Pseudoxanthomonas sacheonensis sp. nov., isolated from BTEX-contaminated soil in Korea, transfer of *Stenotrophomonas dokdonensis* Yoon *et al.* 2006 to the genus *Pseudoxanthomonas* as *Pseudoxanthomonas dokdonensis* comb. nov. and emended description of the genus *Pseudoxanthomonas*

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A Gram-negative, strictly aerobic, rod-shaped bacterium, designated strain BD-c54^T, was isolated from BTEX (benzene, toluene, ethylbenzene and xylenes)-contaminated soil in Sacheon, Korea. Growth of strain BD-c54^T was observed at 15–35 °C (optimum 25–30 °C) and pH 6.0–9.5 (optimum pH 7.0–8.0). The predominant fatty acids were iso-$C_{15:0}$, iso-$C_{17:1}\omega9c$, iso-$C_{11:0}$ 3-OH, iso-$C_{16:0}$, iso-$C_{11:0}$ and iso-$C_{17:0}$. The strain contained large amounts of phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol and a small amount of an unknown amino-group-containing polar lipid as polar lipids. The major quinone was ubiquinone-8 (Q-8) and the G+C content of the genomic DNA was 67.5 mol%. A phylogenetic analysis based on 16S rRNA gene sequences showed that strain BD-c54^T formed a tight phylogenetic lineage with *Pseudoxanthomonas yeongjuensis* GR12-1^T within the genus *Pseudoxanthomonas* and was most closely related to *P. yeongjuensis* GR12-1^T and [*Stenotrophomonas*] *dokdonensis* DS-16^T, with 98.3 and 96.6 % 16S rRNA gene sequence similarity, respectively. The DNA–DNA relatedness between strain BD-c54^T and *P. yeongjuensis* GR12-1^T was 24.5 %. On the basis of chemotaxonomic data and molecular properties, strain BD-c54^T represents a novel species within the genus *Pseudoxanthomonas*, for which the name *Pseudoxanthomonas sacheonensis* sp. nov. is proposed. The type strain is BD-c54^T (=KCTC 22080^T =DSM 19373^T). In addition, the transfer of *Stenotrophomonas dokdonensis* to *Pseudoxanthomonas* as *Pseudoxanthomonas dokdonensis* comb. nov. and an emended description of the genus *Pseudoxanthomonas* are proposed.

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Abbreviation: BTEX, benzene, toluene, ethylbenzene and xylenes.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BD-c54^T is EF575564.

A transmission electron micrograph of cells of strain BD-c54^T and results of TLC of its polar lipids are available as supplementary material with the online version of this paper.

The genus *Pseudoxanthomonas* Finkmann *et al.* 2000 belongs to the family *Xanthomonadaceae* in the order *Xanthomonadales* of the *Gammaproteobacteria*. This genus is closely related phylogenetically to the genera *Xanthomonas*, *Xylella* and *Stenotrophomonas*. These genera share the presence of branched-chain fatty acids and ubiquinone-8 (Q-8), but it has been reported that

members of the genus *Pseudoxanthomonas* can be differentiated from members of the genera *Xanthomonas* and *Stenotrophomonas* by their ability to reduce nitrite but not nitrate to N_2O and by the absence of the fatty acid iso-$C_{13:0}$ 3-OH (Assih *et al.*, 2002; Thierry *et al.*, 2004). However, more recently, *Pseudoxanthomonas kaohsiungensis* (Chang *et al.*, 2005) has been reported as being able to reduce nitrate and *Pseudoxanthomonas spadix* (Young *et al.*, 2007) contains the fatty acid iso-$C_{13:0}$ 3-OH.

In the course of the screening of micro-organisms from contaminated soil samples, we isolated a novel bacterium, designated strain BD-c54^T, that represents a novel species of the genus *Pseudoxanthomonas*. Phylogenetic analysis based on 16S rRNA gene sequences showed that the isolate is closely related to *Pseudoxanthomonas yeongjuensis* GR12-1^T and [*Stenotrophomonas*] *dokdonensis* DS-16^T. Although [*S.*] *dokdonensis* DS-16^T can reduce nitrate, we found that it should be classified within the genus *Pseudoxanthomonas* on the basis of phylogenetic and phenotypic characteristics (for example, iso-$C_{13:0}$ 3-OH was not detected).

Strain BD-c54^T was isolated from BTEX (benzene, toluene, ethylbenzene and xylenes)-contaminated soil in Sacheon, Republic of Korea. The soil sample was contaminated to a level of about 1 g total hydrocarbons (kg soil)$^{-1}$. The soil sample was serially diluted with 1 % (w/v) saline solution, spread on R2A agar (Difco) and incubated at 25 °C for 5 days. Subcultivation was done on R2A agar at 30 °C for 3 days. Although strain BD-c54^T was isolated from BTEX-contaminated soil, it did not grow on minimal agar medium (Stanier *et al.*, 1966) containing BTEX as the sole carbon source, which means that the strain may not be a BTEX degrader.

Four type strains were used as reference strains for DNA–DNA hybridization and phenotypic characterization: [*S.*] *dokdonensis* KCTC 12543^T was purchased from the Korean Collection of Type Cultures (Daejeon, Korea), *P. yeongjuensis* KACC 11580^T, *Pseudoxanthomonas kalamensis* KACC 12354^T and *Pseudoxanthomonas broegbernensis* KACC 10898^T were purchased from the Korean Agricultural Culture Collection (Suwon, Korea) and *P. spadix* IMMIB AFH-5^T was a gift from Dr A. F. Yassin (Young *et al.*, 2007). Physiological characteristics of strain BD-c54^T were examined by growing the isolate on R2A agar at different temperatures (5–50 °C at 5 °C intervals) and in R2A broth adjusted to different pH values (pH 5.0–10.0 at 0.5 pH unit intervals). R2A medium with different pH was prepared as described by Gomori (1955). Gram staining was performed using a bioMérieux Gram stain kit according to the instructions of the manufacturer. Oxidase activity was tested by oxidation of 1 % (w/v) tetramethyl *p*-phenylenediamine (Merck) and catalase activity was evaluated by the production of oxygen bubbles in 3 % (v/v) aqueous hydrogen peroxide solution. Cell morphology and motility were studied using phase-contrast microscopy and transmission electron microscopy (JEM-1010; JEOL) as described by Jeon *et al.* (2004). Hydrolysis of casein, gelatin, Tweens 20 and 80, aesculin, urea, tyrosine and starch was investigated on R2A agar after 5 days of incubation according to previously described methods (Lányí, 1987; Smibert & Krieg, 1994). Nitrate reduction was assayed according to the method of Lányí (1987) and acid production from carbohydrates was tested as described by Leifson (1963). Additional enzyme activities and biochemical features were determined using API ZYM and API 20NE kits at 30 °C as recommended by the manufacturer (bioMérieux).

Growth of strain BD-c54^T was observed at 15–35 °C, with optimum growth at 25–30 °C, and at pH 6.0–9.5, with optimum growth at pH 7.0–8.0. All cells that were observed were non-motile rods, 0.3–0.5 μm wide and 0.8–1.6 μm long at 30 °C on R2A agar (see Supplementary Fig. S1 in IJSEM Online). Cells of strain BD-c54^T stained Gram-negative and were oxidase- and catalase-positive. Anaerobic growth was not observed after 15 days at 30 °C on R2A agar. The strain did not reduce nitrate to nitrite. Other phenotypic features of strain BD-c54^T, [*S.*] *dokdonensis* DS-16^T and other *Pseudoxanthomonas* type strains are presented in Table 1 and in the description of the novel species. Some features are in accordance with characteristics of members of the genus *Pseudoxanthomonas*, whereas others allow differentiation from closely related *Pseudoxanthomonas* species (Table 1).

For analysis of fatty acid methyl esters, cells of strain BD-c54^T were harvested from agar plates after incubation for 3 days on tryptic soy agar (TSA; Difco) at 30 °C. Analysis of fatty acid methyl esters was performed according to the instructions of the Microbial Identification System (MIDI Inc.). Analyses of polar lipids and isoprenoid quinones were carried out using the methods described by Komagata & Suzuki (1987) and Batrakov *et al.* (1997), respectively. The DNA G+C content of strain BD-c54^T was determined using HPLC with a reversed-phase column (GROM-SIL 100 ODS-2FE; GROM) according to the method of Tamaoka & Komagata (1984). The major cellular fatty acids of strain BD-c54^T were iso-$C_{15:0}$ (30.6 %), iso-$C_{17:1}\omega 9c$ (12.3 %), iso-$C_{11:0}$ 3-OH (11.2 %), iso-$C_{16:0}$ (9.5 %), iso-$C_{11:0}$ (9.4 %) and iso-$C_{17:0}$ (8.6 %), and 3-OH fatty acids were the major hydroxylated components; this fatty acid profile is similar to those of *Pseudoxanthomonas* species and [*S.*] *dokdonensis* DS-16^T (Table 2). The fatty acid iso-$C_{13:0}$ 3-OH was not detected from strain BD-c54^T, which is a general property of *Pseudoxanthomonas* species. The polar lipids of strain BD-c54^T were dominated by large amounts of phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol, but a small amount of an unknown amino-group-containing polar lipid (AL1) was also present (Supplementary Fig. S2). The major respiratory lipoquinone of strain BD-c54^T was ubiquinone-8 (Q-8). The G+C content of strain BD-c54^T was 67.5 mol%. The major fatty acids, major lipoquinone and G+C content are in accordance with those of members of the genus *Pseudoxanthomonas* (Finkmann *et al.*, 2000;

Table 1. Phenotypic characteristics of strain BD-c54^T, [*S.*] *dokdonensis* DS-16^T and type strains of some related *Pseudoxanthomonas* species

Strains: 1, strain BD-c54^T (data from this study); 2, *P. yeongjuensis* GR12-1^T (unless indicated, data from Yoo *et al.*, 2007); 3, [*S.*] *dokdonensis* DS-16^T (Yoon *et al.*, 2006); 4, *P. spadix* IMMIB AFH-5^T (Young *et al.*, 2007); 5, *P. kalamensis* JA40^T (Harada *et al.*, 2006); 6, *P. broegbernensis* B1616/1^T (Finkmann *et al.*, 2000). All strains were positive for catalase and aesculin hydrolysis and negative for starch hydrolysis, assimilation of D-mannitol and malate and growth at 42 °C.

Characteristic	1	2	3	4	5	6
Motility and flagellation	−	+	−	+	−	+
Urease*	+	−	−	−	−	−
Optimum temperature (°C)	25–30	28	30	37	30–37	30
Growth at 10 °C	−	+	+	−	+	+
Nitrate reduction	−	−	+	−	−	−
β-Galactosidase*	+	+	−	−	−	+
Hydrolysis of:*						
Gelatin	+	+	+	+	−	−
Tween 80	−	+	+	−	−	+
Casein	+	+	+	+	−	−
Assimilation of:*						
D-Glucose	+	+	+	−	+	+
L-Arabinose	−	−	−	−	+	+
D-Mannose	+	−	+	−	−	+
N-Acetylglucosamine	+	+	+	−	+	+
Maltose	+	+	+	−	+	+
Citrate	−	−	−	−	−	+
DNA G+C content (mol%)	67.5	63.4	65.1	68.5	64.0	66.5

*These results (and catalase activity, hydrolysis of aesculin and starch and assimilation of D-mannitol and malate) were obtained in this study with the strains indicated in the text.

Chen *et al.*, 2002; Thierry *et al.*, 2004; Chang *et al.*, 2005; Yang *et al.*, 2005; Harada *et al.*, 2006; Weon *et al.*, 2006; Yoo *et al.*, 2007; Young *et al.*, 2007). [*S.*] *dokdonensis* DS-16^T has similar chemotaxonomic properties to members of the genus *Pseudoxanthomonas*, but it can reduce nitrate to nitrite, in contrast to previously reported general properties of *Pseudoxanthomonas* species (Harada *et al.*, 2006; Thierry *et al.*, 2004; Yoon *et al.*, 2006; Young *et al.*, 2007).

Sequencing and assembly of the 16S rRNA gene of strain BD-c54^T were carried out as described previously (Lane, 1991). The resultant 16S rRNA gene sequence (1509 nt) was compared with available 16S rRNA gene sequences from GenBank using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/) to determine an approximate phylogenetic affiliation, and the sequence was aligned with those of closely related species using the CLUSTAL W software program (Thompson *et al.*, 1994). Phylogenetic trees were constructed using three methods, the neighbour-joining (NJ), maximum-likelihood (ML) and maximum-parsimony (MP) algorithms; these methods are available in PHYLIP software, version 3.6 (Felsenstein, 2002). Sequence similarities between the novel strain and other related strains were computed using the FASTA3 program in EBI (http://www.ebi.ac.uk/fasta33/nucleotide.html). Bootstrap analysis was performed according to the algorithm of Kimura's two-parameter model (Kimura, 1980) of the NJ method in the PHYLIP package. DNA–DNA hybridization was carried out to evaluate the genomic DNA relatedness between strain BD-c54^T and *Pseudoxanthomonas yeongjuensis* GR12-1^T using the fluorometric microplate method (Ezaki *et al.*, 1989). Fluorometric data recorded after 30 min incubation were used for the calculation of a DNA–DNA hybridization value. The DNA relatedness value is the mean of five values.

Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain BD-c54^T formed a tight phylogenetic lineage with *P. yeongjuensis* GR12-1^T with a 100 % bootstrap value within the genus *Pseudoxanthomonas* (Fig. 1). [*S.*] *dokdonensis* DS-16^T also formed a distinct phyletic lineage within the genus *Pseudoxanthomonas*. The overall tree topology of the NJ tree was supported by trees built using the ML and MP algorithms (Fig. 1). Comparative 16S rRNA gene sequence analysis showed that strain BD-c54^T was most closely related to *P. yeongjuensis* GR12-1^T and [*S.*] *dokdonensis* DS-16^T, with 98.3 and 96.6 % 16S rRNA gene sequence similarity, respectively. The level of DNA–DNA relatedness between strain BD-c54^T and *P. yeongjuensis* KACC 11580^T was 24.5 %, which is clearly below the 70 % threshold generally accepted for species delineation (Rosselló-Mora & Amann, 2001; Wayne *et al.*, 1987).

Table 2. Cellular fatty acid compositions of strain BD-c54^T, [*S.*] *dokdonensis* DS-16^T and type strains of some related *Pseudoxanthomonas* species grown on TSA

Strains: 1, strain BD-c54^T (data from this study); 2, *P. yeongjuensis* GR12-1^T (Yoo *et al.*, 2007); 3, [*S.*] *dokdonensis* DS-16^T (Yoon *et al.*, 2006); 4, *P. spadix* IMMIB AFH-5^T (Young *et al.*, 2007); 5, *P. kalamensis* JA40^T (Harada *et al.*, 2006); 6, *P. broegbernensis* B1616/1^T (Chen *et al.*, 2002). Data are expressed as percentages of total fatty acids. −, Not detected.

Fatty acid	1	2	3	4	5	6
Saturated						
$C_{10:0}$	0.2	–	–	–	0.5	–
$C_{14:0}$	0.2	–	–	3.4	0.6	12.0
$C_{15:0}$	–	–	–	–	0.2	–
$C_{16:0}$	1.5	2.3	1.8	16.5	2.9	6.9
$C_{17:0}$	–	–	–	0.2	0.4	1.5
$C_{17:0}$ cyclo	0.2	–	–	2.8	0.2	1.1
Branched						
iso-$C_{10:0}$	0.2	–	–	–	–	–
iso-$C_{11:0}$	9.4	5.5	5.6	1.3	6.9	1.4
iso-$C_{12:0}$	0.2	–	–	–	–	–
iso-$C_{13:0}$	0.4	–	–	0.5	–	–
anteiso-$C_{13:0}$	–	–	–	0.5	–	–
iso-$C_{14:0}$	1.6	1.4	2.1	1.0	0.6	–
iso-$C_{15:0}$	30.6	32.0	19.6	16.1	40.0	32.4
iso-$C_{15:1}$ AT5	1.3	1.0	–	–	–	–
anteiso-$C_{15:0}$	–	4.4	3.4	1.2	4.6	31.8
iso-$C_{16:0}$	9.5	13.3	27.6	6.4	6.9	–
iso-$C_{17:0}$	8.6	6.4	–	16.1	6.7	–
iso-$C_{17:1}\omega 7c$	–	–	–	–	9.0	5.5
iso-$C_{17:1}\omega 8c$	–	–	–	0.9	–	–
iso-$C_{17:1}\omega 9c$	12.3	14.6	11.9	2.4	–	–
anteiso-$C_{17:0}$	–	1.5	1.5	23.6	1.8	–
anteiso-$C_{17:1}$	1.3	–	–	–	–	–
anteiso-$C_{17:1}\omega 9c$	4.4	–	–	–	–	–
10-Methyl $C_{17:0}$	0.3	–	–	–	–	–
iso-$C_{18:0}$	0.2	–	–	–	–	–
Unsaturated						
$C_{15:1}\omega 9c$	–	–	–	–	0.3	–
$C_{16:1}\omega 5c$	–	–	–	–	1.2	–
$C_{16:1}\omega 7c$	–	–	–	–	–	1.0
$C_{16:1}\omega 7c$ alcohol	2.4	1.6	1.0	–	–	–
$C_{16:1}\omega 9c$ alcohol	–	–	–	–	1.9	–
$C_{16:1}\omega 11c$	0.4	–	–	–	–	–
$C_{17:1}\omega 8c$	–	–	0.9	–	–	–
$C_{18:1}\omega 7c$	0.4	–	1.5	0.4	–	–
$C_{18:1}\omega 9c$	0.1	–	–	–	–	–
Hydroxylated						
$C_{11:0}$ 2-OH	0.3	–	–	–	–	–
$C_{10:0}$ 3-OH	–	–	–	–	0.2	–
$C_{13:0}$ 2-OH	–	–	–	–	0.1	–
iso-$C_{11:0}$ 3-OH	11.2	7.7	4.1	0.4	8.3	1.0
iso-$C_{12:0}$ 3-OH	0.3	–	2.8	–	0.4	–
iso-$C_{13:0}$ 3-OH	–	–	–	0.6	–	–
iso-$C_{14:0}$ 3-OH	0.3	–	–	–	–	–
Summed features*						
3	1.4	4.0	7.8	4.0	–	–
4	0.6	–	–	–	–	–

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contains $C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH. Summed feature 4 contains iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B.

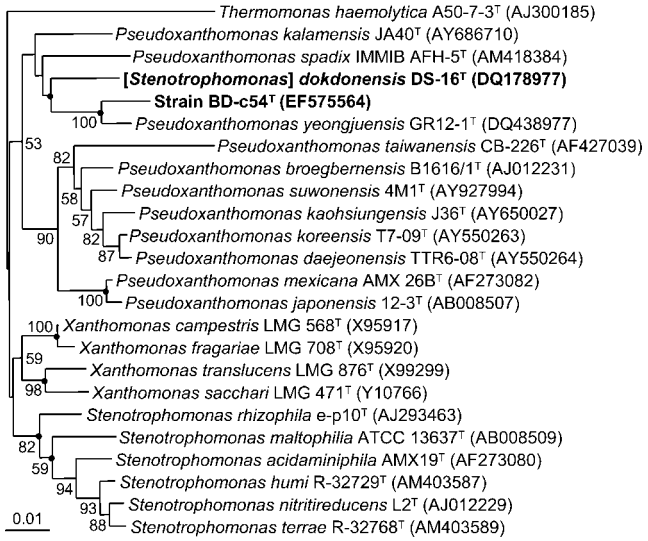

Fig. 1. NJ tree based on 16S rRNA gene sequences showing the phylogenetic relationships of strain BD-c54^T, [*S.*] *dokdonensis* DS-16^T and related taxa. Bootstrap values are shown as percentages of 1000 replicates when greater than 50 %. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the ML and MP algorithms. *Thermomonas haemolytica* A50-7-3^T was used as an outgroup. Bar, 0.01 changes per nucleotide position.

The chemotaxonomic and molecular characteristics described here show that strain BD-c54^T and [*S.*] *dokdonensis* DS-16^T are members of the genus *Pseudoxanthomonas* distinct from known species. We therefore propose the transfer of *Stenotrophomonas dokdonensis* to the genus *Pseudoxanthomonas* as *Pseudoxanthomonas dokdonensis* comb. nov. In addition, strain BD-c54^T represents a novel species in the genus *Pseudoxanthomonas*, for which the name *Pseudoxanthomonas sacheonensis* sp. nov. is proposed.

Description of *Pseudoxanthomonas sacheonensis* sp. nov.

Pseudoxanthomonas sacheonensis (sa.che.on.en′sis. N.L. fem. adj. *sacheonensis* pertaining to Sacheon, Korea, the location of the soil sample from which the type strain was isolated).

Colonies are yellow, opaque, circular and convex with entire margins after 3 days on R2A agar. Cells are strictly aerobic, Gram-negative, short, non-motile rods, 0.3–0.5 μm wide and 0.8–1.6 μm long. Optimal growth occurs at 25–30 °C and pH 7.0–8.0. Catalase- and oxidase-positive. Nitrate is not reduced to nitrite. Hydrolyses aesculin, urea, tyrosine, casein and gelatin, but not Tweens 20 or 80 or starch. Produces acids from raffinose, lactose, L-arabinose, melibiose, D-fructose, D-mannose, D-mannitol, arbutin and salicin, but not from *myo*-inositol or D-galactose. Positive for glucose fermentation, but negative for indole production and arginine dihydrolase activity. Positive for assimilation of D-glucose, D-mannose, *N*-acetylglucosamine and maltose, but negative for assimilation of L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid (API 20NE). Produces alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase and *N*-acetyl-β-glucosaminidase, but not α-galactosidase or β-glucuronidase. Weak activities are observed for lipase (C14), valine arylamidase, cystine arylamidase, α-mannosidase and α-fucosidase. Contains large amounts of phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol and a small amount of an unknown amino-group-containing polar lipid. The major isoprenoid quinone is Q-8. The major cellular fatty acids are iso-$C_{15:0}$, iso-$C_{17:1}\omega9c$, iso-$C_{11:0}$ 3-OH, iso-$C_{16:0}$, iso-$C_{11:0}$ and iso-$C_{17:0}$. The DNA G+C content of the type strain is 67.5 mol% (HPLC).

The type strain is BD-c54^T (=KCTC 22080^T =DSM 19373^T), isolated from BTEX-contaminated soil in Sacheon, Republic of Korea.

Description of *Pseudoxanthomonas dokdonensis* (Yoon *et al.* 2006) comb. nov.

Pseudoxanthomonas dokdonensis (dok.do.nen′sis. N.L. fem. adj. *dokdonensis* of Dokdo, from where the type strain was isolated).

Basonym: *Stenotrophomonas dokdonensis* Yoon *et al.* 2006.

The description is as given by Yoon *et al.* (2006). The type strain is DS-16^T (=KCTC 12543^T =CIP 108839^T).

Emended description of the genus *Pseudoxanthomonas* Finkmann *et al.* 2000 emend. Thierry *et al.* 2004

The description of the genus *Pseudoxanthomonas* is as given by Finkmann *et al.* (2000) and emended by Thierry *et al.* (2004) with the following changes. Type strains of all species except *Pseudoxanthomonas kaohsiungensis* and *Pseudoxanthomonas dokdonensis* have no nitrate reduction ability. The fatty acid iso-$C_{13:0}$ 3-OH is detected only from *Pseudoxanthomonas spadix* and 3-OH fatty acids are the major hydroxylated components of the fatty acid profile.

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