Calidifontibacter indicus gen. nov., sp. nov., a member of the family *Dermacoccaceae* isolated from a hot spring, and emended description of the family *Dermacoccaceae*

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During the course of a study on the bacterial diversity in Western Ghats, India, an actinobacterial strain, designated PC IW02^T, was isolated and characterized by a polyphasic taxonomic approach. Strain PC IW02^T was a non-motile, Gram-positive, short rod that formed creamish white to yellow coloured colonies. 16S rRNA gene sequence analysis showed that the novel strain showed highest sequence similarity with type strains of members of the genus *Dermacoccus*: *Dermacoccus barathri* (96.6%), *Dermacoccus profundus* (96.5%), *Dermacoccus abyssi* (96.4%) and *Dermacoccus nishinomiyaensis* (95.9%). The phylogenetic tree suggested that strain PC IW02^T could represent a member of a new genus of the family *Dermacoccaceae* with the genus *Demetria* as closest clade. Pairwise sequence alignment with *Demetria terrigena* HKI 0089^T and *Kytococcus sedentarius* DSM 20547^T showed similarities of 94.2 and 93.7%, respectively. Strain PC IW02^T had MK-8(H₄) as the major menaquinone. The major fatty acids were iso-C_{16:0} (43.4%), iso-C_{16:1} H (17.2%) and anteiso-C_{17:0} (9.9%). The diagnostic cell-wall amino acid at position 3 of the peptide subunit was lysine; the interpeptide bridge consisted of Gly–Ser–Asp. The polar lipids present were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides and phosphatidylserine, along with two unknown phospholipids. The genomic DNA G+C content of the isolate was 77 mol%. On the basis of phenotypic characteristics, including chemotaxonomic data, and 16S rRNA gene sequence similarities, strain PC IW02^T represents a novel species in a new genus of the family *Dermacoccaceae* for which the name *Calidifontibacter indicus* gen. nov., sp. nov. is proposed. The type strain of *Calidifontibacter indicus* is PC IW02^T (=MTCC 8338^T =DSM 22967^T=JCM 16038^T). An emended description of the family *Dermacoccaceae* is provided.

The genus *Dermacoccus* was proposed by Stackebrandt *et al.* (1995) to accommodate actinomycetes that had been previously classified as members of the genus *Micrococcus*. The genus *Dermacoccus* is the type genus of the family *Dermacoccaceae* (Stackebrandt & Schumann, 2000), which,

at the time of writing, also included the genera *Kytococcus* (Stackebrandt *et al.*, 1995) and *Demetria* (Groth *et al.*, 1997). Members of this family are usually associated with terrestrial habitats or have been isolated from human skin. In this paper, strain PC IW02^T, isolated from a warm spring in the Western Ghats region, India, was characterized using a polyphasic approach and it is proposed that this isolate represents a novel species in a new genus of the family *Dermacoccaceae*.

Strain PC IW02^T was isolated on plate count agar (Hi Media) by dilution plating of a water sample taken from a hot spring in the village of Irde in Puttur Taluka (N 12.77° E 75.22°), Mangalore district, Karnataka State, India. For morphological and biochemical characterization, strain PC

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Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PS, phosphatidylserine.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain PC IW02^T is EF187228.

A supplementary table and figure are available with the online version of this paper.

IW02^T was grown on tryptic soy agar for 3 days at 30 °C. Colony morphology, motility and various biochemical tests were conducted according to Smibert & Krieg (1994). Biochemical tests were also performed using API ZYM and API NE test strips (bioMérieux). Oxidation of various substrates as sole carbon, nitrogen and energy sources was tested using Biolog GP2 MicroPlates in accordance with the manufacturer's instructions, except that tryptic soy broth (TSB) medium was used instead of Biolog universal growth agar medium (Mayilraj *et al.*, 2006); all type species in the three genera of the family *Dermacoccaceae* were also tested. Oxidase and catalase activities were determined using oxidase discs (Hi Media) and 3 % (v/v) H₂O₂ according to the methods described by Smibert & Krieg (1994). Acid production from carbohydrates was studied in peptone water (Lányi, 1987). Fatty acid methyl ester analysis was performed by GC according to the instructions of the Sherlock Microbial Identification System (MIDI; Sasser, 1990) as described previously (Pandey *et al.*, 2002). For analyses of isoprenoid quinones and polar lipids, strain PC IW02^T and *Dermacoccus nishinomiyaensis* DSM 20448^T were cultivated on TSB, grown for 3 days in a rotary shaker (200 r.p.m.) and harvested by centrifugation at 3000 × g. Isoprenoid quinones were extracted and purified as described by Saha *et al.* (2005). Purified quinones were separated by reversed-phase HPLC (SCL-10AVP; Shimadzu) using the solvent system of acetonitrile/2-propanol (65:35) at a flow rate of 1 ml min⁻¹ and monitored at a wavelength of 269 nm. For reference, analysis of the menaquinones of *Dermacoccus nishinomiyaensis* DSM 20448^T, *Nocardioideis albus* DSM 43364, *Chryseobacterium gleum* MTCC 1916^T and *Bacillus subtilis* MTCC 121^T was also carried out. Polar lipids were extracted based on the modified protocol of Bligh & Dyer (1959) and identified by two-dimensional TLC according to procedures described by Komagata & Suzuki (1987). Lipid spots were detected using the spray reagents molybdatophosphoric acid (5 %, w/v) in absolute ethanol, molybdenum blue spray reagent (1.3 %; Sigma), ninhydrin (0.2 %, w/v) in acetone and anisaldehyde reagent (Sigma) for detection of total lipids, phospholipids, aminolipids and glycolipids, respectively. The cell-wall peptidoglycan structure of strain PC IW02^T was determined at DSMZ, Braunschweig, Germany, using established procedures (Schleifer & Kandler, 1972; Schleifer, 1985).

The genomic DNA of the strain was isolated according to the method described by Pitcher *et al.* (1989). The 16S rRNA gene was amplified by PCR using the universal primers 8-27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') as described previously (Pandey *et al.*, 2002). The amplified 16S rRNA gene was sequenced by the dideoxy chain termination method using the BigDye terminator kit followed by capillary electrophoresis on an ABI 310 Genetic Analyzer (Applied Biosystems). Primers used for sequencing were 357F (5'-CTCCTACGGGAGGCAGCAG-3'), 685R (5'-TCTACGCATTTACCGCTAC-3'), 926F (5'-AACTC-

AAAGGAATTGACGG-3') and 907R (5'-CCGTCAATT-CMTTTRAGTTT-3'). The 16S rRNA gene sequences of strain PC IW02^T and those of members of the genera *Dermacoccus*, *Demetria* and *Kytococcus* and type species of other closely related genera retrieved from GenBank were aligned using CLUSTAL_X software (Thompson *et al.*, 1997); alignments were edited manually. 16S rRNA gene sequence similarities were calculated from the alignment. Gaps at the 5' and 3' ends of the alignment were omitted from further analysis. Neighbour-joining and maximum-parsimony trees were constructed using MEGA4 (Tamura *et al.*, 2007).

Colonies of strain PC IW02^T were creamish white to yellow in colour, circular and opaque. Cells were Gram-positive-staining, non-motile, short rods. Physiologically, the isolate differed from its neighbours as follows: salt tolerance; positive for acid production from sugars such as D-fructose, lactose, mannose, raffinose and sucrose; absence of DNase production; and absence of growth at 10 °C. Other detailed differential biochemical characteristics are given in Table 1 and Supplementary Table S1 (available in IJSEM Online). Detailed fatty acid profiles of the isolate and all type strains of members of genera of the family *Dermacoccaceae* are presented in Table 2. It is clear that there are both quantitative and qualitative differences in the fatty acid profiles of strain PC IW02^T and those of the members of the other three genera. Analysis of cellular fatty acids of strain PC IW02^T revealed that iso-C_{16:0} (43.4 %) and iso-C_{16:1} H (17.2 %) were the predominant fatty acids followed by anteiso-C_{17:0} (9.9 %). It is significant here to note that the fatty acid iso-C_{16:1} H was present in high quantities in strain PC IW02^T compared with the other strains studied, whereas C_{17:1ω8c} was present only in traces in strain PC IW02^T. Analysis of the menaquinone system of strain PC IW02^T revealed that MK-8(H₄) was the only menaquinone present. The presence of MK-8(H₄) was confirmed by HPLC using the type strain of *N. albus*, which is reported to have MK-8(H₄) as the major menaquinone, as a standard. Analysis of two-dimensional polar lipid profiles of the isolate showed the absence of phosphatidylethanolamine (PE), which was markedly different from members of the genus *Demetria*. In addition, there were some unique and common lipids that could differentiate strain PC IW02^T from members of the other genera studied (Supplementary Fig. S1, available in IJSEM Online; Table 3). Diphosphatidylglycerol (DPG), phosphatidylglycerol (PG) and phosphatidylinositol (PI) were present in all reported species of the genera *Kytococcus*, *Demetria* and *Dermacoccus* as well as in strain PC IW02^T. In addition, strain PC IW02^T possessed phosphatidylserine (PS) and phosphatidylinositol mannosides (PIM) along with two phospholipids (PL1 and PL2) and two unknown lipids. PIM was present only in strain PC IW02^T. The diagnostic cell-wall amino acid was found to be lysine and the isolate had a distinct peptidoglycan linkage containing Lys-Gly-Ser-Asp compared with members of the other three genera (Table 3).

Chemotaxonomic characteristics (menaquinone type, fatty acids, peptidoglycan type and polar lipid profiles) of

Table 1. Differential characteristics of strain PC IW02^T and members of genera in the family *Dermacoccaceae*

Strains: 1, PC IW02^T; 2, *Dermacoccus nishinomiyaensis* DSM 20448^T; 3, *Dermacoccus abyssi* MTCC 10629^T; 4, *Dermacoccus barathri* MTCC 10630^T; 5, *Dermacoccus profundus* MTCC 10631^T; 6, *K. sedentarius* DSM 20547^T; 7, *K. schroeteri* MTCC 10632^T; 8, *K. aerolatus* MTCC 10633^T; 9, *Demetria terrigena* MTCC 6407^T. All strains are positive for H₂S production.

Characteristic	1	2	3	4	5	6	7	8	9
Colour*	CW-Y	CW-YB	Y	LY	BY	CW	Y	B	WPY
Isolation source†	W	S	M	M	M	S	HB	A	SL
Growth at 42 °C	—	—	—	—	—	—	—	+	—
Growth at pH									
5.2	—	—	+	—	+	—	—	—	+
6.0	+	—	+	+	+	—	—	—	+
9.0	+	+	+	+	+	+	+	+	—
10.0	—	+	+	+	+	—	—	—	—
Growth on NaCl (w/v)									
2 %	+	+	+	—	+	+	+	+	+
5 %	—	+	+	—	+	+	—	+	+
7 %	—	+	+	—	+	+	—	+	—
10 %	—	+	—	—	—	—	—	—	—
Oxidase	+	+	—	+	—	+	—	+	+
Casein hydrolysis	+	—	+	+	+	+	+	+	+
Citrate utilization	—	+	—	—	—	—	—	—	—
Gelatin liquefaction	—	+	—	+	—	—	+	—	—
Urease	—	+	—	—	—	—	—	—	—
Nitrate reduction	+	+	—	—	—	+	—	—	+
Methyl red	+	—	—	—	+	—	—	—	—
Voges-Proskauer	—	+	—	—	—	—	—	—	—
Starch hydrolysis	—	+	—	—	—	+	—	—	—
DNase	—	+	—	—	—	—	—	—	—

*CW, Creamish white; Y, yellow; YB, yellow brown; LY, light yellow; BY, bright yellow; B, beige; WPY, white to pale yellow.

†W, Water; S, skin; M, marine sediments; HB, human blood; A, air; SL, soil.

members of all three reference genera and strain PC IW02^T are given in Table 3. It is quite clear that strain PC IW02^T differs from members of the family *Dermacoccaceae* even though they all share a number of common lipids (Supplementary Fig. S1; Table 3). The fatty acid profile of strain PC IW02^T clearly differed from those of members of all phylogenetically closely related genera. Strain PC IW02^T was closely related at the 16S rRNA gene sequence level to members of the genus *Dermacoccus*, with sequence similarities in the range 95.9–96.6 %. Strain PC IW02^T showed sequence similarities of 94.2 and 93.7 % with the type strains of *Demetria terrigena* and *Kytococcus sedentarius*. The phylogenetic tree showed that strain PC IW02^T fell within the radius of family *Dermacoccaceae* in the suborder *Micrococccineae* and formed a distinct clade with *Demetria terrigena* with a bootstrap value of 58 % (Fig. 1). This was also evident in the phylogenetic tree constructed using the maximum-parsimony method (shown as closed circles at the nodes) where the strain was recovered as a separate clade apart from the genus

Dermacoccus, even though pairwise sequence similarity data gave a different conclusion. The genomic DNA G + C content of the isolate was 77.0 mol%, which was quite high when compared with those of the type species of the other three genera. Therefore, considering all the above phenotypic characteristics, especially the chemotaxonomic characters such as fatty acid profile, polar lipid and cell wall peptidoglycan structure, and sequence similarities, strain PC IW02^T is proposed as a representative of a novel species in a new genus of the family *Dermacoccaceae*.

Stackebrandt *et al.* (1995) delineated the genera *Dermacoccus* and *Kytococcus* from the genus *Micrococcus* and, subsequently, emendation of the families of the suborder *Micrococccineae*, including the family *Dermacoccaceae*, was carried out by the same authors (Stackebrandt & Schumann, 2000). When the family *Dermacoccaceae* was emended, the genus *Demetria* (Groth *et al.*, 1997) was also included. This emendation was based on 16S rRNA gene signature sequences as well as

Table 2. Cellular fatty acid compositions of strain PC IW02^T and members of genera in the family *Dermacoccaceae*

Strains: 1, PC IW02^T; 2, *Dermacoccus nishinomiyaensis* DSM 20448^T; 3, *Dermacoccus abyssi* MTCC 10629^T; 4, *Dermacoccus barathri* MTCC 10630^T; 5, *Dermacoccus profundus* MTCC 10631^T; 6, *K. sedentarius* DSM 20547^T; 7, *K. schroeteri* MTCC 10632^T; 8, *K. aerolatus* MTCC 10633^T; 9, *Demetria terrigena* MTCC 6407^T. ND, Not detected.

Fatty acid	1	2	3	4	5	6	7	8	9
C _{12:0}	ND	ND	ND	ND	ND	ND	ND	4.2	ND
iso-C _{14:0}	0.4	ND	2.4	ND	1.8	ND	ND	ND	0.8
iso-C _{15:0}	4.3	5.9	2.6	9.9	2.4	2.7	4.3	4.0	2.2
anteiso-C _{15:0}	1.6	0.4	0.9	ND	0.8	1.1	1.2	10.6	0.6
iso-C _{16:1} H	17.2	1.1	28.6	18.1	33.1	0.4	0.6	ND	2.0
iso-C _{16:0}	43.4	6.7	43.5	23.1	37.6	3.9	5.5	4.4	18.8
C _{16:0}	3.6	4.0	1.1	2.0	2.6	3.8	3.2	8.4	7.7
iso-C _{17:1} ω9c	5.7	15.4	3.1	10.5	3.0	22.1	33.4	12.3	1.3
anteiso-C _{17:1} ω9c	4.9	0.7	2.9	ND	3.0	5.3	5.3	ND	2.0
iso-C _{17:0}	3.9	18.6	1.5	6.4	1.2	12.7	19.7	15.5	2.8
anteiso-C _{17:0}	9.9	3.3	3.6	1.3	3.1	20.6	17.3	17.5	7.1
C _{17:0} 10-methyl	1.4	5.6	4.4	3.8	3.9	0.7	1.0	ND	1.1
C _{17:1} ω8c	0.7	12.4	2.2	11.7	2.2	13.3	2.2	2.8	8.8
C _{17:0}	0.6	1.4	ND	ND	ND	4.3	0.9	2.1	2.9
C _{18:1} ω9c	0.3	9.2	0.8	3.4	1.1	0.5	0.6	ND	26.3
C _{18:1} ω7c	ND	1.4	ND	0.7	ND	0.3	0.4	ND	2.5
iso-C _{18:0}	0.6	ND	0.7	ND	ND	ND	0.7	ND	1.2
C _{18:0}	1.0	1.0	ND	0.9	1.8	1.7	1.1	8.3	3.2
Summed feature 3*	ND	7.1	1.1	5.0	1.3	1.8	1.9	3.4	5.8

*Summed feature 3 comprised C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

chemotaxonomic characters. However, many novel species of the genus *Dermacoccus* (Pathom-aree *et al.*, 2006a, b) and *Kytococcus* have been proposed since this emendation. In the present study, all type strains belonging to the genera *Dermacoccus*, *Kytococcus* and *Demetria* were characterized. Test results revealed that there were many additional differentiating characteristics among these organisms. Therefore, at this stage, we feel that there is need to emend the description of the family *Dermacoccaceae*.

Description of *Calidifontibacter* gen. nov.

Calidifontibacter (Ca.li.di.fon.ti.bac'ter. L. adj. *calidus* hot; L. n. *fons*, *fontis* spring, fountain; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Calidifontibacter* a rod isolated from hot spring).

Cells are Gram-positive-staining, short rods and do not form endospores. The peptidoglycan structure is of type A4x with the linkage Lys–Gly–Ser–Asp. The menaquinone

Table 3. Chemotaxonomic characteristics that distinguish strain PC IW02^T from related strains

Strains: 1, PC IW02^T; 2, *Dermacoccus nishinomiyaensis* DSM 20448^T (Stackebrandt *et al.*, 1995); 3, *K. sedentarius* DSM 20547^T (Stackebrandt *et al.*, 1995); 4, *Demetria terrigena* MTCC 6407^T (Groth *et al.*, 1997). Fatty acid data are from the present study.

Characteristic	1	2	3	4
Quinone system	MK-8(H ₄)	MK-8(H ₂)	MK-8, MK-9, MK-10	MK-8(H ₄)
Polar lipids*	DPG, PG, PI, PS, PIM, UL1–4	DPG, PG, PI	DPG, PG, PI	DPG, PG, PI, PE
Peptidoglycan type	L-Lys–L-Gly–L-Ser–D-Asp	L-Lys–L-Ser _{1,2} –D-Glu or L-Lys–L-Ser _{1,2} –L-Ala–D-Glu	L-Lys–Glu ₂	L-Lys–L-Ser–D-Asp
Major fatty acids	iso-C _{16:0} , iso-C _{16:1} H, anteiso-C _{17:0} , iso-C _{17:1} ω9c	iso-C _{15:0} , iso-C _{16:0} , iso-C _{17:0} , C _{17:1} ω8c, iso-C _{17:1} ω9c, C _{18:1} ω9c	iso-C _{17:0} , anteiso-C _{17:0} , C _{17:1} ω8c, iso-C _{17:1} ω9c	iso-C _{16:0} , C _{16:0} , anteiso-C _{17:0} , C _{18:1} ω9c, C _{17:1} ω8c
DNA G + C content (mol%)	77	66–71	68–69	66

*PL, phospholipids; UL, unknown lipid.

Fig. 1. Phylogenetic tree constructed using the neighbour-joining method showing the relationships between strain PC IW02^T and other related taxa. Bootstrap values (percentages of 1000 replications) are shown at the branch points. The 16S rRNA gene sequence from *Micrococcus luteus* DSM 20030^T (GenBank accession no. AJ536198) was used as the outgroup. Bar, 0.005 substitutions per site. Branches recovered in the maximum-parsimony algorithm are indicated by filled circles.

present is MK-8(H₄). The predominant fatty acid is iso-C_{16:0}, followed by iso-C_{16:1} H and anteiso-C_{17:0}. The major polar lipids are DPG, PG, PI, PIM and PS. The type species is *Calidifontibacter indicus*, a member of the family *Dermacoccaceae*.

Description of *Calidifontibacter indicus* sp. nov.

Calidifontibacter indicus (in.di'cus. L. masc. adj. *indicus* Indian, referring to the isolation of the type strain from India).

Has the following characteristics in addition to those given in the genus description. Cells are 1–2 µm long, non-motile and aerobic. Catalase-positive. Colonies are creamish white to yellow in colour and opaque with rough margins. Grows at 20–37 °C. The optimum growth temperature is 30 °C. Does not grow at 42 °C. Tolerates a maximum of 4 % (w/v) NaCl. Grows at pH 5.0–8.0. Positive for nitrate reduction and casein, gelatin and Tween 80 hydrolysis. Negative for H₂S production, starch, DNase production and aesculin hydrolysis. Data for acid production from various sugars, oxidation of carbon and nitrogen sources using Biolog GP2 MicroPlates and sensitivity to different antibiotics are given in Supplementary Table S1. According to the API ZYM enzyme assay, positive for esterase, esterase lipase, leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase.

The type strain is PC IW02^T (=MTCC 8338^T=DSM 22967^T=JCM 16038^T), isolated from a warm spring, Western Ghats (N 12.77° E 75.22°), India. The genomic DNA G + C content of the type strain is 77 mol%.

Emended description of the family *Dermacoccaceae*

Dermacoccaceae (Der.ma.coc.ca'ce.ae. N.L. masc. n. *Dermacoccus* type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. *Dermacoccaceae* the *Dermacoccus* family).

The family contains the type genus *Dermacoccus* (Stackebrandt *et al.*, 1995) and the genera *Kytococcus* (Stackebrandt *et al.*, 1995), *Demetria* (Groth *et al.*, 1997) and *Calidifontibacter* (this study). Besides the characteristics mentioned above, members of the family are capable of growing on 2–10 % NaCl. Do not hydrolyse Tween 20 or Tween 40. Do not liquefy gelatin. Most species are sensitive to streptomycin, polymyxin B and gentamicin. Negative for the oxidation of the following sugars in Biolog GP2 MicroPlates: α-cyclodextrin, β-cyclodextrin, glycogen, inulin, mannan, N-acetyl-D-glucosamine, N-acetyl-β-D-mannosamine, amygdalin, arbutin, cellobiose, D-fructose, m-inositol, α-lactose, lactulose, D-mannitol, D-mannose, methyl α-D-galactoside, methyl β-D-galactoside, 3-methyl-D-glucose, methyl α-D-glucoside, methyl β-D-glucoside, methyl

α -D-mannoside, palatinose, D-psicose, salicin, D-sorbitol, stachyose, D-tagatose, AMP, UMP, D-fructose 6-phosphate, α -D-glucose 1-phosphate and D-glucose 6-phosphate. The major fatty acids include iso-C_{16:0}, iso-C_{16:0} H and anteiso-C_{17:0}. The peptidoglycan structure is of the A4 α pattern with the linkage Lys-Gly-Ser-Asp. The common polar lipids are DPG, PG and PI; in addition, PIM and PS are also present.

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