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TAXONOMIC DESCRIPTION

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Sphingobium estronivorans sp. nov. and *Sphingobium bisphenolivorans* sp. nov., isolated from a wastewater treatment plant

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ABSTRACT

Two Gram-stain-negative, aerobic, motile and rod-shaped bacteria, one designated as strain AXB^T, capable of degrading estrogens, and another, YL23^T, capable of degrading estrogen and bisphenol A, were isolated from activated sludge in Xiamen City, PR China. The optimum temperature and pH of both strains were 25–35 °C and pH 7.0–8.0. While strain AXB^T could tolerate 3% (w/v) NaCl, YL23^T could only grow between 0–1 % (w/v) NaCl. They contained ubiquinone-10 as the major quinone, spermidine as the major polyamine, summed feature 8 (comprising $C_{18:1}\omega 6c$ and/or $C_{18:1}\omega 7c$) as the major fatty acids and diphosphatidylglycerol, phosphatidylcholine, phosphatidyldimethylethanolamine, phosphatidylethanolamine, phosphatidylglycerol and sphingoglycolipid as the major polar lipids. The DNA G+C contents of strains AXB^T and YL23^T were 63.6 and 63.7 mol%, respectively. Based on the results of 16S rRNA gene sequence analysis, strains AXB^T and YL23^T belonged to the genus *Sphingobium*. Strain AXB^T was most closely related to *Sphingobium chlorophenolicum* NBRC 16172^T (97.5%) and *Sphingobium chungbukense* DJ77^T (97.2%), and strain YL23^T was most closely related to *S. chlorophenolicum* NBRC 16172^T (97.4%) and *S. quisquiliarum* P25^T (97.1%). Average nucleotide identity values between these two strains and *S. chlorophenolicum* NBRC 16172^T, *S. chungbukense* DJ77^T, *Sphingobium chinhatense* IP26^T, *Sphingobium quisquiliarum* P25^T and *Sphingobium japonicum* UT26S^T were from 80.7 to 85.8%. In conclusion, strains AXB^T and YL23^T represent novel species of the genus *Sphingobium*, for which the names *Sphingobium estronivorans* sp. nov. and *Sphingobium bisphenolivorans* sp. nov. are proposed, respectively. The type strains of *S. estronivorans* and *S. bisphenolivorans* are AXB^T (=MCCC 1K01232^T=DSM 102173^T) and YL23^T (=MCCC 1K02300^T=DSM 102172^T), respectively.

The genus *Sphingobium* (family *Sphingomonadaceae*, order *Sphingomonadales*, class *Alphaproteobacteria*, phylum *Proteobacteria*) was first proposed by Takeuchi *et al.* [1] with *Sphingobium yanoikuyae* as the type species (original name *Sphingomonas yanoikuyae* [2]) and at the time of writing contained 50 species with validly published names (www.bacterio.net/sphingobium.html and www.ezbiocloud.net/identify). Members of this genus share many chemotaxonomic and phenotypic characteristics, such as staining behaviour, mesophilic, strictly aerobic and chemoorganotrophic, having $C_{18:1}\omega 6c$ and/or $C_{18:1}\omega 7c$ as major fatty acids, containing ubiquinone Q-10 as the main respiratory quinone and spermidine as the major polyamine [1–3]. In addition, species in this genus were mostly isolated from contaminated soil and wastewater, known for their roles in bioremediation and biodegradation of pollutants, such as carbaryl, hexachlorocyclohexane, pyrethroid and other xenobiotic compounds [3–6].

In this report, we describe two estrogen-degrading strains, AXB^T and YL23^T, which belong to genus *Sphingobium* by determining their phenotypic properties and taxonomically characterizing them based on their 16S rRNA gene and genome sequences. This is also the first report of species in this genus capable of degradation of estrogens.

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Abbreviations: ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridization; E2, 17β-estradiol; WWTP, wastewater treatment plant.

The GenBank/EMBL/DDBJ accession numbers for the genome and 16S rRNA sequences of strain AXB^T are LFCT01000000 and KM925003.
The GenBank/EMBL/DDBJ accession numbers for the genome and 16S rRNA sequences of strain YL23^T are ASTG01000000 and KU997640.
Four supplementary figures and one supplementary table are available with the online version of this article.

ISOLATION AND ECOLOGY

Strains AXB^T and YL23^T were isolated from activated sludge, and samples were collected from the oxidation ditch of a wastewater treatment plant (WWTP) in Xiamen, PR China to study estrogen-degrading bacteria. After sampling, the sludge was enriched with 17β-estradiol (E2; 2 mg l^{-1}) as the only carbon and energy source in ammonia mineral salts medium (2%, w/v; see supplementary information for detailed composition) [7] in a 250 ml sterile flask and incubated in a rotary shaker at 150 r.p.m. at 30 °C. After 3 days of cultivation, we transferred the enriched sample to fresh ammonia mineral salts medium with E2 (2 mg l^{-1}) and repeated this procedure five times. Strains were isolated by directly plating the serially diluted enrichment culture on DSMZ medium 830 [R2A agar; containing 0.05% (w/v) yeast extract, 0.05% peptone, 0.05% casamino acids, 0.05% glucose, 0.05% starch, 0.03% sodium pyruvate,1.5% agar; pH 7.2) and incubated at 30 °C for a week. After numerous single-colony picks and transfers, two strains designated as AXB^T and YL23^T were isolated and found to be able to degrade both E2 and estrone [7]. In addition, YL23^T also could degrade bisphenol A [8]. For long-term maintenance, they were preserved at −70 °C in R2A broth supplemented with 20% (v/v) glycerol [9] and routinely cultured by streaking on R2A agar and incubating at 30 °C for 48 h before use.

16S rRNA GENE PHYLOGENY

Genomic DNA of strains AXB^T and YL23^T was extracted and purified using TIANamp Bacteria DNA kit (Tiangen Biotech Co.), according to the manufacturer's instructions, from cells grown in R2A broth for 48 h at 30 °C. The 16S rRNA gene sequences were amplified by PCR using primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). Two nearly full-length 16S rRNA gene sequences of strains AXB^T and YL23^T were determined and confirmed by the genome sequences described below. These sequences were compared with all reported type strains using the Nucleotide Similarity Search program in the EzTaxon-e server (www.ezbiocloud.net/identify) [10]. Results indicated that strain AXB^T shared the highest sequence similarity with *Sphingobium chlorophenolicum* NBRC 16172^T (ex *Sphingomonas flava* DS2^T, 97.5%) [11], *Sphingobium chungbukense* DJ77^T (97.2%), *Sphingobium japonicum* UT26S^T (97.0%), *Sphingobium chinhatense* IP26^T (96.9%), and strain YL23^T shared the highest sequence similarity with *S. chlorophenolicum* NBRC 16172^T (97.4%), *Sphingobium quisquiliarum* P25^T (97.1%), *S. japonicum* UT26S^T (97.0%) and *S. chinhatense* IP26^T (97.0%). Those closely related 16S rRNA gene sequences were retrieved from the EzBioCloud server and aligned using the CLUSTAL X (2.1) program. Phylogenetic analysis was performed with the MEGA 7 software [12] by using the Kimura two-parameter model and neighbour-joining [13] (Fig. 1), maximum-likelihood [14] and minimum-evolution methods [15] (Figs S1 and S2, available in the online version of this article) with bootstrap values based on 1000 replications.

GENOME FEATURES

The draft genome of AXB^T was determined by using an Illumina Hiseq 2000 instrument with 300 bp paired-end reads. A total of 4.51 Mb high-quality sequencing data were obtained, having approximately 210-fold coverage assembled *in silico* using SOAPdenovo version 2.04 [16], resulting in 79 contigs (>1000 bp) with an N50 length of 149156 bp. The whole genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number LFCT00000000. The version described in this paper is version LFCT01000000. The whole genome shotgun project of strain YL23^T was deposited at DDBJ/EMBL/GenBank under the accession number ASTG00000000 [8]. The average nucleotide identity (ANI) was calculated at the website www.ezbiocloud.net/tools/ani according to the method described previously [17]. Digital DNA–DNA hybridization (dDDH) values between strains AXB^T and YL23^T and the reference strains were calculated using the Genome-to-Genome Distance Calculator (GGDC) 2.0 server (http://ggdc.dsmz.de/distcalc.php) by means of genome-to-genome sequence comparison [18]. Based on the 16S rRNA gene sequences similarities of strains AXB^T and YL23^T and the phylogenetic analysis, *S. chlorophenolicum* NBRC 16172^T, *S. chungbukense* DJ77^T, *S. japonicum* UT26S^T and *S. chinhatense* IP26^T were selected as reference strains for physiological tests and chemotaxonomic analysis, and *S. chlorophenolicum* NBRC 16172^T, *S. chungbukense* DJ77^T, *S. chinhatense* IP26^T, *S. quisquiliarum* P25^T and *S. japonicum* UT26S^T were selected as reference strains for ANI and dDDH analysis because the genome of *S. japonicum* UT26S^T was not available.

PHYSIOLOGY AND CHEMOTAXONOMY

For cell size, motility and morphology studies, exponential phase cells of strains AXB^T and YL23^T were harvested after growth on R2A agar for 48 h at 30 °C and observed by transmission electron microscopy (Carl Zeiss) at an acceleration voltage of 80 kV. Gram-staining was determined using the bioMérieux kit according to the manufacturer's instructions [19]. Catalase and oxidase activities were tested according to Cowan and Steel [20]. The temperature range for growth was determined at 4, 15, 25, 30, 35, 40 and 45 °C in R2A broth medium. The pH range was assessed in R2A broth with the pH adjusted to pH 4.5–10.0 (at intervals of 0.5 pH unit) prior to sterilization using citrate/Na_2HPO_4 buffer (for pH range 4.5–8.0) or $NaHCO_3$/Na_2CO_3 buffer (for pH range 9.0–10.0) and pH checked again before inoculation. Tolerance to various NaCl concentrations was also tested in R2A broth supplemented with 0–9% (w/v) NaCl (at 1% unit intervals). Anaerobic growth was tested on R2A agar at 30°C for 3 weeks using a GasPak Plus system (BBL) in an anaerobic operation chamber. Hydrolysis of starch was tested as described previously, and Tween 40, Tween 80 and aesculin hydrolysis tests were conducted according to Tindall and Smibert [21, 22]. Other biochemical and physiological characteristics and enzyme activities were assessed using API ZYM and API 20NE strips (bioMérieux) and the Biolog GN2 MicroPlate according to the manufacturers' instructions.

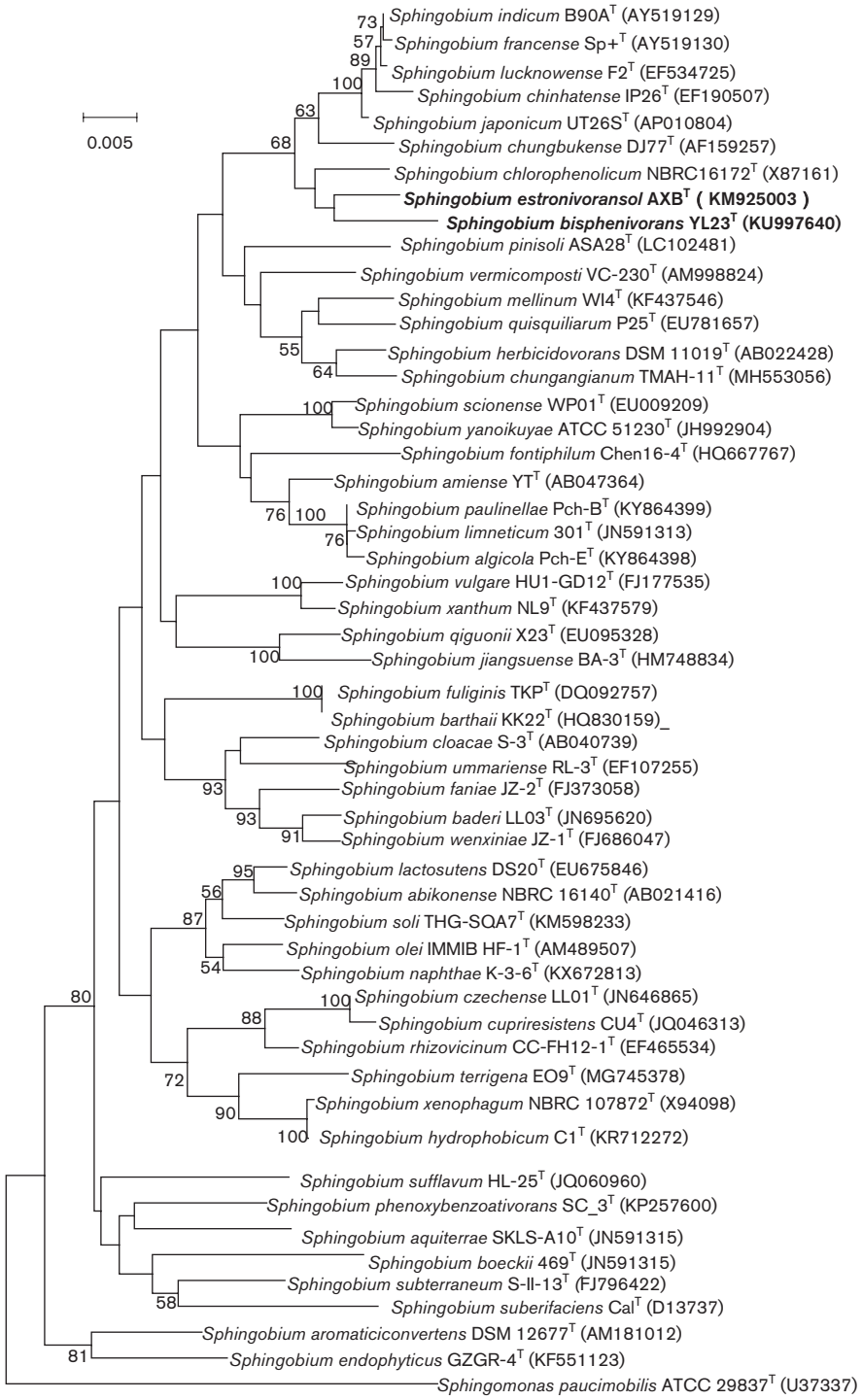

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strain AXB^T and YL23 ^{T} and other related taxa based on 16S rRNA gene sequences. Bootstrap values (expressed as percentages (%) of 1000 replications) above 50% are shown at branch points. Bar, 0.005 nucleotide substitution rate (K_{nuc}) units. Thicker branches indicate that the corresponding branches were also recovered in the trees reconstructed by the maximum-likelihood and minimum-evolution algorithms. *Sphingomonas paucimobilis* ATCC 29837^T (U37337) was used as the outgroup.

For fatty acid analysis, cells of strains AXB^T and YL23^T were cultured on R2A agar medium for 48 h (exponential phase) at 30 °C. Then the fatty acids were extracted and prepared according to the standard protocol of the MIDI Microbial Identification System and determined by gas chromatograph (6850, Agilent), and peaks were identified using MIDI software (version 6.0) [23]. Polar lipids were separated using a chloroform/methanol system and analysed by two-dimensional TLC using Merck silica gel 60 F254 aluminum-backed thin-layer plates [24]. The first solvent was chloroform–methanol–water (65:25:4, by vol.), and the second solvent was chloroform–methanol–acetic acid–water (85:12:15:4, by vol.). We used four plates for TLC simultaneously, and four kinds of spray reagents were used to detect the corresponding lipids including molybdophosphoric acid for total lipids, ninhydrin reagent for aminolipids, molybdenum blue for phosphorus-containing lipids and α-naphthol reagent for glycolipids. The major respiratory quinones were extracted from cells of strains AXB^T and YL23^T with chloroform/methanol (2:1, by vol) and, after being dried at 40 °C, the extracts were resuspended in chloroform/methanol (2:1, by vol), purified by TLC on GF254 silica gel plates (Qingdao Haiyang) with methylbenzene, and analysed by HPLC–mass spectrometry (ABI 6500Q TRAP, SCIEX) with methyl alcohol:isopropyl alcohol (2:1, by vol) as the mobile phase [25]. Polyamines of both strains were analysed by HPLC after extraction [26, 27].

Cells of AXB^T and YL23^T were Gram-stain-negative and motile short rods with one or two polar flagella (Fig. S3). Cells of strain AXB^T were approximately 0.4–0.5 µm wide and 0.8–1.0 µm long, and cells of strain YL23^T were 0.3–0.4 µm wide and 0.8–1.0 µm long. Both strains formed smooth colonies with regular edges and 2–3 mm in diameter after 48 h incubation on R2A agar medium at 30 °C. However, the colony of strain AXB^T was off-white while that of YL23^T was yellow. Growth of both strains was observed at 15–40 °C (optimum, 25–35 °C) and at pH 5.0–9.0 (optimum, pH 7.0–8.0). Strain AXB^T could tolerate 3% (w/v) NaCl, while YL23^T was only able to grow at between 0–1% (w/v) NaCl. Strain AXB^T was positive for oxidase and catalase activity, and strain YL23^T was positive for oxidase activity and weakly positive for catalase activity. Both strains were unable to grow on R2A agar after incubation at 30 °C in anaerobic conditions. In addition, both strains could not reduce nitrate to nitrite or nitrite to nitrogen, which was similar to the reference strains [1, 28, 29]. Strains AXB^T and YL23^T could hydrolyse starch and Tween 80. Cells of strain AXB^T were positive for fermentation of glucose, urea, alkaline phosphatase, acid phosphatase and naphthol-AS-BI-phosphohydrolase in the API ZYM test kit, assimilating capric acid in the API 20NE test kit, and utilizing dextrin, Tween 40, D-fructose, α-D-glucose, D-psicose, methyl pyruvate, β-hydroxy butyric acid, L-glutamic acid, glycyl-L-glutamic acid, L-proline and D-serine in the Biolog GN2 MicroPlate. Cells of strain YL23^T were positive for alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase in the API ZYM test kit, assimilating D-glucose and L-arabinose in the API 20NE test kit, and utilizing dextrin, D-galactose, α-D-glucose, methyl pyruvate, β-hydroxy butyric acid, α-keto butyric acid, L-alaninamide, D-alanine, L-alanine, L-alanyl-glycine and L-glutamic acid in the Biolog GN2 MicroPlate. Phenotypic characteristics allowed the differentiation of strains AXB^T and YL23^T from the closely related species in the genus *Sphingobium* (Table 1) and are presented in the species description.

The major fatty acids (>5%) detected in both strains AXB^T and YL23^T were summed feature 8 (comprising $C_{18:1}\omega6c$ and/or $C_{18:1}\omega7c$), summed feature 3 (comprising $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$), $C_{16:0}$, $C_{14:0}$2-OH and $C_{18:1}\omega7c$ 11-methyl (Table 2). The overall fatty acid features of both strains were similar to the most closely related type strains except for some differences in the respective compositions of some fatty acid components. The major polyamine in both strains was spermidine, which was the same in the related type strains [1, 27, 30]. The predominant respiratory quinone detected in both strains was ubiquinone-10 (Q-10), which was in line with all members of the family *Sphingomonadaceae* [29]. Diphosphatidylglycerol, phosphatidylcholine, phosphatidyldimethylethanolamine, phosphatidylethanolamine, phosphatidylglycerol and sphingoglycolipid were identified as the major polar lipids in both strains. Three unidentified phospholipids were only detected as minor polar lipids in strain AXB^T, while in strain YL23^T, five unknown lipids were detected as minor polar lipids (Fig. S4). The absence of phosphatidylmonomethylethanolamine could be used to differentiate strains AXB^T and YL23^T from the most closely related strains *S. chungbukense* DJ77^T [30], *S. japonicum* UT26S^T [30] and *S. chinhatense* IP26^T [30]. The DNA G+C contents of strains AXB^T and YL23^T were 63.6 and 63.7 mol%, respectively, which were in the range of DNA G+C contents in *Sphingobium* species [31, 32]. ANI analysis showed that strains AXB^T and YL23^T shared a lower degree of similarity ranging from 80.7 to 85.8% with the three related strains (Table S1), indicating a lower taxonomic relatedness with other type strains in the genus *Sphingobium*. In addition, the ANI value between strains AXB^T and YL23^T was 81.9%, also much lower than the 94% considered as the borderline between the same species [17, 33]. The dDDH value between strains AXB^T and YL23^T was 26.9±1.8%. The dDDH values of strains AXB^T and YL23^T with other related strains were from 24.5±0.5 to 32.3±1.0% (Table S1), which were clearly below the 70% threshold generally considered for species delineation [34]. From the phylogenetic, physiological and chemotaxonomic features, strains AXB^T and YL23^T could be assigned to different novel species of the genus *Sphingobium*, for which the names *Sphingobium estronivorans* sp. nov. and *Sphingobium bisphenolivorans* sp. nov. are proposed, respectively.

DESCRIPTION OF *SPHINGOBIUM ESTRONIVORANS* SP. NOV.

Sphingobium estronivorans (es.tro.ni.vo′rans. N.L. neut. n. *estronum* estrone; L. pres. part. *vorans* eating, devouring; N.L. part. adj. *estronivorans* estrone-eating).

Table 1. Characteristics differentiating strains AXB^T and YL23^T from their closely related *Sphingobium* strains

Strains: 1, AXB^T; 2, YL23^T; 3, *Sphingobium chlorophenolicum* NBRC 16172^T; 4, *Sphingobium chungbukense* DJ77^T; 5, *Sphingobium japonicum* UT26S^T; 6, *Sphingobium chinhatense* IP26^T. These analyses were conducted under the same conditions in this study. All strains were negative for indole prodution, L-arginine, gelatin, D-mannose, D-mannitol, *N*-acetyl-glucosamine, maltose, potassium gluconate, adipic acid, trisodium citrate nd phenylacetic acid in the API 20 NE test. All strains were positive for alkaline phosphatase, acid phosphatase and naphthol-AS-BI-phosphohydrolase, negative for valine arylamidase, *β*-galactosidase, *β*-glucuronidase, *N*-acetyl-*β*-glucosaminidase, α-mannosidase and α-fucosidase. +, Positive; –, negative; w, weakly positive.

Characteristics	1	2	3	4	5	6
Colony colour	Off-white	Yellow	Yellow	Yellow	Yellow	Yellow
Oxidase activity	+	+	+	+	+	+
Catalase activity	+	w	+	+	+	+
Reduction of nitrate to nitrite	–	–	–	–	–	–
Hydrolysis of:						
Starch	+	+	–	–	+	–
Tween 80	+	+	–	+	+	–
API 20NE results:						
Urea	+	–	–	–	–	–
Aesculin ferric citrate	–	–	+	+	+	–
β-Galactosidase	–	–	+	+	+	–
D-glucose	–	+	+	–	–	+
L-Arabinose	–	+	–	+	+	+
Capric acid	+	–	+	–	–	–
Malic acid	–	–	–	+	–	+
API ZYM results:						
Esterase (C4)	–	+	+	+	+	+
Esterase lipase (C8)	–	+	+	+	+	+
Lipase (C14)	w	–	+	–	–	–
Leucine arylamidase	+	+	w	w	+	–
Cystine arylamidase	–	w	w	w	w	–
Trypsin	–	w	w	w	w	–
α-Chymotrypsin	–	w	w	w	w	–
α-Galactosidase	–	–	–	+	–	–
α-Glucosidase	–	–	+	+	+	+
β-Glucosidase	–	–	+	+	+	–

Cells are Gram-stain-negative, aerobic, rod-shaped (0.4–0.5 µm wide and 0.8–1.0 µm long), motile, oxidase- and catalase-positive, estrone- and 17*β*-estradiol -degrading. Colonies on R2A agar are off-white, smooth with regular edges and 2–3 mm in diameter after 48 h incubation at 30 °C. Growth occurs at 15–40 °C (optimum, 25–35 °C), at pH 5.0–9.0 (optimum, pH 7.0–8.0) and in 0–3.0% (w/v) NaCl (optimum, 0–1.0%). Starch and Tween 80 are hydrolysed, but Tween 40 and aesculin are not. It neither reduces nitrate nor produce indole. Cells are positive for fermentation of glucose and urea. Capric acid is assimilated in the API 20NE test and positive results are obtained for alkaline phosphatase, acid phosphatase and naphthol-AS-BI-phosphohydrolase in API ZYM tests, negative for other enzymes and substrates in the two tests. Cells can utilize dextrin, Tween 40, D-fructose, *α*-D-glucose, D-psicose, methyl pyruvate, *β*-hydroxy butyric acid, L-glutamic acid, glycyl-L-glutamic acid, L-proline and D-serine in Biolog GN2 MicroPlate assays. Major fatty acids are summed feature 8 (comprising $C_{18:1}\omega6c$ and/or $C_{18:1}\omega7c$), summed feature 3 (comprising $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$),

Table 2. Cellular fatty acid compositions (%) of strains AXB^T and YL23^T and other closely related type strains of the genus *Sphingobium*

Strains: 1, AXB^T; 2, YL23^T; 3, *Sphingobium chlorophenolicum* NBRC 16172^T; 4, *Sphingobium chungbukense* DJ77^T; 5, *Sphingobium japonicum* UT26S^T; 6, *Sphingobium chinhatense* IP26^T. All data were from this study. Values are percentages of the total fatty acids. Fatty acids amounting to less than 0.5% in all strains is not shown. –, Not detected or <0.5%.

Fatty acid	1	2	3	4	5	6
Saturated:						
$C_{16:00}$	11.8	12.0	12.0	15.5	7.8	13.7
$C_{18:00}$	1.1	–	1.1	1.1	–	1.8
$C_{20:00}$	–	–	–	0.8	–	–
Unsaturated:						
$C_{16:1}\omega5c$	0.6	0.6	1.1	1.1	–	1.8
$C_{18:1}\omega9c$	0.8	–	0.6	0.6	–	–
$C_{18:1}\omega5c$	1.5	2.0	1.8	1.4	1.6	2.8
$C_{18:1}\omega7c$ 11-methyl	5.7	5.1	3.4	2.4	1.4	4.6
$C_{19:0}$ cyclo $\omega8c$	0.8	–	–		–	–
$C_{20:4}\omega6,9,12,15c$	–	–	0.6		–	–
Hydroxyl:						
$C_{14:0}$ 2OH	8.1	8.2	11.5	7.1	7.7	8.8
$C_{16:0}$ 2OH	–	0.6	–	0.7	–	–
Summed features:*						
3	9.1	7.2	10.0	6.3	9.8	8.6
8	58.7	60.3	56.0	62.0	60.7	55.3

*Summed features represent groups of one or more fatty acids that cannot be separated. Summed feature 3, $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$; summed feature 8, $C_{18:1}\omega6c$ and/or $C_{18:1}\omega7c$.

$C_{16:0}$, $C_{14:0}$2-OH and $C_{18:1}\omega7c$ 11-methyl. Major polar lipids are diphosphatidylglycerol, phosphatidylcholine, phosphatidyldimethylethanolamine, phosphatidylethanolamine, phosphatidylglycerol, sphingoglycolipid and three unidentified phospholipid (PL1, PL2, PL3). The major respiratory quinone and polyamine are Q-10 and spermidine, respectively.

The type strain is AXB^T (=MCCC 1K01232^T=DSM 102173 ^{T}), isolated from the oxidation ditch of a WWTP in Xiamen, PR China. The DNA G+C content of the type strain is 63.6 mol%. The GenBank/EMBL/DDBJ accession numbers for the genome and 16S rRNA gene sequences of strain AXB ^{T} are LFCT01000000 and KM925003.

DESCRIPTION OF *SPHINGOBIUM BISPHENOLIVORANS* SP. NOV.

Sphingobium bisphenolivorans (bis.phe.no.li.vo′rans N.L. neut. n. *bisphenol* bisphenol; L. pres. part. *vorans* eating, devouring; N.L. part. adj. *bisphenivorans* bisphenol A-eating).

Cells are Gram-stain-negative, aerobic, rod-shaped (0.3–0.4 µm wide and 0.8–1.0 µm long), motile, oxidase-positive and weakly positive for catalase, estrone, 17β-estradiol and BPA-degradation. Colonies on R2A agar are yellow, smooth with regular edges and 2–3 mm in diameter after 48 h incubation at 30 °C. Growth occurs at 15–40 °C (optimum, 25–35 °C), pH 5.0–9.0 (optimum, pH 7.0–8.0) and 0–1.0% (w/v) NaCl. Starch and Tween 80 are hydrolysed, but Tween 40 and aesculin are not. It does not reduce nitrate or produce indole. Cells are negative for all the reactions and assimilates D-glucose and L-arabinose in the API 20NE test, positive for alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase in the API ZYM test. Cells can utilize dextrin, D-galactose, α-D-glucose, methyl pyruvate, β-hydroxy butyric acid, α-keto butyric acid, L-alaninamide, D-alanine, L-alanine, L-alanyl-glycine and L-glutamic acid in the Biolog GN2 MicroPlate. Major fatty acids are summed feature 8 (comprising $C_{18:1}\omega6c$ and/or $C_{18:1}\omega7c$), summed feature 3 (comprising $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$), $C_{16:0}$, $C_{14:0}$2-OH and $C_{18:1}\omega7c$ 11-methyl. Major polar lipids are diphosphatidylglycerol, phosphatidylcholine, phosphatidyldimethylethanolamine, phosphatidylethanolamine, phosphatidylglycerol, sphingoglycolipid and five unknown lipids (L1-L5). The major respiratory quinone and polyamine are Q-10 and spermidine, respectively.

The type strain is YL23^{T} (=MCCC 1K02300^{T}=DSM 102172 ^{T}), isolated from the oxidation ditch of a WWTP in Xiamen, PR China. The DNA G+C content of the type strain is 63.7 mol%. The GenBank/EMBL/DDBJ accession numbers for the genome and 16S rRNA sequences of strain YL23 ^{T} are ASTG01000000 and KU997640.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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