Archives of Microbiology (2019) 201:443–449
https://doi.org/10.1007/s00203-018-1577-0

ORIGINAL PAPER

Caballeronia ginsengisoli sp. nov., isolated from ginseng cultivating soil

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Received: 27 April 2018 / Revised: 15 September 2018 / Accepted: 19 September 2018 / Published online: 4 October 2018

Abstract

A Gram-stain-negative, strictly aerobic, non-motile, ivory colored and rod-shaped bacterium (designated Gsoil 652^T) isolated from ginseng cultivating soil, was characterized using a polyphasic approach to clarify its taxonomic position. Strain Gsoil 652^T was observed to grow optimally at 30 °C and at pH 7.0 on R2A agar medium. Phylogenetic analysis, based on 16S rRNA gene sequences similarities, indicated that Gsoil 652^T belongs to the genus *Caballeronia* of the family *Burkholderiaceae* and was most closely related to *Caballeronia choica* LMG 22940^T (98.9%), *Caballeronia udeis* LMG 27134^T (98.9%), *Caballeronia sordidicola* LMG 22029^T (98.2%) and *Caballeronia humi* LMG 22934^T (98.1%). The DNA G+C content was 62.1 mol% and Q-8 was the major isoprenoid quinone. The main polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, unidentified aminophospholipid, and unidentified phospholipid. The predominant fatty acids were $C_{16:0}$, $C_{17:0}$ cyclo and $C_{19:0}$ cyclo $\omega 8c$. The DNA–DNA relatedness value between strain Gsoil 652^T and closely related type strains of *Caballeronia* species were less than 36.0%. Moreover, strain Gsoil 652^T could be distinguished phenotypically from the recognized species of the genus *Caballeronia*. The novel isolate, therefore, represents a novel species, for which the name *Caballeronia ginsengisoli* sp. nov. is proposed, with the type strain Gsoil 652^T (= KACC 19441^T = LMG 30326^T).

Keywords *Caballeronia ginsengisoli* · 16S rRNA *gene sequence* · *Polyphasic taxonomy*

Introduction

The genus *Caballeronia* was first described by Dobritsa and Samadpour in 2016 (Dobritsa and Samadpour 2016). At the time of writing the genus *Caballeronia* comprises 25 species with validly published names (http://www.bacterio.net/caballeronia.html) and accommodate 24 species of the genus *Burkholderia* and one species (*Pseudomonas glathei*) of the genus *Pseudomonas*. Generally members of this genus are Gram-stain-negative, ovoid or rod-shaped, nonspore-forming, occurring singly, in pairs or in chains. Members of the genus are mesophilic and some species growth is inhibited at 37 or 42 °C. The predominant ubiquinone is Q-8. Affiliates of the genus show $C_{18:1}$ $\omega 7c$, summed feature 3 ($C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ ω 6c) and $C_{16:0}$ as major fatty acids. The DNA G+C contents of members of the genus *Caballeronia* range from 59 to 65 mol% (Dobritsa and Samadpour 2016).

To study the culturable aerobic, anaerobic, facultative and ginsenoside transforming bacterial strains living in the soil of a ginseng field Pocheon province [(37° 91′ 96″N, 127° 22.4′ 59.1″E) South Korea], a number of novel bacterial strains including novel genera *Pseudobacter* (Gsoil

Communicated by Erko Stackebrandt.

Xiao-Tian Quan and Qing-Zhen Liu contribute equally.

The GenBank accession number for the 16S rRNA gene sequence of strain is KY078844.

The DPD taxon number for strain Gsoil 652^T is TA00566.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00203-018-1577-0) contains supplementary material, which is available to authorized users.

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221^T, Siddiqi and Im 2016a), *Anseongella* (Gsoil 524^T, Siddiqi et al. 2016b), *Panacibacter* (Gsoil 1550^T, Siddiqi et al. 2016c), and novel species [*Lysobacter pocheonensis* (Gsoil 193^T, Siddiqi and Im 2016d), *Arachidicoccus ginsenosidivorans* (Gsoil 809^T, Siddiqi et al. 2017), *Mucilaginibacter ginsenosidivorans* (Gsoil 3017^T, Kim et al. 2017), *Aeromicrobium panacisoli* (Gsoil 137^T, Siddiqi et al. 2018), respectively] were isolated on R2A and 1/2 R2A agar plates.

Materials and methods

Isolation of bacterial strain

In this study, we describe a novel bacterial strain, designated Gsoil 652^T isolated from the soil of ginseng field in Pocheon province (37°91′96″N, 127°22.4′59.1″E), South Korea, appeared to be a member of the genus *Caballeronia*. Strain Gsoil 652^T was cultured routinely on R2A agar at 30 °C and preserved as a glycerol suspension (R2A broth with 25% (v/v)), at -80 °C. The type strains *Caballeronia choica* LMG 22940^T, *Caballeronia glathei* LMG 14190^T, *Caballeronia humi* LMG 22934 ^{T}, *Caballeronia sordidicola* KCTC 12081^T and *Caballeronia udeis* LMG 27134T, were obtained from two different culture collections, grown under the same conditions and used as reference strains for Gsoil 652^T.

Phylogenetic analysis, DNA–DNA hybridization and DNA G+C content

The genomic DNA of strain Gsoil 652^T was extracted with DNA-extraction kit (Solgent) and was used in a PCR assay with the universal bacterial primer pair 9F and 1512R to amplify the 16S rRNA gene sequence (Weisburg et al. 1991). The amplified PCR purified products were then sequenced by Solgent Co. Ltd. The almost complete 16S rRNA gene sequence of strain Gsoil 652^T (1453 nt) was assembled using the SeqMan software (DNASTAR) program and then the sequence of strain Gsoil 652^T was compared with the 16S rRNA gene sequences of related taxa, which were obtained from the EzBiocloud server [http://www.ezbiocloud.net/eztaxon] and GenBank data base. CLUSTAL_X program was used for multiple sequence alignments and the gaps were edited via BioEdit program (Thompson et al. 1997; Hall 1999). Evolutionary distances were calculated using Kimura two-parameter model (Kimura 1983). Maximum-parsimony (Fitch 1971), neighbor-joining (Saitou and Nei 1987) and maximum-likelihood algorithms were used to construct the phylogenetic trees using the MEGA6 program (Tamura et al. 2013) with bootstrap values based on 1000 replications (Felsenstein 1985). The DNA–DNA hybridization experiment was carried out as previously described (Ezaki et al.1989).

To measure the DNA G+C content (mol%) of the strain Gsoil 652, the genomic DNA was extracted and purified by the described method of Moore and Dowhan (1995). Then the DNA was enzymatically degraded into nucleosides and the DNA base composition was determined using a reverse-phase HPLC (Mesbah et al. 1989).

Physiological and biochemical characteristics

The Gram reaction was determined as described by Buck (1982). For electron microscopic (LEO 912AB; Carl Zeiss) analysis, strain Gsoil 652^T was grown on R2A agar medium (Difco) for 2 days at 30 °C. Gliding motility was investigated using the hanging drop method described by Bernardet et al. (2002). Catalase and oxidase tests were performed as described by Cappuccino and Sherman (2002). Biochemical tests were carried out using API 20NE, API ID 32GN and API ZYM kits according to the instructions of the manufacturer (bioMérieux). Tests for degradation of DNA (using DNase agar), casein, starch, Tween 80, Tween 20 and CM-cellulose were performed and evaluated after 7 days of incubation at 30 °C as described previously (Ten et al. 2004). Growth at different temperatures (4, 10, 15, 20, 25, 30, 37, 40, 42 °C) and various pH values (pH 4–10 at intervals of 1.0 pH units) was assessed after 5 days incubation at 30 °C. Three different buffers (final concentration, 50 mM) were used to adjust the pH of R2A broth. Acetate buffer was used for pH 4.0–5.5, phosphate buffer was used for pH 6.0–8.0 and Tris buffer was used for pH 8.5–10.0. Salt tolerance was tested on R2A medium supplemented with 1–10% (w/v at intervals of 1% unit) NaCl, and growth assessed after 7 days of incubation. Growth on nutrient agar (NA, Difco), trypticase soy agar (TSA, Difco), LB agar (Difco) and MacConkey agar (Difco) was also evaluated at 30 °C.

Chemotaxonomic analysis

Analysis of respiratory quinone

For quinone analysis, dry cells were dissolved in chloroform/methanol (2/1, v/v) and the quinone was extracted. Afterward, the quinone was concentrated and subsequently extracted with *n*-hexane/water (1/1, v/v). Then, the crude *n*-hexane-quinone solution was purified and analyzed by HPLC (Hiraishi et al. 1996).

Fatty acids analysis

For cellular fatty acid methyl ester (FAME) analysis, strains were on R2A agar (Difco) medium for 48 h at 30 °C. All analysis parameters such as growth medium, temperature

and physiological age were similar for all strains. A full loop of well-grown cells was harvested and fatty acids were extracted, methylated and saponified by the described method of Sherlock Microbial Identification system (MIDI). Then it was analyzed by capillary GLC (Hewlett Packard 6890) using the TSBA library (version 6.1) (Sasser 1990).

Results and discussion

Phylogenetic tree and DNA G+C content

Almost complete 16S rRNA gene sequence of strain Gsoil 652^T (1453 bp), was uploaded to EzTaxon-e server and compared with the closest strains. Based on the EzTaxon-e server analysis strain Gsoil 652^T show highest sequence similarity with *Caballeronia choica* LMG 22940^T (98.9%), *Caballeronia udei* LMG 27134^T (98.9%), *Caballeronia sordidicola* LMG 22029^T (98.2%), and *Caballeronia humi* LMG 22934^T (98.1%). However, in the phylogenetic tree analysis, *C. humi* LMG 22934^T clusters with *C. terrestris* LMG 22937^T and *C. glathei* LMG 14190^T as shown in Fig. 1. This phylogenetic relationship was also confirmed in the trees generated with the maximum-likelihood and maximum-parsimony algorithms.

The genomic DNA G+C content of strain Gsoil 652^T was 62.8 mol%, which lies within the range observed for members of the genus *Caballeronia* (59.0–65.0 mol%, respectively) (Dobritsa and Samadpour 2016).

Physiological biochemical tests

Cells of strain Gsoil 652^T were Gram-reaction-negative, aerobic, and non-motile. Cells were rod shaped (measuring 0.3–0.4 µm and 0.8-2.0 µm) shown in Supplementary Fig. S1. Morphological, physiological, and biochemical characteristics of strain Gsoil 652^T are given in the species description and Table 1. Some physiological characteristics of strain Gsoil 652^T are compared with those of the three reference type strains are in Table 1. List of all negative traits of commercial kits is shown in Table S1.

DNA–DNA hybridization

The DNA–DNA hybridization between strain Gsoil 652^T and *Caballeronia choica* LMG22940^T, *Caballeronia udeis*

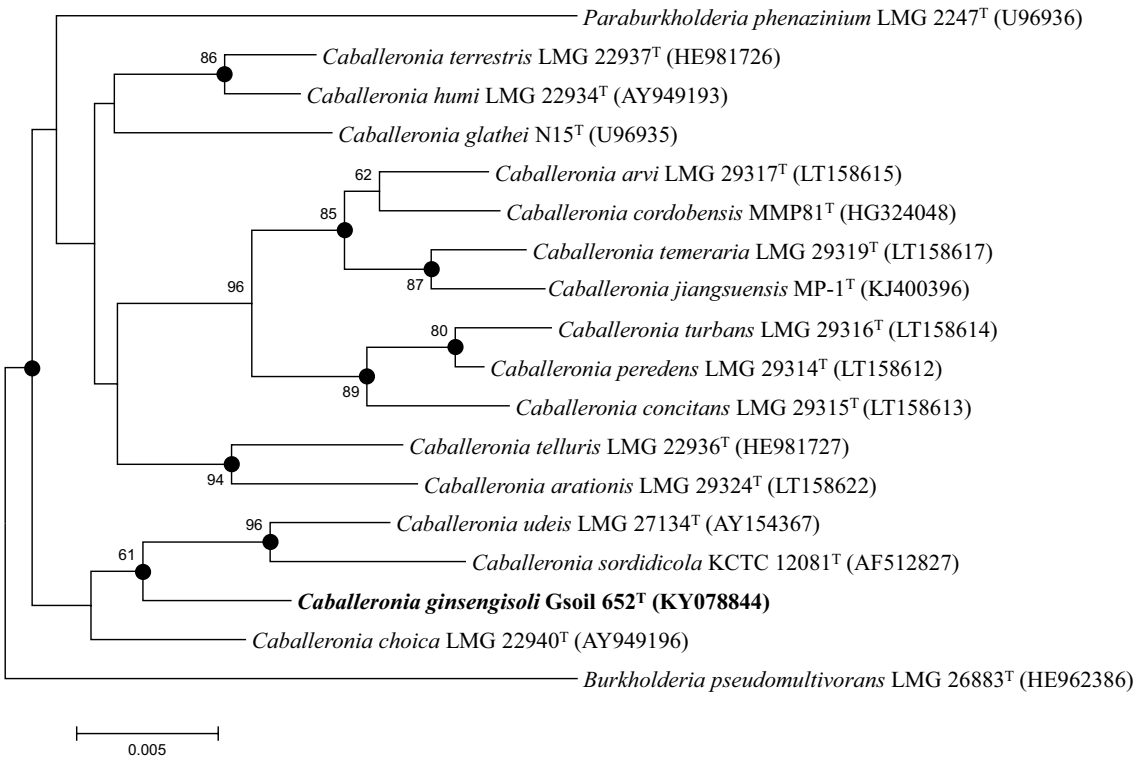

Fig. 1 Phylogenetic relationship of strain Gsoil 652^T with recognized *Caballeronia* species. The tree was constructed using the neighbor-joining method based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages of 1000 replications) greater than 60 % are shown at branch points. *Burkholderia pseudomultivorans* LMG 26883^T (HE962386) was used as an out group. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with maximum-parsimony algorithms and maximum likelihood. Bar 0.005 substitutions per nucleotide position

Table 1 Physiological and biochemical characteristics of strain Gsoil 652^T and related type species of the genus *Caballeronia*

Characteristic	1	2	3	4	5	6
Isolation source	Soil	Soil[a]	Soil[b]	Soil[c]	Soil[d]	Soil[e]
Colony colour	Beige	Beige[a]	Shiny[b]	Beige[c]	Cream[d]	Beige[e]
Temperature range (°C) for growth	18–37	28–40[a]	28–30[b]	28–30[c]	28–30[d]	28–30[e]
pH range	6–8	7–8[a]	6–8[b]	5–7[c]	5–7[d]	5–8[e]
Nitrate reduction	−	−	−	−	+	+
Glucose acidification	−	−	−	−	−	+
Arginine dihydrolase	−	+	−	+	−	+
Urease	−	−	+	−	−	+
Esculin hydrolysis	−	+	−	+	+	+
L−Arabinose	−	−	−	−	+	+
Gluconate	+	+	+	+	+	−
Adipate	+	+	−	−	−	−
Malate	+	+	+	+	−	−
Phenyl-acetate	+	−	−	+	−	−
Salicin	−	+	−	−	−	−
D-Melibiose	+	+	−	−	−	+
L-Fucose	−	−	+	+	+	−
L-Arabinose	+	+	+	−	+	+
Propionate	−	+	+	+	+	−
Caprate	+	−	+	+	+	−
Valerate	+	−	−	+	+	−
Citrate	−	−	+	+	−	−
L-Histidine	−	-	+	+	+	−
2-Ketogluconate	+	+	+	+	+	−
3-Hydroxy-butyrate	−	−	+	+	+	−
4-Hydroxy-benzoate	−	−	+	+	−	−
L-Proline	−	−	+	+	+	−
L-Rhamnose	+	−	+	+	+	−
D-Ribose	+	+	−	+	+	+
Inositol	−	−	+	+	+	−
D-Sucrose	+	+	−	−	−	+
D-Maltose	−	+	−	−	−	+
Itaconate	−	−	+	−	−	−
Malonate	−	−	+	−	−	−
Acetate	−	−	+	+	−	−
Lactate	+	+	+	−	+	−
L-Alanine	+	−	+	+	+	−
5-Ketogluconate	−	−	−	+	−	−
Glycogen	−	−	+	−	−	−
3-Hydroxy-benzoate	−	−	−	+	−	−
L-Serine	−	−	+	+	+	−
Alkaline phosphatase	+	+	+	+	+	−
Esterase (C4)	+	+	+	+	+	−
Esterase Lipase (C8)	−	−	+	+	−	−
Lipase (C14)	−	−	+	+	−	−
Valine arylamidase	−	−	+	+	−	−
Cystine arylamidase	−	−	+	+	−	−
Trypsin	+	+	−	−	−	+
Acid phosphatase	+	+	+	+	+	−
NaphtHol-AS-BI-phosphohydrolase	+	+	+	+	+	−
β-Galactosidase	+	+	+	+	+	+

Table 1 (continued)

Characteristic	1	2	3	4	5	6
β-Glucuronidase	–	–	+	–	–	–
α-Glucosidase	+	+	+	–	–	+
β-Glucosidase	+	+	–	–	–	+
N-Acetyl-β-glucosaminidase	+	+	–	–	–	+
DNA G+C content (mol%)	62.1	63.0	ND	63.0	61.3	60.0

Strains: 1, Gsoil 652^{T}; 2. *C. choica* LMG 22940^{T}; 3. *C. glathei* LMG 14190^{T}; 4. *C. humi* LMG 22934^{T}; 5. *C. sordidicola* KCTC 12081^{T}; 6, *C. udeis* LMG 27134^{T}. All strains were negative for indole production, gelatin hydrolysis, suberate, α-chymotrypsin, α-galactosidase, α-mannosidase, α-fucosidase and positive for D-glucose, D-mannose, D-mannitol, D-sorbitol, *N*-acetyl-D-glucosamine and leucine arylamidase

+ positive, – negative, *ND* no data available/not determined

[a, b, c, d], and [e], Data are taken from Vandamme et al. (2013); Lim et al. (2003); Zolg and Ottow, (1975); Dobritsa and Samadpour (2016)

LMG 27134^{T} and *Caballeronia sordidicola* LMG 22029^{T} was 34.4 ± 0.8, 35.6 ± 0.5 and 24.1 ± 0.9%, respectively. On the basis of DNA–DNA hybridization, strain Gsoil 652^{T} did not belong to the species *Caballeronia choica* LMG22940^{T} and other closest strain according to the recommendation of threshold value of 70% (Wayne et al. 1987). Therefore, our result shows that the strain Gsoil 652^{T} represents a distinct genomic species of the genus *Caballeronia*.

Quinone and fatty acids

The major cellular fatty acids were $C_{16:0}$ (30.1%), $C_{19:1}$ cyclo *ω8c*, (20.6%), and $C_{17:0}$ cyclo (20.3%) as shown in (Table 2). The cellular fatty acids (CFA) analyses indicate that the novel isolate shares similar major CFA with members of the genus *Caballeronia* (Vandamme et al. 2013; Dobritsa and Samadpour 2016). However, variances in the fatty acid content could be observed between strain Gsoil 652^{T} and its phylogenetically closest type strains. Strain Gsoil 652^{T} contained ubiquinone Q-8 as the major respiratory quinone. Our results are in good agreement with those of other members of the genus *Caballeronia* (Dobritsa and Samadpour 2016; Vandamme et al. 2013).

The results obtained from the phenotypic and phylogenetic characterizations indicated that strain Gsoil 652^{T} belongs to the genus *Caballeronia*. The phylogenetic distinctiveness and low value of DNA–DNA hybridization confirmed that this isolate represent a species that is distinct from recognized species of the genus *Caballeronia*. There are some phenotypic differences were found between strain Gsoil 652^{T} and phylogenetically related *Caballeronia* type strains (Table 1). Therefore, on the basis of the data presented, strain Gsoil 652^{T} should be classified within the genus *Caballeronia* as representing a novel species, for which the name *Caballeronia ginsengisoli* sp. nov. is proposed.

Description of Caballeronia ginsengisoli sp. nov

Caballeronia ginsengisoli (gin.seng.i.so′li N.L. N.L. n. *ginsengum* ginseng; L. n. *solum* soil; N.L. gen. n. ginsengisoli of soil of a ginseng field).

Cells are Gram-reaction-negative, aerobic, non-motile, non-spore-forming, rod-shaped (0.3–0.4 µm in diameter and 2.5-5 µm in length), catalase and oxidase positive. Colonies grown on the R2A agar plates for 2 days at 30 °C were circular and beige colour. Growth occurs at 18–37 °C with pH 6–8 (optimum at 30 °C with pH 6–7.0) without additional NaCl supplement. Growth is inhibited in the presence of 1.5% (w/v) NaCl. Does not hydrolyze gelatin casein, starch, CM-cellulose, Tween 80, Tween 20 and DNase. Using API kits (API 32 GN, API ZYM and API 20 NE) the following substrates are utilized: d-glucose, D-mannose, D-mannitol, gluconate, adipate, malate, D-sorbitol, propionate, caprate, valerate, 2-ketogluconate, L-rhamnose, *N*-acetyl-D-glucosamine, D-ribose, phenyl-acetate, D-sucrose, lactate, L-alanine, alkaline phosphatase, esterase, leucine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase and *N*-acetyl-β-glucosaminidase. Ubiquinone 8 (Q-8) is the predominant quinone. The major fatty acids are $C_{16:0}$, $C_{17:0}$ cyclo and $C_{19:0}$ cyclo *ω8c*. The G+C content of genomic DNA is 62.1 mol%.

The type strain, Gsoil 652^{T} (= KACC 19441^{T} = LMG 30326^{T}) was isolated from soil from a ginseng field in Pocheon Province, South Korea.

Table 2 Fatty acid profiles of strain Gsoil 652^T and related species of the genus *Caballeronia*

Fatty acid	1	2	3	4	5	6
Saturated						
$C_{12:0}$	tr	–	–	4.3	3.0	2.5
$C_{14:0}$	1.7	3.9	3.0	1.63	0.85	
$C_{16:0}$	30.1	33.0	25.0	25.7	33.9	12.8
$C_{18:0}$	2.3	1.1	tr	2.8	tr	6.0
Branched-chain fatty acid						
iso-$C_{13:0}$ 3-OH	1.1	–	–	–	–	1.0
iso-$C_{17:0}$	2.1	–	–	–	–	3.3
$C_{18:1}$ ω7c 11-methyl	–	–	tr	42.2	tr	–
$C_{10:0}$ 3-OH	1.1	–	–	1.5	–	–
$C_{16:0}$ 2-OH	3.1	–	5.7	–	–	–
$C_{16:0}$ 2-OH	–	1.4	–	–	3.3	–
$C_{16:0}$ 3-OH	4.1	4.5	5.4	–	5.0	–
$C_{16:1}$ 2-OH	–	–	1.5	–	tr	–
$C_{18:0}$ 3-OH	–	–	–	3.2	–	–
Cyclo						
$C_{17:0}$ cyclo	20.3	27.3	19.4	3.9	28.0	1.7
$C_{19:0}$ cyclo ω8c	20.6	9.1	25.1	9.7	14.7	20.9
Summed feature[a]						
2; $C_{12:0}$ aldehyde	3.1	4.4	5.3	–	3.6	–
3; $C_{16:1}$ ω6c/$C_{16:1}$ ω7c	4.5	2.2	tr	–	1.2	1.1
8; $C_{18:1}$ ω7c/$C_{18:1}$ ω6c	2.6	10.2	3.9	4.3	1.9	48.1

1 Strains: 1, Gsoil 652^T; 2. *C. choica* LMG 22940^T; 3. *C. glathei LMG* 14190^T; 4. *C. humi* LMG 22934^T; 5. *C. sordidicola* KCTC 12081^T; 6, *C. udeis* LMG 27134^T (all data are from this study). Cells of all strains were collected after 48 h of incubation on R2A medium (Difco) at 30 °C. All data were obtained from this study. The data shown in this table are more than 1% for all strains

tr traces < 1%; – not detected

[a]Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system

Acknowledgements This work was supported by the project on survey and excavation of Korean indigenous species of the National Institute of Biological Resources (NIBR) under the Ministry of Environment and by the support of Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ012283) "Rural Development Administration and by the Intelligent Synthetic Biology Center of Global Frontier Project funded by the Ministry of Science and ICT" (2011–0031955), Republic of Korea.

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