Solitalea koreensis gen. nov., sp. nov. and the reclassification of [*Flexibacter*] *canadensis* as *Solitalea canadensis* comb. nov.

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A Gram-negative, rod-shaped, non-spore-forming bacterium, designated strain R2A36-4^T, was isolated from greenhouse soil and subjected to a polyphasic taxonomic analysis. 16S rRNA gene sequence analysis revealed that the strain represented a novel member of the family *Sphingobacteriaceae*. Its nearest phylogenetic neighbour was the type strain of [*Flexibacter*] *canadensis* CIP 104802^T (93.2% 16S rRNA gene sequence similarity). Strain R2A36-4^T and [*F.*] *canadensis* CIP 104802^T fell in a distinct cluster within the family *Sphingobacteriaceae*. Strain R2A36-4^T contained MK-7 as the predominant quinone. Strain R2A36-4^T and [*F.*] *canadensis* CIP 104802^T had iso-C_{15:0}, iso-C_{17:0} 3-OH, summed feature 3 and C_{15:1}ω6c as the major fatty acids. Strain R2A36-4^T could be distinguished from [*F.*] *canadensis* on the basis of several physiological properties and fatty acid compositions. Based on phenotypic characterization and 16S rRNA gene sequence analysis, strain R2A36-4^T represents a novel species within a new genus, for which the name *Solitalea koreensis* gen. nov., sp. nov. is proposed. The type strain of *Solitalea koreensis* is strain R2A36-4^T (=KACC 12953^T=DSM 21342^T). It is also proposed that [*F.*] *canadensis* be transferred to this genus as *Solitalea canadensis* comb. nov. (type strain UASM 9D^T=ATCC 29591^T=CIP 104802^T=DSM 3403^T=NBRC 15130^T=JCM 21819^T=KACC 13276^T=LMG 8368^T).

The genus *Flexibacter* was first proposed by Soriano (1945), and many species were reported for isolates that originated from soil and freshwater. Later, several species of the genus *Flexibacter* were classified into other genera on the basis of phylogenetic and genotypic data. [*Flexibacter*] *aggregans* was reclassified as *Flexithrix dorotheae* (Hosoya & Yokota, 2007), [*Flexibacter*] *columnaris* as *Flavobacterium columnare* (Bernardet & Grimont, 1989), [*Flexibacter*] *psychrophilus* as *Flavobacterium psychrophilum* (Bernardet *et al.*, 1996), [*Flexibacter*] *filiformis* as *Chitinophaga filiformis* (Reichenbach, 1989), [*Flexibacter*] *japonensis* as *Chitinophaga japonensis* (Fujita *et al.*, 1996), [*Flexibacter*] *sancti* as *Chitinophaga sancti* (Kämpfer *et al.*, 2006), [*Flexibacter*] *maritimus* as *Tenacibaculum maritimum* and [*Flexibacter*] *ovolyticus* as *Tenacibaculum ovolyticum* (Suzuki *et al.*,

2001). However, according to the phylogenetic tree (Nedashkovskaya *et al.*, 2003; Weon *et al.*, 2006; Xie & Yokota, 2006), some among the remaining species are still in need of taxonomic reconsideration.

A soil sample was collected from greenhouse soil cultivated with cucumber (*Cucumis sativus* L.) from the Yongin region, Korea. The sample was serially diluted with 0.85% NaCl (w/v) and suitable 10-fold dilutions were plated onto R2A agar (Difco). The plates were incubated at 28 °C for 4 days and strain R2A36-4^T was subsequently isolated. According to the phylogenetic data, strain R2A36-4^T was closely related to [*Flexibacter*] *canadensis* CIP 104802^T (Christensen, 1980).

For the phenotypic and genotypic tests, strain R2A36-4^T was routinely cultivated in R2A medium. Gram reaction, oxidase and catalase reaction and hydrolysis of casein, CM-cellulose, DNA, starch, Tween 80 and tyrosine were determined according to the methods of Smibert & Krieg (1994). Cell morphology was examined by using phase-contrast microscopy (AXIO; Zeiss) and transmission electron microscopy with cells grown for 1 day. For transmission electron microscopy, cells were negatively

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain R2A36-4^T is EU787448.

A transmission electron micrograph of cells of strain R2A36-4^T and a table showing the cellular fatty acid compositions of strain R2A36-4^T and [*Flexibacter*] *canadensis* CIP 104802^T are available as supplementary material with the online version of this paper.

stained with 0.5 % (w/v) uranyl acetate and examined with a LEO model 912AB electron microscope. Gliding motility was observed via oil-immersion phase-contrast microscopy of the edge of colonies in exponential growth phase. The pH range (pH 4.0–10.0 at intervals of 1.0 pH units) for growth was determined in R2A broth that was buffered with citrate/phosphate buffer or Tris/hydrochloride buffer (Breznak & Costilow, 1994). Growth at 0, 1, 1.5, 2, 3 and 5 % NaCl (w/v) was investigated in R2A broth. Growth at various temperatures (5–45 °C) was determined on R2A agar. Tests in the commercial systems API ZYM, API 20E, API 20NE and API ID 32 GN (bioMérieux) were generally performed according to the manufacturer's instructions. The API ZYM test strip was read after 4 h incubation at 37 °C, the API 20E test strip was read after 7 days, and the other API strips were examined after 4 days at 28 °C.

Strain R2A36-4^T grew well on R2A medium and weakly on nutrient agar (Difco), but did not grow on trypticase soy agar or MacConkey agar (Difco). Cells were aerobic, Gram-negative, non-flagellated, gliding and rod-shaped, 0.5–

0.6 µm wide and 1.3–30 µm long (Supplementary Fig. S1, available in IJSEM Online). Colonies were light yellow and irregular. Phenotypic data among strain R2A36-4^T, [*F.*] *canadensis* CIP 104802^T and related taxa are compared in Tables 1 and 2. Strain R2A36-4^T and [*F.*] *canadensis* CIP 104802^T differed from most of their close relatives by their inability to grow in concentrations of NaCl greater than 1 %, and to hydrolyse aesculin (Table 1). Strain R2A36-4^T could be differentiated from [*F.*] *canadensis* CIP 104802^T on the basis of several properties, including colony colour, anaerobic growth, glucose fermentation, nitrate reduction and indole production (Table 2).

Fatty acid methyl esters were extracted and prepared by using the standard protocol of the Microbial Identification system (MIDI; Microbial ID) from cells grown on R2A for 24 h at 28 °C. Isoprenoid quinones were analysed by HPLC as described by Groth *et al.* (1996). The DNA G+C content was determined by HPLC analysis of deoxyribonucleosides as described by Mesbah *et al.* (1989) using a reversed-phase column (Supelcosil LC-18 S; Supelco). The

Table 1. Differential phenotypic characteristics among *Solitalea* gen. nov. and related genera of the family *Sphingobacteriaceae*

Taxa: 1, *Solitalea* gen. nov.; 2, *Mucilaginibacter* (data from Pankratov *et al.*, 2007); 3, *Nubsella zeaxanthinifaciens* TDMA-5^T (Asker *et al.*, 2008); 4, *Olivibacter* (4 species) (Ntougias *et al.*, 2007; Wang *et al.*, 2008); 5, *Parapedobacter* (2 species) (Kim *et al.*, 2007, 2008); 6, *Pedobacter* (20 species) (Baik *et al.*, 2007; Hwang *et al.*, 2006; Gallego *et al.*, 2006; Kwon *et al.*, 2007; Muurholm *et al.*, 2007; Shivaji *et al.*, 2005; Steyn *et al.*, 1998; Ten *et al.*, 2006b; Vanparys *et al.*, 2005; Yoon *et al.*, 2006, 2007a, b, c, d); 7, *Sphingobacterium* (7 species) (Kim *et al.*, 2006; Shivaji *et al.*, 1992; Steyn *et al.*, 1998; Takeuchi & Yokota, 1992). +, Positive; –, negative; v, variable; ND, no data available.

Characteristic	1	2	3	4	5	6	7
Relation to oxygen	v	Facultatively anaerobic	Strictly aerobic	Strictly aerobic	Strictly aerobic	Strictly aerobic	Facultatively anaerobic
Growth at:							
5 °C	–	+	–	v	–	+*	v
40 °C	v	–	+	v	v	–	v
Growth at >1 % NaCl	–	–	ND	+	+†	+	+
Glucose fermentation	v	+	–	+‡	+†	v	+
Nitrate reduction	v	ND	–	ND	–	–	v
Arginine dihydrolase	–	ND	–	+‡	–	v*	ND
Hydrolysis of:							
DNA	v	ND	+	ND	ND	v	v
Gelatin	+	ND	+	+‡	–	v	v
Starch	+	+	+	–§	ND	v	v
Aesculin	–	–	+	+‡	+†	v	v
Urea	–	ND	–	+‡	–	–	v
Assimilation of:							
Arabinose	v	v	–	+‡	v	v	v
Mannitol	–	–	–	v	+	v	v
Maltose	+	+	+	v	+	v	+
Malate	–	–	+	–‡	–	–	–
DNA G+C content (mol%)	37–38.1	42–46	38.6	39.2–45.6	45.6–50.1	36.9–44.2	37.7–44.2

*No data available for *Pedobacter caeni*, *Pedobacter africanus* or *Pedobacter saltans*.

†Data only for *Parapedobacter koreensis*.

‡Data only for three species, *Olivibacter soli*, *Olivibacter ginsengisoli* and *Olivibacter terrae*.

§Data only for *Olivibacter sitiensis*.

||Data only for strain R2A36-4^T.

Table 2. Phenotypic comparison between strain R2A36-4^T and [*F.*] *canadensis* CIP 104802^T

Both strains are positive for catalase, oxidase, Voges–Proskauer reaction, hydrolysis of starch and gelatin, and alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, *N*-acetyl- β -glucosaminidase and α -fucosidase, but negative for CM-cellulose hydrolysis, arginine dihydrolase, urease, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H₂S production, tryptophan deaminase, lipase (C14), β -glucuronidase and α -mannosidase. Both strains assimilate D-glucose, maltose, D-mannose and salicin, but do not assimilate D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, phenylacetic acid, L-rhamnose, D-ribose, inositol, sucrose, itaconic acid, suberic acid, sodium malonate, sodium acetate, lactic acid, potassium 5-ketogluconate, 3-hydroxybenzoic acid, D-sorbitol, propionic acid, valeric acid, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid and 4-hydroxybenzoic acid. +, Positive; –, negative.

Characteristic	Strain R2A36-4 ^T	CIP 104802 ^T
Colony colour	Light yellow	White*
Anaerobic growth	–	+
Glucose fermentation	–	+
pH range for growth	5–8	5–10*
Nitrate reduction	–	+
Indole production	–	+
Hydrolysis of:		
Aesculin	–	+
Casein	–	+
DNA	–	+
Assimilation of:		
L-Arabinose	–	+
<i>N</i> -Acetylglucosamine	+	–
L-Alanine	–	+
Glycogen	–	+
L-Serine	–	+
Melibiose	–	+
L-Fucose	–	–
L-Proline	–	+
Enzymic activities		
Trypsin	–	+
α -Chymotrypsin	–	+
α -Glucosidase	–	+
β -Glucosidase	–	+
DNA G + C content (mol%)	38.1	37

*Data from Christensen (1980).

DNA G + C content of strain R2A36-4^T was 38.1 %. The major respiratory quinone of strain R2A36-4^T was MK-7. The major fatty acids of strains R2A36-4^T and [*F.*] *canadensis* CIP 104802^T were iso-C_{15:0} (33.3–36.2 %), iso-C_{17:0} 3-OH (9.8–13.0 %), summed feature 3 (comprising C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH; 9.2–10.2 %) and C_{15:1} ω 6c (5.2–7.4 %) (Table 3). The two strains could be distinguished from the other taxa studied by means of the

presence of C_{17:1} ω 6c, a lower amount of summed feature 3 and a higher amount of C_{15:1} ω 6c as compared with closely related genera. The differences in the fatty acid compositions of strain R2A36-4^T and [*F.*] *canadensis* CIP 104802^T were also notable (see Supplementary Table S1, in IJSEM Online).

The 16S rRNA gene sequence was determined by PCR amplification (Kwon *et al.*, 2003) and direct sequencing (Hiraishi, 1992). By using the EzTaxon server (<http://www.eztaxon.org/>; Chun *et al.*, 2007) the novel isolate was shown to be closely related to [*F.*] *canadensis* CIP 104802^T. The 16S rRNA gene sequences retrieved were aligned with that of the novel strain (approximately 1440 bases), using the program MEGALIGN (DNASTAR). Strain R2A36-4^T showed the highest sequence similarity (93.2 %) to [*F.*] *canadensis* CIP 104802^T (GenBank accession no. AB078046). The sequence similarities among the type strains of all other species studied were less than 90 %. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 3.0 (Kumar *et al.*, 2004), clustering was determined using the neighbour-joining and maximum-parsimony algorithms and bootstrap analysis (1000 replications) was performed to determine the stability of the clusters. The neighbour-joining tree is shown in Fig. 1. A similar result (not shown) was obtained using the maximum-parsimony algorithm. The novel isolate formed a cluster with [*F.*] *canadensis* CIP 104802^T with 100 % bootstrap support. This cluster was grouped into another larger compact cluster (100 % bootstrap value support) that was composed of the genera *Pedobacter*, *Parapedobacter*, *Nubsella*, *Mucilaginibacter*, *Sphingobacterium* and *Olivibacter*. However, strain R2A36-4^T and [*F.*] *canadensis* CIP 104802^T were clearly separated from the type species of the genus *Flexibacter*, *Flexibacter flexilis* NBRC 15060^T. From the phylogenetic tree (Fig. 1), it is clear that the novel isolate and [*F.*] *canadensis* CIP 104802^T should be affiliated with the family *Sphingobacteriaceae*, not the family *Flexibacteraceae*. However, the cluster including strain R2A36-4^T and [*F.*] *canadensis* CIP 104802^T was distinct from taxa with validly published names. Their unique phylogenetic position and low sequence similarity level with respect to other recognized bacterial species studied demonstrates that the two strains represent a novel genus. Therefore, we propose that strain R2A36-4^T should be considered to represent a novel species within a new genus, for which the name *Solitalea koreensis* gen. nov., sp. nov. is proposed. It is also proposed that [*F.*] *canadensis* be transferred to this genus as *Solitalea canadensis* comb. nov.

Description of *Solitalea* gen. nov.

Solitalea (So.li.ta'le.a. L. n. *solum* soil; L. fem. n. *talea* a rod; N.L. fem. n. *Solitalea* a rod isolated from soil).

Cells are non-flagellated rods or elongated rods and motile by gliding, Gram-negative and obligately aerobic or facultative anaerobic. No spores are formed. Mesophilic and non-halotolerant. Catalase- and oxidase-positive.

Table 3. Cellular fatty acid compositions of *Solitalea* gen. nov. and related genera of the family *Sphingobacteriaceae*

Genera: 1, *Solitalea* gen. nov. 2, *Mucilaginibacter* (2 species) (date from Pankratov *et al.*, 2007); 3, *Nubsella* (Asker *et al.*, 2008); 4, *Olivibacter* (4 species) (Ntougias *et al.*, 2007; Wang *et al.*, 2008); 5, *Parapedobacter* (2 species) (Kim *et al.*, 2007, 2008); 6, *Pedobacter* (20 species) (Baik *et al.*, 2007; Hwang *et al.*, 2006; Gallego *et al.*, 2006; Kwon *et al.*, 2007; Muurholm *et al.*, 2007; Shivaji *et al.*, 2005; Steyn *et al.*, 1998; Ten *et al.*, 2006b; Vanparys *et al.*, 2005; Yoon *et al.*, 2006, 2007a, b, c, d); 7, *Sphingobacterium* (11 species) (Liu *et al.*, 2008; Mehnaz *et al.*, 2007; Steyn *et al.*, 1998; Ten *et al.*, 2006a; Yoo *et al.*, 2007). Values are percentages of total fatty acids. tr, Less than 1 % of the total fatty acids.

Fatty acid	1	2	3	4	5	6	7
anteiso-C _{13:0}	tr	tr	tr	tr	tr to 11.4	tr	tr
iso-C _{13:0}	tr to 2.5	tr	tr	tr	tr	tr	tr
C _{14:0}	tr	tr	tr	tr to 2.0	tr to 1.1	tr to 2.5	tr to 3.9
iso-C _{14:0}	tr to 1.8	tr	tr	tr	tr	tr	tr
C _{15:0}	tr	3.2–4.5	3.9	tr	tr	tr to 4.2	tr
C _{15:0} 2-OH	tr	tr	1.0	tr	tr	tr to 1.5	tr
C _{15:0} 3-OH	tr to 2.1	tr	1.3	tr	tr to 1.7	tr	tr
anteiso-C _{15:0}	2.0–7.7	tr	2.3	tr	tr	tr to 3.9	tr to 4.1
iso-C _{15:0}	33.3–36.2	15.7–32.2	29.4	29.3–35.9	29.3–33.6	15–44.7	17.7–45.6
iso-C _{15:0} 3-OH	3.3–3.5	1.8–3.2	3.2	1.9–3.3	2.3–3.7	1.5–4.1	1.3–4.3
C _{15:1} ω6c	5.2–7.4	tr to 1.2	3.6	tr	tr to 1.1	tr to 5.8	tr
anteiso-C _{15:1}	tr	5.8–21.2	tr	tr to 1.1	tr	tr	tr
iso-C _{15:1} G	tr	tr	tr	tr	tr	tr	tr to 1.8
C _{16:0}	1.7–2.4	tr to 3.1	tr	5.5–10.4	2.7–6.0	tr to 15.5	2.1–10.9
C _{16:0} 2-OH	tr	tr	tr	tr to 2.6	tr to 1.1	tr	tr to 3.2
C _{16:0} 3-OH	tr to 1.5	tr	tr	tr to 2.0	1.9–2.3	tr to 4.5	tr to 6.4
iso-C _{16:0}	2.3–3.1	tr to 1.5	tr	tr	tr	tr to 2.0	tr to 2.3
iso-C _{16:0} 3-OH	tr to 1.0	tr	1.4	tr	tr	tr to 3.1	tr to 1.6
C _{16:0} 10-methyl	tr	tr	tr	tr	tr to 7.1	tr	tr to 9.7
iso-C _{16:1} H	1.0–1.1	tr	tr	tr	tr	tr to 2.5	tr to 1.0
C _{16:1} ω5c	1.6–3.5	2.6–5.8	tr	tr to 1.3	tr	tr to 4.7	tr to 1.5
C _{17:0} 2-OH	tr	tr	tr	tr	tr	tr to 8.9	tr to 2.4
iso-C _{17:0}	tr to 1.4	tr	tr	tr	tr	tr to 1.8	tr
anteiso-C _{17:0}	tr	tr to 4.5	tr	tr	tr to 3.1	tr	tr
iso-C _{17:0} 3-OH	9.8–13.0	8.9–13.1	15.9	11.2–12.4	14.4–16.3	6.0–20.4	5.9–19.7
C _{17:1} ω6c	1.0–2.4	tr	tr	tr	tr	tr	tr
C _{17:1} ω8c	tr to 1.2	tr to 1.5	1.2	tr	tr	tr to 1.1	tr
iso-C _{17:1} ω9c	1.8–5.9	tr to 2.7	5.1	tr to 2.0	tr to 7.1	1.6–7.6	tr to 3.5
anteiso-C _{17:1} ω9c	tr	tr	tr	tr	tr	tr to 4.7	tr
C _{18:0}	tr	tr	tr	tr to 1.5	tr to 1.2	tr	tr to 1.2
C _{18:1} ω5c	tr	tr	tr	tr	tr	tr to 1.2	tr
C _{18:1} ω7c	tr	tr	tr	tr	tr	tr	tr to 1.0
Unknown 13.565	1.1–3.5	tr	3.1	tr	tr	tr to 5.0	tr to 1.4
Unknown 13.961	tr	tr	tr	tr to 2.1	tr	tr to 1.4	tr
Unknown 14.959	tr	tr	tr	tr	tr	tr to 1.5	tr
Unknown 16.582	tr	tr	1.4	tr to 1.1	tr to 1.1	tr to 1.7	tr
Summed features*							
1	tr to 1.2	tr	tr	tr	tr	tr	tr
3	9.2–10.2	21.5–24.7	20.1	31.6–43.2	19.4–27.8	8.0–48	14.9–56.0
4	tr to 1.5	tr	tr	tr	tr	tr to 1.7	tr
5	tr	tr	tr	tr	tr	tr	tr to 1.1
6	tr	tr	tr	tr	tr to 1.2	tr	tr

*Summed feature 1 comprises C_{13:0} 3-OH and/or iso-C_{15:1} I/H; summed feature 3 comprises C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; summed feature 4 comprises iso-C_{17:1} I and/or anteiso-C_{17:1} B; summed feature 5 comprises C_{16:0} 10-methyl and/or iso-C_{17:1}ω9c; summed feature 6 comprises C_{19:1}ω11c and/or unknown 18.756.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strain R2A36-6^T. Numbers at nodes indicate percentages based on 1000 bootstrap resamplings. Filled circles indicate that the corresponding branches were also recovered in the maximum-parsimony tree. Bar, 0.02 substitutions per nucleotide position.

Major respiratory quinone is MK-7. The G + C contents of the genomic DNA are 37.0–38.1 mol%. Phylogenetically, the genus is affiliated to the family *Sphingobacteriaceae*. The type species is *Solitalea koreensis* sp. nov.

Description of *Solitalea koreensis* sp. nov.

Solitalea koreensis (ko.re.en'sis. N.L. fem. adj. *koreensis* pertaining to Korea, from where the organism was isolated).

Exhibits the following properties in addition to those given in the genus description. Cells are 0.5–0.6 µm wide and 1.3–30 µm long. Colonies are light yellow and irregular. Grows at 10–35 °C, in the presence of 0–1 % (w/v) NaCl and at pH 5–8. Optimum growth occurs at 28–30 °C and pH 6.0–7.0. Hydrolyses starch and tyrosine. Does not hydrolyse casein, CM-cellulose or DNA. Positive for gelatin hydrolysis, β-galactosidase and Voges–Proskauer test. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, aesculin hydrolysis, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H₂S production and tryptophan deaminase (API 20NE and API 20E test strips). Assimilative reactions and enzymic activities are shown in Table 2. Predominant fatty acids are iso-C_{15:0}, iso-C_{17:0} 3-OH, summed feature 3 and C_{15:1}ω6c. The DNA G + C content of the type strain is 38.1 mol%.

The type strain, R2A36-4^T (=KACC 12953^T=DSM 21342^T), was isolated from a greenhouse soil sample collected from Yongin region, Republic of Korea.

Description of *Solitalea canadensis* comb. nov.

Solitalea canadensis (ca.na.den'sis. N.L. fem. adj. *canadensis* pertaining to Canada, from where the organism was isolated).

Basonym: *Flexibacter canadensis* Christensen 1980.

The description is identical to that given for [*F.*] *canadensis* by Christensen (1980). In addition, grows in the presence of 0–1 % (w/v) NaCl. Positive for glucose fermentation, aesculin hydrolysis, gelatin hydrolysis, β-galactosidase and Voges–Proskauer test, and negative for arginine dihydrolase, urease, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H₂S production and tryptophan deaminase (API 20NE and API 20E test strips). Assimilative reactions and enzymic activities are shown in Table 2. Predominant fatty acids are iso-C_{15:0}, summed feature 3, iso-C_{17:0} 3-OH, anteiso-C_{15:0} and C_{15:1}ω6c.

The type strain is UASM 9D^T (=ATCC 29591^T=CIP 104802^T=DSM 3403^T=NBRC 15130^T=JCM 21819^T=KACC 13276^T=LMG 8368^T).

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