Phycicoccus endophyticus sp. nov., an endophytic actinobacterium isolated from *Bruguiera gymnorhiza*

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A novel endophytic actinobacterium, designated strain IP6SC6^T, was isolated from surface-sterilized bark of *Bruguiera gymnorhiza* collected from Zhanjiang Mangrove Forest National Nature Reserve in Guangdong, China. Cells of strain IP6SC6^T were Gram-stain-positive, aerobic, non-spore-forming, non-motile rods. Strain IP6SC6^T grew at 20–42 °C (optimum, 37 °C), at pH 6.0–9.0 (optimum, pH 7.0) and in the presence of 0–8 % (w/v) NaCl (optimum, 0–2 %). Chemotaxonomic analyses showed that the isolate possessed *meso*-diaminopimelic acid as the diamino acid of the peptidoglycan, galactose and glucose as whole-cell sugars, and MK-8(H_4) as the predominant menaquinone. The major polar lipids were diphosphatidylglycerol, phosphatidylinositol and an unknown lipid. The major fatty acids were iso-$C_{15:0}$, anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and iso-$C_{16:0}$. The G+C content of the genomic DNA was 72.5 mol%. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain IP6SC6^T belonged to the genus *Phycicoccus* and shared the highest sequence similarity with *Phycicoccus jejuensis* NRRL B-24460^T (96.97 %). On the basis of phylogenetic analysis and phenotypic and chemotaxonomic characteristics, strain IP6SC6^T represents a novel species of the genus *Phycicoccus*, for which the name *Phycicoccus endophyticus* sp. nov. is proposed. The type strain is IP6SC6^T (=DSM 100020^T=CGMCC 4.7300^T).

The genus *Phycicoccus*, a member of the family *Intrasporangiaceae*, suborder *Micrococcineae* (Stackebrandt *et al.*, 1997), was created by Lee (2006) and the description was subsequently emended by Zhang *et al.* (2011). At the time of writing, the genus comprises eight species with validly published names: *Phycicoccus jejuensis* (Lee, 2006) as the type species, *P. dokdonensis* (Yoon *et al.*, 2008), *P. aerophilus* (Weon *et al.*, 2008), *P. bigeumensis* (Dastager *et al.*, 2008), *P. cremeus* (Zhang *et al.*, 2011), *P. ginsenosidimutans* (Wang *et al.*, 2011), *P. badiiscoriae* (Lee, 2013) and *P. soli* (Singh *et al.*, 2015). Members of the genus *Phycicoccus* are Gram-stain-positive, aerobic coccoid- or rod-shaped bacteria isolated from diverse environments, such as dried seaweed, air, soil and scoria. The characteristics of the genus *Phycicoccus* include *meso*-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan, MK-8(H_4) as the major menaquinone, and iso-$C_{15:0}$ and iso-$C_{16:0}$ as the predominant fatty acids. The G+C content of the genus *Phycicoccus* is in the range 69.7–74.0 mol% (Lee, 2013).

During an investigation of the culturable actinobacterial diversity associated with endophytic actinomycetes from mangrove plants, strain IP6SC6^T, the first endophytic actinobacterium affiliated with the genus *Phycicoccus*, was isolated from surface-sterilized bark of *Bruguiera gymnorhiza* collected from Zhanjiang Mangrove Forest National Nature Reserve (21° 34′ 14″ N 109° 45′ 21″ E) in Guangdong, China. Based on polyphasic taxonomic studies, strain IP6SC6^T was distinguished from previously described species of the genus *Phycicoccus* and is considered to represent a novel species. In this paper, the taxonomic position of this strain is reported.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IP6SC6^T is KT327645.

Two supplementary tables and four supplementary figures are available with the online Supplementary Material.

Treatment of the plant sample was processed as described by Qin *et al.* (2009). After sterilization, the sample was ground to a powder by using a micromill and distributed on International Streptomyces Project (ISP) 2 agar (Shirling & Gottlieb, 1966) supplemented with 1 % (v/v) plant tissue extract. After 4 weeks of incubation at 28 °C, a pure culture was isolated and then subcultured on trypticase soy agar (TSA; BD). The purified isolate was maintained at 4 °C on TSA slants and preserved in aqueous glycerol suspensions (20 %, v/v) at −80 °C.

The extraction of genomic DNA from strain IP6SC6^T and PCR amplification of the 16S rRNA gene were performed as described by Li *et al.* (2007). The purified PCR products were cloned using the pEASY-T1 Cloning kit (TransGen Biotech) according to the manufacturer's instructions, and sequenced using an ABI PRISM 3730XL DNA Analyser. Levels of 16S rRNA gene sequence similarity between strain IP6SC6^T and related species were determined via the EzTaxon e-server (http://eztaxon-e.ezbiocloud.net/; Kim *et al.*, 2012). Multiple alignments were generated using the CLUSTAL X program (Thompson *et al.*, 1997). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura, 1983). Phylogenetic trees were reconstructed using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods with MEGA version 6.0 (Tamura *et al.*, 2013). The topologies of the phylogenetic trees were evaluated by using the bootstrap method of Felsenstein (1985) with 1000 repeats.

The nearly full-length 16S rRNA gene sequence (1487 bp) of strain IP6SC6^T was obtained to determine its phylogenetic position. Comparative 16S rRNA gene sequence analyses showed that strain IP6SC6^T was phylogenetically most closely related to members of the family *Intrasporangiaceae*. Strain IP6SC6^T shared highest 16S rRNA gene sequence similarity with *Phycicoccus jejuensis* NRRL B-24460^T (96.97 %), followed by *Tetrasphaera duodecadis* ATCC 13347^T (96.90 %), *Phycicoccus badiiscoriae* Sco-B23^T (96.65 %), *Tetrasphaera veronensis* Ver1^T (96.62 %), *Phycicoccus bigeumensis* MSL03^T (96.46 %), *Phycicoccus dokdonensis* DS-8^T (96.45 %), *Tetrasphaera japonica* ACM 5116^T (96.32 %) and *Tetrasphaera elongata* Lp2^T (96.28 %). Lower sequence similarities (<96.0 %) were found with all other recognized species of the family *Intrasporangiaceae*. The phylogenetic tree reconstructed using the neighbour-joining algorithm (Fig. 1) showed that strain IP6SC6^T was separate from members of the genus *Tetrasphaera* and fell into the cluster of the genus *Phycicoccus* with a relatively high bootstrap value (71 %), forming a separate clade within the family *Intrasporangiaceae*. This topology was also observed in the trees based on the maximum-likelihood and maximum-parsimony algorithms (Figs S1 and S2, available in the online Supplementary Material).

Studies of the cultural, physiological and biochemical characteristics of strain IP6SC6^T were carried out under the same conditions with *P. jejuensis* NRRL B-24460^T. *P. jejuensis* NRRL B-24460^T, the closest phylogenetic neighbour of strain IP6SC6^T in the genus *Phycicoccus*, was obtained from Agricultural Research Service Culture Collection (Peoria, USA) and used as a reference strain. Cultural characteristics were determined by observing growth of the strain at 28 °C for 1–2 weeks on ISP 2, 3, 4, 5 and 7 agars (Shirling & Gottlieb, 1966), nutrient agar (Waksman, 1961), R2A agar (BD), TSA, yeast-starch agar (Ara & Kudo, 2007) and Bennett's agar (Gordon & Smith, 1955). The ISCC-NBS colour charts (Kelly, 1964) were used to assess colony colour and diffusible pigment. Cell morphology and motility were observed by transmission electron microscopy (JEM-1400; JEOL) after incubation on TSA at 28 °C for 3 days. The Gram-stain test was performed as described by Magee *et al.* (1975). Growth under anaerobic conditions was determined after incubation in the BBL GasPak Anaerobic System (BD) at 28 °C for 14 days. The temperature range for growth was determined by incubation of the strain on TSA at 4, 15, 20, 25, 28, 30, 37, 42, 45 and 50 °C for 14 days. The pH range for growth was measured in trypticase soy broth (TSB; BD) with various pH values (pH 4.0–12.0, at intervals of 1.0 pH unit) for 14 days. For the pH experiments, the different buffers described by Xu *et al.* (2005) were used. Salt tolerance was tested in TSA supplemented with 0, 1, 2, 3, 5, 7, 8, 9 or 10 % (w/v) NaCl for 14 days. Catalase activity was determined by bubble production in 3 % (v/v) H_2O_2. Oxidase activity was assessed by using 1 % (w/v) tetramethyl-*p*-phenylenediamine (Cappuccino & Sherman, 2002). Hydrolysis of starch, cellulose, gelatin and Tweens 20, 40 and 80, production of H_2S, and milk coagulation and peptonization were examined as described by Gonzalez *et al.* (1978). Acid production from carbon sources was tested using the API 50CH (bioMérieux) system according to the manufacturer's instructions. Other biochemical characteristics and enzyme activities were tested by using the API 20NE and API ZYM kits (bioMérieux) according to the manufacturer's instructions.

Cells of strain IP6SC6^T were aerobic, Gram-stain-positive, non-spore-forming and non-motile. Colonies grown on TSA plates were circular, smooth, opaque and greyish yellow. Cells of strain IP6SC6^T were rod-shaped (0.5–0.8 μm in diameter and 1.2–2.0 μm in length) on TSA after incubation at 28 °C for 3 days (Fig. S3). Strain IP6SC6^T displayed good growth on TSA, R2A agar, ISP 2 and ISP 3 agars, Bennett's agar and yeast-starch agar, poor growth on ISP 5 and ISP 7 agars, and no growth on ISP 4 agar or nutrient agar. Substrate and aerial mycelia were not observed and no diffusible pigment was produced on any of the media tested. The strain was capable of growth on TSA containing 0–8 % NaCl; it grew at temperatures from 20 to 42 °C and at pH 6.0–9.0. Optimum growth occurred at 37 °C, at pH 7.0 and with 0–2 % (w/v) NaCl. No growth occurred at 15 or 45 °C, at pH 5.0 or 10.0, or in the presence of 9 % (w/v) NaCl. Detailed physiological and biochemical characteristics of strain IP6SC6^T are given in Table 1 and the species description.

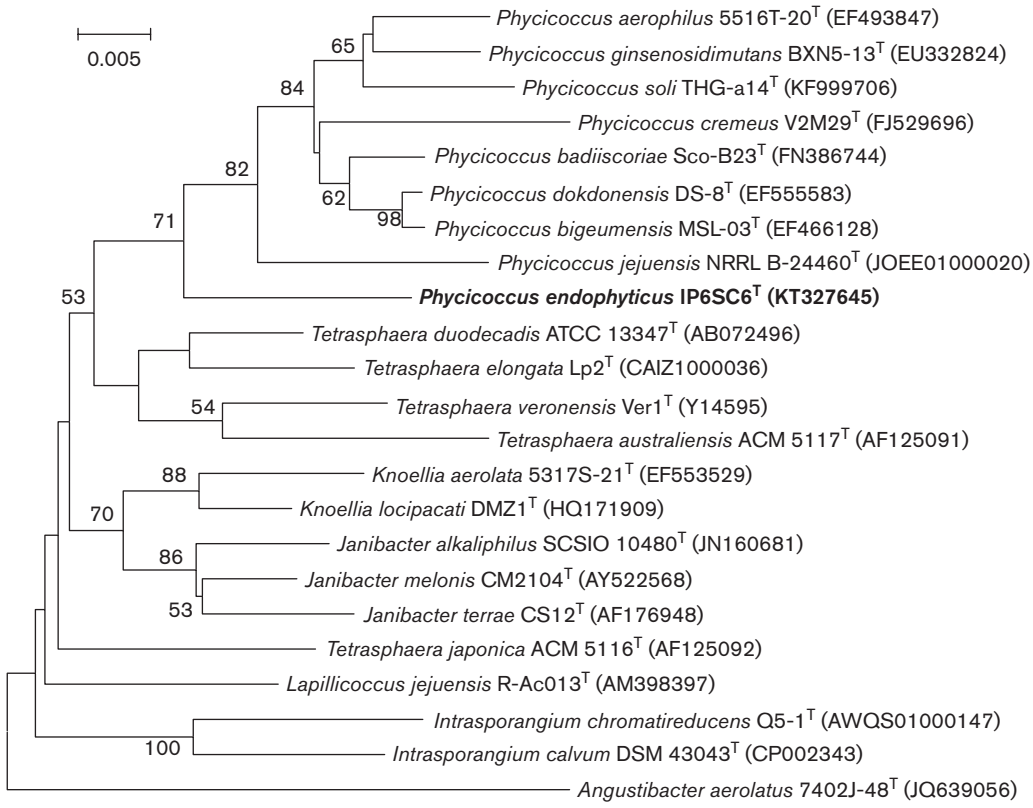

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain IP6SC6^T and the type strains of related species in the family *Intrasporangiaceae*. *Angustibacter aerolatus* 7402J-48^T was used as an outgroup. Numbers at nodes refer to bootstrap values (based on 1000 replicates; only values >50 % are shown). Bar, 5 nt substitutions per 1000 nt.

For the chemotaxonomic investigations, including polar lipids, menaquinones, diagnostic diamino acids and sugars, biomass of both strain IP6SC6^T and the reference strain *P. jejuensis* NRRL B-24460^T was obtained after incubation in TSB at 28 °C, 180 r.p.m., for 3 days. The polar lipids were extracted and analysed by two-dimensional TLC on silica gel 60 F_{254} plates (Merck) as described by Minnikin *et al.* (1984). The solvent systems of the first and the second dimension were chloroform/methanol/water (64 : 27 : 5, by vol.) and chloroform/methanol/acetic acid/water (80 : 18 : 12 : 5, by vol.), respectively. Menaquinones were isolated and purified according to the method of Collins *et al.* (1977), then analysed and identified using an HPLC system coupled to a single quadrupole mass spectrometer (Guo *et al.*, 2015). The diagnostic diamino acids and sugars in whole-cell hydrolysates were identified by TLC as described by Schleifer & Kandler (1972) and Staneck & Roberts (1974), respectively. For the analysis of whole-cell fatty acids, cell mass of strain IP6SC6^T and *P. jejuensis* NRRL B-24460^T were harvested from both TSA and R2A agar plates grown at 28 °C for 3 days, when the bacterial communities reached the late-exponential stage of growth according to the four quadrants streak method. The whole-cell fatty acids were saponified, methylated and extracted according to the standard protocol described by Sasser (1990), and analysed according to the method described by Tuo *et al.* (2015). For G+C content assessment, genomic DNA of strain IP6SC6^T was prepared according to the method described by Marmur (1961) and the G+C content was determined by reversed-phase HPLC as described by Mesbah *et al.* (1989).

Strain IP6SC6^T contained *meso*-diaminopimelic acid as the diagnostic diamino acid in the cell-wall peptidoglycan, and galactose and glucose as whole cell-wall sugars. The predominant menaquinone was identified as MK-8(H_4) (98.5 %) and the minor component as MK-7(H_4) (1.5 %). The polar lipids were diphosphatidylglycerol, phosphatidylinositol and an unknown lipid (Fig. S4). Although there were some differences in proportions, the profiles of fatty acids in both TSA and R2A agar medium were quite similar. Four major components (>10 % of the total), iso-$C_{15:0}$, anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and iso-$C_{16:0}$, and some minor components (<10 %), $C_{18:1}\omega9c$, $C_{16:0}$, iso-$C_{17:0}$, $C_{18:0}$, $C_{16:1}\omega7c$, iso-$C_{14:0}$, 10-methyl $C_{18:0}$, $C_{14:0}$ and 10-methyl $C_{16:0}$, were detected in both TSA and R2A agar medium. The comparative cellular fatty acid compositions of strain IP6SC6^T and *P. jejuensis* NRRL B-24460^T in both

Table 1. Differential characteristics between strain IP6SC6^T and the type strains of recognized *Phycicoccus* species.

Strains: 1, IP6SC6^T (data from this study); 2, *P. jejuensis* NRRL B-24460^T (data from this study, except where indicated); 3, *P. badiiscoriae* Sco-B23^T (Lee, 2013); 4, *P. bigeumensis* MSL-03^T (Dastager *et al.*, 2008); 5, *P. dokdonensis* DS-8^T (Yoon *et al.*, 2008); 6, *P. cremeus* V2M29^T (Zhang *et al.*, 2011); 7, *P. ginsenosidimutans* KCTC 19419^T (Wang *et al.*, 2011); 8, *P. aerophilus* 5516T-20^T (Weon *et al.*, 2008); 9, *P. soli* THG-a14^T (Singh *et al.*, 2015). All were positive or weakly positive for catalase, naphthol-AS-BI-phosphohydrolase and β-glucosidase, and assimilation of D-glucose, malate, maltose and *N*-acetyl-D-glucosamine. All were negative for glucose fermentation, arginine dihydrolase and α-fucosidase, and assimilation of caprate and citrate. +, Positive; −, negative; (+), weakly positive; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9
Source of isolation	Bark of *Bruguiera gymnorhiza*	Seaweed*	Scoria	Soil	Soil	Soil	Soil	Air	Soil
Cell morphology	Rods	Cocci*	Cocci	Cocci	Cocci	Rods	Cocci	Short rods	Cocci
Colony colour	Greyish yellow	Moderate yellow	Moderate yellow	Yellow	Greyish yellow	Cream	Greyish yellow	White	White
Temperature for growth (°C)	20–42	15–37	20–35	20–37	10–36	14–35	10–37	5–37	10–35
pH for growth	6.0–9.0	5.0–10.0	5.1–11.1	7.0–12.0	5.0–8.5	4.1–10.0	5.0–10.0	5.0–9.0	5.5–8.5
NaCl tolerance for growth (%)	0–8.0	0–8.0	0–1.0	0–5.0	0–5.0	0–7.0	0–5.0	0–7.0	0–4.5
Oxidase	(+)	−	−	−	+	−	+	−	+
Hydrolysis of starch	−	−	ND	+	+	−	ND	+	+
Hydrolysis of gelatin	+	+	+	−	+	+	+	+	+
Indole reduction	−	−	−	−	−	−	−	−	+
Nitrate reduction	−	+	−	+	−	+	+	−	+
Enzyme activity (API ZYM)									
Alkaline phosphatase	−	−	+	+	+	(+)	−	−	+
Esterase (C4)	+	+	−	+	+	−	−	−	+
Esterase lipase (C8)	+	+	(+)	+	+	+	−	+	+
Lipase (C14)	−	+	−	−	−	−	−	−	+
Leucine arylamidase	+	+	(+)	+	+	+	−	+	+
Valine arylamidase	(+)	(+)	(+)	−	+	+	−	−	+
Cysteine arylamidase	+	(+)	−	−	+	−	−	−	+
Trypsin	−	−	(+)	−	−	+	−	−	−
α-Chymotrypsin	−	+	−	−	−	−	−	+	+
Acid phosphatase	+	+	+	+	+	+	−	+	+
α-Galactosidase	−	+	−	−	+	+	−	−	+
β-Galactosidase	−	+	−	+	+	+	+	+	+
β-Glucuronidase	−	−	−	+	−	−	−	−	+
α-Glucosidase	+	+	+	−	+	+	−	+	+
N-Acetyl-β-glucosamidase	−	−	−	−	−	+	−	−	−
α-Mannosidase	−	−	−	+	(+)	+	−	−	+
Assimilation of (API 20NE):									
D-Arabinose	−	−	−	+	−	−	−	−	+
D-Mannose	+	+	(+)	+	+	+	−	+	(+)
D-Mannitol	+	+	−	+	+	+	(+)	+	−
Aesculin	+	+	+	−	+	+	+	+	+

Table 1. cont.

Characteristic	1	2	3	4	5	6	7	8	9
Gluconate	+	+	–	+	+	+	+	+	+
Adipate	–	(+)	–	(+)	–	–	–	–	–
Phenylacetate	–	+	–	–	–	–	–	–	–
Polar lipids†	DPG, PI, L	DPG, PE, PI, PL, L	DPG, PI, PIM, PL, L	DPG, PG, GL	DPG, PG, PI, PL	DPG, PI, GL	DPG, PC, PG, PE, PI	DPG, PE, PI	DPG, PG, PI, PAGL, PL, L
DNA G+C content (mol%)	72.5	74*	69.7	73.4	70.7	72	70.8	70.5	71.6

*Data from Lee (2006).
†DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannoside; PL, unknown phospholipid; PAGL, phosphoaminoglycolipid; GL, unidentified glycolipid; L, unknown lipid.

TSA and R2A agar medium are shown in Table S1. The DNA G+C content of strain IP6SC6^T was 72.5 mol%. The major fatty acids and polar lipids of *P. jejuensis* NRRL B-24460^T were similar to those previously reported (Lee, 2006). The differences in the proportion of fatty acids and slight differences in the types of polar lipids may be due to the different experimental conditions used.

The chemotaxonomic characteristics of IP6SC6^T, such as *meso*-diaminopimelic acid as the diagnostic diamino acid of the cell-wall peptidoglycan, MK-8(H_4) as the predominant menaquinone, and diphosphatidylglycerol and phosphatidylinositol as major polar lipids, were consistent with those of members of the genus *Phycicoccus*. The DNA G+C content of strain IP6SC6^T was also within the range of values (69.7–74.0 mol%) reported for recognized species of the genus *Phycicoccus* (Lee, 2013). The results of the phylogenetic analysis based on 16S rRNA gene sequences suggested that strain IP6SC6^T belonged to the genus *Phycicoccus*. However, the relatively low levels of sequence similarity (<97 %) between strain IP6SC6^T and recognized species of the genus *Phycicoccus* indicated that strain IP6SC6^T represents a novel species. Strain IP6SC6^T showed a range of characteristics that differentiated it from other species of the genus *Phycicoccus* (Table 1), including differences either in cultural, physiological and biochemical characteristics, such as cell morphology, temperature and pH range for growth, oxidase activity, hydrolysis of starch, nitrate reduction, carbon source assimilation and enzyme production, or in some chemotaxonomic characteristics, such as the polar lipid pattern and major fatty acid components. Strain IP6SC6^T contained diphosphatidylglycerol, the diagnostic phospholipid of the genus *Phycicoccus* (Zhang *et al.*, 2011), and phosphatidylinositol, which is found in all members of the genus *Phycicoccus* except *P. bigeumensis* MSL-03^T (Dastager *et al.*, 2008); but the absence of, among others, phosphatidylglycerol and phosphatidylethanolamine clearly distinguished it from the other members of the genus *Phycicoccus* (Table 1). The most abundant cellular fatty acids in strain IP6SC6^T were iso-$C_{15:0}$, anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and iso-$C_{16:0}$ (Table S1). The presence of iso-$C_{15:0}$ and iso-$C_{16:0}$ as the major fatty acids in strain IP6SC6^T and *P. jejuensis* NRRL B-24460^T was a common characteristic shared by all members of the genus *Phycicoccus* (Table S2) and was also in agreement with the emended description of the genus *Phycicoccus* given by Zhang *et al.* (2011). Among the major fatty acids in strain IP6SC6^T, anteiso-$C_{15:0}$ was also detected as a main component in *P. ginsenosidimutans* KCTC 19419^T (Wang *et al.*, 2011), whereas antesio-$C_{17:0}$ was only detected as a main component in the novel strain, representing a key characteristic differentiating it from all members of the genus *Phycicoccus*.

Despite the high 16S rRNA gene sequence similarity between strain IP6SC6^T and several species of the genus *Tetrasphaera*, the new isolate is not affiliated to the genus *Tetrasphaera* because it is not only grouped into a different clade in all three tree-making methods, but also shows an

absence of the following diagnostic characteristics for species of the genus *Tetrasphaera*. (1) Cells of *Tetrasphaera* species are cocci, occurring singly or in pairs but predominantly as tetrads or clusters, or exhibit a morphological change from rod to coccus shapes (Ishikawa & Yokota, 2006). (2) Members of the genus *Tetrasphaera* grow very slowly and utilize only a limited number of substrates (Maszenan *et al.*, 2000; Ishikawa & Yokota, 2006). (3) *Tetrasphaera duodecadis* IAM 14868^T indispensably requires vitamin B_{12} for robust growth (Lochhead, 1958; Ishikawa & Yokota, 2006) and isoprenoid quinones cannot be detected in *Tetrasphaera veronensis* Ver1^T (McKenzie *et al.* 2006).

In conclusion, on the basis of phylogenetic analyses, and phenotypic and chemotaxonomic characteristics, strain IP6SC6^T represents a novel species of the genus *Phycicoccus*, for which the name *Phycicoccus endophyticus* sp. nov. is proposed.

Description of *Phycicoccus endophyticus* sp. nov.

Phycicoccus endophyticus (en.do.phy′ti.cus. Gr. pref. *endo* within; Gr. n. *phyton* plant; L. masc. suff. *-icus* adjectival suffix used with the sense of belonging to; N.L. masc. adj. *endophyticus* within plant, endophytic, pertaining to the isolation from plant tissues).

Cells are Gram-stain-positive, aerobic, non-spore-forming, non-motile rods (0.5–0.8 × 1.2–2.0 μm). Substrate and aerial mycelia are not observed, and no diffusible pigment is produced on any of the media tested. Colonies on TSA are circular, smooth, opaque and greyish yellow. Displays good growth on TSA, R2A agar, ISP 2 agar, ISP 3 agar, Bennett's agar and yeast-starch agar, poor growth on ISP 5 agar and ISP 7 agar, and no growth on ISP 4 agar or nutrient agar. Growth occurs at 20–42 °C (optimum, 37 °C), at pH 6.0–9.0 (optimum, pH 7.0) and in the presence of 0–8 % (w/v) NaCl (optimum, 0–2 %). Cells are positive for catalase and weakly positive for oxidase. Tweens 20, 40 and 80, casein and gelatin are hydrolysed, but not starch. Nitrate reduction and H_2S production are negative. Urease and arginine dihydrolase activities are absent. Glucose fermentation does not occur. Milk peptonization and coagulation and aesculin degradation are observed. Assimilates D-glucose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, maltose, gluconate and malate. Does not assimilate D-arabinose, caprate, adipate, citrate or phenylacetate (API 20NE). Positive for esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase (weakly), cysteine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase and β-glucosidase, but negative for alkaline phosphatase, lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase (API ZYM). Acid is produced from D-arabinose (weakly), D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, mannitol, methyl α-D-glucoside, *N*-acetylglucosamine (weakly), amygdalin (weakly), arbutin, aesculin, salicin, cellobiose, maltose, lactose, sucrose, trehalose, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose and D-arabitol (API 50CH). The cell-wall peptidoglycan contains *meso*-diaminopimelic acid as the diagnostic diamino acid. The whole-cell-wall sugars are galactose and glucose. The predominant menaquinone is MK-8(H_4). The polar lipids comprise diphosphatidylglycerol, phosphatidylinositol and an unknown lipid. The major fatty acids (> 10 %) are iso-$C_{15:0}$, anteiso-$C_{15:0}$, anteiso-$C_{17:0}$ and iso-$C_{16:0}$.

The type strain, IP6SC6^T (=DSM 100020^T=CGMCC 4.7300^T), was isolated from surface-sterilized bark of *Bruguiera gymnorhiza* collected from Zhanjiang Mangrove Forest National Nature Reserve in Guangdong, China. The G+C content of the genomic DNA of the type strain is 72.5 mol%.

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