

Fusobacterium nucleatum subsp. *fusiforme* Gharbia and Shah 1992 is a Later Synonym of *Fusobacterium nucleatum* subsp. *vincentii* Dzink et al. 1990

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Received: 25 October 2012 / Accepted: 3 December 2012 / Published online: 24 December 2012
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Abstract On the basis of the DNA–DNA hybridization patterns and phenotypic characteristics, *Fusobacterium nucleatum* was classified into five subspecies. Previous studies have suggested that *F. nucleatum* subsp. *vincentii* is genetically similar to *F. nucleatum* subsp. *fusiforme*. The aim of this study was to investigate the possibility of classifying these two subspecies into a single subspecies by phylogenetic analysis using a single sequence (24,715 bp) concatenated 22 housekeeping genes of eight *F. nucleatum* strains including type strains of five *F. nucleatum* subspecies. The phylogenetic analysis indicated that *F. nucleatum* subsp. *vincentii* and *F. nucleatum* subsp. *fusiforme* were clustered in the same group and each strain of other *F. nucleatum* subspecies were also separated into the same

cluster. These results suggested that *F. nucleatum* subsp. *fusiforme* and *F. nucleatum* subsp. *vincentii* can be classified into a single subspecies. *F. nucleatum* subsp. *vincentii* was early published name; therefore, *F. nucleatum* subsp. *fusiforme* Gharbia and Shah 1992 can be regarded as a later synonym of *F. nucleatum* subsp. *vincentii* Dzink et al. 1990.

Introduction

Fusobacterium nucleatum is Gram-negative, non-motile, obligately anaerobic rod bacteria, which is frequently isolated from the oral cavity and may play an important role in periodontal disease [1, 2]. Based on the PAGE pattern of the whole-cell proteins and DNA homology [3] or electrophoretic patterns of glutamate dehydrogenase and 2-oxoglutarate reductase and DNA–DNA hybridization patterns [4], *F. nucleatum* was classified into five subspecies (*nucleatum*, *polymorphum*, *vincentii*, *animalis*, and *fusiforme*). Previous studies have suggested that *F. nucleatum* subsp. *vincentii* is genetically similar to *F. nucleatum* subsp. *fusiforme* [5, 6]. Therefore, this study was designed to investigate the possibility of classifying these two subspecies into a single subspecies by phylogenetic analysis of 22 housekeeping genes: *rpoB* (DNA-dependent RNA polymerase beta-subunit), *atpC* (F0F1-type ATP synthase gamma subunit), *exoA* (exodeoxyribonuclease III), *pfkA* (6-phosphofructokinase), *pyrB* (aspartate carbamoyltransferase), *gapA* (glyceraldehyde 3-phosphate dehydrogenase), *pgk* (phosphoglycerate kinase), *gpmA* (phosphoglycerate mutase), *pckA* (phosphoenolpyruvate carboxykinase), *ldh* (L-lactate dehydrogenase), *ung* (uracil-DNA glycosylase), *tpi* (triosephosphate isomerase), *oadA* (oxaloacetate decarboxylase alpha subunit), *ddl* (D-alanine-D-alanine ligase), *murA*

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Table 1 Information of the nucleotide sequences of the genes used in this study

Genes (size, nts)	GenBank accession no./location									
	FNN 25586	FNN 23726	FNP 10953	FNP F0401	FNV 49256	FVF 51190	FNA 51191	FNA 0419	FP 33693	
<i>rpoB</i> (2,402)	GQ274958/ 18..2419	NZ_ADVK01000054/ 11636..14037	GQ274957/ 18..2419	ADDB01000013/ 18995..21396	GQ274959/ 18..2419	GQ274960/ 1..2402	GQ274961/ 18..2419	AGEH01000022/ 81304..84040	GQ274990/ 18..2420	
<i>atpC</i> (849)	NC_003454/ 990085..990933	NZ_ADVK010000/ 88895..89743	NW_002062357/ 780014..780862	ADDB01000002/ 65782..66630	NZ_AABF02000062/ 4527..5375	AKX010000003/ 16053..16901	GL985144/ 22764..23612	AGEH01000010/ 51580..52428	GG665893/ 562578..563426	
<i>exoA</i> (762)	NC_003454/ 684567..685328	NZ_ADVK010000/ 27212..27973	NW_002062357/ 417992..418753	JH376782/ 49968..50729	NZ_AABF02000090/ 1790..2551	AKX010000060/ 5492..6253	GL985141/ 989..1750	AGEH01000022/ 58016..58777	GG665897/ 95699..96460	
<i>pfkA</i> (969)	NC_003454/ 1047391..1048359	NZ_ADVK010000/ 14540..15508	NW_002062357/ 2319115..2320083	JH376784/ 19220..20188	AABF01000005/ 3230..4198	AKX010000024/ 3233..4201	GL985146/ 2275..3243	AGEH01000026/ 16790..17758	NZ_ACIY01000027/ 16015..16983	
<i>pyrB</i> (891)	NC_003454/ 1059877..1060767	NZ_ADVK010000/ 11054..11944	NW_002062357/ 2315367..2316257	JH376784/ 13312..14202	AABF01000005/ 21981..22871	AKX010000024/ 6043..6933	GL985146/ 11426..12316	AGEH01000026/ 19594..20484	NZ_ACIY01000098/ 1336..2226	
<i>gapA</i> (1,008)	NC_003454/ 1310502..1311509	NZ_ADVK010000/ 104940..105947	NW_002062357/ 1641524..1642531	JH376781/ 60766..61773	AABF01000017/ 18981..19988	AKX010000011/ 33128..34135	GL985153/ 2921..3928	AGEH01000006/ 174896..175903	ACJY01000018/ 64902..65909	
<i>pgk</i> (532)	NC_003454/ 1312247..1312778	NZ_ADVK010000/ 106685..107216	NW_002062357/ 1642616..1643147	JH376781/ 59485..60016	AABF01000017/ 20074..20605	AKX010000011/ 34221..34752	GL985153/ 1638..2169	AGEH01000006/ 173613..174144	GG665892/ 66655..67186	
<i>gpmA</i> (687)	NC_003454/ 1386494..1387180	NZ_ADVK01000020/ 28367..29053	NW_002062357/ 1744011..1744697	ADDB01000006/ 249236..249922	NZ_AABF02000064/ 3378..4064	AKX010000002/ 62285..62971	AFQD01000272/ 795..1481	AGEH01000009/ 117101..117787	ACJY01000009/ 20267..20953	
<i>pekA</i> (1,584)	NC_003454/ 1761578..1763161	NZ_ADVK01000025/ 61380..62963	NW_002062357/ 1052871..1054454	ADDB01000001/ 95279..96862	NZ_AABF02000083/ 3579..5162	AKX010000061/ 3556..5139	AFQD01000128/ 4174..5757	AGEH01000006/ 79612..81195	ACJY01000096/ 47006..48589	
<i>ldh</i> (957)	NC_003454/ 1827670..1828266	NZ_ADVK01000030/ 7877..8833	CM0000440/ 985716..986672	ADDB01000001/ 163945..164901	AABF01000008/ 9075..10031	AKX010000014/ 12920..13876	AFQD01000120/ 252..1208	AGEH01000006/ 51164..52120	ACJY01000102/ 1284..2240	
<i>ung</i> (678)	NC_003454/ 1884913..1885590	NZ_ADVK01000018/ 5958..6638	NW_002062357/ 921184..921861	ADDB01000001/ 280734..281414	AABF010000031/ 8730..9407	CM0000440/ 4845..5522	AFQD01000146/ 5956..6633	AGEH01000009/ 199487..200167	ACJY01000065/ 10315..10992	
<i>tpi</i> (756)	NC_003454/ 2016853..2017608	NZ_ADVK01000014/ 11379..12134	CM0000440/ 2352020..2352775	ADDB01000016/ 52798..53553	NZ_AABF02000057/ 6041..6796	AKX01000010/ 13024..13779	AFQD01000158/ 3470..4225	AGEH01000027/ 32443..33198	ACJY01000083/ 5049..5804	
<i>oadA</i> (1,347)	NC_003454/ 2024019..2025365	NZ_ADVK01000014/ 3622..4968	NW_002062357/ 2359399..2360745	ADDB01000016/ 60177..61523	NZ_AABF02000098/ 1561..2907	AKX010000010/ 5069..6415	AFQD01000157/ 4323..5669	AGEH01000027/ 22857..24203	ACJY01000025/ 17809..19155	
<i>ddl</i> (864)	NC_003454/ 2134886..2135749	NZ_ADVK01000017/ 44685..45548	NW_002062357/ 1981039..1981902	ADDB01000015/ 11817..12680	NZ_AABF02000042/ 3233..4096	AKX010000033/ 3227..4090	AFQD01000060/ 129..992	AGEH01000025/ 179997..180860	ACJY01000036/ 33640..34503	
<i>murA</i> (1,272)	NC_003454/ 24892..26163	NZ_ADVK01000048/ 4288..5559	NW_002062357/ 51183..52454	ADDB01000015/ 75001..76272	AABF01000050/ 412..1683	AKX01000006/ 11485..12756	AFQD01000090/ 4351..5622	AGEH01000025/ 100285..101556	ACJY01000051/ 96602..97873	
<i>eno</i> (1,302)	NC_003454/ 256783..258084	NZ_ADVK01000055/ 169891..171192	CM0000440/ 1543495..1544796	ADDB01000003/ 401128..402429	NZ_AABF02000019/ 22320..23621	AKX010000001/ 62777..64078	GL985150/ 35356..36657	AGEH01000018/ 10089..11390	GG665894/ 14457..15758	
<i>dnaB</i> (1,341)	NC_003454/ 315989..317329	NZ_ADVK01000027/ 56972..58312	CM0000440/ 11005..12345	ADDB01000003/ 143391..144731	NZ_AABF02000027/ 8821..10161	AKX010000020/ 8573..9913	GL985154/ 3733..5073	AGEH01000012/ 119647..120987	GG665893/ 138502..139842	
<i>pgi</i> (1,122)	NC_003454/ 562901..564022	NZ_ADVK01000033/ 1808..2929	CM0000440/ 305486..306607	ADDB01000013/ 10027..11148	NZ_AABF02000065/ 269..1390	AKX010000048/ 532..1653	GL985141/ 97114..98235	AGEH01000022/ 63858..64979	GG665894/ 46138..47259	
<i>recA</i> (1,134)	NC_003454/ 1199783..1200916	NZ_ADVK01000056/ 2385..3518	CM0000440/ 2165344..2166477	ADDB01000011/ 12365..13498	NZ_AABF02000048/ 4453..5586	AKX010000029/ 4828..5961	AFQD01000208/ 2103..3236	AGEH01000036/ 93358..94491	GG665893/ 295622..296755	
<i>gyrA</i> (1,452)	NC_003454/ 633815..635266	NZ_ADVK01000042/ 75028..76479	NW_002062357/ 377937..379388	ADDB01000012/ 123685..125136	NZ_AABF02000051/ 4601..6052	AKX01000009/ 33350..34801	GL985141/ 3477..4928	AGEH01000021/ 87773..89224	GG665894/ 74486..75937	

Table 1 continued

Genes (size, nts)	GenBank accession no./location	FNN 25586	FNN 23726	FNP 10953	FNP F0401	FNV 49256	FVF 51190	FNA 51191	FNA 0419	FP 33693
<i>gyrB</i> (1,634)	NC_003454/ 636537..638170 1..1634	HQ008303/ 379543..381176	HQ008294/ 1..1634	HQ008030/ 379543..381176	ADDB01000012/ 120897..122530	HQ008296/ 1..1634	AKXJ01000009/ 36213..37846	HQ008306/ 418..2051	AGEH01000021/ 90652..92285	GG665894/ 71498..73131
<i>groEL</i> (1172)	NC_003454/ 1330561..1331732	NZ_AADV01000010/ 125001..126172	NZ_CM000440/ 1685305..1686476	NZ_CM000440/ 1685305..1686476	ADDB01000007/ 18187..19358	NZ_AABF02000238/ 194..1365	AKXJ010000149/ 19..1190	GL985145/ 72217..73388	AGEH01000006/ 143345..144516	GG665898/ 110848..112019

FNN Fusobacterium nucleatum subsp. *nucleatum*, *FNP Fusobacterium nucleatum* subsp. *polymorphum*, *FNV Fusobacterium nucleatum* subsp. *vincentii*, *FNF Fusobacterium nucleatum* subsp. *fusiforme*, *FNA Fusobacterium nucleatum* subsp. *animalis*, *FP Fusobacterium periodonticum*, *rpoB* DNA-dependent RNA polymerase beta-subunit, *atpC* F0F1-type ATP synthase gamma subunit, *exoA* exodeoxyribonuclease III, *pfkA* 6-phosphofructokinase, *pyrB* aspartate carbamoyltransferase, *gapA* glyceraldehyde 3-phosphate dehydrogenase, *pgk* phosphoglycerate kinase, *gpmA* phosphoglycerate mutase, *pcrA* phosphoenolpyruvate carboxykinase, *ldh* L-lactate dehydrogenase, *ung* uracil-DNA glycosylase, *tpi* triosephosphate isomerase, *oadA* oxaloacetate decarboxylase alpha subunit, *ddl* D-alanine-D-alanine ligase, *nurA* UDP-N-acetylglucosamine 1-carboxyvinyltransferase, *eno* phosphopyruvate hydratase, *dnaB* DNA helicase, *pgi* glucose-6-phosphate isomerase, *recA* recombinase A, *gyrA* DNA gyrase subunit A, *gyrB* DNA gyrase subunit B, and *groEL* molecular chaperone GroEL

(UDP-N-acetylglucosamine 1-carboxyvinyltransferase), *eno* (phosphopyruvate hydratase), *dnaB* (DNA helicase), *pgi* (glucose-6-phosphate isomerase), *recA* (recombinase A), *gyrA* (DNA gyrase subunit A), *gyrB* (DNA gyrase subunit B), and *groEL* (molecular chaperone GroEL) for phylogenetic analysis of the five *F. nucleatum* subspecies type strains. Table 1 showed the GenBank accession numbers and analyzed nucleotide location of the 22 housekeeping genes.

A 24,715-bp single sequence was generated by integrating individual genes in the mentioned order. Phylogenetic analysis was conducted using MEGA version 5 [7]. Phylogenetic trees were constructed using the neighbor-joining method and bootstrap analysis was carried out using 1,000 replications. Sequence similarities were calculated using MegAlign program (Lasergene™ 8.0, DNASTar, Inc., Madison, WI, USA).

The phylogenetic tree showed that all of the *F. nucleatum* strains were clearly separated into four distinct clusters with 100 % bootstrap value (Fig. 1a). The genome sequence of eight strains used in this study has been deposited in GenBank. According to a dendrogram generated by the National Center for Biotechnology Information (NCBI; <http://www.ncbi.nlm.nih.gov/genome?term=Fusobacterium%20nucleatum>; NIH, Bethesda, MD, USA), *F. nucleatum* subsp. *vincentii* ATCC 49256^T and *F. nucleatum* subsp. *fusiforme* ATCC 51190^T were clustered in the same group and each strain of other *F. nucleatum* subspecies were also separated into the same cluster. Our data is consistent with that of NCBI except for the similarity between the four clusters composed of each *F. nucleatum* subspecies strain, including the one consisting of *F. nucleatum* subsp. *vincentii* ATCC 49256^T and *F. nucleatum* subsp. *fusiforme* ATCC 51190^T. The sequence similarity of the concatenated 22 housekeeping genes between *F. nucleatum* subsp. *vincentii* ATCC 49256^T and *F. nucleatum* subsp. *fusiforme* ATCC 51190^T was 98.5 % (Fig. 1b). The sequence similarities between the strains of the others *F. nucleatum* subspecies ranged from 98.3 to 99.5 %. The phylogenetic tree also delineated the *F. nucleatum* groups from *Fusobacterium periodonticum* (Fig. 1a). The sequence similarities between *F. nucleatum* strains and *F. periodonticum* ATCC 33369^T ranged from 88.9 to 90.1 % (mean 89.4 %) (Fig. 1b). This is in agreement with our previous report, in that *F. nucleatum* subsp. *vincentii* and *F. nucleatum* subsp. *fusiforme* could not be distinguished from one another based on the analyses of the sequences of 16S rRNA gene, *rpoB*, and the zinc protease gene [6]. These findings suggest that these two subspecies can be classified into a single subspecies. *F. nucleatum* subsp. *vincentii* was early published name; therefore, *F. nucleatum* subsp. *fusiforme* Gharbia and Shah 1992 can be considered as a later synonym of *F. nucleatum* subsp. *vincentii* Dzink et al. 1990.

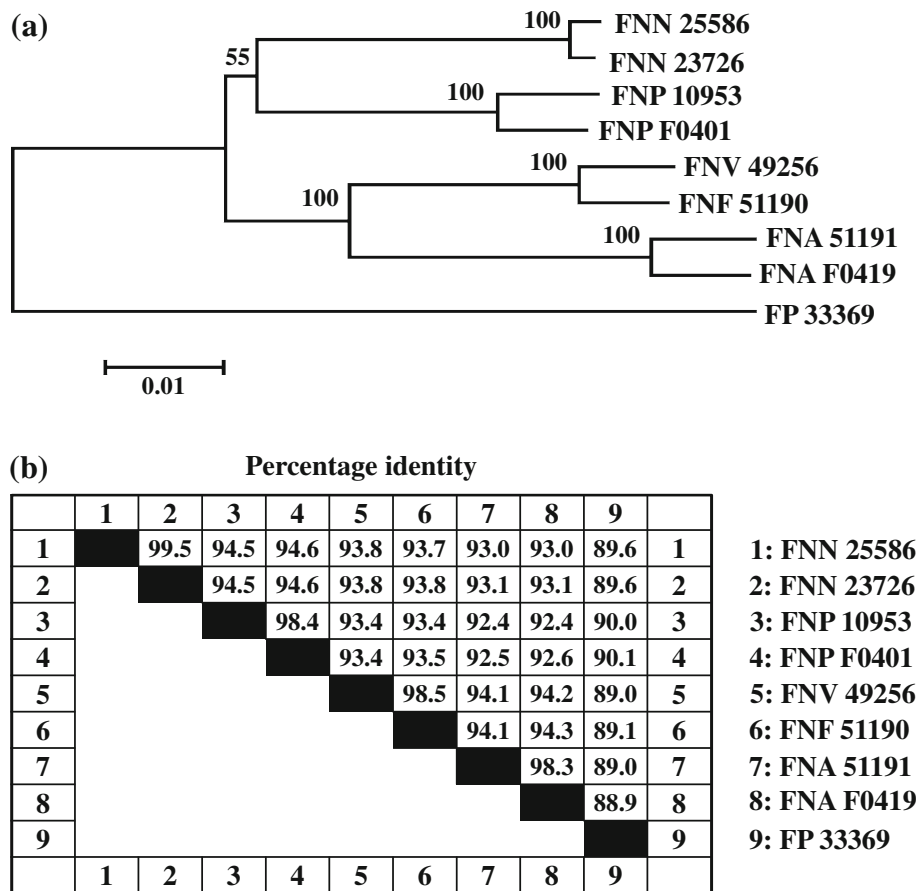


Fig. 1 **a** Phylogenetic tree based on a single sequence (24,715 bp) by concatenating 22 housekeeping genes of eight *Fusobacterium nucleatum* strains, including five type strains of *F. nucleatum* subspecies. The resulting tree topology was evaluated by bootstrap analyses of the neighbor-joining tree based on 1,000 replications using *MEGA* version 5 [7]. **b** Percentage identity among the eight strains of *F. nucleatum* and *F. polymorphum* ATCC 33369^T. Sequence similarities were calculated using the MegAlign program (DNASar LasergeneTM 8.0, DNASar, Inc.)

Acknowledgments This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012-003324) and in part by a grant from the KRIBB Research Initiative program.

Conflict of interest The authors declare that they have no conflict of interest.

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