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TAXONOMIC DESCRIPTION
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Chryseomicrobium excrementi sp. nov., a Gram-stain-positive rod-shaped bacterium isolated from an earthworm (*Eisenia fetida*) cast

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Abstract

A Gram-stain-positive, rod-shaped, slightly halotolerant, nitrate-reducing bacterial strain, designated ET03^T, was isolated from the cast of an earthworm (*Eisenia fetida*) reared at the Centre of Floriculture and Agribusiness Management, University of North Bengal at Siliguri, West Bengal, India. On the basis of 16S rRNA gene sequence phylogeny, the closest relative of strain ET03^T was *Chryseomicrobium palamuruense* PU1^T (99.1 % similarity). The DNA G+C content of strain ET03^T was 42.9 mol%. Strain ET03^T contained menaquinone-8 as the most predominant menaquinone and phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannoside and phosphatidylglycerol as the main polar lipids. The diagnostic diamino acid was meso-diaminopimelic acid. Major cellular fatty acids were iso-$C_{15:0}$, $C_{16:1}\omega7c$ alcohol and iso-$C_{16:0}$. Other biochemical and physiological analyses supported genotypic and phenotypic differentiation of the strain ET03^T from its nearest taxonomic neighbours: *Chryseomicrobium palamuruense, Chryseomicrobium amylolyticum, Chryseomicrobium imtechense, Chryseomicrobium aureum* and *Chryseomicrobium deserti*. The draft genome of strain ET03^T consisted of 2.64 Mb distributed in 14 scaffolds (N_{50} 894072). A total of 2728 genes were predicted and, of those, 2664 were protein-coding genes including genes involved in the degradation of polychlorinated biphenyl and several aromatic compounds. The isolate, therefore, represents a novel species, for which the name *Chryseomicrobium excrementi* sp. nov. is proposed. The type strain is ET03^T (=KCTC 33943^T=LMG 30119^T=JCM 32415^T).

The genus *Chryseomicrobium* was established by Arora *et al.* [1], within the family *Planococcaceae* of the order *Bacillales* and class *Bacilli* within the phylum *Firmicutes*, for some non-sporulating, non-motile, Gram-stain-positive rods. At present, five species isolated from diverse habitats, ranging from tropical soil, sewage sediment to desert soil, have been reported in this genus. These are *Chryseomicrobium imtechense* [1], *Chryseomicrobium amylolyticum* [2], *Chryseomicrobium aureum* [3], *Chryseomicrobium palamuruense* [4] and *Chryseomicrobium deserti* [5]. In the present study, we have characterized strain ET03^T, which represents a novel member of the genus *Chryseomicrobium*. The organism was isolated from the freshly liberated cast of an earthworm (*Eisenia fetida*) reared at the Centre of Floriculture and Agribusiness Management of University of North Bengal at Siliguri (26.7072° N, 88.3558° E), West Bengal, India.

For isolation of strain ET03^T, *Eisenia fetida* earthworms were washed several times with sterile distilled water and left on sterile tissue paper. Freshly liberated cast pellets were collected aseptically, serially diluted (in PBS, pH 7.2), plated on Luria agar (LA; M575, HiMedia) and incubated aerobically overnight at 37 °C. Single colonies were picked and purified by streaking on LA plates. Golden yellow coloured colonies that developed on LA plates were isolated and stored for taxonomic analyses. The strain was also capable of growing in other media such as nutrient broth (HiMedia) and tryptone soy broth (HiMedia). LA was used for

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Keywords: *Chryseomicrobium excrementi*; ET03T; cellular fatty acids; 16S rRNA gene sequence; COFAM; draft genome.
Abbreviations: AAF, assembly and alignment-free; ANI, average nucleotide identity; COFAM, Center of Floriculture and Agribusiness Management; DDH, DNA–DNA hybridization; DSM, Deutsche Sammlung von Mikroorganismen; EMBL, European Molecular Biology Laboratory; FAME, fatty acid methyl esters; GGDC, genome-to-genome direct comparison; JCM, Japan Collection of Microorganisms; KCTC, Korean Collection for Type Cultures; LMG, Laboratory of Microbiology-UGent; MK, menaquinone; NCBI, National Center for Biotechnology Information; ONPG, *ortho*-Nitrophenyl-β-galactoside; PE, phosphatidyl-ethanolamine; PI, phosphatidyl-inositol; PIM, phosphatidylinositol mannoside; PG, phosphatidyl-glycerol; TSB, tryptone soy broth; VP, Voges–Proskauer.
The GenBank accession number for the 16S rRNA gene sequence of ET03^T is KU230523.2. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number PCGR00000000. The version described in this paper is version PCGR01000000.
Three supplementary tables and four supplementary figures are available with the online version of this article.

maintenance of strain ET03^T and for the determination of phenotypic and chemotaxonomic characteristics.

Cell morphology and motility were determined with a phase-contrast microscope (Olympus CH2); details of the cell shape were ascertained with the help of a scanning electron microscope (EVO LS10, Zeiss). The Gram reaction was performed by the KOH lysis method [6] and further confirmed by the Gram-staining method of Claus [7]. The growth range of strain ET03^T was determined at 4, 10, 20, 28, 30, 37, 40 and 45 °C (±1 °C). For salt tolerance tests, 2, 4, 6, 8, 10, 15 and 20 % (w/v) NaCl was added to peptone–yeast extract medium (composition: 10 g peptone, 5 g yeast extract) devoid of NaCl or KCl. To assess growth at different pH levels, the pH of the sterile Luria–Bertani medium was adjusted from pH 3.0 to 12.0 by using either 0.1 M HCl or 0.1 M NaOH. Results were obtained after 48 h incubation at 37 °C. Catalase activity was examined by the production of oxygen bubbles after the addition of few drops of 3 % (v/v) H_2O_2. The ability to hydrolyse starch was determined by assessing the development of clear zones (after treatment with Gram's iodine solution) around the streaked culture on starch agar plates (nutrient agar 2.3 %; soluble starch 0.5 %; pH 7.2). The Voges–Proskauer test was performed by observation of colour development after the addition of alpha-naphthol and potassium hydroxide to the Voges–Proskauer broth of strain ET03^T culture. Urease test was performed by observing the development of a deep red colour at the periphery of the bacterial colonies on Christensen's urea agar plates [8]. Other biochemical characteristics such as the presence of amylase, cellulase, gelatinase, caseinase and phosphatase, tests such as H_2S production, indole test, methyl red, citrate utilization, ortho-nitrophenyl-β-galactoside test, nitrate reduction, ability to ferment glucose, lactose fermentation, and maltose fermentation ability were examined by following standard methods [9–11]. Phenotypic characterization of strain ET03^T was performed using the Biolog GEN III MicroPlate following the manufacturer's instructions. Briefly, bacterial suspension, prepared in a special 'gelling' inoculating fluid, was transferred onto the GEN III MicroPlate (100 µl per well). Incubation was carried out in an aerobic atmosphere for 48 h. Increased respiration due to the growth of bacteria using the single carbon source provided in each well caused reduction of the tetrazolium redox dye, forming a purple colour. The reactions were read using the fully automated OmniLog system. Carbon source utilization assays were also determined by using HiCarbohydrate kit parts A, B and C (HiMedia) according to the manufacturer's protocol. Antibiotic susceptibility (specific for oligotrophic bacteria) was determined according to the method described by Kumar *et al.* [12]. Susceptibilities to some of the antibiotics were also tested using the Biolog GEN III MicroPlate (columns 10–12) and the results were interpreted according to the manufacturer's instructions.

For the study of quinones and polar lipids, two-stage lipid extraction using methanol–hexane (2 : 1 v/v) described by Tindall *et al.* [13] was undertaken with modifications. Briefly, the menaquinone part was purified by running the hexane fraction on thin-layer chromatography (TLC) silica gel 60 F254 (Merck) using petroleum benzene: di-ethyl-ether (0.85 : 0.15) as the solvent. Further development of menaquinone components was performed using acetone–water (0.99 : 0.01) as the solvent and observed under UV light. The presence of *men* genes, coding enzymes for menaquinone biosynthesis, was identified in the genome of strain ET03^T to validate the results. Polar lipids were extracted from the methanolic phase using chloroform–methanol–0.3 % NaCl (1 : 2 : 0.8) as the extraction medium [14–16]. Polar lipids were separated by two-dimensional TLC on silica gel. In the first dimension, chloroform–methanol–water (65 : 25 : 4, v/v), and in the second dimension, chloroform–methanol–acetic acid–water (80 : 12 : 15 : 4, v/v) were used as the solvents. Lipid functional groups were identified using spray reagents specific for phospholipids (Mb-Blue), free amino groups (ninhydrin) and sugars (α-naphtol). The peptidoglycan structure was determined by using a hydrolysate of purified cell wall following methods described by Schleifer and Kandler [17] and subsequent TLC analyses as described by Staneck and Roberts [18], and Hancock [19]. The presence of genes coding for the specificity of Mur ligases (the enzymes responsible for the synthesis of the peptide stem of the peptidoglycan structure), were identified in the genome of strain ET03^T to validate the results. For analysis of fatty acids, fatty acid methyl esters were extracted from 36 h old (exponentially growing) cells grown in tryptone soy agar (M290; HiMedia) at 37 °C. They were then analysed by gas chromatography (Hewlett Packard 5890 II plus) and the Sherlock Microbial Identification System using version 4.10 of the TSBA40 library (Microbial ID).

The 16S rRNA gene of strain ET03^T was amplified from the genomic DNA prepared by the standard method [20], purified and sequenced according to Kumar *et al.* [12]. The obtained 16S rRNA gene sequence was compared with entries in the updated GenBank and EMBL databases by using the BLASTn program [21–23]. To determine the phylogenetic affiliation, the 16S rRNA gene sequence of strain ET03^T was aligned with the sequences of members of the genus *Chryseomicrobium* by using the CLUSTAL_W program [24]. Evolutionary relationships of members of genus *Chryseomicrobium* were inferred using three different tree-making algorithms (neighbour-joining [25], maximum-likelihood [26] and maximum-parsimony [27]) in MEGA 6.0 [28]. Phylogenetic analyses and the fidelity of the tree topologies were evaluated by bootstrap analysis with 1000 replicates [29, 30].

The genome of strain ET03^T was sequenced using NextSeq 500. Briefly, approximately 200 ng DNA was fragmented by covaris M220 to generate ~400 bp segments. End-repaired products were size-selected by AMPure XP beads, PCR amplified with index primers and analysed in a 4200 Tape Station system (Agilent). After obtaining Qubit concentration, PE Illumina libraries were loaded onto the NextSeq 500 for cluster generation and sequencing. The copied reverse strands were then used to sequence from the opposite end of the fragment. Thus adapter-free data of 1.1 Gb

was generated, which is required for the genome to be used for taxonomic purpose [31]. The high-quality reads were then *de novo* assembled by using the SPAdes genome assembler. Prokka [32] was used to predict the genes from final scaffolds. The NCBI annotation pipeline was used to annotate the whole genome sequence (WGS) of strain ET03^T. G+C content was estimated by using the genomic dataset. Since an earlier study has indicated that the G+C content varies no more than 1 % within species and by using 'logistic regression model based on all pairs of genomes, the probability of [digital DNA–DNA hybridization (DDH)] $\geq$70 % was 0.8443 for a 0 % difference in G+C content, 0.05 for 0.7271 %, 0.009 for 1 %, and virtually zero for 3 and 5 %' [33], DDH between ET03^T and other species of the genus *Chryseomicrobium* was not required because the difference in G+C content with the other five species is >5.0. An assembly and alignment-free (AAF) method [34] was used to reconstruct phylogeny from next-generation sequencing data. We calculated the BLAST-based average nucleotide identity (ANI) scores with the genome sequences of strain ET03^T and other related WGSs available in the databases using the JSpecies WS program with default parameters [35]. Analysis was also performed using all three equations in the Genome-to-Genome Distance Calculator (GGDC) online program, version 2.1 [33].

Cells of strain ET03^T were Gram-stain-positive, non-motile rods, measuring 1.5±0.5 µm long and 0.5 µm wide (Fig. S1, available in the online version of this article). Differential physiological and biochemical properties of strain ET03^T and its closest phylogenetic neighbours are shown in Table 1. Strain ET03^T contained menaquinone-8 (MK-8) as the most predominant menaquinone (Fig. S2). This phenotype corroborated with the genotype derived from WGS analyses of ET03^T. In the pathway of MK-8 biosynthesis, enzymes encoded by *men* genes (*men*F, *men*D, *men*H, *men*C, *men*E, *men*B and *men*A) and the *ubi*E (menG) gene have been duly annotated in the NCBI database. The enzymes engaged in MK-8 biosynthesis are: MenF – isochorismate synthase (accession no. PJK17315), MenD – 2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate synthase (PJK17316), MenH – SHCHC synthase (PJK17317), MenC – o-succinyl benzoate synthase (PJK17077), MenE – o-succinylbenzoic acid-CoA synthase PJK17319), MenB – 1,4-dihydroxy-2-naphthoyl-CoA synthase (PJK17318), MenA – 1,4-dihydroxy-2-napthoate octaprenyl-transferase (PJK16692) and UbiE (MenG) – 2-methoxy-6-polyprenyl-1,4-benzoquinol methylase (PJK17772). Phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl glycerol and phosphatidyl inositolmannoside were identified as the phosplolipids present in strain ET03^T (Fig. S3). The cell-wall peptidoglycan of strain ET03^T contained L-alanine, D-glutamic acid, and meso-diaminopimelic acid in the peptide stem. In the bacterial peptidoglycan structure, the variation of the peptide stem is due to the specificity of the Mur ligases, the enzymes responsible for its synthesis. The first, second and third amino acids of the peptide stem are added by the MurC, MurD and MurE ligases, respectively. In the genome of strain ET03^T, *mur*C, *mur*D, and *mur*E gene was analysed to code for UDP-*N*-acetylmuramate-alanine ligase (accession no. PJK17354), UDP-*N*-acetylmuramoylalanine-D-glutamate ligase (PJK17947) and UDP-N-acetylmuramoyl-L-alanyl-D-glutamate-2,6-diaminopimelate ligase (PJK166217 and PJK15783), respectively.

The major cellular fatty acids were 13-methyltetradecanoic acid or iso-$C_{15:0}$ (45 %), (9Z)-9-hexadecenoic acid or $C_{16:1}$ $\omega7c$ alcohol (13 %) and 14-methylpentadecanoic acid or iso-$C_{16:0}$ (11.8 %). Trace amounts of 12-methyltridecanoic acid or iso-$C_{14:0}$ (6.2 %), 12-methyltetradecanoic acid or anteiso-$C_{15:0}$ (3.4 %), 15-methylhexadecanoic acid or iso-$C_{17:0}$ (2.2 %) and 14-methylhexadecanoic acid or anteiso-$C_{17:0}$ (1.6 %) were present, which is typical of members of the genus *Chryseomicrobium*, but the proportions differed from those reported for other members of the genus (Table S1).

An almost-complete 16S rRNA gene sequence comprising 1517 bp was obtained, which was reliable for delineation of taxonomic hierarchy [36]. According to the comparison of 16S rRNA gene sequences (using NCBI, BLASTN), the closest relatives of strain ET03^T were *C. palamuruense* strain PU1^T (99.1 %), *C. aureum* strain BUT-2 (99.0 %), *Psychrobacillus psychrodurans* strain BAB-2243 (99.0 %), *C. amylolyticum* strain ID4 (98.9 %), *C. imtechense* strain HWG-A7 (98.4 %), *C. deserti* strain THG-T1 (96.3 %), *Planococcus rifietoensis* strain M8 (95.7 %), *Planococcus plakortidis* strain DSM 23997 (95.6 %) sequentially followed by other members of the Family *Planococcaceae*. A tree depicting the phylogenetic position of strain ET03^T within the genus *Chryseomicrobium* is shown in Fig. 1. Based on 16S rRNA gene sequence comparison, strain ET03^T forms a distinct subcluster with *C. palamuruense* PU1^T and other members of the genus *Chryseomicrobium*. Using BLASTn, it was revealed that the *gyr*B sequence of strain ET03^T produced maximum identity (83 %) with the *gyr*B gene sequence of *C. imtechense* MW10 (accession no. HM989964; the only *gyr*B sequence from any strain of the genus *Chryseomicrobium* available in the database) followed by *Planococcus* species PAMC 21323 (accession no. CP009129; 76 %) and *Planococcus maritimus* strain Y42 (accession no. CP019640; 74 %) showing greater sequence divergence, compared to the BLASTn results with the 16S rRNA gene sequence of ET03^T.

The draft genome of strain ET03^T includes 2 644 068 bp distributed in 14 scaffolds (N_{50} 894072). Based on whole-genome data, the DNA G+C content of ET03^T was calculated as 42.9 mol%. The difference of G+C content between strain ET03^T and the five previously described species was >5 %, which is well within the range consistent with species belonging to the same genus [33]. A total of 2728 genes were predicted, of which, 2664 were protein-coding genes including 121 *de novo* genes with no BLAST hit. Of the protein-coding genes, there were at least six genes coding for several dioxygenases [2-nitropropane dioxygenase (accession no. PJK17992), biphenyl 2,3-dioxygenase (PJK16435), glyoxalase/bleomycin resistance/estradiol dioxygenase (PJK16272) and three ring-cleaving dioxygenases (PKJ15803, PKJ15871 and

Table 1. Characteristics that differentiate strain ET03^T from other members of the genus *Chryseomicrobium*

Strains: 1, ET03^T; 2, *Chryseomicrobium imtechense* MW 10^T; 3, *Chryseomicrobium amylolyticum* JC16^T; 4, *Chryseomicrobium aureum* BUT-2^T; 5, *Chryseomicrobium palamuruense* PU1^T; 6, *Chryseomicrobium deserti* THG-T1.1B^T. +, Positive; –, negative. AL, unidentified aminolipid; DPG, diphosphatidylglycerol; GL, unidentified glycolipid; PE, phosphatidyl ethanolamine; PG, Phosphatidyl-glycerol; PI, phosphatidyl inositol; PIM, phosphatidylinositol mannoside; PL, unidentified phospholipid; m-DAP, meso-diaminopimelic acid; ND, not detected.

Characteristic	1	2	3	4	5	6
Cell size (μm) (length×diameter)	1.5–2.2×0.5–0.6	1.7–2.9×0.3–0.7	2.0–3.0×1.0	1.5–2.0×0.5–0.86	1.6–2.0×0.6–0.7	2.4–2.7×0.5–0.7
Motility	Non-motile	Non-motile	Non-motile	Non-motile	Motile	Non-motile
Growth temperature range (optimum)	20–40 °C (35–37)	4–45 °C	25–40 °C (30–37)	20–35 °C	18–40 °C	20–35 °C (28–30)
pH range (optimum)	6–9	6–9	7–11 (7–8)	7–10	7 to 10	5–7 (7)
NaCl tolerance limit (%, w/v)	8	6	5	7	9	3
Catalase reaction	–	–	+	–	+	+
Nitrate reduction	+	–	–	–	–	–
Voges–Proskauer reaction	–	+	–	–	–	–
Urease activity	–	–	–	+	+	+
Oxidase	–	–	–	–	+	–
Hydrolysis of:						
Starch	–	–	+	–	+	–
Gelatin	+	–	–	+	–	– ?
Organic substrates utilized for growth:						
Citrate	–	+	–	–	+	+
Glycerol	–	+	+	–	+	–
Acid production from various carbohydrates:						
Glucose	+	+	–	–	+	–
Salicin	+	+	–	+	–	–
Mannose	–	–	+	–	+	+
Fructose	+	+	–	–	–	–
Maltose	+	+	–	–	+	+
Sucrose	–	+	–	–	–	+
Inulin	–	+	–	+	–	–
Trehalose	–	–	+	–	–	+
Melibiose	–	+	–	–	+	+
Cellobiose	–	+	–	–	+	–
Menaquinones (descending abundance)	MK-8, MK-7, MK-6	MK-7, MK-8, MK-7$_{H2}$, MK-6	MK-7, MK-8, MK-6	MK-7, MK-6, MK-8	MK-8	MK-7, MK-8, MK-6
Polar lipids	PE, PI, PIM, PG	DPG, PG, PE, PC, GL	DPG, PG, PE, AL, PL,	DPG, PG, PE, PL	DPG, PG, PE	DPG, PE, PG, GL, AL
Peptidoglycan type	m-DAP	L-Lys-D-Asp	L-Orn-D-Glu	L-Orn-D-Glu	L-Orn	L-Orn-D-Glu
DNA G+C content (mol%)	42.9	53.4	57.6	48.5	48.5	50.4

PJK15941). These microbial genes are responsible for degradation of biphenyls such as polychlorinated biphenyl and several xenobiotic aromatic compounds in the environment. The phylogenetic tree reconstructed from next-generation sequencing data using the AAF method depicts the distinction of strain ET03^T from its taxonomic neighbours (Fig. S4). ANI scores generated during global comparisons of the genome sequence [37] of strain ET03^{T}with previously deposited WGSs in databases indicates sufficient distance from *Bhargavaea cecembensis* T14, *Jeotgalibacillus malaysiensis* D5, *Planomicrobium glaciei* UCD-HAM, *Solibacillus silvestris* StLB046, *Paenisporosarcin*a species HGH0030, *Planococcus kocurii* ATCC 43650, *Planococcus maritimus* MKU009, *Planococcus plakortidis* DSM 2399739, *Planococcus rifietoensis* M8 and *Sporosarcina psychrophila* DSM 6497, which supports the findings of 16S rRNA gene phylogeny (Table S2). The ANI score for strain comparisons between EAG3^T and *Paenisporosarcin*a species HGH0030 was the highest 68.6 % (coverage 38.8 %), which is far below 95–96 % cut-off value for novel species determination by this approach. The GGDC results with BLAST+ for strain EAG3^T and other taxonomic neighbours in the family *Planococcaceae* gave DNA–DNA homology values of <30 % by any of the three models used, assuring sufficient distance among the genomes taken for the analysis (Table S3).

Strain ET03^T can be readily differentiated from its closest relative, *C. palamuruense* PU1^T, with reference to

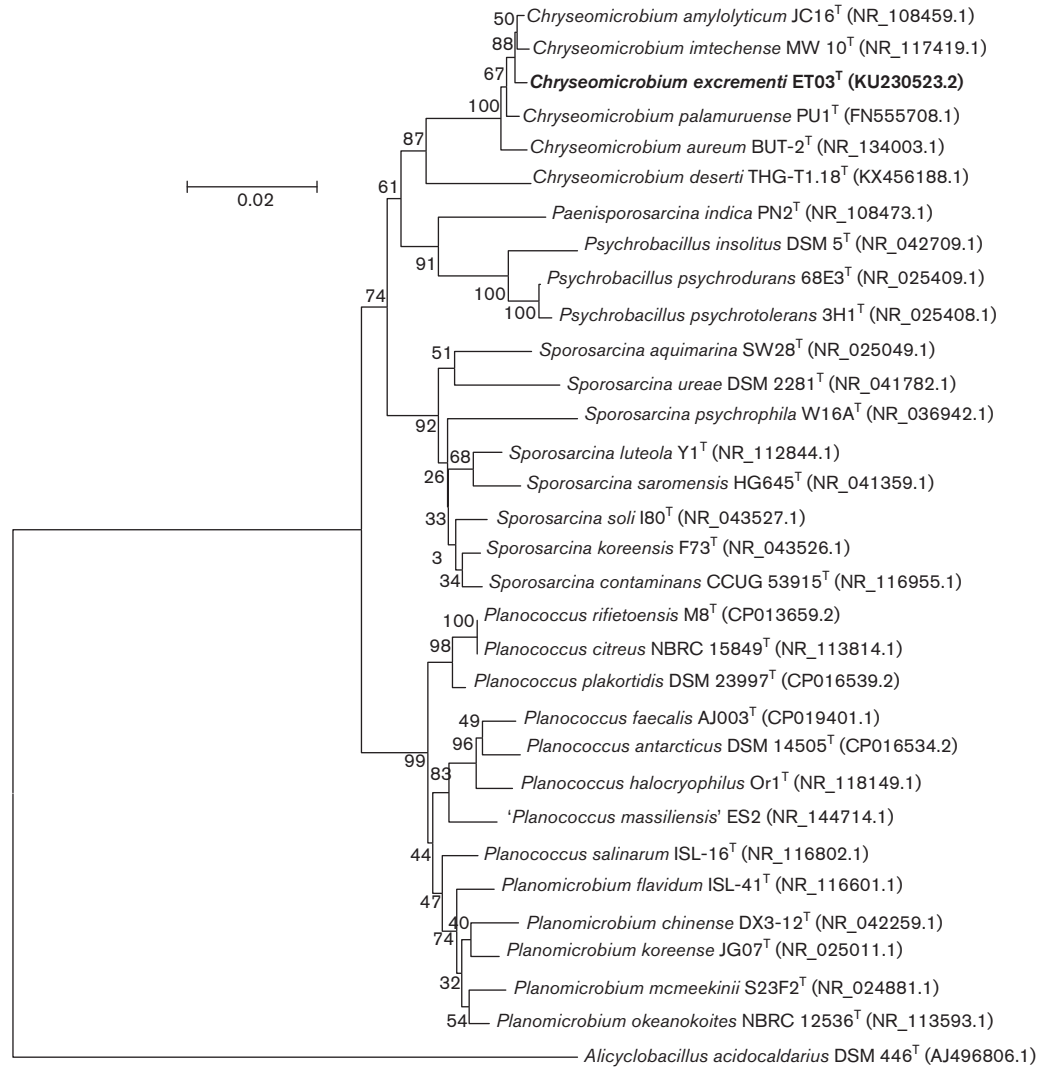

Fig. 1. Phylogenetic tree reconstructed by the neighbour-joining method based on 16S rRNA gene sequences showing the phylogenetic relationship between strain ET03^T and closely related species. Bootstrap percentages (based on 1000 replications) are shown at the nodes. The GenBank accession numbers for 16S rRNA gene sequences are shown in parentheses. *Alicyclobacillus acidocaldarius* strain DSM 446 (AJ496806.1) was used as an outgroup. Bar, 2 nt substitution per 100 nt.

its physiological and biochemical characteristics (e.g. strain ET03^T is negative for catalase, urease and oxidase but PU1^T is positive for those whereas ET03^T is positive for nitrate reduction but PU1^T is negative), cellular fatty acids, 16S rRNA gene sequence and DNA G+C content (Table 1). On the basis of the data obtained from our study using a polyphasic taxonomic approach, strain ET03^T merits recognition as a novel species of the genus *Chryseomicrobium*, for which we propose the name *Chryseomicrobium excrementi* sp. nov.

DESCRIPTION OF *CHRYSEOMICROBIUM EXCREMENTI* SP. NOV

Chryseomicrobium excrementi (ex.cre.men'ti. L. gen. n. *excrementi* - of excreta).

Cells stain Gram-positive and are non-motile, spore-forming rods, 1.5±0.5μm long and 0.5 μm wide. Colonies are golden yellow, flat, circular with a smooth margin when grown on LA plates for 16 h at 37 °C. Growth is observed at 20–40 °C (optimum, 35–37 °C), pH 6–9 (optimum, 7.0) and can tolerate concentrations of NaCl up to 8 % (optimum, 2 %). Negative for catalase and oxidase. Can utilize D-salicin, D-fructose, 3-methyl glucose, L-rhamnose, D-arabitol, myo-inositol, D-glucose-6-PO4, L-arginine, L-aspartic acid, L-glutamic acid, L-pyroglutamic acid, D-gluconic acid, quinic acid, L-malic acid, acetoacetic acid and acetic acid; but is unable to utilize trehalose, cellobiose, gentiobiose, sucrose, turanose, stachyose, raffinose, lactose, melibiose, methyl α-D-glucoside, *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, *N*-acetyl neuraminic acid, D-mannose,

D-galactose, D-fucose, L-fucose, Inosine, D-sorbitol, D-mannitol, glycerol, D-fructose-6-PO_4, D-aspartic acid, D-serine, gelatin, glycyl-L-proline, L-alanine, L-histidine, L-serine, pectin, D-galacturonic acid, D-glucuronic acid, glucuronamide, mucic acid, D-saccharic acid, methyl pyruvate, D-lactic acid methyl ester, L-lactic acid, citric acid, keto-glutaric acid, D-malic acid, bromo-succinic acid, Tween 40, amino-butryric acid, hydroxy-butyric acid, keto-butyric acid, propionic acid and formic acid. The strain is sensitive to fusidic acid, D-serine, troleandomycin, minocycline, lincomycin, guanidine HCl, Niaproof 4, vancomycin and tetrazolium blue. The strain is resistant to 1 % sodium lactate, nalidixic acid, lithium chloride, potassium tellurite, aztreonam and sodium butyrate.

Strain ET03^T contains MK-8 as the predominant menaquinone and phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannoside and phosphatidylglycerol as the main polar lipids. The diagnostic diamino acid is meso-diaminopimelic acid. The major cellular fatty acids are iso-$C_{15:0}$, $C_{16:1}\omega7c$ alcohol and iso-$C_{16:0}$.

The type strain, ET03^T (=KCTC 33943^T=LMG 30119^T =MCC378^T), was isolated from cast of an earthworm (*Eisenia fetida*) reared at the Centre of Floriculture and Agribusiness Management of University of North Bengal at Siliguri (26.7072° N, 88.3558° E), West Bengal, India. The DNA G+C content of strain ET03^T is 42.9 mol%.

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

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