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Full Length Research Paper

Characterization of a new species of *neisseria* isolated from the liver of the gaoyou sheldrake

Wang yanhong*, Wang xiaoquan, Liu wenbo, Yang yuefei, Zhu jie, Shao hui-jun, Zhang bin-bin, Han tao, and Liu Xiufan

Key Laboratory of Animal Infectious Diseases of Ministry of Agriculture, College of Veterinary Medicine, Yangzhou University, Yangzhou, Jiangsu 225009, China.

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A strain of Gram-negative diplococci was isolated from the liver of the Gaoyou Sheldrake. The 16S rRNA gene sequences of this organism was most similar to Bacterium 'New Zealand A' (GenBank accession no. AY721627), with 97% similarity to *Neisseria animaloris* in the *Neisseria* genus. It showed a diplococci morphology by electron microscopy. The biochemical reactions of this strain resembles *N. mucosa*, and it grew on nutrient agar and acidified dextrose, maltose, sucrose and did not acidified lactose. It was positive for oxidase and nitrate reduction. These results suggest that this organism belongs to the genus *Neisseria*, for which the name *Neisseria tadorna* sp. nov. is proposed. The strain was sensitive to Fleroxacin, Amikacin, etc and resistant to spectinomycin, sulfamethoxazolum-trimethoprimum and nalidixic acid.

Key words: Gaoyou sheldrake, 16S rRNA, *Neisseria.*

INTRODUCTION

The genuses *Neisseria* are composed of Gram-negative cocci, diplococci and bacilli. There are currently 12 *Neisseria* species of human origin, with *N. meningitidis* and *N. gonorrhoeae* being important patho-gens and the others being opportunistic (Janda and Knapp, 2003.). These opportunistic pathogens include *Neisseria sicca*, *N. lactamica*, *N. cinerea*, *N. flavescens*, *N. subflava*, *N. mucosa*, *N. polysaccharea*, and *N. elongata*, which are common commensals of the upper respiratory tract (Bovre and Holten, 1970; Smith et al., 1999).

Also, there are some *Neisseria* species of animal origin with *N. canis*, *N. dentiae*, *N. zoodegmatis*, *N. animaloris. N. weaveri*, *N. animalis*, *N. macacae* and *N. iguana* being opportunistic (Andersen et al., 1993; Sneath and Barrett, 1996). *N. animaloris* and *N. weaveri* are commonly isolated from dog and cat bites (Andersen et al., 1993; Holmes et al., 1993). Furthermore, there may be existing new species of the genus *Neisseria* that have not isolated or identified. Such as Bacterium 'New Zealand A, it was isolated from the duck faeces, which was most similar to *Neisseria canis* (Murphy et al., 2005).

In this study, we reported the isolation and characterization of one strain of *Neisseria* species from the liver of the Gaoyou Sheldrake. The 16S rRNA gene sequences of this organism was most similar to Bacterium „New Zealand A" (GenBank accession no. AY721627), with 97% similarity to *N. animaloris* in the *Neisseria* genus. It was most similar to *N. mucosa* in phenotypic characterization which, lead us to propose the name "*Neisseria tadorna* sp. nov" for this strain.

MATERIALS AND METHODS

Bacterial strains of isolation and culture conditions

Free-living ducks are important backyard waterfowl in Gaoyou Lake

*Corresponding author. E-mail: wyh7405@163.com. Tel: 86-514-87979036. Fax: 86-514-87972218.

Table 1. Sources and accession numbers for 16S rRNA data.

Organism	Strain	GenBank accession no.	Country	host	Isolation source	Reference
'New Zealand A'	A	AY721627	New Zealand	mallard duck	feces	Murphy et al., 2005
N. animaloris	LMG 23011	DQ006842	USA	Human	Thumb wound	Vandamme et al., 2006
N. canis	ATCC 14687	L06170		Human	oral	unpublished
Neisseria sp.	BNO09-3	GU121411	China	captive wild goose	brain	unpublished
N. mucosa	LNP405	AJ239282	UK	human	commensal	Smith et al., 1999
N. shayeganii	WC 04-12337	FJ654665	USA	human	arm wound	Wolfgang et al., 2011
N. zoodegmatis	3033/04	GU797849	Switzerland	human	Wound (cat bite)	Keller et al., 2010
N. pharyngis	NCTC4590	AJ239281	UK	human	commensal	Smith et al., 1999
N. macaca	ATCC 33926	L06169	USA	human	periodontal	Dewhirst et al.,1993; Wolfgang et al., 2011
N. sicca	Q28	AJ239293	UK	human	commensal	Smith et al., 1999

in China at autumn and winter each year. Ducks that have been dead were presented to the Veterinary Hospital, College of Veterinary Medicine, Yangzhou University, Yangzhou, China, for examination. This strain was isolated from the liver of the Gaoyou Sheldrake, and grown on chicken blood-agar plates at 35°C for 24 h with 5% CO_2. Subcultures were plated on Luria-Bertani (LB) agar (Oxoid) and MacConkey agar (Shanghai China Academy Of Sciences Shanghai Hexapod Technology Development Co., Ltd.) and incubated aerobically at 35°C.

16S rRNA sequencing and phylogenetic analysis

The 16S rRNA gene of this isolated strain was amplified enzymatically by polymerase chain reaction (PCR) with the forward primer and reverse primer 1 of TakaRa 16S rDNA Bacterial Identification PCR kit (TaKaRa Biotechnology (Dalian) Company Limited, China) according to the manufacturer"s recommendations, The expected size of amplicon was approximately 500 bp.

Aliquot obtained was evaluated by agarose gel electrophoresis. The PCR product was purified by the use of TaKaRa Agarose Gel DNA purification kit Ver.2.0 (TaKaRa Biotechnology (Dalian) Company Limited, China) following the manufacturer"s instructions. Sequencing reactions were done by GenScript Corporation (NanJing) of China Business Dept. Sequence of 16S rDNA was submitted to BLAST alignment (http://www.ncbi.nlm.nih.gov/BLAST) against other sequences available in GenBank. 16S rRNA sequences of 10 species were obtained from GenBank (Table 1). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4 (Tamura et al., 2007). The evolutionary history was inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analyzed. Codon positions included were 1st+2nd+3rd+Noncoding.

Characterization of the isolated strain

This isolated strain was cultured in LB Broth (Oxoid) at 35°C, 150 rpm for16 h. The morphology of the strain was observed by transmission electron microscope (TEM) observation with negative staining, and were visualized and photographed with Philips Tecnai 12 TEM (Eindhoven, the Netherlands). Biochemical tests were performed in conventional tube media (Hanzhou Microbial Reagent Company Limited, China). Antimicrobial suscepti-bility tests were done on pure, 1-day-old cultures of all the aerobic bacteria, using the Kirby-Bauer disk diffusion test and Antimicrobial susceptibility slip (Hanzhou Microbial Reagent Company Limited, China), according to the manufacturer"s recommendations. The following antimicro-bials were tested: Vancomycin, amikacin mezlocillin, fleroxacin, levofloxacin, lincomycin, tobramycin, norxacin, furazolidone, aerosporin, sulfamethoxazolum-trimethoprimum, lomefloxacin, chloramphenicol, cefoxitin, spectinomycin and nalidixic acid.

Nucleotide sequence accession numbers

The 16S rRNA sequence for 20101216Y2 has been deposited in the GenBank data base under accession number JN001182. Accession numbers and references for all other 16S rRNA sequences used in this study are listed in Table 1.

RESULTS

Bacterial strains of isolation and culture conditions

This strain was isolated from the liver of the Gaoyou Sheldrake, and grown on chicken blood-agar plates at 35°C for 24 h with. Incubated aerobically at 35°C with 5% CO2, this strain grew well on LB agar plates. The colonies were round, smooth, glistening, and light gray and measured 0.5 to 1 mm at 24 h. No growth was observed on MacConkey agar. The organisms were Gram negative and diplococci. An electron photomicro-graph of 20101216Y2 is shown in Figure 1; the

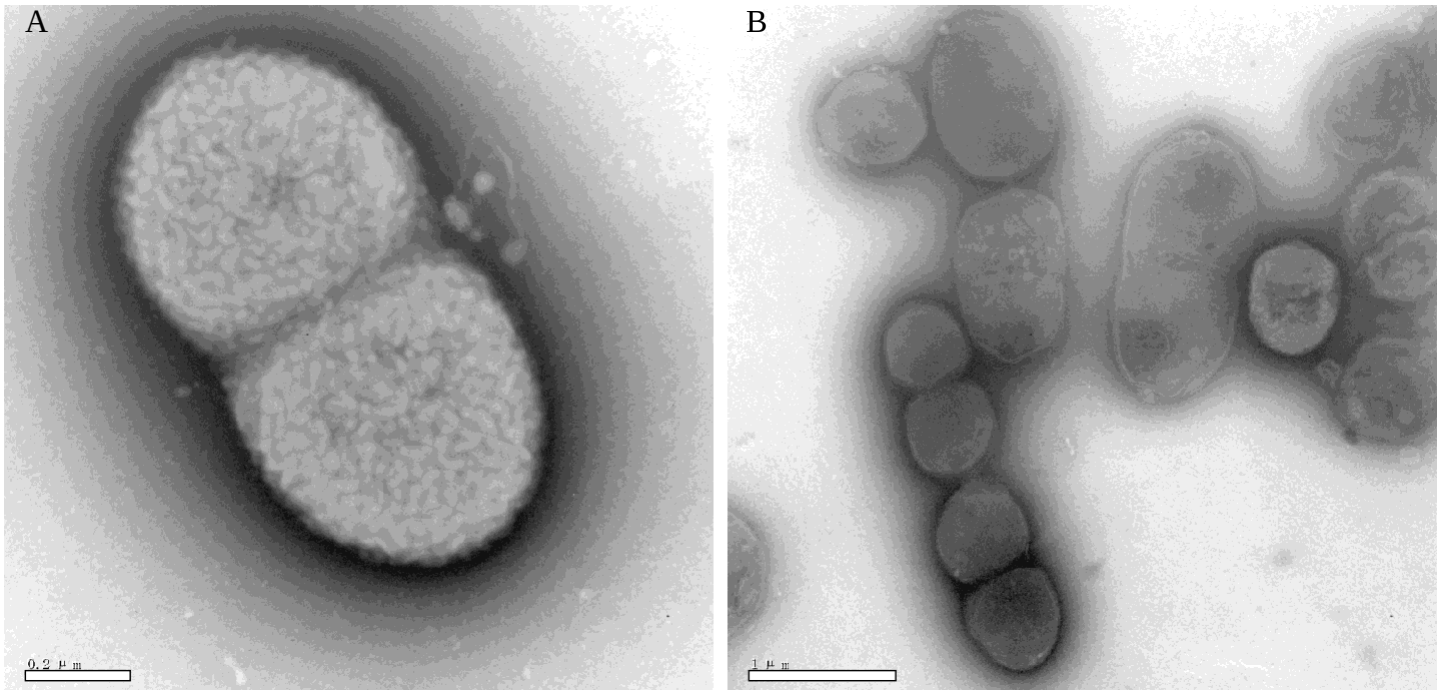

Figure 1. Electron microscopy (A,bar,0.2 μm. B,bar,1 μm) (bottom) of 20101216Y2.

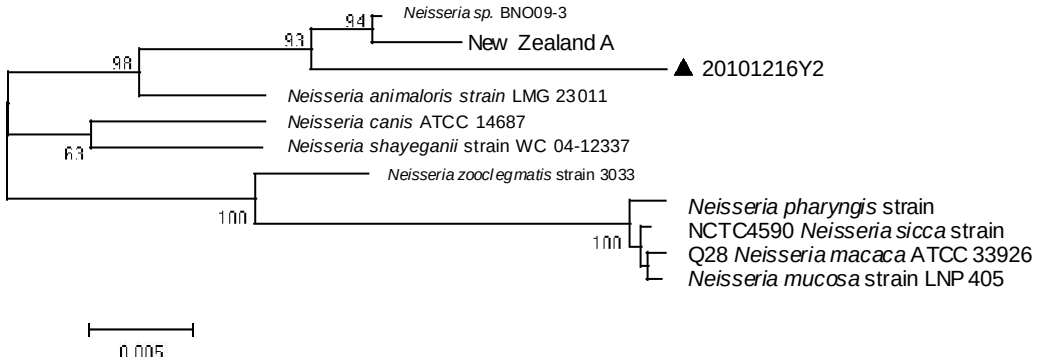

Figure 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences.

organism measures 0.5 μm by 0.5 to1 μm.

Phylogenetic relationships and evolutionary divergence inferred from 16S rRNA gene sequences

The sequences of 16S rRNA genes have been used frequenly to define the phylogenetic relationships between bacterial species. The sequence of 500 bp of sequence of the 16S rRNA gene of *Neisseria* was available from 11 strains. Figure 2 shows a neighbor-joining phylogenetic tree for the 11 nucleotide sequences of 16S rRNA gene sequences. Isolate 20101216Y2 were attributed to the same clade according to 16S rRNA sequence phylogeny results, with *Neisseria* sp. BNO09-3 (GenBank accession no. GU121411) and 'New Zealand A' (GenBank accession no. AY721627) as the nearest neighboring species (97.7 and 97.2% sequence homology). Isolate 20101216Y2 had 89.4% sequence homology *Neisseria macaca* ATCC 33926 as the distant species (Table 2). These results suggest that this organism is likely identical and belong to the genus *Neisseria*.

Characterization of the isolated strain

The biochemical reactions of this strain resembles *N. mucosa*, and it grew on nutrient agar and acidified dextrose, maltose, sucrose and did not acidified lactose. It was positive for oxidase and nitrate reduction. These results suggest that this organism belongs to the genus

Table 2. Evolutionary divergence between sequences.

Organism	20101216Y2	23011	14687	33926	LNP405	NCTC4590	Wc04-12337	Q28	BN009-3	3033	New zealand A
20101216Y2	100										
23011	97.1	100									
14687	95.5	97.2	100								
33926	89.4	91.1	90.6	100							
LNP405	93.9	95.9	95.1	95.2	100						
NCTC4590	93.8	95.7	95.1	95.0	99.6	100					
Wc04-12337	95.3	96.7	97.8	91.2	95.9	95.6	100				
Q28	93.8	95.8	95.0	95.2	99.8	99.6	95.8	100			
BN009-3	97.7	97.3	96.7	90.9	95.4	95.5	96.7	95.6	100		
3033/04	94.7	96.4	96.7	92.9	97.6	97.6	97.4	97.8	96.5	100	
New zealand A	97.2	96.9	96.2	90.3	94.6	94.8	96.2	94.9	99.3	95.8	100

Neisseria, for which the name *Neisseria tadorna* sp. nov. is proposed. The strain was sensitive to Vancomycin, Amikacin, Lincomycin, Levofloxacin, Tobramycin, chloramphenicol, Furazolidone, Cefoxitin and resistant to Mezlocill, Spectinomycin Nalidixic Acid, Norxacin, Aerosporin, Fleroxacin, Sulfamethoxazolum-Trimethoprimum and Lomefloxacin.

DISCUSSION AND CONCLUSION

16S ribosomal DNA (rDNA) bacterial sequencing is fast and reliable identified method of microbial isolates in clinical microbiology, and suitable for the molecular diagnosis of *Neisseriaceae* and *Moraxellaceae* and that a reference database should include more than one strain of each species (Harmsen et al., 2001). In this study, a strain of Gram-negative diplococci isolated from the liver of the Gaoyou Sheldrake. In order to identify this isolated strain, 16S rDNA bacterial sequencing was performed. Homology values showed that it was most similar to *N. animalis* and *N. canis*, therefore, there 16S rRNA sequence analysis demonstrated that 20101216Y2 belongs to the genus *Neisseria* (Figure 2).

The 16S rRNA gene sequences of 20101216Y2 matched at 97% that of Bacterium 'New Zealand A' (GenBank accession no. AY721627). Bacterium 'New Zealand A' is potentially duck-specific bacteria and the mucosa-dwelling *Neisseria* species, which can be opportunistic pathogens (Murphy et al., 2005). Moreover, the biochemical features of 20101216Y2 are similar to those of *N. mucosa*. *N. mucosa* is one *Neisseria* species of opportunistic pathogens, which is common commensals of the upper respiratory tract. These results suggest that 20101216Y2 belongs to the genus *Neisseria*, having the opportunistic nature, and for which the name *Neisseria tadorna* sp. nov. is proposed.

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