Full Paper

Pseudomonas japonica sp. nov., a novel species that assimilates straight chain alkylphenols

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A bacterial strain, WL^T, which was isolated from an activated sludge, was able to degrade alkylphenols. 16S rDNA sequence analysis indicated that strain WL^T belonged to the genus *Pseudomonas* (sensu stricto) and formed a monophyletic clade with the type strain of *Pseudomonas graminis* and other members in the *Pseudomonas putida* subcluster with sequence similarity values higher than 97%. Genomic relatedness based on DNA-DNA hybridization of strain WL^T to these strains is 2–41%. Strain WL^T contained ubiquinone-9 as the main respiratory quinone, and the G+C content of DNA was 66 mol%. The organism contained hexadecanoic acid (16:0), hexadecenoic acid (16:1) and octadecenoic acid (18:1) as major cellular fatty acids. The hydroxy fatty acids detected were 3-hydroxydecanoic acid (3-OH 10:0), 3-hydroxydecanoic acid (3-OH 12:0) and 2-hydroxydodecanoic acid (2-OH 12:0). These results, as well as physiological and biochemical characteristics clearly indicate that the strain WL^T represents a new *Pseudomonas* species, for which the name *Pseudomonas japonica* is proposed. The type strain is strain WL^T (=IAM 15071^T=TISTR 1526^T).

Key Words—activated sludge; alkylphenols degrading bacteria; endocrine disrupter chemicals; *Pseudomonas*; *Pseudomonas japonica* sp. nov.

Introduction

Microorganisms that are isolated on the basis of their ability to degrade natural or man-made organic

compounds very often belong to the genus *Pseudomonas*. Many recalcitrant chemicals can be degraded by various *Pseudomonas* species or related bacteria isolated from nature. Because of the inherent capacity of *Pseudomonas* to catabolize or break down various xenobiotics, many new species with novel catabolic enzymes for a large number of chemicals have been isolated (Gibson and Subramanian, 1984). Alkylphenols (APs) represent a class of metabolic derivatives that are produced during the biodegradation of widely used non-ionic surfactants, alkylphenol polyethoxylates (APEs). These ubiquitous compounds of

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The genebank accession number of the 16S rDNA: AB126621.

The type strain is WL^T (=IAM 15071^T=TISTR 1526^T).

APs have been designated as endocrine disrupters, due to their hormone mimic property that interferes with the reproductive systems of humans and wildlife. Scarce information is at present available on the possible routes for the complete biodegradation of APs in polluted environments (Rudling and Solyom, 1974) and only a few microorganisms capable of attacking APs have been isolated in pure cultures and characterized so far. Those are a yeast strain *Candida aquatextoris* sp. nov. (Vallini et al., 1997), a bacterial strain *Sphingomonas cloacae* sp. nov. (Fujii et al., 2001) and a proposed new subspecies of *Pseudomonas veronii* subsp. *inensis* subsp. nov. (Ajithkumar et al., 2003).

In this paper, we report phylogenetic characterization of strain WL^T, a potent AP-degrading *Pseudomonas* strain that was isolated from an activated sludge. This strain has the ability to utilize APs with *para*-substituted straight alkyl chains from 1 to 9 carbons as a sole carbon source. On the basis of phenotypic characterization, 16S rDNA sequence analysis, G+C base composition and DNA-DNA hybridization, this strain could not be placed in any known species and is proposed as a new species of the genus *Pseudomonas*, namely *Pseudomonas japonica*.

Materials and Methods

Bacterial strains. The bacterial strains used in this study were obtained from the Institute of Applied Microbiology (IAM), the University of Tokyo, Tokyo, Japan, the Japan Collection of Microorganisms (JCM), Saitama, Japan, Collection de Bacteries de l'Institut Pasteur (CIP), Paris, France and the National Institute of Agrobiological Resources, Ministry of Agriculture, Forestry and Fisheries (MAFF), Tsukuba, Japan. Recommended media and known growth conditions by the culture collection of each strain were used to revive these freeze-dried cultures. Strain WL has been deposited in the IAM, as strain IAM 15071^T and in TISTR, Thailand Institute of Scientific and Technological Research Culture Collection Bangkok MIRCEN, Bangkok, Thailand as strain TISTR 1526^T.

Enrichment and isolation of the AP-degrading strains. AP-degrading strains were isolated from an activated sludge that was originally collected from the municipal sewage treatment plant in Tokyo. Significant AP-degrading activities were developed in the activated sludge as a result of prolonged cultivation in a

laboratory scale bioreactor to which Carbon-Free Minimum Medium (CFMM) (Omori et al., 1992) containing a mixture of APs was supplied at a constant flow. Further enrichment of bacteria that were able to grow on APs as the sole carbon source was achieved by repeated transfer and cultivation in flasks containing CFMM supplemented with 0.1% (w/v) APs. All plate and liquid cultures were incubated at 30°C for at least 3 days. The APs used are *p*-*n*-heptylphenol (C₇) and *p*-*n*-octylphenol (C₈) that are of HPLC grade from Kanto Chemical (Japan) and *p*-*n*-nonylphenol (C₉) that is the pure standard product of Dr. Ehrenstorfer (Germany). For determining the substrate utilization capability, *p*-cresol (C₁), *p*-*n*-ethylphenol (C₂), *p*-*n*-propylphenol (C₃), *p*-*n*-butylphenol (C₄), *p*-*n*-pentylphenol (C₅), *p*-*n*-hexylphenol (C₆) from Kanto Chemical (Japan) were used.

Morphological characterization. Cell morphology was investigated by using a differential interference-contrast microscope. The cells were also stained according to the Gram-stain procedure modified by Hucker and Conn (Hucker and Conn, 1923). Motility was investigated by the hanging drop method and bacterial flagellation was examined under a transmission electron microscope.

Physiological and biochemical characterization. Commercially available identification kits were used according to manufacturers' recommendations for physiological and biochemical characterization; those are GN microplates from BIOLOG, and API50CH, APIZYM and API20NE from BioMerieux. Oxidase and Catalase tests were carried out with the Poremedia oxidase test indicator (Eiken Chemical) and 3% hydrogen peroxide, respectively. Fluorescent pigment formation was observed on King medium A and King medium B (Eiken Chemical) (King et al., 1954).

DNA base composition and lipid analysis. Extraction and isolation of bacterial DNA were performed by the modified method of Marmur (Marmur, 1961; Saito and Miura, 1963). DNA base composition was determined by the method of Tamaoka and Komagata (1984) and Mesbah et al. (1989) using HPLC with a Cosmosil 5C₁₈-AR column (4.6×250 mm; Nacalai Tesque, Japan) and a 40:0.5 (v/v) mixture of 0.2 M NH₄H₂PO₄ (pH 4.0) and acetonitrile as the mobile phase. The isoprenoid quinones were extracted from bacterial cells and purified on TLC by the method of Yamada and Kuraishi (1982) and Yamada et al. (1982). Ubiquinone isoprenologues were quantitatively

determined by reverse-phase HPLC. Analysis of whole cell fatty acids was performed on a culture of strain WL^T grown for 24 h in TSA medium (Merck) at 28°C. Cellular fatty acid composition was analyzed as methyl esters by GLC according to the instructions for the Microbial Identification System (MIDI) (Microbial ID, Inc.). The fatty acids that could not be instantly identified by the MIDI system, as exemplified in the summed feature 3, were analyzed as follows: fatty acid samples were first separated into non-polar fatty acids and hydroxyl fatty acids by thin layer chromatography on a TLC plate (Silica-gel F254; Merck) with hexane/ethyl ether (1:1) as a developing solvent. Spots of fatty acids were detected under UV light after spraying 0.02% dichlorofluorescein ethanol solution, scraped from the plate, and extracted with ethyl ether. The separated non-polar fatty acids and hydroxyl fatty acids were analyzed by the MIDI system.

16S rDNA sequencing and phylogenetic analysis. DNA fragments coding for 16S rRNA were amplified with PCR using a universal primer set corresponding to positions 8–27 (forward primer) and 1492–1510 (reverse primer) by the *Escherichia coli* 16S rRNA nucleotide numbering system (Weisburg et al., 1991). The amplified DNA fragments were directly sequenced with an ABI PRISM[®] BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems), according to the manufacturer's instructions on an ABI PRISM[®] 310 automatic sequence analyzer. Then the nucleotide sequences obtained were analyzed with DNASIS-Mac software (Hitachi). Similarity of 16S rDNA sequence was compared with all known sequence data in the GenBank, EMBL and DDBJ databases using the BLAST algorithm (Altschul et al., 1990). The DDBJ accession number for the 16S rDNA sequence of strain WL^T is AB126621. Multiple alignments of the sequences were performed with CLUSTAL W software (Thompson et al., 1994) and the evolutionary distance trees were constructed using the neighbor-joining method (Saitou and Nei, 1987). The robustness for individual branches was estimated by bootstrapping with 1,000 replications (Felsenstein, 1985).

Results and Discussion

Isolation and characterization of AP-degrading bacterium

Strain WL^T was isolated from an activated sludge

that was originally collected from the municipal sewage treatment plant, Odai, Adachi-ku, Tokyo, Japan. This strain indicated the highest growth and was able to assimilate APs and various related compounds. It could utilize a wide range of APs with *para*-substituted straight chains from 1 up to 9 carbon atoms, with a preference for the longer straight alkyl chains. In addition, this strain could also utilize straight-chain alkanes and grow in the presence of short to medium-chain-length alcohols but not branched-chain APs, benzene, toluene, phenol or benzoic acid.

Phenotypic characterization

Cells of strain WL^T were Gram-negative, non spore-forming, rod shaped (2.0–3.5×1.3–1.7 µm), usually present singly or in pairs, but not in long chains and were motile by means of polar multitrichous flagella (Fig. 1). On nutrient agar plates, this strain formed flat creamy white colonies with slightly spreading edges after 24 h of incubation at 30°C. It required about 3 days to form visible colonies on CFMM/APs agar at this temperature with a clear halo zone surrounding the colonies.

Comparative 16S rDNA gene sequence analysis showed affiliation of strain WL^T to the genus *Pseudomonas*. Almost the entire sequence of 16S rDNA (1,500 bp) showed 98% similarity with those of *Pseudomonas plecoglossicida*, *Pseudomonas putida* and other unidentified *Pseudomonas* sp. strains. The phylogenetic tree derived from these 16S rDNA sequences was constructed to illustrate the position of strain WL and 12 other *Pseudomonas* species in Fig. 2, indicating that this strain was placed in the genus *Pseudomonas sensu stricto*, under the subcluster of the *Pseudomonas putida* group (Anzai et al., 2000). Its closest relative is *Pseudomonas graminis*, a newly described species in the *Pseudomonas putida* group, which was isolated from grasses (Behrendt et al., 1999). Differentiation is further confusing among members of *Pseudomonas sensu stricto* due to high levels of 16S rDNA similarity among the species approved to date (sequence similarity >99%). The difficulty in separating these species only on the basis of 16S rDNA sequence similarities has already been pointed out by some recent reports (Moore et al., 1996; Yamamoto et al., 2001). The usefulness of 16S rDNA sequences for the characterization of phylogenetic relationships between species is limited, when their homologies exceed 97% (Stackebrandt and Goebel, 1994). For this

Fig. 1. Morphological figures of strain WL^T.

(a) Differential interference-contrast microscopy indicated rod-shaped cells distributed singly or in pairs. Bar length=1 μm. (b) Transmission electron micrograph showing polar multitrichous flagellation. Bar length=0.2 μm.

Fig. 2. The phylogenetic tree derived from 16S rRNA gene sequences of *Pseudomonas* strain WL^T and other *Pseudomonas* type strains using the neighbor-joining method.

Numbers indicate bootstrap values greater than 500. Scale bar represents an evolutionary distance (K_{nuc}) of 0.002.

reason, the levels of DNA-DNA reassociation were determined fluorometrically by the method of Ezaki et al. (1989) using photobiotin-labeled DNA probes and microdilution plates. The hybridization reaction was replicated five times for each sample. Of the data obtained, the highest and the lowest values in each sample were excluded and the remaining three measurements were used to calculate the mean relatedness value. DNA-DNA reassociation values between strain WL^T and other *Pseudomonas* species are presented in Table 1.

These data were obtained by using DNA from strain WL^T as a labeling probe and were cross-checked with a biotinylation *Pseudomonas graminis* DNA probe. These values indicate relatively low levels of hybridization. The extent of DNA-DNA reassociation between the strain WL^T and the reference *Pseudomonas* species were all less than 50% and within the range from 2 to 41%, which were well below the 70% cut-off point recommended by Wayne et al. (1987) for the recognition of a different genomic species. Therefore,

Table 1. DNA base composition and levels of relatedness between strain WL^T and DNAs of other closely related *Pseudomonas* type strains.

Bacterial strains	G+C content (mol%)	Reassociation (%) with labeled DNA from:	
		Strain WL ^T	<i>P. graminis</i>
<i>Pseudomonas japonica</i> WL ^T	66	100	10
<i>Pseudomonas putida</i> IAM 1236 ^T	63	41	13
<i>Pseudomonas plecoglossicida</i> CIP 106495 ^T	64	36	14
<i>Pseudomonas mosselii</i> CIP 105259 ^T	64	31	12
<i>Pseudomonas monteilii</i> CIP 104883 ^T	62	17	8
<i>Pseudomonas oryzihabitans</i> IAM 1568 ^T	67	15	8
<i>Pseudomonas fulva</i> IAM 1529 ^T	63	18	7
<i>Pseudomonas cremoricolorata</i> JCM 11246 ^T	62	3	2
<i>Pseudomonas parafulva</i> JCM 11244 ^T	63	4	2
<i>Pseudomonas fuscovaginae</i> MAFF 301177 ^T	61	9	5
<i>Pseudomonas graminis</i> CIP 105897 ^T	61	11	100

these results strongly suggest that strain WL^T is distinct from other known *Pseudomonas* species and could be proposed as a new strain.

The G+C composition of strain WL^T and other related *Pseudomonas* type strains are also indicated in Table 1. The DNA base compositions of all strains tested ranged from 61 to 67 mol%. For strain WL^T, the G+C content of genomic DNA was determined as 66 mol%. This value also falls within the range of G+C contents observed for other members of the genus *Pseudomonas*, namely 58–70 mol% (Palleroni, 1984). The major respiratory quinone of strain WL^T was ubiquinone Q-9 and the main non-polar fatty acids detected were hexadecenoic acid (16:1), hexadecanoic acid (16:0) and octadecenoic acid (18:1). The hydroxyl fatty acids detected were 3-hydroxydecanoic acid (3-OH 10:0), 3-hydroxydodecanoic acid (3-OH 12:0) and 2-hydroxydodecanoic acid (2-OH 12:0), as shown in Table 2. This quinone system and fatty acid profile are characteristic of strains from rRNA group I (Oyaizu and Komagata, 1983).

The summarization of differential phenotypic characteristics between strain WL^T and phenotypically similar standard *Pseudomonas* type strains are shown in Table 3. According to the resultant data, all the strains were Gram-negative, aerobic, rod-shaped organisms with motility. Cells were able to grow in nutrient broth at 30°C and 37°C, but not at 42°C. They did not produce pyocyanin on King A medium, even though some strains can produce pigment on nutrient agar and tryptic soy agar. The strains tested were positive for cata-

lase activity, and almost all of them were oxidase positive. From a nutritional standpoint, strain WL^T showed a broad spectrum. Many different compounds served as a sole source of carbon and energy. It had a preference for monomeric sugars but not for most disaccharides tested.

On the basis of phylogenetic, chemotaxonomic and phenotypic results, we conclude that strain WL^T should be classified as a new species of the genus *Pseudomonas*, for which the name *Pseudomonas japonica* sp. nov. is proposed.

Description of Pseudomonas japonica sp. nov.

Pseudomonas japonica (ja. po' ni. ca. N.L. adj. japonica pertaining to Japan, from where the isolate originated).

Cells are Gram-negative, aerobic, motile by means of polar multitrichous flagella, non spore-forming, straight rods (2.0–3.5 by 1.3–1.7 µm) and usually occur singly or in pairs. Colonies are circular, flat, opaque, creamy white to pale yellow with slightly spreading edges on nutrient agar. Pyocyanin and fluorescent pigments are not produced on either King A or King B medium. Cells are able to grow in nutrient broth at 30 and 37°C, but not at 4 or 42°C. Oxidase and catalase tests are positive. This strain cannot reduce nitrate to nitrite nor denitrificate nitrite to nitrogen gas. It utilizes the following substrates as sole carbon sources for growth: glucose, caprate, malate, citrate, phenyl-acetate, D-xylose, D-fucose, α-cyclodextrin, glycogen, tween 40, tween 80, L-arabinose, D-fructose,

Table 2. Cellular fatty acid composition of strain WL^T and other closely related *Pseudomonas* type strains.

Fatty acid	Percentage composition in strains										
	1	2	3	4	5	6	7	8	9	10	11
<i>Saturated fatty acid</i>											
10:0	—	—	0.1	—	—	—	—	0.1	0.2	0.2	0.1
10:0 3-OH	4.3	1.9	3.1	4.0	3.3	2.2	4.2	4.1	4.0	2.9	3.0
12:0	1.7	4.3	2.9	1.6	2.2	4.0	0.8	1.5	1.4	2.5	2.3
12:0 2-OH	4.8	2.5	3.9	5.5	4.5	3.1	6.3	5.4	5.6	4.1	4.1
12:0 3-OH	3.7	3.1	3.4	4.4	3.8	3.4	4.1	3.9	4.1	3.4	3.7
14:0	0.2	0.3	0.3	0.4	0.3	0.3	0.5	0.4	0.7	0.2	0.2
15:0	0.3	—	0.3	0.2	0.2	—	0.3	0.3	0.4	—	0.2
16:0	26.4	29.9	28.2	29.6	25.4	20.9	32.2	27.5	28.9	23.9	20.8
17:0	0.3	0.2	0.4	0.3	0.4	—	0.2	0.3	0.2	0.2	0.3
18:0	0.7	0.8	1.1	0.9	0.7	0.8	0.5	0.7	0.4	1.4	1.2
<i>Unsaturated fatty acid</i>											
12:1 3-OH	0.1	—	—	—	—	—	—	—	0.1	0.1	—
13:1 at 12-13	0.1	—	—	—	—	—	—	—	—	—	—
17:1 ω8c	0.2	—	0.3	—	0.2	—	—	0.2	0.1	—	0.2
18:1 ω5c	—	—	—	—	—	—	—	—	—	—	0.2
18:1 ω6c	—	—	—	—	—	—	—	—	—	3.4	—
18:1 ω7c	22.3	17.9	22.1	14.6	34.1	45.7	23.2	20.0	18.0	25.4	36.8
20:1 ω7c	—	—	—	—	—	—	—	—	—	—	0.2
Summed feature 3 ^a	65.1 ^b	75.0	65.1	75.1	43.5	38.8	50.1	62.1	61.4	60.3	36.8
<i>Branched fatty acid</i>											
11:0 ISO 3-OH	—	—	—	—	0.2	—	—	—	—	—	0.1
11 methyl 18:1 ω7c	—	—	—	0.1	—	—	—	—	—	0.1	—
16:0 ISO	—	0.1	—	—	0.1	—	—	—	—	—	—
17:0 ISO	—	0.2	—	—	0.5	0.2	—	—	—	0.1	0.3
17:0 CYCLO	2.4	1.0	1.4	0.5	2.1	—	2.4	4.1	5.0	1.9	6.7
19:0 CYCLO ω8c	—	—	—	—	0.3	—	0.2	0.1	—	0.1	0.8

^aSummed features are groups of two fatty acids that cannot be separated by GLC with the MIDI system. The summed feature 3 contained one or more of the following fatty acids; 16:1 ω5c and 15:0 ISO 2-OH.

^bThe strain WL^T did not contain 15:0 ISO 2-OH and the summed feature 3 of this strain was 16:1 ω5c by the analysis described in MATERIALS AND METHODS.

1, *Pseudomonas japonica* WL^T; 2, *Pseudomonas graminis* CIP 105897^T; 3, *Pseudomonas putida* IAM 1236^T; 4, *Pseudomonas fuscovaginae* MAFF 301177^T; 5, *Pseudomonas cremoricoloranta* JCM 11246^T; 6, *Pseudomonas oryzihabitans* IAM 1568^T; 7, *Pseudomonas fulva* IAM 1529^T; 8, *Pseudomonas parafulva* JCM 11244^T; 9, *Pseudomonas plecoglossicida* CIP 106495^T; 10, *Pseudomonas montellii* CIP 104883^T; 11, *Pseudomonas mosselii* CIP 105259^T.

L-fucose, maltose, β-methyl-D-glucoside, D-psicose, L-rhamnose, turanose, xylitol, methyl pyruvate, mono-methyl succinate, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-glucuronic acid, β-hydroxybutyric acid, *p*-hydroxyphenyl acetic acid, itaconic acid, α-keto butyric acid, α-keto glutaric acid, α-keto valeric acid, D-lactic acid, L-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, alanine amide, D-alanine, L-alanine, L-alanyl-glycine, L-as-

paragine, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, hydroxyl-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglyutamic acid, D-serine, L-serine, D-carnitine, L-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, phenyl ethylamine, putrescine, 2-amino ethanol, glycerol, glucose-1-phosphate and glucose-6-phosphate. The following enzymes are produced: protease, esterase (C₄), esterase lipase (C₈), lipase (C₁₄), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-

Table 3. Differential characteristics of strain WL^T and *Pseudomonas* species.

Characteristics	Strains										
	1	2	3	4	5	6	7	8	9	10	11
<i>Activity of enzymes</i>											
arginine dihydrolase	—	—	+	—	—	—	+	+	+	+	—
protease	+	+	—	+	+	+	—	—	+	+	—
alkaline phosphatase	—	+	+	+	+	+	+	—	+	+	+
trypsin	—	—	—	+	—	+	+	—	—	+	—
<i>Assimilation of</i>											
arabinose	—	+	+	+	—	+	—	—	—	+	—
mannose	—	—	—	+	—	+	+	+	—	—	+
mannitol	—	+	—	+	—	+	—	—	—	—	+
phenyl-acetate	+	—	+	—	—	—	—	+	+	+	+
L-arabinose	—	—	+	+	—	+	+	+	—	—	—
ribose	—	—	+	+	—	+	+	+	—	+	+
D-fucose	+	—	+	—	+	+	—	+	—	+	—
2-keto-gluconate	—	—	+	—	—	+	+	+	+	+	+
<i>Carbon sources</i>											
α-cyclodextrin	+	—	+	+	+	—	—	—	+	—	+
dextrin	—	—	+	—	+	+	—	—	+	—	+
D-galactose	—	+	+	+	—	+	—	—	—	—	+
turanose	+	+	+	—	+	+	—	+	—	—	—
D-galacturonic acid	—	+	+	+	+	+	—	—	—	+	—
D-glucosamic acid	—	+	—	+	+	+	—	—	—	—	—
D-glucuronic acid	+	+	—	+	+	+	—	—	—	+	—
α-hydroxybutyric acid	—	+	—	—	+	+	—	—	—	—	+
γ-hydroxybutyric acid	—	—	—	—	+	+	—	+	+	—	—
p-hydroxy phenylacetic acid	+	—	+	+	+	—	—	+	—	—	+
itaconic acid	+	+	—	—	+	+	—	—	+	—	+
α-keto butyric acid	+	+	—	—	+	+	—	—	—	—	+
malonic acid	+	+	—	+	+	+	—	—	—	+	—
succinamic acid	+	+	+	—	+	+	—	—	—	—	+
glucuronamide	+	+	+	+	—	+	—	—	—	+	—
glycyl-L-aspartic acid	+	—	+	—	+	+	—	+	+	—	—
L-phenylalanine	+	—	—	+	—	—	—	+	—	+	+
D-serine	+	+	+	—	+	+	—	—	+	—	+
urocanic acid	+	+	—	+	+	+	—	+	+	+	+
inosine	+	+	—	+	+	+	—	+	—	+	+
uridine	+	+	—	+	+	+	—	+	—	—	+
phenyl ethylamine	+	—	+	—	—	—	—	+	+	+	+
2-amino ethanol	+	+	—	+	+	+	—	+	+	+	+
2,3-butanediol	—	—	—	+	+	+	—	—	—	—	—
D,L-α-glycerol phosphate	—	+	—	+	—	+	—	—	+	—	+
glucose-6-phosphate	+	—	+	+	—	+	—	+	—	—	+

+, positive reaction; —, negative reaction; 1, *Pseudomonas japonica* WL^T; 2, *Pseudomonas graminis* CIP 105897^T; 3, *Pseudomonas putida* IAM 1236^T; 4, *Pseudomonas fuscovaginae* MAFF 301177^T; 5, *Pseudomonas cremoricoloranta* JCM 11246^T; 6, *Pseudomonas oryzihabitans* IAM 1568^T; 7, *Pseudomonas fulva* IAM 1529^T; 8, *Pseudomonas parafulva* JCM 11244^T; 9, *Pseudomonas plecoglossicida* CIP 106495^T; 10, *Pseudomonas monteillii* CIP 104883^T; 11, *Pseudomonas mosselii* CIP 105259^T.

AS-BI-phosphohydrolase. This strain can utilize a wide range of *para*-substituted straight chain alkylphenols, namely from 1 up to 9 carbon atoms as a carbon source for growth. The G+C content of its DNA is 66 mol%. The major non-polar fatty acids are C_{16:0}, C_{16:1} and C_{18:1}, while the major hydroxylated fatty acids are 3-OH C_{10:0}, C_{12:0} and 2-OH C_{12:0}. Its major isoprenoid quinone is ubiquinone of Q-9. The type strain is WL^T (=IAM 15071^T=TISTR 1526^T). Its 16S rDNA sequence has been deposited at the DDBJ database under accession number AB126621. This strain was isolated in the year 2000 from an activated sludge seed collected from the municipal sewage treatment plant, Odai, Adachi-ku, Tokyo, Japan.

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