Morococcus cerebrosus is a later heterotypic synonym of *Neisseria mucosa*

Min-gyung Baek[1,*]
[1]Institute for Biomaterials, Korea University, Seoul, Korea

Abstract

Objectives: The taxonomic relationship of *Morococcus cerebrosus* CIP 81.93^T and *Neisseria mucosa* ATCC 19696^T was re-evaluated.

Methods: 16S rRNA gene sequence analysis, genome-based phylogenetic analyses using UBCG and GBDP, overall genome relatedness analyses including ANI and dDDH, and comparative phenotypic characterization using API NH, API ZYM, and fatty acid profiling were performed.

Results: *M. cerebrosus* CIP 81.93^T and *N. mucosa* JCM 12992^T shared 99.93% 16S rRNA gene sequence similarity and formed a tight monophyletic cluster in both 16S rRNA gene and genome-based phylogenetic trees. The two strains showed 96.76% ANI and 72.90% dDDH values, exceeding the commonly accepted species delineation thresholds. Comparative phenotypic and chemotaxonomic analyses revealed no single property that differentiated *M. cerebrosus* CIP 81.93^T from *N. mucosa* JCM 12992^T.

Conclusion: Based on the rules of priority, *M. cerebrosus* CIP 81.93^T should be reclassified as a later heterotypic synonym of *N. mucosa* ATCC 19696^T.

keywords: *Morococcus cerebrosus, Neisseria mucosa*

Introduction

The genus *Morococcus* was proposed in 1981 *by* Long *et al.* with *Morococcus cerebrosus* as the type species[1]. To date, no further isolates have been reported, and *M. cerebrosus* remains the only species within the genus. Initially, when *Morococcus* was proposed, the type strain (= UQM 858^T = ATCC 33486^T = NCTC 11393^T) was compared with *Neisseria mucosa* due to similarities in colonial morphology between the two species. However, subsequent studies revealed significant differences in physiological, cultural, serological, and morphological characteristics between the two strains, leading to the establishment of the novel genus *Morococcus*. At that time, no other *Neisseria* species were considered for comparison with this strain. However, with the advent of 16S rRNA gene sequencing, the phylogenetic similarity of *M. cerebrosus* to some species within the genus *Neisseria* was noted, particularly clustering with *N. mucosa* JCM 12992^T. In the present study, we re-evaluated the taxonomic relationship between *M. cerebrosus* CIP 81.93^T and *N. mucosa* JCM 12992^T through genome comparison.

Methods

16S rRNA phylogeny

The 16S rRNA gene sequences of the *M. cerebrosus* CIP 81.93^T along with those of related strains obtained from the EzBiocloud database[2], were aligned using the EzEditor program[3]. Sequence similarity was calculated using identification tools embedded in the EzBiocloud web service (www.ezbiocloud.net)[2]. Phylogenetic trees were inferred using Molecular Evolutionary Genetic Analysis (MEGA 7) software[4].

Genome analyses

The whole genome sequence of *M. cerebrosus* CIP 81.93^T and *N. macacae* ATCC 33296^T were determined in this study. Genomic DNA was extracted using commercial microbial DNA isolation kit (MP Biomedicals), and sequencing libraries were prepared using the Nextera DNA sample prep kit (Illumina). Sequencing was performed using PacBio Sequel I (Pacific Biosciences) technology. The complete genomes were annotated using the NCBI Prokaryotic Genome Annotation Pipeline, deposited in the GenBank database under the accession numbers CP094242 and CP094241, respectively. Additionally, genome sequences of 28 strains, including *N. mucosa*

*** Corresponding author:** Min-gyung Baek (bmg0330@korea.ac.kr)
Institute for Biomaterials, Korea University, 145 Anam-ro, Seongbuk-gu, Seoul 02841, Korea

JCM 12992^T (CP028150), *N. sicca* ATCC 29256^T (CP079820), *N. subflava* U37^T (CP039887), *N. perflava* U15^T (JAMDHR0000000), *N. flavescens* ATCC 13120^T (CP039886), *N. cinerea* ATCC 14685^T (LS483369), *N. polysaccharea* ATCC 43768^T (ADBE00000000), *N. weaveri* LMG 5135^T (AFWQ01000032), *N. meningitides* MC58 (AE002098), *N. weixii* 10022^T (CP023429), N. *zoodegmatis* NCTC 12230^T (LT906434), *N. lactamica* ATCC 23970^T (ACEQ00000000), *N. gonorrhoeae* NCTC 8375^T (QNRU00000000), *N. iguanae* NVSL 85737^T (PXYY00000000), *N. animaloris* DSM 21642^T (LR134440), *N. dumasiana* 93087^T (MTAC00000000), *N. shayeganii* 871^T (AGAY00000000), *N. animalis* NCTC 10212^T (LR134287), *N. elongata* subsp. *glycolytica* ATCC 29315^T (CP007726), *N. canis* ATCC 14687^T (LR134313), *N. elongata* subsp. *elongata* ATCC 25295^T (UGQW00000000), *Eikenella longinqua* NML02-A-017^T (LXSL00000000), *Bergeriella denitrificans* NBTC 102155^T (UGQS00000000), *N. wadsworthii* 9715^T (AGAZ00000000), *N. arctica* KH1503^T (JTDO00000000), and *N. zalophi* CSL 7565^T (CP031700) were obtained from public databases.

Genome-based phylogenetic trees were reconstructed using up-to-date bacterial core gene (UGCG) set through the UBCG pipeline[5] and Genome Blast Distance Phylogeny (GBDP) method via the Type Strain Genome Server (TYGS; https://tygs.dsmz.de)[6].

Overall genome relatedness indices, including average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH), were calculated using the OrthoANI[7] and Genome-to-Genome Distance Calculator (GGDC) website service[8], respectively.

Phenotype characterization

A comparative analysis of phenotypic characteristics was conducted for *M. cerebrosus* CIP 81.93^T, *N. mucosa* JCM 12992^T, *N. macacae* ATCC 33926^T and *N. sicca* ATCC 29256^T in this study. Cells of the four type strains were collected from 5% Columbia based sheep blood agar at 37°C. Biochemical and physiological properties, including carbohydrate assimilation and enzyme activities, were assessed using API NH and API ZYM strips (bioMérieux) following the manufacturer's instructions. Fatty acid methyl esters were extracted and separated by gas chromatography using the Instant FAME method of the Microbial Identification System (MIDI) version 6.3 and RTSBA6 6.21 database (Agilent technologies).

Results

16S rRNA gene and genomic trees

M. cerebrosus CIP 81.93^T and *N. mucosa* JCM 12992^T exhibited 99.93% similarity in their 16S rRNA gene sequences and formed a monophyletic branch in phylogenetic tree (Fig. 1). Analysis revealed that the two strains are phylogenetically closely related.

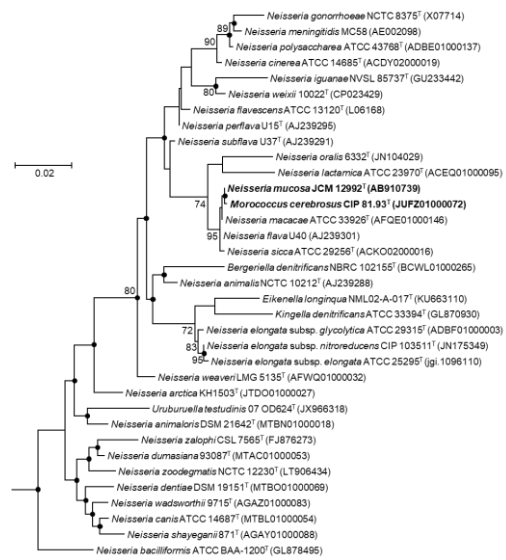


Figure 1. Maximum-likelihood tree showing the 16S rRNA gene sequence relationship of *M. cerebrosus* CIP 81.93^T and *N. mucosa* JCM 12992^T among the radiation of the genus *Neisseria* and closely related genera.
Solid circles indicate nodes recovered in the Neighbor-joining tree. Numbers at the nodes represent bootstrap support (>70%) based on 1000 resampled datasets. *Leeia oryzae* DSM 17879^T (AQXU01000006) was used as an outgroup. Bar, 0.02 nucleotide substitutions per position.

In both UBCG (Fig. 2) and GBDP (Fig. S1) trees, *M. cerebrosus* CIP 81.93^T and *N. mucosa* JCM 12992^T formed a tight cluster independent of their close neighbor, *N. macacae* ATCC 33926^T.

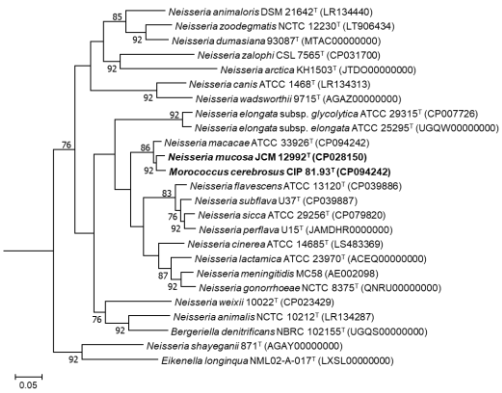
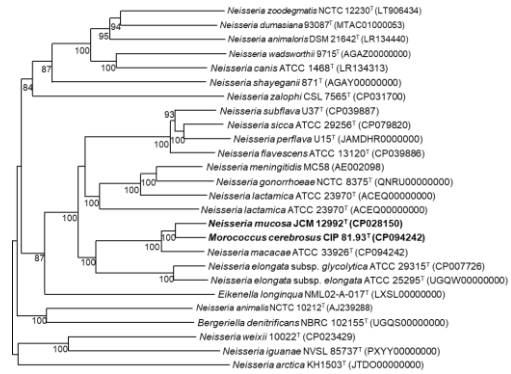


Figure 2. Genome-based phylogenetic tree constructed using the up-to-date bacterial core gene set.

The concatenated alignment of 92 core genes was analyzed using the UBCG pipeline. Numbers at the nodes represent bootstrap support (>70%) based on 1000 resampled datasets. *Leeia oryzae* DSM 17879^T (AQXU01000006) was used as an outgroup. Bar, 0.05 nucleotide substitutions per position.



Supplementary Figure 1. Genome-based phylogenetic tree constructed using Genome Blast Distance Phylogeny and the Type Strain Genome Server.

Numbers at the nodes represent bootstrap support (>70%) based on 100 resampled datasets.

Genomic characteristics

ANI and dDDH values between the *M. cerebrosus* CIP 81.93^T and *N. mucosa* JCM 12992^T were determined to be 96.76% and 72.9%, respectively (Table 1). These values exceeded the recommended thresholds of 95-96% for ANI and 70% for dDDH, which are commonly used for bacterial species delineation.

Table 1. The sequence similarities between *Morococcus cerebrosus* CIP 81.93^T and closely related type strains for which genome data are available in public databases.

Species	16S rRNA gene	ANI	dDDH
N. mucosa JCM 12992^T	99.93	96.76	72.9
N. macacae ATCC 33926^T	99.79	94.54	63.2
N. sicca ATCC 29256^T	99.66	83.54	30.2
N. subflava U37^T	98.15	84.16	31.5
N. perflava U15^T	97.56	82.97	29.1
N. flavescens ATCC 13120^T	97.31	83.57	30.5
N. cinerea ATCC 14685^T	97.27	85.01	35.3
N. polysaccharea ATCC 43768^T	97.13	96.93	61.6
N. weaveri LMG 5135^T	97.13	91.88	43.1
N. meningitides MC58	97.13	84.76	34.9
N. weixii 10022^T	96.92	77.13	22.4
N. zoodegmatis NCTC 12230^T	96.51	77.18	23.1
N. lactamica ATCC 23970^T	96.45	84.31	33.4
N. gonorrhoeae NCTC 8375^T	96.31	84.37	32.8

N. iguana NVSL 85737^T	96.31	76.98	22.8
N. animloris DSM 21642^T	96.10	76.74	22.8
N. dumasiana 93087^T	96.10	76.64	23.9
N. shayeganii 871^T	96.04	74.9	22.9
N. animalis NCTC 10212^T	96.01	78.29	24.1
N. elongate subsp. *glycolytica* ATCC 29315^T	95.83	81.69	37.6
N. canis ATCC 14687^T	95.76	75.26	25.2
N. elongate subsp. *elongate* ATCC 25295^T	95.76	81.36	38.4
Eikenella longinqua NML02-A-017^T	95.35	76.51	25.6
Bergeriella denitrificans NBRC 102155^T	95.22	79.28	24.5
N. wadsworthii 9715^T	95.01	74.62	23.9
N. arctica KH1503^T	94.94	73.24	22.0
N. zalophi CSL 7565^T	94.93	74.78	22.5
N. mucosa JCM 12992^T	99.93	96.76	72.9
N. macacae ATCC 33926^T	99.79	94.54	63.2
N. sicca ATCC 29256^T	99.66	83.54	30.2
N. subflava U37^T	98.15	84.16	31.5
N. perflava U15^T	97.56	82.97	29.1
N. flavescens ATCC 13120^T	97.31	83.57	30.5

Phenotypic characteristics

Strains *M. cerebrosus* CIP 81.93^T and *N. mucosa* JCM 12992^T, along with closely related type strains, exhibited similar fatty acid profiles (Table 2), characterized by a predominance of $C_{16:1}$ ω7c and/or $C1_{6:1}$ ω6c (summed feature 3) and $C_{16:0}$, although detailed compositions varied. In API NH tests, all strains were positive for acidification of D-glucose, D-fructose, D-maltose, and sucrose, as well as for proline arylamidase activity, while all strains were negative for penicillinase, ornithine decarboxylase, urease, lipase, alkaline phosphatase, β-galactosidase, and indole production. In API ZYM tests, all strains were negative for esterase (C4), leucine arylamidase, cysteine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-chymotrypsin, α-galactosidase, α-glucosidase, α-mannosidase, α-fucosidase, β-glucuronidase, and *N*-acetyl-β-glucosaminidase activities. Consistent with these results, no single phenotypic property differentiated *M. cerebrosus* CIP 81.93^T from *N. mucosa* JCM 12992^T. However, *N. macacae* ATCC 33926^T and *N. sicca* ATCC 29256^T could be distinguished by the presence of gamma glutamyl transferase activity or the absence of valine arylamidase activity.

Table 2. Differential characteristics of *Morococcus cerebrosus* and closely related type strains. Strains: 1, *M. cerebrosus* CIP 81.93^T; 2, *N. mucosa* JCM 12992^T; 3, *N. macacae* ATCC 33926^T; 4, *N. sicca* ATCC 29256^T. +, positive; w, weakly positive; -, negative; ND, Not detected. Values are percentages of total fatty acids. Fatty acids representing more than 1 % are shown. All data listed were obtained in this study.

Characteristics	1	2	3	4
API NH test				
Gamma glutamyl transferase	-	-	+	-
API ZYM test				
Esterase lipase (C8)	w	+	+	w
Lipase (C14)	w	+	w	w
Valine arylamidase	w	+	+	-
Major fatty acids (%)				
Saturated				
$C_{12:0}$	3.5	14.7	4.4	9.2
$C_{14:0}$	5.4	3.9	3.6	3.2
$C_{16:0}$	36.6	17.8	33.2	28.4

$C_{18:0}$	0.4	0.1	0.6	2.1
Hydroxy				
$C_{12:0}$ 3-OH	3.1	9.6	3.4	7.5
$C_{16:0}$ 2-OH	ND	ND	2.1	3.2
Sum In Feature*				
2	2.5	5.0	3.1	4.5
3	36.9	38.0	30.2	27.3
8	9.1	7.9	16.1	11.6

*Sum In Feature2 was listed as $C_{14:0}$ 3-OH and/or $C_{16:1}$ iso I; Sum In Feature 3 was listed as $C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c*; Sum In Feature 8 was listed as $C_{18:1}$ ω7*c* and/or $C_{18:1}$ ω6*c*.

Conclusion

On the basis of high genomic relatedness (96.76% ANI and 72.90% dDDH), exceeding the commonly accepted species delineation thresholds (95-96% ANI and 70% dDDH), as well as high 16S rRNA gene sequence similarity (99.93%), and indistinguishable chemotaxonomic and phenotypic characteristics, it is clear that the two type strains belong to the single species. Thus, we propose that *Morococcus cerebrosus* CIP 81.93^T is a later heterotypic synonym of *Neisseria mucosa* JCM 12992^T.

References

1. Long P, Sly L, Pham A, Davis G. Characterization of *Morococcus cerebrosus* gen. nov., sp. nov. and comparison with *Neisseria mucosa*. International journal of evolutionary microbiology. 1981;31(3):294-301.

2. SH Yoon, SM Ha, S Kwon, J Lim, YKim, H Seo *et al.* Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. International journal of evolutionary microbiology. 2017;67(5):1613-1617.

3. YS Jeon, K lee, SC Park, BS Kim, YJ Cho, SM Ha *et al.* EzEditor: a versatile sequence alignment editor for both rRNA-and protein-coding genes. International journal of evolutionary microbiology. 2014;64:689-691.

4. Kumar, S., G. Stecher, and K. Tamura. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular biology and evolution. 2016;33(7):1870-1874.

5. SI Na, YO Kim SH Yoon, S Ha, I Baek, J Chun. UBCG: up-to-date bacterial core gene set and pipeline for phylogenomic tree reconstruction. Journal of microbiology. 2018;56(4):280-285.

6. Meier-Kolthoff JP, Carbasse JS, Peinado-Olarte RL, Göker M. TYGS and LPSN: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. Nucleic acids research. 2022;50: D801-D807.

7. I Lee, Y Ouk Kim, SC Park, J Chun. OrthoANI: An improved algorithm and software for calculating average nucleotide identity. International journal of evolutionary microbiology. 2016;66(2):1100-1103.

8. Meier-Kolthoff JP, Auch AF, Klenk H-P, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics. 2013;14(1): 60.