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Nonencapsulated *Neisseria meningitidis* Strain Produces Amylopectin from Sucrose: Altering the Concept for Differentiation between *N. meningitidis* and *N. polysaccharea*

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***Neisseria meningitidis* is the causative agent of meningococcal sepsis and meningitis. *Neisseria polysaccharea* is a nonpathogenic species. *N. polysaccharea* is able to use sucrose to produce amylopectin, a starch-like polysaccharide, which distinguishes it biochemically from the pathogenic species *N. meningitidis*. The data presented here indicate that this may be an insufficient criterion to distinguish between these two species. The nonencapsulated *Neisseria* strain 93246 expressed a phenotype of amylopectin production similar to that of *N. polysaccharea*. However, strain 93246 reacted with *N. meningitidis* serotype 4 and serosubtype P1.14 monoclonal antibodies and showed the *N. meningitidis* L1(8) lipo-oligosaccharide immunotype. Further analyses were performed on four genetic loci in strain 93246, and the results were compared with 7 *N. meningitidis* strains, 13 *N. polysaccharea* strains, and 2 *N. gonorrhoeae* strains. Three genetic loci, *opcA*, *siaD*, and *lgt-1* in strain 93246, were the same as in *N. meningitidis*. Particularly, the *siaD* gene encoding polysialyltransferase responsible for biosynthesis of *N. meningitidis* group B capsule was detected in strain 93246. This *siaD* gene was inactivated by a frameshift mutation at the poly(C) tract, which makes strain 93246 identical to other nonencapsulated *N. meningitidis* strains. As expected, the *ams* gene encoding amylosucrase, responsible for production of amylopectin from sucrose, was detected in strain 93246 and all 13 *N. polysaccharea* strains but not in *N. meningitidis* and *N. gonorrhoeae* strains. These data suggest that strain 93246 is nonencapsulated *N. meningitidis* but has the ability to produce extracellular amylopectin from sucrose. The gene for amylopectin production in strain 93246 was likely imported from *N. polysaccharea* by horizontal genetic exchange. Therefore, we conclude that genetic analysis is required to complement the traditional phenotypic classification for the nonencapsulated *Neisseria* strains.**

Neisseria meningitidis is a pathogenic species of the genus *Neisseria* causing meningococcal septicaemia and meningitis (15). *N. meningitidis* expresses different capsular polysaccharides that determine the meningococcal serogroups (17, 18). *Neisseria polysaccharea* is a nonpathogenic species that has been isolated from the throats of healthy children (19, 23). The most prominent biochemical feature of *N. polysaccharea* is the production of α-D-glucan from sucrose, distinguishing it phenotypically from *N. meningitidis* (5, 23, 24). The gene coding the extracellular amylosucrase that uses sucrose to produce this amylopectin has been cloned and sequenced (7, 9).

Initially we examined 14 strains of *N. polysaccharea* from bacterial culture collections of the National Microbiology Laboratory of Health Canada for the *opcA* gene coding an outer membrane protein (20, 33). The results revealed that two *N. polysaccharea* strains, 85322 and 89357, contained a novel *opcA* orthologous gene (*N. polysaccharea opcA*) that differed from the *opcA* genes reported for *N. meningitidis* (20) and *Neisseria gonorrhoeae* (33). Interestingly, we found that an *N. polysaccharea* strain, 93246, contained an *N. meningitidis opcA* gene. PCR-restriction fragment length polymorphism analysis of 20 single colonies of this culture yielded the same results for *opcA*, suggesting that the culture of strain 93246 was not likely to be a mixture of *N. polysaccharea* and *N. meningitidis*. This suggested that strain 93246 might be *N. meningitidis* rather than *N. polysaccharea*. Therefore, the phenotypic and genetic characteristics of strain 93246 have been further investigated by several methods. Strain 93246 showed phenotypic characteristics similar to those of *N. polysaccharea*, and its genome also contained the gene encoding amylosucrase. Nevertheless, the results from three genetic loci, *opcA*, *siaD*, and *lgt-1*, showed that these genes in strain 93246 were the same as those in *N. meningitidis*. This suggests that strain 93246 has the basic genome framework of *N. meningitidis* and acquired an amylosucrase gene from *N. polysaccharea*.

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MATERIALS AND METHODS

Bacterial strains, growth conditions, and DNA isolation. The bacterial strains used in this study are shown in Table 3. Five *N. meningitidis* strains, M986, 126E, M158, S4383, and 6304, were obtained from the culture collections of C. E. Frasch, Center for Biologics Evaluation and Research (CBER), U.S. Food and Drug Administration (FDA), Bethesda, Md. Other strains were from authors' collections. Among them, strain 93246 was isolated from the vagina of a 27-year-old female in 1993. The isolate was submitted to the National Microbiology Laboratory of Health Canada for differentiation between *N. meningitidis* and *N. polysaccharea*. This isolate was classified as *N. polysaccharea* because it behaved typically for *N. polysaccharea*, including production of amylopectin from sucrose as well as the ability to grow on Thayer-Martin selective medium containing the antimicrobial agents vancomycin, colistin, and amphotericin B. The growth and

TABLE 1. Primers for PCR amplification of the *opcA, siaD,* and *ams* loci

Primer	Sequence (5′ - 3′)	Specificity[a]	Strand[b]	Position[c]	Accession no.	PCR size[d]
P103	TTCGTTACCTCCGGCATCCG	*opcA* (E)	F	1356–1375	AJ242839	2,744 (P103/P104)
P61	ACCATCAAATGAATATCCAT	*opcA* (E)	R	4080–4099	AJ242839	
P51	GCCGCCATCTCCGGTACTGC	*opcA* (I)	F	2976–2995	AJ242839	662 (P51/P52)
P52	TGACGGTGTTTGTAGAACGG	*opcA* (I)	R	3618–3637	AJ242839	
P124	GTCGCTGCTTGCAATATTCG	*siaD,* (E)	F	2991–3010	M95053	1,770 (P124/P123)
P125	TACAGCAGCTCTGTTGTCGA	*siaD,* (E)	R	4741–4760	M95053	
P63	CTCTCACCCTCAACCCAATGTC	*siaD* (I)	F	4141–4160	M95053	453 (P63/P64)
P64	TCGGCGGAATAGTAATAATGTT	*siaD* (I)	R	4572–4593	M95053	
P114	CGCCGGTCGGAAACTTCAGA	*ams* (E)	F	101–120	AJ011781	1,985 (P114/P115)
P115	CCGTCTGAAACGGTTCAGAC	*ams,* (E)	R	2066–2085	AJ011781	
P116	CAAGTCGGCGGCGTGTGCTA	*ams* (I)	F	451–470	AJ011781	1,300 (P116/P117)
P117	CTGCGGTCGACGGATCGTTG	*ams* (I)	R	1731–1750	AJ011781	

[a] E, external primer at the flanking region; I, internal primer at the coding region.
[b] F, forward; R, reverse.
[c] Position on the sequence from the following strains: AJ242839 from strain FA1090 (33); AJ011781, strain ATCC 43768 (25); M95053, strain B1940 (11).
[d] Numbers indicate the expected size of the PCR product (base pairs). External or internal primer pairs are indicated in parentheses.

biochemical characteristics of strain 93246 were confirmed by the standard method. The bacterial strains were grown on brain heart infusion agar plates at 37°C in 5% CO_2 for 16 h. The chromosomal DNA was isolated using a modified phenol-chloroform extraction method (20, 26).

Phenotypic analysis. (i) Amylopectin production from sucrose. Bacterial strains were grown on tryptic soy broth (Difco) agar containing 1% sucrose at 37°C for 48 h and tested for the production of amylopectin with Lugol's iodine solution using the Centers for Disease Control and Prevention's method (http://www.cdc.gov/ncidod/dastlr/gcdir/NeIdent/Polysacc.html).

(ii) Capsular production. Bacterial strains were grown on antiserum agar containing 6% *N. meningitidis* serogroup B serum against capsular polysaccharide (horse 46 globulin; CBER, FDA) at 37°C for 24 h, and the presence of capsular polysaccharide was detected by immunoprecipitation (8). Serogroup B antiserum was used because the *siaD* gene detected in strain 93246 was specific for the group B capsule (see below) (2, 28).

(iii) Immunotype of lipooligosaccharide (LOS). LOS samples were analyzed by using the sodium dodecyl sulfate-polyacrylamide gel electrophoresis and silver staining method (30). Immunoblotting was performed using the antibodies specific to *N. meningitidis* LOS (31, 32).

(iv) Serotyping and subtyping. *N. meningitidis* serotyping and subtyping were performed using monoclonal antibodies and whole-cell enzyme-linked immunosorbent assay (1).

Genetic analysis. (i) PCR. Twelve primers for PCR are shown in Table 1. The primers for the *opcA* locus were designed from an alignment of four *opcA* sequences from *N. meningitidis* strains Z2491 (GenBank accession number AJ242841) and MC58 (accession number AE002456) and *N. gonorrhoeae* strains FA1090 (accession number AJ242839) and MS11 (accession number AJ242840) (21, 29, 33). The primers at the *siaD* region were designed from the reported sequence of *N. meningitidis* strain B1940 (M95053) (11). Five primer pairs for detecting *siaD* and *orf-2* loci in *N. meningitidis* were the same as those described by Taha (serogroups A, W135, and Y) (28) and Arreaza et al. (serogroups B and C) (2) except for the reverse primer for group B *siaD* (P64) (Table 1). For the purpose of this study, the gene encoding amylosucrase is designated *ams*. The primers at the *ams* region were designed from the reported *ams* sequence of *N. polysaccharea* strain ATCC 43768 (accession number AJ011781) (9). The primers for detecting seven *lgt* genes at the *lgt-1* locus were the same as in our previous report (35, 36). Seven *N. meningitidis* reference strains (Z2491, MC58, M986, 126E, M158, S4383, and 6304Y) and two *N. gonorrhoeae* reference strains (FA1090 and F62) served as controls in the PCR analysis.

The PCR mixtures contained 1 μl of 10 mM deoxynucleoside triphosphates, 10 pmol of each primer, 0.1 μg of chromosomal DNA, 5 μl of 10× PCR buffer, and 1.5 U of *Taq* DNA polymerase (Perkin-Elmer), and sterile redistilled H_2O in a final volume of 50 μl. PCR amplification was performed using the following protocol: denaturation at 94°C for 2 min, 30 cycles of amplification at 94°C for 30 s, 56°C for 30 s and 72°C for 2 min, and a final extension at 72°C for 4 min. The PCR products were analyzed by electrophoresis on a 1% agarose gel and stained with ethidium bromide.

(ii) RT-PCR. Bacteria were grown in a 10-ml liquid brain heart infusion culture that was shaken at 160 rpm for 6 h. Bacteria cells were collected by centrifugation at 3,000 × *g* for 20 min, and total bacterial RNA was isolated from the pellet using TRIzol Reagent (Life Technologies) using the manufacturer's protocol. The total RNA sample was treated using DNase I (Life Technologies) to remove the genomic DNA, and reverse transcription-PCR (RT-PCR) was performed using the Titan One tube RT-PCR system (Roche Molecular Biochemicals) as described by the manufacturer. Two internal primers, P51 and P52, were used for RT-PCR analysis of *opcA* expression. The genomic DNA from strain 93246 was used as a positive control, and RNA from 93246 without RT was used as a negative control. The template of reverse transcription from RNA was used for examination of *opcA* expression.

(iii) DNA sequencing. The whole regions of *opcA*, *siaD*, and *ams* were amplified using the flanking primers and internal primers (complete list available on request to the corresponding author). DNA sequences were determined from both strands of three independent PCR products for each strain as described previously (36). DNA sequences were analyzed with the Genetics Computer Group package (GCG10.2-Unix; University of Wisconsin) (10) and Molecular Evolutionary Genetics Analysis software (MEGA2.1; Arizona State University) (16).

Nucleotide sequence accession numbers. The nucleotide sequences of the *opcA*, *siaD*, and *ams* regions from strain 93246 were determined and have been submitted to GenBank under accession numbers AY099332 to AY099334. The sequence of *ams* from strain 85322 was determined as well (GenBank accession no. AY099335).

RESULTS

Phenotypic features of strain 93246. The phenotypic features of strain 93246 were tested and compared with those of *N. meningitidis* strain M986 and *N. polysaccharea* strain 89357 (Table 2). Strain 93246 showed the same characteristics as *N. polysaccharea* strain 89357, including production of amylopectin from sucrose, negative growth in Catlin meningococcal defined medium, and negative reaction with serogroup B horse serum. Strain 93246 reacted with *N. meningitidis* serotype 4 and serosubtype P1.14 monoclonal antibodies. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis showed that strain 93246 has two LOS bands. The estimated size of the major band is approximately 3.8 kDa, and that of the minor band is approximately 3.6 kDa. The major LOS band reacted with *N. meningitidis* L1 antibody and the minor band reacted with *N. meningitidis* L8 antibody in immunoblotting analysis, indicating that strain 93246 expresses a *N. meningitidis* LOS of L1(8) pattern.

PCR amplification of selected genes. Eleven genes at four loci, whose specificity for *Neisseria* has been well characterized in a natural diverse population, were chosen for this study. These genes in strain 93246 were evaluated by PCR, and the

TABLE 2. Phenotypic characteristics of strain 93246

Strain	Amylopectin from sucrose[a]	Growth in Catlin medium	Precipitation of B capsule	Serotype, serosubtype[b]	LOS immunotype[c]
N. meningitidis					
M986	−	+	+	2a, P1.2,5	L3,7
93246	+	−	−	4, P1.14	L1, (8)
N. polysaccharea					
89357	+	−	−	−	UD

[a] 12 *N. polysaccharea* strains showed the same phenotype as strain 89357; 2 *N. meningitidis* control strains, A1 (serogroup A) and 126E (group C) and *N. cinerea* control strain 81176 showed the same phenotype as strain M986 (B).
[b] Serotyping and serosubtyping using monoclonal antibodies against *N. meningitidis* PorB and PorA proteins.
[c] Strain 93246 showed major L1 and minor L8 bands as LOS prototype strain 126E. UD, undetermined.

results were compared with those for 13 *N. polysaccharea* strains, 8 *N. meningitidis* strains, and 2 *N. gonorrhoeae* strains whose genes were partially or fully described previously (12, 14, 21, 29, 33, 36, 37) (Table 3). The PCR products of *opcA*, *siaD*, *ams*, *lgtZ, lgtC, lgtD*, and *lgtH* genes were detected in strain 93246. The results of this gene pattern suggest that strain 93246 is a group B *N. meningitidis* strain.

Among the strains from the different species, a striking diversity in distribution of genes was observed for *siaD*/*orfA*, *ams*, *lgtZ*, and *lgtE*. A PCR product of *siaD*/*orfA* was amplified in all 7 *N. meningitidis* control strains, but not in the 13 *N. polysaccharea* strains or the 2 *N. gonorrhoeae* control strains, using a set of primer pairs previously reported (2, 28). The results from *N. meningitidis* control strains were consistent with their serogroup classification except that a group C *siaD* product was detected from strain M158, originally reported as the prototype serogroup D strain (6). The *ams* gene was detected in all 13 *N. polysaccharea* strains but not in the 7 *N. meningitidis* strains and 2 *N. gonorrhoeae* strains. The *lgtZ* gene is rare and was only found in *N. meningitidis*. The *lgtE* gene was not amplified in 13 *N. polysaccharea* strains.

TABLE 3. The genetic characteristics of strain 93246

Strain	*opcA*[a]	*siaD*/*orf2*[b]	*amS*	*lgt-1*						
				lgtA	*lgtB*	*lgtC*	*lgtD*	*lgtE*	*lgtZ*	*lgtH*
N. meningitidis										
Z2491	+ (m)	+ (A)	−	+	+	−	−	−	−	+
MC58	+ (m)	+ (B)	−	+	+	−	−	+	−	−
M986	−	+ (B)	−	+	+	−	−	−	−	+
126E	+ (m)	+ (C)	−	−	−	+	+	+	+	−
M158	−	+ (C)	−	+	+	−	−	+	−	−
S4383	+ (m)	+ (W135)	−	+	+	−	−	−	−	+
6304Y	−	+ (Y)	−	+	+	−	−	+	−	−
93246	+ (m)	+ (B)	+	−	−	+	+	−	+	+
N. polysaccharea										
85321	−	−	+	+	+	−	−	−	−	+
85322	+ (p)	−	+	+	+	−	−	−	−	+
85323	−	−	+	+	+	−	−	−	−	+
87042	−	−	+	+	+	−	−	−	−	+
87043	−	−	+	+	+	−	−	−	−	+
87188	−	−	+	+	+	+	+	−	−	+
87190	−	−	+	+	+	+	+	−	−	+
89353	−	−	+	+	+	−	−	−	−	+
89354	−	−	+	+	+	−	−	−	−	+
89355	−	−	+	+	+	−	−	−	−	+
89357	+ (p)	−	+	+	+	−	−	−	−	+
90400	−	−	+	+	+	−	−	−	−	+
91275	−	−	+	+	+	−	−	−	−	+
N. gonorrhoeae										
FA1090	+ (g)	−	−	+	+	+	+	+	−	−
F62	+ (g)	−	−	+	+	+	+	+	−	−

[a] m, p, and g indicate three *opcA* orthologous of *N. meningitidis, N. polysaccharea,* and *N. gonorrhoeae* (20, 33, 36).
[b] A, B, C, W135, and Y indicate PCR-based prediction for serogroups of *N. meningitidis* (2,28). This is consistent with the serogroup classification except that group C *siaD* was detected in group D strain M158.

A 3,088-bp region of *opcA* from strain 93246 showed a genetic organization similar to that of *N. meningitidis* strain MC58, with a single copy of the IS*1106* element upstream of *opcA* (29). Strain 93246 had an *opcA* coding region identical to that of MC58, but they differed at the promoter region. Strain 93246 has a C_9 poly(C) tract, which suggests that *opcA* is not expressed (26). RT-PCR analysis showed no transcript of *opcA* in strain 93246, consistent with the poly(C) sequence prediction. The 2,122-bp upstream region in 93246 had 96.9% homology to the corresponding region in MC58. A phylogenetic tree was constructed from the nucleotide sequences of the *opcA* coding region for strain 93246 and six representative strains, including *N. polysaccharea* 85322 and 89357, *N. meningitidis* Z2491 and MC58, and *N. gonorrhoeae* FA1090 and MS11 (Fig. 1). The *opcA* from strain 93246 is identical to MC58 within the *N. meningitidis opcA* branch.

A 1,715-bp sequence, containing 1,489 bp of the *siaD* coding region and a 226-bp flanking region from strain 93246, was identical to the corresponding region of *siaD* in *N. meningitidis* serogroup B strain B1940 (accession no. M95053) (11), except that strain 93246 had one more cytidine nucleotide at the poly(C) tract (G_8) in the coding region. This G_8 tract would cause a frameshift mutation and therefore inactivate the *siaD* gene in strain 93246.

A 1,967-bp sequence, containing 1,911 bp of the *ams* coding region and a 56-bp flanking region from strain 93246, had 96.4% homology to the corresponding region previously reported in *N. polysaccharea* strain ATCC 43768 (accession no. AJ011781) (9). To understand the natural variation of *ams* gene in *N. polysaccharea* species, the 1,967-bp *ams* region from *N. polysaccharea* strain 85322 was sequenced as well (accession no. AY099335). The *ams* sequence from strain 89322 has 96.2 and 98.5% homology to the sequences from strain ATCC 43768 and strain 93246, respectively. Among the three sequences, 83 sites are polymorphic (4.3%) in the 1,911-bp coding region (Fig. 2A), resulting in 17 substitution sites in 636 amino acids of the Ams protein (2.7%) (Fig. 2B). Fifty-six nucleotide polymorphisms (67.5%) were located at two regions of 133 and 220 bp at positions 251 to 383 and 1317 to 1536 from the ATG start codon. These two fragments accounted for only

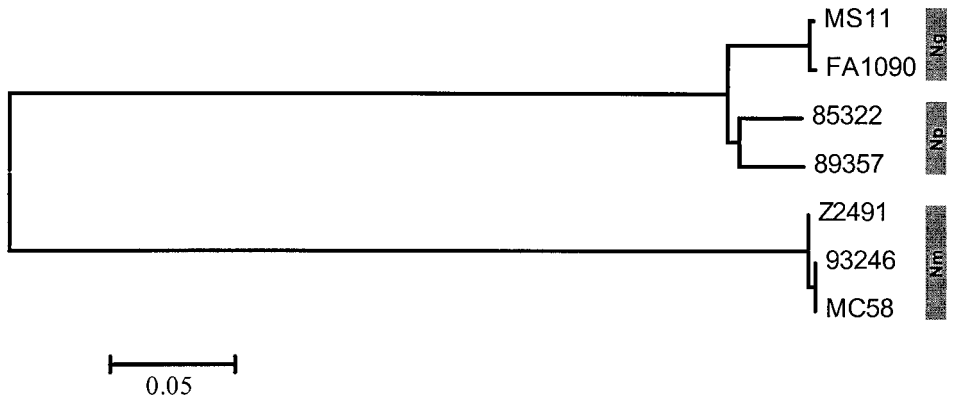


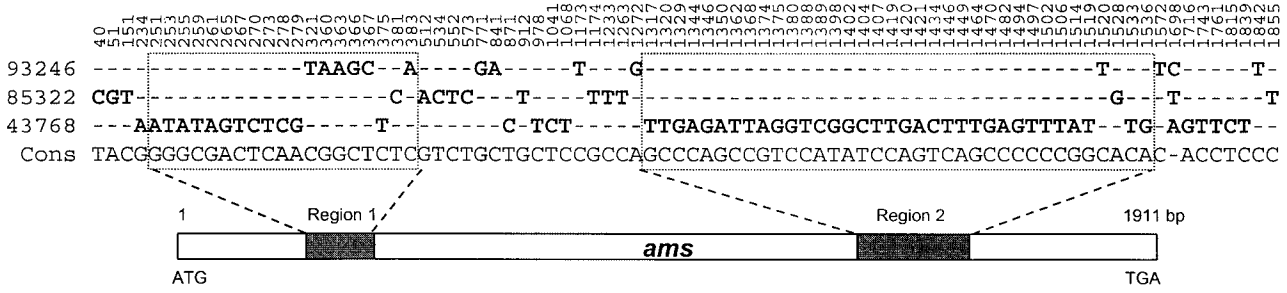
FIG. 1. Phylogenetic relationships of the *opcA* genes. The diagram shows an unrooted tree constructed using neighboring-joining methods (MEGA2.1 program) from the nucleotide sequences of the coding region of the *opcA* gene.

18.5% of the coding region, suggesting that they might be two hypervariable regions in *ams* or hot spots that serve for intragenic recombination. Seventeen amino acid substitutions were observed among three Ams protein variants (Fig. 2B), but they were not at the five critical positions for the enzymatic activity (25).

DISCUSSION

In 1983, Riou et al. described 13 isolates of a new taxon of *Neisseria* sp. provisionally named *Neisseria polysaccharea* (23). Characteristics that differentiated *N. polysaccharea* from meningococcal isolates were D-glucan production when cultured in the presence of 5% sucrose, gamma-glutamylaminopeptidase activity, and a requirement for cysteine or cystine to be able to grow in the Catlin defined medium (5, 23, 24). Among these characteristics, the amylopectin production from starch-free medium containing sucrose is considered the most critical feature for differentiating between *N. meningitidis* and *N. polysaccharea*. However, the data from strain 93246 indicate that results from this kind of phenotypic test may not accurately speciate strains.

A



B

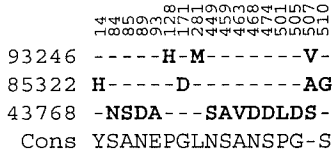


FIG. 2. Details of polymorphic sites in the aligned *ams* genes (A) and the deduced proteins (B) from *N. meningitidis* strain 93246, *N. polysaccharea* strains 85322 (accession no. AY099335), and ATCC 43768 (accession no. AJ011781) (25). Numbers above indicate the position. Strain names are on the left. Cons indicates the consensus sequence, and a dash (-) indicates the nucleotide and amino acid identical to Cons. Each polymorphic site is in bold. Two highly polymorphic regions of 133 and 220 bp (Region 1 and Region 2) are indicated with a shadowed box within the *ams* gene.

The orthologous *opcA* genes have been described for three *Neisseria* species, including *N. meningitidis*, *N. gonorrhoeae*, and *N. polysaccharea* (20, 33, 37). Most *N. gonorrhoeae* strains have an *opcA* gene, and about 60% *N. meningitidis* strains have *opcA* (27, 33). However, the *opcA* gene in *N. polysaccharea* species is rare, and interspecies diversity of *opcA* distinguishes *opcA* from different *Neisseria* species (37). The *opcA* gene was flanked by the IS*1106* element at the upstream region in *N. meningitidis* but with *orfX* and *orfY* in *N. gonorrhoeae* and *N. polysaccharea* (33, 34, 37). Strain 93246 contains not only the *N. meningitidis opcA* gene but also an IS*1106* element at the upstream region. Therefore, both the coding region and the genetic organization at the *opcA* locus identify strain 93246 as belonging to *N. meningitidis*.

The classification of *N. meningitidis* serogroups is based on the structural and immunological differences in the capsular polysaccharide. Traditionally, *N. meningitidis* serogrouping has been done by immunologic methods using polyclonal and monoclonal antibodies. However, the serogroups of some nonencapsulated and nongroupable *N. meningitidis* strains can be determined by PCR using a set of primers for the *siaD* gene and *orf-2* (2, 28). Strain 93246 contains a *siaD* gene of serogroup B *N. meningitidis*, whereas all 13 *N. polysaccharea* strains tested do not contain *siaD* or *orf-2*, which strongly suggests that strain 93246 is *N. meningitidis*. Hammerschmidt et al. (13) reported that capsule phase variation in *N. meningitidis* serogroup B is regulated by a slipped-strand mispairing mechanism in the *siaD* gene. For all nonencapsulated strains analyzed, they found an insertion or deletion of cytidine at the poly(C) stretch within *siaD*, resulting in a frameshift and loss of capsule formation. The sequence analysis in this study revealed that the *siaD* gene in strain 93246 has also been inactivated by a frameshift mutation at the poly(C) tract, make it genetically identical to other nonencapsulated *N. meningitidis*.

The genetic organization of the *lgt-1* locus, responsible for biosynthesis of the α chain of lipooligosaccharide, has been investigated in number of strains representing 14 *Neisseria* species (36). The genetic composition and arrangement at the *lgt-1* locus in strain 93246 (*lgtZCDH*) is similar to those for *N. meningitidis* L1 prototype strain 126E (*lgtZCDE*) but differ from those for all 13 *N. polysaccharea* strains (*lgtABH* and *lgtABCDH*). Strain 93246 differs from 126E in that it has an *lgtH* gene at the 3′ end of the *lgt-1* locus whereas strain 126E has a *lgtE* gene. However, the *lgtE* and *lgtH* genes probably have the same function (35). Use of immunoblotting in this study further showed that strains 93246 and 126E have the same major L1 LOS phenotypes. This suggested that both the LOS genotype and phenotype of strain 93246 are classified as *N. meningitidis* species. The expressed LOS pattern of strain 93246 is the same as that for *N. meningitidis* L1 prototype strain 126E.

In addition, our study confirms that strain 93246 does produce amylopectin from sucrose and also shows other phenotypic characteristics of *N. polysaccharea*. We also detected the *ams* gene encoding amylosucrase in strain 93246 but not in the other *N. meningitidis* and *N. gonorrhoeae* strains tested. Nonetheless, the GC content of the *ams* region (56.7%) in strain 93246 was slightly higher than the average GC contents of two meningococcal genomes (51.8 and 51.5%) (21, 29). The *ams* gene in strain 93246 was probably imported from *N. polysaccharea* species through horizontal genetic exchange, as the data from serotyping, serosubtyping, and three other genetic loci, *opcA*, *siaD*, and *lgt-1*, suggest that strain 93246 is *N. meningitidis* rather than *N. polysaccharea*. Strain 93246 was isolated from a vaginal specimen from a 27-year-old female in Canada in 1993. Isolation of *N. meningitidis* from the urogenital tract was considered unusual, but an increasing number of such cases were reported in the past years (3, 4, 22). This study added further information that *N. meningitidis* strain 93246 isolated from the vagina was able to produce amylopectin from sucrose.

In summary, the *opcA*, *siaD*, and *lgt-1* loci are scattered on the meningococcal genome, with large distances of approximately 321 to 996 kb, respectively, separating these genes (21, 29), and therefore the basic framework of strain 93246 reassembles the *N. meningitidis* genome rather than *N. polysaccharea*. The amylosucrase gene was probably imported into strain 93246, which altered the strain's phenotypic characteristic. Strain 93246 represents an interesting example of the potential discrepancy between genotype and phenotype in bacterial classification. Thus, we conclude that the genetic analysis using multiple genetic loci is required to complement the traditional phenotypic classification for complete identification of atypical bacterial strains.

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