelson, M. L. Gwinn, R. DeBoy, J. D. Peterson, E. K. Hickey, D. H. Haft, S. L. Salzberg, O. White, R. D. Fleischmann, B. A. Dougherty, T. Mason, A. Ciecko, D. S. Parksey, E. Blair, H. Cittone, E. B. Clark, M. D. Cotton, T. R. Utterback, H. Khouri, H. Qin, J. Vamathevan, J. Gill, V. Scarlato, V. Masignani, M. Pizza, G. Grandi, L. Sun, H. O. Smith, C. M. Fraser, E. R. Moxon, R. Rappuoli, and J. C. Venter.** 2000. Complete genome sequence of *Neisseria meningitidis* serogroup B strain MC58. Science **287:**1809–1815.
11. **Welborn, P. P., C. T. Uyeda, and N. Ellison-Birang.** 1984. Evaluation of Gonochek-II as a rapid identification system for pathogenic *Neisseria* species. J. Clin. Microbiol. **20:**680–683.