**Mycobacterium paraintracellulare** sp. nov., for the genotype INT-1 of *Mycobacterium intracellulare*

So-Young Lee,1 Byoung-Jun Kim,1 Hong Kim,1 Yu-Seop Won,1 Che Ok Jeon,2 Joseph Jeong,3 Seon Ho Lee,3 Ji-Hun Lim,3 Seung-Heon Lee,4 Chang Ki Kim,4 Yoon-Hoh Kook1 and Bum-Joon Kim1

1Department of Biomedical Sciences, Microbiology and Immunology, Cancer Research Institute, Institute of Endemic Diseases, and Liver Research Institute, Seoul National University Medical Research Center (SNUMRC), Seoul National University College of Medicine, Seoul, Republic of Korea

2School of Biological Sciences, Chung-Ang University, Seoul, Republic of Korea

3Department of Laboratory Medicine, Ulsan University Hospital, University of Ulsan College of Medicine, Ulsan, Republic of Korea

4Korean Institute of Tuberculosis, Chungbuk, Republic of Korea

Three mycobacterial strains, isolated from independent Korean patients with pulmonary infections, belonging to the *Mycobacterium intracellulare* genotype 1 (INT-1) were characterized using a polyphasic approach. The sequences of the 16S rRNA gene and internal transcribed spacer 1 (ITS1) of the INT-1 strains were identical to those of *Mycobacterium intracellulare* ATCC 13950T. However, multilocus sequence typing (MLST) analysis targeting five housekeeping genes (*hsp65*, *rpoB*, *argG*, *gnd* and *pgm*) revealed the phylogenetic separation of these strains from *M. intracellulare* ATCC 13950T. DNA–DNA hybridization values of >70% confirmed that the three isolates belong to the same species, while the values of <70% between one of them and the type strains of *M. intracellulare* and *Mycobacterium chimaera* confirmed their belonging to a distinct species. In addition, phenotypic characteristics such as positive growth on MacConkey agar and in acidic broth culture, unique matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS profiles of lipids, and unique mycolic acids profiles further supported the taxonomic status of these strains as representatives of a novel species of the *Mycobacterium avium* complex named *Mycobacterium paraintracellulare*. The type strain is MOTT64T (=KCTC 29084T=JCM 30622T).

Non-tuberculous mycobacteria (NTM) are common in the environment and can be opportunistic pathogens. Recently, the application of molecular techniques to the taxonomy and identification of isolates from environmental sources and clinical specimens has led to an increased awareness of the diversity within NTM (Magee & Ward, 2012). Because combined molecular and conventional taxonomic methods have been applied to the isolates from Korean patients, we have successfully identified several novel NTM species to date (Kim et al., 2012d, 2013a, b; Lee et al., 2010a, b; Mun et al., 2008, 2007).

Recent advances in molecular taxonomy have fuelled the identification of novel species within the *Mycobacterium avium* complex (MAC) (Bang et al., 2008; Ben Salah et al., 2009; Murcia et al., 2006; Saini et al., 2009; Tortoli et al., 2004; van Ingen et al., 2009). *M. avium* and *Mycobacterium intracellulare* are well known as two representative species of the MAC. Additionally, another eight MAC species, including *Mycobacterium colombiense* (Murcia et al., 2006),...
Mycobacterium vulneris (formerly sequevar MAC-Q) (van Ingen et al., 2009), Mycobacterium arosense (Bang et al., 2008), Mycobacterium bouchedarhoumense, Mycobacterium marseillense, Mycobacterium timonense (Ben Salah et al., 2009) Mycobacterium chimaera (formerly sequevar MAC-A) (Tortoli et al., 2004) and Mycobacterium yongonense (Kim et al., 2013b), have recently been described.

Recently, via sequence analysis targeting three independent chronometer molecules, hsp65, the internal transcribed spacer 1 region (ITS1) and the 16S rRNA gene, we have reported that there exist five genetically distinct groups, INT-1 to INT-5, in M. intracellulare-related strains from Korean patients (Park et al., 2010). Our previous study demonstrated that INT-1 strains have an hsp65 sequence identical to that of M. chimaera (HG1 genotype) but an ITS1 sequence identical to that of M. intracellulare (MIN-A genotype). In this study, we sought to characterize the taxonomic status of the M. intracellulare INT-1 genotype, which is most prevalent among M. intracellulare-related groups in Korean patients as well as among Hanwoo, a native Korean cattle species (Bos taurus coreanae) (Kim et al., 2014), using a polyphasic taxonomic approach.

The three INT-1 strains (MOTT64 from a 60-year-old male patient, Asan 29591 from a 55-year-old male patient and Asan 37128 from a 71-year-old male patient) and two INT-2 strains (Asan 37016 from a 62-year-old female patient and Asan 38402 from an 89-year-old male patient) used in this study were collected from different pulmonary patients of Asan Medical Center (Seoul, Republic of Korea) during 2010 and identified by sequencing the partial hsp65 gene. M. intracellulare ATCC 13950 and M. chimaera JCM 14737 were also used for comparison with the three INT-1 strains including MOTT64. These strains were cultivated on Middlebrook 7H10 supplemented with 10% oleic acid-albumin-dextrose-catalase (OADC) and 0.5% glycerol at 37°C under 5% CO₂ for 2 weeks.

The phenotypic characteristics of M. intracellulare ATCC 13950 and M. chimaera JCM 14737 and the three INT-1 strains were examined and compared (Table 1). To compare colony morphology and growth patterns at 25°C and 37°C, strains were tested on 7H10 agar plates supplemented with OADC. For analysis of biochemical enzyme activities, arylsulfatase, heat-stable catalase, nitrate reductase, pyrazinamidase, urease, tellurite reductase and Tween 80 hydrolysis were tested (Kent & Kubica, 1985). In addition, inhibition tests of tolerance to thiophene-2-carboxylic acid hydrazide (TCH), p-nitrobenzoic acid (PNB), ethambutol and 5% NaCl on 7H10 agar medium (supplemented with OADC), and the ability to grow on MacConkey agar without crystal violet were performed. Growth in acidic (pH 5.5) conditions was also tested in Middlebrook 7H9 broth supplemented with 10% Albumin-Dextrose-Catalase (ADC), 0.2% glycerol and 0.02% Tween80. Antimicrobial susceptibility was determined by the agar proportion method on Muller-Hinton agar medium (supplemented with OADC) (Kent & Kubica, 1985). The MICs of the strains to nine antimicrobial compounds were determined in 7H9 broth supplemented with ADC. The production of 19 enzymes [alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosamine, α-mannosidase and α-fucosidase] was also analysed with the API ZYM kit (bioMérieux) following the manufacturer’s recommendations.

The optimal growth temperature of INT-1 strains was 37°C, and mature colonies were observed after 1 week on 7H10 agar plates. Colonies appeared smooth and white—yellow, unlike those of M. intracellulare ATCC 13950 showing a white and intermediately smooth-rough type of colonies. INT-1 strains showed positive responses for heat-stable catalase (68°C), pyrazinamidase and tellurite reductase, and negative results for arylsulfatase, nitrate reductase, urease activity and Tween 80 hydrolysis. In inhibition tests, INT-1 strains had resistance to 10 µg TCH ml⁻¹, 500 µg PNB ml⁻¹ and grew on MacConkey agar without crystal violet; however, no growth on media with ethambutol (8 µg ml⁻¹) or 5% NaCl was observed. In addition, these strains showed tolerance to acidic environments. The production of lipase (C14), valine arylamidase and cystine arylamidase was weakly positive, whereas the production of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and β-glucosidase was positive. Although INT-1 strains were generally similar to M. intracellulare ATCC 13950, some unique phenotypic traits— including positive growth in MacConkey agar and acidic 7H9 broth (pH 5.5)— were observed.

Although the drug susceptibility tests did not generally provide decisive information regarding mycobacteria taxonomy, it should be noted that INT-1 strains have generally higher MIC values than M. intracellulare ATCC 13950 for most of the antibiotics. Except for imipenem (>64 µg ml⁻¹) and tobramycin (8 µg ml⁻¹), MIC values of INT-1 strains were higher than those of M. intracellulare ATCC 13950 for seven other antibiotics, amikacin (16 or 32 µg ml⁻¹), cefotixin (256 µg ml⁻¹), ciprofloxacin (16 µg ml⁻¹), clarithromycin (2 µg ml⁻¹), doxycycline (32 µg ml⁻¹), moxifloxacin (2 or 4 µg ml⁻¹) and rifampicin (4 µg ml⁻¹). Detailed information of phenotypic characteristics, biochemical activities and susceptibilities to antimicrobial agents for M. intracellulare ATCC 13950, M. chimaera JCM 14737 and the three INT-1 strains is shown in Table 1.

HPLC was used to analyse mycolic acids from the three type strains MOTT64, M. intracellulare ATCC 13950 and M. chimaera JCM 14737. For HPLC analysis, the strains were cultured on 7H10 agar plates for 1 week at 37°C. HPLC were processed as described previously using the standard method for sample preparation and UV spectrometric analysis (Butler & Guthertz, 2001).
The mycolic acid chromatograms from these mycobacteria evinced a different pattern (Fig. S1, available in the online Supplementary Material). MOTT64T showed two representative peaks ranging from 3.2–3.82 min and from 6.1–6.4 min, respectively. The major peaks were generally detected in all three strains; however, the intensity of absorption units (AU) differed among strains. The AU of the first major peak from MOTT64 was about 0.20–0.50 (Fig. S1a) and those of *M. intracellulare* ATCC 13950T (Fig. S1b) and *M. chimaera* JCM 14737T (Fig. S1c) were 0.15–0.40 and 0.15–0.60, respectively. In addition, the AU of the second major peak from MOTT64 was 0.25–0.30; however, those of *M. intracellulare* ATCC 13950T and *M. chimaera* JCM 14737T were 0.45–0.5 and 0.3–0.5, respectively. The HPLC results also showed a characteristic peak ranging from 5.1 to 6.0 min in MOTT64 and *M. intracellulare* ATCC 13950T that was not found in *M. chimaera* JCM 14737T. The intensity of AU was also different between the two species, with those of MOTT64 and *M. intracellulare* ATCC 13950T ranging from 0.05 to 0.10 and 0.15 to 0.30, respectively.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used to compare total mass of the cell-wall lipid extracted with CHCl₃/CH₃OH (2:1, v/v) by a Voyager-DETM STR mass spectrometer (Applied Biosystems) with a pulse laser emitting at 337 nm (Rhoades et al., 2003). The MALDI-TOF MS profiles from Asan 29591 (Fig. 1a), Asan 37128 (Fig. 1b) and MOTT64T (Fig. 1c) were very similar to each other, but were different from those of *M. intracellulare* ATCC 13950T (Fig. 1d) and *M. chimaera* JCM 14737T (Fig. 1e). INT-1 strains exhibited two clusters, the first major peaks ranging from ∼m/z 1199.9 to ∼m/z 1476.1 and the second peaks from ∼m/z 1869.3 to ∼m/z 1967.4. The first major peaks were also detected in *M. intracellulare* ATCC 13950T and *M. chimaera* JCM 14737T; however, the second peaks were distinct only in INT-1 strains. These peaks were similar to serotype glycopeptidolipid (GPL) 7 ranging from ∼m/z 1869.0 to ∼m/z 1964.0 (Fujiwara et al., 2007; Nakata et al., 2008).

Using the whole genome sequences of strain MOTT64T (Kim et al., 2012a) and two strains belonging to the INT-2 group, *M. intracellulare* ATCC 13950T (Kim et al., 2012b)
Fig. 1. MALDI-TOF MS analysis of extracted cell-wall lipids from strains Asan 29591 (a), Asan 37128 (b), MOTT64<sup>T</sup> (c), M. intracellulare ATCC 13950<sup>T</sup> (d) and M. chimaera JCM 14737<sup>T</sup> (e). The arrows indicate a unique cluster of peaks in the MALDI-TOF MS profiles of INT-1 strains.
and MOTT-02 (Kim et al., 2012c), a phylogenetic tree was inferred by the Mauve alignment algorithm and visualized by using the TreeViewX program.

The reconstructed tree revealed that strain MOTT64$^T$ was clustered with the MAC complex strains, whereas in the M. intracellulare ATCC 13950$^T$-related cluster, strain MOTT64$^T$ was phylogenetically separated from a cluster of two INT-2 strains, M. intracellulare ATCC 13950$^T$ and MOTT-02, as an independent branch (Fig. S2). This result suggests that strain MOTT64$^T$ may be a representative of an independent species that is distinct from M. intracellulare ATCC 13950$^T$.

For the phylogenetic analysis, partial 16S rRNA, ITS-1, hsp65, rpoB, gnd, argG and pgm genes were amplified by PCR from chromosomal DNA extracted by the bead-beater phenol method (Jeon et al., 2014; Kim et al., 2005; Macheras et al., 2011); the primers for the target genes are shown Table S2. The PCR amplicons were compared with sequences in the GenBank database using the BLAST program (NCBI) and aligned using the multiple alignment algorithm in the MegAlign software package as previously described (Kim et al., 2005). Evolutionary distance matrices were generated according to the Jukes & Cantor model (Jukes & Cantor, 1969). Phylogenetic trees were inferred from the seven target gene sequences by the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods in the MEGA version 4.0 software (Kumar et al., 2008).

The BLAST analysis results of the 16S rRNA gene sequence (1305 bp) and ITS1 (280 bp) sequence showed that the sequences of INT-1 strains had 100% sequence similarity with those of M. intracellulare strain ATCC 13950$^T$. 

**Fig. 1.** (cont.)

(d) Voyager spec #1 [BP = 1296.1, 8683]

(e) Voyager spec #1 [BP = 1326.1, 2062]

---

International Journal of Systematic and Evolutionary Microbiology 66

S.-Y. Lee and others

---

Downloaded from www.microbiologyresearch.org by IP: 54.70.40.11
On: Tue, 18 Dec 2018 04:17:40
(GenBank accession nos GQ153276.1 and JQ411532.1, respectively) with a high bootstrap value (100) (Figs. 2a and S3a).

In addition, PCR amplicons of five housekeeping genes for hsp65, rpoB, argG, gnd and pgm gene sequences were further analysed for phylogenetic analysis. The partial hsp65 gene sequence (603 bp) of the three INT-1 strains showed a complete match (100%) to that of M. chimaera DSM 44623\textsuperscript{T} (GenBank accession no. JF795578.1) but a 4 bp difference (those corresponding to the 192nd, 249th, 279th and 285th nucleotides) with the hsp65 gene sequence of M. intracellulare ATCC 13950\textsuperscript{T} (GenBank accession no. JF795578.1), resulting in 99.2% similarity (Fig. 2b). The three INT-1 strains had identical rpoB (711 bp) sequences but showed a 5 bp difference corresponding to 2490th, 2526th, 2541st, 2544th and 2865th nucleotides of the rpoB gene sequence from M. intracellulare ATCC 13950\textsuperscript{T} (GenBank accession no. GQ153276).

---

**Fig. 2.** Phylogenetic relationships of strain MOTT64\textsuperscript{T} and two clinical isolates, Asan 29591 and 37128, among other species of the genus *Mycobacterium*, based on the partial 16S rRNA gene (1305 bp) (a), hsp65 gene (603 bp) (b), rpoB gene (711 bp) (c) and concatenated five-gene (hsp65, rpoB, gnd, argG and pgm; 3093 bp) (d) sequences. These trees were reconstructed using the neighbour-joining method. The bootstrap values were calculated from 1000 replications. Bootstrap values of <50% are not shown. Filled circles indicate that the corresponding groups were supported in the maximum-parsimony trees. Bars, numbers of substitutions per nucleotide position.
Fig. 2. (cont.)

(a)

(b)

(c)

(d)

Rapid growing mycobacteria

Tuberculosis, paratuberculosis, K. TGC 5821T (AY299153)

International Journal of Systematic and Evolutionary Microbiology 66
corresponding to 2445th, 2526th, 2544th, 2865th and 
3048th nucleotides) compared with the rpoB gene sequence 
from M. chimaera DSM 44623\textsuperscript{T} (GenBank accession no. EF521908.1), resulting in 99.3 % and 99.2 % similarity, 
respectively (Fig. 2c , Table S3). The result of partial gnd 
gene analysis was generally similar to the phylogenetic tree 
based on rpoB. The gnd gene sequences (633 bp) from the three INT-1 strains were perfectly matched but showed a 
6 bp difference (99.1 % similarity) with the sequence from 
M. intracellulare ATCC 13950\textsuperscript{T} and an 11 bp substitution 
(98.3 % similarity) with the sequence from M. chimaera 
JCM 14737\textsuperscript{T} (Fig. S3, Table S4). The phylogenetic tree 
based on argG sequences (513 bp) demonstrated that 
strains MOTT64\textsuperscript{T} and Asan 29591 had the same argG 
sequence, and differed by only 1 bp from another clinical 
isolate (Asan 37128) with 99.8 % similarity. The argG gene 
from MOTT64\textsuperscript{T} showed sequence similarities of 99.2 % 
(6 bp difference) with M. intracellulare ATCC 13950\textsuperscript{T} and 
98.8 % (4 bp difference) with M. chimaera JCM 14737\textsuperscript{T} 
(Fig. S3c, Table S5). The results of pgm analysis (633 bp) of 
these mycobacteria were similar to those obtained using 
argG. The partial gene from MOTT64\textsuperscript{T} and Asan 29591 had 
the same sequence, which differed by 1 bp from Asan 37128 
(99.8 % similarity). In comparison with the sequence of 
MOTT64\textsuperscript{T}, there were differences of 5 bp with 
the pgm sequence of M. intracellulare ATCC 13950\textsuperscript{T} and 
19 bp with the pgm sequence of M. chimaera JCM 14737\textsuperscript{T}, 
resulting in similarities of 99.2 % and 97.0 %, respectively 
(Fig. S3d, Table S6).

Finally, a phylogenetic tree based on five concatenated 
housekeeping genes [hsp65 (603 bp) + rpoB (711 bp) + gnd 
(633 bp) + argG (513 bp) + pgm (633 bp)] was reconst- 
structed. The sequence similarity of the concatenated genes 
(3093 bp) among the three INT-1 strains showed that there 
were no nucleotide differences between MOTT64\textsuperscript{T} and 
Asan 29591 and only a 2 bp difference with Asan 37128 
(99.9 % similarity). The analysis based on concatenated 
sequences also revealed that the three INT-1 strains were 
more closely related to M. intracellulare ATCC 13950\textsuperscript{T}, 
showing a similarity value of 99.2 % (26 bp differences), 
than to M. chimaera JCM 14737\textsuperscript{T}, which had a similarity 
value of 98.6 % (40 bp differences) (Fig. 2d, Table S7).

DNA–DNA hybridization (DDH) was performed to verify 
the DNA relatedness among the three type strains 
(MOTT64\textsuperscript{T}, M. intracellulare ATCC 13950\textsuperscript{T} and 
M. chimaera JCM 14737\textsuperscript{T}) and the other INT-1 and INT-2 strains 
(Table 2), as previously described (Sambrook & Russell, 
2001; Park et al., 2007; Lee et al., 2011, 2012; Choi et al., 
2013; Lo et al., 2014). Briefly, the genomic DNA extracted 
was blotted onto Hybond-N+ nylon membranes (Amer- 
sham Pharmacia Biotech) in triplicate after denaturation 
using NaOH solution and heating at 80 °C. Each DNA sam- 
ple (4 µg) was digested by HaeIII restriction enzyme to label 
DNA probe for cross-hybridization, and the hybrids were 
detected on nylon membrane by using the DIG High Prime 
DNA Labeling kit (Roche Applied Science) according to 
the manufacturer's instructions. The hybridization signals were 
detected by a scanner (HP Scanjet 3770) and analysed using 
Adobe Photoshop (version 7.0). The signal produced by the 
hybridization of the probe to the homologous target DNA 
was taken to be 100 %, and the signal intensities arising 
from the self-hybridization of the series of dilutions were 
used to calculate the levels of DNA relatedness.

When MOTT64\textsuperscript{T} DNA was used as a probe, the DDH 
values between MOTT64\textsuperscript{T} and M. intracellulare ATCC 13950\textsuperscript{T} 
and between MOTT64\textsuperscript{T} and M. chimaera JCM 14737\textsuperscript{T} were 
57.9 ± 4.0 % and 49.1 ± 4.9 %, respectively. When the type 
strains of the two recognized species were used as probes 
for cross check, the DDH values between MOTT64\textsuperscript{T} and 
M. intracellulare ATCC 13950\textsuperscript{T} and between MOTT64\textsuperscript{T} and 
M. chimaera JCM 14737\textsuperscript{T} were 53.1 ± 0.2 and 47.2 ± 1.6, 
respectively, which were below the 70 % that is generally accepted 
as a standard for species delineation (Wayne et al., 1987). 
The DDH values between MOTT64\textsuperscript{T} and Asan 29591 and 
between MOTT64\textsuperscript{T} and Asan 37128 were 85.9 ± 7.9 % 
and 72.3 ± 2.8 %, respectively. In addition, to check the fidelity 
of the DDH assay, it was further applied for the analysis 
of DDH values between the INT-2 strains and M. intracel- 
lulare ATCC 13950\textsuperscript{T}. The DDH values between M. intracel- 
lulare ATCC 13950\textsuperscript{T} and the two INT-2 strains, Asan 37016 
and Asan 38402, were 86.6 ± 6.23 % and 81.9 ± 2.48 %, 
respectively, suggesting that they could be members of the 
same species. The DDH results are shown in Table 2 and 
the membrane images of DDH are shown in Fig. S4.

The genomic distances between MOTT-64\textsuperscript{T} and M. intra-
cellulare ATCC 13590\textsuperscript{T} were determined from fully 
sequenced genomes using the average nucleotide identity 
(ANI; CLgenomics version 1.52; http://www.chunlab.com/) 
(Goris et al., 2007; Konstantinidis & Tiedje, 2005) and 
the web-based Genome-to-Genome Distance Calculator 
(GGDC version 2.1; http://ggdc.dsmz.de/).

The ANI values calculated between M. intracellulare ATCC 
13950\textsuperscript{T} and MOTT-64\textsuperscript{T} were 98.56 %. Also, according to 
the Genome Blast Distance Phylogeny (GBDP) DDH pre-
prediction, the predicted DDH values between M. intracellu-
lare ATCC 13950\textsuperscript{T} and MOTT-64 were 89 %.

Taken together, the distinct sequence characteristics of the 
five independent genes [hsp65, rpoB, gnd, argG and pgm] 
together with biochemical characteristics, unique HPLC 
profiles of mycolic acids, MALDI-TOF profiles of lipids and 
DDH values of >70 % with the type strains of M. intracellu-
lare and M. chimaera strongly support the conclusion that 
the INT-1 strains belong to a distinct mycobacterial species 
within the M. avium complex for which the name Mycobac-
terium paraintracellulare sp. nov. is proposed.

Description of Mycobacterium paraintracellulare sp. nov.

\textit{Mycobacterium paraintracellulare} [pa.ra.in.tra.cell.lu.la’re]. 
Gr. prep. para next to, resembling; N.L. neut. adj.
intracellulare a bacterial epithet (intracellular); N.L. neut. adj. paraintracellulare next to (Mycobacterium intracellulare).

A rod-shaped and acid-fast bacteria without spores or filaments under the microscope. The optimum temperature for growth is 37 °C on Middlebrook 7H110 agar medium, which results in smooth and white–yellow colonies after more than 7 days. Growth is observed on not only MacConkey agar without crystal violet but also in acidic environments (pH 5.5). Positive for heat-stable catalase (68 °C). Strongly resistant to seven antibiotics (aminicin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, moxifloxacin and rifampin). The lipid profile by MALDI-TOF MS and mycacid analysis by HPLC are distinctive and different from those of M. intracellulare and M. chimaera. Unique sequences for five other housekeeping genes (hsps65, rpoB, gnd, argG and pgm) differentiate the species from M. intracellulare.

The type strain, MOTT64T (=KCTC 29084T=JCM 30622T), was isolated from human sputum in Seoul, Korea.

Acknowledgements

This work was supported by a National Research Foundation of Korea (NRF) grant (no. NRF-2014R1A1A2004008), Republic of Korea.

References


